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The Synthetic-Technical Development of Oseltamivir Phosphate Tamiflu™: A Race against Time

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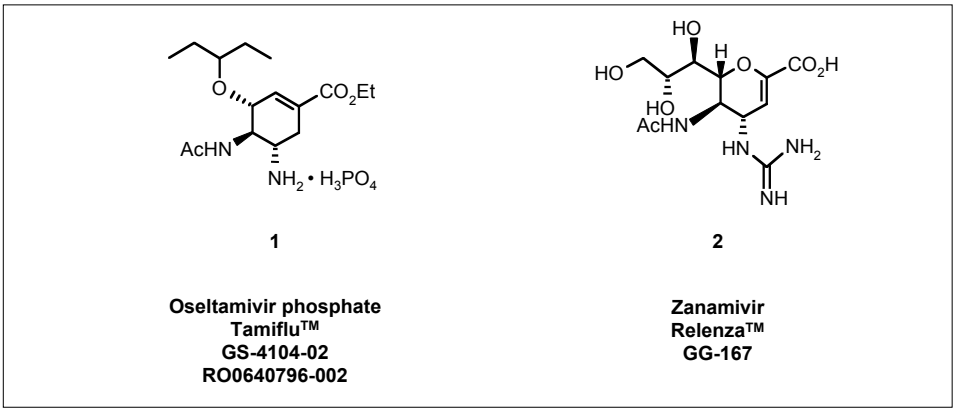
Abstract: The clinical development of the first orally available neuraminidase inhibitor prodrug oseltamivir phosphate (Tamiflu™) proceeded very fast. In order to support this program an unprecedented team effort in chemical process research, development, piloting, production and analytics took place, which allowed the successful launch of Tamiflu™ in 1999, only two and a half years after it was licensed from Gilead Sciences. This article describes selected aspects of the commercially used synthesis route and a brief summary of alternative syntheses devised by Roche chemists.

Keywords: Analytics · Influenza neuraminidase inhibitor · Oseltamivir phosphate · Production · Synthesis · Technical development

1. Introduction

In September 1996 a license agreement between Gilead Sciences, Foster City, California and F. Hoffmann-La Roche Ltd, Basel was signed for the co-development of the novel orally available neuraminidase inhibitor prodrug molecule **1** (Fig. 1) patented by Gilead Sciences in February 1995,[1] today known as oseltamivir phosphate, trade name Tamiflu™. In April 1999, after only two and a half years of development time, a new drug application (NDA) for **1** was filed with the US Food and Drug Administration (FDA) for the use of **1** for the treatment of influenza virus infections. This ambitious development program was in part triggered by the parallel development of zanamivir (**2**), trade name Relenza™ by GlaxoSmithKline. This neuraminidase inhibitor emerging from the research efforts of Prof. von Itzstein, Monash University, Australia[2] was already patented in 1990 and is equipotent *in vitro* to **1** but shows low oral bioavailability and was filed with the NDA for the topical treatment of influenza using disc inhaler technology in October 1998.



1

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

2

Zanamivir
Relenza™
GG-167

Fig. 1. Marketed neuraminidase inhibitors

Due to the exceptionally fast clinical development program for **1**, the standard chemical-technical development plans including trouble-shooting of the discovery synthesis, synthesis & process research and technical development activities leading to commercial route selection followed by piloting and including all related analytical activities had to be challenged in order to finally allow for a successful and timely launch production of several metric tonnes. An unprecedented and overlapping team effort regarding decisions on critical milestones was required throughout the functions involved.

