

Edited by
Clemens Lamberth and Jürgen Dinges

Bioactive Carboxylic Compound Classes

Pharmaceuticals and Agrochemicals



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Preface

Three years ago, shortly after the publication of our first book “Bioactive Heterocyclic Compound Classes,” we had been contacted by several readers, but also contributors, who praised the concept of combining all important heterocyclic active ingredient classes with short, educational, similarly structured chapters within one book. This positive feedback encouraged us to apply the same concept also to the second major group of functional groups, which play an important role in pharmaceuticals and agrochemicals. These are, besides the already covered heterocycles, the derivatives of carboxylic acids, which means not only the acids themselves but also their esters, amides, ureas, carbamates, hydrazides, thioesters, and so on.

According to our definition, such a carboxylic compound class is made up of three or more commercialized active ingredients that (i) bear the same carboxyl functionality, means acyclic C=O functions except aldehydes and ketones; (ii) possess the same or at least a similar scaffold and a similar substitution pattern; and (iii) have the same mode of action. To be able to cover the most important carboxylic active ingredient families in medicine and crop protection within one book, we had to focus. Therefore, we declared the following functional groups or compound classes to be “out of scope”: (i) all functional groups based on a C=S motif; (ii) cyclic carboxylic compounds with a C=O group (lactones, lactams, diketopiperazines), as they have been covered already in “Bioactive Heterocyclic Compound Classes”; (iii) sulfonic acid derivatives with a SO₂ function; and (iv) peptides and macrocycles.

As it was already the case for “Bioactive Heterocyclic Compound Classes,” also the chapters of this book are divided into the same five sections: (i) introduction, (ii) history, (iii) synthesis, (iv) mode of action, and (v) structure–activity relationship. Only the agrochemical chapters possess an additional section “biological activity” to describe the target spectrum of the active ingredients.

We are very grateful to the authors of the 36 chapters of this book, all of them expert in their field and several of them already contributing to “Bioactive Heterocyclic Compound Classes,” for spending their scarce time summarizing the historical background, typical chemical syntheses, biochemical modes of action, biological activities, and structure–activity relationships of their area of interest.

It was again Anne Brennführer of Wiley-VCH to whom we are thankful for her help in starting also this book, from a first discussion at the Wiley booth during the German Chemical Society conference “Wissenschaftsforum Chemie 2013” in Darmstadt, our alma mater, through different phases such as creating the concept, finding the authors, and writing the chapters.

Finally, one more big thank you to our wives Annette and Petra, who again accepted that science sometimes took over too much of our spare time, you are the greatest!

Stein and North Chicago, March 2016

*Clemens Lamberth
Jürgen Dinges*

1

Different Roles of Carboxylic Functions in Pharmaceuticals and Agrochemicals

Clemens Lamberth and Jürgen Dinges

1.1

Introduction

Explaining the importance of carboxylic acid and its related derivatives in medicine and crop protection is best achieved by examining the number of endogenous processes and molecules that rely on the chemical nature of this functional group. From amino acid conjugation via peptide synthesis to proteins and posttranslational protein acylation to triglycerides, bile acids, and prostanoids, it is evident that carboxylic acid, ester, and amide functions contribute to the physiology of many living systems [1]. Not surprisingly then, there exists an extensive number of active ingredients bearing such functions. Roughly 25% of all commercialized pharmaceuticals contain a CO_2H group [2]; a similar portion (25%) is reported to bear an amide [3]. A similar ratio is true for agrochemicals; at least 40% of all marketed crop protection agents bear a carboxylic function [4]. Several sets of criteria for the definition of the preferred chemical composition leading to optimal bioavailability for active ingredients, such as Lipinski's "Rule of Five" for oral drugs [5], Astex's "Rule of Three" for fragment-based lead discovery [6], and Brigg's "Rule of Three" for agrochemicals [7], contain the need for the presence of hydrogen-bond donors and hydrogen-bond acceptors for ideal drugs, a requirement that several carboxylic functions fulfill. This book chapter tries to highlight the most important roles that carboxylic functions play in pharmaceuticals and agrochemicals.

1.2

Solubilizer

The introduction of carboxylic acid into a biologically active compound positively impacts the water solubility of the compound. Acids are generally highly ionized at physiological pH values and therefore solvated to a greater degree and display more favorable aqueous solubilities than neutral molecules of similar lipophilicity do. In addition, the counterion influences solubility and

physicochemical properties of active ingredients bearing a carboxylate [8]. The presence of charges plays a significant role in modulating solubility, lipophilicity, and thus cell permeation. However, acidic compounds are also often associated with poor permeability because they are mainly present in the deprotonated state in this pH range and cannot readily cross negatively charged lipid membranes [2]. Such solubilizing effects are even more pronounced in the presence of two or more ionized groups, especially zwitterions. For example, because of the pH gradient unique to the gastrointestinal tract, it is the piperazine moiety in the quinolone antibiotic ciprofloxacin that governs the charge state within the acidic upper gastrointestinal tract (gastric region). The elevation of the pH value in the subsequent proximal intestine results in the zwitterionic state **2** (Figure 1.1) [1, 9]. In crop protection, carboxylic acids are known for their pronounced phloem mobility, which includes the basipetal movement of an active ingredient from the leaves to the roots within a plant.

Further proof for the effect of carboxylic acid functions on the solubility of pharmaceuticals is found in the history of antihistaminic drugs [10]. Several first-generation derivatives, such as hydroxyzine (**3**), were rather lipophilic compounds, which were able to cross the blood–brain barrier and had a sedating effect because they were no substrates for P-glycoproteins (P-gps). Owing to the replacement of the hydroxyl function by a carboxylic acid, cetirizine (**4**), a second-generation antihistaminic, is less lipophilic and therefore a P-gp substrate that limits the CNS exposure (Figure 1.2) [11, 12].

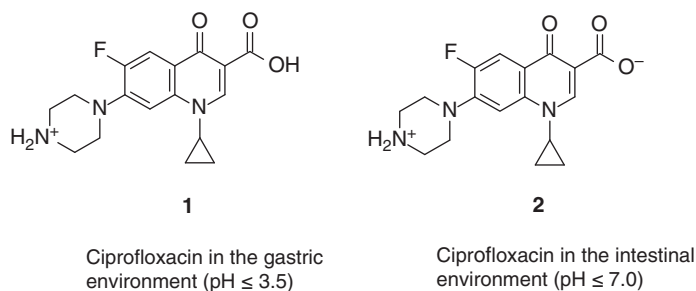


Figure 1.1 Ionization state of ciprofloxacin in the gastrointestinal tract [1].

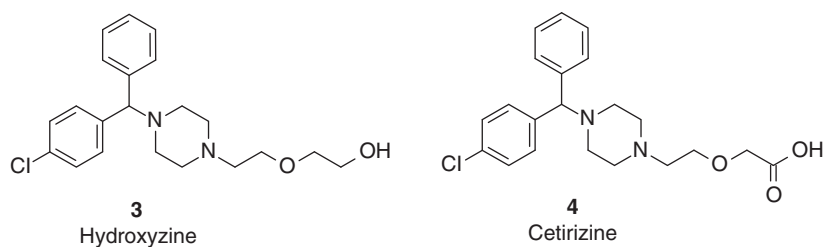


Figure 1.2 Increasing solubility of antihistaminic compounds by carboxylic acids.

1.3

Pharmacophore

A pharmacophore is the group of atoms and, therefore, also the ensemble of steric and electronic features of an active ingredient ensuring optimum molecular interactions with an enzyme and responsible for triggering or blocking its biological response [13]. The acidity of carboxylic acids, combined with the ability of all carboxylic acid derivatives to establish relatively strong electrostatic interactions and single or bifurcated hydrogen-bond bridges with the protein target, conferring both binding affinity and specificity to the drug–target interaction, is the reason that carboxylic acid functions are often the key determinant of pharmacophores [2]. Figure 1.3 shows only four examples of many pharmaceuticals and agrochemicals that rely on the presence of a carboxylic function in their pharmacophore. The terminal carboxylate of atorvastatin (**5**), a blood cholesterol-reducing blockbuster drug, forms a salt bridge with Lysine735 of its target enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase [14]. The carbonyl oxygen atom of the carboxylic acid function in the nonsteroidal anti-inflammatory acid flurbiprofen (**6**) builds a hydrogen-bond bridge with the phenol group of Tyrosine355 of cyclooxygenase-1 [15]. The broad-spectrum activity of azoxystrobin (**7**), the world's biggest selling fungicide, is due to the interaction of the carbonyl oxygen atom of its ester function with an amine proton of Glutamine272 in cytochrome bc1, the complex III of the respiratory

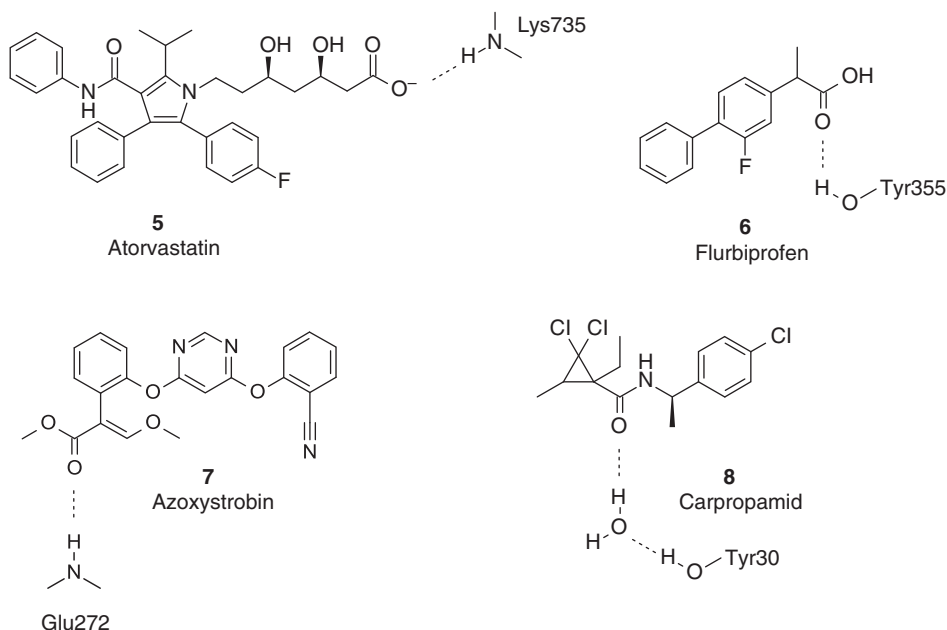


Figure 1.3 Examples for pharmacophores of active ingredients based on carboxylic acids, esters, and amides.

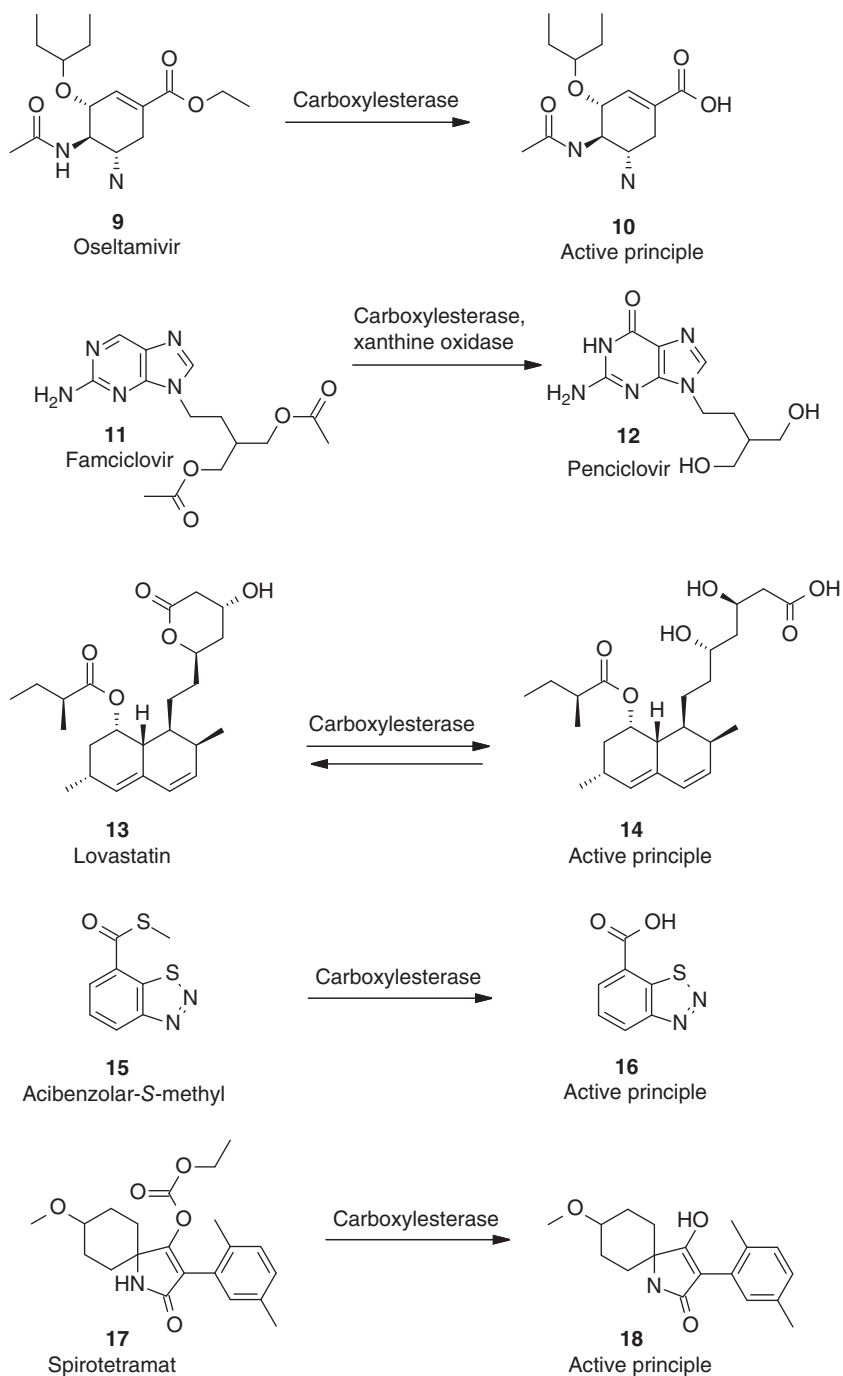
chain in the mitochondria of the fungi [16]. The carbonyl oxygen of the amide function of carpropamid (**8**), a melanin biosynthesis-inhibiting rice fungicide, accepts a hydrogen bond from a water molecule coordinated to Tyrosine30 of scytalone dehydratase [17].

1.4

Prodrug

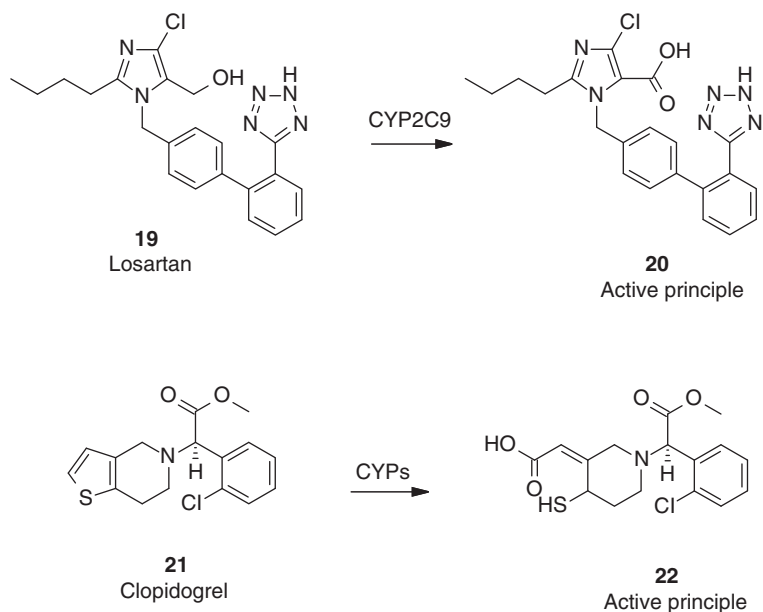
A carboxylic acid group, being usually ionized in the physiological pH range, adds to the hydrophilicity and polarity of the active ingredient. As a result, a large number of biologically active carboxylic acids display unfavorable pharmacokinetic properties such as low bioavailability because of limited uptake. Thus, the improvement of pharmacokinetic properties can be achieved by transferring the drug into a prodrug. A prodrug is a compound that itself is not biologically active, but is converted by enzymes, heat, moisture, or UV light into an intrinsically active derivative. Because of the ubiquitous availability of esterases and peptidases in many species, including human, the *in vivo* hydrolysis of esters and related carboxylic functions to the corresponding acids is one of the classical prodrug cases [18–23]. Such ester derivatives are called carrier prodrugs, because they often facilitate the adsorption and distribution of pharmaceuticals or agrochemicals to the desired location, followed by release of the active principle by cleavage of the carrier group through a hydrolytic reaction [19]. The ethyl ester in oseltamivir (**9**) increases the oral bioavailability in humans from less than 5% for the carboxylic acid parent **10** to 80% and, therefore, allows this anti-influenza antiviral agent to be administered orally [18, 20, 21]. Ester prodrugs that release a biologically active alcohol species instead of a carboxylic acid-containing drug are known to a lower content. The reason for this may be that the improvement of pharmacokinetic features is generally greater when masking the highly polar carboxylate rather than the less polar hydroxyl group [22]. An important example is the antiherpes virus agent famciclovir (**11**), which delivers *in vivo* penciclovir (**12**) by enzymatic ester cleavage and purine oxidation. The oral bioavailability of 4% for penciclovir is increased to 75% for famciclovir [20]. A special case of ester prodrugs are lactones, which are formed by intramolecular cyclization of hydroxyl acids and which liberate this function after cleavage. An example is the reversible ring opening of the lactone in 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor lovastatin **13** to its β -hydroxy acid open form **14** [21, 22]. The carbothioic acid *S*-methyl ester in the plant activator acibenzolar-*S*-methyl (**15**) [24] and the carbonate function in the insecticide spirotetramat (**17**) [25] are further examples of carboxylic acid derivatives, which can be employed as carrier prodrugs, and also carbamates have been used in this context (Scheme 1.1) [18, 19].

Another kind of prodrugs are bioprecursors, which deliver the biologically active compound via an *in vivo* transformation without the need to cleave a carrier moiety. Also here, the formation of carboxylic acids seems to play an important role. The angiotensin II receptor antagonist losartan (**19**), which



Scheme 1.1 Some examples of ester, carbonate, and carbothioic S-ester prodrugs.

is used in antihypertensive medication, can be seen as bioprecursor prodrug, because its primary alcohol is oxidized *in vivo* by the cytochrome P450 enzyme CYP2C9 to the carboxylic acid **20**, which represents the active principle [18, 19, 21]. In addition, the antithrombotic drug clopidogrel (**21**) is metabolized by cytochrome P450 enzymes to its active form **22** containing a carboxylic acid (Scheme 1.2) [18, 19].



Scheme 1.2 Losartan (**19**) and clopidogrel (**21**) as bioprecursors of carboxylic acid derivatives.

1.5

Bioisosteric Replacement

We have seen so far that carboxylic functions are often important constituents of active ingredients; however, the presence of such groups can also be responsible for significant drawbacks, such as metabolic instability, toxicity, and limited passive diffusion across membranes. To avoid some of these shortcomings while retaining the desired attributes of the carboxylic moiety, its replacement by a carboxylic bioisostere might be considered. The concept of bioisosterism is based on the notion that the exchange of single atoms or whole groups that exhibit similar size, shape, charge distribution, and physicochemical properties creates a new compound with similar biological properties to the parent active ingredient [13]. The same type of strategy can also be applied effectively for other purposes, for example, to increase the potency or selectivity of a biologically active compound or

to create own intellectual property. However, the outcome of any isosteric replacement cannot be readily predicted as the result is generally found to be dependent on particular properties of the drug and its target. As a result, screening of a panel of different isosteres is typically required; the more isosteres of one defined functional group are known, the higher the chances to find one with full preservation or even enhancement of the desired biological activity. As a matter of fact, several groups with an isosteric relationship to carboxylic functions are known, especially surrogates of carboxylic acids are really high in number [26, 27]. For example, several organic heteroatom acids, such as phosphonic acids, phosphinic acids, and sulfonic acids, are proven replacements of carboxylic acids, as demonstrated by the phosphonic acid phaclofen (**24**) and the sulfonic acid saclofen (**25**), two γ -aminobutyric acid (GABA)_B antagonistic bioisosteres of the GABA_B-agonistic antispasmodic drug baclofen (**23**) (Figure 1.4) [26].

Another group of well-established bioisosteric functions of the carboxylic acid group are certain heterocycles, such as hydroxyisoxazole [28], hydroxyisothiazoles [29], thiadiazolidinediones [30], oxadiazolones [31], and of course especially tetrazoles [32]. The tetrazole moiety in losartan (**19**) mimics a carboxylic acid function. 3-Hydroxyisoxazole has been extensively applied as carboxylic acid bioisostere in the case of GABA and glutamate signaling drugs, which mimic GABA and glutamic acid, two essential neurotransmitters in the mammalian central nervous system [26, 29]. The GABA_A agonists muscimol (**27**) and gaboxadol (**28**) are close analogs of GABA (**26**); their hydroxyisoxazole moiety resembles the acid group of **26** [26]. The naturally occurring heterocyclic amino acid ibotenic acid (**30**) and its synthetic analog thioibotenic acid (**31**) are hydroxyisoxazole and hydroxyisothiazole bioisosteres of glutamic acid (**29**), and, therefore, also potent glutamate receptor ligands [29]. The thiazolidinedione in the antidiabetic drug rosiglitazone (**33**) is probably required as carboxylic acid surrogate, because several other compounds with comparable activity against the targeted peroxisome proliferator-activated receptor, such as ragaglitazar (**32**), bear an acid function in the same region of the molecule (Figure 1.5) [30].

Several bioisosteric groups of other carboxylic functions, such as esters and amides, are clearly linked to related acid surrogates. Because 5-substituted tetrazoles are acid replacements, logically 1,5-disubstituted tetrazoles are effective bioisosteres for the amide bond [32]. In addition, the ability of 1,2,4-oxadiazoles to function as heterocyclic surrogate of the amide bond [33] is linked to the known

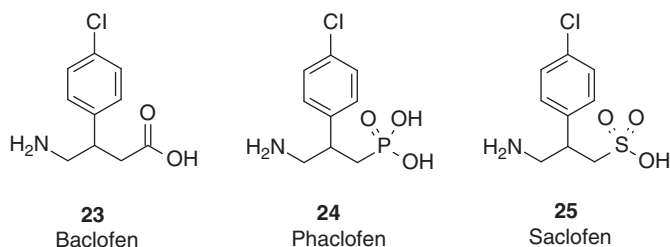


Figure 1.4 Baclofen (**23**) and its bioisosteres phaclofen (**24**) and saclofen (**25**) [26].

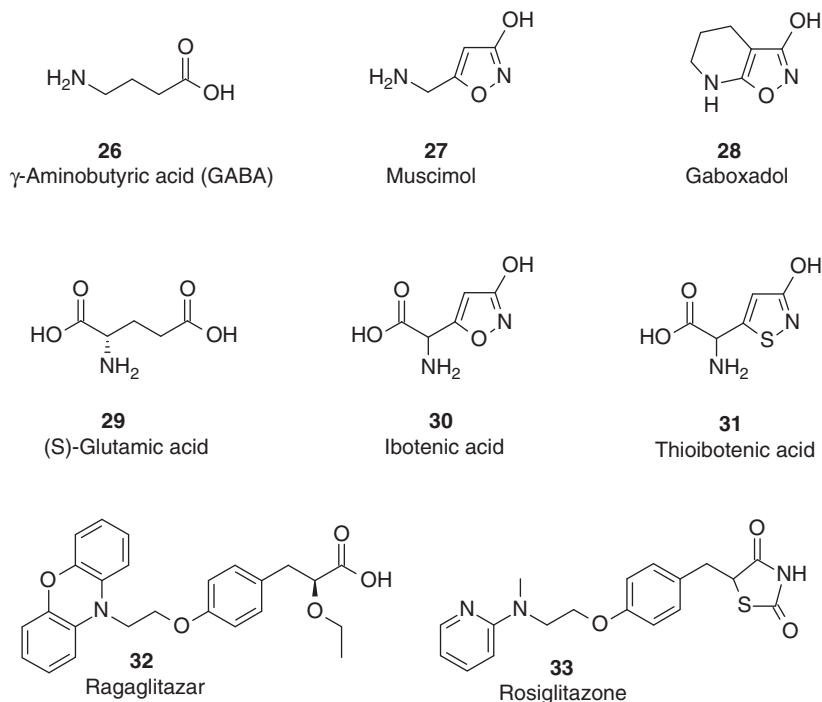


Figure 1.5 Heterocyclic bioisosteres of carboxylic acid derivatives.

track record of related 1,2,4-oxadiazol-5-ones as good acid replacement [31]. As 2,2,2-trifluoroethan-1-ol is a highly viable carboxylic acid bioisostere [26], it is no surprise that α -trifluoromethyl-substituted amines can mimic amides [34].

1.6

Scaffold

Amides as stable and relatively neutral carboxylic acid derivatives play an important role in providing a three-dimensional scaffold required for optimum binding of the active ingredient to the target enzyme by linking elaborated acid and amide moieties together. Not only the potential of the amide group as hydrogen-bond donor as well as acceptor is an advantage, another reason for the vast amount of amides among pharmaceuticals and agrochemicals is their straightforward synthetic accessibility from carboxylic acids, chlorides, or esters with appropriate amines. The tyrosine kinase inhibitor imatinib (**34**) [35], the dopamine antagonist amisulpride (**35**) [36], the cellulose synthase inhibitor mandipropamid (**36**) [37], and the succinate dehydrogenase inhibitor (SDHI) isopyrazam (**37**) [38] are four examples of many pharmaceuticals and agrochemicals relying on an amide bridge (Figure 1.6).

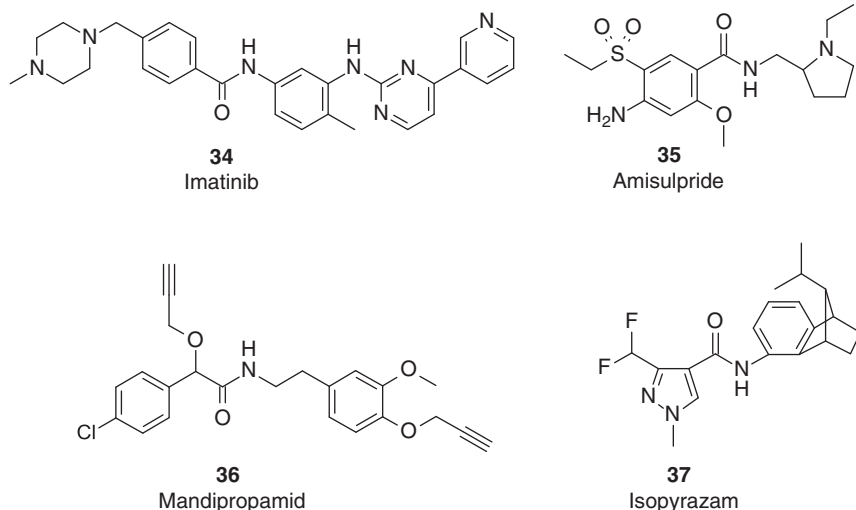


Figure 1.6 Four active ingredients, which rely on an amide function linking important parts of the molecule.

1.7

Conclusion

Carboxylic acid-containing drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs), β -lactam antibiotics, and statins, have revolutionized the progress in the medical treatment of pain and diseases in the twentieth century. The history of chemical weed control and therefore the start of industrial agriculture would be unimaginable without carboxylic acid herbicide classes such as auxin mimics and sulfonylureas. Two out of the three currently most important fungicide classes, the inhibitors of complex II and complex III of the fungal respiratory chain (SDHIs and quinone outside inhibitor (QoI)s), are based on carboxylic functions. All these facts are proof for the fundamental importance of carboxylic functions for pharmaceuticals and agrochemicals.

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Part I

Neurology

2

Carboxylic Ester Containing Norepinephrine–Dopamine Reuptake Inhibitors (NDRIs)

David J. O'Neill

2.1

Introduction

Norepinephrine–dopamine reuptake inhibitors (NDRIs) block the action of the norepinephrine transporter (NET) and the dopamine transporter (DAT) by inhibiting the reuptake of the neurotransmitters norepinephrine (**1**) and dopamine (**2**) (Figure 2.1), respectively. This leads to increased extracellular concentrations of both norepinephrine (**1**) and dopamine (**2**) and thus an increase in noradrenergic and dopaminergic neurotransmission. NDRIs would not be expected to impact serotonergic transmission or the action of the serotonin transporter (SERT).

Compounds described as NDRIs in the literature have been investigated as treatments for clinical depression [1], smoking cessation [2], attention deficit disorder (ADD) [3], attention deficit hyperactivity disorder (ADHD) [3], narcolepsy [4], Parkinson's disease [5] and as analgesics [6].

While many different structural classes of NDRIs have been identified, this chapter focuses primarily on racemic-methylphenidate (($\pm$)-*threo*-**3**, Ritalin®), its (+)-*threo*-enantiomer dexamethylphenidate ((+)-*threo*-**3**, Focalin®), and analogs, such as ($\pm$)-ethylphenidate (($\pm$)-**4**) (Figure 2.2).

2.2

History

Methylphenidate (**3**) was first synthesized in 1944 by Panizzon [7] and was initially used as a treatment for barbiturate coma and then for the treatment of depression and convalescence [8]. Originally, **3** was marketed as Centedrin™ (Rickter Works, Hungary), as a mixture of the racemic ($\pm$)-*threo* (20%) and ($\pm$)-*erythro* (80%) isomers (Figure 2.3) [9].

It was shown subsequently that the erythro isomers exhibit very little therapeutic effect and contribute mainly to the toxic hypertensive effects of this mixture

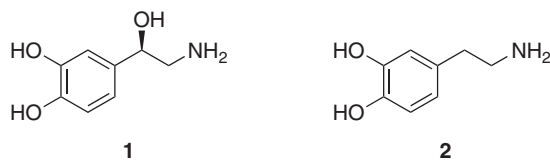


Figure 2.1 Structures of norepinephrine (1) and dopamine (2).

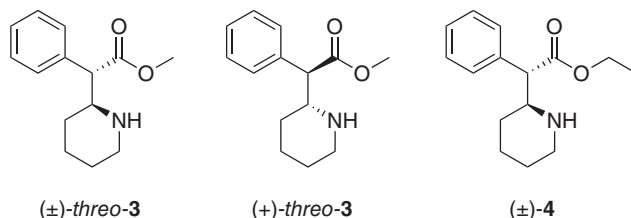


Figure 2.2 Structures of racemic-methylphenidate ((±)-threo-3, Ritalin[®]) and its (+)-threo-enantiomer dextromethylphenidate ((+)-threo-3, Focalin[®]) and (±)-ethylphenidate ((±)-4).

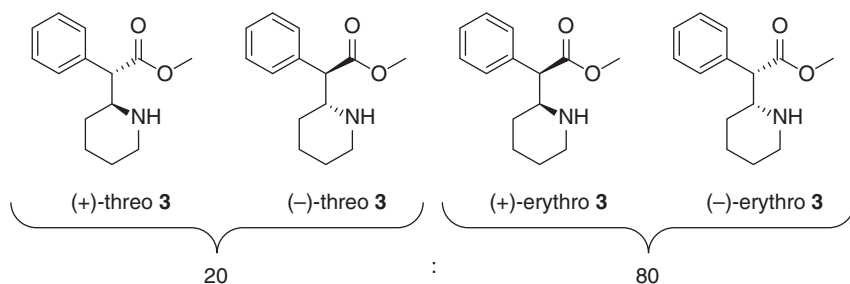


Figure 2.3 Structures of the *threo* and *erythro* isomers found in Centedrin[™] (3).

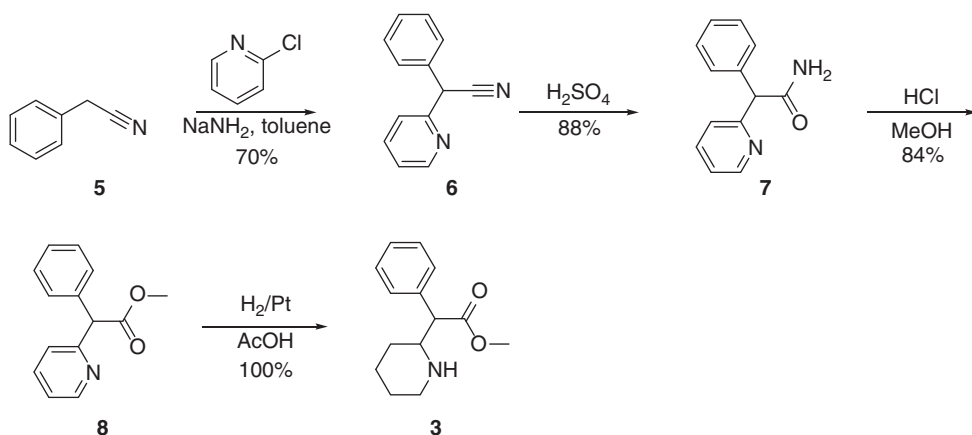
[10]. Routes to the (±)-*threo* isomers were developed and this mixture was marketed as Ritalin ((±)-*threo*-3) by Ciba-Geigy (now Novartis) in 1955.

The mixture of (±)-*threo*-3 is considered a mild CNS stimulant and is a widely prescribed treatment for ADHD [11, 12]. It has become increasingly heavily prescribed since the 1990s, when the diagnosis of ADHD itself became more widely accepted [13]. ADHD and related conditions are thought to be linked to functional impairment of the dopamine and norepinephrine functions in the brain, primarily in the prefrontal cortex [14]. The stimulant properties of (±)-*threo*-3 have been linked to its dopamine reuptake inhibition and thus increased levels of synaptic dopamine (2) [15, 16] with norepinephrine reuptake inhibition being a secondary, but potentially also important, effect [17, 18]. This stimulation is thought to regulate impulsivity and attention in children with ADHD. For patients treated with (±)-*threo*-3, side effects have been observed, for example, insomnia, anorexia, dizziness, weight loss, and dysphoria. It also has the potential for substance abuse in patients, especially when administered through inhalation or intravenously as it

can produce a euphoric effect. It is thought that this is primarily due to the action of the (–)-*threo* enantiomer as patients with ADHD who were treated with the (+)-*threo* isomer exhibited reduced side effects and euphoric effects and had enhanced relief [19]. In addition, it has been shown that (+)-*threo*-3 has a higher affinity for the norepinephrine and dopamine transporters than (–)-*threo*-3 and is more potent in the induction of locomotor activity [20]. Studies of radiolabel binding of MPH enantiomers in animal models have demonstrated that binding of (+)-*threo*-3 to DAT is selective, saturable, and reversible, while binding of the (–)-*threo*-enantiomer is largely nonspecific [21]. In addition, positron emission tomography (PET) images of a human brain after administration of [^{11}C](+)-*threo*-3 and [^{11}C](–)-*threo*-3 demonstrated that the [^{11}C](+)-*threo*-3 was concentrated in the basal ganglia, where it bound to DAT, whereas the [^{11}C](–)-*threo*-3 did not bind [22]. This, therefore, led to an increased interest in (+)-*threo*-3, and it was approved for the treatment of ADHD in early 2002. Extended release and long-acting formulations of both (±)-*threo*-3 and (+)-*threo*-3 have also been explored and have shown extended durations of efficacy, prolonging the effect from several hours, owing to the relatively rapid metabolism of the ester to the biologically inactive acid, to all day coverage [23].

2.3 Synthesis

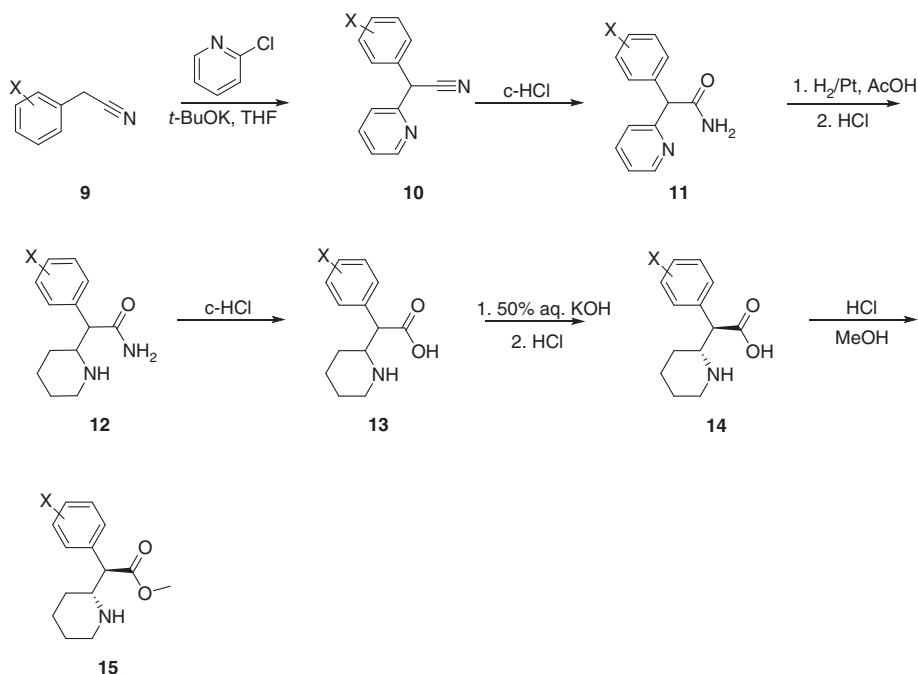
The initial route to methylphenidate (3) as reported by Panizzon [7] is shown in Scheme 2.1.



Scheme 2.1 Initial route toward methylphenidate (3).

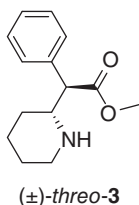
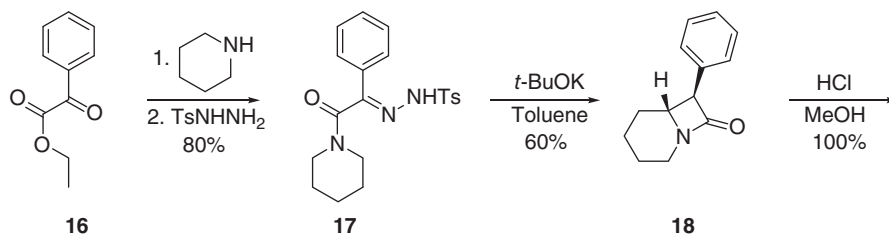
Treatment of a solution of phenylacetonitrile (5) and 2-chloropyridine in anhydrous toluene with sodamide followed by heating to 110°C for 1 h provided the desired product 6 in 70% yield. Subsequent heating with concentrated

sulfuric acid resulted in formation of amide **7**, which was readily converted to methyl ester **8** with HCl gas and MeOH. Finally, hydrogenation of the pyridyl ring was accomplished in quantitative yield utilizing platinum in acetic acid at room temperature providing **3**. It should be noted that Panizzon also reported the synthesis of ethylphenidate (**4**) in the same communication, substituting ethanol for methanol in the third step of the reaction sequence. Both compounds were synthesized as a mixture of the *erythro* and *threo* diastereomers. A similar route was later reported for the synthesis of analogs of methylphenidate (**3**) to investigate their potential as cocaine antagonists, Scheme 2.2, with modifications such as use of KO^tBu/THF in step 1, concentrated HCl for hydrolysis of the nitrile in step 2, and altered epimerization conditions in the formation of **13** with an increase in the efficiency of the route [24].



Scheme 2.2 Modified route toward methylphenidate analogs.

A third approach has also been reported, which provides ($\pm$)-*threo*-methylphenidate (($\pm$)-*threo*-**3**) selectively, Scheme 2.3 [25]. Beginning with ethyl phenylglyoxalate (**16**), reaction with piperidine provides a ketoamide, which is condensed with tosylhydrazine, to give the tosylhydrazone **17**. Treatment with potassium *tert*-butoxide in refluxing toluene then affords, after crystallization, the β -lactam **18**. The equilibrating reaction conditions provide predominantly the *exo* product, where the phenyl ring is oriented on the convex face. Hydrolysis of **18** with acidic methanol provides ($\pm$)-*threo*-**3** as a single diastereomer.

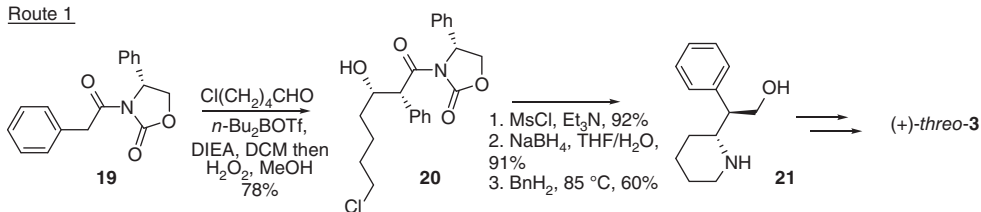


Scheme 2.3 Stereoselective route to $(\pm)\text{-threo-3}$.

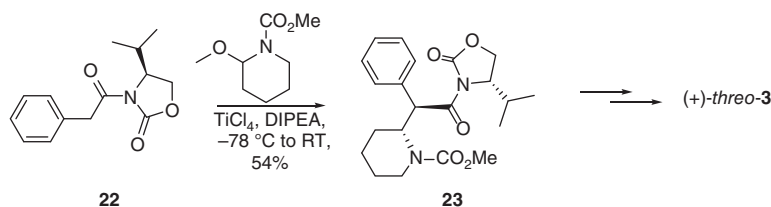
While other approaches to $(\pm)\text{-threo-3}$ have been reported [26], significant effort has been expended in the preparation of enantiomerically pure $(+)\text{-threo}$ methylphenidate ($(+)\text{-threo-3}$) [27]. Initial preparations focused on the resolution of an advanced intermediate using D-(–)-tartaric acid to provide access to enantiomerically pure precursors that could then be converted to $(+)\text{-threo-3}$ [28, 29]. Further work utilized a classic resolution of $(\pm)\text{-threo-3}$ with (*R*)-(–)-binaphthyl-2,2'-diyl hydrochloride followed by recrystallization to provide $(+)\text{-threo-3}$ in high enantiopurity [20, 30, 31]. Additional resolving agents have been reported [30–33] as well as the enhancement of the enantiomeric purity of enantiomerically enriched mixtures of $(\pm)\text{-threo-3}$ via specific recrystallization conditions [20, 32, 34]. Resolution of $(\pm)\text{-threo-3}$ with enzyme-based approaches has also been explored as has the resolution of precursors of $(\pm)\text{-threo-3}$ [19, 32]. Finally, use of enantioselective syntheses to directly provide $(+)\text{-threo-3}$ has been described. These generally fall into two categories: first, incorporation of a chiral auxiliary and second, use of a chiral ligand, to impart the desired stereoselectivity. As an example for the first category (Scheme 2.4, route 1), Novartis [35] utilized an asymmetric aldol condensation of the (*Z*)-boron enolate of *N*-phenylacetyl-(*R*)-4-phenyl-2-oxazolidinone (**19**) with 5-chlorovaleraldehyde to provide the two key stereocenters of $(+)\text{-threo-3}$. Mesylation, removal of the chiral auxiliary, and ring closure provided **20**. Compound **21** was subsequently obtained in >99% enantiomeric excess (e.e.) and the intermediate was then converted to $(+)\text{-threo-3}$. A similar approach utilizing a titanium enolate was also reported [36], with the coupling partner being a substituted piperidine (Scheme 2.4, route 2). This method provided intermediate **23** in >96% e.e. before its transformation to $(+)\text{-threo-3}$.

Examples of chiral ligand utilization are illustrated in Scheme 2.5. Route 1 [37] utilizes a rhodium-mediated C–H insertion of methyl phenyldiazoacetate

Route 1

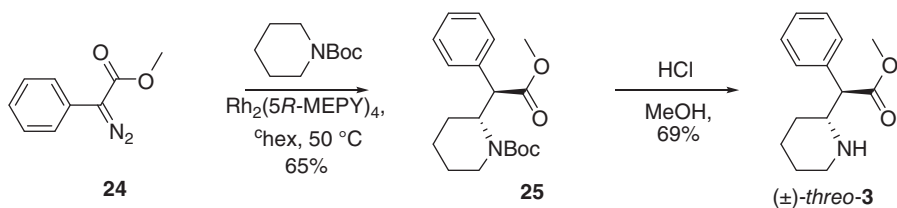


Route 2

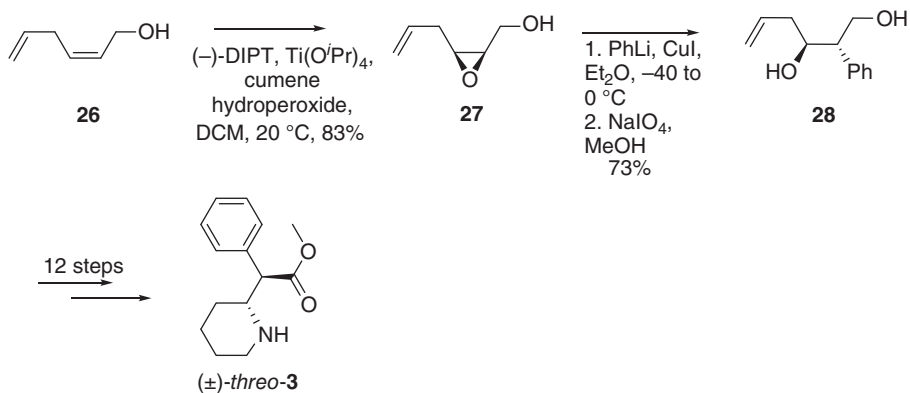


Scheme 2.4 Stereoselective routes to (+)-threo-3 utilizing a chiral auxiliary.

Route 1



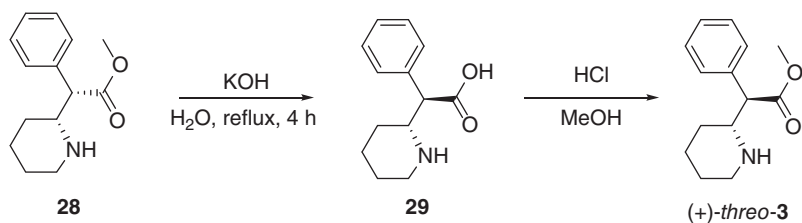
Route 2



Scheme 2.5 Stereoselective routes to (+)-threo-3 utilizing a chiral ligand.

(**24**) with *N*-Boc piperidine. This leads to the selective formation of *N*-Boc-*D*-*threo*-methylphenidate (**25**), which was then deprotected with HCl gas in methanol to provide crude (+)-*threo*-**3** with 94% diastereomeric excess (d.e.) and 69% e.e. Further recrystallization improved the purity to 95% d.e. and 96% e.e. Route 2 [38] utilizes a Sharpless asymmetric epoxidation of the *Z*-alkene **26** with cumene hydroperoxide in the presence of (–)-diisopropyl *D*-tartrate and titanium(IV)isopropoxide to provide epoxide **27** in 92% e.e. Ring opening with PhLi in the presence of CuI led to diol **28** as the major product. Additional steps then provided (+)-*threo*-**3** in enantiopure manner.

Additional work [39, 40] has also targeted a diastereomer of (+)-*threo*-**3** for synthesis, as the epimerization of (+)-*erythro*-methylphenidate (**28**) at the benzylic stereocenter has been shown to provide direct access to (+)-*threo*-**3**, Scheme 2.6 [41].



Scheme 2.6 Epimerization to provide (+)-*threo*-**3**.

2.4

Mode of Action

When a neuron is stimulated, the action potential travels down the axon; see Figure 2.4. When this signal reaches the end of the axon, it triggers a series of chemical changes in the axon terminal. Calcium ions flow into the axon terminal, which initiates the release of neurotransmitters (dopamine, norepinephrine, serotonin, acetylcholine, γ -aminobutyric acid (GABA), glutamate, or glycine) from vesicles via exocytosis after fusing with the neuronal membrane. The neurotransmitters are released into the synaptic cleft and diffuse across to the postsynaptic neuron. The chemical binding of neurotransmitters with their respective receptors initiates changes in the postsynaptic neuron that may facilitate or inhibit an action potential. After binding to their receptors on the postsynaptic neuron, the neurotransmitters are released from the receptors and diffuse back into the synaptic space. The released neurotransmitters, as well as any neurotransmitters that did not bind to a receptor, are either taken back up into the presynaptic axon terminal by active transport through a transporter or reuptake pump or degraded by enzymes in the synaptic cleft. Once the neurotransmitter has been transported back inside the axon terminal, it is either degraded or repackaged into new vesicles for later release.

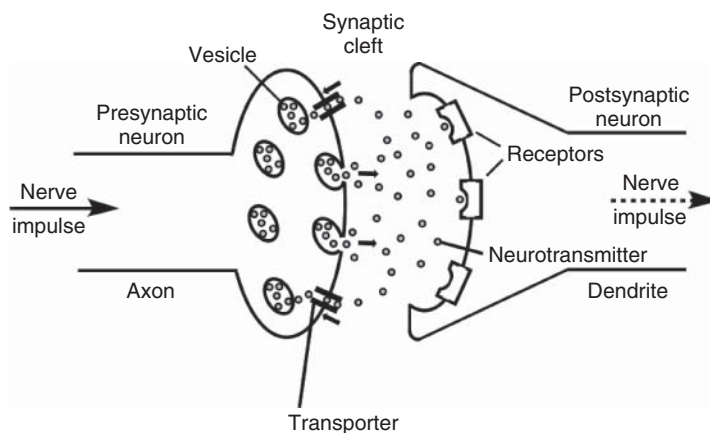


Figure 2.4 Schematic diagram of a synapse.

NDRIs block the action of NET and DAT by inhibiting the reuptake of the neurotransmitters norepinephrine (1) and dopamine (2), respectively, in noradrenergic and dopaminergic neurons. This leads to increased extracellular concentrations of both norepinephrine (1) and dopamine (2) and thus an increase in noradrenergic and dopaminergic neurotransmission. It has been reported that ($\pm$)-*threo*-3 inhibits the synaptosomal reuptake of norepinephrine (1) with an IC_{50} of 51 ± 21 nM, for dopamine (2) the IC_{50} of 20 ± 8 nM. Dexmethylphenidate ((+)-*threo*-3) has an IC_{50} of 39 ± 7 nM for norepinephrine (1) and an IC_{50} of 23 ± 6 nM for dopamine (2), whereas (–)-*threo*-3 has an IC_{50} of 980 ± 300 nM for norepinephrine (1) and an IC_{50} of 1600 ± 740 nM for dopamine (2), demonstrating the eutomer/distomer relationship between the two [42]. It has been shown that increases in dopamine (2) in the striatum decrease background firing rates and increase the signal-to-noise ratio of striatal cells [43]. The resulting increase in dopamine (2), owing to the inhibition of its reuptake, may act to improve attention and decrease distractibility. Sympathomimetic activity in the CNS, including the prefrontal cortex, has also been shown to increase [44]. This leads to improved attention span, increased ability to follow directions or complete tasks, decreased distractibility, and decreased impulsivity and aggression [45]. In the periphery, the effects of ($\pm$)-*threo*-3 or (+)-*threo*-3 are minimal at therapeutic doses. In addition, ($\pm$)-*threo*-3 and (+)-*threo*-3 have been demonstrated to be safe and effective in the treatment of pediatric ADHD in a double blind, controlled comparison with placebo, with (+)-*threo*-3 having equal effects to ($\pm$)-*threo*-3 at half the dose [46].

2.5

Structure–Activity Relationships

There has been significant interest both in analogs of (+)-*threo*-3 [47] to improve the compound's relatively short duration of action [48] and in the potential of those

analogs to act as cocaine abuse treatment agents [24, 49]. This work is summarized in Figure 2.5.

An early report [50] on substitution of the aryl ring of **3** described the effects of *para*-hydroxylation on both the *erythro* and *threo* racemates with a resulting increase in maximal locomotor response implying increased synaptic transmission. This was followed by additional exploration around the scaffold; see Figure 2.6. In general, substitution at the 2-position of the aryl ring yielded less potent analogs than the corresponding derivatives with the same substituent at the 3- or 4-position. The size of this effect roughly correlated with the size of the substituent [24]. Analogs with 3-, 4-, and 3,4-substitution showed interesting activity, particularly where the substituents were electron withdrawing. As

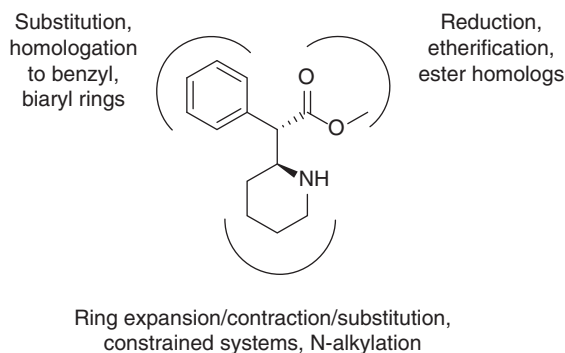


Figure 2.5 Summary of SAR exploration around the methylphenidate scaffold.

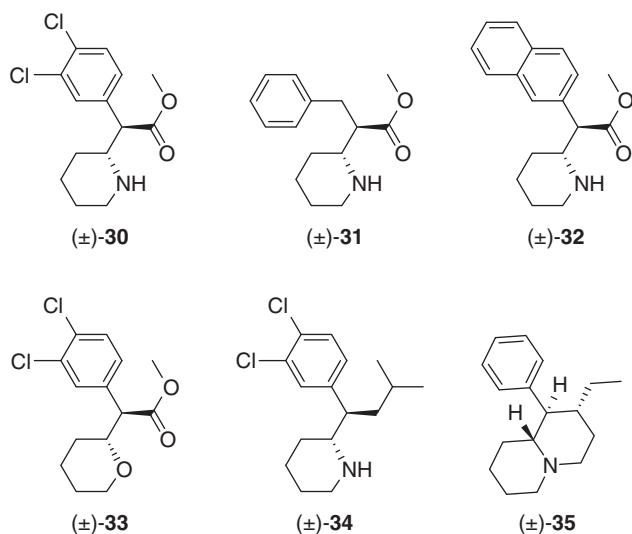


Figure 2.6 SAR exploration around the methylphenidate scaffold.

such, 3-Cl-, 3-Br-, and 3,4-di-Cl-methylphenidate (($\pm$)-**30**) showed improved DAT binding compared to (+)-*threo*-**3** (NET activity not reported). Similarly to ($\pm$)-*threo*-**3**, for analogs that were separated into the constituent enantiomers, the (+)-*threo* analogs showed increased potency relative to the (–)-*threo* analogs for both norepinephrine (**1**) and dopamine (**2**) [51]. Other strategies to probe the pharmacophore around the aryl ring include homologation to a benzyl group and incorporation of extended ring systems such as naphthyl [25]. The extension to benzyl (($\pm$)-**31**) significantly decreased DAT binding, while compounds containing the naphthyl ring (($\pm$)-**32**) were more potent than ($\pm$)-*threo*-**3**. The 2-naphthyl analog was almost sevenfold more potent at binding to DAT and fivefold more potent at inhibiting dopamine reuptake. For analogs of (+)-*threo*-**3** with variations around the piperidine ring, N-methylation consistently provided compounds with lower potency than the corresponding N–H analogs. It was speculated that this could be due to steric effects [52]. Deutsch, however, reported that the *N*-Bn analog demonstrated improved binding to DAT relative to ($\pm$)-*threo*-**3** [53]. Converting the piperidine ring to a morpholine, decreasing the size of the ring to the pyrrolidine, or increasing it to the azepane or azocane resulted in a significant loss of DAT binding activity. Interestingly, Davies reported that pyrrolidine analogs of ($\pm$)-*threo*-**3** that contained the 2-naphthyl group in place of the phenyl ring displayed high binding affinity and selectivity for SERT [54]. Other variations around the piperidine ring include the carbo- and oxo-cyclic analogs [55]. The direct tetrahydropyranyl analog of ($\pm$)-*threo*-**3** was approximately 175-fold less potent at DAT than ($\pm$)-*threo*-**3**; however, tetrahydropyranyl analogs of, for example, the 3,4-di-Cl phenyl analog ($\pm$)-**33** demonstrated significant potency albeit lower than the parent piperidine compound. The 5- and 6-carbocyclic analogs with the 3,4-di-Cl phenyl group also had activity at DAT, demonstrating that the piperidine N is not absolutely essential for activity. SAR around the ester group has also shown some flexibility in the pharmacophore. While ester homologues are generally lower in activity compared to ($\pm$)-*threo*-**3** (e.g., ($\pm$)-ethylphenidate (**4**) is fivefold less active than ($\pm$)-*threo*-**3** for dopamine reuptake inhibition and tenfold less active for norepinephrine reuptake) [42], there has been interest in these compounds as they are more dopamine selective than ($\pm$)-*threo*-**3**, which could be a useful pharmacological profile for patients. However, there are concerns around the safety and potential recreational abuse of **4** [56]. It has been shown that the ester group can be removed entirely and can be replaced with alkyl groups such as isobutyl. In combination with potency enhancing groups such as the 3,4-di-Cl phenyl (($\pm$)-**34**), this can lead to potent analogs with an IC_{50} for monoamine reuptake of 19 ± 3 nM for norepinephrine (**1**) and 31 ± 11 nM for dopamine (**2**) [48]. Finally, Kim *et al.* [49] demonstrated that this alkyl substituent could be cyclized around onto the piperidine N to create bicyclic “restricted rotation” analogs of ($\pm$)-*threo*-**3**, for example, compound ($\pm$)-**35**. Depending on the substitution pattern on the bicyclic amine and the phenyl, potent DAT and NET inhibitors could be obtained.

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3

Analgesic and Anesthetic Amides

Thomas Erhard

3.1

Introduction

Fentanyl (**1**) and its reported close analogs **2–6** are some of the most widely applied opioid analgesics in clinical practice (Table 3.1). Both the 2-phenylethyl side chain and the phenylamido piperidine are characteristic structural elements of fentanyl (**1**). Chemical modifications on the piperidine and its axial 4-position as well as isosteric replacements of the phenyl group led to the discovery of carfentanyl (**2**), sufentanyl (**3**), lofentanyl (**4**), remifentanyl (**5**), and alfentanyl (**6**), all of which bear the same propionic amide functionality [1–3].

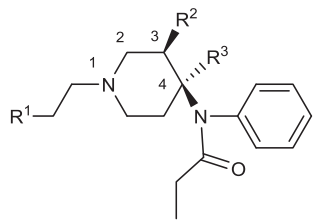
The local anesthetic lidocaine (**7**) [4] and the mild analgesic acetaminophen (APAP for *acetyl-para*-aminophenol, **8**) [5] both share a common phenyl amide moiety but differ completely in their mechanism of action from those previously mentioned opioid agents (Figure 3.1). Acetaminophen (**8**) is a fairly simple acetanilide from a chemical point of view, while **7** belongs to the group of 2,6-dimethylphenyl-*N*-carboxamides.

This chapter focuses on selected examples from the large class of fentanyls and in a second part looks at the smaller group of lidocaine (**7**) and acetaminophen (**8**).

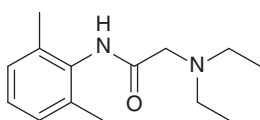
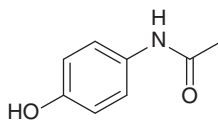
3.2

History

Opioids are the oldest class of compounds used to relieve pain, and administration of opium derived from poppy plants dates back to the Ancient Egyptians. Since Serturmer isolated morphine in 1804/1805 [6], chemical modifications leading to semisynthetic derivatives dominated the chemical and pharmaceutical development until World War I. Stimulated by the need for a fully synthetic opioid, which could replace morphine in order to alleviate potential supply shortages, and fostered by the German government during the political events leading to World War II, German researchers of I.G. Farben in the 1930s invented meperidine (**9**, launched 1938/1939) and methadone (launched 1939). Paul Janssen, born in 1926

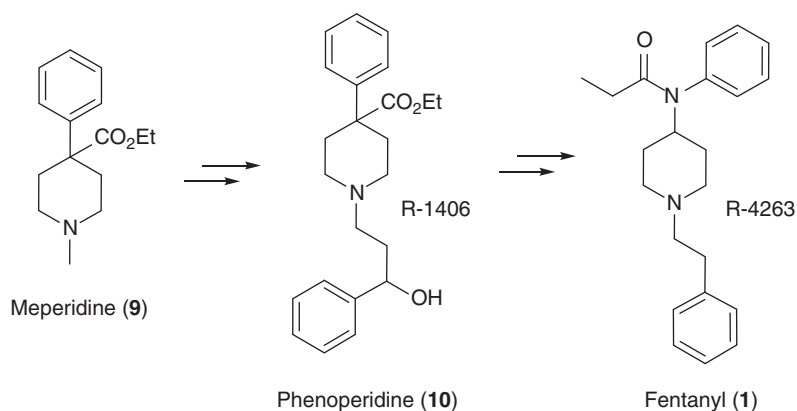
Table 3.1 Fentanyl (1) and important drug analogs.


Compound	R ¹	R ²	R ³
Fentanyl (1)	Ph	H	H
Carfentanil (2)	Ph	H	CO ₂ CH ₃
Sufentanil (3)	2-Thienyl	H	CH ₂ OCH ₃
Lofentanil (4)	Ph	CH ₃	CO ₂ CH ₃
Remifentanyl (5)	CO ₂ CH ₃	H	CO ₂ CH ₃
Alfentanil (6)	1-(4-Ethyl-tetrazolin-5-one)	H	CH ₂ OCH ₃

**Lidocaine (7)****Acetaminophen (8)****Figure 3.1** Representative anilides lidocaine (7) and acetaminophen (8).

(2003¹⁾) and founder of Belgian Janssen Pharmaceutica (1953), was interested in creating a potent, safer, and more effective pain treatment. Chemical structures of morphine and meperidine (9, Scheme 3.1) were known at that time, and Janssen well understood the relationship between structure and activity among the existing opioids. His research team started to modify meperidine (9), which had strong advantages with respect to structural complexity and synthetic tractability. One of his working hypotheses for the poor action of meperidine (9) compared to morphine was attributed to the poorer fat solubility. Concentrated efforts in the replacement of the *N*-methyl group [7] led to phenoperidine (10) in 1956, whereas the installation of the phenyl propionic amide at the piperidine 4-position ultimately paved the way for the discovery of fentanyl (1) in 1960, which was approved later in 1968. It is worthwhile to note that the propiophenone analogs of R-1406 could tell their own story in the discovery of haloperidol [8]. Subsequent research activities guided the Janssen team to sufentanil (3, 1974; launched EU 1979, US 1985), carfentanil (2, 1974; launched as a veterinary product 1986), and alfentanil (6, 1976; launched EU 1983, US 1987). In contrast to the commercial disappointment of Janssen with its analogs of fentanyl (1), Glaxo recognized an opportunity for an ultrashort-acting opioid and devoted a team of chemists to work toward this goal. Ultimately, the team came up with remifentanyl (5) in the late 1980s (launched EU and US 1996/7) [1, 2, 9].

1) Deceased.



Scheme 3.1 Discovery of fentanyl (1) via sequential modifications of meperidine (9).

Despite the success in the production of fully synthetic opioids with stronger potency than morphine (Table 3.2) and tailor-made profiles, the lack of commercial success of the fentanyl follow-ups was disappointing for Janssen. This substantially influenced the decision by Janssen to stop research activities into opioids for anesthesia in the early 1990s [2]. Nevertheless, application of and developments in noninvasive delivery systems have ensured fentanyl's (1) continued clinical success for patients suffering pain to the present day [1].

The discovery of lidocaine (7) is a story of serendipity. Early observations during cocaine administration indicated local anesthesia as a side effect before its target had been mechanistically identified. Synthetic chemistry led to the “-caine” class of local anesthetics, which generally consists of three components – (i) a lipophilic aromatic ring, (ii) an ester or amide linkage, and (iii) usually a tertiary amine – and which could be clustered into two categories according to those linkages. Cocaine, benzocaine (11), and procaine (12), for instance, belong to the amino ester group, whereas bupivacaine (13) and lidocaine (7) represent the amino amides (Figure 3.2). Lidocaine (7) was first synthesized by Swedish

Table 3.2 Potency comparisons for various fentanyl derivatives in rat tail withdrawal reflex [2, 3].

Compound	ED ₅₀ (mg/kg)	Potency ratio
Meperidine (9)	6.15	0.002
Morphine	3.15	0.003
Phenoperidine (10)	0.12	0.09
Alfentanil (6)	0.044	0.25
Fentanyl (1)	0.011	1
Remifentanyl (5)	0.0044	2.5
Sufentanil (3)	0.00067	16.4
Lofentanil (4)	0.0006	18.3
Carfentanil (2)	0.00037	29.7

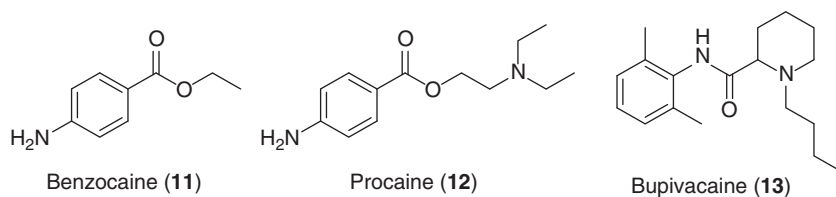
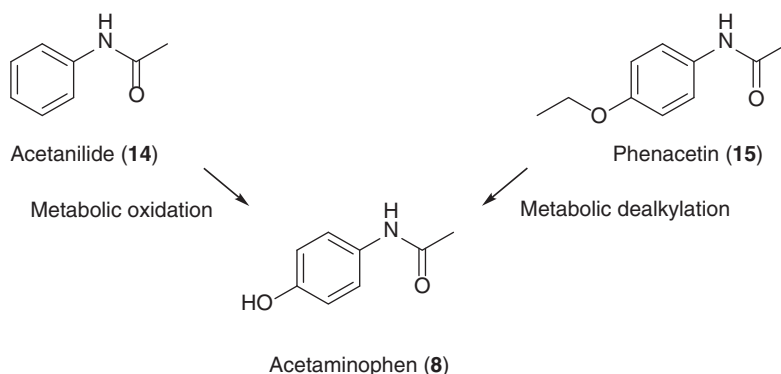


Figure 3.2 Selected representatives of the “-caine” family of local anesthetics.

Löfgren in 1944. His group member Lundqvist recognized its huge potential, so amide **7** quickly became known as LL30 and was introduced to the market in 1948 (Sweden) and 1949 (US) [4, 10, 11].

The history of acetaminophen (**8**) is also shaped by serendipitous observations and is related to acetanilide (**14**) and phenacetin (**15**), both of which showed analgesic effects but appeared to be quite toxic. Among others, the groups of Brodie and Axelrod [12, 13] investigated the metabolism of acetanilide (**14**) and phenacetin (**15**). As it turned out, both compounds were metabolized to acetaminophen (**8**), which was responsible for the observed analgesia, whereas the toxicity was attributed to the corresponding deacetylated anilines (Scheme 3.2) [5].



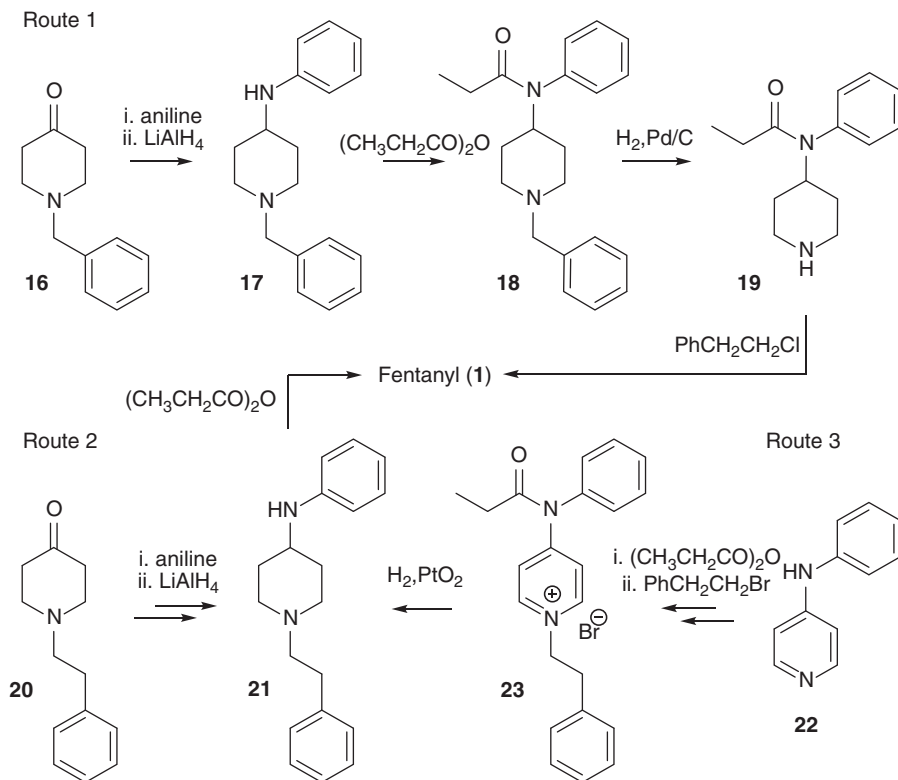
Scheme 3.2 Chemical structures of acetanilide (**14**) and phenacetin (**15**) and the pathways involved in the metabolic production of acetaminophen (**8**).

3.3

Synthesis

The general routes for the synthesis of fentanyl (**1**) are shown in Scheme 3.3. It is worth emphasizing that **1** itself and its 4-anilido-4-carboxylated or -alkylated derivatives do not require asymmetric transformations or isomeric separations since they do not bear any stereogenic center. Condensation of 1-benzylpiperidin-4-one (**16**) and aniline yielded the corresponding imine, which was subsequently

reduced with LiAlH_4 . Propionylation of the secondary amine **17** gave the propionic amide **18**. Hydrogenation-induced debenzylization and alkylation of the thereby liberated amine functionality of **19** completed the synthesis of fentanyl (**1**, route 1) [3, 14].

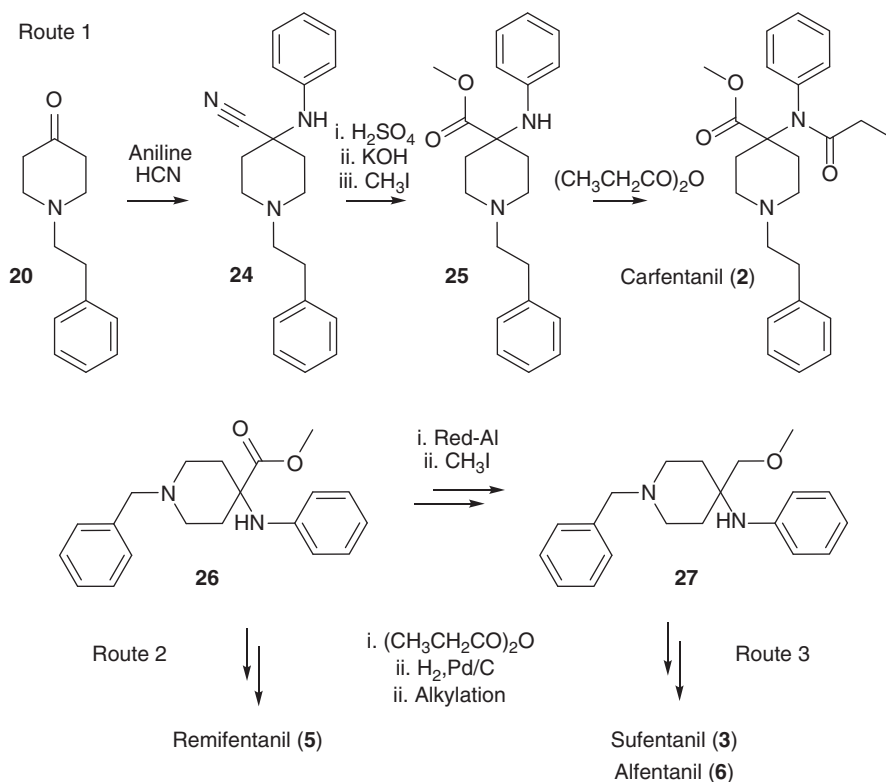


Scheme 3.3 General approaches toward the synthesis of fentanyl (**1**).

This particular benzyl protecting strategy is routinely applied if incorporation of chains different from 2-phenylethyl are envisaged. A shorter approach started with 1-(2-phenylethyl)piperidin-4-one (**20**) avoiding the need for the dealkylation step (route 2) [15]. Another route was based on the reduction of the alkylated pyridinium salt **23**, which led to the very same fentanyl precursor **21** (route 3) [16]. The preparation of 3-methylfentanyl analogs follows the same approaches (route 1 or 2) except that 3-methylpiperidin-4-ones are used as slightly modified starting materials [17].

Synthetic paths toward the 4,4-disubstituted piperidine scaffolds related to compounds **2–6** follow a very similar sequence as illustrated in Scheme 3.4. Given the need for an additional carbon linkage, Strecker synthesis gave access to the α -amino nitrile **24**, which was converted to the methylester **25** by hydration, amide hydrolysis, and methylation of the corresponding potassium carboxylate.

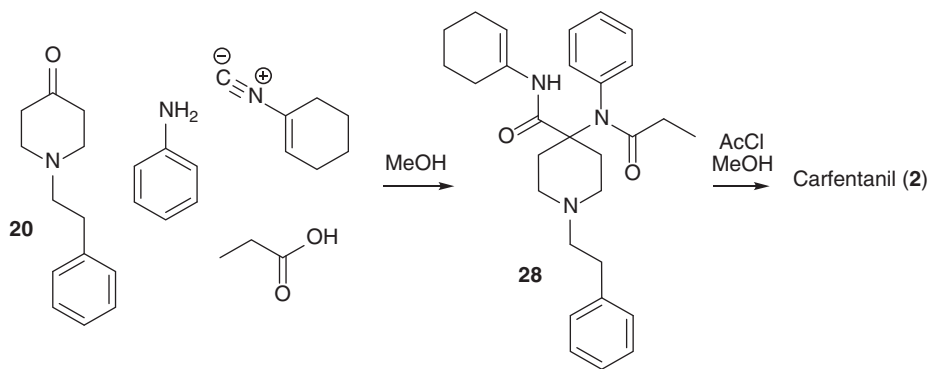
Propionylation readily completed the synthesis of carfentanil (**2**, route 1) [18]. Further attempts to improve the overall yield were devoted to primary amide methanolysis via cyclic imide [19] or imine [20] intermediates, which were thought to increase the reactivity of the amide moiety. The use of the benzyl protected amine **26** allowed modifications at the piperidine by alkylation (route 2). The 4-methoxymethyl group of **27** is prepared by Red-Al reduction of the methylester **26** and subsequent methylation of the primary alcohol (route 3) [21].



Scheme 3.4 Modified pathways toward 4,4-disubstituted fentanyl analogs.

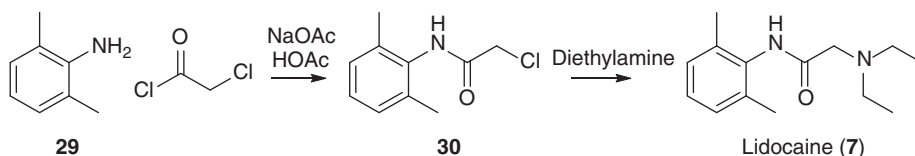
A sophisticated retrosynthetic analysis proposed a four-component Ugi reaction as superior alternative for the synthesis of carfentanil (**2**), which would reduce the number of steps from five to two compared to route 1 in Scheme 3.4. Heating of piperidinone **20**, propionic acid, aniline, and 1-cyclohexenyl isonitrile under optimized conditions in MeOH gave the desired amide **28** in a single step with 82% yield. Carfentanil (**2**) could then be obtained by acidic methanolysis in overall two steps (Scheme 3.5) [22].

A general route to lidocaine (**7**) and its analogs involves the acylation of 2,6-dimethylaniline (**29**) with chloroacetyl chloride, followed by nucleophilic displacement with diethylamine (Scheme 3.6) [23]. A synthetic process with



Scheme 3.5 Ugi synthesis of carfentanil (2).

acetone and sodium carbonate allowed more environmentally friendly reaction conditions in order to achieve the alkylation [24]. Reductive acylation approaches starting with 2,6-dimethylnitrobenzene were also discussed as interesting alternatives [25].



Scheme 3.6 General process for the synthesis of lidocaine (7).

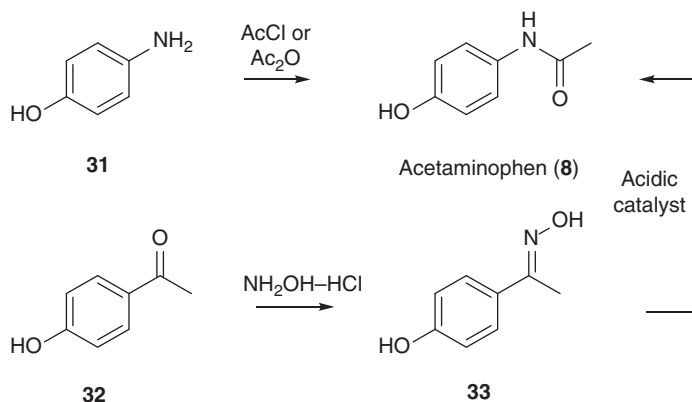
Protocols for the acetaminophen (8) synthesis are common place in chemistry student's textbooks, and the author assumes that most chemists are familiar with its preparation. Acetaminophen (8) can be readily prepared from aminophenol (31), which is accessed by nitration and reduction of phenol, by treatment with either acetyl chloride or acetic anhydride in water (Scheme 3.7) [26].

A Celanese process patent reported the synthesis starting from acetophenone 32. The corresponding oxime 33 is formed with hydroxylamine, and Beckmann rearrangement in the presence of an acidic catalyst produced the acetanilide moiety in a single step [27].

3.4

Mode of Action

Fentanyl (1) and its analogs are centrally acting agents and mediate their effects through action at the opioid receptors, which can be divided into four receptor subtypes. In particular, MOPR (*mu* opioid receptor), KOPR (*kappa*), DOPR (*delta*), and most recently NOPR (*nociceptin-orphanin*), which all belong to the class of membrane-bound G-protein coupled receptors and differ in their



Scheme 3.7 Short synthesis of acetaminophen (8).

pharmacological profiles [28]. As most recent technical breakthrough, crystal structures of all four subtypes with antagonists bound were available since 2012 [29]. Agonistic activation of the MOPR is important for the production of analgesia, which is fentanyl's (1) primary site of action, but this also produces side effects such as respiratory depression. The binding affinity to the *mu*-site is generally high (e.g., 1: 1.2 nM, 2: 0.024 nM) with good selectivity for the *delta*-receptor (e.g., 1: 150-fold, 2: 138-fold) and even better selectivity for the *kappa*-subtype (e.g., 1: 242-fold, 2: 1792-fold) [30].

Lidocaine (7) acts as a peripheral sodium-channel blocker, thus inhibiting signal propagation in neuronal cell membranes at the cytosolic site. Sodium ion influx is impaired by blocking the inner channel pore. These anesthetics are usually administered locally and insensitivity is generated in the regions of the body treated [4, 31].

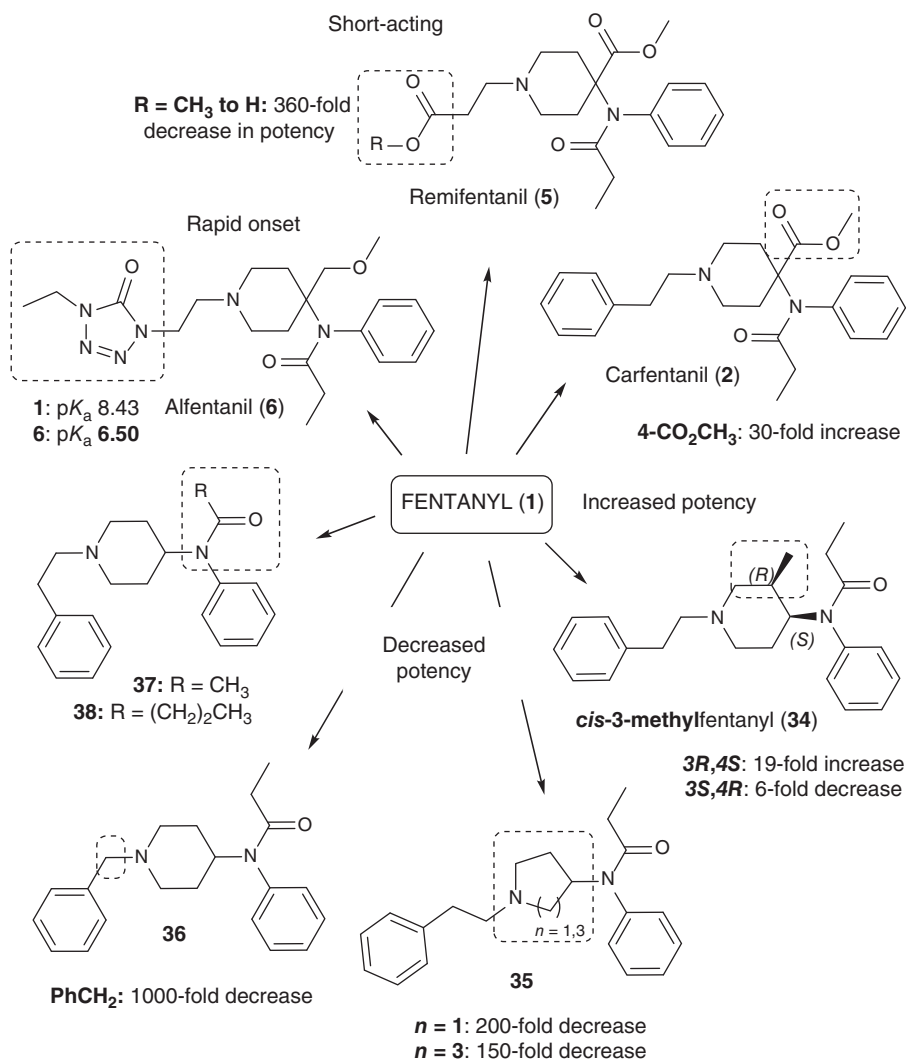
To date, the mechanism of action of acetaminophen (8) is not fully understood. Inhibition of the peroxidase enzymes cyclooxygenase (COX-1) and COX-2 is generally accepted although this might not be the sole mechanism responsible for analgesia [5, 32].

3.5

Structure–Activity Relationships

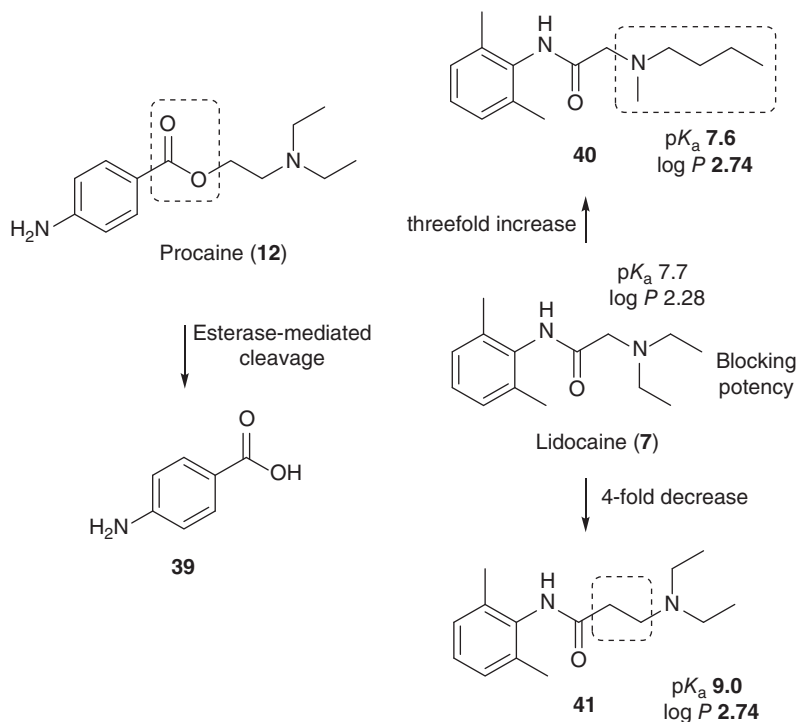
Over the past decades, any number of modifications of the fentanyl skeleton was pursued, but only a few results have been chosen for discussion here (Scheme 3.8). Optimizing the substituent in 4-position of fentanyl (1) significantly increased potency. A methyl scan around the piperidine core identified the 3-position as most sensitive in order to improve potency, whereas the single *cis*-enantiomer 34 is clearly preferred [3, 33].

In addition, neither piperidine ring expansion to perhydroazepine nor its contraction to pyrrolidine seemed to change potency significantly. An exception



Scheme 3.8 Summary of selected SAR results derived various modifications of fentanyl (1). Potency ratios with respect to ED_{50} in rat tail withdrawal reflex.

is analog **35**, bearing the preferred 2-phenylethyl side chain, which shows a significant decrease in potency. However, commonly higher potency is achieved with the 2-phenylethyl chain compared to the shortened analog **36** [34]. Variations of the propionic amide were also investigated and both acetyl fentanyl (**37**) and butyryl fentanyl (**38**) showed reduced activity [35]. Structure–activity relationship (SAR) efforts were not solely directed on improving potency, but also on pharmacokinetic properties as required. Alfentanil (**6**) has the fastest onset among



Scheme 3.9 Effects of selected modifications in the “-caine” family.

compounds **1–6**, which is caused by electronic effects of the ethyltetrazolin-5-one. Piperidine basicity is reduced by approximately two log units compared to fentanyl (**1**) (**6**: pK_a 6.50, **1**: pK_a 8.43), which results in a much larger fraction of the nonprotonated state at physiological pH 7.4. As a consequence, a faster onset due to rapid brain penetration is observed [36]. Remifentanyl (**5**) was specifically designed as an ultrashort-acting analog. Drug inactivation was enabled by replacing the arene with a methylester moiety, which is still tolerated by the receptor site. Rapid esterase-mediated cleavage to the corresponding carboxylic acid *in vivo* led to an approximately 360-fold decreased potency and is responsible for remifentanyl’s (**5**) very short duration of action [3, 37].

Among the “-caine” family, the general lower stability of the amino esters compared to the amino amides in solution reflects an important SAR feature. Owing to the rapid cleavage of the ester moiety in procaine (**12**), its duration of action is limited and allergic reactions caused by the liberated *para*-amino benzoic acid (**39**) are not uncommon (Scheme 3.9) [38]. In the lidocaine template, lipid solubility and thus potency could be modulated by both tertiary amine substituents and linker variations. Given the fact that sodium-channel blockade is produced by the protonated species, a balanced pK_a profile is equally important in order to ensure sufficient membrane permeability. While an increased hydrophobicity

improved tonic block (7 vs 40), extension of the carbonyl-amine proximity caused an opposite effect although having equal logP (40 vs 41) [39].

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Part II

Cardiovascular Diseases

4

Fibrate Acids and Esters for the Treatment of Hyperlipidemia (PPAR α Activators)

Gavin O'Mahony

4.1

Introduction

The fibrates are a class of carboxylic acid derivatives that have been used in the treatment of hyperlipidemia since the 1960s. They were discovered before the currently established paradigm of single target-directed drug discovery, and their biological target was not identified until the discovery of the peroxisome proliferator-activated receptors (PPARs) in the 1990s. They have been largely superseded by the statins for use as a monotherapy in the treatment of hyperlipidemia and are generally not recommended as first-line treatment in patients with high plasma lipid levels. However, in certain patient subpopulations that are either refractory to or intolerant of statin therapy, fibrates still represent a useful therapeutic option. In addition, certain patients already receiving the maximum dose of a statin but not reaching sufficiently low cholesterol levels or patients with severe hypertriglyceridemia (>500 mg/dL) may benefit from receiving a fibrate as an adjunct to statin therapy. Structurally, the fibrates are derivatives of fibric acid (2-phenoxyisobutyric acid (**1**), Figure 4.1), with the minor exception of gemfibrozil (**6**), which replaces the phenoxy moiety with a short alkyl chain.

Clofibrate (**2**), developed by Imperial Chemical Industries (ICI), was the first fibrate that was introduced for the clinical treatment of hyperlipidemia in the 1960s (Table 4.1). Currently, generic versions of four fibrates are available in various European countries: bezafibrate (**3**), ciprofibrate (**4**), fenofibrate (**5**), and gemfibrozil (**6**). In the United States, only fenofibrate (**5**) and gemfibrozil (**6**) are currently approved for use, with fenofibrate (**5**) in its various forms and formulations (e.g., fenofibric acid (**5a**)) currently being the most-prescribed member of the class [1].

A small number of fibrates and fibrate derivatives were only launched in a limited number of markets via national marketing authorizations, for example, the atypical symmetrical diacid clinofibrate (**7**) [2], which is only available in Japan and South Korea. A number of diesters of clofibrate (**2**) and nicotinic acid (see

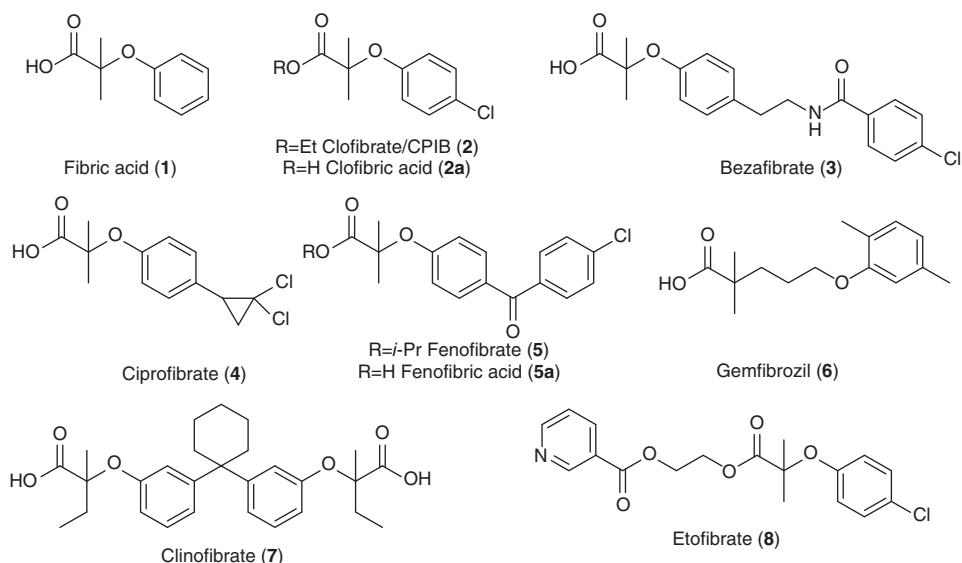


Figure 4.1 Chemical structures of fibrate acid (1), clofibrate (2), clofibric acid (2a), and the currently commercially available fibrates and fibrate derivatives (3–8).

Table 4.1 Currently available fibrate-derived hypolipidemics.

International Nonproprietary Name (INN)	Selected proprietary name(s)	Originating company	First patent disclosure
Clofibrate (2)	Atromid, Atromid-S	ICI (now AstraZeneca)	1961
Gemfibrozil (6)	Lopid	Parke-Davis (now Pfizer)	1969
Bezafibrate (3)	Bezalip, Bezatol	Boehringer Mannheim (now Roche)	1973
Clioquinol (7)	Lipoclin	Sumitomo (now Dainippon Sumitomo)	1973
Ciprofibrate (4)	Modalim, Hyperlipen	Sterling Drug (now Sanofi)	1975
Fenofibrate (5/5a)	Tricor, Trilipix	Groupe Fournier (now AbbVie)	1975
Etofibrate (8)	Lipo-Merz, Tricerol	Merz	1971

Section 4.4) have also been developed, for example, etofibrate (8) [3], and ronifibrate (9) and binifibrate (10), both of which have been withdrawn from use.

4.2

History

The history of the discovery of the fibrates has previously been reviewed by others, including Lalloyer [4, 5] and Oliver [6, 7], but a brief summary is presented here as the story is illustrative of the nature of drug discovery in the

pregenomic age. The initial discovery of the potential of acetic acid derivatives for the treatment of elevated cholesterol levels was brought about by the serendipitous observation of unusually low plasma cholesterol levels in hospitalized French farm workers, who had been accidentally exposed to an aerially applied insecticide (phenyl ethyl acetic acid or 2-phenylbutanoic acid) developed by ICI. At this time, the connection between elevated cholesterol levels and coronary heart disease (CHD) was being established [8], and pharmaceutical agents capable of lowering plasma cholesterol were of interest to the pharmaceutical industry.

The potential of this class of acetic acid derivatives in the treatment of elevated cholesterol levels and resultant CHD was recognized by Jeff Thorp, a chemist at ICI. He initiated a course of research which ultimately led to the discovery of the prototypical member of the fibrate class, ethyl- α -*p*-chlorophenoxyisobutyrate (**2**, CPIB, later given the International Nonproprietary Name (INN) clofibrate). The compound was discovered by extensive *in vivo* testing of structural analogs of phenyl ethyl acetic acid [9]. It was recognized early on in the course of this research that the ester moiety was hydrolyzed *in vivo* to afford the biologically active component, clofibric acid (**2a**).

Initial *in vivo* studies and clinical experiments with clofibrate (**2**) were performed using a combination of clofibrate (**2**) and (3 α ,5 α)-3-hydroxy-androstan-17-one (androsterone), given the name *Atromid* or *Atromid-S*. This was due to ICI's research interests in the effects of steroid hormones on serum lipid profiles and an observation that clofibrate (**2**) potentiated the known hypolipidemic effect of androsterone. The potentiation effect was attributed to clofibrate-mediated displacement of androsterone from plasma proteins leading to an increased free concentration of androsterone. Preliminary studies of *Atromid*'s cholesterol-lowering effect in rats were followed by clinical trials in healthy male volunteers, which showed lowering of both plasma cholesterol and triglycerides. The hypothesis that *Atromid* was mainly an orally active androsterone formulation [10] had to later be revised following conflicting results [11], as it was established that administration of clofibrate (**2**) alone led to comparable effects on the reduction of plasma lipids as the combination with the steroid [12].

In a large-scale 5-year primary prevention trial investigating the use of clofibrate (**2**) to decrease plasma cholesterol levels, clofibrate (**2**) treatment led to a mean 9% reduction in plasma cholesterol and a 25% decrease in nonfatal myocardial infarction compared to placebo [13]. However, the group receiving clofibrate (**2**) experienced an excess of deaths due to intestinal and biliary side effects, as well as an increase in the occurrence of gall stones and cholecystectomies. A 6-year follow-up of this study concluded that no excess cardiovascular mortality was observed upon discontinuation of clofibrate (**2**) treatment [14].

It was not until the discovery and clinical assessment of other structurally different fibrates, and the later discovery of the LDL (low-density lipoprotein) receptor and statins, that it was established that the negative effects of clofibrate (**2**) were

compound-related and not due to lipid lowering *per se*. In spite of the demonstrated risks, clofibrate (**2**) was approved in the United States in 1969, as it was the only lipid-lowering agent available. Clofibrate (**2**) use declined upon the introduction of the statins, and the drug was eventually withdrawn from sale in the United States in 2002.

Many pharmaceutical companies were spurred on by the clofibrate (**2**) observations to find compounds with a more attractive safety profile. Parke-Davis filed the initial patent application claiming gemfibrozil (**6**) in 1969, and the compound received US market approval in 1982. Gemfibrozil (**6**) was used as the pharmacological intervention in the Helsinki Heart trial, a primary prevention trial that investigated the effect of modifying plasma cholesterol levels on CHD [15]. In the study, gemfibrozil (**6**) treatment was associated with decreases in triglycerides and LDL cholesterol and an increase in HDL (high-density lipoprotein) cholesterol, which was in turn associated with a decreased risk of CHD [16]. Despite these positive results, the extent of gemfibrozil (**6**) use is somewhat limited, mainly due to an increased risk of rhabdomyolysis (a form of muscle toxicity) when prescribed in combination with a statin [17].

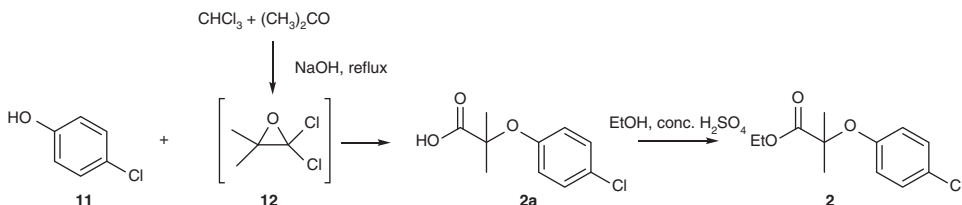
Fenofibrate (**5**) has a long development history since its initial discovery by the French company Groupe Fournier, [18] and has been in clinical use in Europe since 1975. In the United States, an initial NDA was rejected by the FDA in 1984, as its clinical benefit was not considered to be sufficient to offset the potentially class-related side effects seen with clofibrate (**2**). The compound was eventually approved in 1993 on the basis of its ability to lower plasma triglycerides, at which point Abbott gained the rights to the compound, marketing it as Tricor.

By this time, the compound was vulnerable to generic competition, leading to the successive launch of four different formulations of fenofibrate/fenofibric acid (**5/5a**), which gave Abbott market exclusivity until 2011. The fourth-generation formulation, marketed as Trilipix, saw a switch from the isopropyl ester prodrug (**5**) to the use of a choline salt of fenofibric acid (**5a**), the active metabolite. The bioavailability of highly lipophilic, poorly aqueous soluble fenofibrate (**5**) is improved by dosing with food; however, choline fenofibrate (Trilipix) exhibits consistently improved bioavailability when dosed without food [19]. Recently, the combination of fenofibrate (**5**) and a statin (simvastatin) in patients with Type 2 diabetes was investigated in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. The combination led to improved plasma lipid profiles, but showed no benefit on the overall composite primary endpoint of major cardiovascular events (i.e., nonfatal myocardial infarction or stroke or death from cardiovascular disease) compared to simvastatin therapy alone [20]. Recent metaanalyses of cardiovascular outcomes trials of fibrates came to the conclusion that the main contribution of fibrate therapy to improved cardiovascular outcomes is mainly due to a reduction in coronary events [21] and that they have little or no effect on all-cause mortality [22].

4.3

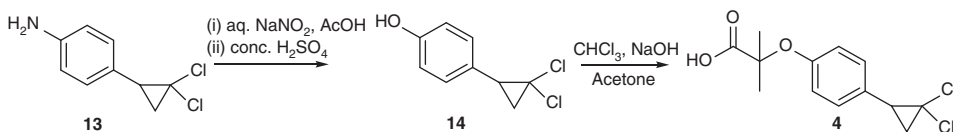
Synthesis

The originally ICI-patented synthesis of clofibrate (**2**) employs a Bargellini reaction of *p*-chlorophenol (**11**) with acetone and chloroform in the presence of sodium hydroxide (Scheme 4.1) [4]. The Bargellini reaction proceeds via attack of the *p*-chlorophenolate anion on the dichloroepoxide species **12** to afford an acyl chloride. The epoxide **12** is formed by nucleophilic attack of the trichloromethyl anion on acetone with subsequent cyclization. The acyl chloride undergoes hydrolysis under the basic reaction conditions to afford clofibric acid (**2a**). This is followed by a standard Fischer esterification to afford clofibrate (**2**).



Scheme 4.1 Synthesis of clofibrate (**2**) employing a Bargellini reaction.

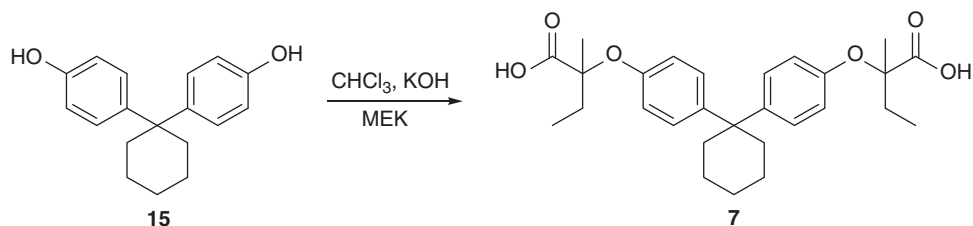
A Bargellini reaction is also used in the originally disclosed synthesis of ciprofibrate (**4**, Scheme 4.2) [23]. Diazotization of aniline **13** and subsequent hydrolysis in hot concentrated sulfuric acid affords the phenol **14**. Bargellini reaction of phenol **14** with chloroform and sodium hydroxide in acetone affords ciprofibrate (**4**). Synthesis of aniline **13** was reported by Sterling Drug in a subsequent, related patent application [24]. Nitration of (2,2-dichlorocyclopropyl)-benzene afforded a mixture of *ortho* and *para* isomers, which after reduction and crystallization afforded the pure *para* isomer **13**. The source of (2,2-dichlorocyclopropyl)benzene was not disclosed, but it is readily accessible by reaction of dichlorocarbene with styrene [25].



Scheme 4.2 Synthesis of ciprofibrate (**4**).

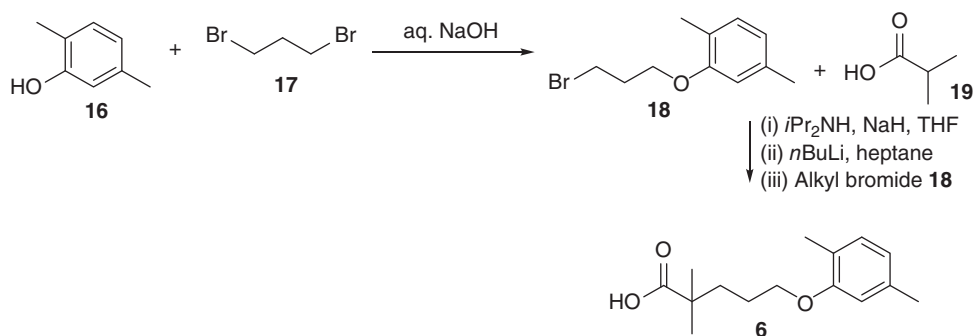
A Bargellini reaction between the symmetrical bisphenol **15** and a dichloroepoxide formed from methyl ethyl ketone and chloroform is employed in the synthesis of *meso* clinofibrate (**7**, Scheme 4.3) [26]. The synthesis of bisphenol **15** is not specified in the patent, but it is readily accessible via a double Friedel–Crafts alkylation between cyclohexanone and 2 equiv. of phenol [27].

The original patent route to gemfibrozil (**6**) is shown in Scheme 4.4 [28]. Treatment of a neat mixture of 2,5-dimethylphenol (**16**) and 1,3-dibromopropane (**17**)



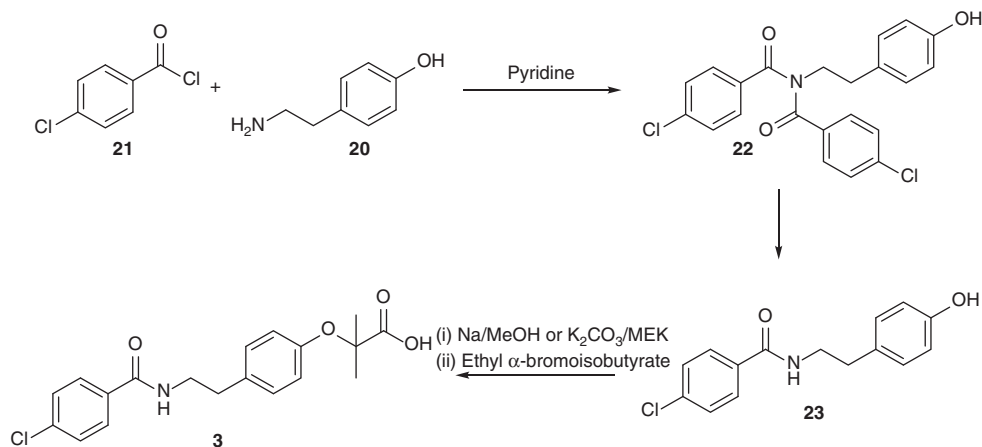
Scheme 4.3 Synthesis of clonofibrate (**7**).

with aqueous sodium hydroxide solution at 100 °C afforded the primary alkyl bromide **18**. The intermediate is then reacted with the dianion of isobutyric acid (**19**) at 0–10 °C to give the final product gemfibrozil (**6**), which can be purified by vacuum distillation. Two methods for the formation of the dianion of isobutyric acid (**19**) are exemplified in the patent: either (as shown in Scheme 4.4) by sequential treatment of isobutyric acid (**19**) with sodium diisopropylamide followed by *n*-butyllithium, or by addition of isobutyric acid (**19**) to magnesium oxide in refluxing toluene with azeotropic removal of water and subsequent addition of lithium diisopropylamide (LDA) to the resulting magnesium carboxylate at 0 °C.



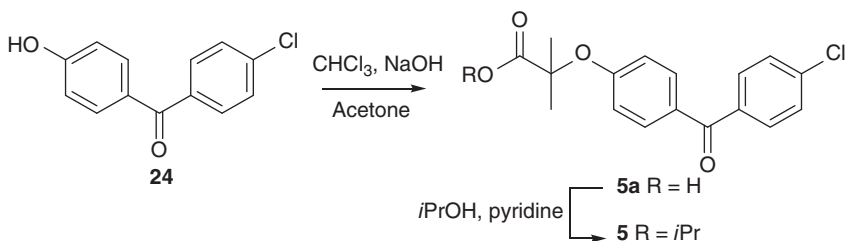
Scheme 4.4 Synthesis of gemfibrozil (**6**).

The initially-patented synthesis route for bezafibrate (**3**) is shown in Scheme 4.5 [29]. Reaction of tyramine (**20**) with 2 equiv. of 4-chlorobenzoyl chloride (**21**) in pyridine at 100 °C yields imide **22**. The imide is hydrolyzed with aqueous potassium hydroxide at 40–45 °C to afford amide **23**. Subsequent phenolate formation by treatment with either sodium methoxide in methanol or potassium carbonate in refluxing methyl ethyl ketone, followed by reaction with ethyl 2-bromoisobutyrate affords bezafibrate (**3**). Interestingly, the sodium phenolate of **23** (but not the potassium phenolate) was isolated as a dry powder by evaporation and azeotropic drying with benzene before reaction with the bromoester. Despite being based on the phenoxyisobutyrate scaffold, a Bargellini-reaction-based route appears not to have been used to produce bezafibrate (**3**), but such a synthetic strategy has recently been described [30].



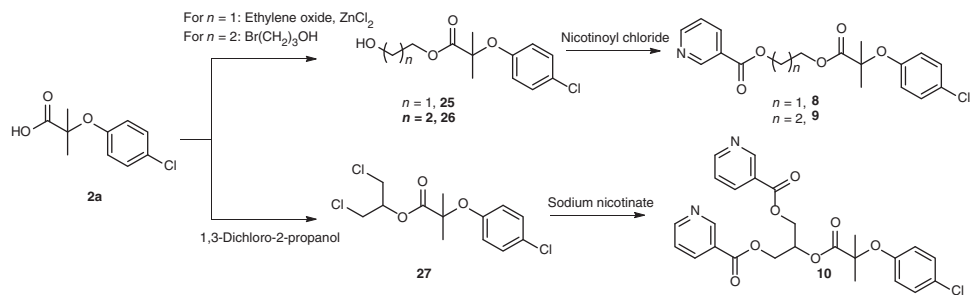
Scheme 4.5 Synthesis of bezafibrate (3).

The synthesis of fenofibric acid (**5a**) and fenofibrate (**5**) is shown in Scheme 4.6 [31]. A Bargellini reaction is employed to install the phenoxyisobutyrate moiety by reaction of the phenoxide anion of phenol **24** with chloroform in refluxing acetone, affording fenofibric acid (**5a**) in 75% yield. Subsequent acyl chloride formation with thionyl chloride and reaction with isopropanol using pyridine as base in benzene or other inert solvent leads to the isopropyl ester, fenofibrate (**5**). It is recommended in the patent to perform the esterification directly on the acid chloride in order to avoid problems with production of traces of foul-smelling SO₂ arising from the thionyl chloride [31].



Scheme 4.6 Synthesis of fenofibric acid (**5a**) and fenofibrate (**5**).

The synthesis of the nicotinic acid-fibrate conjugates etofibrate (**8**) [3, 32], ronifibrate (**9**) [33], and binifibrate (**10**) are shown in Scheme 4.7. Clofibric acid (**2a**) is esterified either with ethylene oxide and zinc chloride in refluxing toluene (for etofibrate (**8**)) or by treatment of the sodium salt of clofibric acid (**2a**) with bromopropanol in refluxing ethanol (for ronifibrate (**9**)) [34]. Subsequent reaction of the hydroxyesters **25** and **26** with nicotinoyl chloride furnishes the final diesters etofibrate (**8**) and ronifibrate (**9**). Esterification of clofibric acid (**2a**) with 1,3-dichloropropan-2-ol affords *bis*-alkyl chloride **27** and subsequent double nucleophilic displacement with sodium nicotinate affords binifibrate (**10**) [35].



Scheme 4.7 Synthesis of etofibrate (8), ronifibrate (9), and binifibrate (10).

4.4

Mode of Action

The fibrates were first identified in the 1950s as lipid-lowering agents by laborious *in vivo* screening, long before the molecular mode of action of the compounds was elucidated. It was not until the early 1990s and the advent of molecular biology techniques that the biological target of the fibrates could be identified. The fibrates had long been known to proliferate peroxisomes in rodents. Efforts at identifying the biological target of the fibrates were therefore directed toward a mechanistic understanding of this effect, which is absent in humans (and nonhuman primates), due possibly in part to 10-fold lower PPAR α expression levels in humans compared to rodents [36]. This work led to the identification of the PPARs via a reverse-endocrinology approach [37].

PPAR α is a member of the PPAR family of nuclear receptors, a family of ligand-modulated transcription factors consisting of three subtypes: PPAR α , PPAR δ , and PPAR γ . PPAR α was the first member of the family to be cloned [38]. It was shown soon afterward that it exerted its action as a functional heterodimer with another nuclear receptor, the retinoid X receptor (RXR) [39, 40], and that this heterodimer is activated by endogenous fatty acids as well as the fibrates [41, 42]. Compelling evidence that PPAR α is the key mediator of the biological effect of the fibrates was eventually provided by a PPAR α knockout mouse [43]. In humans, PPAR α is expressed mainly in the liver, PPAR γ mainly in the adipose tissue, and PPAR δ is expressed in most tissues.

The PPAR proteins consist of three main domains: a DNA-binding domain (DBD), a ligand-binding domain (LBD), and an N-terminal domain. Within the members of the PPAR family, there is a high degree of sequence identity in the DBD (about 85%), with a lower degree of sequence identity between the LBDs (about 70%), which introduces the possibility of the development of subtype-selective ligands. Endogenous agonists/activators of PPAR α include a range of saturated and unsaturated fatty acids such as oleic, linoleic, palmitic, and arachidonic acids [44, 45], leading to the proposed role of PPAR α as sensor of nutrient status.

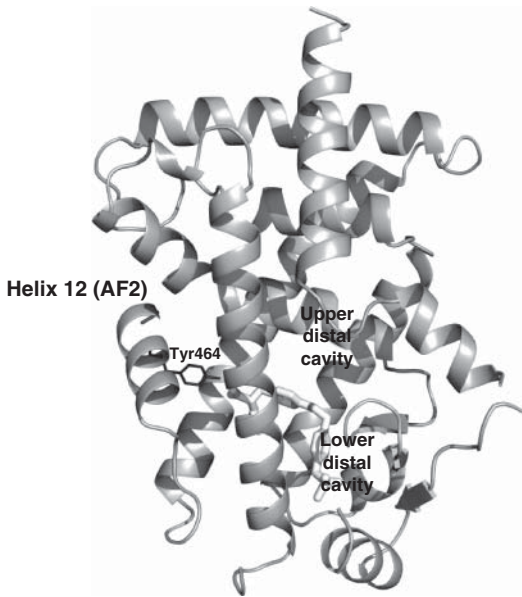


Figure 4.2 Overall structure of the PPAR α LBD bound with AZ242/tesaglitazar (shown as sticks). Key agonism-related hydrogen-bonding residue (Tyr464) highlighted. Picture courtesy of Anders Hogner, generated from pdb 117G [46].

As with the other PPAR subtypes, the PPAR α LBD consists of 12 helices arranged in an antiparallel helix sandwich, with the ligand-binding site located in the central core of this structure [46]. Helix 12 (AF-2) exhibits significant conformational mobility, which is intimately connected to receptor activation (see Figure 4.2, Section 4.5).

PPAR:RXR heterodimers bind to DNA sequences known as PPAR response elements (PPREs) in the promoter regions of PPAR target genes and either repress or activate gene transcription. In the absence of a receptor agonist/activator, corepressor proteins are associated with the PPAR:RXR heterodimer, leading to repression of gene transcription by formation of a “closed” chromatin structure. In contrast, PPAR activators/agonists lead to recruitment of coactivator proteins to the PPAR:RXR heterodimer. This occurs by a ligand-induced change in the conformation of the receptor, whereby helix 12 is stabilized in an “activated” conformation that creates a docking surface for coactivator proteins, ultimately leading to initiation of gene transcription. In the case of PPAR α activators, many of the genes that are upregulated are involved in lipid uptake and oxidation, such as lipoprotein lipase (LPL), liver-type fatty acid-binding protein (L-FABP), and acyl-coA oxidase (ACOX).

Fibrate treatment leads to a lowering of elevated plasma triglycerides and cholesterol, with the most profound effect being a lowering of triglyceride-rich lipoproteins. Increases in HDL and decreases in LDL are generally observed. The

increase in HDL is most pronounced in patients with initially low baseline HDL concentrations [47].

The clofibrate–nicotinic acid conjugates etofibrate (**8**), ronifibrate (**9**), and binifibrate (**10**), of which only etofibrate (**8**) is currently in limited use, have an additional mode of action. Upon administration, they are hydrolyzed to release the two active components, clofibric acid (**2a**) and nicotinic acid. Nicotinic acid is an antilipolytic agent (a GPR109A agonist), which acts by reducing free fatty acid levels and thereby suppressing VLDL (very low density lipoprotein) production [48]. This is complementary to the mainly triglyceride lowering effect of the fibrate component. In addition, these conjugates act as slow-release formulations of clofibric acid (**2a**) and nicotinic acid [49, 50], thereby helping to avoid the gastrointestinal effects and facial flushing which are side effects of nicotinic acid treatment that limit its prescription as a hypolipidemic monotherapy.

4.5

Structure–Activity Relationships

Since the identification of PPAR α as the target of the fibrates, much drug discovery effort has been directed toward the generation of more potent PPAR α activators. This has largely been without success, mainly due to extensive preclinical and clinical safety liabilities [51]. It is therefore perhaps not surprising that the most recently discovered of the fibrate-based PPAR α activators that is still in clinical use was first launched in 1975.

As can be seen from Table 4.2, the fibrates are comparatively low-potency activators of PPAR α , a fact that is reflected in the typical doses required to exert the desired hypolipidemic effect (up to 2000 mg/day).

Within the class, the prototypical clofibric acid (the active metabolite of clofibrate (**2**)) is the least potent PPAR α activator. Increasing lipophilicity (as described by clogP) generally leads to increased potency at PPAR α , with a concomitant reduction in dose. However, the overall pharmacokinetic properties of the compounds also have an effect on dose [52]. Both gemfibrozil (**6**) and

Table 4.2 *In vitro* PPAR agonist potency data, human plasma half-life, and doses of selected fibrates.

Compound	clogP	PPAR α EC ₅₀ (μ M) ^a	PPAR γ EC ₅₀ (μ M) ^a	PPAR δ EC ₅₀ (μ M) ^a	t _{1/2} (h) [52]	Typical human dose (mg/day)
Clofibric acid (2a) [53,]	2.8	55	>300	Inactive	15	2000
Fenofibric acid (5a) [53, 54]	4.1	30	300	50	19–27	200
Gemfibrozil (6) [53]	3.9	45	Inactive	75	1.3	900–1200
Ciprofibrate (4) [55]	3.1	20	>300	Inactive	81	100
Bezafibrate (3) [53]	3.7	50	60	20	1.5–3	600

a) Values from GAL4 transactivation assays.

bezafibrate (3) have shorter plasma half-lives than fenofibrate (5) and ciprofibrate (4), a fact reflected in their higher overall doses. The relatively high potency and long half-life of ciprofibrate (4) contribute to it requiring the lowest clinical dose within the class. Ciprofibrate (4) is also the only member of the fibrate class possessing a stereogenic center. However, it is marketed as a racemic mixture, as the *in vivo* pharmacokinetics (PK)/pharmacodynamics (PD) properties of the pure enantiomers are similar to those of the racemate [56].

As mentioned in Section 4.4, ligand-induced conformational changes in the receptor lead to the activation of gene transcription by recruitment of coactivator proteins. Structurally different nuclear receptor ligands can lead to subtle differences in the nature of the conformational change. This leads to a different profile of the coactivator proteins recruited, which in turn can lead to differences in the overall gene expression profile and clinical outcome. Clinically, gemfibrozil (6) differs from the chlorinated fibrates clofibrate (2), ciprofibrate (4), and fenofibrate (5) in that, although it leads to an overall increase in HDL, it has little or no effect on levels of apoA-I (typically a major component of HDL particles). This has been shown to be caused by a different gene expression profile for gemfibrozil (6), which is due to differential recruitment of coactivator proteins [57].

The PPAR α ligand-binding cavity is generally described as Y- or T-shaped, Figure 4.2 [46]. PPAR α activators generally exhibit the modular structure shown in Figure 4.3, with an acidic, hydrophilic head group and two lipophilic groups attached in series by linkers. The PPAR (α , γ , δ) subtype selectivity from subtype selective to pan-subtype activators is generally determined by modifications to the lipophilic groups and linkers [58].

The carboxylic acid head group of PPAR α activators makes a key hydrogen-bonding interaction with Tyr464 in helix 12 (AF-2), thereby stabilizing a conformation of the receptor that permits coactivator protein recruitment and subsequent initiation of gene transcription. Only the lower distal cavity is occupied by ligand in public domain crystal structures of ligand-bound PPAR α [59].

Early structure–activity relationships in fibrate drug discovery were deduced from *in vivo* measurements of plasma lipids and thus reflected the overall PK/PD properties of the compounds. However, in order to understand the

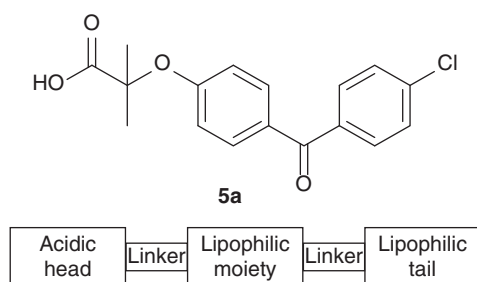


Figure 4.3 General structure of PPAR α activators as exemplified by fenofibric acid (5a).

structure–activity relationship (SAR) of the fibrates, target-based SARs derived from *in vitro* PPAR α assays are instructive.

For example, modifications of the hypolipidemic PPAR α activator and potent rodent peroxisome proliferator Wy-16463 (**28**) (also known as pirinixic acid) demonstrate how PPAR subtype selectivity can be modulated by structural modifications in the vicinity of the acidic head group (Table 4.3). Pirinixic acid (**28**) is a modestly selective PPAR α activator. Introduction of an ethyl group in the α -position of the acid head group gives the more potent dual PPAR α / γ full agonist **29**, while introduction of the much bulkier 4-phenylnaphth-1-yl moiety leads to PPAR γ selective activator **30** [60].

Work carried out at Glaxo Wellcome (now GlaxoSmithKline (GSK)) to identify more potent and selective PPAR α activators led to the discovery of the ureidofibrates, exemplified by GW2331 (**31**), see Table 4.4 [61]. These compounds demonstrate how bulky lipophilic substituents can be accepted by the distal arms of the PPAR α -binding pocket, leading to increased PPAR α binding affinity, 600-fold improved functional potency compared to fenofibric acid ((**5a**), PPAR α EC₅₀ = 30 μ M), and sixfold selectivity for PPAR α over PPAR γ . Replacing the α -ethyl group of GW2331 (**31**) with a methyl group, to give compound **32**, leads to a reduction in overall potency and selectivity, again demonstrating the functional sensitivity of this region of the receptor binding site. However, replacing the oxygen of **32** with sulfur (to give GW9578 (**33**)) results in regained PPAR α potency and enhanced selectivity (20-fold) toward PPAR γ and PPAR δ

Table 4.3 SAR for pirinixic acid derivatives: EC₅₀ values and relative activation compared to a positive control (pioglitazone for PPAR γ and GW7647 for PPAR α) from GAL4 reporter gene assays [60].

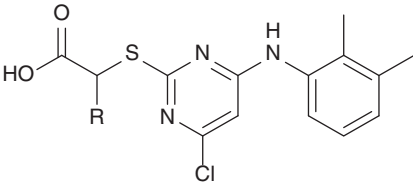
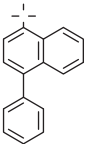
				
Compound	R	PPAR α EC ₅₀ (μ M)/% activation	PPAR γ EC ₅₀ (μ M)/% activation	
Pirinixic acid (28)	H	36.3/100	53.6/79	
29	Et	1.0/100	3.6/100	
30		Inactive	4.5/45	

Table 4.4 SAR and PPAR selectivity profiles for ureidofibrates and ureido-thioisobutyric acids.

Compound	X	R ₁	R ₂	R ₃	PPAR α EC ₅₀ (μ M)	PPAR γ EC ₅₀ (μ M)	PPAR δ EC ₅₀ (μ M)
GW2331 (31)	O	Et	<i>n</i> -Heptyl		0.05	0.3	>1
32	O	Me	<i>n</i> -Heptyl		0.79	0.2	1.4
GW9578 (33)	S	Me	<i>n</i> -Heptyl		0.05	1.0	1.4
GW2433 (34)	O	Me			0.12 ^{a)}	>10 ^{a)}	0.04 ^{a)}

EC₅₀ values from GAL4 reporter gene assays.a) K_D values from competition binding assays.

[62]. GW9578 (**33**) was the first highly potent, truly PPAR α -selective compound reported.

Screening of a combinatorial chemistry library of ureidofibrates carried out by the same group at GSK led to the discovery of GW2433 (**34**), the first high-affinity PPAR δ ligand to be disclosed, which also displays PPAR α affinity [63]. From this combinatorial library, the group was able to deduce that, in spite of overall similarities in the binding pockets of the various PPAR subtypes, subtle differences in structure could be exploited to introduce PPAR subtype selectivity.

As the SAR examples demonstrate earlier, significant PPAR subtype ($\alpha/\gamma/\delta$) selectivity is achievable for fibrate derivatives through relatively minor structural modifications in the vicinity of the carboxylic acid head group. With respect to the PPAR subtype selectivity of the clinically used fibrates, bezafibrate (**3**) is unique in that it is a pan-PPAR activator at clinically achievable concentrations (Table 4.2), even though it possesses the same phenoxyisobutyric acid head

group as fenofibric acid (**5a**) and ciprofibrate (**4**). This illustrates the difficulty in predicting PPAR subtype selectivity based on simple inspection of a compound's chemical structure. Even though the LBDs of the various PPAR subtypes display only moderate sequence identity, an overall similarity in the tertiary structure between the subtypes means that subtle differences in ligand structure can have an unexpectedly significant impact on PPAR subtype selectivity. The pan-PPAR profile of bezafibrate (**3**) demonstrates the fact that modifications to the ligand far from the region of the acidic head group have considerable influence on PPAR subtype selectivity.

Finally, even though it has been shown that the fibrates exert their lipid-lowering effect via PPAR α activation, they exhibit some differences in the clinical lipid profiles achieved. Systematic review of fibrate clinical trials has shown that bezafibrate (**3**) treatment causes a slightly greater HDL increase than other fibrates, whereas fenofibrate (**5**) leads to a greater reduction in total cholesterol compared to other fibrates [22]. However, these lipid profile differences are generally not reflected in significantly different clinical outcomes.

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5

Antiplatelet 2-Hydroxy Thienopyridine Ester Derivatives for the Reduction of Thrombotic Cardiovascular Events

Modesto de Candia, Nunzio Denora, and Cosimo D. Altomare

5.1

Introduction

Thienopyridine (TP) refers to the common structure – that is, 5-benzyl-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine – of a class of platelet aggregation inhibitors. Ticlopidine (**2**), clopidogrel (**3**), and the acetate ester of 2-hydroxy TP prasugrel (**4**, Figure 5.1) are the marketed drugs of the class. The TPs are bioprecursor prodrugs, which, after oral administration, undergo a cytochrome-mediated activation to active metabolites. Those in turn reduce or block *in vivo* platelet aggregation through irreversible inhibition of the purinergic receptor P2Y₁₂ located on platelet cell membranes [1]. TPs are currently used to decrease the risk of thrombotic cardiovascular events related to the adenosine diphosphate (ADP)-induced platelet aggregation, occurring in several pathologies (e.g., cerebral ischemia, acute coronary syndrome (ACS), and arterial thrombosis) [2, 3]. After the discovery of ticlopidine (**2**), a number of analogs have been developed in order to improve efficacy and safety. An emerging favorable feature in antiplatelet TP analogs has been the acetate ester group in position 2 of the TP scaffold, which proved to significantly affect bioactivation and pharmacokinetics (PKs), ultimately making the newest TPs more efficacious and safer. This chapter focuses on the development of prasugrel (**4**), whose 2-OCOCH₃ group plays an essential role in modulating the *in vivo* antiplatelet activity of TPs.

5.2

History

Ticlopidine (**2**, Figure 5.1) was discovered in 1972. Looking for new anti-inflammatory and analgesic drugs related to tinoridine (**1**), research groups at Sanofi synthesized a number of analogs, which were assayed in different animal models of human pathologies. While none of the tested compounds showed anti-inflammatory effects, some of them, including **2**, surprisingly showed antiplatelet and antithrombotic activities upon oral administration into rats.

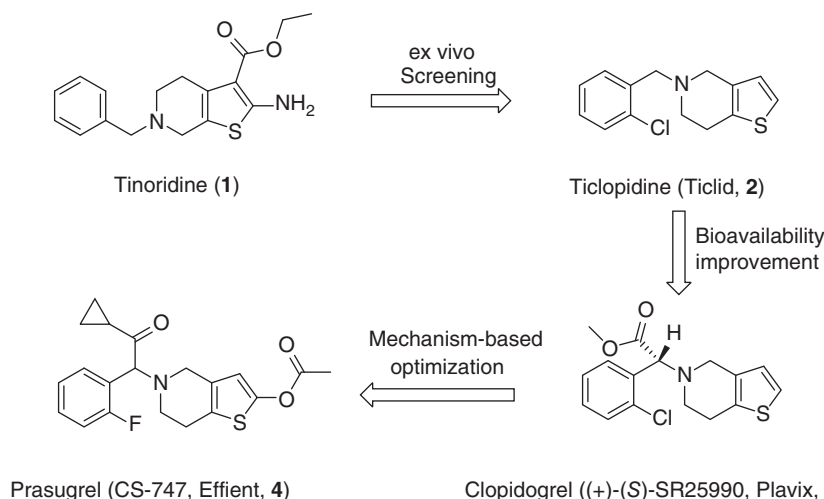


Figure 5.1 Development pathway of antiplatelet thienopyridines leading to the discovery of prasugrel (4).

In the early 1970s, the link between platelet aggregation and thrombosis had not been clearly understood, but ticlopidine (2) was selected and evaluated in pathological conditions associated to thrombotic complications, such as cardiac surgery with extracorporeal circulation, or hemodialysis. Due to the promising results, compound 2 was marketed in France in 1978 under the brand name Ticlid® and approved with limited clinical indications, such as thrombosis, transient ischemic attack, stroke, and peripheral arterial disease. Once its efficacy in patients with high thrombotic risk was proved in larger clinical trials, ticlopidine (2) was approved by the FDA in 1991 and marketed in the United States and worldwide. However, postmarketing surveillance of ticlopidine (2), in addition to rash and diarrhea, revealed a number of severe reversible hematological side effects (including leucopenia, thrombocytopenia, agranulocytosis, and pancytopenia). Many analogs of 2 were thus synthesized with the aim of improving efficacy and safety. Among the more than 1000 derivatives produced, 8 compounds were investigated up to Phase-I trials. Among those, clopidogrel (3, PCR4099, Plavix®), marketed in 1998 by Sanofi and Bristol-Myers Squibb, proved to be more potent and better tolerated than 2, especially in patients experiencing previous thrombotic events, as shown by Phase-II studies. The compound was also superior in reducing the risk of restenosis associated with stent implantations [3, 4]. Clopidogrel (3) immediately appeared as a powerful inhibitor of ADP-induced platelet aggregation, and *ex vivo* experiments in animals combined with PK studies contributed to elucidate its mechanism of action [5]. Compared to ticlopidine (2), the only structural change in 3 was the introduction of a methyl carboxylate (COOCH₃) group on the benzyl carbon in an attempt to increase bioavailability. Indeed, clopidogrel (3) showed better oral absorption, even if the peak of the antiplatelet effect was achieved for both drugs only after 3–5 days [1]. Because

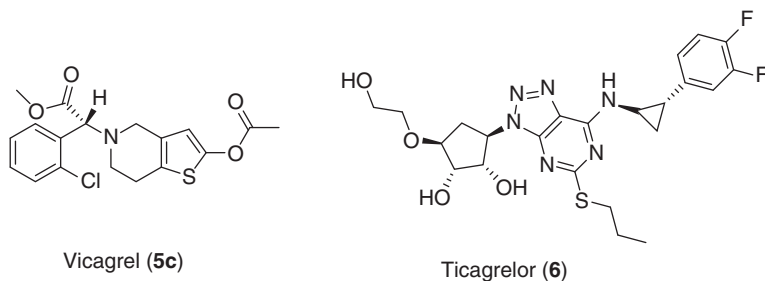


Figure 5.2 Structures of newer antiplatelet agents acting as irreversible (**5c**) and reversible (**6**) antagonists of the P2Y₁₂ receptor.

TGs did not show any detectable antiplatelet activity *in vitro*, it became clear that **2** and **3** behave as prodrugs. In the years 2000–2005, *in vitro* metabolism studies with microsomes revealed the oxidative opening of the thiophene ring, resulting in the formation of reactive thiol metabolites, which act as irreversible antagonists of the P2Y₁₂ receptors. The two enantiomers of clopidogrel (**3**) showed different biological effects, with the (*S*)-(+)-enantiomer (eutomer) exhibiting antiplatelet activity and the (*R*)-(–)-enantiomer (distomer) being inactive and less tolerated [6]. However, in some patient populations, reduced antiplatelet activity and resistance to treatment were reported. Interindividual variability in the inhibition of platelet aggregation might be related to a number of factors, such as adherence to therapy, variable absorption of the parent drug, and different rates of active metabolite formation. It was shown that up to 30% of Caucasian patients were nonresponsive or poorly responsive to clopidogrel (**3**), because of a polymorphism of CYP2C19 (and CYP2C9) associated with decreased exposure to the active metabolite and consequent loss of activity. In 2010, the FDA pointed out a high risk of treatment failure for CYP2C19 poor metabolizers. Possible therapeutic approaches aimed at overcoming clopidogrel (**3**) resistance could include (i) higher doses of clopidogrel (**3**); (ii) addition of cilostazol (a selective inhibitor of phosphodiesterase-3, which acts as direct vasodilator and platelet aggregation inhibitor); and (iii) the use of new antiplatelet agents, such as prasugrel (**4**) and ticagrelor (**6**), the latter being a reversible P2Y₁₂ receptor antagonist. Besides prasugrel (**4**), a novel TG (**5c**, vicagrel; Figure 5.2) was proposed in 2012, which proved equipotent to **4** in preventing platelet aggregation. In addition, the compound was able to overcome response variability due to CYP2C19 polymorphism [7, 8].

The third-generation TG prasugrel (**4**; trade names: Effient® in the United States and India, and Efient® in European countries) was approved in 2009 for the reduction of thrombotic events in patients with ACS. The structure of prasugrel (**4**) differs from that of **3** by: (i) a 2-fluorobenzyl replacing the 2-chlorobenzyl group in position 5, (ii) a hydrolytically stable cyclopropylcarbonyl moiety instead of the COOCH₃ group, and even more importantly, and (iii) an acetyl ester group (OCOCH₃) at C(2). These structural features were supposed to achieve, and in fact did achieve, improved bioavailability and bioactivation of the third-generation

Table 5.1 Effects of antiplatelet thienopyridines on platelet aggregation parameters in Phase-I studies.^{a)}

Drug	Onset of action (h)	Time to full platelet inhibition (d) ^{b)}	Persistence of action after stopping therapy
Ticlopidine (2)	24–48	3–5	>72 h
Clopidogrel (3)	~48	3–5	3–5 d
Prasugrel (4)	0.5	3	5–7 d

a) Results from multiple-dose studies with *ex vivo* testing (ADP as the platelet aggregation inducer) in healthy volunteers.

b) Without using loading doses.

Adapted from Ref. [2].

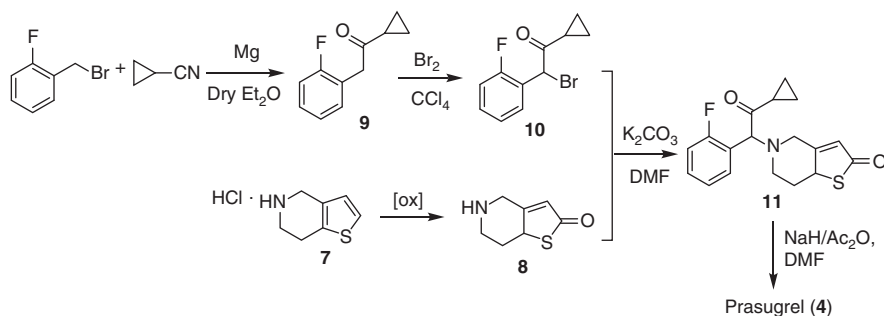
TPs. The *in vivo* effects on platelet aggregation of the three TP (2–4), as assessed in healthy human volunteers, are summarized in Table 5.1.

The data highlight the progress achieved with prasugrel (4), over clopidogrel (3) and ticlopidine (2), in terms of reduction of the onset of action and trend prolongation of the effects after the end of therapy.

5.3

Synthesis

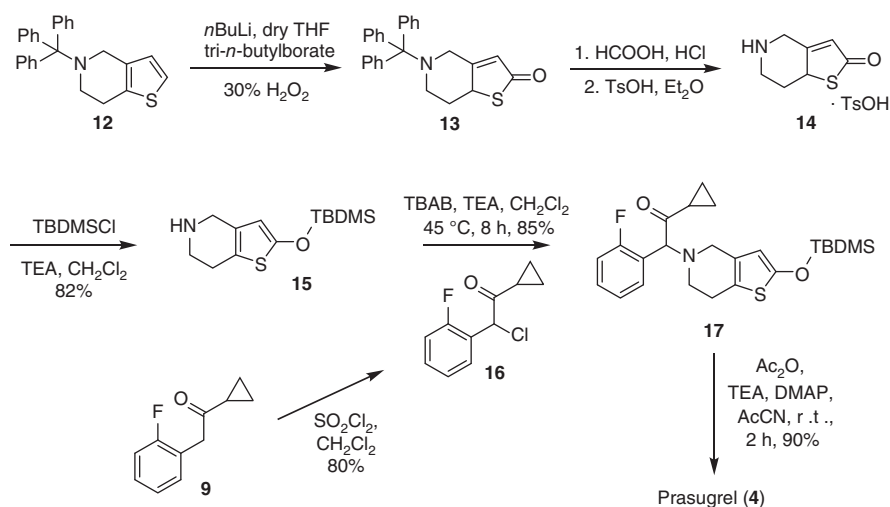
The first original synthesis of prasugrel (4) was patented by Kioke *et al.* at Daiichi Sankyo Co. in 1993 (Scheme 5.1) [9].

**Scheme 5.1** The Daiichi Sankyo synthesis pathway of prasugrel (4).

The commercially available 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride (**7**) was oxidized at position 2 yielding thien-2(4H)-one **8**, which was then N-alkylated with 2-bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone (**10**) to afford compound **11**. Compound **10** was in turn synthesized through α-bromination of the ketone intermediate **9** resulting from a Grignard reaction between the 2-fluorobenzyl magnesium bromide and cyclopropylcyanide. In the

final step, compound **11**, which undergoes keto-enol tautomerism, was acetylated to prasugrel (**4**) and at this stage could be resolved into the pure enantiomers by chiral high-performance liquid chromatography (HPLC) during early studies. Unfortunately, the key reaction between **8** and **10** occurred in only 32% yield (process: total yield is about 10%). In an attempt to increase the yield in this step, Stepankova and Hajicek also used the methanesulfonate analog of the Br-alkyl intermediate **10**, but could not obtain any appreciable improvement [10].

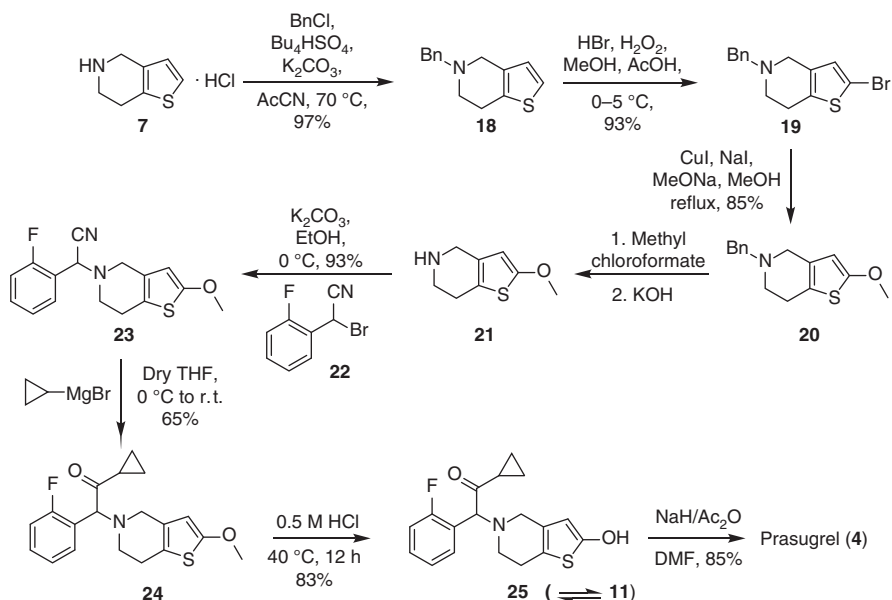
An alternative synthesis of prasugrel (**4**) was patented in 1999 by Kikuo *et al.* [11] (Scheme 5.2), who claimed a significant increase of the total yield up to 72%.



Scheme 5.2 Synthesis pathway for prasugrel (**4**) proposed by Kikuo *et al.* [11].

2-((*t*-Butyldimethylsilyl)oxy)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (**15**) was reacted with 2-chloro-1-cyclopropyl-2-(2-fluorophenyl)ethanone (**16**), prepared in turn by chlorination of **9** with SO_2Cl_2 . The use of a suitable OH protecting group proved to be important, and as a matter of fact, the use of groups different from *t*-butyldimethyl silyl (TBDMS) caused significantly lower yields, as afterwards shown by Padi [12]. As reported in Scheme 5.2, the synthesis of **15** was accomplished through oxidation (*n*-BuLi and borate ester) of **12**, that is, the *N*-trityl-protected derivative of **7**, to the thienone compound **13**. Removal of the trityl protecting group under acidic conditions furnished the tosylated salt **14**, which was transformed into the silyl ether **15** (>80% yield). In the final step, the treatment of the TBDMS-protected intermediate **17** under mild basic conditions with Ac_2O provided prasugrel (**4**) in 90% yield. Unfortunately, due to safety issues, and the costs of the protecting reagents as well, this synthesis was not applicable on an industrial scale.

Most recently, Pan *et al.* proposed a new linear efficient synthesis of prasugrel (**4**) applicable to production level (Scheme 5.3) [13]. By considering



Scheme 5.3 High-yield synthetic pathway to prasugrel (4) [13].

the relevance of the 2-OH protecting group to achieve good yields, a 2-OMe substituent was introduced into the 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine scaffold. N-Benzylation of the starting compound **7** yielded **18**, which was subsequently brominated at position 2 under mild conditions. 5-Benzyl-2-bromo-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (**19**) was obtained on gram scale and was converted (>90% yield) into the 4,5,6,7-tetrahydro-2-methoxythieno[3,2-*c*]pyridine (**21**) through CuI/NaI-promoted substitution of the bromine with NaOMe (**20**), followed by N-debenzylation.

Compound **21** was reacted with 2-bromo-2-(2-fluorophenyl)acetonitrile (**22**). The key intermediate **23** was the substrate in the Grignard reaction with cyclopropyl magnesium bromide to furnish **24**. The yield in this step was significantly improved (65%) by adding a large excess (about 50% equiv.) of the Grignard reagent. Demethylation of **24** (0.5 M HCl, 40 °C), formed the 2-OH derivative **25** (the tautomer of **11**) in 83% yield, and subsequent acetylation (85% yield) provided prasugrel (**4**).

