1
From Milligrams to Tons: The Importance of Synthesis and Process Research in the Development of New Drugs

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1.1
Introduction

Synthetic chemistry plays a key role in the multidisciplinary development process of new small molecule pharmaceuticals. In this context, organic synthesis is not only the essential tool to find potential drug candidate molecules but is also in charge of the subsequent creation, exploration, and evaluation of short, efficient, safe, reproducible, scalable, ecological but still economical syntheses for the selected clinical candidates. This second activity generally named *synthesis and process research* or just *process research* is the indispensable link between discovery chemistry and technical development heading toward future large-scale industrial production. In addition to solving the gradually rising synthetic problems associated with the ever increasing structural complexity of new potential drug molecules, the resulting synthesis has to show technical potential and has, particularly, to take into account the basic requirements and limitations of a prospective technical process.

In this chapter, the role and importance of synthesis and process research in the development process of new drugs from discovery chemistry (medicinal chemistry) synthesis up to the technical route will be outlined and exemplified with specific examples also taking into account large-scale production requirements. The chapter concentrates on the synthetic strategies and tactics applied to drug candidates in order to create efficient chemical syntheses with technical potential suitable for further technical optimization aiming at the large-scale industrial production of new pharmaceuticals.

Owing to the permanently changing environment of the pharmaceutical industry and the tremendous advancements of science, neither general rules nor final or permanent principles and recipes for the successful transformation of a synthetic process from milligrams to tons or from discovery chemistry to production can be provided. Synthetic organic chemists know too well that molecules frequently behave incalculably, and that they are usually hard to control and therefore deserve individual treatment.

Pharmaceutical Process Chemistry. Edited by Takayuki Shioiri, Kunisuke Izawa, and Toshiro Konoike

ISBN: 978-3-527-32650-1

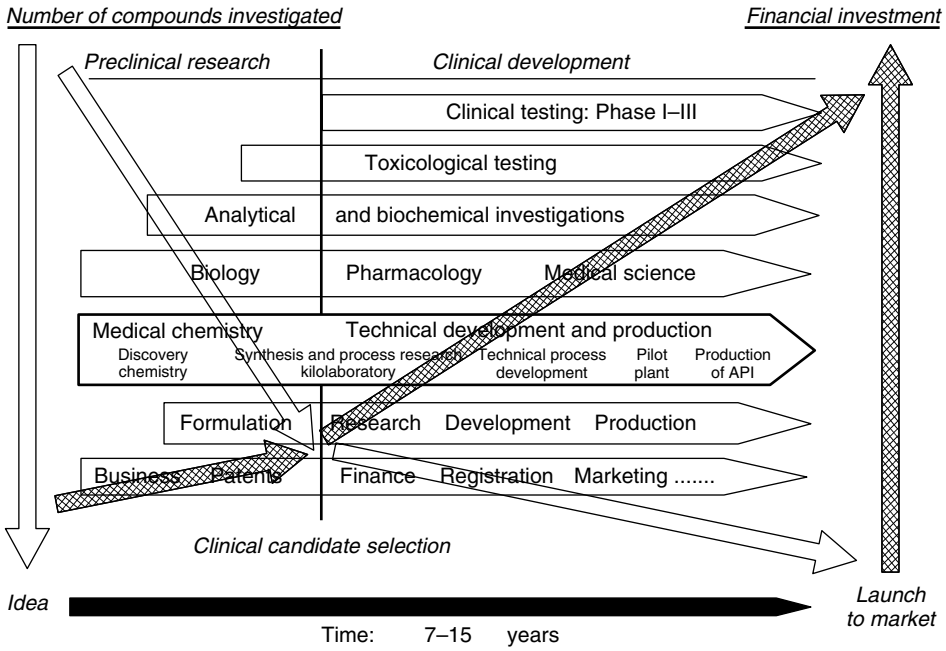


Figure 1.1 The drug development process (overview).

After a short introduction to the environment of chemists working in a synthesis and process research environment, the topic will be illustrated by four specific examples of innovative pharmaceuticals on their way to production for the market.

The classical development process of a new drug is sketched as a very coarse overview in Figure 1.1 and starts with the idea of which disease to treat and – in the best case – ends up with the introduction of a new pharmaceutical to the market. This multifaceted process easily takes 7–15 years, requires financial investments of up to $1–$2 billion and starts with a high number of up to many thousand compounds to be tested parallely by sophisticated methods such as high-throughput screening to finally come up with one or two clinical candidates to be further evaluated. In this overall research and development process, synthesis and process research – together with a scale-up or a kilolaboratory – represents a central activity at the important borderline linking preclinical research with clinical development at the stage of the clinical candidate selection. At this point in time, the so-called ''clinical candidate'' molecule emerging from discovery chemistry is selected, and first larger, sometimes up to kilogram amounts are immediately required to start clinical development and all related activities mentioned in Figure 1.1 including extended toxicological programs, analytical and biochemical investigations, as well as formulation research and development to find the appropriate pharmaceutical dosage form for the potential drug.

The role of synthesis and process research at this stage is twofold; namely, first to support the scale-up or kilolaboratory in troubleshooting and scaling up of the

discovery chemistry route to allow for an initial small-scale production of the new drug candidate as quickly as possible, material urgently required to start the clinical development activities mentioned in Figure 1.1.

If the project continues – but the attrition rate at this point is still high – this activity is followed by a partial or full synthetic redesign to finally identify a synthetic route with technical potential to be handed over to the technical development department, which then has the task to transform the new synthesis into an efficient production process that will later be used for the manufacture of the active pharmaceutical ingredient (API) in commercial amounts.

An important question that regularly comes up is: why is synthesis and process research needed since there already exists a synthesis established by discovery chemistry about the way to find the clinical candidate? The answer to this question is emblematized in Figure 1.2 and relates to the different synthetic strategies applied by discovery chemistry on the one hand against synthesis and process research on the other hand.

The goal of discovery chemistry – the task of medicinal chemists – is to synthesize as many new compounds as quickly as possible, which will then be tested by biologists against the chosen biological target. Therefore, the synthetic strategy of discovery chemistry is an overall diversity-oriented process, allowing for finding

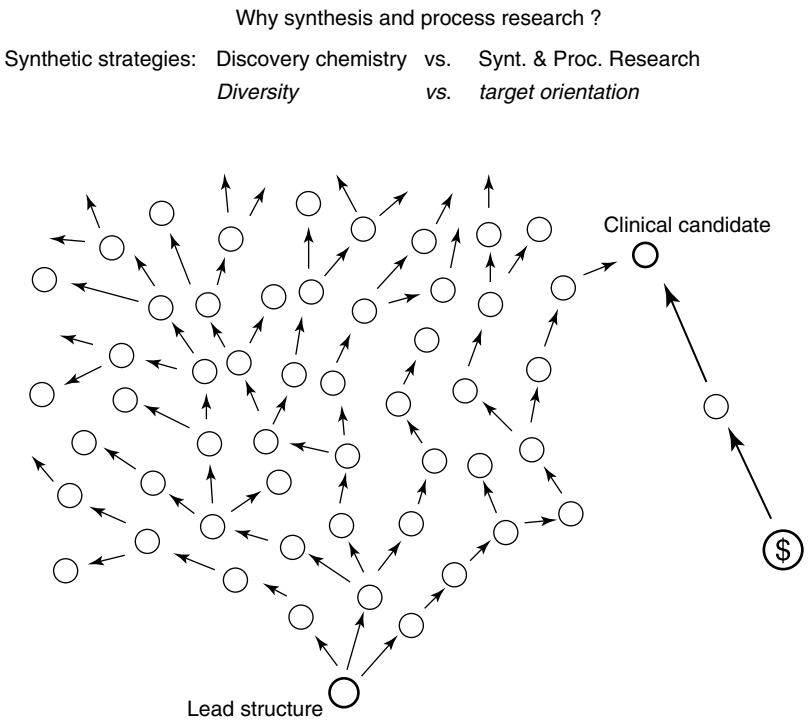


Figure 1.2 Synthetic strategies used by drug discovery versus synthesis and process research.

Prof. Ryoji Noyori
Nobel Laureate 2001

"Chemical synthesis with practical elegance"

Key requirements:

Absolute efficiency using perfect chemical reactions
► 100% selectivity & 100% yield

Economical processes ► No unwanted wastes

Environmentally friendly ► Resource and energy-saving

"The need for efficient and practical synthesis remains one of the greatest intellectual challenges with which chemists are faced in the 21st Century"

Figure 1.3 Chemical synthesis with practical elegance [1].

access to a large number of new compounds as quickly as possible. Starting from a hit compound obtained by high-throughput screening, automated parallel chemistry, or related techniques, lead structures are selected, which will further be optimized regarding the key parameters using multidimensional optimization to finally reach viable clinical candidates, which meet the criteria set regarding activity, selectivity, toxicology, safety, and so on. Despite all the modern and rational methods of contemporary drug discovery, a large number of compounds still have to be synthesized and tested.

After the identification of a new clinical candidate, the situation regarding the synthetic strategy changes entirely from diversity to target orientation. The task now is to create for the selected clinical candidate molecule, a specific synthesis with the potential to be later technically developed by the technical development department into a large-scale production process.

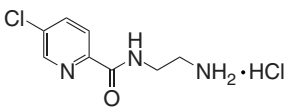
In this context, it is motivating for synthetic organic chemists working in the field of synthesis and process research and related departments to recall the definition of a ''synthesis with practical elegance'' introduced by Prof. Noyori, Nobel laureate, 2001 (Figure 1.3). Although the key requirements for a ''synthesis with practical elegance'' are highly challenging regarding efficiency, environmental impact, and economy, it is the obligation of responsible chemists to intensively and persistently strive for these goals.

To illustrate the synthetic development process for new pharmaceuticals at F. Hoffmann-La Roche Ltd, Basel, Switzerland, the four examples shown in Figure 1.4 will be discussed and commented, examples already patented and/or published for which syntheses with technical potential were created. Three of them (Xenical™, Saquinavir™, Tamiflu™) finally reached the market place. Tempium, a monoamine oxidase type B (MAO-B) inhibitor, which finally was dropped, but is included in the discussion since substantial amounts have already been produced according to the final one-step process starting off from a nine-step discovery synthesis. Taking into account the fundamental differences of the synthetic strategies, it is

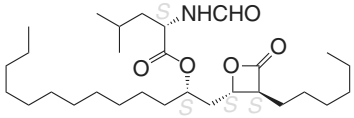
Synthesis and process research at roche

"Comparing" number of synthetic steps and overall yield :

Tempium™ (Alzheimers disease)
Lazabemide:

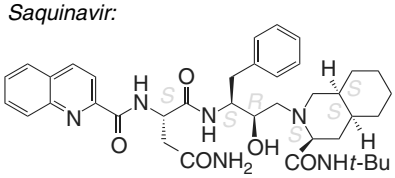


Xenical™ (obesity)
Tetrahydrolipstatin:

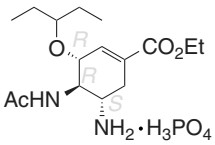


Discovery chemistry:	9 (8%)	12 (2%)
Synthesis and process research:	1 (75%)	8 (22%)

Invirase™ (HIV)
Saquinavir:



Tamiflu™ (Influenza)
Oseltamivir phosphate:



Discovery chemistry:	25 (5%)	16 (5%)
Synthesis and process research:	10 (50%)	10 (35%)

Figure 1.4 Selected examples of synthesis and process research at F. Hoffmann-La Roche Ltd, Basel, Switzerland.

very important to stress that a ''comparison'' of syntheses between the discovery chemistry route and the resulting synthesis created by synthesis and process research only with regard to the number of steps and overall yields is not admissible, since the key tasks, goals, and strategies of both areas are fundamentally different. As discussed above, the key task of discovery chemistry is to synthesize in a diversity-oriented manner – and as fast as possible – small amounts of new, biologically active molecules to be tested as potential clinical candidates. To this end, all available and sophisticated synthetic methods and separation techniques of modern organic chemistry should be applied. In contrast and after clinical candidate selection, it is the distinguished task of synthesis and process research chemists to create and evaluate in a target-oriented manner, a synthesis with technical potential for the selected clinical candidate to subsequently be technically developed for large-scale production.