2. A Swift Start

In September 1996, chemists from the Synthesis & Process Research depart-

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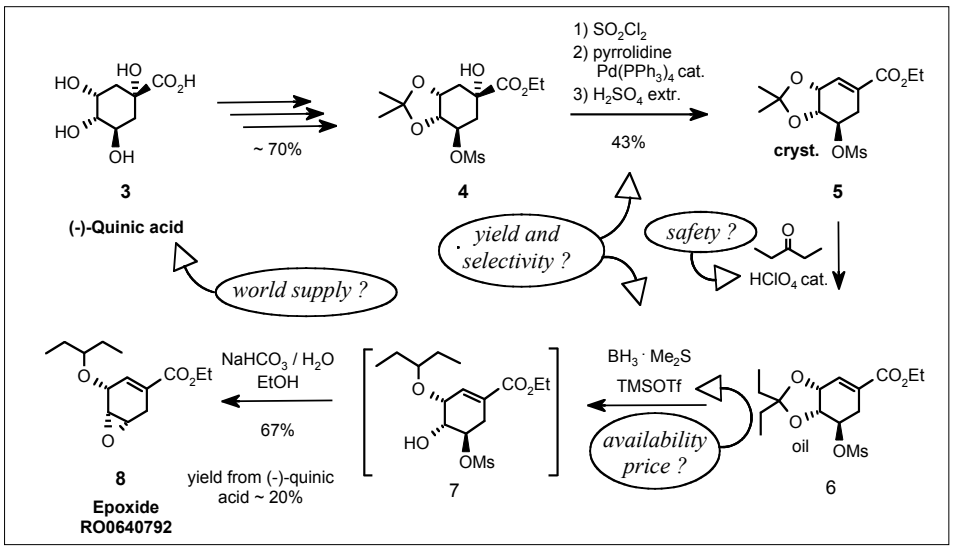
ment of F. Hoffmann-La Roche Ltd were first confronted with the synthetic pathway shown in Schemes 1 and 2 (taken from refs[3,4]) created and used by Rohloff *et al.*, Gilead Sciences for the scale-up of the transformation of quinic acid (**3**) to oseltamivir phosphate (**1**), published in 1998.[3] Due to the limited information available on the majority of issues circled in the two Schemes **1** and **2** (*e.g.* the availability of the starting material **2** on a large scale, the feasibility, safety and scalability of several synthetic steps) the immediately required assessment was largely based on the confidence in the skills of the chemists who were to be involved.



Scheme 1. Synthesis of epoxide **8** according to Rohloff *et al.*[3]

3. The Access to the Key Epoxide 8: Where to Start from?

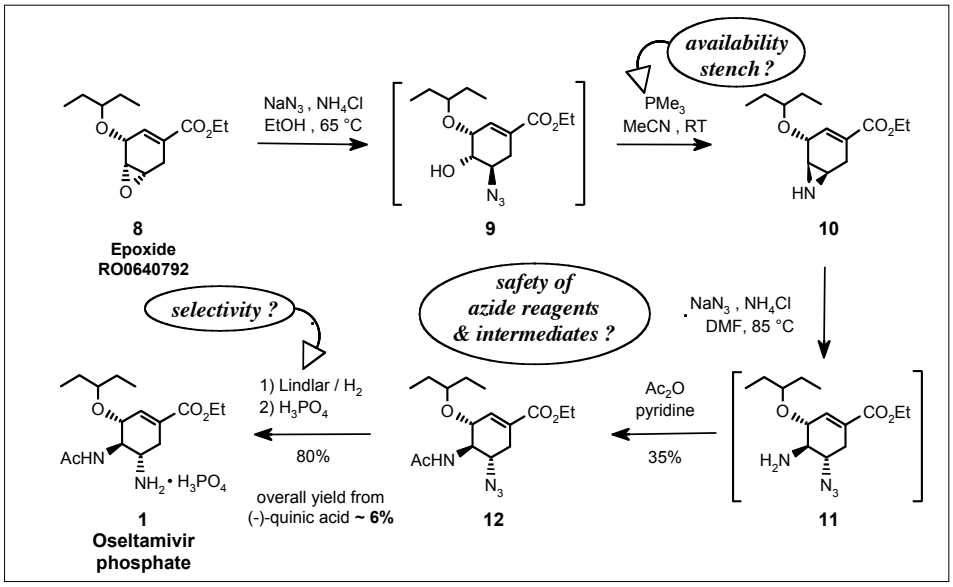
Initially quinic acid (**3**) was used as the raw material which is isolated from the bark of the cinchona tree as a side product during the extraction of cinchona alkaloids. The cinchona tree is indigenous predominantly in Central Africa, Zaire. The worldwide yearly capacity for quinic acid was estimated to be approx. 80 tonnes. Potential suppliers at the time offered to provide us with max. 15 tonnes per year translating into approx. 5 tonnes of drug substance **1**, which was clearly below the quantities forecasted. Therefore for large-scale production an alternative raw material had to be found.