5.4

Mode of Action

The antiplatelet TPs are bioprecursor prodrugs. Inactive *in vitro*, they are converted *in vivo* into active metabolites, which irreversibly inhibit the purinergic receptor P2Y₁₂, conducting to block platelet aggregation. Examining the biological events, which occur in the platelet aggregation processes in more detail, ADP

activates two different platelet G-protein coupled receptors (GPCRs), which act synergistically, namely the $P2Y_1$ and $P2Y_{12}$ receptors coupled with a G_q and a G_i protein, respectively [2, 14]. Activation of $P2Y_1$ receptors mediates the platelet shape change and adhesion in the early reversible stage of platelet activation. Through a G_q -coupled protein, $P2Y_1$ receptors induce activation of phospholipase C, cleavage of membrane phospholipids and production of intracellular second messengers, such as the hydrophobic diacylglycerol (DAG) and the hydrophilic inositol-1,4,5-triphosphate (IP_3). IP_3 increases the cytosolic Ca^{2+} concentration essential for aggregation and clot formation, whereas DAG is converted by phospholipase A_2 into arachidonic acid. The latter, in turn, is transformed by prostaglandin synthase into the strong platelet aggregation agonist thromboxane A_2 . Activation of the $P2Y_{12}$ receptors, through a G_i -coupled protein, leads to inhibition of adenylate cyclase, consequently lowering the cAMP (cyclic adenosine monophosphate) concentration, the release of aggregation agonists contained in the cytoplasm dense granules, as well as the secondary irreversible phase of platelet aggregation. The $P2Y_{12}$ receptors are selectively expressed on platelets, thereby representing an attractive target for antithrombotic agents, as widely demonstrated clinically [1, 2, 5].

Following oral administration, the second-generation TP_s, such as *S*-(+)-clopidogrel (**3**), undergo a two-step cytochrome P450 (CYP)-mediated metabolic activation as shown in Figure 5.3 (the first-generation drug ticlopidine (**2**) undergoes a similar metabolic activation). Clopidogrel (**3**) is rapidly absorbed and converted (~85%) by plasma esterases into the carboxylic acid **26**. This main inactive metabolite, which shows peak plasma concentrations 1 h after administration, is excreted as glucuronide conjugate [5]. The unhydrolyzed fraction is

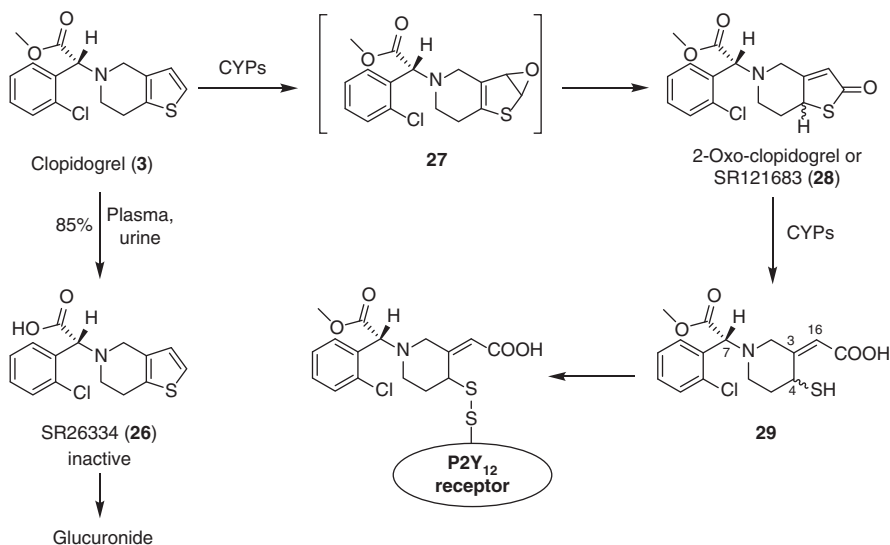


Figure 5.3 Activation and metabolic pathway of clopidogrel (**3**); CYPs: cytochromes P450.

oxidized by CYPs to the thiolactone metabolite SR121683 (2-oxo-clopidogrel, **28**) via an epoxy intermediate (**27**) [5]. SR121683 (**28**) is then rapidly converted by a ring-opening reaction into a mixture of inactive and active thiol metabolites (**29**). The structure of **29** has three stereochemically relevant sites, that is, two chiral centers at C4 and C7 and the C3–C16 ethylenic bond, which may lead to the formation of eight stereoisomers. Metabolomics studies (MS) and NMR studies on stereoisomers formed by incubating 2-oxo-clopidogrel (**28**) with human liver microsomes suggested that the active metabolite has *Z* configuration at C3–C16 double bond, *S* configuration at C7, and, possibly, *R* configuration at C4. However, due to the very high reactivity of the thiol function, the configuration at C4 could not be fully established [15]. The active metabolite irreversibly blocks P2Y₁₂ receptors, by forming a disulfide covalent bond with receptor Cys residues, which is consistent with the platelet inhibition still detectable several days after suspension of therapy and recovery of the normal platelet functions through cell turnover [1, 5].

Unlike clopidogrel (**3**), prasugrel (**4**) is orally administered as racemic mixture and, due to different structural features, shows different bioactivation pathways (Figure 5.4). Prasugrel (**4**) is rapidly absorbed with high bioavailability and activated in a one-step process. The active metabolites are detectable in plasma 15 min after administration and show peak plasma concentrations within half an hour, whereas the unchanged drug is no longer detectable after 30 min [1, 16]. The acetate ester undergoes rapid intestinal and serum metabolism via carboxylesterase-mediated hydrolysis to form the 2-hydroxy TP derivative, which tautomerizes toward the thermodynamically stable thiolactone derivative R-95913 (**11**) [17]. Compound **11** is rapidly converted into the active thiol metabolite R-138727 (**30**) through a ring-opening reaction catalyzed by CYPs in the liver. After its generation, **30** forms a covalent disulfide bond with the Cys residues of the P2Y₁₂ receptors. PK studies in healthy volunteers revealed that the active metabolite **30** has a very short half-life, because it is rapidly inactivated through a disulfide bond formation with free cysteine (R-119251, **31**) or by –SH methylation (R-106583, **32**). R-106583 is further converted to other metabolites, two of which are glucuronides [18–21].

Two different routes for the ring-opening reaction of the thiolactone intermediates have been proposed, based on detailed *in vitro* study of the metabolism of **4** by human liver microsomes and human sera [22]. The first route leads to the active 4-mercapto-3-piperidinyliden acetic acid metabolite **30** and is similar to the bioactivation of clopidogrel (**3**) shown earlier. It involves the CYP-mediated oxidative ring-opening of **11** through the formation of a thioester sulfoxide, which rapidly reacts with water to form a sulfenic acid intermediate. The sulfenic acid is then reduced by glutathione (GSH) to **30**. Most likely, the sulfenic acid conjugates with GSH or a Cys side chain and the generated disulfides are directly reduced by GSH or the glutaredoxin–thioredoxin system [23].

A second possible ring-opening route, occurring in serum and liver microsomes both for prasugrel (**4**) and clopidogrel (**3**) thiolactones, has recently been discovered. The metabolite **33**, bearing the double bond within the piperidine ring, is

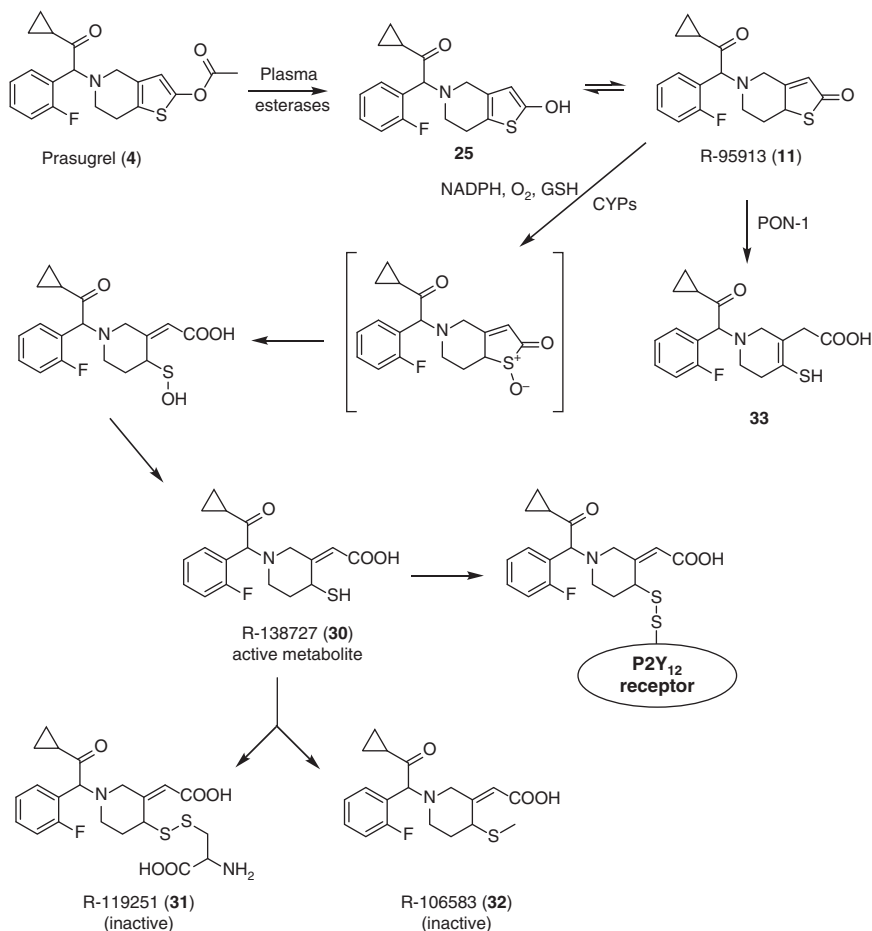


Figure 5.4 Activation and metabolic pathways of prasugrel (**4**) [1, 2, 16–20]. CYPs: cytochromes P450; PON-1: paraoxonase-1; and GSH: glutathione.

produced in much lower amount by a nonoxidative ring-opening reaction of key metabolite R-95913 (**11**). Under these conditions, the hydrolysis of the thioester **11** could be mainly catalyzed by paraoxonase-1 (PON-1), whose polymorphism could affect the reaction rate and thus the amount of metabolite **33** formed.

Many studies proved that the ring-opening reaction in prasugrel (**4**) is mainly catalyzed by CYP3A4 (low affinity, high capacity of substrate conversion) and CYP2B6 (high affinity, low substrate conversion) and only to a lesser extent by CYP2C9 and CYP2C19. As a matter of fact, genetic variation in CYP2C19 significantly determines the clinical clopidogrel response, whereas it does not seem to have an effect on the efficacy of prasugrel (**4**). The first oxidative step of clopidogrel (**3**) activation is enantioselective. Only the *S*-(+)-enantiomer undergoes oxidation, mostly catalyzed by CYP2C19 and to lesser extent by other

CYP isoforms (1A2, 2B6, 2C9). CYP2C19 undergoes a wide gene polymorphism and many drug–drug interactions, which can affect the activity of clopidogrel (**3**) (i.e., reduced conversion of the thiolactone metabolite) [24]. The formation of R-138727 (**30**) from prasugrel (**4**) is mediated by CYP3A4, an enzyme catalyzing the oxidation metabolism of many drugs. Despite this fact, only a limited amount of drug–drug interactions have been described in healthy volunteers treated with prasugrel (**4**) and CYP3A4 inhibitors such as ranitidine or the antifungal ketoconazole. In contrast, thiolactone R-95913 (**11**) seems to weakly inhibit some CYP isoforms (2C9, 2C19, 2D6, 3A4). The clopidogrel metabolite **29** does not show any appreciable inhibition of its activating CYPs (absence of interactions with drugs metabolized by CYP2B6, such as cyclophosphamide, bupropion, nevirapine, halothane, and propofol), whereas inhibition of CYP2C9 and CYP2C19 has been observed only at concentrations much higher than the ones that are used clinically. CYP2C9 and CYP2C19 are involved in the bioactivation of clopidogrel (**3**), but not prasugrel (**4**), and are inhibited by proton pump inhibitors (e.g., omeprazole, esomeprazole) [16].

The third-generation antiplatelet prasugrel (**4**) is the acetate ester prodrug of 2-hydroxy TP. It was designed and synthesized to overcome the resistance (non- or poor responsiveness) to the clopidogrel (**3**), which is mainly caused by CYP2C19 loss-of-function polymorphism. The P2Y₁₂ antagonism of prasugrel (**4**) and the consequent inhibition of ADP-induced platelet aggregation were proved by a number of clinical studies. Prasugrel (**4**) is used to reduce thrombotic cardiovascular events, including stent thrombosis, in patients with ACS, and to prevent thrombosis after percutaneous coronary intervention. Table 5.2 summarizes the main pharmacological properties of prasugrel (**4**) compared with those shown by clopidogrel (**3**).

Prasugrel (**4**) produces rapid and long-lasting inhibition of platelet function, has a greater potency than clopidogrel (**3**), and shows additive effects when used in combination with aspirin to prevent blood clots after angioplasty or coronary bypass graft [25]. The improved biological properties of prasugrel (**4**) relate to the increased production of the active metabolite R-138727 (**30**). Accumulating PK and drug–drug interaction data suggest a lower risk of clinically relevant interactions with other drugs. Despite the limited interactions with (es)omeprazole, no clinically relevant effects of the proton pump inhibitors on the formation of the prasugrel (**4**) active metabolite, and consequent platelet aggregation inhibition, have been observed.

Bleeding remains a major risk associated to the use of prasugrel (**4**). It should be used with caution and not be prescribed to patients with active pathological bleeding (e.g., peptic ulcer, history of transient ischemic attack or stroke). Clinical studies that focused on bleeding risk as a major endpoint showed a favorable safety/efficacy balance for prasugrel (**4**) [1, 23].

Adverse effects, occurring with decreasing frequency, include cardiovascular side effects (hypertension, hypotension, atrial fibrillation, bradycardia, noncardiac chest pain, thrombotic thrombocytopenic purpura), gastrointestinal side effects

Table 5.2 Pharmacological properties in humans of clopidogrel (3) and prasugrel (4).

	Clopidogrel (3)	Prasugrel (4)
Administration route	Oral	Oral
Frequency	Once daily	Once daily
Absorption	Rapid	Rapid
Metabolism	Liver	Intestine, plasma, liver
Half-life (h)	7–8	~4
Elimination route	Renal, biliary	Primarily renal
Pharmacokinetics	Linear	Linear
Mean platelet inhibition (%) ^{a)}	45	79
Onset of action (h)	2–8	0.5–4
Offset of action (d)	7–10	7–10
Drug–drug interactions	Extensive ^{b)}	Limited ^{c)}

a) Data (%) refer to inhibition of 5 μ M ADP-induced platelet aggregation on the seventh day after oral administration of clopidogrel (3) (75 mg/day) or prasugrel (4) (15 mg/day); results are from studies on 30 healthy volunteers and 101 patients with coronary artery disease.

b) Phenytoin, tamoxifen, tolbutamide, torasemide, fluvastatin, warfarin, unfractionated and low-molecular-weight heparins, dipyridamole, streptokinase, urokinase, omeprazole, esomeprazole.

c) Minimal interactions with the proton pump inhibitors omeprazole and esomeprazole.

Adapted from Refs [1, 2].

(nausea, diarrhea, gastrointestinal hemorrhage), hyperlipidemia, headache, dizziness, fatigue, fever, extremity pain, rash, leucopenia, anemia, and hypersensitivity reactions. The TP thiol metabolites could covalently react with cellular and/or circulating macromolecules (proteins, nucleic acids). Although not all the observed toxic effects depend upon the reactivity of the metabolites, there is evidence suggesting that their production and/or inadequate detoxification can cause idiosyncratic reactions.

5.5

Structure–Activity Relationships

The antiplatelet potency of prasugrel (4) relates to the production rate of the active metabolite R-138727 (30). R-138727 (30) is formed through a CYP-catalyzed oxidative ring-opening reaction of the thiolactone metabolite R-95913 (11), in turn produced through esterase-mediated hydrolysis of the 2-OCOCH₃ group in the parent drug 4. *Ex vivo* platelet aggregation and bleeding time data, determined in female rats orally administered with 1 mg/kg doses of prasugrel analogs [9], allow the following structure–activity relationships (SARs) to be drawn: (i) the 2-hydroxy ester moiety has a significant impact on potency, taking into account that analogs lacking the 2-OCOR moiety are inactive; (ii) the steric hindrance of the R group in the alkyl esters is optimal for methyl, but is tolerated up to the size of *n*-propyl; (iii) the prasugrel thiolactone metabolite 30 has antiplatelet

activity *in vivo*, although it is slightly less potent than the parent prodrug; and (iv) the 2-fluoro substituent appears to be important for the potency of prasugrel analogs, since replacement with 2-Cl resulted in loss of potency.

As for the stereochemistry, unlike clopidogrel (**3**), where only the *S*-(+) enantiomer is active, prasugrel (**4**) is orally administered as a racemic mixture. The CYP-mediated thiolactone ring-opening reaction to afford the active metabolite R-138727 (**30**) is stereoselective, and in human plasma, the (*R,S*) and (*R,R*) diastereoisomers (Figure 5.5) together account for 84% of the active thiol metabolites. *In vitro* platelet aggregation data proved that the antiplatelet activity of **30** is due only to the (*R,S*) and (*R,R*) isomers, whereas the (*S,R*) and (*S,S*) isomers are inactive, suggesting that the *R*-configuration of the piperidine C(4) chiral center is critical for blocking P2Y₁₂ receptors [1, 17]. Other studies report better antiplatelet activity for the (*R,S*) isomer over the (*R,R*) isomer of the prasugrel active metabolite [15]. Stereochemical characterization of the clopidogrel metabolites (Figure 5.3) suggested that the active thiol metabolite **29** has an (*S*)-configuration at the benzyl C7 and a (*Z*)-configuration at the piperidine C3 exocyclic double bond [26].

In 2012, Shan *et al.* [7] published a study aimed at investigating a number of 2-OH TP ester derivatives as prodrugs of the clopidogrel thiolactone (**28**) in order to overcome clopidogrel (**3**) resistance. This study not only showed that the concentration of the thiolactone **28** generated from **5c** (vicagrel, Figure 5.2) was six-fold higher than that formed from clopidogrel (**3**) *in vivo* but also significantly added to our knowledge on the SAR of 2-hydroxy TP derivatives (Table 5.3). *Ex vivo* data of the effects on ADP-induced platelet aggregation in rats showed that (*S*)-vicagrel (**5c**) is a strong inhibitor, only slightly less potent than prasugrel (**4**). As a further confirmation that the (*S*)-configuration on the benzyl carbon is critical for the activity of clopidogrel analogs, the (*R*)-**5c** enantiomer proved to be inactive. In contrast to prasugrel (**4**), the 2-chloro substituent appears to be essential for the potency of these ester derivatives. The 2-OH alkyl esters, showing a trend of activity similar to the carbonic acid esters (carbamate esters proved inactive in the aggregation assay), revealed that the potency decreases as the size of R² increases. This suggests that the steric hindrance at the 2-OCOR² groups does decrease the rate of hydrolysis to produce the thiolactone metabolite **28**. The aromatic esters, with the only exception of nicotinate **5n**, are less potent than the alkyl esters.

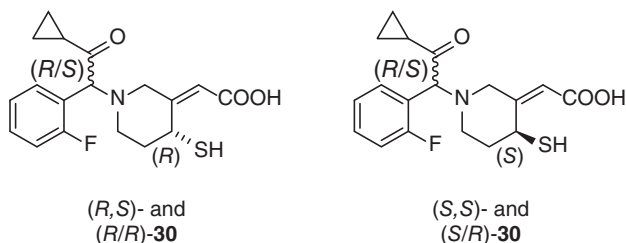


Figure 5.5 Stereoisomers of the prasugrel active metabolites **30**.

Table 5.3 Inhibition of ADP-induced platelet aggregation in rats^{a)} and structure–activity relationships of 2-hydroxytetrahydropyridine ester derivatives.

Compound ^{b)}	R ¹	R ²	Relative potency ^{c)}	SAR
3	Cl	—	—	Prasugrel (4) is the most potent compound; clopidogrel thiolactone (28) is a potent antiplatelet agent
4	F	Me	2.3**	
28	Cl	—	1.5**	
5a	H	Me	0.9	R¹ : 2-Cl > 2-F > 2-H The acetate ester (vicagrel, 5c) is only slightly less potent than 4
5b	F	Me	1.2*	
5c	Cl	Me	2.1**	
5d	Cl	Et	1.6**	R² : 2-OH alkyl esters Potency decreases as the R ² size increases: 5c > 5d > 5e > 5f > 5g
5e	Cl	<i>n</i> -Pr	1.4**	
5f	Cl	<i>t</i> -Bu	1.2*	
5g	Cl	<i>t</i> -Amyl	1.1	
5h	Cl	Ph	1.2**	
5i	Cl	4-NO ₂ -Ph	1	Aromatic esters (5h–k) are less potent than the alkyl esters, with the exception of nicotinate 5n
5j	Cl	4-MeO-Ph	0.9	
5k	Cl	2-AcO-Ph	0.9	
5l	Cl	Bn	1.4**	
5m	Cl	Styryl	0.9	
5n	Cl	Pyridin-3-yl	1.6**	R² : 2-OH carbonic acid esters Potencies similar to those of the alkyl esters; similar trend between antiplatelet potency and R ² size
5o	Cl	PhOCH ₂	1	
5p	Cl	MeO	1.9**	
5q	Cl	EtO	1.4**	
5r	Cl	<i>i</i> -PrO	1.5**	
5s	Cl	<i>i</i> -BuO	1.1	R² : carbamates are inactive
5t	Cl	BnO	1	
5u	Cl	Me ₂ N	1.1	
5v	Cl	Pyrrolidine-3-yl	0.9	Enantioselectivity : (R) enantiomer is inactive
(R)- 5c	Cl	Me	1.1	
(R,S)- 5c	Cl	Me	1.2*	

a) The *ex vivo* platelet aggregation data are taken from Ref. [7].

b) Compounds **5a–v**, only the optically active (*S*)-configured isomers of clopidogrel (**3**) and thiolactone metabolite (**28**) were tested; prasugrel (**4**) was tested as racemic mixture.

c) Relative potency versus clopidogrel (**3**) was calculated from platelet aggregation data referring to *ex vivo* measurements in male Wistar rats (200–250 g weight) 2 h after oral administration (3 mg/kg); **P* < 0.05, ***P* < 0.01 versus vehicle.

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6

Carboxylic Acids and Lactones as HMG-CoA Reductase Inhibitors

Xiang-Yang Ye and Pratik Devasthale

6.1

Introduction

Research and development of carboxylic acids and lactones as inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a key enzyme in biosynthesis of cholesterol (1), began over 25 years ago [1]. The developed class of compounds, called statins (“stat” in Latin means “to halt or arrest”), has now been proved to be highly effective in lowering low-density lipoprotein (LDL) cholesterol (1) and decreasing the incidence of heart attacks and strokes. Across 14 clinical trials involving over 90 000 patients with atherosclerotic disease, a 30% reduction in heart attacks after treatment with statins was observed [2]. Surveys have indicated that the majority of Americans “know” their cholesterol (1) numbers, which is a testament to the transformational success of statins as a drug class. In 2013, total US and global sales of statins amounted to ~\$10 and \$30 billion, respectively [3]. In 2006, Lipitor® alone had worldwide sales of almost \$13 billion and was the top-selling drug in history until it lost its patent protection in 2011 [4]. To date, 13 Nobel prizes have been awarded to work primarily focused on some aspect of cholesterol (1) research [5].

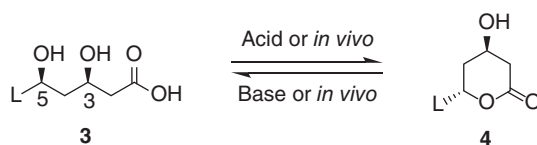
The discovery and development of carboxylic acid-based statins has been one of the most successful and exemplary stories in the pharmaceutical industry. It is one that was initiated through the unrelenting efforts of a handful of scientists against significant setbacks and then brought to fruition through a collaborative effort between industry, academia, and the regulatory agencies. The therapeutic and commercial success of statins also provided a strong impetus for the development of greener and more cost-effective biocatalytic methods for statin production, discussed later in this chapter. Epidemiological studies with statins alone or in combination with nonstatin therapy continue to strengthen the case for the routine use of statins to reduce cardiovascular risk. Recently published results from the IMPROVE-IT trial, evaluating the simvastatin (2)–ezetimibe combo therapy, suggested that the additional LDL-cholesterol-lowering achieved from the use of the combo relative to a statin alone resulted in a greater benefit in cardiovascular outcomes, lending further support to the “lower is better” viewpoint [6]. This

bodes well for highly potent current and future LDL-cholesterol-lowering agents such as Proprotein Convertase Subtilisin Kexin 9 (PCSK9) inhibitors, two of which (alirocumab from Sanofi/Regeneron and evolocumab from Amgen) received FDA approval in July and August 2015 respectively. In addition, prospects for therapies that effect potent LDL lowering with the combination of a low-dose statin to reduce statin-related adverse events and a PCSK9 inhibitor are likely to be explored in the future.

6.2

History

All statins contain a (3*R*,5*R*)-3,5-dihydroxypentanoic acid (**3**) moiety or its corresponding δ -lactone **4**, equivalent to a (4*R*,6*R*)-4-hydroxy-tetrahydro-2H-pyran-2-one, as a key pharmacophore (Scheme 6.1). The preferred absolute stereochemistry of the two hydroxyl groups matches that of the corresponding centers in the mevaldyl-CoA transition state intermediate **5** (Figure 6.2). The interconversion of the acid and the lactone forms can happen chemically under basic, acidic, or thermal conditions, or *in vivo* in the liver.



Scheme 6.1 General structure of statins and the interconversion of acid **3** and lactone **4**. L is a hydrophobic group.

A large body of human genetic and epidemiological studies have established a causal relationship between high cholesterol (**1**) levels and atherosclerosis and coronary heart disease. As researchers began to explore pharmacological approaches to reduce cholesterol (**1**), HMG-CoA reductase, the rate-limiting enzyme in cholesterol (**1**) biosynthesis, became a natural target, particularly given that the majority of the circulating cholesterol (**1**) in the body is biosynthesized in the liver.

In the late 1960s, a Japanese microbiologist at Sankyo Research Laboratories named Akita Endo speculated that fungi such as molds and mushrooms could produce compounds that inhibited HMG-CoA reductase [7]. Inspired by the work of Alexander Fleming on the discovery of penicillin from the blue-green mold in 1928, Endo decided to explore culture broths of thousands of fungi. After evaluating 3800 strains of fungi, he identified a culture broth that showed potent HMG-CoA reductase activity. The active component in the broth was identified to be a known substance, citrinin, which was also shown to lower serum cholesterol (**1**) levels in rats. However, while citrinin did not advance further due to kidney toxicity, the discovery provided impetus to continue to explore culture broths

to identify inhibitors. By mid-1972, Endo found another active culture broth of blue-green mold, *Penicillium citrinum* Pen-51, isolated from a rice sample at a Kyoto grain shop. After much labor, in July 1973, he isolated the first statin, compactin, also called mevastatin (**6**, Scheme 6.3), from the broth following solvent extraction, silica gel chromatography, and crystallization. The discovery and characterization of compactin (**6**) were reported in 1976 [8]. Interestingly, compactin (**6**) did not reduce serum cholesterol (**1**) in rats. This was later found to be due to rat-specific HMG-CoA reductase enzyme induction of 8–10-fold following repeat dosing with compactin (**6**), which renders the initial stoichiometry of the drug versus the enzyme to be inadequate for efficacy. The molecule was efficacious in hens, dogs, and monkeys. The development work on compactin (**6**) at Sankyo was nearly derailed by findings of microcrystalline structures in the livers of rats after treatment with high doses of the compound. These structures were later identified as nontoxic cholesterol (**1**). Spurred by this result, Endo initiated a collaboration with Akira Yamamoto from the Osaka University Hospital, who noted significant cholesterol-lowering without severe side effects in patients with familial hypercholesterolemia treated with compactin (**6**). While this initial clinical work was not sanctioned by Sankyo, the company later decided to proceed with the development of compactin (**6**) based on the success of Yamamoto's studies. When the compound was in Phase II, further development was discontinued by Sankyo due to findings of lymphoma in dogs treated with high doses (200-fold over projected human efficacious dose) of compactin (**6**). By this time, other pharmaceutical companies had begun intensive efforts to identify alternative statins. Merck identified mevinolin, later termed lovastatin (**7**), from the fungus *Aspergillus terreus* [9]. On hearing about the dog toxicology findings with compactin (**6**) at Sankyo, the CEO of Merck Research Laboratories, Roy Vagelos, decided to suspend clinical trials with lovastatin (**7**). About 3 years later, Merck's new CEO, Edward Scolnick, decided to make a significant investment in a comprehensive nonclinical safety evaluation of lovastatin (**7**), leading to an improved understanding of the toxicological findings. Based on an anticipated favorable benefit-to-risk profile, Merck resumed the development of lovastatin (**7**) and successfully launched it in 1987. The first three approved statins, obtained via fermentation broths, have a decalin core and are classified as Type I statins (Figure 6.1) [10]. Sandoz was the first company to discover and develop a statin, fluvastatin (**8**), that had a nondecalin core (Type II) [11]. Subsequently, a number of new Type II inhibitors reached the market, the most successful of them being atorvastatin (Lipitor, **9**) [12], which became the world's most widely used statin and the best-selling drug at its peak [4].

6.3 Synthesis

All Type I statins are produced from fermentation or semisynthesis, thus fully importing the natural decalin framework. For example, many filamentous fungi

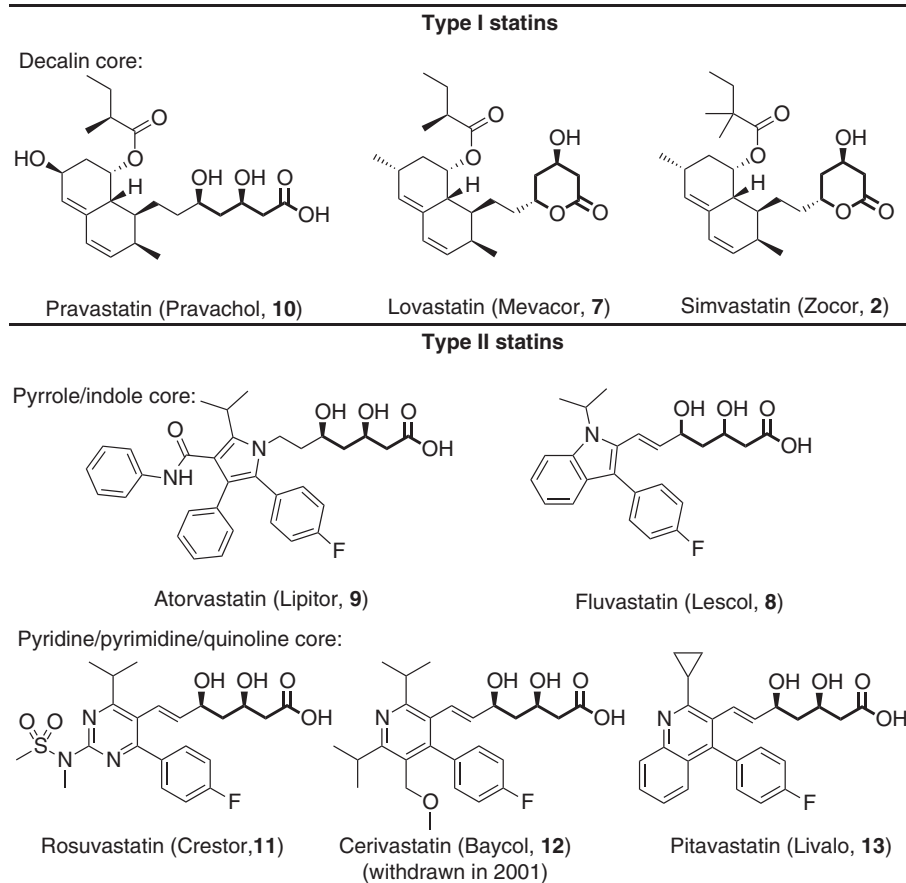
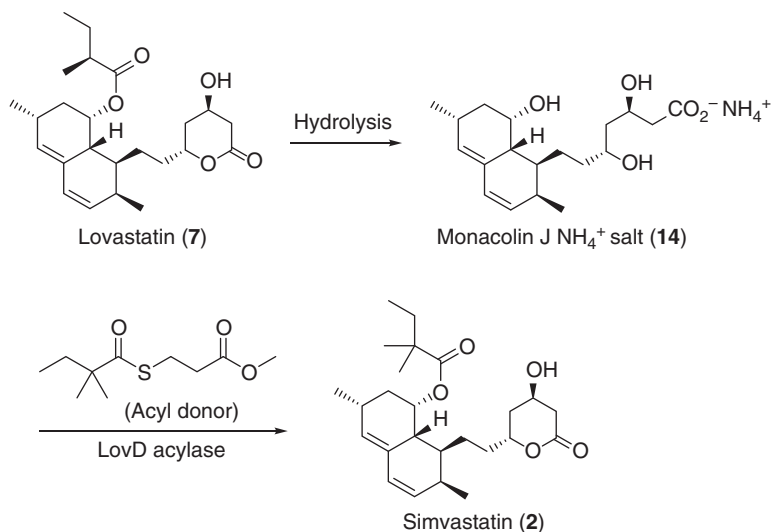


Figure 6.1 Marketed statins [10].

can produce lovastatin (7) in various concentrations. Studies suggest that the biosynthesis of lovastatin (7) involves several enzymes and polyketide synthases through a fungal polyketide pathway. On an industrial scale, lovastatin (7) is produced from *A. terreus* through submerged fermentation [13]. Microorganism strain selection, medium composition, and cultivation parameters play pivotal roles in the production yield. The biotechnology and patents in this area have been extensively reviewed recently [14].

On the other hand, simvastatin (2) can be produced from lovastatin (7) using a chemical process. Two manufacturing routes were being used before 2012. The first route entailed the ester hydrolysis of lovastatin (7), followed by re-esterification to install a different side chain. The second route involved the direct methylation of lovastatin (7). Both routes have the demerits of low overall yield (<70%), a need for excess hazardous and toxic reagents, and large amounts of solvents. Professor Yi Tang and his group from UCLA won the

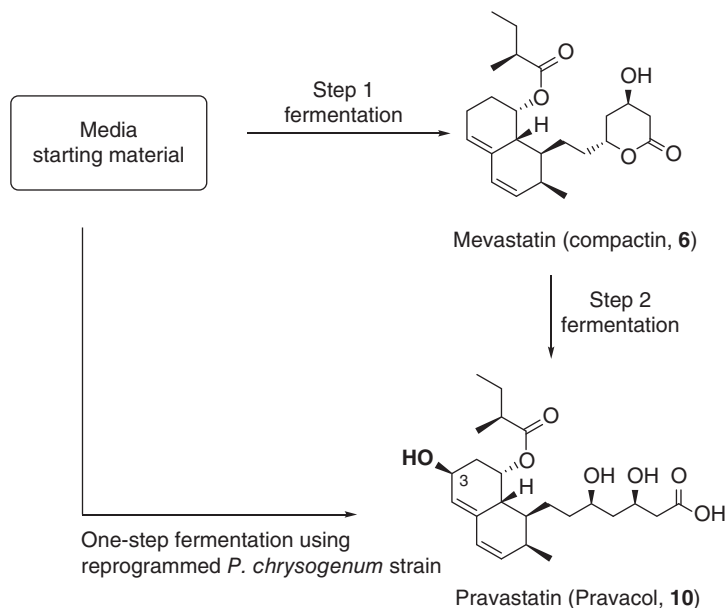
2012 Presidential Green Chemistry Challenge Award for developing an efficient biosynthetic technology to overcome the deficiencies in the existing chemical synthesis routes. In their new route (Scheme 6.2), lovastatin (**7**) is hydrolyzed and converted into the water-soluble ammonium salt of monacolin J (**14**). Then, a genetically evolved variant of LovD acyltransferase from *Escherichia coli* uses methyl 3-((2,2-dimethylbutanoyl)thio)propanoate as the acyl donor to make the water-insoluble ammonium salt of simvastatin (**2**). The overall yield of this new process was improved to 97%, with a significantly reduced requirement for solvents and chemicals [15].



Scheme 6.2 Production of simvastatin (**2**) from lovastatin (**7**).

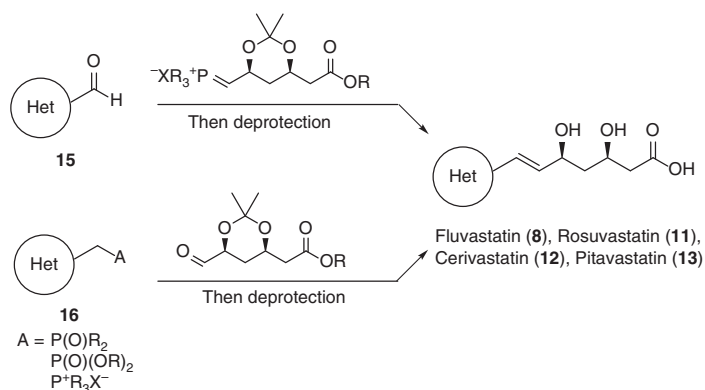
The original manufacturing route to pravastatin (**10**) involved a two-step fermentation process: the first step to produce the natural product compactin (**6**) and the second step to regio- and stereoselectively hydroxylate compactin (**6**) in 3-position to generate pravastatin (**10**). Recently, scientists from the University of Manchester, together with industrial partner DSM Biotechnology Center (Netherlands), have developed a single-step fermentation method for the production of pravastatin (**10**) (Scheme 6.3). The technology involves reengineering the antibiotics-producing fungus *Penicillium chrysogenum*, by deleting the gene responsible for the degradation of compactin (**6**) and incorporating a P450 enzyme that is capable of selective 3-hydroxylation. This breakthrough technology has significantly driven down the production cost of pravastatin (**10**) [16].

Type II statins share a common structural feature: the active pharmacophore (3*R*,5*R*)-3,5-dihydroxypentanoic acid (**3**), which is linked to a template (indole, pyrimidine, pyridine, or quinoline) through an (*E*)-carbon–carbon double bond spacer. Atorvastatin (**9**) is the only exception, since it has a carbon–carbon single



Scheme 6.3 Production of pravastatin (**10**).

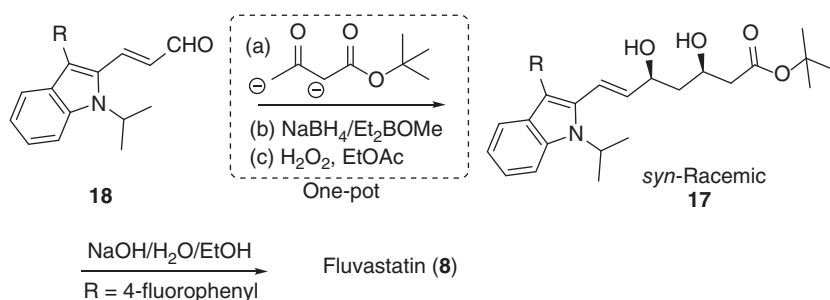
bond as a spacer. Many synthetic methods have been developed using a convergent strategy to build the (*E*)-carbon–carbon double bond at a late stage via Wittig or Horner–Wadsworth–Emmons reactions between aldehyde **15** (or phosphorousylide precursors **16**) with appropriate chiral side-chain partners (Scheme 6.4) [17].



Scheme 6.4 Convergent synthesis of some Type II statins.

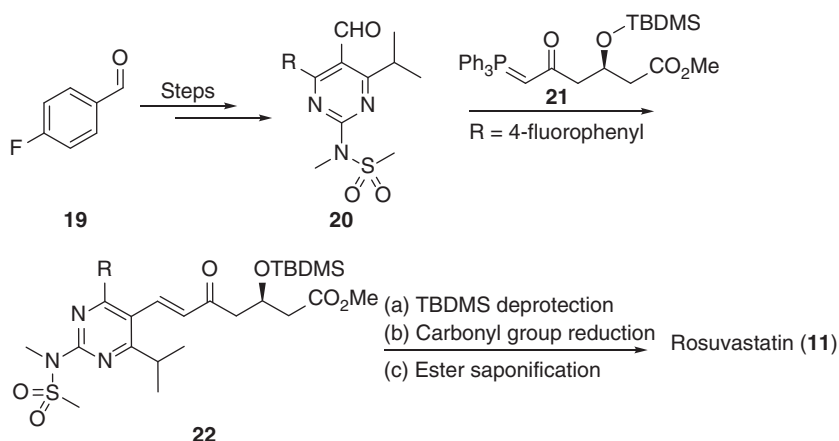
On the industrial production scale, each Type II statin is prepared using a slightly different route based on considerations of overall cost of goods, reaction conditions, and ease of purification. Fluvastatin (Lescol, **8**) is the first

synthetic statin approved by the FDA, and it is the only statin marketed as a racemic mixture, even though the therapeutic activity is significantly higher for the more active enantiomer (+)-(3*R*,5*S*)-fluvastatin ((+)-**8**) [18]. Its industrial production focuses on the stereoselective generation of *syn*-diol **17** in racemic form (Scheme 6.5). Given that the potency of the racemate competed favorably with the three previously approved statins and the technical and resource challenges associated with the generation of the more active enantiomer at the time, fluvastatin (**8**) was progressed as a racemate rather than a single enantiomer [1]. The key one-pot process involves (i) addition of the bis-anion of *tert*-butyl 3-oxobutanoate to aldehyde **18** and (ii) a boron-mediated stereoselective ketone reduction to *syn*-diol **17** [19]. The enantioselective synthesis of the more active (+)-(3*R*,5*S*)-enantiomer of fluvastatin ((+)-**8**) as well as the less active (–)-(3*S*,5*R*)-enantiomer (–)-**8** has been reported [20].



Scheme 6.5 Industrial production of fluvastatin (**8**).

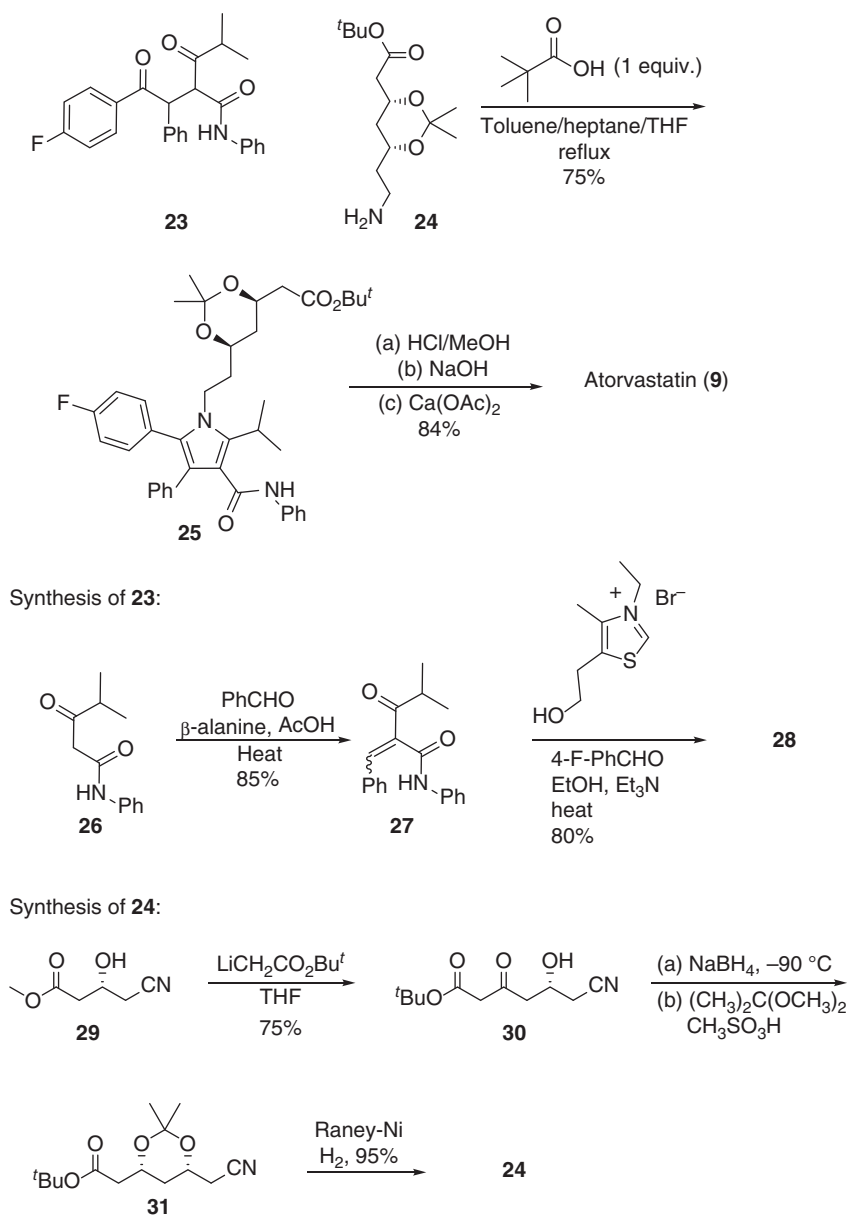
Rosuvastatin (**11**) is produced in a convergent manner from aldehyde **20** and the enantiomerically pure ylide **21** (Scheme 6.6), followed by deprotection of the



Scheme 6.6 Industrial production of rosuvastatin (**11**).

TBDMS group, reduction of carbonyl group, and ester saponification of the resultant (*E*)-olefin **22** [21].

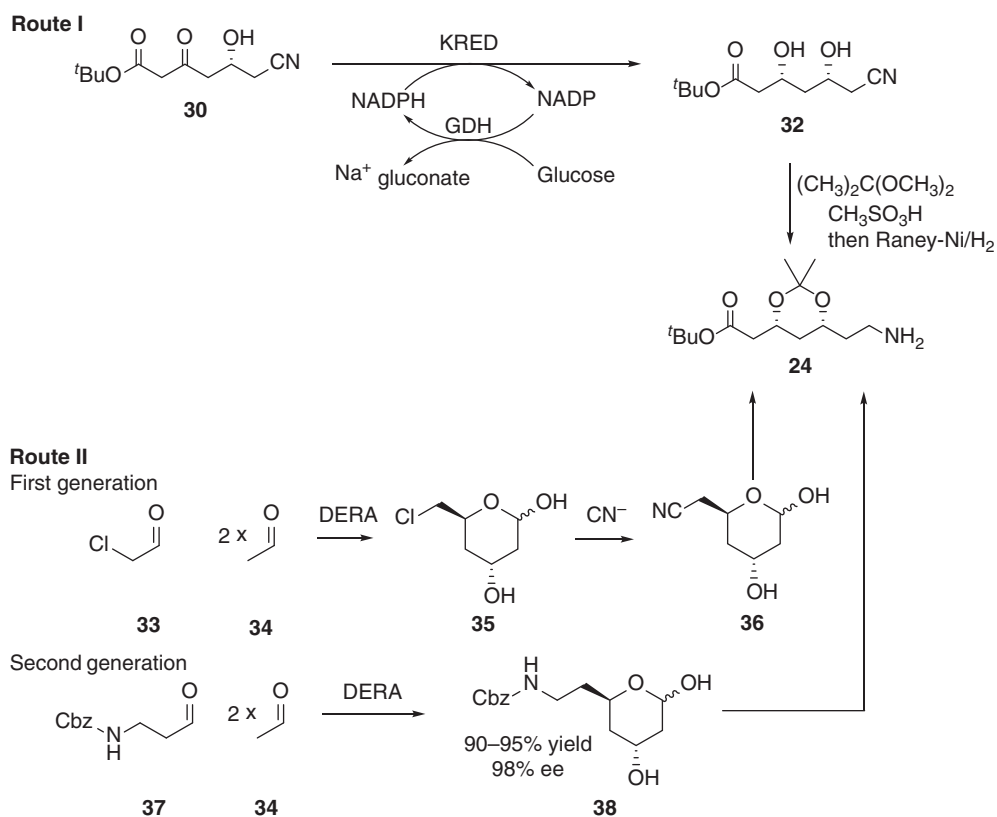
Pfizer's commercial route for atorvastatin (**9**) is also highly convergent (Scheme 6.7). Paal–Knorr pyrrole synthesis between fully functionalized



Scheme 6.7 Industrial production of atorvastatin (**9**).

diketone **23** and the chiral amine **24** forms the tetrasubstituted pyrrole **25**, which is then deprotected to afford atorvastatin (**9**) in 84% yield. The diketone **23** is prepared from **27** and **28** via a two-step process (Knoevenagel condensation and Stetter reaction) in 68% overall yield. Separately, Claisen condensation of (*R*)-4-cyano-3-hydroxybutyrate (**29**) and the anion of *t*-butyl acetate yields **30** in 75% yield. After the reduction of **30** at low temperature (-90°C), followed by diol protection and nitrile reduction, the chiral amine **24** is obtained with excellent enantioselectivity (99.9% ee) and good overall yield (60–65%) [22].

In the past two decades, tremendous advances in biocatalysis have been achieved largely owing to a more in-depth understanding of DNA sequencing, gene synthesis, as well as the ability to reprogram or engineer natural enzymes to realize certain chemical transformations more effectively than by chemical synthesis [23]. The chemical syntheses shown in Scheme 6.7 have the disadvantage that it requires a cryogenic reactor, high energy consumption, and the chiral starting material **29**. Scientists have developed two biocatalysis approaches to overcome these issues (Scheme 6.8). Route I employs ketoreductase (KRED) in



Scheme 6.8 Biocatalysis approaches for the synthesis of the chiral side chain **24**.

the presence of cofactors to convert ketone **30** to diol **32**. In the first generation of route II, deoxyribose-5-phosphate aldolase (DERA) converts a mixture of 2-chloroacetaldehyde **33** and 2 equiv. of acetaldehyde **34** to chloromethyl dihydroxypropan **35**, which is further converted into **24** via cyanation (**36**), hydrogenation, and protecting group manipulation. In the second generation of route II, benzyl (3-oxopropyl)carbamate **37** is used to replace 2-chloroacetaldehyde in the aforementioned transformation to form **38** in high yield and high enantioselectivity. This new route saves two steps and eliminates the hazardous cyanation chemistry. In both cases, specially engineered KERDs and DERAs were screened and selected to achieve the highest enantioselectivity, reaction rate, and the stability to high substrate concentrations [24]. It is worth noting that **35** has also been used as key intermediate in the rosuvastatin (**11**) synthesis [24].

6.4

Mode of Action

Approximately 70% of the circulating cholesterol (**1**) in our body is produced by the liver. The first committed step in the biosynthesis of cholesterol (**1**) via the mevalonate pathway is the reduction of HMG-CoA (**39**) to mevalonate

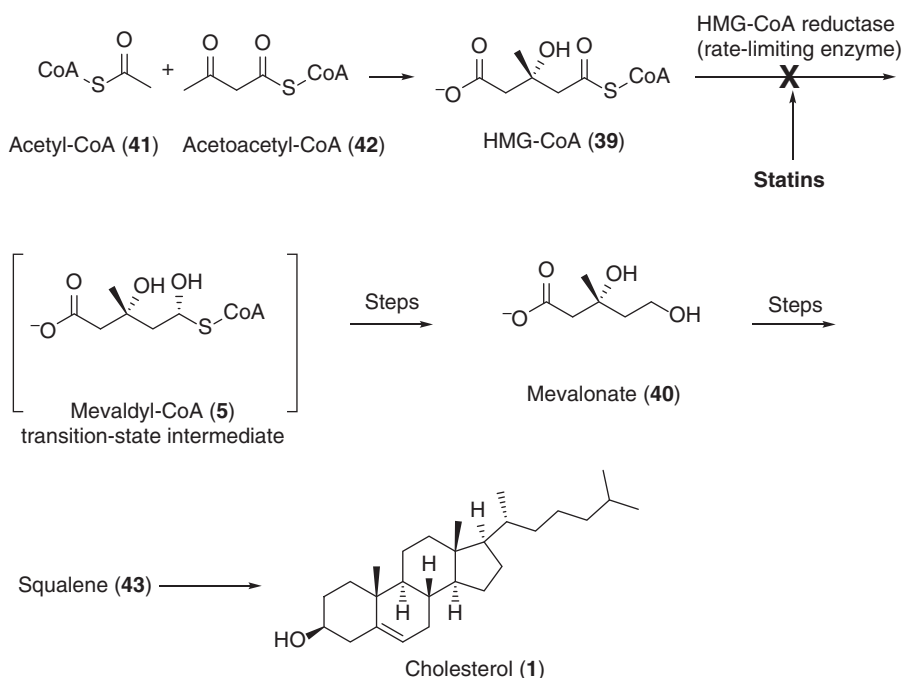


Figure 6.2 Biosynthesis of cholesterol (**1**).

(**40**), catalyzed by the rate-limiting enzyme HMG-CoA reductase (Figure 6.2). Subsequent downstream processing leads to the synthesis of squalene (**43**), a 30-carbon open-chain triterpene, which serves as a biochemical precursor for the entire family of sterols, including cholesterol (**1**) [25].

All statins possess a (3*R*,5*R*)-3,5-dihydroxypentanoic acid (**3**) moiety attached to a hydrophobic group as a common pharmacophore. The dihydroxypentanoic acid piece mimics the half-reduced mevaldyl-CoA transition state intermediate (**5**) with a preference for the (3*R*,5*R*) stereochemistry at the alcohol centers [26]. Lovastatin (**7**) and simvastatin (**2**) are lactone prodrugs, which rapidly convert to the active free (3*R*,5*R*)-3,5-dihydroxypentanoic acids *in vivo*, which in turn inhibit HMG-CoA reductase.

The recommended primary indication for the use of statins is for lowering LDL cholesterol (**1**) and triglycerides in patients who are unable to meet their lipid-lowering goals through diet and exercise alone. All statins are potent, reversible inhibitors of HMG-CoA reductase and bind competitively with substrate HMG-CoA (**39**). The LDL-cholesterol-lowering potencies of approved statins have been well documented [10]. In addition to inhibiting cholesterol (**1**) synthesis, the reduction in risk of cardiovascular disease by statin therapy has been purported to be due to improvement in endothelial function, modulation of inflammatory responses, maintenance of plaque stability, and prevention of thrombus formation [27]. The most common side effects include muscle pain, elevation of liver enzymes, and an increased risk of diabetes [28]. Cerivastatin (**12**) was voluntarily withdrawn from the market by Bayer in 2001 due to reports of fatal rhabdomyolysis [29]. The risk of myopathy for the currently marketed statins is significantly lower than that for cerivastatin (**12**). In addition to and independent of their lipid-lowering effects, statins are also reported to have other pleiotropic effects relating to their anti-inflammatory activity and their ability to modulate bone metabolism [30].

6.5

Structure–Activity Relationship

The weak inhibitory activity ($IC_{50} > 10 \mu\text{M}$) of mevalonic acid (**40**) suggests that the high potency of the FDA-approved statins accrues primarily from (3*R*,5*R*)-3,5-dihydroxypentanoic acid (**3**) and the hydrophobic groups attached to it through a “template” (Figure 6.3). The hydroxylactone **4** or its open acid form is an essential fragment, with a requirement of the (3*R*,5*R*) stereogenic centers, similar to those in HMG-CoA. Its counterpart (3*S*,5*S*) enantiomer is much less active (up to 30-fold). The two-atom spacer (carbon–carbon single or double bond) has been identified as optimal between the pharmacophore and the template. Longer or shorter spacers attenuate inhibitory activity. All Type I statins (lovastatin (**7**), simvastatin (**2**), and pravastatin (**10**)) are produced from natural sources or derived semisynthetically and have decalin as the template. On the basis of an improved understanding of the molecular mechanism and

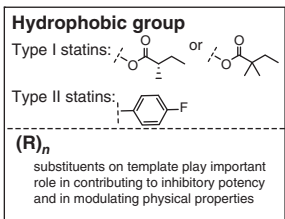


Figure 6.3 Structural features of potent statins.

structural requirement of HMG-CoA reductase inhibitors, the decalin template was successfully replaced with a variety of 5–10-membered ring heterocycles (or bicycles) to derive Type II statins. Pyrrole, pyrimidine, pyridine, quinoline, and indole templates are exemplified in Figure 6.3. An optimized spatial relationship between the hydrophobic group and the pharmacophore is crucial for potent inhibitory activity. All Type II statins have a 4-fluorophenyl group that closely overlays with the ester side chain from Type I statins, thus significantly contributing to potency. All Type II statins also have an isopropyl group attached to the template, with the exception of pitavastatin (**13**), which has a cyclopropyl group instead.

Crystal structures of several statins complexed with human HMG-CoA reductase reveal that most of the statins have a similar binding orientation to the enzyme that includes several polar interactions with residues located in the cis-loop of the enzyme. The terminal carboxylic group in statins forms a salt bridge with a positively charged Lys⁷³⁵ residue in the active site. The isopropyl or cyclopropyl is believed to have a hydrophobic interaction with Leu⁵⁶² in the enzyme hydrophobic binding pocket (Figure 6.4). The fluorine atom shows a polar interaction with Arg⁵⁹⁰. Rosuvastatin's (**11**) sulfone group forms additional polar interactions with Arg⁵⁶⁸ and Ser⁵⁶⁵ residues, while atorvastatin's (**9**) carbonyl group only interacts with Ser⁵⁶⁵. In fact, rosuvastatin (**11**) has the greatest number of binding interactions with HMG-CoA reductase, resulting in superior binding affinity to the enzyme compared to other statins, which in turn translates to its superior efficacy in human (up to 63% LDL cholesterol lowering) [31].

The differences of the templates as well as the substituents attached to them affect physicochemical properties and pharmacological properties in the following aspects: (i) affinity to the enzyme; (ii) rates of entry into hepatic and non-hepatic tissues; (iii) systemic circulation; and (iv) metabolism profiles [32]. The profile of an optimal statin would ideally include high enzyme affinity and liver-selective exposures to favor potent inhibition in the target tissue at a minimal dose

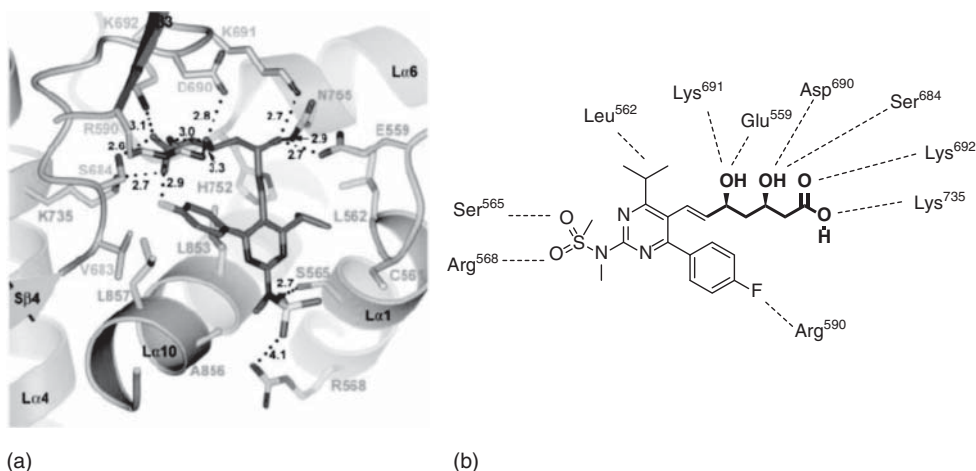


Figure 6.4 (a) X-ray cocrystal structure of rosuvastatin (**11**) with human HMG-CoA reductase. (b) The key interactions of rosuvastatin (**11**) with enzyme residues [31]. (Istvan [31]. Reproduced with permission of AAAS.)

and to mitigate extrahepatic activity, particularly in some vulnerable tissues such as skeletal muscle by minimizing systemic bioavailability. In general, hydrophilic statins such as pravastatin (**10**) and rosuvastatin (**11**) show higher exposures in the liver, aided by the uptake transporter Organic Anion Transfer Protein (OATP). The most lipophilic statin, cerivastatin (**12**), is able to permeate across multiple cell types resulting in the most serious side-effect profile among all approved statins that led to its ultimate withdrawal in 2001.

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7

Angiotensin II Receptor Antagonists with Carboxylic Functionalities in Cardiovascular Disease

Steve Swann and Simone Bigi

7.1

Introduction

Sartans are a class of compounds that originated from 2-(1-benzyl-1*H*-imidazol-5-yl)acetic acid (**1**, Figure 7.1). Originally, the class was comprised of an imidazole core, substituted with a benzyl group on the *N*¹-nitrogen and differing substituents on the various positions of the core heterocyclic ring.

In 1982, molecules of this type were first disclosed by Takeda Chemical Industries [1] as having anti-hypertensive effects in rats. This research group would later demonstrate that the blood-pressure-lowering activity of molecules CV-2198 (**2**) and CV-2973 (**3**) (Figure 7.2) were a result of inhibition of the angiotensin II (AII)-induced hemodynamic pathway, also known as the renin–angiotensin system (RAS) [2]. Takeda went on to conduct a clinical trial with CV-2973 (**3**), which was ultimately halted because of poor oral efficacy.

Despite the modest potency and limited oral bioavailability of this class of compounds, their discovery served as a launching point for more than two decades of drug discovery around nonpeptidic AII antagonists. This field of research has since yielded a large number of marketed angiotensin receptor blockers (ARBs), thereby improving the lives of millions of people suffering from hypertension and chronic heart failure. To date, there are eight marketed ARBs in the United States (Figure 7.3): losartan (Cozaar[®], **4**, Merck, 1995), valsartan (Diovan[®], **5**, 1990), irbesartan (Avapro[®], **6**), candesartan cilexetil (Atacand[®], Blopess[®], Atacand, Amias[®], Ratacand[®], **7**, AstraZeneca, and Takeda, 1995), olmesartan medoxomil (Benicar[®], Olmetec[®], **8**), eprosartan (Teveten[®], **9**, Abbott Laboratories), telmisartan (Micardis[®], **10**), and azilsartan medoxomil (Edarbi[®], **11**).

This chapter describes the discovery of the nonpeptidic AII receptor blockers, the synthetic routes that have been developed for this chemotype, as well as the structure–activity relationships (SAR) and key modifications to the core that have been explored in the past 25 years.

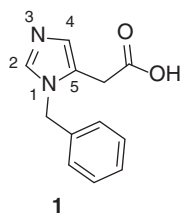


Figure 7.1 Basic structure and numbering of 2-(1-benzyl-1H-imidazol-5-yl)acetic acid (**1**).

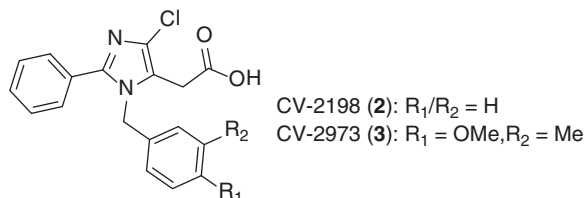


Figure 7.2 Structure of compounds CV-2198 (**2**) and CV-2973 (**3**).

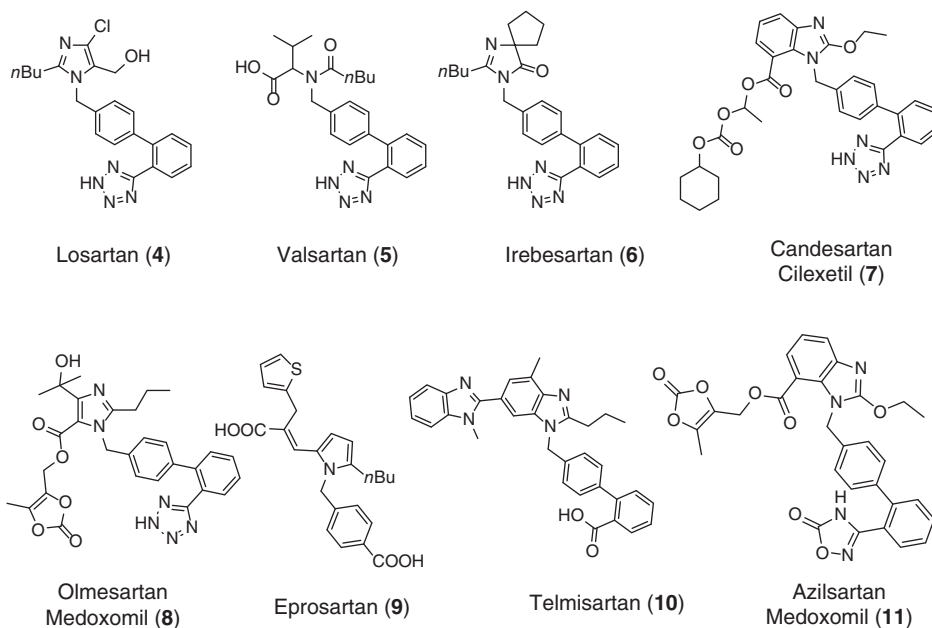


Figure 7.3 Structures of angiotensin II receptor blockers marketed in the United States: losartan (**4**), valsartan (**5**), irebesartan (**6**), candesartan cilexetil (**7**), olmesartan Medoxomil (**8**), eprosartan (**9**), telmisartan (**10**), and azilsartan medoxomil (**11**).

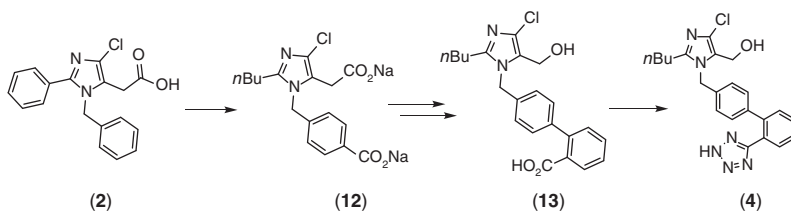
7.2

History

Discovered in the early 1970s, the first angiotensin-converting enzyme (ACE) inhibitor, captopril, directly inhibited the formation of AII [3]. This discovery solidified the role of angiotensin in the pathogenesis of hypertension and general homeostasis of the cardiovascular system. However, adverse events, such as rash development and taste disturbances, prompted the exploration into other targets within the RAS. Peptidomimetic inhibitors of AII, such as saralasin, were known to be efficacious when administered intravenously, but their lack of bioavailability limited their clinical value [4]. In the early 1980s, Takeda reported a series of *N*-benzyl-5-imidazole acetic acids that lowered blood pressure in hypertensive rats [1]. These compounds were later characterized as selective antagonists of AII. Despite this seminal achievement, these compounds demonstrated only moderate potency, a limited duration of action, and limited bioavailability.

The DuPont Pharmaceuticals Division was pursuing similar compounds during a simultaneous and independent campaign for identification of orally efficacious AII receptor antagonists [5]. Their initial attempts at increasing oral exposure of 2-(1-benzyl-1*H*-imidazol-5-yl)acetic acids (**1**) by modifying the 5-acetic acid moiety with other acid bioisosteres, such as trifluorosulfonamides and sulfates, yielded no orally active inhibitors.

Using computer modeling, it was hypothesized that the carboxylate and the imidazole in the original Takeda compounds served as bioisosteres for a phenylalanine and a histidine in the AII binding pocket. DuPont pursued further substitutions on the benzyl group and revealed that the 4-carboxyl group in **12** resulted in enhanced potency. Moving this acid group into the 2-position of the phenyl ring and reducing the acetic acid moiety on the core imidazole to a methyl alcohol ultimately yielded a potent AII inhibitor (**13**, $EC_{50} = 0.280$ nM) that reduced hypertensive activity in rats upon oral administration. Bioisosteric replacement of the carboxylate with a tetrazole yielded a compound with an additional 10-fold increase in oral activity. This compound went on to become losartan (**4**) [5–7], the first successful AII antagonist approved in the United States in 1995 and ultimately marketed by Merck (Scheme 7.1).



Scheme 7.1 Evolution of original 2-(1-benzyl-1*H*-imidazol-5-yl)acetic acid scaffold into losartan (**4**).

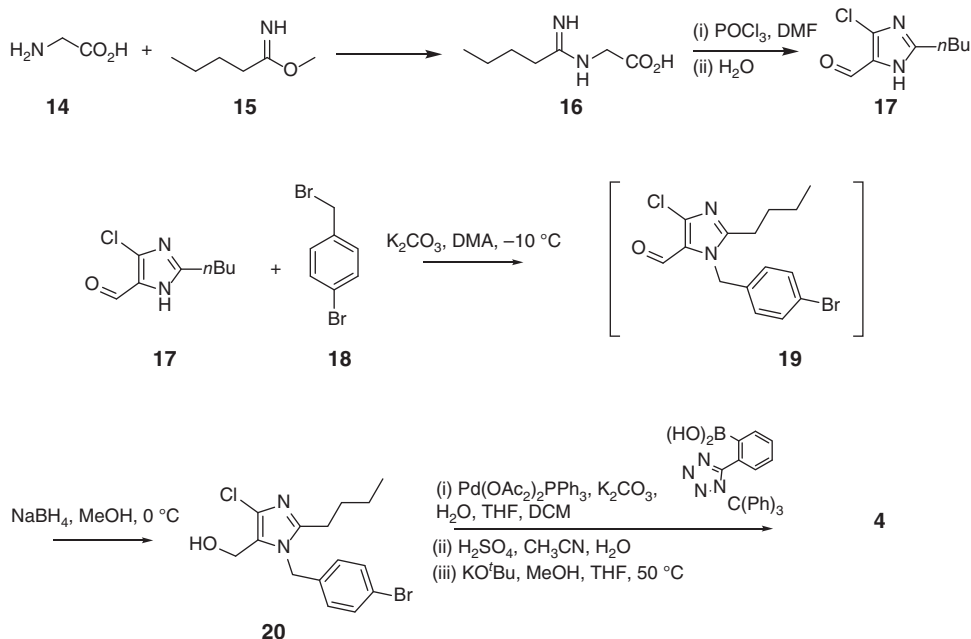
The discovery of nonpeptidic AII receptor antagonists represented a major breakthrough in the treatment of cardiovascular diseases. These agents are still considered first-line therapies for several indications including mild hypertension, chronic heart failure, and stroke prevention. Compared to ACE inhibitors and other hypertensive agents, the clinical superiority of these compounds is a result of their direct inhibition of the AII receptor resulting in limited side effects.

7.3

Synthesis

The classical method for the syntheses of imidazoles was reported in 1882 where Radziszewski *et al.* [8] described the condensation of an appropriate aldehyde with an 1,3-dicarbonyl compound and ammonia to, what was known then as, a glyoxaline ring. This general approach led to the genesis of an overwhelming number of synthetic procedures published in the past century for the generation of imidazoles, as well as other heterocycles.

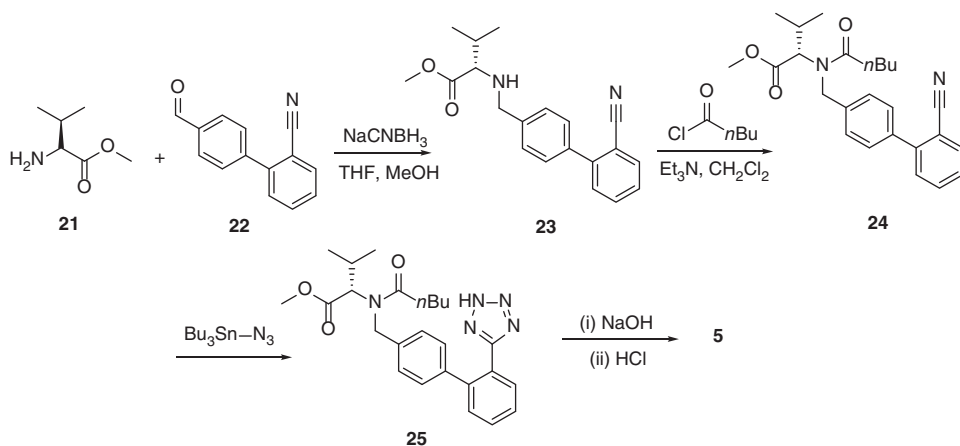
The synthesis of losartan potassium, as reported by Merck [9, 10], was the first disclosure describing a general approach to the core imidazolyl-methylbiphenyl-tetrazole scaffold found in many of the currently marketed ARBs. The synthesis is shown in Scheme 7.2, where glycine (**14**) was first condensed with methyl



Scheme 7.2 Synthesis of losartan (**4**) from key intermediate **17**.

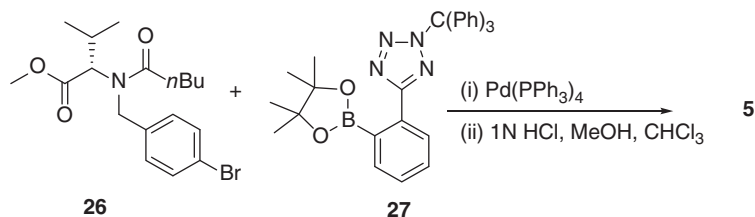
pentanimidate (**15**) to form imino-glycine **16**, which cyclized to **17** under Vilsmeier–Haack conditions. Intermediate **17** was then alkylated with 4-bromobenzyl bromide (**18**) to form **19** and afterward reduced *in situ* to give **20**. A Suzuki coupling was used to install the protected phenyl-tetrazole, which was subsequently deprotected to losartan (**4**) and converted to the sodium salt using sodium hydroxide.

Valsartan (**5**) is a nonheterocyclic ARB where the imidazole is replaced with an acylated amino acid. Ciba-Geigy [11] first reported the synthesis of valsartan (**5**) through the coupling of *L*-valine methyl ester (**21**) with 4'-formyl-[1,1'-biphenyl]-2-carbonitrile **22**, summarized in Scheme 7.3. Intermediate **23** was then acylated with *n*-pentanoyl chloride to produce **24**. Triazole formation to **25** was affected using tri-*n*-butyltinazide and upon saponification, valsartan (**5**) was formed.



Scheme 7.3 Synthesis of valsartan (**5**).

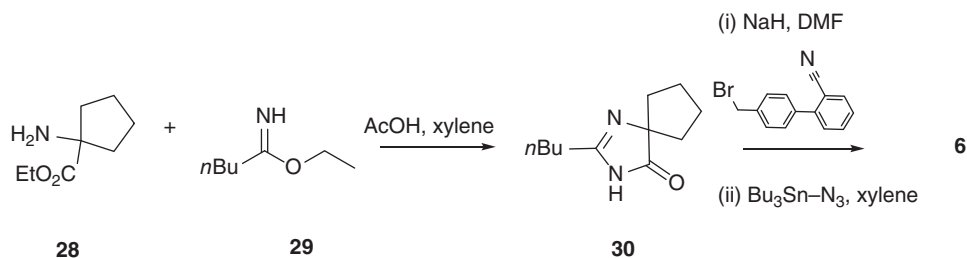
The aforementioned process suffered from a number of complications including the use of a highly toxic tin reagent, contamination in the final product, and an overall yield of less than 10%. Since the original synthesis of valsartan (**5**) was released in the literature, an extensive number of patents have been published [12] to address the issues of the original process, the majority of which address the reactivity of the tetrazole and associated protection strategies. Two alternative, convergent approaches were reported that incorporated a trityl-protected tetrazole, which eliminated the use of toxic tin reagents later in the synthesis (Negishi [13] and Suzuki coupling [14]). The most recent example was reported by Tsiperman *et al.* [15] where the phenyl tetrazole was incorporated in the final step with a Suzuki coupling using palladium tetrakis triphenylphosphine (Scheme 7.4). However, this process required a large catalyst loading and the isolation of the final product using silica gel chromatography. To date, no commercial syntheses



Scheme 7.4 Modified synthesis of valsartan (**5**) using a Suzuki coupling reaction between the intermediate **26** and the trityl-protected tetrazole-boronic acid ester **27**.

have been disclosed that eliminate the use of tin reagents in the preparation of the tetrazole found in the active pharmaceutical ingredient (API) of valsartan (**5**).

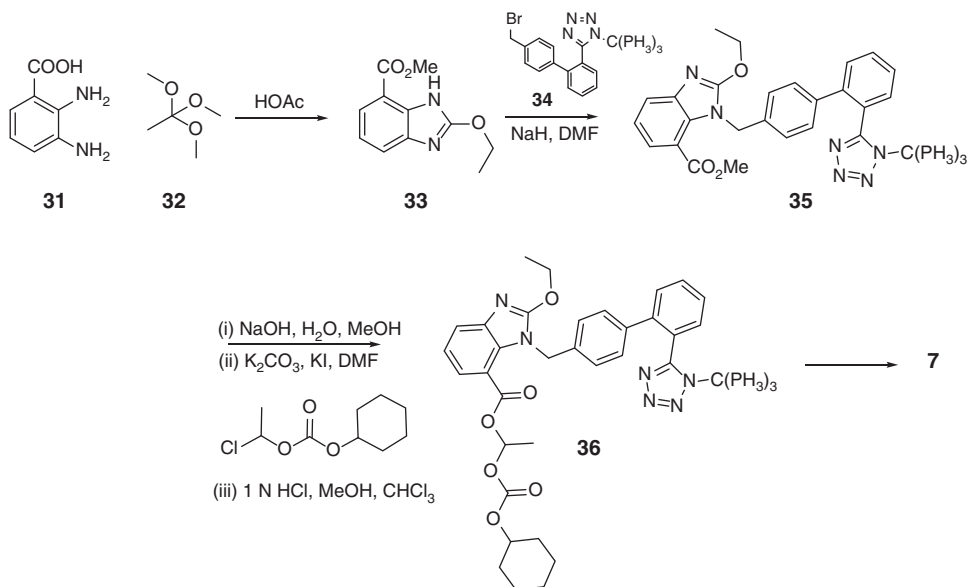
The synthesis of irbesartan (**6**) was originally described by Sanofi [16, 17] (Scheme 7.5). The spirocyclic 1*H*-imidazol-5(4*H*)-one core of **30** was assembled by the condensation of ethyl 1-aminocyclopentane carboxylate (**28**) with ethyl pentanimidate (**29**). This intermediate was then directly alkylated with the appropriate 4-arylbenzyl bromide and was further elaborated to irbesartan (**6**).



Scheme 7.5 Synthesis of irbesartan (**6**).

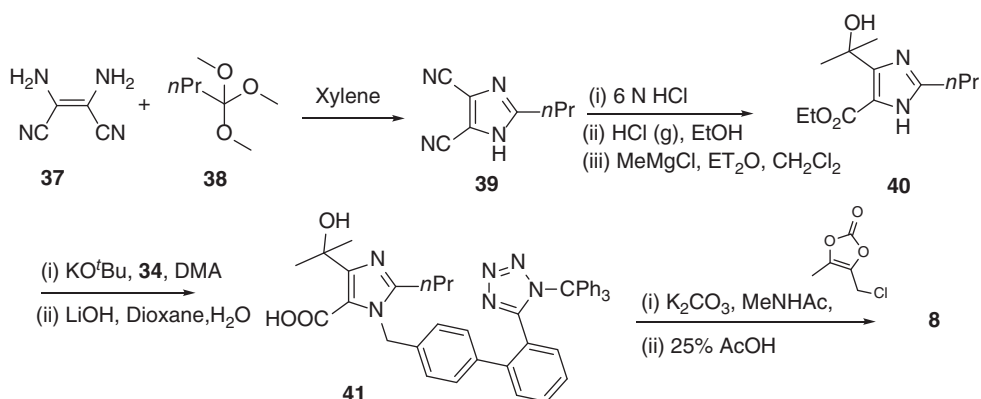
Candesartan (**7**) is marketed as a prodrug where the carboxylic acid in the molecule is protected as the cyclohexyl 1-hydroxyethyl carbonate (cilexetil) ester. As a prodrug, the generally poor absorption characteristics of carboxylic acids can be overcome. Additionally, the cilexetil ester is completely metabolized by esterases during absorption of the drug in the intestinal wall to give the active species. Candesartan (**7**) contains a more unique bis-benzimidazole core, synthesized [18, 19] from the cyclization of methyl-2,3-diaminobenzoate **31** with trimethyl orthoacetate **32** (Scheme 7.6). Intermediate **33** was then alkylated with the protected bromomethylbiphenyltetrazole **34**. The ester **35** was subsequently hydrolyzed and alkylated with the 1-chloroethyl cyclohexyl carbonate to form **36**, before deprotection of the tetrazole yielded the prodrug candesartan cilexetil (**7**).

The structure of olmesartan (**8**) is very similar to the one of losartan (**4**), but the compound is also marketed as a prodrug, where the 5-carboxyl group is protected as a (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester. The reported



Scheme 7.6 Synthesis of candesartan cilexetil (7).

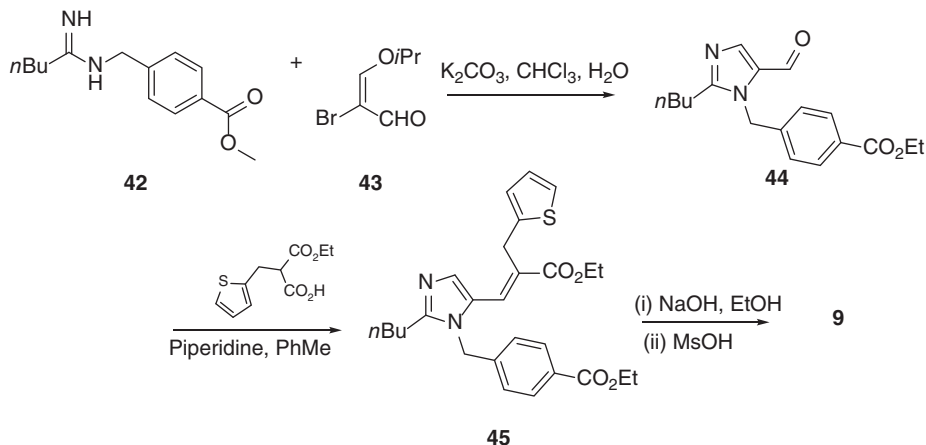
synthesis [20] of olmesartan (8) described the assembly of the imidazole core through condensation of 2,3-diaminomaleonitrile (37) with trimethyl orthobutyrate (38), as seen in Scheme 7.7. The bis-cyano imidazole intermediate 39 was hydrolyzed to the diester and treated with two equivalents of methyl magnesium chloride to form the tertiary alcohol 40. The imidazole was then alkylated with the protected bromomethylbiphenyltetrazole 34, followed by saponification to the free acid 41 using lithium hydroxide. Esterification of the carboxylic acid



Scheme 7.7 Synthesis of olmesartan medoxomil (8).

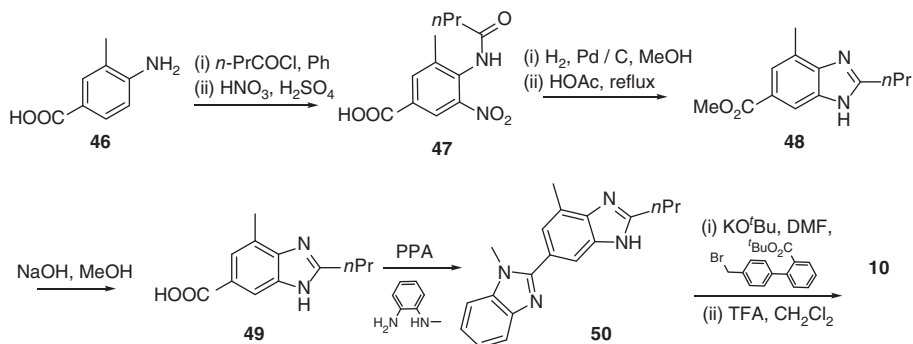
with 4-(chloromethyl)-5-methyl-1,3-dioxol-2-one (medoxomil chloride) and deprotection of the tetrazole provided olmesartan medoxomil (**8**).

The synthesis of eprosartan (**9**) reported [21, 22] by Smith Kline Beecham Pharmaceuticals was different than syntheses reported for other marketed ARBs (Scheme 7.8). The salient step was the assembly of the core imidazole ring from cyclization of amidine **42** with the enol ether **43**. The pendant aldehyde **44** then served as a handle for a Knoevenagel-type condensation with 2-carbethoxy-3-(2-thienyl)propanoic acid yielding the diester **45**, followed by saponification to form eprosartan (**9**).



Scheme 7.8 Synthesis of eprosartan (**9**).

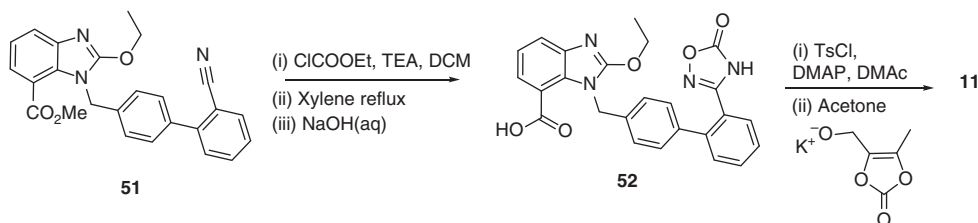
Boehringer Ingelheim first reported the synthesis (Scheme 7.9) of telmisartan (**10**) [23] starting from commercially available 4-amino-3-methyl benzoate (**46**). This material was acylated followed by nitration to form **47**. The nitro intermediate was reduced to the diamine, which cyclized to **48** under acidic conditions. The methyl ester of **49** was hydrolyzed and a second benzimidazole ring was formed



Scheme 7.9 Synthesis of telmisartan (**10**).

through condensation with *N*-methyl-phenylenediamine. Alkylation of the resulting benzimidazole **50** with the protected bromomethyl-biphenylcarboxylic acid followed by ester hydrolysis furnished telmisartan (**10**).

With its FDA approval in 2011, azilsartan (**11**) is the most recent commercialized AII antagonist in the United States. The compound features a novel oxadiazole ring as an acid bioisostere to the carboxylic acid or tetrazole seen in other ARBs. The synthesis of azilsartan (**11**) [24] is similar to the other AII antagonist from Takeda, candesartan (**7**), where **33** was alkylated with the appropriate biaryl benzylbromide to form **51**. The unique oxadiazolone ring was formed from the nitrile, which was converted to the transient oxime and cyclized using ethyl chloroformate (Scheme 7.10). The carboxylic acid **52** was then esterified with medoxomil alcohol in the presence of tosyl chloride to form azilsartan medoxomil (**11**).



Scheme 7.10 Synthesis of azilsartan medoxomil (**11**).

7.4

Mode of Action

The RAS and specifically its primary effector peptide, AII, have been linked to vasoconstriction, the pathogenesis of hypertension, and congestive heart failure. Renin, produced predominantly by the kidneys, catalyzes the conversion of circulating angiotensinogen to an inactive form, angiotensin I (AI). In lung endothelium, ACE cleaves AI to active AII, which then binds to either one of the two AII receptors: AT₁ and AT₂ [25]. These distinct G-protein-coupled receptors (GPCRs) affect many intracellular signaling cascades, with the AT₁ receptor localized throughout the adrenal cortex, myocardium, and renal and lung tissue [26, 27]. Binding of AII to the AT₁ receptor is responsible for the majority of peripheral effects of the RAS system and, more specifically, the traditional symptoms associated with hypertension and heart disease (Figure 7.4).

Therapeutic manipulation has focused on blocking AII production by inhibiting renin or ACE, as well as inhibiting the interaction of AII with the AT₁ receptor. Nonpeptidic AT₁ antagonists can be highly efficacious at blocking the enzymatic cascade of RAS since AII can be produced through other pathways independent of ACE [28–31]. Therefore, regardless of AII production, RAS can be inhibited by focusing on the AT₁ receptor responsible for an increase in blood pressure and

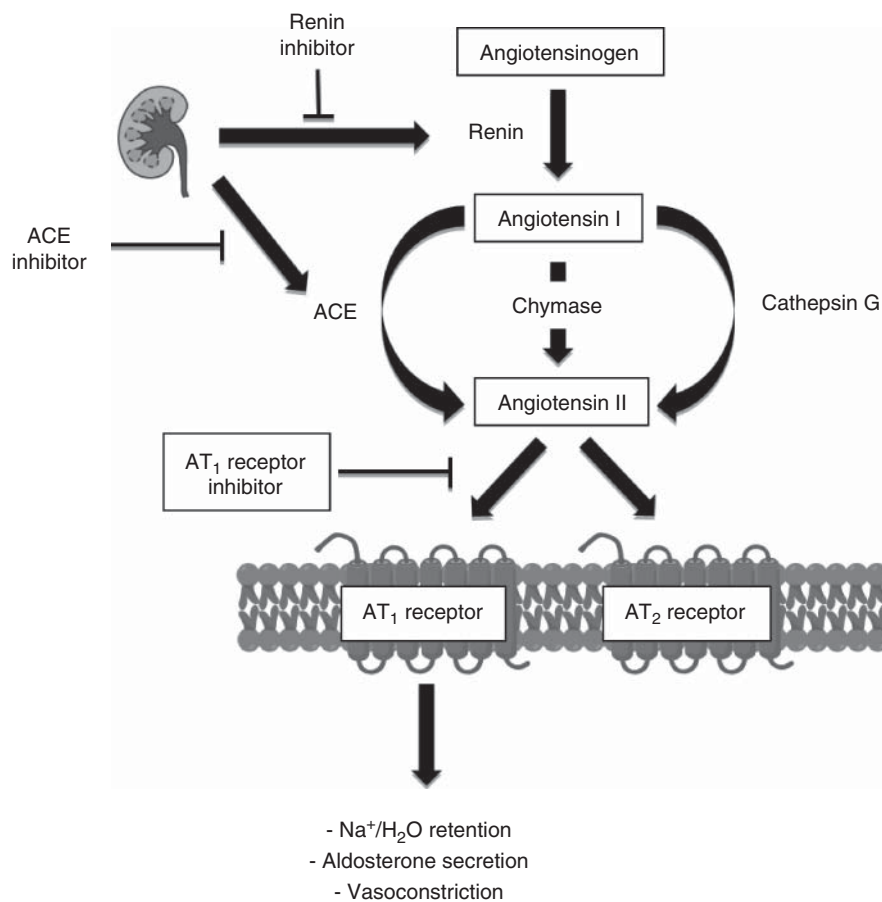


Figure 7.4 The renin–angiotensin system (RAS) pathway.

the classical actions of heart disease: renal reabsorption of sodium, constriction of blood vessels, and aldosterone release (Figure 7.5) [32].

7.5

Structure–Activity Relationships

In 1991, a publication [5] broadly described the SAR around the biphenyl imidazole core of losartan (**4**), including the impact of changes on *in vitro* potency, as well as *in vivo* anti-hypertensive activity, through intravenous and oral dosing.

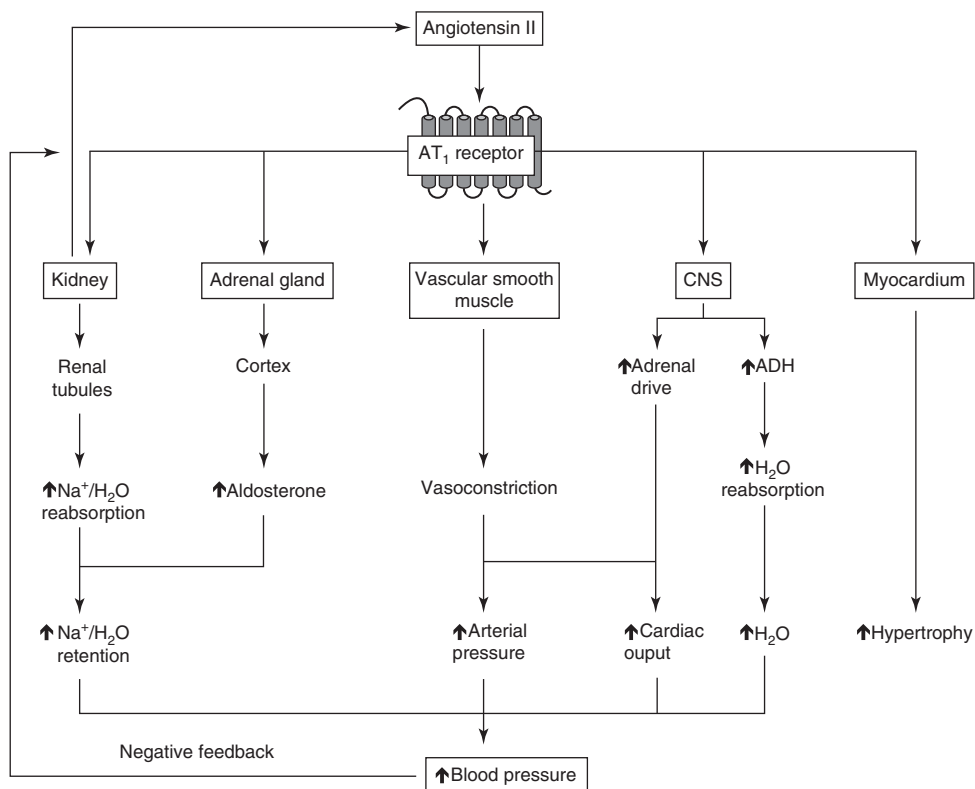
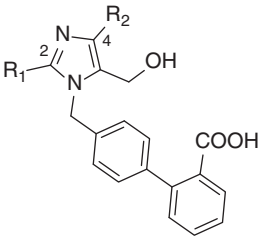


Figure 7.5 The interaction of angiotensin II with the AT₁ receptor.

Modifications to the imidazole ring were explored highlighting the steep SAR at the 2-position of the imidazole, specifically a propyl side chain exhibiting 10-fold greater potency than the corresponding ethyl and butyl chains. This preference for an alkyl chain at this position of the scaffold is consistent in other marketed ARBs, including irbesartan (**6**), olmesartan (**8**), and telmisartan (**10**). Halogenated modifications at the 4-position identified numerous compounds, **53–58**, with a possible 10-fold increase in activity relative to the methyl-containing comparator **61** (Table 7.1).

Early exploration of the 1-benzyl-1*H*-imidazole core revealed that a 1-methyl-biphenyl-2-carboxylic acid, as in **13**, actually increased *in vivo* activity upon oral dosing. It is therefore not surprising that this side chain has been maintained in all of the currently marketed ARBs, except for eprosartan (**9**). This significant early discovery established the precedent that a nonpeptidic, orally bioavailable AII inhibitor was achievable.

Table 7.1 SAR for 2- and 4-disubstituted imidazole analogs of compound **14**.

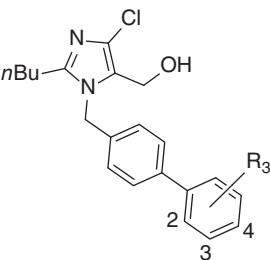
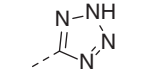
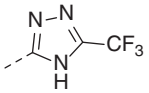


Compounds	R ₁	R ₂	IC ₅₀ (μM)	<i>In vivo</i> ED ₅₀ po (mg/kg)
4	–C ₂ H ₅	–Cl	1.7	100
53	–C ₃ H ₇	–Cl	0.16	30
13	–C ₄ H ₉	–Cl	4.1	ED ₃₀ = 11
54	–C ₅ H ₁₁	–Cl	0.98	30
55	–C ₆ H ₁₃	–Cl	1.3	>30
56	–Ph	–Cl	24	100
57	–C ₄ H ₉	–Br	0.09	30
58	–C ₄ H ₉	–I	0.061	100
59	–C ₄ H ₉	–CF ₃	0.062	30
60	–C ₄ H ₉	–NO ₂	0.26	>30
61	–C ₄ H ₉	–CH ₃	1.8	>30

Continued optimization efforts focused on bioisosteric replacements of the carboxylate to increase *in vivo* activity (see **64**–**73** in Table 7.2). A number of compounds, including the trifluoroacetylsulfonamide **68** and the trifluorosulfonamide **71**, maintained potency against the AII receptor but lacked *in vivo* activity upon oral dosing. Replacement of the acid with a tetrazole **72** showed both a 20-fold improvement in potency, as well as *in vivo* activity with an ED₃₀ = 0.6 mg kg^{–1}. The identification of the tetrazole and its impact on *in vivo* activity have since influenced the majority of the marketed ARBs. Although eprosartan (**9**) and telmisartan (**10**) contain a carboxylic acid, most of the current ARBs on the market contain a tetrazole or similar acid bioisostere, such as an oxadiazolone (e.g., **11**).

An initial discovery by Takeda Chemical Industries demonstrated that analogs of 2-(1-benzyl-1*H*-imidazol-5-yl)acetic acid (**1**) can display blood-pressure-lowering activity through inhibition of the AII hemodynamic pathway. The discovery served as a launching point for more than two decades of drug discovery research around nonpeptidic AII antagonists. This class of compounds has since yielded a large number of marketed ARBs, which have improved the lives of millions of people suffering from hypertension and chronic heart failure. The discovery of their novel mode of action has served as a seminal advancement in the field of drug discovery. Their comprehensive use and effectiveness have revolutionized the standard of care for patients worldwide.

Table 7.2 SAR for bioisosteric replacements of the carboxylate on the biphenyl imidazole core.

				
Compounds	Position	R ₃	IC ₅₀ (μM)	In vivo activity po (mg/kg)
13	2	–COOH	4.1	ED ₃₀ = 11
62	3	–COOH	0.49	100
63	4	–COOH	11.0	>30
64	4	–CONH ₂	35.0	No data
65	4	–CONHOH	4.1	>30
66	4	–CONHOCH ₃	2.9	>30
67	4	–CONHOCH ₂ Ph	4.9	>30
68	4	–CONHSO ₂ CF ₃	0.2	>30
69	4	–NHCOCF ₃	6.3	>30
70	4	–CH ₃ –COOH	1.9	10
71	4	–NHSO ₂ CF ₃	0.083	100
72	4		0.019	ED ₃₀ = 0.6
73	4		0.37	10

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8

Carboxylic Acid Containing Direct Thrombin Inhibitors for the Treatment of Thromboembolic Diseases

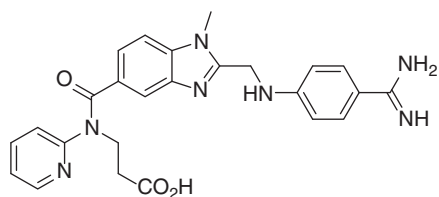
Harry R. Chobanian and Mathew M. Yanik

8.1

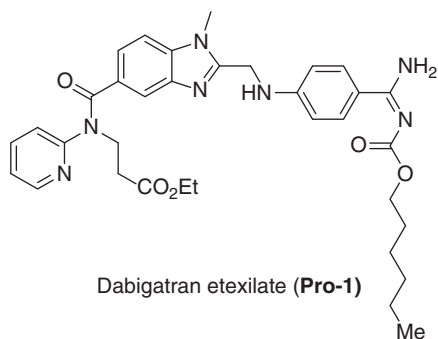
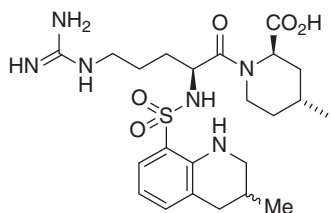
Introduction

Thromboembolic diseases continue to remain the leading cause of preventable hospital deaths in the United States. Venous thromboembolism is estimated to affect 1–2 million people, progressing to pulmonary embolism in about 600 000 of these patients and becoming fatal in about 100 000 patients annually [1, 2]. Thrombin is a serine protease that plays a central role in the blood coagulation cascade by mediating the conversion of fibrinogen to fibrin. Thrombin also affects arterial thrombosis via activation of the protease-activating receptor PAR1 [3]. Primary clinical indications for prevention of blood coagulation are postsurgical deep vein thrombosis (DVT) after joint replacement (knee and hip) and in stroke prevention in a predisposed subset of patients with atrial fibrillation (AF). Several classes of oral and injectable anticoagulants are in clinical use and include the vitamin K antagonist warfarin, heparin, and low-molecular-weight heparins. These compounds target points of the tightly regulated coagulation cascade upstream of prothrombin, which upon proteolysis is converted into the active enzyme thrombin. Thrombin acts directly on fibrinogen, converting it into fibrin, which aggregates into blood clots. Several direct thrombin inhibitors possessing a carboxylic acid pharmacophore have been approved for the treatment of thromboembolic disease.

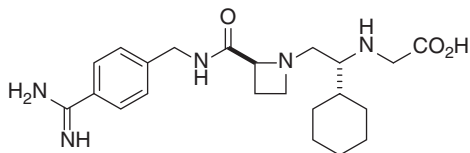
All of the small-molecule direct thrombin inhibitors share two functionally retained moieties (Figure 8.1). At one terminus is a carboxylic acid and at the distal end is an amidine. Argatroban (**2**), developed by both Mitsubishi and Dai-ichi pharma, has a free carboxylic acid and a free alkyl amidine resulting in poor absorption [4]. The drug therefore requires intravenous (*i.v.*) administration [5]. Dabigatran etexilate (**Pro-1**), marketed and developed by Boehringer Ingelheim [6], and ximelagatran (**Pro-3**), marketed and developed by Astra-Zeneca [7], respectively, are both prodrugs with the carboxylic acid masked as an ethyl ester and the benzamidine protected as either a hexylcarbamate or hydroxylamidine functionality.



Dabigatran (1)

Dabigatran etexilate (**Pro-1**)

Argatroban (2)



Melagatran (3)

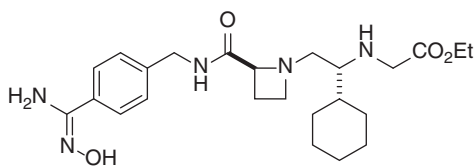
Ximelagatran (**Pro-3**)

Figure 8.1 Carboxylic-acid-containing direct thrombin inhibitors dabigatran (1) dabigatran etexilate (**Pro-1**), argatroban (2), melagatran (3), and ximelagatran (**Pro-3**).

8.2

History

Direct inhibition of thrombin was originally observed with the biological peptide hirudin, a component of leech saliva, which facilitates feeding [8]. The commercially available analog of hirudin is bivalirudin (**4**, trade name Angiomax or Angiox, Figure 8.2). The drug has a rapid onset of action as well as rapid clearance from circulation by a combination of renal excretion and proteolytic cleavage. It acts in a bivalent manner by binding not only to the active site of thrombin via the carboxylic acid moiety but also to exosite-1. The typical half-life in healthy individuals is approximately 25 min, and a return of coagulation to normalcy occurs 1 h after halting administration of the drug. This makes it suitable for use only in emergency surgical interventions [9].

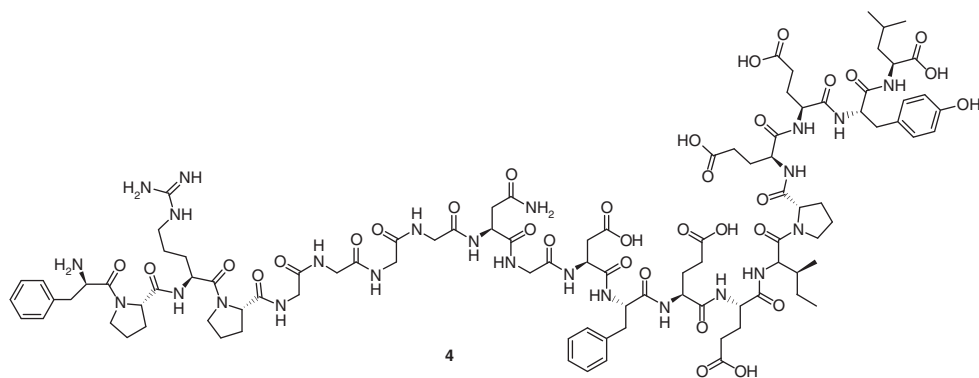


Figure 8.2 Hirudin analog bivalirudin (4).

Following the success of a peptide-based direct thrombin inhibitor, several pharmaceutical companies sought to develop a small-molecule direct thrombin inhibitor with an acceptable time of onset. The success of hirudin as a direct thrombin inhibitor spurred significant research into attempting to make a monovalent and hence less complex thrombin inhibitor. This would take advantage of the carboxylic acid moiety without the extraneous peptidyl portion of hirudin. It had been known in the literature that *N*- α -L-arginine methyl ester had been reported to show satisfactory thrombin inhibition but lacked the protease specificity to be further developed. Along these lines, chemists at Mitsubishi Chemical Industry in the late 1970s began to explore structure–activity relationship (SAR) for *N*- α -tosyl-L-arginine as a starting point [4, 10]. A classical SAR approach to modify the *N*-aryl substituent as well as the methyl ester gave rise to a potent, monovalent, and reversible direct thrombin inhibitor known as argatroban (2). In 2000, argatroban (2) was approved by the FDA for the treatment of thrombosis in patients with heparin-induced thrombocytopenia (HIT) [11]. HIT is a severe disease in which heparin reverses its own effect and may lead to thromboses. Argatroban (2) has been shown to be a safe and effective treatment in patients with HIT. Argatroban (2), however, lacked the oral bioavailability desired from a direct thrombin inhibitor.

Along these lines, Astra-Zeneca began to feverishly work to develop a new direct thrombin inhibitor devoid of the sulfonamide moiety but maintaining the carboxylic acid pharmacophore. Melagatran (3) belongs to a group of peptidomimetic thrombin inhibitors that mimic the D-Phe-Pro-Arg sequence. A benzamidine side chain replaces the arginine residue at P1 [12] and the central scaffold is restrained to an azetidine, which ultimately led to the discovery of melagatran (3). Additionally, a prodrug approach was taken to synthesize an orally bioavailable thrombin inhibitor unlike argatroban (2). **Pro-3** is a double prodrug of melagatran (3) resulting from modification of the carboxylic acid to the ethyl ester and the benzamidine into a hydroxyl-benzamidine. The ester was shown to undergo conversion to the acid by esterase activity. Hence, ximelagatran (**Pro-3**, Exanta or Exarta) was the first member of the direct thrombin inhibitors to exhibit

any appreciable level of bioavailability [13]. The compound was prescribed for BID dosing and is rapidly absorbed by the small intestine. However, after its approval in 2004, liver toxicity was reported in several markets in Europe and South America. Astra-Zeneca soon withdrew ximelagatran (**Pro-3**) after DILI (drug-induced liver injury) was detected in ongoing clinical evaluations [14].

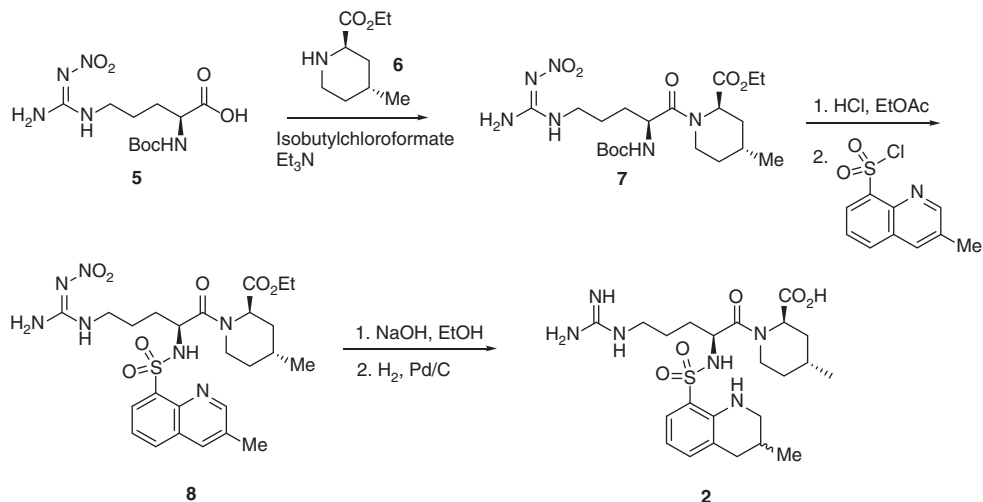
Following up on the successful implementation of a prodrug approach to a direct thrombin inhibitor by Astra-Zeneca, in 2002, Boehringer-Ingelheim reported on their own medicinal chemistry efforts to arrive at **1** and **Pro-1** [15]. FDA approval was ultimately granted to **Pro-1** for use as a direct thrombin inhibitor in 2009.

8.3

Synthesis

The discovery and optimization of argatroban (**2**) was conducted by Mitsubishi Chemical Industries in the early 1970s. U.S. Patent 4,201,863 disclosed the structure and synthesis of argatroban (**2**) and related analogs in the arylsulfonyl-L-arginine amide class and established the utility of the class for direct antagonism of thrombin [16, 17]. The chemical name for argatroban (**2**) is 1-[5-[(aminoiminomethyl) amino]-1-oxo-2-[[[(1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]amino]pentyl]-4-methyl-2-piperidinecarboxylic acid monohydrate. The commercial product is sold as a mixture of the 21(*R*) and 21(*S*) methyl diastereomers, which are introduced in the final hydrogenation step of the 3-methylquinoline into the 1,2,3,4-tetrahydroquinoline in a typical ratio of 65:35 with the *R* diastereomer as the predominant form.

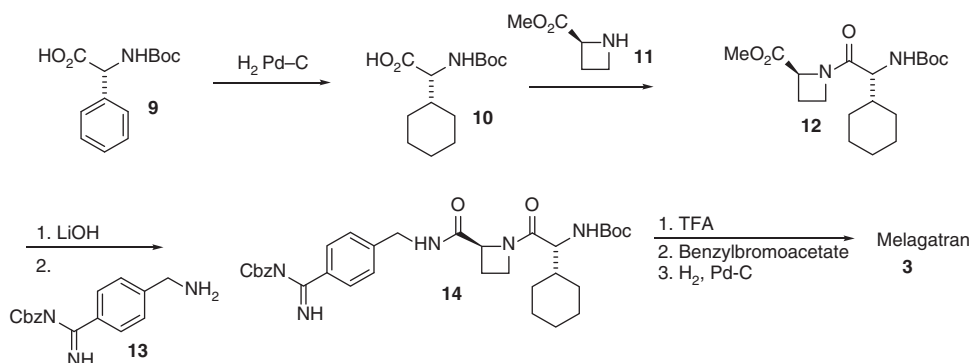
The synthesis of argatroban (**2**) commenced from a protected form of the amino acid L-arginine **5** (Scheme 8.1). The guanidine functionality was altered by *N*-nitration to reduce its reactivity, and the amino group was protected



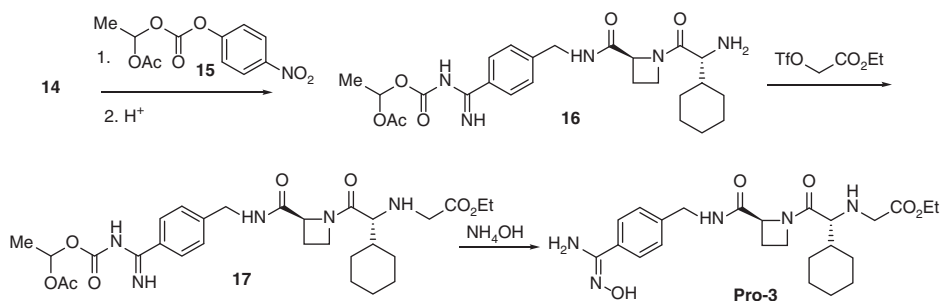
Scheme 8.1 Synthesis of argatroban (**2**) from *N*-nitro-Boc-L-arginine.

with a *tert*-butyl carbamate (Boc) group. Amide coupling with ethyl (2*R*,4*R*)-4-methyl-2-pipecolic acid ester (**6**) afforded piperidine amide **7**. Activation of the carboxylic acid was achieved by formation of the mixed carbonate anhydride with isobutylchloroformate. Multiple routes have been disclosed to the asymmetric pipecolic ester **6**, which is resolved using *L*-tartaric acid [18]. Acid-catalyzed deprotection of the Boc group and subsequent condensation with quinoline sulfonylchloride afforded sulfonamide **8**. Basic hydrolysis liberated the free carboxylic acid adjacent to the piperidine ring, followed by exhaustive hydrogenation to reduce the nitroguanidine to the amidine functionality. Concomitantly, hydrogenation serendipitously reduced the quinoline ring into the 1,2,3,4-tetrahydroquinoline affording the 21(*R*) and 21(*S*)-methyl diastereomers of argatroban (**2**).

The routes shown in Scheme 8.2 to melagatran (**3**) and Scheme 8.3 to the prodrug ximelagatran (**Pro-3**) are condensed from the discovery efforts detailed in the patent literature [19, 20]. Modifications to the route facilitating clinical development and commercial manufacture were not disclosed. The synthesis of melagatran (**3**) commenced with a hydrogenation to convert phenylglycine **9** into the cyclohexylglycine **10**. Amide coupling with methyl (*S*) 2-azetidinecarboxylate **11** easily provided dipeptide **12**. Saponification of the methyl ester at the C-terminus and condensation with 4-benzylamino-Cbz-benzamidine **13** afforded



Scheme 8.2 Synthesis of melagatran (**3**).



Scheme 8.3 Discovery synthesis of ximelagatran (**Pro-3**).

the key differentially protected intermediate **14** [21]. Acidic deprotection of the *N*-Boc terminus of **14** and alkylation with benzyl bromoacetate followed by global deprotection of the Cbz-amidine and benzyl ester by hydrogenolysis completed the preparation of melagatran (**3**).

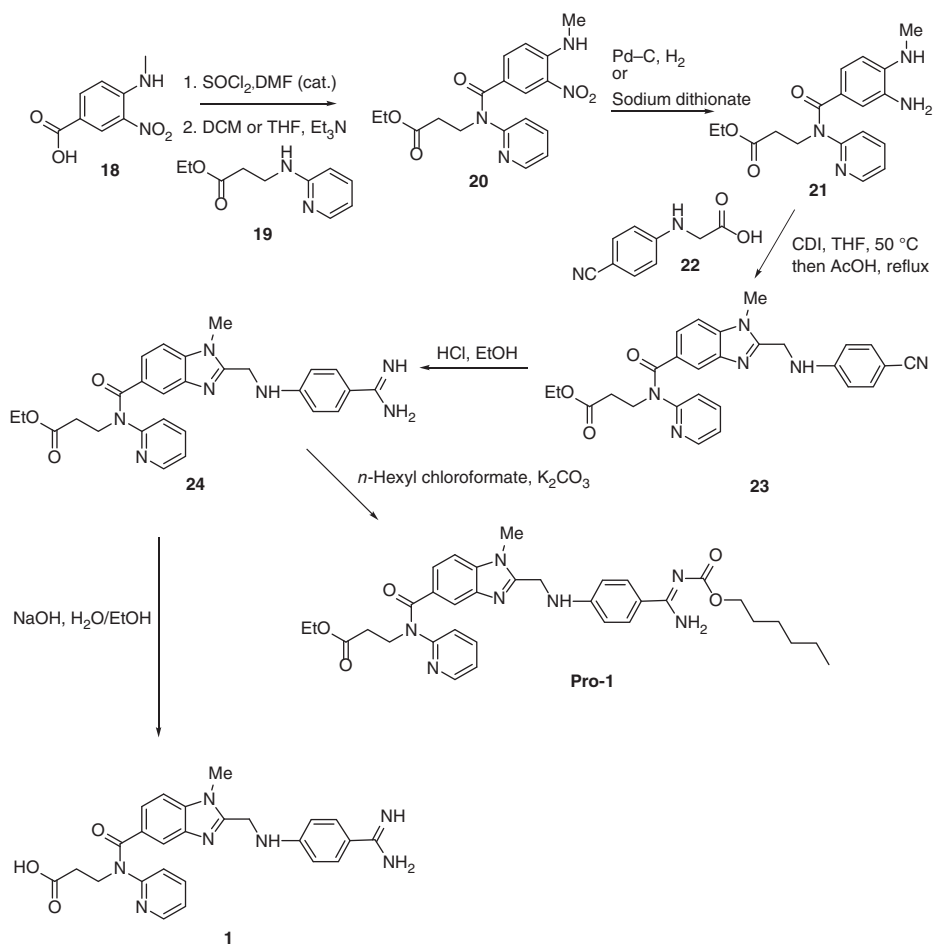
Ximelagatran (**Pro-3**) was prepared from the common intermediate **14** by hydrogenolysis of the Cbz-amidine. The benzamidine was next condensed with the *para*-nitrophenol acetoxycarbonate **15** followed by acid-mediated deprotection of the Boc group affording amine **16**. Alkylation of the amine with ethyl trifluoromethanesulfonyl acetate to the ethyl glycine **17** was followed by the conversion of the carbamate to the hydroxyl-benzamidine with hydroxylamine resulted in ximelagatran (**Pro-3**).

The synthetic routes to dabigatran (**1**) have evolved as the compound progressed from preclinical optimization, through clinical trials, and finally onto the market. Since the FDA approval, additional refinements and alternative routes have been published. The medicinal chemistry route starts with conversion of the trisubstituted benzoic acid **18** to the acid chloride using thionyl chloride (Scheme 8.4) [22]. This was followed by amide formation with **19** giving rise to intermediate **20**. Nitro group reduction to the aniline by hydrogenation with Pd-C afforded the phenylenediamine intermediate **21**. Alternative conditions using sodium dithionite to achieve the nitro reduction are claimed to yield the diamine in superior purity following isolation [22]. Condensation of **21** and 4-cyanophenylglycine **22** was accomplished using carbonyl diimidazole (CDI) in tetrahydrofuran (THF) at elevated temperature, followed by refluxing in acetic acid giving rise to the benzimidazole **23**. Pinner reaction using HCl/EtOH at 0 °C quickly furnished the requisite amidine **24**. The key intermediate **24** could either be hydrolyzed directly to dabigatran (**1**) or converted into dabigatran etexilate (**Pro-1**) by treatment of the ester **24** with *n*-hexylchloroformate.

8.4

Mode of Action

Upon injury to a vessel wall, a tissue factor can be exposed on the surface of the compromised endothelium. The interaction of tissue factor with plasma factor VII activates the corresponding coagulation cascade pathway. Through this cascade, thrombin is produced in a stepwise manner through activation of a series of proenzymes as shown in Figure 8.3. Thrombin is chief among the clotting enzymes due to its inherent mode of action in converting soluble fibrinogen into fibrin [23]. This process then leads to the activation of factors V, VIII, and XI, which thereby generates additional thrombin with subsequent platelet aggregation. Through activation of Factor XIII, thrombin favors the formation of cross-linked bonds among the fibrin residues, which stabilize the clot. Thrombin inhibitors block the action of thrombin by binding to three distinct domains, the active site or the catalytic site, which has two exosites (exosites-1 and 2). Thrombin itself can be inhibited indirectly by low-molecular-weight



Scheme 8.4 Synthesis of dabigatran (1) and dabigatran etexilate (Pro-1).

heparins, because these drugs strongly catalyze the function of antithrombin. Direct thrombin inhibitors act independently of antithrombin and can therefore inhibit thrombin bound to fibrin or fibrin degradation products. Monovalent direct thrombin inhibitors such as melagatran (3), dabigatran (1), and argatroban (2) bind only to the active site, thereby inhibiting platelet aggregation through inactivation of fibrin-bound thrombin.

8.5

Structure–Activity Relationship

The pioneering work on argatroban (2) was derived from what had already been well understood regarding synthetic arginine esters and their efficaciousness

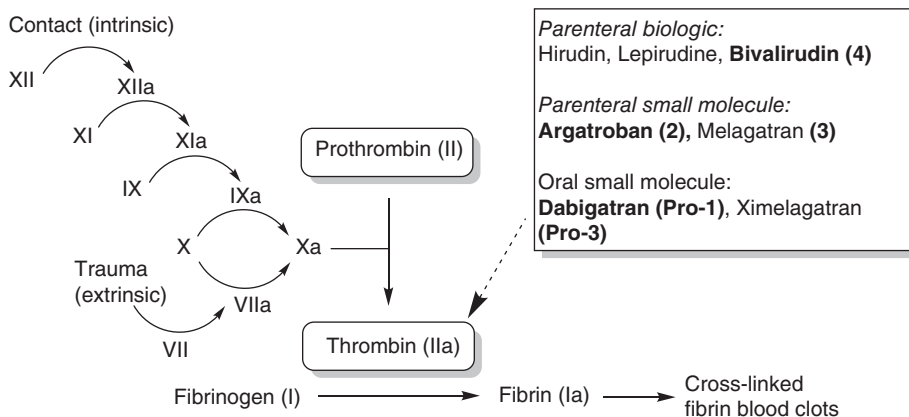
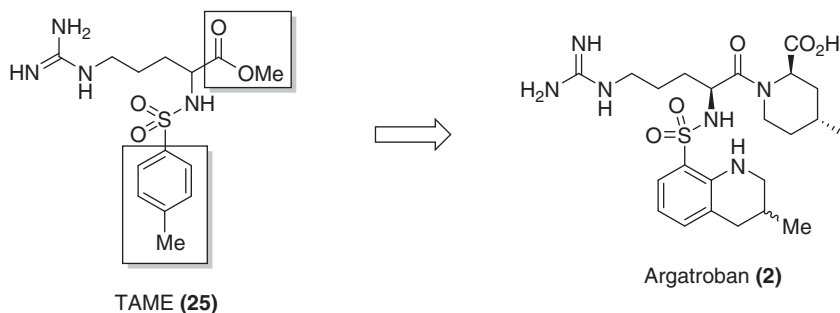


Figure 8.3 Coagulation cascade pathway.

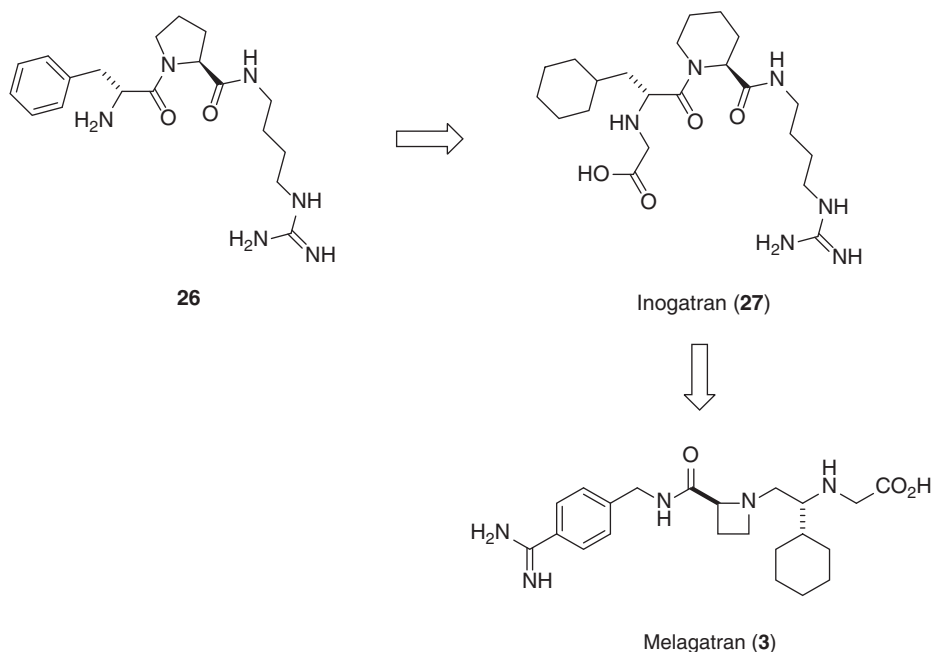
as direct thrombin inhibitors (Scheme 8.5). Specifically, *N*- α -tosyl-L-arginine methyl ester (TAME) (**25**) as a starting point led to modifications of both the aryl sulfone as well as the methyl ester [4, 10]. These point changes led to analogs of increased potency as well as increased specificity over related serine proteases giving rise to **2** [10].



Scheme 8.5 *N*- α -Tosyl-L-arginine methyl ester (TAME) (**25**) as a starting point for argatroban (**2**).

Argatroban (**2**) is dosed *i.v.* and its onset of action is observed very quickly; however, the carboxylic acid is rapidly excreted with a half-life of only 35 min, presumably through formation of an acyl glucuronide [23].

With regard to melagatran (**3**), Astra-Zeneca began their foray into direct thrombin inhibitors starting from the agmatine-derived inhibitor (**26**), which was modified to ultimately give inogatran (**27**) (Scheme 8.6) [24]. Replacement of the benzylic substituent by a fully reduced cyclohexane ring as well as replacement of the requisite proline central scaffold with a piperidine ring led to Inogatran **27** which upon further modification of led to the discovery of melagatran (**3**). Further modification of inogatran (**27**) led to the discovery of melagatran (**3**).



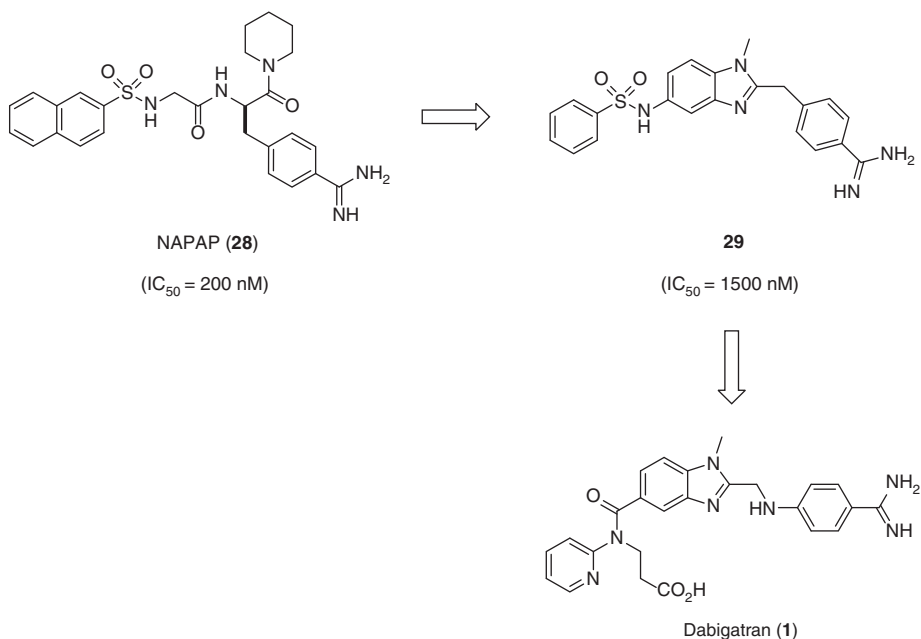
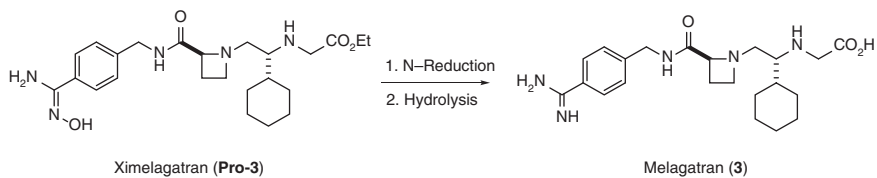
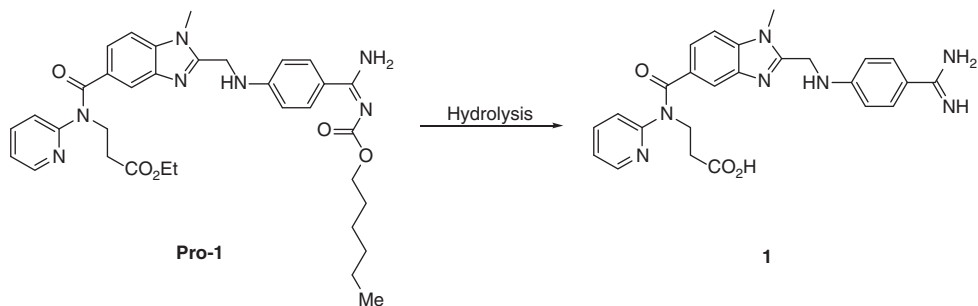
Scheme 8.6 Historic path to melagatran (3).

Melagatran (3) does, however, inhibit trypsin ($K_i = 4 \text{ nM}$) and suffers from poor oral absorption. On the heels of the work on melagatran (3), Boehringer-Ingelheim embarked on a journey to use structure-based design to develop novel thrombin inhibitors starting from the known, peptide-like benzamidine-based thrombin inhibitor, NAPAP (28) (Scheme 8.7) [15, 24].

Using NAPAP (28) as a template, the team quickly identified the key areas of the compound that could be modified to further enhance potency as well as selectivity over related proteases. Starting from 29, the benzimidazole portion was maintained while replacement of the sulfonamide was achieved with a tertiary amide moiety. The tertiary amide introduced the carboxylic acid moiety as well as a directly linked 2-pyridyl substituent. Additionally, the benzylic linker at the 2-position was now substituted as a benzylic amine [15]. The benzylic amine imparted the most improved potency profile over related carbon- and oxygen-linked derivatives while continuing to improve physical properties of the molecule. The totality of these modifications led to the identification of 1, which showed an excellent overall profile both *in vitro* and *in vivo*.

Melagatran (3) is dosed as a double prodrug in the form of ximelagatran (**Pro-3**) as an attempt to improve the overall bioavailability of the drug (Figure 8.4). The N-reduction and subsequent hydrolysis occur very rapidly *in vivo* to liberate the active form of the drug [13].

Dabigatran etexilate (**Pro-1**) is also dosed as a double prodrug and is rapidly converted by serum esterase into dabigatran (1) *in vivo*. However, the oral

Scheme 8.7 Historic path to dabigatran (**1**).Figure 8.4 Conversion of ximelagatran (**Pro-3**) into melagatran (**3**).Figure 8.5 Conversion of dabigatran etexilate (**Pro-1**) into dabigatran (**1**).

bioavailability of dabigatran etexilate (**Pro-1**) remains an issue since it is reported to be only in the 3–7% range (Figure 8.5) [25].

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Part III

Infectious Diseases

9

Tetracycline Amide Antibiotics

Ingo Janser

9.1

Introduction

Tetracyclines are a group of broadband-spectrum antibiotics that comprise a distinct family of substituted hydronaphthacene compounds. Antibiotics are chemical substances produced by microorganisms that are able to kill other microorganisms without being toxic to the person, animal, or plant being treated. “Broadband-spectrum antibiotics” refer to compounds that act against a wide range of disease-causing bacteria. As the name indicates, tetracyclines are derived from a basic system of four linearly annulated six-membered rings (**1a**, Figure 9.1), namely, octahydronaphthacene or tetracene (naphthacene), containing a characteristic arrangement of double bonds. All tetracyclines contain two distinct chromophoric regions, ring A and rings BCD, which are separated by the sp^3 carbon 12a (**1b**, Figure 9.1). Ring D is always aromatic, while the other ones (BCD) are partially saturated. The carbon atoms 4, 4a, 5, 5a, 6, and 12a become asymmetric upon appropriate substitution. Generally, tetracyclines contain five to six asymmetric centers, which make the compounds optically active. The absolute configuration of tetracyclines was determined by X-ray structure analysis [1–3] and supplemented by NMR [4, 5]. The members differ mostly in the substitution pattern at positions 5, 6, 7, and 9.

Tetracyclines are produced by strains of *Streptomyces* bacteria, or made in the laboratory through biosynthesis from naturally occurring tetracyclines, such as chlortetracycline (**2**) or oxytetracycline (**3**) (Figure 9.2). Others are produced semisynthetically (Figure 9.3), and some are even synthesized from scratch (total synthesis), for example, tigecycline (**10**) (Figure 9.4)[6]. Tetracyclines were first discovered in 1948 by Duggar and coworkers with the isolation of chlortetracycline (**2**). Two years later, Finlay and coworkers reported the isolation of oxytetracycline (**3**). In the 1950s, when most of the tetracyclines were discovered, their antimicrobial spectrum was broader than that of any other antibiotic known by then. They, together with penicillin, became the most widely used antibiotics.

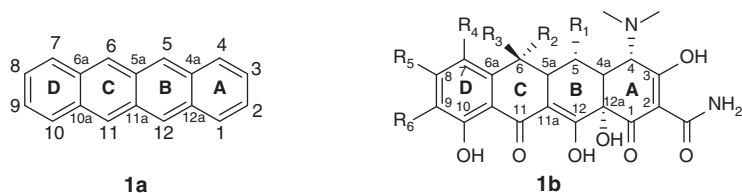


Figure 9.1 The naphthalene ring system (1a) and the basic structure and numbering conventions for tetracyclines (1b).

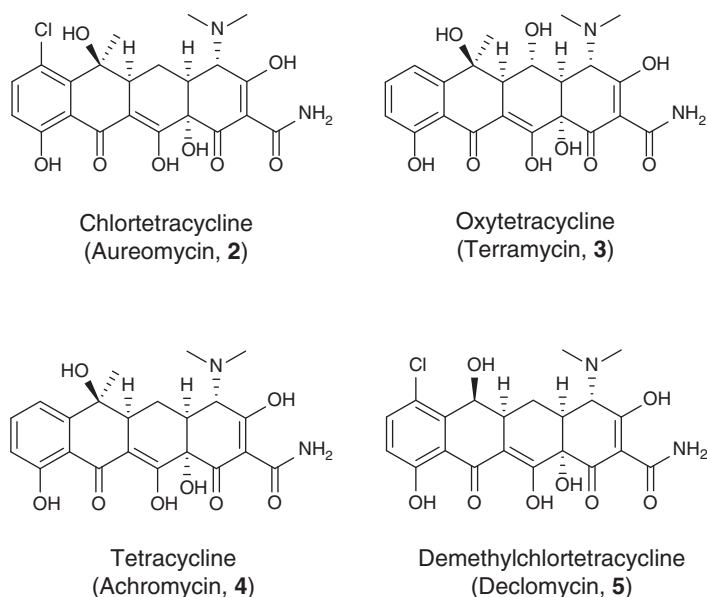


Figure 9.2 Examples of tetracycline natural products.

Tetracyclines are characterized by their exceptional chemotherapeutic efficacy against a wide range of Gram-positive and Gram-negative bacteria. The main indications for the use of tetracyclines are infections due to *Escherichia coli* and *Haemophilus influenzae*, infections of the bile duct, bacterial respiratory disorders including bronchitis prophylaxis, mixed infections arising from the mouth, pharynx, or intestinal tract, brucellosis, tularemia, plague and other pasteurelloses, leptospirosis, lymphogranuloma inguinale, cholera, and rickettsiosis [7]. Mycobacteria, protozoa, fungi, and yeasts are resistant. The individual tetracyclines, including the semisynthetic tetracyclines, differ less in their potency than in their pharmacokinetic properties such as resorption, tissue diffusion, and elimination [7].

All tetracyclines are yellow, odorless, bitter tasting, light-sensitive, crystalline compounds. Naturally occurring tetracyclines are only slightly soluble (~1 mg/ml)

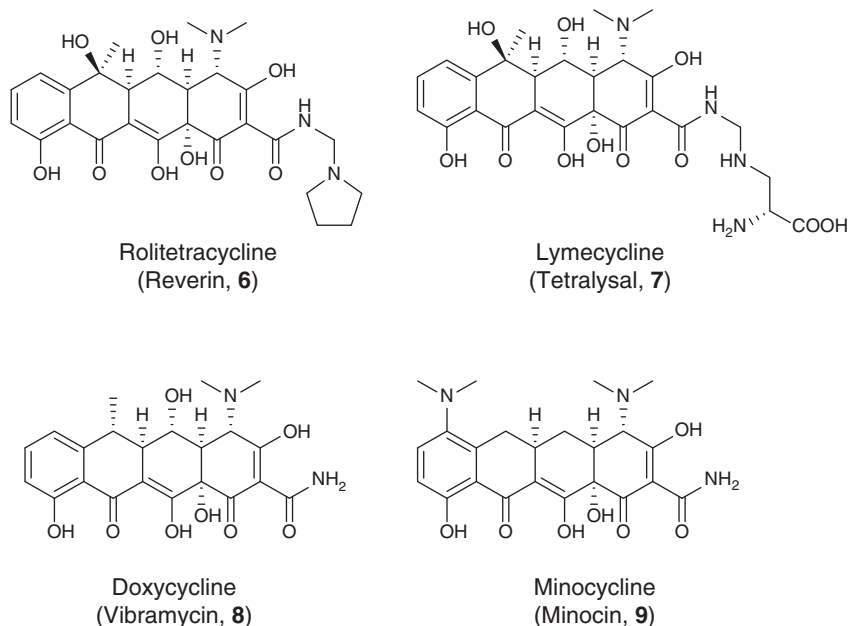


Figure 9.3 Examples of semisynthetic tetracyclines.

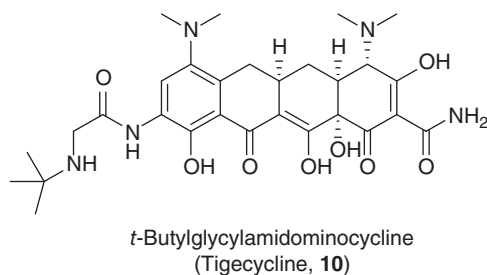


Figure 9.4 Structure of tigecycline (10), an example of a fully synthetic tetracycline.

in the physiological pH region. They show amphoteric behavior because of the acidic substituents and the basic dimethylamino group on C-4. The UV spectra of tetracyclines are highly characteristic; the BCD ring chromophore absorbs at 225, 285, 320, and 360 nm and the ring A chromophore at 262 nm; a band at 275 nm is composed of several absorptions [8].

Historically, tetracyclines are divided into three generations. They are considered first generation if they are obtained by biosynthesis, for example, chlortetracycline (2), oxytetracycline (3), tetracycline (4), and demethylchlortetracycline (5) (Figure 9.2). Second generation if they are semisynthetic antibiotics, such as rolitetracycline (6), lymecycline (7), doxycycline (8), and minocycline (9) (Figure 9.3).

Third generation if they are obtained from a total synthesis, for example, tigecycline (**10**) (Figure 9.4).

9.2

History

9.2.1

First-Generation Tetracyclines – The Discovery

Chlortetracycline (Aureomycin, **2**) (Figure 9.2) was the first tetracycline discovered. It was isolated in the late 1940s from a Missouri soil sample by Benjamin Duggar from Lederle Laboratories [9]. Duggar named the compound Aureomycin because of its yellow color and the gold-colored *Streptomyces* strain from which it was extracted. In 1948, the drug was approved by the FDA for clinical use. It was an immediate success in the clinic, and it saved tens of thousands of lives against a broad spectrum of infectious diseases. Within a short period of time after the Lederle discovery of Aureomycin (**2**), other companies started to announce their own discoveries of new antibiotics. Alexander Finlay and colleagues at Charles Pfizer Co., Inc. had gathered thousands of soil samples. Soil samples were collected from deserts, jungles, oceans, and even mountaintops. As a result, Finlay isolated a compound from the soil bacterium *Streptomyces rimosus*, which was very similar in color to Aureomycin (**2**), but it was more water-soluble and had a better bioactivity [10]. The compound, nowadays known as oxytetracycline (**3**) (Figure 9.2), was named Terramycin in reference to *terra*, Latin for *earth*, and perhaps its origin, Terre Haute, Indiana, where it was collected on the grounds of a factory, owned by Pfizer. Shortly after its discovery, Terramycin (**3**) was approved by the FDA in 1950. From the beginning, this new drug was competing against Aureomycin (**2**). Terramycin (**3**) actually was the subject of the first mass-marketing campaign by a modern pharmaceutical company. Pfizer advertised it in medical journals and spent twice as much money on its marketing than it did on its discovery and development. Eventually, it turned a small company into a pharmaceutical giant. Although both drugs, Aureomycin (**2**) and Terramycin (**3**) were used to successfully treat a broad spectrum of infectious diseases, their chemical structures were hard to solve and remained elusive for both companies. This was mostly due to the fact that, at the time, chemical characterization was limited to ultraviolet–visible (UV–vis) spectroscopy and infrared (IR) spectroscopy [11]. Structural proof mostly relied on chemical modifications and degradation studies, but only a few laboratories worldwide were equipped to perform these kinds of experiments. Scientists at Pfizer, led by Karl Brunings, in collaboration with the legendary Harvard University Professor Robert Woodward finally succeeded in determining the structure of both compounds for the most part in 1952 [12]. The core scaffold of this new family of antibiotics became known as tetracycline. The major structural features of the molecules were published by the Pfizer–Woodward group in a

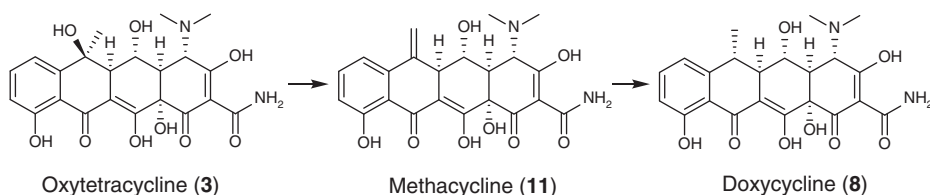
paper in 1954 titled “The Structure of Aureomycin” [13]. This publication now opened the doors for the first semisynthetic derivatives of this new family of antibiotics.