Since a detailed discussion on the chemistry of all these four projects is out of scope of this review, the focus has been on the strategic principals and solutions, which has finally led in all four cases to practical technical solutions. Additional details are easily accessible through the chemical literature and through patents referred to in the corresponding schemes.

1.2
The Synthetic Development of the Monoamine Oxidase-B Inhibitor Lazabemide™

Lazabemide, an MAO-B inhibitor developed for the treatment of Alzheimer's disease, was first synthesized following a classical pathway starting from cheap "aldehyde collidine" (5-ethyl-2-methylpyridine). The key intermediate was 5-chloro-2-picolinic acid that was further converted to a wide variety of the corresponding amides to finally select the 2-aminoethyl-amide as the clinical candidate. The synthesis completed in a nine linear sequence and about 2–8% overall yield in the discovery chemistry stage, as shown in Scheme 1.1.

This classical approach required about 26 kg of the starting material to produce 1 kg of the active substance. An additional immense challenge for the kilolaboratory was the barely selective permanganate oxidation of the starting material at the first step, which was confronted with the cumbersome filtration of a large amount of manganese dioxide.

Although a troubleshooting of the discovery chemistry route enhanced the overall yield to about 10%, it was not essential for further scaling up. Efforts on the search for an alternative synthesis allowed the catalysis group of synthesis and process research to identify commercially available 2,5-dichloropyridine as an ideal starting material, which underwent Pd-catalyzed Sonogashira reaction with various acetylenes to afford the corresponding acetylenic pyridines in a highly efficient and selective manner. Although a permanganate oxidation was still required to gain access to the 5-chloro-picolinic acid intermediate, the overall yield of this scalable four-step process was already improved to 58% and only 1.1 kg of the starting material was required.

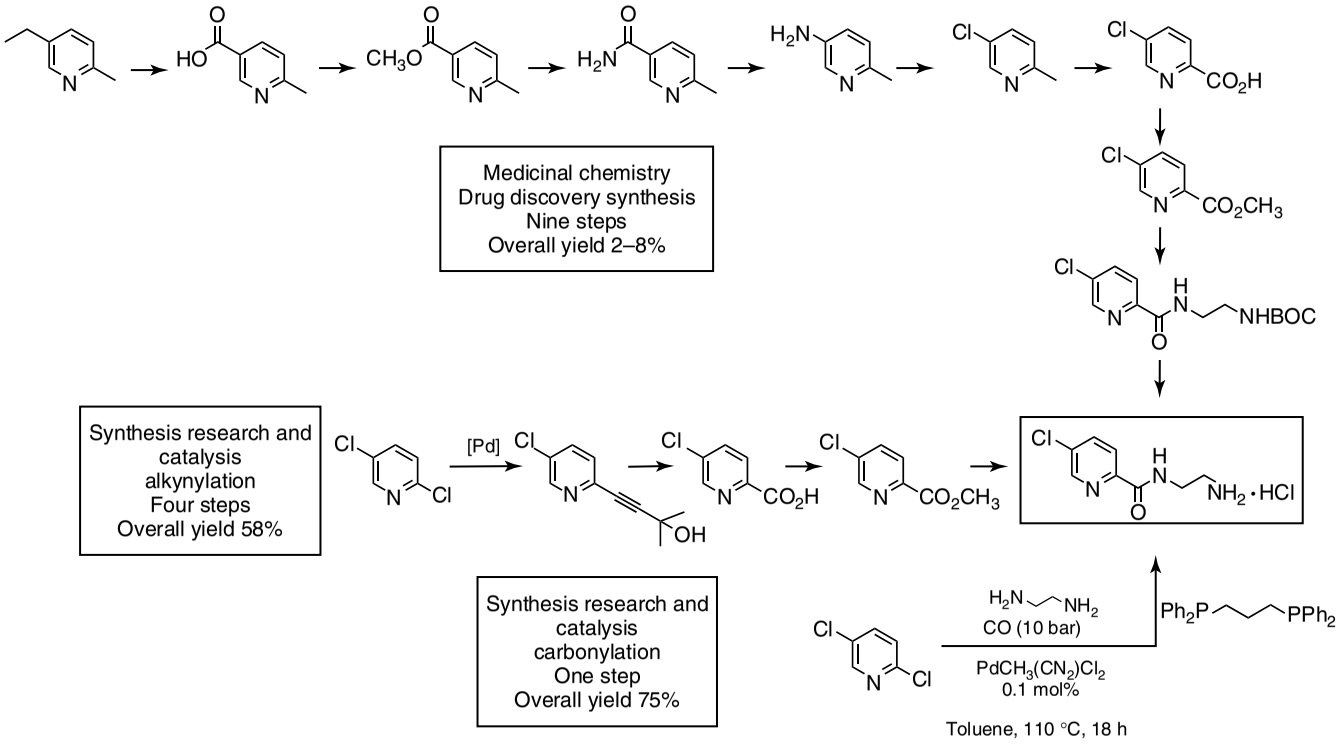
Finally, a direct, one-step amido carbonylation process was introduced using the same starting material, which provided the API in one step and 75% yield. The reaction was later developed to the 100 kg scale, requiring only 0.8 kg of the starting material to obtain 1 kg of the API.

As shown in Scheme 1.1, the reaction with an excess of ethylenediamine and carbon monoxide at 10 bar using only 0.1 mol% of the Pd(0) precursor and a phosphine ligand in toluene at reflux led after appropriate workup directly to the pure API in 75% yield. Interestingly, ethylenediamine used in excess acted as a reagent and as the base without deactivating the catalytically active Pd(0) species.

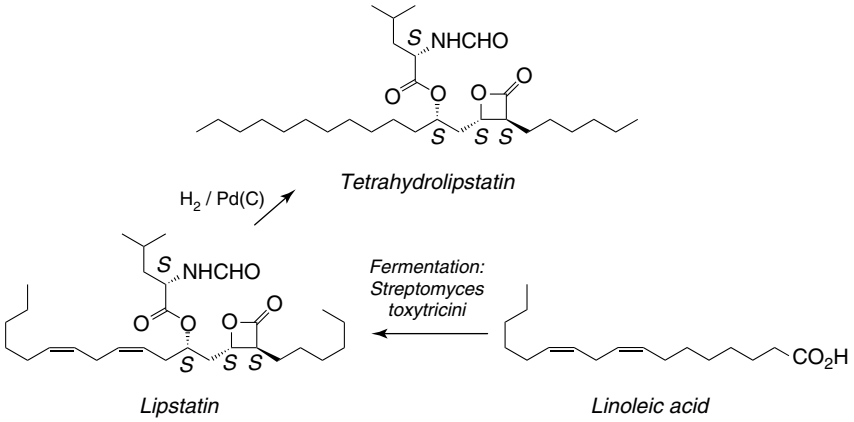
Although a one-step solution looks very favorable from a synthesis and process research chemist's point of view, the potential issues regarding the registration of such a short approach with the health authorities should not be neglected.

1.3
The Synthetic Development of the Lipase Inhibitor Tetrahydrolipstatin (Xenical™)

The second case to be discussed concerns tetrahydrolipstatin, a very potent and irreversible inhibitor of pancreatic lipase found and developed at Roche for the



Scheme 1.1 The synthetic development of the monoamine oxidase-B inhibitor, lazabemide [3].



Scheme 1.2 Tetrahydrolipstatin [4].

treatment of overweight is shown in Scheme 1.2. This molecule represents the tetrahydro derivative of the natural product lipstatin, a secondary metabolite produced by *Streptomyces toxytricini*. Tetrahydrolipstatin as well as its natural counterpart lipstatin effectively inhibit the hydrolysis of triglycerides in food, thereby reducing digestion and uptake of dietary fats.

Although it finally became possible to produce lipstatin by fermentation using a mutant strain and employing linoleic acid as an auxiliary material, the search for an organic chemical synthesis with technical potential was first allowing for the production of the API at many a hundred ton scale.

From a synthetic standpoint, two intriguing structural features have to be synthetically controlled, also at large scale, namely a rather labile and quite reactive trans-substituted β-lactone moiety embedded in a C21 aliphatic chain and four stereocenters. Only one of them is commercially available in high optical purity in the form of the amino acid, leucine. Therefore the three stereocenters embedded in the aliphatic C21 chain had to be created by stereoselective synthesis.

From a stability standpoint it was essential to introduce the rather labile β-lactone moiety toward the end of the synthesis. Therefore, all practical syntheses of tetrahydrolipstatin proceeded through a common intermediate, the 2*S*,3*S*,5*R*-configured α-hexyl-β-hydroxy-δ-benzyloxy acid, shown in Scheme 1.3.

This key intermediate was then converted to the final product tetrahydrolipstatin by β-lactone formation, followed by debenzylation and introduction of the *N*-formyl leucine side chain under Mitsunobu conditions with complete inversion at the reacting stereo center.

The control of configuration of the three stereocenters in the chain was first achieved using two independent stereoselective transformations; namely, first an enantioselective hydrogenation of the starting β-keto-ester followed by O-benzylation and reduction to the aldehyde. Second, the aldehyde was condensed at low temperature in a Mukayama-type antialdol manner according to

Scheme 1.3 Early access to tetrahydrolipstatin [6, 7].

Gennari and Scolastico with the (trimethylsilyl) TMS-ketene acetal bearing the *N*-methylephedrine auxiliary group to induce the two additional stereo centers in the required absolute configuration.

From a technical standpoint, this 12-step discovery route, however, is clearly not deemed for future technical purposes, not only due to the large number of transformations involved including low temperature steps, but also due to the large number of chromatographic purification steps required and leading to a modest overall yield. Nevertheless, in a heroic effort, the kilolaboratory succeeded in producing kilogram amounts of tetrahydrolipstatin according to gradually troubleshooted versions of this initial approach, since the material was urgently needed for starting clinical development.

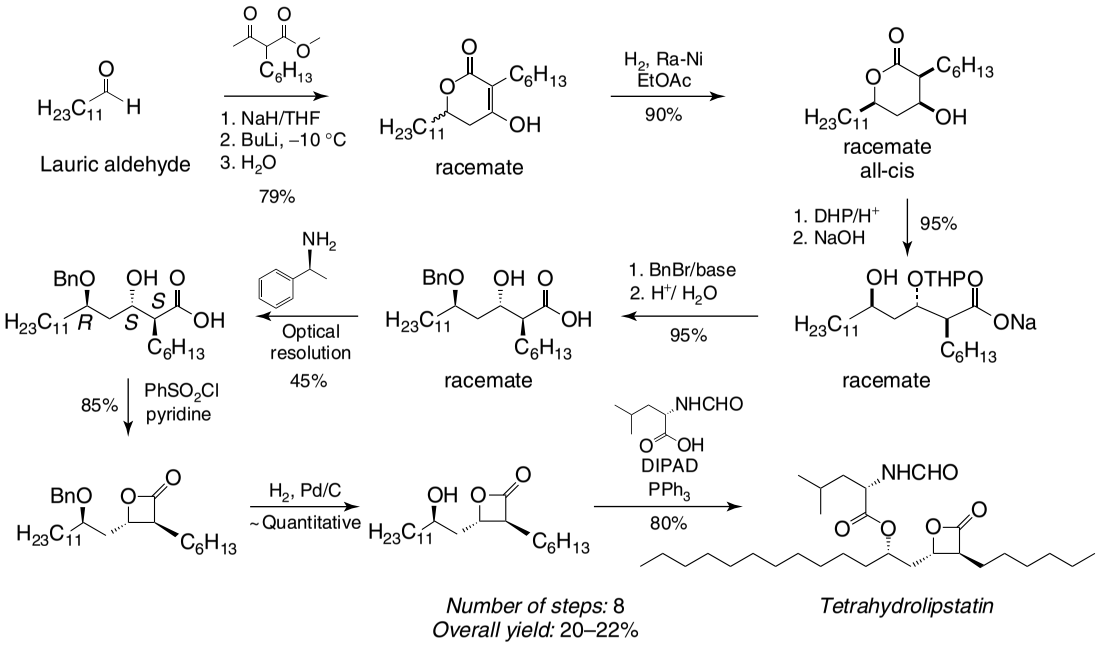
In order to take advantage of a preexisting stereo center in 3-position of the optically pure β-hydroxy ester to induce additional stereo centers in a diastereoselective manner, synthesis and process research first investigated the path shown on the right section of Scheme 1.3. Starting from the optically pure *O*-benzylated β-hydroxy ester a two-carbon chain elongation using Masamune's protocol followed by debenzylation, cyclization, and alkylation directly led to the dihydropyrone which now, by heterogeneous hydrogenation over Ra-Ni was transformed to the β-hydroxy-δ-lactone with high diastereoselective induction of the two additional stereo centers. Subsequent opening of the resulting β-hydroxy-δ-lactone unit provided access to the key intermediate, the optically pure α-hexyl-β-hydroxy-δ-benzyloxy acid.