3.1. *Problems when Starting from Quinic Acid 3*

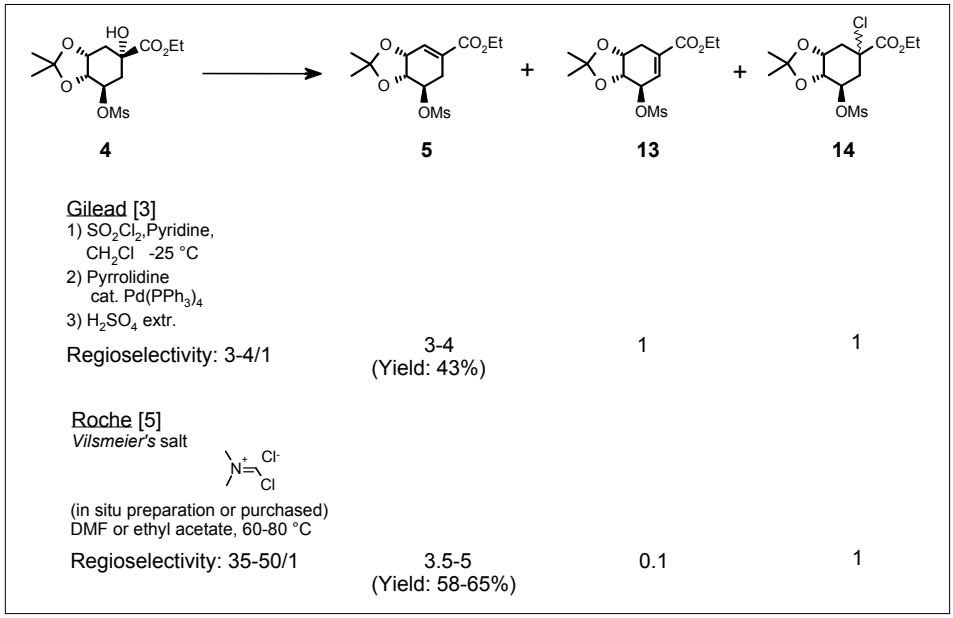
The key problematic step in the transformation of quinic acid (**3**) to the ethylmesylshikimate (**5**) was the dehydration of hydroxymesylate **4** (Scheme 3). The original method using SO_2Cl_2/pyridine was not particularly regioselective resulting in a 3–4:1 mixture of the regioisomers **5** and **13** together with smaller amounts of the chloro derivative **14**. In order to separate the desired crystalline isomer **5** without chromatography the mixture had to be subjected to a Pd(0)-catalyzed substitution with pyrrolidine, whereby only the allylic mesylate in the undesired isomer **13** reacted, followed by extractive removal of the corresponding pyrrolidine derivative. A much higher regioselectivity of 35–50:1 was achieved using Vilsmeier's salt.[5] The desired product **5** was isolated in 60% yield after crystallization from MeOH.

3.2. *Starting from Shikimic Acid (15)*

Shikimic acid (**15**), the ideal raw material for the synthesis of **1**, was commercially only available in small research quantities by mid 1997. However, the newly generated high demand for this compound



Scheme 2. Synthesis of oseltamivir phosphate (**1**) according to Rohloff *et al.*[3]



Scheme 3. Regioselective elimination: from quinic acid derivative **4** to the corresponding shikimic acid derivative **5**