In this scientific era, it was believed that chemical modification of antibiotics would ultimately decrease their activity. It was Lloyd Conover from Pfizer who took the opposite view and claimed that the C-7 chlorine in chlortetracycline (Aureomycin, **2**) is not responsible for the biological activity of the drug. He postulated that the chloro group can be removed or modified leading to an even higher biological activity. In order to prove his hypothesis, he treated Aureomycin (**2**) with hydrogen in the presence of a palladium metal catalyst (catalytic hydrogenation) and synthesized the C-7 deschloro derivative. This compound was named tetracycline (**4**) (Figure 9.4). It indeed showed an improved potency, a better solubility in water, and a more favorable pharmacological activity [14] and was therefore approved by the FDA for clinical use in 1954. Tetracycline (**4**) was the first novel tetracycline obtained through modification of a natural product, and it was one of the first commercially successful semisynthetic antibiotics used in medicine.

9.2.2

Second-Generation Semisynthetic Tetracyclines

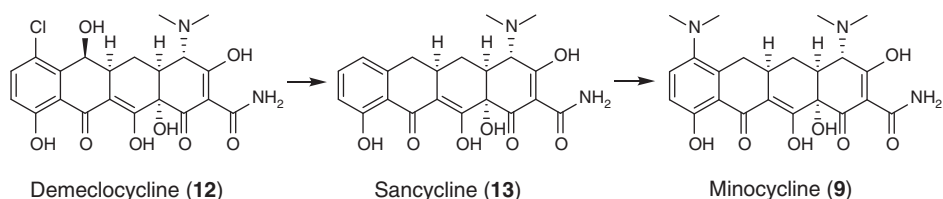
Lederle and Pfizer, independent from each other, further tried to modify the tetracycline scaffold in order to generate more potent and active tetracycline antibiotics. The Pfizer chemists, led by Robert Blackwood, chose to modify the C ring of oxytetracycline (**3**) to increase chemical stability, and halogenation together with C-6-dehydration yielded the antibiotic methacycline (**11**) [15]. Even though **11** showed improved pharmacological properties compared to its precursor oxytetracycline (**3**), it was never submitted for approval within the United States. However, it was used as a starting material by Charlie Stephens to produce an analog with remarkable activity, namely doxycycline (**8**) (Scheme 9.1), which was approved in 1967 [16]. Doxycycline (**8**) is still widely used as an antibiotic with activity against a broad spectrum of bacterial infections, and a diverse range of microbes, from the causative agent of anthrax infections, *Bacillus anthracis*, to malaria caused by the intracellular schizont of *Plasmodium falciparum* [17]. Both companies also studied other antibiotic-producing soil bacteria and created



Scheme 9.1 Historic development of tetracycline antibiotics at Pfizer.

biochemical mutants of their *Streptomyces* strains in an effort to induce higher yields of products as well as to discover other novel tetracyclines.

One bioengineered strain by Lederle scientists produced a new tetracycline, which was named demeclocycline (**12**) [18]. Due to its low bioactivity, **12** was further stripped down to its minimal pharmacophore sancycline (**13**) (Scheme 9.2). Robert Church produced a novel C-7 and C-9 derivative of sancycline (**13**) [19] that carried a C-7-dimethylamino group. This analog was found to exhibit far greater antibacterial and pharmacological activity against a larger range of bacteria compared to the first-generation compounds and doxycycline (**8**). The compound was named minocycline (**9**) and was approved for clinical use in 1971. It became one of the most widely used tetracyclines to date and also was the last tetracycline to enter the clinic for the next 35 years.



Scheme 9.2 Historic development of tetracycline antibiotics at Lederle.

9.2.3

Tetracycline Resistance

Already within the first year after the discovery of chlortetracycline (**2**), the first evidence of bacterial resistance was reported [20]. It was shown that the extent of this resistance varies from region to region and is not the same for the various causative organisms. However, all investigations show that the increase in resistance runs parallel to frequent use of tetracyclines. The situation is particularly unfavorable in view of the extensive cross-resistance between the individual tetracyclines. Microorganisms that are not inhibited at concentrations of 3 µg/ml are considered to be tetracycline resistant [21]. There are three types of resistance: (i) primary or natural resistance, equivalent to a natural insensitivity of the given strain to the drug; (ii) acquired chromosomal resistance, in which a bacterial population is caused by spontaneous gene mutation in bacterial chromosomes. This resistance is directed only against the selective antibiotic. (iii) Transferable resistance, which is transferred from one microorganism to another by chromosomal or extrachromosomal DNA elements (R factors, plasmids, episomes). Such a DNA element generally contains several resistance genes, so that the carrier bacteria become resistant simultaneously against chemically differentiable, non-cross-resistant antibiotics. The greatest multiple resistance observed affects six antibiotics, namely, tetracycline streptomycin, chloramphenicol, kanamycin, neomycin, and ampicillin, as well as sulfonamides [7, 21].

9.2.4

Third-Generation Tetracyclines

Due to the increased antibiotic resistance, pharmaceutical companies were prompted to reenter or expand their antibiotic discovery programs. In the late 1980s, Wyeth launched a program to chemically modify the minocycline scaffold, producing hundreds of C-9-amino derivatives that possess an amide functionality with a glycine subunit attached (glycylcyclines) for evaluation. Many of the compounds were active against Gram-positive and Gram-negative bacteria. Soon their lead compound tigecycline (**10**) (Figure 9.4) entered clinical studies and was approved by the FDA for hospital use in 2006. This was the first new third-generation tetracycline to enter market in over 35 years and one specifically designed to confront tetracycline-resistant mechanisms. While tigecycline (**10**) has been proven active against a broad range of bacterial strains, pharmaceutical companies have continued their quest for novel antibiotic tetracyclines. In the late 1980s, Tufts University School of Medicine began to screen tetracycline derivatives as inhibitors of the tetracycline efflux proteins. The goal was to block the efflux mechanism of resistance, allowing a clinically used tetracycline to enter the cell and inhibit protein synthesis and growth. In 1996, Paratek Pharmaceuticals, Inc. was launched in Boston. There, many new classes of compounds that possessed antibiotic activity were synthesized utilizing transition metal chemistry, and thousands of compounds against tetracycline-susceptible and -resistant bacteria were tested. More recent developments have involved the total synthesis of tetracycline derivatives. Harvard University Professor Andrew Myers reported a facile and versatile route to produce numerous novel tetracyclines with modifications at positions that cannot be accessed semisynthetically. Currently, this pathway for tetracycline synthesis has been used by Tetraphase Pharmaceuticals of Watertown, Massachusetts, and has resulted in the generation of a C-7-fluorotetracycline derivative coded as TP-434 (eravacycline), which is currently entering Phase II clinical trials against pathogens and indications related to intra-abdominal infections.

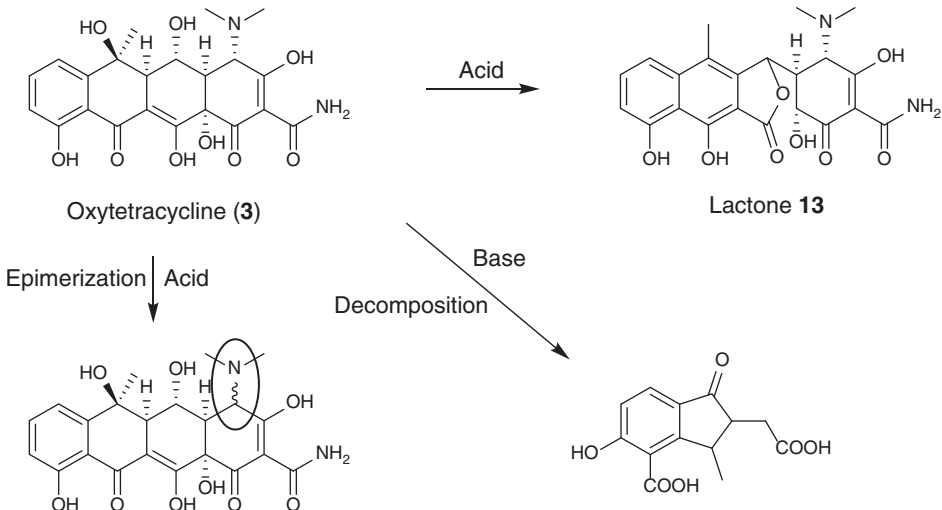
9.3

Synthesis

In 1979, Muxfeldt and colleagues pointed out the challenges and basic obstacles of achieving a total synthesis of any of the natural tetracyclines [22].

- 1) *Stereochemical complexity*: There are up to five adjacent asymmetric centers (e.g., Terramycin (**3**)), which must be established. Because of the complicated stereochemistry and substitution, Woodward once described these compounds as a “diabolical concatenation of reactive groupings” [23].
- 2) *Chemical sensitivity*: Many substituents in the tetracyclines are partially very sensitive to acidic, basic, and/or reducing reagents. This made it hard to find

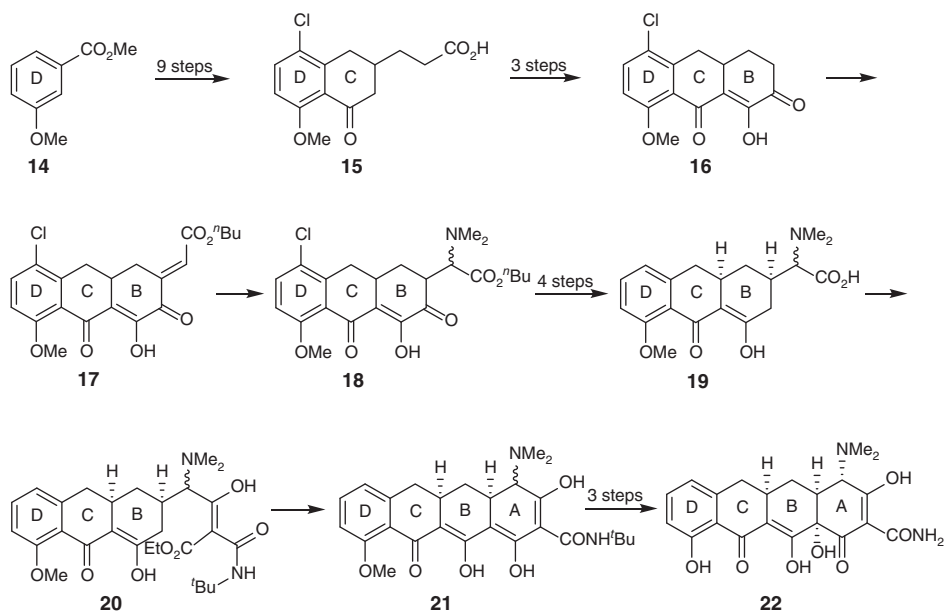
a stereospecific synthesis for this type of molecules, especially since no synthetic prototype was known. For the 6-methyl-6-hydroxy tetracyclines (e.g., oxytetracycline (**3**)), mild acid rapidly catalyzes dehydration, ketalization, and a retro-aldol reaction to produce lactone **13** as shown in Scheme 9.3. Mildly basic conditions result in deprotonation of the C-5 and C-6 hydroxyls, initiating a cascade of events, which lead to the decomposition of the molecule. Finally, the C-4 stereocenter is readily epimerized upon exposure to acetic acid or aqueous buffers [22].



Scheme 9.3 Chemical sensitivity of 6-methyl-6-hydroxy tetracyclines toward acids and bases.

In 1962, after many years of intensive research and experimentation, Woodward and a research team at Pfizer succeeded in the first total synthesis of a biologically active tetracycline, ($\pm$)-6-demethyl-6-deoxytetracycline (**22**). An initial communication [24] and a personal account [25] were published in 1962 and 1963, respectively, followed by a full article describing the total synthesis in 1968 [26]. Even though the route suffered from very low yields (25 steps, $\sim 0.002\%$ overall yield), this synthesis is of historical significance because it was the first preparation of a member of the tetracycline family. In 1965, Muxfeldt and Rogalski succeeded in obtaining ($\pm$)-6-demethyl-6-deoxytetracycline (**22**) in a more elegant and much simpler way [27].

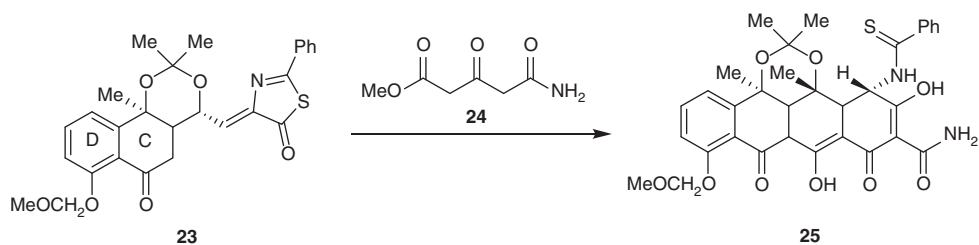
Woodward's synthesis starts with the aromatic ring **D**, onto which rings **C**, **B**, and **A** are built on stepwise by condensation reactions (Scheme 9.4). Methyl *m*-methoxybenzoate (**14**) is converted in nine steps into the tetralone (**15**), which is condensed to the tricycle (**16**) by oxalic ester and sodium hydride. Stepwise construction of ring **A** was the most difficult and challenging problem, since all the ring elements are substituted and C-4, C-4a, and C-12a are asymmetric. *n*-Butyl glyoxylate is reacted with **16** to afford **17**, and dimethylamine then adds



Scheme 9.4 Woodward's total synthesis of (±)-6-demethyl-6-deoxytetracycline (**22**).

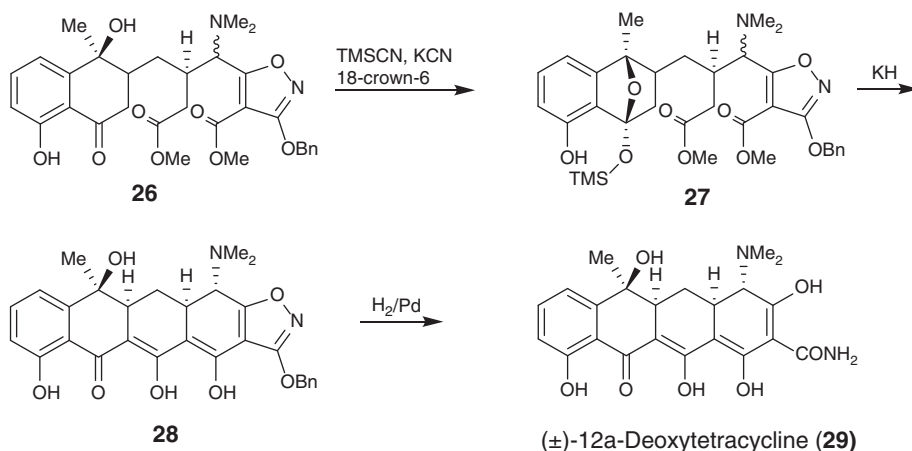
stereospecifically to the exocyclic double bond, giving **18**. The bulky side chain takes up the equatorial, and the hydrogen the axial position in this reaction. The acid **19** is obtained from **18** by reduction of the keto group in ring B and hydrolysis of the ester. After reductive dehalogenation, the intermediate is converted into a mixed anhydride, which yields **20** upon reaction with ethyl *N*-(*tert*-butyl)malonamate. Cyclization of **20** by use of sodium hydride as a condensation agent gives the tetracycline (**21**). Stereospecific introduction of the hydroxyl group at C-12a by autooxidation, inversion of the 4-dimethylamino group from the β - to the natural α -configuration, and removal of the protecting groups afforded (±)-6-demethyl-6-deoxytetracycline (**22**). When tested biologically, (±)-6-demethyl-6-deoxytetracycline (**22**) had only 50% of the potency of the optically active 6-demethyl-6-deoxytetracycline obtained from natural material. This shows that only the enantiomer with the natural configurations at the asymmetric centers is biological active.

In 1979, the Muxfeldt group [22] achieved the total synthesis of (±)-oxytetracycline (Terramycin (**3**)) by utilizing a different approach that started from a CD ring precursor and built out to the A ring. The key step involved the generation of the thiazolone intermediate **23**, which was then condensed with the glutamate derivative **24**. This low-yielding but elegant condensation created three new C–C bonds in one step and afforded the (±)-oxytetracycline precursor **25** with most of the positions already having the appropriate substitution pattern (Scheme 9.5) [7].



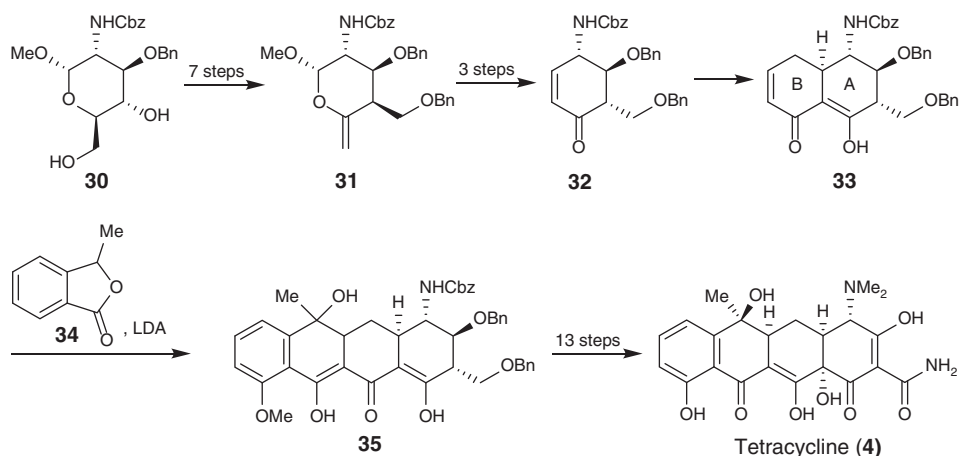
Scheme 9.5 Key step in Muxfeldt's synthesis of (±)-oxytetracycline (Terramycin (**3**)).

In 1996, a landmark synthesis carried out by Stork and coworkers [28] produced (±)-12a-deoxytetracycline (**29**) (17 steps, 22% overall yield, Scheme 9.6). The key step in this synthesis involved a double Claisen cyclization reaction to generate the A and B rings of the tetracycline nucleus. This involves generating ketal **27** from the C ring hydroxyl group in **26**, followed by deprotonation at the α -position. For this synthesis, Stork did develop a method to protect the vinylogous carbamide functionality of the tetracyclines as an isoxazole, a strategy that would be utilized again later [29].



Scheme 9.6 Key steps in Stork's total synthesis of (±)-12a-deoxytetracycline (**29**).

Despite significant synthetic efforts, an asymmetric total synthesis of a tetracycline was not achieved until 2000 by Tatsuta and coworkers (Scheme 9.7) [30]. Rather than beginning with a D or DC ring precursor, Tatsuta and coworkers started from an A ring precursor. Thus, the D-glucosamine derivative **30** was converted into **31** in 47% yield over seven steps. Ferrier rearrangement led to the conversion of the exocyclic enol ether in **31** to the thermodynamically stable cyclohexanone **32** and the elimination of the anomeric methoxy group. The electron poor alkene in **32** then underwent a regioselective Diels–Alder cycloaddition with a diene to construct the AB ring system. The obtained



Scheme 9.7 First asymmetric total synthesis of natural (–)-tetracycline (4) by Tatsua.

α,β -unsaturated enone **33** was treated with the isobenzofuran derivative **34** to generate the tetracycline skeleton **35** in 48% yield over three steps via a tandem Michael–Dieckmann reaction [30]. Finally, compound **35** could be successfully converted into (–)-tetracycline (**4**) in 13 steps.

9.4

Mode of Action

Tetracyclines inhibit many enzyme reactions that are important for the vital processes of bacterial and mammalian cells. Examples are oxidative phosphorylation and electron transport [7]. The most sensitive biochemical reaction that is inhibited by tetracyclines, however, is the biosynthesis of proteins [31, 32].

Tetracyclines bind to the 30S subunit of microbial ribosomes. During protein biosynthesis, the new tRNA that carries an amino acid attempts to bind to the A-site of the ribosome. However, this site is blocked by the tetracycline [33, 34], which prevents the introduction of new amino acids into the nascent peptide chain. By inhibiting protein biosynthesis, tetracyclines cause cell death of the bacterial cell. The tetracyclines (tetracycline (**4**), doxycycline (**8**), demeclocycline (**12**), minocycline (**9**), etc.) block bacterial translation by binding reversibly to the 30S subunit and distorting it in such a way that the anticodons of the charged tRNAs cannot align properly with the codons of the mRNA. Mammalian cells are less vulnerable to the effect of tetracyclines, despite the fact that the tetracyclines bind to the small ribosomal subunit of both prokaryotes and eukaryotes (30S and 40S, respectively). This is because bacteria actively pump the tetracyclines into their cytoplasm, even against a concentration gradient, whereas mammalian cells do not. This accounts for the relatively small side effect of tetracyclines on human cells. The tetracyclines enter the bacterial cell wall in two ways: passive diffusion

and an energy-dependent active transport system, which is probably mediated in a pH-dependent manner. Once inside the cell, protein synthesis is ultimately inhibited, leading to a bacteriostatic effect.

9.5

Structure–Activity Relationships

Analysis of the relationships between the molecular structures of tetracycline and the *in vivo* biological activities of these compounds shows that only little modifications to the tetracycline skeleton are tolerated in order to maintain their biological activity. The linear arrangement of the four rings and the two chromophoric keto-enol systems in ring A and in rings BCD cannot be altered at all since they constitute an important prerequisite for antibiotic activity. All partially or totally synthetic tetracycline derivatives containing fewer than four rings are inactive.

Figure 9.5 shows the structural requirements for tetracyclines to be biologically active. Only the groups R_1 to R_4 can be varied without causing a substantial decrease in antimicrobial activity. The simplest tetracycline structure, which embodies all the elements necessary for full biological activity, is the partially synthetic 6-demethyl-6-deoxytetracycline (sancycline (13)), so this structure may be regarded as the minimum pharmacophore [21].

Functional groups at positions 5–7 may be removed without drastically altering the antimicrobial properties. The correct configurations at C-4 and C-5a are essential for biological activity. Keto-enol tautomerization involving C-4 leads to the relatively inactive 4-epi-tetracycline (quartrimycins). There is a strong belief that the principal active center is the C-11, C-12 diketone system of rings B and C. Other features that are essential for antibacterial activity are maintaining the stereochemistry at C-4a, C-12a (AB ring junction), and C-4 (dimethylamino group). The conservation of the C-10 phenol and the keto-enol systems at positions C-11 and C-12, in conjugation with the C-12a-OH group, are also important. Tetracyclines are strong chelating agents [33], and their biological activities are influenced by chelation of metal ions. Chelation sites include the enol system in position C-1 and C-3, the carboxamide (position C-2), and the β -diketone system at position C-11 and C-12. Replacement, for example, of the C-2 carboxamide moiety with other functional groups, generally resulted in a reduced antibacterial activity of the corresponding tetracyclines [35]. However, the addition of substituents to the amide nitrogen can significantly increase the solubility of the compound (e.g., lymecycline (7) and rolitetracycline (6)) in water. These prodrugs, once hydrolyzed

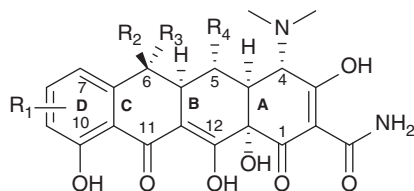
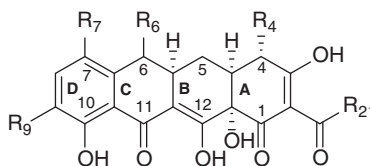


Figure 9.5 Structural requirements for biological activity of tetracyclines.

in vivo, will liberate free tetracycline (4) [21]. Besides substituents at positions C-1, C-3, C-4a, C-10, C-11, and C-12, which are invariably detrimental for the antibacterial activity of tetracyclines, other substituents can be replaced in order to increase or alter their biological activities. Positions C-5 to C-9, located in the so-called upper peripheral region of the compound, can be chemically modified, which generates derivatives with varying antibacterial activity. Substituents R_2 , R_3 , and R_4 can be altered in order to give more selectivity to the biological target in antifungal tetracyclines, but not for the antibacterial activity. Whereas modification of the substituent R_4 did not appear to make any differences in the biological activity of the tetracycline [36], modifications at position 6 (R_2 and R_3) showed a significant increase. Elimination of the 6-hydroxy group led to more lipophilic, acid-stable tetracyclines such as doxycycline (8) and methacycline (11). Ring D is most amenable to changes. Modifications of substituent R_1 and the other positions are tolerated to give high bacterial specificities and significant variations in the pharmacokinetic profiles. It is remarkable that the activity of tetracyclines is enhanced both by substituents that are strong electron donors and strong electron acceptors. Substitution is more advantageous at position 7 than at position 9. A nitro or dimethylamino group in position 7 provides the greatest potency improvement. For example, minocycline (9) with a dimethylamino group in position 7 is two to eight times more active as tetracycline (4) against tetracycline-sensitive strains [36].

Extensive structure–activity studies have also revealed that with one exception, each of the rings, ABCD, must be six-membered and purely carbocyclic for the compounds to retain their antibacterial activity. The exception to this rule is 6-thiatetracycline, which possesses a sulfur atom in position 6 of the C ring. Analogs of this series have potent antibacterial properties [37]. It is worth mentioning that these thiatetracyclines are nowadays referred to as atypical tetracyclines since they exhibit a different structure–activity relationship [33, 38]. The stereochemical and substitution requirements for optimal antibacterial activities are summarized in Figure 9.6.



Rings (ABCD)
= linear-fused rings

Rings (BCD): C-10, C-11, C-12
= phenolic keto-enol system

Ring (A): C-1, C-2, C-3
= tricarboxyl-type keto-enol system

R_2 = NH_2 or NHR , R = aminoalkyl

R_4 = (α)-dimethylamino group most superior

R_6 = combination of (β)-OH and/or (α)- CH_3 or unsubstituted, or methylene group

R_7 = H, Cl, dialkylamino, NO_2

Figure 9.6 Stereochemical and substitution requirements for optimal antibacterial activity.

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10

Carboxylic-Acid-Based Neuraminidase Inhibitors

Stacy Van Epps

10.1

Introduction

Seasonal influenza, a severe viral infection of the respiratory system, annually affects 20% of the world population and is the cause of 250 000–500 000 annual deaths [1]. Currently, there are two main strategies employed to control the influenza virus: vaccination and antiviral treatment. There are two major classes of antivirals available for the treatment and prevention of influenza, the M2 protein inhibitors (amantadine and rimantadine) and the neuraminidase inhibitors (NAIs), also known as sialidase inhibitors [2]. NAIs have an advantage over other mechanisms, such as M2-inhibitors, as they act against both influenza A and B. Currently, four drugs were approved in various countries for the treatment of influenza, zanamivir (Relenza, **1**) oseltamivir (Tamiflu®, **2**), laninamivir (Inavir, **3**), and peramivir (Rapivab, **4**) (Figure 10.1). A common structural motif found in each of these drugs is a carboxylic acid functional group mimicking that of sialic acid, the natural cellular receptor for viral neuraminidase. The carboxylic acid functional group is a common pharmacophore for a variety of therapeutic agents that have been marketed worldwide. This chapter focuses on the synthesis, structure–activity relationship (SAR), and mode of action for NAIs, emphasizing targets that encompass the carboxylic acid moiety.

10.2

History

The carboxylic acid functional group is becoming a more prevalent pharmacophore in new age drug design [3]. However, the presence of this moiety in pharmaceutical drugs can also lead to significant drawbacks, including metabolic instability, toxicity, and limited passive diffusion into cells. Carboxylic acids have the ability to establish strong electrostatic interactions and hydrogen bonds, which can enhance the binding affinity to their respective targets. Most carboxylic acids have a pK_a value of about 3.5–4.5 and therefore exist in their ionized form

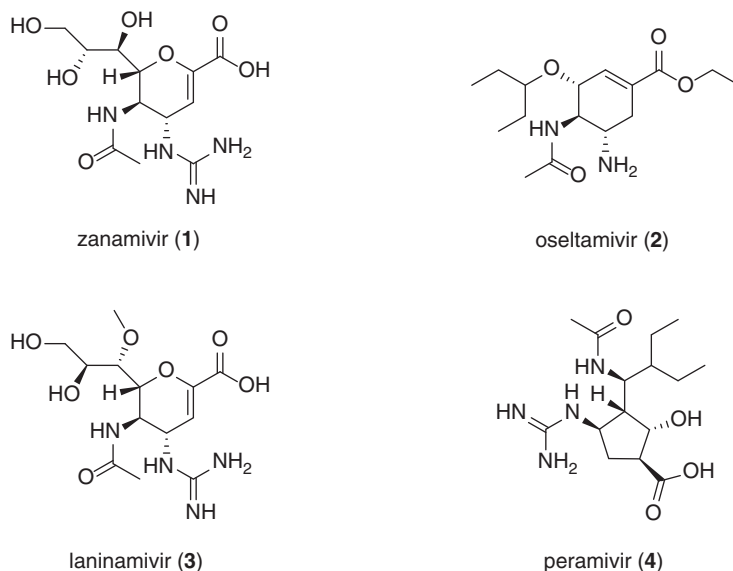


Figure 10.1 Neuraminidase inhibitors: zanamivir (1), oseltamivir (2), laninamivir (3), and peramivir (4).

under physiological pH conditions. This section provides an overview of the most popular examples of carboxylic-acid-containing drugs that demonstrate utility for the treatment of influenza.

NAIs for the treatment of influenza were first identified in the 1960s. Early on, two classes of synthetic inhibitors of this enzyme were reported. The first is based on high-molecular-weight azo dyes, such as Congo red and Trypan blue reported by Becht and Drzeniek in 1967 [4]. The second focused more on lower-molecular-weight compounds, containing carboxylic acid functionalities, reported by Edmond and coworkers [5]. They found that *N*-substituted oxamic acid derivatives exhibited neuraminidase inhibitory properties. Second-generation inhibitors included substituted β -aryl- α -mercaptoacrylic acids as well as benzimidazoles reported by Haskell *et al.* in the 1970s [6]. These compounds showed varying levels of activity against neuraminidase; however, they proved to be nonspecific enzyme inhibitors.

In contrast to these previously reported compounds, by 1974, derivatives of sialic acid (Figure 10.2) were discovered as potent NAIs and were found to exhibit a high degree of specificity for the enzyme [7]. In the absence of structural information, the initial focus on the discovery of NAIs was on substrate-like derivatives of sialic acid (5). From these studies, the most potent synthetic analog prepared was the dehydrated derivative of sialic acid (5), 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (DANA, 6) [8]. This compound was the first of this class of substrates to demonstrate efficacy in a mouse model of influenza infection [9].

This was a major breakthrough in the field at the time, considering that the crystal structure of the enzyme had not yet been reported. By the early 1990s,

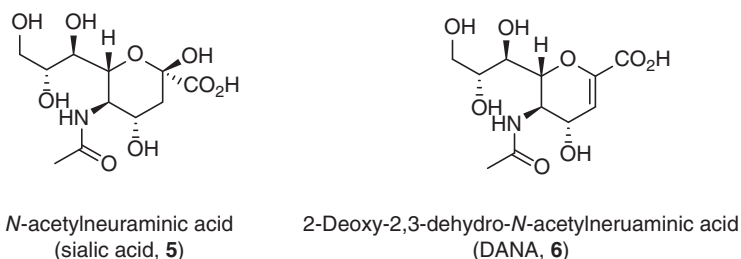


Figure 10.2 Natural substrate sialic acid (**5**) and first neuraminidase inhibitor 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid (DANA, **6**).

the determination of the crystal structure of influenza virus surface protein was revealed by Burmeister *et al.*, albeit with low resolution [10]. Several years later, a more suitable crystal structure was obtained and resulted in the structure determination of the influenza virus neuraminidase [11]. The crystal structure confirmed that DANA (**6**) binds with identical interactions as the natural substrate sialic acid (**5**).

Improved structural information initiated widespread efforts toward structure-based drug design in the field. The first remarkable breakthrough was the synthesis of zanamivir (**1**), which arose from an extensive program led by Colman and Varghese [12] at Biota in 1989. Biota, a small Australian biotechnology company, did not have the resources to bring zanamivir (**1**) to market on its own. The molecule was licensed to GlaxoSmithKline in 1990 for clinical development and approved by the FDA for commercialization in the United States by 1999 for the treatment of influenza A and B under the trade name Relenza.

The carboxylic acid moiety adds to the hydrophilicity and of the molecule, which may ultimately impede its bioavailability when dosed orally. Due to the extremely low oral bioavailability (2–3%) of zanamivir (**1**), this drug must be administered either topically or directly to the respiratory tract by intranasal spray. Evaluation of synthetic approaches toward the next generation of drugs, such as oseltamivir (**2**), relied on a prodrug approach to improve on the oral bioavailability of the compound. The phosphoric acid salt of the ethyl ester prodrug, oseltamivir (**2**) phosphate, is hydrolyzed in the liver by hepatic esterases to deliver the active carboxylate. As a result of its improved bioavailability, this drug is administered orally. Oseltamivir (**2**), marketed as Tamiflu®, was discovered at Gilead Sciences in 1995. One year later, a contract with Hoffmann-La Roche Ltd was signed for the codevelopment of the drug, and by 1999, the compound was commercially launched.

Furthermore, peramivir (**4**), an intravenous NAI for the treatment of H1N1 was recently reported by BioCryst Pharmaceuticals and was approved for the treatment of hospitalized and pediatric patients when other methods were ineffective [13]. H1N1 is a subtype of the influenza A virus. It is an orthomyxovirus that contains both of the glycoproteins, hemagglutinin and neuraminidase. Unlike zanamivir (**1**) and oseltamivir (**2**), peramivir (**4**) has shown to perform

effectively against all neuraminidase subclasses (N1–N9). In 2009, it was granted an Emergency Use Authorization (EUA) by the FDA for the treatment of H1N1 influenza, and in 2014, the FDA approved peramivir (**4**), marketed as Rapivab to treat influenza infection in adults.

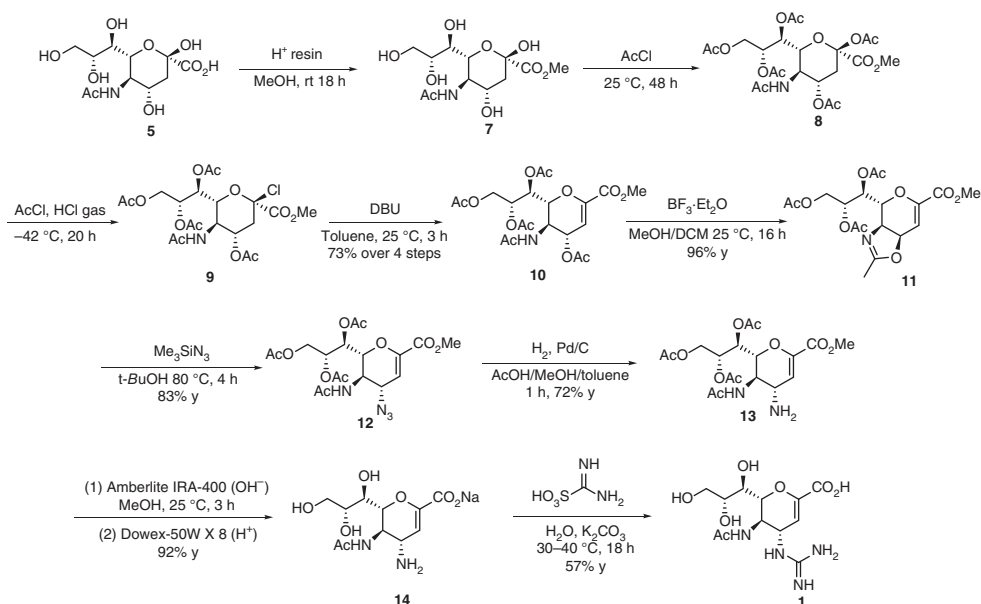
The discovery of zanamivir (**1**) provided a platform for the pursuit of carbohydrate-based compounds as NAIs. Subsequent efforts were refocused to identify and develop inhibitors based on noncarbohydrate templates. Potent and selective targets for neuraminidase were identified based on a range of core templates including cyclohexene carboxylates, such as oseltamivir (**2**), and cyclopentane carboxylates, such as peramivir (**4**).

10.3

Synthesis

There are a multitude of approaches known in the literature for the preparation of carboxylic-acid-containing NAIs. This section focuses on the initial synthetic routes toward zanamivir (**1**), oseltamivir (**2**), and peramivir (**4**), as well as the various synthetic developments that enabled the production of multikilogram quantities of these marketed drugs. Despite the simplicity of the monocyclic structures of zanamivir (**1**), oseltamivir (**2**), and laninamivir (**3**), each ring system is highly decorated with functional groups that form five contiguous stereogenic centers, making these drugs challenging synthetic targets. Until recently, manufacturing of these compounds relied solely on the expensive starting material sialic acid (**5**). However, the relative low overall yields for producing these drugs on scale made the cost of these processes extremely high. Synthetic efforts have focused on implementing asymmetric routes to build in the stereocenters of the carbohydrate skeleton using cheaper and readily available starting materials [14, 15].

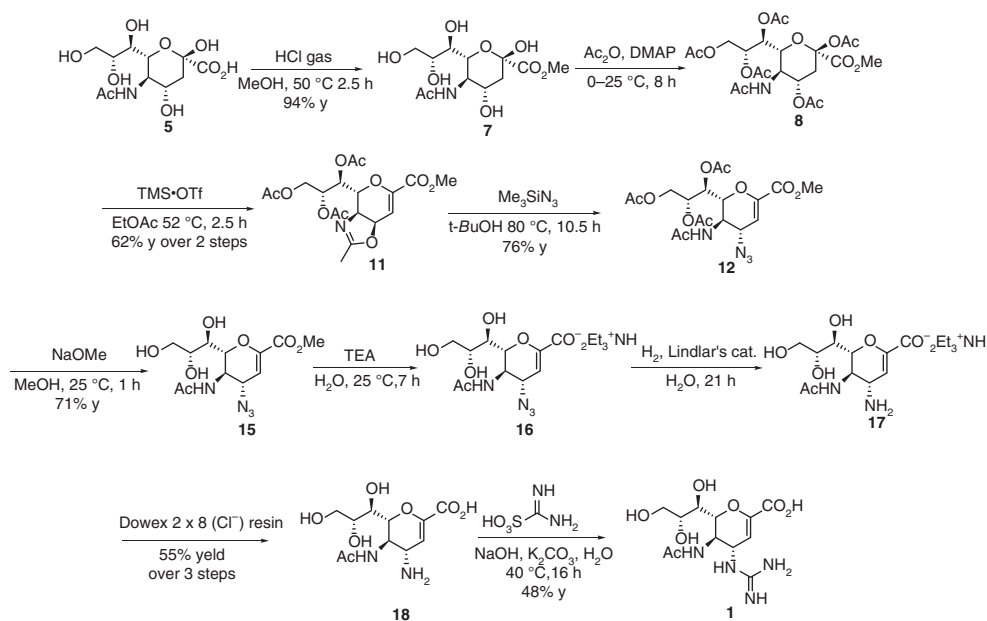
The first synthetic preparation of zanamivir (**1**) [16] represents a milestone in the development of reported NAIs, since it was the first member of its class that culminated in an FDA approval for the treatment of influenza A and B. The medicinal chemistry approach only produced milligram quantities of the drug and was disadvantaged by the use of unsafe reagents such as trimethylsilyl azide and chromatography for intermediate isolation, which would preclude it from becoming a practical synthesis on scale. The synthesis commences with the manipulation of *N*-acetylneuraminic acid (**5**) to compound **10** by esterification in dry methanol with a cation exchange resin (**7**), followed by acetylation of all the free hydroxyl groups (**8**, Scheme 10.1). Subsequent displacement of the 2-acetoxy group by chloride in a pressurized vessel afforded the penta-acetylated intermediate **9** and chloride elimination via treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene readily produced the desired intermediate **10** in 73% overall yield for the four steps. Treatment of acetate **10** with $\text{BF}_3 \cdot \text{Et}_2\text{O}$ provided the allylic oxazoline **11**, which was susceptible to nucleophilic attack by azidotrimethylsilane, yielding azide **12** stereoselectively. Hydrogenation of azide **12** at atmospheric pressure with 10% Pd/C gave amine **13** in 72% yield. Ester hydrolysis with Amberlite-IRA



Scheme 10.1 First synthesis of zanamivir (**1**) from sialic acid (**5**).

400 (OH[−]) resin and aqueous NaOH followed by neutralization with Dowex resin provided the desired sodium salt **14**. The reaction sequence was completed by reacting amine **14** with aminoiminomethanesulfonic acid to produce zanamivir (**1**) in 57% isolated yield.

Although there are several reported synthetic methods for the improved synthesis of zanamivir (**1**) [17, 18], the only published process on large scale was executed by the process chemistry group at GlaxoSmithKline [19]. While this report only produces 1.28 g of compound, several steps were run on hundreds of grams scale, including the introduction of the azide functional group at the C4-carbon (Scheme 10.2), ultimately succeeding in an improved route to achieve an 8.3% overall yield for the nine-step sequence. The process route also began with the sialic acid (**5**), which was converted into the methyl ester **7** with catalytic HCl gas in methanol. Acylation of the free alcohols with acetic anhydride provided a selective route to the β-OAc isomer of the penta-acetoxy intermediate **8**. Cyclic oxazoline **11** was obtained through an improved procedure by using trimethylsilyl triflate in warm EtOAc, followed by trimethylsilyl azide addition in hot *tert*-butanol to afford azide **12** stereoselectively in 76% yield. To further improve the synthetic route from the previous reported methods, the acetate-protecting groups were hydrolyzed with catalytic NaOMe to increase the water solubility of the product. The methyl ester was treated with aqueous triethylamine to generate the triethyl ammonium salt **16**, followed by hydrogenation of the azide with Lindlar's catalyst, and subsequent neutralization to yield the free amino acid **18** in 55% yield over three steps.

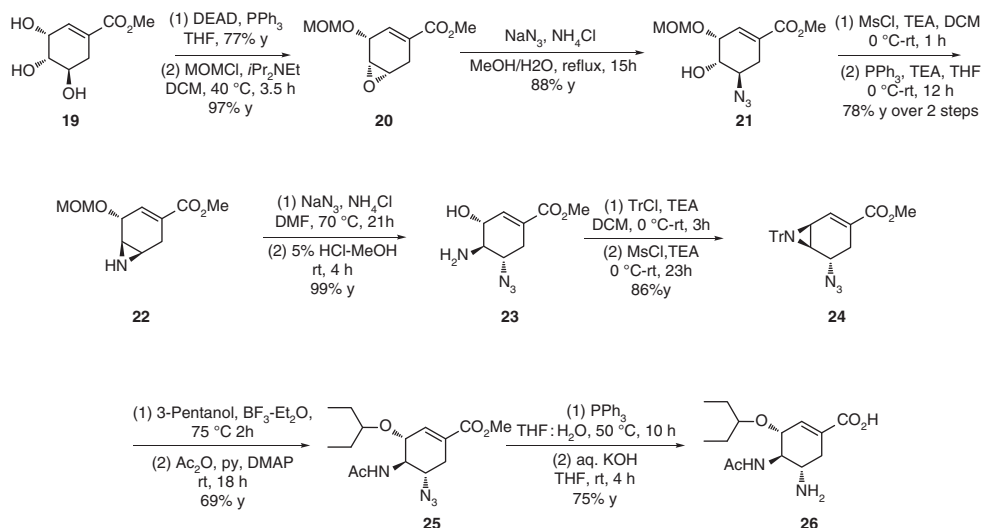


Scheme 10.2 Optimized scalable synthesis of zanamivir (**1**).

The final product **1** was prepared using aminoiminomethanesulfonic acid as exemplified previously to introduce the guanidine functional group in 48% yield after ion-exchange chromatography.

The synthesis of oseltamivir (**2**) relied on significant developments in organic synthesis to provide practical routes on scale. The medicinal chemistry group at Gilead Sciences was the first group to synthesize oseltamivir (Tamiflu, **2**) starting from the known natural product, (–)-methyl shikimate **19** (Scheme 10.3) [20]. Treatment of **19** under Mitsunobu conditions, followed by protection of the C3-hydroxyl group, produced epoxide **20** in 97% yield. Subsequent epoxide ring-opening with NaN_3 occurred selectively at the least sterically hindered carbon, yielding the azido alcohol **21** in 88% yield. The aziridine **22** was prepared by O-mesylation, followed by Staudinger reduction with triphenyl phosphine. Regioselective aziridine-opening with sodium azide and cleavage of the MOM group afforded amino alcohol **23**. The amino group was protected with trityl chloride followed by mesylation of the hydroxyl group to yield intermediate **24**. Selective ring-opening of aziridine **24** with 3-pentanol in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$, followed by acetylation of the resulting amine, gave amido ether **25** in 69% yield. The synthesis was completed by reducing the azido group as before with triphenyl phosphine and hydrolyzing the methyl ester to yield oseltamivir carboxylate **26** in 75% yield.

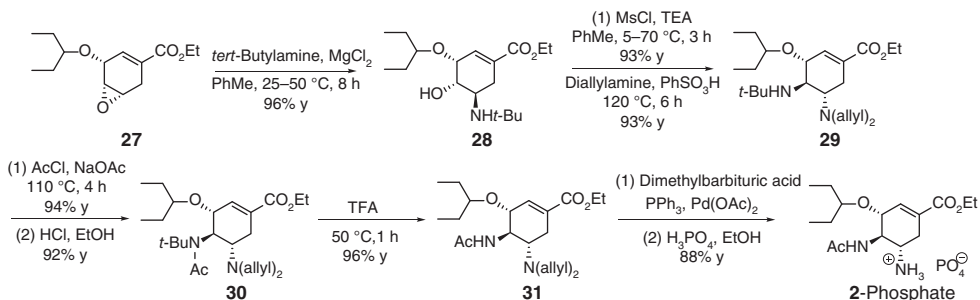
Although this route was executed only on milligram scale, the 14-step linear sequence remarkably produced oseltamivir (**2**) in a 15% overall yield. There are two



Scheme 10.3 Initial synthetic route toward oseltamivir carboxylate **26** by Gilead Sciences.

drawbacks to this synthesis: the use of the potentially explosive azide-containing intermediate and the limited availability of (–)-shikimic acid.

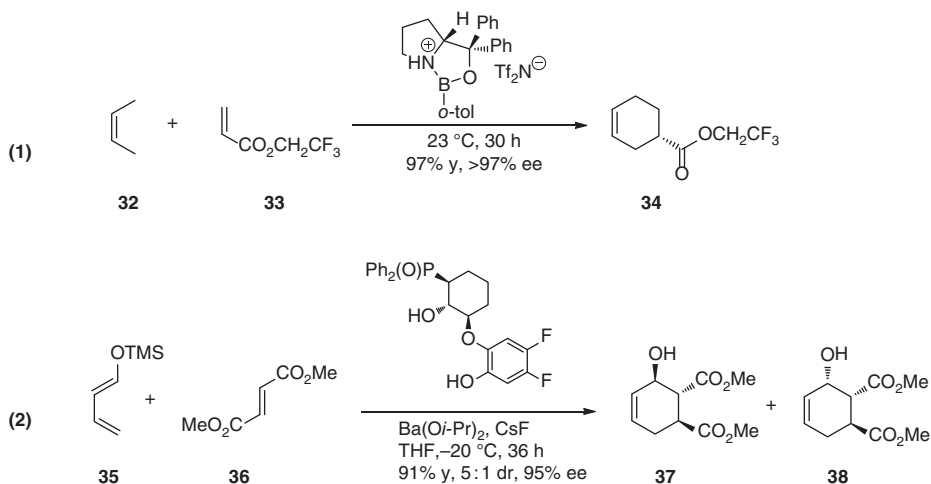
Due to the high cost and limited availability of the naturally occurring (–)-shikimic acid for the preparation of oseltamivir (**2**) on scale, several different strategies were investigated by other groups. Hoffmann-La Roche identified a second-generation process [21] for oseltamivir (**2**) phosphate that employs the use of readily available (–)-quinic acid and eliminates the use of NaN_3 (Scheme 10.4). The optimized preparation of epoxide **27** was previously reported as a key intermediate, and it is readily synthesized in six steps from (–)-quinic acid [22]. To avoid the use of hazardous NaN_3 , *t*- $\text{BuNH}_2\text{-MgCl}_2$ complex was used as an alternative amine source for the regioselective opening of epoxide **27** to produce amino alcohol **28**. The bulky *tert*-butyl group on the amine enabled selective O-mesylation to afford an aziridine, which was subsequently opened with diallylamine in the presence of PhSO_3H to give **29** in 93% yield.



Scheme 10.4 Hoffmann-La Roche improved process route to oseltamivir (**2**) phosphate.

The secondary amine was acetylated under forcing conditions and treated with HCl to precipitate compound **30** as the corresponding HCl salt. Impressively, this precipitation is the only purification required throughout the entire sequence from epoxide **27** to desired product **2**. Final cleavage of the *N*-*tert*-butyl group was facilitated with trifluoroacetic acid (TFA), and the removal of the allyl protecting groups was efficiently carried out through Pd-catalyzed allyl transfer to dimethylbarbituric acid. This protocol allowed for the direct transfer of the reaction mixture into the salt-forming step to give oseltamivir (**2**) phosphate in 88% yield.

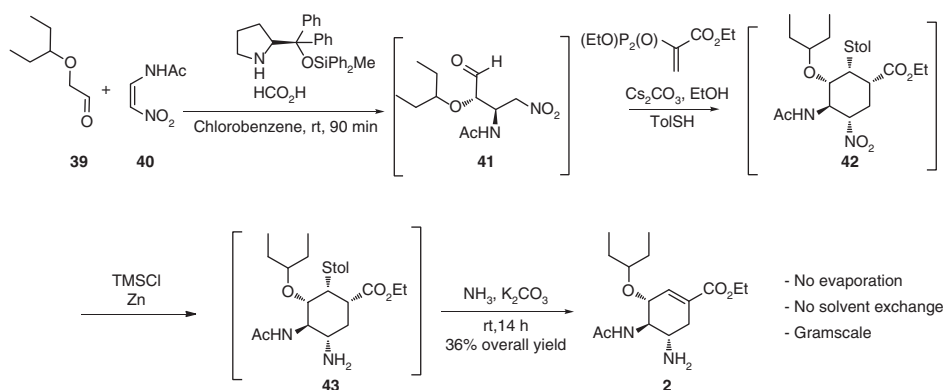
The search for novel synthetic routes independent of (–)-shikimic acid and (–)-quinic acid as starting points has received wide attention [23]. Several innovative approaches have recently been reported in the literature. In 2006, Corey's group at Harvard reported a concise azide-free synthesis of **2** employing their enantioselective Diels–Alder reaction from 1,3-butadiene and 2,2,2-trifluorethyl acrylate (Scheme 10.5, route 1) [24]. This transformation has been run on multigram scale at ambient temperature to provide adduct **34** in 97% yield and >97% e.e. and culminated in a 27% overall yield for an 11-step sequence toward oseltamivir (**2**). Shortly after Corey's report, Shibasaki described a route to set the stereochemistry of the two chiral centers of the molecule via an asymmetric, barium-catalyzed Diels–Alder reaction (Scheme 10.5 route 2) [25]. The use of Danishefsky's diene had previously been reported in Lewis-acid-catalyzed Diels–Alder reactions; however, diene **35** had never been used in this type of transformation due to its acid lability. The optimized catalyst system resulted in a mixture of adducts **37** and **38** (*dr* = 5 : 1) in 91% yield and 95% e.e. on a 58 g scale.



Scheme 10.5 Asymmetric Diels–Alder reactions toward the core of oseltamivir (**2**).

Recently, the groups of Hayashi [26, 27], Ma [28], Sebesta [29], and Lu [30] have independently reported shorter syntheses of (**2**), highlighting asymmetric Michael reactions to install the respective stereogenic centers. The Hayashi group reported

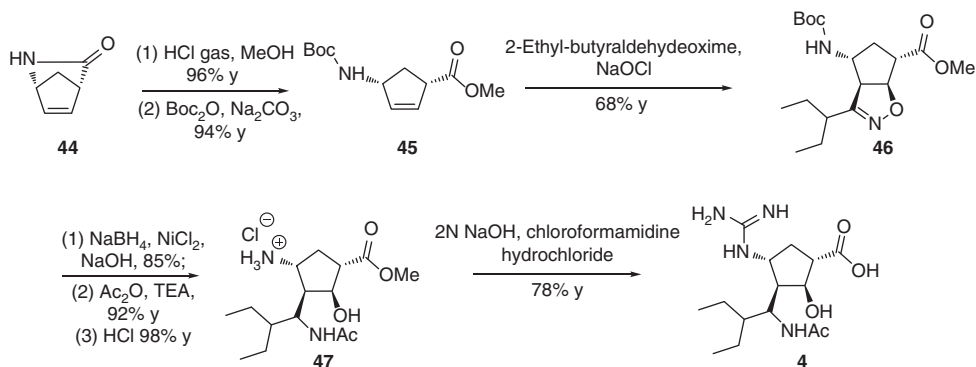
the shortest route and highest yield to date for the synthesis of oseltamivir (**2**) by implementing a one-pot domino-reaction sequence with a 28% overall yield on gram scale (Scheme 10.6) [31]. Alkoxyaldehyde **39** and nitroalkene **40** underwent an asymmetric Michael reaction to yield nitroaldehyde **41** with a dr of 9 : 1 and 99% e.e. in 90% yield. With the optimized reaction conditions in hand, the complete one-pot sequential synthesis of (**2**) was tested. The Michael reaction of **39** and **40** produced **41**. Addition of an ethyl acrylate derivative in the presence of Cs_2CO_3 in EtOH, followed by addition of toluenethiol, produced the desired thiol Michael adduct **42** with the desired stereochemical configuration. By addition of Zn and trimethylsilyl chloride to the same vessel, reduction of the nitro group yielded amine **43**, from which a retro-Michael reaction of the thiol group proceeded with treatment of base to afford (**2**) in a single pot.



Scheme 10.6 Hayashi's one pot synthesis of oseltamivir (**2**).

Many synthetic developments have been reported for the improved synthesis of the cyclohexyl-carboxylate skeleton of oseltamivir (**2**); however, less attention has focused on the cyclopentyl-carboxylic acid derivative of peramivir (**4**). Although several studies regarding the synthesis peramivir (**4**) have been reported [32, 33], this is still an area in need of improvement.

As shown in Scheme 10.7, the improved synthetic route to peramivir (**4**) commences with the treatment of (–)-(1*R*,4*S*)-2-azabicyclo[2.2.1]hept-5-en-3-one **44** with HCl gas and subsequent Boc-protection to afford compound **45** in excellent yield. The selective 1,3-dipolar cycloaddition between compound **45** and the nitrile oxide derived from 2-ethyl-butylaldehyde oxime proceeded with the desired regiochemistry as previously reported [34]. The reductive ring opening of isoxazoline **46** was successfully carried out in the presence of NaBH_4 and NiCl_2 . The intermediate was then acylated and subsequent Boc deprotection afforded the HCl salt **47** in 98% yield. The final step implemented a new method to introduce the guanidine by employing chloroformamidinium hydrochloride as the amidino reagent; this avoided the use of the highly toxic reagent HgCl_2 used in previous reports. In this improved approach for the preparation of **4**,



Scheme 10.7 Facile synthesis of peramivir (**4**).

inexpensive and commercially available materials were used and the overall yield for the process was enhanced to 34%.

10.4

Mode of Action

NAIs are antivirals used for the treatment and the prophylaxis of influenza. zanamivir (**1**), oseltamivir (**2**), laninamivir (**3**), and peramivir (**4**) are currently the mainstay of anti-influenza treatments [35]. Influenza viruses are members of the *Orthomyxoviridae* family and are classified into three types: influenza A, B, and C. Influenza viruses are further subdivided by characterization of the two major surface glycoproteins anchored in the viral envelope, hemagglutinin (HA), and neuraminidase (NA). There are nine different subtypes of NA that circulate in human and animals; however, only viruses with the HA 1, 2, or 3 types together with neuraminidase type 1 or 2 are human pathogens [36]. Both surface glycoproteins play a role in recognizing sialic acid in the upper respiratory tract and play a crucial role in the lifecycle of the virus (Figure 10.3) [37]. HA is a sialic acid receptor-binding molecule and mediates the entry of the virus into the target cell. Once the virus is endocytosed, the acidic conditions in the endosome release the viral RNA, which is trafficked to the nucleus of the host cell. Viral proteins are synthesized from viral mRNA and directed to the cell membrane for virion assembly. After a full genome of eight segments is incorporated into the virus particle, budding of the virus initiates at the cell membrane. NA is responsible for cleavage of the cellular-receptor sialic acid residues to which the newly formed virus particles are attached, thus allowing release of the progeny virus from the host cell. NAIs are virus-specific drugs that bind to the active site of the NA on influenza A or B, thereby interfering with the functions of the protein. NAIs inhibit the release of budding viral particles from the infected host cells, preventing the spread of the virus from infected cells to uninfected cells.

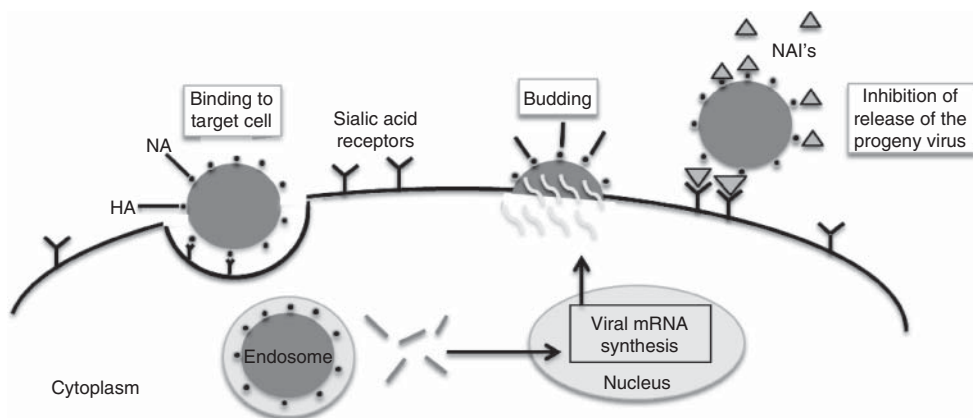


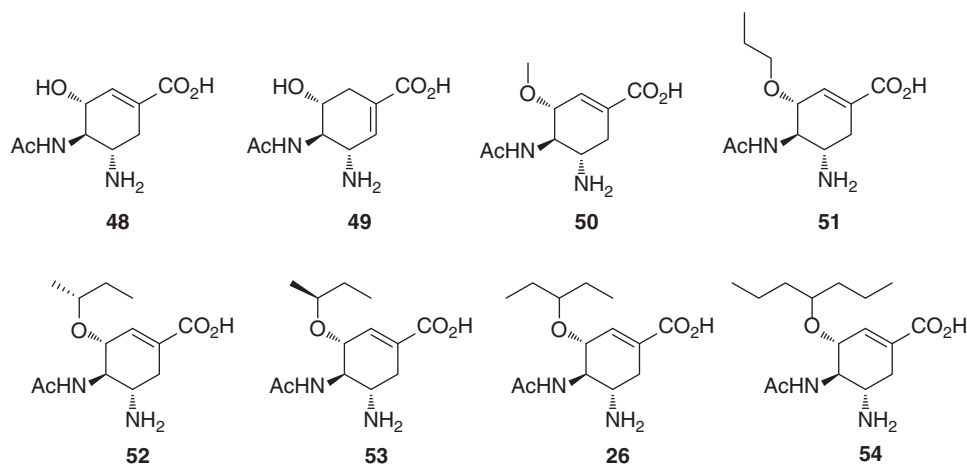
Figure 10.3 Mechanism of action of neuraminidase inhibitors against influenza virus replication. Hemagglutinin (HA), Neuraminidase (NA), Neuraminidase inhibitor (NAI).

Based on this mechanism, it is vital to administer drug within 48 h of the onset of symptoms for effective treatment.

10.5

Structure–Activity Relationships

NAIs have successfully been developed as effective treatments for influenza A and B infections. However, the occurrence of highly pathogenic influenza strains along with the concern of the increased mutagenic rates of the virus has contributed to the rapid development of resistance. In response to the emerging problem around drug resistance with the currently marketed NAIs, the search for alternative novel anti-influenza drugs has gained momentum. Much attention has focused on SAR around oseltamivir (**2**) analogs. Early work by Gilead led to the discovery [38] of several analogs to identify regions of the compound that could improve neuraminidase potency and selectivity. First, relocating the C–C double bond in the cyclohexene scaffold of oseltamivir (**2**) core was evaluated (Table 10.1, **48** and **49**). While **48** proved to be a potent NAI with an inhibitory concentration of 6.3 nM, **49** showed no inhibitory activity, demonstrating the importance of the position of the double bond in the cyclohexyl scaffold. The length, size of branching, and geometry of the 3-pentyl alkyl group of oseltamivir (**2**) also profoundly influenced the NA inhibitory activity. A series of linear alkyl analogs (Table 10.1) demonstrated a 20-fold increase in NA potency for compound **51** compared to the methyl counterpart **50** alluding to a significant hydrophobic interaction of the *n*-propyl group. Further extension of the branching with compound **54** resulted in a decrease of NA activity suggesting that the 3-pentyl group of **26** exhibits the optimum hydrophobic contact within the NA active site.

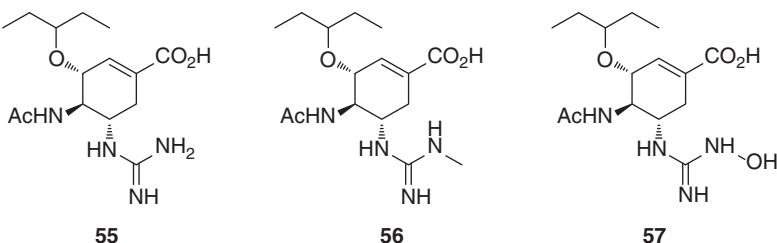
Table 10.1 Influenza neuraminidase inhibition by oseltamivir carboxylate analogs.

Compound	48	49	50	51	52	53	26	54
NA enzyme IC ₅₀ (nM)	6.3	No inhibition	3700	180	10	9	1	16

All of the clinically relevant NAIs suffer from distinct drawbacks. Despite the recent success of oseltamivir (**2**), N1-subtype resistance is a rising concern [39]. By comparison, this mutation has little effect on the antiviral activity of zanamivir (**1**), laninamivir (**3**), and peramivir (**4**), which contain an exocyclic guanidine moiety [40]. New oseltamivir (**2**) analogs, described by Martin *et al.* [41], incorporate various *N*-substituted guanidines in place of the C-5 amino group with improved NA activity. It was previously reported by Gilead that introduction of the guanidine moiety at the C-5 position (**55**, Table 10.2) significantly improved neuraminidase activity compared to the oseltamivir carboxylate **26** [42]. The results of the *N*-substituted analogs indicate that the size of the substituent on the guanidine is limited to methyl or hydroxyl to maintain potency, as illustrated by compounds **56** and **57**. When the guanidine was substituted with ethyl, propyl, allyl, isopropyl, or cyclopropyl, the compounds displayed little or no inhibition.

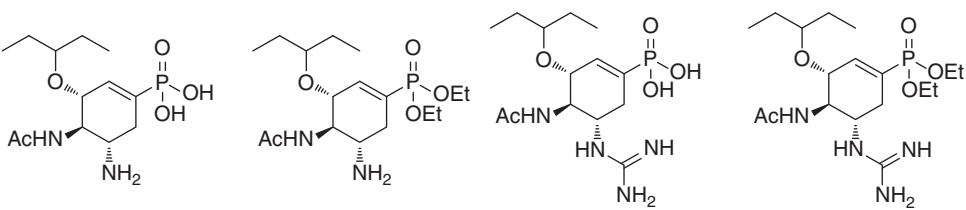
Most guanidine derivatives have not been further pursued due to their lack of oral bioavailability, which was reported to be 4% for oseltamivir guanidine **55** [43]. Several groups have attempted to improve the ADME properties of guanidines by implementing different prodrugs [44, 45]. The work described by Fang and Wong [46] attempted to further increase the binding affinity of oseltamivir (**2**) through bioisosteric replacement of the carboxylic acid with a phosphoric acid (Table 10.3). As expected, oral bioavailability of these compounds was also limited. However, compared to **26**, the phosphonate bioisosteres improved potency against H1N1 and wild-type H1N1 using the H275 mutant (**58–61**).

Table 10.2 Inhibitory activities against wild-type and mutant influenza neuraminidases.

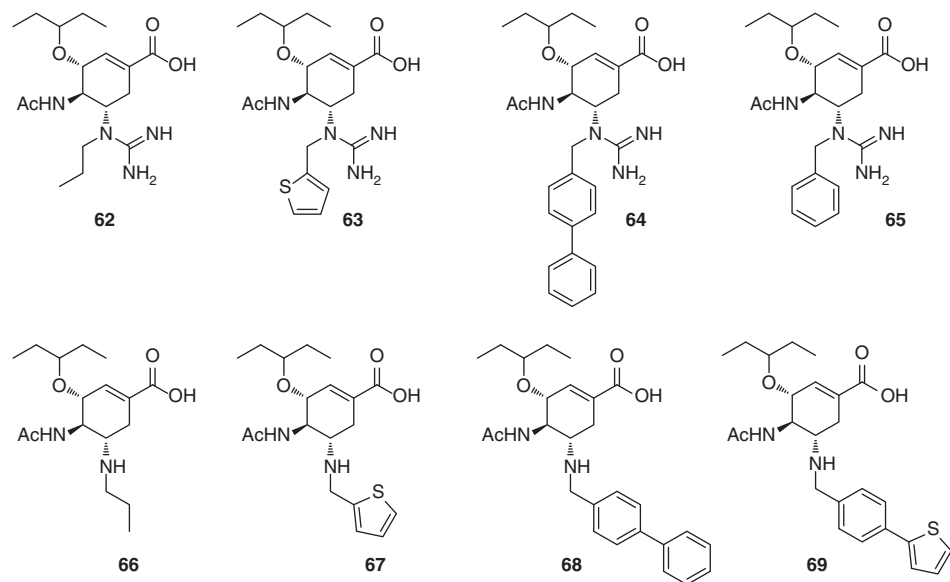
				
	55	56	57	
Compound	H1N1 wild type IC ₅₀	H1N1 mutant IC ₅₀	H5N1 wild type IC ₅₀	H5N1 mutant IC ₅₀
26	2.1	250	250	330
55	0.87	1.7	1.32	1.9
56	1.73	4.2	3.6	4
57	3.7	43	33	38

Neuraminidase inhibition, IC₅₀ (nM). IC₅₀ values reported based on triplicate measurement.

Table 10.3 IC₅₀ values (nM) of influenza neuraminidase inhibition for phosphonic acid bioisosteres.

					
	58	59	60	61	
Virus	26	58	59	60	61
A/WSN/33 (H1N1) IC ₅₀ (nM)	2.6	2.4	3.0	0.009	1.1
A/WSN/33 H275Y (H1N1) IC ₅₀ (nM)	477	993	1679	0.4	25.1

With the recent disclosure of the “150-cavity” reported from a high-resolution crystal structure, several groups have exploited this area of the protein to improve selectivity and potency for N1-neuraminidase inhibitors. The X-ray structure shows a large cavity adjacent to the active site that is only found in subtype 1 NAs. Recently, the work reported by Xie and Xu [47] uncovered interesting SAR around oseltamivir carboxylate derivatives that take advantage of the 150-cavity as an auxiliary binding site by modifying the C-5 amine. Earlier reports by Lin proved that acylguanidine-modified zanamivir (**1**) analogs locked the 150-cavity of group-1 NAs but resulted in overall decreased potencies [48].

Table 10.4 SAR of N-substituted oseltamivir (**2**) derivatives as potent and selective inhibitors of H5N1 NA.

Virus	26	62	63	64	65	66	67	68	69
H5N1-1220 IC ₅₀ (nM)	17	1140	200	150	1170	19	15	1.9	2.1
H9N2-415 IC ₅₀ (nM)	2.8	1180	210	470	2600	2280	740	1200	3520

Xu's approach developed compounds to occupy the new potential binding site, while maintaining a basic moiety for increased potency. Several secondary amine derivatives were found to increase potency compared to the previously reported *N*-substituted guanidines (Table 10.4). Compound **68** possesses an IC₅₀ value of 1.9 nM against three types of H5N1 virus strains and proved to be eightfold more potent than oseltamivir carboxylate **26**. Moreover, compound **69** exhibited increased activity against subtype N1 with excellent selectivity over subtype N2. Considering the interesting antimutant potential of compound **69**, further derivatization and SAR of these types of compounds might lead to more effective drug-resistant NAIs.

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11

Oxazolidinone Amide Antibiotics

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11.1

Introduction

Oxazolidinones are a class of antibiotics that are potent against multidrug-resistant Gram-positive bacteria, which are responsible for skin and soft tissue infections, pneumonias, and bacteremias [1]. These bacteria include methicillin-resistant *Staphylococcus aureus* (MRSA) [2], penicillin-resistant *Streptococcus pneumoniae* [3], and vancomycin-resistant *Enterococcus faecalis* (VRE) [4]. Additionally, some oxazolidinones exhibit activity against multidrug-resistant *Mycobacterium tuberculosis* strains [5]. The pharmacophore of oxazolidinone-based antibacterial agents typically consists of the 1,3-oxazolidin-2-one A ring, carrying an *N*-methylacetamido group (or bioisosteric replacements) at C-5, a phenyl B ring, and, in many cases, a C ring based on an aliphatic heterocycle or a phenyl group (Figure 11.1) [6].

The first member of this antibacterial class approved by the FDA in 2000 was linezolid (**1**), marketed as Zyvox® (Figure 11.2) [7, 8]. Other antibacterial agents that are currently at various stages of clinical development include sutezolid (**2**) [9], radezolid (**3**, Phase-II completed) [10], and posizolid (**4**, Phase-II for tuberculosis) [11]. In 2014, a second generation oxazolidinone antibiotic, tedizolid phosphate (**5**) [12], was approved by the FDA and is marketed as Sivextro®.

Oxazolidinone-based compounds have also been developed for other applications. For instance, they have been known as monoamine oxidase (MAO) inhibitors since the late 1950s, and toloxatone (**6**, Humoryl®) is a selective reversible inhibitor of monoamine oxidase-A (RIMA) launched as antidepressant in 1984 [13]. More recently, rivaroxaban (**7**, Xarelto®) has been discovered and developed by Bayer (2008) as the first orally active direct factor Xa (fXa) inhibitor, approved by the FDA and other regulatory agencies for prophylaxis of venous thromboembolism (VTE) and in patients with nonvalvular atrial fibrillation [14].

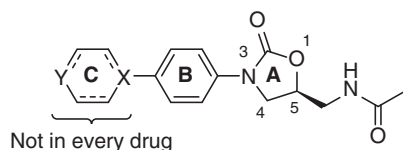


Figure 11.1 General structure of oxazolidinone-based antibacterial agents.

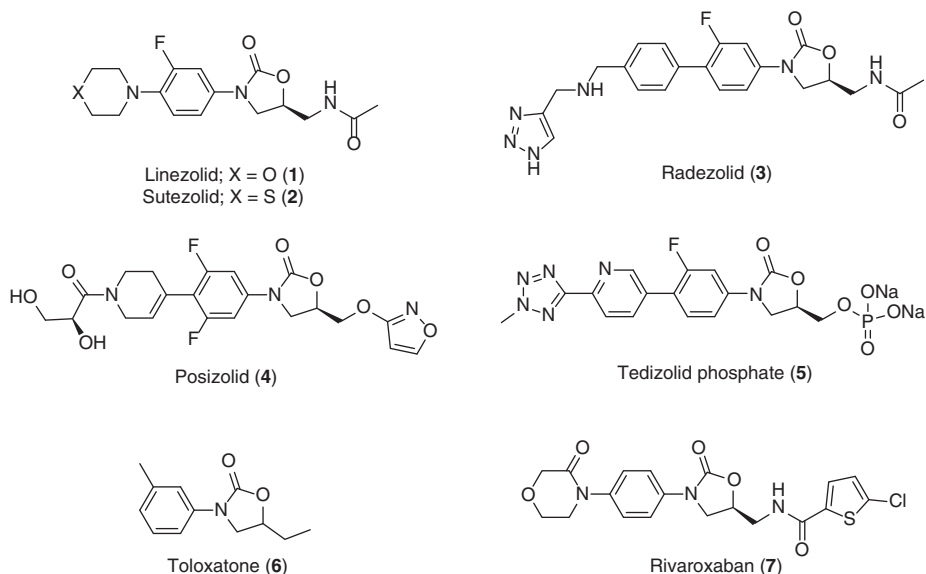


Figure 11.2 Structures of representative oxazolidinone-based drugs.

11.2

History

The first antibacterial oxazolidinones described in the patent literature by DuPont in 1978 were the racemic 5-halomethyl-3-phenyloxazolidinones **8–10** (Figure 11.3) [15]. Initial studies revealed that two members of this series, 5-chloromethyl-3-(4-methylsulfinylphenyl)-2-oxazolidinone (**8**) and 5-chloromethyl-3-(4-methylsulfonylphenyl)-2-oxazolidinone (**9**), displayed good activity against plant bacterial and fungal diseases, with some analogs (**9** and **10**) also being active against *Staphylococcus* pathogens in *in vitro* and *in vivo* animal models [16]. Optimization of this series led to the discovery of 5-hydroxymethyl analogs such as S-6123 (**11**) with a (5*R*) configuration, which was shown to be essential for antibacterial activity [17, 18]. S-6123 (**11**) displayed only modest *in vitro* activity against several Gram-positive and Gram-negative organisms, but it demonstrated good *in vivo* efficacy against *Escherichia coli* in a mouse lethal infection model [17].

From subsequent work at DuPont, two active oxazolidinones, DuP 105 (**12**) and DuP 721 (**13**), containing an (*S*)-5-acetamidomethyl side chain emerged. Both compounds were more potent than the predecessor oxazolidinones and displayed

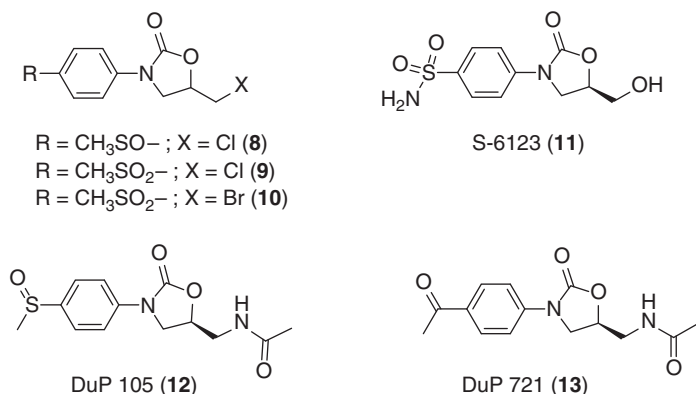


Figure 11.3 Structures of the first oxazolidinone antibiotics.

potent *in vitro* and *in vivo* activity against a variety of pathogens such as staphylococci, enterococci, and streptococci. Additionally, they demonstrated favorable pharmacokinetic profiles and efficacy in animal models and did not generate resistant mutants *in vitro* [19]. While both DuP 105 (**12**) and DuP 721 (**13**) entered Phase-I clinical trials, they were quickly terminated due to animal toxicity issues. The oxazolidinone program at DuPont was terminated with the failure of the two drug candidates [1].

After the disclosure of the DuPont analogs, the oxazolidinone work was pursued further at the Upjohn company (Pharmacia, now Pfizer) and led to the identification of the indanone (PNU-82965, **14**) [20] and tetralone (PNU-85055, **15**) [21] analogs, both synthesized in racemic form, in which the 4-substituted phenyl was replaced by a fused ring system (Figure 11.4). A comparison between PNU-82965 (**14**) and DuP 721 (**13**) revealed that the indanone did not exhibit the toxicity of the original DuPont analogs [22].

Following this discovery, extensive structure–activity relationship (SAR) development at the Upjohn group identified three series: oxodihydroindenyl oxazolidinones such as PNU-97456 (**16**), tropone fluorophenyl oxazolidinones such as PNU-97786 (**17**), and piperazinyl 3-fluorophenyl oxazolidinones such as PNU-97665 (**18**) [23]. The oxodihydroindenyl oxazolidinone PNU-97456 (**16**) exhibited good levels of antibacterial activity and showed no significant adverse events in a 30-day rat toxicology study. However, analogs in this series displayed overall lower antibacterial activity than previous series [24]. The introduction of a 3-fluoro substituent into the central phenyl ring resulted in an increase in antibacterial potency [25, 26]. The piperazine series exhibited not only excellent *in vitro* and *in vivo* activity but also good water solubility and pharmacokinetic properties [27].

Optimization of the piperazine oxazolidinones led to the identification of eperezolid (**19**), while replacement of the piperazine with a morpholine bioisostere provided linezolid (**1**). Similar to DuP 721 (**13**), both compounds had excellent *in vitro* and *in vivo* activities, comparable with vancomycin, but without the toxicity of the original DuPont analogs [22]. Both linezolid (**1**) and eperezolid (**19**) entered

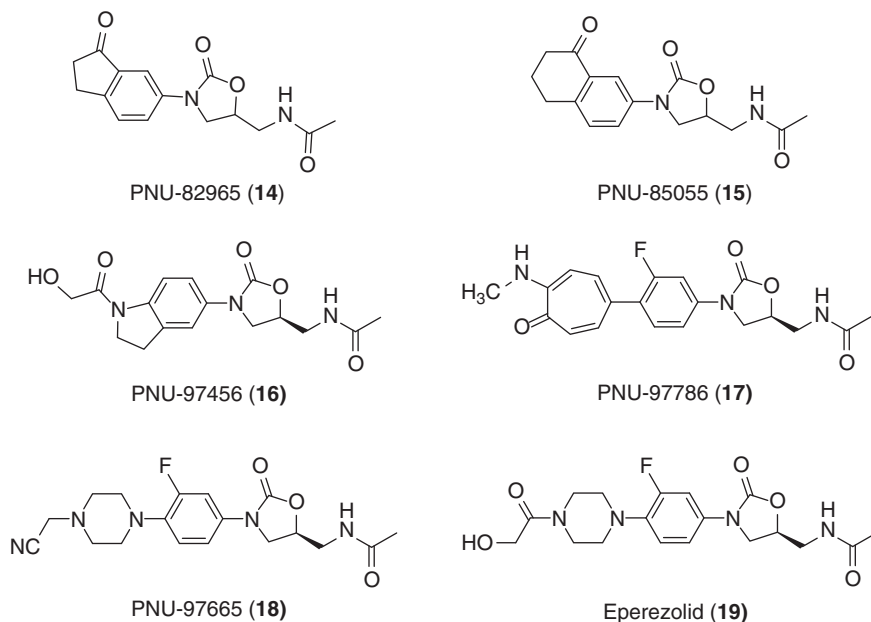


Figure 11.4 Representative oxazolidinone analogs from Upjohn.

Phase-I clinical trials; however, only linezolid (**1**) was selected to advance into Phase-II, due to a better pharmacokinetic profile, and was ultimately approved by FDA in April 2000 [8, 9]. The approval of linezolid (**1**) marked an important milestone in the treatment of Gram-positive infections, being the first antibiotic to gain regulatory approval in over 30 years. Several other regulatory agencies (in the United Kingdom, Japan, Canada, Europe, Latin America, and Asia) approved linezolid (**1**) in 2000–2001.

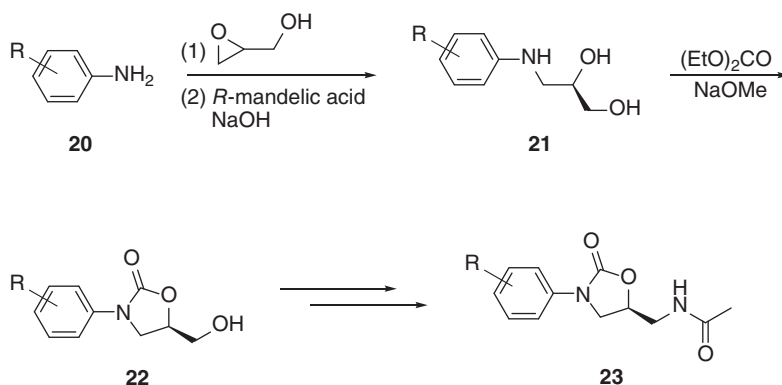
The replacement of the piperazine moiety with a thiomorpholine resulted in the identification of a novel subclass of oxazolidinone antibiotics. One member of this class, sutezolid (**2**) was shown to exhibit very potent *in vitro* activity against Gram-positive bacteria and several strains of *M. tuberculosis* [28]. Sutezolid (**2**) has undergone several Phase-I and -II clinical trials for the treatment of tuberculosis. Another oxazolidinone, posizolid (**4**), in which the (*S*)-5-acetamidomethyl side chain is substituted with a bioisostere, an O-linked isoxazole, was evaluated by AstraZeneca in Phase-II clinical trials for the treatment of tuberculosis. Results from this study are currently pending [29]. Radezolid (**3**) is twofold more potent than linezolid (**1**) against staphylococci and 4- to 16-fold more potent against streptococci and enterococci. Radezolid (**3**) has been evaluated in Phase-II clinical trials for community-acquired pneumonia and uncomplicated skin and soft tissue infections [30]. Rib-X (now Melinta) reported positive results from both trials. Tedizolid phosphate (**5**) was developed by Cubist Pharmaceuticals following the acquisition of Trius Therapeutics and was approved in 2014 under the trade name

of SivextroTM for the treatment of complicated skin and skin-structure infections. Tedizolid phosphate (**5**), the second oxazolidinone to be approved by the FDA, is a monophosphate prodrug that is four- to eightfold more potent *in vitro* than linezolid (**1**) against staphylococci, streptococci, and enterococci [12, 30].

11.3

Synthesis

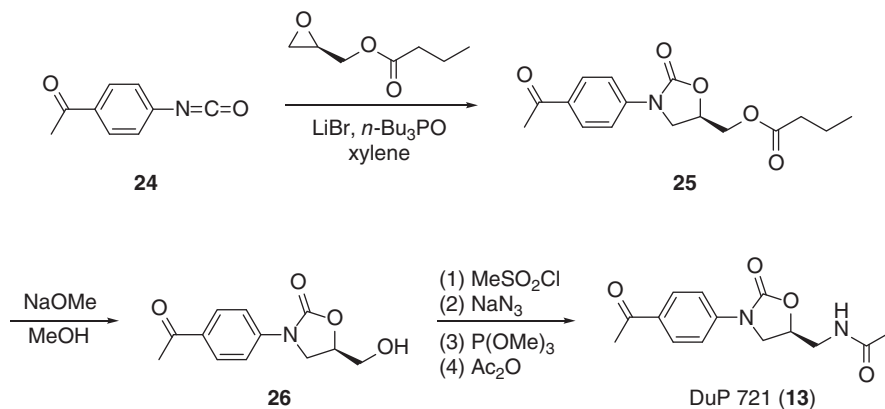
The initial route to optically active 5-substituted oxazolidinones, developed by the DuPont researchers, consisted of the alkylation of aniline **20** with glycidol followed by resolution of the amino diol with (*R*)-mandelic acid (Scheme 11.1). Cyclization of the desired stereoisomer **21** with diethyl carbonate and sodium methoxide afforded the oxazolidinone **22**, which was further elaborated to the acetamide **23** [17, 31].



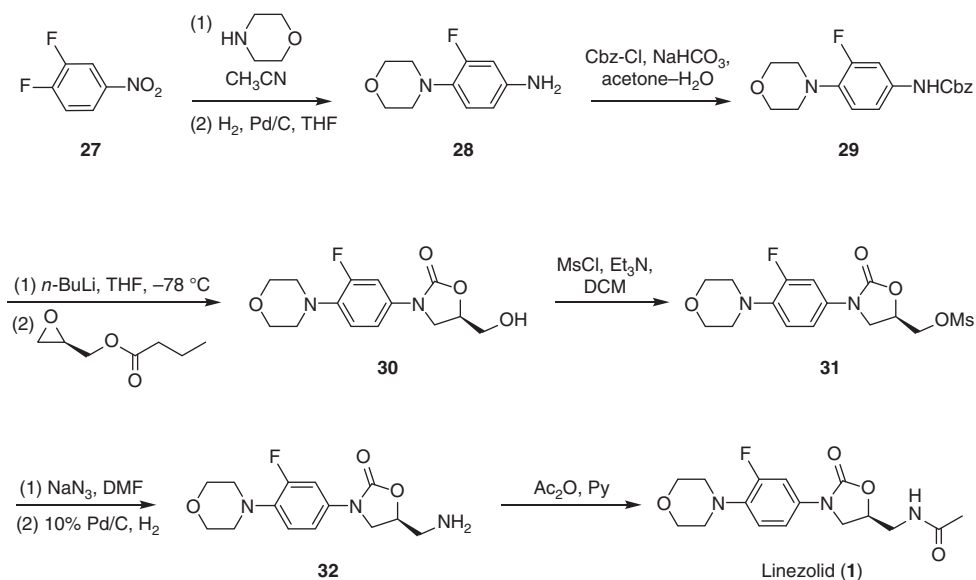
Scheme 11.1 DuPont synthesis of oxazolidinones via chiral resolution.

An alternative approach to oxazolidinones, also utilized by the researchers at DuPont, did not require a chiral resolution. It involved the lithium bromide-catalyzed cycloaddition of the isocyanate **24** with (*R*)-glycidyl butyrate to the oxazolidinone **25**. Methanolysis of the butyrate resulted in the alcohol **26**, which was mesylated and displaced by sodium azide. Reduction and ultimately acetylation afforded DuP 721 (**13**) (Scheme 11.2) [31].

A more versatile method was developed by Manninen and Brickner at Upjohn (Scheme 11.3) [32]. The synthesis started with an S_NAr displacement of 3,4-difluoronitrobenzene (**27**) with excess morpholine to selectively provide the 4-substituted nitrophenyl derivative, which was reduced to the aniline **28** [33]. Subsequent reaction with benzylchloroformate afforded the carbamate intermediate **29**. Deprotonation with *n*-BuLi and addition of (*R*)-glycidyl butyrate provided the (5*R*)-(hydroxymethyl)-2-oxazolidinone **30** in more than 80% yield, thus achieving a greater overall economy of chemical steps compared with the DuPont approach [34].



Scheme 11.2 Synthesis of DuP 721 (13).

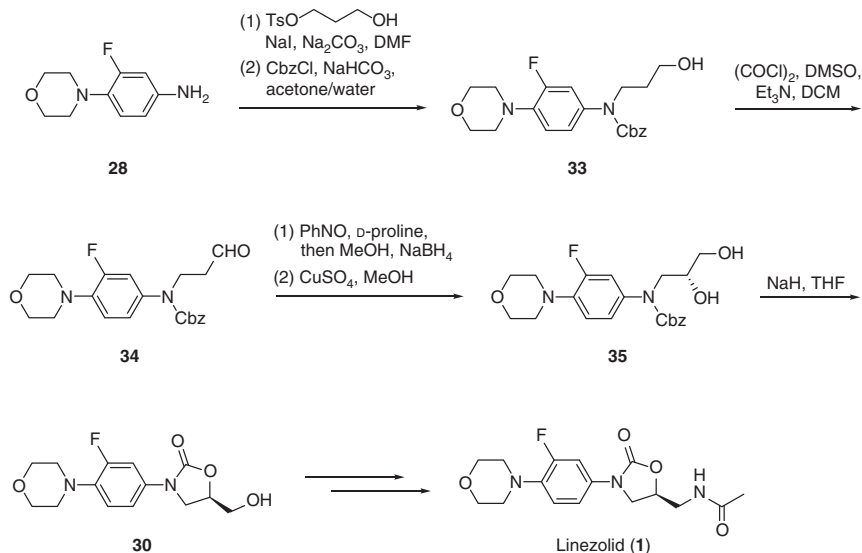


Scheme 11.3 The Upjohn synthesis pathway of linezolid (1).

Activation of **30** as mesylate (**31**) and displacement with NaN₃ followed by catalytic hydrogenation of the azide intermediate yielded the 5-aminomethyl compound **32**, which was finally acetylated to provide linezolid (**1**) (69% overall yield from **29**, >99.8% e.e.). However, due to the cryogenic conditions (−78 °C) and air-sensitive base (*n*-BuLi), the key step **29** → **30** to form the 2-oxazolidinone ring is not feasible for the industrial preparation of **1**. Lohray *et al.* [29] therefore reported an alternative synthesis via an asymmetric bis-epoxide, using D-mannitol as the starting material. Despite high yields, this synthetic pathway proved to be long (nine steps) and quite laborious.

In a more proficient process, transformation of **29** to linezolid (**1**) was achieved in 72% yield by using (*S*)-*N*-[2-(acetyloxy)-3-chloropropyl]acetamide and LiOt-Bu in dimethylformamide (DMF)/MeOH, in lieu of (*R*)-glycidyl butyrate [35, 36].

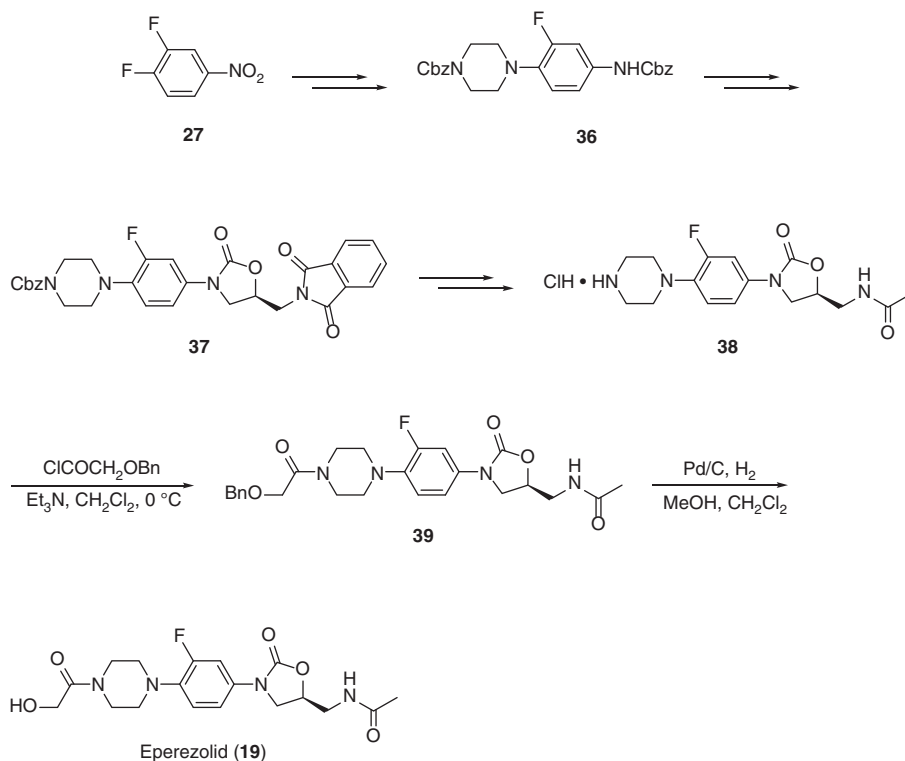
The enantioselective syntheses of linezolid (**1**) was achieved with 56% overall yield (Scheme 11.4) [28, 37].



Scheme 11.4 Alternative enantioselective synthesis of linezolid (**1**).

The aniline derivative **28** was reacted with 3-hydroxypropyl toluenesulfonate, followed by protection of the secondary amine with a Cbz group to give **33** in 85% overall yield. The primary alcohol is then oxidized to the aldehyde derivative **34** using standard Swern conditions. The conversion to the corresponding diol **35** through a D-proline-catalyzed asymmetric α -aminooxylation is accomplished in two steps: (i) reaction of the –CHO group using nitrosobenzene as the oxygen source in the presence of 25 mol% D-proline in CH₃CN at –20 °C, followed by treatment with NaBH₄ in MeOH to provide the crude aminooxy alcohol and (ii) subsequent reduction of the crude product with 30 mol% CuSO₄ to the chiral diol **35** in 86% yield. The regioselective intramolecular cyclization using sodium hydride in tetrahydrofuran (THF) at 0 °C provided the desired oxazolidinone **30** in 96% yield and 99% e.e.

The same synthetic methodology as described in Scheme 11.3 was applied for the syntheses of eperezolid (**19**) and sutezolid (**2**) (Schemes 11.5 and 11.6). In the synthetic sequence for eperezolid (**19**), intermediate **36** was obtained by using Cbz-protected piperazine instead of morpholine (Scheme 11.5). The next steps involved several protecting group manipulations to afford compound **37**, which was further converted to the piperazine HCl salt **38**. Acylation with (benzyloxy)acetyl chloride and debenzoylation provided eperezolid (**19**) [33].



Scheme 11.5 Synthesis of eperezolid (19).

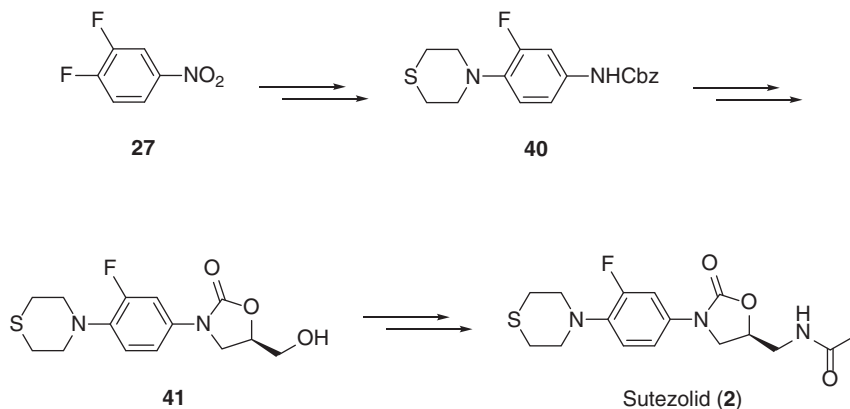
Sutezolid (2) was prepared in a similar manner by replacing morpholine with thiomorpholine in the reaction with 3,4-difluoronitrobenzene **27** to give intermediate **40** (Scheme 11.6) [28, 37]. Conversion of the hydroxymethyl side chain in **41** to the corresponding acetamidomethyl moiety then provided sutezolid (2).

Radezolid (3), developed by Rib-X Pharmaceuticals, contains a 1,2,3-triazole moiety, which was assembled as shown in Scheme 11.7. Suzuki coupling of intermediate **42** with 4-hydroxymethylphenylboronic acid was followed by mesylation and chlorination to yield **43**. The chloride was displaced with propargyl amine and subjected to a [2+3] cycloaddition reaction with sodium azide to provide radezolid (3) [38].

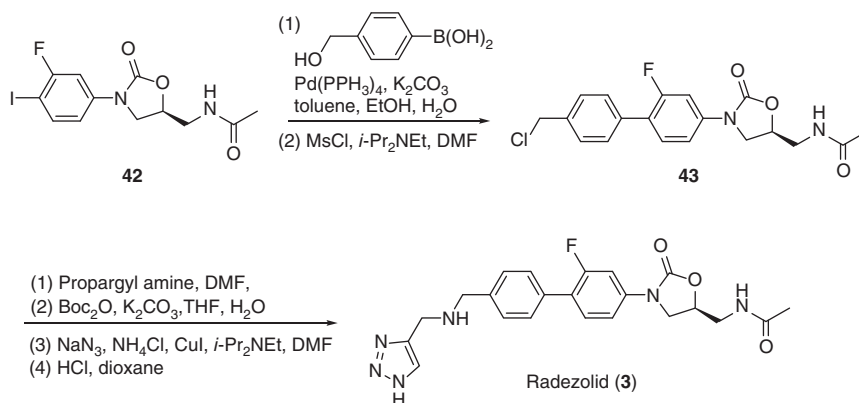
11.4

Mechanism of Action

The oxazolidinone antibiotics have a unique mechanism of action, targeting an early event in the synthesis of bacterial proteins [39, 40]. This mechanism is in contrast to other known antibiotics (e.g., chloramphenicol, macrolides,



Scheme 11.6 Synthesis of sutezolid (2).



Scheme 11.7 Synthesis of radezolid (3).

lincosamides, tetracyclines) that act by inhibiting the elongation step of protein synthesis [41]. Oxazolidinones have been shown to inhibit the formation of the 70S initiation complex, which is composed of the 30S subunit of the bacterial ribosome, formylmethionyl-tRNA (fMet-tRNA), mRNA, guanosine-5'-triphosphate (GTP), and initiation factors (IFs) (Figure 11.5).

While earlier studies indicated that oxazolidinones bind to the 30S ribosome subunit [42], Lin *et al.* demonstrated that eperezolid (**19**) interacts with the 50S ribosomal subunit at the peptidyl transferase center (PTC) and competes with chloramphenicol and lincomycin, known inhibitors of the PTC [43]. Colca *et al.* have used a radioactive photo affinity probe attached to eperezolid (**19**) to investigate the site of action for oxazolidinones. These studies illustrate that oxazolidinones bind in the proximity of the ribosomal peptidyl transferase near the P site [44]. Linezolid (**1**) has also been shown to potently bind to the PTC in the 50S subunit. The PTC is located in the middle of the 50S subunit at the bottom of

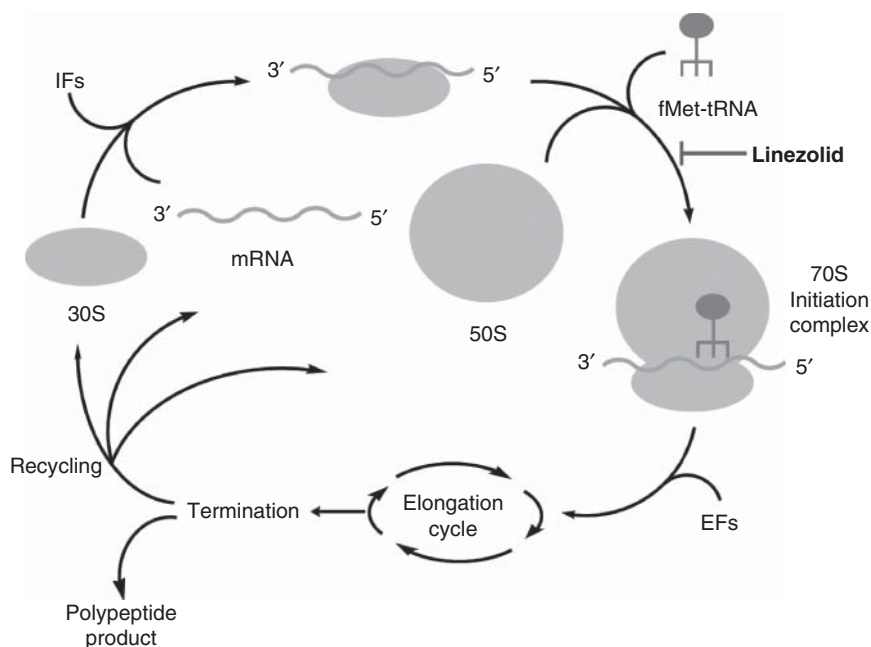


Figure 11.5 Schematic representation of the mode of action of oxazolidinones, which bind to the peptidyl transferase center (PTC) of the 50S ribosomal subunit, preventing the formation of the 70S initiation complex and blocking the protein synthesis. IFs: initiation factors; EFs: elongation factors; and fMet-tRNA: formylmethionyl-tRNA.

the cleft where the sites for aminoacyl-tRNA (A site) and peptidyl-tRNA (P site) are positioned. The binding of linezolid (**1**) to the PTC was supported by mutagenesis studies [45]. The PTC is almost exclusively composed of rRNA, and the nucleotides in this region are highly conserved.

Due to this unique mechanism, target-site mutation is substantially the only mechanism of resistance to linezolid (**1**) known so far. The binding pocket of linezolid (**1**) is lined with the highly conserved nucleotides G2061, A2451, C2452, A2503, U2504, G2505, U2506, and U2585 [46]. Resistant strains undergo mutations either in nucleotides of the 23S rRNA involved in the linezolid (**1**) binding or in nucleotides located distally, ultimately resulting in a reduced affinity of the antimicrobial drug to the 50S subunit. The pattern of resistance is organism-specific (with only little overlap) between various *Enterococcus* species, *E. coli*, *M. tuberculosis*, and *S. aureus*. The most frequently reported site of mutation in resistant clinical isolates is G2576U [47], which is associated with prolonged treatment [48]. However, the activity of linezolid (**1**) is unaffected by the rRNA methylases that modify the 23S rRNA so as to block the binding of known antibiotics, such as macrolides and lincosamides. Since 2003, attention has been paid to mutations in the ribosomal L3 protein as further determinant of linezolid (**1**) resistance; these

bacterial mutations have been also associated with resistance to other antibacterials, such as tiamulin and anisomycin, which bind to sites that overlap with that of linezolid (**1**) [49].

The potency and antimicrobial spectrum of linezolid (**1**) have been continuously monitored through international surveillance programs as multiple Zyvox® Antimicrobial Potency Study (ZAPS), Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS), and Linezolid Experience and Accurate Determination of Resistance (LEADER) [51–53]. During Phase-III clinical trials, resistance to linezolid (**1**) emerged in a few patients infected with *Enterococcus faecium* as well as in a compassionate use program. After FDA approval, case reports of resistance among clinical isolates revealed modifications of the target site PTC [53]. These earlier cases of resistance occurred during prolonged therapy (>21 days) [54]. The rare clinical occurrence of resistance to linezolid (**1**) was corroborated by the LEADER program results, which reported nonsusceptibility rates between 0.03 and 1.83% against more than 40 000 clinical isolates of staphylococci and enterococci across nine consecutive surveillance years (2004–2012) in the United States [55]. However, some emergences of methicillin-resistant *Staphylococcus epidermidis* (MRSE) clinical isolates were reported very recently [56].

Linezolid (**1**) was shown to be active against the most important Gram-positive pathogens, whereas Gram-negative bacteria, with a few exceptions (e.g., *E. coli*), are oxazolidinone-resistant, apparently because oxazolidinones are recognized and excreted by endogenous efflux pumps [57]. The main therapeutic indications of linezolid (**1**) and other oxazolidinone antimicrobials are uncomplicated and complicated infections of the skin and skin structure infections, as well as hospital- and community-acquired pneumonia caused by *S. pneumoniae* with concurrent bacteremia or caused by *S. aureus*. Linezolid (**1**) was further approved for the treatment of VRE and MRSA, including infections with concurrent bacteremia [57]. During investigational studies, linezolid (**1**) displayed *in vitro* inhibitory activities against numerous clinically relevant bacteria, including staphylococci (methicillin-susceptible and resistant), streptococci, enterococci (vancomycin-susceptible and -resistant), *Corynebacterium* spp., *M. tuberculosis*, and some species of anaerobic bacteria [58].

In vitro testing has shown the minimum inhibitory concentration (MIC₉₀) of linezolid (**1**) against most Gram-positive bacteria to be <4 µg/ml [58]. In general, linezolid (**1**) was less active against Gram-negative bacteria, displaying weak activity against *Haemophilus influenzae* (MIC₉₀ = 16 µg/ml) and modest activity against *Moraxella catarrhalis* (MIC₉₀ = 4 µg/ml), both organisms that cause respiratory tract infections. Additionally, linezolid (**1**) has been shown to have a high antibacterial activity (MIC₉₀ = 0.125–0.5 µg/ml) against *M. tuberculosis* [59]. Studies in animal models demonstrated that linezolid (**1**) administered orally was effective in systemic infections caused by a variety of Gram-positive pathogens with activity comparable to vancomycin.

Linezolid (**1**) is commercially available in either oral (PO) or intravenous (IV) dosages. An oral dose of 600 mg in humans resulted in maximum serum levels of 19 µg/ml with an average of 6 µg/ml after 12 h. The bioavailability of linezolid

(1) is 100% following PO administration with no food effects. The drug is only moderately bound to plasma proteins and is distributed to several body tissues such as skin, soft tissue, heart, lung, liver, bladder, and intestines, therefore having the potential of curing Gram-positive infections regardless of localization. The same dose of linezolid (1) (600 mg, twice a day) is recommended for both PO and IV administration, thus allowing for flexibility in patient treatment [30, 60]. Linezolid (1) does not inhibit the most important cytochrome P450 isoforms, and it is mainly metabolized through morpholine ring oxidation [61]. The acetamido group is quite stable. In fact, the enzyme-catalyzed hydrolysis of NHCOCH_3 , followed by deamination, affords metabolites only in trace levels. The main metabolic pathways are depicted in Figure 11.6.

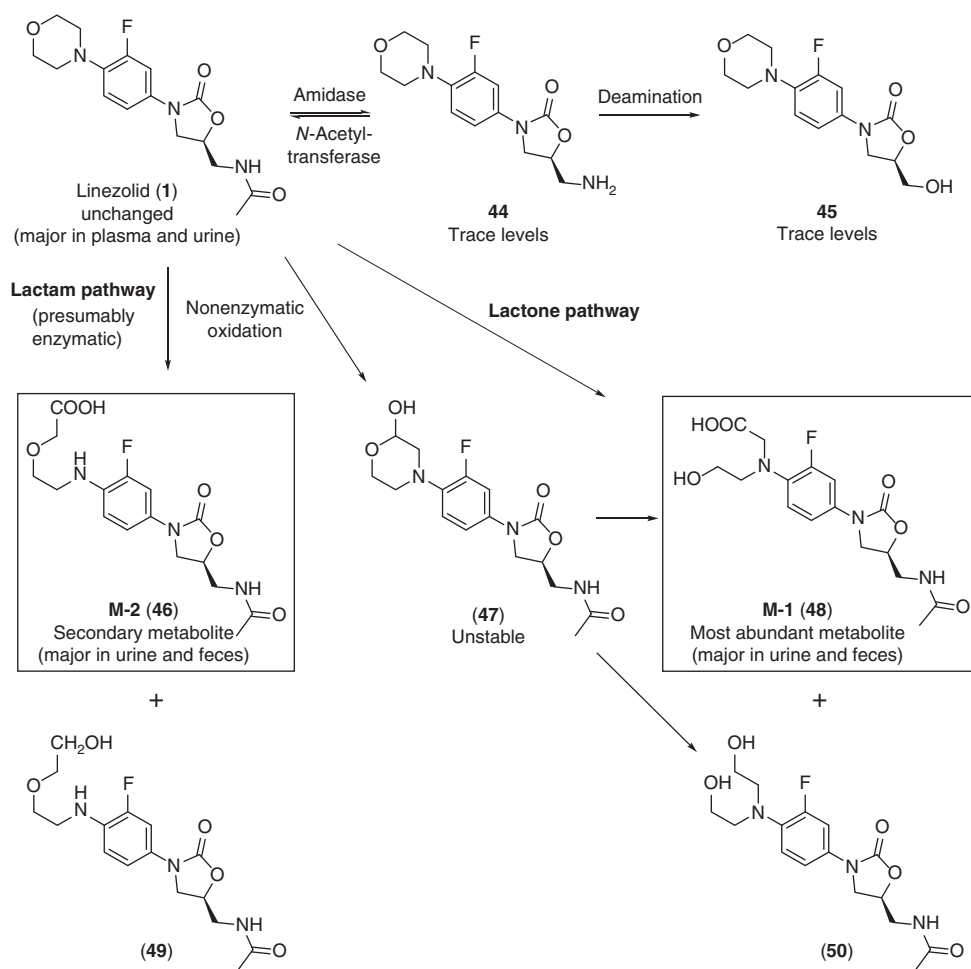


Figure 11.6 Scheme of the main metabolic pathways of linezolid (1).

Studies with radioactive ^{14}C -labeled drug in humans showed that about 30% of the administered linezolid (**1**) dose is excreted unchanged in the urine. *In vitro* studies with human liver microsomes [62] suggested that linezolid (**1**) is nonenzymatically oxidized to the hemiacetal metabolite **47**, which is unstable and oxidized to the most abundant metabolite **48** (M-1, about 45% of dose in human excreta), through the so-called lactone pathway. A secondary metabolite **46** (M-2, about 11% of dose) is a δ -amino acid formed through the (presumably) enzymatic lactam pathway. The two main metabolites have no significant toxicity or antimicrobial activity. Other minor metabolites (**44**, **45**, **49**, and **50**) account totally for approximately 5% of dose. While the metabolites detected in dog and rat excreta are qualitatively comparable to those revealed in humans, the relative proportions of the metabolism products vary from species to species. In humans, the lactone pathway is favored (4:1), the dog metabolizes the drug by the two pathways about equally, whereas the rat favors the lactam pathway (1:4) [63].

Linezolid (**1**), which shares the 3-aryl-oxazolidin-2-one pharmacophore with the RIMAs toloxatone (**2**), befloxatone (**51**), and cimoxatone (**52**) (Figure 11.7), is a weak MAO inhibitor.

As such, linezolid (**1**) should not be administered in combination with other MAO inhibitors, large amounts of tyramine-rich foods [64], or serotonergic drugs, particularly selective serotonin reuptake inhibitors (SSRIs), such as paroxetine and sertraline. Linezolid (**1**) may also enhance the blood-pressure-increasing effects of sympathomimetic drugs and should not be administered in combination with pethidine (meperidine) under any circumstance, due to the risk of serotonin syndrome. In clinical studies, no life-threatening adverse events were reported. Gastrointestinal side effects (nausea and, less frequently, diarrhea and vomiting) were reported in 10% cases. Other less frequent adverse effects include headache, tongue discoloration, and oral monilia, whereas thrombocytopenia [64], gastrointestinal bleeding, and decreased hemoglobin levels are among the effects resulting from discontinuation of linezolid therapy. Reversible myelosuppression [65] and neuropathy [66] have been observed in some cases of long-term treatment.

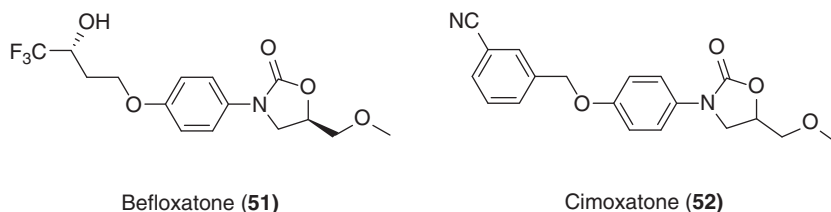


Figure 11.7 The reversible inhibitors of MAO-A (RIMAs) befloxatone (**51**) and cimoxatone (**52**).

11.5

Structure–Activity Relationships

The development of new oxazolidinones has remained challenging, due to the need of maintaining favorable absorption, distribution, metabolism, excretion (ADME), and solubility, while improving safety and efficacy. Linezolid (**1**), which is in neutral form at physiological pH, has a water solubility of about 3 mg/ml and a logP of 0.55. The structure of oxazolidinone antibiotics allows for facile chemical modifications to generate analogs. As described in the introduction, oxazolidinones are composed of A, B, and C rings with a C-5 side-chain unit on the A-ring substructure (Figure 11.1). The main SAR data, summarized in Figure 11.8, highlight the effects of structural variations useful in the design and optimization of new oxazolidinone antimicrobials [20, 22].

SARs have shown the importance of the (*S*) stereochemistry at the C-5 position of the oxazolidinone ring for activity. Additionally, the C-5 acetamide is believed to interact with the ribosome binding site by acting as a hydrogen bond donor. Minor changes to the C-5 acetaminomethyl side chain to formylamino, ethylamino or dichloroacetamide, methyl carbamate, and simple urea resulted in a decrease in *in vitro* potency [31]. The key role of the NHAc can be highlighted by comparison with other non-antimicrobial oxazolidinone-based drugs. It is worthy of note that the anticoagulant rivaroxaban (**7**), and its metabolites as well, albeit being structurally similar to linezolid (**1**), did not show any antibacterial effect against Gram-positive pathogens. Both drugs share the 3-phenyl-1,3-oxazolidin-2-one pharmacophore, showing two minor differences (i.e., a morpholinone instead of a morpholine group as the C ring, no fluorine atom on B phenyl ring) and one major difference in the 5-substituent, that is, a 5-chlorothiophene-2-carboxamide

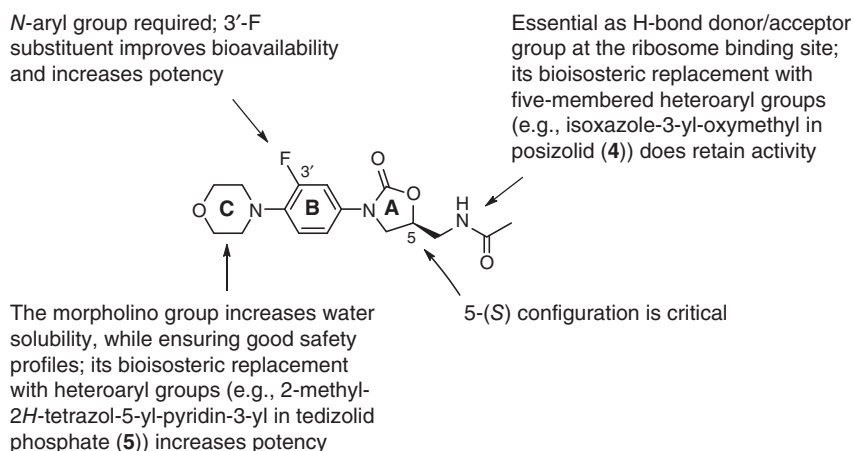


Figure 11.8 Structure–activity relationships of 2-oxazolidinone-based antimicrobials around the structure of linezolid (**1**).

moiety in rivaroxaban (7) instead of the acetamide moiety of linezolid (1). On the one hand, it is the 5-chlorothiophene-2-carboxamide group that prevents rivaroxaban (7) to interact with the ribosome target site of the oxazolidinone antibacterial drugs, while it is this moiety that allows rivaroxaban (7) to efficiently inhibit the blood coagulation factor Xa (as shown by the X-ray crystal structure, chlorothiophene is buried inside the S1 pocket of factor Xa, with chlorine pointing toward the center of the Tyr228 aromatic ring), ultimately achieving good anticoagulant properties [14, 67].

Researchers at Bayer and Pharmacia have also shown that sulfur isosteres of the acetamidomethyl side chain such as thioamide generated highly potent analogs [68]. In the case of tedizolid phosphate (5), the C-5 acetamide was replaced with a hydroxyl group. Interestingly, the antimicrobial potency of derivatives carrying the hydroxymethyl and the 1,2,3-triazole group in C5 is unaffected by the rRNA methylases, while compounds containing the acetamide substituent demonstrate a two- to eightfold loss in activity against strains whose resistance is mediated through target-site mutations [69]. Replacement of the acetamide with five-membered heterocycles such as the O-linked isoxazole in posizolid (4) maintained comparable antibacterial activity. Many other heterocyclic substitutions such as 1,2,3-triazol-2-yl-methyl and pyrid-2-yl-oxymethyl groups are generally poorly tolerated, although 1,2,3-triazoles showed good activity, most likely due to different binding modes to the target site [70]. In general, it was found that five-membered ring heterocycles were preferred over larger rings possibly due to steric concerns and the need for hydrogen bond donor substituents at the C-5 position [6].

Modifications at the A ring focused on oxazolidinone ring surrogates such as butenolide, isoxazolines, and isoxazolidinone that maintained good antibacterial activity [1].

The fluorophenyl moiety of the B ring is crucial for activity since it is believed to be engaged in an aromatic stacking interaction with the 50S ribosomal subunit [31]. The only successful variant of the B ring is the 3,5-difluorophenyl group in posizolid (4).

Several modifications of the C ring were reported without a significant loss in activity. One notable finding was the need for a suitable electron-donating amino substituent on the phenyl ring to maintain *in vitro* potency and a good safety profile. Modifications of the C ring include (i) fusion of B and C rings, (ii) novel extensions on established C rings, (iii) separation of B and C rings by a spacer, and (iv) novel C rings [31]. Among these modifications, the most notable one is the introduction of the biaryl ring system such as in radezolid (3) and tedizolid phosphate (5).

A deeper understanding of the interactions of oxazolidinones with the bacterial and mitochondrial ribosome will help the design of analogs with improved potency and tolerability. While linezolid (1) and tedizolid phosphate (5) are the only currently marketed oxazolidinones, there is still an unmet need for antibiotics with a broad spectrum of activities that include Gram-negative bacteria, long-term tolerability, and reduced MAO inhibition.

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12

Sovaldi, an NS5B RNA Polymerase-Inhibiting Carboxylic Acid Ester Used for the Treatment of Hepatitis C Infection

Alastair Donald

12.1

Introduction

Sovaldi (**1**, sofosbuvir, GS-7977, formerly PSI-7977, Figure 12.1) is a carboxylic acid ester used in the treatment of human hepatitis C virus (HCV) infection [1]. It is representative of a group of viral nonstructural protein 5B (NS5B) RNA-dependent RNA polymerase inhibitors based on the phosphoramidate pronucleotide (“ProTide”) concept pioneered by McGuigan [2]. A number of similar ProTide esters such as INX-08189 (**2**) (Inhibitex), PSI-353661 (**3**) (Pharmasset), and AZT analog **4** have also been extensively investigated.

A second inhibitor of the NS5B polymerase has also been commercialized (Dasabuvir, Exviera), but this molecule comes from a distinct, nonnucleoside chemotype [3]. Sovaldi (**1**) is far from being a standard or conventional carboxylic acid ester prodrug. Carboxylic acid esters are most frequently deployed as prodrugs to overcome adverse overall physicochemical properties that impede upon bioavailability of the corresponding acid such as excessive ionization and the lowering of $\log D$ below what is usually believed to be required for cell permeability and good oral absorption. In these cases, the charged carboxylate group forms specific interactions with the biological target that make it indispensable for activity. In addition, carboxylic acid esters are also often used as soft drugs, where limitation of the pharmacological action to a specific location following topical administration or administration by inhalation is desired [4]. Upon entry into the systemic circulation, the hydrolysis of the soft drug ester yields pharmacologically inactive or cell impermeant metabolites. In contrast to this, ProTide esters share a common mechanism of activation, whereby cleavage of the ester promoiety by cellular hydrolases reveals the carboxylate, which then spontaneously cyclizes onto the phosphoramidate group with expulsion of an alkoxide (normally a phenoxide). Subsequent cleavage of the phosphoramidate phosphorus–nitrogen bond generates a prephosphorylated nucleoside analog, elegantly bypassing the usual requirement for intracellular enzymatic phosphorylation (see Section 12.4 for

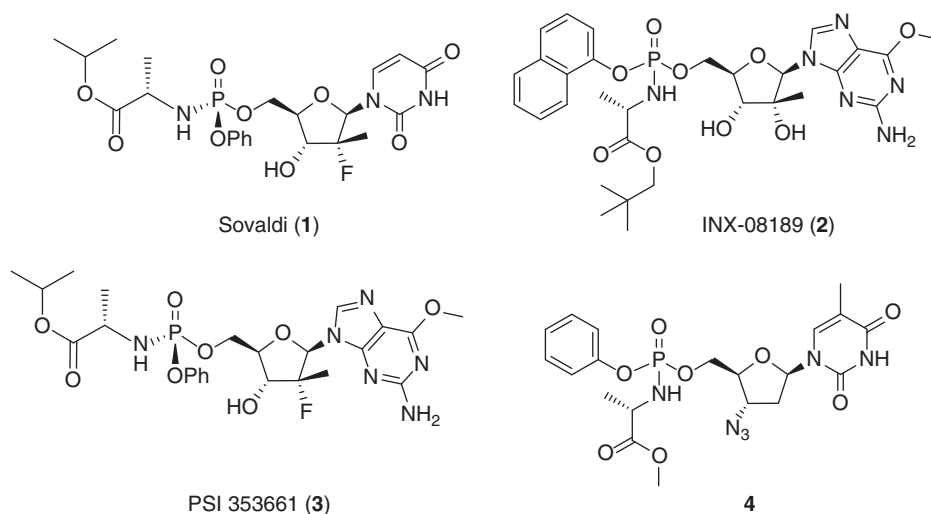


Figure 12.1 Sovaldi (1) and other ProTide-based antiviral agents.

further details) [5]. Thus, the carboxylate group is extruded from the molecule after fulfilling its purpose and has no further role in binding to the biological target.

12.2

History

The antiviral activities of nucleoside and nucleotide analogs can be traced back to the pioneering early work of Hitchings and Elion [6, 7]. The ProTide concept was first described in 1992 with the initial publication from the McGuigan group, outlining the approach as directed toward analogs of the anti-HIV agent AZT [8]. In this article, the first published ProTide analog (**4**, Figure 12.1) bears a striking similarity to Sovaldi (**1**). Sovaldi (**1**) was first prepared by researchers at Pharmasset in the early 2000s. The patent application WO2005/003147 (derived from US7429572, filed in April 2004) discloses the base nucleotide of **1** (**5**, GS-606965, Figure 12.2), while International Patent Application WO2008/121634 discloses a number of phosphoramidate prodrugs of nucleoside analogs including Sovaldi (**1**) itself (Example 25, p. 683). Of special note in these applications is the claimed selectivity of the 2'-disubstituted nucleosides against HCV.

Sovaldi (**1**) was introduced to the US market in December 2013 and the European Union in January 2014. The invention pathway is shown in Figure 12.2 [9]. Starting from the previously described β -D-2'-deoxy-2'- α -F-2'- β -C-methyluridine **5**, Pharmasset researchers systematically examined a variety of ProTide analogs. In adopting the ProTide approach, they hoped to take

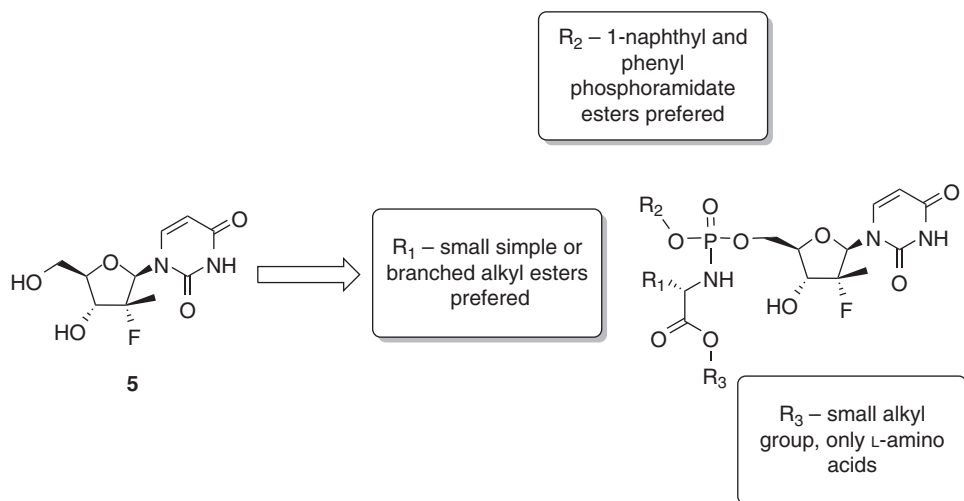


Figure 12.2 Invention pathway of Sovaldi (1).

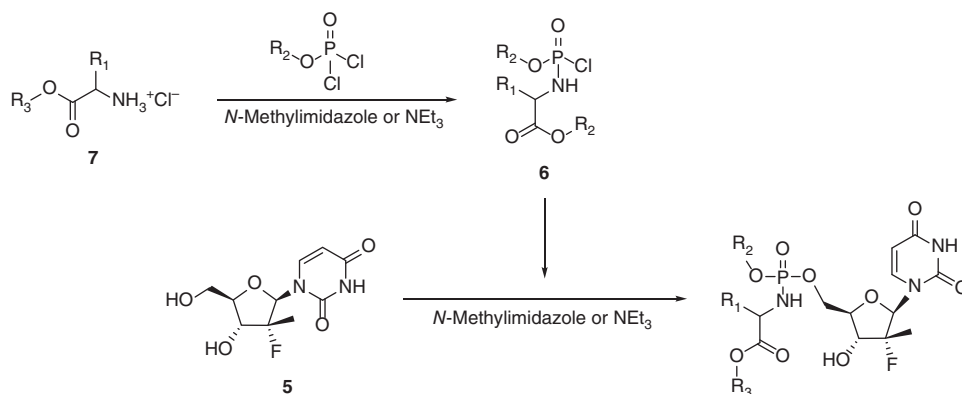
advantage of extensive hepatic first-pass metabolism of the amino acid ester group, predicting that this would lead to the delivery of high concentrations of active species to the desired site of action (the liver).

Initial focus on modification of the ester motif of the amino acid (R_3 , Figure 12.2) indicated that small simple alkyl esters (branched or unbranched) and small cycloalkyl esters were preferred, providing molecules with submicromolar activity in cellular assays. Subsequent investigation of toleration to structural variation of the phosphoramidate ester moiety (R_2) showed that phenolic and naphthyl esters were optimal in terms of potency and that alkyl esters were not effective at this position. Despite showing excellent levels of potency, naphthyl and polyhalophenolic esters were disregarded as they demonstrated significant cytotoxicity in this chemical series. Optimal R_1 (amino acid side chain) substituents were found to be small alkyl-substituted L-amino acids such as L-alanine. *In vitro* studies were conducted to determine relative stability of these congeners in plasma, simulated gastric, and simulated intestinal fluids. On the basis of this structure–activity relationship (SAR) and stability data, several compounds were advanced into *in vivo* studies to determine which agent was capable of delivering the greatest quantity of active uridine triphosphate derivatives to the liver (successfully surviving the absorption phase and arriving as desired at the site of action largely unchanged). When considered alongside pharmacokinetic and isolated cell studies it was found that the isopropyl ester of alanine, in combination with a simple, unsubstituted phosphoramidate phenol ester was optimal in terms of delivering the highest quantity of parent prodrug and active metabolite in both dog and monkey, as well as superior levels of uridine triphosphate derivative in primary human hepatocytes.

12.3

Synthesis

A very concise discovery synthesis of Sovaldi was achieved from the uridine nucleoside **5** by condensation with the monochlorophosphoramidate **6**, prepared from the respective amino acid ester salt, **7**, in the presence of *N*-methylimidazole or triethylamine [10] (Scheme 12.1).

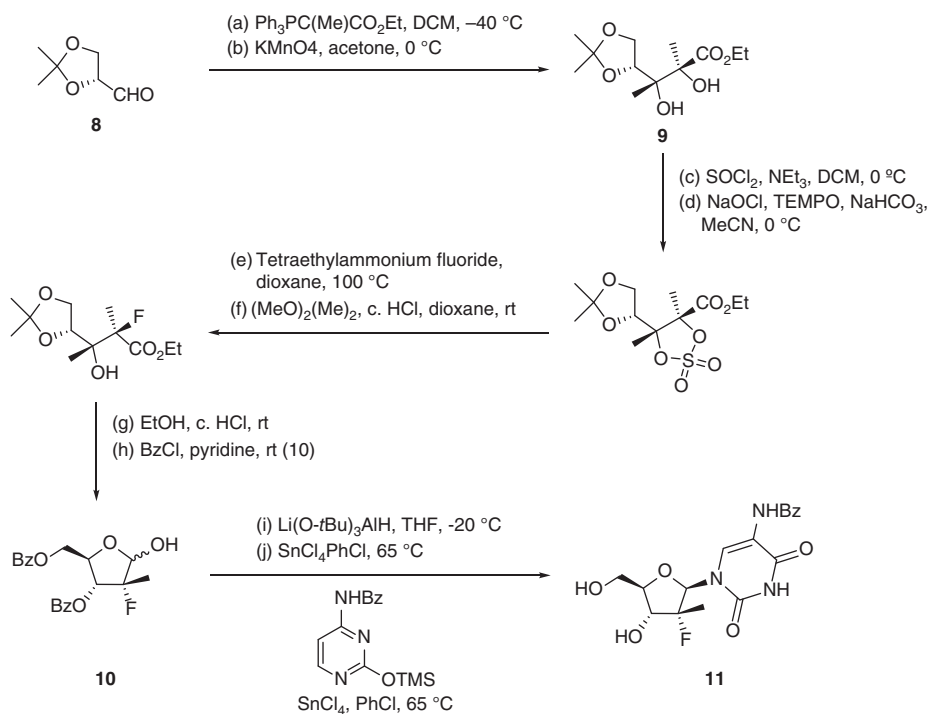


Scheme 12.1 Discovery-stage synthetic route to Sovaldi (**1**) and analogs.

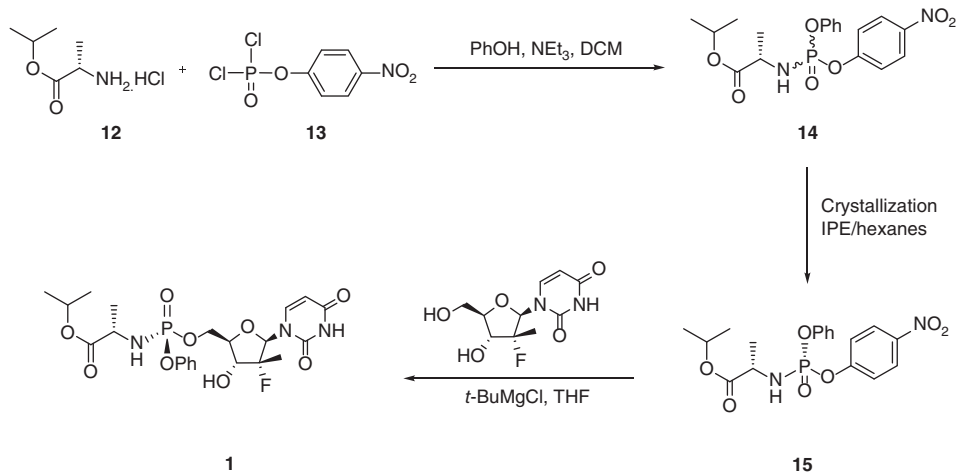
An elegant and efficient large-scale synthesis of a uridine nucleoside building-block precursor **11** has also been reported: this route was developed to overcome the severe limitations of the initial discovery synthesis of **5**, such as the poor yield of a fluorination step (15%), and the necessity for multiple chromatographic purifications (Scheme 12.2) [11]. Starting from the enantiomerically pure and commercially available isopropylidene-protected *D*-glyceraldehyde **8** (derived from *D*-mannitol), **11** is prepared in significantly improved yield (2.9% vs 6.4%), with no chromatographic purifications required. Several intermediates including **9–11** could be isolated in solid form. It has also been reported that further optimization of the final glycosylation step resulted in a significantly improved yield for this transformation (59% vs 29%) [12]. **11** can be readily converted into **5** in good yield by treatment with methanolic ammonia (not shown) [11].

In the discovery-stage synthesis, Sovaldi (**1**) was separated into the diastereomeric pair of phosphoramidate esters by chiral HPLC [9]. On a large scale, Sovaldi (**1**) is prepared in diastereoisomerically pure form through recrystallization of a mixture of diastereoisomeric phosphoramidates (**14**), prepared from *L*-leucine isopropyl ester **12** and the 4-nitrophenol dichlorophosphonate ester **13** as shown in Scheme 12.3 [13].

Building block **15** is subsequently stereoselectively reacted with the magnesium salt of the nucleoside to give the desired enantiomerically pure product (**1**).



Scheme 12.2 Large-scale synthesis of uridine nucleoside building block (11).

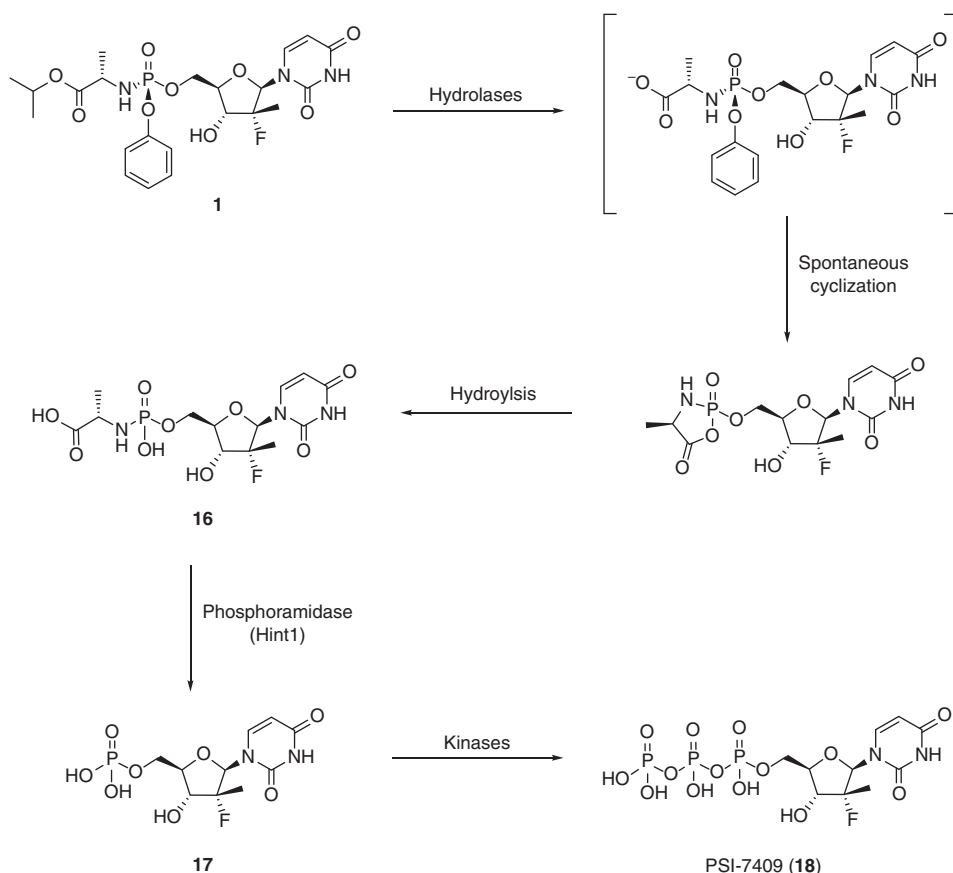


Scheme 12.3 Stereoselective preparation of **1** via phosphoramidate ester building block **15**.

12.4

Mode of Action

Sovaldi (**1**) acts to disrupt the normal function of the hepatitis C NS5B RNA polymerase, a 591 amino-acid viral protein critical for the replication of the RNA genome. Using the positive strand of the viral RNA genome as its template, the polymerase catalyzes the production of a duplicate RNA strand from nucleoside triphosphate building blocks. Sovaldi (**1**) acts as a nonobligate chain-terminating antimetabolite. Serving as a recognized substrate for the enzyme, its incorporation into the growing RNA strand leads to premature termination. Prior to incorporation *in vivo*, Sovaldi is metabolized to the active species by a sequence of transformations as shown in Scheme 12.4.



Scheme 12.4 *In vivo* activation of Sovaldi (**1**) to the uridine triphosphate PSI-7409 (**18**) [9].

Upon absorption into the liver, the hydrolases and peptidases cathepsin A (CatA) and carboxylesterase-1 (hCE1) rapidly cleave the isopropyl ester, with

the generated carboxylate spontaneously cyclizing onto the phosphoramidate moiety. This hydrolysis reaction was found to be highly stereospecific with the S_p diastereoisomer of Sovaldi (GS-7977) being more rapidly hydrolyzed compared to the R_p isomer (GS-7976) [5]. It is believed that the cyclic phosphoramidate is then spontaneously chemically hydrolyzed to intermediate **16**, which is then further processed by as yet incompletely characterized phosphoramidases (including Hint1), yielding the free nucleoside analog (**17**) [5]. This adduct is then phosphorylated to the diphosphate ester (PSI-7410, not shown) by UMP-CMP kinase and then to the triphosphate ester (**18**, PSI-7409) by nucleoside diphosphate kinase, with PSI-7409 being the species that competes for the NS5B nucleoside binding site.

12.5

Structure–Activity Relationships

Analysis of the SARs of ProTide congeners such as Sovaldi (**1**) is very complex due to the many metabolism and activating steps required prior to engagement with the eventual biological target. Alongside this, the predominant use of cell-based assays introduces additional considerations and complications, such as assessing the potential impact of varying degrees of cell permeability and the influence and importance of active transport and efflux mechanisms. Nonetheless, several striking trends are evident. There is a strong preference for small alkyl esters on the phosphoramidate amino acid motif, consistent with the known preference of hCE1 for such substrates (Figure 12.1, R_3 position) [14]. However, CatA is believed to show broader substrate specificity when hydrolyzing amino acid esters [15], and as yet it is unclear as to the relative contribution of hCE1 and CatA to this step. A small number of alternate ester groups are also tolerated; these can be used to modify the overall chemical stability and physicochemical characteristics of the lead molecule (especially log P). As such, there are reports that cyclohexyl, benzyl as well as propyl, butyl, and isopropyl ProTide esters generally offer an optimal “window” balancing sufficient stability to reach the liver intact, but also with sufficient lability to allow enzymatic cleavage at the site of action. It has been noted that *tert*-butyl esters of ProTide conjugates are generally too stable (i.e., insufficiently hydrolyzed) to be active in cellular assays. However, this stands in contrast to other reports on amino acid ester (non-ProTide) prodrugs, where it has been found that *tert*-butyl esters of certain amino acids can in fact deliver high concentrations of acid into cell-based systems because they are substrates for hCE1 [16, 17].

There is a significant preference for the amino acid motif to carry a small hydrophobic side chain, again consistent with what is known about hCE1 selectivity [14]. In contrast to the α,α -dimethyl-substituted analog (**19**), the cyclopropyl congener (**20**) was shown to be inactive (Figure 12.3). The unusual stability of cyclopropyl substituted esters has been documented, and it is likely that this unusual stability prevents activation of the ProTide [18]. Surprisingly, Pharmasset did not report further ring-expanded analogs: given the structural

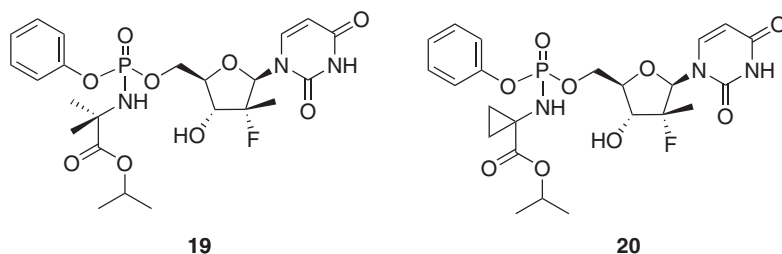


Figure 12.3 Geminal dimethyl substituted (19) and cyclopropyl congener (20) of Sovaldi (1).

tolerance to α,α -disubstitution, it seems likely that larger rings (lacking the special stability of the cyclopropyl analog) would be active as has been reported for other ProTide series [19].

Esters of nonnatural D-amino acids have been found to be relatively poor analogs of Sovaldi (1) [9]. This trend has also been noted in other ProTide analog series [20]. Pharmasset reported that the 2'- β -methyl-2'- α -fluoro ribofuranose analogs demonstrated particularly high levels of selectivity compared their 2'- α -hydroxy counterparts. Consistent with this finding is the reported SAR around the nucleoside core of NS5B polymerase inhibitors [21]. A 3'- α -hydroxyl substituent is required for whole cell activity without cell toxicity. While the 2'- α -fluoro analogs are reported to lack cell activity, the 2'- α -fluoro-2'- β -methyl analogs are active with cytosine as the nucleobase. On the other hand, the 2'- α -hydroxy-2'- β -methyl ribofuranose analog is active in cells in connection with all bases. However, the uridine triphosphate of the 2'- α -fluoro-2'- β -methyl analog is a potent inhibitor in isolated enzyme assays and also shows an extremely long half-life. These data indicate that the limitation in this series is the key sequence of phosphorylation events, a limitation efficiently overcome by the ProTide approach.

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Part IV

Metabolic Diseases

13

Dipeptidyl Peptidase-4 (DPP-4)-Inhibiting Amides for the Treatment of Diabetes

Naomi S. Rajapaksa and Xiaodong Lin

13.1

Introduction

Inhibitors of dipeptidyl peptidase-4 (DPP-4 DPP-IV, CD26), also called gliptins, comprise a class of compounds that is used to treat Type 2 diabetes (T2D) [1–3]. This chapter reviews the six amide-containing DPP-4 inhibitors that are approved for the treatment of T2D, with an emphasis placed on the first three to reach the market (Figure 13.1). The gliptins covered in this chapter all share a common amide bond linkage but have significant differences in their binding modes to the active site of the enzyme.