From a technical perspective, this procedure not only had the advantage to be devoid of low temperature reactions but also had the additional advantage that the cyclic intermediates are crystalline compounds and therefore easy to purify by recrystallization. However, this approach – although without chromatographic purifications involved – is even more demanding than the troubleshot discovery route regarding the number of steps involved, already requiring five steps for the access to the dihydropyrone key intermediate.

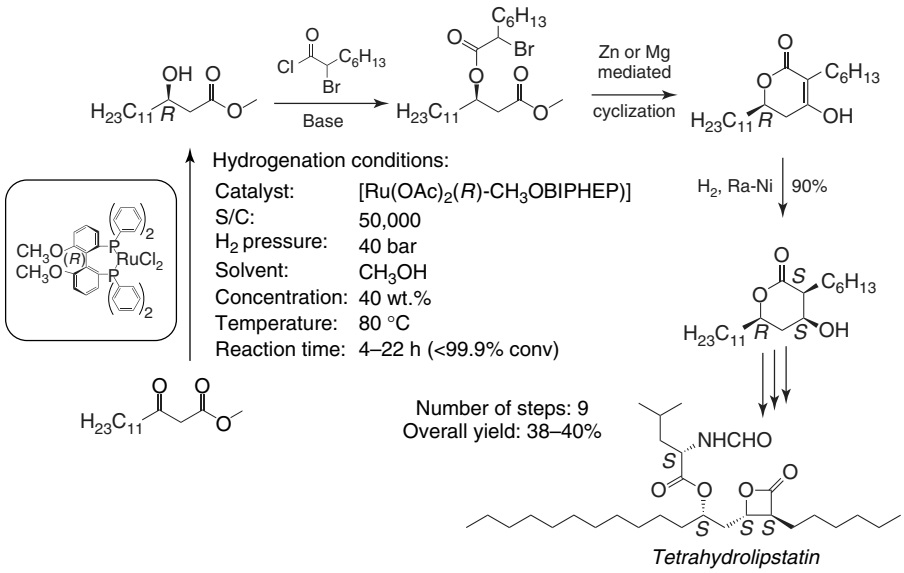
The problem was finally solved in a very pragmatic way when it was found that access to the racemic dihydropyrone requires only one step that is achievable by addition of the double anion of 2-hexyl-methyl-acetoacetate to laurinic aldehyde followed by spontaneous cyclization at workup as summarized in Scheme 1.4.

Since the diastereoselectivity argument also holds true for the racemic dihydropyrone, the current technical synthesis of tetrahydrolipstatin proceeds through racemates followed by classical optical resolution. Diastereoselective hydrogenation provided the still racemic, but all-cis β-hydroxy-δ-lactone in 90% yield.

Tetrahydropyranyl (THP) protection of the β-hydroxy group followed by lactone ring opening through basic hydrolysis provided the sodium salt of the corresponding acid, which allowed for the selective O-benzylation in δ-position providing the key intermediate as a pure racemate. Optical resolution using economical (−)-phenyl-ethylamine was performed with high yield furnishing the desired α-hexyl-β-hydroxy-δ-benzyloxy acid in optically pure form ready for the final sequence including β-lactone formation, hydrogenolysis, and introduction of



Scheme 1.4 The technical synthesis of tetrahydrolipstatin [7].



Scheme 1.5 Synthesis of tetrahydrolipstatin via enantioselective access to the optically pure α-hexyl-β-hydroxy-δ-lactone [8].

the *N*-formyl leucine side chain under Mitsunobu-type conditions with complete inversion of configuration.

This eight-step synthesis was developed by technical development to a large-scale process by which many hundred tons of the active principle have been synthesized so far.

Evidently, optical resolution at an intermediate or even at the late stage of a technical synthesis is clearly not the preferred option regarding efficiency, in the case that the undesired enantiomer cannot be recycled and has to be discarded. Therefore, various routes toward an efficient enantioselective access to the optically active α-hexyl-β-hydroxy-δ-lactone were investigated. A successful approach as shown in Scheme 1.5 is based on earlier findings and started with the O-acylation of the previously mentioned optically pure β-hydroxy ester with α-bromo caprylic acid chloride followed by Zn- or Mg-mediated Reformatzky-type ring closure. Raney nickel hydrogenation then provided the optically active β-hydroxy-δ-lactone moiety ready for the transformation to tetrahydrolipstatin in analogy to the transformation shown above, but without requiring optical resolution.

To this end, a high-yielding and efficient enantioselective hydrogenation procedure for the β-keto ester starting material in order to obtain the optically pure β-hydroxy ester was required. This method was developed by the catalysis group of synthesis and process research and uses a ruthenium catalyst with the Roche owned (*R*)-CH_3OBIPHEP-ligand and applies the conditions summarized in Scheme 1.5 providing the optically pure β-hydroxy ester in almost quantitative yield and a high optical purity of more than 99% ee.

In summary, this enantioselective protocol provided access to tetrahydrolipstatin with a nearly doubled overall yield compared to the racemic approach shown in Scheme 1.4.

1.4 The Synthetic Development of the HIV Protease Inhibitor Saquinavir (Invirase™)

Saquinavir (Figure 1.5) represents a very potent HIV protease inhibitor found and developed at Roche for the treatment of AIDS. The speedy development of this peptidomimetic molecule allowed entering the market first in 1995, followed by Merck's Indinavir™.

A coarse retrosynthetic view presented in Scheme 1.6 depicts the essential starting materials required to be finally combined to the API. Quinaldic acid as well as L-asparagine representing the left-hand part are commercially available in large amounts. Both the isoster subunit, assumed to be mainly responsible for the inhibitory activity by mimicking a peptide bond, as well as the decahydroamide part are derived from another abundant amino acid, namely L-phenylalanine by diastereoselective reactions.

Despite enormous efforts put into the evaluation of alternative enantioselective approaches to the decahydroamide building block, the diastereoselectivity based route evolved as the most efficient strategic one. The overview on the discovery chemistry synthesis presented in Scheme 1.7 summarizes the enormous effort of discovery chemistry in the initial access to the key intermediates, the so-called phthaloyl epoxide and the decahydroamide both derived from L-phenylalanine. Combination of these key fragments by epoxide ring opening was then followed by the stepwise introduction of pentafluorophenol activated L-asparagine and *N*-hydroxy-succinimidyl activated quinaldic acid to obtain – after 25 steps and about the same number of chromatographic purifications – the active compound in an overall yield of about 5%. It is important to mention at this point that discovery chemistry synthesized several hundred compounds of related structure and a kilolaboratory was able to produce several 10 kg of the API applying a gradually developing route, material urgently required to start clinical development.

After stepwise improvement, a synthesis with technical potential was handed over to the chemists of the technical development department in order to develop the large-scale technical process shown in Scheme 1.8.

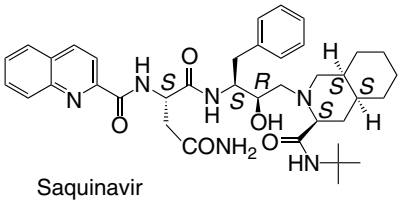
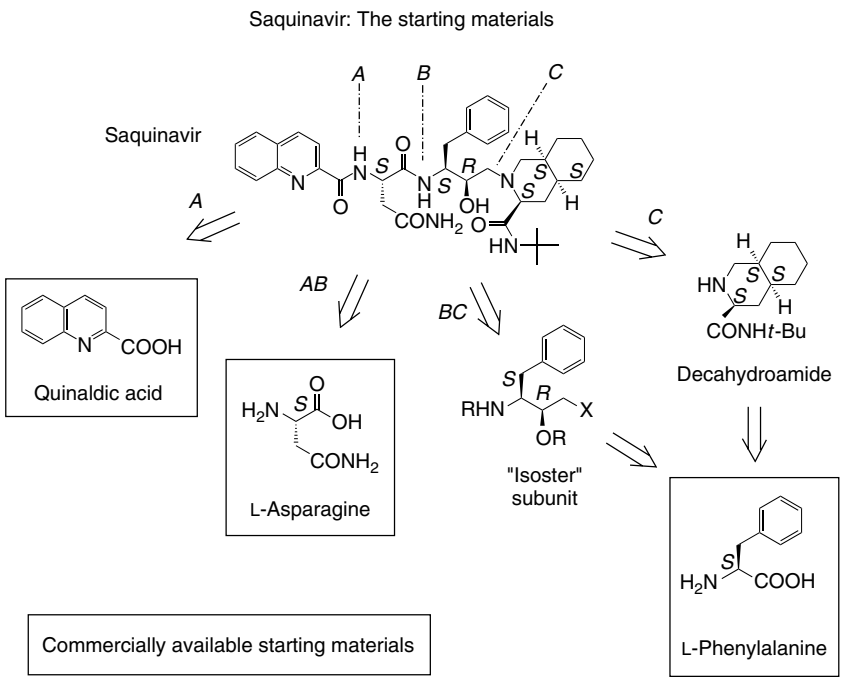


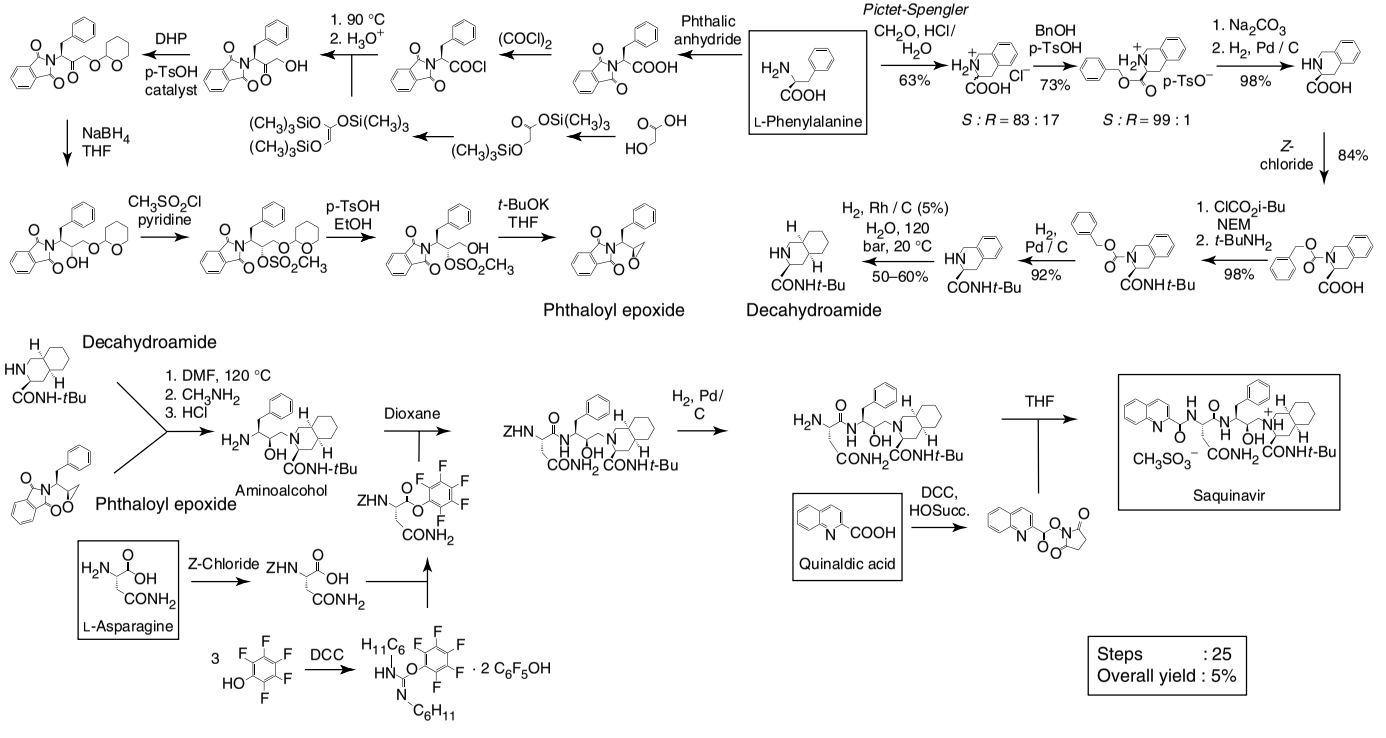
Figure 1.5 The structure of Saquinavir (Invirase™).