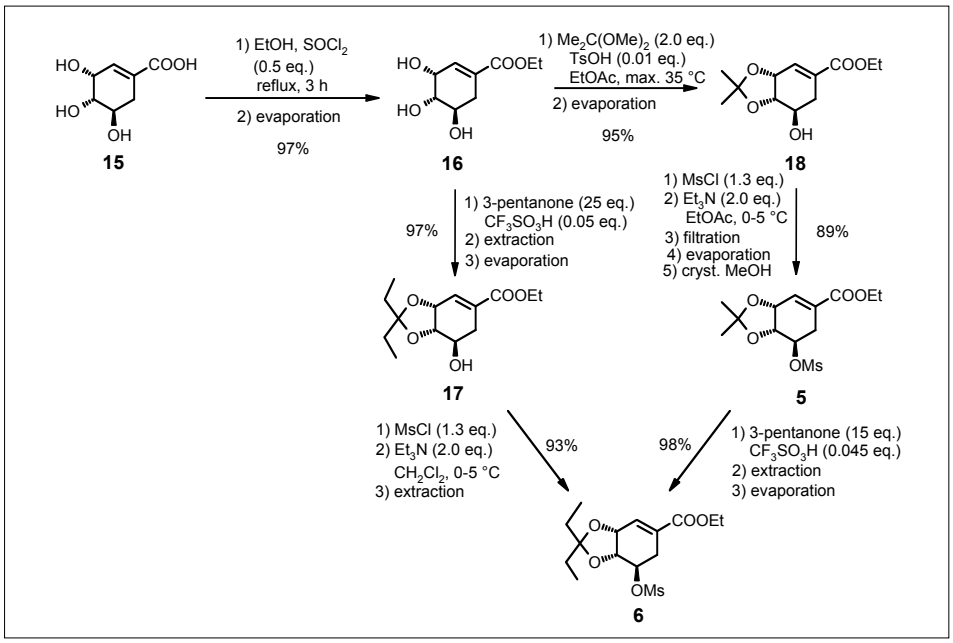
quickly changed the situation. The two sources were and still are production by fermentation using a recombinant *E. coli* [6] strain and extraction of **15** from star anise. Therefore the supply of sufficient quantities of shikimic acid (**15**) is secured even in the face of the dramatically increased demand for Tamiflu (**1**) caused by the threat of an influenza pandemic.

The conversion of shikimic acid (**15**) to the ketal **6** is outlined in Scheme 4. For the efficient production of **6** the longer route *via* the crystalline acetonide **5** is preferred since in the one step shorter route *via* the ketal **17** all intermediates are oils, which are difficult to purify efficiently on large scale.

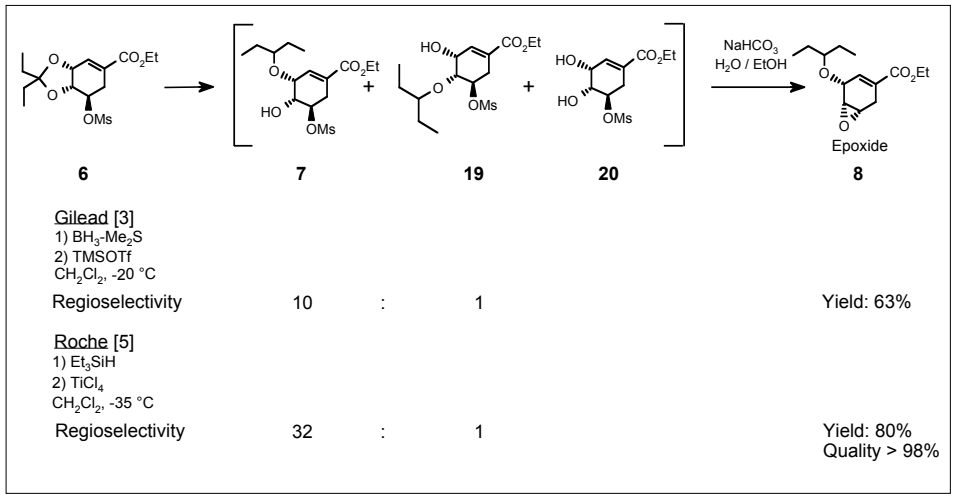
Clearly shikimic acid (**15**) is the starting material of choice giving access to the ethylmesylshikimate intermediate **6** in about 80% overall yield compared to about 40% overall yield starting from quinic acid **3**.