13.2

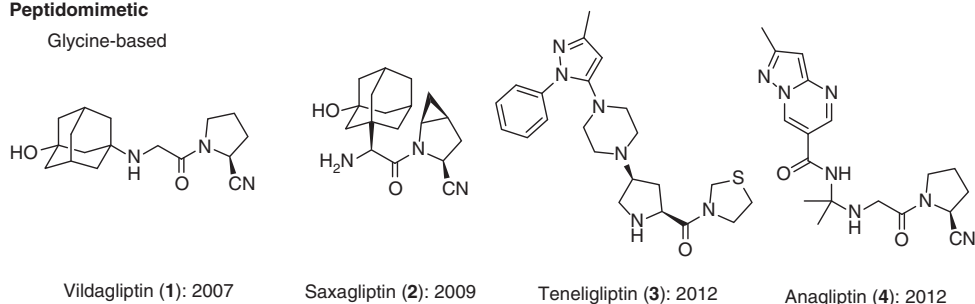
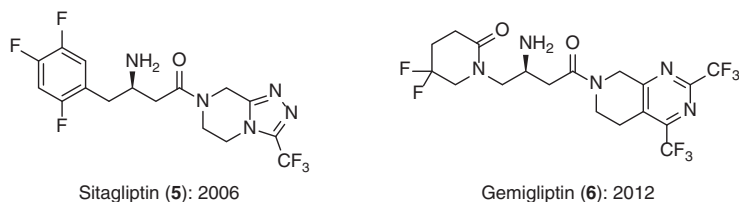
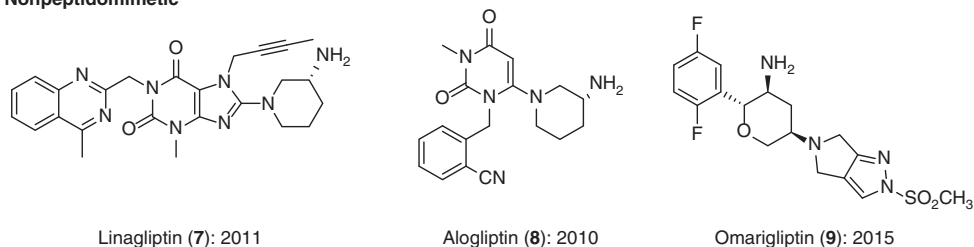
History

DPP-4 is a serine protease that, since its discovery in 1966 [4], has emerged as an important therapeutic target for T2D. DPP-4 cleaves N-terminal dipeptides from multiple regulatory proteins including glucagon-like peptide-1 (GLP-1, GLP-1(7-36) amide) [5]. GLP-1 is a hormone that is released in response to nutrients and that induces a decrease in blood glucose levels. The half-life of GLP-1 is very short, as DPP-4 rapidly cleaves its N-terminal dipeptide to afford inactive GLP-1(9-36) amide. DPP-4 inhibition prolongs the effects of endogenous GLP-1 and has proven to be an effective strategy to treat T2D.

To date, nine DPP-4 inhibitors have been approved for the treatment of T2D (Figure 13.1) [2]. Inhibitors can be classified by structure as peptidomimetic or nonpeptidomimetic, and the former, which are the focus of this review, are further categorized as glycine- or β -alanine-based. Glycine-based inhibitors mimic the natural substrates of DPP-4, and the first β -alanine-based inhibitors were derived from high-throughput screening (HTS) hits. The four approved glycine-based inhibitors are vildagliptin (Galvus[®], **1**, Novartis), saxagliptin (Onglyza[®], **2**, Bristol-Myers Squibb (BMS)), teneligliptin (Tenelia[®], **3**, Mitsubishi Tanabe Pharma), and

Peptidomimetic

Glycine-based

 β -Alanine-based**Nonpeptidomimetic****Figure 13.1** The nine approved DPP-4 inhibitors with the year of first approval.

anagliptin (Suiny[®], **4**, Sanwa Kagaku Kenkyusho/Kowa). The two β -alanine-based inhibitors are sitagliptin (Januvia[®], **5**, Merck & Co.) and gemigliptin (Zemiglo[®], **6**, LG Life Sciences). Finally, the three nonpeptidomimetic inhibitors, which will not be discussed further, are linagliptin (Tradjenta[™], **7**, Boehringer Ingelheim/Lilly), alogliptin (Nesina[®], **8**, Takeda/Furiex), and omarigliptin (Marizev[®], **9**, Merck & Co.).

DPP-4 is also known as CD26 and has a role in T-cell activation. In the early-to-mid 1990s, before the role of DPP-4 in glucose regulation was elucidated, inhibitors were investigated as potential immunomodulating agents. DPP-4 cleaves N-terminal dipeptides from substrates that have a free N-terminus and contain either proline or, to a lesser extent, alanine at the penultimate P1 position. Based on this substrate specificity, many early inhibitors were designed to have a free amine, an acylated five-membered heterocycle to occupy the S1 pocket, and an electrophilic warhead to covalently interact with the serine residue of the catalytic triad (Ser630) (**10**, Figure 13.2).

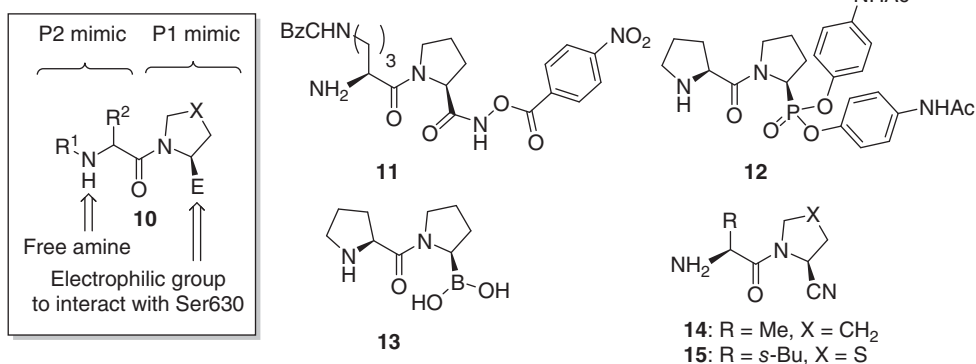
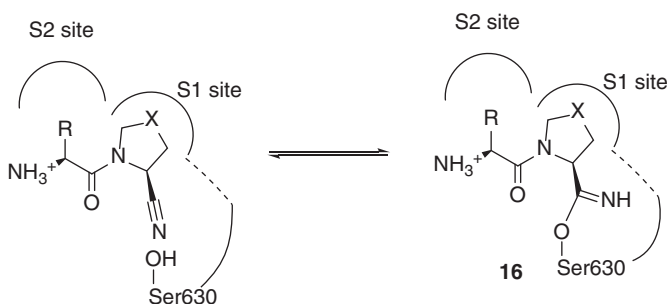


Figure 13.2 DPP-4 substrate specificity and the design of early inhibitors.

N-Acylpyrrolidines with diacyl hydroxylamine (e.g., **11**) [6] or phosphate (e.g., **12**) [7] substituents were found to be weakly potent irreversible covalent inhibitors (Figure 13.2). Boronic acids, such as **13**, which are proposed to irreversibly generate tetrahedral transition-state analogs upon complexation with the Ser630 hydroxyl group, were very potent but suffered from solution instability [8]. The free amine, which is required for inhibitory activity, was also found to undergo an intramolecular cyclization with the electrophile, resulting in an inactive boronate product. For similar reasons, reversible covalent inhibitors bearing aldehyde or ketone warheads lacked solution stability [9]. In 1995, scientists at Mount Sinai School of Medicine and Ferring Research independently reported the first DPP-4 inhibitors bearing nitrile warheads as potential immunomodulators (**14** and **15**, respectively) [10–12]. Addition of the Ser630 hydroxyl group to the nitrile is proposed to generate an imidate adduct (**16**, Scheme 13.1) [13]. While the nitrile warhead is also susceptible to intramolecular cyclization, it was found to have improved solution stability. The 2-cyanopyrrolidine motif is present in all launched covalent DPP-4 inhibitors.



Scheme 13.1 Reversible imidate formation of nitrile-containing inhibitors with Ser630.

Landmark publications from a number of groups in the late 1990s revealed that in addition to its role in the immune system, DPP-4 also degrades GLP-1 [5]. Furthermore, while the continuous intravenous infusion of GLP-1 in T2D patients was effective in controlling glucose [14], single injections were ineffective due to the rapid degradation of endogenous and exogenous GLP-1 by DPP-4 [15–17]. These studies led to the proposal of increasing GLP-1 levels by inhibiting the peptidase [17]. The first inhibitors used in this context also incorporated five-membered heterocycles to occupy the S1 pocket but lacked an electrophilic warhead. In 1998, researchers at the University of Copenhagen and Novartis jointly reported that L-valine-pyrrolidide (**17**, Figure 13.3) reduced DPP-4 activity in non-fasted anesthetized pigs by 90%, resulting in a threefold increase in the amount of full-length GLP-1 and increased insulin concentrations [18]. In the same year, a collaborative effort between academic groups led by Hans-Ulrich Demuth and Raymond A. Pederson showed that L-*threo*-isoleucine thiazolidide (**18**, P32/98) suppressed DPP-4 activity in both obese and lean Zucker^{fa/fa} rats, resulting in enhanced insulin secretion [19]. This compound was patented by Probiobdrug, a company cofounded by Demuth, and was advanced into clinical trials. P32/98 (**18**) showed an increase of GLP-1 levels in healthy volunteers and an increase of insulin levels in diabetic patients [20, 21]. Merck subsequently in-licensed P32/98 (**18**) and the diastereomeric thiazolidide **19** from Probiobdrug but discontinued development in 2001 due to animal toxicity findings.

Based on literature indicating that DPP-4 recognizes substrates with a terminal *N*-methyl-glycine [22], Novartis then studied a series of inhibitors containing an *N*-substituted glycine 2-cyanopyrrolidine [23]. This scaffold provided potent inhibitors and two important benefits: (i) it represented unpatented chemical space, as all previously reported DPP-4 inhibitors were derived from α -branched amino acids, and (ii) the relatively hindered secondary amine resulted in improved solution stability compared to analogous primary-amine-containing cyanopyrrolidines. NVP-DPP728 (**20**) was identified as a potent inhibitor with efficacy in animal models and was advanced into clinical trials. From these trials, it was shown for the first time that inhibiting DPP-4 in diabetic patients was a safe and effective means of reducing blood glucose levels [24]. Novartis' second clinical candidate, vildagliptin (**1**) [25], had superior potency, a longer half-life, and a longer-lasting pharmacodynamic effect than **20** and was approved for the treatment of T2D in 2007 by the EMA (European Medicines Agency).

Saxagliptin (**2**), developed by BMS and approved in the United States by the FDA (Food and Drug Administration) in 2009, became the second glycine-based

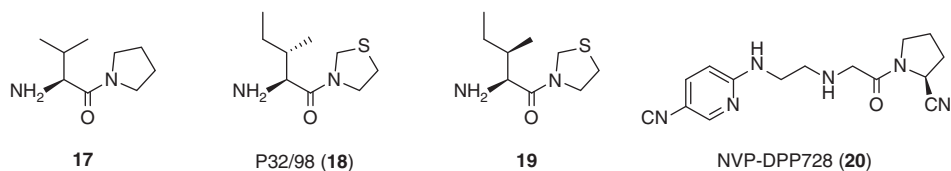


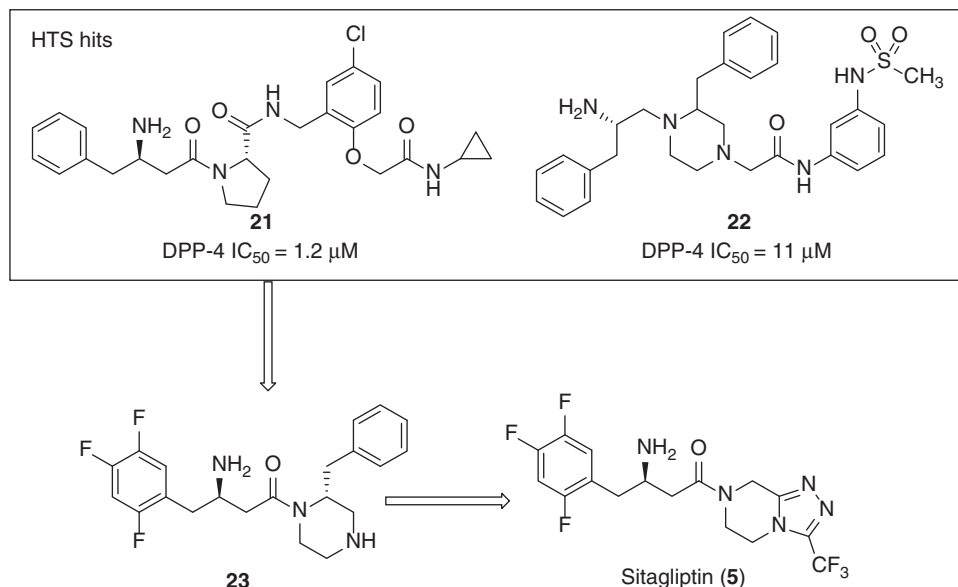
Figure 13.3 Early inhibitors used to probe the role of DPP-4 in glucose regulation.

DPP-4 inhibitor to reach the market. BMS scientists returned to the primary aminoacyl-2-cyanopyrrolidine scaffold and identified key structural modifications that greatly improved solution stability. In particular, the *cis*-methano-fused cyanopyrrolidine and adamantyl group at the α -position of **2** significantly hindered the intramolecular amine–nitrile cyclization deactivation pathway [26, 27]. The enhanced solution stability allows for once-daily oral dosing in the treatment of T2D.

Two other glycine-based DPP-4 inhibitors have reached the market. Teneligliptin (**3**), approved in 2012, was developed by Mitsubishi Tanabe. Unlike the other launched glycine-based inhibitors, and reminiscent of P32/98 (**18**), teneligliptin lacks an electrophilic warhead and acts as a noncovalent inhibitor. This inhibitor also takes advantage of the S2 extensive subsite of the binding pocket [28]. These two properties are thought to contribute to its high potency and selectivity for DPP-4. Anagliptin (**4**), also approved in 2012, contains a 2-cyanopyrrolidine motif and, similarly to vildagliptin (**1**), has an *N*-substituted glycine amide [29].

Sitagliptin (**5**), discovered by Merck, was the first β -alanine-based inhibitor discovered and the first approved DPP-4 inhibitor for the treatment of T2D. After early clinical proof-of-concept studies by Novartis and Probiobdrug validated the inhibition of DPP-4 as a means of regulating blood glucose levels in diabetic patients, Merck also embarked on a program to develop DPP-4 inhibitors [30]. They profiled the compounds in-licensed from Probiobdrug, P32/98 (**18**) and **19**, in order to better understand the root cause of animal toxicities. The activity and toxicology differences between the two compounds suggested that the observed adverse effects were due to off-target inhibition of DPP-8/9, related proteases with lesser known functions, rather than DPP-4 inhibition [31]. This hypothesis, although later called into question by additional studies [32], led Merck to target a DPP-4 inhibitor with a large selectivity window over these related enzymes [30]. An HTS conducted with the Merck compound collection led to the identification of **21** (DPP-4 IC_{50} = 1.9 μ M) and **22** (DPP-4 IC_{50} = 11 μ M) (Scheme 13.2). Through the course of optimizing those hits, the Merck scientists determined that these DPP-4 inhibitors had distinct SAR from the glycine series (*vide infra*) and that they generally had >1000-fold selectivity over DPP-8/9. The optimization efforts merged with the discovery of **23** [33], a nanomolar inhibitor that is a hybrid of the two initial leads as it contains the β -amino-amide moiety from **21** and the benzyl-substituted piperazine ring from **22**. Finally, exchanging the right-hand portion of **23** with a trifluoromethyl-substituted triazolopiperazine ring system improved metabolic stability and provided sitagliptin (**5**) [34].

In 2012, LG Life Sciences gained approval from the Korean FDA for gemigliptin (**6**), a β -alanine-based inhibitor that bears structural similarity to sitagliptin (**5**) [35]. Although the binding mode of gemigliptin (**6**) has not been elucidated, it is proposed to participate in many of the same enzyme–inhibitor interactions as **5** (*vide infra*, Figure 13.8).

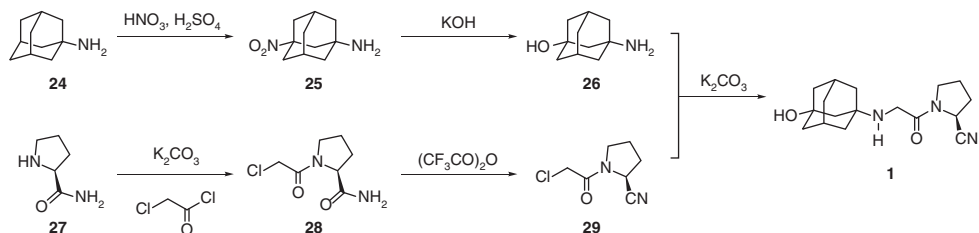


Scheme 13.2 The development of sitagliptin (**5**) from the HTS hits **21** and **22**.

13.3

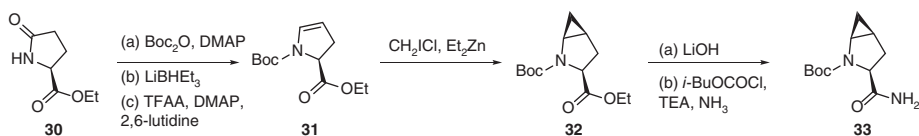
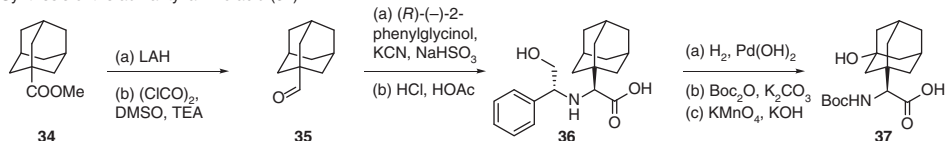
Synthesis

Vildagliptin (**1**) is obtained by alkylating the amine **26** with chloride **29** (Scheme 13.3) [25, 36]. The 3-aminoadamantan-1-ol fragment **26** is prepared in two steps from aminoadamantane **24** via nitration followed by hydrolysis. Acylation of L-prolinamide (**27**) with chloroacetyl chloride, followed by dehydration using trifluoroacetic anhydride, leads to (*S*)-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile (**29**).

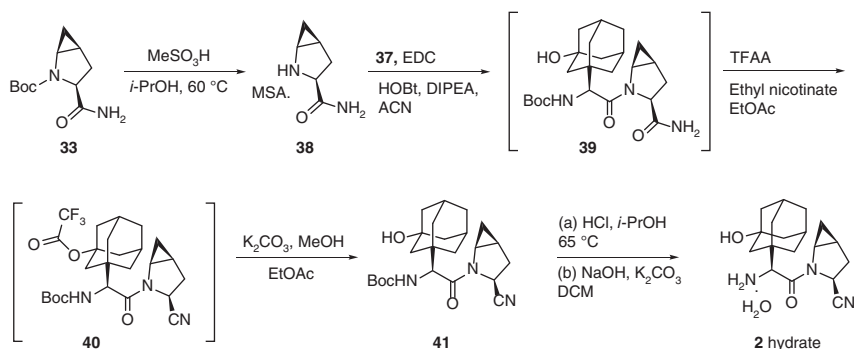


Scheme 13.3 Synthesis of vildagliptin (**1**).

The synthesis of saxagliptin (**2**) requires the coupling of the two unnatural amino acid derivatives **33** and **37** (Scheme 13.4). Methanoprolinamide **33** is obtained from L-pyroglutamic acid (**30**) [27]. Thus, after Boc-protection of the lactam, the amide is reduced with lithium triethylborohydride. Dehydration of

Synthesis of methanoprolineamide (**33**)Synthesis of the adamantyl amino acid (**37**)

Final stages of the saxagliptin synthesis

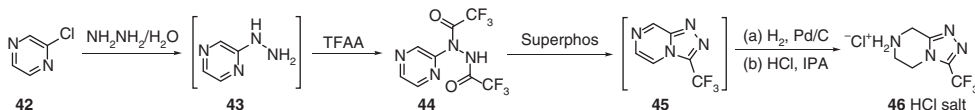
Scheme 13.4 Synthesis of saxagliptin (**2**).

the resulting hemiaminal using trifluoroacetic anhydride yields the enamine **31**. A subsequent Simmons–Smith cyclopropanation with chloriodomethane and diethylzinc gives the bicyclic intermediate **32**. Saponification using LiOH leads to the corresponding carboxylic acid, which is converted into primary amide **33** via activation with isobutyl chloroformate, followed by reaction with ammonia. The synthesis of the second coupling fragment involves the conversion of the methyl adamantane-1-carboxylate **34** to the aldehyde **35**. In this two-step procedure, the ester is reduced with lithium aluminum hydride and the resulting alcohol is oxidized (Swern oxidation) [26]. The aldehyde is condensed with (R) -(-)-2-phenylglycinol, and an asymmetric Strecker reaction followed by acidic hydrolysis of the resulting nitrile affords the amino acid **36**. Removal of the chiral auxiliary by hydrogenolysis is followed by Boc-protection of the amine. Finally, oxidation of the adamantyl group with potassium permanganate under basic condition provides the desired amino acid **37**. In the process route, deprotection of the Boc-amine **33** with methanesulfonic acid yields **38** as a crystalline salt, which is condensed with acid **37** using EDC and HOBT [37]. The amide **39** is taken forward and dehydrated to the nitrile **40** with trifluoroacetic anhydride and ethyl nicotinate, which was chosen to replace pyridine due to concerns of

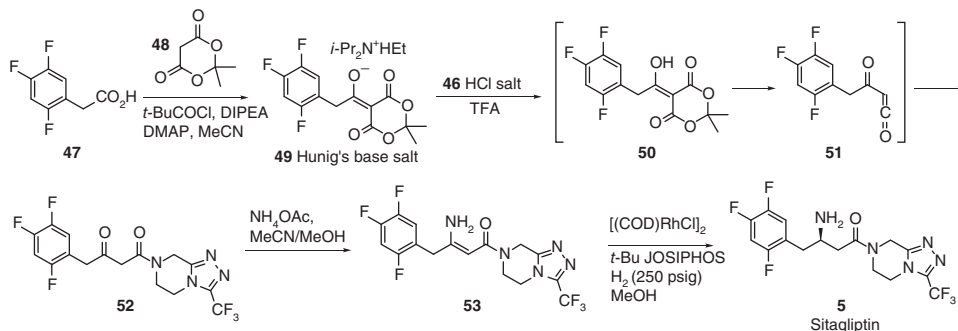
odor and toxicity. This step also results in the trifluoroacetate of the adamantane alcohol, which is subsequently hydrolyzed under mildly basic conditions using potassium carbonate to give **41**. The final deprotection using hydrochloric acid affords the HCl salt of saxagliptin (**2**), which is free-based to yield its monohydrate form.

Both the Merck medicinal chemistry [34] and process routes [38, 39] to sitagliptin (**5**) use triazolopiperazine **46** for a late-stage amide bond formation (Scheme 13.5). The synthesis of this building block begins with a nucleophilic displacement of the chloro substituent in **42** with hydrazine. Without isolation, **43** is reacted with trifluoroacetic anhydride to afford the bis-trifluoroacetohydrazide **44**. This intermediate is heated with superphosphoric acid to close to the triazolopyrazine **45**, which, in turn, is hydrogenated and treated with HCl to furnish the triazolopiperazine HCl salt **46**. One key difference to all the synthetic routes is the strategy used to set up the β -amino chiral center [40]. In order to be cost-effective as well as environmentally friendly, Merck developed a highly efficient asymmetric synthesis as the second-generation route for its manufacturing process. For this work, the company received the Presidential Green Chemistry Challenge Award in 2006. As shown in Scheme 13.5, trifluorophenyl acetic acid **47** is activated by forming the mixed anhydride with pivaloyl chloride and is then condensed with Meldrum's acid (**48**). The resulting adduct, **49**, is stable as a salt with Hunig's base. Treatment of **49** with a substoichiometric amount of trifluoroacetic acid liberates the free acid **50**, which decomposes to ketene **51**. The triazolopiperazine HCl salt **46** intercepts the ketene **51** to yield the β -ketoamide **52**, and the crude product is converted exclusively into the desired *Z*-enamine **53**.

Synthesis of triazolopiperazine (**46**)



Synthesis of sitagliptin (**5**) (second-generation process route)



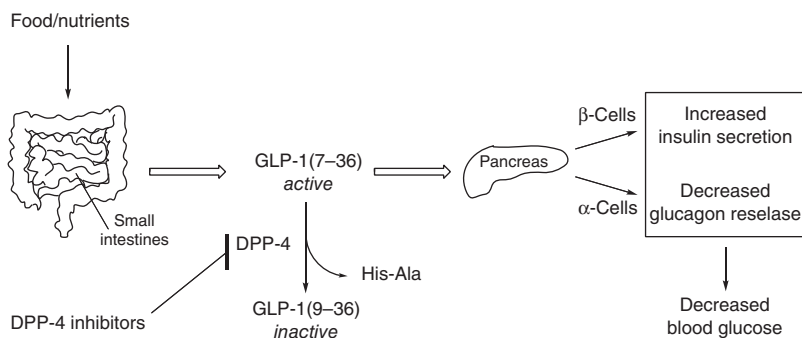
Scheme 13.5 Synthesis of sitagliptin (**5**).

with ammonium acetate. A simple filtration at the end of this three-step, one-pot sequence affords **53** in 82% overall isolated yield and with 99.6 wt% purity. Conditions to effect hydrogenation of **53** ($[\text{Rh}(\text{COD})\text{Cl}]_2$ (0.15 mol%) and *t*-Bu JOSIPHOS (0.155 mol%)) were identified through an exhaustive screening effort, and these reproducibly afford sitagliptin (**5**) in 98% yield and 95% e.e. Recently, a joint effort by Codexis and Merck resulted in a groundbreaking biocatalytic asymmetric synthesis of sitagliptin (**5**) from its β -keto-amide precursor **52** by using an engineered transaminase in the presence of diisopropylamine to obtain **5** in 99.95% e.e. [41].

13.4

Mode of Action

In response to food, and as part of the glucose regulation process, L-cells of the small intestine produce GLP-1(7-36) amide (Scheme 13.6) [42]. Upon binding to GLP-1 receptors in the pancreas, this 30-amino acid protein stimulates secretion and gene expression of insulin and inhibits glucagon release. These two hormones are central to blood glucose regulation: insulin induces glucose storage as glycogen and glucose uptake into the liver, muscle, and fat, and glucagon promotes the depolymerization of glycogen to glucose. Hence, GLP-1 indirectly results in a lowering of blood glucose. GLP-1 demonstrates additional antidiabetic effects including delayed gastric emptying, increased β -cell proliferation, and decreased appetite. However, GLP-1 is short-lived, as DPP-4 rapidly ($t_{1/2} < 2$ min) cleaves its N-terminal dipeptide, His-Ala, to afford the inactive GLP-1(9-36) amide.



Scheme 13.6 The roles of GLP-1 and DPP-4 in blood glucose regulation.

T2D is characterized by chronic hyperglycemia resulting from impaired insulin sensitivity and secretion. Gliptins inhibit the degradation of endogenous GLP-1 by occupying the active site of DPP-4 either via covalent or noncovalent interactions with the enzyme. In this way, gliptins potentiate the antidiabetic effects of GLP-1. Because GLP-1 stimulates insulin secretion in a strictly glucose-dependent manner, DPP-4 inhibition has a very low risk of inducing hypoglycemia. This

is in contrast with glucose-independent T2D treatments, such as insulin or sulfonylureas, which are known to induce hypoglycemia. Another advantage of DPP-4 inhibitors over other T2D therapeutic agents (insulin, sulfonylureas, thiazolidinediones, and GLP-1 analogs) is that they are weight-neutral. Finally, there has been success in coadministering gliptins with metformin, a biguanide insulin sensitizer, and combination therapies of metformin with vildagliptin (**1**), saxagliptin (**2**), sitagliptin (**5**), linagliptin (**7**), and alogliptin (**8**) have been approved.

13.5

Structure–Activity Relationships

It is noteworthy that vildagliptin (**1**), saxagliptin (**2**), and anagliptin (**4**) share an α -aminoacylpyrrolidine core and a nitrile (Figure 13.4). These inhibitors contain a proline mimic at the P1 site and resemble the cleavage product of a P2–P1 dipeptidyl substrate. The cyano group forms a covalent bond with Ser630 in the S1 pocket, and the amine tail group engages in hydrogen bonds with Glu205 and Glu206 in the S2 pocket [13]. The amide bond in these structures not only serves as a linker between the cyano group and the α -amino tail, the oxygen of the amide also forms an important hydrogen bonding network with residues Arg125 and Asn710.

This scaffold has significant steric restrictions for the P2 site regarding substitution on the amine (R^1) and the α -position (R^2). Although substitution at either position is tolerated, simultaneous substitution at the two positions (both $R^1, R^2 \neq H$) is not. This observation is exemplified by a series of inhibitors from Novartis, in which a cyclohexyl group at either the α -position (**54a**) or amine (**54b**) provides low nanomolar inhibition, but substitution at both positions (**54c**) significantly reduces potency (Figure 13.5). Novartis pursued a series of *N*-substituted glycines ($R^1 \neq H, R^2 = H$, Figure 13.4), which ultimately led to the discovery of vildagliptin (**1**). The aforementioned substitution restrictions were also confirmed by BMS, and their study into α -substituted 2-cyanopyrrolidine amides ($R^1 = H, R^2 \neq H$, Figure 13.4) led to the discovery of saxagliptin (**2**). The

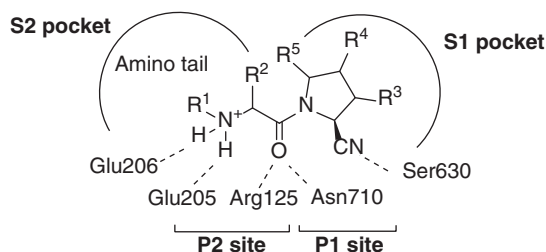


Figure 13.4 General structural features of glycine-based DPP-4 inhibitors.

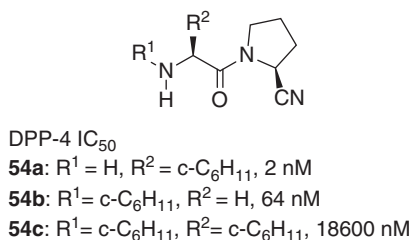
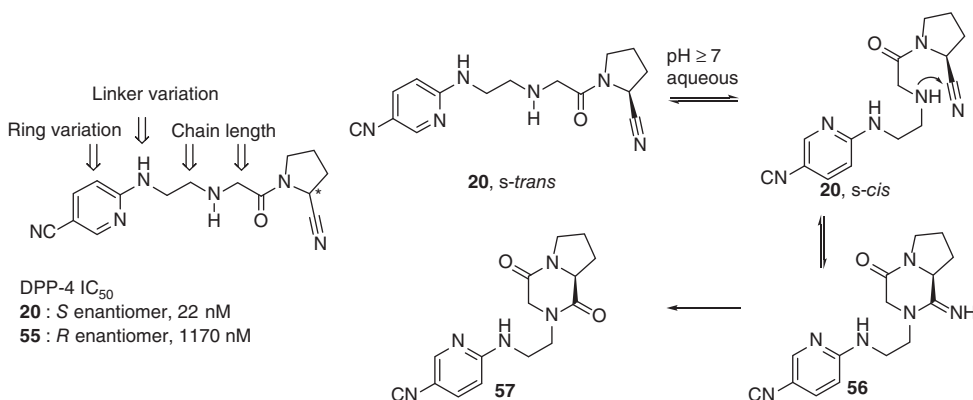


Figure 13.5 Steric restrictions at the P2 site of glycine-based inhibitors.

main exceptions to the substituent trend are cases where R¹ and R² form a ring (e.g., teneligliptin (**3**)).

The first clinical candidate from Novartis, NVP-DPP728 (**20**), is a low-nanomolar DPP-4 inhibitor that contains an amino pyridine (Scheme 13.7) [22]. SAR built up around this compound reveals that changes to the chain lengths, linker composition, and identity of the six-membered aromatic ring generally lead to less-potent inhibitors. The *S* configuration of the pyrrolidine stereocenter also proved important for activity as demonstrated by a 53-fold loss in potency for the enantiomer **55**.



Scheme 13.7 NVP-DPP728 (**20**) and issues of solution stability.

One issue associated with this scaffold is its solution stability. Under neutral or basic aqueous conditions, the nitrile of the 2-cyanopyrrolidine undergoes a nucleophilic attack by the P2 site amine. This intramolecular cyclization results in amidine **56**, which is rapidly hydrolyzed to diketopiperazine **57** [25]. Consequently, although inhibitor **20** improved oral glucose tolerance *in vivo*, its relatively short half-life in humans ($t_{1/2}$ = 0.85 h, 100 mg, p.o.) precluded its development.

Improved potency is observed with increasing branching of the N-substituent, as demonstrated by a series of isomeric inhibitors: **58a** (linear), **58b** (cyclic), and **58c** (branched) (Figure 13.6). This steric effect is enhanced with bridged polycyclic substituents, as seen with 2-aminonorbornane (**58d**, IC₅₀ = 15 nM) and 1-adamantylamine (**59**, IC₅₀ = 3 nM). Examination of primary metabolites of

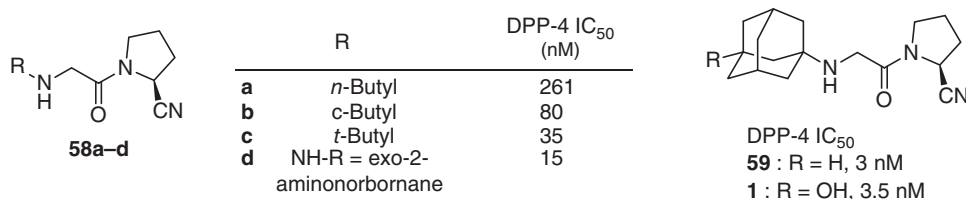


Figure 13.6 SAR development leading to vildagliptin (**1**).

59 suggested that close analogs containing a monohydroxylated adamantyl ring might also be potent inhibitors. The 3-hydroxylated-1-aminoadamantane analog **1** (vildagliptin), which avoids incorporating an additional chiral center, has not only great potency for DPP-4 but also superior selectivity, especially over two closely related peptidases, post-proline-cleaving enzyme (PPCE, IC₅₀ = 210 μM) and DPP-2 (IC₅₀ > 500 μM). Furthermore, it showed a desirable pharmacokinetic profile (*F*% > 90%, CL = 1.51 l/h/kg, *V*_{ss} = 0.7 l/kg, *t*_{1/2} = 90 min, in monkeys) and its terminal elimination half-life is 2.6-fold longer than that of the early development candidate **20** (*t*_{1/2} = 35 min, in monkeys). This PK improvement correlates well with the observed pharmacodynamic effect. When studying the effect of DPP-4 inhibition in normal cynomolgus monkeys, maximum inhibition of plasma DPP-4 activity (~95%) was observed ~2 h postdose of **1** (1 μmol/kg, p.o.) while ≥50% inhibition was observed within 30 min postdose and persisted for ≥10 h. This longer PD effect provided a better profile for a less-frequent dosing regimen.

Saxagliptin (**2**) was discovered by BMS scientists [26, 27], who investigated whether a cyclopropane-bridged pyrrolidine derivative could serve as a proline surrogate in α-substituted inhibitors. They reasoned that a methano bridge on the pyrrolidine may occupy the region typically occupied by a methylene group of a saturated pentacycle in the puckered or “envelope” conformation. As seen in Figure 13.7, the regio- and stereochemical disposition of the cyclopropyl group has profound effects on the potency and stability of inhibitors (**61a–d**). Compared to unsubstituted pyrrolidine **60**, a methano bridge *trans* to the nitrile group such as in **61a** and **61b** significantly reduces inhibitory potency. In contrast, only a slight loss in potency is observed when the methano bridge is *cis* to the nitrile, such as in **61c** and **61d**. These two inhibitors are differentiated by their solution stability. Stability half-life measurements at 39.5 °C in a pH 7.2 phosphate buffer show that the *cis*-4,5-methano-bridged analog **61d** is much more stable than both proline nitrile **60** and the *cis*-3,4-methano-bridged analog **61c**. Consequently, more thorough SAR studies were carried out on a series of *cis*-4,5-methano-bridged inhibitors. Increasing the degree of branching at the α-position improves both potency and solution stability (**62b** vs **62a**). Aliphatic medium rings at the α-position generally lead to inhibitors with good potency (e.g., **62c**), and β-branching increases solution stability while maintaining potency (e.g., **62d**). Bridged polycyclic systems such as adamantyl show significantly improved *in vitro* potency and extended duration of action, as measured by plasma DPP-4

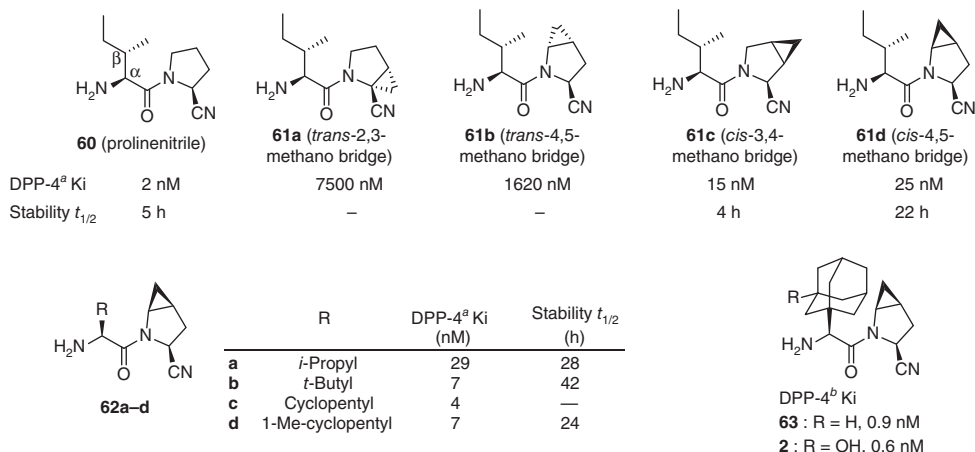


Figure 13.7 SAR development leading to saxagliptin (**2**). ^aData obtained using porcine DPP-4. ^bData obtained using human DPP-4.

levels, after oral dosing in rats. Although compound **63** showed potent and prolonged plasma DPP-4 inhibition in rats (84% at 0.5 h, 83% at 4 h), it exhibited low oral bioavailability ($F = 2\%$), poor microsome stability, and weak CYP3A4 inhibition ($IC_{50} = 20 \mu\text{M}$). The likely metabolite, the bridgehead-hydroxylated analog **2** showed identical *in vitro* potency and plasma DPP-4 inhibition (87% at 0.5 and 4 h), had no CYP3A4 inhibition up to $100 \mu\text{M}$, and gave a good pharmacokinetic profile ($F\% = 75\%$, $t_{1/2} = 2.1 \text{ h}$, $V_{ss} = 5.21/\text{kg}$). This compound, later developed as saxagliptin (**2**), demonstrated favorable efficacy in the oral glucose tolerance test in both Zucker^{fa/fa} rats and *ob/ob* mice (measured by enhancements in glucose clearance and elevated insulin levels). Its extended pharmacodynamic response enabled once-daily dosing in humans.

Merck's development of sitagliptin (**5**) clearly shows that the SAR of β -alanine-based inhibitors is distinct from that of the glycine-based inhibitors. For example, replacement of the β -aminoacyl group in the pyrrolidine-based HTS hit **21** (Scheme 13.2) with an α -aminoacyl group leads to a two- to threefold loss in potency [30]. The Merck scientists also found that the right-hand portion of compound **21** could be replaced with a thiazolidine group without any loss of potency, and some optimization efforts were pursued into that direction [43]. While changes to the side chain length (**64**, Figure 13.8) or replacement of the phenyl ring with heteroaromatic or cycloalkyl groups results in a loss of potency, fluorination of the phenyl ring improves potency compared to the parent compound **64a**. The impact of the fluorine substitution is demonstrated by compounds **64b–f**. Interestingly, although monofluorination has a modest or no effect on potency (**64b–d**), the appropriately difluorinated compound **64e** improves potency >10-fold and the 2,4,5-trisubstituted compound **64f** even boosts potency 25-fold.

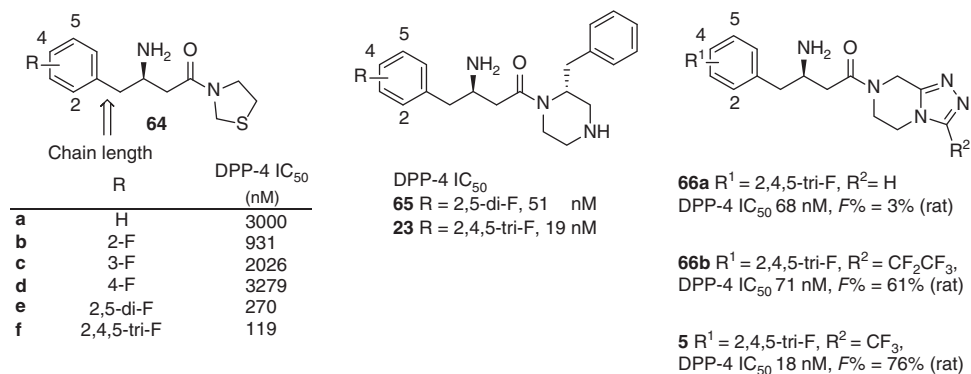


Figure 13.8 SAR development leading to sitagliptin (**5**).

These analogs have good selectivity against related peptidyl proteases but suffer from poor pharmacokinetic properties (e.g., **64f** CL = 120 ml min⁻¹ kg⁻¹, $t_{1/2}$ = 1.1 h, F = 3%, 1/2 mg/kg i.v./p.o. in rat). The SAR knowledge obtained from series **64** was applied to the structurally distinct screening lead **22**. Once again, the additive fluorine substitution effect was observed for this scaffold (**65**, **23**), but the compounds were also unsuitable for development due to poor *in vivo* pharmacokinetic profiles [33]. Metabolite identification studies using rat liver microsomes indicated extensive metabolism, primarily via piperazine ring oxidation. Replacement of the piperazine ring with several 5,6-fused heterocyclic alternatives improves metabolic stability and pharmacokinetic properties [34, 44–48]. The 1,3,4-triazolopiperazine-based system was further evaluated *in vivo*, and 2,4,5-tri-fluoro phenyl substitution again proved optimal. Small hydrophobic groups at R₂ impact both potency and the pharmacokinetic profile as illustrated by compounds **66a**, **66b**, and **5**. The unsubstituted analog **66a** has poor oral bioavailability (rat F = 3%). In contrast, both pentafluoroethyl- (**66b**) and trifluoromethyl-substituted (**5**) analogs show improved bioavailability at 61% and 76%, respectively. Moreover, trifluoromethyl substitution improves potency nearly fourfold relative to **66a**. Compound **5** maintains the desired selectivity profile (e.g., IC₅₀: QPP > 100 μM, DPP-8 48 μM, DPP-9 > 100 μM), shows excellent oral bioavailability in other preclinical species (F = 100% in dogs, 68% in monkeys), and was eventually developed as sitagliptin (**5**).

An enzyme-inhibitor X-ray cocrystal structure shows that sitagliptin (**5**) binds in the DPP-4 active site with the amide moiety in the opposite orientation compared to the glycine-based inhibitors. The key interactions are depicted in Figure 13.9. The 2,4,5-trifluorophenyl moiety fully occupies the S1 hydrophobic pocket. The β-amino group forms three hydrogen-bonding interactions with the side chains of Tyr662, Glu205, and Glu206. The amide oxygen forms a water-mediated interaction with the hydroxyl of Tyr547, and the triazolopiperazine is stacked against the side chain of Phe357. Last but not least, the trifluoromethyl substituent interacts with the side chains of Arg358 and Ser209 in the S2 extensive subsite.

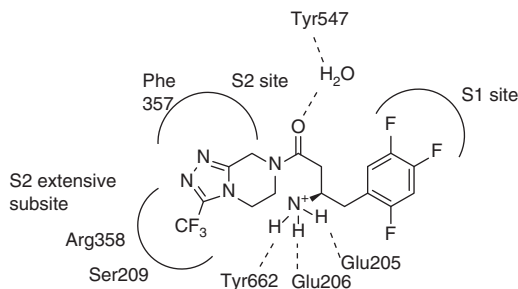


Figure 13.9 Key interactions of sitagliptin (5) with the DPP-4 active site.

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Part V

Oncology

14

Ibrutinib, a Carboxylic Acid Amide Inhibitor of Bruton's Tyrosine Kinase

Timothy D. Owens

14.1

Introduction

Bruton's tyrosine kinase (Btk) is a critical enzyme for the development and function of B cells. It is believed to play an important role in a variety of hematological malignancies and cancers and is implicated in a variety of autoimmune diseases and inflammatory conditions. As such, great efforts have been made by the medicinal chemistry community to identify potent and selective inhibitors of this enzyme. Ibrutinib (**1**) (Figure 14.1), the first commercialized Btk inhibitor, is marketed as Imbruvica® and has now been approved for the treatment of a variety of cancers including chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), and Waldenström's macroglobulinemia (WM) [1, 2]. Compound (**1**) contains an acrylamide designed to irreversibly form a covalent bond to a specific cysteine present in Btk, and the carboxylic acid amide moiety plays a critical role in both the positioning and reactivity of this cysteine-targeting element.

14.2

History

The origin of interest in Btk as a therapeutic target stems from the identification of X-linked agammaglobulinemia (XLA) in 1952 by pediatrician Ogden Bruton [3]. This inherited condition is manifested by the absence of antibodies and results in a susceptibility to bacterial infection and sepsis early in childhood. In 1993, Btk was identified as the gene that codes the tyrosine kinase and is responsible for XLA [4–6]. Btk has since been found to play a critical role in various stages of B-cell development and proliferation. Moreover, anti-CD20 antibodies, such as rituximab, effectively deplete B cells. They have shown clinical efficacy and are approved for the treatment of various leukemias and lymphomas, as well as rheumatoid arthritis. Therefore, Btk has emerged as a target of considerable interest since it is amenable to the development of an oral therapy by targeting the kinase activity with small-molecule inhibitors.

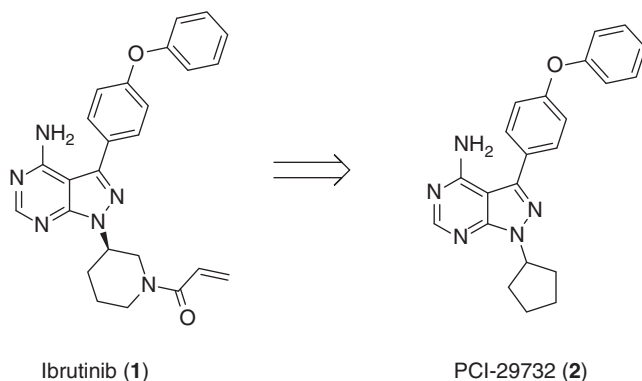


Figure 14.1 The chemical structure of ibrutinib (**1**) and optimization starting point PCI-29732 (**2**).

The majority of kinase inhibitors have targeted the ATP binding site; however, given their highly conserved nature, the development of selective kinase inhibitors has proven quite difficult. In 2007, researchers at Celera Genomics published their discovery of **1** [7]. After screening a variety of scaffolds deemed capable of inhibiting Btk, PCI-29732 (**2**) emerged as an active inhibitor (IC_{50} of 8.2 nM in a Förster resonance energy transfer (FRET)-based competition assay). Upon further profiling, the team discovered that compound **2** was also a potent inhibitor of the tyrosine kinase Lck. Based on known ligand structures of these kinases, the compound was docked into a homology model, which aligned known X-ray structures of the Btk kinase domain (1K2P) and Lck (1QPE). After inspecting the amino acid residues in close proximity, Cys481 was identified as a potentially nucleophilic residue, which could be employed to design a highly selective Btk inhibitor. In Lck, the corresponding amino acid is a serine, and in the known kinome, only 10 kinases share a cysteine in the same location. Of these, Cys773 in epidermal growth factor receptor (EGFR) had previously been targeted by irreversible kinase inhibitors, which had already advanced into clinical trials. A series of electrophilic groups were then attached through various linkers, eventually culminating in the identification of **1**, which incorporates an acrylamide moiety.

Compound **1**, based on the connection of Btk and XLA, was originally tested in a mouse arthritis model to probe its potential in autoimmune indications. Mice were stimulated with anticollagen antibodies and lipopolysaccharide (LPS) and **1** was administered orally once per day. Complete inhibition of arthritis was seen at doses as low as 10 mg/kg. Critically, due to the irreversible nature of the inhibition, this corresponded to plasma concentrations, which reached a high of 0.6–1.7 μ M at T_{max} ($\sim$ 2 h) but did not need to be maintained throughout the 24 h period between doses.

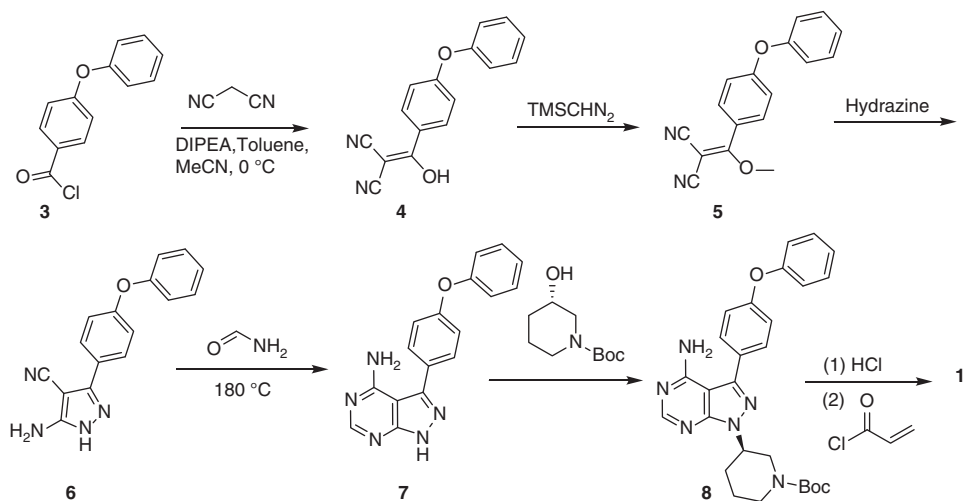
The carboxamide moiety is now well established as an electron-withdrawing group, which is able to sufficiently activate adjacent alkenes (or alkynes) to allow engagement of accessible cysteines on proteins. However, in contrast

to more reactive vinyl sulfonamides, for example, these functional groups are sufficiently unreactive that Michael addition occurs nearly exclusively at cysteine moieties in close proximity to the targeted binding pocket of the protein of interest. By effectively increasing the local concentration of the reactive species through noncovalent interactions, very high selectivity can be achieved. Due to this unique reactivity, as will be discussed later, this class of carboxamides has provided utility not only as important therapeutics in oncology but also as molecular probes for gaining understanding of cellular processes and quantifying pharmacodynamic inhibition of enzymes in clinical and *in vivo* experimental settings [8].

14.3

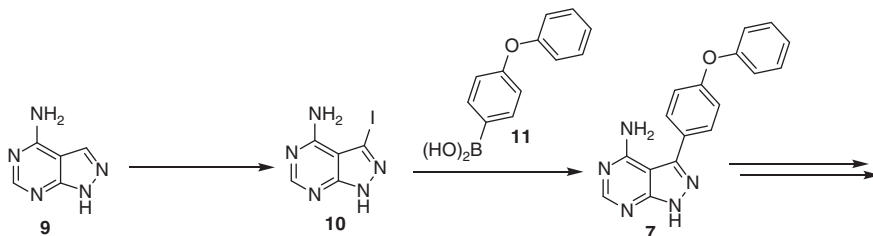
Synthesis

The original synthesis of ibrutinib (**1**) commences with the preparation of **8** (Scheme 14.1), which had originally been reported by researchers at Abbott for the preparation of the 1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine class of kinase inhibitors [9]. The benzoyl chloride **3** is treated with malononitrile and the resultant enol **4** is methylated with trimethylsilyldiazomethane to afford compound **5**. Formation of the aminopyrazole **6** is accomplished by treatment with hydrazine, and the key intermediate **7** is produced by the subsequent thermal cyclization with acetamide at 185° for 4 h. The piperidine group is installed via Mitsunobu reaction with inversion of stereochemistry to afford Boc-protected (*R*)-**8**. After deprotection, the resultant piperidine is reacted with acryloyl chloride to furnish compound **1** [10].



Scheme 14.1 Original synthesis of the carboxylic acid acrylamide ibrutinib (**1**).

An alternate synthesis of **1**, described in the same patent, commences with iodination of 1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**9**) to afford **10**, followed by Suzuki coupling with the boronic acid **11** to install the diarylether component of compound **7** (Scheme 14.2). This route appears more amenable to establishing structure–activity relationship (SAR) around the diaryl ether moiety and has been utilized in several subsequent publications with this scaffold.



Scheme 14.2 Alternate synthesis of ibrutinib (**1**) and related analogs.

14.4

Mechanism of Action

Btk is a nonreceptor tyrosine kinase of the Tec kinase family. It is expressed primarily in hematopoietic cells, particularly B cells, but not in normal plasma cells or T cells. B cells are a type of white blood cells and play a critical role in the body's adaptive immune system, protecting the body from infectious agents. The outer surface of B cells is expressing transmembrane receptor proteins called B-cell receptors (BCRs), which recognize foreign antigens and activate immune responses. Btk plays a central role in both BCR signaling and B-cell development [11]. Upon activation of B cells by a cognate antigen, Btk is activated by the upstream kinases Lyn and Syk and phosphorylates phospholipase $\text{C}\gamma$ ($\text{PLC}\gamma$). Phosphorylation of $\text{PLC}\gamma$ leads to calcium mobilization and activation of the NF κ B and MAP kinase pathways, among others. In short, Btk plays a critical role in the proliferation, differentiation, and activation of B cells upon antigen recognition and the resulting inflammatory response. In addition to B cells, Btk is present in myeloid cells, and it is thought to play an important role in $\text{Fc}\gamma$ -receptor signaling in macrophages, dendritic cells, and natural killer cells, as well as $\text{Fc}\epsilon$ -receptor signaling in mast cells. The role of Btk in multiple cell types associated with autoimmune disorders and the positive effect of Btk inhibitors dosed in animal models of inflammation make it an intriguing therapeutic target.

The critical role of Btk in B-cell signaling in most B-cell malignancies made it a rational target for use in the oncology setting [12]. Interestingly, it has been found that treatment of CLL with ibrutinib (**1**) appears to greatly affect the tumor microenvironment and integrin-mediated cell adhesion of tumor cells in the bone marrow and lymph nodes [13]. Clinically, and counterintuitively, this can lead to a transiently high peripheral lymphocyte count as malignant cells are driven

into circulation, which is not indicative of disease burden [14]. It appears that Btk inhibition is not directly cytotoxic, but that the translocation of lymphocytic tumor cells out of the supporting tumor microenvironment leads to impaired proliferation and survival.

The rich biology of Btk inhibition in oncology and other indications (autoimmune disease) will continue to be defined as compounds such as ibrutinib (**1**) continue to be studied both clinically and experimentally. The covalent irreversible inhibition of Btk by **1** has proven to be of immense clinical benefit, even while the exact modes of action continue to be discovered. For example, although treatment with ibrutinib (**1**) shows a profound effect on disease remission and survival in patients, reports of a C481S mutation of the critical cysteine in Btk and subsequent loss of activity have surfaced [15]. As described later in the section regarding structure–activity relationships, targeting cysteine as in **1** has allowed for the development of highly selective Btk inhibitors. However, despite high selectivity achieved with cysteine targeting, it also appears that strong inhibition of other kinases sharing the cysteine, notably Itk, may indeed play a critical therapeutic role, particularly in the area of immuno-oncology [16]. The development of additional clinical Btk inhibitors with differentiated selectivity profiles may help define the therapeutic role, if any, of these other kinases.

14.5

Structure–Activity Relationships

The SARs surrounding the development of ibrutinib (**1**) is largely driven by the presence of the acrylamide linkage and its irreversible binding to Cys481 of Btk. The corresponding cyclopentane analog **2** is a relatively potent ($IC_{50} = 8.2$ nM) inhibitor of Btk, but is an even more potent inhibitor of Lck ($IC_{50} = 4.6$ nM) and Lyn ($IC_{50} = 2.5$ nM). In contrast, the racemic version of **1** strongly boosted Btk activity ($IC_{50} = 0.7$ nM) while decreasing the potency against Lck ($IC_{50} = 97$ nM) dramatically, validating the effectiveness of the approach to gain selectivity. In addition to acrylamides, a variety of other electrophiles were profiled in the initial report (Figure 14.2). In general, excellent activity was found in the apparent

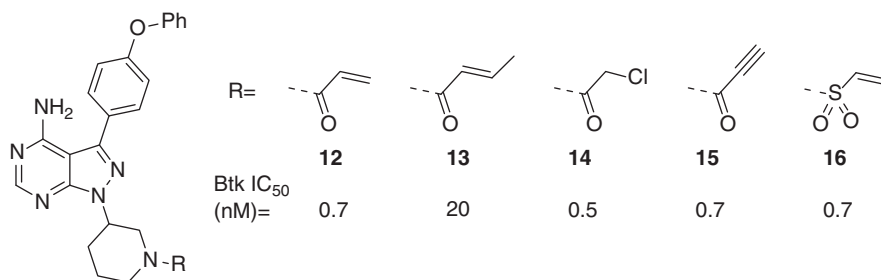


Figure 14.2 Btk potency of inhibitors incorporating different electrophiles.

IC_{50} for most of the electrophiles studied, with the exception of the substituted acrylamide **13**, which was significantly less potent.¹⁾

Various linkers besides the 3-piperidine were also reported in the initial Celera publication, including 4-piperidines, 3-pyrrolidines, and 4-aminocyclohexanes, but the piperidine substituted at the 3-position appears to be preferred for Btk inhibition. With an apparent IC_{50} of <0.5 nM, the (*R*) enantiomer **1** was found to be more potent than the corresponding (*S*) enantiomer (IC_{50} = 1.6 nM).

The selectivity of **1** in standard biochemical assays likely underrepresents the high selectivity within the kinome due to the irreversible nature of the interaction with Btk's Cys481 combined with the rapid clearance of the drug from systemic exposure. Even for kinases where the apparent IC_{50} is quite low, the concentration of drug is only transiently above this level and the effects are therefore mitigated. Within the 10 kinases, which contain a homologous cysteine, however, the selectivity is significant. Among these 10, EGFR is known to be associated with side effects, such as often severe rashes on the face and upper body, and indeed is inhibited by **1**. As described in the mechanism of action section, the inhibition of other kinases may possibly be of clinical benefit (Itk IC_{50} = 2.2 nM) [17]. The selectivity might be due to the fact that engagement of the cysteine is driven by other binding events that keep the electrophile in close proximity.

The acrylamide functionality of ibrutinib (**1**) has been used not only for targeting Cys481 but also as a functional handle for incorporating various molecular probes [8]. The compound PCI-33380 (**17**) (Figure 14.3) incorporates a boron-dipyrromethene (BODIPY) fluorescence probe. This cell-permeable, irreversible Btk inhibitor allowed researchers to quantitate the amount of Btk occupied by inhibitors in various settings. For instance, in pulse studies in primary B cells, a 10 nM concentration of **1** was found to fully occupy the active site of Btk, in accord with the concentration required to inhibit B-cell activation

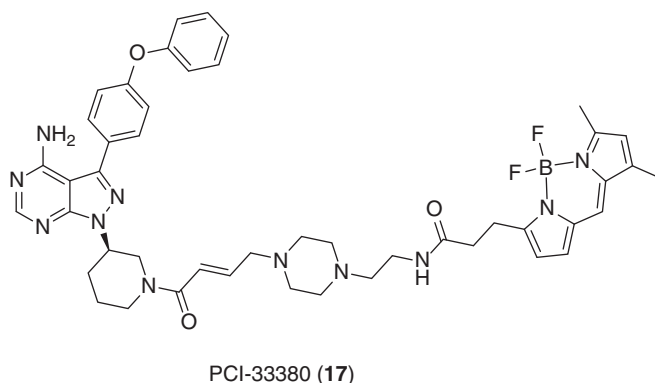


Figure 14.3 Irreversible fluorescence probe PCI-33380 (**17**) used in Btk occupancy studies.

1) Note that apparent IC_{50} values reported for irreversible inhibitors are dependent upon incubation time. Please see individual references for specific conditions used to measure the inhibitory activity.

(using a CD69 readout). The same probe was utilized to connect Btk occupancy with efficacy in a mouse model of arthritis, where a 12.5 mg/kg dose was sufficient to fully occupy the target enzyme in splenocytes for 12 h. Since the probe can be utilized in peripheral blood mononuclear cells (PBMCs), it also provides an excellent means to measure pharmacodynamic (PD) effects *ex vivo* in preclinical and clinical settings.

The rate of reaction of standard acrylamides with other endogenous thiols is relatively low, as seen by the maintenance of potent activity in the presence of added thiol or in cell-based assays [18]. Cravatt and coworkers [19] have recently utilized activity-based protein profiling (ABPP) to demonstrate that at high concentrations in cells, a large number of kinases and nonkinase off-target proteins can react with seemingly very selective acrylamide-based inhibitors. Structural analogs **18** and **19** (Figure 14.4), maintaining similar Btk activity to **1** and **13**, incorporate an alkyne to allow coupling to an azide-rhodanine tag via click chemistry. After incubation of **18** or **19** in cells or cell lysates at varying concentrations, incorporation of the tag allows one to visualize the proteins to which the probes bound via sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and in-gel fluorescence scanning [20]. Using this method, Cravatt and coworkers uncovered multiple expected bands for the covalent binding of the probes to Btk and EGFR as well as unexpected bands, indicating the attachment to other enzymes that also contain reactive cysteine moieties. Utilizing tools of this sort to determine selectivity of irreversible inhibitors in cells may prove useful in determining the full selectivity profile of irreversible inhibitors.

Additional irreversible inhibitors have entered clinic trials for oncology in recent years. Avila has initiated clinical trials for CC-292, formerly AVL-292, (**20**) (Figure 14.5), an acrylamide targeting the same Btk Cys481 residue as ibrutinib (**1**) [21]. Notably in this case, the acrylamide is connected via an aniline linkage in contrast to the tertiary amide in **1**. Compound **20** is a very potent Btk inhibitor

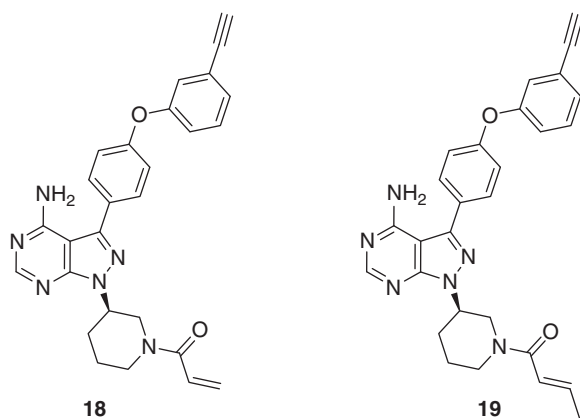


Figure 14.4 ABPP probes developed to study the selectivity profile of irreversible Btk inhibitors.

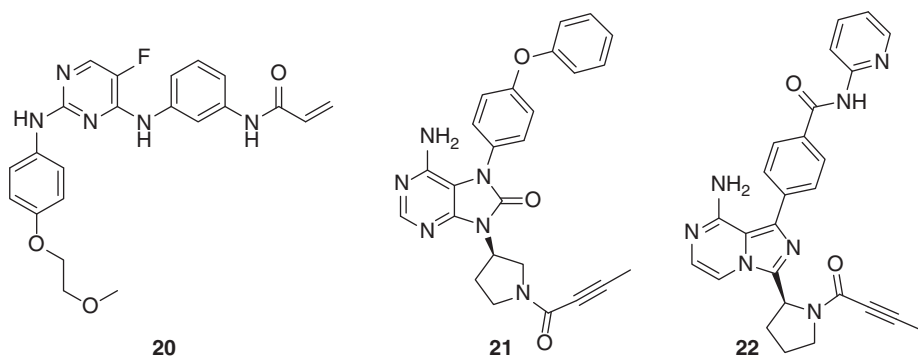


Figure 14.5 Irreversible Btk inhibitors incorporating carboxamide moiety.

with an apparent $IC_{50} < 0.5$ nM and with an excellent selectivity profile. Within the family of kinases containing an analogous cysteine, it was reported to only covalently modify the kinases Bmx, Itk, Tec, and Txk. Interest in Btk inhibitors has risen dramatically since the initial clinical reports with ibrutinib (**1**), and many companies have moved into clinical trials with their own drug candidates. A second Btk inhibitor, ONO-4059 (GS-4059) has reportedly completed phase I studies and is being evaluated in B-cell malignancies. A newer patent application from Ono describes the use of the Btk inhibitor **21** in conjunction with anti-CD20 antibodies [22]. Interestingly, this molecule incorporates an acyl alkyne as the likely electrophile targeting Cys481 of Btk. Acerta has recently published the use of Btk inhibitor **22**, which also incorporates an alkynyl amide moiety [23]. Further details of this emerging electrophile class compared to the acrylamides exemplified by **1** and **20** will likely emerge over time. Nonetheless, the variety of inhibitors underscores the key role of the carboxylic amide as a lynchpin for incorporation of electrophilic groups in Btk inhibitors.

Taunton and coworkers have pioneered the development of cyanoacrylamide-based covalent inhibitors, which are reversible in nature [24]. The off-rates of these inhibitors are determined by the fundamental reactivity of the electrophile

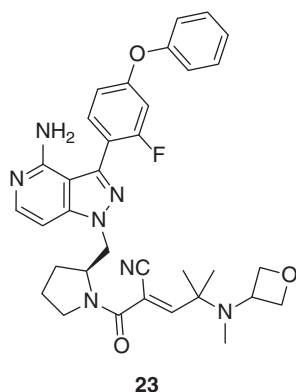


Figure 14.6 The cyanoacrylamide-based covalent reversible Btk inhibitor **23**.

combined with the various stabilizing interactions between the target protein and the inhibitor. Therefore, endogenous thiols such as glutathione, which lack a drug-gable binding pocket, should exhibit fast off-rates compared to the binding of inhibitor on a protein target. This approach was applied to Btk inhibitors in a recent publication by Bradshaw *et al.* [25], where he showed that compound **23** (Figure 14.6) displayed a residence time on Btk of approximately 1 week in biochemical occupancy studies.

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Part VI

Anti-Inflammatory Drugs

15

Fumaric Acid Esters

Tony S. Gibson

15.1

Introduction

Fumaric acid esters (FAEs, **1**) also known as fumarates (Figure 15.1), are an example of elegant medicinal chemistry solutions in the treatment of disease. FAEs (**1**) are relatively simple chemical compounds that are characterized by *trans*-(*E*) α,β -unsaturated carboxylic acid esters. The unsaturated carboxylic ester moiety lends this class of compounds to be highly electrophilic and therefore able to readily undergo Michael-type addition reactions. The molecular properties of FAE (**1**), as any other drug, are critical to the pharmacokinetics in the human body, which includes absorption, distribution, metabolism, and excretion (ADME). Thus, the simplicity of FAE (**1**) allows these pharmaceutical agents to follow the well-publicized Lipinski rule of five; molecular mass <500 Da, $-2.0 < \text{clogP} < 5$, hydrogen bond donors <5, number of hydrogen bond acceptors <10 [1]. While the relative simplicity of FAE (**1**) may not invoke the inner passion of drug design from medicinal chemists, this class of compounds has been proven to be successful in the treatment of disease.

15.2

History

FAEs (**1**) were first proposed in 1959 by the German biochemist Schweckendiek for the treatment of Psoriasis vulgaris [2]. Psoriasis vulgaris is a chronic inflammatory skin disease that is often characterized by scaly plaques that can affect large portions of the skin and is estimated to affect approximately 1–3% of the Caucasian population [3]. Schweckendiek, who was afflicted with psoriasis, hypothesized that the disease occurred as a result of low levels of fumaric acid (**1c**) in the citric acid cycle, which would lead to immune dysregulation [4, 5]. Thus, Schweckendiek proposed that fumaric acid supplementation would stimulate the immune system, providing relief to his symptoms. The treatment regimen involved fumaric acid (**1c**) in both topical and oral form. Due to the acidity of

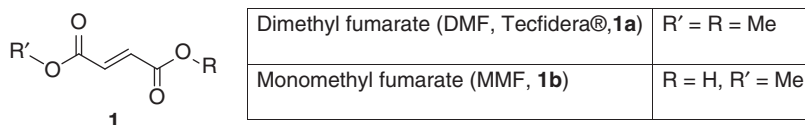


Figure 15.1 Basic structure of fumaric acid esters (**1**).

fumaric acid (**1c**), Schweckendiek assumed that its oral administration would likely cause increased gastrointestinal irritation for patients; he therefore decided to first test his hypothesis by self-administration of a mixture of FAEs (**1**). While Schweckendiek's original hypothesis regarding decreased levels of fumaric acid (**1c**) in the citric acid cycle was never verified, patients that were stricken with psoriasis appeared to benefit from the oral treatment of FAE (**1**) with dimethyl fumarate (DMF, **1a**) and mono ethyl fumarate (MEF, **1d**) salts. During the 1970s, the German general practitioner Gunther Schäfer implemented a standard treatment for psoriasis that included FAE (**1**) as both topical and oral supplementation. However, many German dermatologists were not convinced of the efficacy FAE (**1**) had on psoriasis, and there were growing concerns that the compounds were associated with acute renal toxicity, all of which eventually led to discontinued usage.

During the 1980s, a renewed interest in FAE (**1**) began to occur among both dermatologists and psoriasis patient groups, which eventually led to the first clinical observational studies conducted in dermatology centers in both Switzerland and the Netherlands. The published results of these studies confirmed both the efficacy of FAE treatment for psoriasis and an acceptable safety profile among psoriasis patients. As a direct result of Schweckendiek's early experimentation, a mixture of DMFs (**1a**) along with the Ca, Mg, and Zn salts of the corresponding monoethylester (ethyl hydrogen fumarate (EHF) or MEF, **1d**) was approved in Germany as oral therapy for severe psoriasis under the brand name Fumaderm® in 1994 (Table 15.1) [6]. Fumaderm is manufactured and codistributed by Biogen (Cambridge, MA) and Almirall-Hermal GmbH (Reinbek, Germany). In 2008, Fumaderm treatment was expanded to include moderate psoriasis patients. While FAEs (**1**) are currently only licensed in Germany for the treatment of moderate-to-severe psoriasis, they are the most prescribed oral systemic treatment for severe psoriasis in Germany.

Table 15.1 Approved drugs that contain dimethyl fumarate (**1a**).

Brand name	Indication	Active ingredient	Approved
Fumaderm®	Psoriasis	Mixture of dimethyl fumarate (1a) and monoethylfumarate (1d) Ca, Mg, and Zn salts	Germany
Tecfidera®	Multiple sclerosis	Dimethyl fumarate (DMF, 1a)	The United States, Canada, Australia, and the European Union

Beginning in 2013, an oral formulation of DMF (**1a**, formerly known as BG-12 and now sold under the brand name Tecfidera® by Biogen) was approved as an oral relapsing-remitting multiple sclerosis (MS) therapy. MS is the most common autoimmune disorder of the central nervous system. As of 2013, there were approximately 2.5 million people afflicted with MS worldwide [7]. MS is characterized by inflammatory demyelination of nerve cells in the brain and spinal cord. The resulting damage disrupts communication between the rest of the body, which results in progressive neurodegeneration and disability. As a result, the nerves themselves may deteriorate, undergoing a process that is irreversible. MS often occurs in a relapsing-remitting form, which is characterized by periods of isolated attacks followed by periods in which symptoms may disappear completely. While symptom-modifying agents exist and are effective at slowing disease progression, there is, to date, no curative therapy.

Tecfidera (**1a**) is administered as an oral capsule BID, beginning as 120 mg dose and moving to the full dose of 240 mg during the second week [8]. The most common side effects associated with taking DMF (**1a**) are flushing of the skin (redness, itching irritation, and burning), nausea and vomiting, heartburn, abdominal pain, and diarrhea.

15.3

Synthesis

The original synthesis of FAE (**1**) involved condensation of fumaric acid with an appropriate alcohol under acidic conditions. DMF (**1a**) is manufactured from readily available feedstock chemicals manufactured, using sulfuric acid as a catalyst. The final product is crystallized and milled, providing an overall yield of 78% for the entire process from fumaric acid (**1c**) to milled drug [9]. DMF exists as a white crystalline solid and has the following molecular properties, molecular mass of 144 Da, $\log P$ 0.7, Fsp^3 0.33, and topological polar surface area 52 Å². Mono methyl fumarate (MMF, **1b**) is formed in a similar manner to that of DMF (**1a**). Fumaric acid (**1c**) is rapidly converted into MMF (**1b**), and the time required to generate MMF (**1b**) depends on the amount of sulfuric acid catalyst used [9].

15.4

Mode of Action

DMF (**1a**) is one of the first small molecules that is responsible for improving two autoimmune diseases, psoriasis and MS, yet the mechanisms underlying the therapeutic efficacy remain unclear. There is strong evidence that DMF (**1a**) and its primary metabolite MMF (**1b**) act on the nuclear factor (erythroid-derived)-like-2 (Nrf2) pathway, which is often thought of as the master regulator of cytoprotective responses such as antioxidant, detoxification, and cell defense gene expression to exogenous stresses caused by reactive oxygen species (ROS)

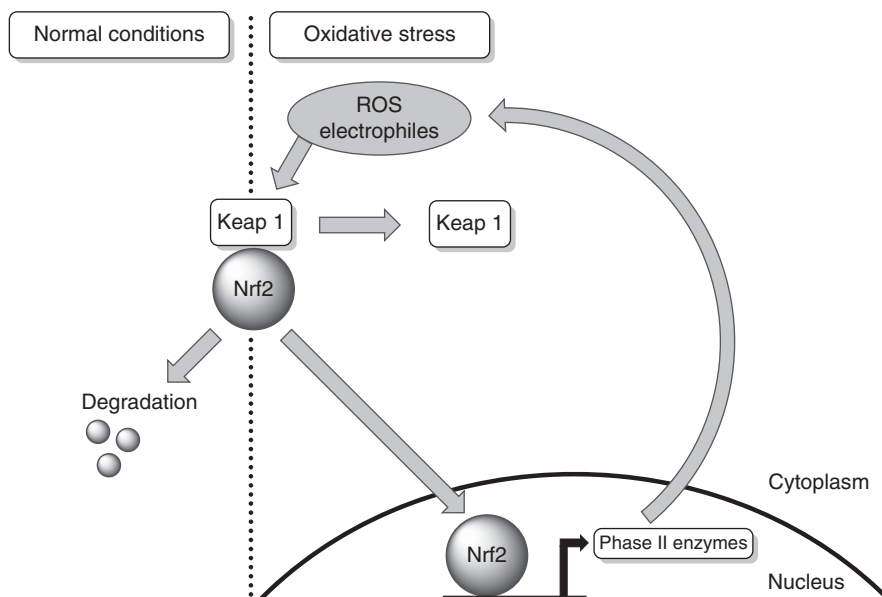


Figure 15.2 Simplified diagram of the Keap1 Nrf2 pathway.

and other electrophiles. Nrf2 is expressed ubiquitously in a range of tissues and, under normal conditions, is associated with cysteine-rich Keap1 (Kelch-like ECH-associated protein 1, Figure 15.2) [10, 11].

Bound Nrf2 is transcriptionally inactive and is degraded in the cytoplasm. Under the influence of oxidative stress, Keap1 is dissociated from Nrf2 by ROS and a variety of electrophiles. Nrf2 accumulates in the nucleus, activating the antioxidant response element (ARE). ARE in turn upregulates phase II antioxidants such as glutathione and detoxifying enzymes, which then act upon ROS and electrophiles such as the α,β -unsaturated carboxylic acid ester moiety of FAE (**1**) in order to relieve oxidative stress [7]. In human and rodent astrocyte cell cultures, Linker *et al.* have successfully demonstrated that both DMF (**1a**) and MMF (**1b**) led to a concentration-dependent increase in cellular levels of Nrf2, accompanied by prototypical phase II metabolizing enzymes such as glutathione [12]. Furthermore, studies on Nrf2 knockout mice showed that most of the therapeutic ability of FAE (**1**) had been diminished in the absence of Nrf2. Michael acceptors such as DMF (**1a**) have been shown to activate Nrf2 through the reaction of carboxylic acid esters that bind to glutathione, the cell's most important scavenger of ROS, and in the process depletes intracellular glutathione levels. Oxidative stress and inflammation are thought to be important in the modulation of immune-mediated inflammatory pathways responsible for both psoriasis and MS. Regulation of redox-sensitive pathways in which DMF (**1a**) reacts with glutathione plays a critical role. The depletion of cellular glutathione may affect the body's capacity to cope with oxidative stress and as a result helps control the cellular response. Ultimately, the activation of the Nrf2 pathway is

thought to contribute to the dual anti-inflammatory and neuroprotective effects associated with FAE (1).

The upregulation of phase II metabolizing enzymes brought about by DMF (1a) and MMF (1b) leads to an inhibitory effect on the activation of nuclear factor kappa B (NF- κ -B), which is a pivotal regulator of immune responses, inflammation, cell proliferation, and apoptosis. NF- κ -B is a crucial regulatory element in a variety of immune and inflammatory pathways and the ability of DMF (1a) to inhibit the production of NF- κ -B-dependent cytokines may be critical in its mode of action and help explain the beneficial effects of treatment of psoriasis patients with DMF (1a). Evidence of this hypothesis is supported when psoriasis patients that were administered DMF (1a) were shown to have increased levels of anti-inflammatory cytokine IL-4 [7].

Figure 15.3 outlines the combined immunomodulatory, anti-inflammatory effects attributed to DMF (1a). This combination of anti-inflammatory and immunomodulatory effects of DMF (1a) is thought to be beneficial in diseased patients with either MS or psoriasis.

15.5

Structure–Activity Relationships

No studies regarding any structure–activity relationships (SARs) toward improving the efficacy of Tecfidera (1a) have been published. This paragraph therefore focuses on the SAR developed with respect to the metabolism of this drug.

There has been an extensive amount of *in vitro* data collected in order to characterize the pharmacokinetics of FAE (1). The partial hydrolysis of DMF (1a) to MMF (1b) occurs rapidly in an alkaline environment (pH 8), mimicking the pH of the small intestine, with a half-life of approximately 1.5 h. There is no effect of hydrolysis in an acidic environment (pH 1) mimicking the pH of the stomach [13].

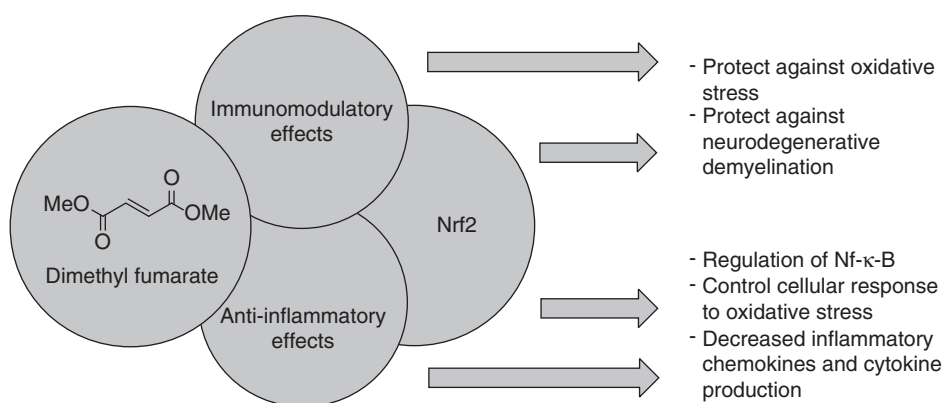


Figure 15.3 Combined immunomodulatory, anti-inflammatory effects attributed to DMF directly or by way of interaction with Nrf2.

It is also interesting to note that the rate of hydrolysis in a buffer of pH 7.4 is slow with a half-life of 12.7 ± 1.0 h, but in human serum, at the same pH, DMF (**1a**) is rapidly hydrolyzed [13]. Furthermore, the half-life of DMF in whole blood is even shorter than that in serum. The metabolic fate of DMF (**1a**) is not entirely understood; however, the inherent reactivity associated with α,β -unsaturated carboxylic acid esters would lead one to believe that rapid metabolism of said functional group occurs *in vivo*. Additionally, *in vitro* data has been generated with DMF (**1a**) and only the MMF (**1b**) hydrolysis product has been detected in peripheral blood of treated MS or psoriasis patients. This is evident in the fact that DMF (**1a**) has a short degradation half-life and is rapidly hydrolyzed by esterases to MMF (**1b**) before reaching systemic circulation.

Exposure to various organ preparations such as pancreatic extract, intestinal perfusate and homogenate, along with liver S9 fraction, results in the complete degradation of DMF (**1a**) within 1 min. It has also been shown that DMF (**1a**) is degraded faster in the intestinal perfusate and homogenate than in the presence of pancreas extract. This might be explained by the fact that enzymes in the pancreas extract such as trypsin, chymotrypsin, lipase, and amylase hydrolyze DMF (**1a**) less effectively compared to esterase's present in intestinal perfusate and homogenate. The monoester derivatives as a whole have a much longer degradation half-life when exposed to the liver S9 fraction. In studies performed by Werdenberg *et al.*, no significant reduction in the concentration of MMF (**1b**) was observed in the intestinal perfusate and homogenate after 120 min [14]. This might lead one to believe that the pharmacological activity of DMF (**1a**) is likely due to the active metabolite MMF (**1b**). Any DMF (**1a**) not hydrolyzed in the small intestine may be converted into MMF (**1b**) in the circulatory system by way of esterases. MMF (**1b**) is then further metabolized through the citrate cycle to fumarate (**1c**) and CO_2 , with exhalation of CO_2 being the primary route of elimination. Additionally, small amounts of MMF (**1b**) are excreted through urine or feces.

The rate of hydrolysis of DMF (**1a**) to MMF (**1b**) suggests that **1b** may be the active pharmaceutical agent. However, there is also contradictory evidence to that hypothesis that suggests the more lipophilic nature of DMF (**1a**) as compared to MMF (**1b**) may play a more pronounced role and therefore **1a** being the active pharmaceutical agent. There is evidence that suggests that the lipophilic nature of DMF (**1a**) allows it to quickly penetrate across cell membranes in larger quantities after *p.o.* administration, as compared to MMF (**1b**). Because of these inconsistencies, the mechanism for which DMF (**1a**) exerts any pharmacological effect upon *p.o.* administration is still unclear. It is also entirely possible that there is a completely unknown mechanism of action or alternate pathway that is responsible for the drugs' pharmacological effects.

The α,β -unsaturated carboxylic acid esters possess electrophilic reactivity and therefore inherently can undergo a Michael-type addition reaction. The activated α,β -unsaturated carbonyl of FAE (**1**) can readily undergo conjugate addition with an acceptable reactive donor. Due to the soft electrophilic nature of Michael acceptors, FAE (**1**) will react most efficiently with soft nucleophiles as that of glutathione. Glutathione, which is present in millimolar concentration

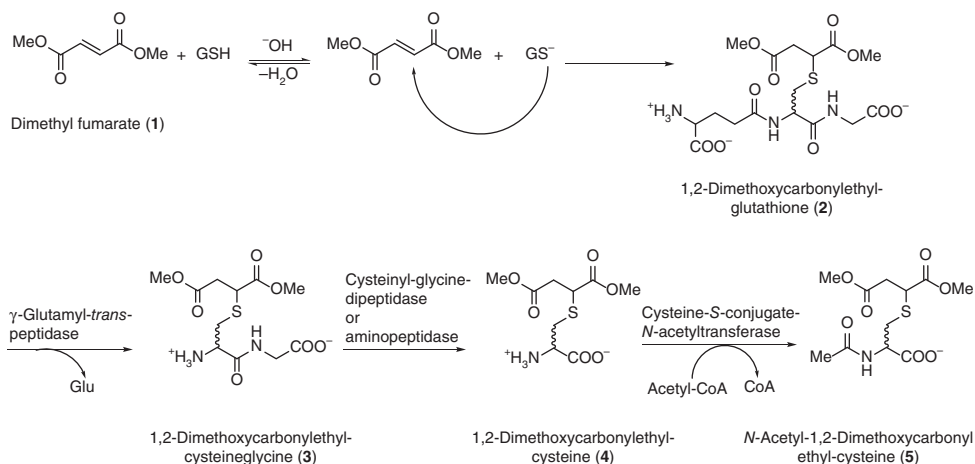


Figure 15.4 Reaction between DMF (**1a**) and glutathione in which the initial rate of reaction is pH-dependent.

in most cells, is a major endogenous antioxidant and participates directly in the neutralization of free radicals along with reactive oxygen compounds. It has been demonstrated by Schmidt *et al.* that glutathione reacts with DMF (**1a**) exhibiting pseudo-first-order kinetics in a pH-dependent manner [15]. Figure 15.4 shows the reaction between DMF (**1a**) and glutathione and the subsequent metabolites formed.

Upon reaction of DMF (**1a**) with glutathione, a series of enzymatically catalyzed reactions occur, resulting in the formation of *N*-acetyl-1,2-dimethoxycarbonyl-ethyl-cysteine (**5**). *In vivo* studies performed by Rostami-Yazdi demonstrated that *N*-acetyl-1,2-dimethoxycarbonyl-ethyl-cysteine (**5**) was present in patient's urine in relatively high concentrations 4 h after oral intake of two tablets of Fumaderm (240 mg DMF (**1a**)) [16]. Moreover, it has been shown that the reaction of glutathione with MMF (**1b**) proceeds at a rate approximately 30 times slower than that of DMF (**1a**), thus implying that MMF (**1b**) is less affected by the promiscuity of spontaneous glutathione conjugation (Figure 15.5) [15]. In rat *in vivo* studies, performed by Rostami-Yazdi *et al.*, the portal vein blood was analyzed for the presence of DMF (**1a**), MMF (**1b**), and glutathione adducts of both MMF (**1b**) and DMF (**1a**) after DMF (**1a**) was given directly into the small intestine [17]. The results of the experiment showed that no free DMF (**1a**) was present in the plasma of portal vein blood, only MMF (**1b**) was found. The glutathione adducts of DMF (**1a**) were present in the portal vein blood. Furthermore, it has been shown that this reaction also occurs *in vivo* by the detection of *N*-acetyl-1,2-dimethoxycarbonyl-ethyl-cysteine (**5**) in the urine of psoriasis patients after oral intake of Fumaderm. Since *N*-acetyl-1,2-dimethoxycarbonyl-ethyl-cysteine (**5**) is the by-product of the DMF-glutathione conjugate, it has been hypothesized that DMF (**1a**) is absorbed from the small intestine and then enters the portal vein where it is then able to undergo conjugate addition to glutathione

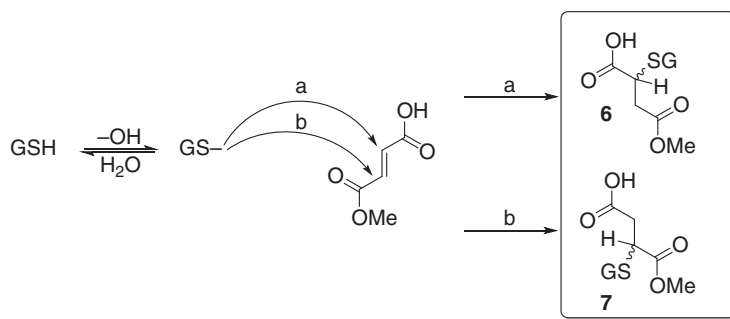


Figure 15.5 Reaction between MMF (**1b**) and deprotonated glutathione provides equimolar amounts of **6** and **7** via path a or b. Glutathione reacting with neither protonated MMF (**1b**) does not occur nor does deprotonated glutathione react upon deprotonated MMF (**1b**).

by penetrating into circulating cells. Only DMF (**1a**) not MMF (**1b**) due to its lipophilic nature would be able to penetrate into circulating cells and undergo conjugate addition to glutathione. Cytochrome P450 (phase I metabolism) is an essential detoxifying enzyme system of the body. There is no evidence that DMF (**1a**) undergoes metabolism by way of cytochrome P450 enzymes in the liver or p-glycoprotein systems.

The permeability of DMF (**1a**) has been studied in the Caco-2 cell system and was found to be approximately 10-fold higher than MMF (**1b**) [14]. DMF (**1a**) has been shown to be quite unstable in biological media due to rapid hydrolysis when compared to MMF (**1b**). However, due to the higher lipophilicity of DMF (**1a**), it is possible that the rapid penetration of **1a** into tissue allows the drug to reach the site of action at higher concentrations than MMF (**1b**). In the target tissue, DMF (**1a**) would then be degraded to MMF (**1b**). MMF (**1b**) has been shown to display protein binding of 50%, while DMF (**1a**) does not appear to bind to serum proteins. The fact that DMF (**1a**) does not appear to bind to serum proteins may contribute to the rapid turnover in circulation.

Discovered over 50 years ago, small-molecule FAEs (**1**) have proven to be viable drugs that are responsible for improving two autoimmune diseases, psoriasis and MS. Low manufacturing cost equated with the drugs' synthetic simplicity combined with acceptable ADME properties have resulted in a successful class of pharmaceutical agents. While the mechanism of action and metabolic fate remain unclear, there is no debate surrounding the success that FAEs (**1**) have had in the pharmaceutical marketplace.

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16

Carboxylic Acid Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

Yan Lou and Jiang Zhu

16.1

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most important drug classes that provide analgesic, antipyretic, and at higher doses, anti-inflammatory effects in various pathological conditions. The term “nonsteroidal” is in comparison to steroids, which possess similar broad anti-inflammatory effects. There are a few dozens of NSAIDs approved, and as a class, these are among the most commonly prescribed drugs. The most well-known members are aspirin (1), ibuprofen (2), and naproxen (3) (Figure 16.1), widely used to treat acute or chronic pain and inflammation. In 2001 alone, over 30 billion doses were sold and there were 7 million prescriptions of NSAIDs in the United States [1].

Depending on their selectivity for the cyclooxygenase COX1/2 isoforms, which are the primary biological targets for NSAIDs (as described in detail later), NSAIDs are divided into classical (non-COX2 selective) and COX2 selective categories. Many older NSAIDs were known before their mechanism of action was elucidated, and thus, they were classified based on their chemical structures or origin. NSAIDs can be grouped into the following: (i) salicylates, for example, aspirin (1); (ii) propionic acid derivatives, for example, ibuprofen (2) and naproxen (3); (iii) acetic acid derivatives, for example, indomethacin (4) and diclofenac (5); (iv) anthranilic acid derivatives; (v) enolic acid derivatives; and (vi) selective COX2 inhibitors, for example, celecoxib (6), rofecoxib (7), and lumiracoxib (8) (Figure 16.1).

This chapter discusses some of the most important drugs that contain a key carboxylic acid moiety, which is critical for their biological activity, specifically non-COX2 selective aspirin (1), ibuprofen (2), naproxen (3), indomethacin (4), diclofenac (5), and COX2 selective lumiracoxib (8).

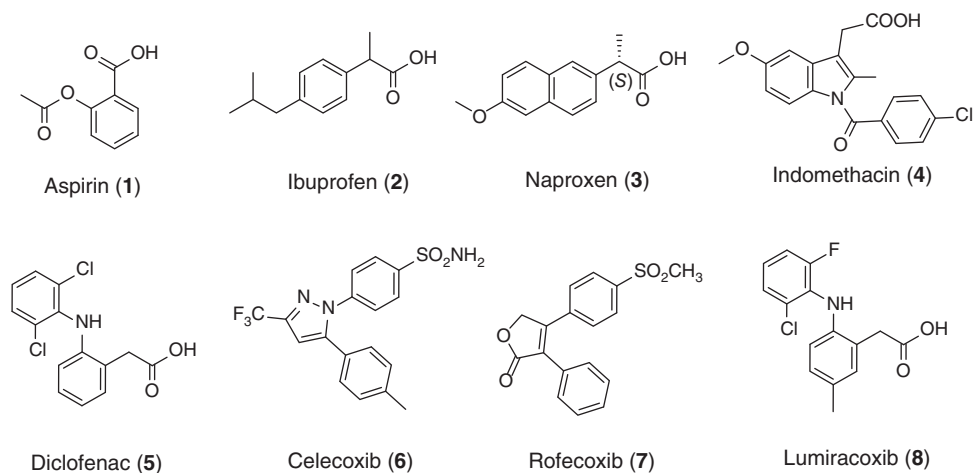


Figure 16.1 Selected examples of NSAIDs.

16.2

History

The evolution of NSAIDs showcases the significant transition of drug discovery from the early days of empirical art with folklore and mythological guidance to the modern days of thorough structure activity analysis against biological targets with powerful tools such as X-ray crystallography, computational modeling, biophysical characterization, biochemical assays.

The history of NSAIDs dates back a few thousand years to early uses of plant extracts containing salicylate. The Greek doctor Hippocrates first reported the use of willow bark as pain reliever. The active ingredient of willow bark, a glycoside called salicin, named after the Latin name for the white willow (*Salix alba*), was later isolated in 1827 by Johann Andreas Buchner. Salicylic acid (**9**) is more effective than salicin, but its strong acidity makes it very irritating to the stomach and its use causes serious gastrointestinal bleeding. In search for a superior salicylic acid derivative, Felix Hoffman at Bayer discovered acetylsalicylic acid (**1**) by acetylating the phenol group of salicylic acid (**9**) in 1897. It was found to be a more potent but less irritating anti-inflammatory agent, and Bayer introduced it to the market as aspirin (**1**) just 2 years later. In contrast to merely being a prodrug of salicylic acid (**9**), aspirin (**1**) has a unique mechanism of action. It covalently inhibits COX enzymes via the transfer of its acetyl group to a key serine residue, which will be discussed later in more details.

The remarkable success of aspirin (**1**) inspired extensive search for more effective and/or safer medicines to treat inflammation, pain, and fever. This effort resulted in the discovery of many NSAIDs, among which are ibuprofen (**2**), naproxen (**3**), and indomethacin (**4**). Ibuprofen (**2**) was originally discovered in 1961 by studying the activity of a novel set of nonsteroidal compounds in

reducing the inflammatory pain caused by UV light. It was first marketed in 1966 by Boots Company as *brufen* and is currently available with notable trade names such as *Advil*, *Motrin*, and *Nurofen*. Its efficacy is comparable to aspirin (1) but with diminished gastrointestinal irritation [2]. *Ibuprofen* (2) was marketed as a prescription drug in the United States in 1974. It became the second NSAID after aspirin (1) to be distributed over the counter later in 1983. A more potent propanoic acid analog, *naproxen* (3), with a naphthalene core, was discovered and marketed by Syntex in 1976. Its improved potency and longer half-life of ~12 h allow convenient once-daily dosing. Soon after its introduction as *Naprosyn* (and later as *Aleve*), the sales exceeded \$1 billion annually, making it arguably one of the first blockbuster drugs. It is also one of the very few propanoic acid NSAIDs marketed as a pure enantiomer [3]. Around the same time, scientists at Merck & Co. discovered *indomethacin* (4), an indole acetic acid analog, as a potent anti-inflammatory drug by screening 350 indole-containing compounds using a cotton string granuloma test as a model of inflammatory pain. *Indomethacin* (4) was approved by the FDA in 1965. It is a potent drug with many side effects including headache in 10–20% patients, which leads to its decreasing popularity nowadays. The discovery of an exceptionally potent and relatively well-tolerated acetic acid NSAID, *diclofenac* (5), was inspired by a pharmacophore model of *indomethacin* (4) [4]. The hypothesis of essential characteristics includes an acidity constant (pH value) between 4 and 5, a partition coefficient ($\log P$, *n*-octanol/aqueous buffer, pH 7.4) of approximately 10, and two aromatic rings twisted in relation to each other. Although the small-molecule conformation was confirmed by X-ray crystallography, interestingly, later protein X-ray structure work showed that *diclofenac* (5) binds to its biological targets with a different orientation from *indomethacin* (4). First registered as *Voltaren/Cataflam* by Ciba-Geigy (now Novartis) in 1973, *diclofenac* (5) is a leading NSAID for relieving symptoms of rheumatic diseases although its use has been linked to liver toxicity.

While these important NSAIDs were discovered using great insights on chemical structures and profiling in animal models, the lack of understanding of the mechanism of action hindered further progress to address the gastrointestinal toxicity until intense studies in this area culminated in a few breakthrough discoveries. The first breakthrough came in 1971 when Vane and others proposed, and confirmed later, that aspirin-like drugs blocked prostaglandin (PG) biosynthesis, which is catalyzed by COX [5]. A purified active COX enzyme was isolated a few years later in 1976 [6]. Another breakthrough in 1991 came with the discovery of a second COX isoform with a high amino-acid sequence homology but a different expression profile [7]. COX1 as the constitutive isoform is mainly responsible for the synthesis of cytoprotective prostaglandins in the gut, while COX2 as the inducible and short-lived isoform is upregulated upon stimulation with proinflammatory factors. Classical NSAIDs are nonselective inhibitors of both COX enzymes. This led to the “COX2 bad: COX1 good” hypothesis, which inspired the hunt for COX2 selective drugs [8]. A substantial drug discovery effort resulted in the introduction of *celecoxib* (*Celebrex*, 6, Pfizer, 1999) and *rofecoxib* (*Vioxx* 7,

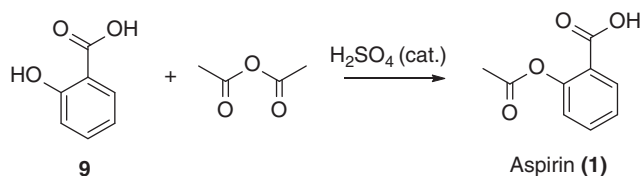
Merck, 1999) as a next generation of NSAIDs with reduced gastrointestinal toxicity [9]. Most COX2 selective drugs do not contain a carboxylic acid moiety due to selectivity issues arising from the acid moiety making productive nonselective interactions with both COX enzymes. Therefore, those drugs are beyond the scope of this chapter. However, the distinctive binding mode of diclofenac (**5**) allowed for selectivity improvements that led to the discovery of lumiracoxib (**8**), a close analog that differs in only two heavy atoms. Lumiracoxib (Prexige, **8**), discovered and developed by Novartis, was first approved in Europe in 2006. Unfortunately, the use of lumiracoxib (**8**) is also linked to hepatotoxicity, similar to diclofenac (**5**).

The great success of COX2 selective NSAIDs was found to have long-term cardiovascular safety issues from the results of two trials with rofecoxib (**7**) and celecoxib (**6**) [10]. This led to the subsequent withdrawal of rofecoxib (Vioxx, **7**) in 2004. The use of celecoxib (Celebrex, **6**) has also been diminished and restricted. Clinical studies have shown that less selective traditional NSAIDs such as naproxen (**3**) may have reduced cardiovascular risk [11]. On the other hand, NSAIDs have been found to increase the survival of various populations of cancer patients [12]. Biology and pharmacology of this fascinating class of drugs continue to evolve, and it is not unimaginable that new breakthroughs may emerge in the future.

16.3

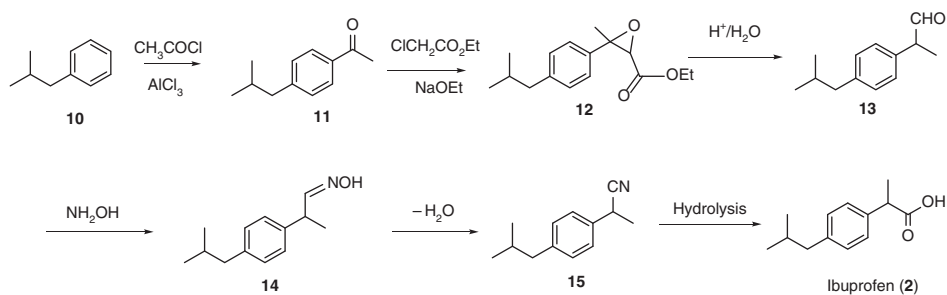
Synthesis

Aspirin (**1**) was simply made by the reaction of salicylic acid (**9**) with acetic anhydride in the presence of a catalytic amount of sulfuric acid as shown in Scheme 16.1. The initial process was modified by using acetic acid as the catalyst and patented in 1961 by Dow Chemical Co. [13].



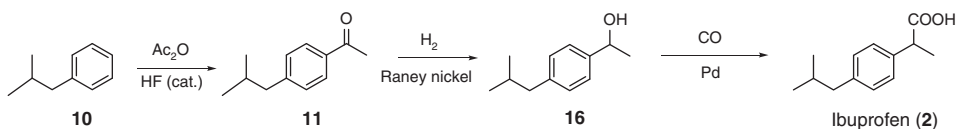
Scheme 16.1 Synthesis of aspirin (**1**).

The first commercial synthesis of Ibuprofen (**2**) was patented by Boots Company [14]. The synthesis was accomplished in six steps as shown in Scheme 16.2. In the process, isobutylbenzene (**10**) was acylated under Friedel–Crafts conditions to give ketone **11**. Treatment with ethyl chloroacetate in the presence of sodium ethoxide formed the epoxide **12**, which was hydrolyzed and decarboxylated to give aldehyde **13**. Aldehyde **13** was then reacted with hydroxylamine to form (**14**), followed by dehydration to form nitrile **15**. Ibuprofen **2** was obtained in racemic form by hydrolysis of **15**.



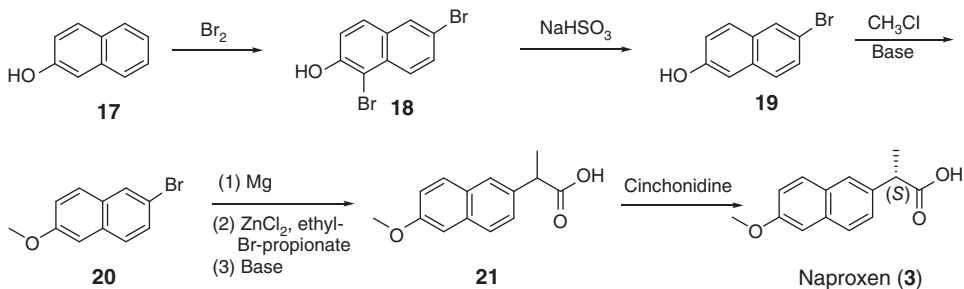
Scheme 16.2 Boots synthesis of ibuprofen (**2**).

In 1990, a much more efficient route was developed by The BHC Company (Scheme 16.3) [15]. This process, called Hoechst Synthesis, also started with isobutylbenzene (**10**). The acylation was carried out with acetic anhydride catalyzed by hydrogen fluoride to give ketone **11**. Reduction of **11** to the alcohol **16**, followed by Pd-catalyzed CO insertion gave ibuprofen (**2**) in only three steps. The BHC company won the prestigious Presidential Green Chemistry Challenge award in 1997 and the prominent Kirkpatrick Chemical Engineering Achievement Award in 1993 for this development.



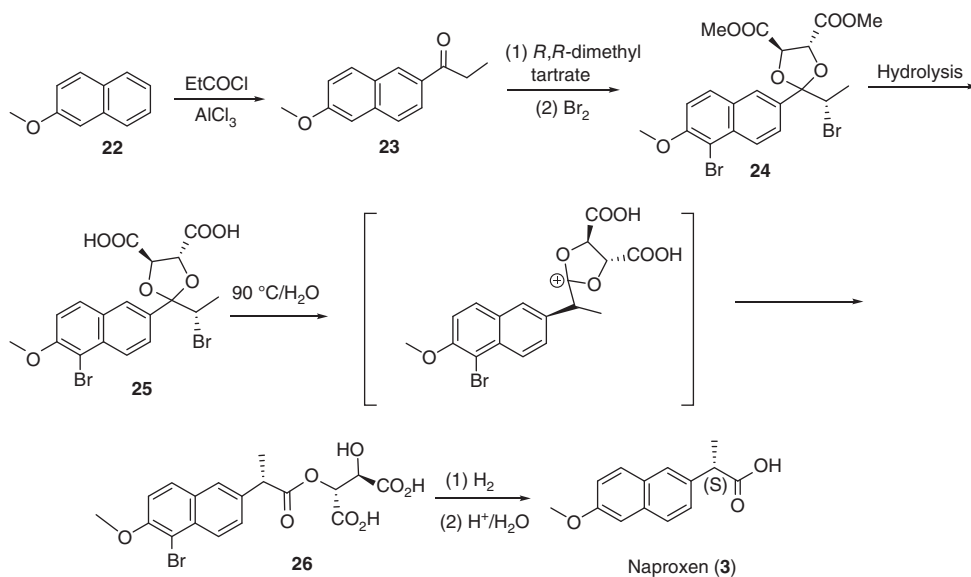
Scheme 16.3 Hoechst synthesis of ibuprofen (**2**).

The first commercial process to prepare naproxen (**3**) used naphthol (**17**) as starting material (Scheme 16.4). Bromination led to the dibromide **18**, which was selectively reduced with sodium bisulfite to give the monobromide **19**. After formation of the methyl ether **20**, the intermediate was converted to a Grignard reagent and reacted with ethyl bromopropionate. Ester hydrolysis to the racemic naproxen (**12**) and subsequent chiral resolution with cinchonidine yielded enantiomerically pure naproxen (**3**) [16].



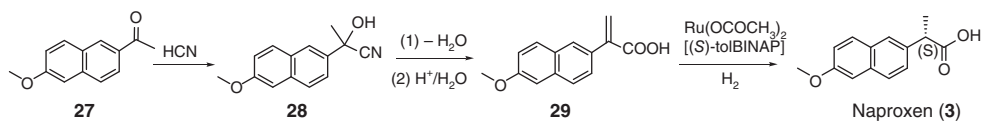
Scheme 16.4 First commercial synthetic route for naproxen (**3**).

The Zambon process is a well-known process, utilizing a chiral auxiliary for the stereoselective synthesis of naproxen (**3**) (Scheme 16.5) [17]. Starting from 2-methoxynaphthalene (**22**), Friedel–Crafts acylation furnished the ketone **23**. After acetalization of **23** with the dimethyl ester of L-(+)-tartrate, highly selective bromination in α -position of the acetal and in 5-position on the naphthyl ring generated the diastereomeric mixture (94:6) of the dibromide **24**. The diester was then hydrolyzed under basic conditions. Subsequently, **25** underwent acid-catalyzed 1,2-aryl shift with complete inversion of configuration at the migration terminus to give the 1-bromonaproxen ester **26**. Reductive removal of the bromo substituent from **26** followed by hydrolysis afforded naproxen (**3**) with >98% e.e. [18].



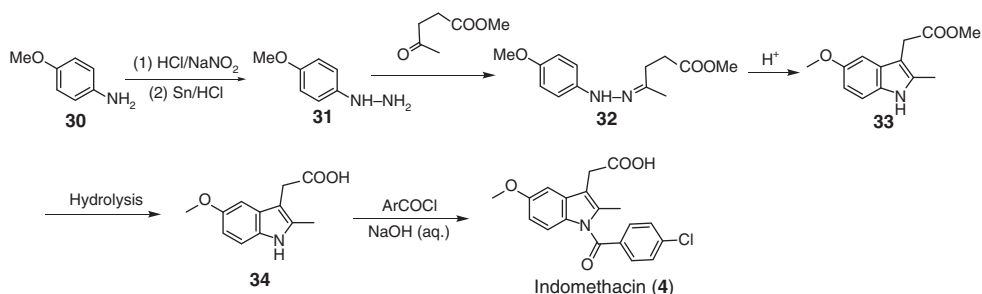
Scheme 16.5 Zambon process for asymmetric synthesis of naproxen (**3**).

Another important evolution is the use of a catalytic asymmetric hydrogenation to manufacture enantiomerically pure naproxen (**3**) (Scheme 16.6). The olefin precursor **29** was generated from ketone **27** through formation of the cyanohydrin **28** followed by elimination of the tertiary alcohol and ester hydrolysis. Naproxen (**3**) was then obtained with high enantiomeric purity (e.e. > 98%) under Noyori hydrogenation conditions [19].



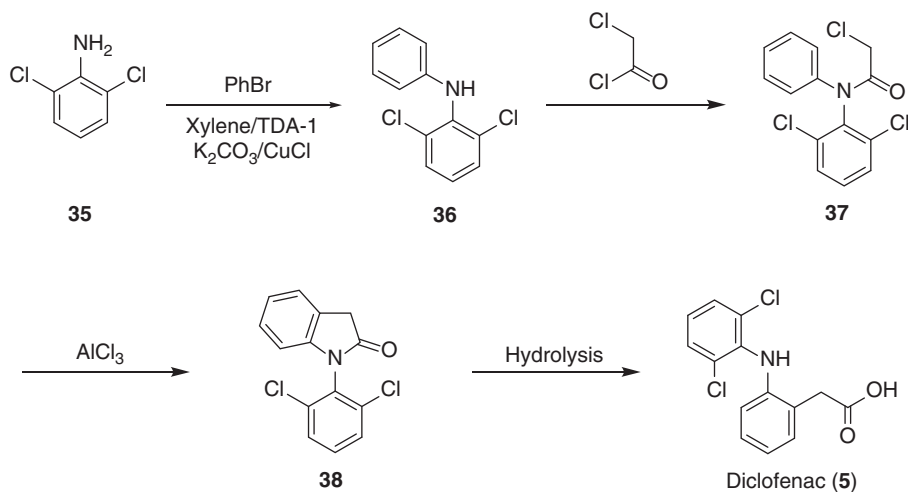
Scheme 16.6 Catalytic asymmetric hydrogenation synthesis of naproxen (**3**).

The production of indomethacin (**4**) via a Fischer Indole synthesis (Scheme 16.7) was developed and patented by Merck & Co. [20]. *p*-Methoxyaniline (**30**) was transformed into the hydrazine **31** via diazonium formation and reduction. Condensation of **31** with methyl levulinate formed the hydrazone **32**, which rearranged to the indole ester **33** under acidic conditions. Ester hydrolysis and subsequent *N*-benzoylation under basic condition gave the desired indomethacin (**4**).



Scheme 16.7 Merck's Fischer Indole synthesis-based route to indomethacin (**4**).

An efficient route to prepare diclofenac (**5**) was patented by Puig Torres and Baro at Esteve Quimica (Scheme 16.8) [21]. Coupling of 2,6-dichloroaniline (**35**) with bromobenzene produced the diphenylamine **36**. Acetylation of **36** with chloroacetyl chloride (**37**), followed by cyclization under Friedel–Crafts alkylation condition led to the oxindole **38**, which upon hydrolytic opening of the lactam ring provided diclofenac (**5**) in four steps. A similar procedure was reported to prepare the closely related lumiracoxib (**8**).

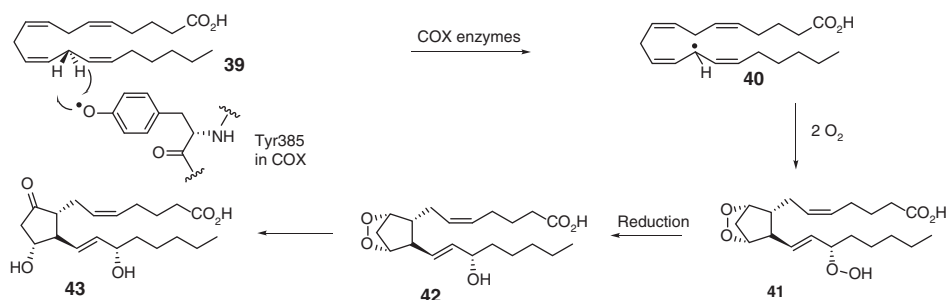


Scheme 16.8 Synthesis of diclofenac (**5**).

16.4

Mode of Action

The profound anti-inflammatory activity of NSAIDs is primarily attributed to the inhibition of the COX enzymes, although some of them have been shown to possess potent activity against other proteins that could contribute to their observed pharmacological effects [22]. COX enzymes, also known as prostaglandin-endoperoxide synthases (PTGS/PGHS), catalyze the rate-limiting conversion of arachidonic acid **39** (AA) to prostaglandins **41–43** (PGs) (Scheme 16.9) [23]. Prostaglandins are short-lived key mediators of inflammation, normally present in very low concentrations. Their synthesis in cell membranes starts with hydrogen abstraction at C₁₃ of AA (**39**) by an activated tyrosine oxygen radical **40**, followed by rapid combination with two molecules of oxygen to give PGG₂ **41**. Further transformations then produce PGH₂ **42** and PGE₂ **43**.



Scheme 16.9 Catalytic reaction of AA to PGs.

X-ray crystallographic studies, together with site-directed mutagenesis work, provided an understanding on the molecular level of how COX enzymes work and how NSAIDs inhibit COX enzymes. Figure 16.2a shows the AA (**39**) substrate bound in the active site of COX1 with Tyr385 in close proximity to the C₁₃ atom [24]. The hydrogen abstraction process is blocked when an inhibitor occupies the same active site and prevents the binding of AA (**39**), as shown in Figure 16.2b, with ibuprofen (**2**) bound to COX1 [25]. Published X-ray structures show that ibuprofen (**2**) [26a], indomethacin (**4**) [26b], diclofenac (**5**) [26c], and lumiracoxib (**8**) [26d] are competitive inhibitors of the COX enzymes.

With the exception of aspirin (**1**), all other approved COX inhibitors associate with the protein in a noncovalent manner. The most potent inhibitors exhibit slow, tight binding and form very stable complexes that are sometimes functionally irreversible. A two-step kinetic model was proposed with an initial rapid, reversible binding of the inhibitor to the enzyme, followed by an essentially irreversible time-dependent change in the enzyme–inhibitor complex. As a representative example, indomethacin (**4**) was shown to require the carboxylic acid as its methyl ester lacks the time-dependent step but retains the fast-reversible step

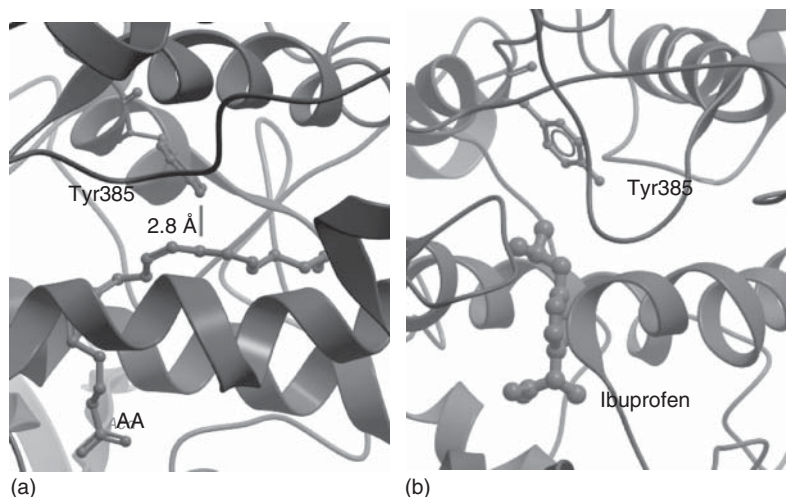


Figure 16.2 (a) Arachidonic acid (AA, **39**) bound to COX1 (pdb code: 1DIY). (b) Ibuprofen (**2**) bound to COX1 (pdb code: 1EQG).

[27]. The kinetics for the inhibitor binding has significant pharmacological implications because the prolonged COX inhibition due to the slow off-rate does not necessarily require sustained target coverage to achieve *in vivo* efficacy. This could provide a means to achieve kinetic selectivity against off-targets with fast off-rates, which would need sustained target engagement to manifest side effects. Naproxen (**3**) serendipitously shows time-dependent inhibition for COX2 but lacks the time-dependent component for COX1, which may contribute to the cardioprotective effects of naproxen (**3**) noted in clinical trials [28]. Optimization of off-rate and residence time has been increasingly recognized in the modern drug discovery effort and a systematic approach of using reversible “tailored covalency” by design generated inhibitors with durable inhibition of multiple kinase targets [29].

Aspirin (**1**) blocks COX via a completely different mechanism as it first binds to the enzyme reversibly but then transfers its acetyl group to the hydroxyl group of Ser530 [30]. Figure 16.3 shows bromoaspirin bound to COX1.

Its mode of action clearly contradicts the original hypothesis that aspirin (**1**) is a simple prodrug of salicylic acid (**9**). Mutagenesis studies of Ser530 to alanine showed that this amino acid is not important for the catalytic activity of COX, suggesting that its covalent modification works by blocking AA (**39**) binding to the COX active site. Aspirin (**1**) is an intriguing drug from many perspectives, and its unique mode of action inspired a lot of research to further elucidate the detailed biochemical action of how the acetylation occurs [31]. Mutagenesis and computational studies helped to establish a hypothesis of the substrate-assisted process shown in Scheme 16.10.

It proceeds in two successive steps: initially, while transferring its hydrogen to the carboxylate of aspirin (**1**), the oxygen atom of the hydroxyl group in Ser530 attacks the carbonyl carbon atom of the acetic ester moiety in aspirin (**1**) to

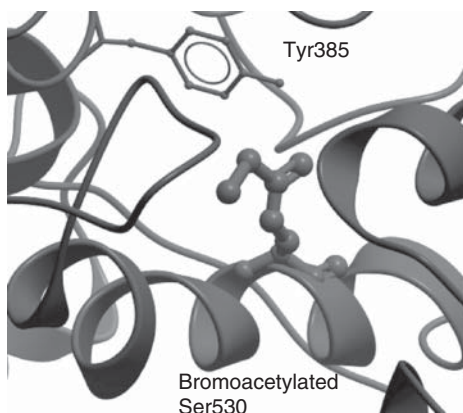
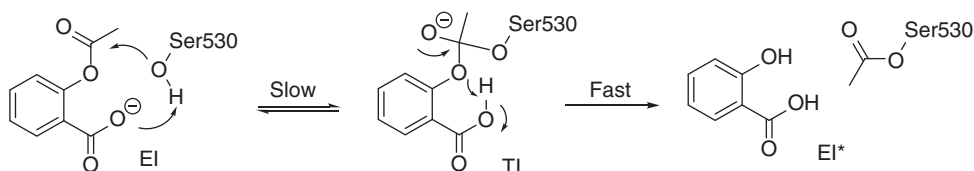


Figure 16.3 COX2 Ser530 modified as bromoacetate by bromoaspirin (pdb code: 1PTH).



Scheme 16.10 Reaction mechanism of aspirin (**1**) acetylating Ser530 of COX.

form TI. Subsequent rearrangement of the lone pair of the formed oxygen anion leads to the break of the C–O bond to yield salicylic acid (**9**) and the inactivated O-acetyl-COX (EI*). This represents a rare example of a covalent inhibition of an enzyme by targeting a noncatalytic serine residue, while targeted covalent inhibition of noncatalytic cysteine has enjoyed much greater success and attention such as the discovery of ibrutinib [32], also discussed in this book. The utilization of the carboxylic acid anion of aspirin (**1**) as an internal base to activate the otherwise relatively unreactive serine –OH group for its covalent addition to the acetyl functionality may inspire future drug discovery efforts to target noncatalytic serine residues.

16.5

Structure–Activity Relationships

As discussed in the previous section, the available evidence suggests that the anti-inflammatory and analgesic properties of traditional NSAIDs are due to the inhibition of COX2, whereas the gastrointestinal side effect is attributed to the inhibition of COX1. Given the different methods and conditions used in profiling, there has been much variability in the reported biochemical IC₅₀s for many of the NSAIDs. Further confounding the issue is that the apparent IC₅₀ does not

always correlate with the potency of inhibitors that are irreversible or have a very slow off-rate as discussed earlier [27]. Assays using intact cells, broken cells, and whole blood were developed in trying to bridge the gap. In particular, the human whole blood (HWB) assay has become a gold standard for assessing differential inhibition of the COX isoforms and later for other autoimmune drug discovery efforts [33]. The most attractive feature of the HWB assay is that the biochemical efficacy is measured in blood samples from humans (including patients) under physiologically relevant conditions. Table 16.1 lists the whole-blood activity for the comparison of COX selectivity, which spans a wide range from the most COX1 selective drug, aspirin (**1**), to the most COX2 selective drug, lumiracoxib (**8**) [26d, 34].

Much effort has been devoted to understand the structure–activity relationship (SAR)/selectivity relationship of NSAIDs. In particular, X-ray structure elucidations of the COX enzymes with and without bound ligands try to explain which role the carboxylic acid plays. Given the high amino-acid sequence homology (60%) of COX1 and COX2, and their common function of AA (**39**) catabolism, it is not surprising that the solution of their crystal structures revealed great similarity of their substrate binding sites. Crystal structures and molecular models of COX1 and COX2 complexed with the AA (**39**) indicate that the carboxylic acid of AA (**39**) forms ion-pair interactions with the guanidinium group of Arg120 and hydrogen bonding interactions with Tyr355 (Figure 16.4) [35]. Many traditional NSAIDs possess a carboxylic acid functionality, which makes similar interactions with the COX enzymes, explaining their lack of selectivity as in the case of ibuprofen (**2**) and indomethacin (**4**). However, recent studies reveal that the ion pairing of the acid moiety of AA (**39**) with R120 is not important for the catalytic activity of COX2 [36]. This discovery led to the general strategy of converting nonselective NSAIDs into selective COX2 inhibitors by neutralizing the acid moiety [37]. It was shown that esters and amides of indomethacin (**4**) indeed are more COX2 selective and possess potent anti-inflammatory effects without being ulcerogenic [37]. Computational studies suggest that a difference in another nearby residue, Arg513 in COX2 versus His513 in COX1, might be accountable

Table 16.1 HWB activity of NSAIDs for COX1/2.

Compound	HWB IC ₅₀ (μM) (COX1)	HWB IC ₅₀ (μM) (COX2)	IC50 ratio (COX2/COX1)
Aspirin (1)	1.7	>100	>100
Ibuprofen (2)	7.6	7.2	0.9
Naproxen (3)	9.3	28	3
Indomethacin (4)	0.013	1.0	80
Diclofenac (5)	0.075	0.038	0.5
Celecoxib (6)	1.2	0.83	0.7
Rofecoxib (7)	63	0.84	0.013
Lumiracoxib (8)	67	0.13	0.002

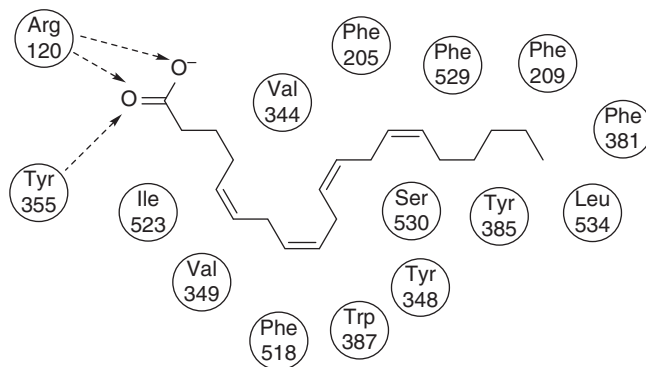


Figure 16.4 Adapted molecular interaction map of AA (39) with COX1 generated by MOE (pdb code: 1DIY).

for this result [31a]. This proposal rationalizes the well-known but seemingly puzzling COX1 selectivity of aspirin (**1**), given its common mechanism of covalent inhibition of both COX enzymes [8a]. The computational results confirmed that aspirin (**1**) would be 10–100 times more potent against COX1 than against COX2 and revealed that the specificity can be attributed mainly to the difference in the kinetics of the covalent inhibition reaction. In the formation of the noncovalent complex state (EI), the presence of Arg513 in COX2 would increase the activation barrier of the acetylation reaction, while the corresponding His513 in COX1 only has a minimal effect on the transition state. The interaction between the positively charged guanidinium group of Arg513 and the negatively charged carboxylate group of aspirin (**1**) would disfavor the protonation of the latter during the acetyl transfer process, thus slowing down the acetylation of COX2 [8a]. The inactivation of COX1 in platelets, coupled with the long lifetime of platelets (8–10 days), forms the basis for the popular usage of low-dose aspirin (**1**) for the prophylactic treatment of thromboembolic disease and myocardial infarction.

The most fruitful approach to achieve COX2 selectivity exploits the presence of a side pocket in COX2 that originates from a single amino-acid difference: the smaller side chain of Val523 in COX2 versus isoleucine in COX1 [9]. The side pocket is located above the Arg120/Tyr355/Glu524 constriction. It is bordered by Val523 and contains an Arg513 (vs His513 in COX1) at the base of the pocket [38]. The solvent accessible surface in the COX2 active site is larger because of the smaller Val523 as well as the Arg513 and Val434 substitution (His513 and Ile434 in COX1) in the secondary shell. Figure 16.5 compares the binding sites of COX1 and COX2. Crystal structures of COX2 selective sulfonamide or methyl sulfone drugs such as celecoxib (**6**) bind in this side pocket, accounting for the selectivity against COX1. Additional evidence from an elegant mutagenesis study shows that the COX2 selectivity is lost for the V523I mutant, whereas classical NSAIDs such as indomethacin (**4**) have no change in their selectivity profile [39].

Another very different approach to accomplish good COX2 selectivity was demonstrated in the discovery of lumiracoxib (**8**). As the only COX2 selective

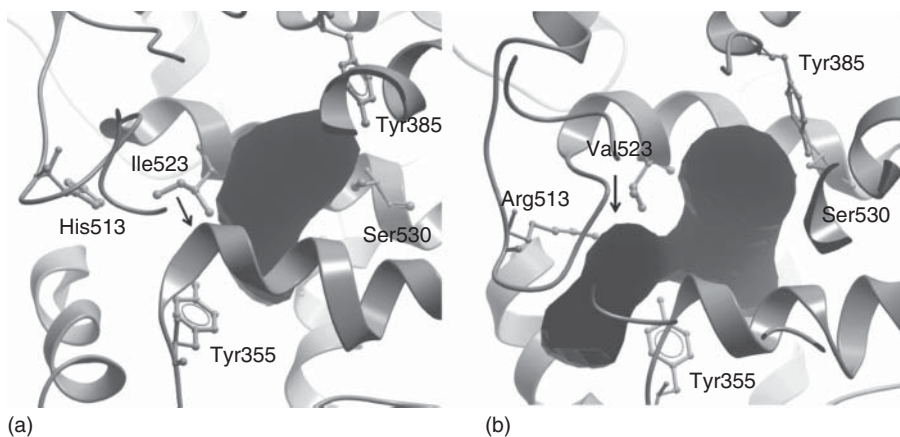


Figure 16.5 Comparison of COX1 ((a), pdb code: 1DIY) and COX2 ((b), pdb code: 1CX2), active site binding pockets, gray shapes, generated with ICM PocketFinder.

inhibitor with a carboxylic acid moiety, it is a close analog to diclofenac (**5**). Although diclofenac (**5**) was designed to mimic indomethacin (**4**), later profiling showed that it has a different binding mode since its inhibition was unaffected by the mutation of Arg120 to alanine but was dramatically attenuated by an S530A mutation. The cocrystal structure of diclofenac (**5**) with COX2 demonstrates that diclofenac binds to COX2 in an inverted conformation with its carboxylate group making hydrogen bonding interactions with Tyr385 and Ser530 (Figure 16.6a) [26c]. This is in contrast to other carboxylic acid NSAIDs whose carboxylic acid groups interact with Arg120 and Tyr355. The crystal structure of lumiracoxib (**8**) with COX2 revealed that it also binds in an inverted orientation, similarly to that of diclofenac (**5**) with its carboxylate group forming hydrogen bonding

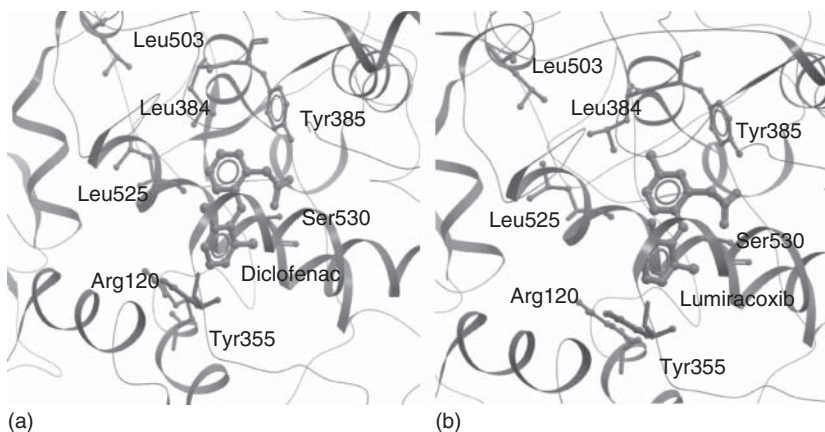


Figure 16.6 (a) Diclofenac (**5**) bound to COX2 (left, pdb code: 1PXX). (b) Lumiracoxib (**8**) bound to COX2 (right, pdb code: 4OTY).

interactions with Ser530 and Tyr385 at the top of the active site [40]. The methyl group on the phenyl acetic acid ring of lumiracoxib (**8**) projects into a small groove near Leu384 by the movement of Leu384 in the COX2 active site (Figure 16.6b). When lumiracoxib (**8**) is modeled into COX1, the methyl group clashes with the side chain of Leu384 of COX1 because a similar movement of this residue is restricted due to the presence of a bulky secondary shell residue behind Leu384 (Ile525 and Phe503 in COX1 versus Val525 and Leu503 in COX2). Detailed SAR studies of diclofenac (**5**) and lumiracoxib (**8**) analogs confirmed that the methyl group in lumiracoxib (**8**) is responsible for its COX2 selectivity as suggested by the crystal structure and by modeling [40].

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17

Carboxylic-Acid-Containing Antihistamines*

Irini Akritopoulou-Zanze

17.1

Introduction

Antihistamines containing a carboxylic acid moiety have been some of the most widely used drugs for allergic reactions and urticaria (hives) [1]. They have been shown in clinical trials to treat the symptoms of allergic rhinitis, such as nasal congestion, sneezing, itching, and rhinorrhea, and the symptoms of allergic conjunctivitis, such as itching, watery, red eyes. They are administered orally or topically as eye drops. Furthermore, they are used to treat acute and chronic urticaria reducing whealing and itching. Some antihistamines have also shown an effect in atopic dermatitis and asthma. The polar carboxylic acid group was introduced into various antihistamine classes as a way to limit CNS penetration and thus to reduce the associated side effects of earlier antihistamines.

17.2

History

Histamine, a biogenic amine, and its role in allergic and anaphylactic reactions were first discovered in the early 1900s and became the subject of intense studies throughout the last century [2]. The first-generation antihistaminic drugs became available in the mid-1940s and were broadly used until the late 1970s for a variety of allergic disorders such as rhinitis, conjunctivitis, and urticaria. However, their ability to easily cross the blood–brain barrier and their poor selectivity over muscarinic, serotonin, and adrenergic receptors resulted in multiple side effects such as sedation and cardiotoxicity [3].

As a result, a second-generation of antihistamines emerged in the late 1970s and early 1980s with higher selectivity over other aminergic receptors and limited blood–brain barrier permeability. They contained polar moieties that limited antihistaminic action to peripheral histamine receptors and avoided

* Dedicated to Prof. Koji Nakanishi on the occasion of his 90th birthday.

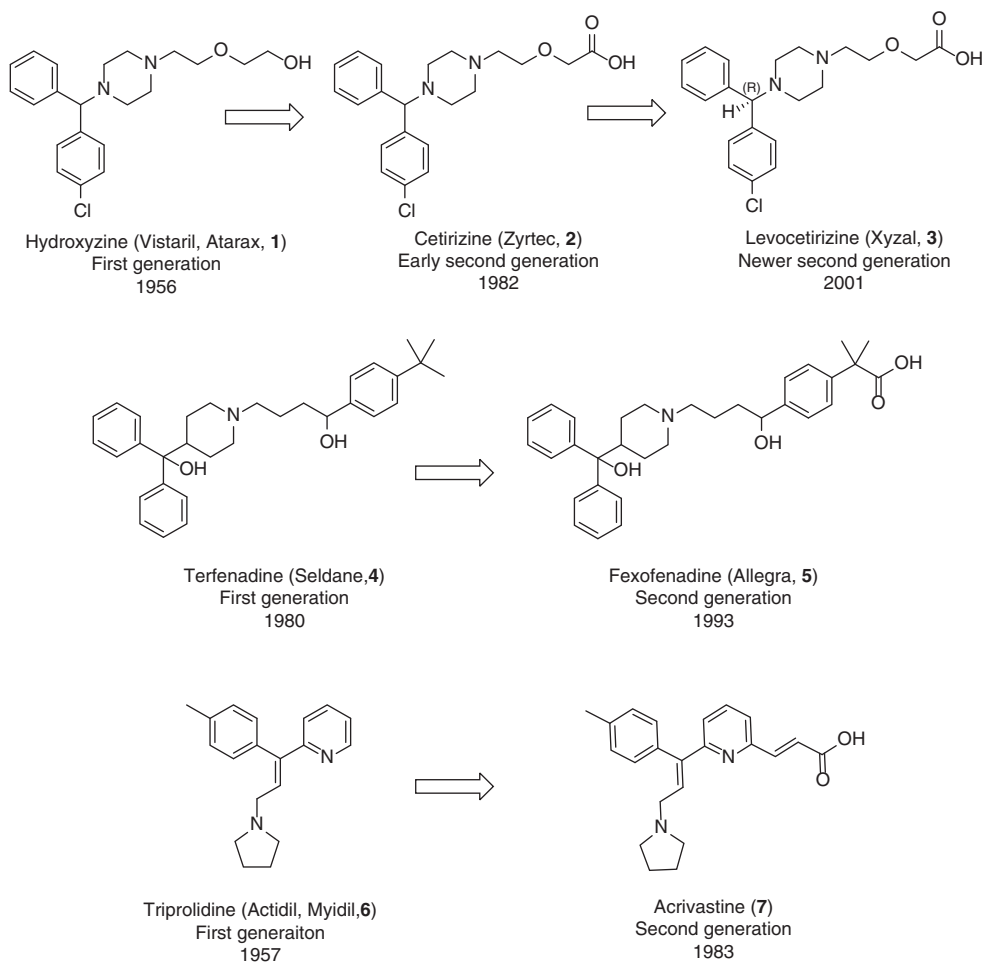


Figure 17.1 Second-generation carboxylic-acid-containing antihistaminic drugs that are metabolites or derivatives of first-generation antihistaminic drugs.

CNS-mediated adverse events. Active metabolites of first-generation antihistamines containing carboxylic acids fell into this category, with the first one disclosed in 1982 [4]. Cetirizine (**2**), the major human metabolite of the first-generation antihistamine drug hydroxyzine (**1**) (Figure 17.1), preserved the antihistaminic properties of the parent drug without the sedative and anticholinergic effects [5]. Cetirizine (**2**), which is a zwitterion, had a lower volume of distribution and lower brain penetration, targeting peripheral histamine receptors while avoiding the CNS [6]. The compound was approved for the treatment of allergic rhinitis and urticaria, and it is currently an over-the-counter drug. Cetirizine's (**2**) active enantiomer, levocetirizine (**3**), was also developed for allergic rhinitis and chronic idiopathic urticaria in adults and children [7], and it is currently available as a generic.

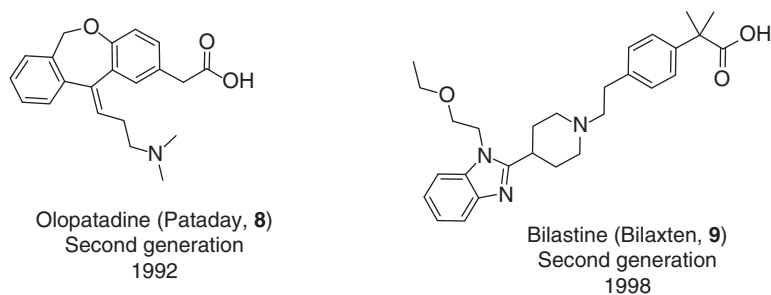


Figure 17.2 Antihistaminic drugs designed to contain a carboxylic acid moiety.

The success of cetirizine (**2**) led to the evaluation of other first-generation antihistamine metabolites as potential candidates with improved properties. Terfenadine (**4**), a first-generation antihistamine initially approved in 1985 for allergic rhinitis, was found to have an increased risk of cardiac arrhythmias due to its lack of selectivity over the human ether-a-go-go related gene (hERG) channel and was removed from the market in 1997. The same year, its active metabolite, fexofenadine (**5**) was approved for allergic rhinitis and urticaria. Fexofenadine (**5**) retained the antihistaminic activity of terfenadine (**4**) and was selective over hERG [8]. Fexofenadine (**5**), which lost exclusivity in 2011, went on to become one of the most commonly used antihistamines [9].

The introduction of a carboxylic acid into known antihistamines to produce drugs with improved selectivity and fewer side effects also led to the preparation of acrivastine [10, 11], (**7**) a derivative of the older antihistamine triprolidine (**6**). Acrivastine (**7**) is currently an over-the-counter drug in the United Kingdom and, in combination with pseudoephedrine, a prescription medication in the United States.

In 1992, a Japanese group, aware of the acrivastine (**7**) research, introduced carboxylic acid moieties into their antihistamine molecules, thus developing olopatadine (**8**) (Figure 17.2) [12, 13], currently available as prescription eye drops.

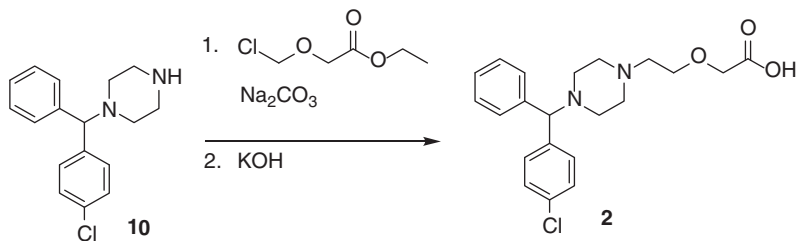
Although most of the earlier carboxylic acid drugs were derivatives or metabolites of first-generation antihistamines, by the mid-1990s, attempts were made to prepare novel drugs incorporating all desirable properties of second-generation antihistamines, such as high selectivity and potency for the H₁ receptor and improved pharmacokinetic (PK) properties. Out of these efforts came bilastine (**9**) [14], currently approved in the European Union for allergic rhinoconjunctivitis and urticaria [15]. Bilastine (**9**) is not distributed in the CNS, has a long half-life, and in clinical trials, exhibited no drowsiness or cardiotoxicity [16].

17.3

Synthesis

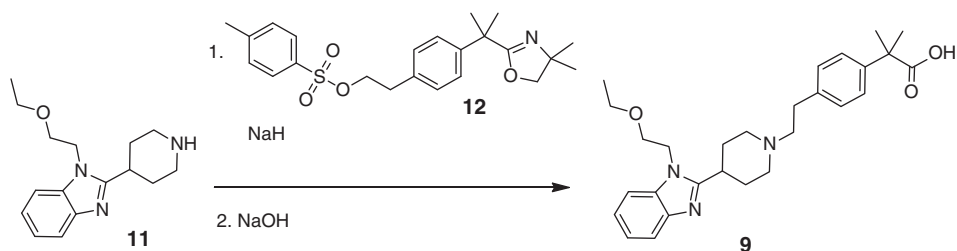
Piperidine- and piperazine-based antihistamines are commonly constructed from the corresponding amine via alkylation reactions with halides or mesylates. The

carboxylic acid moiety is usually protected as an ester and deprotected at the final stages of the synthesis. Thus, cetirizine (**2**) is prepared in two steps from piperazine **10** (Scheme 17.1) [17], while the *R*-enantiomer (levocetirizine (**3**)) is prepared from the corresponding chiral piperazine [18].

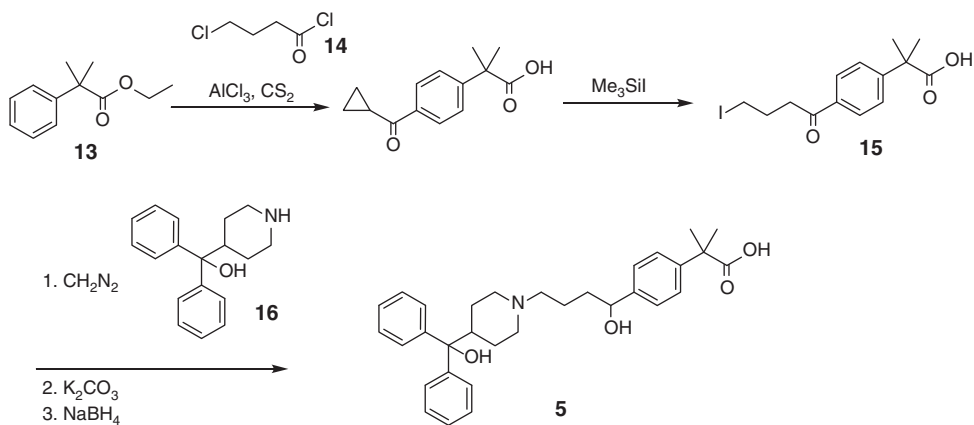


Scheme 17.1 Synthesis of cetirizine (**2**).

Similarly, bilastine (**9**) [19] is prepared from amine **11** (Scheme 17.2) and mesylate **12** and fexofenadine (**5**) via alkylation of amine **16** (Scheme 17.3). A key Friedel–Crafts reaction of ester **13** with acyl chloride **14** is used to construct intermediate iodide **15** [20].

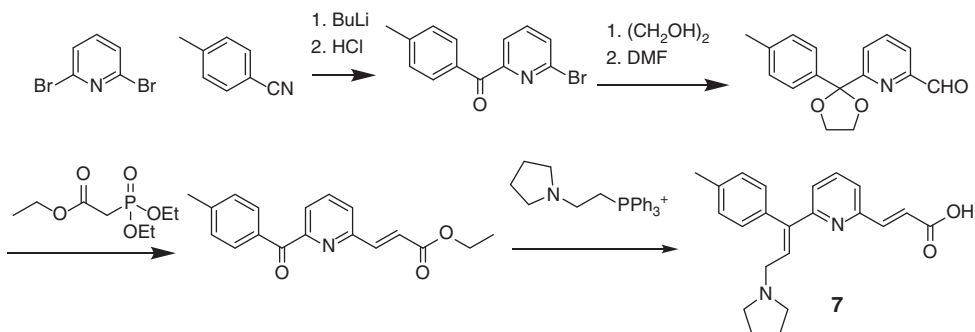


Scheme 17.2 Synthesis of bilastine (**9**).



