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The Synthetic Development of the Anti-Influenza Neuraminidase Inhibitor Oseltamivir Phosphate (Tamiflu®): A Challenge for Synthesis & Process Research

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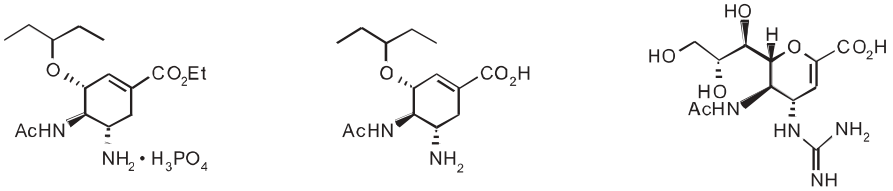
Abstract: The evolution of the synthesis of oseltamivir phosphate (Tamiflu®), used for the oral treatment and prevention of influenza virus infections (viral flu) is described. Oseltamivir phosphate is the ethyl ester prodrug of the corresponding acid, a potent and selective inhibitor of influenza neuraminidase. The discovery chemistry route and scalable routes used for kilo laboratory production as well as the technical access to oseltamivir phosphate from (–)-shikimic acid proceeding *via* a synthetically well-developed epoxide building block followed by azide transformations are reviewed. Synthesis and process research investigations towards azide-free conversions of the key epoxide building block to oseltamivir phosphate are discussed. The search for new routes to oseltamivir phosphate independent of shikimic acid including Diels-Alder approaches and transformations of aromatic rings employing a desymmetrization concept are presented in view of large-scale production requirements.

Keywords: Biotechnology · Influenza neuraminidase inhibitor · Process research · Synthesis

1. Introduction

The highly water-soluble phosphate salt of the trisubstituted cyclohexene ethyl carboxylate **1** (oseltamivir phosphate; Tamiflu®) is the orally available prodrug of the corresponding acid **2** (Fig. 1) which is a very selective and potent inhibitor of influenza neuraminidase already at nanomolar concentrations [1][2]. This compound, with a serum half-life of about 3 h, is used for the oral treatment and the prevention of influenza virus infections, a disease which affects several million people each winter. In contrast and due to its low oral bioavailability and its short half-life the *in vitro* equipotent heterocyclic competitor compound **3** (zanamivir; Relenza™, GlaxoSmithKline) (Fig. 1) requires topical application using disk inhaler technology [3]. Various compounds inhibiting influenza virus neuraminidase and their syntheses have been reviewed [4].

The active compound **1** was found at Gilead Sciences, Foster City, California and patented in 1995. In late 1996, a co-development contract was signed with F. Hoffmann-La Roche Ltd and a fast track development program was initiated in both companies. After only 2.5 years of development time the US NDA was filed and after accelerated review and approval Tamiflu® was launched in November 1999.



1 **2** **3**

Oseltamivir phosphate
Tamiflu™
GS-4104-02
RO0640796-002

GS-4071
RO0640802-000

Zanamivir
Relenza™
GG-167

Fig. 1. Marketed neuraminidase inhibitors and prodrug

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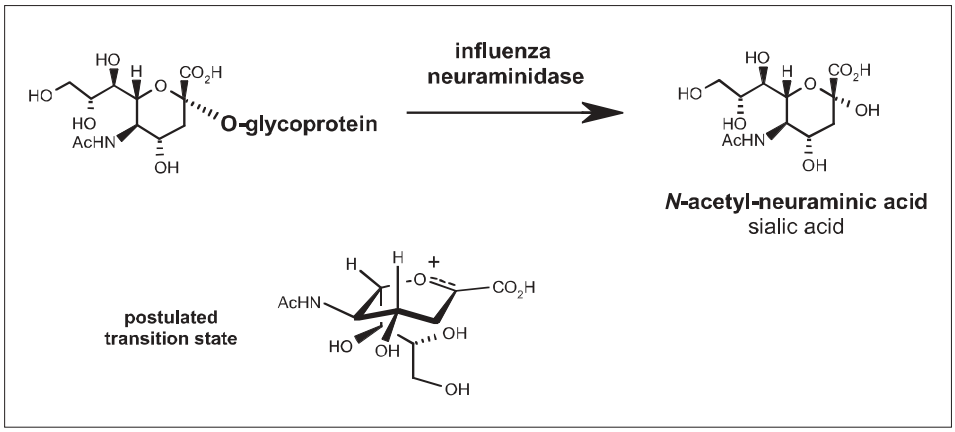
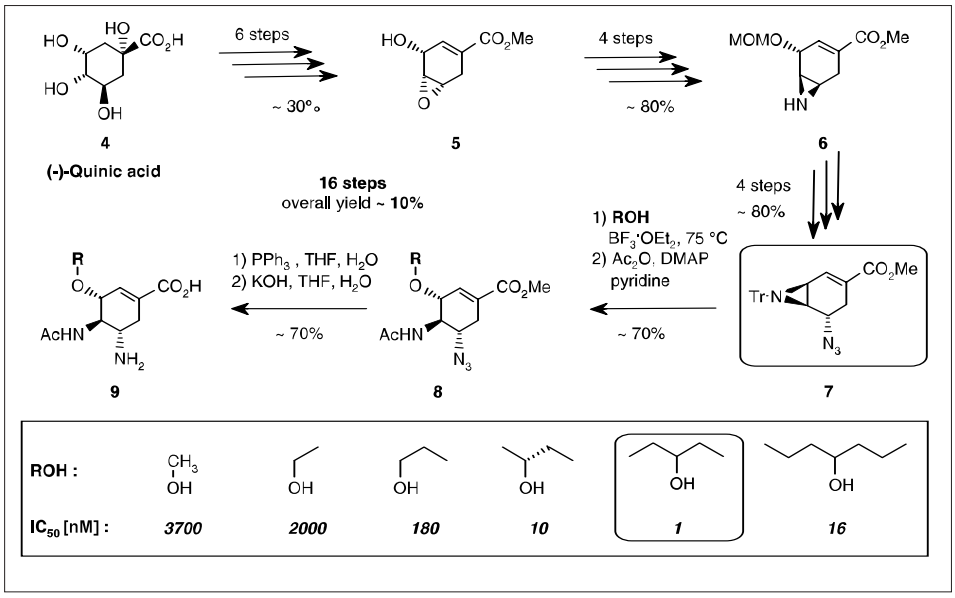


Fig. 2. The postulated role of neuraminidase in influenza virus replication



Scheme 1. Overview of the discovery chemistry synthesis [1][2]

The influenza neuraminidase is 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. This cleavage process as outlined in Fig. 2 is assumed to allow the newly formed viral particles to escape from the 'sialic acid glue' on the infected cells surface and to infect new host cells. Inhibition of this process is considered to hold back the evading viruses from the destroyed cell, leading to their aggregation and inactivation thereby efficiently stopping the infective cycle. The compounds active as neuraminidase inhibitors are presumed to be mimics of the postulated oxonium type transition state of the cleavage process [5].

2. The Evolution of the Current Technical Synthesis of Oseltamivir Phosphate (1)

2.1. *Discovery Chemistry Synthesis of Oseltamivir Phosphate (1)*

The discovery chemistry synthesis of the active principle **2** and its analogues as described in detail by Kim *et al.* [1][2] is summarized in Scheme 1 and represents a typical diversity-oriented discovery chemistry approach with the goal to efficiently explore a new class of biologically active molecules aiming at the most potent representative. To this end the N-trityl aziridine azide intermediate **7** was targeted as an advanced synthetic branching point allowing for the fast access to a variety of drug candidates for biological testing.