The original reductive opening[3] of the ketal **6** with BH_3-SMe_2/TMSOTf proceeded with only moderate regioselectivity and the reagents were considered unsuitable for large-scale production. A much higher selectivity was observed using $Et_3SiH/TiCl_4$. At –34 °C a 32:1 mixture of the desired hydroxyether **7** and its regioisomer **19** was obtained along with 2–4% diol **20**.

The crude hydroxyether **7** was converted to the epoxide **8** with $NaHCO_3$ in $EtOH/H_2O$ at 60 °C. Epoxide **8** crystallized from hexane as a white, crystalline solid in 80% yield and typically 99% assay (Scheme 5).



Scheme 4. Synthesis of ketal **6** from shikimic acid (**15**)



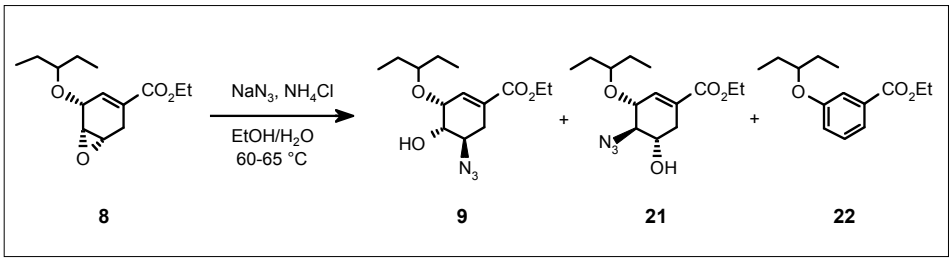
Scheme 5. Regioselective reduction of the ketal **6**

4. The Azide Based Transformation of the Epoxide 8 to Oseltamivir Phosphate (1)

Based on the synthesis of Rohloff *et al.*[3] development work at Roche was performed in close collaboration with several contract manufacturers experienced in azide chemistry. In-depth investigations of process safety were required for the whole sequence.

For the isolated azidoalcohols **9** and **21** safety tests recommended 40 °C as the highest permissible safe process temperature whereas in solution a safety limit of 70 °C was recommended. The opening of the epoxide **8** proceeded highly stereospecifically with 9:1 regioselectivity. Both regioisomers **9** and **21** formed the desired aziridine **10** in the subsequent step (Scheme 8). An excess of sodium azide was required in order to push the reaction to completion and to keep the formation of the undesired by-product **22** below 3%.

For the formation of the aziridine **10** trimethylphosphine was replaced by triphenylphosphine, which is cheaper and much easier to handle on industrial scale production. Trimethylphosphine is hardly available in large quantities, exceptionally malodorous, pyrophoric and highly toxic. Mechanistically the first step of the transformation is interpreted as a Staudinger type reaction which forms iminophosphorane and oxazaphospholidine intermediates at room temperature. These intermediates are sensitive to traces of water. In order to avoid formation of undesired aminoalcohol **23**, solvents and reagents have to be carefully checked for absence of water before use.



Scheme 6. Optimized conditions for the epoxide ring opening

The direct conversion of the intermediates to the aziridine **10** requires high reaction temperatures and long reaction times. The progress of the reaction was followed by ^{31}P-NMR spectroscopy: the signals for the iminophosphorane and oxazaphospholidine showed up at 10.99 ppm (mostly broad) and –55.28 ppm, respectively, and disappeared in the course of the reaction. The triphenylphosphine oxide signal occurred at 28.49 ppm and grew in intensity

and the signal for excess triphenylphosphine at –4.95 ppm remained constant. The mechanism of the aziridine formation is outlined in Scheme 7.