Scheme 1.6 Starting materials for Saquinavir.

The same primary starting material, L-phenylalanine, was used to propagate configuration and induce additional stereo centers. The chlorohydrine, which was employed as the phthaloyl epoxide equivalent as well as the decahydroamide were both accessible in only three steps. Coupling of these building blocks followed by N-deprotection gave access to the so-called amino alcohol building block, which was joined in the last step with quinargine, the coupling product of quinaldic acid and asparagine efficiently obtained via the mixed anhydride of pivalic acid.

The contribution of synthesis and process research to the process regarding ecological factors such as materials and solvents required is summarized in Table 1.1. The amount of materials and solvents hypothetically required to produce 1 metric ton of Saquinavir according to the discovery chemistry synthesis would be huge (calculation based on the experimental description of the discovery chemistry publication presented in Scheme 1.7), whereas these amounts were already considerably reduced by troubleshooting and optimization work toward a first scalable 16-step route providing additional active material required for the further clinical development program. The technically developed process based on the 10-step synthesis uses considerably less materials and solvents. However, it is important to stress that the main contribution of synthesis and process research concerns the evaluation of a short, efficient 10-step synthesis with technical potential. The key contribution to the reduction of materials involved is mainly



Scheme 1.7 Discovery chemistry synthesis of Saquinavir [9, 10].

Table 1.1 Contribution of synthesis and process to the production route of Saquinavir.

Synthesis	Steps	Overall yield (%)	Required for 1 ton active drug	
			Reagents (tons)	Solvents
Discovery route	25	5	700	176 
Troubleshooting	25	20	88	23 
Scalable synthesis	16	26	80	17 
Commercial synthesis	10	50	13	3 

based upon the work of technical development chemists by streamlining and optimizing all process steps to the optimum.

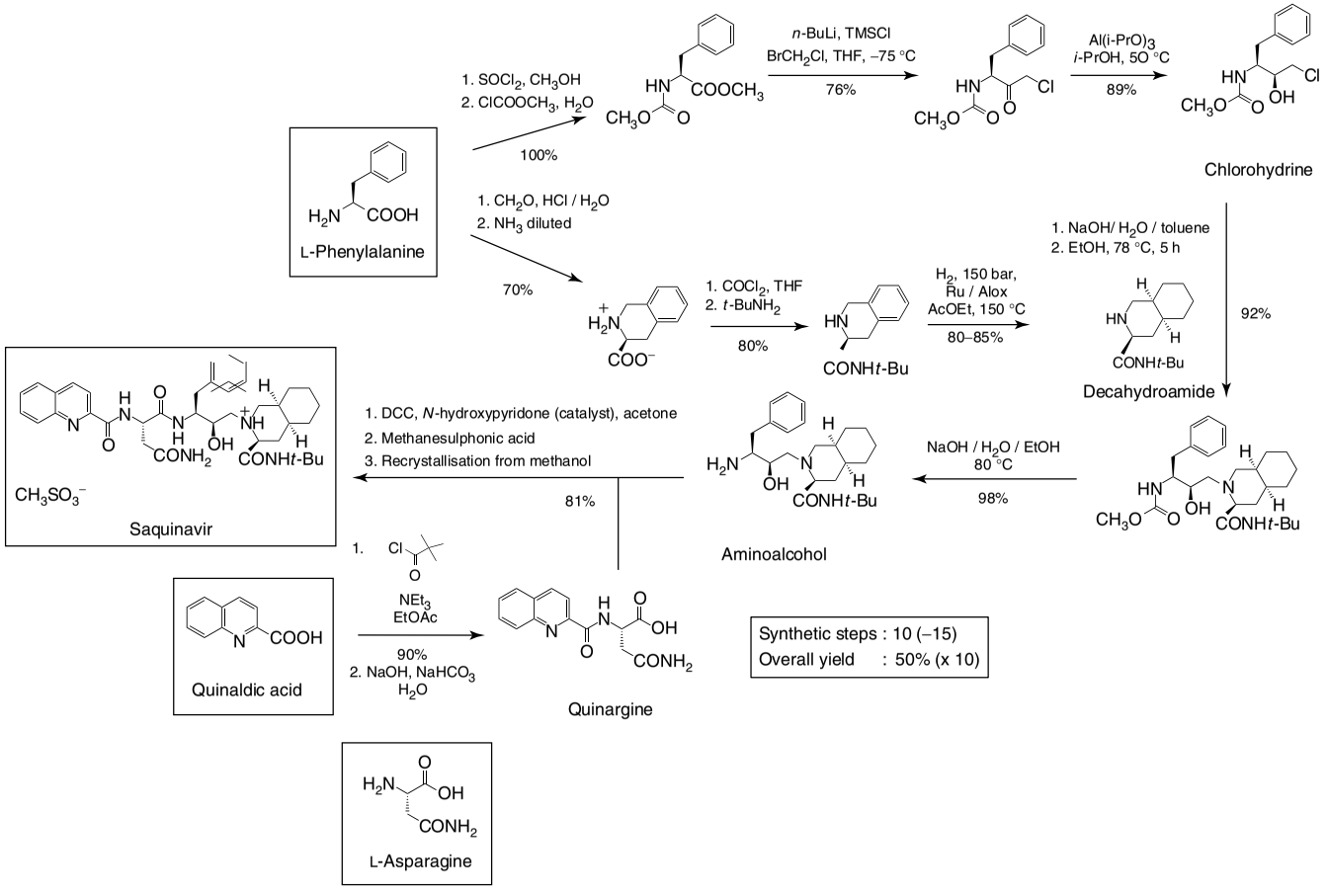
1.5 The Synthetic Development of the Influenza Neuraminidase Inhibitor Oseltamivir Phosphate (Tamiflu™)

1.5.1 Introduction

The Roche/Gilead influenza neuraminidase inhibitor oseltamivir phosphate (Tamiflu™) (Figure 1.6), a trisubstituted cyclohexene ethyl carboxylate, is the orally available prodrug of the corresponding acid, which in turn is a very selective and potent inhibitor of influenza neuraminidase at nanomolar concentrations with an ideal half-life of about 3 h. The highly water-soluble phosphate salt is now used for the oral treatment and prevention of influenza virus infections, a disease that affects several million people each winter and providentially, the compound is also active against the H5N1 bird flu as well as the H1N1 swine flu virus that spreadduring 2009.

The inhibitor was found at Gilead Sciences, California, and a codevelopment contract was signed with Roche in 1996 followed by one of the fastest development programs culminating, after only three years of chemical and clinical development, in the launch of Tamiflu in as early as November 1999.

The ambitious program was triggered by the competitive situation since GlaxoSmithKline was concurrently developing their neuraminidase inhibitor zanamivir (Relenza™) (Figure 1.6). Even though their heterocyclic guanidino substituted dihydropyrane carboxylic acid derivative is also a very potent inhibitor of influenza neuraminidase, the compound shows low oral bioavailability and a short half-life



Scheme 1.8 Commercial synthesis of Saquinavir [11].

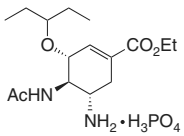
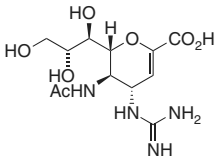
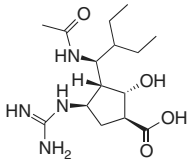
Oseltamivir phosphate Tamiflu™		Zanamivir Relenza™		Peramivir Rapiacta™ (*Japan*)	
					
Use	Oral treatment and prevention of influenza virus infections	Use	Topical treatment of influenza virus infections by application via disk inhaler	Use	Treatment of influenza virus infections by intravenous application
Originator:	Gilead Sciences, California	Originator:	Biota holdings, Australia	Originator:	BioCryst
Licensee:	F. Hoffmann-La Roche Ltd.	Licensee:	Glaxo SmithKline	Licensee:	Shionogi and others
Launch:	November 1999	Launch:	July 1999	Launch:	October 2009 (USA, for emergency use) January 2010 (Japan)

Figure 1.6 Marketed anti-influenza neuraminidase inhibitors.

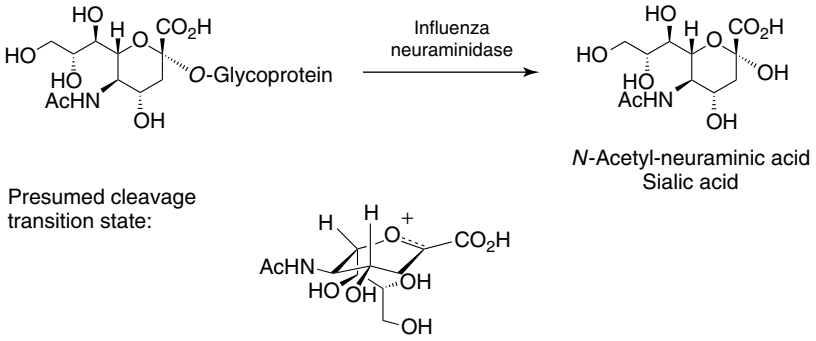
allowing only for its topical application via disk inhaler technology compared to an easy to administer capsule for oseltamivir phosphate. Zanamivir originates from the laboratories of Monash University in Australia and was licensed to GlaxoSmithKline via Biota Holding.

A third compound peramivir (Figure 1.6), found at BioCryst Pharmaceuticals, Inc. entered the US market in October 2009, when the FDA authorized its emergency use as an intravenous antiviral for certain patients and was introduced in January 2010 in Japan by the Shionogi & Co., Ltd under the trade name Rapiacta™.

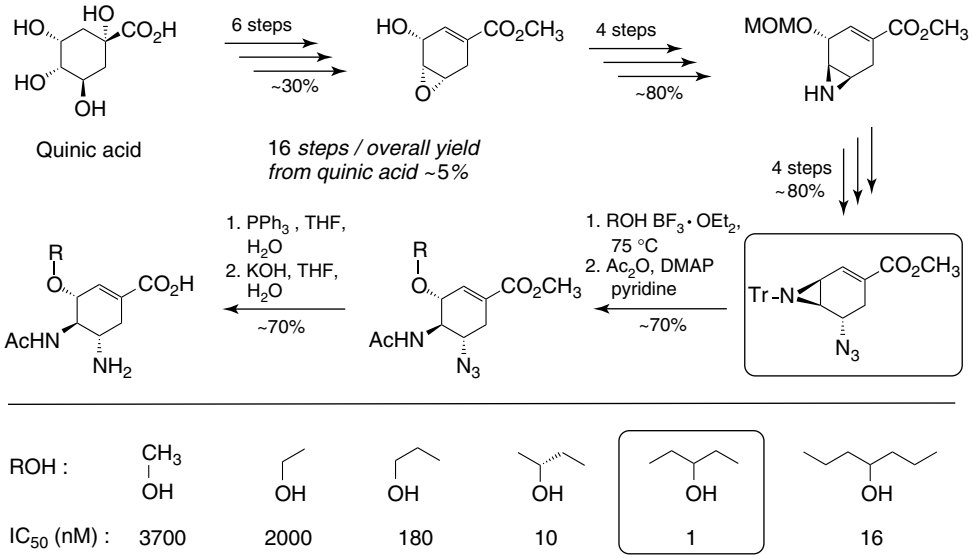
The influenza neuraminidase represents a viral surface protein with the important role of cleaving the sialic acid end groups of the glycoproteins present on the surface of the infected cell. According to current knowledge this cleavage process allows the newly formed viral particles to escape from the ''sialic acid glue'' of the infected cells' surface and to infect new host cells. Inhibition of this cleavage process schematically depicted in Scheme 1.9 leads to the aggregation of the emerging viral particles on the surface of the destroyed cell, thereby efficiently stopping the infective cycle. Oseltamivir-free acid as well as zanamivir are thought to be effective mimics of the postulated oxonium-type transition state of this cleavage process.