Scheme 17.3 Synthesis of fexofenadine (**5**).

The synthesis of olopatadine (**8**) [21] and acrivastine (**7**) [10] start from the corresponding ketones via Grignard or Wittig reactions to introduce the double bonds. As an example, subsequent Horner Wadsworth Emmons and Wittig reactions provided acrivastine (**7**) in a few steps (Scheme 17.4).



Scheme 17.4 Synthesis of acrivastine (**7**).

17.4

Mode of Action

Carboxylic-acid-based antihistamines exert their action primarily via modulation of the H1 receptor, a G protein-coupled receptor (GPCR) responsible for many of the symptoms of allergic reactions. Although for many years, antihistamines have been described as H1-antagonists, advances in our understanding of GPCR function led to the currently accepted mode of action of all known antihistamines as inverse agonists [22].

Furthermore, carboxylic acid antihistamines have been shown to downregulate activation of NF- κ -B [23], a transcription factor regulating the production of many proinflammatory cytokines and adhesion proteins.

17.5

Structure–Activity Relationship

Carboxylic-acid-based antihistamines are divided into several subclasses such as alkylamines (Table 17.1), piperidines (Table 17.2), and piperazines (Table 17.3). Although they all act as inverse agonists of the H1 receptor, their structural differences result in differentiated effects on the receptor's signaling pathways as well as on other inflammatory mechanisms. Olopatadine (**8**) has high affinity for the H1 receptor and high selectivity over H2, H3, α -adrenergic, dopaminergic, and muscarinic receptors. In addition to its antihistaminic activity, it is believed to exert its action via multiple additional mechanisms, such as inhibition of mediators from mast cells, reduction of arachidonic acid release, and inhibition of

Table 17.1 Alkylamines.

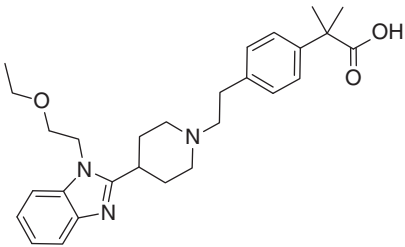
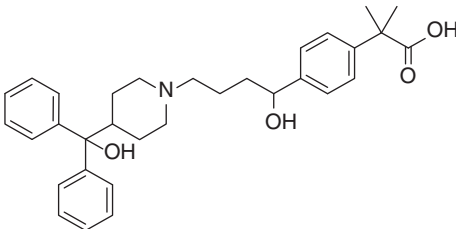
Drug name (Company)	Structure	Indications/current status
Acrivastine (7) (Wellcome Foundation Ltd, UK)		Allergic rhinoconjunctivitis/FDA approved in 1994 [24] in combination with pseudoephedrine
Olopatadine (8) (Kyowa Hakko Kogyo)		Allergic conjunctivitis decongestant nasal spray formulation/FDA approved as ophthalmic solution

Table 17.2 Piperazines.

Drug name (Company)	Structure	Indications/current status
Cetirizine (2) (UCB)		Seasonal allergic rhinitis, urticaria/FDA approved for urticaria in 1995 and allergy in 1996. Currently available over the counter
Levocetirizine (3) (UCB/Sepracor then Sanofi-Aventis)		Seasonal allergic rhinitis and perennial allergic rhinitis/FDA approved for allergy and hives in 2007 and as a generic in 2010

tumor necrosis factor (TNF)- α release [25]. Similarly, fexofenadine (5), in addition to its antihistaminic activity, has an effect on multiple pathways associated with inflammatory responses, such as decreases in leukotriene and prostaglandin production, inhibition of cyclooxygenase-2 and decreases in cytokine levels [26].

Table 17.3 Piperidines.

Drug name (Company)	Structure	Indications/current status
Bilastine (9) (FAES Farma)		Allergic rhinoconjunctivitis, allergic rhinitis, and urticaria/approved in the European Union but not in the United States
Fexofenadine (5) (Sepracor, then Sanofi-Aventis)		Seasonal allergic rhinitis and chronic idiopathic urticaria/approved in EU in 2000 and in the United States in 2006. Became an over-the-counter drug in 2011

The PK profiles of carboxylic acid antihistamines are characterized by good to excellent absorptions, high protein binding, low volumes of distribution, and significant renal clearance. Piperidine and piperazine carboxylic-acid-based antihistamines are not metabolized by the liver. Acrivastine (7) is metabolized to the propionic acid analog. Piperazine analogs are P-glycoprotein and organic anion-transporting polypeptides (OATPs) substrates, and their oral bioavailability decreases when taken with grapefruit, orange, or apple juices [27, 28].

Although carboxylic-acid-based antihistamines have very few side effects, alkylamine and piperidine analogs have sedating effects. Piperazines are devoid of these effects. None exhibit cardiac toxicity, drug abuse, or toxicity from overdose.

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18

Corticosteroid Carboxylic Acid Esters

Maurizio Franzini

18.1

Introduction

The focus of this chapter is on glucocorticosteroid carboxylic acid ester (GCE) analogs with the general structure of an 11 β ,17 α -dihydroxy-2,4-pregnadiene-3,20 dione (**1**), containing carboxylic esters of the hydroxyl groups in 17 α and/or 21-position, where R²¹ can be a carbon or a heteroatom (prototypical examples are hydrocortisone (HC)) butyrate (**2a**), acetate (**2b**), and aceponate (**2c**), Figure 18.1. Analogs with carbalkoxy functionalities at C-6, C-16, or elsewhere on the steroidal scaffold are not covered here.

GCEs began being distributed on the market in the early 1950s. Originally developed as topical steroids, the most frequently used drugs in dermatological practice, they were later adapted for intranasal delivery. Table 18.1 lists, in alphabetical order, marketed GCEs with one or two ester moieties, their route of administration and indications (two-letter abbreviations refer to the base steroid, three-letter abbreviations to esters). Compounds **6**, **9**, **16**, and **26** deviate from general structure **1** at the D ring, and they are represented in Figure 18.2, along with structural details for two GCEs (**21s** and **24f**) featuring long-chain C²¹-esters.

Research in this arena has for a long time aimed at optimizing anti-inflammatory and immunosuppressive potency and at minimizing adverse effects from systemic action. A safe GCE must permeate the *stratum corneum* or the *alveolar mucosa*, reach adequate concentration in the target tissue, but should not be distributed systemically. Although the ideal topical corticosteroid has not yet been discovered, progress is still ongoing. The increased lipophilicity, obtained with esterification, has enabled a better permeation of these steroidal molecules, while reducing plasma concentration and systemic exposure. The most desirable pharmacokinetic/pharmacodynamic profile would allow for once-daily application (to boost compliance), prevent induction of cross-sensitivity, and not induce atrophogenicity (wasting of dermal or epidermal tissue).

Inhaled corticosteroids (ICSs) are the mainstay preventative therapy for patients with persistent asthma or allergic rhinitis. Among them, mometasone furoate (MMF, **23**) has been available for clinical use for more than 25 years. In

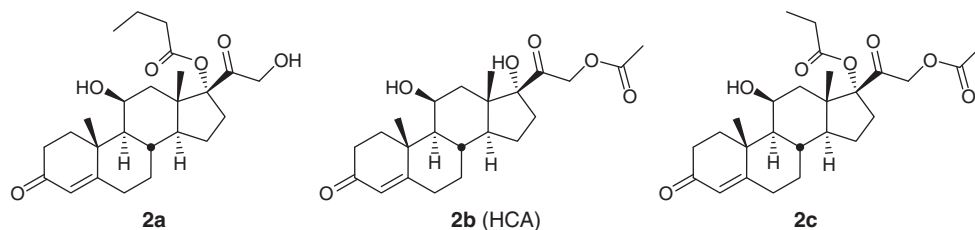


Figure 18.1 Hydrocortisone monoesters (**2a**, **2b**) and diester (**2c**).

the United States, it is also prescribed to children for its favorable risk–benefit profile, although it is not completely devoid of systemic side effects [1]. MMF has a very low oral bioavailability (~1%). Ciclesonide (CIC, **6**) is used in the preventative treatment of persistent bronchial asthma and is also efficacious in the pediatric population. CIC was approved in 2006 and represents the first example of a “soft” prodrug – in the lungs, it is converted into the much more active metabolite, *des*-isobutyryl-CIC [2]. Its pulmonary effects can be measured with an alleviation of small airway obstruction and bronchoconstriction in response to allergens or exercise.

Fluticasone propionate (FTP, **18p**) in fixed combination with the long-acting β_2 -adrenoceptor agonist (LABA) formoterol fumarate in twice-daily single, metered-dose aerosol inhalation is a recent addition to the arsenal of treatments for patients with severe asthma [3]. The combination drug allows for more effective management of asthma symptoms than with the two agents taken separately and for an overall favorable tolerability profile [4]. The formulation of FTP (**18p**) with antihistaminic azelastine hydrochloride has become one of the cornerstones of first-line therapy for allergic rhinitis [5], which often precedes asthma and represents a risk factor for its development [6]. Intranasal steroids have been shown in meta-analyses to be superior to oral or intranasal antihistamines [7]. The later conceived fluticasone furoate (FTF, **18f**) has a longer duration of action in several cell systems, as well as enhanced oxidative stress insensitivity, compared with FTP (**18p**), with significant clinical implications [8]. A new once-daily FTF (**18f**)/vilanterol combination [9], Breo[®] Ellipta[™] by Theravance/GSK, has recently been approved by the FDA for use in COPD (chronic obstructive pulmonary disease) (in 2013) and asthma (2015) for adult patients [10]. The reduced dosing frequency is expected to increase adherence [11] and to help asthmatic patients control the disease better than with preexisting albeit efficacious treatment [4].

Hydrofluoroalkane propellants for intranasal steroidal treatment were approved by the FDA in 2012 [12], thus simplifying the formulation for ICS administration via nebulization (only aqueous solutions were previously permitted) into an aerosol of smaller particles that are more likely to reach further into the small airways of the lungs [13].

Topical glucocorticoids can also be designed safely [14] and used for the treatment of Crohn’s disease and ulcerative colitis [15] or for eosinophilic

Table 18.1 Currently approved or marketed glucocorticoids carboxylic acid esters.

GCE of general structure 1		Structural details				Launch		Route
		X ⁶	X ⁹	16-Me	R ¹⁷	R ²¹	Notes	
3	Alclometasone dipropionate (ACP)	H	H	α	EtCO	CH ₂ OCOEt	X ⁷ = Cl	T
4	Beclomethasone dipropionate (BDP)	H	Cl	β	EtCO	CH ₂ OCOEt		Or, I
5p	Betamethasone (BM)	H	F	β	EtCO	CH ₂ OCOEt		T
5v	dipropionate (BMP) valerate (BMV)				BuCO			T
6	Ciclesonide (CIC)	H	H	—	—	CH ₃	Figure 18.3	I
7	Clobetasol propionate (CBP)	H	F	β	EtCO	CH ₂ OCO/Pr		T
8	Clobetasone butyrate (CBB)	H	F	β	PrCO	CH ₂ Cl	11-keto	T
9	Deflazacort (DFZ)	H	H	—	—	CH ₂ Cl	Figure 18.3	Or
10	Dexprodone propionate	H	H	—	EtCO	CH ₂ OCOMe	No 16-Me	T
11c	Dexamethasone (DX)	H	F	α	cPrCO	CH ₃		I
11v	cipecilate (DXC) valerate (DXV)				BuCO	CH ₂ OCOcHx		T
12	Diflorasone diacetate	F	F	α	MeCO	CH ₂ OH		T
13	Difluprednate	F	F	—	PrCO	CH ₂ OCOMe	No 16-Me	T, Op
14	Etiprednol dicloacetate (EPC)	H	H	β	Cl ₂ CHCO	CH ₂ OCOMe		Or, I
15	Flumethasone (FM) pivalate (FMP)	F	F	α	H	CH ₂ OCOtBu		T
16	Fluocinolone (FC) acetate (FCA) (or fluocinonide)	F	F	—	—	CH ₂ OCOMe	Figure 18.3	T, Op, j
17	Fluocortin butyl	H	F	α	—	CO ₂ Bu		T

(continued overleaf)

Table 18.1 (continued)

GCE of general structure 1	Structural details					Notes	Launch	Route
	X ⁶	X ⁹	16-Me	R ¹⁷	R ²¹			
18f	F	F	α	2-FuCO	SCH ₂ F	Figure 18.3	2003, GSK (UK)	I
18p				propionate (FTP)	EtCO		1993, GSK (UK)	T, I, Op
19	F	F	β	EtCO	CH ₂ Cl		1991, Novartis (US)	T
20	F _β	F	—	MeCO	CH ₂ OCOMe	27 (2-Br)	1988, Sumitomo Dainippon (J)	IV
2c	H	H	—	EtCO	CH ₂ OCOMe	No Δ ¹ Figure 18.2	1991, Galderma (F)	T
2b				acetate (HCA)	CH ₂ OCOMe		2011, Skinvisible (US)	T
2a				butyrate	CH ₂ OH		1984, Astellas (UK)	T
21	H	H	—	EtOCO	OCH ₂ Cl	No 16-Me	1998, Bausch & Lomb (US)	Op
22a	Me	H	—	EtCO	CH ₂ OCOMe	No 16-Me	1992, Bayer (ZA)	T
22s				sulpeptanate	See Figure 18.3		1991, Pfizer (US)	Or, Ij
23	H	Cl	α	2-FuCO	CH ₂ Cl		1988, Merck (US)	T, Or, I, Ij
24	H	H	—	EtOCO	CH ₂ OCOEt	No 16-Me	1986, Sanofi (D)	T
25f	H	H	—	H	See Figure 18.3	No 16-Me, Figure 18.3	1998, Kuraray (J)	T
25a				acetate	CH ₂ OCOMe		(unknown)	Op
26	H	F	—	—	CH ₂ OCOMe	Figure 18.3	2009, Foamix (unknown)	T, Ij

Bu = butyl; Et = ethyl; Fu = furyl; cHx = cyclohexyl; Me = methyl; Pr = propyl; and cPr = cyclopropyl.
T (Topical): dermatitis, pruritus, (plaque) psoriasis, eczema (atopic and seborrheic), aphthous ulcers, aphthous stomatitis, oral lichen planus, and discoid lupus erythematosus.
I (Inhaled/Intranasal): asthma, allergic rhinitis, airway eosinophilia, sinusitis, and COPD (chronic obstructive pulmonary disease).
Ij (injectable), IV (i.v.), Or (Oral): asthma, rheumatoid arthritis, lupus erythematosus, Crohn's disease, autoimmune hepatitis, Duchenne muscular dystrophy, nephrotic syndrome, and immunosuppressant for organ transplants.
Op (Ophthalmic): uveitis, postoperative pain, cataract, xerophthalmia, diabetic macular edema, and seasonal allergic conjunctivitis.



esophagitis [16]. Antenatal administration of GCEs (such as betamethasone propionate (BMP, **5p**)) can accelerate the fetal lung maturation and significantly decrease neonatal mortality and morbidity in infants born before 34 weeks of gestation [17]. Deflazacort (DFZ, **9**), an oxazoline derivative, is a potent immunosuppressive agent, with fewer adverse effects than many other GCEs [18], indicated for autoimmune diseases and for muscular dystrophy [19].

History

Although the isolation, structure determination, synthesis, and endocrine characterization of the rare adrenal hormones (which feature an oxygen atom at C-11) have been ongoing since the mid-1930s [20], the anti-inflammatory corticosteroid era started in earnest with the announcement in 1949 at the Mayo Clinic that cortisone had a dramatic beneficial effect on bedridden patients suffering from rheumatoid arthritis. Commercial quantities of cortisone became available in 1950 from Merck and Schering [21]. Whereas cortisone acetate was efficacious systemically, hydrocortisone (Table 18.1) could also be used topically for the treatment of dermatitis [22]. The impracticality of the first developed synthesis of hydrocortisone 11,21-diacetate spurred the hunt for an enzymatic solution to the selective hydrolysis of the 11 β -acetate [23]. These efforts led to the serendipitous discovery of prednisolone (PD, **25**) [24], which showed an enhanced corticosteroid activity with a reduced mineralocorticoid interference, hence with a better therapeutic index (TI). After an accelerated preclinical safety evaluation, this

new GCE was administered first in humans in 1954 for the treatment of arthritis [25]. Although the microbial hydrolysis of the 11β -acetate was accomplished using the novel *Flavobacterium dehydrogenans* [26], the real breakthrough came in 1952 with the microbial 11β -hydroxylation of 17α -hydroxyprogesterone by the fungus *Cunninghamella blakesleeana* to provide hydrocortisone directly [27]. A complete first synthesis of HCA (hydrocortisone acetate) (**2b**) from 11α -hydroxyprogesterone, also obtained by microbial oxidation (with another fungus, *Rhizopus nigricans*), was accomplished by the scientists at Upjohn in the following year [28]. Other approaches were successfully explored around the same time until a preferred path starting from 11-ketoprogesterone via “diglyoxylation” was devised. HC (**2**) and PD (**25**) [29] were subsequently identified with the new $\Delta^{1,4}$ -dien-3-one functionalized A ring that would become a consolidated motif in GCEs. The tremendous efforts in this vast area of research and development had a profound effect on drug discovery as it led to the application of steric and electronic first principles to medicinal chemistry and contributed to the rise of process chemistry as highly sophisticated branch of applied science [30].

The synthesis of the first highly potent nonnatural steroid analog, 9α -fluorohydrocortisone, was achieved in 1953, starting from 11-epicortisol [31]. However, the Δ^1 double bond in prednisolone could not curb the increased salt retention typically observed in more potent 9α -fluorinated derivatives [32]. Introduction of a 6α -fluorine followed 5 years later, at Upjohn [33] and Syntex [34].

The 16α -methyl group was introduced in 9α -fluoroprednisolone with the expectation that potency would be enhanced by shielding the C-20 ketone from metabolism [35]. Because substituents at C-16 are on a five-membered ring, therefore neither equatorial nor axial, the choice of either position could not have been made based on structural reasons. Serendipity assisted in the discovery of DX (dexamethasone) (**11**), the first derivative of a 9α -fluorocortisol with increased anti-inflammatory potency but devoid of mineralocorticoid activity. The discovery of betamethasone (BM, **5**, Merck and Schering), with a 16β -methyl group, followed in 1958 [36], which is slightly more potent and causes less salt retention than DX. The original Merck synthesis started from diosgenin as raw material, while Schering's started from a $\Delta^{16(17)}$ hydroprogesterone [36]. It was not until 1968 that the introduction of the 17α -hydroxy group into a 16β -methyl-20-ketone substrate could be optimized when the α -ketol was obtained by reacting the 20-ketone with oxygen and trialkyl phosphite/sodium *tert*-butoxide at -25°C , instead of the older procedure via the hydroperoxide [37]. With 6α -methylprednisolone (MP, **22**), a more active steroid with a better TI with respect to salt retention was also obtained by Upjohn [38]. It would be just a matter of time for a yet more potent analog, flumethasone (FM, **15**), to be simultaneously claimed by Upjohn and Syntex [35c, 39].

Initially, stigmasterol, derived from soybean, and diosgenin, a sapogenin obtained by degradation of extracts from yam tubers, had been the primary sources of progesterone during the first phase of the corticosteroid production. However, the cheaper sitosterol, which is found in a multitude of plants, became the primary source of corticosteroids in the early 1970s, thanks to another

microbial-mediated transformation (9 α -hydroxylation by *Mycobacterium fortuitum*) [40], followed by dehydration to a Δ^9 -derivative that can be further elaborated into HCA (**2b**) [41].

In 1962, a new human-based pharmacologic test came to light, allowing an easier identification of the topical potency of steroidal anti-inflammatory agents [42]. The screening of a variety of esters of betamethasone (BM, **5**) against the new standards triamcinolone acetonide (TC, **26**) and fluocinolone acetonide (FC, **16**) led to the discovery of BMV (betamethasone valerate) (**5v**) and BDP (beclomethasone dipropionate) (**4**), which were 10-fold more potent than BM (**5**), and their commercialization by Glaxo and Schering in the late 1960s and early 1970s, respectively. BDP (**4**) would become the mainstay of anti-inflammatory therapy for asthma for more than 40 years [43].

The inability to improve the TI of prednisolone (**25**) in a significant way, despite prolonged efforts, led many researchers to the conclusion that the search for safer and highly active glucocorticoids would have to be beyond the mere exploration of analogs. Electrolyte imbalance is the main systemic effect associated with topical corticosteroids and is presumably caused by the mechanism of transactivation (as discussed in Section 18.4). Additional side effects include bodyweight gain and growth retardation [18], especially serious for the pediatric population; adrenal atrophy and Cushing's syndrome; dyslipidemia, hypertension, thrombosis, vasculitis; diabetes mellitus; pseudoprimary aldosteronism; osteoporosis; peptic ulcer, gastritis, pancreatitis; cerebral atrophy; and other deleterious changes in the central nervous system [44]. Topical corticosteroids can also cause a delayed-type hypersensitivity reaction [45], which is rarely induced by some of the more recently introduced esters – BMV (**5v**), CBB (clobetasone butyrate) (**8**), MMF (**23**), FTP (**18p**) – as well as a slate of other local adverse effects such as skin atrophy, rosacea, acne, delayed wound healing, erythema, hypotrichosis, hypopigmentation, cataracts, ocular hypertension, and glaucoma [44]. Long-term use of topical GCEs may also lead to tachyphylaxis. The first 16 α -methyl 17 α -acyloxy steroid, MMF (**23**), with great potency and effective separation of topical from systemic activity, was only introduced in the late 1980s [46]. The notion consolidated that 17 α - and 21-esterification and replacement of the 21-hydroxy group with a halogen would confer higher skin permeability and increased affinity for the glucocorticoid receptor (GR) [47].

The 21-methyl ester of prednisolonic acid, a topical anti-inflammatory agent introduced in the 1980s, was equipotent to preexisting PD (**25**), but weaker systemically, in that it would not suppress the pituitary–adrenal function or cause glycogen depletion in rats [48]. With a labile ester at C-21, the concept of “ante-drug” was thus introduced in 1982 [49], and an intact 20-ketol side chain was not any longer an absolute requirement for the anti-inflammatory activity. Upon entry into the circulatory system, the ester group would be swiftly hydrolyzed and the corresponding carboxylic acid was devoid of any significant activity.

Investigations followed into aromatic heterocyclic ester derivatives of the 17 α -hydroxy function of the side chain of 9,11-dichloro corticosteroids, inspired by earlier interesting results with betamethasone (**5**) 17-benzoate [50]. In particular,

2-furoyl esters emerged from the Schering research division as the most potent analogs compared to BMV (**5v**) [46]. Although this finding was relatively unexpected, it was already known that the replacement of the 21-acyloxy function with chlorine can lead to highly potent topical corticosteroids such as clobetasol propionate (CBP) (**7**) [51]. Analogous results emerged from the 17-heteroaryl esters of the 9 α -chloro-11 β -hydroxycorticosteroid series, with DX (**11**) 17 α -(2-furoate) as the most potent representative [46].

ICSs came to the fore in 1972 with BDP (**4**) [52] and are still the most effective treatment available for allergic airway diseases, as asthma and rhinitis are reaching epidemic proportions, especially among children [53]. The topical delivery of the active drug to the lungs allowed reduction of the dose, which resulted in a dramatic improvement of the unwanted systemic side-effect profiles. With the newer generations of lipophilic, esterified corticosteroids, most of the serious side effects previously discussed were virtually eliminated [54]. Since with ICSs about 60–90% of the administered dose is swallowed, intestinal absorption and systemic circulation cannot be avoided. Extensive development work has demonstrated that the particle size of the inhaled powder or aerosol droplet must have a mass median aerodynamic diameter of 1–5 μm to reach the alveoli [55], where the GRs are mostly concentrated [56]. Even the 10–40% fraction absorbed deep in the lungs will eventually enter circulation, where it can trigger side effects with a steep dose–response curve (as it has been observed with FTP, **18p**) [57]. A low oral bioavailability, increased plasma protein binding, and high clearance define a desirable pharmacokinetic profile for the GCEs of more recent design – FTP (**18p**), MMF (**23**), CIC (**6**) [55]. Fluticasone (FT) (**18**) is 91% protein-bound in plasma and is primarily metabolized to an inactive carboxylic acid by CYP3A4 [58]. The potentially dangerous liability linked to systemic exposure has spurred an ongoing interest in R&D to develop “soft” corticosteroids, a concept introduced in 1976 and of similar meaning to that of the later introduced synonym “antedrug,” [59] which refers to the identification of therapeutic agents that have a mechanistic deactivation option built into their molecular structure, that is, the hydrolysis of an ester, leading to systemically inactive steroidal carboxylic acids or alcohols [60]. Such inactivation must take place in a single, low-energy step that is mediated by one of the ubiquitous esterases, as further discussed in Section 18.4.

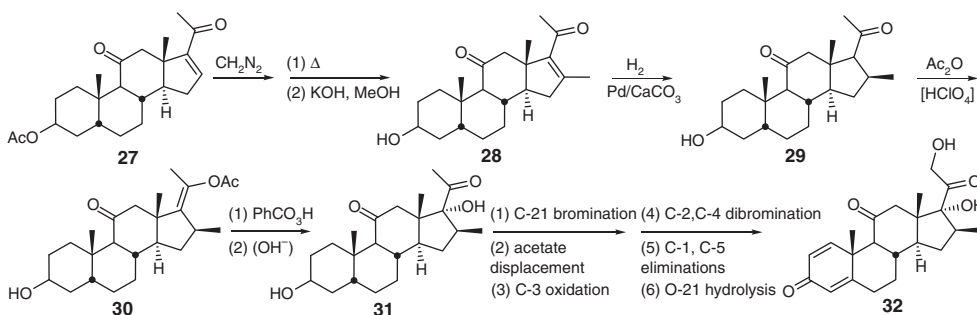
18.3

Synthesis

Although synthetic efforts on corticosteroids had been the benchmark for modern organic chemistry since the 1940s, this section focuses primarily on the methodologies implemented in order to introduce the structural modifications on ring D that enable the formation of the ester functionalities in pursuit of the ultimate “soft” drug.

The first comprehensive description of the elaboration of the 3 α -acetoxy-16-pregnene-11,20-dione **27** into a 16 β -methyl corticosteroid came from Merck

Sharp & Dohme in 1959 [36]. The 16 β -methyl group was introduced by reacting **27** with diazomethane to give a pyrazoline, which upon pyrolysis delivered **28** and upon diastereoselective hydrogenation delivered to **29** Scheme 18.1.

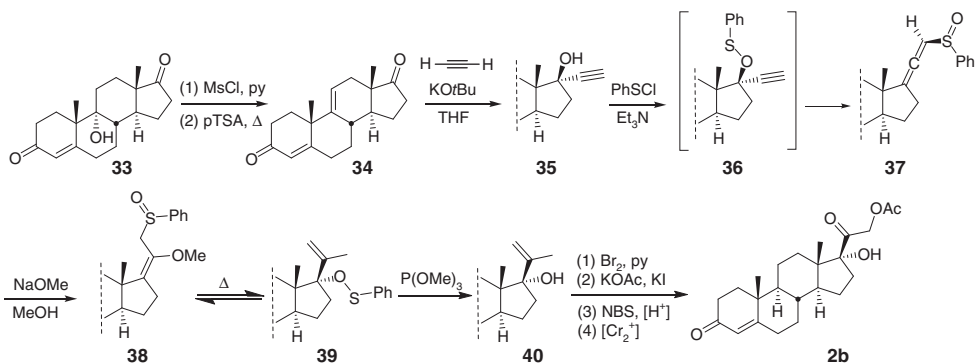


Scheme 18.1 First synthesis of a 16 β -methyl corticosteroid (**32**).

The introduction of the 17 α -hydroxyl was achieved by enolacetylation to **30**, and treatment with perbenzoic acid followed by basic cleavage of the hydroperoxide to give **31**. These 16 β -methyl-17 β -acetyl-17 α -hydroxy-20-ketosteroids are more prone to undergo D-homoannulation than the corresponding 16 α -methyl isomers. The D-homoannulation, an acyloin rearrangement, is a Lewis-acid – or –base-catalyzed reverse aldol cleavage of the 16,17-bond, followed by an intramolecular aldol reaction linking C-16 with C-20 [61]. The further transformation of the key intermediate **31** into 16 β -methylcortisone **32** was accomplished without difficulty by a sequence previously optimized [62].

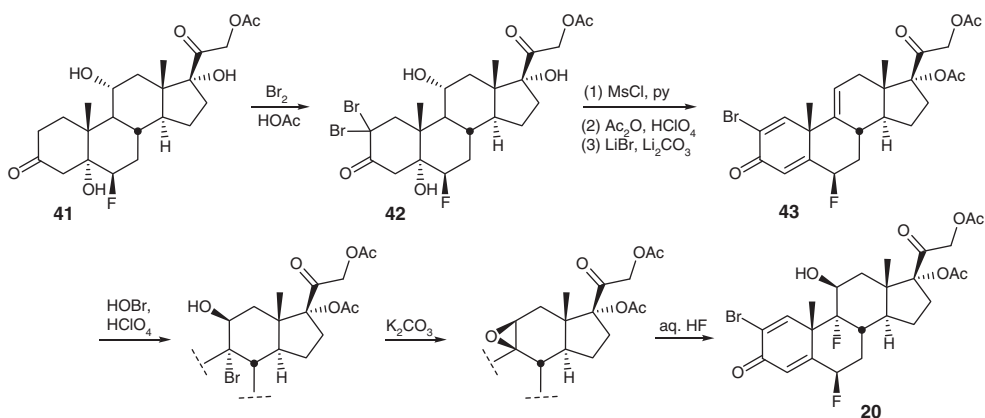
An efficient synthesis of HCA (**2b**) that took advantage of a sulfoxide-sulfenate rearrangement of the β -sitosterol-biodegradation product **33** (by a *M. fortuitum* mutant) was described by Upjohn's scientists in 1979 [41]. Acid-catalyzed elimination of the 11 α -mesylate of **33** to the $\Delta^{9(11)}$ -dione **34** was followed by nucleophilic attack of potassium acetylide to give **35**, without the need for protection of the Δ^4 -en-3-one due to the stability of the corresponding dienolate, Scheme 18.2. Reaction with phenylsulfenyl chloride provided the 17-sulfenate ester **36**, which upon slow warming above -40°C resulted in a [2,3]-sigmatropic rearrangement to the allene sulfoxide **37**. Treatment of **37** with sodium methoxide gave a mixture of ether sulfoxides **38** through conjugated addition. Refluxing of **38** in methanol induced the stereoselective equilibration to 17 α -sulfenate **39**, which could be captured by reaction with thiophilic trimethylphosphite to provide the corresponding tertiary alcohol **40**. This elegant sequence was concluded with well-precedented chemistry [63], which took advantage of the known intramolecular acetate migration from a 20-acetylacetal to the C-21 alcohol [64], to deliver HCA (**2b**).

Early investigations into the effects of fluorination on several positions of the steroid scaffold unfolded the potency-boosting effects of 6 α - and 9 α -substitutions [34]. The optimized methods for these regioselective halogenations paved the way to more sophisticated derivatizations, such as the production



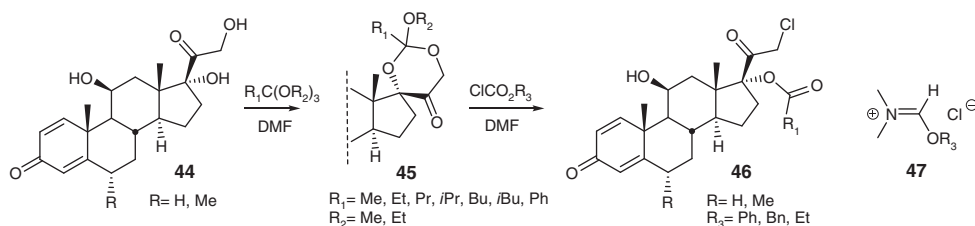
Scheme 18.2 The sulfoxide-sulfenate rearrangement to introduce a 17 α ,21-dihydroxy acetone side chain.

of hypothalamic-pituitary-adrenal (HPA) (**20**) from 6 α -fluorocorticosteroid **41** obtained at Pierrel, Milan [65]. Regioselective bromination to **42**, followed by mesylation of the 2° alcohol, acetylation of the tertiary alcohols, and base-promoted elimination, afforded Δ^9 -triene **43**, Scheme 18.3. The final stretch to HPA (**20**) took advantage of existing chemistry [66].



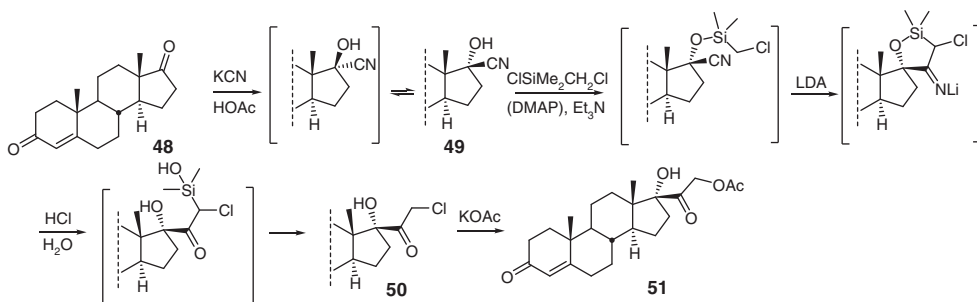
Scheme 18.3 Synthetic approach to halopredone acetate.

An industry-academia joint effort from Japanese scientists improved the conversion of prednisolone analogs (**44**) into 17 α -acyloxy-21-chloro-21-deoxy corticosteroid derivatives (**46**) in a two-step protocol, also via cyclic orthoesters (**45**), Scheme 18.4 [67]. This improved methodology overcame the poor-yielding direct conversions of 21-hydroxy to 21-chloro via routine mesylation/chloride displacement and took advantage of the activation of chloroformates by amines to iminium salts **47**, which would be productively utilized later for the preparation of analogs with a 17 α -methylsuccinate group [68].



Scheme 18.4 Optimized access to 17 α -acyloxy-21-chloro corticosteroids.

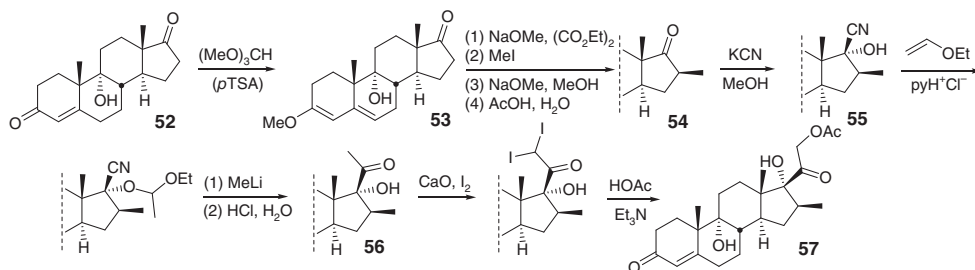
On another path to the D-ring functionalization, scientists at Upjohn strived to exploit androst-4-ene-3,17-dione (**48**), an inexpensive natural product [69]. Under appropriate crystallization conditions, the 17 β -cyanohydrin **49** could be prepared in high yield, leaving the Δ^4 -ene-3-one unmasked. Transient protection of the 17 α -hydroxy group with a bifunctional chloromethylsilane, followed by deprotonation and intramolecular nucleophilic attack on the cyano group, could lead to the 17 β -chloroacetyl intermediate **50**, which can be easily converted into cortexolone acetate **51**, Scheme 18.5, an important commercial precursor for a variety of corticosteroids through microbial 11 β -hydroxylation by *Curvularia lunata*.



Scheme 18.5 Expedient synthesis of cortexolone acetate (**51**).

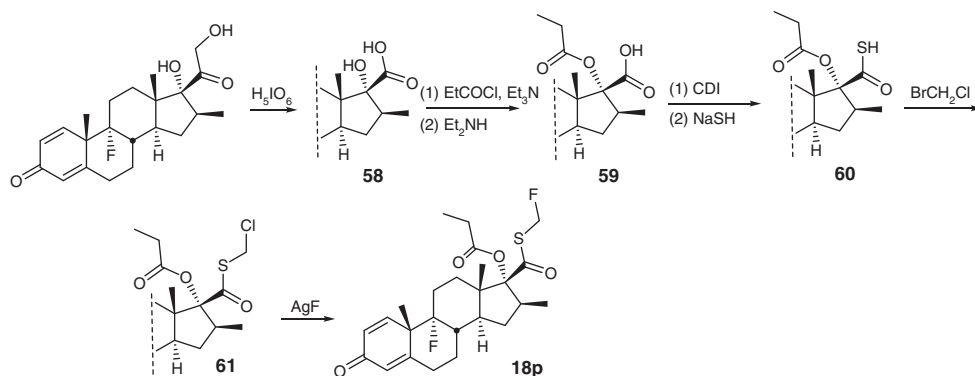
A variation to the route to 16 β -methyl corticosteroids via cyanohydrins was fruitfully achieved at Schering-Plough shortly thereafter [70]. After acid-catalyzed enolization of cheap enedione **52** and **53**, the diastereoselective methylation that utilizes the “oxalate-blocking method” gave 16 β -methyl derivative **54** after temporary protection of the 3-ketone, Scheme 18.6 [71]. Well-known conversion to the 17 β -cyanohydrin **55**, followed by transient masking of the 17 α -hydroxyl group and nucleophilic methylation gave the 17 β -acetyl derivative **56**, which could be diiodinated at C-21 to afford the target 21-acetyl compound **57**.

Improved access to 17 β -thioesters enabled scientists at Glaxo to expand the structure–activity relationship (SAR) in this region of the molecule and to hone in on FTP (**18p**) [72]. Starting from readily accessible 17 α -hydroxyandrostane-17 β -carboxylic acid **58**, selective 17 α -acylation gave **59**. Treatment with capacitive deionization (CDI) followed by sodium hydrosulfide produced the carbothioester



Scheme 18.6 β -Cyanohydrins as precursors to 17-dihydroxyacetone-corticosteroids.

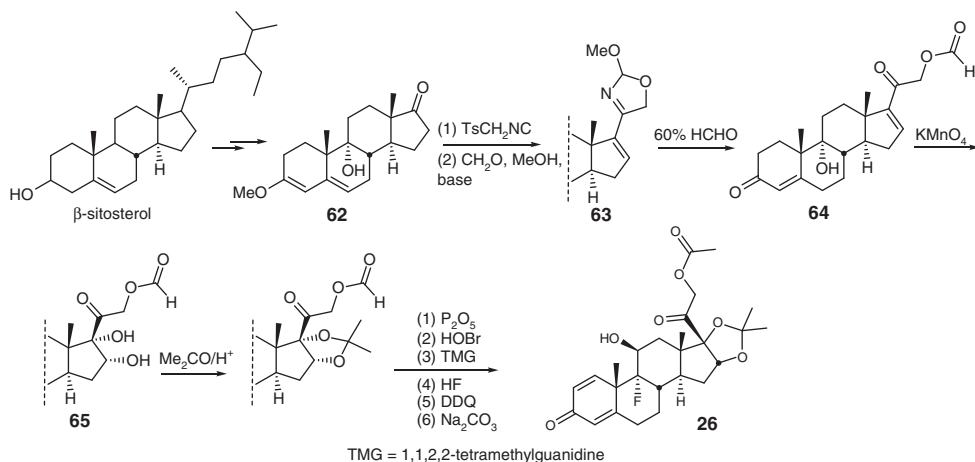
acid **60**, which could be converted into chloromethylcarbothioate **61** and then into the corresponding fluoride **18p** (FTP), Scheme 18.7 [73].



Scheme 18.7 Discovery of fluticasone propionate (**18p**).

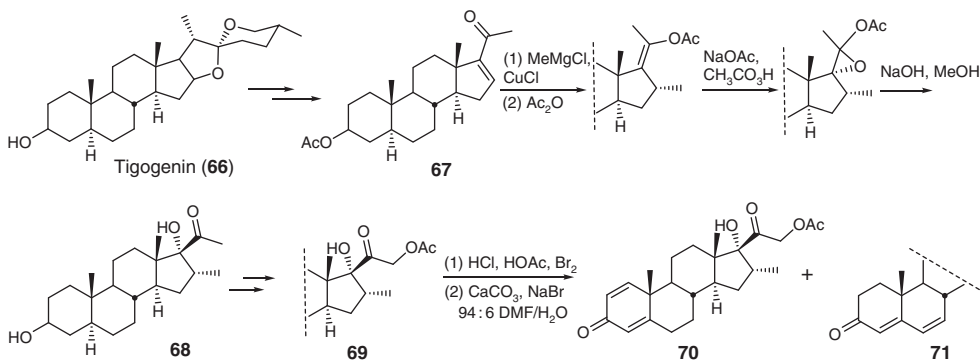
An incremental improvement on the existing commercial-scale manufacturing process of FTP (**18p**), which starts from flumethasone (**15**), was very recently proposed with attention to principles of green chemistry by scientists at Zhejiang University in Hangzhou, whereby *N,N*-dimethylthiocarbamoyl chloride was utilized as the thioesterification agent [74]. A synthesis of triamcinolone acetate (TCA) (**26**) was described, starting from the oxazolinyl derivative **63**, obtained from β -sitosterol-derived dienone **62** [75]. The conversion of **62** to **63** via Knoevenagel-type condensation with tosylmethyl isocyanide, followed by reaction with formaldehyde and concomitant allylic deprotonation at C-16, had been previously described by the same group, Scheme 18.8 [76]. Partial hydrolysis of **63** in 60% aqueous formic acid gave formate **64**, which underwent KMnO_4 -bis-hydroxylation to the diol **65**; routine steps (formation of the $16\alpha,17\alpha$ -acetonide, functionalization at C-9, C-11, and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ)-mediated dehydration to Δ^1) delivered TCA (**26**).

DX (**11**) is one of the few 16α -methyl corticosteroids on the market, and one of the most heavily used with an annual multiton demand. An optimized



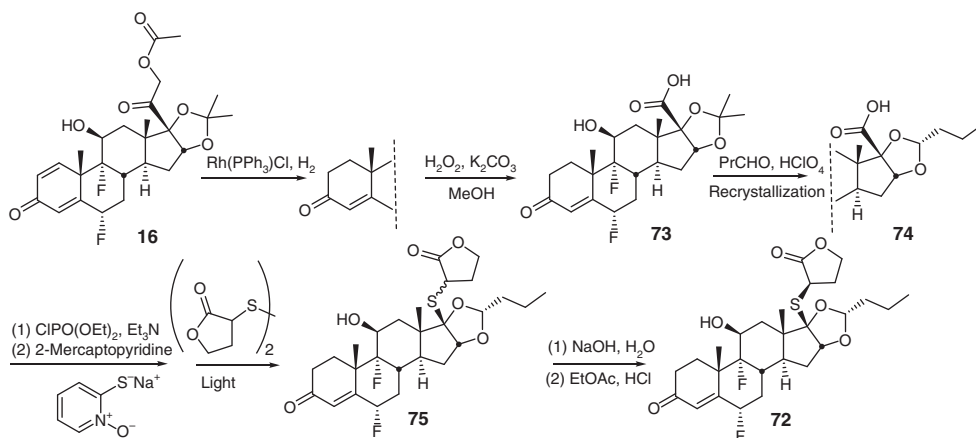
Scheme 18.8 A new synthetic approach to triamcinolone acetate (TCA, **26**).

synthesis of *des*-fluoro **DX** starts from the raw material tigogenin (**66**). This source, now more heavily tapped than diosgenin and other natural steroids, was recently devised by the researchers at Doshisha University near Kyoto [77]. The new protocol starts with the introduction of the 17 α -hydroxy-16 α -methyl onto pregn- $\Delta^{16(17)}$ -ene-20-ketone **67**, executed in four steps: methylation, acetylation, epoxidation (with diluted peracetic acid in a buffer solution), and hydrolysis, Scheme 18.9. The C-21 position of the thus obtained 16 α -methyl-17 α -hydroxy compound **68** is easily elaborated into **69** by known chemistry [78]. The outcome of the key oxidation of ring A (**69**–**70**) via traditional double bromination/base-promoted elimination was significantly improved by tweaking the polarity of the reaction mixture, so that only the desired E2 mechanism would occur and E1, leading to side-product **71**, would be suppressed.



Scheme 18.9 An improved synthetic approach to the DX-type scaffold from commodity raw material.

The incorporation of γ -butyrolactones into the glucocorticoid scaffold, which was attained by the researchers at Glaxo Wellcome with the development candidate **72**, is a more recent example of a “soft” drug [79]. Fluocinonide (FCA, **16**) was used as starting material. As illustrated in Scheme 18.10, a selective hydrogenation of the Δ^1 -olefin with Wilkinson’s catalyst followed by hydrogen-peroxide-induced C-20 oxidation provided the acid **73**. The 16,17 α -acetonide was then stereoselectively exchanged with the 16,17 α -(*R*)-butyl acetal **74**. The acid **74** can be activated as a mixed phosphoryl anhydride and can be subjected to Barton ester formation, followed by decarboxylation in the presence of butyrolactone disulfide to give a 1 : 1 epimeric mixture of the γ -lactone sulfide **75**, which can be equilibrated to **72** [80].



Scheme 18.10 Synthesis of **72** from fluocinonide (**16**).

18.4

Mode of Action

Calor, dolor, rubor, tumor, and functio laesa have been used to describe the acute symptoms of inflammation, at least since the 1600s by an English physician (Thomas Sydenham) or the 1800s by a German doctor (Rudolf L.C. Virchow) [81]. Glucocorticoids are essential in limiting and resolving the inflammatory process. Their pleiotropic effects on pathways controlled by GRs can also trigger a variety of adverse effects, in addition to alleviating inflammation [82]. The GR, a member of the steroid hormone receptor family, is maintained in the cytoplasm as an inactive multiprotein complex and is activated by strong binding with the hormone cortisol to form a complex that (i) migrates to the nucleus and binds to DNA sequences named “glucocorticoid-responsive elements” (GREs), located in the promoter region of responsive genes, eventually initiating transcription by RNA polymerase II; (ii) interacts with other transcription factors, such as nuclear

factor- κ -B (NF- κ B); and (iii) triggers signaling through membrane-associated receptors and second messengers via a “nongenomic” pathway (such as the activation of endothelial nitric oxide synthetase). All three events contribute to the anti-inflammatory effects of the GCEs, with inhibition of the synthesis of several profibrotic cytokines, such as interleukin-1 α (IL-1 α), and other mediators. GCEs can trigger a negative feedback loop that stimulates the HPA axis, which can also have an impact on the expression and activity of multiple immunomodulators (such as lipocortin I and p11/calpactin binding protein, involved in suppressing the release of arachidonic acid) [44] and on their ability to induce apoptosis in lymphocytes [83]. GCEs have direct inhibitory effects on or interfere with macrophages, mast cells, T-lymphocytes, eosinophiles, granulocytes, fibroblasts, and airway epithelial cells that are directly involved with inflammation in asthma. They also inhibit plasma exudation and mucus secretion [83]. The reduced inflammation lessens airway hyperresponsiveness to histamine, cholinergic agonists, and allergens in asthma patients, thus preventing, in the long run, the irreversible airflow obstruction.

The anti-inflammatory effects of glucocorticoids are primarily mediated by the inhibition of NF- κ B and activator protein 1 (AP-1) through negatively regulated transcription, and by “transrepression,” a much less understood mechanism whereby a single GR (monomer) binds to transcription factors or coactivators. Conversely, their side effects result from the so-called transactivation agonistic mechanism [84], which requires binding of receptor dimers to specific palindromic sequences in the cis-regulatory region of the GREs after translocation to the nucleus [85]; for example, the increase in expression of genes that upregulate gluconeogenesis. The HPA axis can also be suppressed by transrepression, via the decreased release of the adrenocorticotrophic hormone (ACTH) [86]. A better understanding of the transcriptional regulation mechanism was gained from the first crystal structure of the human GR ligand-binding domain (LBD) bound to DX (**11**) and a coactivator motif, derived from the transcriptional intermediary factor 2. The structure revealed features that explain the selectivity for endogenous hormones [87]. The steroid-shaped pocket was also found in very similar form in progesterin-, androgen-, and estrogen receptors, which all descend from a common ancestor [88] and bind to DNA response elements with the same degenerate consensus sequence, hence the promiscuity of their ligands [89]. The GR pocket has an additional branch that can specifically accommodate the 17 α -substituents of DX (**11**) and many other GCEs, with a concomitant boost of binding affinity primarily associated with unspecific van der Waals-type interactions [90]. The recently determined X-ray crystal structure of FTF (**18f**) in the GR LBD shows that the 17 α -(2-furoate) ester occupies this pocket more extensively, a possible reason for the increased relative binding affinity of this glucocorticoid compared to its closest analog, FTP (**18p**), and to DX (**11**), in a 29:18:1 ratio, respectively [91]. On the other hand, most of the 17 β -esters are inactive (as in CIC (**6**) and BDP (**4**)), but they can be activated by hydrolytic cleavage. The selectivity against mineralocorticoid receptors (MRs) can instead be attributed to the differences in hydrogen bonding patterns between the receptor

and the ligands. As further discussed in the next section, ligands with different structures (especially around ring D) impart a ligand-specific volume to the binding pocket [92], thus inducing different conformations of the receptor by virtue of its flexibility [93], resulting in divergent transcriptional activities [82]. The dissociation of the two activation mechanisms has long been pursued in the arena of research for safer GCEs.

CIC (**6**), BDP (**4**), and DXC (dexamethasone cipeclate) (**11c**) are prodrugs that can be activated through hydrolysis of the 21-ester by enzymes present in the lungs but not in the oropharynx [94]. BDP (**4**) is relatively stable in the plasma but is rapidly converted into the more active monopropionate BMP (**5p**) by the action of esterases in the lungs [95]. When CIC (**6**) is hydrolyzed to *des*-CIC, it forms reversible, lipophilic fatty acid conjugates in the lung tissue, which facilitate its pulmonary retention [96]. The more favorable safety profile is linked with a stronger preference for high lung deposition [13], as opposed to oropharyngeal absorption, than in FTP (**18p**), thus significantly reducing oral bioavailability [97]. *In vitro*, *des*-CIC is effective in inhibiting the release of inflammatory mediators, such as granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein-1 (MCP-1), interferon (IFN)- γ , IL-2, IL-4, IL-5, IL-8, and tumor necrosis factor (TNF)- α , and inhibited the proliferation of immune system cells. CIC (**6**) significantly reduced eosinophilia, a hallmark of asthma, and attenuated the allergen-induced reduction in IFN- γ -positive CD₄⁺ T cells, which play an anti-inflammatory role. CIC (**6**) becomes highly protein-bound when it reaches the plasma, which reduces its systemic exposure, and undergoes extensive first-pass metabolism in the liver. As a result, to date, no clinical evidence of HPA-axis suppression has been recorded for CIC (**6**). Partly due to its minimal oropharyngeal deposition, even high-dose inhaled CIC (**6**) has a significantly lower suppressive effect on serum cortisol levels than PD (**25**) [98], lower HPA-axis suppression than FTP (**18p**) [99], and a decreased effect on markers of bone formation [100]. However, FTP (**18p**), with a long inhalation half-life ($t_{1/2} \sim 14$ h) [55], is often preferred over CIC by patients (**6**) because of its scent and soothing feel.

The efficacy and safety of glucocorticoid esters as intranasal agents are in part the result of a “retrometabolic” design of drugs that integrate structure–activity and –metabolism relationships according to the concept of “soft” drugs as previously introduced [60]. Soft drugs should be inactivated by ubiquitously distributed enzymes that exert their action preferably in the plasma or in the peripheral tissue where the GCEE is delivered (i.e., the lungs). LPE (loteprednol etabonate) (**21**) and EPC (etiprednol dicloacetate) (**14**) are two recent examples of intentionally designed “soft” analogs, as they are hydrolyzed to cortienic acid, the inactive metabolite of hydrocortisone (**2**), and have been referred to as “dissociated” GCEEs because they do not exert any transactivation [60]. The enhanced TI of LPE (**21**) stems from its long-lasting action and the complete lack of activity of its metabolites [101]. EPC (**14**) then came as a second-generation cortienic-acid-based soft steroid, with a binding affinity higher than LPE (**21**) [60].

The rapid metabolism of FTP (**18p**) occurs not by hydrolysis of the ester group in the plasma but by oxidation of the sulfide in the liver [102]; the high first-pass

metabolism removes oral bioavailability, but not systemic exposure after subcutaneous administration, which can lead to accumulation (with cortisol suppression) because of the long terminal half-life (13 h) after inhalation [103].

Multiple randomized controlled trials have demonstrated that the addition of a LABA to the existing ICS therapy (as fixed-dose combinations, from FTP (**18p**) + formoterol fumarate to the latest FTF (**18f**) + vilanterol) provides more effective clinical outcome than an increase in the dose of ICS monotherapy, also thanks to an increased real-world patient adherence [104]. The synergistic effect unleashed by these combinations can be explained by the observation that the β 2-receptor gene is transcriptionally upregulated by glucocorticoids, whereas long-term administration of a LABA would lead to downregulation of the same genes. On the other hand, LABAs complement the effects of corticosteroids via interaction with glucocorticoid signal transduction, by activation of GRs and enhancement of the transcription of anti-inflammatory mediators.

Despite all the recent advances in ICS-based therapies, 5–10% of the asthmatic population is unresponsive to corticosteroids, possibly because of the expression of IFN γ - and TNF α -inducible protein-10 (CXCL10), a chemokine known to mediate mast-cell migration into asthmatic airway smooth muscle bundles, that is found in elevated level in patients suffering from viral exacerbation and from COPD [105]. Glucocorticoids also perform poorly in patients with acute respiratory distress syndrome and lung fibrosis and in the case of blunted lung development in preterm infants, pathologies that along with COPD are mediated by the transforming growth factor (TGF)- β family of polypeptide growth factors, whose signaling pathway is potentiated by DX (**11**), MP (**22**), and FT (**18**) (among others) [106]. Although asthma has been typically described as a Th2-associated eosinophil-predominant disease, alternative models have more recently called into action a Th17-mediated neutrophil-predominant phenotype that causes more severe disease and less responsiveness to steroids [107].

18.5

Structure–Activity Relationships

The Δ^1 double bond in the general structure **1** was introduced early on and maintained because of the increased potency (Figure 18.3) [90]. The presence of the 11 β -hydroxyl (instead of an 11-ketone), the 17 α -hydroxyl, the 16 α - or (preferably) the 16 β -methyl, or the 16 α -alkoxyl (in the case of acetonides) groups also emerged as an indispensable structural feature to ensure potency [108]. For example, DX (**11**) analogs with 16-methylidene, 16-*gem*-dimethyl, or Δ^{15} -16-methyl all turned out to be inactive [30].

A 7-halogen substitution, as seen in ACP (alclometasone dipropionate) (**3**), can also yield compounds in the prednisolone series of potency comparable to that of BMV (**5v**) (for 7-Br and 7-Cl) [109]. Also seldom investigated, more recent 12 β -acyloxy analogs of betamethasone (**5**) were less topically active than comparable

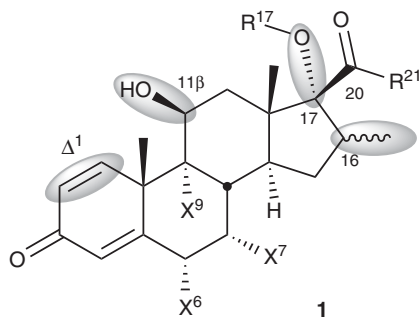


Figure 18.3 The general structure of GCEs with essential functionalities for activity highlighted.

BMP (**5p**) and BDP (**4**), but they did not display any systemic side effect, nor were they atrophogenic [110].

For the first three decades or so in the history of topical anti-inflammatory corticosteroids, analog deviations from the winning $17\alpha,21$ -dihydroxy- 20 -keto pattern were rare. Its replacement with a 17α -alkynyl- 17β -hydroxy, with all else equal, is one of the few early examples where not only activity was retained but also the relative affinity toward GR versus MR, the progesterone receptor (PR), and the androgen receptor (AR) was comparable or better than with DX (**11**) [111]. Esterification at positions 17 and 21 had been long known to shift activity from oral to topical; simple benzoate mono- and diesters or higher homologs of acetate contributed to the decreased systemic activity in halopredone (HP) (**20**), for instance [112]. The 17α side-chain propionate and 2-furoate (including 3-furoate and a few alkylated furoates) [113] render agents such as MMF (**23**) and FTP (**18p**) highly lipophilic, which may facilitate their absorption through nasal mucosa and uptake across phospholipid cell membranes. These compounds demonstrate negligible systemic absorption, owing to the fast hepatic and intestinal metabolism [114], unlike older corticosteroids such as BDP (**4**) and TC (**26**) [115]. The high lipophilicity (as in FTP (**18p**)), along with 6α - or 9α -halogenation, translates into higher receptor affinity for GCEs [116], which is directly related to the clinical efficacy of ICSs [117], with fast association and slow dissociation from the GCE receptor [118]. A 9α -fluorine also retards the oxidation at the proximal 11β -hydroxyl group. Along with light halogens at 6α or 9α (bromine already decreases activity) [90], the additional Δ^1 double bond confers the higher potency of the corticosteroid not only against GR but also against MR. This cross-reactivity can be alleviated by the presence of a 16-methyl group [44]. A 6α -methyl substitution is known to be favorable, as demonstrated by $17,21$ -diester analogs of betamethasone (**5**) [119]. Equally relevant, the introduction of $16\alpha,17\alpha$ -acetonides, as in FCA (**16**) and TCA (**26**), also contributed to the jump in the lipophilicity that proved useful for topical applications [90]. Similarly, the $[16\alpha,17\alpha-d]$ -oxazoline in DFZ (**9**) or the regioisomeric $[16\alpha,17\alpha-d]$ -isoxazoline analogs provided potent compounds with improved safety profiles [120]. Interest in $16\alpha,17\alpha$ -acetals, including aryl acetals [121], stemmed from their facile hepatic metabolism that degrades these functionalities into inactive fragmentation products [122]. A combination of

21-alkylthio-16 α ,17 α -acetonide can be found in the Glaxo compound **71** (Section 18.3, Scheme 18.10) that incorporates a lactone readily hydrolyzable in the plasma by paraoxonase (EC 3.1.8.1), whereas it remains stable in the lungs, an excellent example of a “soft” drug [79].

In an effort to extend the paradigm of “soft” drugs, positionally reversed (20*R/S*)-hydroxy, 21-carboxamide analogs were proposed and tested for local and systemic activity [123]. Not surprisingly, amides with larger N-substituents had higher binding affinity, in good correlation with increased lipophilicity. However, these amide analogs were not devoid of undesirable systemic side effects, presumably because of the slower rate of hydrolysis compared to esters.

The early exploration of 21-chloro analogs, which would eventually lead to the discovery of CBP (**7**) [68], made evident that an intact 17 β -ketol side chain is not an absolute requirement for activity [48]. For instance, 21-chloro-17-methylsuccinate analogs of CBP (**7**) demonstrated vasoconstrictive activity, a good predictor of topical anti-inflammatory potency, comparable or better than BMV (**5v**) [124]. Similarly, 21-chloro-17 α -(methylthio)acetoxy derivatives of BMV (**5v**) also retained comparable activity [125]. The halogenation at C-21, along with the 16 β -methyl group, seems to reduce the paradoxical allergenicity by systemic contact dermatitis, reported with many earlier corticosteroids – indeed most of the modern GCE esters rarely induce sensitization [126].

On further exploration of the 20-keto side chain, 21-thio analogs of BMV (**5v**) also proved active when combined with an ester at position 17 α [127]. More recently, SAR studies on 21-sulfurated derivatives of hydrocortisone converged on 21-(2-mercaptobenzothiazole)-17 α -(2-furoate) as a promising candidate for the dissociated side effects from the anti-inflammatory *in vivo* potency in rodent models, which resulted in a TI 15-fold higher than FTP (**18p**) [128]. The addition of a 16 α -methyl group further enhanced potency in this series [129].

The modification of the 20-keto moiety into a 20-ester, as in LPE (**21**) and similar analogs [113], or the 20-thioester, as in FTP (**18p**) [73], likely contributed to enhanced safety. For instance, “nonketotic” LPE (**21**) is less likely to elevate intraocular pressure and is less cataractogenic, presumably for its inability to form Schiff base intermediates with lens proteins [130].

In the absence of structural information on GR, early geometry optimization calculations provided some rationale on the preferred conformations of selected glucocorticoids, which would then be confirmed by the small-molecule crystal structure [131]. D rings would range from a 13 β -envelope to a 13 β ,14 α -half chair, depending on the substitution pattern and on the possibility of intramolecular H-bonds between 17-OH and the 21-ketone, leading to the hypothesis that a specific conformation of the 17-side chain is needed for receptor binding. The convexity of the steroidal scaffold is increased by the introduction of the Δ^1 double bond and the 11 β -hydroxy group, compared to cortisone and its derivatives, and it is decreased by the 17-hydroxy group [131]. In 9 α -fluorocortisol, the A ring is bent under the average plane of the steroidal scaffold more than in the nonhalogenated parent compound, a conformational change similar to that caused by the Δ^1 double bond [132].

The potential of C-ring substitutions, such as the 9- or 11-halogens, to markedly alter the preferred conformation of the steroid side chain was illustrated in X-ray crystallographic studies on a series of pregnanes [133].

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Part VII

Ophthalmology

19

Prostaglandins with Carboxylic Functionalities for the Treatment of Glaucoma

Fabrizio Carta and Claudiu T. Supuran

19.1

Introduction

The prostaglandins (PGs) are fatty acid derivatives, belonging to the autocrine- and paracrine-acting autacoids with important physiologic functions in many tissues/organs. They are synthesized in all nucleated cells from arachidonic acid (AA) [1]. There are several different representatives of this class of biologically active lipids, obtained from AA through the cyclooxygenase or lipoxygenase pathways, which lead to PGs, thromboxanes, or leukotrienes [1, 2]. For the purpose of discussion in this chapter, only the most important member of the PGs connected to glaucoma is considered, that is, $\text{PGF}_{2\alpha}$ **1** (Figure 19.1), which is involved in many physiologic processes such as uterus contraction, bronchoconstriction, and aqueous humor outflow within the eye [3].

19.2

History

The observation that topical administration of $\text{PGF}_{2\alpha}$ (**1**) directly into the eyes of primates or other experimental animals leads to a significant hypotensive effect dates back to the early 1980s [4, 5]. This led to the hypothesis that antiglaucoma drugs based on PGs may be effective in the control of elevated intraocular pressure (IOP) typical of this disease. The main problems arose from the severe side effects provoked by $\text{PGF}_{2\alpha}$ (**1**), such as strong conjunctival hyperemia and irritation, at doses that produced the maximal effect on IOP [6]. Subsequently, the idea of developing $\text{PGF}_{2\alpha}$ analogs, which should possess the potent IOP-lowering effects of the natural autacoids without their side effects, triggered a lot of research in the field. Thus, the large medicinal chemistry efforts in the 1980s and 1990s allowed the development of at least five clinically used agents, which were launched in the market between the late 1990s and early 2000 [6]. These drugs are latanoprost (**2**), bimatoprost (**3**), travoprost (**4**), tafluprost (**5**), and unoprostone (**6**) (Figure 19.2). [6, 7]

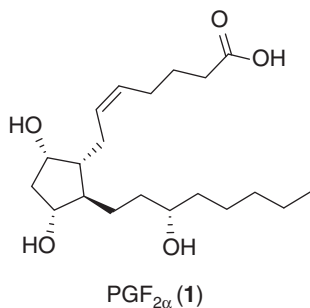


Figure 19.1 Structure of PGF_{2α} (1).

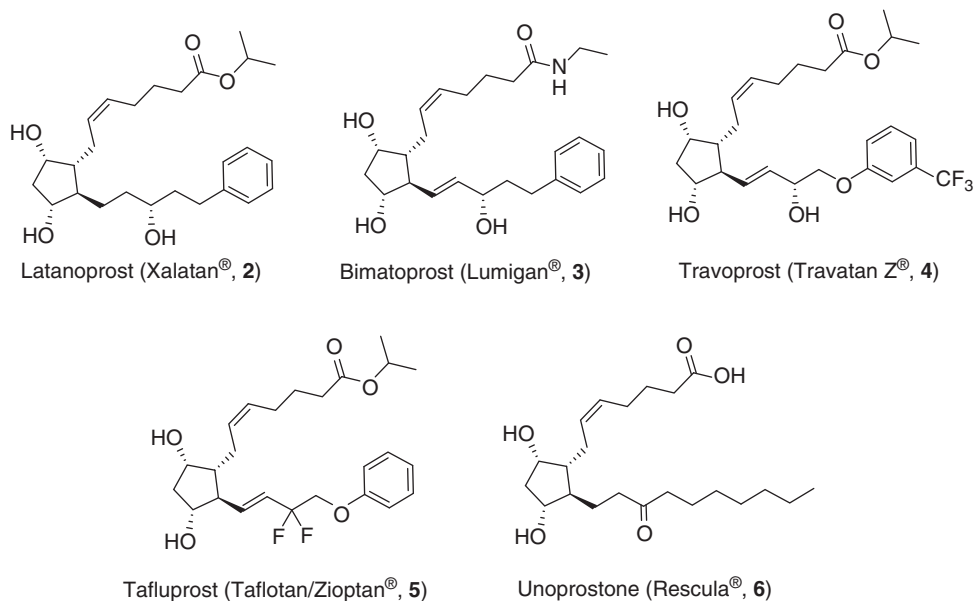


Figure 19.2 Marketed prostaglandins for the treatment of glaucoma.

They are among the most used antiglaucoma agents presently used, alone or in combination with other drugs such as the carbonic anhydrase inhibitors and β -adrenergic antagonists [8, 9].

19.3

Synthesis

As PGF_{2α} (1), the PG-based antiglaucoma agents herein reported are C20 fatty acids that are cyclized between C8 and C12 to form a cyclopentane ring. The cyclopentane ring is 9,10-*cis*-dihydroxylated. The side chains are *trans* to each other and are functionalized in a variety of ways (Figure 19.3).

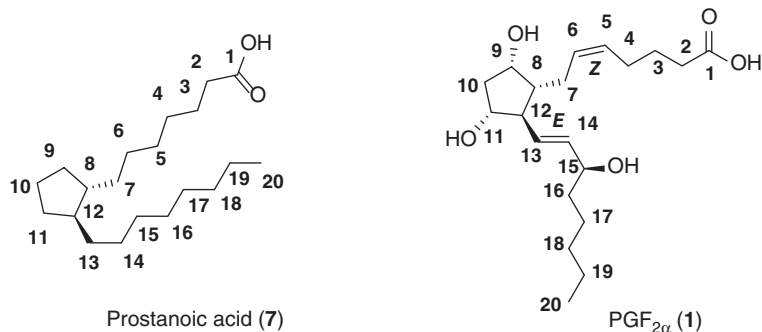
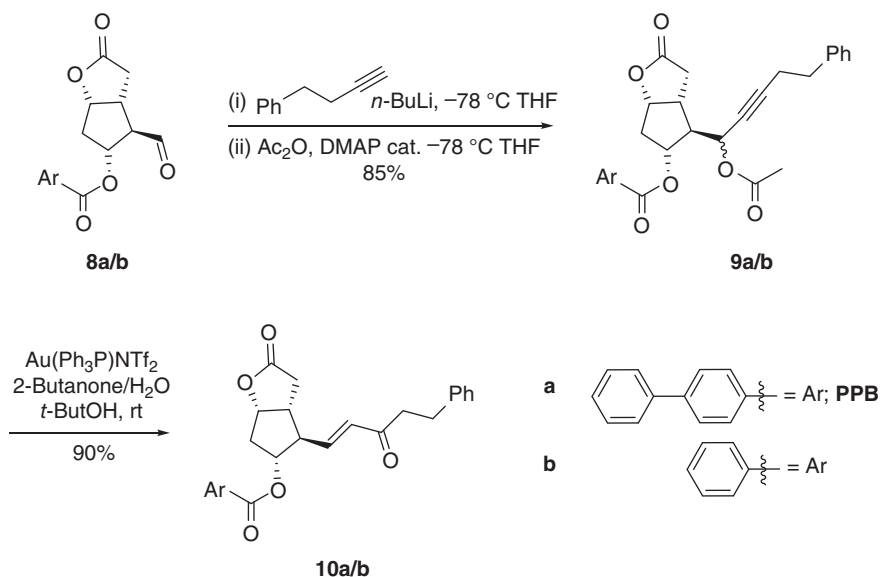


Figure 19.3 Structures and numbering of prostanoic acid (7) and PGF_{2α} (1).

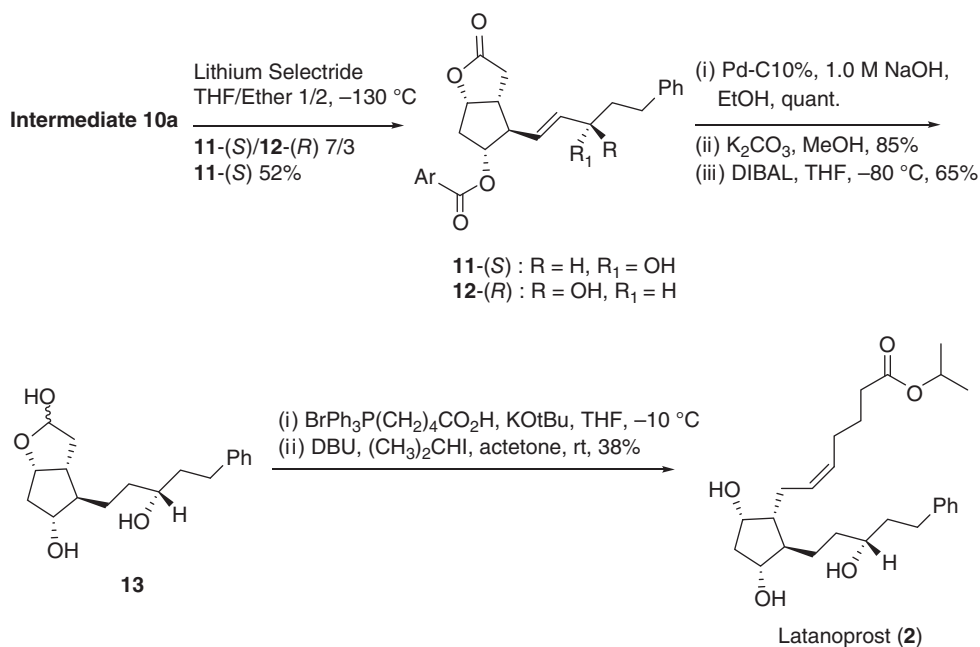
The synthesis of these compounds proved to be rather challenging as different aspects had to be taken into consideration, including the introduction of various functional groups, preservation of the stereochemistry as well as scalability and reproducibility. Herein, we report the most significant contributions in the field, most notably the innovative and revolutionary contribution of Corey *et al.* with lactone **8** (Scheme 19.1). This compound was specifically designed to allow the introduction of the appropriate functional groups with the required stereochemistry into the final PG scaffolds and, thus, significantly simplified the chemistry approaches that had to be used [11–13]. In particular, Corey's approach consisted of the sequential introduction of the C12 side chain into the lactone **8** via classical Horner–Wadsworth–Emmons (HWE) reaction, followed by Wittig condensation to introduce the C8 side chain. Recently, Zanoni *et al.* greatly improved Corey's approach by substitution of the HWE coupling with the gold-promoted Meyer–Schuster (M–S) rearrangement on the propargyl acetate **9** [10]. Zanoni's synthetic approach proved to be easier to conduct and, more importantly, free of epimerization events occurring at the labile stereogenic centers when compared with the HWE couplings. The syntheses of the common intermediate **10** as well as its use for the synthesis of latanoprost (**2**) and bimatoprost (**3**) are reported in Schemes 19.1–19.3 [10].

Treatment of the protected Corey lactones **8a** or **8b** with 4-phenyl-butyne lithium salt, generated *in situ*, followed by acetylation, led to **9a/b**. The alkyne was subjected to the M–S rearrangement with a 0.6 mol% Au(I)-based catalyst in 2-butanone/water (90/1) leading to the key intermediate **10a/b**, which showed 100% retention of its *E* configuration (Scheme 19.1) [10].

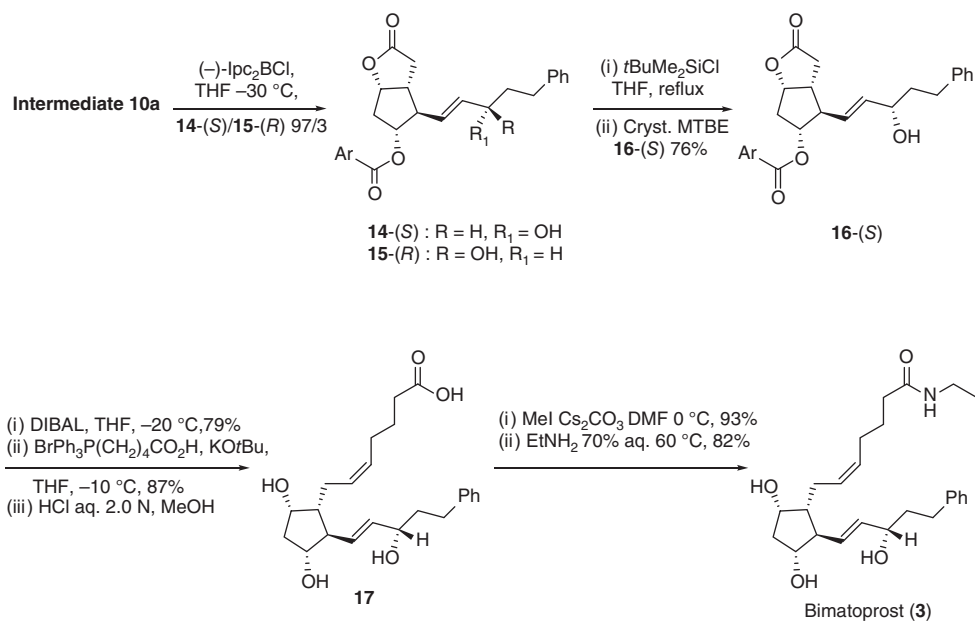
Thus, the Meyer–Schuster rearrangement represents the winning tool on Corey's synthetic strategy leading to prostaglandins and their drug analogs such as latanoprost (**2**, Scheme 19.2) and bimatoprost (**13**, Scheme 19.3) [14, 15]. To obtain latanoprost (**2**), the key intermediate **10a** was treated according to the reported procedure of Resul *et al.* [14] by reducing the ketone group in the lateral chain with lithium selectride, thus leading to a 7/3 mixture of epimeric alcohols **11**-(*S*) and **12**-(*R*), which were separated by flash chromatography to afford the desired **11**-(*S*) in 52% yield. Then, reduction of the (*E*) alkene with 10%



Scheme 19.1 Synthesis of intermediate **10a/b** using the gold-catalyzed Meyer–Schuster (M–S) rearrangement [10].



Scheme 19.2 Synthesis of latanoprost (Xalatan[®], **2**) [14].

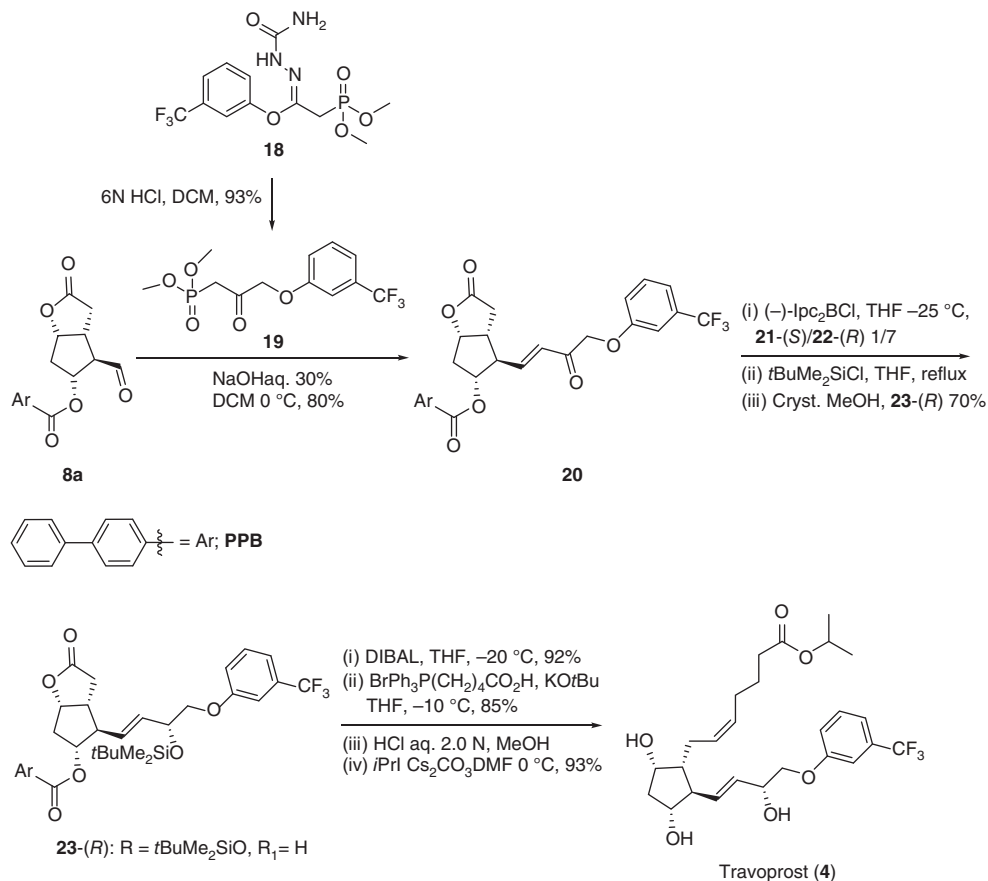


Scheme 19.3 Synthesis of bimatoprost (Lumigan[®], **3**) [15].

Pd–C in alkaline medium, followed by removal of the 4-phenylbenzyl (PPB)-protecting group and reduction of Corey's lactone with diisobutylaluminum hydride (DIBAL), afforded the saturated alcohol **13**. The insertion of the C8 chain with the correct (*Z*) geometry was ensured by subjecting **13** to Wittig coupling conditions with the appropriate phosphonium salt. Finally, esterification of the carboxylic group as isopropyl derivative afforded latanoprost (**2**).

A similar procedure was employed for the synthesis of bimatoprost (**3**, Scheme 19.3) [15]. The key ketone intermediate **10a** was subjected to asymmetric reduction in the presence of (–)-B-chlorodiisopinocampheylborane ((–)-DIP-Chloride) to afford a mixture of the epimeric alcohols **14-(S)** and **15-(R)** in a 97/3 ratio, which were silyl-protected and the desired **16-(S)** derivative was isolated by crystallization from methyl *tert*-butyl ether (MTBE) in excellent yield. Then, DIBAL reduction of the Corey lactone and Wittig coupling allowed the upper chain to be installed. Finally, PPB cleavage afforded the derivative **17**, which in turn was converted into the corresponding ethylamide **3** (bimatoprost) by routine procedures [15].

For the synthesis of travoprost (**4**), the classical Corey approach was used (Scheme 19.4). Corey's lactone **8a** was converted into intermediate **20** by HWE condensation with phosphonate **19**. Asymmetric reduction of the α,β -unsaturated ketone **20** in the presence of (–)-DIP-Chloride, as previously shown in Scheme 19.3, afforded a mixture of the epimeric alcohols **21-(S)** and **22-(R)** in a 1/7 ratio. The alcohols were silyl-protected and the desired **23-(R)** epimer was isolated through crystallization in methanol in 70% yield. The insertion of the C8 chain



Scheme 19.4 Synthesis of travoprost (Travatan Z[®], **4**) [15].

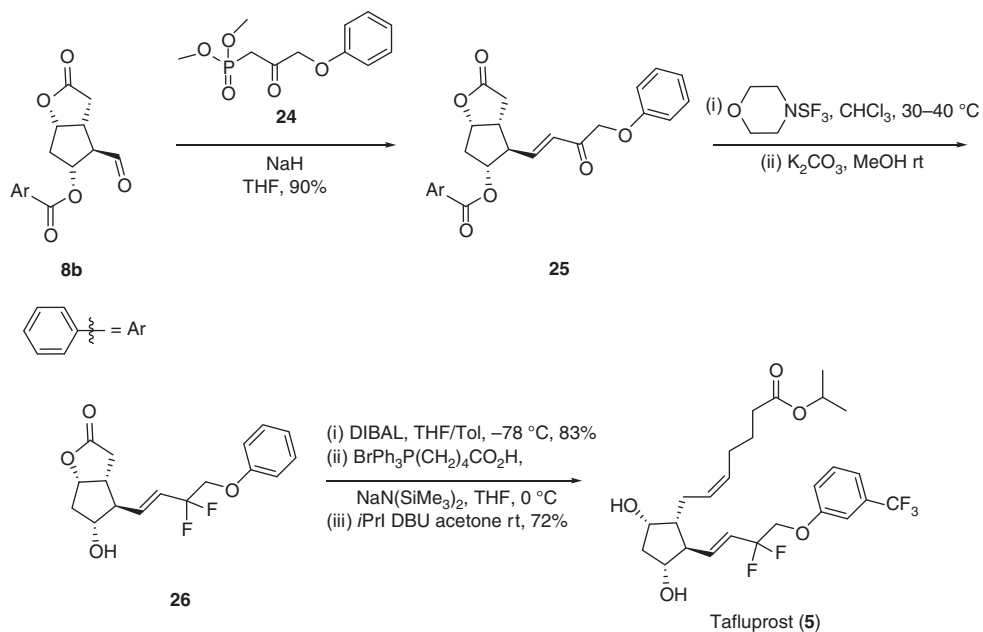
proceeded through a Wittig condensation after the DIBAL reduction of Corey's ketone, followed by PPB cleavage and esterification of the carboxylic acid to afford travoprost (**4**) [15].

The same approach as for the travoprost (**4**) was also followed for the synthesis of tafluprost (**5**) and unoprostone (**6**) (Schemes 19.5 and 19.6, respectively) [16–18].

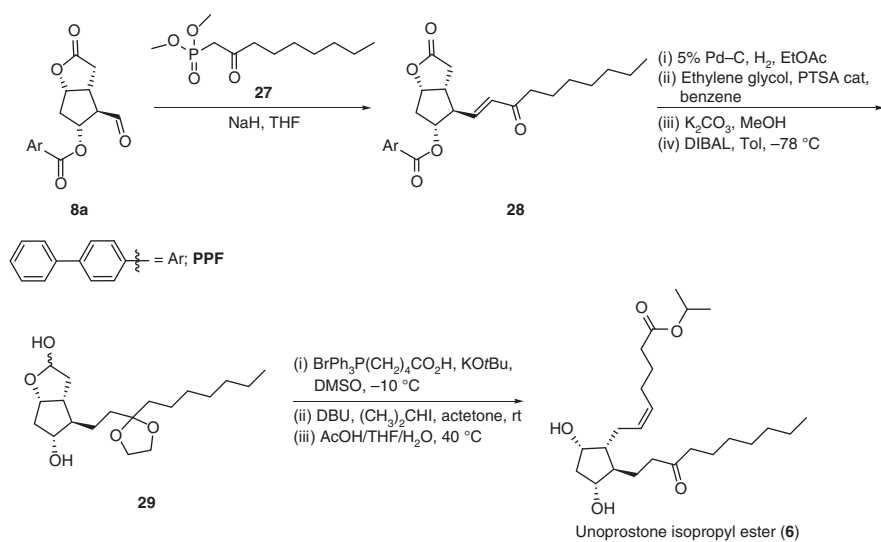
19.4

Mode of Action

The effective antiglaucoma activity of the PGF_{2α} derivatives is due to the reduction of elevated IOP, which in turn is caused by an increased outflow of the aqueous humor [6] mainly through the uveoscleral routes. This represented a new pharmacological principle for the treatment of glaucoma [6]. The drugs act through G-protein-coupled receptors specific of each PG type, in this case,



Scheme 19.5 Synthesis of tafluprost (5) [16, 17].



Scheme 19.6 Synthesis of unoprostone isopropyl ester (6) [18].

the prostaglandin F (FP) receptors [3, 6]. After topical administration, they reach systemic concentrations, which are below the level expected to stimulate FP-receptors outside the eye and are rapidly eliminated with a short half-life in plasma. For example, the half-life of latanoprost (**2**) is 17 min [6], which explains why clinical trials have not revealed any systemic side effects for the drug. The most frequent side effect observed with latanoprost (**2**) and the other PG analogs is an increased pigmentation of the iris, mainly in eyes with irides that are already partly brown. This effect is seen with several naturally occurring prostaglandins and is due to stimulation of melanin production in the melanocytes of the iridial stroma [6, 7]. The mechanism of action for the other PG analogs discussed in this chapter is the same as for latanoprost (**2**), the most used antiglaucoma drug of this class of pharmacological agents [6]. Latanoprost (**2**) and the other PG analogs were shown to possess significant additive effects when used in combination with non-PG-type antiglaucoma medications, such as carbonic anhydrase inhibitors or β -blockers [7–9]. The fixed combination of latanoprost (**2**) plus timolol was significantly more efficacious than either of its individual components and more effective than the fixed combination of dorzolamide and timolol [19].

19.5

Structure–Activity–Relationship (SAR)

Many $\text{PGF}_{2\alpha}$ analogs have been prepared and evaluated *in vivo* and *in vitro* for their antiglaucoma activity [20, 21]. Their IOP-lowering properties and potential side effects have been tested in cats, monkeys, and rabbits, widely used animal models for glaucoma. The Structure-Activity-Relationship (SAR) investigations were rather straightforward:

- 1) The esters (usually isopropyl esters) were more active and led to less side effects compared to the corresponding free carboxylic acids. In fact, four of the five clinically used drugs are isopropyl esters at the terminal COOH moiety. In some cases (e.g., bimatoprost (**3**)), the amides also showed very good IOP-lowering effects, comparable to the isopropyl esters [20].
- 2) Inversion of the configuration of the hydroxyl groups on carbon-9 or carbon-11 resulted in either a potency-lowering effect or turning of the $\text{PGF}_{2\alpha}$ FP agonist profiles to antagonists.
- 3) Replacement of parts of the C12 chain of $\text{PGF}_{2\alpha}$ (**1**) with a benzene ring changed the potency and receptor profile as FP antagonists. The optimal position of the benzene ring was on carbon-17, that is, 17-phenyl-18,19,20-trinor $\text{PGF}_{2\alpha}$ -isopropyl ester. These derivatives exhibited a much higher therapeutic index in the eye compared to $\text{PGF}_{2\alpha}$ (**1**) or its esters.
- 4) The biological activities of different substituents on the C8 benzene ring have also been studied. Introduction of a methyl group at position 2 or 3 afforded compounds that were more active than those incorporating the methyl group at position 4 [20].

In conclusion, the PGF_{2α} analogs represent a successful story of drug design, which led to antiglaucoma drugs with a completely new mechanism of action, very high clinical efficacy, and a favorable side-effect profile. This made drugs belonging to this pharmacological class, such as latanoprost (2), bimatoprost (3), travoprost (4), tafluprost (5), and unoprostone isopropyl ester (6), widely used therapeutic agents for the management of glaucoma.

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Part VIII

Weed Control

20

Herbicidal Carboxylic Acids as Synthetic Auxins

Paul Schmitzer, Jeffrey Epp, Roger Gast, William Lo, and Jeff Nelson

20.1

Introduction

Seven classes of auxin mimic herbicides (commonly referred to as synthetic auxins), all containing carboxylic acid functionality, have been introduced for commercial use throughout nearly seven decades. These auxin herbicide classes consist of the phenoxy-carboxylic acids (Figure 20.1), benzoic acids (Figure 20.2), pyridinecarboxylic acids (Figure 20.3), pyridyloxy-carboxylic acids (Figure 20.4), quinolinecarboxylic acids (Figure 20.5), pyrimidinecarboxylic acids (Figure 20.6), and aryl pyridinecarboxylic acids (Figure 20.7). Synthetic auxins were the first selective organic compounds developed for weed control.

20.2

History

In the early 1940s, researchers in the United Kingdom and the United States synthesized novel compounds that provided the same plant growth regulator activity as 1-naphthalene acetic acid, but showed the ability to selectively control dicot species in monocot crops. 2,4-Dichlorophenoxyacetic acid (2,4-D, **1**) and 2-methyl-4-chlorophenoxyacetic acid (MCPA, **2**) (Figure 20.1) were among the compounds that were synthesized and tested [1]. The research eventually led to the commercialization of both compounds in the late 1940s.

Dichlorprop (**3**) and mecoprop (**4**) were both patented by The Boots Co. Ltd and were introduced as herbicides in 1961 and 1956, respectively [2]. The growth regulatory properties of 2,4-dichlorophenoxybutyric acid (2,4-DB, **5**) and 2-methyl-4-chlorophenoxybutyric acid (MCPB, **6**) were first described in 1947 and 1955, respectively [2]. The phenoxy-carboxylic-acid auxin herbicides represented the first selective organic herbicides commercially available to farmers.

The plant growth regulating properties of substituted benzoic acids were identified in the early 1940s [3]. A number of substituted benzoic acids were developed

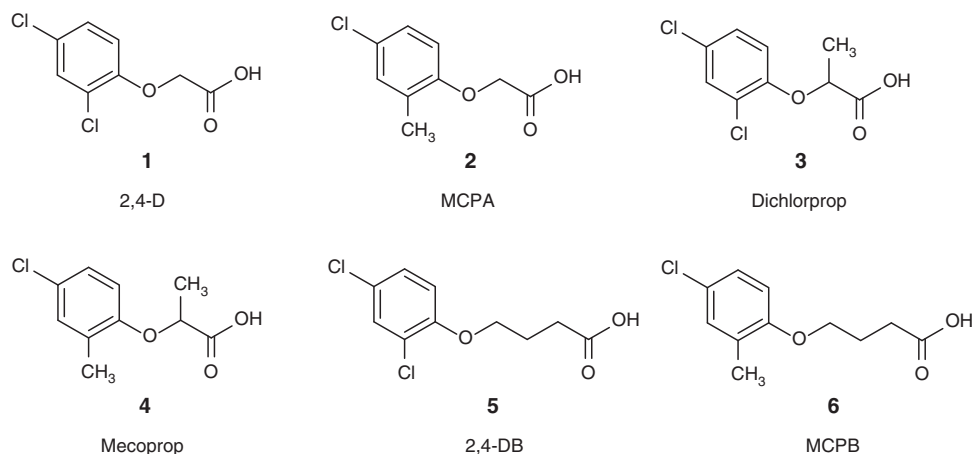
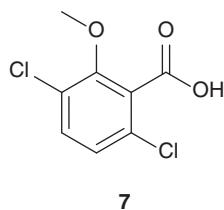


Figure 20.1 The phenoxy-carboxylic acid auxin herbicides 2,4-D (1), MCPA (2), dichlorprop (3), mecoprop (4), 2,4-DB (5), and MCPB (6).



Dicamba

Figure 20.2 The benzoic acid auxin herbicide dicamba (7).

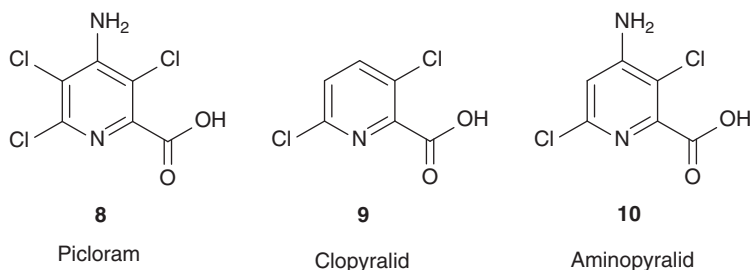


Figure 20.3 The pyridinecarboxylic acid auxin herbicides picloram (8), clopyralid (9), and aminopyralid (10).

for use as herbicides including the most commercially successful benzoic acid herbicide, dicamba (7). The structure and synthesis of dicamba were first published in 1961 in US patent 3,013,054. Dicamba was invented by Sidney Richter in 1958, and the patent was assigned to Velsicol Chemical Company (Figure 20.2) [4].

The pyridinecarboxylic acid herbicides (also known as the picolinic acid herbicides) were first discovered due to crop injury caused by soil metabolites in field trials of pyridine-based nitrification inhibitors [5]. Picloram (8) and clopyralid (9) were synthesized as analogs of the originally identified herbicidally active soil

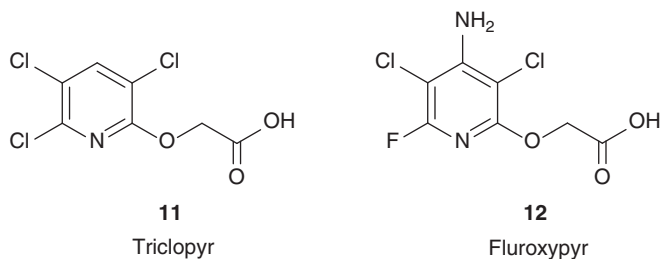


Figure 20.4 The pyridyloxy-carboxylic acid auxin herbicides triclopyr (**11**) and fluroxypyr (**12**).

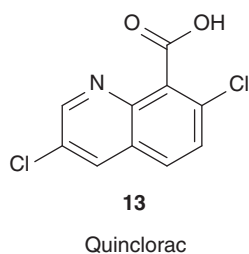


Figure 20.5 The quinolinecarboxylic acid auxin herbicide quinclorac (**13**).

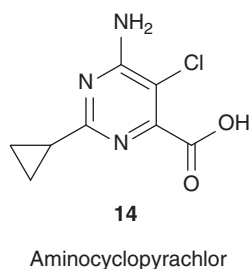
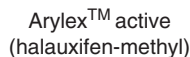


Figure 20.6 The pyrimidinecarboxylic acid auxin herbicide aminocyclopyrachlor (**14**).

metabolites. The structure and herbicidal activity of picloram were published in 1963 [6, 7]. Clopyralid (**9**) was patented in 1964, but its herbicidal utility was not described until 1975 [8, 9]. Aminopyralid (**10**) was identified as a potential process impurity while chemists at The Dow Chemical Company were researching the electrochemical synthesis of clopyralid. The herbicidal utility of aminopyralid was first identified in 1998 and patented in 2001 (Figure 20.3) [10].

Pyridyloxy-carboxylic acids, including triclopyr (**11**), were first synthesized in the late 1950s to determine if pyridine analogs of the phenoxy-carboxylic acids would provide the same hormonal activity on higher plants [11]. The herbicidal utility of DOWCO 233 (triclopyr, **11**) was not described until 1975 [12]. The pyridyloxy-carboxylic acids with a 4-amino substitution were first introduced as herbicides in US Patent 3,761,486 assigned to The Dow Chemical Company in 1973 [13]. The herbicidal activity of DOWCO 433 (fluroxypyr, **12**) was first described in 1981 (Figure 20.4) [14].

Synthetic efforts focused on substituted quinolines as antimalarial drugs at BASF eventually led to the discovery of the quinolinecarboxylic acid auxins [15].



Rinskor™ active

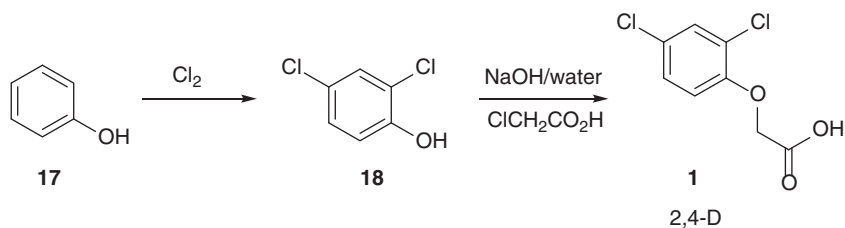
(Figure 20.5) [16].

clopyrachlor (**14**) (Figure 20.6).

(Figure 20.7) [20, 21].

Synthesis

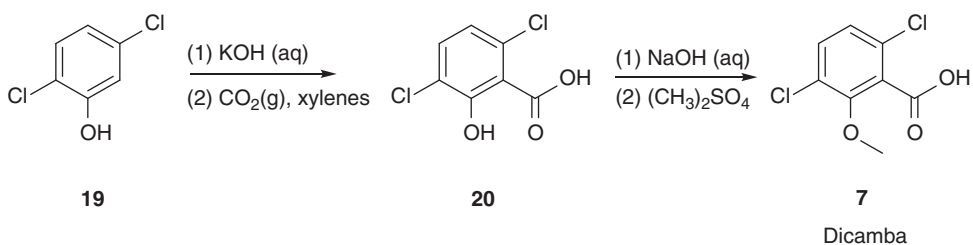
simple and can be exemplified by the synthesis of 2,4-D (**1**) shown in Scheme 20.1.



Scheme 20.1 Synthesis of phenoxy-carboxylic acid auxin herbicide 2,4-D (**1**) [22].

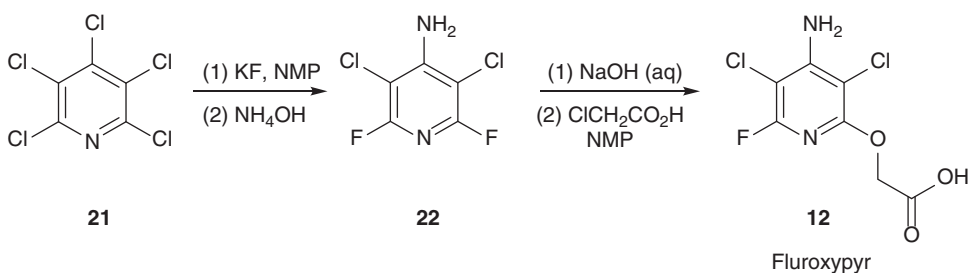
Accordingly, key dichlorophenol **18** can be produced via chlorination of phenol (**17**). Subsequent alkylation of **18** with chloroacetic acid affords 2,4-D (**1**) [22].

The synthesis of the benzoic acid auxin herbicide dicamba (**7**) is shown in Scheme 20.2. Accordingly, key benzoic acid **20** can be produced from 2,5-dichlorophenol (**19**) by an unusual reaction of potassium 2,5-dichlorophenoxide with carbon dioxide under high pressure. Subsequent alkylation of benzoic acid **20** with dimethylsulfate affords dicamba (**7**) directly [4].



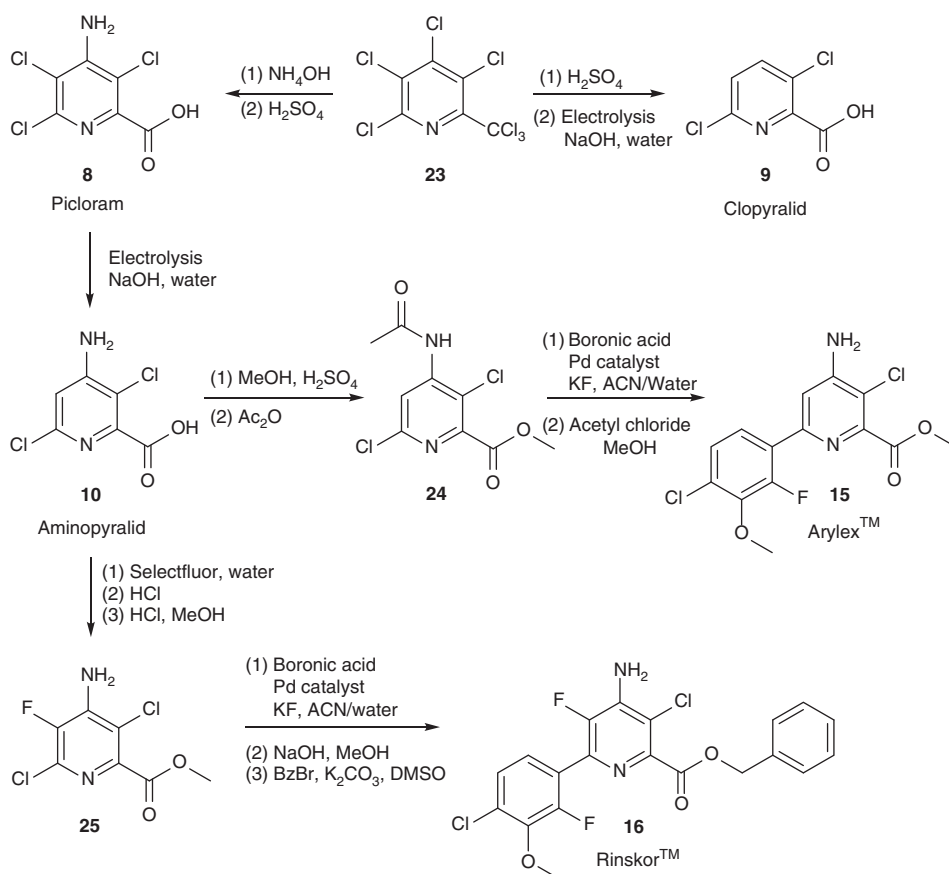
Scheme 20.2 Synthesis of benzoic acid auxin herbicide dicamba (**7**) [4].

The synthesis of pyridyloxy-carboxylic acid auxin herbicide fluroxypyr (**12**) is illustrated in Scheme 20.3. Key 4-aminopyridine **22** can be produced in two steps (nucleophilic fluorination followed by amination) starting with pentachloropyridine **21** [23, 24]. Subsequent hydrolysis of **22** with aqueous base and alkylation with chloroacetic acid affords fluroxypyr (**12**) [25].



Scheme 20.3 Synthesis of pyridyloxy-carboxylic acid auxin herbicide fluroxypyr (**12**) [23–25].

A synthesis of the pyrimidinecarboxylic acid auxin aminocyclopyrachlor (**14**) was recently described in the nonpatent literature [18]. The pyridinecarboxylic acid auxins picloram (**8**), clopyralid (**9**), aminopyralid (**10**) and the aryl pyridinecarboxylic acid auxins Arylex (**15**) and Rinskor (**16**) can be synthesized by the sequences shown in Scheme 20.4. Accordingly, clopyralid (**9**) can be produced by hydrolyzing tetrachloropyridine **23** under acidic conditions followed by electrolysis under basic conditions [26]. Picloram (**8**) can be produced from tetrachloropyridine **23** by amination followed by acidic hydrolysis [27]. Aminopyralid (**10**) can be produced from **8** by electrolysis under basic conditions [28]. Arylex (**15**) can be prepared in two steps (Suzuki coupling followed by deacetylation) via intermediate **24**, which in turn can be prepared in two steps (esterification followed by amidation) from **10** [20]. Rinskor (**16**) can be prepared from intermediate **25** by Suzuki coupling followed by hydrolysis and esterification



Scheme 20.4 Synthesis of pyridinecarboxylic acid auxin herbicides picloram (**8**), clopyralid (**9**), and aminopyralid (**10**) and the aryl pyridinecarboxylic acid auxin herbicides Arylex™ active (**15**) and Rinskor™ active (**16**) [18, 20, 21, 26–29].

[21]. Key intermediate **25** can be produced from intermediate **10** by electrophilic fluorination followed by esterification [29].

20.4

Mode of Action

Initially, the mode of action for synthetic auxins in sensitive plant species was described as the plants growing themselves to death [30]. Indeed, the phenotypic response of sensitive plants to auxin herbicides includes stem and petiole twisting (epinasty), leaf malformations, swelling and splitting of the stem, root growth inhibition, and eventually plant death. For decades, the mode of action investigation of synthetic auxin herbicides focused on morphological, physiological, and biochemical responses in plants treated with specific auxins. Biochemical responses in plants affected by synthetic auxin herbicides include elevated levels of 1-aminocyclopropane-1-carboxylate leading to increased levels of ethylene, cyanide, and abscisic acid. The relatively recent identification of TIR1 and AFB5 F-box proteins as auxin receptors [31–33] has enabled research to understand the transcriptionally mediated downstream effects of auxin binding. Differences in binding specificity to these auxin receptors for various classes of auxins have recently been identified [34]. Ultimately, this differential binding may help explain the differences in weed control spectrum and crop selectivities observed for the various synthetic auxin herbicide classes and compounds.

20.5

Biological Activity

The auxin mimic herbicides are generally known for their ability to selectively control annual and perennial broadleaf weeds in grass crops, yet members of the auxin herbicides are used for the control of some annual sedges (i.e., triclopyr (**11**)) and annual warm-season grass weeds (i.e., quinclorac (**13**) and Rinskor active (**16**)) as well. A comparison of the individual commercial auxin herbicides shows that each is characterized by a unique profile that includes application rates, application types, weed control spectrums, crop tolerance, environmental fate, and physical chemistry characteristics. These differences enable a variety of crop uses for weed control around the world (Table 20.1).

The weakly acidic carboxylic acid moiety of auxin herbicides confers plant phloem mobility and systemic herbicidal activity. Most auxin herbicides are applied as foliar treatments, but many provide some degree of soil residual control through root uptake that is dependent on application rate and environmental conditions. In most cases, auxin herbicides are formulated as either salts or various esters to achieve the desired biological activity [2]. De-esterification of esters in the plant apoplast is necessary to provide the active carboxylic acid moiety. Over time, discovery efforts have resulted in a general trend

Table 20.1 Herbicides, weed control spectrum, and major uses for each auxin family [2].

Herbicide family	Active ingredient	Weed control ^{a)}	Typical use rate (g ha ⁻¹)	Major use
Phenoxy-carboxylic acid	2,4-D	BL	280–4 000	Wheat, barley, corn, turf
	MCPA	BL	260–1 800	Wheat, barley, corn, turf, sorghum
	Dichlorprop	W	1 000–10 000	ROW ^{b)}
	Mecoprop	BL	2 240–3 360	Turf, wheat, barley
	2,4-DB	BL	140–1 700	Soybean, peanut, forage legumes
	MCPB	BL	560–1 700	Peas
Benzoic acid	Dicamba	BL	70–560	Corn, wheat, barley, R&P, turf
Pyridinecarboxylic acid	Picloram	BL, W	125–560	R&P ^{c)} , ROW
	Clopyralid	BL	80–560	Corn, R&P, wheat, barley, canola, sugar beet
Quinolinecarboxylic acid	Aminopyralid	BL	5–120	R&P, ROW
Pyridyloxy-carboxylic acid	Quinclorac	BL, G	280–560	Rice, ROW, turf
	Triclopyr	BL, S, W	280–1 120	R&P, rice, ROW, turf
Pyrimidinecarboxylic acid	Fluroxypyr	BL	70–280	Wheat, barley, corn
Aryl pyridinecarboxylic acid	Aminocyclopyrachlor	BL, W	70–180	ROW
Aryl pyridinecarboxylic acid	Arylex TM Active	BL	5–10	Wheat, barley
	Rinskor TM Active	BL, G, S	5–30	Rice

a) BL – broadleaf, G – grass, S – sedge, and W – woody.

b) ROW – right-of-way.

c) R&P – range and pasture.

toward higher levels of active ingredient potency and more targeted weed control spectrums. For example, phenoxy-carboxylic acid herbicides are typically used at 560–1120 g ha⁻¹, pyridine herbicides at 60–280 g ha⁻¹, and aryl pyridinecarboxylic acid herbicides at 5–30 g ha⁻¹ (Table 20.1).

Of the phenoxy-carboxylic acid herbicides, 2,4-D (**1**) is the most widely used and has the most diverse set of uses, providing selective postemergence control of broadleaf weeds in monocot crops and noncropland. Other herbicides in this class are generally less active but confer improved crop tolerance for specific crop uses. While the major uses of the benzoic acid herbicide dicamba (**7**) are similar to those of 2,4-D, dicamba is generally more active than 2,4-D with use rates ranging from 70 to 560 g ha⁻¹ in row crops and higher use rates in range and pasture (R&P), turf, and fallow. Dicamba is often used to control weeds that are not readily controlled by 2,4-D, including a number of biennial and perennial broadleaf species.

The pyridinecarboxylic acid herbicides picloram (**8**), clopyralid (**9**), and aminopyralid (**10**) are especially effective at controlling perennial broadleaf species. Picloram and aminopyralid both are mainly used in R&P and noncrop

sites but do have utility in certain grass and broadleaf crops. Discovered almost 40 years after picloram, aminopyralid provides improved spectrum and control of many important species at lower use rates. Clopyralid has a narrower control spectrum but is uniquely selective in certain broadleaf crops such as canola (oil seed rape), sugar beets, and number of specialty crops. It is also more suitable for cropping systems due to its lower soil residuality. The related pyridyloxy-carboxylic acid herbicides triclopyr (**11**) and fluroxypyr (**12**) are also used in R&P and noncrop sites, but for vastly different purposes. Triclopyr is particularly effective for woody plant control as well as certain broadleaf weeds. Fluroxypyr, very selective in R&P and cereal crops, controls economically important weeds such as docks (*Rumex* spp.), sericea lespedeza (*Lespedeza cuneata*), kochia (*Kochia scoparia*), and cleavers (*Galium aparine*), which are prevalent in cereals. The pyrimidinecarboxylic acid herbicide aminocyclopyrachlor (**14**) is also effective in annual and perennial broadleaf weed control but is registered only for noncrop uses.

A unique feature of quinclorac (**13**) is its activity on certain grass species; however, the activity of quinclorac on grass species is thought to be through a different mode of action than in broadleaves. Quinclorac is primarily used in rice (*Oryza sativa* L.) for the control of *Echinochloa* spp. and some dicot weeds, but it has also been used in turfgrass, cereals, canola, and fallow applications [2].

The aryl pyridinecarboxylic acid herbicides Arylex active (**15**) and Rinskor active (**16**) are primarily designed for use in cereals and rice, respectively, but also have potential for use in other crops including some broadleaf crops. They demonstrate highly desirable characteristics such as very low effective use rates and rapid degradation in the environment to nonherbicidal residues [5]. Arylex is typically used at 5–10 g ha⁻¹ for control of annual broadleaf weeds in cereal crops. Rinskor is used at slightly higher rates (5–30 g ha⁻¹) for control of grasses, sedges, and annual broadleaf weeds in seeded and transplanted rice [35].

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21

Chloroacetamide Herbicides

Clemens Lamberth

21.1

Introduction

The chloroacetamides are one of the most widely used groups of herbicides, which are characterized by an excellent efficacy against many annual grass weeds and certain, mainly small-seeded, dicotyledonous weeds in a variety of major crops, such as corn, cotton, rice, and soybean. They are mainly used in preemergent and early postemergent treatment. The herbicidal activity of the chloroacetamides is due to their capability to inhibit the biosynthesis of nonsphingolipid very-long-chain fatty acids (VLCFAs), resulting in a lack of lipids, proteins, and lignin for the plant. Fourteen different members of this compound class have been commercialized, which all are tertiary chloroacetic acid amides. With the exception of the aminothiophene derivative dimethenamid (**6**) [1] and the aminostyrene derivative pethoxamid (**9**) [2], the amine moieties of all other chloroacetamide herbicides are secondary phenylamines, for which reason the members of this compound class are also called the chloroacetanilides. Besides propachlor (**11**) [3] and prynachlor (**13**) [4], all of these phenyl-based chloroacetamides bear a methyl or an ethyl group in each of the two *ortho* positions of the amine function as characteristic structural feature (2,6-dialkylated phenyl). Although 45 years have passed since the commercialization of alachlor (**2**) [5], the very first chloroacetamide on the crop protection market, this herbicide class still plays a significant role in weed control. In 2013, acetochlor (**1**) [6] and metolachlor (**8**) [7] were among the six biggest-selling herbicides, each with annual sales of about 600–650 million USD [8]. Other commercially successful chloroacetamides with annual sales of more than 100 million USD are the four market products metazachlor (**7**) [9], pretilachlor (**10**) [10], dimethenamid (**6**) [1], and butachlor (**3**) [11] (Figure 21.1).

21.2

History

The discovery of the chloroacetamide herbicide class took place in the early 1950s at Monsanto, where Hamm and Speziale, two scientists in the Organic

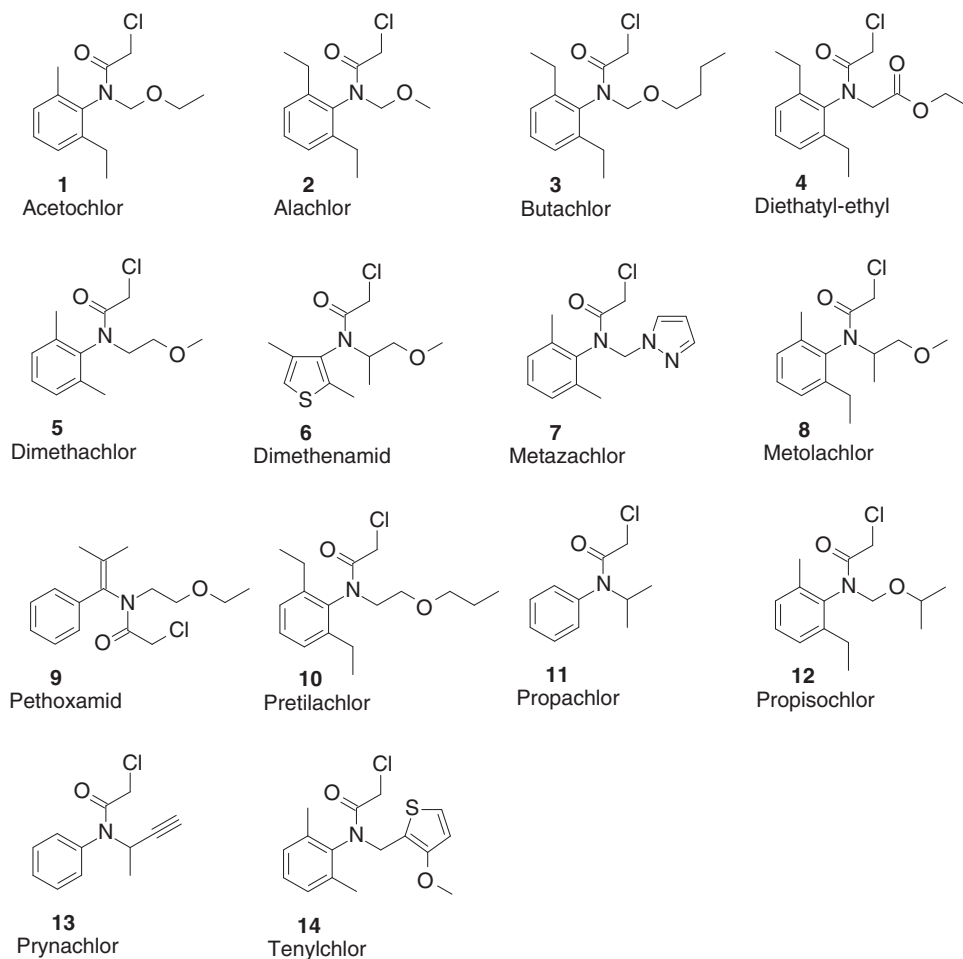
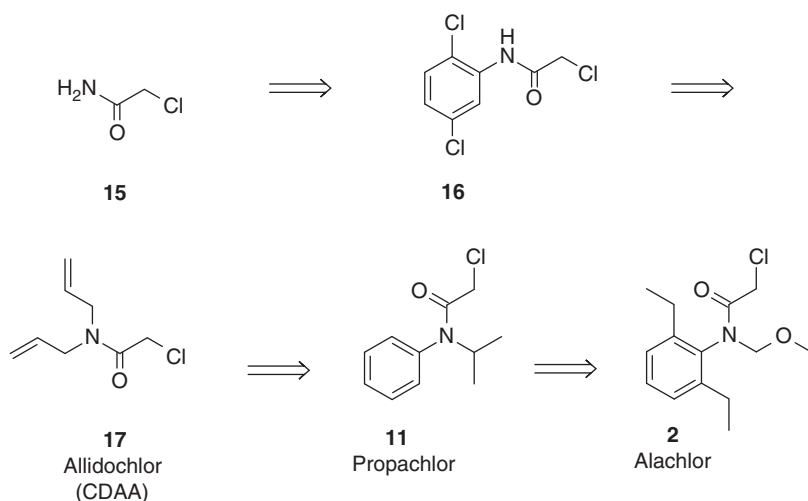


Figure 21.1 The 14 commercialized chloroacetamide herbicides.

Division of that company, realized that chloroacetamide (**15**) was “severely inhibitory to germinating seeds at relatively high rates, but only weakly active at lower use rates” [12]. Soon after followed the observation that *N*-(2,5-dichlorophenyl)chloroacetamide (**16**) strongly prevented the grass seed germination with little or no effect against broadleaf seeds; this sign of selectivity marks the birth of the chloroacetamide herbicides. At this time, more than 60 years ago, the need for annual grass control was acute. *Setaria faberi* (giant foxtail), *Echinochloa crus-galli* (barnyardgrass), and *Digitaria sanguinalis* (large crabgrass) were fierce competitors in many of the corn and soybean growing areas of the world. Mostly these weeds had to be removed manually from the fields or by spraying postemergent herbicides. A chemical that would be applied

at planting time reliably controlling the weeds, but allowing the crop to grow normally, would have been a fabulous new economic tool for the farmer. This long-standing dream came true in 1954 when Monsanto pushed allidochlor (**17**, CDAA) into development to be launched 2 years later to the market as first successful preemergent herbicide. However, allidochlor was eye- and skin-irritating as well as soil-dependent with quite low performance on sandy loam soils. Therefore, propachlor (**11**) was launched by Monsanto in 1965, which had similar properties compared to allidochlor (**17**), but reduced irritation. Finally, the progress in the preparation of sterically hindered anilines led, still at Monsanto, to the identification of the 2,6-dialkylated chloroacetanilides and in 1969 to the commercialization of alachlor (**2**) as the very first example of this group of compounds, which was not irritating at all and worked in all kinds of soils for the reliable preemergent broad-spectrum control of both grasses and broad-leaved weeds in corn and soybeans. This was a remarkable breakthrough in weed control and a milestone on the way to modern farming practices (Scheme 21.1) [12]. Acetochlor (**1**) and metolachlor (**8**), the two most important chloroacetamide herbicides of today were launched on the market in 1994 and 1976, respectively. The commercial replacement (also called chiral switch) of metolachlor (**8**) by its active principle (*S*)-metolachlor, which contains only the two herbicidally active forms of the four stereoisomers of metolachlor (see Section 21.6), hereby significantly reducing the chemical load to the environment, occurred in 1996 and was a paramount milestone for the whole crop protection industry as it is till today the world's largest industrial application of asymmetric catalysis [13–16].

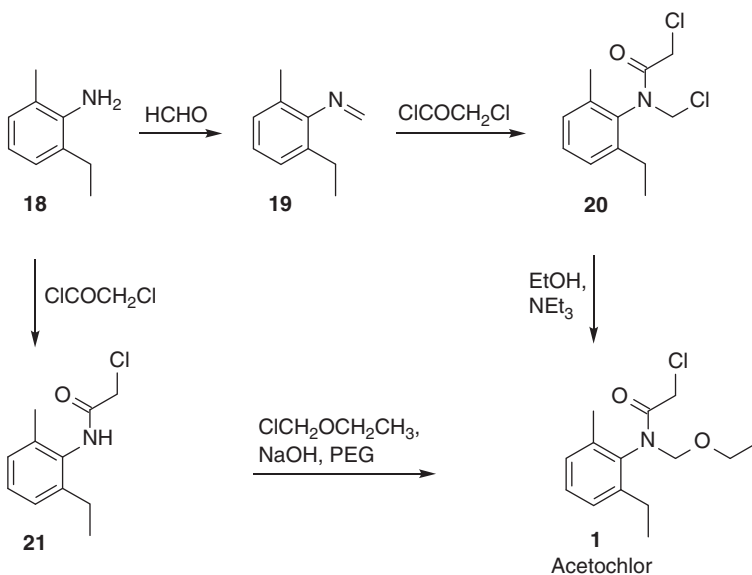


Scheme 21.1 Invention pathway of alachlor (**2**), the first 2,6-dialkylated chloroacetanilide [12].

21.3

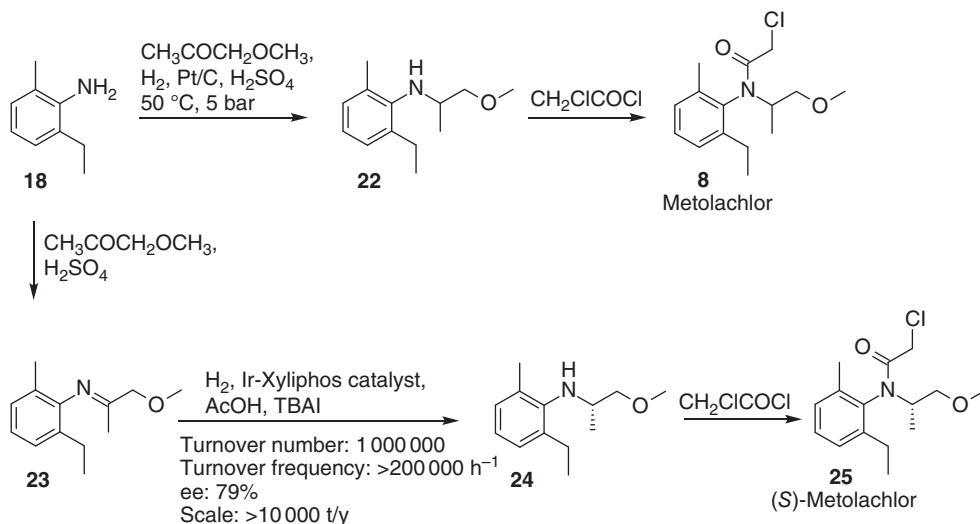
Synthesis

The original synthesis route to acetochlor (**1**) starts from 2-ethyl-6-methylaniline (**18**), which is converted with formaldehyde into the imine **19**. This Schiff base reacts with chloroacetyl chloride to the chloroacetanilide **20**, which delivers upon treatment with ethanol and triethylamine acetochlor (**1**) [17, 18]. Alternatively, the aniline **18** can also be acylated with chloroacetyl chloride to the amide **21**, which is then alkylated with ethoxymethyl chloride under phase-transfer catalysis to acetochlor (**1**) (Scheme 21.2) [19].



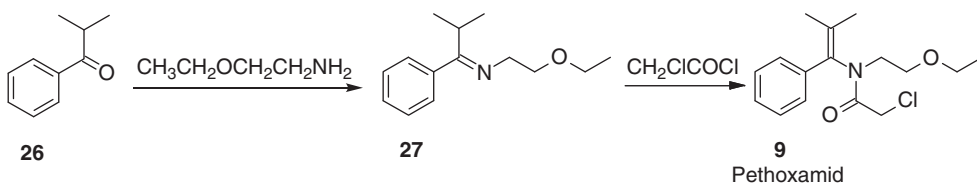
Scheme 21.2 Synthesis of acetochlor (**1**).

Both metolachlor (**8**) and its active principle (*S*)-metolachlor (**25**) are prepared from 2-ethyl-6-methylaniline (**18**). In the technical synthesis route to metolachlor, this starting material is converted with methoxyacetone under reductive conditions to the secondary amine **22**, which is then acylated with chloroacetyl chloride to metolachlor (**8**). The large-scale manufacturing of (*S*)-metolachlor (**25**) starts from the same two building blocks, 2-ethyl-6-methylaniline (**18**) and methoxyacetone, which are reacted to the imine **23**. The Ir-catalyzed enantioselective hydrogenation of this Schiff base to the chiral amine **24** is facilitated by a chiral ferrocenyl diphosphine ligand. This process, a true masterpiece of large-scale agrochemistry and till today the largest application of asymmetric catalysis, is used on a production scale of >10 000 tons/year, and for the synthesis of each 10 tons of **24** only 34 g of Iridium and 70 g of Xyliphos ligand are required. The last step is, in analogy to the racemate **8**, is the amidation with chloroacetyl chloride delivering (*S*)-metolachlor (**25**) (Scheme 21.3) [13–16, 20–22].



Scheme 21.3 Synthesis of metolachlor (8) and enantioselective synthesis of (S)-metolachlor (25).

The transformation of isobutyrophenone (26) with ethoxyethylamine to the imine 27 and its subsequent reaction with chloroacetyl chloride deliver pethoxamid (9) in only two steps (Scheme 21.4) [2].



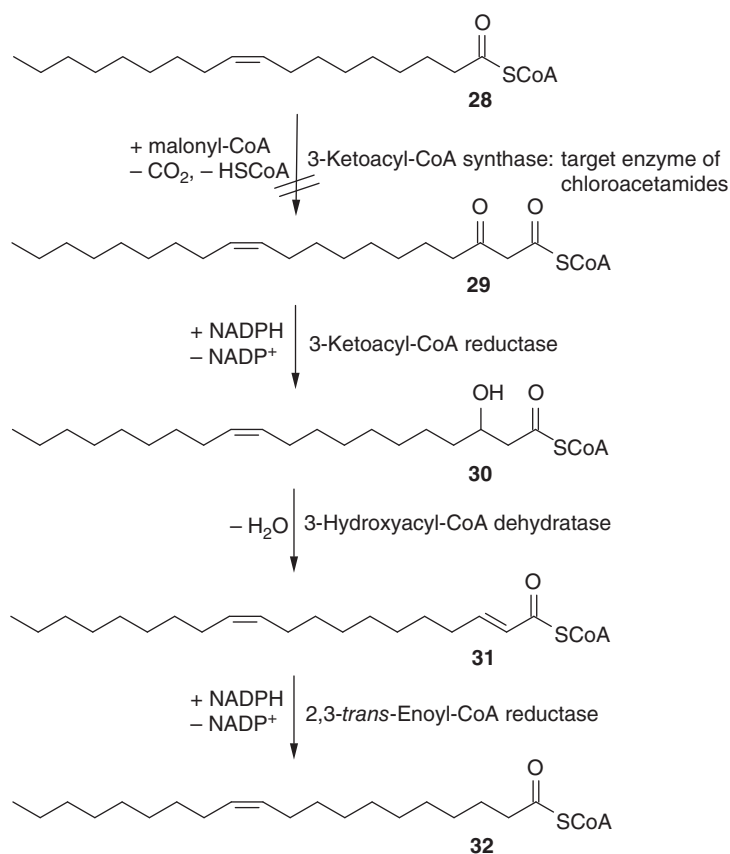
Scheme 21.4 Synthesis of pethoxamid (9).

21.4

Mode of Action

The mode of action of the chloroacetamides at the time of the market launch of most of the products was not clear. First, it was proposed that acetyl-CoA would play a role [23], but later it could be shown that lipid synthesis was impaired by inhibiting the incorporation of acetyl- and malonyl-CoA precursors into lipids [24]. Then it was found that cell division and cell enlargement are blocked [25], until finally it was established that these compounds inhibit the elongation of fatty acids to VLCFAs (20–26 carbon atoms) in plants [26–31]. VLCFAs mainly serve as stabilizing components of the plasma membrane in higher plants, but they also occur in epicuticular waxes, as energy storage system in seeds; as precursors of hydrocarbons, aldehydes, and alcohols for the plant surface; as components of the

protective barrier at the plant–environment interface; they play a role in reproductive organ development [26]. Without VLCFAs, the membrane loses stability and becomes leaky, resulting in severe problems for the plant. The VLCFAs are usually produced in the endoplasmic reticulum of the cytosol by stepwise C2-elongation of fatty acids with malonyl-CoA, which is facilitated by a membrane-bound, multienzyme acyl-CoA elongase system containing four distinct enzymes (3-ketoacyl-CoA synthase, 3-ketoacyl-CoA reductase, 3-hydroxyacyl-CoA dehydratase, 2,3-trans-enoyl-CoA reductase) (Scheme 21.5) [26, 27]. The 3-ketoacyl-CoA synthase, which catalyzes the reaction of malonyl-CoA with the activated substrate and which depends on a reactive cysteine unit in its reaction center, is inhibited irreversibly by nucleophilic attack of the cysteine sulfur atom to the halogenated chloroacetamide carbon atom [26, 27]. Herbicides that inhibit the VLCFA biosynthesis not only impair the function of plasma membranes but also have a notable effect on the synthesis of lipids, flavonoids, isoprenoids, epicuticular waxes, and proteins. The chloroacetamides share the mode of action of



Scheme 21.5 Four-step reaction sequence for the elongation of fatty acids to very long-chain fatty acids [26, 27].

VLCFA biosynthesis inhibition with the acetamides diphenamid, naproanilide, and napropamide, the oxyacetamides flufenacet and mefenacet, the tetrazolinones ipfencarbazone and fentrazamide as well as the miscellaneous herbicides anilofos, cafenstrole, piperophos, and pyroxasulfone.

21.5

Biological Activity

The chloroacetamides are characterized by an excellent selective activity against many major annual grass weeds and certain (mostly small-seeded) dicotyledonous weeds in cabbage, corn, cotton, oilseed rape, peanuts, potatoes, rice, sorghum, soybean, sugar beet, sugarcane, sunflower, tobacco, and many further crops. Combined with a safener, their use can be extended to other crops by selectivity improvement. Metolachlor (8), for instance, has been used for selective weed control in more than 70 different crops [7]. They demonstrate pre- and early-postemergent activity and long-lasting weed control by inhibition of the early plant development. Chloroacetamides are taken up through roots, shoots, or cotyledons and are xylem-transported by acropetal movement [30]. The primary site of absorption and action of these herbicides on broadleaf species is the roots, whereas the primary site of absorption and action on grasses is the emerging shoot. They inhibit the early development of susceptible weeds. Their seeds usually germinate but growth is stopped, the seedlings remain stunted and do not emerge at all. The first leaves coming out of the coleoptile and the cotyledons of dicotyledonous weeds are misformed and small. Especially the compounds taken up by the soil provide a strong effect on germination and development of young tissue without damaging pre-existing tissue. Most important grass weeds controlled by chloroacetamides are *D. sanguinalis* (large crabgrass), *E. crus-galli* (barnyardgrass), *Setaria* sp. (foxtail), and *Sorghum halepense* (Johnsongrass).

The crop tolerance (selectivity) of chloroacetamide herbicides is dependent on a rapid detoxification in crop plants by glutathione *S*-transferases. In this regard, the molecular mechanisms of both phytotoxicity and tolerance are based on essentially the same nucleophilic reaction (*S*-alkylation) of the herbicide, with a cysteine residue either as part of the active-site of 3-ketoacyl-CoA synthase or as part of glutathione or homoglutathione [26, 32].

Despite over 45 years of widespread chloroacetamide use in agriculture, resistance to these herbicides in the field has occurred on extremely rare occasions only. A possible reason for this phenomenon is that target-site (cysteine) mutation would abolish herbicide binding, but would also be lethal for the plant [26].

An important requirement of a preemergent, soil-acting herbicide is that its residual activity should be sufficiently long to prevent competition from weeds during the early stages of crop establishment. However, soil persistence should not be so long that carryover of residues into the succeeding crop season can take place. Some chloroacetamide herbicides persist for a long time in soil, and therefore, the residues can potentially injure subsequent rotation crops, especially in sandy soils with low organic matter.

21.6

Structure–Activity Relationship

Some general structure–activity relationships of the chloroacetamide herbicides have been observed relatively early [12]. The chemical chloroacetamide itself (R and R' in I = hydrogen) does not display useful herbicidal activity (Figure 21.2). If both R and R' in I are alkyl groups, then the resulting compounds are often highly efficient seed germination inhibitors. When in I R is aryl, especially bis-*ortho* substituted aryl, and R' is alkyl, the compounds have the potential to be potent herbicides. In this case the chain length of three carbon atoms appears to be optimum for R' , but also longer aliphatic chains are well tolerated if broken up by oxygen linkers. When in I R is unsubstituted aryl and R' is hydrogen, then the compounds are essentially inactive, which is also the case for any kind of nonchlorinated acetamide analogs II, even if they carry the best possible combinations for R and R' (Figure 21.2 [12]. For *N*-chloroacetyl-*N*-phenylglycine esters such as diethatyl-ethyl (4) a detailed structure–activity relationship study analyzed the influence of the substituents in the phenyl ring. In general, *ortho*-substituted compounds, in particular those substituted in both *ortho* positions, show the highest herbicidal activity, while the efficacy of *meta*- and *para*-substituted derivatives is considerably lower [33].

The presence of a chiral axis due to hindered rotation around a C–N bond and an asymmetrically substituted carbon atom in both dimethenamid (6) and metolachlor (8) yields four different stereoisomers. No major difference in the biological activity of the atropisomers *aR* and *aS* isomers has been found; however, the configuration of the stereogenic center next to the nitrogen atom plays a big role for the efficacy as about 95% of the herbicidal activity of the racemic metolachlor resides in the two 1'S diastereoisomers 33 and 34 and therefore has been commercialized as (*S*)-metolachlor (25) [1, 7, 13–16, 20, 22, 34–36]. Figure 21.3 shows the four stereoisomers 33–36 of metolachlor.

Some chloroacetamide analogs, in which the chlorine atom has been replaced by an inorganic ester, for example, a thiophosphate as in the prynachlor analog 37 or a sulfonate as in the propachlor analog 38, also showed herbicidal activity [37]. Also, the exchange of the chloro substituent by other halogen atoms is possible, as demonstrated by the experimental bromoacetamide herbicide 39 [37].

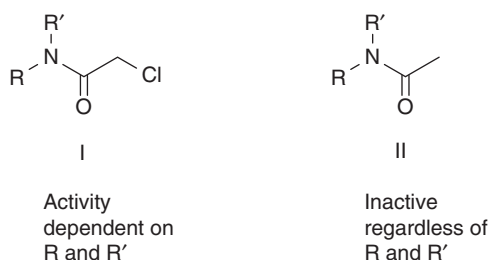
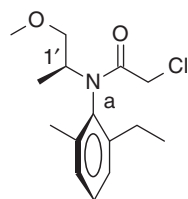
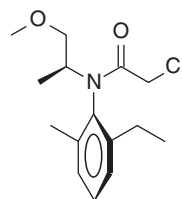


Figure 21.2 The general structure of chloroacetamide herbicides I and their acetamide analogs II [12].

Herbicidally active:

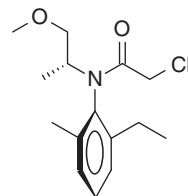


33
aR,1'S-Metolachlor

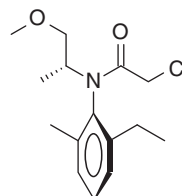


34
aS,1'S-Metolachlor

Herbicidally inactive:

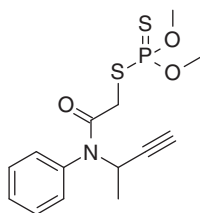


35
aR,1'R-Metolachlor

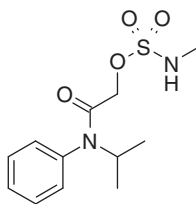


36
aS,1'R-Metolachlor

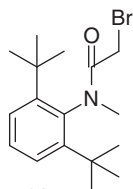
Figure 21.3 The four different stereoisomers of metolachlor (**8**).



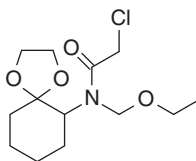
37



38



39



40

Figure 21.4 Some herbicidally active chloroacetamide analogs.

The chloroacetylated 6-amino-1,4-dioxaspiro[4,5]decane derivative **40**, in which the bis-*ortho*-substituted phenyl ring of acetochlor (**1**) has been replaced by a saturated cyclohexyl analog with a bulky *ortho*-substituent, displays herbicidal activity against *Sinapis alba* (white mustard), but on a weaker level compared to acetochlor (Figure 21.4) [38].

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22

Carboxylic-Acid-Containing Sulfonylurea Herbicides

Atul Puri and Paul H. Liang

22.1

Introduction

Sulfonylureas are a well-known major class of herbicides that are potent inhibitors of plant growth. They incorporate a sulfonylurea bridge that usually links an *ortho*-substituted phenyl or heteroaromatic aryl ring to a pyrimidine or 1,3,5 triazine ring. The importance of the *ortho*-group R, specifically the carboxylic acid derivatives such as esters and amides, will be the focus of this short review, which is a continuation of a more general prior review of herbicidal sulfonylureas (Figure 22.1) [1].

For sulfonylureas, *ortho*-R groups are generally required for good herbicidal activity and a large variety of R substituents lead to highly active herbicides. The *ortho*-esters and amides are among the most active substituents. Representative examples of sulfonylurea herbicides containing an *ortho*-carboxylic acid derivative include the pyridine *ortho*-diethylamide nicosulfuron (**1**, Accent®, Milagro®) [2] and the benzene *ortho*-methyl ester mesosulfuron-methyl (**2**, Mesomaxx®) [3] (Figure 22.2). These two compounds represent the leading sulfonylurea herbicides sold in 2012 [4], the latest year for which this information is available, with sales of \$580 million out of a total \$2300-million sulfonylurea market. Sulfonylurea herbicides control weeds at low use rates in all of the major agronomic crops and in many specialty uses such as vegetation management and rangeland.

22.2

History

Highly active sulfonylurea herbicides were discovered by Dr George Levitt at DuPont [5] in the mid-1970s. This discovery signaled a new low-dose era in herbicide research where selective weed control could be achieved at use rates significantly less than 100 g/ha (and often below 10 g/ha), which was greater than

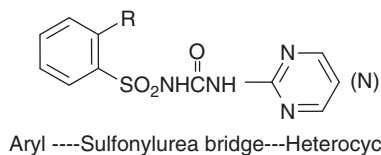


Figure 22.1 General structure of sulfonylurea herbicides.

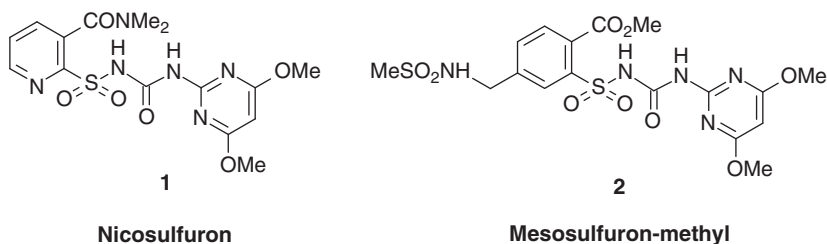


Figure 22.2 The sulfonylurea herbicides nicosulfuron (1) and mesosulfuron-methyl (2).

a 50–100-fold increase in herbicidal activity over previous chemistry resulting in a significant reduction in the impact on the environment including low risk to mammals and nontarget organisms. Sulfonylureas act by inhibition of the biosynthesis of branched-chain amino acids with selectivity based on rapid metabolic inactivation in wheat, barley, corn, soybeans, rice, oilseed rape, and other crops. They degrade in soil by chemical and biological processes [6, 7].

The chemical scope of sulfonylureas is immense [8, 9]. To date, about 34 sulfonylureas have been commercialized by eight agrichemical companies [4]. The challenge in sulfonylurea research has been to sort through the enormously broad scope of highly active compounds and other data to select the best analogs that meet the needs of farmers and have favorable environmental effects.

Optimization of the herbicidal activity of the sulfonylureas focused on the three main structural areas: the phenyl or heteroaromatic aryl left-hand portion of the molecule, the sulfonylurea bridge, and the heterocyclic portion. It became apparent early on that an *ortho*-substituent on the benzene ring was one of the prerequisites for good herbicidal activity, and many *ortho*-groups, usually electron withdrawing, led to significant boosts in activity compared to the *ortho*-unsubstituted analogs. These substituents included the carboxylic acid derivatives and other groups such as nitro, alkyls, trifluoromethyl, halogens, sulfonamides, sulfones, ketones, ethers, and thioethers.

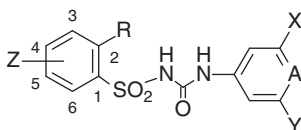
However, among the commercial sulfonylureas, the carboxylic acid derivatives are the predominant *ortho*-substituents. Of the 34 commercial sulfonylurea herbicides, over half of them contain the carboxylic acid derivatives (mostly methyl esters but also a few ethyl esters and dimethyl amides). Figure 22.3 on the next two pages lists the sulfonylureas containing an *ortho*-carboxylate derivative that have been commercialized [9]. The sulfonylureas are divided into three groups: (i) the phenyl sulfonylureas, (ii) the heteroaromatic aryl sulfonylureas, and (iii) the bridge-modified sulfonylureas.

22.3

Synthesis

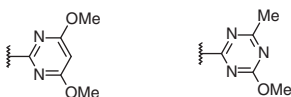
Sulfonylureas are most often prepared by reacting a sulfonylisocyanate with an amino pyrimidine or triazine (route A) as shown in Scheme 22.1. Most commercial sulfonylureas are produced using this route. Alternatively, sulfonylureas can be prepared by reacting a sulfonamide with a heterocyclic isocyanate (route B). The sulfonyl or heterocyclic isocyanates are often used in a protected form as alkyl or phenyl carbamates (routes C and D, respectively). The sulfonyl isocyanates or heterocyclic isocyanates can then be formed from the carbamates *in situ*. In route E, a sulfonylchloride, isocyanate salt, and amino pyrimidine or triazine are combined without preforming an isocyanate intermediate to produce sulfonylureas [9–11].