Thus **7** obtained from (–)-quinic acid (**4**) was efficiently transformed into the potential candidates by Lewis-acid catalyzed opening of the aziridine ring using diverse hydroxy components ROH and subsequent acetylation to **8** followed by azide reduction and saponification. As easily deducible from the typical representatives of products shown, variation of the ether side chain led to a tremendous effect on *in vivo* activity as indicated by their inhibitory concentrations against influenza virus neuraminidase [1]. However, this 16 step synthesis starting from (–)-quinic acid (**4**) and taking six known steps to the hydroxy-epoxide **5**, four steps to the aziridine **6** and four additional steps to reach the branching point **7** was hardly amenable to the production of larger quantities of the clinical candidate.

2.2. *First Scalable Synthesis of Oseltamivir Phosphate (1)* [6]

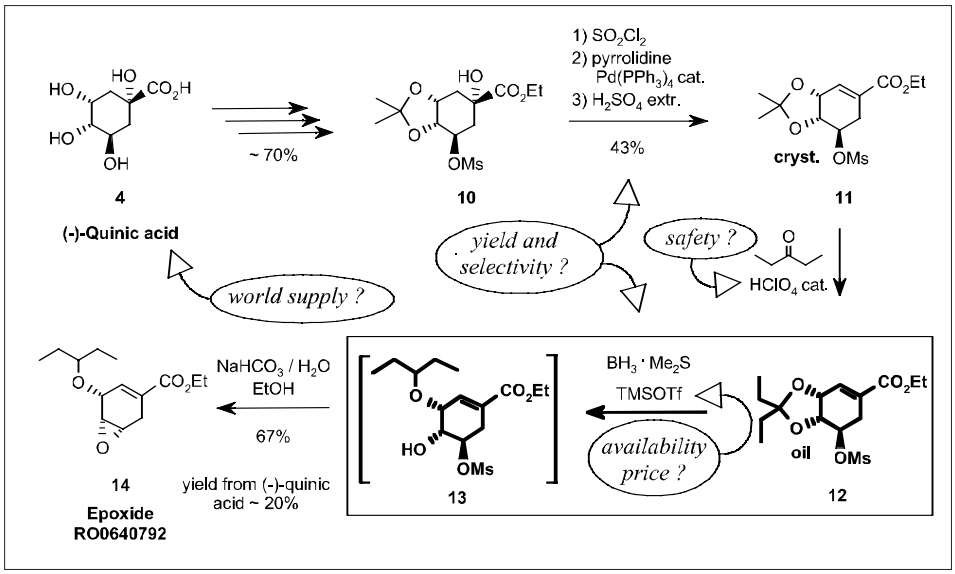
Synthesis and process research started at Gilead Sciences in the group of Rohloff *et al.* [6] concentrated now on the selected 3-pentyloxy substituted ethyl ester prodrug **1** and led to a scalable process depicted in Schemes 2 and 3 which was further elaborated and developed at Roche towards the current commercial synthesis. This approach is based on the elegant introduction of the 3-pentylether side chain by the regioselective reductive opening of ketal intermediate **12** directly followed by base-induced epoxide ring closure leading to the key precursor epoxide **14**. This route still used (–)-quinic acid (**4**) as the starting material which was easily converted to the acetonide mesylate **10**. The elimination step **10** → **11**, however, was low yielding due to the lack of selectivity and the formation of the undesired '1,6'-double-bond isomer, an allylic mesylate which had to be removed by Pd-catalyzed transformation into the pyrrolidino derivative followed by extraction into acidic medium. Transketalization of the crystalline acetonide **11** then led to the key diethyl ketal **12** used for the key step.

The transformation of the key precursor epoxide **14** to the drug substance **1** essentially deals with the transformation of an epoxide to a 1,2-diamino derivative extensively involving potentially hazardous azide reagents and intermediates. The sequence started with the epoxide ring opening using sodium azide at 65 °C to obtain **15** followed by its direct transformation to the aziridine employing the extremely malodorous and toxic trimethylphosphine. Although hardly available in larger commercial quantities, this reagent had the advantage of allowing the removal of the corresponding water soluble phosphine oxide by simple extraction.

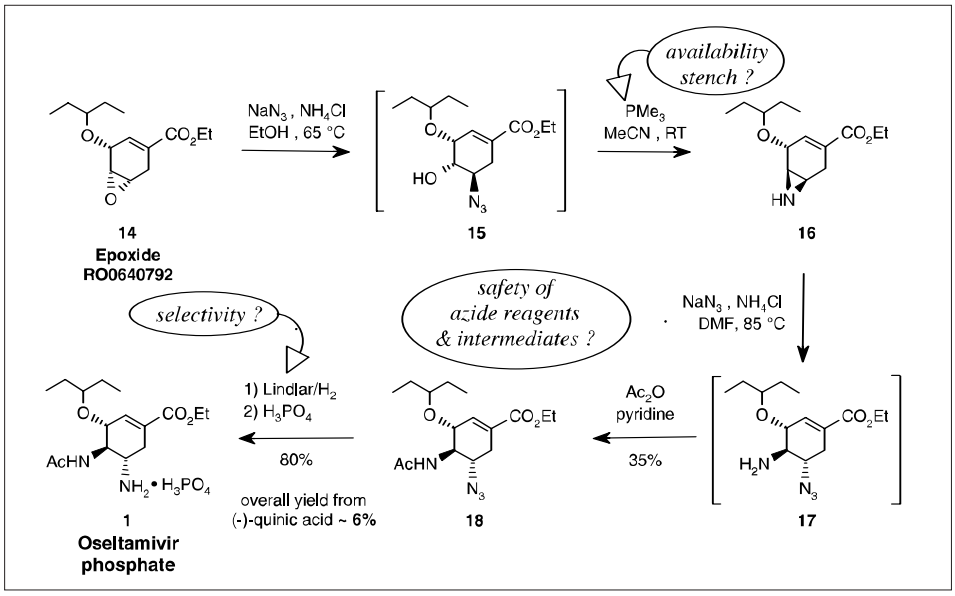
Opening of the aziridine ring of **16** with sodium azide under acidic conditions at 85 °C led to the amino-azide intermediate **17**, which – after acetylation to **18** followed by reduction of the azido group and phosphate salt formation – yielded the drug substance **1** in about 27 to 29% overall yield from the epoxide **14**. Although the overall yield of the route shown in Schemes 2 and 3 was still rather low it made the production of kg amounts possible without chromatographic purifications. This material was urgently required to start non-clinical development activities and later clinical trials.

2.3. *Current Technical Synthesis of Oseltamivir Phosphate (1)*

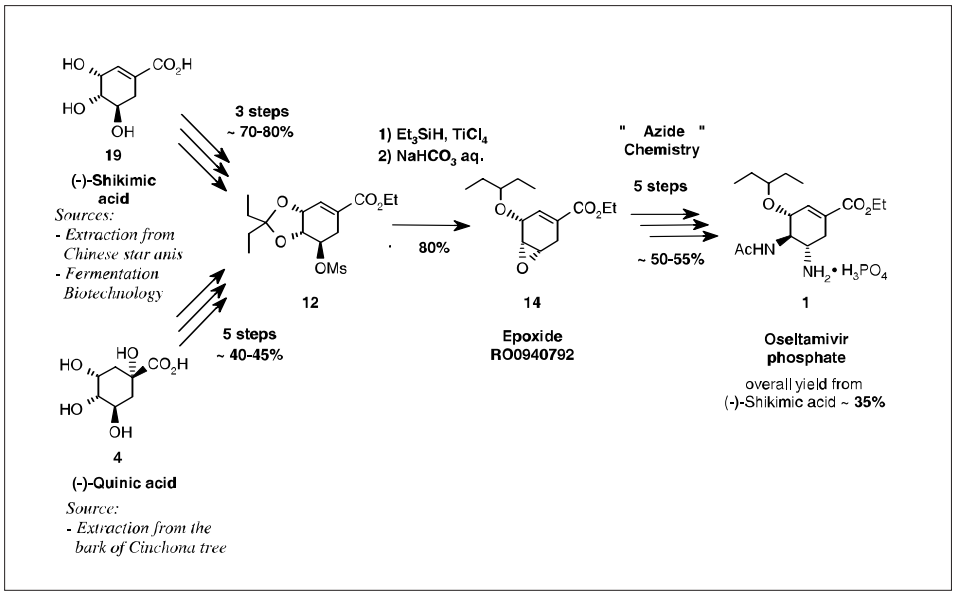
Extensive development by Gilead Sciences, by Roche, and by third parties was crucial to guarantee the safe large-scale supply of