1.5.2
The Development of the Current Technical Synthesis of Oseltamivir Phosphate

To illustrate the general remarks provided in the introduction regarding the task and role of synthesis and process research, the discussion starts with an overview of the Gilead discovery chemistry route as a typical example demonstrating the



Scheme 1.9 The role of the neuraminidase in the life cycle of the influenza virus.



Scheme 1.10 The Gilead drug discovery synthesis [12, 13].

different synthetic strategies of drug discovery on the one hand compared to synthesis and process research on the other.

For discovery chemistry, the trityl aziridine azide was synthesized as the branching intermediate allowing for a fast transformation to a variety of potential drug candidates by regio- and stereoselective opening of the aziridine ring at the allylic position using various hydroxy components under Lewis acid catalysis followed by N-acetylation, azide reduction, and saponification. As indicated with a small selection of derivatives in Scheme 1.10 variation of the ether side chain led to a tremendous effect on activity, starting with the methoxy derivative still in the micromolar range passing through the ethyl and propyl derivative and finally arriving at the nanomolar activity range with the 3-pentyloxy derivative as the most active derivative.

This discovery chemistry route obviously was hardly amenable to scale-up due to a number of issues starting with (−)-quinic acid, a compound that is scarcely available in larger amounts. The access to the hydroxy-epoxide took six known steps, another four steps to the aziridine, and four additional steps to reach the branching trityl aziridine azide. All together about 16 steps required with an overall yield of roughly 10% including numerous chromatographic purifications.

After choosing the 3-pentyl-ether derivative as the most active inhibitor and the ethyl ester as the ideal prodrug, synthesis and process research activities were already initiated at Gilead Sciences for making oseltamivir phosphate available at least in kilogram amounts.

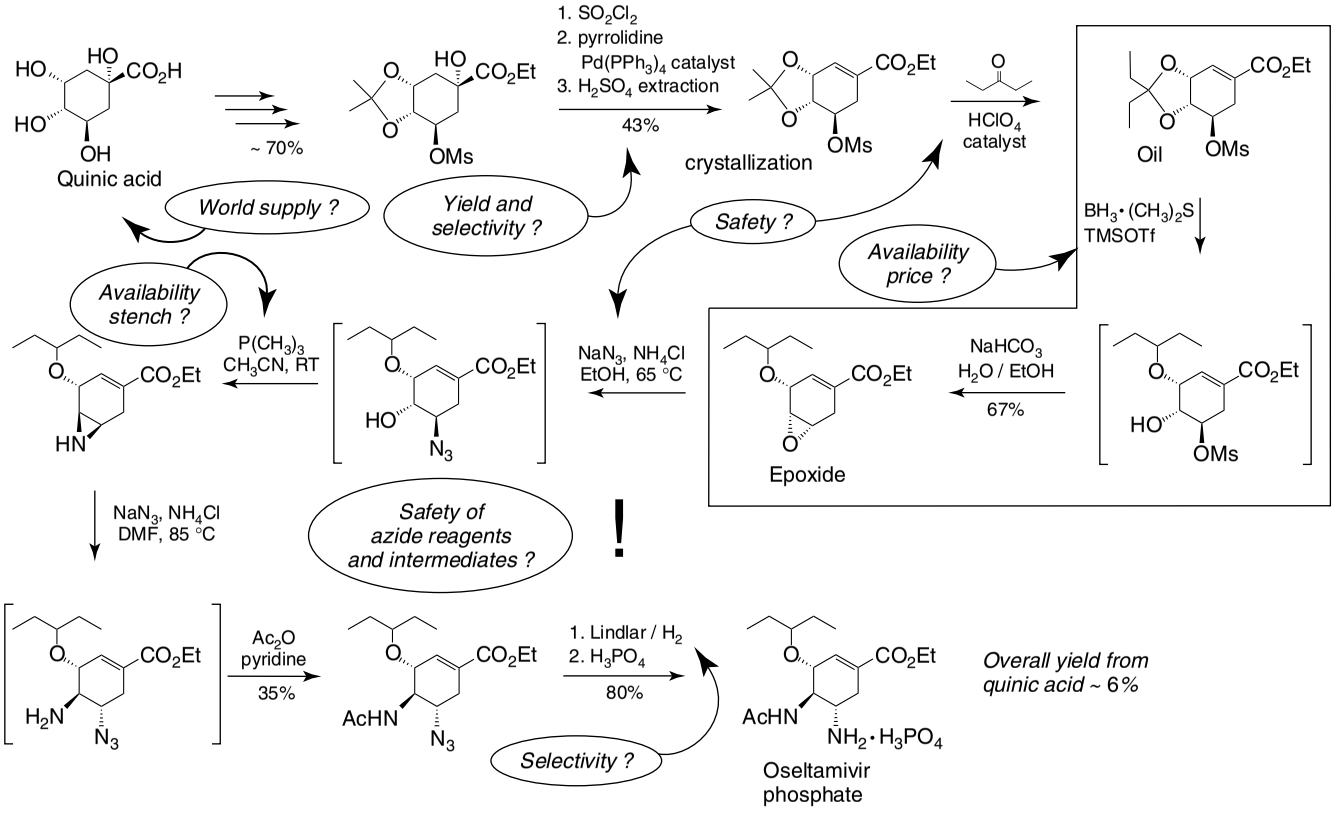
The first scalable synthesis shown in Scheme 1.11 was based on the elegant and early introduction of the 3-pentyl-ether side chain achieved by the regioselective reductive opening of the 3-pentanone ketal intermediate directly followed by the base-induced epoxide ring closure leading to the key precursor epoxide. This approach still required (−)-quinic acid as the starting material, which was easily converted to the acetonide mesylate. However, the dehydration step turned out to be particularly problematic both regarding yield and regioselectivity. Purification and isolation of the required cyclohexene intermediate became possible by the selective transformation of the accompanying but undesired ′1, 6′-double bond isomer by Pd-catalyzed allylic substitution of the mesylate group and subsequent extraction of the resulting pyrrolidino derivative into the acidic aqueous phase upon workup. Trans-ketalization of the resulting crystalline acetonide purified by crystallization then led to the oily key ketal ready to be used for the reductive ketal opening step followed by epoxide formation.

The subsequent transformation of the key precursor epoxide to the drug substance essentially represents the transformation of an epoxide to a 1,2-diamine derivative, a transformation involving azide reagents and intermediates. The sequence started with the epoxide ring opening using sodium azide at 65 °C followed by the direct transformation to the aziridine via a Staudinger phosphine imine using the extremely irritating and barely available reagent trimethylphosphine but with the advantage of allowing the removal of the formed trimethylphosphine oxide by extraction into the aqueous phase.

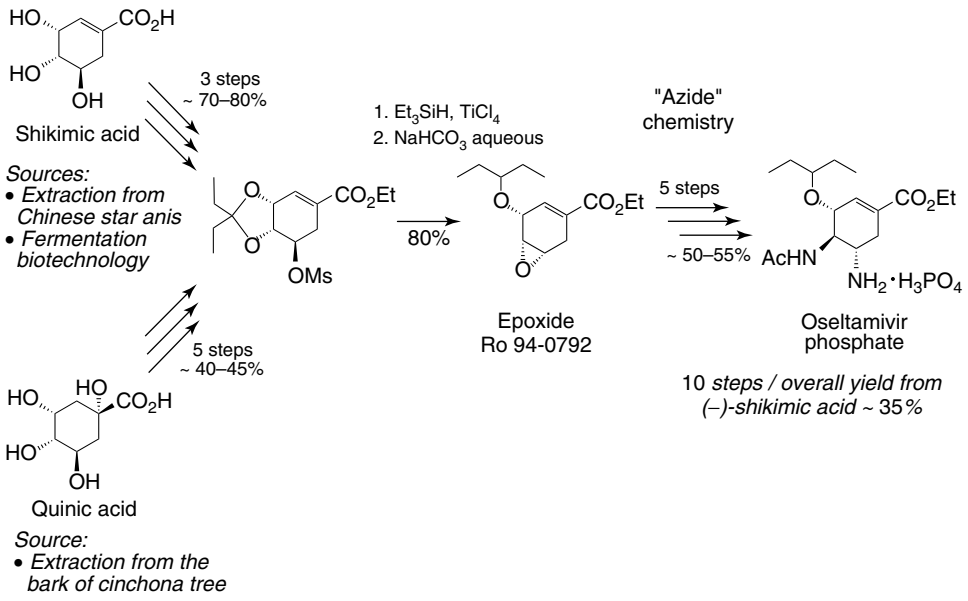
Opening of the aziridine ring with sodium azide under slightly acidic conditions at 85 °C led to the amino-azide intermediate, but with the hazard of forming hydrazoic acid, a low boiling liquid with a known tendency to detonate bond and therefore requiring stringent safety measures even for small-scale production.

Acetylation and reduction of the azido group with Lindlar's catalyst led after phosphate salt formation to the drug substance in an overall yield lower than the discovery synthesis but already with the advantage to proceed without chromatographic purifications and therefore allowing for the production of the first kilogram quantities directly required to continue clinical and biological investigations.

With the prospect of an upcoming multihundred-ton production for oseltamivir phosphate, a far reaching development of the process by Gilead, Roche, and also by third parties was critical to ensure the future market supply of the API. Owing to the ambitious timelines set by management, intensive troubleshooting of this



Scheme 1.11 Gilead scalable synthesis of the prodrug [14].



Scheme 1.12 Current commercial synthesis of oseltamivir phosphate [14–16].

synthesis finally led to the current industrial synthesis shown in Scheme 1.12, which preferentially starts from (−)-shikimic acid, since this raw material already contains the cyclohexene 1,2-double bond and therefore allows a much more efficient access to the key ketal intermediate.

For the reductive ketal opening a new cheap and very selective reagent combination triethylsilane/$TiCl_4$ was introduced. The ''azide chemistry'' was sourced out and developed by specialists in the field together with companies dealing with this type of chemistry in a safe way for many decades. This process now allows for the safe transformation of the key epoxide intermediate to the drug substance in an overall yield of 50–55% resulting in an overall yield of about 35% from (−)-shikimic acid, a route that now secures – together with the support of worldwide partners – a yearly production of several hundred metric tons for pandemic stockpiling.

Although (−)-shikimic acid was only available in research quantities at a very high price at the start of this project, it is now obtainable in ton quantities either by extraction from Chinese star anise or by fermentation using a genetically engineered *Escherichia coli* strain. Thus, the production of oseltamivir phosphate represents a noteworthy example for the proficient combination of biotechnology and synthetic organic chemistry.

In contrast, the overall yield to the epoxide intermediate starting from (−)-quinic acid never exceeded 50% due to the selectivity problem encountered in the dehydration step. Furthermore, the world supply of (−)-quinic acid is very limited since it is just a by-product of the extraction of quinine for tonic waters from the bark of the African cinchona tree.

1.5.3 The Search for Alternative Routes to Oseltamivir Phosphate

Although the main problems of the synthesis were solved and the supply of the API for clinical studies and its launch were secured, the price and availability of the starting material and the required outsourcing of the ''azide chemistry'' prompted synthesis and process research on the one hand to establish ''azide-free'' transformations of the key precursor epoxide in order to establish an independent, safe, and efficient alternative route amenable to a risk-free large-scale production. On the other hand, a search for shikimic acid independent new syntheses departing from cheap and abundant starting materials was initiated.

1.5.3.1 The Development of Azide-Free Transformations of the Key Epoxide Intermediate to Oseltamivir Phosphate

The first step was to identify an appropriate nitrogen nucleophile for replacing azide and the appropriate conditions for the epoxide ring opening. After an extensive search summarized in Scheme 1.13, allylamine was identified as the best substitute and magnesium bromide diethyletherate as a cheap catalyst which was new and not yet described in the chemical literature for this purpose.