1. Phenyl sulfonylureas



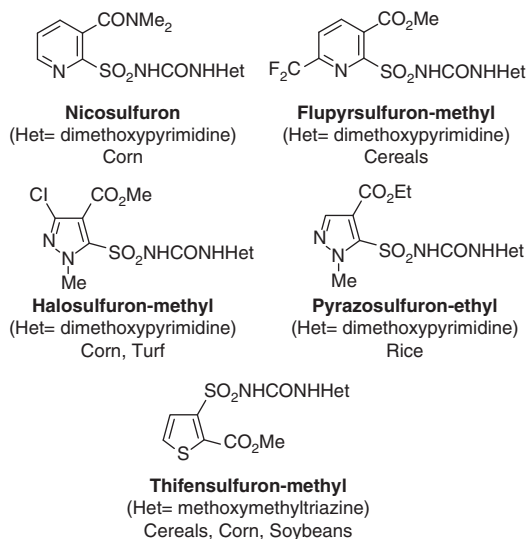
R	Z	A	X	Y	Common name	Crops
CO ₂ Et	H	CH	Cl	OMe	Chlorimuron-ethyl	Soybeans
CO ₂ Me	H	N	NHCH ₃	OEt	Ethametsulfuron-methyl	Oilseed Rape
CON(Me) ₂	5-NHCHO	CH	OMe	OMe	Foramsulfuron	Corn
CO ₂ Me	5-I	N	OMe	Me	Iodosulfuron-methyl	Cereals
CO ₂ Me	5-CH ₂ NHSO ₂ Me	CH	OMe	OMe	Mesosulfuron-methyl	Cereals
CO ₂ Me	H	N	OMe	Me	Metsulfuron-methyl	Cereals, Rice
CO ₂ - 	H	CH	Me	Me	Oxasulfuron	Soybeans
CO ₂ Me	H	CH	OCF ₂ H	OCF ₂ H	Primisulfuron-methyl	Corn
CO ₂ Me	H	CH	Me	Me	Sulfometuron-methyl	Vegetation Management
CO ₂ Me	6-Me	N	N(Me) ₂	OCH ₂ CF ₃	Triflurosulfuron-methyl	Sugarbeet

2. Heteroaromatic aryl sulfonylureas



In the following structures, **Het** = dimethoxypyrimidine or methoxymethyltriazine

Figure 22.3 Commercial sulfonylureas containing an *ortho*-carboxylate derivative. (Continued on next page).



3. Bridge modified sulfonylureas

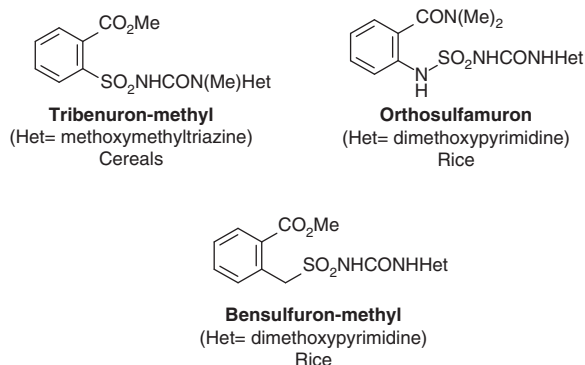
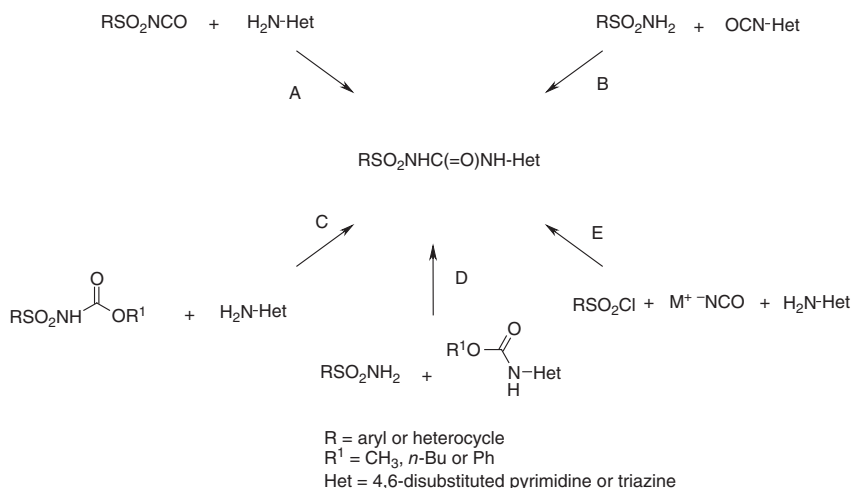


Figure 22.3 (Continued).

22.4

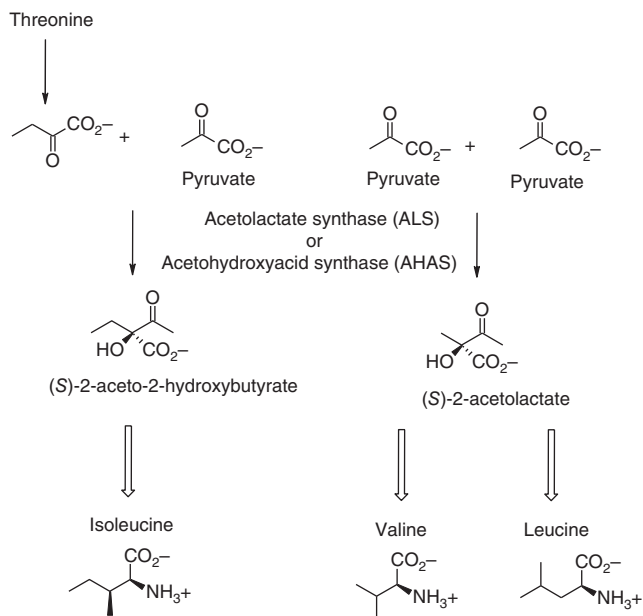
Mode of Action

The mode of action of the sulfonylurea herbicides is the inhibition of the biosynthesis of branched-chain amino acids. Acetolactate synthase (ALS) (EC 4.1.3.18; also known as acetohydroxyacid synthase) is the first enzyme in the pathway for biosynthesis of branched-chain essential amino acids valine, leucine, and isoleucine. ALS is the common target site of five herbicide chemical classes, that is, sulfonylureas, imidazolinone, triazolopyrimidines, pyrimidinyl-thiobenzoates, and sulfonyl-aminocarbonyl-triazolinones. These herbicides act as potent



Scheme 22.1 Methods for the synthesis of sulfonyleureas.

inhibitors of the enzyme ALS resulting in plant starvation of valine, leucine, and isoleucine, ultimately leading to plant death in plants [12–15] as shown in Scheme 22.2 [16, 17]. The sulfonyleurea herbicides inhibit plant root and shoot growth, with further visual symptoms including vein reddening, leaf chlorosis, terminal bud death, and necrosis slowly developing several days after treatment



Scheme 22.2 AHAS or ALS biochemical pathway [16, 17].

[13, 18]. Inhibition of ALS leads to starvation of the plant for these amino acids, and it is this starvation that is thought to be the primary mechanism by which these herbicides cause plant death. Other secondary effects of ALS herbicides, such as buildup of 2-ketobutyrate, disruption of protein synthesis, and disruption of photosynthate transport also result in growth inhibition and plant death [19]. The biochemistry of branched-chain amino acid pathway and sulfonylurea inhibition of ALS is reviewed in detail elsewhere [20–22].

More recently, Pang *et al.* and McCourt *et al.* [23, 24] have published the crystal structure of plant ALS bound with sulfonylureas. For an in-depth discussion of the ALS binding site and how it relates to sulfonylurea-resistant weeds, see Gutteridge and Thompson's review [25], where they compare the crystal structure of the binding site with mutations known to confer resistance.

22.5

Biological Activity

Sulfonylurea herbicides have both pre- and postemergence activity. Postemergence activity is often significantly enhanced by a nonionic surfactant in the spray solution. These herbicides can be taken up by both roots and shoots and are readily translocated in both the xylem and phloem. Sulfonylurea herbicides are weak acids and are well suited to concentration in the neutral symplast from acidic apoplast by an acid-trapping mechanism and translocate to meristems and other metabolic sinks [26].

The sulfonylurea herbicides have proven to be the most flexible and widely adaptable class of herbicidal chemistry yet discovered. Broad-spectrum sulfonylurea herbicides have been commercialized with selectivity in numerous crops including wheat, barley, rice, soybeans, corn, oilseed rape, cotton, sugar beet, potatoes, forestry, plantation crops, pastures. The margin of crop tolerance can be quite wide. For example, wheat and barley are over 1000-fold more tolerant of chlorsulfuron than sensitive species such as sugar beet [10]. With few exceptions, the crop selectivity and weed control spectra of these herbicides are due to differential active-site sensitivity, and metabolic inactivation by the tolerant crops.

Sulfonylureas are susceptible to various primary transformations leading to herbicidal inactivation in both tolerant crops and weed species. These transformations include ester hydrolysis, aryl and aliphatic hydroxylation followed by carbohydrate conjugation, O- and N-demethylation, glutathione conjugation, and cleavage of the sulfonylurea linkage [11, 27–29]. Metsulfuron-methyl and thifensulfuron-methyl are rapidly metabolized by wheat, accounting for its tolerance to these herbicides. Metsulfuron-methyl is subject to the same metabolic pathway in wheat as chlorsulfuron, namely, aryl hydroxylation and glucose conjugation [30].

Barley plants readily catalyze the urea-bridge cleavage of chlorsulfuron and metsulfuronmethyl (in addition to aryl hydroxylation and glucose conjugation).

Rice tolerance to bensulfuron-methyl also results from rapid metabolic inactivation [31].

Since their introduction in the early 1980s, persistent and widespread use of ALS herbicides has consequently resulted in the rapid evolution of many ALS herbicide-resistant weed populations.

Worldwide, there are biotypes of 139 weed species with evolved ALS herbicide resistance. In many cases, evolved resistance is target-site-based, due to resistant plants having one or more specific resistance-endowing single point mutations in the target ALS gene. A total of 26 resistance-endowing gene mutations at eight conserved amino acid residues in the ALS gene have so far been identified in field-evolved resistant weed biotypes over the last 20 years [32–34]. A variety of crops, including corn (*Zea mays* L.), canola (*Brassica napus* L.), rice (*Oryza sativa* L.), and wheat, resistant to ALS-inhibiting herbicides have been developed by many approaches such as somatic cell selection, mutation breeding, plant transformation, and interspecific crossing [35–40]. These herbicide-resistant crops have allowed the expansion of selective weed control with currently registered, highly efficacious active ingredients into new crops and protected rotational crops from possible carryover injury. However, the development of ALS-inhibiting herbicides resistant crops has resulted in even greater use of these herbicides, thereby increasing the problem of ALS resistant weed species.

22.6

Structure–Activity Relationship

The structure–activity relationship (SAR) of the sulfonylureas can be broken down into three regions: the *ortho*-substituted aryl or heterocyclic portion of the sulfonamide, the bridge, and the right-hand amino heterocycle (Figure 22.4). The SAR of the three regions has been reviewed [11]. The greatest structural variation is found in the aryl or heterocyclic portion of the sulfonamide. An *ortho*-group is necessary for optimal activity, and all of the commercial sulfonylureas have at least one *ortho*-substituent. Other substituents on the aryl or heterocyclic sulfonamide help to modify the physical properties, crop selectivity, and metabolism of the sulfonylurea. The SAR for sulfonylureas where the phenyl sulfonamide portion is replaced by a heterocyclic or bicyclic sulfonamide has also been reviewed [41–43].

All of the commercial sulfonylureas retain the typical $-\text{SO}_2\text{NHC}(=\text{O})\text{NH}-$ bridge. On the right-hand side, all of the commercial sulfonylureas have either



R Bridge Heterocycle

Figure 22.4 The three regions of sulfonylurea herbicides.

R = an *ortho* substituted aryl or heterocycle
Het = 4,6-disubstituted pyrimidine or triazine

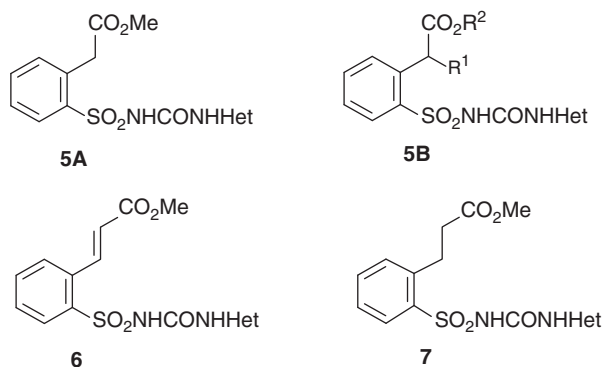


Figure 22.5 Linkers to the *ortho*-esters.

a pyrimidine or triazine as the heterocycle. These heterocycles are typically 4,6-di-substituted with methoxy/methyl groups. Other heterocycles are generally less active.

Although many sulfonylureas have been commercialized, countless more have been synthesized by various agrichemical companies and other research organizations in their R&D efforts. Hundreds of patents have been filed covering a broad range of unique structures and creative discoveries. Many of those discoveries were part of the normal iterative R&D process that often led to highly active sulfonylureas. Although most never became commercial products, the work did aid in a better understanding of the SAR. The following briefly summarizes some examples of one such area where the herbicidal activity of certain sulfonylureas containing the carboxylic ester groups was enhanced when the carboxylate moiety was conformationally restricted by incorporation into a ring.

As mentioned, many of the commercial sulfonylureas contain a carboxylic acid derivative, generally a methyl ester, at the crucial *ortho*-position. Inserting a methylene linkage between the carboxylate and the phenyl ring as in phenyl acetic esters **5A** (Figure 22.5) also produced highly active herbicides especially in postemergence applications. However, loss of activity occurred in **5B** with the addition of both larger alkyl esters ($R^2 > 3$ carbons) and an alkyl group attached to the methylene linker ($R^1 = \text{Me}$). Increasing the linkage to two carbons between the carboxylate and the phenyl ring as in the cinnamate esters **6** and its dihydro analogs **7** also reduced activity.

However, limiting both the conformational flexibility and the steric bulk of the carboxylate of **5B** by “tying up” the R^1 and R^2 groups of the phenyl acetic esters **5B** into butyrolactones **5C** (Figure 22.6), restored the herbicidal activity lost by the alpha substitution and the larger alkyl esters and, in fact, significantly increased the preemergence activity of **5C** compared to the corresponding phenylacetic esters **5A** [44].

Similarly, the “tied-up” cinnamate esters **8** (Figure 22.7) were significantly more herbicidal than the open-chain cinnamate esters **6**, both pre- and postemergence,

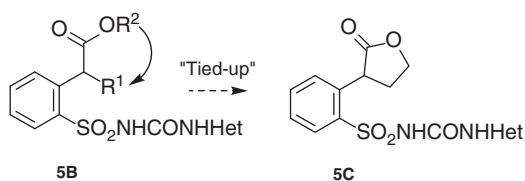


Figure 22.6 “Tied-up” phenylacetic esters.

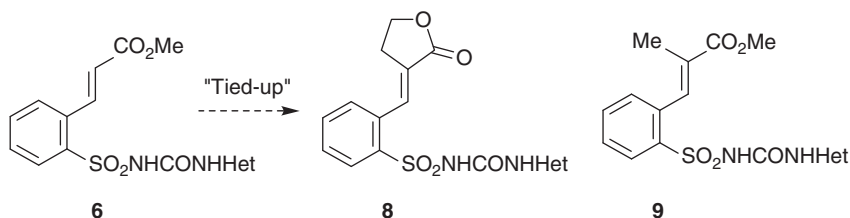


Figure 22.7 “Tied-up” cinnamate esters.

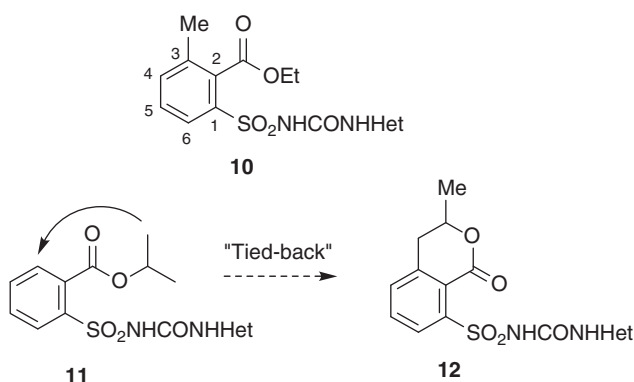


Figure 22.8 “Tied-back” esters.

and surprisingly were even more active compared to the *alpha*-methylcinnamate esters **9**.

Another example [45] of highly active conformationally restricted sulfonylureas (Figure 22.8) began with the observation that sulfonylureas **10** containing a methyl substituent at the 3-position of the phenyl ring, combined with the usual *ortho*-ester groups at the 2-position, were also highly active. This led to programs to “tie-back” the *ortho*-groups to the 3-position of **10** as shown in **11** to form a fused bicyclic ring **12**. This limited the steric effects and rotation of the *ortho*-ester group, which again was a successful strategy in improving biological activity. The isocoumarins **12** were clearly more active on both broadleaves and grasses than the open-chain analogs **10**.

This strategy was also effective in “tying-back” other *ortho*-groups such as ethers, thioethers, sulfones, sulfonamides, and ketones. In general, a variety of

these bicyclic sulfonylureas containing different “tied-back” *ortho*-groups were highly active and in some cases displayed crop selectivity.

In summary, the *ortho*-carboxylate group has proven to be a major contributor to the sulfonylurea story, a functional group that has stood out in a class that created a new era of low use rate and safe herbicides.

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23

Amino Acids as Nonselective Herbicides

Stephane Jeanmart

23.1

Introduction

The amino acid herbicides are well-established classes of agrochemical active ingredients, which target a broad range of weeds and are considered to be nonselective herbicides [1]. This group of herbicides consists of two different subclasses: inhibitors of the 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS) including glyphosate (**1**) and sulfosate (**2**) and inhibitors of glutamine synthetase (GS) including bilanafos (**3**) and glufosinate (**4**) (Figure 23.1) [1].

Glyphosate (**1**) and sulfosate (**2**) are extremely close in chemical structure and biological activity because the latter is the trimethylsulfonium salt of glyphosate [2]. Both compounds are used to control annual and perennial grasses and broadleaf weeds [2, 3]. Glyphosate (**1**), a nonselective foliar herbicide, is widely applied in trees, nuts, and vine crops [1a, 3–5]. It is also used in noncropland and postplanting/preemergence in cereals, vegetables, and other crops [3–5]. Glyphosate (**1**) is widely used in a selective way on genetically modified crops embedded with glyphosate-resistant protein [1a, 4–6]. The global glyphosate herbicide market was valued at 5.46 billion USD in 2012, and it is predicted to grow to 8.79 billion USD by 2019 [7].

Bilanafos (**3**), also called bialaphos, is a natural product isolated from the soil bacteria *Streptomyces hygroscopicus* and *Streptomyces viridochromogenes* [1b, 3, 8, 9]. The natural product is a tripeptide composed of two molecules of alanine and an unusual amino acid containing a phosphine group [3, 8]. The tripeptide does not inhibit the GS enzyme as it is a proherbicide and is only active after its metabolic hydrolysis in the plant to its active principle L-phosphinothricin (**5**) [3, 8]. Although only this (*S*)-enantiomer, L-phosphinothricin, is herbicidally active, its racemate (**4**) has been launched on the market under the common name glufosinate and is primarily sold as the ammonium salt. L-Phosphinothricin (**5**) is a phosphinic acid mimic of (*S*)-glutamic acid, the natural substrate of GS. Glufosinate (**4**) and bilanafos (**3**), similarly to glyphosate (**1**), are broad-spectrum foliar herbicides, which provide control of practically all annual and perennial grasses as well as broad-leaved weeds [1b, 3, 8]. Glufosinate is also used in a selective way in

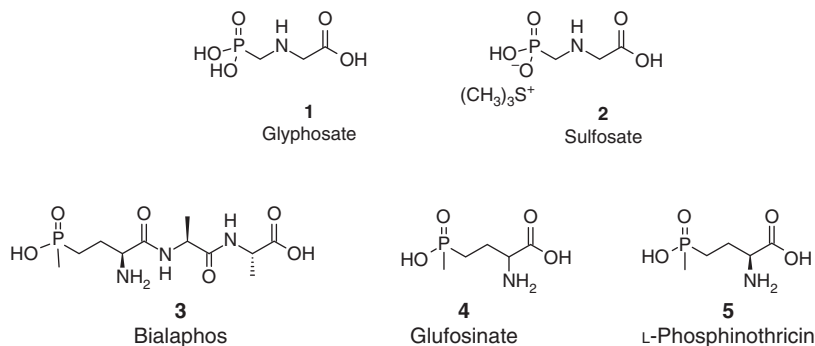


Figure 23.1 Examples of amino acid herbicides.

vineyards and orchards since it is possible to direct the spray away from the leaves of the trees [3, 8]. Sales of glufosinate were 510 million USD in 2013 [7]. In 2013, Bayer CropScience announced its plan to construct a plant in the United States to double the production volume of glufosinate. The plant should be ready to supply glufosinate (**4**) for the 2016 season [10].

23.2

History

Glyphosate (**1**) was, as reported by Franz *et al.*, first synthesized in 1950 by Henri Martin at Cilag in Switzerland but was not tested or patented for herbicidal use [4, 5]. Twenty years later, at Monsanto, John E. Franz prepared a sample of glyphosate and found its herbicidal activity. This discovery was based on two early herbicidal hits from a research program on aminomethylphosphonic acids as water-softening agents [4, 5]. The molecule went quickly through the development process and was introduced on the market in 1974 under the brand name Roundup™. In 1996, Monsanto introduced the Roundup Ready™ crops, which are crops genetically modified to be tolerant to glyphosate [1b, 4, 5].

In 1972, Bayer *et al.* reported the isolation of a new tripeptide from *S. viridochromogenes*, which consisted of a new amino acid, 2-amino-4-methyl-phosphinobutyric acid, and two alanines [11]. The new amino acid structure was confirmed by synthesis and called phosphinothricin. In their original paper, Bayer *et al.* had already identified some biological activity for the molecule as antibiotic and antifungal activity [11]. Through synthesis, they confirmed that the biological activity was in fact coming from the newly isolated amino acid. By analogy to glutamine, they proposed and confirmed that the biological activity was due to the inhibition of the GS enzyme [11].

D,L-Phosphinothricin was synthesized at the Hoechst central research laboratories in the mid-1970s. They confirmed the weak antibiotic activity. The agrochemical division tested the molecule and realized its potential as a herbicide.

The molecule went quickly through development, and the ammonium salt of the compound was introduced to the market under the common name glufosinate ammonium [3, 8].

Also in the 1970s, scientists from Meiji Seika independently discovered the same natural product **3** from the soil bacteria *Streptomyces hygroscopicus*. The compound was named bialaphos. Meiji Seika discovered the herbicidal activity of bialaphos and developed it as a nonselective herbicide. It was introduced to the Japanese market in 1984 [3, 8].

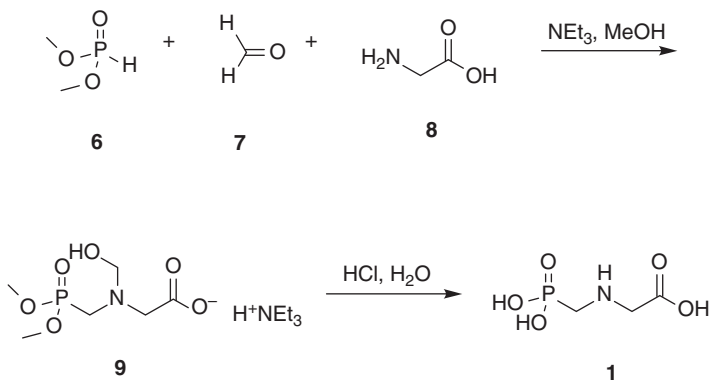
In 1995, canola tolerant to glufosinate was commercialized by Bayer Crop-Science under the brand Liberty Link™ [1b]. This was the first commercial crop that was tolerant to a nonselective herbicide.

The amino acid herbicides, in combination with the genetically modified crops, have undoubtedly changed the herbicide crop protection market: In 2015, 94% of the soybean, 89% of the corn, and 89% of cotton planted in the United States were herbicide-tolerant [12].

23.3

Synthesis

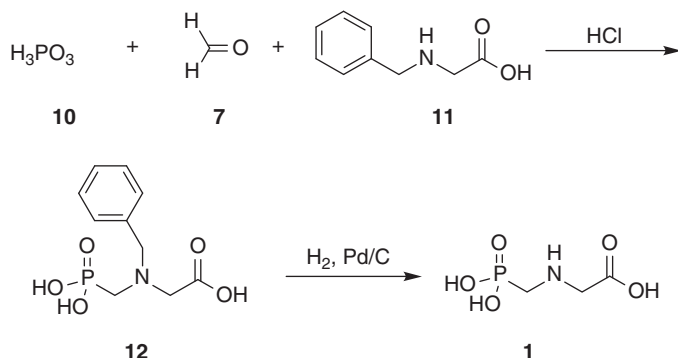
Glyphosate (**1**) can be prepared in many ways. Due to its economic importance, many syntheses have been reported in the literature. A practical synthesis involves the condensation of dimethyl phosphite (**6**), formaldehyde (**7**), and glycine (**8**) in the presence of a catalyst followed by hydrolysis of the condensed product to provide glyphosate (**1**). This synthetic pathway has been reported in the presence of triethylamine and methanol for the condensation step while the hydrolysis was performed in the presence of hydrochloric acid (Scheme 23.1) [13]. A similar sequence has been reported with only hydrochloric acid as the catalyst for both the condensation step and the hydrolysis reaction [13]. It has also been reported that glycine can react with formaldehyde and phosphorus acid with the formation of



Scheme 23.1 Synthesis of glyphosate (**1**) involving dimethyl phosphite (**6**) and glycine (**8**).

N,N-bis(phosphonyl)-glycine, which can be converted into *N*-phosphonomethylglycine by either oxidation or electrolytically [14].

The synthetic procedure reported in Scheme 23.1 can have many variations. As an example, the synthesis can be performed in a similar way by converting *N*-benzyl glycine (**11**) as a starting material and phosphorous acid (**10**) and formaldehyde (**7**) to the intermediate **12**, which delivers glyphosate (**1**) by hydrogenation (Scheme 23.2) [15].

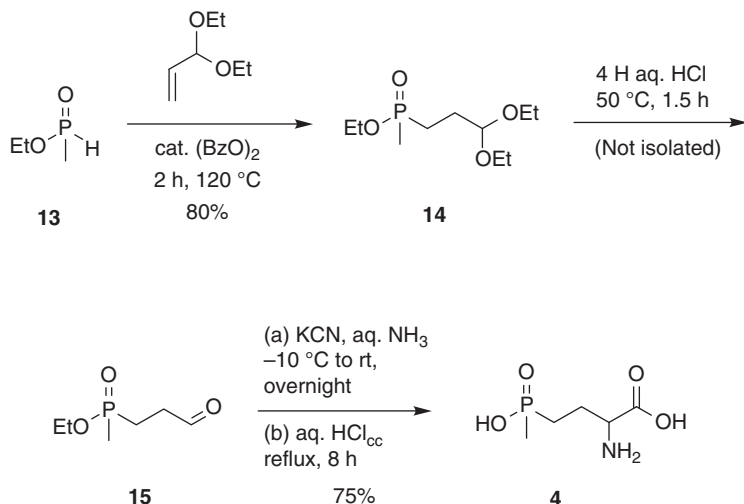


Scheme 23.2 Synthesis of glyphosate (**1**) involving phosphorous acid (**10**) and *N*-benzyl glycine (**11**).

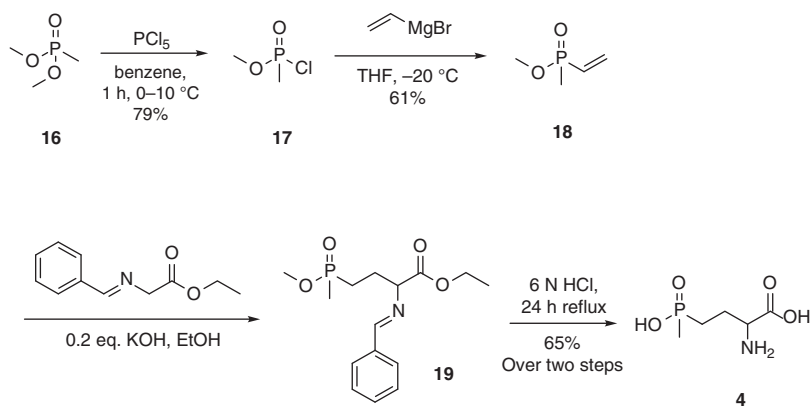
Glufosinate (**4**) can be synthesized in two formal steps. The first step is the free-radical addition of ethyl methyl phosphonite (**13**) onto an acrolein acetal. The acetal **14** is then hydrolyzed to the aldehyde **15**, which is submitted without isolation to typical Strecker reaction conditions and a further hydrolysis to provide the desired product **4** (Scheme 23.3) [16]. Both steps are high yielding and are involving cheap reagents. The Strecker approach toward **4** was already reported in the original Hoechst patent [17].

In another approach, the vinylphosphonite **18** was prepared by addition of vinyl Grignard to [chloro(methyl)phosphoryl]oxymethane (**17**), which itself was prepared by the reaction of dimethyl methylphosphonate (**16**) with phosphorous pentachloride in benzene [18]. Addition of a glycine Schiff base under basic condition to the Michael acceptor **18** provided the adduct **19**, which was successfully hydrolyzed to glufosinate (**4**) (Scheme 23.4) [19].

Many nonproteinogenic α -amino acids have been prepared by Schöllkopf's bis-lactim ether methodology [20] and the *L*-phosphinothricin (**5**) is no exception. The (3*R*)-bis-lactim ether **20**, which is derived from (*R*)-valine, is metalated with *n*-butyllithium at -78°C in tetrahydrofuran followed by alkylation with isobutyl 2-chloroethyl-methylphosphinate. Further hydrolysis with 0.25 N HCl provides the acid ester **22**. Harsher hydrolysis conditions are required to transform the *iso*-butyl ester into the corresponding acid **23**. This is readily done with 6 N hydrochloric acid. Finally, propylene oxide in ethanol is used to free up the amine of the hydrochloride salt to provide *L*-phosphinothricin (**5**) (Scheme 23.5) [21].



Scheme 23.3 Synthesis of glufosinate (4) involving a Strecker reaction.

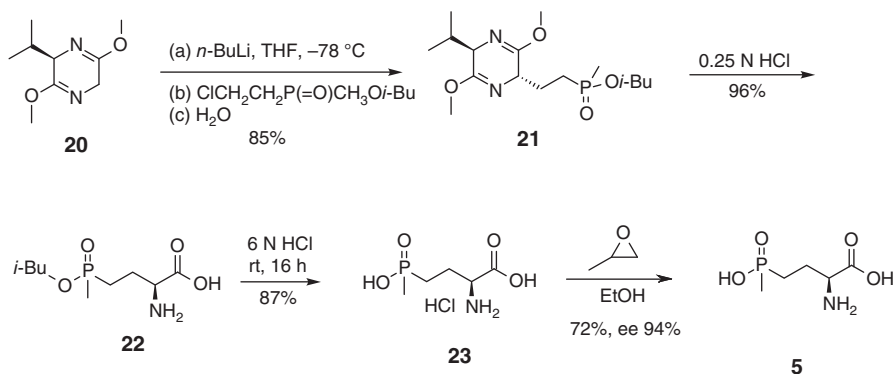


Scheme 23.4 Synthesis of glufosinate (4) involving a Schiff base.

23.4

Mode of Action

Glyphosate (1) inhibits the enzyme EPSPS. The EPSPS enzyme catalyzes the transfer of phosphoenolpyruvate to shikimate 3-phosphate to provide 5-enolpyruvyl shikimate 3-phosphate. Glyphosate (1) is in fact mimicking the transition state of phosphoenolpyruvate, one of the substrates of EPSPS. EPSPS is the sixth enzyme in the shikimate pathway involved in the synthesis of the aromatic amino acids phenylalanine, tryptophan, and tyrosine. The lack of aromatic amino acids to produce the necessary proteins is what finally leads to the plant death. It has also been proposed that the deregulation of the shikimate pathway by the inhibition of the



Scheme 23.5 Enantioselective synthesis of L-phosphinothricin (**5**).

EPSPS resulted in an increased carbon flow for the pathway, which in turn results in a shortage of carbon for other pathways [1a, 4, 5].

The EPSPS enzyme is only present in fungi, bacteria, and plants. The fact that the enzyme is not present in mammals, birds, and fishes makes the mode of action particularly safe for these species [1a].

Bilanafos (**3**), as stated in the introduction, does not inhibit GS, it must first be metabolized in plant or in bacteria to produce L-phosphinothricin (**5**), the active principle of both bilanafos (**3**) and glufosinate (**4**). Bayer *et al.* already proved that the effects of the bilanafos (**3**) on *Escherichia coli* were reversed by the addition of L-glutamine [11]. Bilanafos (**3**), also, does not produce any effect when the bacteria are grown on a media rich in glutamate. GS catalyzes the formation of L-glutamine from L-glutamate [1b]. This enzyme enables a very rare transformation, the incorporation of inorganic nitrogen into organic forms by production of glutamine for ammonia detoxification. The glutamine is subsequently used as the substrate for the glutamate synthase enzyme. The end product of GS and glutamate synthase is used as an amine donor for the synthesis of amino acids, purine, and pyrimidines. Irreversible inhibition of GS induces the accumulation of ammonia in plant cells up to toxic levels. This lack of nitrogen metabolism in the plant disrupts photosynthesis, the structure of the chloroplast, stroma vesiculation, and glyoxylate accumulation, ultimately causing the plant death. In plants, there are three different types of GS and the homology between them is high [1b, 8].

23.5

Biological Activity

Glyphosate (**1**) and glufosinate (**4**) are broad-spectrum foliar herbicides, which provide an efficacious control of annual and perennial grasses and broad-leaved weeds [1–3]. Due to the physical properties of the compounds, they are absorbed through the leaves but not through the roots or stems. Because of the strong adsorption of the molecules to the soil and their low level of mobility into water,

the compounds are not mobile in the soil and therefore cannot be translocated to the root system of plants. This latter fact provides a window of selectivity for application in orchards and vineyards [5, 22].

Glyphosate (**1**) is commercially used at rates ranging between 1 kg/ha for control of annual weeds between crops and up to 4 kg/ha for control of perennial weeds. Speaking of the biological spectrum of glyphosate, it is easier to state what the molecule does not control rather than making a list of what the molecule actually does control. Out of 78 important weeds, glyphosate controls 76 weeds [23]. Only *Equisetum arvense*, the field horsetail or common horsetail, and fern are unaffected by glyphosate at conventional rates. It has been reported that an overexpression of the 5-enolpyruvyl shikimate 3-phosphate synthase is the reason for the lack of susceptibility of these two weeds toward glyphosate and that they are metabolizing glyphosate (**1**) [24, 25].

Intensive use of glyphosate as the only herbicide has created a selection pressure for resistant weeds. Between 1998 and 2013, 24 species were reported to have developed resistance to glyphosate. Due to its commercial importance and subsequent commercial impact, the resistance mechanisms to glyphosate have been the most studied in the field of herbicide resistance. These mechanisms of resistance include target-site mutation, target-site gene duplication, active vacuole sequestration, limited cellular uptake, and a rapid necrosis response [26].

Use of glufosinate as a herbicide has created a selection pressure that leads to some resistance weeds. There are four mechanisms of resistance that have been reported in the literature: altered uptake, reduced translocation, a GS overproduction, and, very recently, target-site mutation [27].

23.6

Structure–Activity Relationships

Thousands of analogs of glyphosate have been prepared across the industry. To date, glyphosate remains the only molecule on the market inhibiting the EPSPS enzyme. Sulfosate delivers *in vivo* the same cidal form as glyphosate although it has been reported that the sulfonium salt enhances the uptake of the active ingredient [2]. Figure 23.2 shows some of the molecules that have been prepared in an attempt to find an alternative EPSPS inhibitor. With the exception of *N*-hydroxy-glyphosate, which showed good herbicidal activity but was less active than glyphosate itself, all other molecules are much weaker or inactive. Substitution of the OH group on the phosphorous atom with alkyl groups, for example, generated much weaker compounds. Alkylation of the nitrogen group also led to much reduced herbicidal activity while substitution on either of the CH₂ linkers is detrimental for activity. Replacement of the acid functionality with aldehyde, tetrazole, or SO₃H produced inactive compounds [28, 29].

The interesting biological activity of L-phosphinothricin prompted many agrochemical companies to search for similar or better compounds. As stated earlier, only the L-amino acid is active, the D-isomer is inactive. Except compounds that

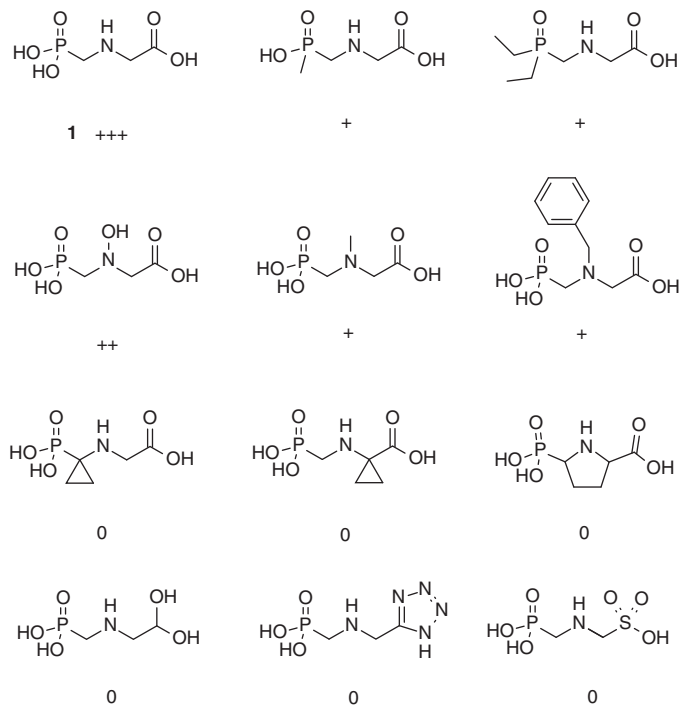


Figure 23.2 Some structure–activity relationships of glyphosate (1).

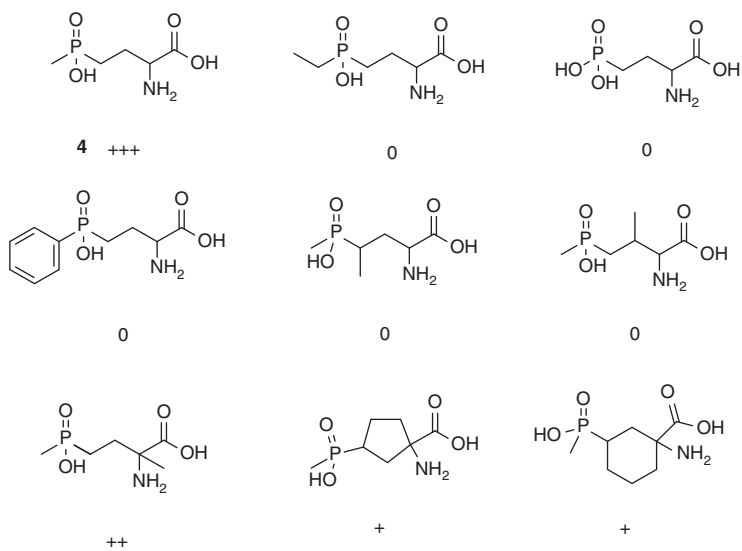


Figure 23.3 Some structure–activity relationships of glufosinate (4).

are rapidly metabolized *in vivo* to the parent compound (e.g., ester of the amino acid), modification of the L-phosphinothricin tends to suppress or decrease the herbicidal activity significantly. For example, modification of the phosphinic acid was detrimental for the activity. Introduction of a methyl group on the alkyl linker also suppresses the biological activity. Only the introduction of a methyl group in between the carboxylic function and the amino group is tolerated, leading to the retention of some activity. The cyclization of the alkyl chain also provides compounds with some level of activity, but they are much weaker than glufosinate (4) (Figure 23.3) [8].

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24

Herbicidal Aryloxyphenoxypropionate Inhibitors of Acetyl-CoA Carboxylase

William G. Whittingham

24.1

Introduction

More than 10 compounds with a 2-[4-(aryloxy)phenoxy]propionate substructure have been commercialized as herbicides. Some of the current major products are shown in Figure 24.1 [1–7].

Although the commercial compounds are sold as esters, it is the acids that are the active herbicides [8]. The esters provide improved uptake (and, in some cases, enhanced selectivity) and are then cleaved in the plant [9, 10].

All of these compounds provide selective postemergence control of grass weeds and are used in a variety of crops. Between them, they had sales of 1217 million USD in 2013 [11].

Members of this class of herbicides act by inhibiting the enzyme acetyl-CoA carboxylase (ACCase) in the chloroplasts of sensitive plants [12].

24.2

History

The aryloxyphenoxypropionate herbicides are known as “fops” and are a subgroup of the acetyl-CoA inhibitor group (HRAC group A) [13]. The first of the fop herbicides, diclofop-methyl (4), was launched in 1975 by Hoechst (now Bayer CropScience), under the trade name Hoelon®, for the control of grass weeds in cereals [14]. This compound arose from pharmaceutical research into hyperlipidemia being carried out at Hoechst [15], although there are obvious structural similarities to the aryloxypropionate herbicides, for example, mecoprop (5) [7], which were developed in the 1950s, and this may also have contributed to the design of diclofop (Figure 24.2), although the biochemical mode of action is different.

The launch of diclofop stimulated considerable further research, and fluazifop-butyl [16, 17] was launched as Onecide® by Ishihara in 1982 [18]. This compound

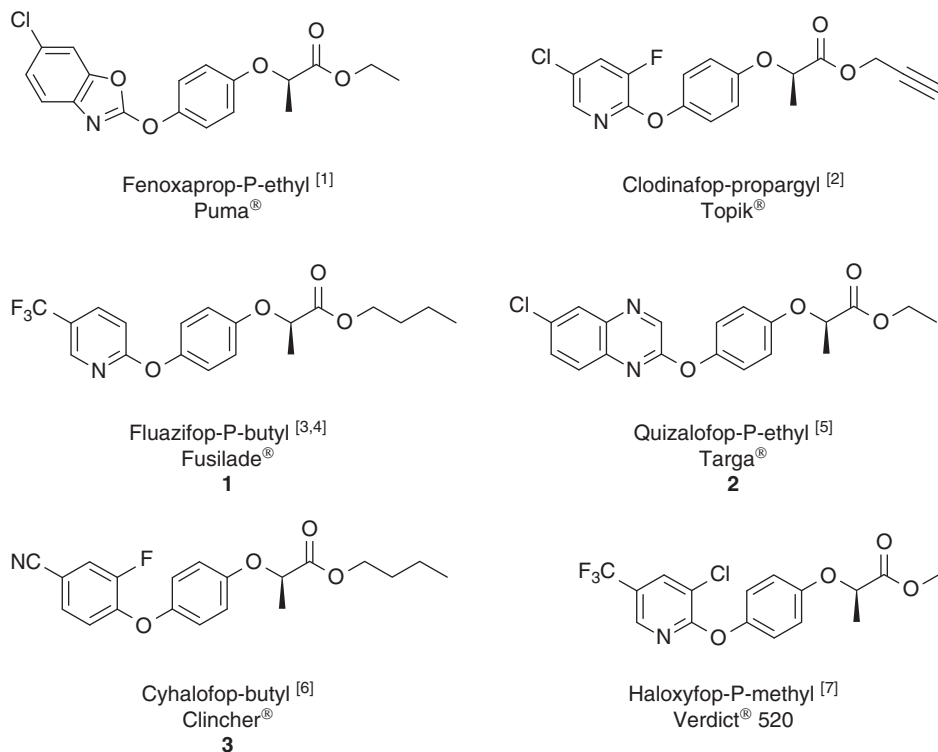


Figure 24.1 Structures of some commercial aryloxyphenoxypropionate herbicides.

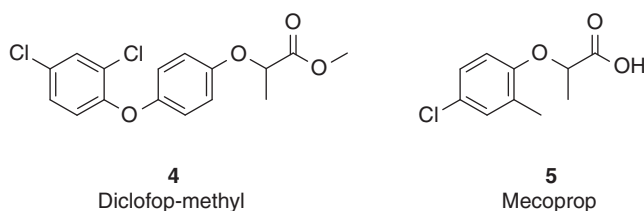


Figure 24.2 The first fop herbicide, diclofop-methyl (4), and the synthetic auxin mecoprop (5).

was codeveloped by ICI (now Syngenta), who marketed it as Fusilade[®] in most countries outside Japan [16]. This was followed by the launch of quizalofop-ethyl as Targa[®] by Nissan in 1984 [19], and haloxyfop, launched by Dow in 1985 as the ethoxyethyl (etotyl) ester, Gallant[®] and the methyl ester, Verdict[®] [7].

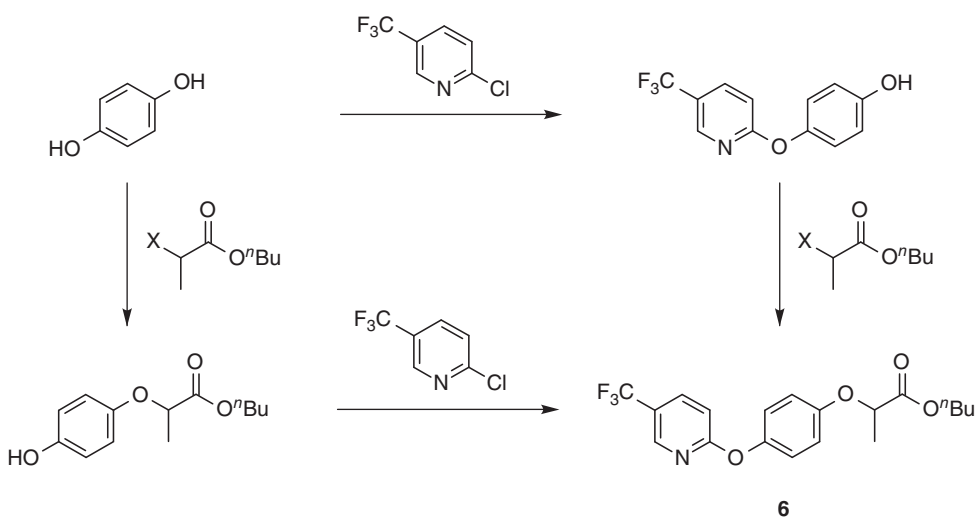
The early fops were launched originally as racemates. However, in many cases these have been replaced by single enantiomers, for example, fluazifop-P-butyl (1), introduced in 1985 [4]. In contrast, the later fops such as clodinafop [2] and

cyhalofop [6] were marketed as single enantiomers from their launches in the late 1980s and early 1990s.

24.3

Synthesis

The aryloxyphenoxypionates are typically prepared from three building blocks: a halo aromatic, hydroquinone, and a propionate ester bearing a leaving group (e.g., a halogen or sulfonate group) in the 2-position [20]. Two alternative routes are possible, depending on which group is attached to the hydroquinone first [21, 22]. These are illustrated for fluazifop-butyl (**6**) in Scheme 24.1 (X is a leaving group).

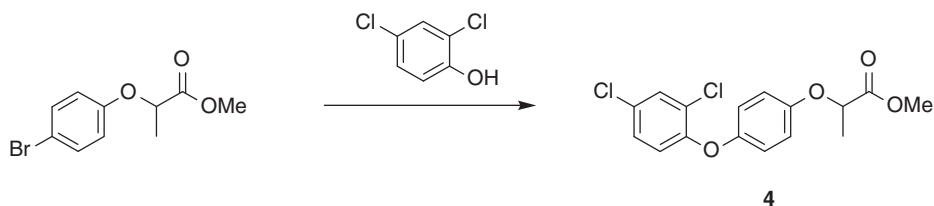


Scheme 24.1 Typical routes for the synthesis of fluazifop-butyl (**6**).

Variants of these routes in which one of the hydroxyl groups of the hydroquinone is protected have also been used [23].

This approach is preferred for most of the fops, as the halo aromatic is activated toward S_NAr chemistry. Where this is not the case, for example, diclofop, more forcing conditions can be used or the reactivity can be reversed [24], as shown in Scheme 24.2.

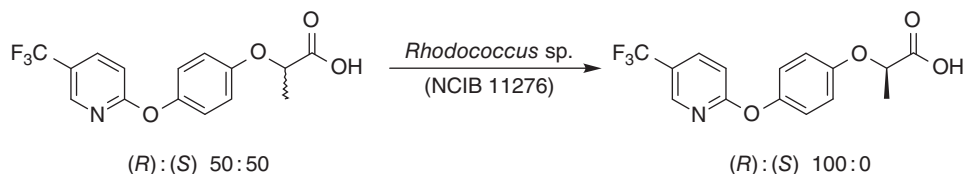
The same routes are applicable to the synthesis of single enantiomers [25, 26]; in this case, an enantiomerically pure propionate derivative is used, the reaction with hydroquinone or the aryloxyphenol proceeding with inversion of the stereocenter. The propionate derivative may be a mesylate or tosylate [25, 27], prepared by sulfonylation of an (*S*)-lactate ester, or a chiral 2-bromo- or 2-chloro-propionate [25, 28]. The halopropionates can be synthesized from lactic acid [28] but are



Scheme 24.2 Alternative route for the synthesis of diclofop-methyl (4).

typically prepared using biocatalytic methods; several complementary approaches have been developed [29–31].

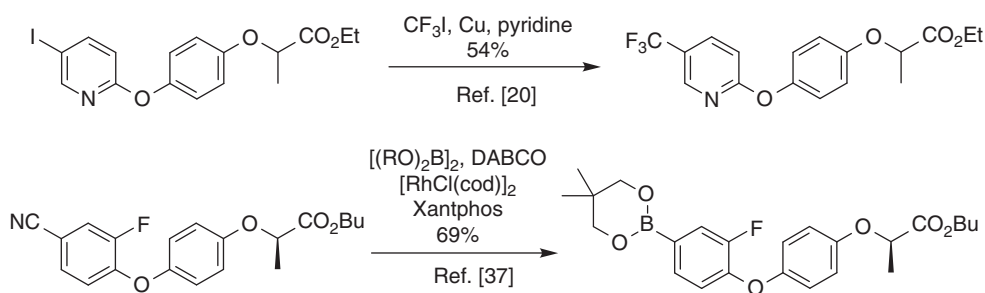
A different strategy for the preparation of enantiomerically enriched compounds is to use a biocatalytic inversion of the undesired enantiomer of the aryloxyphenoxypropionic acid, as demonstrated for fluazifop in Scheme 24.3 [32].



Scheme 24.3 Biocatalytic resolution of fluazifop.

Alternatively, a more classical resolution may be carried out using a stereospecific biocatalytic cleavage of the ester of the aryloxyphenoxypropionate [33].

The aryloxyphenoxypropionate core structure is quite robust, allowing the late-stage modification of substituent groups even under forcing conditions. This approach has been widely used for variation of the ester group [34] but has also been applied to altering substitution on the aromatic rings. Some examples are shown in Scheme 24.4, others include Balz–Schiemann fluorination to prepare clodinafop [36] and hydrolysis of the nitrile in cyhalofop, which involved heating at 100 °C under strongly basic conditions [37].



Scheme 24.4 Late-stage modifications of substitution.

Several other approaches to the fops have been described [38, 39], but these have not been widely used.

24.4

Mode of Action

Diclofop was first shown to inhibit the biosynthesis of lipids in sensitive plants in 1981 [40]. Further studies pinpointed the site of action of haloxyfop as inhibition of ACCase, the first committed step in the biosynthesis of fatty acids [12]. This work also demonstrated that a second class of herbicides, known as the “dims,” shared this biochemical mode of action, although they are structurally distinct. More recently, the herbicide pinoxaden (7), from a new structural class known as “dens,” has been shown to have the same mode of action (Figure 24.3) [41].

Further studies have identified the carboxyl transferase (CT) domain of ACCase as the site of interaction with the enzyme [42]. Crystal structures of diclofop and haloxyfop bound to the CT domain of yeast ACCase have been reported and show how the fops bind into the pocket that would otherwise be occupied by acetyl-CoA [43]. Interestingly, binding of these compounds results in significant movement of amino acid side chains in the enzyme active site to accommodate the terminal aromatic ring; pinoxaden and most dims do not elicit such a large conformational change [44, 45].

Most plants possess two classes of ACCase enzymes, one localized in the chloroplasts and the other in the cytosol. In grasses, both of these are eukaryotic-type enzymes, consisting of a single protein, which contains the three functional domains of the enzyme. In most other plants, the cytosolic enzyme is a eukaryotic type but the chloroplastic ACCase is a so-called prokaryotic form, with four separately coded proteins combining to form the complete enzyme complex [46]. It has been shown that the fop herbicides (and the dims and pinoxaden) act by selectively inhibiting the eukaryotic-type ACCase in the chloroplasts of sensitive grasses, thus blocking the biosynthesis of fatty acids and causing plant death [47]. These compounds have little or no inhibitory activity against the cytosolic enzyme or the prokaryotic-type enzyme in the chloroplasts of dicotyledonous

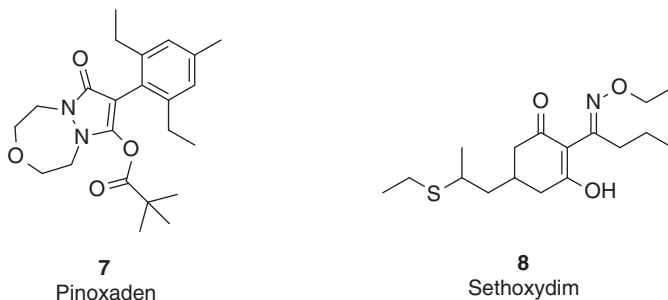


Figure 24.3 Structures of pinoxaden (7) and sethoxydim (8), a typical dim herbicide.

plants [47]. This selectivity explains the restriction of the herbicidal activity of these compounds to grasses [47].

24.5

Biological Activity

As mentioned earlier, the aryloxyphenoxypropionates provide selective control of grass weeds, while causing little or no damage to broad-leaved crops. They control a wide range of annual and perennial grasses in cool and warm temperate regions when applied postemergence (i.e., onto the foliage of the growing plants) but have much less activity when applied to the soil before emergence of the weed [48].

Many of the major fops, including fluazifop, quizalofop, and haloxyfop, are used for grass control in a range of broad-leaved crops, notably soya, oilseed rape, sugar beet, sunflower, cotton, grape vines, and potatoes [49, 50]. The use of these compounds was in a slow decline, due to both the increasing use of herbicide-tolerant soya and the emergence of resistance [11]. However, since 2010, sales have increased significantly, in part due to the appearance of glyphosate-resistant weeds and the resulting need for mixture partners to control them.

In contrast to these compounds, diclofop and clodinafop are used to provide selective grass control in cereal crops. Fenoxaprop is also widely used in this market as well as in broad-leaved crops. In these cases, the selectivity is a result of increased metabolism in the crop compared to the weeds [51], rather than selectivity at the biochemical target. Clodinafop and fenoxaprop are largely used in combination with herbicide safeners (cloquintocet-mexyl and mefenpyr-diethyl, respectively), which enhance metabolism [51], to ensure that the selectivity in cereal crops is robust [52].

Cyhalofop-butyl is unusual in that it is used for grass control in rice, a crop that is sensitive to most other fops. This unexpected selectivity is a result of differential uptake and metabolism, with activation by esterases being slower and subsequent metabolism to inactive compounds faster in rice than in weeds [53]. Metamifop (**9**) [54] and isoxapyrifop (**10**) [55] (Figure 24.4) are also selective to rice. The reason for this selectivity is not clear, but it may be due to the replacement of the ester with an amide in these compounds.

As these compounds are selective grass killers, they are often used in combination with other classes of herbicides that control broad-leaved weeds. However, it has been observed that the activity of the aryloxyphenoxypropionates is often antagonized by mixture partners. This effect has been seen for several classes of herbicide, but most notably for synthetic auxins [59]. The reason for this antagonism is not completely clear, although a number of explanations have been put forward, including the reduction of uptake and translocation of the fops in the presence of the auxin [60].

Within a few years of their introduction, weeds showing resistance to aryloxyphenoxypropionates were reported [61]. Resistance has spread steadily and has been observed in many species [62]. Considerable effort has been expended to

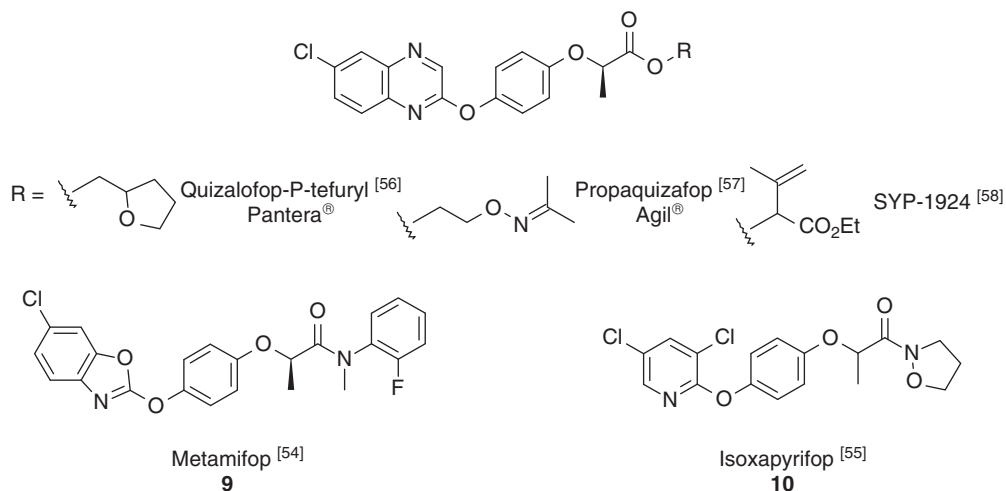


Figure 24.4 Structures of some ester variations.

understand the basis of the resistance and several different mechanisms have been identified [63]. These include increased metabolism [64] and impaired uptake [65], but a major resistance mechanism is mutation of the ACCase enzyme, rendering it less sensitive to the inhibitor [66]. At least six point mutations, all within the acetyl-CoA binding site, that result in resistance to multiple fops are known [67], although some of these confer a significant fitness penalty on the weed [68]. A combination of protein crystal structure information [44] and computational studies [69, 70] has been used to rationalize why certain point mutations confer resistance to both fops and dims, whereas others are series- or even compound-specific.

Maize that is tolerant to sethoxydim (**8**) has been developed [71]; in this case, the tolerance is a result of increased expression of ACCase. This maize also shows some tolerance to fops, but not at levels that are commercially useful. A number of reports of crops rendered resistant to the fops have appeared, and the Enlist™ range of crops developed by Dow are resistant to fops as well as to aryloxyacetate auxins [72]. The herbicide tolerance is a result of either increased metabolism of the herbicides [73], as for Enlist, or the presence of a modified ACCase enzyme [74, 75].

24.6

Structure–Activity Relationships

The structures of the commercialized aryloxyphenoxypionate herbicides (Figure 24.1) illustrate some of the variations of structure that can be tolerated while maintaining high levels of biological activity. Since the discovery of diclofop, all parts of the aryloxyphenoxypionate structure have been explored in detail

and many highly active analogs have been made but not commercialized. An overview of the SAR from the early patent literature has been published [76].

A wide range of esters show good activity [17, 20, 34]; for example, in addition to the ethyl ester, both the tetrahydrofurylmethyl [56] and an oxime-containing [57] ester of quizalofop have been commercialized and a further variant, SYP-1924 [58], is being developed (Figure 24.4).

It is known that the acid is the species that binds at the ACCase active site [8] and the ester acts as a procidal group to aid uptake [77]. Few other groups in this position have been developed; notable exceptions are metamifop (**9**) [54], in which the ester is replaced by an aryl amide, and isoxapyrifop (**10**) [55] and related hydroxamate esters [78]. In the case of metamifop, it has been reported that the amide itself inhibits ACCase [54], but it is not clear if some degree of hydrolysis to the acid occurs in plants and contributes to the herbicidal activity. A number of other variants and bioisosteres of the acid or ester group have been reported as giving good activity, but none has yet reached the market. Some examples are illustrated in Figure 24.5 [79–84].

The phenoxypropionate portion of the molecules is common to all commercialized fop herbicides. It has been shown that only the (*R*)-enantiomer inhibits ACCase [85, 86], and it is only this enantiomer that exhibits herbicidal activity when the compounds are applied as a foliar spray [4, 25, 86]. However, both enantiomers show similar activity when applied to the soil [25, 87]; this is due to selective conversion of the (*S*)-enantiomer to the (*R*)-enantiomer by soil microbes [4, 86–88].

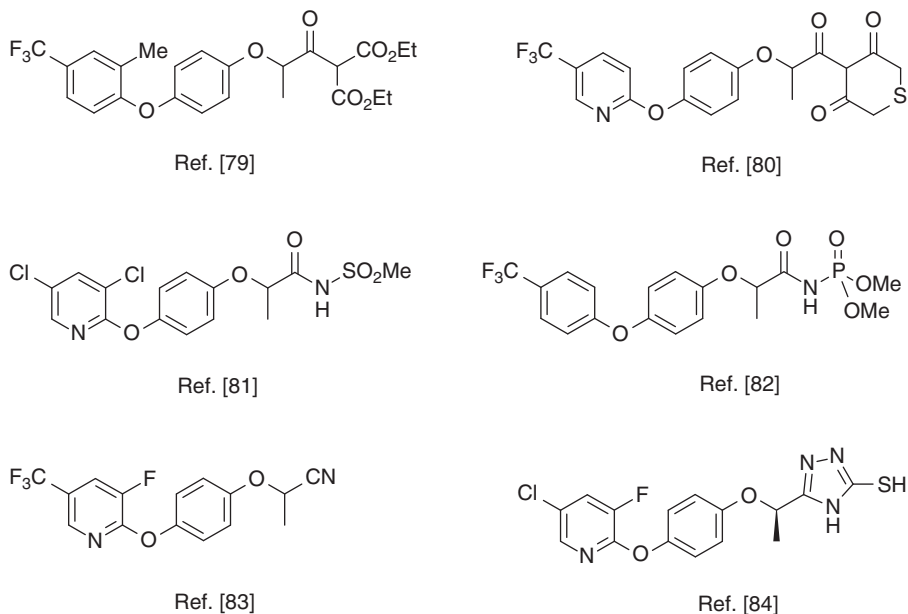


Figure 24.5 Compounds with ester replacements that show herbicidal activity.

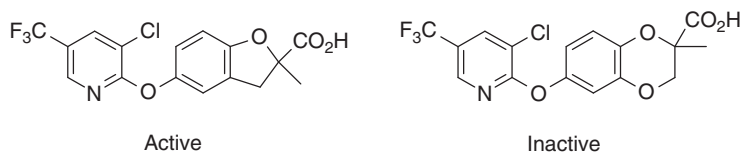
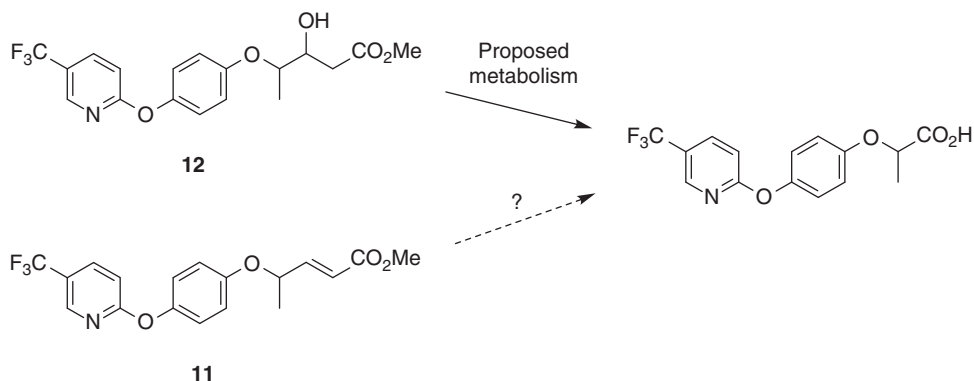


Figure 24.6 Cyclic analogs of haloxyfop.

A modeling study exploring the role of the chiral methyl group has suggested that this has a function in controlling the conformation of the acid group as well as in binding to the enzyme [89]. On the basis of this modeling, a series of conformationally restricted, cyclic analogs were designed and prepared. Several of the compounds with a five-membered ring showed activity both against the ACCase enzyme and as herbicides (Figure 24.6).

Systems in which the propionate group is extended, for example, to a 4-aryloxy-pent-2-enoate such as (**11**) [90], have also shown good herbicidal activity. It is likely that these compounds are metabolized to generate the propionic acid as is proposed to be the case for 3-hydroxypentanoates (**12**) (Scheme 24.5) [91].



Scheme 24.5 Extended analogs of fluazifop and possible routes for their metabolism.

Many other changes to this part of the molecule have been explored; they tend to result in a reduction or complete loss of activity or a switch in mode of action from inhibition of ACCase to activity as synthetic hormone herbicides with broad-leaved weed control [76].

The central phenyl ring with groups attached in the *para* positions is also common to all commercialized members of the fop class of herbicides, and in all cases, there is no further substitution on this ring. Analogs with the groups attached in both the *meta* and *ortho* positions have been reported, and in both cases, these have much less herbicidal activity or a different biochemical mode of action [76].

There is a report of a naphthyl ring system, with the oxygen substituents in the 1- and 5-positions, showing activity [92] (see Figure 24.7).

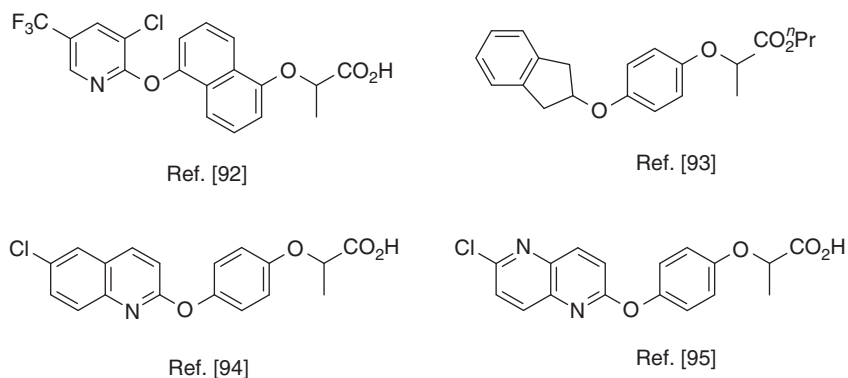


Figure 24.7 Further analogs that show herbicidal activity.

The oxygen linker between the two aromatic rings is another structural feature that is maintained in all commercial fops. Most variations in this portion of the molecule, for example, replacing the oxygen atom with sulfur or a carbonyl group, result in a loss of activity [76]. However, an N–Me (but not an N–H) linker provides good activity in a subset of compounds [96].

A fully aromatic group at the “left-hand side” of the molecule is generally required for good activity, although compounds with indane or tetralin ring systems show some activity [93] (see Figure 24.7). Both mono- and bicyclic ring systems can provide high levels of activity [96]. Many of the compounds possess ring systems containing one or two heteroatoms, perhaps favored because of the ease of their synthesis, but this is not essential, as demonstrated by diclofop-methyl (**4**) and cyhalofop-butyl (**3**).

In the bicyclic series, considerable variation of the ring system is tolerated, although the substitution pattern around the rings is important if activity is to be maintained [94, 97]. Sensitivity to small changes in this part of the molecule is also shown in a series of naphthyridines, with only the 1,5-isomers showing activity [95].

In monocyclic systems, substitution in the 4-position (relative to the oxygen linker) is essential, in the 2-position substitution is beneficial but not critical [76]. On the other hand, substituents in the 3- and 5-positions generally lead to a reduction in activity. It has been shown that pyridine *N*-oxides are active [98].

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25

Pyridines Substituted by an Imidazolinone and a Carboxylic Acid as Acetohydroxyacid-Synthase-Inhibiting Herbicides

Dale Shaner

25.1

Introduction

The imidazolinone herbicides consist of six commercial products (**1–6**) with varying levels of crop selectivity and herbicidal activity depending on the chemistry (Figure 25.1) [1]. These herbicides share the imidazolinone ring and vary in the attached ring structure. The imidazolinones kill plants by inhibiting acetohydroxyacid synthase (AHAS, also known as acetolactate synthase), which is the first enzyme in the branched-chain amino acid pathway leading to the synthesis of valine, leucine, and isoleucine [2]. The imidazolinones have low mammalian toxicity and offer excellent control of many important broadleaf and grassy weeds. The development of imidazolinone-resistant varieties of wheat, rice, canola and sunflower, and other crops provides control of weeds that are closely related to crops such as *Oryza sativa* (red rice) in rice and *Aegilops cylindrica* (jointed goatgrass) in wheat [3].

25.2

History

The discovery of the imidazolinone herbicides started with the screening of a phthalimide **7** (Scheme 25.1), which was originally prepared as a potential anticonvulsant and which controlled a sufficient number of weeds at 4 kg/ha to warrant further investigation [4]. Unknown at the time, this compound was an inhibitor of AHAS [5, 6]. A synthesis program around **7** resulted in the preparation of the chlorophthalimide AC 94 377 (**8**), a plant growth stimulant that mimicked gibberellic acid and led to a development project [7]. However, during the production of several hundred kilograms of **8** for field studies, a novel imidazoisindole **9**, was isolated, which was slower acting than **7** but the biological activity was high enough to further explore this area of chemistry [4]. One of the earliest compounds prepared in this new series of imidazoisindoles was **10**, which can be

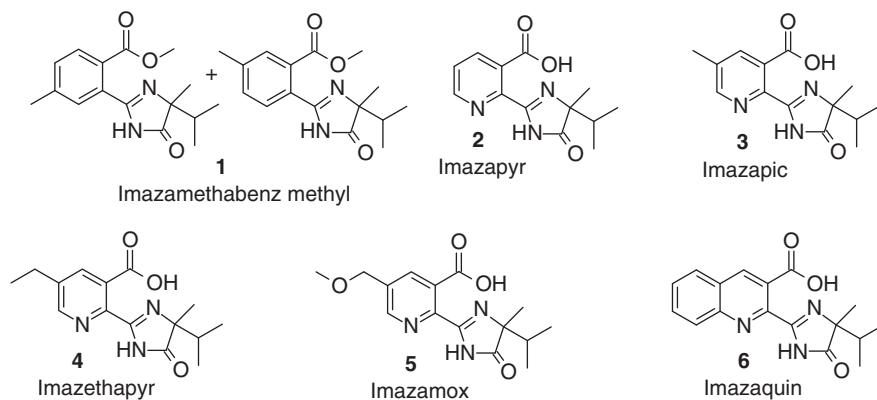
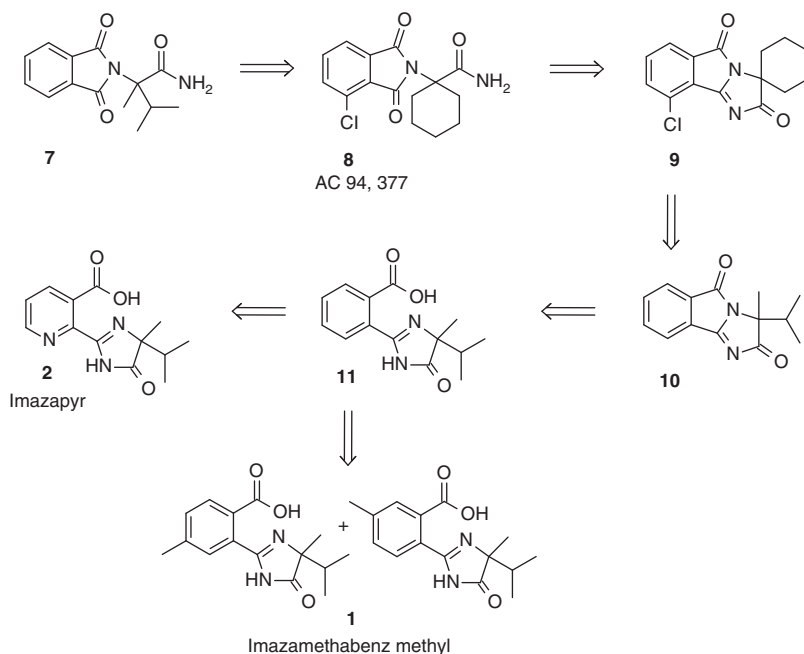


Figure 25.1 The imidazolinone herbicides imazamethabenz methyl (1), imazapyr (2), imazapic (3), imazethapyr (4), imazamox (5), and imazaquin (6).



Scheme 25.1 Invention pathway of the herbicidal imidazolinones.

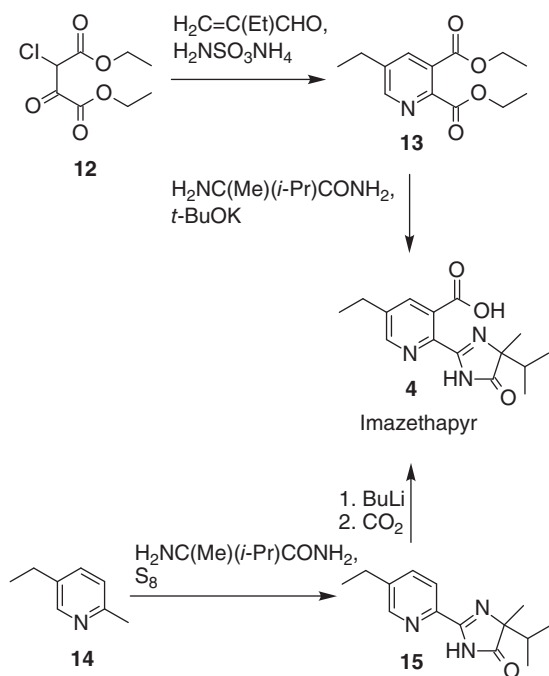
obtained from **7** under basic conditions [4]. Surprisingly, **10** did not have any GA-like activity but was active on many weeds, including perennial weeds such as *Cyperus* sp. and *Convolvulus* spp., although the activity was not high enough to warrant commercial development [4]. As a result of this investigation, it was found that **10**, an analog of **9** with different substitution pattern in the phenyl ring, had herbicidal activity. A study of the chemistry of the tricyclic compound **10**, led

via transformation with sodium methoxide in methanol to the imidazolinybenzoate **11**, the first imidazolinone with herbicidal, however, primarily nonselective activity [4]. The introduction of a methyl group into the phenyl ring of **11** turns a nonselective herbicide into the product imazamethabenz-methyl (**1**), the only one of numerous analogs made within the benzene imidazolinones, which has been commercialized to control *Avena fatua* and a limited spectrum of broadleaf weeds in wheat. The real breakthrough in the commercial success of the imidazolinones was the exchange of the phenyl ring of **11** by a pyridine resulting in imazapyr (**2**) and its analogs **3–6**, which possess an order of magnitude higher activity than their benzene analogs and which are selective in several crops [5, 8].

25.3

Synthesis

There are various routes available for the synthesis of the imidazolinone herbicides, as demonstrated for imazethapyr (**4**). It can be efficiently prepared in only two steps by condensation of ethyl chlorooxalacetate (**12**) and an enal, in the presence of a buffered ammonia source, to the pyridine diester **13** and subsequent regioselective transformation with potassium tert-butoxide and α -methylvalinamide (Scheme 25.2). The same amino acid amide is applied in an



Scheme 25.2 Synthesis of imazethapyr (**4**).

alternative two-step procedure to imazethapyr (**4**), where it is directly converted into the imidazolinone **15** with 5-ethyl-2-methylpyridine (**14**) together with an excess of sulfur in a Willgerodt–Kindler reaction. Due to the *ortho*-directing effect of this five-membered ring, a subsequent metalation/carbonylation sequence leads regioselectively to imazethapyr (**4**) [5].

25.4

Mode of Action

The imidazolinones kill plants by inhibiting AHAS, the first common enzyme in the biosynthesis of valine, leucine, and isoleucine, the branched-chain amino acid [2]. This enzyme is the target site for a number of commercial products including sulfonylureas, pyrimidinylthiobenzoates, sulfonylaminocarbonyltriazolinone, and triazolopyrimidines [9]. AHAS catalyzes the condensation of either two pyruvate molecules to produce acetolactate, which leads to the synthesis of valine and leucine, or a molecule of pyruvate and 2-ketobutyrate to produce 2-aceto-2-hydroxybutrate, which is needed to produce isoleucine. Partitioning between these two reactions is determined by the concentration of the substrates [10]. AHAS requires thiamine pyrophosphate, a divalent metal ion, and flavin adenine dinucleotide [11]. The imidazolinones are extraneous target-site inhibitors because they bind to the enzyme outside of the active site of the enzyme.

The versatility of the imidazolinones and other AHAS inhibitors resulted in their widespread adoption by farmers. However, within 3 years after the introduction of the AHAS inhibitors, weed populations evolved and became resistant to these herbicides [12]. It is now known that there are at least 10 sites within the AHAS gene where changes in the amino acid sequence result in resistance to one or more classes of AHAS inhibitors [13]. In 2011, there were over 100 species globally that have evolved resistance to an AHAS inhibitor [9].

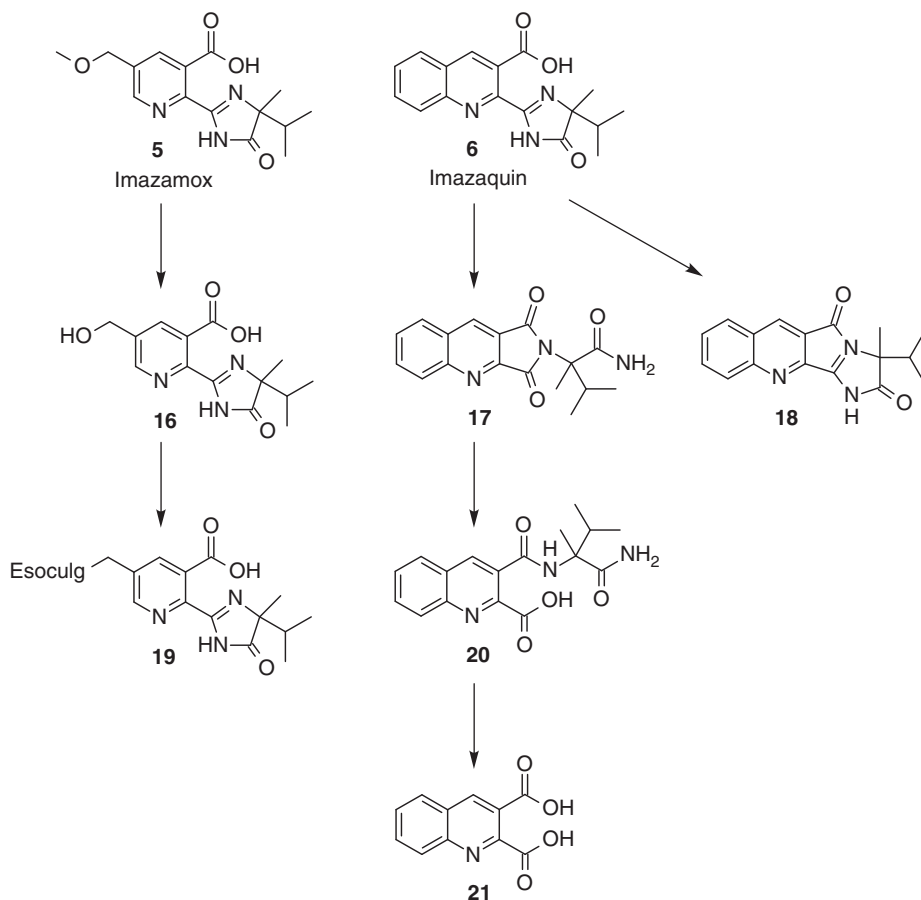
25.5

Biological Activity

The imidazolinones kill annual and perennial broadleaf and grassy weeds when applied to the foliage or soil. The spectrum of activity depends on the chemistry and the ability of the weed to metabolize the herbicide to nontoxic forms [14]. The biological activity of these herbicides is dependent on the translocation of the herbicide to the growing points of the plant. AHAS, the target site, is most highly expressed and important in meristematic tissue such as the growing points and reproductive tissue [6]. Imazapyr (**3**) is the broadest spectrum of all the imidazolinones. It translocates rapidly through the phloem and accumulates in the growing points. It is also not rapidly metabolized by many species [1]. Imazamethabenz methyl (**1**) has the narrowest spectrum of activity due to limited translocation. Imazamox (**5**) has the highest activity on grassy species due to the

inability of grasses to rapidly hydroxylate the methoxymethyl substituent on the pyridine ring [14].

The natural crop selectivity of the imidazolinones is due to the ability of the crops to rapidly metabolize the herbicides to nontoxic forms [1]. For **1**, **3–5**, this metabolism is through a mixed-function oxidase that hydroxylates the substitution on the pyridine or benzene ring followed by conjugation to glucose (Scheme 25.3) [1].



Scheme 25.3 General route of metabolism of imidazolinone herbicides in plants, with example of imazamox (**5**) and imazaquin (**6**).

Imidazolinone-resistant crops were selected by challenging mutanized seeds or tissue culture with the imidazolinones [3]. Resistant biotypes were obtained and were subsequently used to develop imidazolinone-resistant crop varieties. All of these biotypes contain mutations within the AHAS gene, which makes the enzyme resistant to the imidazolinones. Since none of these crops was developed

through genetic engineering, the crops do not have the restrictions placed on them as for other transgenic-herbicide-resistant crops. These imidazolinone-resistant crop cultivars have been commercially developed in wheat, rice, canola, maize, and lentils [3].

25.6

Structure–Activity Relationship

The basic structure of the imidazolinones is shown in Figure 25.2. This structure is divided into three elements: the imidazolinone ring, the backbone, and the acid equivalent site. All three of these elements are necessary for biological activity. Major deviation away from this generic structure results in complete loss of herbicidal activity. For example, the acid equivalent site and the imidazolinone ring have to be *ortho* to each other. Any other arrangement is inactive [8, 15].

The carboxylic acid is necessary for herbicidal activity. Imazamethabenz methyl (1) is the only commercial product that is applied as a methyl ester, which is quickly converted in the plant into the carboxylic acid. All of the other imidazolinones are applied as a salt of the acid. The unique compounds **22** and **23** have been published, in which the carboxylic acid is replaced by a labile oxime ester or by a phosphonic acid [16] (Figure 25.3).

The imidazolinone ring is required for activity. The best substitutions for R¹ and R² are a methyl (R¹) and an isopropyl (R²), respectively. This site is also capable of optical isomerism. The (*R*)(+)-isomer has about twice the activity of the racemic mix and the (*S*)(–)-isomer is much less active, with about one-tenth of the activity of the racemic mixture [8]. An oxygen at the W site and a hydrogen at the B site are the most active herbicides.

The substitutions on the backbone are very interesting, particularly when comparing the whole plant activity to the inhibition of AHAS. A simple benzene ring

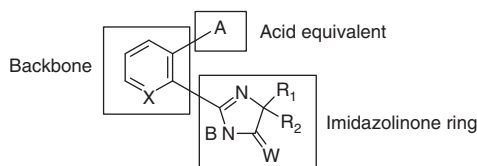


Figure 25.2 General structure of the imidazolinone herbicides.

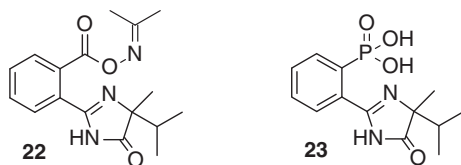


Figure 25.3 Carboxylic acid mimics of imidazolinone herbicides.

with no substitutions is the most inhibitory analog at the enzyme level, but the pyridine analog where N is located at X is the most active at the whole plant level. At the enzyme level, the position of N in the ring in relationship to the imidazolinone and carboxylic acid functionalities was not important. All of the analogs were equally inhibitory. However, at the whole plant level, the only active analog was when N was *meta* to the carboxylic acid and *ortho* to the imidazolinone ring. The differences in whole plant activity are due to the ability of the compound to enter the plant cell and to translocated within the plant [8, 17].

Substituents on the heterocyclic ring provide the selectivity of the imidazolinones. Most of these substituents are in the *meta* position from the carboxylic acid function. Of commercial products, the only exceptions are imazaquin (**6**) where the backbone consists of a quinoline ring and imazamethabenz methyl (**1**), which is a 50 : 50 mixture of the 5-methyl and 4-methyl analogs. Substitutions at the *ortho* position to the carboxylic acid are inactive.

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26

Carboxylic-Acid-Containing Protoporphyrinogen-IX-Oxidase-Inhibiting Herbicides

George Theodoridis

26.1

Introduction

A wide variety of protoporphyrinogen-IX-oxidase (Protox)-inhibiting herbicides containing a carboxylic acid, or a carboxylic acid derivative, have reached the commercial stage of development. Examples include Protox inhibitors such as saflufenacil (1) [1, 2], benzfendizone (2) [3], fluazolate (3) [4], acifluorfen-sodium (4) [5], carfentrazone-ethyl (5) [6, 7], and flupropacil (6) [8] (Figure 26.1).

Weed shifts observed in genetically modified crops, caused by the development of weed resistance to the widely used glyphosate herbicide [9], will continue to offer market opportunities for herbicides with alternative modes of action, such as Protox-inhibiting herbicides.

26.2

History

The early Protox-inhibiting herbicides did not contain a carboxylic acid functional group. These early Protox herbicides were the diphenyl ether nitrofen (7) [10] introduced in 1963 by Rohm and Haas, now Dow AgroSciences, and the oxadiazolinone oxadiazon (8) [11], introduced in 1968 by Rhône-Poulenc (Figure 26.2). While chemically quite different, the two molecules share a common mode of action, inhibition of the protoporphyrinogen oxidase enzyme, although this mode of action was not well understood until the late 1980s. These early examples of Protox-inhibiting herbicides generated intensive work from the 1960s through the 1990s, which resulted in numerous diverse chemistries, from which many useful commercial products were obtained. With the exception of the diphenyl ether class of compounds, most other Protox-inhibiting chemistry classes can be broadly described as having a heterocyclic ring attached to a substituted aromatic ring via a nitrogen or carbon atom.

The heterocyclic ring could be any of several dozen heterocyclic rings, such as oxadiazolinone [12], tetrahydrophthalimide [13], tetrazolinone [14],

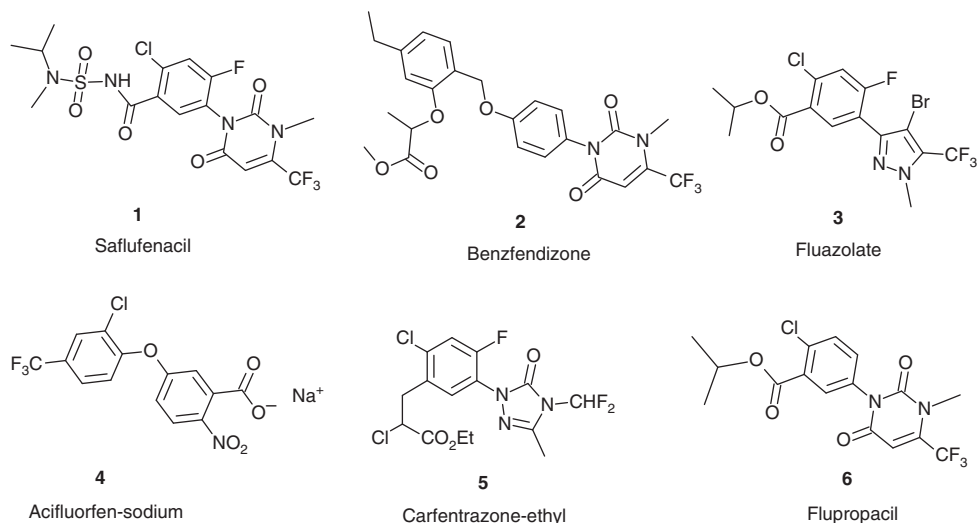


Figure 26.1 Examples of Protox herbicides containing the carboxylic acid functional group.

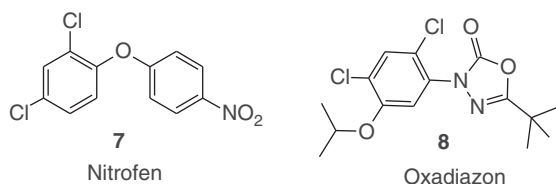


Figure 26.2 Chemical structures of two early examples of Protox inhibitors.

triazolinone [15], pyrazole [4], or oxazolidinedione [16]. Extensive reviews of these heterocyclic systems, their properties, and their synthesis have been published [17–21]. Of the several dozen new heterocyclic systems introduced during the period from 1990 to 2005, the one that stands out as having the greatest impact in terms of significant increase in biological activity is the 6-trifluoromethyl-2,4(1H,3H)-pyrimidinedione ring – commonly referred to simply as 6-trifluoromethyluracil – initially introduced by Hoffmann-La Roche and Uniroyal in flupropacil (**6**) [8].

We can broadly organize the carboxylic-acid-containing Protox herbicides into two groups:

- 1) Carboxylic acid functional group directly attached to aromatic ring of Protox herbicide
- 2) Carboxylic acid functional group present but not directly attached to aromatic ring of Protox herbicide.

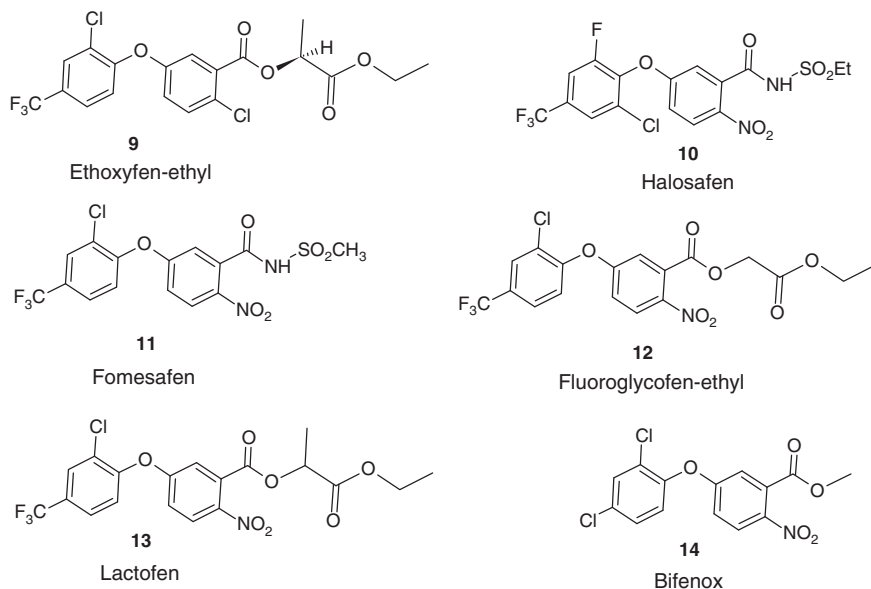


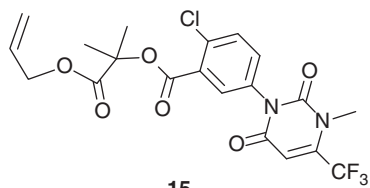
Figure 26.3 Diphenyl ether Protox herbicides with a carboxylic acid group directly attached to the aromatic ring.

26.2.1

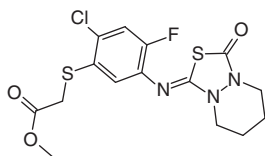
Carboxylic Acid Functional Group Directly Attached to Aromatic Ring of Protox Herbicide

There are a number of significant commercial Protox herbicides currently on the market in which the carboxylic acid, or one of its derivatives, is directly attached to the aromatic ring. One of the earliest examples of this group was the diphenyl ether acifluorfen-sodium (**4**) [5] shown in Figure 26.1. A number of other carboxylic-acid-containing diphenyl ether commercial herbicides quickly followed (Figure 26.3), such as ethoxyfen-ethyl (**9**) [22], the sulfonamides halosafen (**10**) [23], and fomesafen (**11**) [23], fluoroglycofen-ethyl (**12**) [24], lactofen (**13**) [25], and bifenox (**14**) [26]. These diphenyl ether herbicides were used primarily for postemergence control of broadleaf weeds, with fomesafen showing good soybean safety.

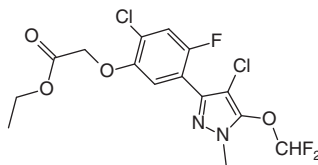
Following the successful introduction of the early diphenyl ether Protox herbicides, a number of newer Protox herbicides were introduced in which the aromatic ring was now directly attached to a heterocyclic ring. Examples of these newer Protox herbicides that are of commercial interest include butafenacil (**15**) [27] (Figure 26.4), the sulfonamide saflufenacil (**1**) [1, 2], and the esters fluazolate (**3**) [4], and flupropanil (**6**) [8] (Figure 26.1).

**15**

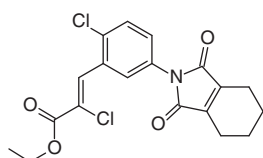
Butafenacil

Figure 26.4 The Protox herbicide butafenacil (15) [27].**16**

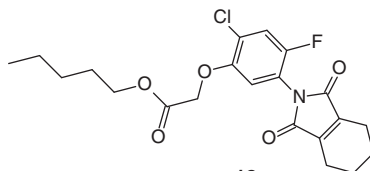
Fluthiacet-methyl

**17**

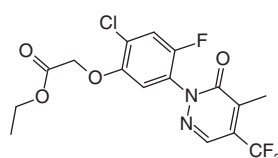
Pyraflufen-ethyl

**18**

Cinidon-ethyl

**19**

Flumiclorac-pentyl

**20**

Flufenpyr-ethyl

Figure 26.5 Protox herbicides with ester functional groups not directly attached to the aromatic ring.

26.2.2

Carboxylic Acid Functional Group Present but Not Directly Attached to Aromatic Ring of Protox Herbicide

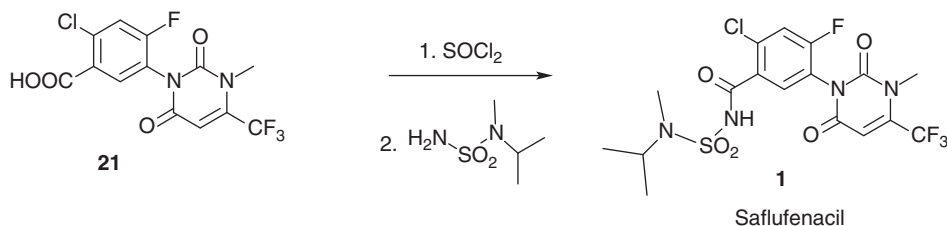
A number of commercial Protox herbicides have been introduced in which a carboxylic acid is attached to the aromatic ring via a variety of different bridging groups. Examples in this group include benzfendazole (2) [3], carfentrazone-ethyl (5) [6, 7], fluthiacet-methyl (16) [28], pyraflufen-ethyl (17) [29], cinidon-ethyl (18) [30], flumiclorac-pentyl (19) [11], and flufenpyr-ethyl (20) [31] (Figure 26.5).

26.3

Synthesis

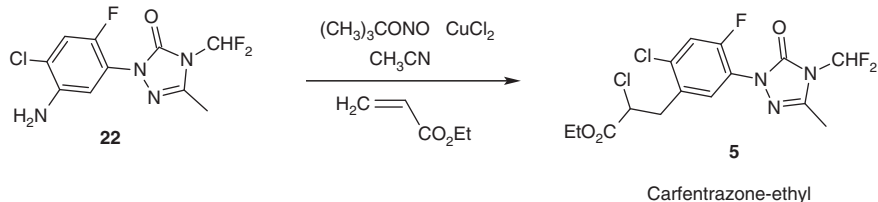
A number of earlier publications discussed the synthesis of a variety of Protox herbicides [17–21]. The synthesis of saflufenacil (1) provides a good example of a commercial Protox herbicide in which the carboxylic acid group is directly

attached to the aromatic ring. The carboxylic acid precursor **21** is converted into the acid chloride by treatment with thionyl chloride, followed by reaction with the corresponding sulfamide (Scheme 26.1) [1, 2]. The carboxylic acid precursor of saflufenacil, compound **21**, can be prepared by the reaction of the corresponding arylisocyanates with ethyl 3-amino-3-trifluoromethylcrotonate in the presence of a base. The uracil heterocycle is then directly N-methylated with methyl iodide in a one-pot reaction [32].



Scheme 26.1 Synthesis of saflufenacil (**1**) [1, 2].

Carfentrazone-ethyl (**5**) is a representative example of the synthesis of a Protox herbicide in which the carboxylic acid functional group is not directly attached to the aromatic ring. In this case, the carboxylic acid functionality is introduced in the final step via a copper-catalyzed Meerwein reaction of the corresponding aniline **22** with ethyl acrylate (Scheme 26.2) [6, 7].



Scheme 26.2 Synthesis of carfentrazone-ethyl (**5**) [6, 7].

26.4

Mode of Action

Protox-inhibiting herbicides act by inhibition of the enzyme protoporphyrinogen oxidase, the last common enzyme to both heme and chlorophyll biosynthesis [33–35]. The enzyme protoporphyrinogen oxidase catalyzes the oxidation of protoporphyrinogen IX to protoporphyrin IX by molecular oxygen. Inhibition of the Protox enzyme results in the accumulation of the enzyme product protoporphyrin IX, but not the protoporphyrinogen IX substrate, via a complex process that has not been entirely elucidated. In the presence of light, protoporphyrin IX generates large amounts of singlet oxygen, which results in the peroxidation of the unsaturated bonds of fatty acids found in cell membranes. The end result of

this peroxidation process is the loss of membrane integrity and leakage, pigment breakdown, and necrosis of the leaf, resulting in the death of the plant. This is a relatively fast process, with leaf symptoms such as a flaccid wet appearance observed within hours of plant exposure under sunlight to the Protox-inhibiting herbicide.

26.5

Biological Activity

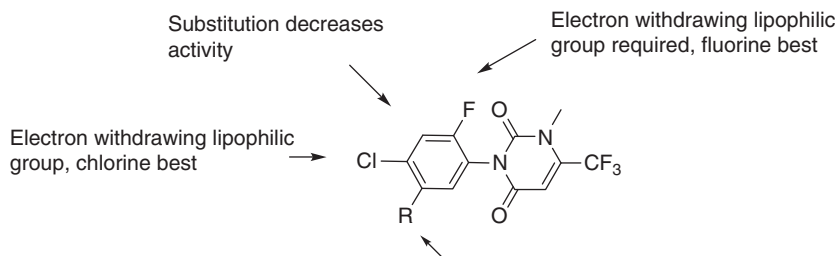
Protox-inhibiting herbicides have found commercial application in a wide range of important crops such as corn, soybean, rice, and sunflower when applied preemergently or postemergently. Preemergent or postemergent application of a herbicide refers to the timing of the placement of a herbicide, with preemergence indicating application prior to the emergence of the plant and postemergence indicating application to weeds after the crop has emerged. Research on Protox-inhibiting herbicides reached a peak in the early 1990s [36] and diminished soon after, following the introduction of genetically modified crops such as glyphosate-resistant crops. Glyphosate, N-(phosphonomethyl)glycine is a broad-spectrum, postemergence, systemic herbicide that has been used extensively over the past 35 years. However, the intense and prolonged use of the glyphosate herbicide has resulted in documented resistance to glyphosate in several weed populations [9], which in turn has encouraged renewed interest in Protox-inhibiting herbicides. Indicative of this continuing interest is the recent introduction by BASF of saflufenacil (**1**) herbicide, which is being developed for broadleaf weed control in several crop and noncrop use patterns. Saflufenacil (**1**) causes rapid burndown of emerged broadleaf weeds when applied in conservation till or no-till soybean management systems. Saflufenacil (**1**) has been shown to effectively control many key broadleaf species, including glyphosate- and acetolactate synthase (ALS)-resistant biotypes, such as *Conyza Canadensis* (horseweed), *Lactuca serriola* (prickly lettuce), *Chenopodium album* (common lambsquarters), *Ambrosia trifida* (giant ragweed), and *Amaranthus hybridus* (smooth pigweed). Research has focused on burndown use rates of 18–25 g a.i./ha [37].

Another example of renewed interest in Protox-inhibiting herbicides is carfentrazone-ethyl (**5**) [6, 7], a postemergence cereal and corn herbicide. At low rates of 20–35 g a.i./ha, carfentrazone-ethyl (**5**) provides excellent control of weeds in commercially important cereal crops – weeds such as bedstraw, speedwell, morning glory, Kochia, spurge, and deadnettle [38].

26.6

Structure–Activity Relationship

Comprehensive modeling and quantitative structure–activity relationship (QSAR) studies of Protox-inhibiting herbicides have been reported [19, 20, 39, 40]. The SAR of the phenyl ring of 2,4-dihalo-5-substitutedphenylheteroaryl



Best overall activity when R is lipophilic group such as OCH_2CCH_3 , but very limited crop selectivity. Potential for greater crop selectivity when R contains acidic group such as $-\text{NHSO}_2\text{CH}_3$ or CONHSO_2R

Figure 26.6 Structure–activity relationships of the 2,4,5-trisubstituted phenyl heterocycles.

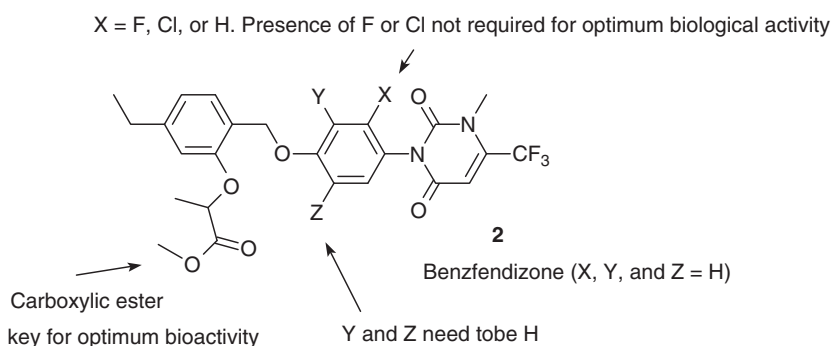


Figure 26.7 Structure–activity of 3-(benzoheterocyclic)-1-methyl-6-trifluoromethyluracil Protox-inhibiting herbicides.

Protox-inhibiting herbicides is shown in Figure 26.6. The phenyl ring is substituted in the 2- and 4-positions by halogens, with fluorine best in the 2-position and chlorine best in the 4-position. A vast number of R substituents exist in the 5-position that results in high biological activity. This is also the position that has the greatest potential to provide the molecule with crop selectivity. For instance, introduction of the NHSO_2CH_3 group in position 5 of the phenyl ring of the aryl triazolinone Protox-inhibiting herbicide sulfentrazone [15] resulted in weed control and high selectivity in soybean. Another example is the presence of the $\text{CONHSO}_2\text{N}(\text{CH}_3)\text{CH}(\text{CH}_3)_2$ group in position 5 of the phenyl ring of saflufenacil (1), which provides corn and soybean crop selectivity when applied as a preemergence burndown material [37].

A different group, 3-(4-benzyloxyphenyl)-1-methyl-6-trifluoromethyluracil, redefined the accepted SAR and QSAR of the aromatic 4-position of aryl heterocyclic Protox herbicides. In this group, the chlorine in the 4-position of the aromatic ring is replaced by the more bulky benzyloxy group. A halogen substituent in the 2-position of the aromatic ring is no longer crucial. Substituents other than hydrogen in the 3- and 5-positions (substituents X and Y) of the aromatic ring resulted in loss of biological activity (Figure 26.7). The

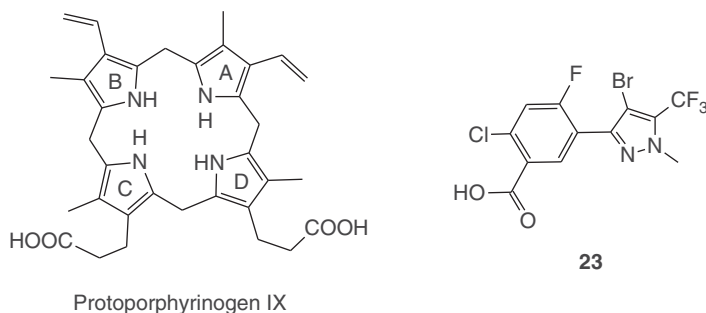


Figure 26.8 Prototoxin inhibitor **23** used in protoporphyrinogen IX oxidase-binding studies.

presence of the carboxylic acid group in the molecule is crucial for optimum activity [20].

In 2004, the crystal structure of the mitochondrial Prototoxin enzyme obtained from tobacco, and complexed with Prototoxin inhibitor phenyl pyrazole **23**, was published [40]. It proposes that the pyrazole ring of compound **23** matches ring A and the phenyl ring matches ring B of protoporphyrinogen IX (Figure 26.8). Molecular modeling studies of Prototoxin inhibitors found good overlap between the diphenyl ether aromatic rings and protoporphyrinogen IX (protopogen) [41] and between a set of imide-type Prototoxin inhibitors and protopogen [42].

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Part IX

Disease Control

27

Phenylamide Fungicides

Laura Quaranta

27.1

Introduction

The control of plant diseases caused by oomycetes has been a major target since the beginning of modern crop protection industry. The arsenal of resources against oomycetes diseases is rich, including broad-spectrum fungicides such as the strobilurin class [1]. However, due to the special taxonomy of the oomycetes between algae and fungi, the chances to identify leads and develop products with high level of selectivity and environmental safety are high, as proven by the phenylamide fungicides (also called acylanilides). The phenylamide class includes a number of structurally related amino-acid derivatives with high activity against all members of the *Peronosporales* and *Pythiales* and an excellent safety profile [2]. Introduced to the market in the 1970s, as a breakthrough technology alternative to the use of metal containing product, the phenylamides despite resistance problems continue to hold market share, leading the oomycetes segment with an overall market value of 435 million USD [3] mostly dominated by metalaxyl (1) (Ridomil®, Syngenta) [4], but also including benalaxyl (2) (Galben®, Isagro, FMC) [5], furalaxyl (3) (Fongarid®, Syngenta) [4c, 6], oxadixyl (4) (Sandofan®, Syngenta) [7], and ofurace (5) (Patafol®, Bayer AG) [1] (Figure 27.1).

27.2

History

The origin of the phenylamide fungicides is linked to the cross-indication screening of chloroacetanilide herbicides, such as metolachlor (7) [8, 9]. The antifungal activity displayed by chloroacetanilide herbicides has been optimized during an intensive optimization program in Ciba-Geigy initiated in 1973 with concurrent elimination of herbicidal activity. Different structural features have proven to be key for fungicidal or herbicidal activity: replacement of the

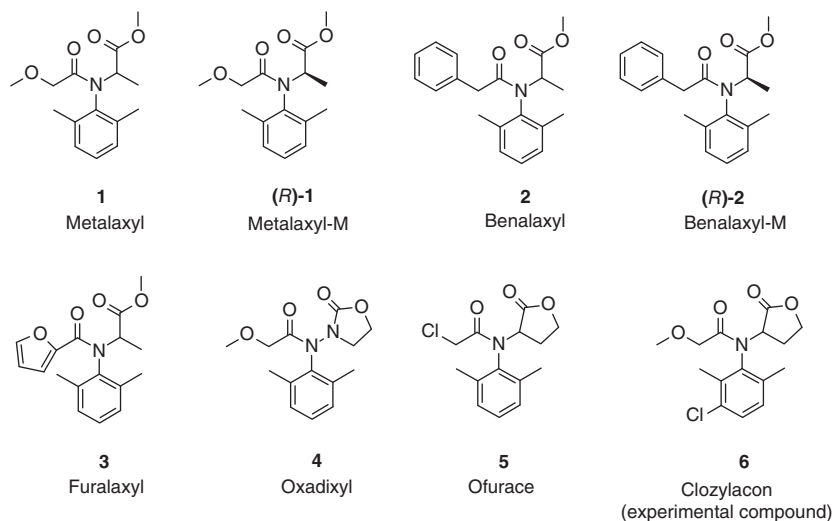
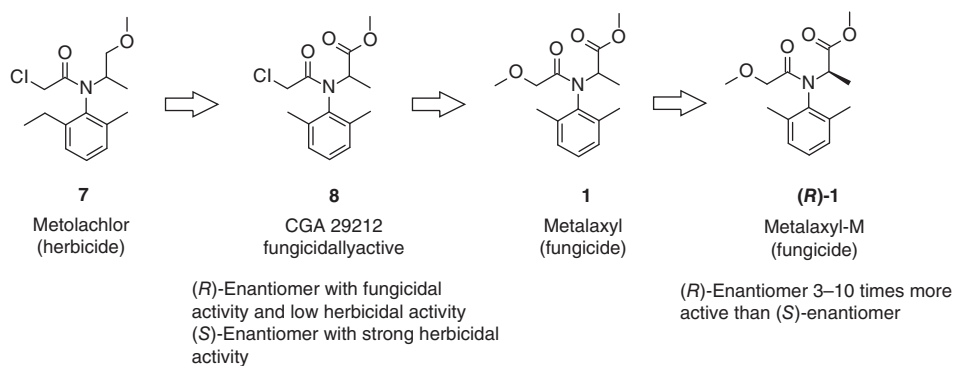


Figure 27.1 Commercial phenylamides fungicides [2, 3].

alkoxyalkyl substituent in metolachlor by an ester function led to compound CGA29212 (**8**) showing good systemic, curative, and preventative activity against *Phytophthora infestans* [8, 9]. Biological testing of resolved enantiomers revealed that the (*R*)-enantiomer is an excellent fungicide with low herbicidal activity, while the (*S*)-enantiomer showed only herbicidal activity. The phytotoxic effect could be banned completely by replacing the chloroacetyl group in CGA29212 (**8**) by other acyl fragments. In particular, exchange of the chloro with a methoxy group led to the highly fungicidally active metalaxyl (**1**) (Scheme 27.1) [4]. Aware of the differential activity of the enantiomers of the prototype compound (**8**), the two optical isomers of (**1**) were resolved. *In vitro* screening against *P. infestans* and *Pythium ultimum* showed that (*R*)-(**1**) was approximately 1000 times more active



Scheme 27.1 Invention pathway of metalaxyl (**1**) and metalaxyl-M (*R*)-(**1**) [8].

than (*S*)-(1). However, in *in vivo* trials, such difference in activity was reduced to 3–10 times depending on the pathogen tested [8]. Because racemic metalaxyl (1) shows high activity and no phytotoxic effects, separation of enantiomers was not mandatory and the compound was first developed as a racemate. Interestingly, early observation that chirality dependency of fungicidal activity is different from that of herbicidal activity (*R*)-enantiomers displaying higher fungicidal activity than (*S*)-enantiomers while in contrast herbicidal activity resides mostly on the (*S*)-enantiomers suggested that the mechanism of action of the phenylamide in fungi is likely different from that in plants [10]. In 1996, Ciba-Geigy (now Syngenta) announced the introduction of the most active (*R*)-(–)-enantiomer of metalaxyl (1) in the market, under the common name of metalaxyl-M and mefenoxam ((*R*)-1) [11, 12]. The introduction of a pure enantiomer replacing the racemic mixture, also named “chiral switch,” had as main goal the reduction of chemicals dispersed in the environment, as application rates could be reduced to half the amount compared with metalaxyl (1). Extensive degradation and metabolism studies of metalaxyl-M (*R*)-(1) in soil, plant, and animals have shown a similar profile to metalaxyl (1) and no specific effects could be attributed to the (*R*)-enantiomer that impair its safety profile. Thus, the metalaxyl “chiral switch” has marked a milestone both in the control of oomycetes by phenylamide fungicide and in general in the use of chiral crop protection agents [11].

Besides metalaxyl (1) and metalaxyl-M ((*R*)-1), other phenylamide fungicides with same spectrum of activity have reached the market. Benalaxyl (2) was introduced by Farmoplant (now Isagro) in 1982 for the control of late blight and downy mildew on a range of crops including ornamentals and control of *Pythium* diseases of turf [5]. Its rights were transferred to FMC in 2009. The active (*R*)-enantiomer benalaxyl-M (*R*)-(2) (also called kiralaxyl) was introduced and currently marketed by Isagro in 2003 [13]. Furalaxyl (3) was first introduced in the market in 1977 by Ciba-Geigy (now Syngenta), being developed especially for the control of oomycetes in ornamentals where it has shown to be safer to certain plants than metalaxyl [4c, 6]. Oxadixyl (4) containing a unique *N*-amino oxazolidinone replacing the amino-acid portion was introduced by Sandoz (now Syngenta) in 1984 [7]. Ofurace (5) containing a butyrolactone was introduced in the market in 1992 by Chevron, the rights were subsequently transferred to Schering (now Bayer AG) [14]. It is closely related to clozylacon (6) an experimental fungicide prepared by Ciba-Geigy chemists, having improved metabolic stability against microbial degradation making it well suited for soil application against oomycetes where protection from the pathogens is required for relatively long periods [15]. The presence of a stereocenter and atropisomerism due to hindered rotation around the aryl carbon–nitrogen bond in (6) generates four stereoisomers. After the finding that the desired fungicidal activity arises mainly from the stereoisomers with absolute configuration ($\alpha S, 3R$), a synthesis campaign was started to identify an enantioselective route amenable to large-scale production. Because undesired biological effects of clozylacon were observed in field tests, the project was discontinued. However, two successful enantioselective syntheses were validated (the chiral pool approach and the enantioselective hydrogenation

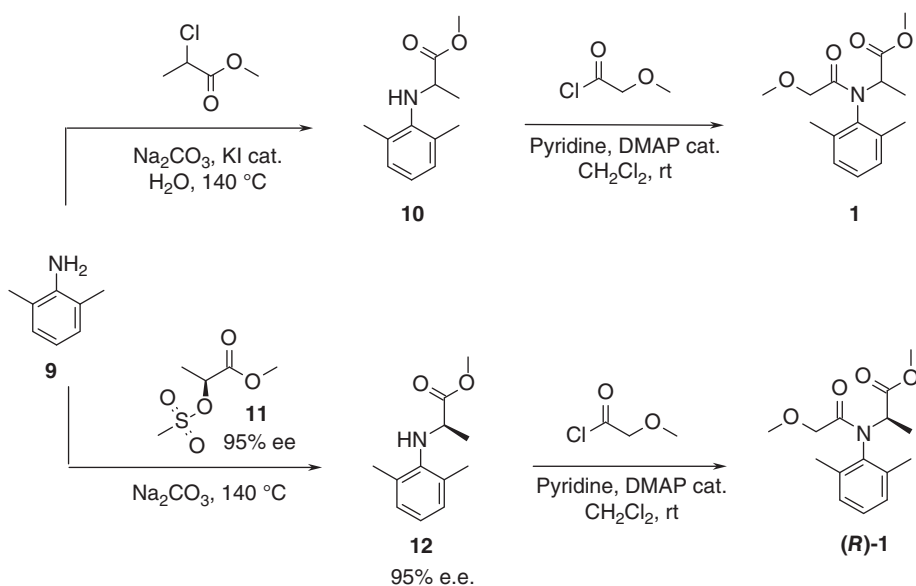
of enamides) [8, 16] and the learning could be successfully transferred to the synthesis of metalaxyl-M, as shown in the next chapter.

27.3

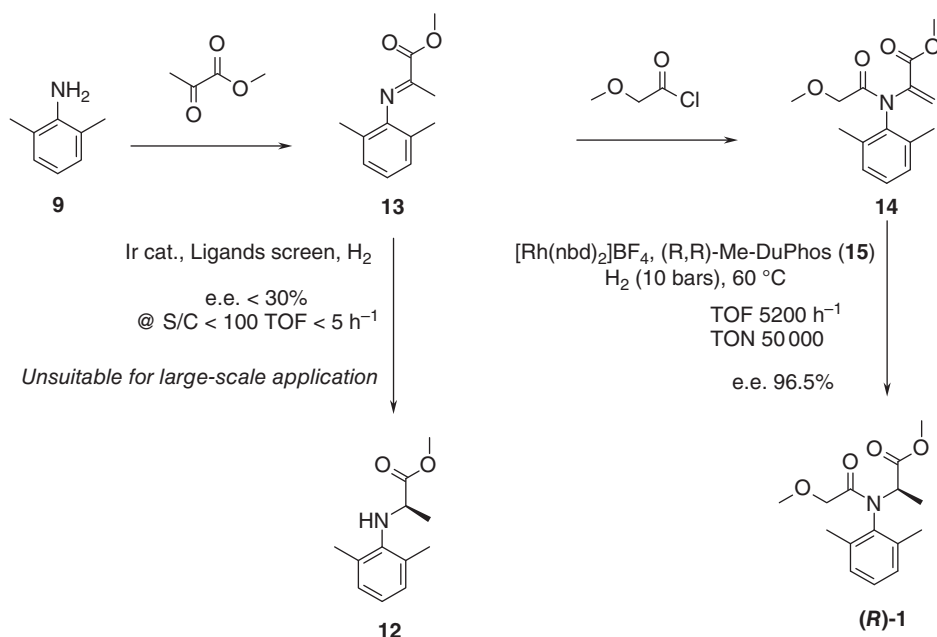
Synthesis

This chapter describes the enantioselective routes identified for the preparation of metalaxyl-M (*R*)-(1), which, together with the synthesis of (*S*)-metolachlor (7), has marked a breakthrough in the industrial application of chiral catalysis for the production of agrochemicals [17].

Both enantiomers of metalaxyl were initially prepared by fractional crystallization of the salt of DL-*N*-(2,6-dimethylphenyl)-alanine with (+) and (–)- α -phenethylamine followed by Fischer esterification and acylation with methoxyacetyl chloride [11]. Such a process would hardly be scalable to industrial production. Selective enzymatic hydrolysis of esters of DL-*N*-(2,6-dimethylphenyl)-alanine to the corresponding D-*N*-(2,6-dimethylphenyl)-alanine could represent an economically viable solution especially if coupled with racemization of the undesired enantiomer. Recent work based on enzymatic resolution has been recently published [18]. A “chiral pool” approach was subsequently developed based on the preparation of methyl D-*N*-(2,6-dimethylphenyl)-alaninate (10) in a *R/S* ratio of 97.5 : 2.5 from the reaction of 2,6-dimethylaniline (9) and methyl (*S*)-2-(methylsulfonyloxy)-propionate (from L-lactic acid) (11) through inversion of the chiral center (Scheme 27.2). No racemization was observed in the following steps leading to (*R*)-(1) [4c, 19].

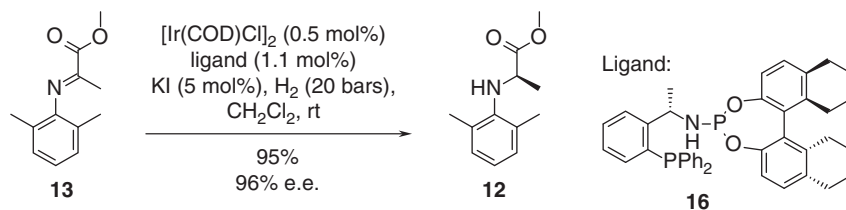


Scheme 27.2 Chiral pool synthesis of metalaxyl-M (*R*)-(1) [11].



Scheme 27.3 Enantioselective hydrogenation routes to metalaxyl-M (*R*)-(1) [17].

Two stereoselective syntheses have been extensively studied, both of which employ an enantioselective hydrogenation as key step (Scheme 27.3). Enamide (**14**) and imine (**13**) were selected as intermediates for introducing the stereogenic center. The easily prepared enamide (**14**) was subjected to catalyst screening. Out of 34 chiral Rh diphosphine catalysts tested, 12 produced enantiomeric excess (e.e.) of >90% of metalaxyl-M. Further efforts were carried out in order to improve the catalyst productivity without affecting activity and enantioselectivity, which led to the identification of the catalyst $[\text{Rh}(\text{nbd})_2]\text{BF}_4/(\text{R},\text{R})\text{-Me-DuPhos}$ (**15**) fulfilling all requirements for a technically feasible production process: 10 bar H_2 , 60 °C, substrate-to-catalyst ratio of 5×10^4 resulting in 95.6% e.e. and a turnover frequency of $5.2 \times 10^3 \text{ h}^{-1}$. The alternative route via enantioselective hydrogenation of imine (**13**) turned out not to be suitable for large-scale production: insufficient enantiomeric purity (e.e. $\leq 30\%$) and catalyst activity (turnover frequency $\leq 5 \text{ h}^{-1}$) at substrate-to-catalyst ratio of 100 were achieved with chiral iridium catalyst [17]. As a demonstration of the power of chiral catalysis, recently, a new enantioselective imine hydrogenation using an iridium catalyst with novel chiral phosphine–phosphoramidite ligand (**15**) has been published, resulting in 95.6% e.e. of (*R*)-(1) at substrate-to-catalyst ratio of 100, which represents a potentially promising hit for the optimization of the enantioselective hydrogenation of imine (**13**) (Scheme 27.4) [20].



Scheme 27.4 Enantioselective imine hydrogenation routes to metalaxyl-M (*R*)-(1) [20].

27.4

Mode of Action

The phenylamide fungicides are highly specific inhibitors of ribosomal RNA biosynthesis, specifically RNA polymerization (polymerase complex I) in the target pathogens, as suggested by studies of metalaxyl in mycelium of *Phytophthora megasperma* [21]. The exact way metalaxyl interferes with rRNA synthesis is not known yet. In *in vitro* experiments, metalaxyl did not inhibit the activity of partial purified polymerase I from *P. megasperma*. However, endogenous RNA polymerase activity of isolated nuclei of sensitive *P. megasperma* and *P. infestans* was highly sensitive to metalaxyl, indicating that metalaxyl only interferes with the intact polymerase-template complex. Endogenous nuclear RNA polymerase activity of resistant strains was significantly less sensitive to metalaxyl than that of the wild-type, suggesting that a mutation in the target site is responsible for resistance. Additional support to the hypothesis was given by the observation that (^3H)-metalaxyl binds to cell-free mycelial extract of sensitive but not resistant strains [22]. The most sensitive developmental stages of oomycetes to phenylamides are hyphal growth and the formation of haustoria and spores. This can be explained because spores contain many ribosomes to support early growth stages; hence, RNA synthesis becomes fully operational after spore germination and, as a consequence, more sensitive to inhibitors [23]. Members of the phenylamide class exhibit different levels of intrinsic activity and rRNA polymerase inhibition; however, similarities in chemical structure, identical action spectra, and the existence of cross-resistance among target fungi suggest that phenylamides have a common mechanism of action [24].

Shortly after commercialization of metalaxyl, resistant isolates were detected in several oomycetes species, such as *P. infestans*, *Plasmopara viticola*, and *Pseudoperonospora cubensis*. Despite many investigations that have been undertaken in the last 25 years, the mechanism of resistance has not been elucidated yet. It may involve one (or two) major gene(s) and potentially several minor genes; however, the target gene(s) and the site of mutation(s) in the genome have not been mapped so far [25]. Therefore, there are no molecular methods available for the detection of resistance. Although phenylamides are considered to bear a high intrinsic resistance risk [26] and have been extensively used in the past 30 years, they have failed to fully eliminate the sensitive subpopulations from nature. The proportions of resistant isolates in *P. infestans* and *P. viticola* vary from year to year and within

the season. This strongly suggests that recommended antiresistance strategies are successful and that biological processes (e.g., sexual reproduction, fitness) of the pathogens may contribute to equilibrate sensitivity in populations.

27.5

Biological Activity

Phenylamide fungicides control all diseases caused by pathogens of the orders of *Peronosporales*, *Sclerosporales*, and *Pythiales* on a wide range of crops without causing phytotoxicity. However, there are strong differences in performance against various target pathogens between the active ingredients of this class, with metalaxyl (and nowadays metalaxyl-M) displaying the most complete spectrum of activity and highest intrinsic potency. The biological profile of phenylamides is characterized by high systemicity, leading to excellent protective and curative activity against most oomycete diseases and high persistence in plant tissue allowing extended spray intervals. Rapid uptake by leaves, stems, and roots and excellent apoplastic transport lead to protection of new growth. In addition, the phenylamides control by seed-dressing application systemic seed- and soilborne diseases, which were not well controlled by chemicals before their launch to the market [23]. The biological site of action of phenylamides in the infection cycle has been studied for several airborne and soilborne oomycetes. In *P. viticola*, metalaxyl did not inhibit spore germination and initial penetration in the grape leaf, but strongly inhibited all growth stages inside the leaf after haustorium formation. This is complementary to the mode of action of protective multisite fungicides, which only affect spores and germ tubes on the leaf surface, making them privileged mixing partners in field applications [27].

27.6

Structure–Activity Relationship

The structure–activity relationship of phenylamides has been studied by Hubele *et al.* [9c] and Gozzo *et al.* [5b] using multiregression analysis (e.g., Hansch equation) to correlate biological activity to structural features of the scaffold. For the analysis, the general molecule has been divided into three segments: the acyl portion, the aryl part, and the alkyl function attached to the nitrogen atom. QSAR analysis of the acyl group reveals that biological activity is dependent on two key parameters: the lipophilicity (optimal $\log P$ calculated as 1.64) and the length of the acyl rest. In particular, activity increases with increase in the length of the linear chain up to five atoms. Moderate-to-excellent activity is retained by some vinyl analogs, while carbonyl-containing derivatives display weak activity. Interestingly, the benzyl analog (benalaxyl (**2**)) shows excellent activity, much higher than that predicted by the equation (due to a higher $\log P$). During the follow-up work that was in part promoted to explain the deviation, two different modes of action have

been found for benalaxyl. The first is common to the other member of the phenylamide class, leading to the inhibition of the mycelial growth and is dependent on the optical purity of the compound. A second mode of action, unusual for phenylamides, has been found operating on the reproductive stages of the pathogen and is nonstereospecific and independent of the strain. Substitution in the aromatic group has been analyzed, also revealing a dependence on the lipophilicity. The 2,6-disubstituted derivatives are superior to the mono- or no-substituted. Electronegativity has little influence, whereas steric factors seem to be relevant (methyl group is the best of the series). Substituents in the *meta*-position contribute mainly by their lipophilic parameters and only little by steric parameters. *para*-Substituents instead cause drastic decrease in activity: the 2,4,6-trimethyl analog of metalaxyl, with almost ideal log *P* value, is inactive. Heterocyclic replacement of the phenyl ring leads to weak or inactive compounds. Tentatively, these facts indicate that in the active molecule, the xylyl ring, besides playing a key role in inducing the appropriate conformation of the other two portions, has a critical role in binding to the receptor site. Chemical manipulations of the alanine group have been the object of intense research in various laboratories aiming at understanding the influence of size, hybridization, and electronic factors. These studies have shown, once more, that a favorable lipophilicity is necessary but not a sufficient prerequisite for high activity. One of the most active analogs identified was the (α -amino)-butyrolactone. It is possible that the good performance in *in vivo* tests is linked to the rigid and almost planar *s-cis* conformation of the cyclic lactone, suggesting that a high population of the *s-cis* versus *s-trans* ester conformation might play an important role for activity (Figure 27.2).

QSAR analysis reveals that two requirements are key for high fungicidal activity:

- Low lipophilicity
- Length of the C(O)R chain

Benalaxyl is an exception (see text)

Best:
CH₂OR, CH₂SR, CH₂Cl
cyclic ethers

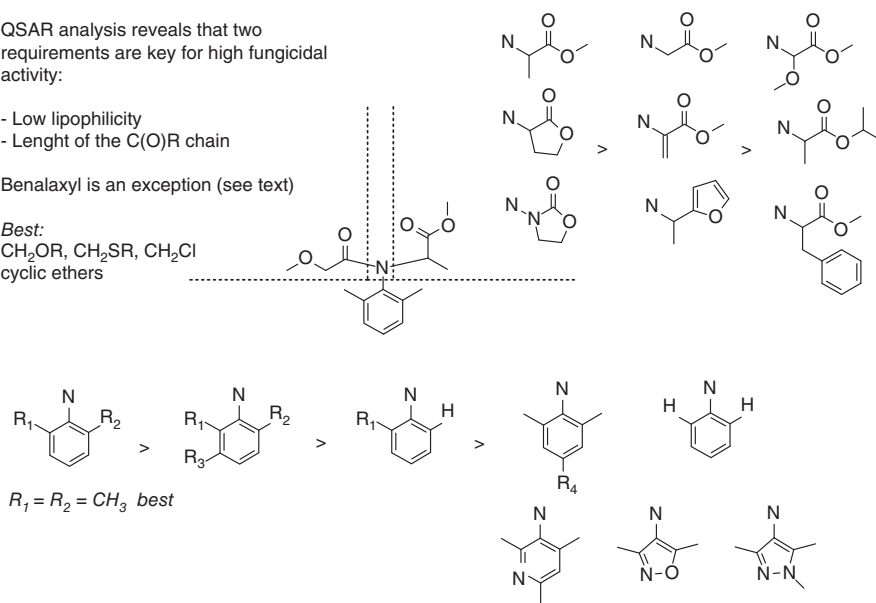
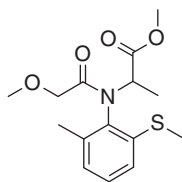


Figure 27.2 SAR of phenylamide fungicides [5b, 9c].



17

Figure 27.3 Alkylthio analog of metalaxyl [28].

The strong effect of chirality on the biological activity of the member of this class has been discussed already in this chapter. Variations of the chiral carbon were carefully considered in view of its discriminating role in the fungicidal activity. As an example, effect of the hybridization of the carbon atom was studied by preparing the corresponding enamide, which shows a weaker but significant activity. The replacement of the carbon atom with a nitrogen atom led to the discovery of a first member in series of a new highly potent subclass, culminating with the development of the isoxazolone analog oxadixyl (4). Some members of this fungicidal class containing a chiral carbon atom and a chiral axis around the aromatic carbon–nitrogen bond exist as mixtures of diastereoisomers. In the case of clozylacon, it was found that the pair of enantiomers ($\alpha R, 3S$)($\alpha S, 3R$) is twice as active as the unresolved mixture and that the single isomer ($\alpha S, 3R$) exhibits four times higher activity. Biological data *in vivo* clearly demonstrate that the single isomer ($\alpha S, 3R$) is mainly responsible for the fungicidal activity of (6) (CGA 80000) [15]. In a separate study, it was found out that the metalaxyl analog (17) (Figure 27.3) in which one of the *ortho*-methyl groups has been replaced by methylthio (and other alkylthio groups) exists as a mixture of separable diastereoisomers. Structure–activity relationship reveals that the rotamers exhibit the same disease spectrum but with very different activities, one being significantly more active against *P. infestans* and *P. viticola* [28].

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28

Complex III Inhibiting Strobilurin Esters, Amides, and Carbamates as Broad-Spectrum Fungicides

Clemens Lamberth

28.1

Introduction

The strobilurins belong undoubtedly to the most important classes of agrochemical fungicides [1–12], because in the recent years, they have been the best-selling group of fungicides as demonstrated by sales over 3.3 billion USD in the year 2013. Azoxystrobin (**1**), the world's largest-selling fungicide with sales over 1 billion USD, is responsible for a third of this sum [13]. Almost all of the so far 17 commercialized strobilurin fungicides belong to the subclasses of methoxyacrylates, such as azoxystrobin (**1**) (Figure 28.1) [14]; methoxyiminoacetates, such as kresoxim-methyl (**7**) [15]; methoxyiminoacetamides, such as orysastrobin (**9**) [16]; methoxyacetamides, such as mandestrobin (**13**) (Figure 28.2) [17]; methoxycarbamates, such as pyraclostrobin (**14**) [18]; and benzylcarbamates, such as pyribencarb (**17**) (Figure 28.3) [19]. These ester, amide, and carbamate functions, which are used for the classification of the strobilurins into different subclasses, are part of the pharmacophore system responsible for the fungicidal activity. The strobilurins are inhibitors of the fungal respiration by blocking complex III of the mitochondrial respiratory chain. This mode of action enables them to be broad-spectrum fungicides for the control of all four major classes of phytopathogenic fungi.

28.2

History

Strobilurin A (**18**) was isolated from *Strobilurus tenacellus* in 1977, a mushroom living on buried pine cones [20]. Structurally related natural products have also been isolated from other basidiomycetes, such as oudemansin A from *Oudemansiella mucida* [21]. The ability of these fungi to synthesize fungicidally active compounds gives them an advantage to compete for nutrients in their natural environment [1, 4, 5, 8, 10, 12, 22]. Although the *in vitro* fungicidal activity of strobilurin A was discovered soon, its agrobiological testing *in vivo*

Methoxyacrylate strobilurins:

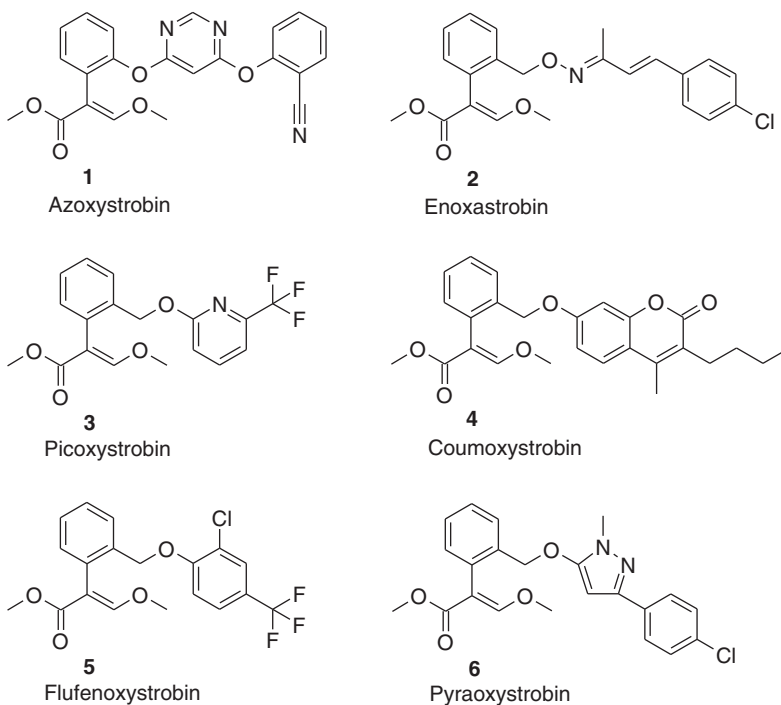
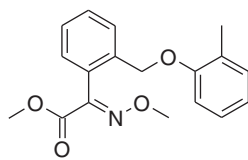


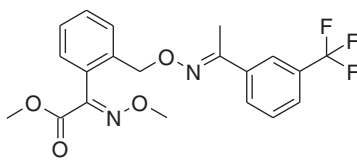
Figure 28.1 The methoxyacrylate strobilurin fungicides azoxystrobin (1), enoxastrobin (2), picoxystrobin (3), coumoxystrobin (4), flufenoxystrobin (5), and pyraoxystrobin (6).

was difficult because of its volatility and the inherent lability of the (*E,Z,E*)-triene system, which resulted in rapid photolytic or metabolic degradation [1–12]. The unusual structural simplicity of this natural product soon made it a compelling inspiration for chemical derivatization. The incorporation of the *Z*-olefinic bond of strobilurin A into a phenyl ring was a first breakthrough in attempts to prepare photostable analogs of strobilurin A, delivering the so-called enol ether stilbene (MOA-stilbene) (**19**) [1–8, 10–12]. Although highly active in greenhouse assays, **19** showed only a poor level of disease control under field conditions because of photoinstability [1, 3, 5–7, 10–12]. The replacement of the stilbene C–C double bond of MOA-stilbene by an ether bridge delivered the systemic diphenyl ether **20**, one of the first strobilurin derivatives combining good fungicidal activity with full photostability, which was an important cornerstone in the discovery approaches of scientists at both Zeneca Agrochemicals and BASF toward the first two commercialized strobilurin fungicides azoxystrobin (**1**) and kresoxim-methyl (**7**) [1, 3–12]. During the attempt of scientists at Zeneca to further improve the fungicidal efficacy, the elongation of the side chain of **20** led to the diphenoxybenzene **21**, which showed even higher activity. However,

Methoxyiminoacetate strobilurins:

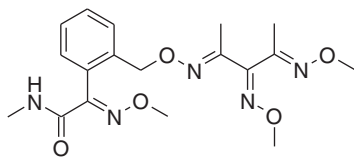


7
Kresoxim-methyl

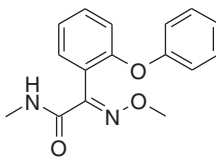


8
Trifloxystrobin

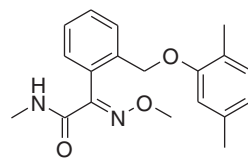
Methoxyiminoacetamide strobilurins:



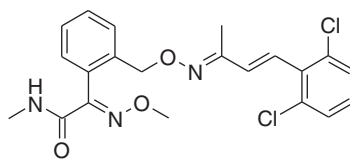
9
Orysastrobin



10
Metominostrobin

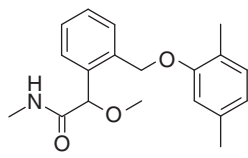


11
Dimoxystrobin



12
Fenaminstrobin

Methoxyacetamide strobilurins:



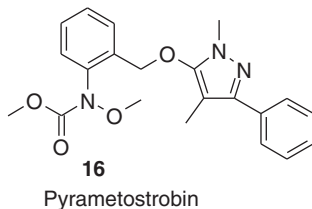
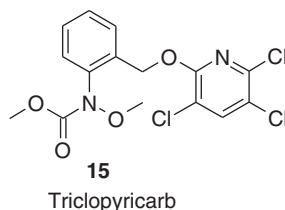
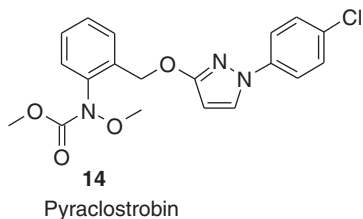
13
Mandestrobin

Figure 28.2 The methoxyiminoacetate strobilurin fungicides kresoxim-methyl (7) and trifloxystrobin (8), the methoxyiminoacetamide strobilurin fungicides orysastrobin

(9), metominostrobin (10), dimoxystrobin (11), and fenaminostrobin (12) as well as the methoxyacetamide strobilurin fungicide mandestrobin (13).

the systemic movement, which was observed for the diphenyl ether **20** was lost, because its lipophilicity prevented **21** from being redistributed effectively within the plant [1, 3–5, 7–10]. The aim to increase the systemicity of the diphenoxybenzene **21** was reached by the replacement of the central phenyl ring by a more hydrophilic pyrimidine ring [1, 3–8, 10], finally delivering azoxystrobin (**1**), today

Methoxycarbamate strobilurins:



Benzylcarbamate strobilurins:

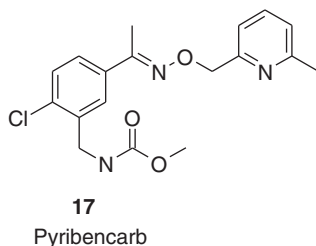
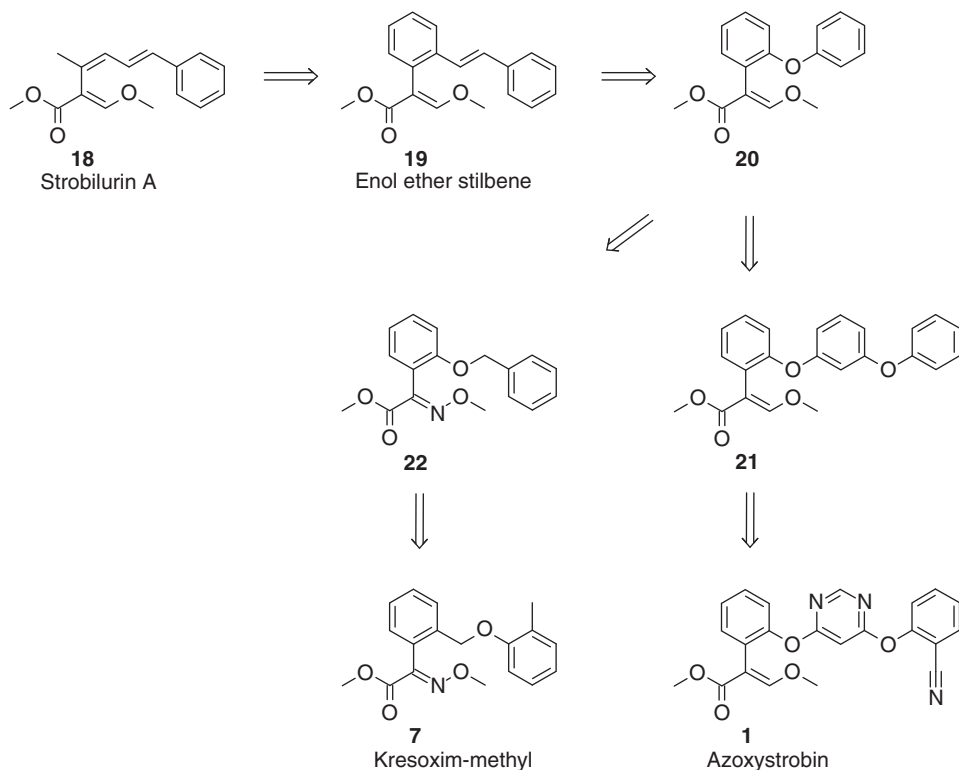


Figure 28.3 The methoxycarbamate strobilurin fungicides pyraclostrobin (**14**), triclopyricarb (**15**), and pyrametostrobin (**16**) as well as the benzylcarbamate strobilurin pyribencarb (**17**).

the world's biggest-selling fungicide with annual sales of more than 1000 million USD (Scheme 28.1). Not only the increase of fungicidal efficacy and crop safety, but especially the improvement of photostability during this invention pathway of azoxystrobin is impressive: in a simulated sunlight test, strobilurin A (**18**) is stable for 12 s, whereas azoxystrobin (**1**) has a half-life of 24 h [1, 6]! To avoid areas already protected by Zeneca, scientists at BASF began to exchange the enol ether function of the methoxyacrylate pharmacophore by an oxime ether unit. The simultaneous replacement of the phenoxy side chain by a benzyloxy group led to **22**, one of the first examples of the methoxyiminoacetate subclass. The C–O inversion of the two-atom bridge between the two phenyl rings of **22**, which turned the benzyloxy side chain into a phenoxymethyl moiety, led to a 10-fold increase in *in vitro* activity. The subsequent introduction of a methyl group delivered kresoxim-methyl (**7**) (Scheme 28.1) [4, 5]. Azoxystrobin and kresoxim-methyl, the two earliest commercialized strobilurin fungicides, both had their first registration for use on cereals in 1996 [5].