Scheme 2. Scalable synthesis of **1** – access to the key precursor epoxide **14**



Scheme 3. Scalable synthesis of **1** (continued) – transformation of the epoxide **14** to oseltamivir phosphate (**1**) [6]



Scheme 4. Technical synthesis of oseltamivir phosphate (**1**)

the final product **1**. These activities finally led to the route summarized in Scheme 4 starting preferably from (–)-shikimic acid (**19**) with the 1,2-double bond already in place and hence enabling a short and efficient access to the diethyl ketal intermediate **12** [7]. Efficient, safe, economic and highly reproducible conditions were established for the reductive opening to **13** as well as for the following azide steps to the active pharmaceutical ingredient **1** allowing the production of ton amounts for launch.

Even though (–)-shikimic acid (**19**) was only available in research quantities at an excessive price at the beginning of the project, it is now available in ton quantities either by extraction of Chinese star anise or alternatively by fermentation using a genetically engineered *E. coli* strain developed by Frost and coworkers [8] and technically established at Roche. Thus, this access to oseltamivir phosphate (**1**) represents an attractive example for the successful combination of biotechnology and organic synthesis affording the mesylate **12** in up to 80% yield. In contrast, the overall yield of **12** from (–)-quinic acid (**4**) never exceeded 50%. Furthermore, the world supply of (–)-quinic acid (**4**) is limited since the compound is the undesired and therefore discarded by-product of the extraction of quinine for tonic waters from the bark of the African cinchona tree.

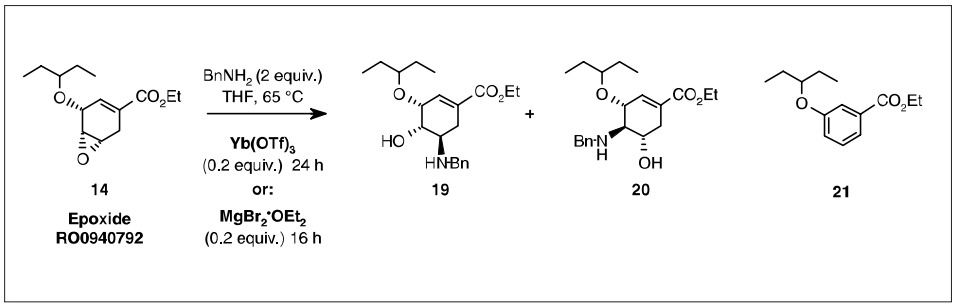
3. Alternative Approaches to Oseltamivir Phosphate (1)

Although the most significant problems of the early synthesis were solved and the supply for clinical studies as well as for launch was secured, the price of the starting material (–)-shikimic acid (**19**) – although accessible – and the costs of the azide chemistry generated new specific questions regarding the economy of the overall process. Therefore synthesis and process research were asked on the one hand to evaluate an azide-free transformation of the key precursor epoxide **14** in order to establish an independent and efficient alternative route for large-scale production of **1**. On the other hand, the search for new syntheses independent of (–)-shikimic acid (**19**) and departing from inexpensive and abundant starting materials was initiated.

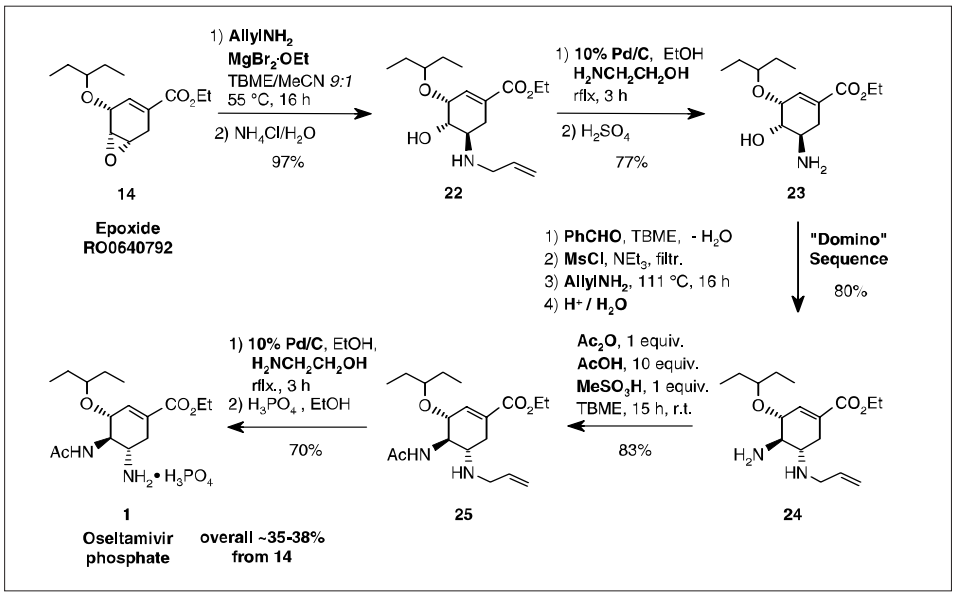
3.1. *Azide-free Transformations of the Epoxide 14 to Oseltamivir Phosphate (1)*

3.1.1. *'Allylamine' Route of the Epoxide 14 to 1* [9]

Although the use of azide chemistry on an industrial scale is well documented [10], the potential hazards related to its application as well as the dependence on third parties prompted us to search for an efficient azide-free transformation [9]. To this end, a suitable non-azide nitrogen nucleophile and



Scheme 5. Metal-ion catalyzed epoxide opening



Scheme 6. Azide-free allylamine route to oseltamivir phosphate (**1**)

appropriate conditions had to be found that were compatible with the functional groups present and the pronounced tendency of such highly functionalized cyclohexene derivatives towards aromatization. For the epoxide ring opening of **14** a substantial number of parallel experiments were performed using several amines and related nitrogen nucleophiles under various non-catalytic and catalytic conditions. All experiments, however, resulted in either no or incomplete conversion accompanied by substantial aromatization leading to variable amounts of **21** along with non-identified by-products. The first positive results were achieved using 2 equiv. of benzyl amine and 0.2 equiv. of ytterbium trifluoromethanesulfonate ($Yb(OTf)_3$) according to [11] providing the aminoalcohol **19** after reflux in THF for 24 h together with its regioisomer **20** (**19**/**20** ~ 85:15) in 93% yield as shown in Scheme 5. Since $Yb(OTf)_3$ is a high molecular weight, very expensive and therefore barely technical catalyst, an extensive search for a more practicable promoter for the oxirane ring opening led to the discovery and implementation of magnesium bromide etherate ($MgBr_2$•OEt_2) for this purpose, affording the same product mixture in only 16 h.