After an intensive search for the most effective way to transform the key epoxide building block into oseltamivir phosphate replacing azide by allylamine, the conditions described in Scheme 1.14 were elaborated. The sequence starts with the regio- and stereoselective opening of the epoxide with allylamine in a nearly quantitative yield followed by Pd-catalyzed deallylation leading to the amino alcohol by acidic workup. Ethanolamine was shown to speed up the deallylation reaction considerably although the role of this promoter is not yet fully understood.

The transformation of the amino alcohol to the aminoallylamine was accomplished by a ''near to one-pot'' protocol discussed below including a reaction cascade without the need to isolate the intermediates encountered.

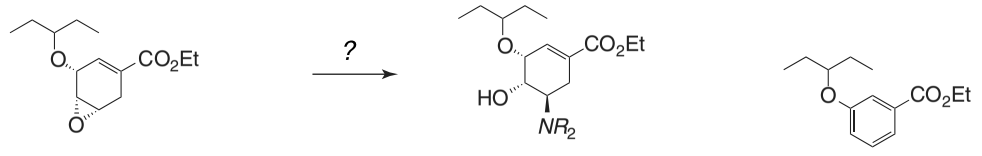
Selectivity in the N-acetylation of the aminoallylamine intermediate was achieved under acidic conditions, namely through transient protonation of the more basic secondary amino function followed by deallylation and phosphate salt formation leading to the drug substance in an overall yield from the epoxide of up to 40%.

This azide-free reaction sequence now compares well with the azide route concerning the number of steps and the number of intermediates isolated.

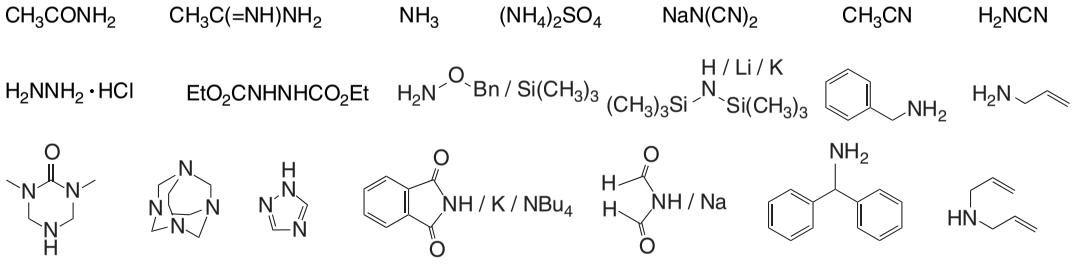
The reaction cascade or ''domino'' sequence mentioned above and depicted in Scheme 1.15 includes six consecutive reaction steps. The sequence started with the benzaldehyde imine formation allowing for the subsequent O-mesylation. After filtering off the triethylamine hydrochloride formed, the resulting iminomesylate was heated with 3 equiv. of allylamine in a Büchi autoclave for 16 h. Tracking the sequence by analytics revealed a first trans-imination to form the amino mesylate, which underwent fast ring closure to the aziridine releasing methanesulfonic acid triggering the aziridine ring opening. Interestingly, after completion of the reaction, the product found in the autoclave was not yet the desired aminoallylamine

How to replace azide reagents and intermediates ?

Attempted epoxide ring opening

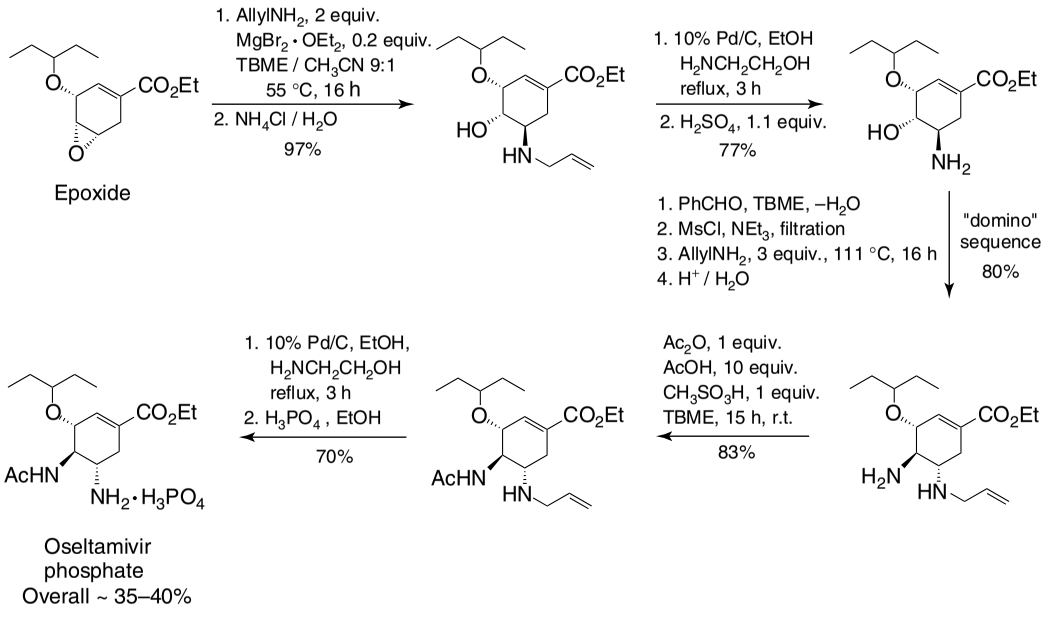


Selection of nitrogen nucleophiles tested:

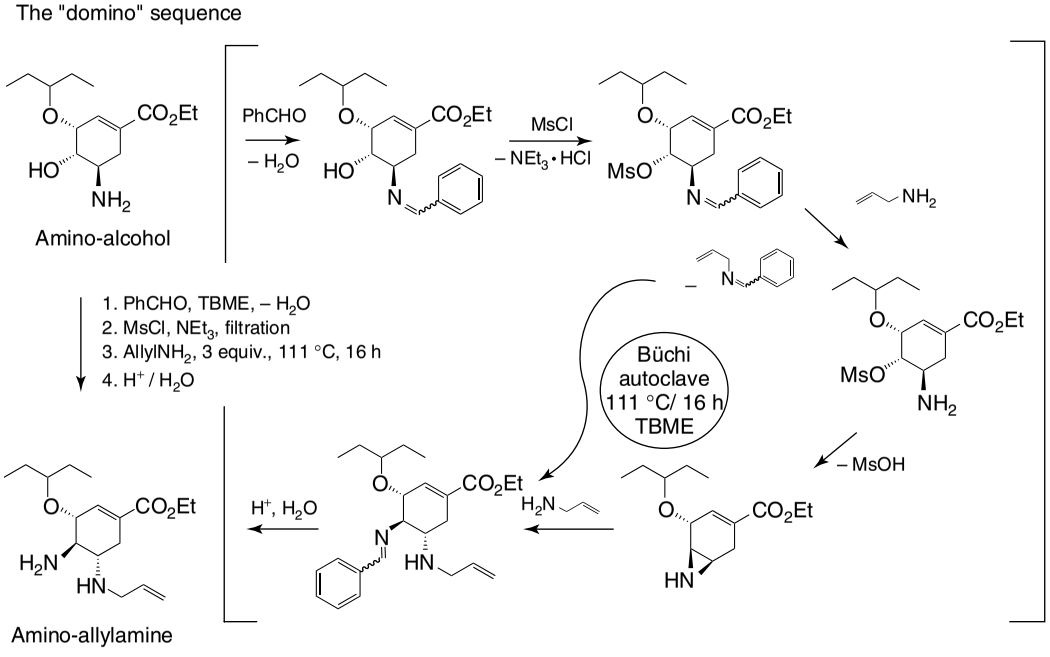


Problems: aromatization, decomposition, no or nonselective reaction

Scheme 1.13 Replacing azide reagents and intermediates.



Scheme 1.14 The allylamine promoted azide-free synthesis of oseltamivir phosphate [17].



Scheme 1.15 The reaction cascade introducing the 5-allylamino functionality [17].

but the corresponding benzaldehyde imine interpreted as the product of a second trans-imination process. Acidic hydrolysis then led to the required aminoallylamine.

This reaction sequence demonstrates effectively the value of reaction cascades as an ideal tool for process improvement, which in this case allowed for transforming the amino alcohol to the aminoallylamine including five chemical transformations but requiring only one isolation and purification step.

This azide-free process compares well with the azide protocol also concerning the overall yield, which was improved up to 50% by technical development.

A related approach for the azide-free transformation of the key epoxide intermediate to oseltamivir phosphate employing *t*-butylamine to promote the epoxide ring opening and the less volatile diallylamine for the succeeding introduction of the 5-amino functionality is shown in Scheme 1.16. This route provides the API in an overall yield of about 60% starting from the epoxide.

1.5.3.2 The Development of Alternative Syntheses for Oseltamivir Phosphate

Since at an early stage of the synthetic development of oseltamivir phosphate, the commercial availability of (−)-shikimic acid in multihundred tons to secure the large-scale production of the API was still under exploration, the evaluation of new and different approaches to the API and potentially independent on (−)-shikimic acid as the raw material was deemed very important. Therefore, synthesis and process research was given the task to also evaluate alternative routes.

Figure 1.7 summarizes the major synthetic challenges of new syntheses of oseltamivir phosphate independent on (−)-shikimic acid. These include, primarily, the installation of the cyclohexene ring with the 1,2-double bond and three stereogenic centers of the required absolute configuration. Concurrently, the formation of the 4,5-amino substituents as well as the formation of the 3-pentylether side chain has to be efficiently controlled. Diels–Alder approaches as well as routes based upon suitably substituted aromatic rings and their transformations are as conceivable as novel ring constructions or even starting from suitable abundant chiral pool materials.

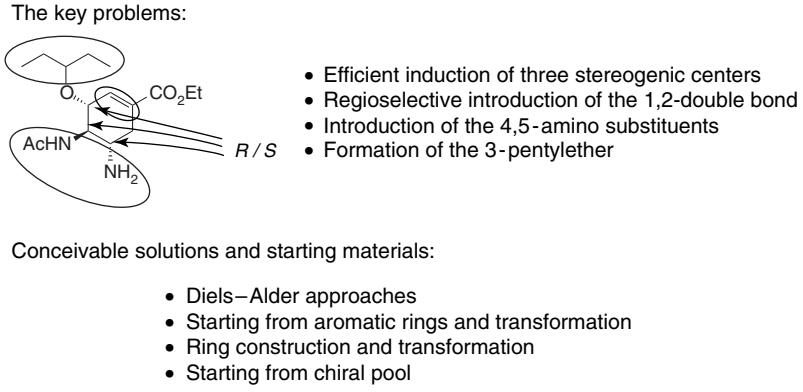
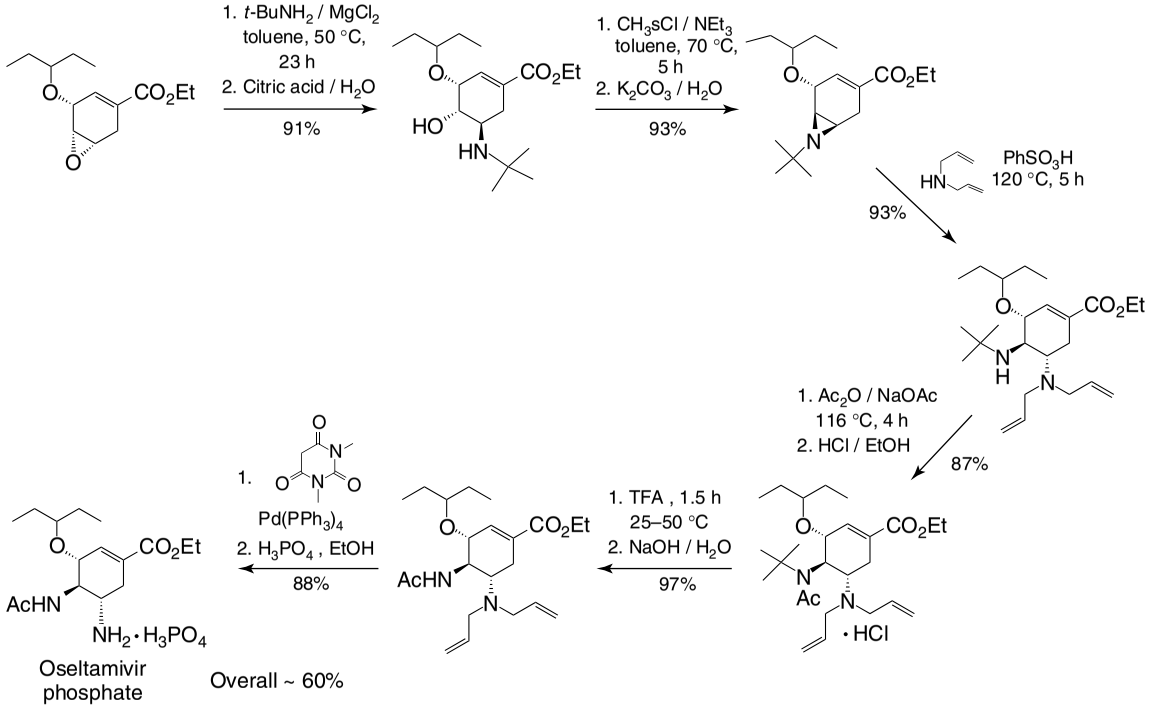
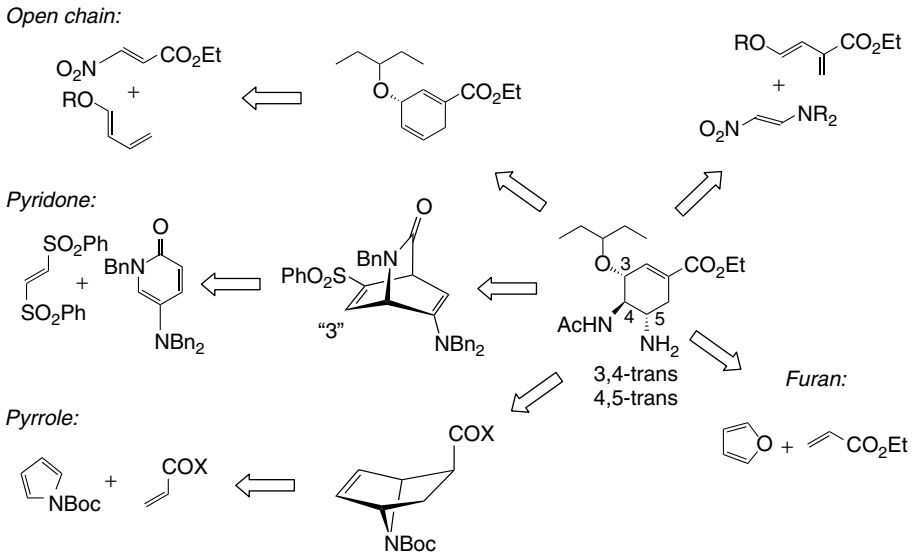