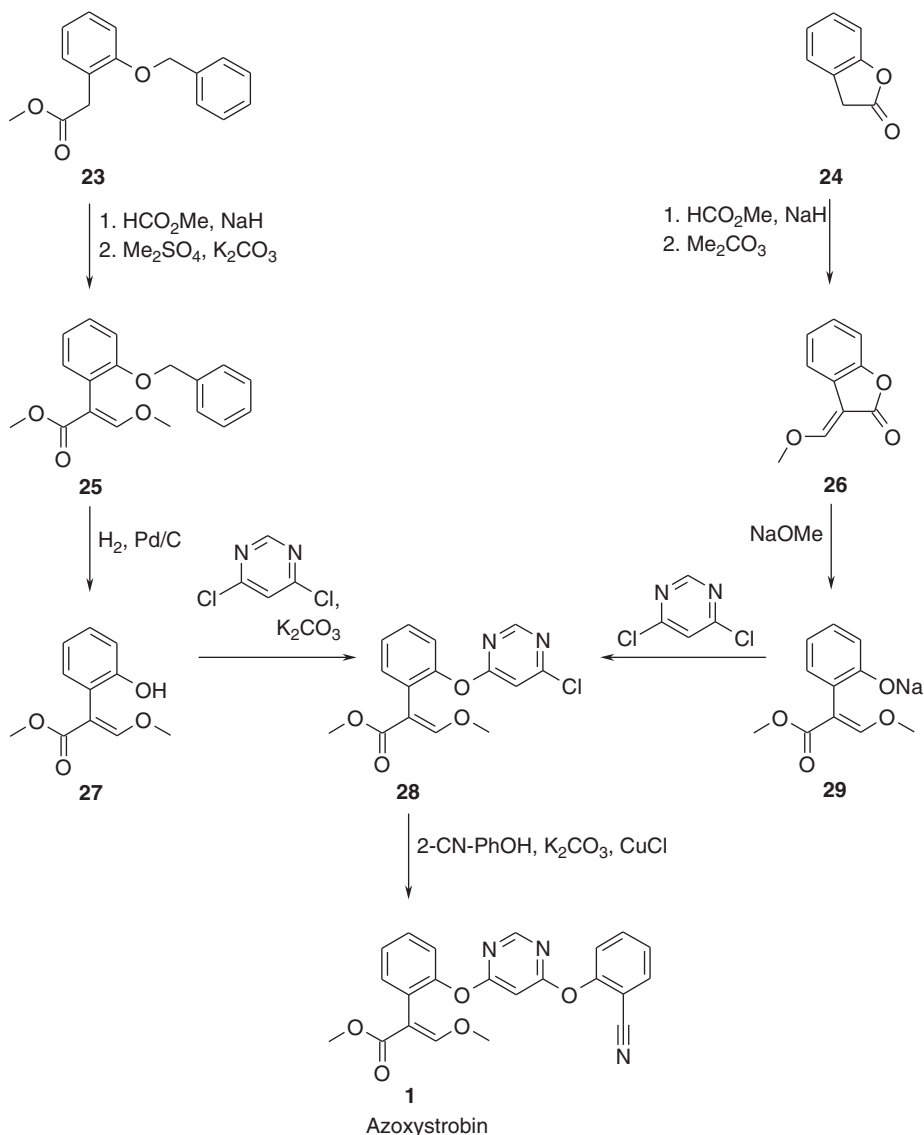
Scheme 28.1 Invention pathway from the natural product strobilurin A (**18**) to azoxystrobin (**1**) and to kresoxim-methyl (**7**) [6, 7, 10].

28.3

Synthesis

Two different possibilities for the synthesis of the methoxyacrylate derivative azoxystrobin (**1**) are demonstrated in Scheme 28.2. One synthesis starts with the formation of the pharmacophore by Claisen condensation of the phenylacetate derivative **23** with methyl formate in the presence of a base, followed by O-methylation of the resulting methyl β -hydroxyacrylate [7, 8]. This formylation is highly stereoselective, leading almost exclusively to the desired (*E*)-isomer **25**. Further steps are the debenzoylation of **25** and two consecutive chloride displacements of 4,6-dichloropyrimidine, directly leading to azoxystrobin (**1**) [7, 8].

Alternatively, phthalide (**24**) is used as starting material for the Claisen condensation instead of methyl 2-benzyloxyphenylacetate (**23**). The resulting methoxymethylene derivative **26** is then ring-opened under basic conditions to the β -methoxyacrylate **29** bearing a sodium phenolate function in the *ortho*-position of the phenyl ring. The same two chloride substitution steps, which have

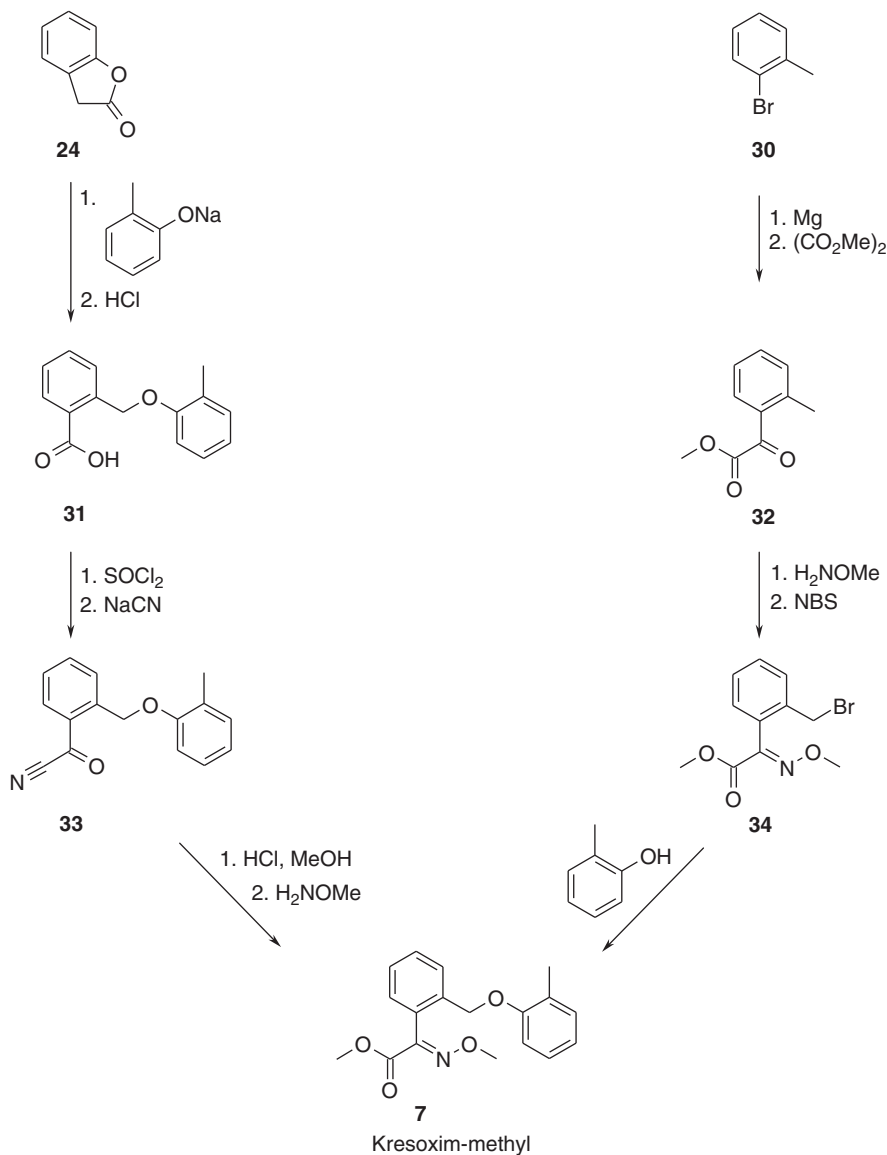


Scheme 28.2 Synthesis of the methoxyacrylate strobilurin azoxystrobin (**1**) [2, 7, 8].

been described above, are used in this alternative approach for the transformation of **29** into azoxystrobin (**1**) (Scheme 28.2) [2].

Phthalide (**24**) is also applied as starting material in the synthesis of kresoxim-methyl (**7**), as it is perfectly suited to deliver the pharmacophore and the *ortho*-connection of the side chain by ring-opening and is inexpensively available by hydrogenation of the bulk chemical phthalic anhydride. Opening of the γ -lactone ring of **24** with sodium 2-methylphenolate leads to the carboxylic acid

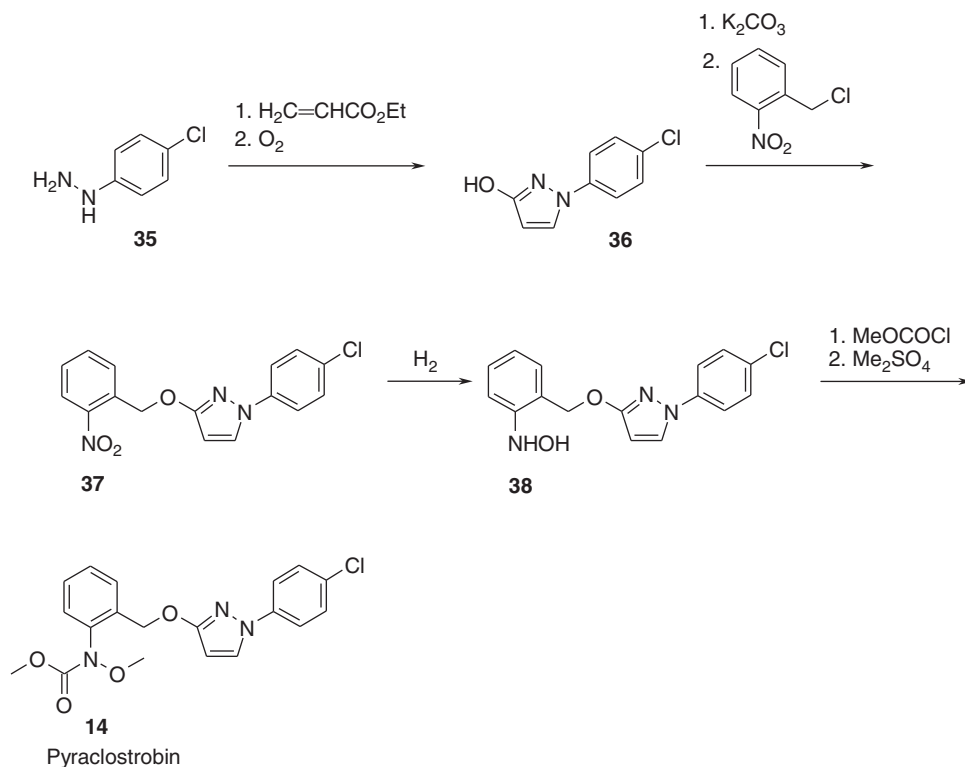
31, which is further converted via the intermediate acid chloride derivative into the α -ketonitrile **33**. The acid-mediated Pinner-type transformation of this acyl cyanide delivers the corresponding α -ketoester, which is easily converted by oximation into the methoxyiminoacetate kresoxim-methyl (**7**) (Scheme 28.3) [2]. Another possibility for the preparation of kresoxim-methyl is metalation



Scheme 28.3 Synthesis of methoxyiminoacetate strobilurin fungicide kresoxim-methyl (**7**) [2].

of 2-bromotoluene (**30**) and the subsequent transformation of the reactive intermediate with an oxalic ester derivative to the α -ketoester **32**. Oximation with O-methylhydroxylamine and benzylic bromination with *N*-bromosuccinimide deliver the versatile intermediate **34**, in which the methoxyiminoacetate pharmacophore is already installed. The introduction of the side chain by nucleophilic substitution of the bromine atom with 2-methylphenol finally leads to kresoxim-methyl (**7**).

The synthesis of pyraclostrobin (**14**) proceeds via a completely different route, as none of the phenylacetic acid and phenylglyoxylic acid derivatives described in Schemes 28.2 and 28.3 would be a suitable starting material or intermediate for this methoxycarbamate strobilurin. The synthesis approach starts instead with the addition/condensation of 4-chlorophenylhydrazine (**35**) and ethyl acrylate to a pyrazoline and its subsequent oxidation to the hydroxypyrazole **36**. Alkylation of the hydroxyl function with 2-nitrobenzylchloride leads to **37**, which is converted into **38** by reduction of the nitro group of **37** into a hydroxylamine under hydrogenation conditions. The acylation and methylation of the hydroxylamine function deliver the methoxycarbamate strobilurin pyraclostrobin (**14**) (Scheme 28.4) [2].



Scheme 28.4 Synthesis of the methoxycarbamate strobilurin fungicide pyraclostrobin (**14**) [2].

28.4

Mode of Action

The fungicidal efficacy of the strobilurins results from their ability to inhibit the mitochondrial respiration by binding to the ubiquinol-oxidation center (Q_o site) of cytochrome *b*. Cytochrome *b* is part of the cytochrome bc_1 complex (complex III), located in the inner mitochondrial membrane of fungi and other eukaryotes. The function of this enzyme is to catalyze the transfer of electrons from ubiquinol to cytochrome *c*. As this takes place, protons are translocated across the membrane in which the complex is embedded. This establishes a proton gradient, which drives the synthesis of ATP. In the presence of a strobilurin, ubiquinol is still able to bind to cytochrome *b*, but it is not oxidized anymore. As a result, the electron transfer between cytochrome *b* and cytochrome c_1 is blocked, which in turn disrupts the energy cycle within the fungus by stopping the oxidative phosphorylation and therefore the production of ATP [3, 7, 10]. This mode of action, which is shared by all strobilurin fungicides, has been specifically proven for azoxystrobin (**1**) [23], kresoxim-methyl (**7**) [24], and metominostrobin (**10**) [25].

Further studies have confirmed that the strobilurins bind at a specific site of cytochrome *b*, which is located on the outer side of the mitochondrial membrane and is therefore called Q_o -center (o standing for outside). Since the strobilurins can displace one another from this Q_o site, it is clear that they are reversibly bound. Famoxadone and fenamidone, two commercial fungicides which are chemically distinct from the strobilurins and belong to the family of azolones, also bind to the Q_o site of cytochrome *b* and therefore are cross-resistant with most of the strobilurins.

Already at the start of the synthesis of the first synthetic strobilurin analogs by derivatization of the natural products, it was assumed that the β -methoxyacrylate group must be the pharmacophoric function, responsible for the activity and binding strongly to the active site, as it is the only commonality of the fungicidally active natural product families of strobilurins, oudemansins, and myxothiazols [4, 7, 11, 12]. Later, an intensive structure–activity relationship study with dozens of different variations of the methoxyacrylate, methoxyiminoacetate, methoxyiminoacetamide, and methoxycarbamate pharmacophores enabled a realistic picture of the molecular requirements of the inhibitor–target interactions with the conclusion that only the carbonyl group of the ester, amide, and carbamate functions is retained in all active derivatives [2, 4, 6]. Of course, the hydrogen bond acceptor role of its oxygen atom was speculated in the beginning to be essential for the activity, but finally, X-ray data of several cocrystallized strobilurin/ bc_1 complexes confirmed that an N–H proton of Glu272 (yeast numbering) is the hydrogen donor for the carbonyl group in the strobilurin pharmacophore (Figure 28.4) [2].

The mitochondrial bc_1 complex, the molecular target of the strobilurins, is common to many eukaryotic species. This is reflected by the fact that, depending on the derivative, fungicidal, phytotoxic, insecticidal, or cytotoxic activities have been observed. Although mitochondrial preparations of rat liver and beef heart have been sensitive to strobilurins, such fungicides are surprisingly not toxic to

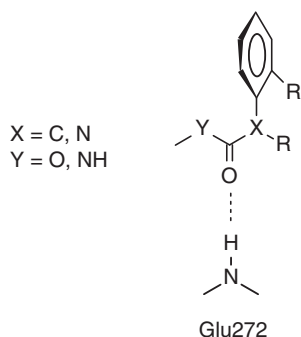


Figure 28.4 Model of the strobilurin target binding via hydrogen bridge between Glu272 and the pharmacophore carbonyl group [2].

rodents and other mammals (acute oral toxicity of azoxystrobin (**1**), picoxystrobin (**3**), kresoxim-methyl (**7**), trifloxystrobin (**8**), and pyraclostrobin (**14**) in rats: $LD_{50} > 5000$ mg/kg) [3, 5, 6]. This selective toxicity could be linked to the fact that either especially energy- and hence respiration-dependent processes such as the fungal spore germination are vulnerable to strobilurins [4] or strobilurins are degraded in mammals, for example, by an esterase, before they reach their target [22].

28.5

Biological Activity

The strobilurins are in general broad-spectrum fungicides with activity against all four important classes of eumycotic plant pathogenic fungi: the Ascomycetes (e.g., powdery mildews), Basidiomycetes (e.g., rusts), Deuteromycetes (e.g., leaf spots), and Oomycetes (e.g., downy mildews) [2, 3, 7]. Some of them, such as the biggest-selling strobilurin azoxystrobin (**1**), have protectant, systemic, translaminar, and eradicant properties, enabling the potential for use as a foliar, paddy water, seed or soil treatment [7]. Azoxystrobin is registered in more than 100 different crops in almost as many countries [3]! This broad activity led to the simplification of disease control programs, for example, in grapes, strobilurins simultaneously control downy (*Plasmopara viticola*) and powdery mildews (*Uncinula necator*) [3, 5, 6]; in rice, they are applied against blast (*Magnaporthe grisea*) and sheath blight (*Rhizoctonia solani*) [5–8]. Based on the mode of action, spore germination and zoospore motility as two of the most energy-demanding stages of fungal development are particularly sensitive to strobilurin treatment [3]. Therefore, strobilurins are most effectively used preventatively, which means prior to infection or in the early stages of the disease development [3, 5]. But several strobilurin fungicides, such as azoxystrobin (**1**), picoxystrobin (**3**), kresoxim-methyl (**7**), trifloxystrobin (**8**), and pyraclostrobin (**14**), also demonstrated strong curative activity against a range of phytopathogens, which means that control of the disease after the infection has occurred but before the symptoms are visible [3]. Some strobilurins seem to be specialized on a limited

range of pathogens, such as orysastrobin (**9**) and metominostrobin (**10**) from the group of methoxyiminoacetamides, which focus on rice diseases.

The surprisingly rapid development of strobilurin resistance in some cereal pathogens, such as *Blumeria graminis* (powdery mildew) and *Mycosphaerella graminicola* (leaf blotch), has limited opportunities in this key segment. Another pathogen, which is meanwhile affected by strobilurin resistance, is *P. viticola* (grape downy mildew). These resistance issues are mainly caused by the mutational exchange of glycine by alanine at amino acid position 143 (G143A) in the active site of cytochrome *b*. However, such losses have been compensated by the outstanding efficacy against *Phakopsora pachyrhizi* (Asian soybean rust), a fungal disease of enormous economic importance, which had been more or less irrelevant at the time the first strobilurins have been developed [2].

A significant yield enhancement can be observed in strobilurin-sprayed wheat and barley fields, if compared to similar treatments with other fungicides, for example, triazoles, giving the same level of visible disease control. This is due to the pronounced “greening effect” and delayed senescence caused by strobilurins. The increased chlorophyll content and a higher photosynthesis index enable the plant to maintain green leaf area until late in the season, thereby maximizing the grain-filling period with resultant yield benefits [2, 3].

28.6

Structure–Activity Relationship

The characteristic feature of all strobilurins with excellent fungicidal activity is an (*E*)-methyl β -methoxyacrylate pharmacophore, or its bioisosteric equivalent, attached to a phenyl ring A with a side chain in an adjacent (*ortho*-)position and usually no further phenyl substituent [5]. The scope for variation of the side chain, while retaining the fungicidal activity, has proven to be very large, including aryloxy or heteroaryloxy side chains, as in azoxystrobin (**1**) or metominostrobin (**10**); aryloxymethyl or heteroaryloxymethyl side chains, as in kresoxim-methyl (**7**) or pyraclostrobin (**14**); and ketoximinomethyl side chains, as in trifloxystrobin (**8**) and orysastrobin (**9**) (Figure 28.5) [3].

As already demonstrated by the commercialized strobilurin fungicides **1–16**, the side chain is always in the *ortho*-position of the pharmacophoric group. If it is connected to the *meta*- or *para*-position of phenyl ring A, then the fungicidal

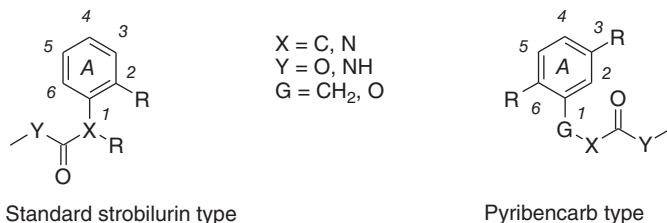


Figure 28.5 The general structural requirements of strobilurin fungicides.

activity drops dramatically [6, 11, 12]. As an exception to this rule, good activity with side chains in the *meta*-position of the toxophore is only achieved, if the pharmacophore is not directly linked to the ring A, but via a one-atom spacer, such as in pyribencarb (17) and in 39 [4, 5] or if the *meta*-side chain is accompanied by an additional small phenyl substituent next to the pharmacophore, such as the methyl group in 40 (Figures 28.5 and 28.6) [26].

Also, strobilurins are known, in which either the whole pharmacophore or a part of it has been cyclized to a heterocyclic ring. The seed treatment agent fluoxastrobin (41) possesses an oximinoacetate-like pharmacophore, in which the ester function is incorporated into a dihydrodioxazine ring, its side chain is closely related to the one of azoxystrobin (1) [27]. The former developmental product DPX KZ165 (42) combines the same side chain as trifloxystrobin (8) with a unique triazolinone pharmacophore (Figure 28.7) [2].

Fluacrypyrim (43) is the only β -methoxyacrylate strobilurin, which has been developed and commercialized as an acaricide for use against spider mites (Figure 28.8) [2].

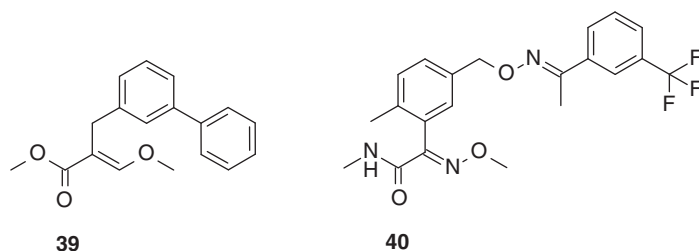


Figure 28.6 Strobilurins with the side chain in the *meta*-position of the pharmacophore.

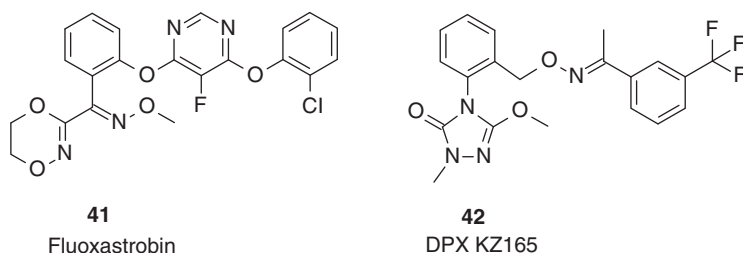


Figure 28.7 Fluoxastrobin (41) and DPX KZ165 (42), two strobilurins with a cyclic pharmacophore.

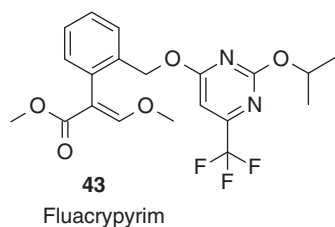


Figure 28.8 The strobilurin acaricide fluacrypyrim (43).

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29

Scytalone-Dehydratase-Inhibiting Carboxamides for the Control of Rice Blast

Andrew E. Taggi

29.1

Introduction

The scytalone-dehydratase-inhibiting carboxamides are a relatively small class of fungicides that act on a vital step in the fungal melanin biosynthesis pathway. While they have a physiological effect on a broad range of fungi, they are only effective at controlling rice blast (*Magnaporthe oryzae*¹⁾) and some pathogens from the genus *Colletotrichum*. The group, also known as melanin biosynthesis inhibitor–dehydratase (MBI-D) is comprised of the commercial agrochemical fungicides carpropamid (**1**), diclocymet (**2**), and fenoxanil (**3**), which share a common amide motif, crucial for binding to the active site in scytalone dehydratase (Figure 29.1).

29.2

History

The story of the discovery of the scytalone-dehydratase-inhibiting fungicides is interesting in that there were no fewer than five companies working concurrently, from different starting points, to ultimately find extremely potent inhibitors. Arguably, the first MBI-D inhibitor in the literature was **4** from Sankyo [1], which was reported to control *M. oryzae* in a Japanese patent filed in 1977 (Figure 29.2). It is unclear if at the time they were aware that this compound inhibited MBI-D, since it is also active at complex I of the mitochondrial electron transport system, which broadens the spectrum of activity and complicates the identification of the mode of action.

The discovery of carpropamid (**1**), the first commercial representative of this class, began at Nihon Bayer Agrochem in the early 1980s with competitive scouting around a 1972 patent from Shell describing WL 28325 (**5**) as a rice

1) The name *Magnaporthe oryzae* is used interchangeably with *Pyricularia oryzae* and *Magnaporthe grisea*.

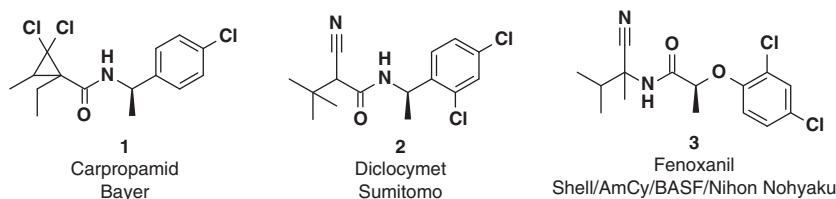


Figure 29.1 The commercial scytalone dehydratase inhibitors (MBI-D).

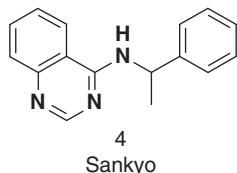
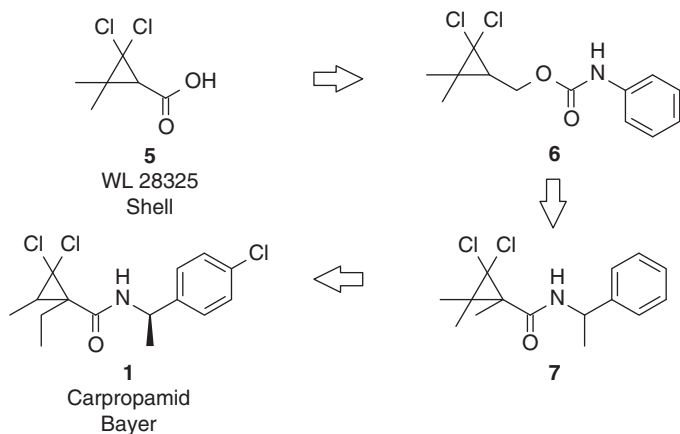


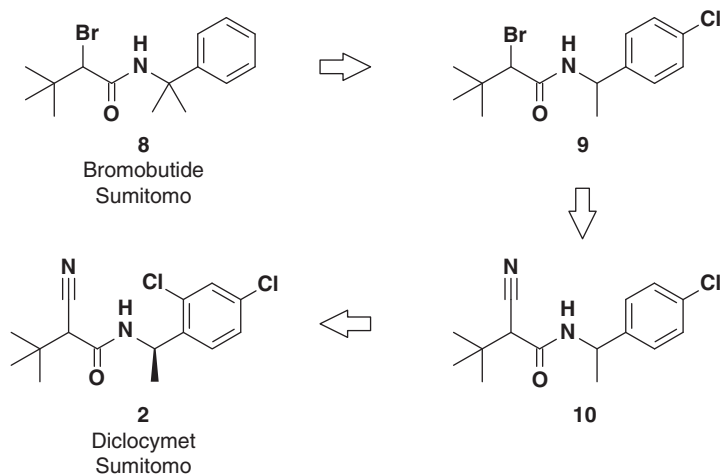
Figure 29.2 An amino-quinazoline melanin biosynthesis inhibitor from Sankyo.

blasticide [2], which was found to induce the production of phytoalexins in rice (Scheme 29.1) [3]. Optimization of the substituted cyclopropyl ring, followed by elaboration to carbamates and α -methyl-benzylamides, ultimately resulted in carpropamid [4, 5], which had field activity superior to the starting compound 5, as well as a different mode of action. Carpropamid launched in the Japanese rice market in 1997.



Scheme 29.1 The discovery of carpropamid (1).

The second entry into this mode of action was diclocymet (2), which was discovered while Sumitomo was investigating reports that their herbicide bromobutide (8), and close derivatives, exhibited moderate control of rice blast (Scheme 29.2) [6–8]. While 9 had foliar rice blast activity, it lacked systemic movement, which is a key attribute for the rice nursery box market. An optimization program was undertaken to improve the physical properties of the molecule,

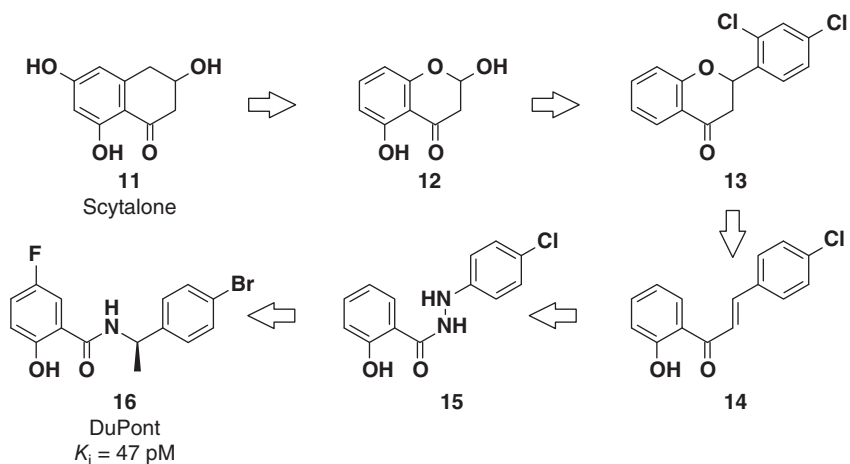


Scheme 29.2 The discovery of diclocymet (**2**) from the herbicide bromobutide (**8**).

resulting in the α -cyano amide **10**. Unfortunately, **10** retained some phytotoxicity from its bromobutide lineage, so further exploration was necessary to optimize the phenyl substitution into what would ultimately become diclocymet. Similarly to carpropamid, it was determined that the *R*-configuration of the α -methyl benzyl amine was ideal. While the *S*-enantiomer at the cyano-bearing center was the most potent, due to the ease of racemization at this center, it was decided that the commercial compound would be racemic at this position.

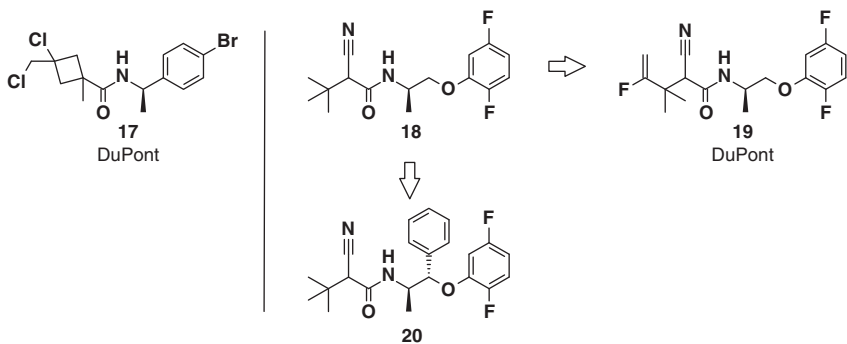
While fenoxanil was initially discovered at Shell, due to industry consolidation, it passed through American Cyanamid and was ultimately codeveloped by BASF and Nihon Nohyaku. Although the launch of fenoxanil in 2001 was 1 year after that of diclocymet, the research program at Shell actually began earlier than Sumitomo's [9]. The literature is sparse regarding the genesis of the fenoxanil program, but based on patent priority dates, even though they share structural similarities, it is unlikely that either compound was discovered through competitive scouting of the other.

Somewhat ironically, although they never commercialized an MBI-D, DuPont may have been the only company to specifically target scytalone dehydratase from the outset. DuPont's work began in 1985 with a desire to make scytalone (**11**) mimics as potential active-site inhibitors (Scheme 29.3). Work started with hydroxyl- and phenyl-substituted chromanones **12** (which were later reported to inhibit human melanin biosynthesis [10]) and **13** and then moved to acyclic analogs **14** and **15**, which showed more promise. The hydrazide **15** was followed by the benzyl amide, and then further optimized into **16**, which had a K_i of 47 pM on scytalone dehydratase. Unfortunately, **16** was not as potent in whole plant assays, as the phenol serves as an excellent handle for detoxification [11]. The program then paused for several years, until 1991, when work was initiated to cocrystallize **16** in the active site of scytalone dehydratase. Using insights from



Scheme 29.3 Scytalone mimics from DuPont.

this and other crystal structures [12], plus learnings about whole plant activity from the soon-to-be-commercial competitive compounds, DuPont revisited the optimization of the MBI-Ds (Scheme 29.4). One avenue of exploration involved the reoptimization of carpropamid using combinatorial chemistry to aid the project. After screening many different acid head groups coupled with amines from various known MBI-D inhibitors, halocyclobutanes such as **17** were identified as leading candidates with laboratory data rivaling carpropamid [13]. Turning to the other side of the molecule, DuPont explored elongation of the common α -methyl-benzylamine motif into a 1-methyl-2-phenoxy-ethylamine as illustrated in **18** [11]. This tactic was successfully employed using both carpropamid and diclocymet as the starting scaffold. Further optimization of the *tert*-butyl terminus into the 1-fluoro-ethene **19** served to boost the greenhouse foliar activity to more than 15 $\times$ that of carpropamid [14]. It was also found that **18** could be elaborated to include a norephedrine moiety as in compound **20**

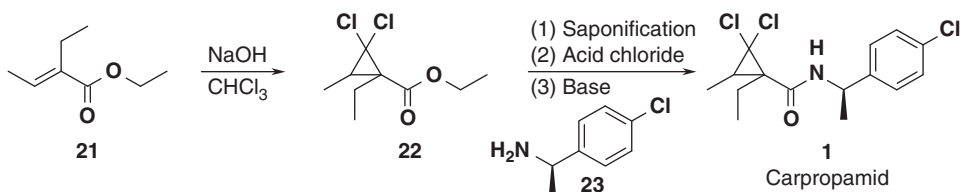


Scheme 29.4 Reoptimized MBI-Ds from DuPont.

[15]. Even though **19** was far superior in the greenhouse, it ultimately proved to be approximately equal to carpropamid in field trials.

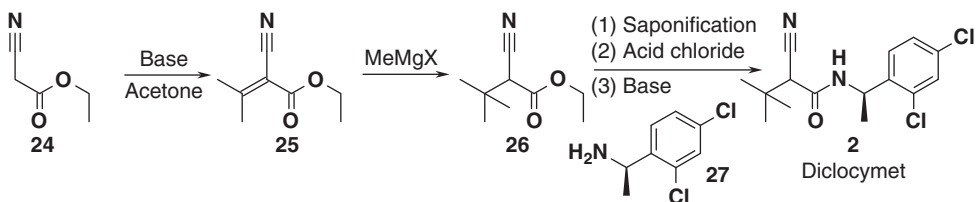
29.3 Synthesis

The synthesis of carpropamid starts with the butenoate ester **21**, which is converted to the dichlorocyclopropane **22** using hydroxide base and chloroform. The ester is then saponified and the resulting acid converted into the acid chloride. Amide coupling with (*1R*)-1-(4-chlorophenyl)ethanamine (**23**) in the presence of a base results in carpropamid (**1**) (Scheme 29.5) [5, 16].



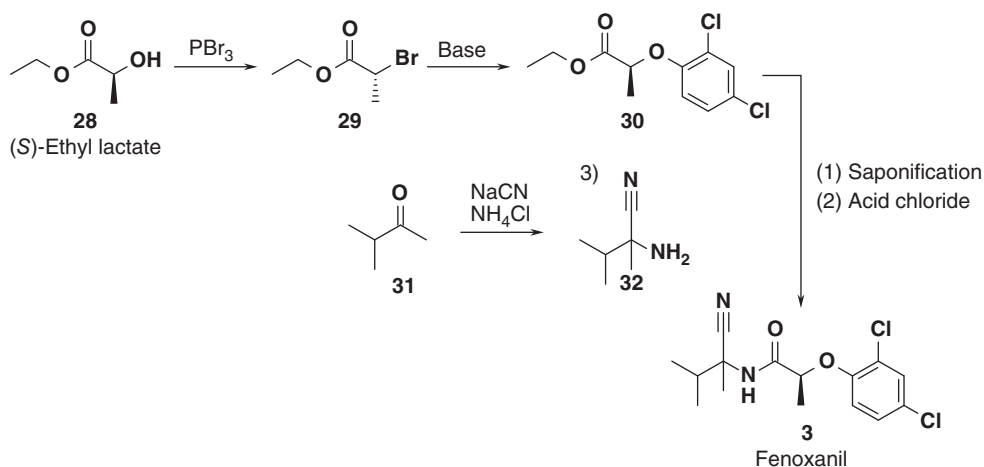
Scheme 29.5 The synthesis of carpropamid (**1**).

Not surprisingly, diclocymet follows a similar synthetic scheme, which starts with ethyl cyanoacetate (**24**) being condensed with acetone resulting in **25**. Treatment with a methyl Grignard reagent installs the *tert*-butyl group adjacent to the cyano as in **26** [17]. The carboxylic acid is then saponified and coupled with the benzyl amine (**27**), resulting in desired product (**2**) (Scheme 29.6).



Scheme 29.6 The synthesis of diclocymet (**2**).

The synthesis of fenoxanil begins with (*S*)-ethyl lactate (**28**), which is converted into the bromide with phosphorus tribromide. 2,4-Dichlorophenol is then used to displace the bromine on **29**, with stereochemical inversion, resulting in ester **30**. After saponification and acid chloride formation, the amide is formed with 2-amino-2,3-dimethyl-butanenitrile (**32**), which was prepared from isopropyl-methyl-ketone (**31**) via a Strecker reaction [18], resulting in fenoxanil (**3**) (Scheme 29.7) [19].



Scheme 29.7 The synthesis of fenoxanil (3).

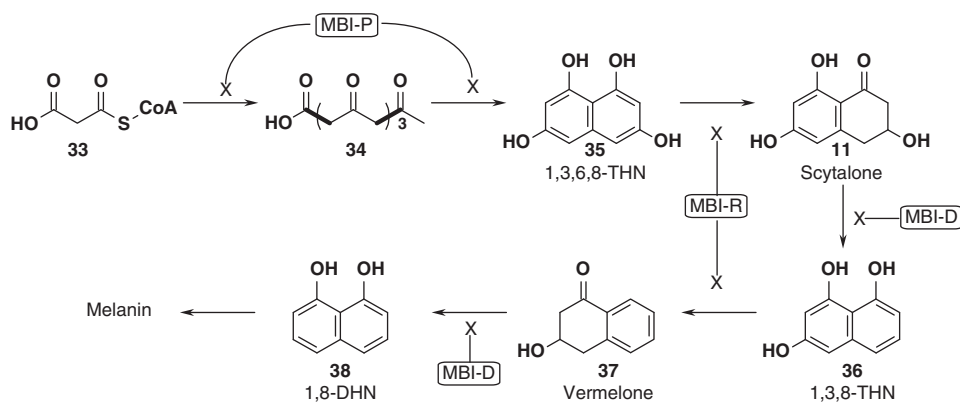
29.4

Mode of Action

From the initial biological evaluation of carpropamid (1), it was evident that the mode of action was different from that of the starting point 5 [3]. When 1 was tested on a broad range of plant pathogens including *M. oryzae*, it was found that *Phytophthora nicotianae* var. *parasitica* was the only species that showed even a slight diminishment in mycelia growth, although this may have been due to crossover into a different mode of action [20]. It was noted, however, that many pathogens exhibited a color change that suggested an absence of melanin [5]. Spore germination studies on *M. oryzae* found normal growth of the appressoria, but they were transparent and unable to penetrate into rice epidermal cells. All of these signs were consistent with melanin biosynthesis inhibition.

In order to determine the site of action in the melanin biosynthesis pathway, a liquid culture of *M. oryzae* was treated with carpropamid and the intermediates from melanin biosynthesis were isolated and characterized to reveal that scytalone (11) was accumulating in the carpropamid-treated broth. This indicated that carpropamid was inhibiting the conversion of scytalone to 1,3,8-trihydroxynaphthalene (36, Scheme 29.8). Furthermore, reversion studies showed that carpropamid also blocks the dehydration of vermeline (37) to 1,8-dihydroxynaphthalene (38) [21]. This demonstrated that the site(s) of action are distinct from that of the previously known melanin biosynthesis reductase (MBI-R) fungicides (tricyclazole, pyroquilon, and phthalide).

Recently, tolprocarb (40), which shows structural similarity with the known MBI-D fungicides, as well as the cellulose-synthase-inhibiting carboxylic acid amide (CAA) fungicides, such as iprovalicarb (39), was found to inhibit the polyketide synthase steps in the melanin biosynthesis pathway [22] (Figure 29.3).



Scheme 29.8 The melanin biosynthetic pathway with the steps inhibited by the three classes of MBIs noted.

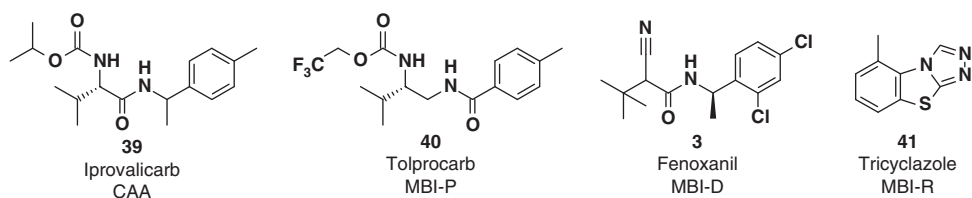


Figure 29.3 Tolprocarb (40) and its structural similarity to both MBI-D and CAA chemistry.

It is worth noting that the MBI-D, MBI-R, and MBI-P compounds do not show cross-resistance with each other.

29.5

Biological Activity

The scytalone-dehydratase-inhibiting carboxamides selectively control fungi, which are dependent on melanization during the infection process: mainly pathogens in the *Magnaporthe* genus as well as some *Colletotrichum* species [23]. In the case of these pathogens, after the spores germinate and produce germ tubes, melanin is used to reinforce the appressorial cell wall. There is subsequently a buildup of hydrostatic pressure in the rigidified appressorium, which allows the pathogen to mechanically force its way through the epidermis of the host. Since the MBI-Ds disrupt the infection process, and, in fact do not kill the pathogen, they are only active preventatively and not after the rice blast infection has entered into the plant. The commercial compounds are all active through foliar application, as well as through a systemic root uptake application or during seed sowing [24], which make them a perfect fit with nursery box rice cultivation methods. On the enzyme level, the MBI-Ds are extremely potent, with K_i values routinely reaching the 10–40 pM range. Only compounds that have been

optimized for active-site potency, as well as excellent systemic movement, excel under field conditions. Within 3 years of introduction into the Japanese market in 1998, field resistance to the MBI-Ds was found due to the replacement of a valine with a methionine in the active site (V75M). In the laboratory, this mutation results in an approximately 20-fold decrease in potency for carpropamid [25]. The rapid appearance of resistance, coupled with the limited disease spectrum, is likely responsible for the limited commercial success of the MBI-D fungicides.

29.6

Structure–Activity Relationships

While there is moderate structural diversity at the termini of the MBI-Ds, the central portions of the molecules are fairly well conserved, with a carboxamide linker adjacent to a chiral-methyl-bearing carbon. The amide carbonyl and NH each provide critical hydrogen-bonding interactions by bridging to the active site via two bound water molecules. Directly to the right of the amide is always a methyl in the *R*-configuration. In all cases, the alternate enantiomer is inactive. The portion of the binding site accommodating the phenyl ring is fairly large, allowing for the ring to be connected to the chiral methyl with 0, 1, or 2 atom linkers as exemplified by fenoxanil (3) and **18–19**. The linker can also tolerate a substituent of significant size, as in **20**. The opposite end of the molecule often has multiple tertiary and quaternary centers adorned with halogens or a cyano. It is postulated that the cyano and chlorines participate in favorable electrostatic interactions with an active-site asparagine (L147) [12c]. While all of the commercial compounds are sold as the *R*-enantiomer at the center bearing the methyl, they are not enantiopure at the chiral centers at the opposite end of the molecule. Studies showed that the chirality at these centers was important [8, 26], but due to the cost of production, as well as some of the centers bearing easily racemizable acidic protons, it was decided to keep this portion of the molecules in racemic form.

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30

Carboxylic Acid Amide Fungicides for the Control of Downy Mildew Diseases

Clemens Lamberth

30.1

Introduction

The carboxylic acid amide fungicides, also called CAA fungicides, are a relatively young family of agrochemical active ingredients, which specifically control foliar Oomycetes diseases, such as downy mildew and late blight by inhibition of *Cel-lulose synthase* [1]. This group of fungicides consists of three different subclasses, the cinnamic acid amides dimethomorph (1) [2], flumorph (2) [3], and pyrimorph (3) [4], the valinamides iprovalicarb (4) [5], bentiavalicarb (5) [6], and valifenalate (6) [7] as well as the mandelamide mandipropamid (7) [8] (Figure 30.1). Although the active ingredients across these three subclasses possess completely different chemical scaffolds and their only commonality is the central amide linkage, they all share not only the same target enzyme but also the same binding site, which causes a general cross-resistance pattern among them (FRAC group 40).

30.2

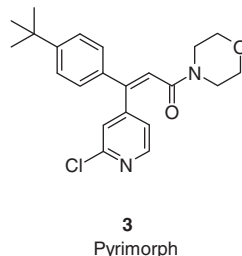
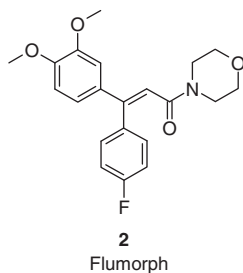
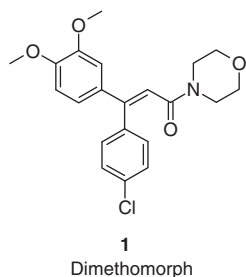
History

The specific fungicidal activity of dimethomorph (1) against Oomycetes diseases was discovered during the 1980s by scientists at Celamerck. It has been the first CAA fungicide, which reached the market in 1988. The two other cinnamic acid amides followed much later, flumorph (2) was introduced by Shenyang in 2000, pyrimorph (3) by China Agricultural University in 2013 [1].

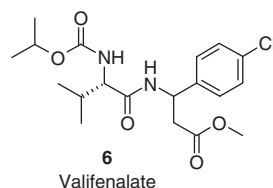
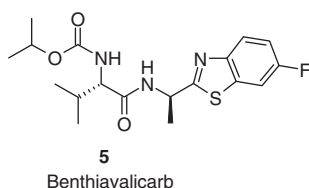
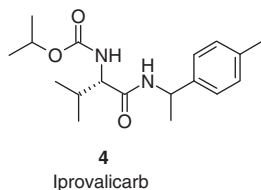
Iprovalicarb (4), the first example of the group of valinamides, was discovered at Bayer, as a result of a combinatorial chemistry program searching for new fungicidal lead structures. It was presented to the public in 1998, several years earlier than the other two commercialized valinamides, because bentiavalicarb (5) was launched by Kumiai in 2003 and valifenalate (6) by Isagro in 2008 [1].

Mandipropamid (7), the only commercialized representative of the subclass of mandelamides, was introduced to the market by Syngenta in 2005. The invention pathway of mandipropamid is shown in Scheme 30.1. *N*-Carbamoylvalinamides

Cinnamic acid amides:



Valinamides:



Mandelamides:

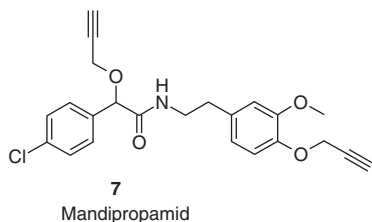
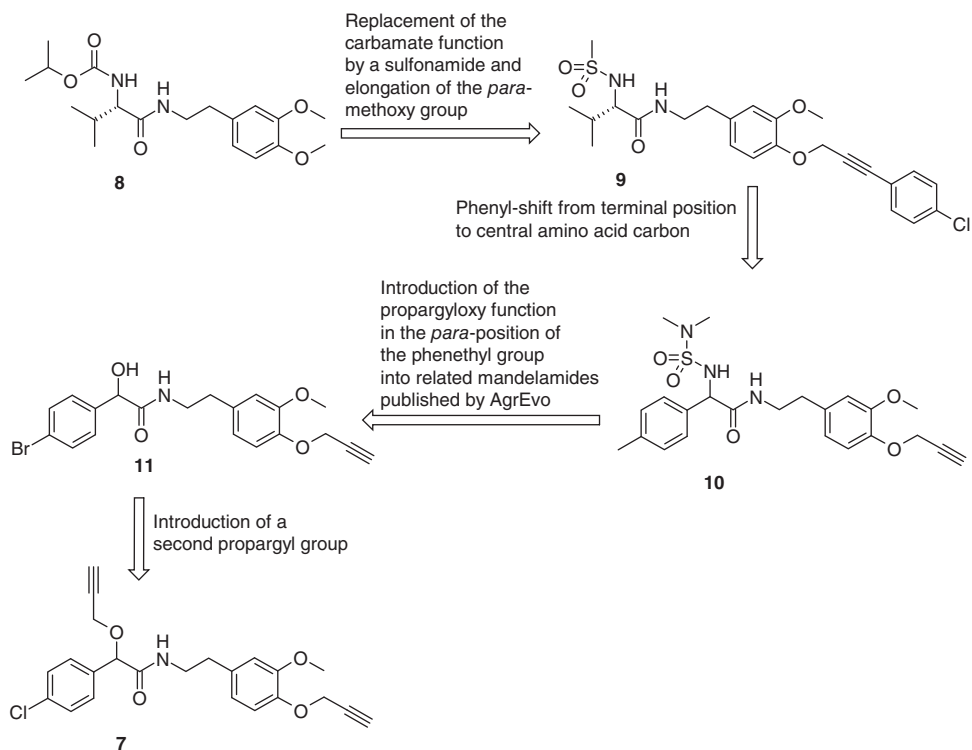


Figure 30.1 The seven commercialized carboxylic acid amide fungicides [1].

such as **8**, which had been patented by American Cyanamid in 1992 and which carry in contrast to iprovalicarb's phenyl-1-ethylamine a phenyl-2-ethylamine moiety [9], attracted the attention of researchers at Syngenta in the 1990s. For reasons related to patentability and because of some in-house experience with *N*-sulfonylated amino acids, they decided to replace the carbamate by a sulfonamide. This manipulation did indeed lead to increased fungicidal activity, but only in combination with the elongation of the methoxy function in the *para*-position of the phenethylamine to a phenylpropargyloxy group, delivering **9** [10]. During further optimization of such *N*-sulfonylvalinamides, it was found that even better biological activity could be achieved, when the phenyl ring was shifted from the terminus of the side chain to the α -carbon atom of the amino acid, turning the valinamide **9** into a phenylglycinamide **10** [8, 10]. However, these *N*-sulfonylphenylglycinamides were still not on the level of commercial fungicides; therefore, the Syngenta research group was looking for further opportunities



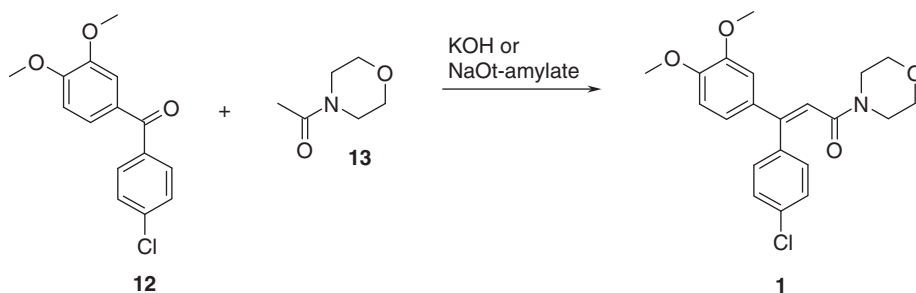
Scheme 30.1 Invention pathway of mandipropamid (**7**) [8].

to enhance the activity. At that time, they became aware of structurally related mandelamides, which carried exactly the same 3,4-dimethoxyphenethylamine than the original lead **8** from American Cyanamid and which had been published by AgrEvo [11]. The Syngenta team had been convinced that at least part of the interesting anti-oomycetes activity of the phenylglycinamide **10** was owing to the propargyloxy function in position 4 of the phenethylamine moiety. Therefore, they prepared the mandelamide **11** as close analog of the AgrEvo compounds, just having a methoxy group replaced by propargyloxy. Its activity was even better than that of the phenylglycinamide **10** but could be even more improved by the introduction of a second propargyl group into the mandelic acid moiety of the molecule, delivering mandipropamid (**7**) [8].

30.3

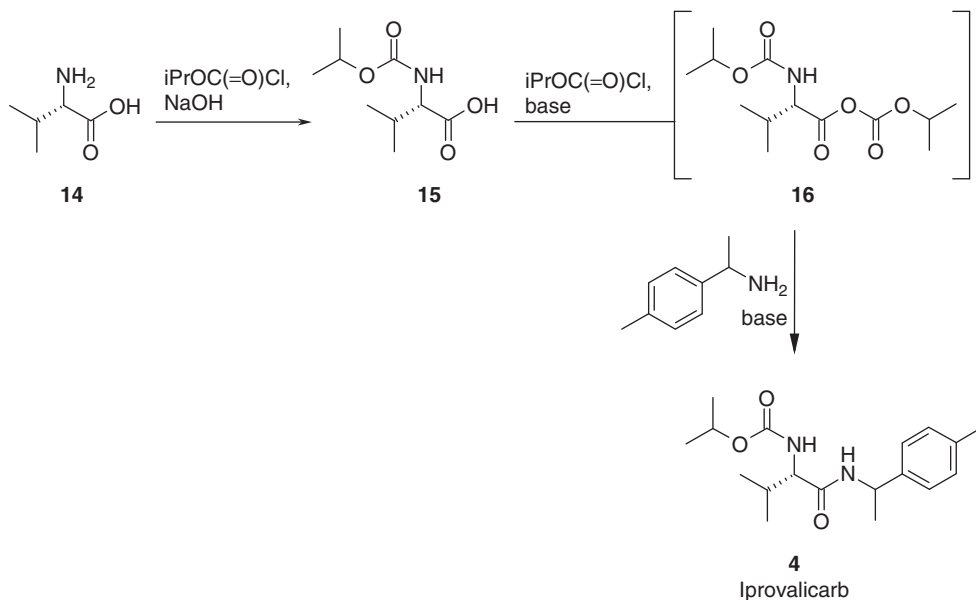
Synthesis

A concise synthesis of the cinnamic acid amide dimethomorph (**1**) is achieved by an aldol condensation of 4-chloro-3',4'-dimethoxybenzophenone (**12**) and *N*-acetylmorpholine (**13**) with the aid of either potassium hydroxide or sodium *tert*-amylate (Scheme 30.2) [1].



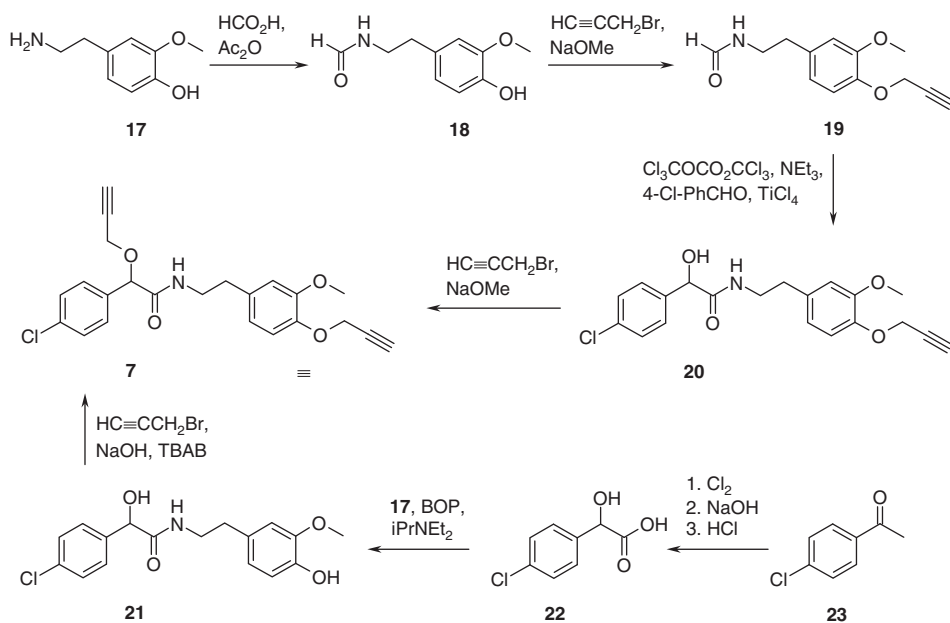
Scheme 30.2 Synthesis of the cinnamic acid amide dimethomorph (**1**) [1].

The synthesis of the valinamide iprovalicarb (**4**) starts with the transformation of L-valine (**14**) with isopropyl chloroformate in aqueous sodium hydroxide solution to give the carbamate **15**. Its carboxylic acid function is activated by treatment with a second equivalent of isopropyl chloroformate, delivering the mixed anhydride **16**, which cannot be isolated. The conversion of this reactive intermediate with *para*-methylphenylethyl-1-amine, which is obtained in only one step from 4-methylacetophenone by reductive amination, directly delivers iprovalicarb (**4**) under elimination of carbon dioxide and isopropanol (Scheme 30.3) [1].



Scheme 30.3 Synthesis of the valinamide iprovalicarb (**4**) [1].

There are several possibilities for the synthesis of mandipropamid (**7**), two of which are shown in Scheme 30.4. An important building block for both



Scheme 30.4 Synthesis of the mandelamide mandipropamid (7) [1, 8, 12].

approaches is 3-*O*-methyldopamine (17), which is available in two steps from vanillin [1, 10]. This special phenethylamine can be converted by standard *N*-formylation and *O*-propargylation into the propargylated formamide 19. After the dehydration of this intermediate with triphosgene and triethylamine, the resulting isocyanide is transformed *in situ* with 4-chlorobenzaldehyde and titanium tetrachloride under the conditions of Seebach's modification of the Passerini reaction into the mandelamide 20. The introduction of a second propargyl group into the hydroxyl function of the mandelic acid moiety of 20 delivers mandipropamid (7) [1, 8, 12]. Alternatively, 4-chloroacetophenone (23) is converted into 4-chloromandelic acid (22) by dichlorination and subsequent Cannizzaro-type transformation of the intermediate dichloromethyl ketone. The reaction of the mandelic acid 22 with the phenethylamine 17 under peptide coupling conditions with Castro's reagent and Hünig's base delivers the mandelamide 21, which bears an alcoholic as well as a phenolic hydroxyl function. Both OH groups are then simultaneously propargylated with propargyl bromide under phase-transfer conditions to deliver mandipropamid (7) (Scheme 30.4) [1, 12].

30.4

Mode of Action

Even when dimethomorph (1), the first cinnamic acid amide, iprovalicarb (4), the first valinamide, and mandipropamid (7) as, so far, the sole mandelamide were

commercially sold already, their mode of action had been unknown and the target enzyme was not identified, but earlier it was relatively clear that they share a common cross-resistance pattern for several tested isolates of *Plasmopara viticola* [13]. It was speculated for quite a long time that the CAA fungicides block the cell wall deposition or the phospholipid biosynthesis, before the inhibition of cellulose biosynthesis by directly targeting the Cellulose synthase 3 (CesA3) enzyme could be determined only recently [14]. The resulting disturbance of cell wall structure is demonstrated by swollen tips of germinated cystospores as visible symptom. Another primary effect that led to the elucidation of the mode of action of CAA fungicides is that in their presence, the incorporation of D-(U-14C)-glucose into the crystalline cellulose fraction is largely reduced.

30.5

Biological Activity

The CAA fungicides control selectively and with high efficacy the most important foliar Oomycetes diseases, such as late blights caused by *Phytophthora infestans* (potato and tomato) and *Phytophthora capsici* (peppers) as well as downy mildews caused by *P. viticola* (grape), *Pseudoperonospora cubensis* (cucurbits), *Peronospora effusa* (spinach), *Peronospora destructor* (onions), *Peronospora tabacina* (tobacco), *Peronospora parasitica* (brassicas), and *Bremia lactucae* (lettuce). They are inactive against all members from the genus *Pythium* and against fungal species outside the Oomycetes family. The CAA fungicides strongly inhibit mycelial growth, sporulation and germination of sporangia and cystospores of these phytopathogens. Their fungicidal efficacy is mainly due to their strong preventative action, but some of them also show curative activity and some eradicated effects, depending on the quantity of the fungicide taken up into the leaf and its further distribution. Most of the CAA fungicides show good translaminar activity, iprovalicarb (4) is even a systemic compound. All CAA fungicides show excellent crop safety and also possess a very favorable human and environmental safety profile. Mandipropamid (7) is rapidly absorbed into the wax layer of the plant surface, providing a rainfast and long-lasting barrier against fungal infections [15].

30.6

Structure–Activity Relationship

The fact that the only common structural motif of the three CAA subclasses is an amide function is really fascinating, as all CAA fungicides are cross-resistant to each other for the vast majority of isolates and therefore seem to use a common binding site, which means that usually we would expect a highly conserved scaffold or substitution pattern. But all three subclasses seem to have their own structural requirements for best fungicidal activity. The C–C double bond of the

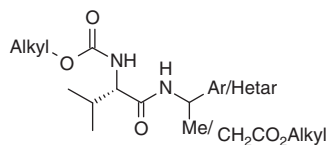
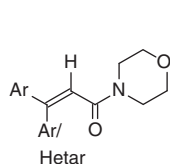
cinnamic acid amides has to be diaryl or heteroaryl substituted in the β -position of the amide function but unsubstituted in the α -position. The fungicidal activity of the valinamides depends on the (*S*)-configuration of the amino acid and on an amine with a one-carbon linker between the amino function and an aryl or heteroaryl ring, which is branched in this benzylic position. The efficacy of the mandelamides depends on the 3-methoxy-4-propargyloxyphenethylamine part, the biggest scope is in the choice of the *para*-substituent of the phenyl ring and the alkylation agent of the benzylic hydroxyl function of the mandelic acid (Figure 30.2).

The two very active *N*-sulfonylated amino acid amides **9** and **10**, which have been already mentioned in Scheme 30.1, demonstrate as missing links the relationship of the valinamide and mandelamide subclasses [10]. The valinamide **9** is related to the commercialized valinamides **4–6**, whereas the phenylglycinamide **10** resembles some structural features of mandipropamid (**7**) (Figure 30.3).

Two α -propargyloxycarboxamides showed a similar impressive level of activity against the key target diseases *P. infestans* and *P. viticola* as their lead compound mandipropamid (**7**). These are **24**, in which the chlorophenyl ring of mandipropamid has been replaced by a chlorothiophene and the glyceric acid amide **25**, which carries an OCH_2 spacer between the phenyl ring and the propargyloxy-substituted carbon atom next to the amide function of mandipropamid (Figure 30.4) [8, 16].

Further interesting mandipropamid analogs with a reduced level of activity are the fluorinated mandelamide **26** [17], the glyoxylic acid amide oxime **27** [8], the peptidomimetic **28**, in which a triazole is mocking the amide linkage

Cinnamic acid amides: Valinamides:



Mandelamides:

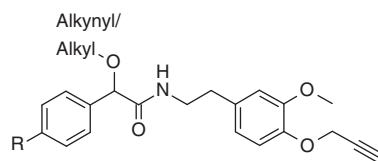


Figure 30.2 The general structural requirements of carboxylic acid amide fungicides.

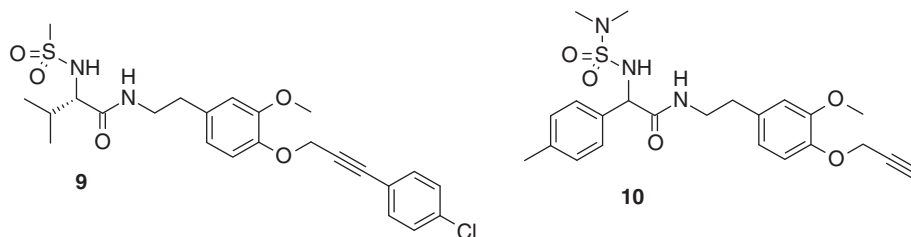


Figure 30.3 The *N*-sulfonylated amino acid amide fungicides **9** and **10** [10].

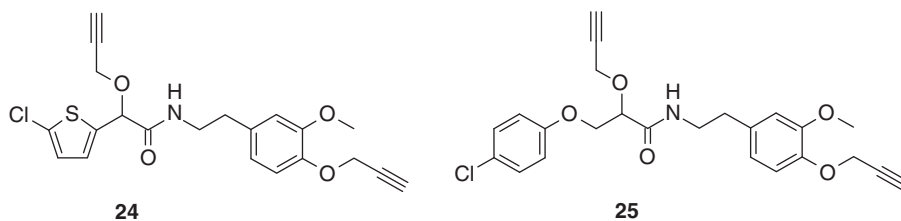


Figure 30.4 Compounds **24** and **25**, two highly active analogs of mandipropamid [8, 15].

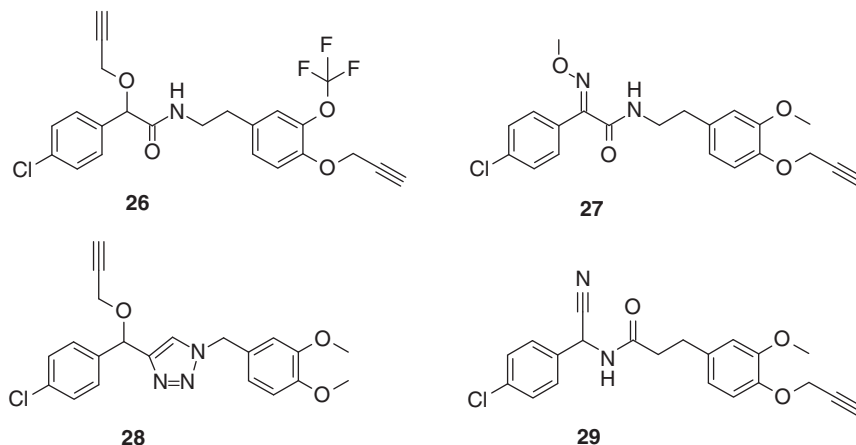


Figure 30.5 The mandipropamid analogs **26–29** [1, 8, 16, 18].

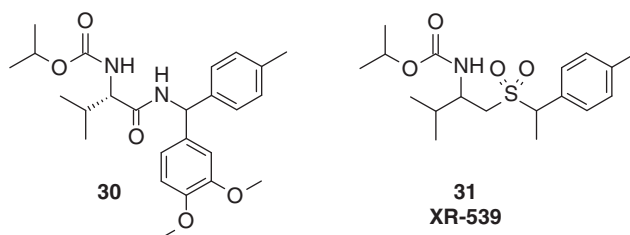


Figure 30.6 The valinamide **30**, a crossover compound with structural features of dimethomorph (**1**) and iprovalicarb (**4**), and the aminosulfone XR-539 (**31**) [19, 20].

[18], and the hydroferulic acid amide **29**, possessing a reversed amide function (Figure 30.5) [1].

The crossover compound **30**, which tries to combine the aromatic portion of dimethomorph (**1**) with the general scaffold of iprovalicarb (**4**), is reported to be active against *P. capsici* and *P. cubensis* [19]. Also, the fungicidally active aminosulfone XR-539 (**31**) shares several structural similarities with iprovalicarb (**4**) (Figure 30.6) [1, 20].

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31

Fungicidal Succinate-Dehydrogenase-Inhibiting Carboxamides

Harald Walter

31.1

Introduction

Inhibition of succinate dehydrogenase (SDH) has been a well-known fungicidal mode of action for more than 45 years, and succinate dehydrogenase inhibitors (SDHIs) are classified under the FRAC Code 7 by the Fungicide Resistance Action Committee (FRAC) [1], a technical industry group reporting to Crop Life International. The FRAC reviews the resistance risk of all currently used fungicidal market compounds (overall 210) based on knowledge of their modes of actions. There are currently 18 SDHI carboxamides listed (15 of them are heterocyclic compounds) by the FRAC, consisting of 8 chemical groups. In this book chapter, the author uses group names **A–G** for the specific chemical classes: oxathiin carboxamides (group **A**), phenylbenzamides (group **B**), furane carboxamides (group **C**), thiazole carboxamides (group **D**), pyridine carboxamides (group **D**), pyrazole-4-carboxamides (group **E**), pyridinyl-ethyl benzamides (group **F**), and phenyl-oxo-ethyl thiophene amides (group **G**). Figures 31.1 and 31.2 show the structures of all carboxamides listed so far by the FRAC. Carboxin (**1**), the first SDHI compound on the market belongs to the oxathiin carboxamide group **A** and was introduced to the market in 1966 by Uniroyal [2]. This compound has a narrow spectrum (mainly smuts and bunts) and is used as a seed treatment compound only, and probably due to its low price, it is still sold today (sales 2014: 75 million USD – all sales figures mentioned in this text are the figures published by Phillips McDougall in 2015). For a long time, the SDHI mode of action was recognized to deliver only compounds with a narrow biological spectrum. This view changed with the market introduction of boscalid (**8**) in 2003 [3]. Boscalid (**8**) was the first SDHI compound with a broader biological spectrum and is until now the best-selling SDHI fungicide (sales 2014: 390 million USD).

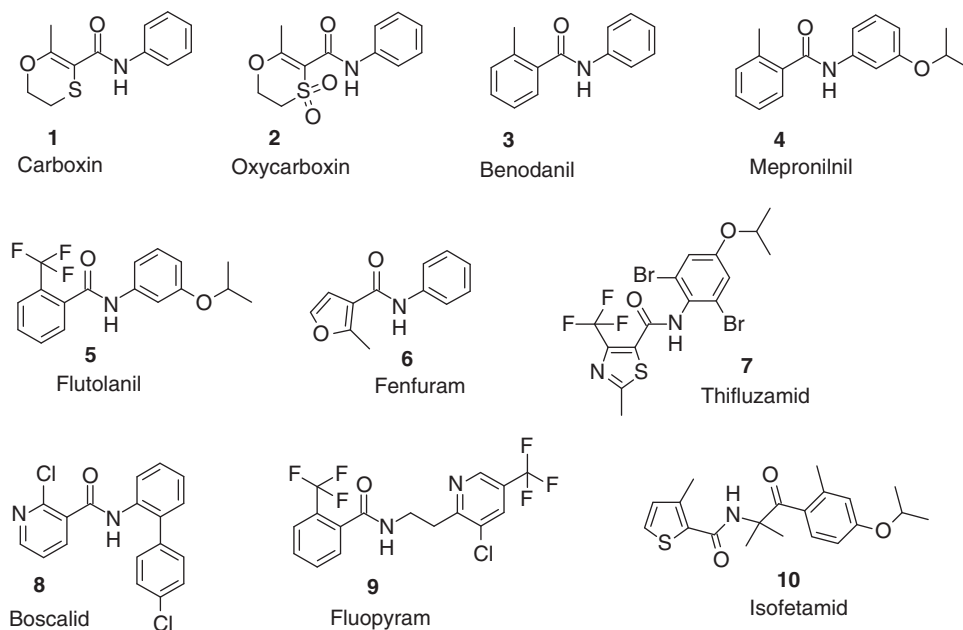


Figure 31.1 The FRAC listed SDHI carboxamides not belonging to the pyrazole-4-carboxamide group (chemical groups A–D and F–G).

31.2

History

As mentioned in the introduction, carboxin (1) was the first SDHI compound on the market and was launched in 1966 [2]. This carboxamide was derived from a six-membered heterocyclic carboxylic acid and belongs to the oxathiin carboxamide class. Many companies tried to broaden the spectrum of carboxin (1) as well as to improve its efficacy. In the 1980s, compounds such as benodanil (3), mepronil (4), and flutolanil (5) were introduced. These compounds belong to the phenyl benzamide class and are the only SDHI anilides derived from benzoic acids. Although the compounds were cheap, their market impact was low due to their unsatisfactory spectrum and efficacy. Another approach to improve spectrum and efficacy of carboxin (1) was to use five-membered heterocyclic acid parts such as pyrazole carboxylic acids. Furametpyr (11) was the first pyrazole carboxamide introduced to the market in 1996. But again a narrow spectrum (mainly *Rhizoctonia* on rice) hindered this compound's success in the market. The breakthroughs in the SDHI area were the introduction of a bigger group in the *ortho* position of the aniline part and the optimization of the pyrazole substitution pattern. BASF and Monsanto were clearly the pioneers in this area [3, 4]. BASF chemists invented the heterocyclic biphenyl-type amides, whereas Monsanto chemists favored the phenyl cycloalkyl amide area (see Figure 31.3). Some of the new pyrazole-4-carboxamides introduced in the last 5 years show a broader spectrum in comparison to boscalid

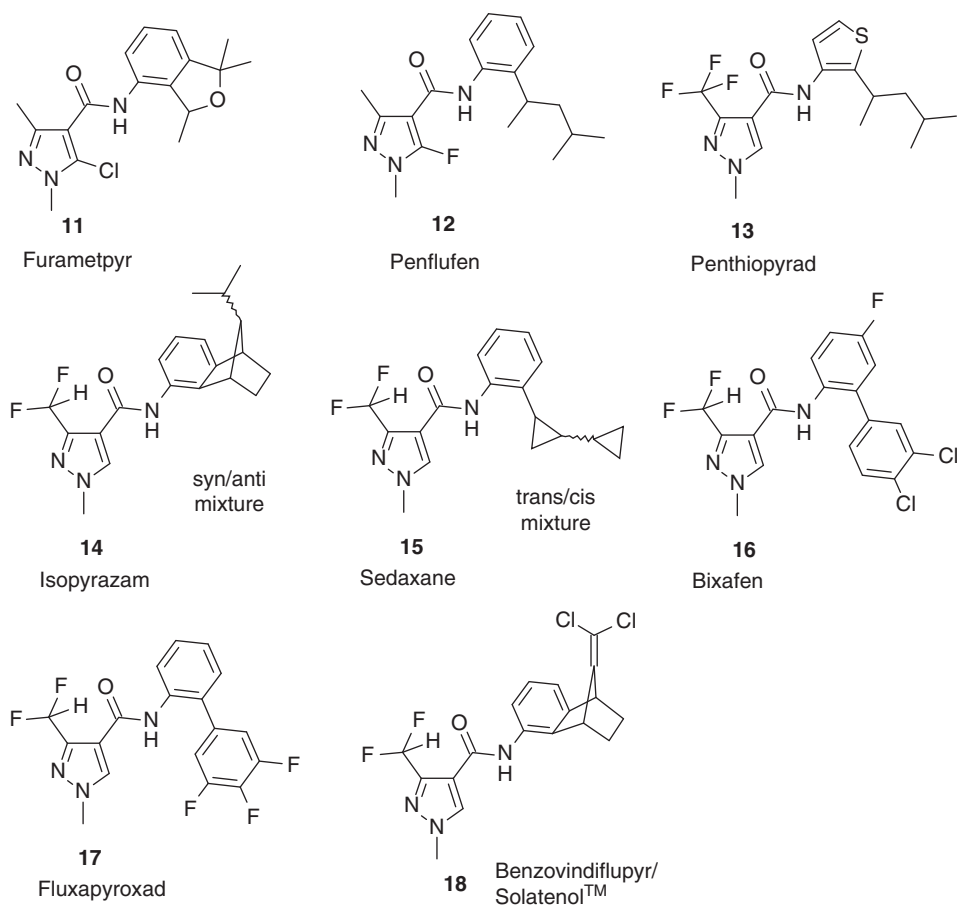


Figure 31.2 The complete list of the pyrazole-4-carboxamides, the most important SDHI carboxamide group (chemical group E).

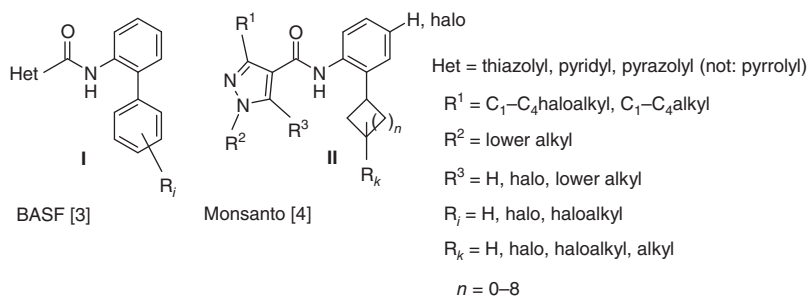
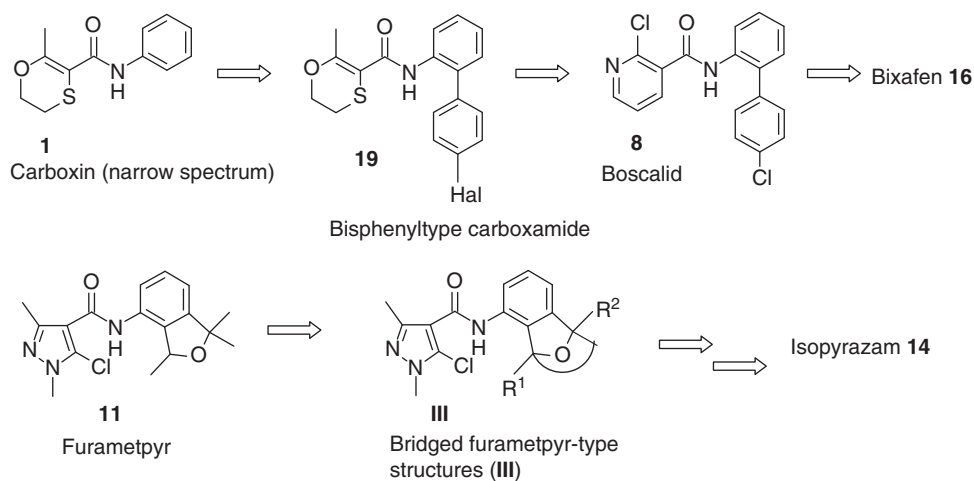


Figure 31.3 General structures of the biphenyl-type and phenylcycloalkyl-type amides.

(8) (e.g., addition of soybean rust control and control of *Zymoseptoria tritici*) as well as an improvement of efficacy in comparison to boscalid (8). Compound 8, a pyridine carboxamide, is mainly used in the fruit and vegetable segment (*Botrytis cinerea*, *Alternaria solani*, and *Sclerotinia sclerotiorum* as major diseases). A full cereal disease spectrum including *Z. tritici* was lacking for boscalid (8). It was obvious that pyridine carboxamides were not able to deliver the full cereal disease spectrum and other chemical classes were needed to control efficiently the major cereal diseases as well as to further broaden the spectrum of boscalid (8). Compounds with an optimized substitution pattern at the pyrazole ring have delivered improved biological spectra. Examples of compounds covering the full cereal spectrum including *Z. tritici* are the recently introduced fungicides isopyrazam (14), bixafen (16), fluxapyroxad (17), and benzovindiflupyr (18) (brand name: Solatenol™). All these compounds belong to the pyrazole-4-carboxamides, by far the most important SDHI group (structures see Figure 31.2) and as a common feature, all these compounds have a difluoromethyl group in 3-position of the pyrazole ring.

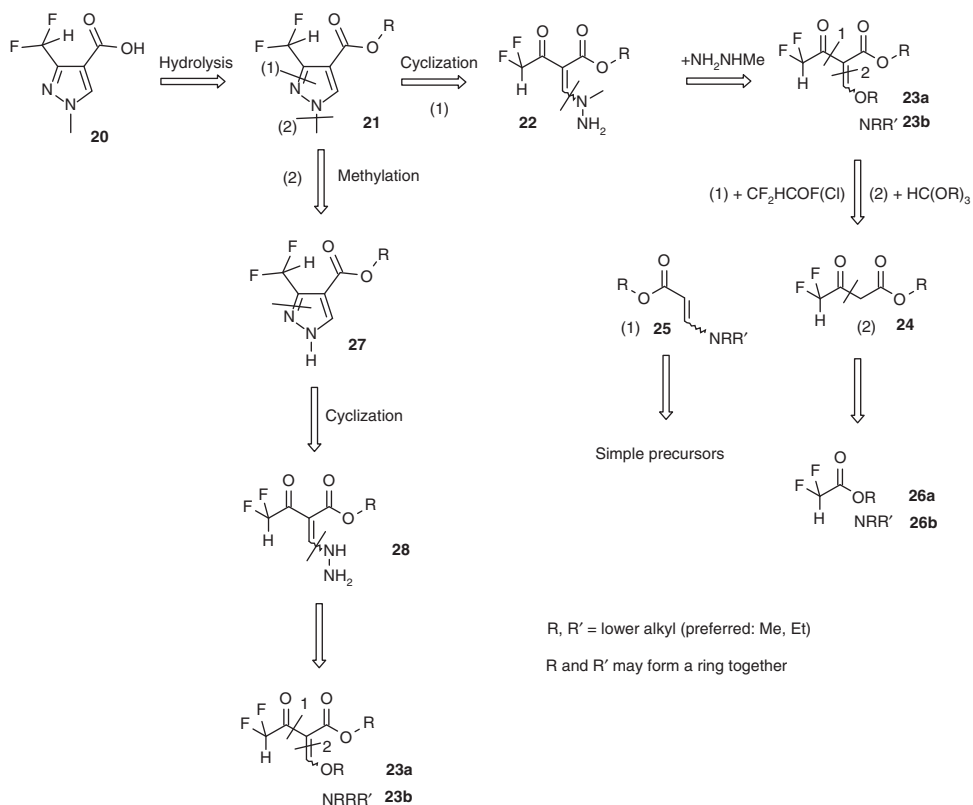
In Scheme 31.1 the evolution of boscalid, bixafen, and isopyrazam structures is shown. Boscalid (8) as well as bixafen (16) could be derived from carboxin (1) (adding an *ortho*-phenyl ring to the aniline part and then substituting the oxathiin part by a chloropyridine lead to boscalid (8); substitution of pyridyl by an appropriately substituted pyrazole and then adding a fluorine to the aniline-phenyl finally lead to bixafen (16)). Isopyrazam (14) could be derived from furametpyr (11), by adding another cyclic moiety to the five-membered oxygen-containing heterocycle, then substituting the oxygen in the ring system by a substituted carbon fragment (this leads to the benzonorbornene moiety). Finally, introducing the most favorable substituents into the pyrazole ring leads to the benzonorbornene pyrazole carboxamide isopyrazam (14).



Scheme 31.1 Evolution of boscalid, bixafen, and isopyrazam structures.

31.3 Synthesis

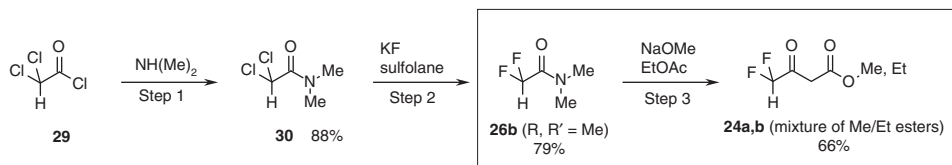
In the first part of the synthesis chapter, synthesis approaches to 3-difluoromethylpyrazole-1-methyl-4-carboxylic acid (**20**) are discussed. The retrosynthetic analysis of 3-difluoro-methylpyrazole-1-methyl-4-carboxylic acid (**20**) clearly reveals the obvious intermediates needed for a cost-efficient large-scale synthesis (see Scheme 31.2).



Scheme 31.2 Retrosynthetic analysis of the pyrazole carboxylic acid **20**.

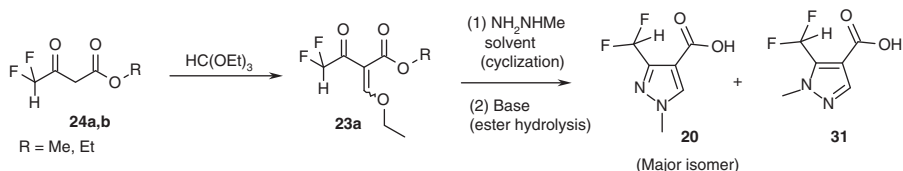
The most critical intermediates are the fluorinated building blocks: difluoroacetyl fluoride, difluoroacetyl chloride, difluoroacetic acid esters or amides thereof. Approaches to introduce the two fluorine atoms at a later stage of the synthesis sequence seemed to be less favorable. When chemists at Syngenta looked into the large-scale synthesis of pyrazole acid **20** in 2004, none of the mentioned fluorinated materials was available in bulk quantities. Following the β -ketoester route, Syngenta chemists had to work out first a route for the synthesis of an appropriate difluoromethyl precursor, which had potential for

upscaling. The Syngenta approach was to use a difluoroacetic acid dialkylamide **26b**, which was thought at the time to be transferable to the β -ketoester **24** via an amido-Claisen approach. This key transformation was unknown at the beginning of the Syngenta project. In Scheme 31.3 the first Syngenta approach to the mixture of β -ketoesters **24a,b** is described, starting from dichloroacetyl chloride (**29**) as a cheap starting material [5]. This route turned out to be robust enough to prepare 4,4-difluoro-3-oxobutyric esters **24a,b** in multikilogram quantities.



Scheme 31.3 First Syngenta synthesis of the CF_2H group containing mixture of β -ketoesters **24a,b** [5].

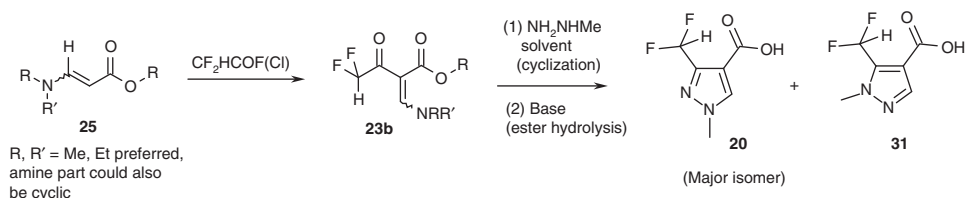
The completion of the β -ketoester route could be accomplished via an acid-catalyzed reaction of a 4,4-difluoro-3-oxobutyric acid alkyl esters **24a,b** with a triethyl orthoformate, which gave the desired alkyl-4,4-difluoro-2-ethoxymethylidene-3-oxobutanoates **23a** in excellent yields (>90%). The transformation to the pyrazole acid **20** was performed by initial treatment with methylhydrazine and then hydrolysis of the resulting ester with sodium hydroxide (Scheme 31.4). This route was also partially described by Monsanto chemists in 1993. In the Monsanto patent, the cleavage of the ester was performed by the treatment with TMSI (trimethylsilyl iodide), which is not the method of choice for an industrial synthesis.



Scheme 31.4 Completion of the β -ketoester route [4, 6].

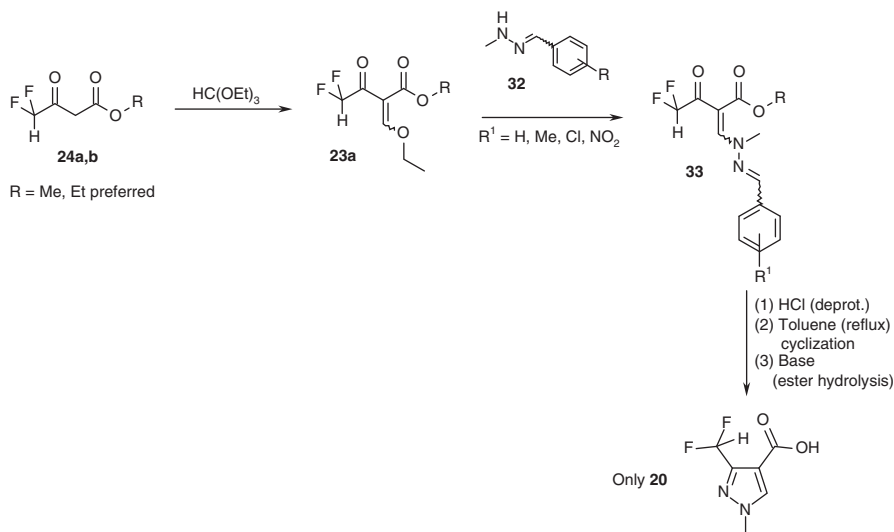
An alternative is the cyclization with hydrazine followed by methylation which was found by Syngenta chemists years later [7]. Both approaches face the problem of regioselectivity and the correct isomer has to be separated at the acid stage by crystallization. While the regioselectivity of the cyclization is one problem, the cheap synthesis of the β -ketoester is another. Due to the progress made in recent years in the large-scale synthesis of difluoroacetyl acid and esters thereof [8–14], these compounds are now available in bulk quantities. As the difluoroacetic acid esters are now available for a reasonable price, the Claisen condensation of a difluoroacetic acid ester with acetic acid methyl or

ethylester seemed to be a useful industrial approach. Chemists from Bayer CropScience, BASF, and Central Glass used a different approach to pass by the β -ketoester using difluoroacetic acid chloride or the respective fluoride in the addition reaction with 3-(dialkylamino)acrylates **25** [15–19]. The resulting 2-difluoroacyl-3-aminoacrylic acid esters **23b** can then easily be transferred to the target pyrazole carboxylic acid **20** (Scheme 31.5). As huge progress had also been made in the industrial synthesis of difluoroacetyl fluoride and the respective chloride [9, 11–14, 20], this approach seems to be promising.



Scheme 31.5 Bayer CS/BASF/Central Glass approach to the pyrazole acid **20** using the 3-(dialkylamino)acrylate route.

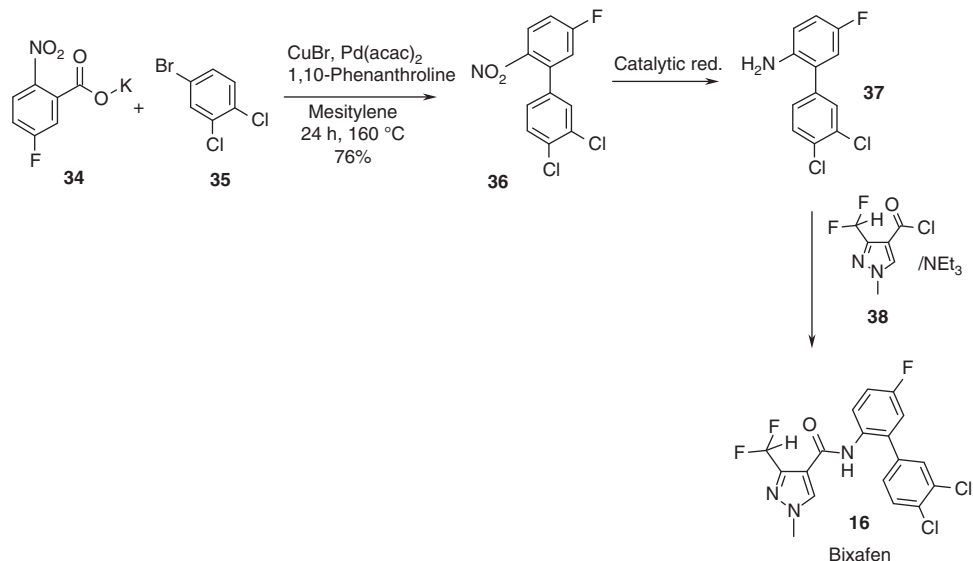
To overcome the regioselectivity problem, BASF chemists are using protected *N*-methylhydrazines such as the hydrazone **32** in reactions with the key enolether intermediate **23a**. After deprotection with hydrogen chloride, the cyclization can only lead to the desired regioisomer **20** of the pyrazole acid (Scheme 31.6) [21].



Scheme 31.6 BASFs protecting group approach for the regioselective synthesis of the pyrazole acid **20** [21].

As the first synthesis example of an SDHI carboxamide, synthetic approaches to bixafen (**16**) [22], especially involving different strategies for the synthesis of

the aniline moiety of bixafen (**16**), will be introduced. A more curious approach leading to *ortho*-substituted biphenyl anilines uses a cycloaddition reaction of an appropriate acetamidophenylacetylene precursor with 3,4-dichlorothiophene-1,1-dioxide as the key step [23]. Another interesting route involves a new Pd/Cu-catalyzed decarboxylative cross-coupling of arylchlorides with potassium carboxylates as the key step [24, 25], which is outlined in Scheme 31.7. Catalytic reduction of the nitro group present in compound **36** and coupling of the resulting aniline **37** with the pyrazole acid chloride **38** in the presence of a base (e.g., triethylamine) gave bixafen (**16**) in good overall yields.

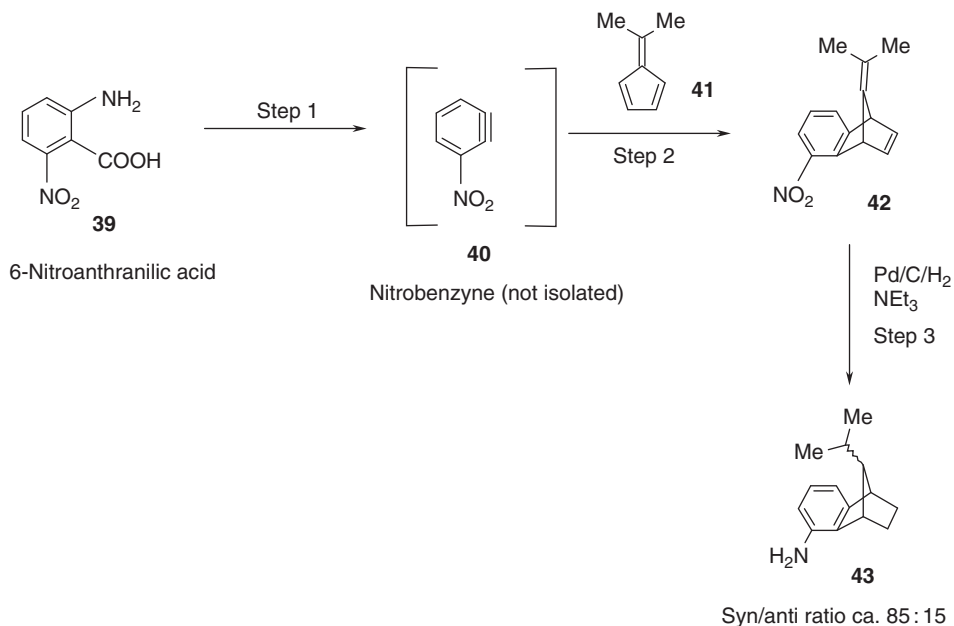


Scheme 31.7 Synthesis of bixafen (**16**) using the Goossen approach for the synthesis of the aniline part [24, 25].

Both aforementioned approaches look good especially from the laboratory perspective but are probably not suitable for a large-scale manufacturing approach. It is more likely that Bayer's production route for the synthesis of 2-(3'4'-dichlorophenyl)aniline **37** involves a Suzuki coupling step [26] similar to the one used by BASF chemists to prepare the aniline part of boscalid (**8**). BASF was the first agrochemical company using Suzuki coupling technology for the large-scale synthesis of an agrochemical intermediate – the aniline part of boscalid, which involves the coupling of 2-chloronitrobenzene with 4-chlorophenyl-boronic acid in the presence of triphenylphosphine [27].

The synthesis of the benzonorbornene amide isopyrazam (**14**) [28] was very challenging for the chemists at Syngenta. One of the earlier approaches started from 6-nitroanthranilic acid (**39**) [29], which was used as the precursor for nitrobenzene (**40**). The tricky cycloaddition reaction of the reactive intermediate **40** with dimethylfulvene (**41**) [29] gave the nitrobenzonorbornadiene **42** in

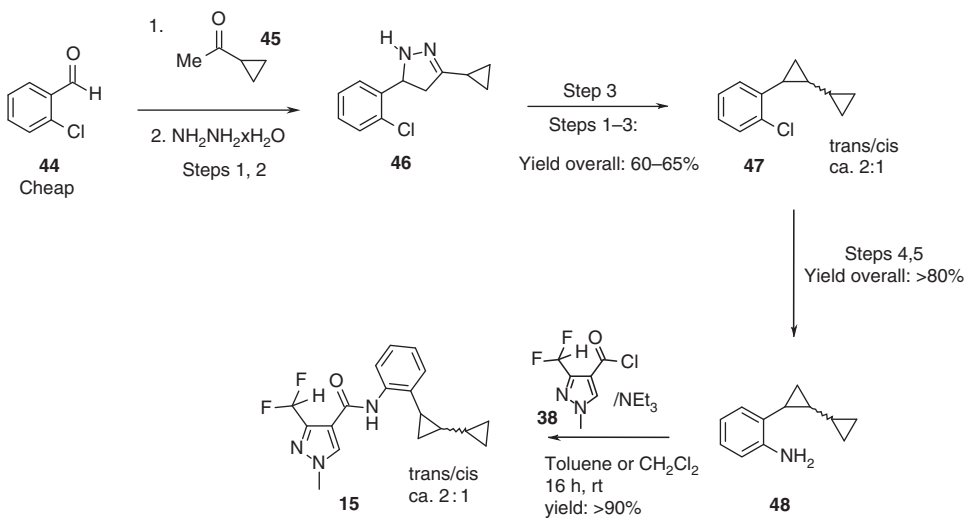
yields of 50–55% and the reduction of **42** using a palladium catalyst in the presence of a base at higher pressures yielded the aminobenzonorbornene **43** as a syn/anti mixture (about 85% syn isomer obtained under specific reaction conditions [30, 31]). The coupling of **43** with the pyrazole carboxylic acid chloride **38** under standard conditions finally leads to isopyrazam (**14**) in good yields (Scheme 31.8).



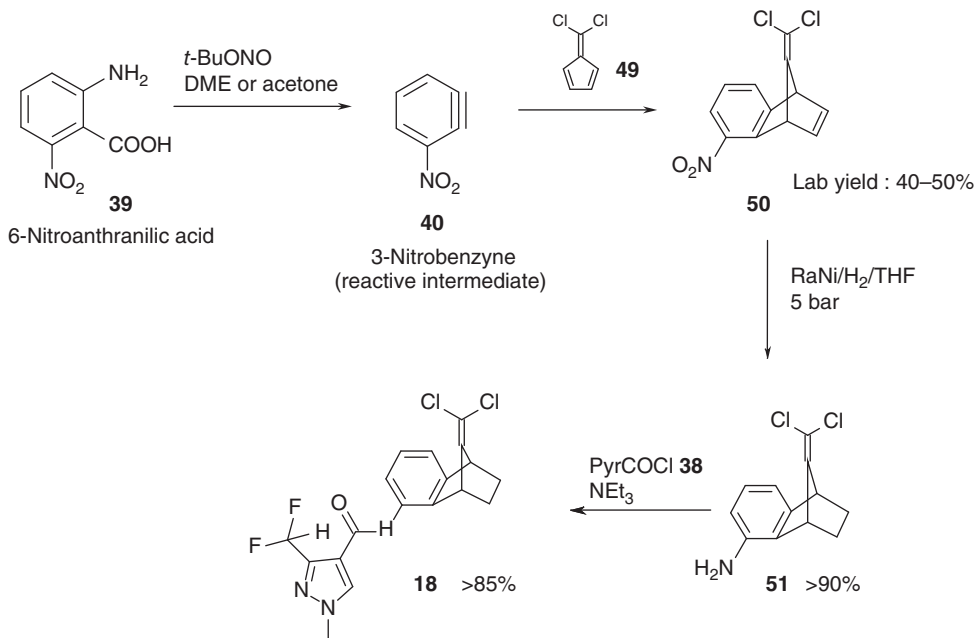
Scheme 31.8 Synthesis of isopyrazam (**14**) (aniline part only) – first optimized synthesis [28–31].

As another example of a pyrazole-4-carboxamide, the synthesis of sedaxane (**15**) [32] will be discussed in more detail in this chapter. The first step is a base-catalyzed crossed-aldol reaction of 2-chlorobenzaldehyde (**44**) with cyclopropyl methyl ketone (**45**). The resulting aldol adduct was not isolated but directly converted into the dihydropyrazole **46** via reaction with hydrazine [32, 33]. Treatment of the dihydropyrazole **46** at higher temperatures (>180°) in the presence of a base, after loss of nitrogen and cyclopropyl formation [34, 35] gave the desired 2-bicyclopropyl-1-chlorobenzene **47** as about 2:1 mixture of *trans* and *cis* isomers. A Pd-catalyzed benzyl-amination reaction followed by a metal catalyzed deprotection step gave the desired 2-bicyclopropylaniline **48** in good yields [36]. Coupling of the aniline **48** with the pyrazole acid chloride **38** under standard conditions finally led to sedaxane (**15**) (e.g., *trans/cis* mixture ratio 2.1) in satisfactory yields (Scheme 31.9).

An example of a synthesis of benzovindiflupyr (**18**) is outlined in Scheme 31.10 and starts from 6-nitroanthranilic acid (**39**) an intermediate already known



Scheme 31.9 Synthesis of sedaxane (**15**) (example: trans/cis ratio about 2 : 1) [32–36].



Scheme 31.10 Synthesis of benzovindiflupyr (**18**) using a new cycloaddition approach [37, 38].

from earlier isopyrazam synthesis routes. The cycloaddition of *in situ* generated nitrobenzynes (**40**) with dichlorofulvene (**49**) gave the nitrobenzonornbornadiene intermediate **50** in reasonable yields. Reduction of the nitro group concomitant hydrogenation of the double bond was accomplished by the use of a Ra–Ni catalyst and generally gave yields >90% of the amine **51**. The final coupling of amine **47** with the pyrazole acid chloride **38** under standard conditions delivered benzovindiflupyr (**18**) in very good yields.

31.4

Mode of Action and Importance of Respiration Inhibitors

Complex II carries out the succinate dehydrogenase function of the tricarboxylic acid (TCA) or Krebs cycle and catalyzes the oxidation of succinate to fumarate, coupled to the reduction of ubiquinone to ubiquinol. Complex II contains four subunits (A–D), the inhibitor binding site being located in a steric binding pocket composed of parts of subunits B, C, and D. The fungicidal effect of all complex II inhibitors relies on the disruption of the TCA cycle.

Inhibitors of the mitochondrial respiration chain play an important role in agrochemical fungicidal research. The most important targets for fungicides in the respiration chain to date are complexes II and III (see Figure 31.4) [39].

Compounds inhibiting complex III such as azoxystrobin (best-selling fungicide in 2014: 1215 million USD) and pyraclostrobin are key players in the fungicide market. The importance of complex II inhibitors has increased in the last years with the discovery of broad-spectrum SDHI compounds. Boscalid (**8**) is currently

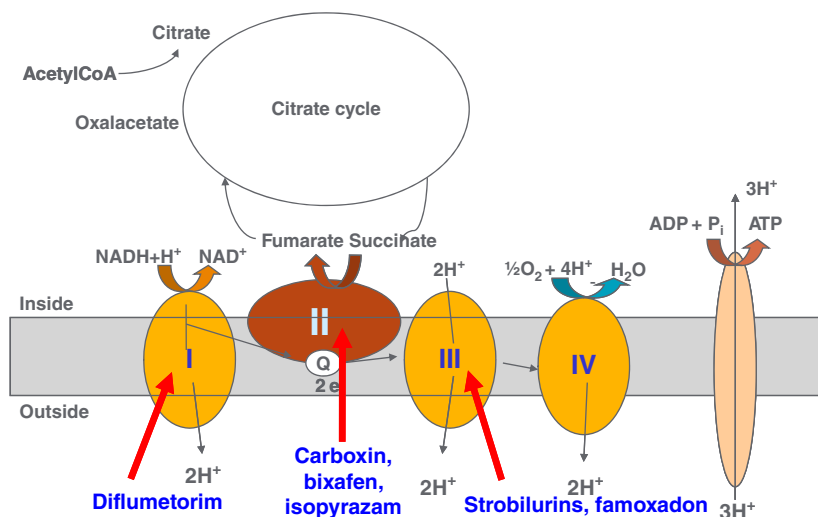


Figure 31.4 Schematic representation of the respiration chain (containing complexes I–IV).

the best-selling SDHI (sales 2014: 390 million USD), but some of the pyrazole carboxamides discussed in this review also have already demonstrated their sales potential (bixafen sales 2014: 200 million USD; fluxapyroxad sales 2014: 285 million USD). Fungal resistance within the strobilurins (powdery mildews and *Zymoseptoria* leaf blotch in wheat) makes this class less attractive for investing further in research. New modes of action especially in cereal applications are needed to close the QoI-(strobilurin) gap. The SDHIs already introduced or close to market introduction will serve as important mixing partners for strobilurins and triazoles and will help to reduce the resistance risk of future cereal and fruit/vegetable solutions [40].

31.5

Biological Activity and Market Impact

31.5.1

The Foliar SDHI Carboxamides

As mentioned earlier, the pyridine carboxamide boscalid (**8**) was the first SDHI, which showed a broader spectrum and good efficacy levels against a range of pathogens in the field [3]. The major strengths of boscalid (**8**) are control of *A. solani* (early blight), *Botrytis* spp. (molds including gray mold), *S. sclerotiorum* (stem rot), and *Pyrenophora teres* (barley net blotch). In mixtures with other fungicides, boscalid (**8**) can be used in a lot of crops including wheat and barley (BASF cereal solution: boscalid/epoxiconazole mixture [41]), but the relatively weak activity against *Z. tritici* (*Zymoseptoria* leaf blotch) and *Puccinia recondita* (brown rust) is clearly a disadvantage of boscalid (**8**). The next generation of foliar SDHI fungicides such as isopyrazam (**14**), bixafen (**16**), and fluxapyroxad (**17**) are clearly more focused on the cereal segments, and all these compounds show excellent activity against leaf spot diseases in wheat and barley [42–45]. In addition, isopyrazam (**14**) is a very strong leaf spot compound outside cereals and controls *A. solani* (early blight), *Venturia inaequalis* (apple scab), *Mycosphaerella fijiensis* (banana black sigatoka), and powdery mildew diseases in fruits and vegetables. The first commercial product based on isopyrazam (**14**) was the mixture with cyprodinil called Bontima®. This mixture was introduced in the UK Q1/2010 in barley [44]. Bixafen (**16**) is also effective against other leaf spot diseases such as *A. solani* (early blight in potatoes and tomatoes) and *M. fijiensis* (banana black sigatoka). Bayer CropScience markets a mixture consisting of bixafen (**16**) and prothioconazole (a very effective cereal fungicide) as XPro technology [43]. This mixture controls a broad cereal disease spectrum including all major leaf spots, brown rust, and Fusarium. Isopyrazam (**14**) and bixafen (**16**) also control *B. cinerea* (gray mold), but to a lower level than boscalid (**8**). BASFs fluxapyroxad (**17**) was launched in 2012 [45] and is so far the most successful biphenyl-type SDHI commercially. Bixafen (**16**) and fluxapyroxad (**17**) are both pyrazole carboxamides derived from biphenyl anilines, whereas isopyrazam (**14**)

has a totally novel structure containing a benzonorbornene ring system. This illustrates the fact that SDHI compounds with potential for commercialization can possess totally different structural elements. Fluxapyroxad (**17**) is a broad-spectrum compound with a strength against leaf spot diseases in many crops [45], used also in mixtures with epoxiconazole and pyraclostrobin. Fluxapyroxad (**17**) also has the potential for use in the seed treatment area. Penthiopyrad (**13**), originally invented by Mitsui chemists, is the only compound that is derived from an aminothiophene. The compound is more a fungicide for the fruit and vegetable segment, with strengths against *A. solani* (early blight), *V. inaequalis* (apple scab), *Podosphaera leucotricha* (powdery mildew on apples), and *Rhizoctonia solani* (root rot) [46]. Penthiopyrad (**13**) is mainly a competitor for boscalid (**8**), but in mixture with picoxystrobin (mixture sold by DuPont), it is also a competitor for the cereal SDHIs isopyrazam (**14**), bixafen (**16**), and fluxapyroxad (**17**). There might also be potential for penthiopyrad (**13**) in the seed treatment area.

The second-generation benzonorbornene carboxamide benzovindiflupyr (**18**) [37] is a broad-spectrum compound that has the full cereal spectrum including *Z. tritici* and also offers outstanding activity against soybean rust (best in class fungicide). This fungicide is sold in mixture with azoxystrobin as Elatus™ in Brazil. Elatus was introduced to the market in Brazil in 2014. Benzovindiflupyr (**18**) is also active against other market relevant diseases in the fruit and vegetable segments (e.g., *Cercospora* in peanuts), and further product introductions in the cereal as well as in other segments are planned for the next years.

Bayer's fluopyram (**9**) is a special case as its structure is not derived from an aromatic amine [47]. It belongs to the new class of pyridinyl-ethyl benzamides. Fluopyram (**9**) is a broad-spectrum compound active against some major diseases in the fruit and vegetable segment. It is especially active against molds as well as powdery mildew species. The mixture with tebuconazole is sold under the brand name Luna® [48].

31.5.2

The Seed Treatment SDHI Carboxamides

Penflufen (**12**) [49] and sedaxane (**15**) [50, 51] are broad-spectrum seed treatment fungicides covering a wide range of diseases in many crops. All sedaxane-based offers will be sold under the global brand name Vibrance®. The first of these offers Vibrance Integral® (mixture with fludioxonil, difenoconazole, and thiamethoxam) was launched in Argentina in May 2011 [50]. Sedaxane (**15**) provides best-in-class protection against *Rhizoctonia* species and *Microdochium nivale* (snow mold) and shows good efficacy against *Ustilago nuda* (loose smut), *Sclerotinia* spp., *Tilletia caries* (common bunt), *Macrophomina*, and other species [51]. Sedaxane (**15**) Vibrance® enables optimized root performance (Rooting Power™) providing protection to the root system under a wide range of environmental stresses, resulting in more efficient use of water and nutrients, finally resulting in higher average yields.

Penflufen (**12**) has a similar spectrum compared to sedaxane (**15**), but overall sedaxane (**15**) technically seems to be superior to penflufen (**12**) (sales 2014: penflufen: 130 million USD and sedaxane: 85 million USD). In summary, the new pyrazole-4-carboxamides, which have entered the market in the last 5 years, currently cover all major agro-relevant fungi classes with the exception of oomycetes. To our knowledge, no SDHI carboxamide showing market relevant activity against *Phytophthora infestans* (late blight) and/or *Plasmopara viticola* (grape downy mildew) has been discovered so far. SDHIs have gained considerable market share in the last 3 years (total sales 2014: 1410 million USD). Only fungicides based on the QoI strobilurins and the DMI mode of action have bigger market penetration than the SDHIs. SDHI fungicides will play an important future role in agrochemical disease control, and further sales growth is expected in the next years. With the use of well-designed product concepts (mixtures) mixing and use strategies, the speed of resistance buildup for the SDHI carboxamides should be reduced.

31.6

Structure–Activity Relationships

31.6.1

General Structure–Activity Relationship Considerations of the Pyrazole Carboxamide Class IV

Compounds of the pyrazole carboxamide class **IV** are active against many important diseases of agricultural crops such as leaf spots, mildews, molds, rusts, smuts. It is not the goal here to present a detailed picture on SAR of the pyrazole carboxamide class **IV**, but instead, a simplified analysis covering *Z. tritici* (*Zymoseptoria* leaf blotch) and *P. recondita* (brown rust) in wheat, *B. cinerea* (gray mold) in grapes, and *A. solani* (early blight) in potatoes (Figure 31.5). Of course, the analysis is not the same for all pathogens, and therefore, some simplifications have to be made in this analysis (the goal is to give a rough summary). The pyrazole ring seems to be well optimized especially for the cereal diseases. CF₂H is better than CF₃ (Me normally is not good, if we only consider the foliar fungicide aspect, an exception is the ST compound penflufen (**12**), where an Me group in 3-position and a fluorine group in 5-position are favorable). CF₂CF₃ leads to very weak or inactive compounds. Introduction of ethyl, propyl, and isopropyl in 3-position also leads to weak or inactive compounds. In the 5-position only H and fluorine are good; chloro, bromo, Me, and CHO are less favorable; and in almost all cases, weak biological activity was the result. An observation here is that all good *Zymoseptoria* compounds have a CF₂H substituent on the pyrazole ring.

Substitution of the amide linker (CONH) by other groups, while maintaining a good level of activity, turned out to be challenging. The only group delivering a good level of activity was the thioamide (CSNH) [52]. Substituents on nitrogen

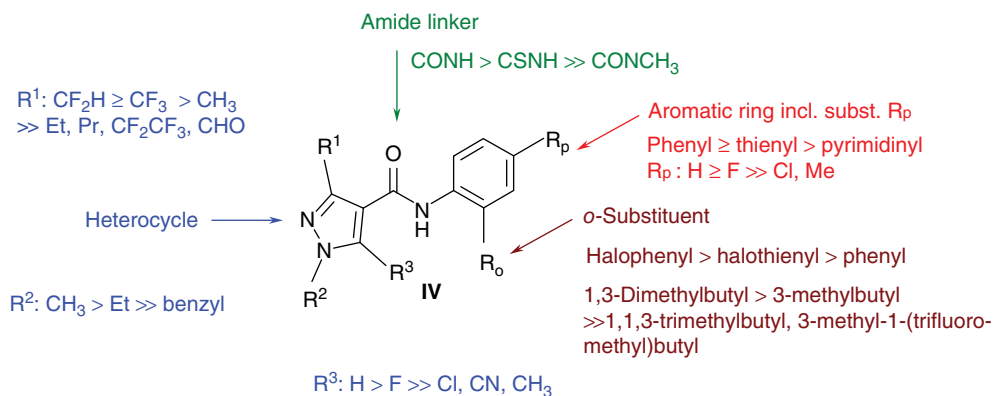


Figure 31.5 Simplified SAR picture for the pyrazole-4-carboxamide class covering brown rust and *Zymoseptoria* leaf blotch on wheat, gray mold on grapes, and early blight on tomato (related to glasshouse data).

in most cases did not lead to significant activity and even the small methyl group gave a dramatic decrease in activity. Only the unsubstituted propargyl group [53] was tolerated, and in field trials, acceptable activity level could be observed for the bisphenyl-type series. Other approaches such as the use of reversed amides, iminoethers, and thioethers were not successful [54]. For the aromatic ring, phenyl and thienyl turned out to be favorable. Other heterocycles (e.g., pyrimidinyl) were not well tolerated, and less active compounds were obtained. For a long time, the unsubstituted phenyl ring was preferred by the chemists. Later it was observed that F was tolerated in the 4-position without losing significant levels of activity (see structure of bixafen (**16**)). Chloro and methyl in the 4-position led to compounds with dramatically lower level of activity. The last part of the SAR analysis deals with the influence of the *ortho*-substituent (R_o) on activity and spectrum. For boscalid (**8**), halophenyl (F and Cl preferred) is a favorable *ortho*-substituent especially in the case of *Alternaria* and *Botrytis*. For the *Zymoseptoria* activity, halophenyl, dihalophenyl, and trihalophenyl as well as alkylacetinyl-substituted phenyl [55] look favorable, and this is also to some extent observed with rust. Some alkyl side chains (containing four to seven carbon atoms) look especially promising for good activity against brown rust. A preferred side chain was 1,3-dimethylbutyl, but the 3-methylbutyl substituent also delivered compounds with reasonable rust activity. Compounds bearing either a trifluoromethyl group or two methyl groups at the benzylic position tend to deliver compounds with much lower activity level in comparison to the unsubstituted or monomethyl-substituted compounds. The side chain also tolerates a silicon atom without losing a good level of activity [56]. Principally specific *o*-cycloalkyl groups (preferred: cyclohexyl for the foliar segment and cyclopropyl for the seed treatment segment) can replace the phenyl ring, leading to compounds with good efficacy. Some *ortho*-silacyclohexanes revealed good

cereal compounds with a pronounced strength against *Z. tritici* (*Zymoseptoria* leaf blotch) [57].

31.6.2

Replacement of the Pyrazole Ring by Phenyl or Pyridyl

The phenylbenzamides **3–5** show a narrow biological spectrum and only have a small impact on the market. Boscalid (**8**) was described earlier and the conclusion here is that compounds derived from pyridine-3-carboxylic acids may have interesting spectrum and efficacy but are not able to deliver the full cereal disease spectrum. Pyraziflumid (**52**), a pyrazine biphenyl-type carboxamide from Nihon Nohyaku, according to what is disclosed in their patent [58], is active against apple scab, gray mold, powdery mildew, and brown rust. Spectrum information about other diseases was not exemplified in the patent text. It is the first compound derived from a pyrazine carboxylic acid (Figure 31.6).

31.6.3

Replacement of the Pyrazole Ring by Other Five-Membered Heterocycles

In Figure 31.7, other five-membered heterocycle replacements for pyrazole in the SDHI carboxamide amide chemistry are summarized. Thifluzamide (**7**), which was originally discovered by Monsanto chemists [59] and since 2010 has been marketed by Nissan Chemical, contains a thiazole ring. This compound also showed a narrow spectrum of activity (sheath blight and black scurf) and the market impact was also rather limited. Compounds **53–55** never reached the level of activity as the corresponding pyrazole compounds. Compound **54** in the glasshouse shows a very interesting biological spectrum and good efficacy level,

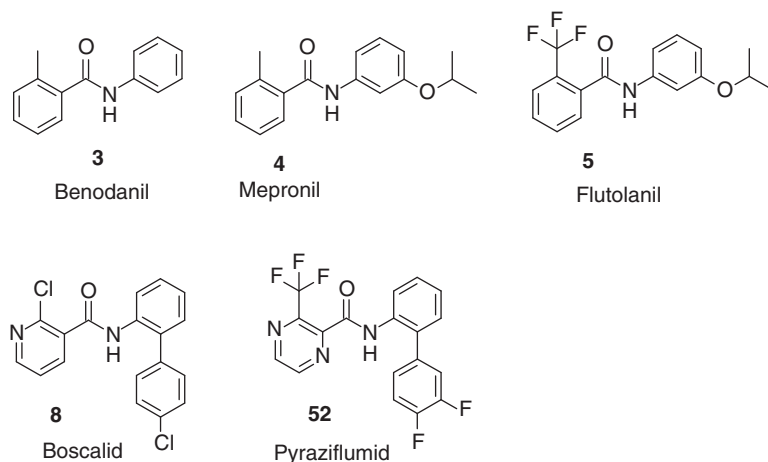


Figure 31.6 Structures of the phenylbenzamides **3–5**, the pyridine-3-carboxamide boscalid (**8**) and pyraziflumid (**52**).

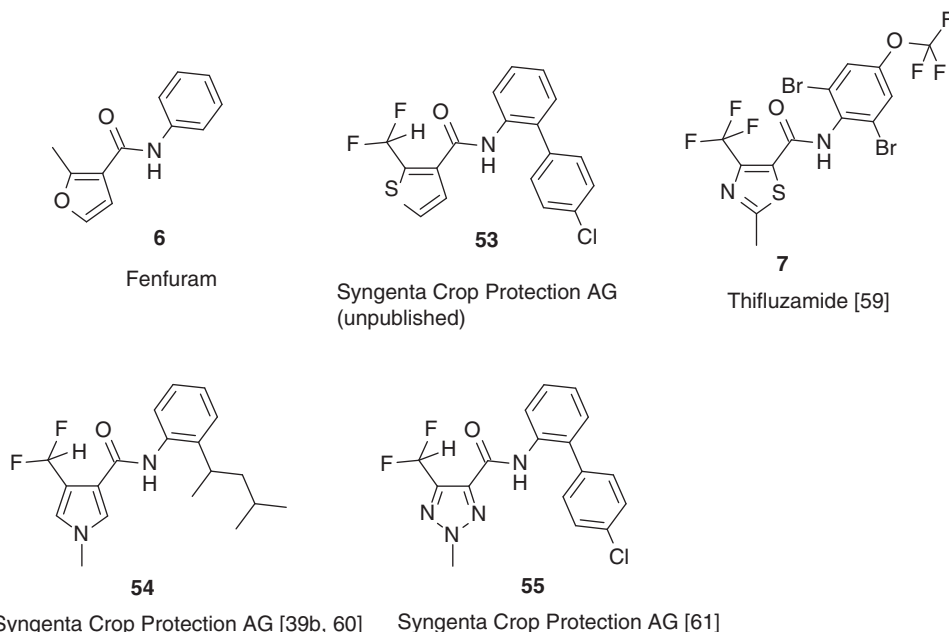


Figure 31.7 Examples of other five-membered ring containing SDHI fungicides.

but probably due to stability issues, a reliable long-lasting market-relevant biological effect was never observed in field trials. Overall, the pyrazole-4-carboxamides derived from adequately *ortho*-substituted anilines deliver the broadest spectrum as well as the best efficacy.

31.6.4

Carboxamides not derived from Aromatic Amines

Fluopyram (**9**), according to the FRAC classification is a pyridyl-ethyl benzamide and was the first marketed SDHI Carboxamides not derived from Aromatic Amines. The compound shows broad-spectrum activity, and its major uses are in the fruit and vegetable segment. The published sales figures for 2014 are 60 million USD. Isofetamid (**10**) [62] is a compound currently developed by Ishihara Sangyo Kaisha and according to the FRAC group classification, belongs to a new subclass. The potential is currently unclear. Gray mold and powdery mildews are published targets. Pydiflumetofen (**56**) represents a new structural type within the SDHI carboxamide class [63] and is currently being developed by Syngenta Crop Protection AG. Its ISO name was published recently. In the Syngenta patent [63], activity in glasshouse trials against the following diseases was exemplified: gray mold (grapes), early leaf spot on groundnut, net blotch (barley), powdery mildew (wheat), snow mold, and *Zymoseptoria* leaf blotch (wheat) (Figure 31.8).

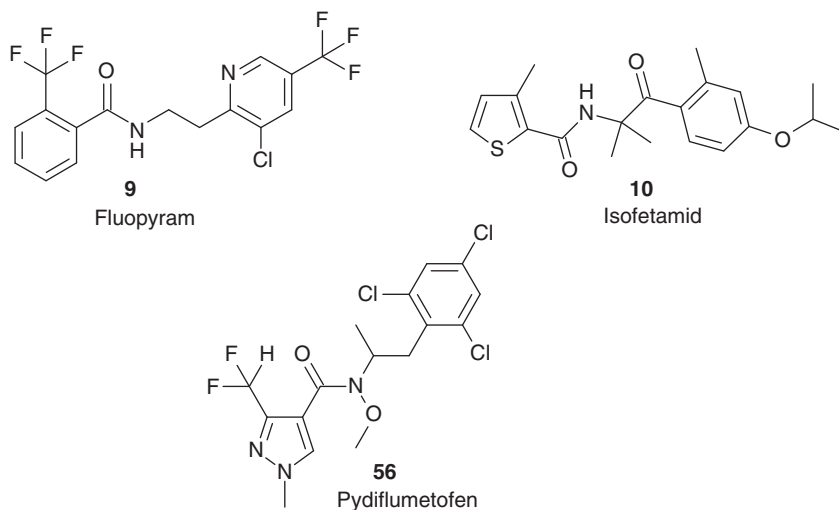


Figure 31.8 Structures of SDHI carboxamides not derived from aromatic amines.

Acknowledgments

The author expresses his great thanks to many colleagues at Syngenta who are engaged in SDHI pyrazole carboxamide research, development, production, and marketing for their excellent contributions to this fascinating field. Particular thanks go to his colleagues Chris Godfrey, Dietrich Hermann, Andy Leadbeater, Xavier Leprince, Christian Schlatter, and Wolfgang Stutz for valuable discussions.

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Part X

Insect Control

32

Esters and Carbamates as Insecticidal Juvenile Hormone Mimics

Sebastian Rendler

32.1

Introduction

Inspired by the discovery of structure and function of insect juvenile hormones, a new class of synthetic analogs possessing insecticidal activity was developed in widespread research activities from the early 1960s to the late 1980s. Juvenile hormones are naturally occurring sesquiterpenoids playing a vital role in metamorphosis, reproduction, and development of insect larvae [1]. The exploration of insect growth regulation for insect and vector control has led to the commercialization of six active ingredients, four of which contain an ester or carbamate moiety: juvenile hormone analogs hydroprene (**1**), kinoprene (**2**), and methoprene (**3**), and a second-generation nonterpenoid fenoxycarb (**4**) (Figure 32.1).

Juvenile hormone mimics are of limited commercial interest in the field of crop protection, yet they are still of some relevance in noncrop use such as mosquito control or protection of stored products. Nevertheless, their history of discovery and development illustrates an inspiring example of insecticide research that has also been expertly reviewed by some of the major contributors to this field [2, 3].

32.2

History

The biochemical function of juvenile hormones was known since the first report in 1934. However, it was only following Röller's isolation and characterization of JH I (**6**) in 1968 [4] that this and six other naturally occurring juvenile hormones have been identified (Figure 32.2) [2, 5]. JH 0 to JH 3 (**5–8**) were found to be mono-epoxidized sesquiterpenoid methyl esters with varying degrees of additional methylation. Later on, bisepoxy juvenile hormones **9** and **11** as well as 4-methylated derivative **10** were isolated. Not surprisingly, all natural juvenile hormones are single stereoisomers [6]. Their biosynthesis scheme follows a modified terpenoid pathway [7].

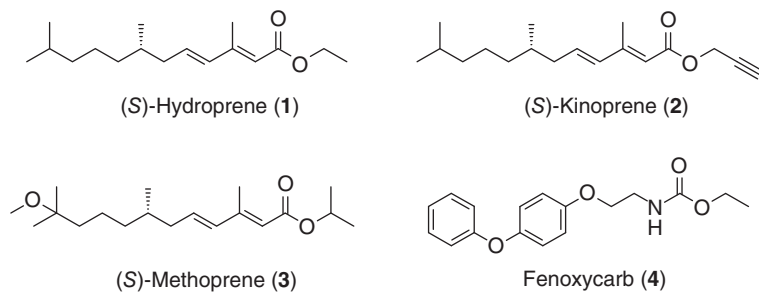


Figure 32.1 Four commercialized ester- or carbamate-bearing juvenile hormone mimics.

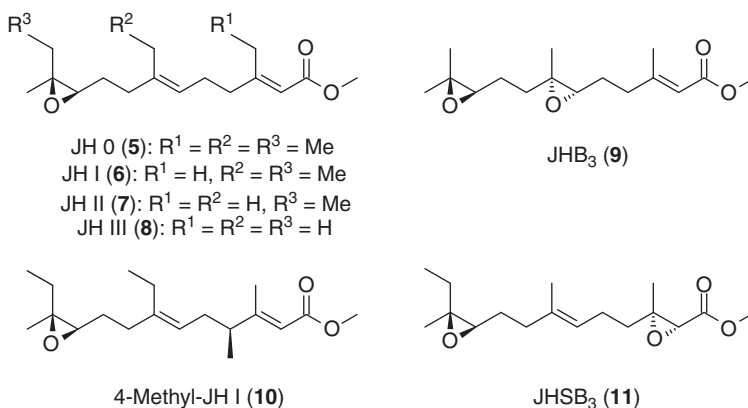


Figure 32.2 Naturally occurring juvenile hormones.

Substantial progress had been made over the decades in the chemical total synthesis of juvenile hormones [6, 8]. Yet, two aspects inspired widespread interest to identify unnatural juvenile mimics: (i) each of these juvenile hormones was found to be specific for a certain type of insect, which accounted for high selectivity but also limited the pest spectrum as potential insecticides; and (ii) natural juvenile hormones suffered from photochemical and hydrolytic instability (due to both epoxide hydration and ester hydrolysis) [1, 2]. Extensive research undertaken at Paul Djerassi-founded company, Zoëcon Corp., later acquired by Sandoz AG, resulted in three synthetic first-generation juvenile hormone mimics that were commercialized around 1975: hydroprene (1), kinoprene (2), and methoprene (3) (Figure 32.1). All of these compounds were initially produced as racemate but were later on substituted by their respective enantiopure active ingredients. The second-generation nonsesquiterpenoid active ingredient fenoxycarb (4) was developed and introduced to the market in 1985 by Dr R. Maag AG, which was later acquired by Ciba-Geigy AG and is now part of Syngenta AG. An additional ester or carbamate-derived commercial compound

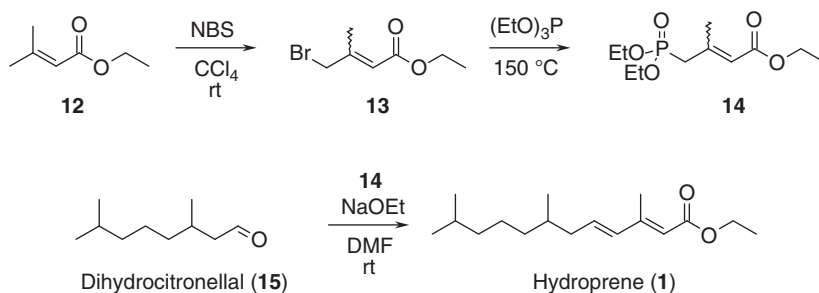
is no longer distributed (diofenolan **28**, Figure 32.5, *vide infra*), while Sumitomo's third-generation pyriproxyfen (**29**, Figure 32.5, *vide infra*) launched in 1995 is the biggest commercial success in this class of insect growth regulators (IGRs) with modest sales of 65 million USD in 2013 [9].

32.3

Synthesis

The synthetic chemistry of juvenile hormone mimics has been pioneered by Zoëcon Corp. using convergent olefination approaches. A typical research route is shown for the synthesis of hydroprene (**1**) (Scheme 32.1) [10, 11]. The required phosphonate **14** could be prepared via bromination of 3-methylbutenoate **12** to give bromide **13**, which was subjected to an Arbuzov reaction. At this stage, a wide range of aldehydes could be used in the Horner–Wadsworth–Emmons olefination to obtain the desired dienoates. As an example, phosphonate **14** could be coupled with aldehyde **15** to give hydroprene (**1**). Many of these aldehydes are available from natural sources or by simple derivatization thereof. Notably, under the reaction conditions, **14** was shown to be configurationally labile allowing for a diastereoconvergent approach [12].

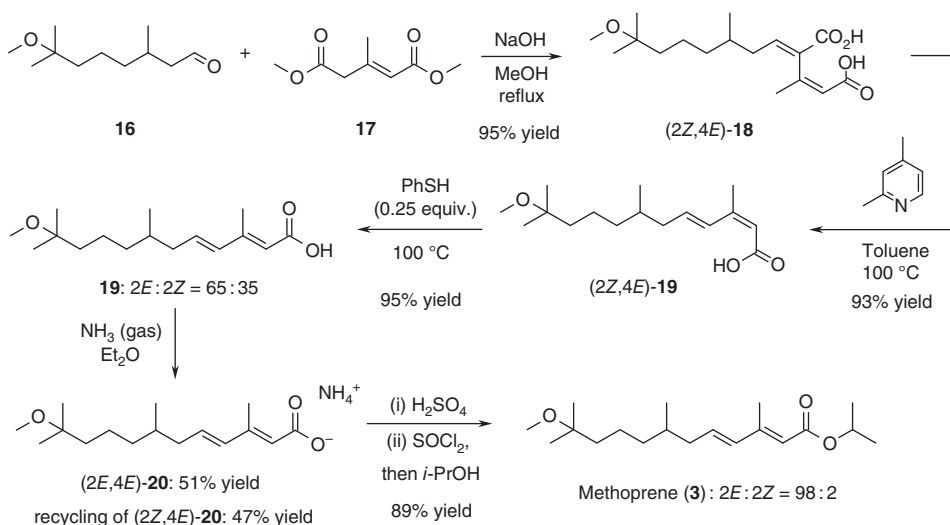
(a) Horner–Wadsworth–Emmons olefination



Scheme 32.1 Key steps in research route to hydroprene (**1**).

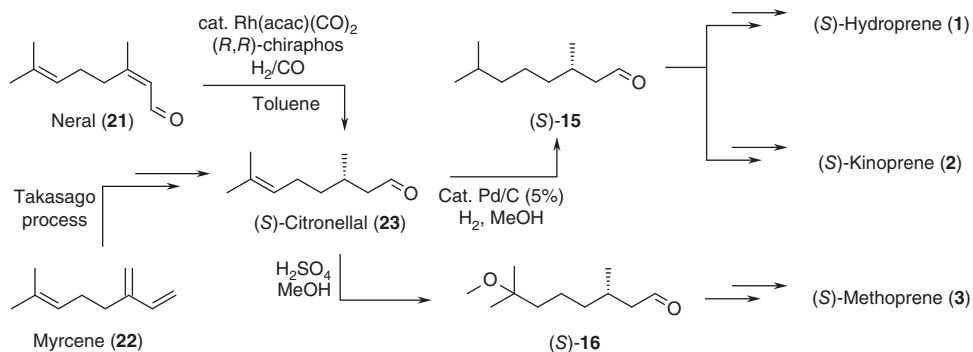
In a subsequent publication, the team around Henrick at Zoëcon Corp. also described a scalable, high-yielding route to methoprene (**3**) eliminating the need of a phosphorus-based olefination step (Scheme 32.2) [13]. Instead, the authors opted for an aldol condensation of isodehydroacetate-derived diester **17** with the prerequisite functionalized citronellal derivative **16** to give diacid (2*Z*,4*E*)-**18** in excellent yield. Lutidine-mediated decarboxylation provided the acid **19** in high yield and diastereomeric ratio, albeit the undesired 2*Z*,4*E*-isomer was exclusively formed. A highly efficient two-step epimerization process including recycling of the undesired isomer allowed to access methoprene (**3**) in excellent overall yield. To this end, a thiol-mediated double-bond isomerization of **19** allowed

(b) Aldol condensation and decarboxylation

**Scheme 32.2** Key steps in a second-generation approach to methoprene (3).

for equilibration in favor of the desired *2E,4E*-isomer. Gratifyingly, the desired isomer could be selectively precipitated as ammonium salt **(2E,4E)-20** while remaining **(2Z,4E)-20** was submitted to re-equilibration. Methoprene (**3**) was then obtained via the acid chloride and esterification in excellent diastereomeric purity.

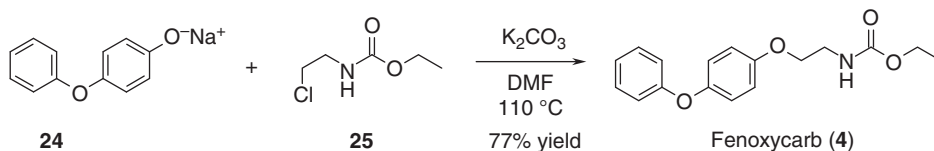
Already, early on, it was recognized that not only geometrical isomerism but also optical purity had a strong impact on the biological activity with the (*S*)-enantiomer being markedly more potent [14]. Using the aforementioned same disconnection, enantioselective approaches could be devised due to the availability of citronellal (**23**) in enantiopure form. Both enantiomers were initially prepared using an *ex-chiral pool* approach – both citronellol enantiomers are naturally occurring [14]. Over the past decades, several catalytic asymmetric industrial-scale approaches to (*S*)-**23** have been described (Scheme 32.3). (*S*)-Citronellal (**23**) is an important intermediate in the fragrance and flavor industry and is used for the synthesis of D-menthol. For example, BASF described a catalytic enantioselective hydrogenation of neral (**21**) using a chiral rhodium–phosphine complex [15]. On the other hand, the Takasago process for the preparation of enantiopure menthol starting from myrcene (**22**) is already a classic example of industrial enantioselective catalysis [16]. Here, the key step is a rhodium–phosphine-catalyzed enantioselective allylic isomerization of an allylic amine to an enamine. (*S*)-Citronellal (**23**) can be used to produce saturated (*S*)-hydroprene (**1**) and (*S*)-kinoprene (**2**) via straightforward alkene hydrogenation [17] leading to (*S*)-dihydrocitronellal (**15**) [14]. (*S*)-Methoprene (**3**) is available via acid- [18] rather than mercury salt-mediated [14] Markovnikov hydroalkoxylation to provide (*S*)-**16**. Initially introduced to the market as racemic



Scheme 32.3 Importance of chirality of juvenile hormone mimics.

mixtures, all juvenile hormone mimics of this class have been substituted later on by their optically pure (*S*)-enantiomers.

The nonsesquiterpenoid juvenile hormone mimic fenoxycarb (4) with its bisaryloxy ether scaffold has been prepared at Dr R. Maag AG following a straightforward sequence (Scheme 32.4) [19]. Key step is an aryl ether synthesis using sodium phenoxide **24** and chloroethyl carbamate **25**. A modification of this process has been published by Novartis Agro AG in 1997 [20].



Scheme 32.4 Synthesis of fenoxycarb.

32.4

Mode of Action

Juvenile hormone mimics (IRAC group 7) are IGRs, which are synthetic analogs of insect-specific hormones **5–11** (*vide supra*). These hormones are produced by a gland, the corpus allatum, and their absence or presence in the insect lifecycle has distinct morphological effects. Generally, the presence of juvenile hormones or its analogs at each molt prevents the insect to go to the next life stage. In nymphs and larvae, metamorphosis to an adult stage is prevented and another larval stage is being produced, often along with varying degrees of deformation. Effects on reproduction have also been observed and are of use in insect control. Adult stages may be unable to reproduce, and true ovicidal activity by disruption of embryogenesis has been observed [1, 21].

The disruption of metamorphosis is of limited utility in crop protection as it is highly dependent on the larval stage, that is, it is challenging for the timing of

application. It has been seen even as detrimental due to the formation of “super larvae.” More importantly, the slow speed of effect and lack of activity on early larval stages is disadvantageous for many applications. Yet, if the adult and not the immature life stages are pests, for example, in mosquito control, juvenile hormone mimics were found to be effective. Beyond these applications, it is mostly the sterilization and ovicidal effect that has proven to be useful in insect control. A principal challenge remains since insects react to very specific juvenile hormones and the synthetic mimics tend to be more generally applicable but still remain comparably narrow in their pest spectrum [1, 21].

32.5

Biological Activity

Juvenile hormone mimics are often considered as green pesticides having an insect-specific mode of action, favorable mammalian toxicology, and are readily degraded in the environment. Based on the aforementioned considerations, their use is mostly limited to noncrop applications [22]: (*S*)-hydroprene (**1**) (Biopren BH™, Gentrol™) and (*S*)-methoprene (**3**) (Altosid™, Biopren BM™) both are used to control cockroach, moth, flea, and fire ant as well as mosquito populations. (*S*)-Methoprene (**3**) is seen as the most potent among the sesquiterpenoid analogs. The use patterns are typically in the protection of stored products, animal, or public health. (*S*)-Kinoprene (**2**) (Enstar II™) is used in glasshouse settings for control of aphids, whiteflies and scales in ornamentals. Fenoxycarb (**4**) (Insegar™) has found noncrop applications (stored product protection, public health, cockroaches, fire ants) but also specialist niches in ornamentals, fruits, and vegetables for control of Lepidoptera and sap-feeding insects.

32.6

Structure–Activity Relationship

The systematic and broad structure-activity relationship exploration program undertaken at Zoëcon Corp. in order to identify synthetic juvenile hormone mimics aimed to address major shortcomings of natural analogs: reduction of susceptibility toward (i) ester hydrolysis and (ii) epoxide hydration. Most importantly, it was, however, to identify less specific juvenile hormone analogs showing good biological activity across a range of insects. Figure 32.3 provides details for some of the most significant aspects of the structure–activity relationship for juvenile hormones and their analogs. It must be stated, however, that subtle differences between individual insect pests have often been observed [23]. For a much more detailed set of data and their interpretation, Clive Henrick’s landmark review is recommended as a source of information [3].