Since selective hydrogenolysis of **19** to the aminoalcohol **23** without affecting the cyclohexene double bond was not achievable, a search for an amine suitable for the overall transformation **14** → **23** was started. This investigation finally led to the selection of allylamine as the reagent of choice not only for the $MgBr_2$•OEt_2 catalyzed epoxide ring opening reaction to **22** and the subsequent Pd/C-catalyzed deallylation to **23**, but also for the short, selective and effective introduction of the second amino function present in **24**. The details of this new and efficient reaction sequence finally leading to the drug substance **1** in good overall yield is summarized in Scheme 6 and will be discussed below.

The deallylation of **22** to **23** was achieved in 77% yield with Pd/C in EtOH in the presence of ethanolamine at reflux for 3 h followed by acidic work-up. The direct conversion of **23** to **24** with concomitant introduction of the second amino function was performed without isolation of the intermediates. The domino sequence shown in Scheme 7 started with the protection of the 5-amino group by formation of the benzaldehyde imine **26** through azeotropic removal of water in *t*-BuOMe directly followed by mesylation. After filtration of NEt_3•HCl, the *t*-BuOMe solution of **27** was treated for 15 h with 4 equiv. of allylamine in an autoclave at 112 °C at 3.5 to 4.5 bar resulting in 80% yield of **24** after acidic hydrolysis. This reaction cascade requires just one filtration and constitutes a new, straightforward and practical conversion of an aminoalcohol into a vicinal diamine employing readily available and safe reagents. The efficiency of this transformation is mainly due to the reversible protection of the amino group as the benzaldehyde imine.

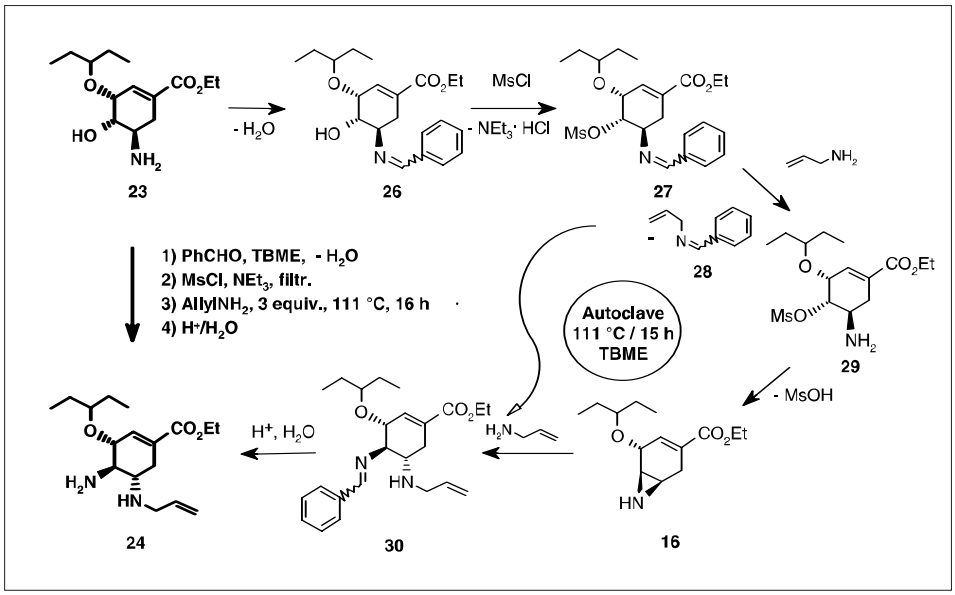
The unexpected presence of **30** as the final product of the reaction sequence before hydrolysis raised the question concerning the actual intermediates in the process. Analytical tracking showed the temporary formation and disappearance of both the imine **28** and of the aziridine **16**. Therefore we assume that the domino sequence starts with the trans-imination reaction of **27** with allylamine to form **28** and the aminomesylate **29**. The fast ring closure to the aziridine **16**, however, prevents **29** from being detected in the reaction mixture. The subsequent aziridine ring opening induced by methanesulfonic acid liberated in the aziridine ring closure step, led initially to the diamine **24** which by trans-imination with **28** – present in the reaction mixture – forms the imino derivative **30**.

The selective acetylation at the 4-amino group of **24** was achieved under acidic conditions with acetic anhydride, a large excess of acetic acid and 1 equiv. of $MeSO_3H$ in EtOAc at room temperature. This selectivity is interpreted by effective protonation of the secondary 5-amino group under acidic conditions reflecting the substantial pK_a difference in **24** of more than 3 units (pK_a^1 4.2, pK_a^2 7.9, measured in 0.1 M aq. KNO_3 containing 3% MeOH). Deallylation of **24** over 10% Pd/C in refluxing EtOH and ethanolamine followed by filtration of the catalyst, acidic hydrolysis and extraction provided the free base of the drug substance which was converted into the phosphate salt yielding drug substance **1** of high purity (99.7%) in an overall 35 to 38% yield based on the epoxide **14**.

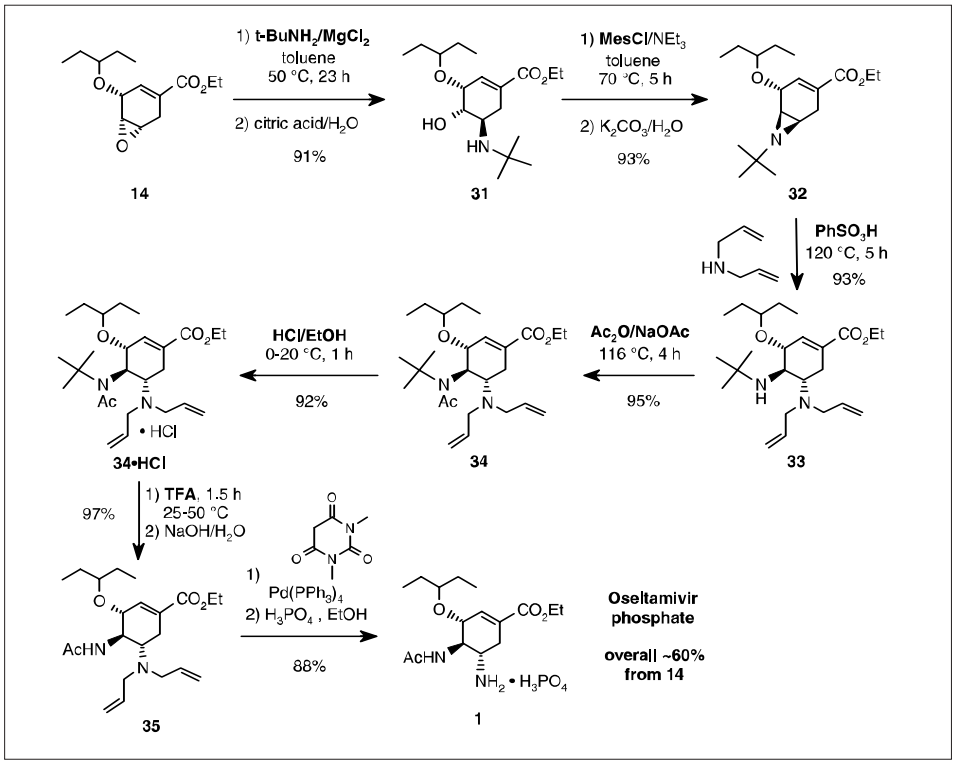
Due to the consequent utilization of the 'domino' concept, namely the execution of consecutive synthetic transformations in 'one pot' without work-up and isolation of intermediates this azide-free reaction sequence now compares well with the azide route shown in Scheme 3 concerning the number of isolated intermediates, a number most relevant for the productivity of a process on a commercial scale.