Figure 1.7 The evaluation of shikimic acid independent syntheses.



Scheme 1.16 The azide-free *t*-butylamine-diallylamine transformation [18].



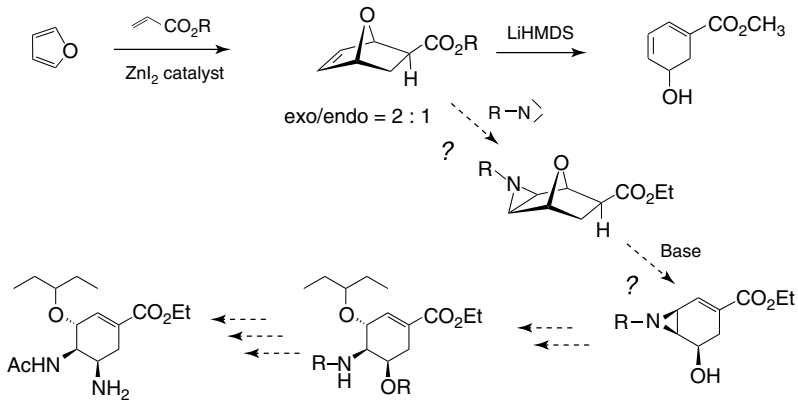
Scheme 1.17 The evaluation of Diels–Alder concepts.

Dealing with a cyclohexene derivative, obviously much effort was devoted to Diels–Alder approaches as shown in Scheme 1.17, summarizing the Diels–Alder concepts evaluated. Several ''open-chain'' concepts tested with the goal to introduce the two amino functions directly with the Diels–Alder reaction at a very early stage of the synthesis had to be abandoned mainly due to the instability of the corresponding dienophiles or the dienes, some of them representing quite unstable and hardly accessible compounds.

Attempts toward a 1,4-cyclohexadiene with the option to attack the more electron-rich nonconjugated 4,5-double bond with a nitrene or its equivalent were stopped due to the same reasons.

The ''pyridone'' Diels–Alder concept was based on the [4 + 2] cycloaddition of the perbenzylated 5-amino-2-pyridone to ethylene diphenyl disulfone. This reaction not only proceeded with high yield but also was also followed by the selective elimination of one of the sulfonyl groups. After exo selective sodium cyanoborohydride reduction of the enamine double bond, access to the bicyclic vinyl sulfone intermediate depicted in Scheme 1.17 was opened. This compound was expected to be ideally suited for conjugate nucleophilic addition at the prospective position ''3.'' Although addition at this position was achievable, the concept had to be abandoned due to the stereoselectivity problem since it was not possible to reach the required 3,4-trans 4,5-trans configuration.

Since acrylic systems are not known or only sluggishly to react with Boc pyrrole, the ''pyrrole'' approach was also abandoned in favor of a classical furan Diels–Alder chemistry, starting with the very cheap and abundant starting materials furan and ethyl acrylate.



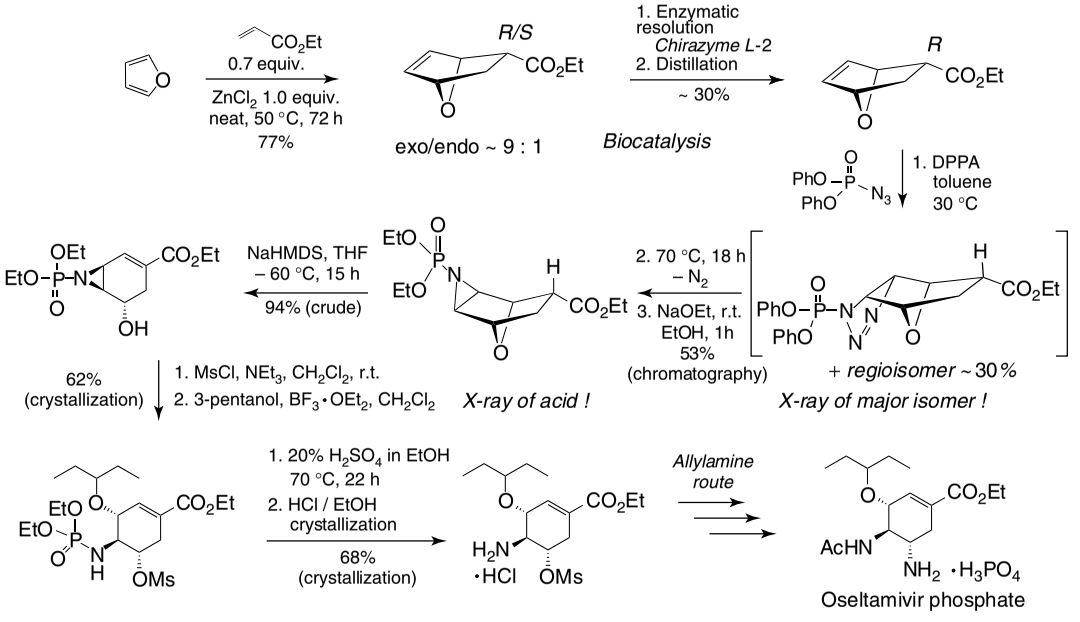
Scheme 1.18 The furan Diels–Alder/nitrene addition concept [19].

The "furan" concept is based on known investigations describing the zinc iodide catalyzed Diels–Alder reaction of furan and acrylates leading preferentially to the exo bicyclic isomer as shown in Scheme 1.18. Base-induced eliminative opening of the oxabicyclic system led to rather reactive 2,4-cyclohexadienols. As a variation of this protocol, we envisaged to first form an aziridine ring by nitrene addition or an equivalent protocol prior to the eliminative opening of the bicyclic system. This approach would lead to a cyclohexene-aziridine intermediate that should facilitate the regio- and stereoselective introduction of the 3-pentylether side chain as already known from the discovery chemistry approach (cf. Scheme 1.10). Further manipulations including the introduction of the amino function in 5-position were planned in order to reach the desired target.

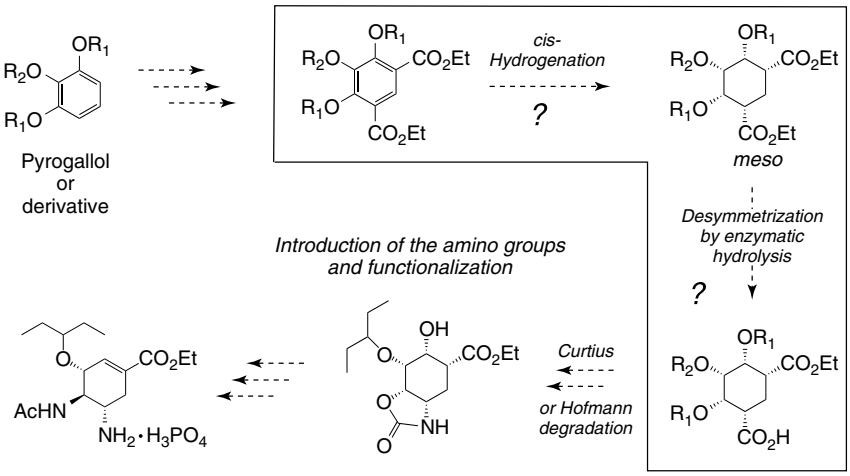
As shown in Scheme 1.19, it was first possible to improve the exo selectivity of the Lewis acid catalyzed Diels–Alder reaction by up to 9 : 1, by replacing zinc iodide by the cheap zinc chloride and driving the reaction to the thermodynamic equilibrium. Second, an enzymatic resolution step allowed obtaining the pure *R*-isomer of the exo oxabicyclic intermediate after removal of the remaining parts of the endo isomer by distillation.

As a nitrene equivalent, one of the most stable, safe, and commercially available azides, namely *Shioiri's reagent* diphenylphosphoryl azide (DPPA) was applied, which at somewhat above room temperature added in an exo manner to the oxabicyclic system leading to a mixture of regioisomeric triazoles. The exo configuration of both isomers was clearly indicated by ^{1}H NMR coupling constants, and for the major isomer it was confirmed by X-ray analysis. After the thermal extrusion of nitrogen from the triazole mixture occurring at about 70 °C followed by trans-esterification at phosphorus (advantageous for a later step), the endo isomer, surprisingly, was isolated as determined by X-ray analysis of the corresponding acid.

This formal but still unexplained exo to endo "inversion," however, paved the way toward a very short and effective synthetic completion since the endo aziridine smoothly underwent eliminative ring opening followed by direct



Scheme 1.19 The furan Diels–Alder/diphenylphosphoryl azide approach [15, 20].



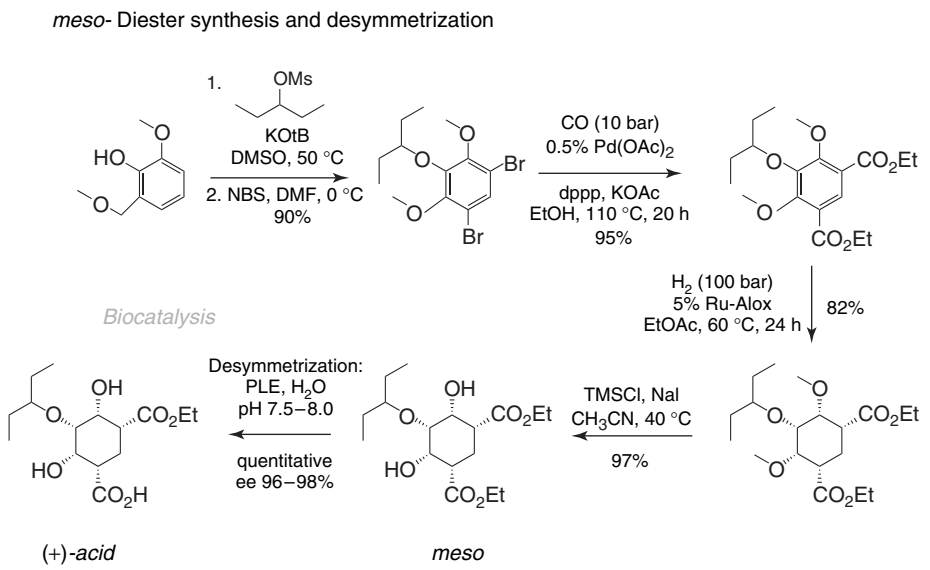
Scheme 1.20 The desymmetrization concept.