In order to prevent deactivation by epoxide hydration, several replacements for the 10,11-epoxide were investigated (Figure 32.3a). While the alkene was very

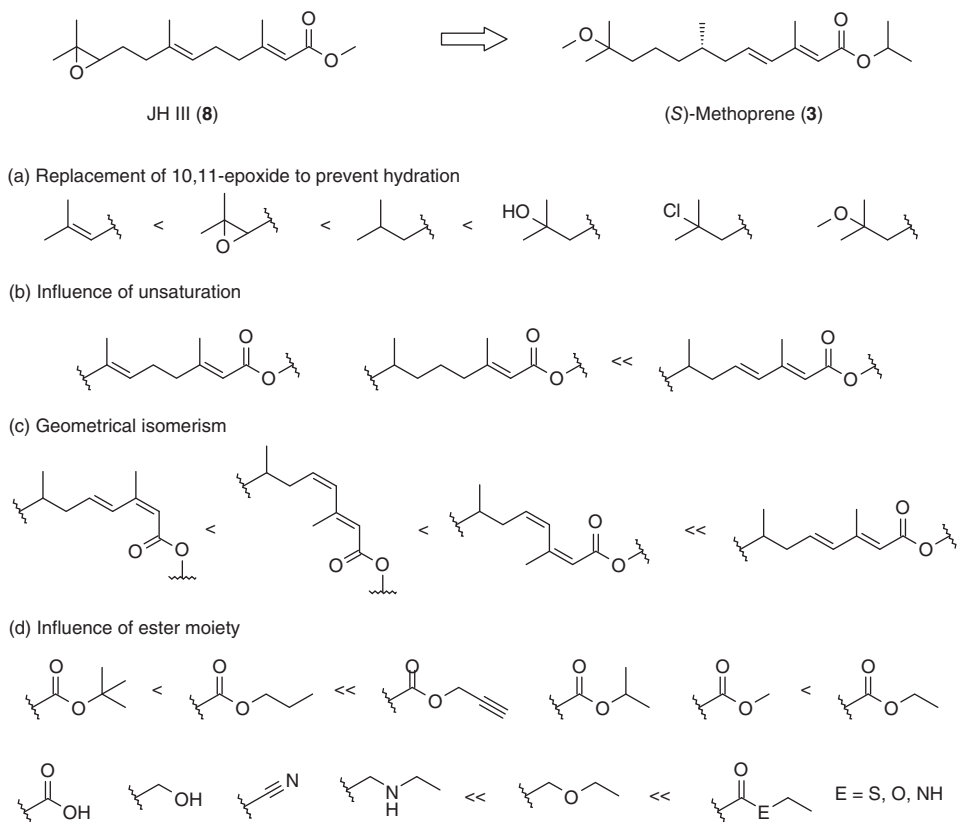


Figure 32.3 SAR of juvenile hormone analogs (a–d).

weak, the 10,11-saturated version was more active than parent epoxide. Among the best compounds were 11-chloro and 11-methoxy analogs with the latter being preferred. The unsaturation at C-6 was also subject to variation (Figure 32.3a). Importantly, conjugated dienoic acid gave a boost in biological activity. It must be noted here that this change did not improve the UV sensitivity of these lead compounds. In fact, compounds **1–3** are readily degraded in UV light. Along with walking the alkene into conjugation, a new stereogenic center at C-7 was generated, the superior biological activity of the *S*-enantiomer was consistently superior across this class. Within the dienoic acid class, only the *E,E*-configured diastereomer showed the appreciable levels of biological activity with the *Z,Z*-configured isomers being most detrimental to obtain satisfying potency (Figure 32.3c). Many variations of the ester group were investigated as part of this research program. The intricate balance between intrinsic biological activity favoring small esters and hydrolytic stability favoring larger esters ultimately led to propargyl, ethyl, and isopropyl esters being preferred (Figure 32.3d). Among ester replacements, thioesters and selected amides had high intrinsic activity but ultimately did not

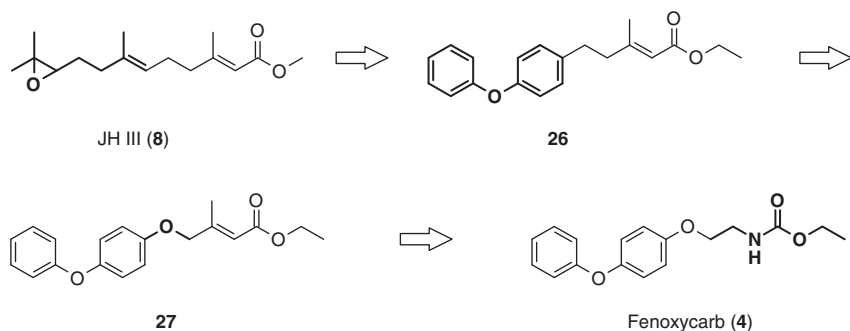


Figure 32.4 From juvenile hormone esters to aryl ethers: discovery of fenoxycarb.

match the best esters – perhaps due to an interplay of hydrolytic stability and volatility. Other functions such as ethers, amines, nitriles, and alcohols turned out to be very weak if not inactive. Also, the free carboxylic acid did not show any biological activity.

The discovery of fenoxycarb (**4**) provides an illustrative example of how addressing photostability and epoxide hydration led to a juvenile hormone mimic with better fit to outdoor crop protection settings (Figure 32.4). Initially, the replacement of the unsaturated terpenoid chain in **8** with a simple and photo-stable bisaryloxy motif by a team at Ciba-Geigy AG led to an active compound **26** [24]. Around the same time, a CH_2 -for-O replacement to bisaryloxyether **27** was identified, which serves as a structural link to other juvenile hormone analogs [25]. Strikingly simple ethyl carbamate fenoxycarb (**4**) identified by the team at Dr R. Maag AG is one endpoint of this successful research direction. The carbamate functionality not only reduces hydrolytic and UV instability but also simplifies the structure by removing a geometrically defined double bond [19].

Another line of research led to juvenile hormone mimics devoid of ester or carbamate functional groups, namely diofenolan (**28**) [26] of Ciba-Geigy AG and pyriproxyfen (**29**) [27] of Sumitomo Chemical Co. (Figure 32.5). Both of them are successful ester replacements that maintain the methyl group as a chiral element. While **28** is no longer in commercial distribution, pyriproxyfen (**29**) is, from a commercial perspective, the most successful product originating from research on juvenile hormone mimics.

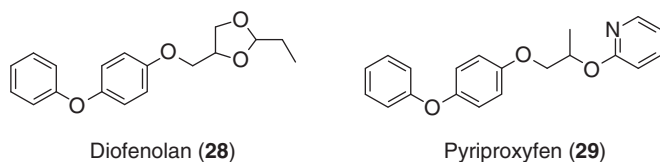


Figure 32.5 Juvenile hormone mimics with ester or carbamate replacements: diofenolan and pyriproxyfen.

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33

***N*-Benzoyl-*N'*-Phenyl Ureas as Insecticides, Acaricides, and Termiticides**

Peter Jeschke

33.1

Introduction

The *N*-benzoyl-*N'*-phenyl ureas, also called BPUs, have been used for almost 40 years as insect growth regulators (IGRs) [1]. According to the Insecticide Resistance Action Committee (IRAC, an expert committee of CropLife International; <http://www.irac-online.org>), the mode of action (MoA) of the Main Group 15 “Inhibitors of chitin biosynthesis” is represented by benzoylurea insecticides. Some BPUs have additional acaricidal or termiticidal activity and can be used as ectoparasitocides in veterinary medicine. This chemical subgroup consists mainly of halogenated BPUs:

- 1) The insecticides/acaricides diflubenzuron (**1**, Dimilin®) [2], flucycloxuron (**2**, Andalin®) [3], and flufenoxuron (**3**, Cascade®) [4],
- 2) The insecticides triflumuron (**4**, Alsystin®) [5], teflubenzuron (**5**, Dart®) [6], chlorfluazuron (**6**, Atabron®) [7], and novaluron (**7**, Rimon®) [8]
- 3) The insecticides/termiticides bistrifluron (**8**, Xterm™) [9], noviflumuron (**9**, Recruit III) [10], hexaflumuron (**10**, Consult®) [11] as well as lufenuron (**11**, Match®) [12] (Figure 33.1).

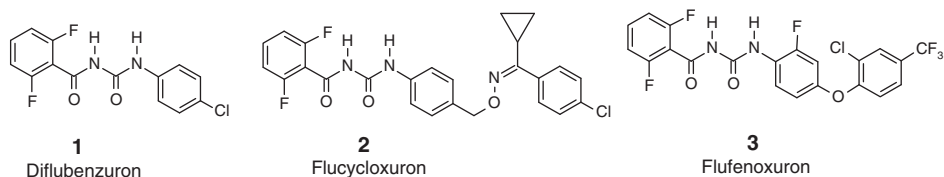
Because of their specific MoA [13], their low risk for *nontarget* organisms including many beneficial arthropods (very low acute and chronic vertebrate toxicity) and the environment, they are an important tool in different Integrated Pest Management (IPM) programs.

33.2

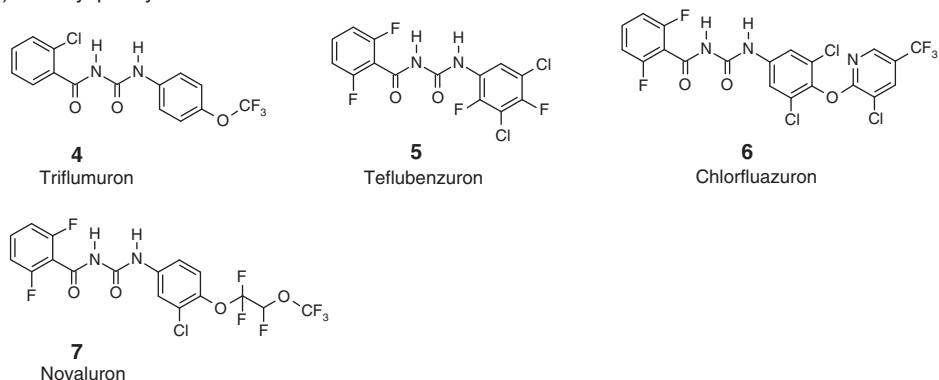
History

The class of BPUs was discovered during syntheses within an herbicide project. Scientists at Philips-Duphar B.V. (later Uniroyal) in the Netherlands combined two herbicide structures with different MoAs, the cellulose-biosynthesis inhibitor

(a) Benzoyl phenyl ureas as insecticides/acaricides:



(b) Benzoyl phenyl ureas as insecticides:



(c) Benzoyl phenyl ureas as insecticides/termiticides:

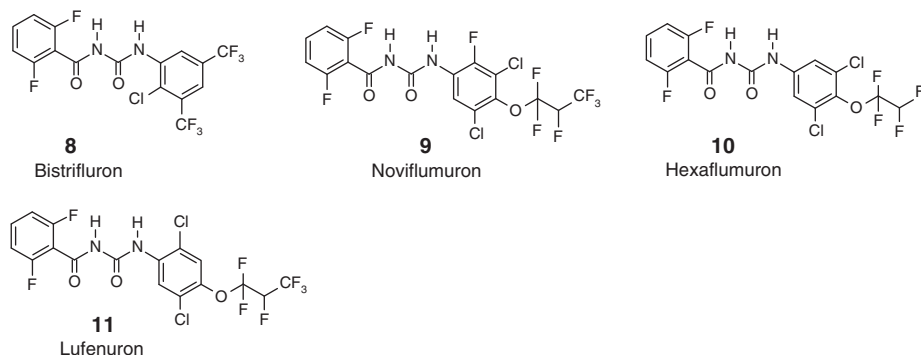


Figure 33.1 The 11 commercialized BPUs mainly used as (a) insecticides/acaricides, (b) insecticides, and (c) insecticides/termiticides.

(CBI), 2,6-dichlorobenzonitrile (**12**, dichlobenil), and the photosystem II (PS II) inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethyl-urea (**13**, diuron), forming *N*-(2,6-dichlorobenzoyl)-*N'*-(3,4-dichlorophenyl)-urea DU19111 (**14**) [14] (Figure 33.2).

The *nonherbicidal* BPU **14** shows insecticidal activity [15]. Although without effects on adult insects, **14** interfered with the molting process of the immature stages of larvae or nymphs causing death due to the IGR activity. In 1974, further optimization of the lead structure at Philips-Duphar B.V. resulted in diflubenzuron

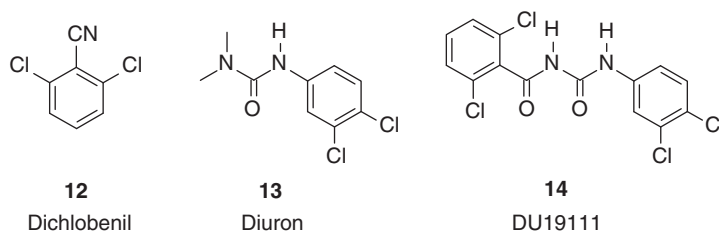


Figure 33.2 Identification of the insecticidal active *N*-(2,6-dichlorobenzoyl)-*N'*-(3,4-dichlorophenyl)-urea (**14**, DU1911) by combination of the herbicide structures dichlobenil (**12**) and diuron (**13**).

(**1**), the first representative of the BPUs, which was brought to the market in 1975 as an *nonsystemic* insecticide for control of lepidopteran and coleopteran pests in fruits, cotton, soybeans, and vegetable crops [16] (Section 33.5, Table 33.1) with slightly acaricidal activity (e.g., mite control on greenhouse ornamentals).

At the beginning, interest in the agrochemical industry was limited, because of the already established, fast-acting neurotoxic acetylcholinesterase (AChE) inhibitors such as organophosphates (OPs), carbamates, and the sodium-channel modulators such as pyrethrins and later the pyrethroids. Because of the slow-acting BPUs (affected larvae may continue to feed until the next molt), there was a conception concerning marketing of IGRs. Generally, for farmers and growers, it is important to see a rapid and efficient insecticidal activity directly after spray application. Further problems with the BPU class were seen in their lack of or limited contact activity including the absence of systemic activity, which has strongly limited their insecticidal pest spectrum and their use against hidden insects. The formulation difficulties of BPUs resulted from their unusual physicochemical properties, as outlined in Section 33.5. However, in recent years, some of the toxicological and environmental problems associated with neurotoxic insecticides have changed this view and led to further efforts to development and commercialize BPU products with improved activities. The discovery of BPU insecticides with additional and improved acaricidal activity, such as flucycloxuron (**2**) and flufenoxuron (**3**), led to enhanced interest, particularly because of mite-resurgence problems caused by declines in the population of their natural enemies due to overuse of sodium-channel modulators such as pyrethroid insecticides.

Today, other BPUs demonstrate their use outside of crop protection as part of a bait matrix for the long-term control of urban pests such as termites, for example, bistrifluron (**8**), noviflumuron (**9**), hexaflumuron (**10**), and lufenuron (**11**). Both **8** and **11** are major active ingredients in subterranean termite long-term protection systems such as Xterm Defence against Termites from Sumitomo (contains 1% **8** as a bait) and in the Sentricon™ Termite Colony Elimination System from Dow AgroSciences LLC. Similarly to the BPUs **8–10**, flufenoxuron (**3**) has also been formulated as a bait (Flurox™) by the Janssen company and can be used in wood protection against subterranean termite infestations.

Table 33.1 Biological profiles of commercialized *N*-benzoyl-*N'*-phenyl urea insecticides (1–11).

BPU insecticides	Launch year	Agricultural crops (selection)	Agricultural pests (selection)
Diflubenzuron (1)	1975	Fruit, cotton, soybean, maize, vegetable/horticultural crops	Lepidoptera, Coleoptera, grasshoppers, larvae of flies spider mites, codling moth, leaf miner
Flucycloxuron (2)	1988	Fruit, vegetable, ornamentals, vine	Lepidoptera, phytophagous spider mites, scale insects
Flufenoxuron (3)	1989	Fruit, vine, citrus, tea, cotton, maize, soybean, vegetable	Lepidoptera, Psyllidae, Diptera, Coleoptera
Triflumuron (4)	1979	Fruit, soybeans, vegetable, cotton, forest trees	Lepidoptera, Coleoptera, Coleoptera
Teflubenzuron (5)	1986	Fruit, potatoes, cotton, vegetable, citrus, sorghum, soybeans	Lepidoptera, Coleoptera, Diptera, Hymenoptera
Chlorfluazuron (6)	1989	Vegetable, tea, cotton, fruit, potatoes, soybean, maize cabbages, cotton, fruit, tomatoes, ornamentals, vegetables cabbage, apples, other crops	Lepidoptera, thrips, whiteflies, beetle larvae
Novaluron (7)	1998		Lepidoptera, Coleoptera, whiteflies, leaf miner
Bistrifluron (8)	2015		Lepidoptera, whiteflies, termites
Noviflumuron (9)	2003	Trees fruit, cotton, potatoes, cotton, apples, citrus, vegetables, tea, turf	Hymenoptera, ants, termites
Hexaflumuron (10)	1989		Lepidoptera, Coleoptera, Diptera, Homoptera
Lufenuron (11)	1993		Lepidoptera, Coleopteran, rust mites, whiteflies

Modified after Sheets [14].

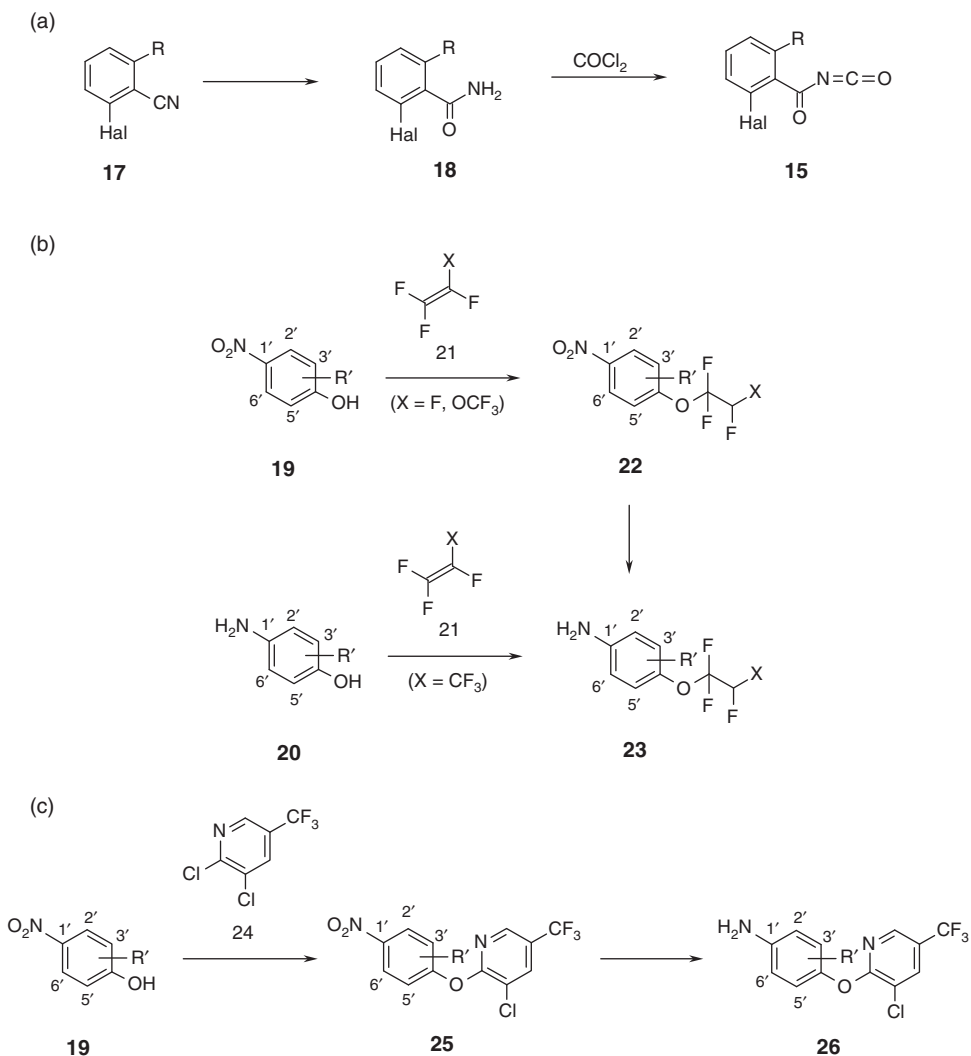
Because of their nontoxicity to vertebrates, BPUs are also used in veterinary medicine for the control of sheep lice, flies, blowflies (larvae) or ticks such as diflubenzuron (**1**; Magnum®), triflumuron (**4**, Starycide®), or lufenuron (**11**, Program®), and in home (e.g., **4** and **9**) or against animal and human health pests such as fleas, ticks, and cockroaches.

33.3

Synthesis

The key intermediates for commercialized BPU insecticides, acaricides, and termiticides are benzoyl isocyanates **15** (R/Hal = F; R = H, Hal = Cl) and substituted arylamines **16**, respectively. Different synthetic pathways have been investigated to develop practical processes for both intermediates based on various

commercially available starting materials such as benzonitriles **17** or benzamides **18** ($R/\text{Hal} = \text{F}$; $R = \text{H}$, $\text{Hal} = \text{Cl}$) and in the most cases halogen-substituted nitro- or amino-phenols **19** and **20** (Scheme 33.1).



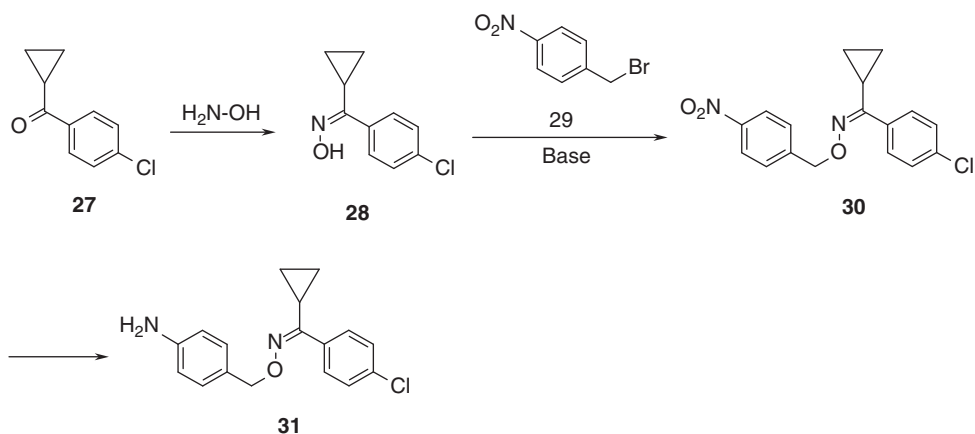
Scheme 33.1 Synthesis of key the intermediates benzoyl isocyanates **15** and substituted arylamines **23** and **26**. (a) Synthesis of benzoyl isocyanates. (b) Synthesis of halogen-substituted amino- or nitrophenols. (c) Synthesis of pyridine-2-yl-substituted aminophenols.

Starting with benzonitriles **17**, the benzamides **18** can be prepared and their carbonylation with phosgene or oxalyl dichloride in toluene (reflux, 5 h) or dichloroethane (reflux, 18 h) leads to benzoyl isocyanates **15**.

The halogen-substituted nitro- or amino-phenols (**19**) and (**20**) can be treated with *X*-substituted trifluoroalkenes **21** (*X* = F, CF₃, OCF₃), for example, base-catalyzed in *N,N*-dimethyl-formamide at room temperature, forming the halogenalkoxy-substituted nitro- or amino-phenols **22** and **23**. The halogenalkoxy-substituted aminophenols **23** can easily be obtained from **22** by hydrogenation. These intermediates are important building blocks for the preparation of novaluron (**7**; *X* = OCF₃), noviflumuron (**9**; *X* = CF₃), hexaflumuron (**10**; *X* = F), and lufenuron (**11**; *X* = CF₃).

On the other hand, coupling of 2,6-difluoro-4-nitro-phenol (**19**; *R'* = 3'-Cl, 5'-Cl) with 2,3-dichloro-5-(trifluoromethyl)-pyridine (**24**) leads to the pyridine-2-yl-substituted nitrophenol (**25**), which can be hydrogenated to the intermediate **26**, useful for the synthesis of chlorfluazuron (**6**).

Starting with (4-chlorophenyl)cyclopropyl-methanone (**27**) and following formation of oxime **28**, the *O*-[(4-nitrophenyl)methyl]oxime **30** can be prepared by a base-catalyzed *O*-benzylation with 4-nitro-benzylbromide (**29**). After hydrogenation of **30**, the *O*-[(4-aminophenyl)methyl]oxime key intermediate **31** for the synthesis of flucycloxuron (**2**) is available as outlined in Scheme 33.2.

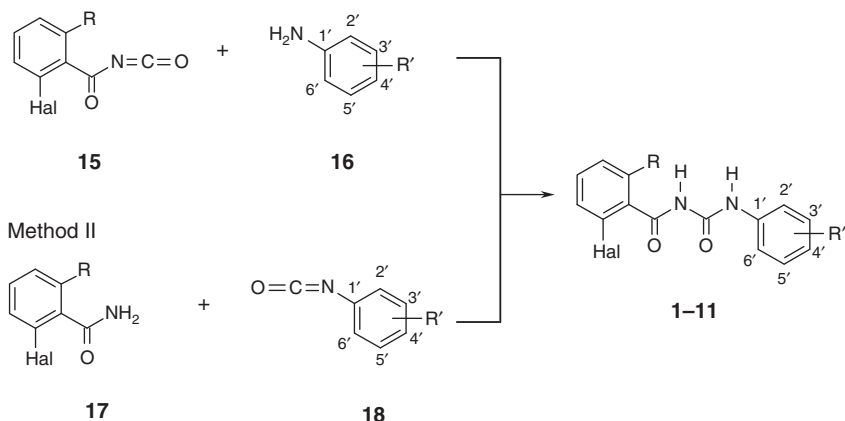


Scheme 33.2 Preparation of the *O*-[(4-aminophenyl)methyl]oxime key intermediate **31** for the synthesis of flucycloxuron (**2**).

The final synthesis of the BPUs flucycloxuron (**2**), triflumuron (**4**), teflubenzuron (**5**), novaluron (**7**), bistrifluron (**8**), and noviflumuron (**9**) can be carried out by treatment of benzoyl isocyanates **15** (*R*/Hal = F for **2**, **5**, **7**–**9**; *R* = H, Hal = Cl for **4**) and substituted arylamines (**16**) in toluene (r.t., 15 h) or dichloroethane (40 °C, 1 h) according to Method I (Scheme 33.3).

Alternatively, the preparation of the BPUs diflubenzuron (**1**), teflubenzuron (**5**), chlorfluazuron (**6**), hexaflumuron (**10**), and lufenuron (**11**) starts with the 2,6-difluoro-benzamide (**17**, *R*, Hal = F) and the substituted arylisocyanates **18** according to Method II (Scheme 33.3).

Method I



Scheme 33.3 Methods I and II for syntheses of *N*-benzoyl-*N'*-phenyl ureas (1)–(11).

For flufenoxuron (3), the use of 2,6-difluoro-benzoyl-isothiocyanate (in **15** $\text{N}=\text{C}=\text{S}$ instead of $\text{N}=\text{C}=\text{O}$; $\text{R}/\text{Hal}=\text{F}$) has been described [17], leading in a first step to a *N*-benzoyl-*N'*-phenyl thiourea, which can be easily transformed into the BPU by desulfuration of the $\text{C}=\text{S}$ -group.

33.4

Mode of Action

The BPU insecticides have a *non*neurotoxic MoA at a target site that is absent in vertebrates and act as IGRs by inhibiting chitin formation in the insect cuticle and thereby causing abnormal endocuticular deposition, abortive molting, and finally, the death of insects [18]. The BPUs control the immature stages of numerous insects from various orders, in which larvae/nymphs are the most damaging stages. For example, in insects and rust mites, this MoA can result in larvicidal and ovicidal effects at the time of molting of the larvae or at hatching of the eggs. The onset of insecticidal activity of BPUs is slow, which means that several days are required before any reduction is detected in larval numbers, although cessation of larval feeding often occurs earlier.

Initial investigations demonstrate that incorporation of uridine diphosphate-*N*-acetyl-glucosamine (UDP-NAGA) to chitin was clearly inhibited *in vivo* or *in situ* (isolated integument incubated in a tissue culture medium). But, all further studies trying to prove the action of BPUs on any part of the chitin synthesis pathway in insects in cell-free systems were unsuccessful [19]. Generally, it is difficult to isolate a soluble enzyme preparation from insects or other arthropods; and where this has been successfully achieved, BPUs do not inhibit it. Furthermore, BPUs demonstrate no inhibitory actions on fungal systems *in vivo* as well as in cell-free systems, although fungi have roughly equivalent chitin synthesis pathways as

insects [19a]. Different postulated mechanisms for BPUs have been described by Cohen [20]. Therefore, the MoA of BPUs remained unresolved. The most recent hypothesis regarding the MoA of BPUs stems from the observation that diflubenzuron (**1**) stimulates the protein-kinase-catalyzed phosphorylation of a 39-kDa protein found in the integument of a newly molted *Periplaneta americana* (American cockroach) [21]. This effect is in common with other compounds that disrupt pH gradients, such as uncouplers, and ionophores, across vacuolar membranes.

A few years ago, it was established that the insect sulfonylurea receptor (SUR) protein in an intracellular vesicle preparation from the cuticle of newly molted *P. americana* as well as *Blattella germanica* (German cockroach) is likely the actual target site of diflubenzuron (**1**) to cause inhibition of chitin synthesis [22]. The rule of SUR has been determined by using the sulfonylurea, 5-chlor-*N*-[2-[4-(cyclohexylcarbamoyl-sulfamoyl)phenyl]ethyl]-2-methoxy-benz-amide (glibenclamide), a typical SUR-specific inhibitor. In this case, both diflubenzuron (**1**) and glibenclamide have been shown to cause the depolarization of the vesicle membrane through inhibition of the potassium channel, which resulted in their inhibition of chitin synthesis [22b]. The SUR is associated with four pore-forming (Kir6.x) potassium channel subunits (four SUR subunits and four Kir6.x subunits), which form the adenosine triphosphate (ATP)-sensitive potassium channels [23].

It could recently be further demonstrated that exposure of *Panonychus citri* (citrus red mite) larvae to diflubenzuron (**1**) for 6 h leads to a significantly high mortality rate of *P. citri*, and the *mRNA* expression levels of a chitin synthase 1 gene (*PcCHS1*; these are expressed 114.8-fold higher in the egg stage than in adult) are significantly enhanced. These results indicate a promising use of (**1**) to control *P. citri* by possibly acting as an inhibitor in chitin synthesis as indicated by the upregulation of *PcCHS1* in this mite after exposure to (**1**) [24].

33.5

Biological Activity

The biological activity and agricultural uses of BPU insecticides are in a broad range of important agricultural crops and insect pests such as Lepidoptera, Coleoptera, Hymenoptera, Diptera species including spider mites and termites (Table 33.1).

However, BPU insecticides have some of the most unusual physicochemical properties of any of the active ingredients used in crop protection. The BPUs are all crystalline, lipophilic solids with high melting points (e.g., diflubenzuron (**1**): 228 °C, chlorfluazuron (**6**): 226.5 °C decomposed). Consequently, these insecticides have extremely low vapor pressure (e.g., flufenoxuron (**3**): 6.5×10^{-12} , hexaflumuron (**10**): 5.9×10^{-9}) and very low water solubility (e.g., flucycloxuron (**2**): <0.001, novaluron (**7**): 0.053).

The log K_{ow} values of BPUs are in the range of 3.9 (for diflubenzuron (**1**)) to 6.9 (for flufenoxuron (**3**)); therefore, they cannot be translocated in plants and have no systemic insecticidal activity. These properties result in both advantages and

disadvantages for crop protection uses with regard to their toxicological behavior (low intrinsic toxicity to vertebrates, low absorption by the mammalian gut or skin, and no accumulation in the body), ease of formulation (but, difficulties in preparing emulsifiable concentrates (ECs)), and interaction with the environment. Because of their strong soil adsorption ($K_{oc} = 760 - 30\,000$), BPUs have no leaching effects and groundwater contamination.

Overall, however, the BPUs (2-R/6-Hal = 2,6-F₂) demonstrate an impressively large pesticidal spectrum (Table 33.1). For example, diflubenzuron (**1**) is effective at 25–75 g a.i./ha against most leaf-feeding insects in forestry; in concentrations of 0.01–0.015% a.i. against *Cydia pomonella* (codling moth), leaf miners, and other leaf-feeding insects in top fruit; in concentrations of 0.0075–0.0125% a.i. against *Phyllocoptruta oleivora* (citrus rust mite) in citrus and at a dosage of 50–150 g a.i./ha against a number of pests in cotton plant, for example, *Anthonomus grandis* (cotton boll weevil) or *Spodoptera frugiperda* (fall armyworm) in soybeans against *Chrysodeixis includens* (soybean looper) as well as in maize against *S. frugiperda*.

Flucycloxuron (**2**) shows a stronger efficacy by ingestion than by contact and is better ovo-larvicidal active against spider mites, such as *Panonychus ulmi* (European red mite) or *Tetranychus* species, than several commercial products. Recommended concentrations for control of mites on fruit crops are 0.01–0.015% a.i. Good activity has been identified against codling moth, leaf miners, and some leaf rollers in pome fruit. In ornamentals grown under glass or plastic foil, lower concentrations (0.005–0.001% a.i.) can be used. In grapes, the recommended dosage is 125–150 g a.i./ha.

Flufenoxuron (**3**) is active against immature stages of many phytophagous mite species after foliar application (40–250 g a.i./ha) and shows strong efficacy against insect pests on various agricultural crops as outlined in Table 33.1. Adult insects exposed to residues are not controlled, but they produce a high proportion of non-viable eggs.

Teflubenzuron (**5**) may affect fertility of female insects after contact or ingestion. It controls Lepidoptera, Coleoptera, Diptera, *Aleyrodidae*, *Hymenoptera*, *Psyllidae*, and *Hemiptera* larvae in various agricultural crops at rates in a range from 10 to 150 g a.i./ha.

Chlorfluazuron (**6**) shows high activity on larval stages of lepidopteran insects such as *Spodoptera litura* (Oriental leafworm moth) or *Plutella xylostella* (diamondback moth) with long residual activity (10–14 days) and low application rates (10–50 g a.i./ha). It has a very little negative impact on natural enemies and pollinator insects and can be used in IPM programs. In addition, (**6**) is active against *Bemisia tabaci* (sweet potato whitefly) as well as Thrips and can also be used on fruit, potatoes, ornamentals, and tea.

Novaluron (**7**) can be applied as a foliar spray broad-spectrum insecticide, and it acts from both ingestion and contact. The BPU is a powerful suppressor of lepidopteran larvae such as *S. frugiperda*, *S. littoralis* (Egyptian cotton leafworm), *Spodoptera exigua* (beet armyworm), *Helicoverpa armigera* (cotton bollworm), all species being major pests of corn, cotton, and vegetables as well as *Tuta absoluta*

(tomato leaf miner). In addition, no cross-resistance has been observed between novaluron (**7**) and other BPU's used against *B. tabaci*, *Spodoptera* spp., and *Lep-tinotarsa decemlineata* (Colorado potato beetle). The total larval mortality was obtained at a concentration of 1 mg a.i./l [25].

Bistrifluron (**8**) is suitable for the control of *Trialeurodes vaporariorum* (greenhouse whitefly) and *B. tabaci* and lepidopteran pests such as *P. xylostella* and *S. exigua* on Chinese cabbages, apples, and other crops. The application rate is in a range from 75 to 400 g a.i./ha.

Noviflumuron (**9**) is especially active against different ants, such as Red Imported Fire Ant *Solenopsis invicta* (red imported fire ant), *Monomorium pharaonis* (pharaoh ant), or *Tapinoma sessile* (odorous house ant). Against these ant pests, (**9**) exhibited effects that were detrimental to the colony by reduced brood development and survival, queen fecundity, and worker mortality. Furthermore, it is active against cockroaches, fleas, and termites.

Hexaflumuron (**10**) is used as insecticide and larvicide with stomach and some contact activity. It demonstrates a fast activity and has been used for control of a broad spectrum of insecticides, especially against major heliothine species. Furthermore, (**10**) is particularly active against young larvae and also has strong ovicidal activity due to its ability to penetrate egg shells.

Lufenuron (**11**) demonstrates more ingestion than contact activity. It has activity against larval and nymphal stages of arthropods, rust mites on citrus, and citrus whiteflies. Lufenuron (**11**) is not plant-systemic, but has some translaminar activity against *S. litura* on cotton leaves.

Finally, the differently substituted molecule triflumuron (**4**) (2-R = H; 6-Hal = 6-Cl) demonstrates a broad insecticidal activity combined with a strong feeding and contact efficacy and is active against chewing insect pests such as *S. frugiperda* (down to 0.064 ppm) and against *Phaedon cochleariae* (mustard leaf beetle) (down to 8 ppm), respectively [26].

33.6

Structure–Activity Relationship

During optimization of DU19111 (**14**), it was already found that there is small scope for variation in substitution pattern on the *N*-benzoyl moiety A, as only BPU's with at least one *ortho*-substituent R such as methyl, methoxy, trifluoromethoxy, or pentafluoroethoxy retained insecticidal activity. Most of the BPU's are halogenated by fluorine and chlorine atoms because Quantitative Structure-Activity Relationship (QSAR) studies of BPU's demonstrated that benzoylurea derivatives with a 2,6-difluoro substitution (2-R/6-Hal) in the *N*-benzoyl moiety A exhibit higher larvicidal activity than other substitutions (Figure 33.3) [27].

Generally, the *ortho*-substituents (2-R and/or 6-Hal) revealed a tendency for the insecticidal activity related to the substituents 2-R/6-Hal in the following order: 2,6-Cl₂ < 2-H, 6-Cl < 2,6-F₂.

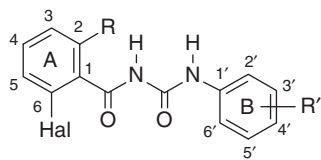


Figure 33.3 The general structures of BPU insecticides, acaricides, and termiticides.

The intense search for active BPUs provided further derivatives containing an α -fluoroalkoxy residue in the 4'-position as exemplified by novaluron (**7**) ($3'$ -R = Cl; $4'$ -R = O-CF₂-CHF-O-CF₃), noviflumuron (**9**) ($2'$ -R = F, $3'$, $5'$ -R = Cl; $4'$ -R = O-CF₂-CHF-CF₃), hexaflumuron (**10**) ($3'$, $5'$ -R = Cl; $4'$ -R = O-CF₂-CHF₂), and lufenuron (**11**) ($2'$, $5'$ -R = Cl; $4'$ -R = O-CF₂-CHF-CF₃). They are all far more potent than diflubenzuron (**1**) against various crop pests [28].

The beneficial steric effects of both fluorine atoms in the 2-R and 6-Hal position on the inhibition of chitin synthase [1] and the difference in environmental stability factors such as soil degradation half-life caused by the presence of these atoms. The chemical and metabolic degradation of BPUs has been described [29]. The *N*-(2,6-difluorobenzoyl)-*N'*-phenyl ureas are stable at acidic pH values but are hydrolyzed at pH 9–10 to give 2,6-difluorobenzoic acid and a 4'-chlorophenyl urea. In contrast to the conformation of the corresponding and less active 2,6-dichlorobenzoyl urea, which degrades in between 6 and 12 months, forming the 2,6-dichloro-benzamide, 4'-chloro-aniline, and carbon dioxide, the 2,6-difluorobenzoyl moiety is in plane with the whole urea structure [30].

Among the currently commercialized BPUs, only triflumuron (**4**; R = H, Hal = Cl, R' = 4'-OCF₃) does not have the 2,6-difluoro substitution pattern. During the structural optimization of triflumuron (**4**) derivatives with activity against *noncrop* insects (mosquitoes, termites, cockroaches) and coleopteran pests such as *P. cochleariae*, the *N*-2-chlorobenzoyl moiety (2-R = Cl) was selected. However, some of the most successful substitution patterns include the use of F₃C and F₃CO groups as the so-called pseudohalogens. As demonstrated for the *N'*-phenylamine moiety, the 2',3',5'-F₃, 4'-F₃C and 2',3'-F₂, 4'-F₃C substitution patterns are most favorable. These compounds have a good activity against *P. cochleariae*, down to 0.1 ppm. Furthermore, BPUs containing *N'*-phenylamine moieties such as 2',3',5'-F₃ or 4'-CF₃ and 2',3'-F, 4'-CF₃ with fluorine atoms show a good activity against two different lepidopteran pests, *S. frugiperda* and *P. xylostella*, down to 0.01 ppm. During the further optimization of the *N'*-phenylamine moiety, an F₃CO residue in the 4'-position was found to be beneficial. One of the most active compounds is a BPU, containing a 3'-Cl, 4'-F₃CO substitution pattern in the *N'*-phenylamine moiety. It was found that the introduction of one fluorine atom at the 5- or 6-position together with an additional substituent such as an F₃C or F₃CO group in the 3'-position strongly influences the selectivity for both coleopteran and lepidopteran pests, such as *P. cochleariae* and *P. xylostella* [29].

In recent years, mostly Chinese research groups designed and synthesized various fluoro-substituted BPUs with new substitution pattern in order to improve

the physicochemical properties of the commercial IGRs. For example, the introduction of a heptafluoroisopropyl residue ($4'\text{-R} = \text{CF}(\text{CF}_3)_2$) in $4'$ -position leads to the BPU **32**, which has a better solubility in acetone (around 120 g/l at 25 °C) [31] than diflubenzuron (**1**) (6.5 g/l at 20–25 °C) (Figure 33.4).

Furthermore, results from field trials indicated that **32** (Figure 33.4) applied as 5% EC exhibited similar efficacy comparable with that of hexaflumuron (**10**) against *P. xylostella* and similar efficacy to that of reference chlorfluazuron (**6**) against this imported cabbage worm.

Surprisingly, further modified BPUs, such as **33**, and **34** ($4'\text{-R} = \text{O-CH}(\text{CF}_3)_2$) (Figure 33.4), exhibited higher activities against the *Mythimna separata* (Oriental armyworm) and mosquito than hexaflumuron (**10**) [32].

Based on the oxime group in flucycloxuron (**2**), novel *O-tert*-butyl oximes such as NK-17 (**35**) ($4'\text{-R} = \text{CH}=\text{N-O-C}(\text{CH}_3)_3$) were prepared (Figure 33.5) [33].

It was found that the insecticidal activity of NK-17 (**35**) against *S. exigua* was 2.69 times and 1.93 times those of chlorfluazuron (**6**) and hexaflumuron (**10**), respectively. The efficacy of **35** against *P. xylostella* was 1.36 times and 1.90 times those of **6** and **10** in laboratory. The insecticidal mechanism of NK-17 (**35**) was explored by utilizing the fluorescence polarization method. NK-17 (**35**) could bind to SUR of *B. germanica* with stronger affinity comparing to diflubenzuron (**1**) and glibenclamide, which suggested that NK-17 (**35**) may also act on the site of SUR, inhibiting the chitin synthesis in insect body [23]. On the basis of SUR, new BPUs containing amide and sulfonate groups (e.g., $4'\text{-R} = \text{SO}_2\text{-O-CH}_2\text{CF}_3$) as shown in the structure **36** were designed and synthesized (Figure 33.5). The BPU displays already a comparable level of insecticidal activity against *M. separate* and *P. xylostella* as the flucycloxuron (**2**) [34].

Several years ago, it was demonstrated that the insecticidal potency and onset of efficacy of biologicals such as *S. litura* nucleopolyhedrovirus (SINPV) can be increased by their coadministration with BPUs such as chlorfluazuron (**6**). Due to its ability to disrupt the peritrophic matrix of insects, an easier entry of the virus

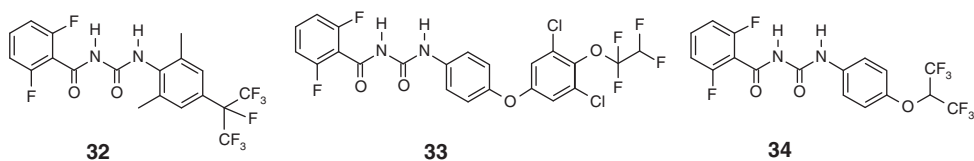


Figure 33.4 Structures of novel BPU insecticides **32–34**.

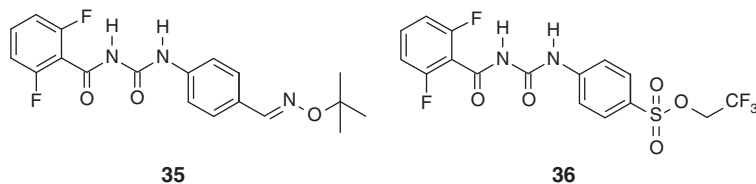


Figure 33.5 Structures of the BPU insecticides NK-17 (**35**) and **36**.

to the gut is possible [35]. This combined approach could open new avenues for the use of BPU with *nonchemical* agents such as SINPV.

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34

Pyrethroid Esters for the Control of Insect Pests

Régis Mondière and Fides Benfatti

34.1

Introduction

The pyrethroid insecticide family is an incredibly prolific class of compounds. According to the IRAC classification, more than 40 active ingredients belong to this sodium-channel modulator family. Their golden age was probably during the 1980s when replacement of older chemistries (organophosphate, carbamates, etc.) was needed and with the invention of particularly potent compounds such as deltamethrin [1]. The introduction of insect-resistant crops in the 1990s, particularly of genetically modified *Bacillus thuringiensis* cotton strains, reduced their market share. However, in 2013, pyrethroids still represented 17% of the global market for insecticides [2]. The two largest products in terms of sales are lambda cyhalothrin (1) (Syngenta) and deltamethrin (2) (Bayer CropScience), both accounting for multihundred million dollar sales in 2013. Some selected pyrethroid representatives are depicted in Figure 34.1.

The pyrethroid family encompasses a large set of molecules with very diverse structures, which actually go beyond the carboxylic ester motif. The following classification can be proposed [3]:

- *Class A*: Carboxylic esters that are very reminiscent of the naturally occurring pyrethrins. These compounds all contain a cyclopropyl carboxylic ester, for example, lambda cyhalothrin (1) and deltamethrin (2).
- *Class B*: Carboxylic esters that do not contain any cyclopropyl moiety such as esfenvalerate (5).
- *Class C*: Compounds that do not contain any carboxylic ester motif, but another linker such as an oxime or ether as in etofenprox (6).

Despite the apparent structural divergences, these three classes all share very similar 3D shapes, which then interact with the insect sodium channel in a similar fashion.

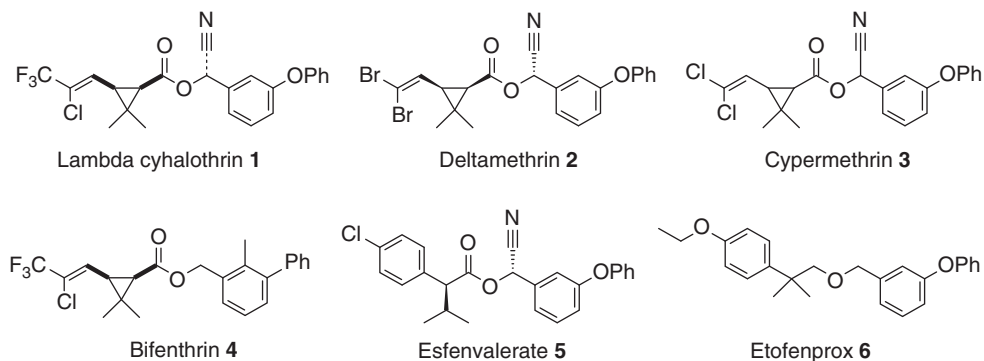


Figure 34.1 Selected commercialized pyrethroids.

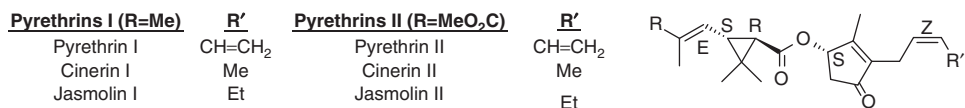


Figure 34.2 The classification of pyrethrins in two classes.

In this chapter, we focus on Classes A and B only (carboxylic esters), even though one acknowledges that Class C compounds can also bring some interesting features, such as reduced fish toxicity for etofenprox (**6**) in rice culture, for example, [4].

34.2

History

The insecticidal power of pyrethrum, the extract of the dried flowers from *Tanacetum cinerariifolium*, has been known for centuries. First cultivated on the Dalmatian coast, its use has spread worldwide. Nowadays, the main producers of pyrethrum are Tasmania, Papua New Guinea, and Kenya. Pyrethrum is composed of six main components, the pyrethrins, which can be divided into two subgroups: “pyrethrins I”, which are chrysanthemic esters (R = Me, Figure 34.2); and “pyrethrins II”, which are pyrethric esters (R = MeO₂C, Figure 34.2), with pyrethrin I (R = Me, R' = CH=CH₂) being the most biologically active molecule. Although the main structural features of pyrethrins were described as soon as 1924 by Staudinger and Ruzicka [5], the absolute stereochemistry of the compounds was only confirmed in the 1970s [6]. The limited stability of pyrethrins toward oxidation and photodegradation impeded their use in crop protection but allowed efficient household use.

Pyrethrin I was also an excellent source of inspiration for the design of improved insecticides. The development of synthetic pyrethroids targeted both

improved alcohol and carboxylic acid moieties. An historical perspective is given in Figure 34.3 [7].

Early research focused on the alcohol variation: the replacement of the complex cyclopentenolone moiety by truncated motifs such as in bioallethrin (**8**) [11] or in prallethrin (**9**) [12] brought the first improvements. The use of simpler aromatic alcohols as in resmethrin (**10**) led to the first synthetic pyrethroid equipotent to pyrethrin I (**7**), and provided a better photostability [13]. The use of the same 5-benzyl-3-furylmethanol with a new cyclopropyl carboxylic unit also led to the discovery of kadethrin (**11**) [14], which showed very good knockdown activity, demonstrating that the *trans* arrangement of the cyclopropyl ring is not a prerequisite for biological activity. The implementation of benzyl alcohols, in particular of the 3-phenoxybenzyl alcohol, or its cyanohydrin analog led to the discovery of even more stable and safer insecticides (compared to organophosphates or carbamates) such as cyphenothrin (**13**) [15].

Variations on the carboxylic acid moiety started with the modification of the isobutenyl group: Farkas *et al.* exchanged the methyl groups with chlorine atoms on the allethrin scaffold [16]. Unfortunately, this compound **18** was inactive. Elliott and his team at the Rothamsted Experimental Station later reinvestigated this option with the best benzylic alcohols described in the previous paragraph and discovered new insecticides with unprecedented potency at the time. This culminated with the discovery of deltamethrin (**2**) [1]. This was a relatively unusual situation where an academic group (the Rothamsted Experimental Station) was at the forefront of industrial insecticidal research [17]. A further refinement was the replacement of a chlorine atom of cypermethrin (**3**) by a CF₃ group to give cyhalothrin (**20**) [18]. The coupling of the cyhalothrinic acid with a refined benzylic alcohol moiety led to the discovery of tefluthrin (**21**), which had the particularity of being an efficient soil insecticide [19]. These halogenated pyrethroids analogs turned out to be particularly suited for agricultural use due to their improved stability toward oxidative or photodegradation [20].

With the presence of heavier atoms on the cyclopropyl substituent, the IUPAC stereodescriptor of the chiral center born on the ring would change, although their relative stereochemistry compared to their natural carbon counterparts would not. For example, the formal replacement of the carbon atoms by chlorine atoms in the isobutenyl moiety of (1*R*,3*R*)-*trans* phenothrin would provide (1*R*,3*S*)-*trans* permethrin (Figure 34.4). In order to eliminate such ambiguity, Elliott proposed to identify the various diastereoisomers with the IUPAC absolute stereodescriptor for the C1 chiral center (*R* or *S*) and then with the relative stereochemistry descriptor at the C3 center (*cis* or *trans*) [21].

Sumitomo has been a major player in the pyrethroid discovery process. To simplify the pyrethroid scaffold further, they designed fenvalerate (**14**) (and its enantio enriched form esfenvalerate (**5**)), a synthetic pyrethroid that did not contain a cyclopropyl carboxylic ester anymore (Figure 34.3) [22]. The concept was further extended by Henrick with fluvalinate (**15**) [23].

More recent developments have focused on household use and on optimized volatility as seen with metofluthrin (**16**), where ambient temperature vaporization

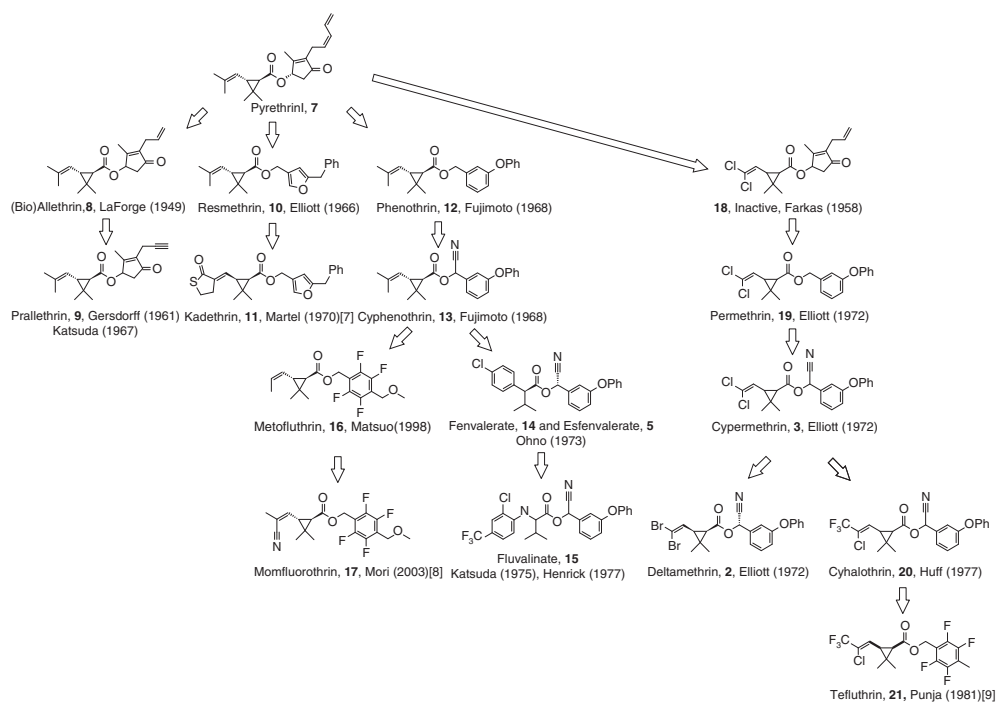


Figure 34.3 The journey to the discovery of pyrethroids.



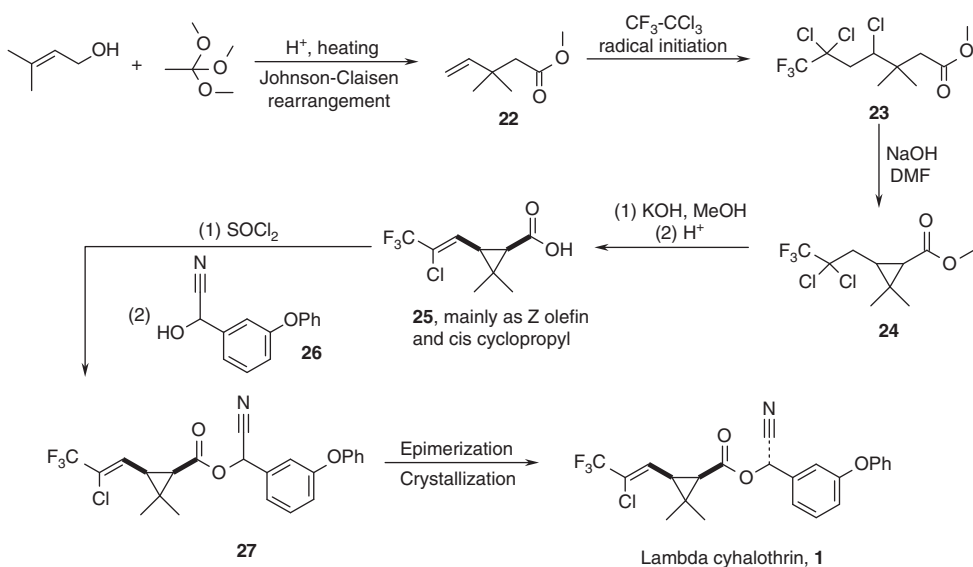
(1R,3R) trans Phenothrin = (1R,trans) Phenothrin (1R,3S) trans Permethrin = (1R,trans) Permethrin

Figure 34.4 The nomenclature convention for the stereochemistry of pyrethroids.

was aimed to provide convenient mosquito control (Figure 34.3) [24]. Momfluorothrin (**17**), a recent analog thereof was recently approved by the American (2015) and Canadian (2014) regulatory agencies for indoor, outdoor, and crack-and-crevice treatments in residential and commercial settings.

34.3 Synthesis

The synthetic routes to pyrethroids have been extensively studied and reviewed [25, 26]. Only three representative syntheses are described in this section. The synthesis of lambda cyhalothrin (an enantiomer pair from the eight possible diastereomers of cyhalothrin (**20**)), the most cost-efficient pyrethroid solution, is shown in Scheme 34.1.

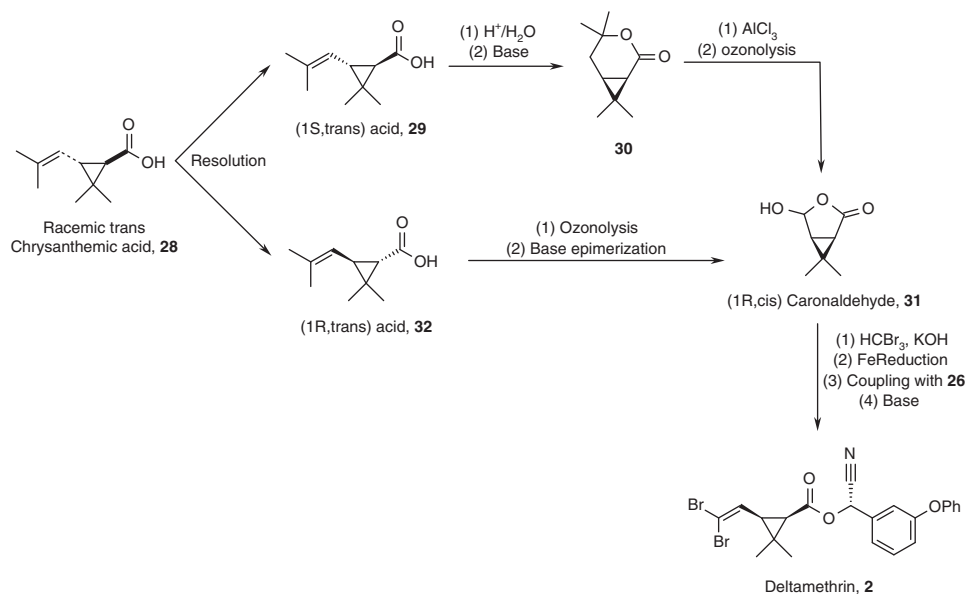


Scheme 34.1 The synthesis of lambda cyhalothrin (**1**).

Prenyl alcohol was reacted with methyl orthoacetate, which gave the unsaturated ester **22** via a Johnson–Claisen rearrangement. Ester **22** was submitted to

the radical addition of $\text{CF}_3\text{--CCl}_3$ to give **23**, which cyclized under basic conditions to the isomeric mixture of esters **24**. Under basic conditions, the mixture **24** underwent elimination to give one major isomer **25** containing a *Z* alkene and a *cis* relative stereochemistry at the C-3 ring. The coupling of the acid **25** with the cyanohydrin **26** gave the mixture of diastereoisomers **27** (undefined stereochemistry at cyanohydrin center), which under suitable epimerization, and crystallization conditions provided the desired lambda cyhalothrin (**1**) as an enantiomer pair.

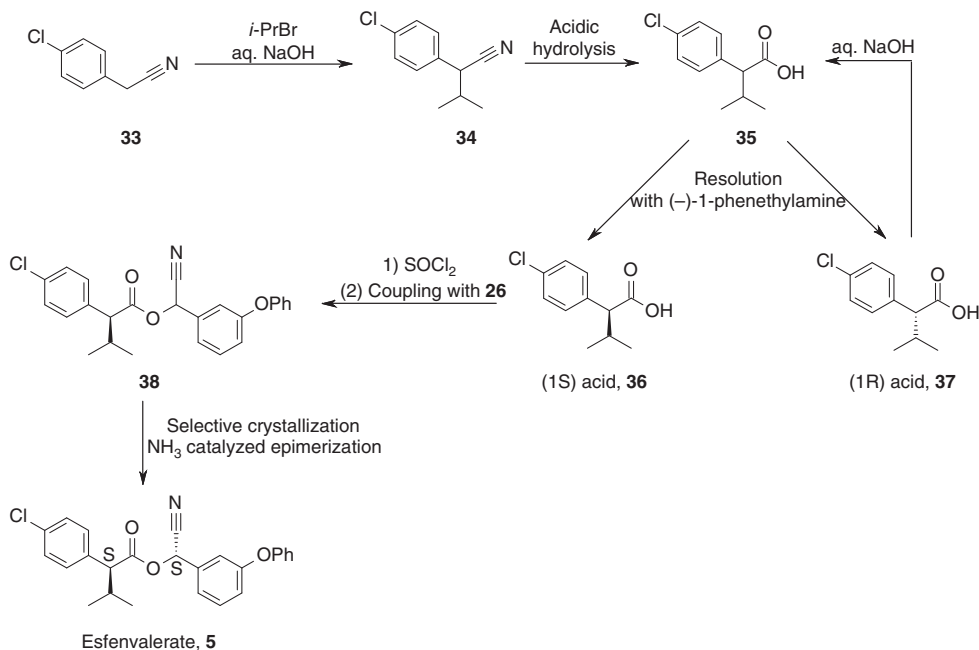
Most pyrethroid insecticides have been introduced in the market as racemates or even as diastereoisomeric mixtures. By contrast, for deltamethrin (**2**), the most active enantiomer (*1R, cis*)-(αS) having been discovered relatively early by Elliott *et al.* [1], this sole enantiomer was produced and introduced in the market by Roussel Uclaf (now Bayer CropScience). Over the last decades, numerous patents and publications have dealt with efficient enantioselective syntheses of deltamethrin (**2**) or its cyclopropyl acid moiety, the deltamethrinic acid [27]. The original Roussel Uclaf industrial synthesis is shown in Scheme 34.2 [28].



Scheme 34.2 The synthesis of deltamethrin (**2**).

Racemic *trans* chrysanthemic acid (**28**) was submitted to resolution (using a chiral amine) to access both enantiomers. Both enantiomers were converted into the same key intermediate (1*R*)-*cis* caronaldehyde (**31**) (Scheme 34.2). (1*R*)-*cis* caronaldehyde was then subjected to the addition of bromoform (avoiding a tedious Wittig alternative), followed by metal reduction, and a final coupling with the racemic cyanohydrin **26** completed by an elegant basic epimerization to reach the desired (1*R, cis*)-(αS) deltamethrin (**2**).

Finally, the synthesis of the class B compound esfenvalerate (**5**) (the enantioenriched version of fenvalerate) is discussed (Scheme 34.3) [22]. The racemic acid **35** was easily obtained from the corresponding benzylic nitrile **33** via alkylation and acidic hydrolysis. Acid **35** was then resolved with (–)-1-phenethylamine, and the undesired (1*R*) enantiomer **37** was racemized back to **35** under basic conditions. Acid **36** was then coupled with the racemic cyanohydrin **26** to give **38** where the cyanohydrin center is racemic. An elegant process finally combined the selective crystallization of the desired (*S*, α *S*) enantiomer (esfenvalerate, (**5**)) and the ammonia-catalyzed epimerization of the (*S*, α *R*) diastereoisomer.



Scheme 34.3 The synthesis of esfenvalerate (**5**).

34.4

Mode of Action

Pyrethroids exert their insecticidal action through an interaction with the insect voltage-gated sodium channels [29]. Voltage-gated sodium channels are integral transmembrane proteins that help establish and control the voltage potential across cell membranes, so their correct functioning is essential for proper nerve activity. Sodium channels open in response to a voltage stimulus, allowing sodium ions to flow into the cell for a few milliseconds, then inactivation occurs. Pyrethroids preferentially bind and stabilize the open state of sodium channels, producing prolonged channel opening and conductance of sodium into the

cell, which ultimately results in paralysis and death of the insect [30]. Based on chemical structure and insect symptomology [30, 31], pyrethroids are classified in two subgroups (Type I and Type II). Typical symptoms induced by Type I pyrethroids include lack of coordination, tremors, and paralysis, whereas insects exposed to Type II pyrethroids show lack of coordination, together with salivation and convulsions.

There are at least 10 different binding sites on voltage-gated sodium channels, which are targeted by natural products (e.g., tetrodotoxin, the puffer fish's poison) and drugs (e.g., local anesthetics such as lidocaine) [32, 33]. Pyrethroids bind to the so-called "site 7," which is predicted to be accessible from the membrane and therefore suggesting that a lipophilic character is required for such insecticides. The exact nature of the pyrethroid-binding site has long been elusive, as the use of a radioligand-binding assay is hampered by the high lipophilicity of these insecticides and the eukaryotic sodium channel is to date an unsolved target for X-ray crystallography. Therefore, the current knowledge on the molecular determinants of pyrethroid interaction with insect sodium channel is largely based on molecular modeling. O'Reilly *et al.* built a homology model of the housefly sodium channel, using the rat brain Kv1.2 potassium-channel crystal structure as template [34]. The docking of fenvalerate (**14**) predicts a hydrogen bond interaction between the carbonyl of the insecticide with the hydroxyl moiety of Threonine 929 in the binding site. Additional interactions made by the lipophilic alcohol portion of fenvalerate (**14**) in the hydrophobic cavity of the sodium channel are believed to enhance the binding (Figure 34.5).

The extensive use of pyrethroids in agriculture and vector control over several decades has, however, led to resistance in many insect populations. One of the major mechanisms of pyrethroid resistance is known as knockdown

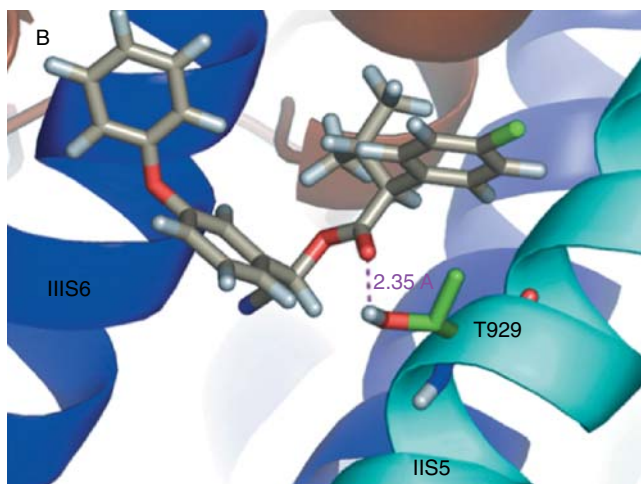


Figure 34.5 Proposed mode of binding of fenvalerate to housefly voltage-gated Na channel. Reproduced with permission, from Ref. [34]. © The Biochemical Society.

resistance (kdr) and consists in a reduction of nerve sensitivity. To date, more than 50 unique resistance-associated mutations or combinations of mutations have been identified in almost all medically and agriculturally significant insect species, and this number is steadily increasing [35, 36]. The impact of combination of mutations can be so pronounced as to almost abolish the sensitivity of the voltage-gated sodium channel toward the pyrethroids. Knockdown resistance is likely due to decreased pyrethroid binding to the voltage-gated sodium channel, as confirmed by electrophysiological and pharmacological assays on mutant sodium channels heterologously expressed in *Xenopus* oocytes [36].

Voltage-gated sodium channels are present in different phyla, including mammals; however, pyrethroids exert selective toxicity toward insect rather than vertebrate sodium channels.

As a matter of fact, mammalian (rat) sodium-channel heterologously expressed in *Xenopus* oocytes are up to 1000-fold less sensitive than insect channel to pyrethroids [37]. Differential metabolism, for example, degradation of the insecticide in mammals by means of esterases and cytochrome P450, also contributes to selective toxicity [38].

34.5

Biological Activity

Pyrethroids are broad-spectrum insecticides effective against a broad range of foliar pests, including Coleoptera (beetles and weevils), Diptera (flies and mosquitoes), Heteroptera (bugs), Homoptera (aphids, whiteflies, and leafhoppers), Lepidoptera (moths and butterflies), Thysanoptera (thrips), and Orthoptera (cockroaches and grasshoppers). Some pyrethroids, such as bifenthrin (**4**) and fluvalinate (**15**), also control species of Acarina (mites). They are generally nonsystemic insecticides with contact and ingestion action. Rapid absorption, especially in the case of the halogenated pyrethroids, results in knockdown and possibly insect death within seconds to minutes. Pyrethroids are used in cereals, citrus, cotton, grapes, maize, oilseed rape, soya beans, ornamentals, potatoes, top fruit, and vegetables among other crops. As an example, application rates of permethrin (**19**) are 100–150 g/ha in cotton, 25–50 g/ha in fruits, and 40–70 g/ha in vegetables [39]. In addition, pyrethroids find an important application in human health, notably in the control of vector-borne diseases, such as malaria [40]. The use of insecticide-treated bed nets constitutes one of the most successful strategies in vector control, and pyrethroids are the insecticides of choice because of their favorable human safety profile.

Overall, the annual usage of permethrin (**19**) in agriculture, animal health, and public health was estimated by the United States Environmental Protection Agency to be 2 million lb (in weight) in 2006 [41].

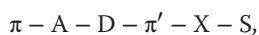
Some pyrethroids, such as fluvalinate (**15**), have remarkably low toxicity toward bees and therefore can be used to control parasitic mites (e.g., *Varroa destructor*) that are responsible for viral transmission inside beehives. Metabolism via

cytochrome P450 has been thought to be the major factor contributing to detoxification of this insecticide by honeybees [42].

34.6

Structure–Activity Relationship

As mentioned in the introduction, some very diverse structures are encompassed in the pyrethroid family. A general description of the bioactive candidates may be the one given by Naumann with the general formula [17]:



where

π is a π system (alkene or aromatic) optionally substituted by further lipophilic groups, for example, halogen or haloalkyl groups;

A is a small lipophilic anchor group containing a gem dimethylmethylene group, for example, a dimethyl cyclopropyl group;

D is a small spacer such as an ester, an ether, or an oxime;

π' is another π system (alkene or aromatic);

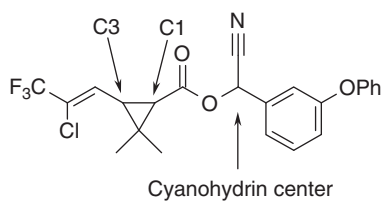
X may or may not be present. X is a flexible pivot such as an oxygen atom or a methylene group;

S is a substituent containing a π system connected to the *meta* position of the π' system.

The natural pyrethrins have very defined absolute stereochemistry both on the chrysanthemic acid and on the cyclopentenolone alcohol (cf. Section 34.2, Figure 34.2). The stereochemistry (1*R*) at C1 center of the chrysanthemic and (4*S*) at the C4 center of the cyclopentenolone turn out to be essential to demonstrate significant biological activity [43]. An inversion at these centers either diminishes or even eliminates insecticidal activity.

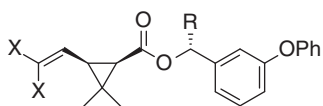
A similar trend was observed for synthetic pyrethroids. This will be exemplified by comparing the insecticidal activity of various diastereoisomers of cyhalothrin (20) (Table 34.1) [44]. The (1*R*) stereochemistry was a prerequisite for activity, the (1*S*) isomers being essentially inactive (entries 1–2 vs 3–4). There was also a strong preference for the (*S*) stereochemistry at the cyanohydrin chiral center, the (*R*) isomers being much weaker (entries 1 and 5 vs 2 and 6). Finally, the stereochemistry at the C3 center also had an influence although less pronounced than for the two previous stereocenters (entry 1 vs entry 5). It is worth noticing that the most potent diastereoisomer of chrysanthemic esters is the (1*R*, *trans*), while it is generally the (1*R*, *cis*) isomer for cyclopropyl esters bearing halogen or haloalkyl groups on the alkenyl moiety.

Beyond stereochemical requirements, some other structural elements also have a significant impact on the insecticidal activity of pyrethroids. For example, the presence of the cyano group on the benzyl moiety really gave a boost in activity as exemplified in Table 34.2 (entries 2, 4, and 6 vs entries 1, 3, and 5 respectively)

Table 34.1 The relation between stereochemistry and insecticidal activity exemplified with cyhalothrin (**20**) diastereoisomers.Diastereoisomers of Cyhalothrin, **20**

Entry	Stereochemistry of isomers		LD ₅₀ houseflies, ^{a)} <i>Musca domestica</i>
	Cyclopropyl acid	Cyanohydrin	
1	(1 <i>R</i> , <i>cis</i>)	(<i>S</i>)	2500
2	(1 <i>R</i> , <i>cis</i>)	(<i>R</i>)	<100
3	(1 <i>S</i> , <i>cis</i>)	(<i>S</i>)	Inactive
4	(1 <i>S</i> , <i>cis</i>)	(<i>R</i>)	Inactive
5	(1 <i>R</i> , <i>trans</i>)	(<i>S</i>)	1500
6	(1 <i>R</i> , <i>trans</i>)	(<i>R</i>)	<16

a) Relative toxicity of permethrin (**19**) (LD₅₀ permethrin = 100).

Table 34.2 The impact of benzyl ester substituents and halogen substituents on insecticidal activity.

Entry	X	R	LD ₅₀ houseflies, ^{a)} <i>M. domestica</i>
1	F	H	170
2	F	CN	350
3	Cl	H	210
4	Cl	CN	2400
5	Br	H	180
6	Br	CN	2800

a) Relative toxicity of (1*R*, *trans*) resmethrin (bioresmethrin, LD₅₀ bioresmethrin = 100).

[17]. With respect to halogen substituents on the alkenyl moiety, a potency ranking $F < Cl < Br$ could be determined.

The ranking of the alkenyl substituents could be further refined (Table 34.3) [17]. The CF_3 group turned out to be beneficial only when used in combination

Table 34.3 The impact of the alkenyl substituents and of the stereochemistry on the α position on insecticidal activity.

Entry	X	Y	Stereochemistry		LD ₅₀ houseflies, ^{a)} <i>M. domestica</i>
			Cyclopropyl acid	Cyanohydrin	
1	Cl	Cl	Racemic <i>cis/trans</i>	α racemic	150–180
2	CF ₃	CF ₃	Racemic <i>cis/trans</i>	α racemic	40
3	Cl	CF ₃	Racemic <i>cis/trans</i>	α racemic	330
4	Br	CF ₃	Racemic <i>cis/trans</i>	α racemic	310
5	Cl	C ₂ F ₅	Racemic <i>cis/trans</i>	α racemic	150
6	Cl	Cl	(1 <i>R</i> , <i>cis</i>)	α <i>S</i>	1300
7	Br	Br	(1 <i>R</i> , <i>cis</i>)	α <i>S</i>	1500
8	Cl	CF ₃	(1 <i>R</i> , <i>cis</i>)	α <i>S</i>	3800

a) Relative toxicity of cyphenothrin (**13**) (LD₅₀ cyphenothrin = 100).

with a halogen atom on the double bond. Indeed, the bis CF₃ derivative (entry 2, Table 34.3) was weaker than a CF₃/Cl or CF₃/Br pair (entries 3 and 4). As mentioned earlier, the most bioactive diastereoisomers were the one combining the (1*R*, *cis*) cyclopropyl acid with the *S* cyanohydrin (α *S*) as exemplified in entries 6–8.

Finally, the physicochemical properties of pyrethroids also influence their performance or their ease of use (Table 34.4) [7, 17]. Pyrethroids are all lipophilic molecules and are generally nonsystemic insecticides, but their log *P* can vary over several units as shown in Table 34.4. As mentioned previously,

Table 34.4 Selected physicochemical properties of some pyrethroids.

Entry	Compound	log <i>P</i>	Vapor pressure (10 ^{−6} mmHg/20 °C)
1	Phenothrin (12)	7.4	4
2	<i>cis</i> Permethrin (19)	6.8	0.019
3	Fenvalerate (14)	6.8	0.001
4	Deltamethrin (2)	6.4	0.001
5	Tefluthrin (21)	6.2	0.19
6	Pyrethrin I (7)	5.9	20
7	Metofluthrin (16)	5.64	13.5 (at 25 °C)
8	Kadethrin (11)	5.4	0.04
9	Pyrethrin II (cf. Figure 34.2)	4.3	20

fine-tuned volatility (exemplified here with vapor pressure) can be a critical factor to enhance activity in soil, as seen with the case of tefluthrin, (**21**) [19], or allow a good diffusion at room temperature as described for metofluthrin (**16**) [24].

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35

Ecdysone Receptor Agonistic Dibenzoyl Hydrazine Insecticides

Ottmar F. Hüter

35.1

Introduction

The market products methoxyfenozide (**1**, Intrepid®, Runner®, Prodigy®, Falcon®) [1, 2], tebufenozide (**2**, Mimic®, Confirm®, Romdan®) [2, 3], halofenozide (**3**, Mach 2®) [2], chromafenozide (**4**, Matric®, Killat®) [4, 5], and fufenozide (**5**) [6] are characteristic examples of dibenzoyl hydrazine insecticides (Figure 35.1).

These dibenzoyl hydrazine insecticides offer an efficient control of several different lepidopteran larvae by acting as nonsteroidal ecdysone agonists. In addition, halofenozide (**3**) also showed activity against coleopteran larvae as reported in the recent review of Dhadialla and Ross [7].

35.2

History

The class of insecticidal dibenzoyl hydrazines was discovered at Rohm and Haas by serendipity in the late 1980s [8]. The prototype RH-5849 (**6**) was well characterized as a nonsteroidal agonist of the receptor of the insect-molting hormone 20-hydroxyecdysone (**7**) in insect cell preparations (Figure 35.2) [9]. Optimization of the substituents led to the development of the three dibenzoyl hydrazine products methoxyfenozide (**1**) [1], tebufenozide (**2**) [3], and halofenozide (**3**) [2]. These products were then commercialized by Dow AgroSciences. In 2010, Dow AgroSciences divested the tebufenozide business to Nippon Soda. The dibenzoyl hydrazine chromafenozide (**4**) was discovered and jointly commercialized by Nippon Kayaku and Sankyo [4, 5]. Fufenozide (**5**) was discovered and developed by Jiangsu Pesticide Research Institute [6].

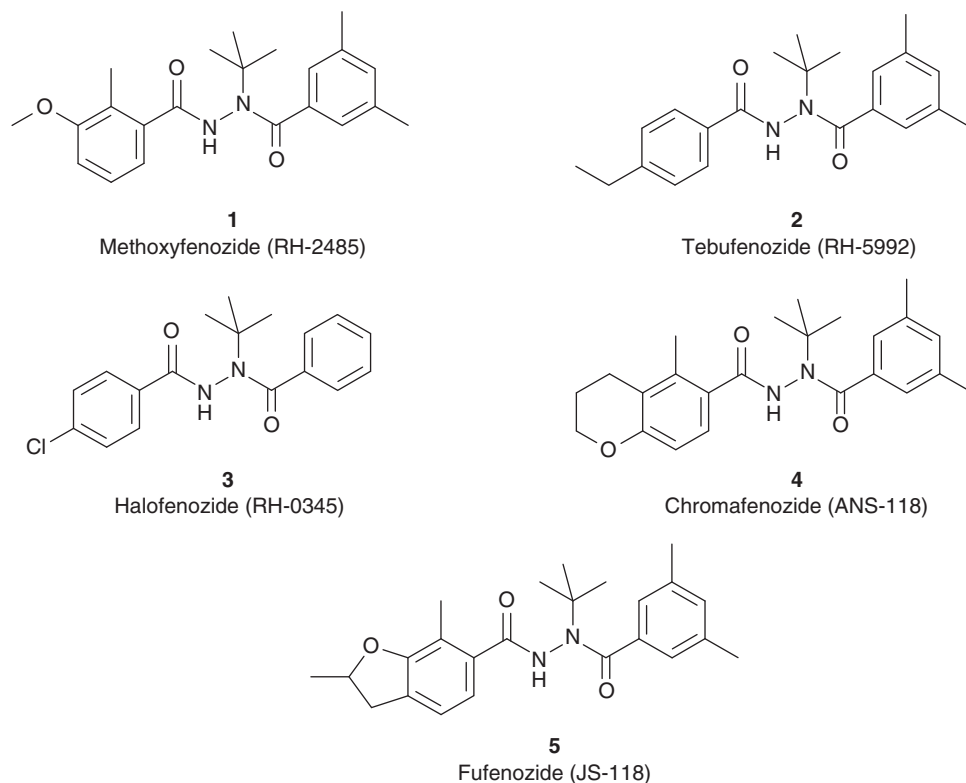


Figure 35.1 The insecticides methoxyfenozide (1), tebufenozide (2), halofenozide (3), chromafenozide (4), and fufenozide (5).

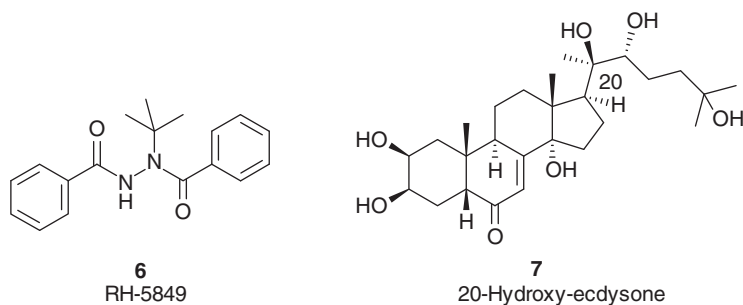
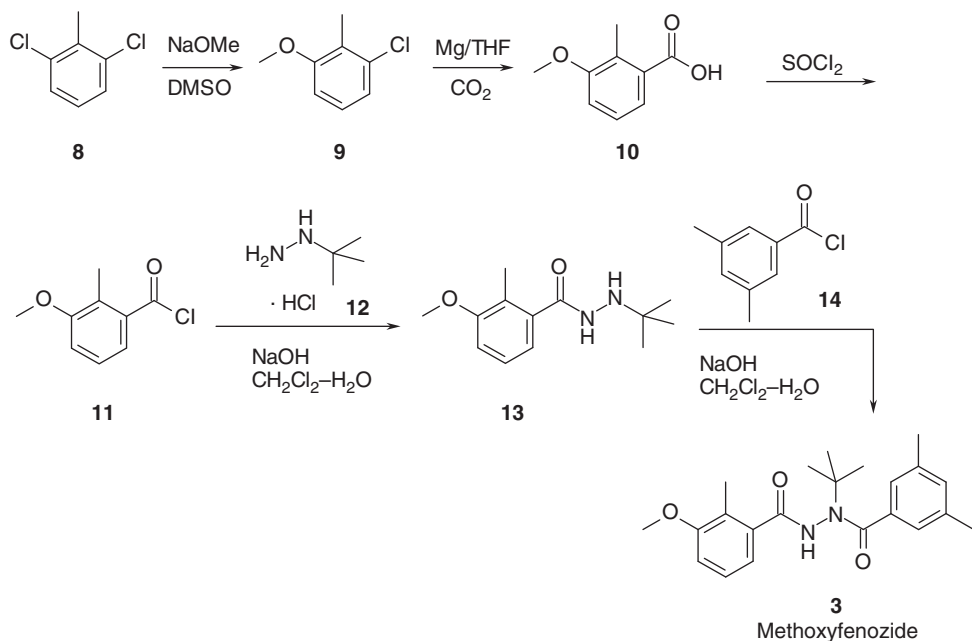


Figure 35.2 The lead compound RH-5849 (6) and the insect-molting hormone 20-hydroxyecdysone (7).

35.3

Synthesis

The preparation of methoxyfenozide (1) is shown in Scheme 35.1. The required acid chloride **11** is synthesized by monomethoxylation of 2,6-dichloro-toluene (**8**)



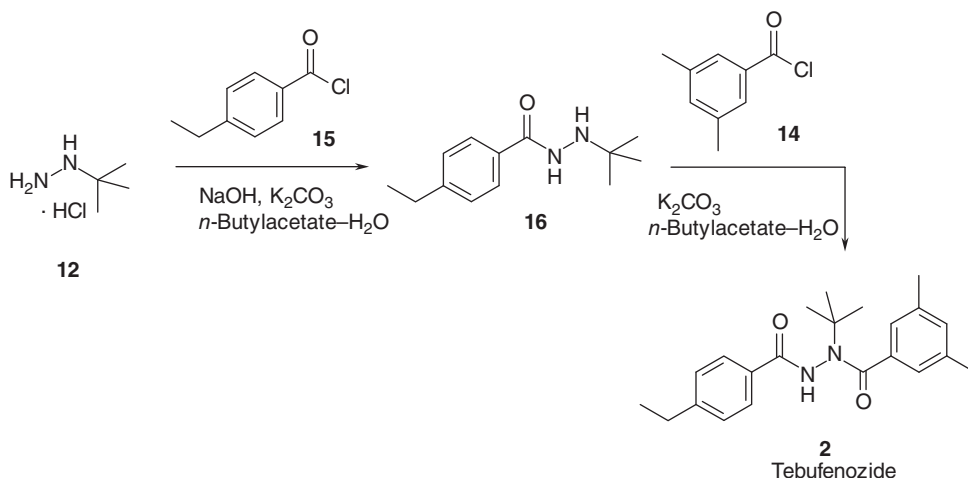
Scheme 35.1 Synthesis of methoxyfenozide (**1**) [7, 10, 11].

with sodium methoxide. Subsequently, the intermediate **9** is transformed to the carboxylic acid **10** by formation of the Grignard reagent followed by quenching with carbon dioxide. Then, the acid **10** is converted to the acid chloride **11** and reacted with *tert*-butyl hydrazine (**12**). Finally, the intermediate **13** is benzoylated with 3,5-dimethyl-benzoyl chloride (**14**) to give methoxyfenozide (**1**) [7, 10, 11].

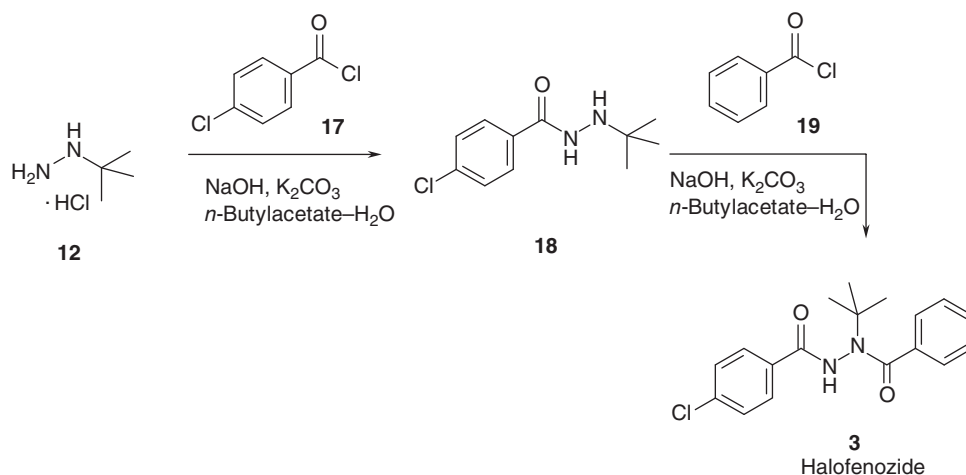
Tebufenozide (**2**) can be synthesized in a few steps starting with the selective benzoylation of *tert*-butyl hydrazine (**12**) at the sterically less-hindered nitrogen with 4-ethyl-benzoyl chloride (**15**) followed by treatment of the intermediate **16** with 3,5-dimethyl-benzoyl chloride (**14**) (Scheme 35.2) [7, 12].

Halofenozide (**3**) can be prepared in a similar way as described earlier by benzoylation of *tert*-butyl hydrazine (**12**) with 4-chloro-benzoyl chloride (**17**) followed by the reaction of the intermediate **18** with benzoyl chloride (**19**) (Scheme 35.3) [7, 12].

Chromafenozide (**4**) has a more complex chemical structure than the dibenzoyl hydrazines **1–3**. The synthesis of the required acid chloride **27** starts with the alkylation of phenol **20**. The resulting intermediate **21** is cyclized by heating in *N,N*-diethyl-aniline, and the double bond in the resulting bicyclic compound **22** is then hydrogenated. Friedel–Crafts acylation with acyl chloride gives the ketone **24**, which is transferred into the acid **25** by bromination and subsequent hydrolysis (haloform reaction). The *tert*-butyl group is then removed with aluminum chloride followed by formation of the acid chloride **27**. Reaction with *tert*-butyl hydrazine (**12**) gives the monobenzoylated intermediate **28**, which is benzoylated



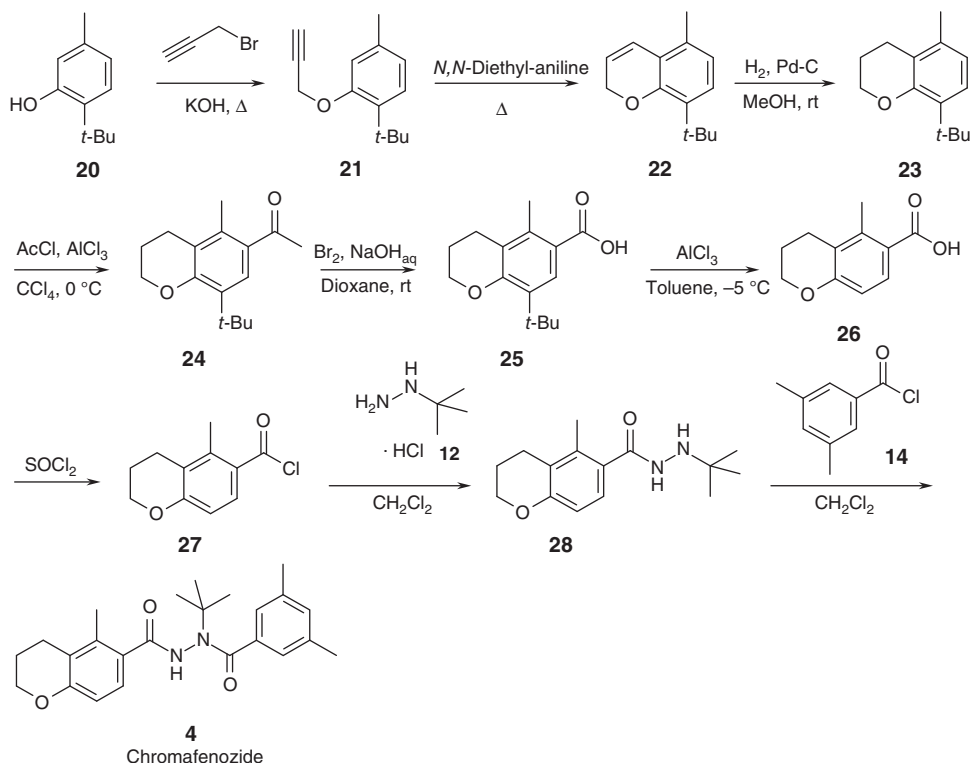
Scheme 35.2 Synthesis of tebufenozide (2) [7, 12].



Scheme 35.3 Synthesis of halofenozide (3) [7, 12].

with 3,5-dimethylbenzoyl chloride (14) to give chromafenozide (4) (Scheme 35.4) [7, 13].

Fufenozide (5) can be synthesized starting from 3-chloro-2-methylaniline (29). Diazotization and heating give the phenol (30), which is subsequently allylated. The allylated intermediate (31) undergoes Claisen rearrangement and cyclization. In the cyclized intermediate (32), the chlorine is substituted by a cyano group. Then, the cyano group is hydrolyzed and transformed into the acid chloride (35). The acid chloride is reacted with *tert*-butylhydrazine (12), and the resulting mono-benzoylated product is then reacted with 3,5-dimethylbenzoyl chloride (14) to give fufenozide (5) (Scheme 35.5) [14, 15].



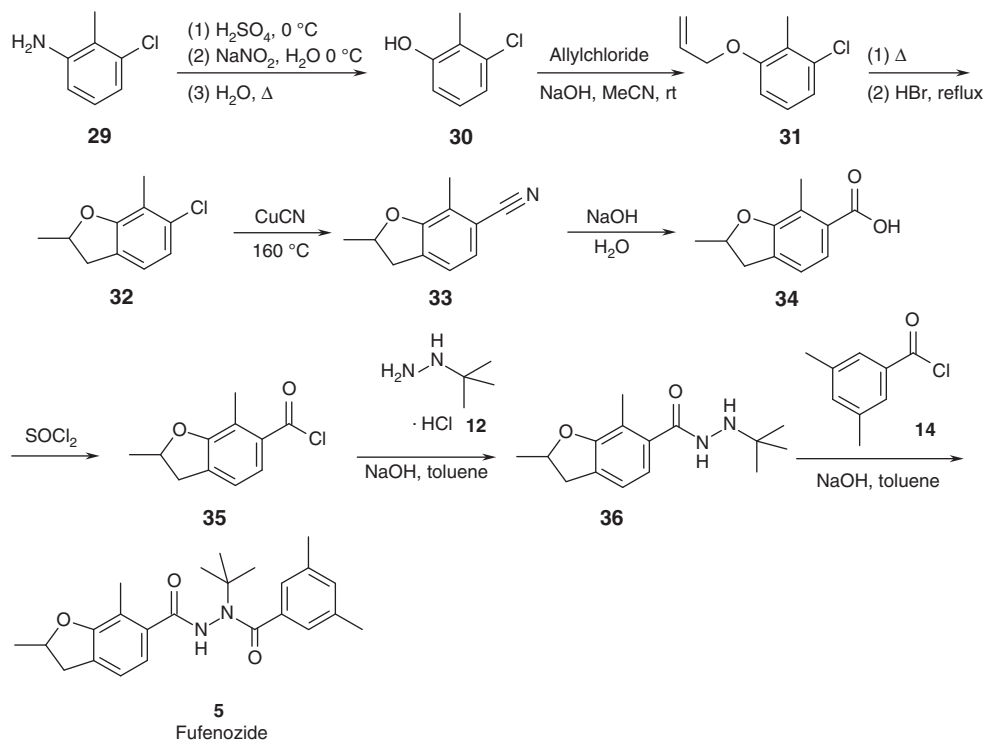
Scheme 35.4 Synthesis of chromafenozide (**4**) [7, 13].

35.4

Mode of Action

Insects develop from the immature stages, such as larvae or nymphs, to the adult stage through metamorphosis and molting. The process is regulated by juvenile hormones and ecdysteroids. 20-Hydroxy-ecdysone (**7**) interacts with the ecdysone receptor (EcR) complex, which is a heterodimer of the EcR protein and the ultraspiracle protein (USP) [16]. The steroidal ligand 20-hydroxyecdysone (**7**), which is found in insects, and the natural product ponasterone A (25-deoxy-ecdysterone) (**37**), which was isolated from plants [17], show very high affinities for the EcRs of all insects. It was shown that the nonsteroidal dibenzoyl hydrazines methoxyfenozide (**1**), tebufenozide (**2**), and chromafenozide (**4**) selectively bind to the EcRs of lepidopteran pests. The binding data correlate well with the biological activity against insects. Interestingly, halofenozide (**3**) showed good biological activity against Lepidoptera and Coleoptera species in spite of weaker binding affinities to the receptors of these insect species [7].

Crystal structures revealed that the steroidal ligand ponasterone A (**37**) and the dibenzoyl hydrazine BY106830 (**38**) occupy different but overlapping cavities



Scheme 35.5 Synthesis of fufenozide (5) [14, 15].

in the EcR binding pocket of *Heliothis virescens*. It was shown that in the crystal structure, the alkyl side chain of ponasterone A (37) and the *t*-butyl group of BY106830 (38) [13] are located in the same region of the binding pocket (Figure 35.3) [18]. Later, the crystal structure of EcR binding pocket of *Bemisia tabaci* was published. Comparing the binding pockets of *H. virescens* and *B. tabaci* demonstrated differences in the region of the binding pocket, which is

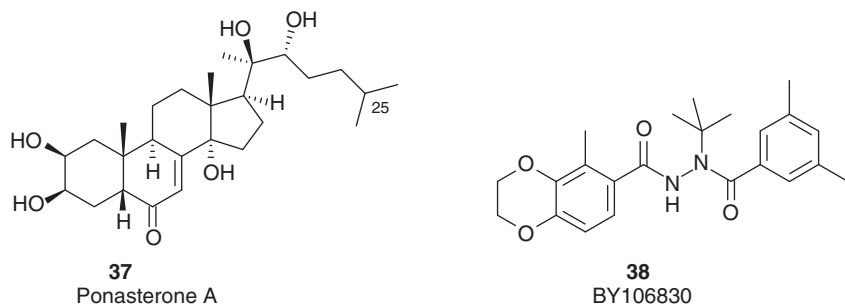


Figure 35.3 Ponasterone A (37) and dibenzoyl hydrazine BY106830 (38).

occupied by the dibenzoyl hydrazines, which could explain the differences in species selectivity [19].

For certain dibenzoyl hydrazines, for example, RH-5849 (**6**), some neurotoxic effects were observed at high concentrations (100 μmol) leading to strong contraction of muscles of larvae of the housefly *Musca domestica*. This was explained by blocking of the K^+ channel [20, 21].

35.5

Biological Activity

The dibenzoyl hydrazine insecticides methoxyfenozide (**1**), tebufenozide (**2**), chromafenozide (**4**), and fufenozide (**5**) are mainly used against larvae of lepidopteran pests by foliar application. The uptake into the larvae is primarily by ingestion and to a lesser extent by contact. They inhibit feeding within a few hours after application, which prevents further damage to the crop. Subsequently, they introduce lethal premature molting. Due to their specific mode of action, they are considered as safe insecticides with no biological activity against natural predators and pollinators as described in previous reviews [7, 22, 23]. Methoxyfenozide (**1**) is the commercially most important active ingredient in the class of dibenzoyl hydrazine insecticides. It has a lower application rate and a broader spectrum than tebufenozide (**2**). Methoxyfenozide (**1**) is registered in more than 60 countries. It is used in fruits, vegetables, cotton, maize, rice, wheat, soybeans, vines, tea, ornamentals, and forestry. More details can be found in the reviews of Smagghe *et al.* [22] and Gomez *et al.* [23]. Tebufenozide (**2**) is predominantly used in fruits, vegetables, and rice. Chromafenozide (**4**) was registered for uses in fruits, vegetables, rice, vines, and tea mainly in Japan. Fufenozide (**5**) has been developed against lepidopteran larvae in fruits, vegetables, tea, cotton, and rice in China. Halofenozide (**3**) is used as a soil insecticide in turf, for example, against scarabaeid grubs in soil [7].

35.6

Structure–Activity Relationship

The structure–activity relationship of dibenzoyl hydrazine insecticides has been thoroughly summarized in several reviews [24, 25]. Comparison of the structures of the different commercial dibenzoyl hydrazine insecticides leads to a simplified pharmacophore model, which is outlined in Figure 35.4. The general structure contains a sterically demanding, lipophilic group connected to one of the hydrazine nitrogens, two hydrogen acceptors (HAs), one hydrogen donor (HD), and two optionally substituted phenyl rings.

There were several attempts to replace the *N-tert*-butyl dicarbonyl hydrazine backbone; but this led only to compounds with weaker biological activity or inactive compounds. The biological activity in Figures 35.5–35.7 is based on larval

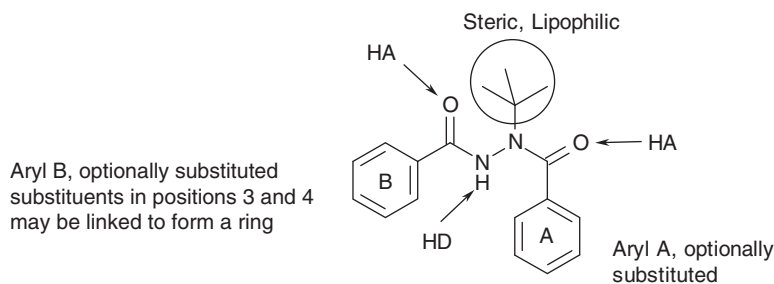
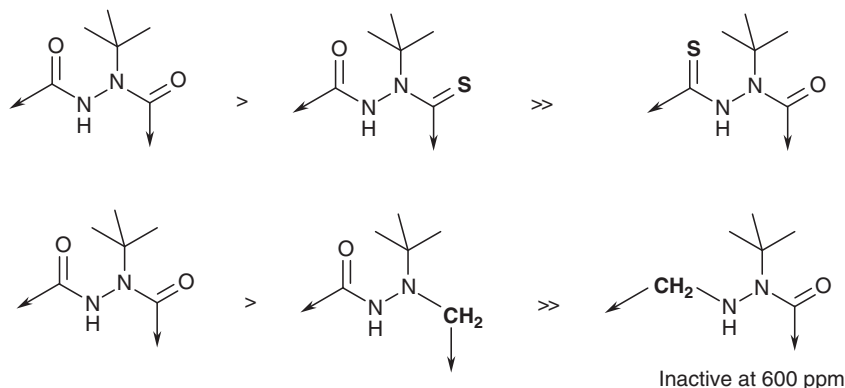


Figure 35.4 Pharmacophore model based on commercial dibenzoyl hydrazine insecticides.



Selection of further replacements with no activity at 600 ppm:

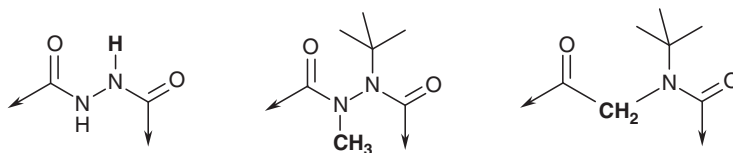


Figure 35.5 Effect of the replacement of the *N*-tert-butyl dicarbonyl hydrazine backbone on the biological activity against *Spodoptera eridania*.

toxicity against *Spodoptera eridania* (southern armyworm) of a leaf-sprayed solution containing the test substance [25].

The replacement of the phenyl rings by aromatic heterocycles or by alkyl groups is shown in Figure 35.6. Compounds containing 2-pyridyl and 3-furyl rings showed interesting levels of activity against larvae of *S. eridania*. Other groups, such as alkyl or cycloalkyl, led to a decrease of biological activity [25].

Several substituents in the A-ring or in the B-ring give better biological activity against lepidopteron larvae than the unsubstituted phenyl rings (Figure 35.7) [7]. However, the activity-enhancing effects of the substituents on the A-ring and the B-ring are not necessarily additive.

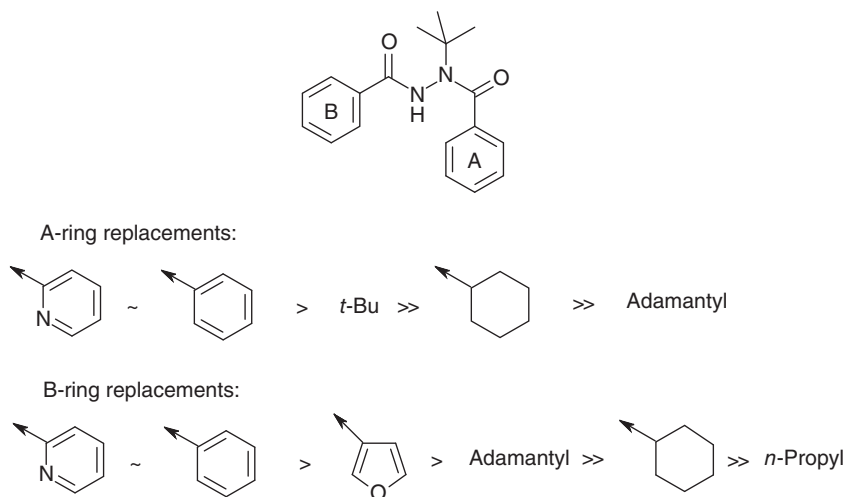


Figure 35.6 Effect of replacements of the A-ring or B-ring on the biological activity against *Spodoptera eridania*.

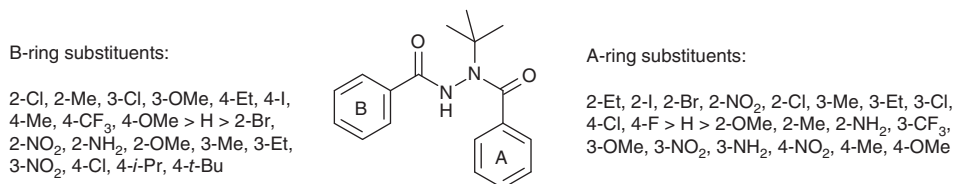


Figure 35.7 Influence of the substituents on the A-ring or B-ring of dibenzoyl hydrazine insecticides on the biological activity against *Spodoptera eridania*.

For good biological activity against lepidopteron larvae, a 3,5-dimethyl substitution pattern on the A-ring seems to be ideal. In contrast, for good coleopteran activity, an unsubstituted A-ring and a 4-chloro substituent on the B-ring seem to be preferred.

Analogues in which the B-ring is extended to a bicyclic system were intensively investigated [13, 26–28]. Figure 35.8 demonstrates the effect of a few selected bicyclic systems on the biological activity against *Spodoptera litura* (F.) (common cutworm). In these bicyclic systems, a substituent in the 2-position of the phenyl, especially a methyl or a chloro substituent, appears to be favored. On the other hand, substituents in the 5- and 6-position lead to biologically inactive analogues.

A few new structural types related to the dibenzoyl hydrazines have been published recently (Figure 35.9). In the amidoketones [29, 30], the nitrogen that bears the *t*-butyl group in the dibenzoyl hydrazines is replaced by a carbon with two alkyl substituents as in compound **39**. One of the carbonylamino groups in

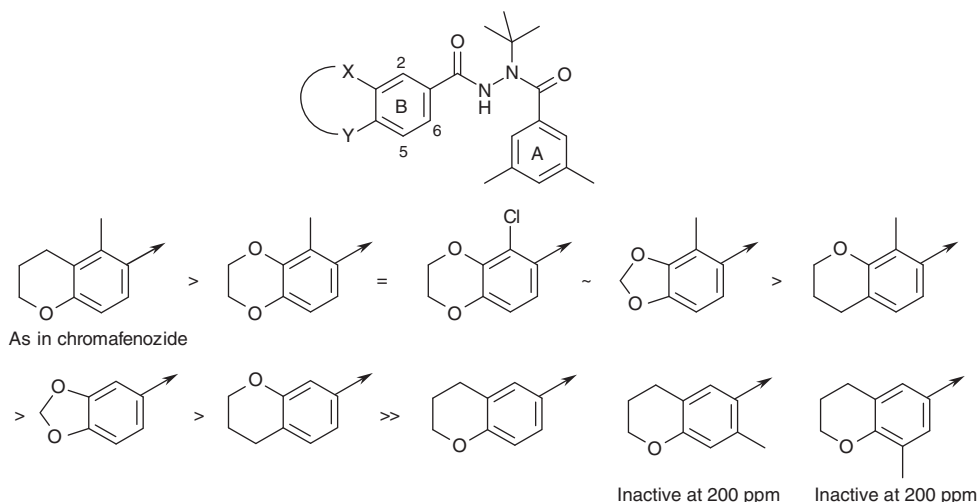


Figure 35.8 Effect of different bicyclic systems on the biological activity against *Spodoptera litura* (F.).

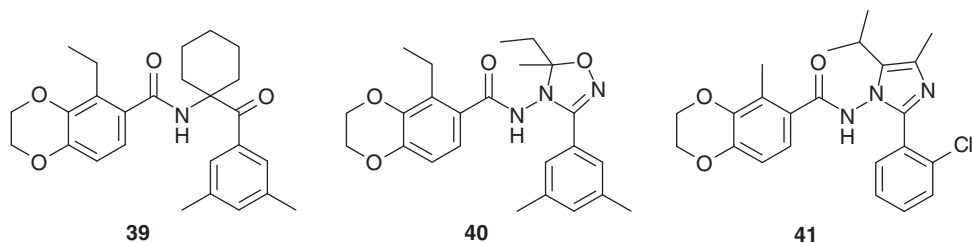


Figure 35.9 New structural types related to the dibenzoyl hydrazine insecticides.

dibenzoyl hydrazines may be replaced by heterocyclic groups such as oxadiazolines [31], for example, compound **40**, or *N*-aminoimidazoles [32, 33], such as compound **41**.

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36

Diamide Insecticides as Ryanodine Receptor Activators

André Jeanguenat

36.1

Introduction

Although the insecticidal activity of ryanodine, a plant metabolite from *Ryania speciosa*, by modulating the calcium channel called the ryanodine receptor (RyR), has been known for a long time, its impact as a commercial insecticide has met with limited success [1]. The recent discovery of the phthalamides and the anthranilamides has, however, demonstrated the potential of this mode of action. Flubendiamide (**1**), chlorantraniliprole (**2**), and cyantraniliprole (**3**) have already captured a significant market share in the insect control business. Further analogs such as cyclaniliprole (**4**), tetraniliprole (**5**), and tetrachlorantraniliprole (**11**) are expected to enter the market soon. The diamides consist of two chemical classes: the phthalamides (flubendiamide) belong to the first-generation diamides and the anthranilamides to the second generation [2]. They share the same mode of action and most probably the same site of action in Lepidoptera species.

36.2

History

During a program devoted to the discovery of herbicidal protoporphyrinogen-IX-oxidase inhibitors, weak insecticidal activity of some phthalamides and phthaldiamides was identified by researchers at the Japanese company Nihon Nohyaku in the 1990s [2]. Looking for selective insecticides devoid of phytotoxic properties, a breakthrough invention was the introduction of a perfluorinated side chain, which led to the discovery of flubendiamide (**1**) in 1998 [3]. The exceptional level of activity against Lepidopteran pests much stimulated industrial research and in a noteworthy achievement, researchers at DuPont identified a novel chemical class, the anthranilamide represented by chlorantraniliprole (**2**) [4]. The modification of the original lead included a classical inversion of an amide bond, the replacement of a phenyl substituted by a heptafluoroisopropyl group by a CF₃- or halo-pyrazole, and the transformation of a methyl substituent to a 3-chloro-2-pyridyl

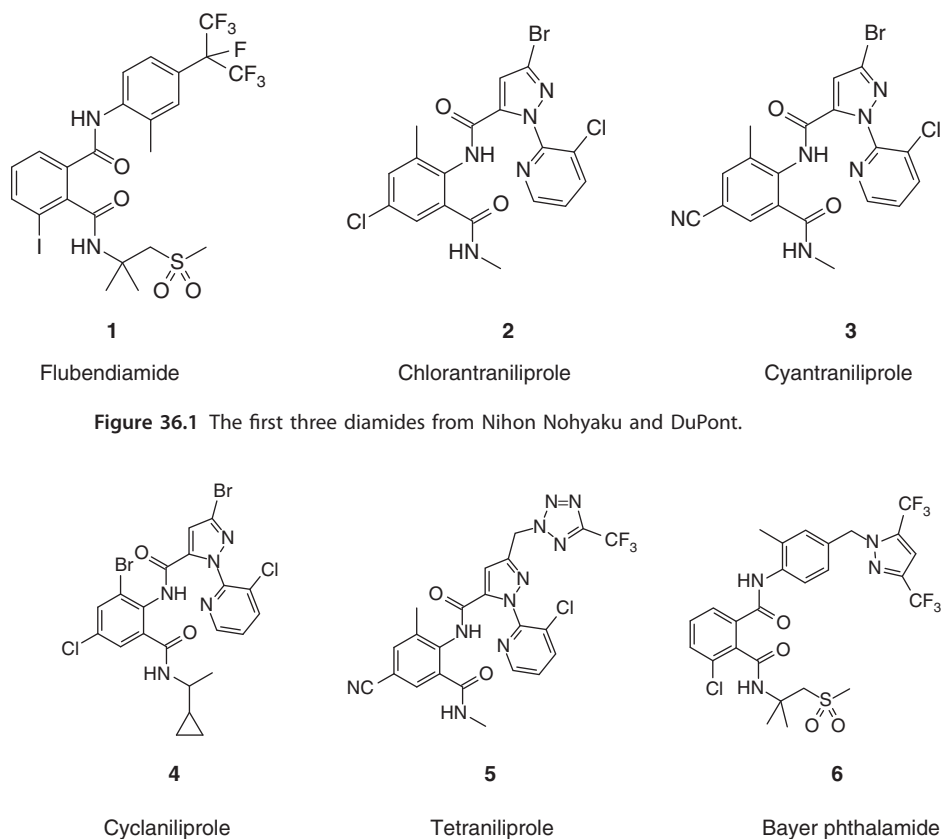


Figure 36.1 The first three diamides from Nihon Nohyaku and DuPont.

Figure 36.2 Diamides from ISK and Bayer.

moiety (Figure 36.1)! A second anthranilamide with broader spectrum of activity followed rapidly; cyantraniliprole (**3**) exhibits improved properties over the older diamides, which is reflected in a better systemicity in the plant and an improved soil mobility. Its insecticidal spectrum is larger and includes Hemipteran pests such as *Myzus persicae* and hoppers in addition to Lepidoptera [5].

Two further analogs of chlorantraniliprole have recently received proposed ISO common names (Figure 36.2). Cyclaniliprole (**4**) has an aliphatic amide side chain featuring a cyclopropyl group. It was discovered by Ishihara Sankyo Kaisha and is currently in registration phase. Tetraniliprole (**5**) was discovered by Bayer Crop Sciences and is related to cyantraniliprole. As a substituent on the pyrazole ring, it has a CF_3 -substituted tetrazole ring linked by a methylene bridge. Interestingly, Bayer claimed insecticidal phthalamides with a similar substitution replacing the heptafluoroisopropyl group (compare **6** and **5** in Figure 36.2). This structure–activity relationship (SAR) analogy is a hint for a common active site at the RyR for the phthalamides and the anthranilamides in Lepidoptera.

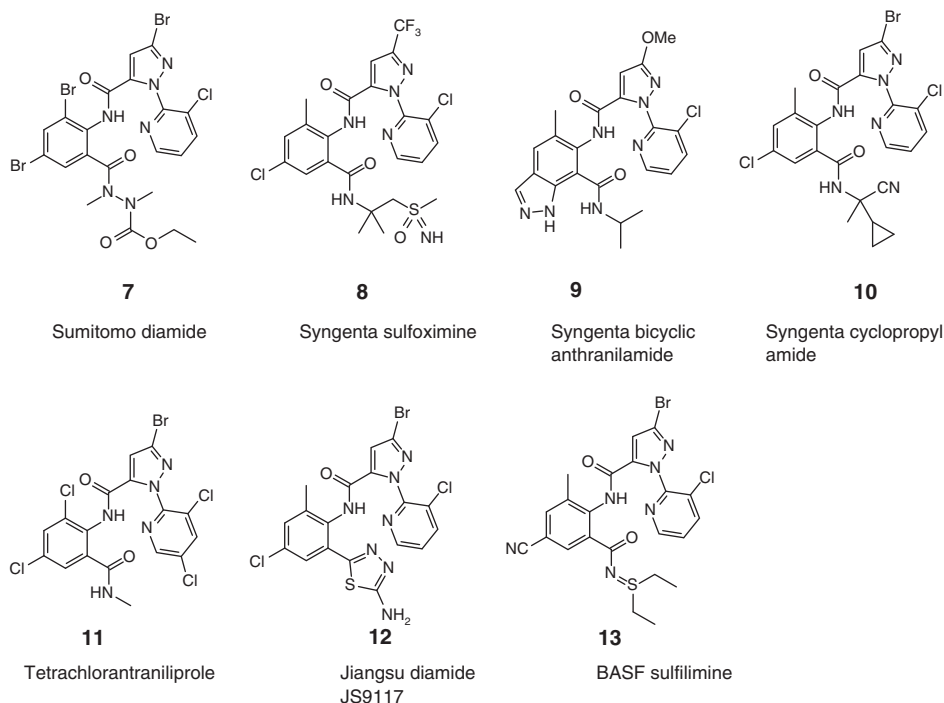


Figure 36.3 Diamide structures from Sumitomo, Syngenta, Bayer, SRICI/Sinochem, Jiangsu, and BASF.

From the patent literature, more analogs are known, sometimes with company codes. Sumitomo hydrazides such as **7** are contemporary of cyclaniliprole, as well as Syngenta sulfoximines (e.g., **8**, [6]), bicyclic anthranilamides (e.g., **9**) or cyclaniliprole analogs (e.g., **10**). Later on, further analogs were disclosed, such as tetrachlorantraniliprole or SYP-9080 (**11**) by SRICI/Sinochem, the amino thiadiazole analog **12** by Jiangsu, and sulfilimine derivatives such as **13** by BASF (Figure 36.3).

Two analogs of flubendiamide are known with a company code: CMP (**14**), disclosed by Nihon Nohyaku and Bayer, and ZJ4042 (**15**), disclosed by ZRICI/Sinochem (Figure 36.4).

36.3

Synthesis

The synthesis of flubendiamide (**1**) is depicted in Scheme 36.1 [3]. The iodine atom, which is a very rare substituent in agrochemistry, can be introduced at an early stage by a Sandmeyer reaction from cheap nitrophthalic acid (**16**). The two amine side chains are subsequently introduced in a regioselective manner

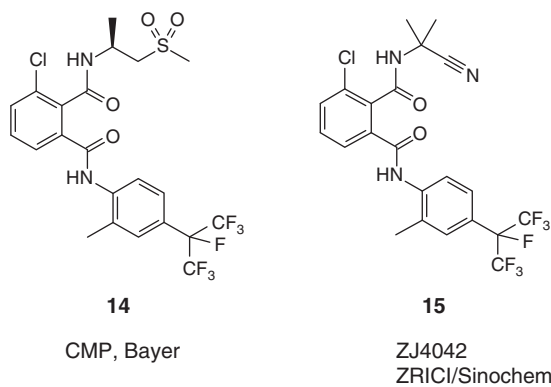
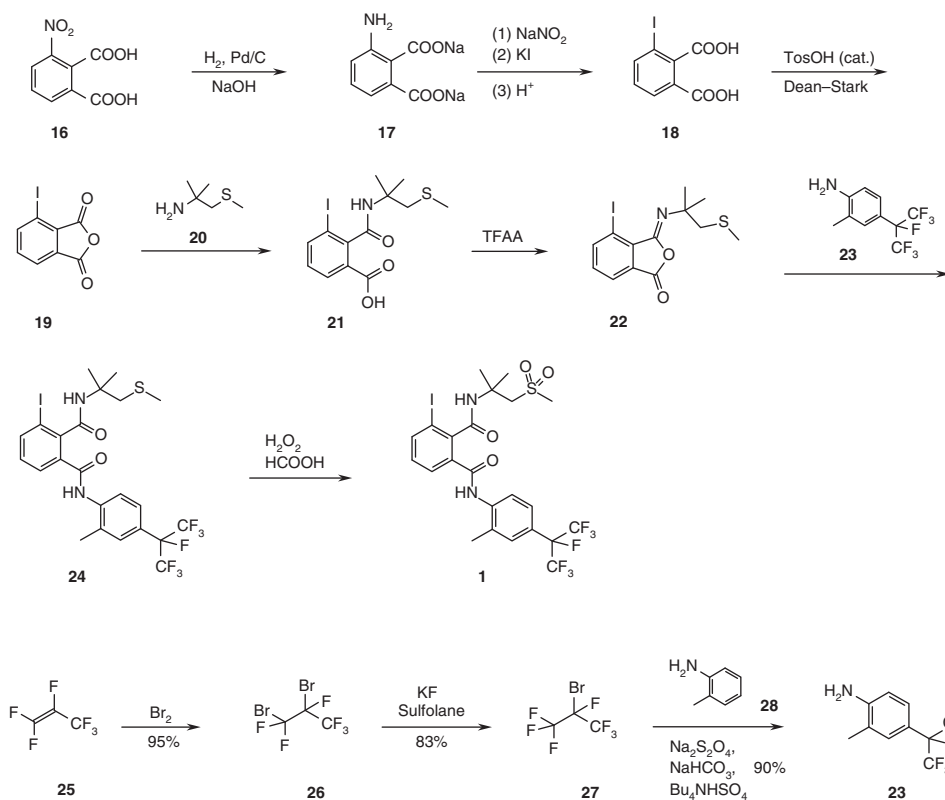


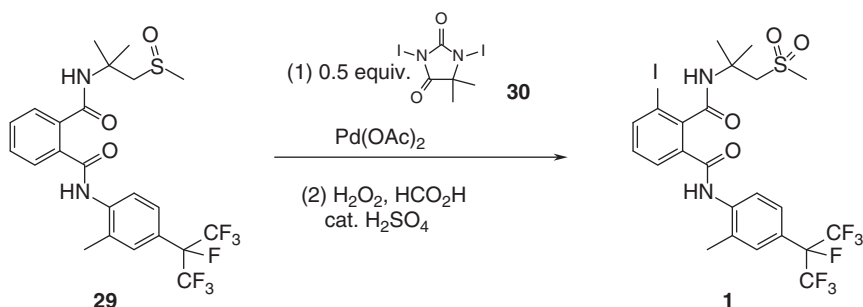
Figure 36.4 Phthaldiamide structures from Bayer and ZRICI/Sinochem.



Scheme 36.1 Early-stage synthesis of flubendiamide.

from the phthalanhydride **19** and the isoimide **22**. The heptafluoroisopropyl substituent is introduced via a radical reaction of 2-bromo-heptafluoropropane (**27**) on the aniline **28**. Bayer process chemists have developed an elegant and cheap synthesis of 2-bromo-heptafluoropropane (**27**), easily available from the commodity chemical hexafluoropropene (**25**).

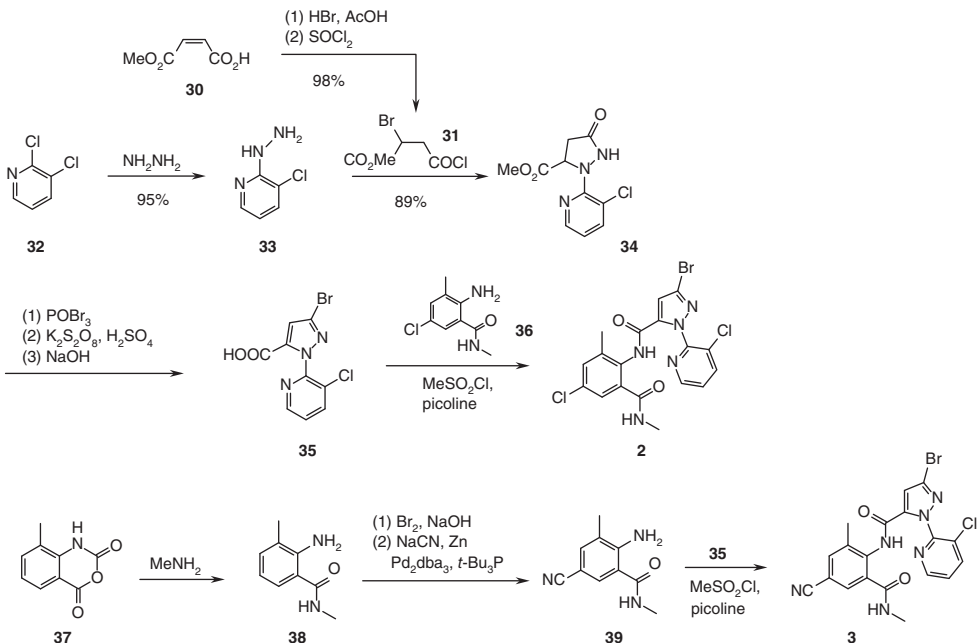
Alternatively, the iodine atom can be introduced at a late stage via a Pd-catalyzed regioselective iodination as depicted in Scheme 36.2. This nowadays classical C–H activation reaction [7] was quite novel when introduced in the patent literature by Nihon Nohyaku researchers [8].



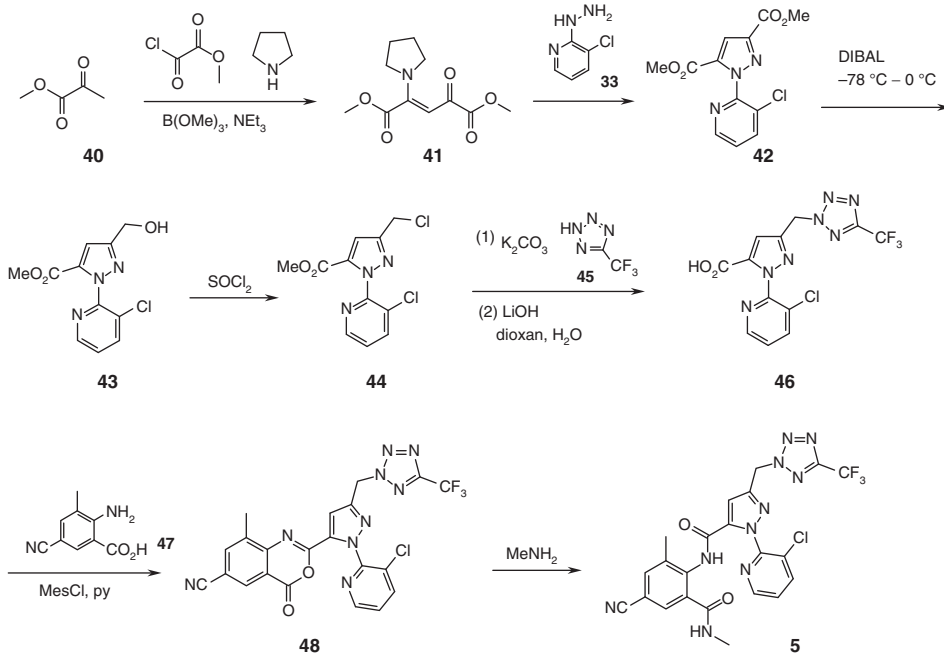
Scheme 36.2 Late-stage introduction of the iodine atom via C–H activation.

There are several possible syntheses of anthranilamides. A convergent approach is depicted in Scheme 36.3 [9]. The pyrazole carboxylic acid moiety is prepared by condensation of the pyridylhydrazine **33** with the maleic derivative **31** (or, alternatively, maleic ester) followed by bromination and oxidation. The acid **35** is then coupled with the amino amide **36** to give chlorantraniliprole (**2**).

In an alternative synthesis, introduced by DuPont researchers at an early stage [4], and exemplified in the synthesis of tetraniliprole (**5**) as depicted in Scheme 36.4, a pyrazole carboxylic acid such as **46** is coupled with an anthranilic acid **47** to give the benzoxazinone intermediate **48**. Benzoxazinones react smoothly with amines to give an anthranilic diamide such as tetraniliprole (**5**). This approach allows a broad variation of the aliphatic amine and was used for SAR investigations by most of the workers active in the field. Although the formation of the benzoxazinone from an acid and an anthranilic acid processes through a number of steps, simply mixing the partner in the presence of an excess of mesyl chloride as an activating agent and pyridine as a base gives the desired compound in high yield. In the synthesis of tetraniliprole (**5**), the regioselective reduction of the diester **42** to the mono alcohol is noteworthy as well as the alkylation of the chloride **44** with the tetrazole **45**, which gives a mixture of regioisomers that are difficult to separate.



Scheme 36.3 Synthesis of chlorantraniliprole (2) and cyantraniliprole (3).



Scheme 36.4 Synthesis of tetraniliprole (5).

36.4

Mode of Action

The diamides have been shown to activate the RyRs, which are ion channels regulating the Ca release from intracellular stores located in the sarcoplasmic reticulum. The regulation of intracellular calcium concentration is fundamental for a series of biological processes such as muscle contraction, neurotransmission, hormonal release, gene expression, cell growth, and cell differentiation [10]. Calcium mobilization through the RyR in muscle cells governs excitation/contraction coupling, and impairment of the RyR channels leads to serious muscle and neuronal disorders such as paralysis. Both phthalamides and anthranilamides show nanomolar affinity for the RyR. The diamide binding site is different from the ryanodine site but is allosterically related. In *Lepidoptera*, binding assay results, as well as SAR analogies, indicate a common active site for both the phthalamides and the anthranilamides. In other species, important differences have been found. In *Musca*, a high affinity site has been found for the anthranilamides but not for the phthalamides [11]. The structure of a 2.2 MDa homotetrameric RyR from rabbit and mouse, determined by cryo-electron microscopy at 3.8 Å resolution, has been published recently [12]. The mammalian RyR and insect RyR have poor homology, which makes the rational design of new insect RyR modulators by homology modeling not practicable. On the other hand, the excellent toxicology profile of chlorantraniliprole (**2**) is explained in part by its very poor affinity for the mammalian RyR receptor. In contrast, the natural product ryanodine has high affinity for the insect as well as for the mammalian RyR and, as a consequence, is toxic. It was not a successful lead for the search for novel RyR modulators.

36.5

Biological Activity

The diamides have a broad spectrum of activity against all *Lepidopteran* pests [9, 13]. They are less effective against adults than against larvae. Contrary to OPs and spinosyns, they have robust activity against larger larvae.

Flubendiamide (**1**) (log *P*: 4.2) is the less systemic compound of the family and has no activity against *Coleoptera*, *Hemiptera*, and *Acarina*. Chlorantraniliprole (**2**) (log *P*: 2.86) controls all major *Lepidopteran* pests and is also active against Colorado potato beetle, some grubs, leaf miners, and termites. It has also ovicidal properties. Cyantraniliprole (**3**) (log *P*: 1.91) is active against a broad range of *Lepidoptera* and a range of sucking pests such as white flies, thrips, aphids, plant- and leafhoppers through population suppression. It has excellent translaminar activity after foliar spray, controlling sucking pests that feed on the untreated side of the leaf. Typical field rates for chlorantraniliprole are 10–100 g ai/ha, for cyantraniliprole they are 10–100 g ai/ha (chewing pests) and 50–200 g ai/ha (sucking pests). Suppression of feeding activity is generally observed within 24 h after exposure. The diamides are generally nontoxic toward beneficial

arthropods such as predators, parasitoids, and pollinators. They have low mammalian toxicity. Due to their remarkable biological and ecological properties, the diamides have reached in a short time market prominence, and this popularity has led to the emergence of a number of resistant pest populations detected in various locations. The mechanism of resistance has been shown to involve single mutations in the case of diamond back moths [14, 15]. Currently, major companies are engaged in multiple insecticide resistance management activities worldwide (IRAC Country Diamide Working Group [16]).

36.6

Structure–Activity Relationship

The structure–activity relationship analysis of the phthalamides has been discussed in the literature based on *in vivo* [3, 13] as well as *in vitro* activity [17] (Ca^{2+} pump stimulation).

On the phthalamide core, a substituent R^1 , *ortho* to the aliphatic amide, is required and is preferentially halogen. Chlorine and iodine are the favorite substituents (Figure 36.5).

On the aromatic amide moiety, the unusual R^2 substituent $\text{CF}(\text{CF}_3)_2$ boosts the activity of a factor 10 over OCF_3 or a factor 100 over Cl. Furthermore, a methyl substituent R^3 is required for good activity.

The most favorable simple aliphatic amide is isopropyl or *tert*-butyl ($\text{R}^4 = \text{H}$ or CH_3). Adding a methylsulfonyl group enhanced the activity by a factor 10.

Detailed structure–activity relationship analyses for the anthranilamides have been published (Figures 36.6–36.9) [4, 18].

On the anthranilamide core, a substituent R^1 , *ortho* to the aromatic amide, is required for activity and is preferably methyl or halogen. Other substituents such as NO_2 , CN, OCH_3 , and acetylene lead to lower activity. Various substituents R^2 are tolerated, such as H, CH_3 , halogen, CN. The anthranilic core can be bicyclic (Types II or III, Type I is inactive), and examples of very potent analogs are the naphthalene derivative of Type II **49** and the indazole derivative of Type III **50** (Figure 36.6). The optimization of the pyrazole and pyridine heterocycles is

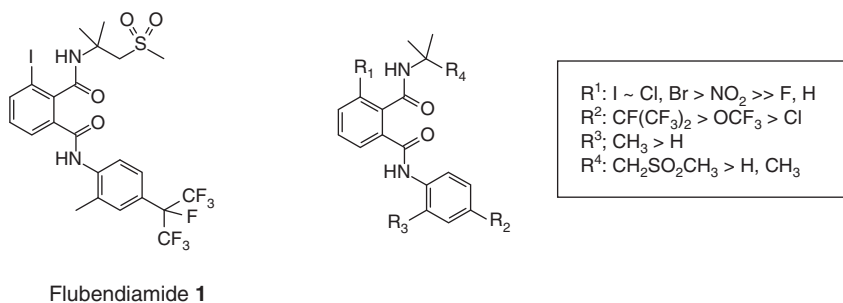
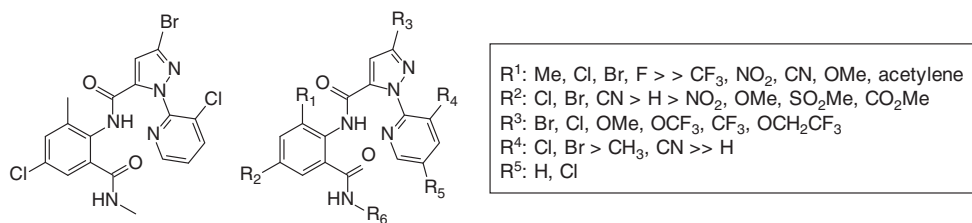
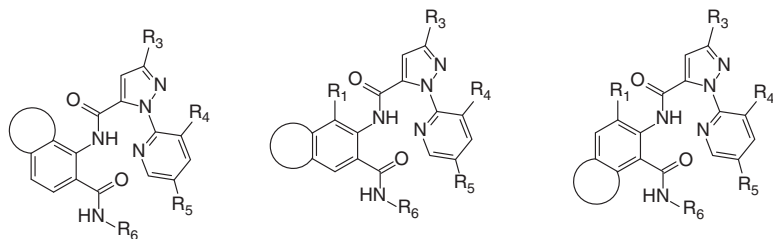


Figure 36.5 SAR of the phthalamides.



Chlorantraniliprole 2



Bicyclic anthranilamide Type I

Bicyclic anthranilamide Type II

Bicyclic anthranilamide Type III

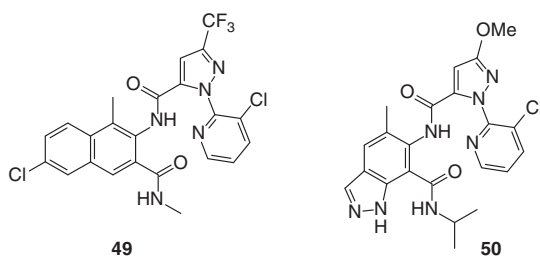


Figure 36.6 SAR of the anthranilamides (1).

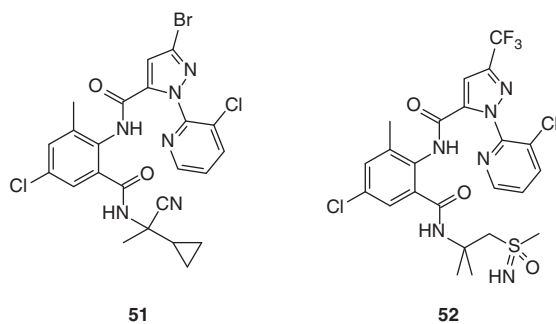


Figure 36.7 SAR of the anthranilamides (2): aliphatic amide variations.

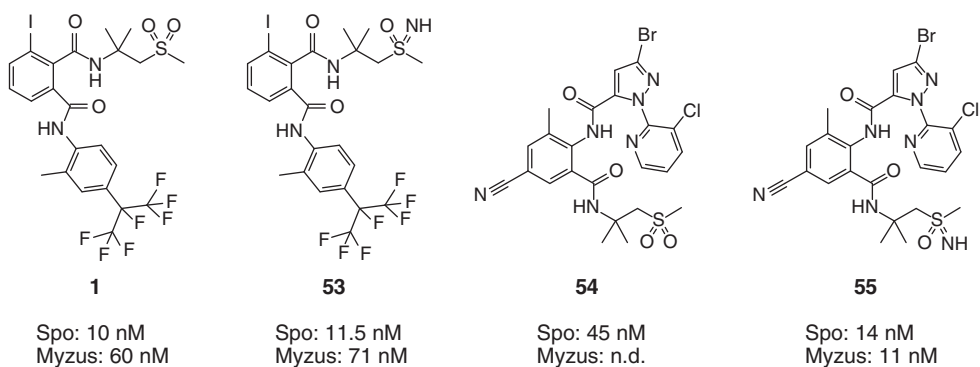


Figure 36.8 SAR of sulfoximine derivatives (binding assay [6], IC₅₀, n.d.: not determined).

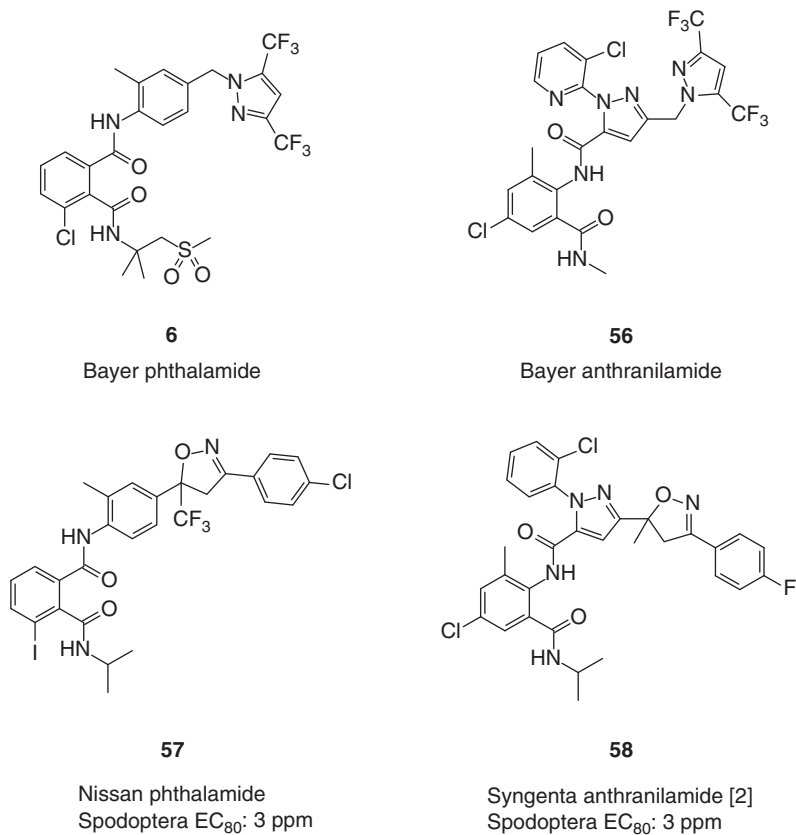


Figure 36.9 Analogy in the SAR of the phthalamides and the anthranilamides.

documented in [4] and in the patent literature. The pyrazole substituent R^3 can be CF_3 , halogen, OCF_3 , OCH_2CF_3 , OCH_3 . Compounds with $R^3 = OCH_3$ tend to have broader insecticidal spectrum due to increased aqueous solubility. The substituent R^4 on the pyridine ring is required for biological activity, twisting the two heterocycles out of planarity. R^5 can be H or halogen. The pyridine can also be replaced by a phenyl group.

The substituent R^6 (aliphatic amide) can be broadly varied. There are probably no strong binding interactions between R^6 and the RyR, and variations at this position rather affect the mobility properties of the diamides. Small alkyl chains, linear or branched, are optimal, the methyl group being the cheapest. Introducing functionalities such as CN in **51** or a sulfoximine in **52** [6] makes the molecule more soluble in water (Figure 36.7).

There are several arguments in favor of a similar SAR in the phthalamide and in the anthranilamide series. The best aliphatic side chains are often similar in both series, for example, the sulfoximines **53** and **55** or the cyano amine side chains (Figure 36.8). However, the side chain of flubendiamide attached to an anthranilamide gives a compound with a reduced *in vitro* activity by a factor 4–10, as shown in Figure 36.8 (compare, e.g., **54** with **1**, binding assay values, displacement of a tritiated analog of chlorantraniliprole (**2**) as described in the supplementary part of [6]).

Similar large substituents on the aromatic amide of the phthalamides and at the pyrazole of the anthranilamides are tolerated (compare the pairs **6/56** and **57/58** in Figure 36.9). This speaks in favor of a similar SAR or even a similar active site on the RyR of key Lepidoptera target pests. The phthalamides are, however, rather poor in a displacement assay in *Myzus* (displacement of a tritiated anthranilamide [6]) as well as in *Musca* [11, 19]. Key differences in the binding site are probably insect-specific [11].

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