3.1.2. *'t-Butylamine-Diallylamine' Route of the Epoxide **14** to **1***

As an interesting variation of the 'allylamine' route of the epoxide **14** to **1** a closely related process has been established and developed [12]. This alternative '*t*-butylamine-diallylamine' process is depicted in



Scheme 7. Domino sequence transforming aminoalcohol **23** into the aminoallylamine **24**



Scheme 8. Azide-free *t*-butylamine-diallylamine route to oseltamivir phosphate (**1**)

Scheme 8 and features a magnesium chloride-amine complex catalyzed opening of the epoxide **14** by *t*-butylamine to **31** followed by O-sulfonylation without requiring N-protection. The subsequent aziridine ring closure to **32** was followed by the selective benzenesulfonic acid-induced opening of the aziridine ring using the less volatile diallylamine at 120 °C leading to the diamine **33**. N-Acetylation using acetic anhydride and sodium acetate followed by hydrochloride formation provided the hydrochloride of **34**, which represents the only purification step of the whole sequence. The cleavage of the *t*-butyl group was then accomplished using neat trifluoroacetic acid. In contrast to the 'allylamine' route described above, where removal of the allyl moiety was achievable by double bond isomerization over Pd on charcoal and subsequent hydrolysis, the removal of both allyl groups in **35** was accomplished by the Pd(0)-catalyzed allyl transfer to N,N-dimethylbarbituric acid leading after work-up and treatment with phosphoric acid to oseltamivir phosphate (**1**) of high purity. Although this route entails a higher number of isolated intermediates than the 'allylamine' process shown above, it is advantageous regarding the overall yield of about 60% from **14**.

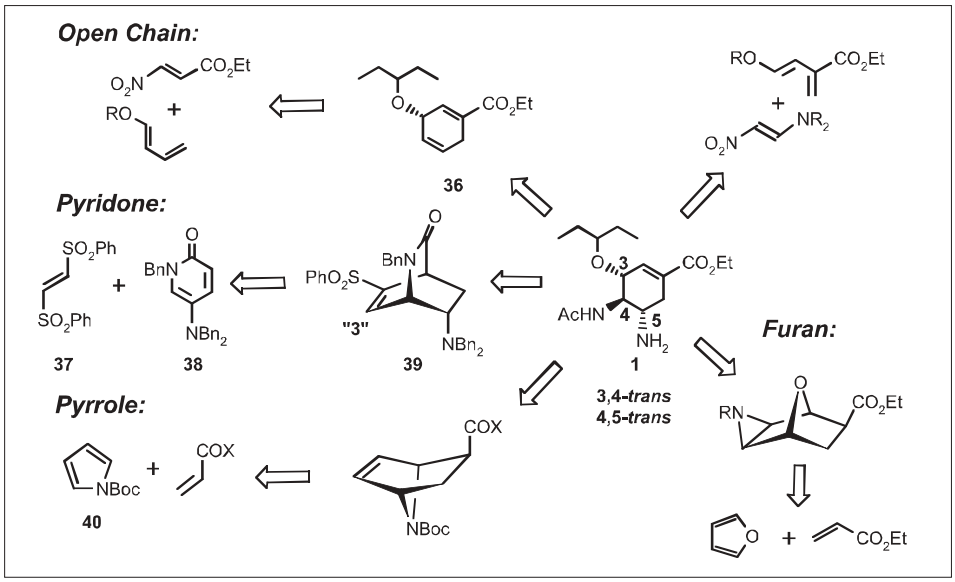
3.2. *Shikimic Acid-Independent Routes to Oseltamivir Phosphate (1)*

The evaluation of new approaches to oseltamivir phosphate (**1**) independent of (–)-shikimic acid (**19**) as the raw material started with the evaluation of the main synthetic problems to be considered and covers the efficient induction of the three stereogenic centers, the regioselective introduction of the 1,2-double bond, the establishment of the two amino groups and last but not least the formation of the 3-pentylether side chain. Since on the one hand we were dealing with a cyclohexene derivative including subtle configurational features, Diels-Alder strategies were obvious. On the other hand, dealing with a highly substituted ring compound, why not to start with a suitably substituted aromatic system? All attempts to solve the problem by trying to build up the cyclohexene ring system from acyclic precursors or to start from new inexpensive sources from the chiral pool failed so far. Therefore, we first comment on Diels-Alder approaches followed by an approach based on the transformation of a suitably substituted aromatic ring employing an enzymatic hydrolytic desymmetrization.

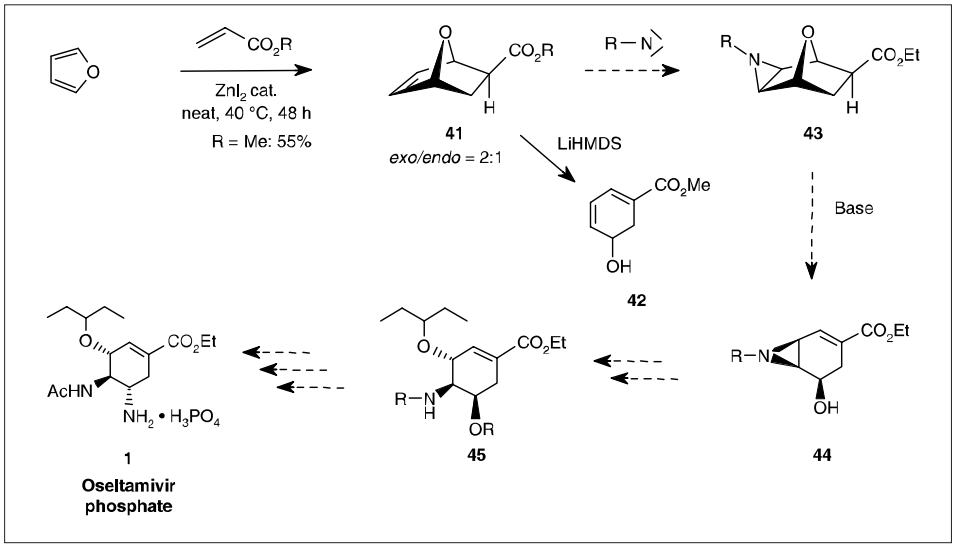
3.2.1. *Diels-Alder Approach to Oseltamivir Phosphate (**1**)* [13]

The Diels-Alder approaches evaluated during the early assessment are generically summarized in Scheme 9. Several 'open chain' concepts were tested with the goal to introduce the amino functions at a very early stage, preferably with the Diels-Alder reaction. However, the concepts pursued had to be abandoned either due to the instability of the corresponding dienes or due to the dienophiles, some of them representing rather unstable, hardly accessible compounds. Attempts to approach the non-conjugated 1,4-cyclohexadiene **36** with the option to attack the more electron rich 4,5-double bond had to be abandoned for similar reasons.