O-mesylation and regio- and stereoselective introduction of the 3-pentyl-ether side chain. Trans-esterification to the diethoxy phosphoryl compound – as mentioned above – was essential for the acidic cleavage of the N,P-bond leading to the amino mesylate isolated as the crystalline hydrochloride. With the specific configuration of this last intermediate, the transformation to the drug substance applying an analogous azide-free ''allylamine'' protocol as described in Schemes 1.14 and 1.15 became feasible, leading to the optically pure drug substance.

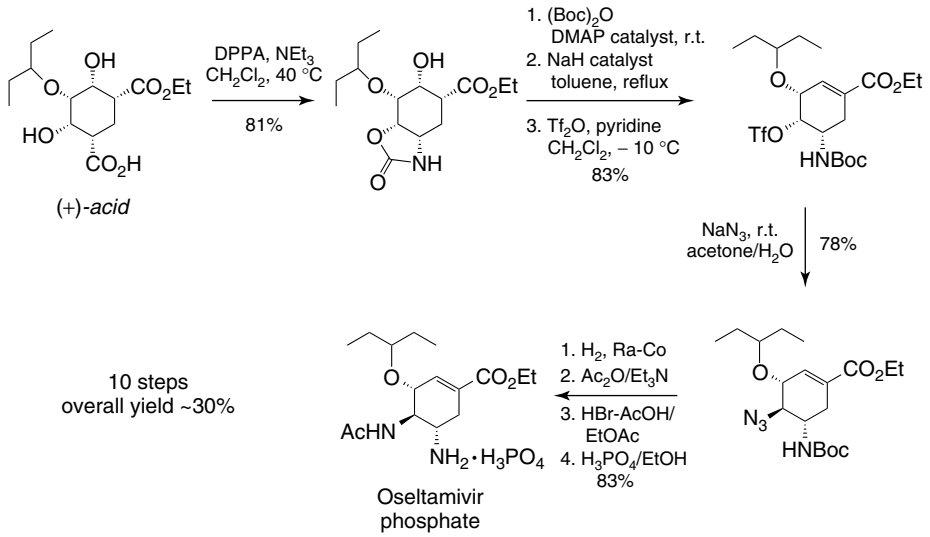
Taking advantage of a desymmetrization protocol over racemate cleavage regarding effectiveness, the synthetic desymmetrization concept sketched in Scheme 1.20 is based on two key steps. First, the possible all-cis hydrogenation of the iso-phthalic diester derivative will expectantly proceed by the use of a pyrogallol-type starting material, leading to an all-cis *meso*-diester. The meso ester will have the potential to undergo an enantioselective enzymatic desymmetrization, giving optimistically an optically pure acid–ester intermediate ready for the introduction of the required nitrogen functionalities in 4- and 5-positions via Hoffmanns- or Curtius-type degradation.

The result of this investigation is presented in Schemes 1.21 and 1.22. The synthesis started from cheap dimethoxyphenol with a sequence of high-yielding reactions. After effective 3-pentylether formation followed by dibromination and Pd-catalyzed double ethoxy carbonylation, the hydrogenation of the appropriately substituted iso-phthalic diester – although at somewhat elevated pressure and temperature – indeed led to the desired *meso*-diester.

Nearly quantitative and highly selective cleavage of the methyl ether groups using *in situ* generated trimethylsilyl iodide then led to the meso dihydroxy intermediate ready for smooth desymmetrization investigated by the biocatalysis group. Using cheap pig liver enzyme, it was possible to obtain the desired (+)-monoacid in quantitative yield.

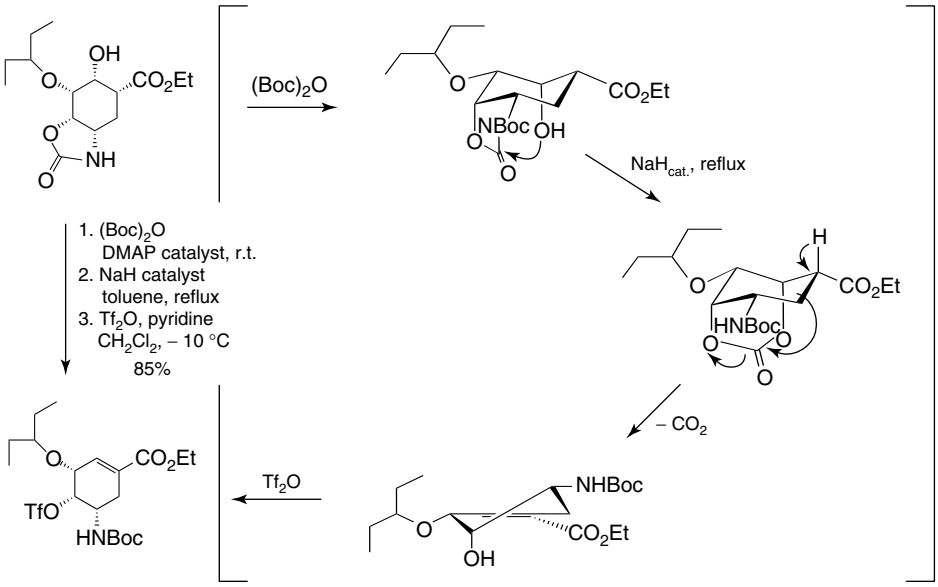


Scheme 1.21 *meso*-Diester synthesis and desymmetrization.



Scheme 1.22 Introducing the amino functions [21].

The conversion of this optically pure key intermediate to the drug substance was straightforward as shown in Scheme 1.22 starting with a Curtius-type degradation of the β-hydroxy-acid allowing introducing the 5-amino group with direct formation of the oxazolidinone. The subsequent reaction cascade takes advantage of the special configuration of this intermediate. The *N*-Boc protected intermediate was



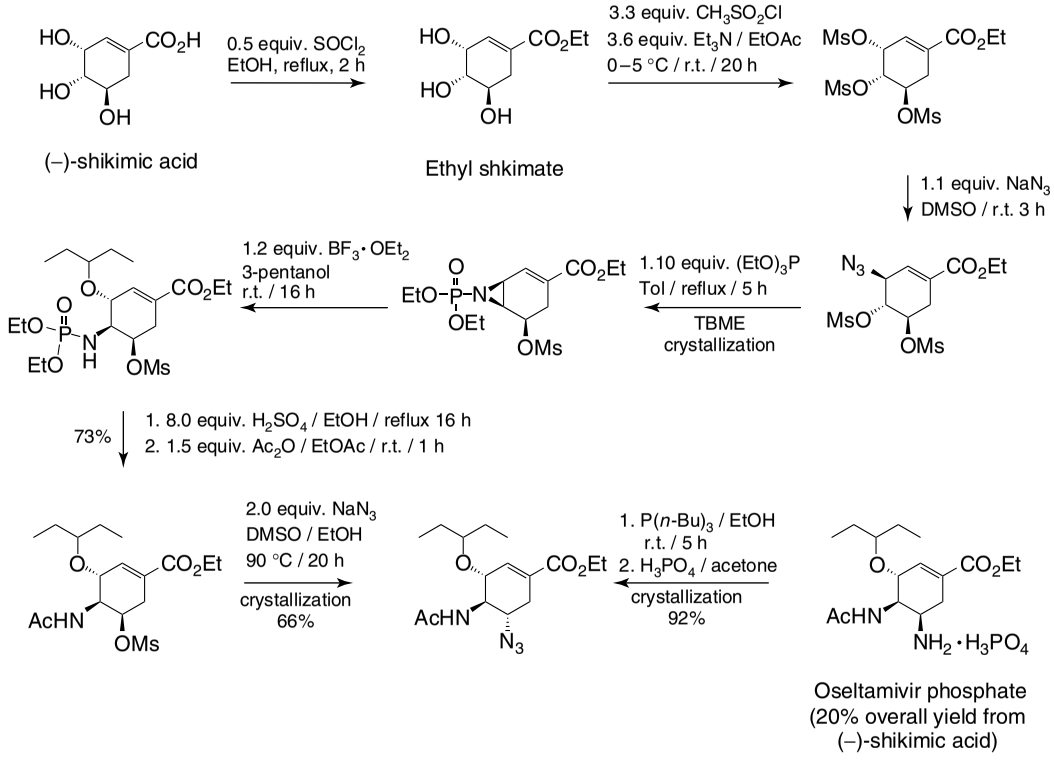
Scheme 1.23 Introducing the amino functions: the oxazolidinone shortcut [21].

treated with catalytic amounts of strong base triggering the effective and selective formation of the 1,2-double bond and – at the same time – the cleavage of the oxazolidinone system, followed by the formation of a triflate leaving group in an overall yield of 83%.

Inversion at position 4 so far still uses sodium azide but at room temperature and under neutral conditions, avoiding the formation of hydrazoic acid and sets the stage for the final sequence, namely, azide reduction, N-acetylation, Boc deprotection, and phosphate salt formation. The overall yield starting from a cheap aromatic starting material accounts for 30%, a result that compares favorably with the – already well developed – shikimic acid route.

To achieve this overall result, substantial fine tuning of all the reaction sequences is a clear requirement. A typical example for this process – the oxazolidinone transformation with concomitant introduction of the 1,2-double bond – is illustrated in Scheme 1.23. The transformation consists of a multistep cascade starting with *N*-Boc protection of the oxazolidinone intermediate followed by sodium hydride promoted deprotonation. An intramolecular attack on the oxazolidinone formed a strained cyclic carbonate stable enough to be isolated, but substantially activated for the subsequent carbon dioxide fragmentation process, directly providing the hydroxy compound ready for activation.

Several groups have published new approaches to oseltamivir phosphate also from alternative sources than (−)-shikimic acid, claiming a high need for routes independent of (−)-shikimic acid often on the basis of unfounded arguments regarding the availability of this acid as a technical starting compound as well as the potential risks involved in handling azide chemistry on an industrial



Scheme 1.24 Synthesis of oseltamivir phosphate via the *O*-trimesylate of ethyl shikimate starting from (–)-shikimic acid [22].

scale. Although commercial sources of large quantities of (−)-shikimic acid were unobtainable at the outset of this project, also prompting us to create and evaluate shikimic acid independent routes as described above, it became widely available in multihundred-ton amounts through this endeavor.

With large amounts of (−)-shikimic acid on hand and the constructive experience with partners performing azide chemistry on a bulk scale, the search for even shorter routes than the current commercial synthesis starting from this now abundant material finally led us to the protocol shown in Scheme 1.24 proceeding via the *O*-trimesylate of ethyl shikimate, obtained in high yield from (−)-shikimic acid by way of ethyl shikimate. Subsequent mesylation and regio- and stereoselective substitution of the allylic *O*-mesylate group with sodium azide at room temperature under nonacidic conditions led to the azide intermediate. Subsequent treatment thereof with triethyl phosphite in toluene at reflux produced the aziridine intermediate, which underwent regio- and stereoselective ring opening at the allylic position. The N,P-bond cleavage afforded the last mesylate, which by azide substitution under neutral conditions furnished the penultimate intermediate of the current commercial route of oseltamivir phosphate. This eight-step route provides the API in 20% overall yield from (−)-shikimic acid, already at a technically undeveloped stage.

The successful synthetic development of new small molecule drugs depends primarily and most vitally on dedicated people willing to cooperate and form teams starting with discovery chemistry and proceeding through synthesis and process research, kilolaboratory, and technical development departments. Only through thorough discussions and with high mutual respect for and acceptance of the individual competencies among the responsible chemists involved, the speedy evaluation and development of routes from milligrams to tons is attainable.

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