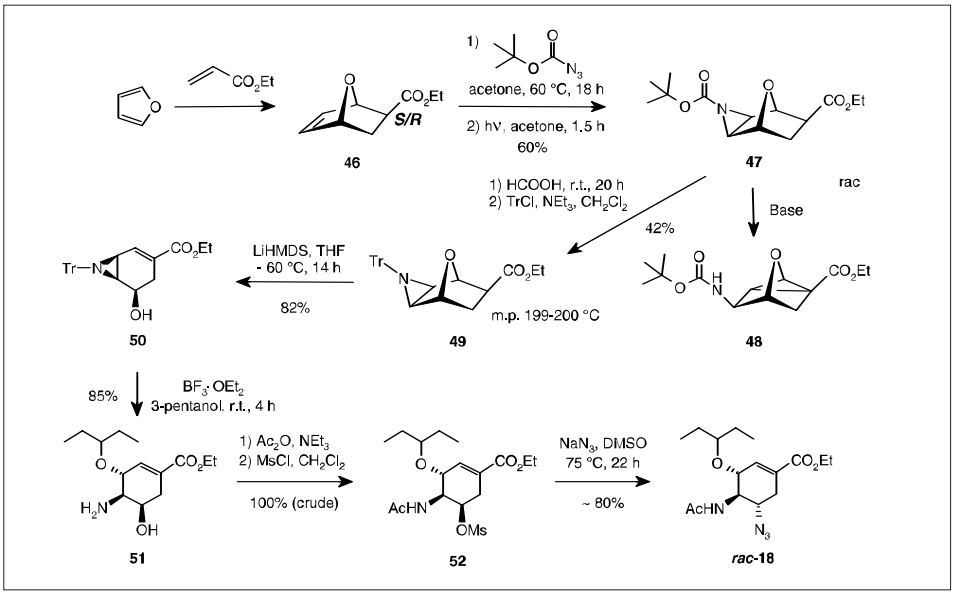
The 'pyridone' Diels-Alder concept based on the [4+2] cycloaddition of the perbenzylated 5-amino-2-pyridone **38** to the ethylene diphenyldisulfone **37** proceeded with high yield and was followed by stereoselective sodium cyanoborohydride reduction of the enamine double bond and the regioselective elimination of the '*exo*'-sulfonyl moiety under aqueous basic conditions providing the vinylsulfone **39** potentially suited for conjugate nucleophilic addition at position '3'. However, all attempts to introduce the 3-pentyloxy substituent in **39** or on succeeding intermediates failed.



Scheme 9. Exploration of Diels-Alder concepts towards oseltamivir phosphate (**1**)



Scheme 10. Furan-acrylate Diels-Alder concept



Scheme 11. Furan-ethyl acrylate Diels-Alder approach – proof-of-concept

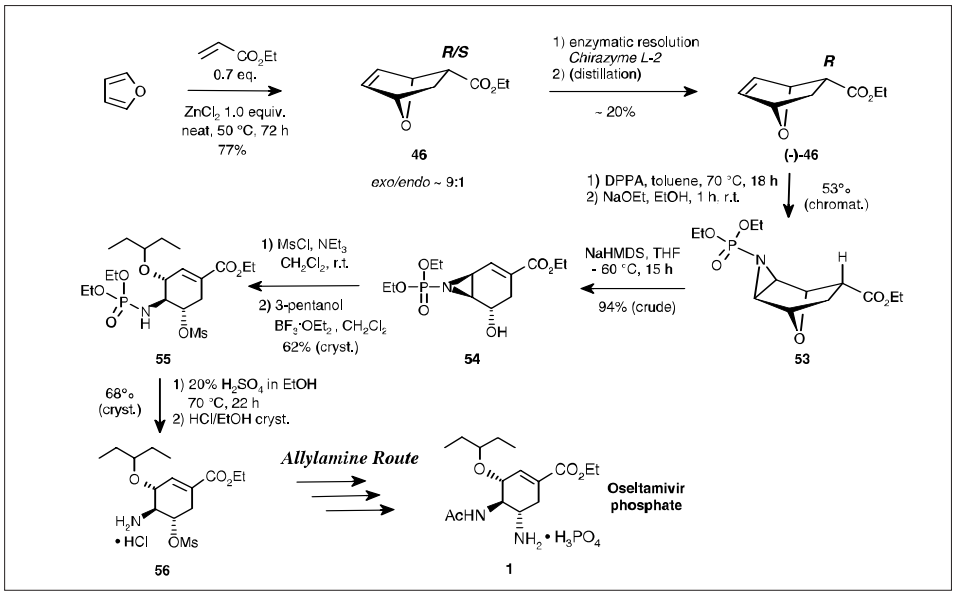
Since acrylic derivatives also refused to react with Boc-pyrrole **40** in the desired fashion, the Diels-Alder chemistry starting from very inexpensive and abundant starting materials furan and ethyl acrylate was investigated.

The concept was based on investigations by Brion [14] describing the *exo*-directed Zn-catalyzed Diels-Alder reaction of furan and acrylates followed by base-induced eliminative opening of the oxabicyclic system of type **41** shown in Scheme 10 to obtain the reactive 1,3-cyclohexadienes of type **42**. As a variation of this procedure, the formation of an aziridine ring by nitrene addition or an equivalent protocol was envisaged prior to the eliminative opening of the bicyclic system. This approach then would lead *via* **43** to a cyclohexene aziridine intermediate of type **44** which should facilitate the regio- and stereoselective introduction of the 3-pentylether side chain as established in the discovery chemistry approach summarized in Scheme 1. Further manipulations *via* **45** including the introduction of the 5-amino functionality were planned in order to reach the desired target **1**.

A first – although still racemic – realization of this concept is shown in Scheme 11. The *exo*-isomer of the Diels-Alder adduct **46** – separated from the *endo*/*exo*-mixture – was treated with Boc-azide to form a non-isolated triazole which was directly converted to the N-Boc-aziridine **47** by extrusion of nitrogen through irradiation.

Attempts to perform the intended base-induced eliminative ring opening on **47** unexpectedly led to the tricyclic derivative **48** by opening of the aziridine ring probably due to the electron-withdrawing properties of the N-Boc protecting group in **47**. Therefore the conversion to the crystalline trityl derivative **49** – although with low yield – was performed. Treatment of **49** with base smoothly led to the desired cyclohexene carboxylate **50** which was transformed to the amino-alcohol **51** by treatment with 3-pentanol under Lewis-acid catalysis. N-acetylation followed by O-mesylation provided the mesylate **52** which by azide substitution under neutral conditions led to the azido acetamide ***rac*-18**, the racemic form of the final intermediate of the current large-scale synthesis.

From a technical viewpoint this overall result although representing the first shikimic acid **19** independent access to the racemic drug substance *rac*-**1** was still burdened by substantial shortcomings. Consequently, an optical resolution preferably at an early stage of the synthesis was required as well as the substitution of the hazardous Boc-azide requiring irradiation to form the aziridine. Furthermore, inefficient protecting group interchanges and additional azide



Scheme 12. Furan-ethyl acrylate Diels-Alder approach – final route

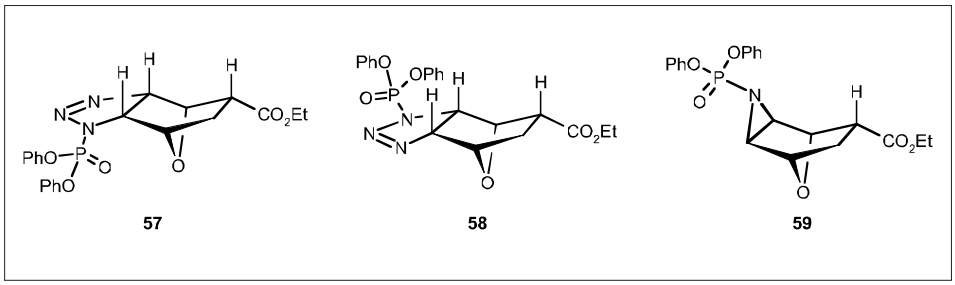


Fig. 3. Configurations of intermediates **57**, **58**, and **59**

steps had obviously to be avoided. The results of these efforts are summarized in Scheme 12.

In order to explore and improve the isomer ratio of the Diels-Alder reaction in favor of the desired *exo*-isomer **46**, zinc- and magnesium-based catalysts were evaluated which proved active enough to promote the cycloaddition without causing significant decomposition or polymerization of the starting materials. These investigations led to the use of inexpensive zinc chloride as the catalyst of choice. Further optimizations revealed a clear dependence of the *exo*/*endo*-ratio with increasing reaction times confirming the kinetically preferred formation of the *endo*-isomer of **46** followed by a steady increase of the share of the thermodynamically favored *exo*-isomer asymptotically reaching the equilibrium ratio of 9:1. This *exo*/*endo*-ratio along with the obtained yield and the simplicity of the process were superior to the best results known in literature for **46** or its methyl ester analogue [15].

For the optical resolution of ***rac*-46** *via* enantioselective ester hydrolysis 83 microorganisms and 50 enzymes were screened. The moderate enantioselectivity of the best enzyme found, namely Chirazyme L-2 was improved by intensive parameter optimization. Low temperatures and non-polar co-solvents favored high enantioselectivity, whereas high pH and high substrate concentration adversely affected selectivity. Best results under technically relevant conditions (5% conc.) were obtained with methylcyclohexane as a biphasic co-solvent in aqueous buffer pH 8.0 at 1°C, providing (–)-**46** in 97% ee at 75% conversion. Almost complete removal of the remaining *endo*-isomer, both enantiomers of which proved to be inert against enzymatic hydrolysis, was then achieved by distillation.

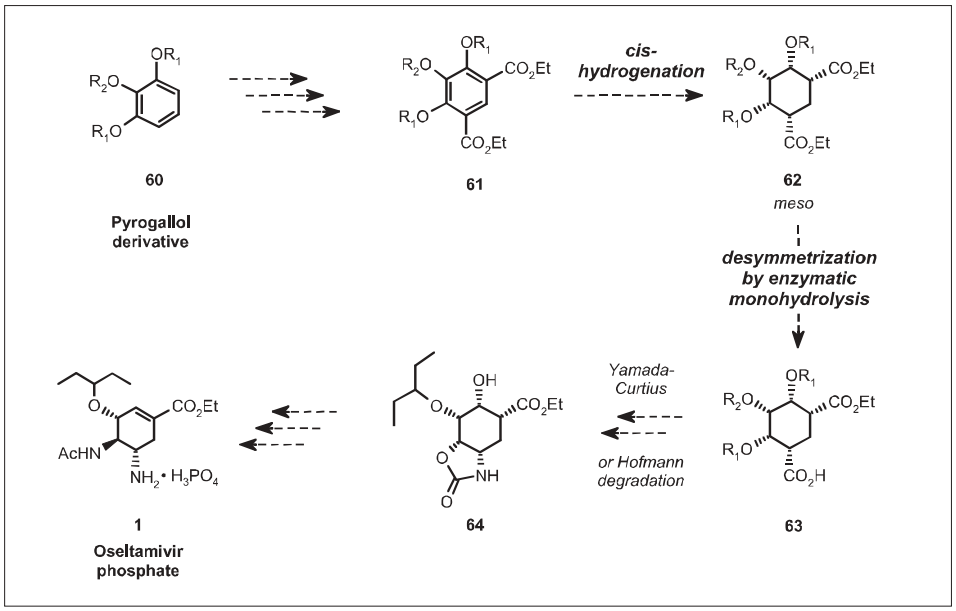
The safety limitations associated with the use of Boc-azide were overcome by the use of diphenylphosphoryl azide (DPPA) which, to our knowledge, represented the first example of its use for the generation of an aziridine moiety from an olefin [16]. The use of DPPA, a commercially available azide with a high decomposition temperature of 190 °C, not only added in [3+2]-fashion to the double bond of (–)-**46** already at 70 °C to form a mixture of the corresponding *exo*-triazoles, but also allowed for the thermal extrusion of nitrogen thus avoiding the technically problematic photochemical nitrogen extrusion step required to produce the Boc-substituted triazole **47** (Scheme 11). The diphenylphosphoryl derivative obtained was transesterified in the same pot to the diethylphosphoryl ester **53**, a conversion which is essential to prevent extensive side product formation in the ensuing P,N-bond cleavage to **56**.

Surprisingly and interestingly, the aziridine intermediate **53** undoubtedly represented the unexpected *endo*-aziridine isomer as confirmed by selective hydrolysis to the corresponding crystalline carboxylic acid (LiOH in THF/MeOH/H_2O at 60 °C) and X-ray crystallography [17]. Attempts to gain insight into the mechanism of this unexpected selectivity, the configuration of the two regio-isomeric precursor triazoles **57** and **58** formed in an about 2:1 ratio by treatment of (–)-**46** with DPPA at 30 °C for 72 h was examined. However, only the major isomer **57** was isolated by chromatography, while **58** decomposed on the column. Crystallization and X-ray analysis of **57** revealed the *exo*-position of triazole moiety, as already suggested for both regioisomers by ^{1}H-NMR analysis of the mixture. Heating the isolated triazole **57** to 70 °C provided the *endo*-aziridine **59** (Fig. 3).

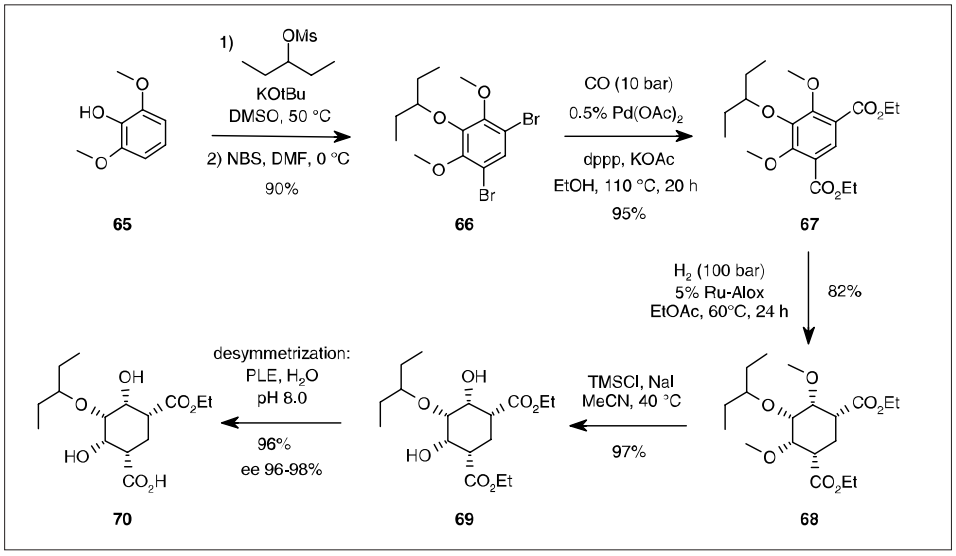
Although a full explanation for the observed formal 'inversion' is still pending [18], this unexpected result opened the door to a very short and effective synthesis of oseltamivir phosphate (**1**) since the *endo*-aziridine **53** smoothly underwent eliminative ring opening to form **54** followed by direct O-mesylation and regio- and stereoselective introduction of the 3-pentyl-ether side chain applying the conditions used in the discovery chemistry synthesis (Scheme 1) efficiently leading to **55**. P,N-bond cleavage in **55** required somewhat harsh conditions and led to the crystalline hydrochloride **56**. With the specific configuration of this intermediate, the transformation to the drug substance **1** applying an analogous azide-free 'allylamine' protocol as described above became feasible, leading to the optically pure drug substance **1** in good overall yield.

3.2.2. *Aromatic Ring Transformations: The Desymmetrization Concept* [19]

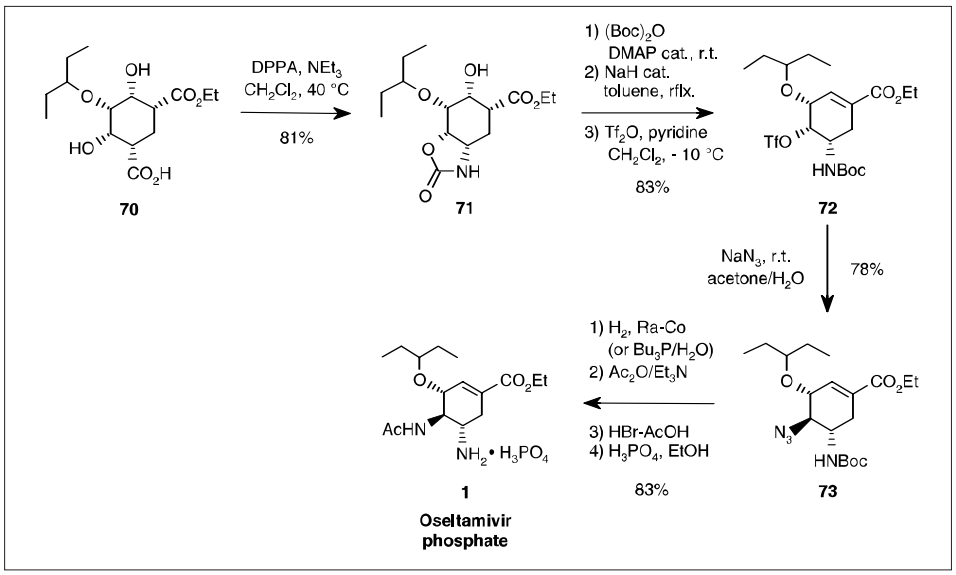
Taking advantage of the desymmetrization protocol over racemate cleavage regarding effectiveness, the 'desymmetrization concept' based on a potential enzymatic monohydrolysis of an all-*cis* *meso*-diester of type **62** to the optically active mono-acid **63** outlined in Scheme 13 was proposed. Besides the desymmetrization step **62** → **63**, the concept is based on the intended transformation of a pyrogallol derivative of type **60** to a diester **62** including a selective *cis*-hydrogenation of the symmetrically substituted aromatic isophthalic diester **61**, as well as on the efficient introduction of the two amino func-



Scheme 13. The desymmetrization concept towards oseltamivir phosphate (**1**)



Scheme 14. Access to the optically active mono-acid (+)-**70**



Scheme 15. Transformation of the optically active mono-acid **70** to oseltamivir phosphate (**1**)

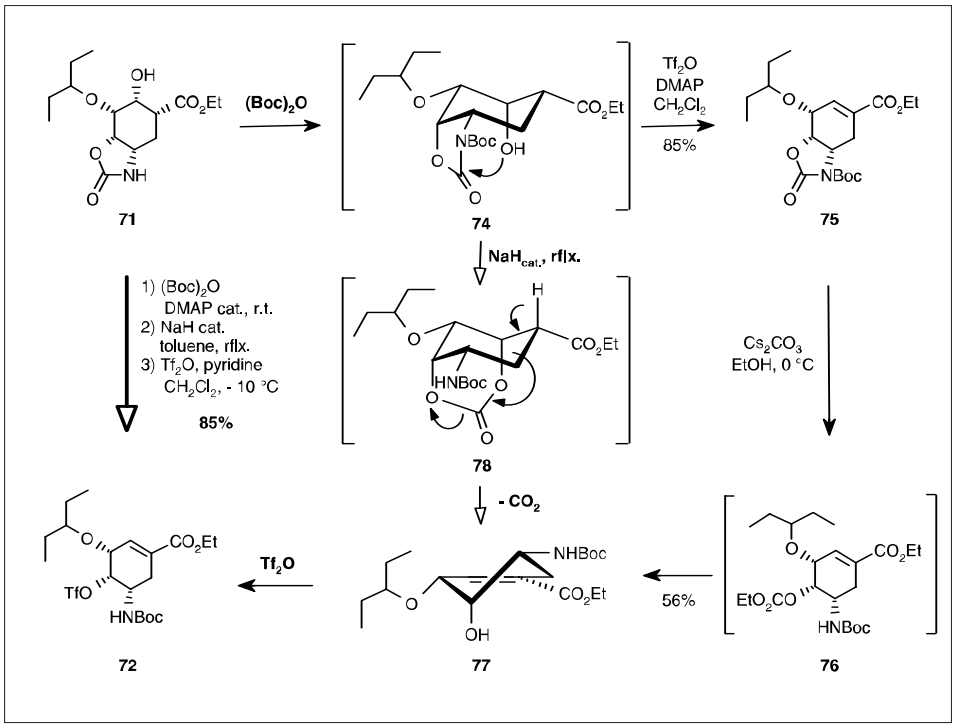
tionalities starting from the mono-acid **63**. The successful realization of this concept is shown in Schemes 14 and 15.

The synthesis started from inexpensive 1,6-dimethoxy phenol (**65**) with a sequence of high-yielding reactions: 3-pentyl-ether formation followed by bromination gave the dibromide **66** in 90% overall yield. The Pd-catalyzed double ethoxycarbonylation furnished the isophthalic diester **67** which was hydrogenated over Ru-Alox at 100 bar leading to the all-*cis meso*-diester **68** with high selectivity and yield.

Nearly quantitative and highly selective cleavage of the methyl ether groups in **68** using *in-situ* generated TMS-iodide provided the *meso*-dihydroxy intermediate **69** ready for the enzymatic hydrolytic desymmetrization. An enzyme screening led to a technical preparation of pig liver esterase (PLE) which afforded the desired (*S*)-(+)-mono-acid **70** in high yield and with 96–98% *ee*. The reaction was significantly accelerated by increasing the reaction temperature to 35 °C with only minor loss of selectivity (96% *ee*). Quite surprisingly, the enzyme tolerated a 10% substrate concentration well, even at 35 °C (96% *ee*; 99% yield; s/e = 10, 2.5 d), probably due to the insolubility of the substrate as well as the hydrophilic nature of the product **70**.

The conversion of the β-hydroxy-acid **70** to the drug substance **1** was straightforward as shown in the Scheme 15 and started with Yamada-Curtius degradation of **70** allowing to introduce the 5-amino-functionality by formation of the oxazolidinone **71**. The subsequent reaction sequence took advantage of the special *cis*-configuration of this intermediate. In an efficient 'one-pot' procedure outlined in Scheme 16 the N-Boc-protected oxazolidinone **74** was treated with catalytic amounts of sodium hydride in refluxing toluene 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 introduction of a good leaving group to provide the triflate **72** in an overall yield of 83%.

This efficient and interesting reaction cascade is assumed to be induced by the base-promoted deprotonation of the hydroxy group in **74** followed by intramolecular attack at the oxazolidinone carbonyl carbon with concomitant opening of the oxazolidinone creating the postulated cyclic carbonate **78** undergoing a fragmentation reaction to the cyclohexenol **77** ready for the introduction of the triflate leaving group. This efficient sequence easily doubling the yield of the originally used stepwise protocol *via* the isolated intermediates **74**, **75**, and **77** clearly demonstrates the power of this decarboxylative fragmentation sequence to effectively shorten a multi-stage transformation (Scheme 16).



Scheme 16. Decarboxylative fragmentation of the Boc-oxazolidinone **74** to the cyclohexenol **77**

The 4-amino functionality was finally introduced *via* S_N2-substitution of the triflate **72** using sodium azide with concomitant inversion of configuration under mild, basic conditions. Azide reduction, N-acetylation, Boc-deprotection, and phosphate salt formation gave the final product oseltamivir phosphate (**1**) in an overall yield of 30%, starting from 1,6-dimethoxy phenol (**65**) comparing favorably with the shikimic acid-based route.

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[18] As a preliminary, experimentally not yet confirmed rationalization we propose the existence of an equilibrium in the [3+2]-cycloaddition of **46** and DPPA leading to small amounts of the corresponding *endo*-triazol isomers prone to considerably faster nitrogen extrusion due to higher steric strain finally producing the *endo*-aziridines.

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