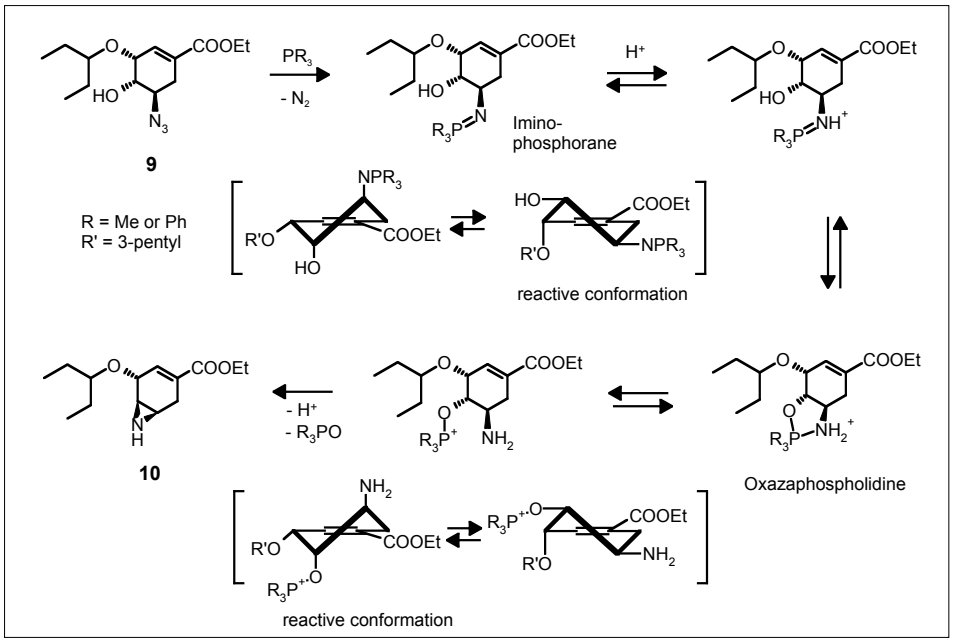
Since the aziridine **10** was found to be thermally unstable acceleration by the addition of catalytic amounts of HOAc as reported[7] was considered. Rohloff *et al.*[3] demonstrated that 5–10 mol% NEt_3-HCl was suitable but the disadvantage of this approach was the opening of the aziridine ring by the chloride ion as the nucleophile generating the by-product **24** (Fig. 2). Depending on the reaction conditions, the amount of the dimer by-product **25** increased. A breakthrough was achieved by using triethylamine and methanesulfonic acid. Acid-catalysis is required for the conversion of azidoalcohol **9** and **21** to aziridine **10** in order to shorten the reaction time and to keep the reaction temperature below 60 °C. Further reaction to the dimer by-product **25** is minimized when using a slight excess of triethylamine.

Originally the aziridine opening to aminoazide **11** was carried out using sodium azide and ammonium chloride in dimethylformamide at 80–85 °C. This procedure turned out to be not technically feasible. Yields of *ca.* 50% were obtained on lab scale. Upon scale-up to 0.5–5 kg yields dropped to 25–30%. Calorimetric measurements showed that aminoazide **11** is unstable at temperatures above 50 °C. With the finding that aziridine **10** opened smoothly with sodium azide in dimethylsulfoxide in the presence of phosphoric acid a much safer process for aziridine opening was available since deposition of ammonium azide sublimating into the cooling system of the equipment was avoided (Scheme 8). In order to prevent unacceptably high levels of HN_3 in the headspace, the reaction was carried out below 37 °C.

The next step was the development of a one-pot reaction by carrying out both aziridine formation and opening to the aminoazide **11** in dimethylsulfoxide as solvent.

For the one-pot procedure the reaction mixture obtained after aziridine formation was treated with sodium azide and heated to 33–37 °C directly followed by aziridine ring opening performed by feeding a solution of sulfuric acid in dimethylsulfoxide at a rate keeping the temperature below 37 °C. An excess of sodium azide is required to suppress the dimerization but is limited due to the solubility of sodium azide in dimethylsulfoxide.

In contrast to the original procedure, solvent exchange between aziridine formation and ring opening was avoided. With the replacement of ammonium chloride by sulfuric acid the temperature of the ring opening reaction was lowered and the time of conversion and cycle times were considerably reduced as well as the number of unit



Scheme 7. Mechanistic considerations of the formation of aziridine **10** from azidoalcohol **9**

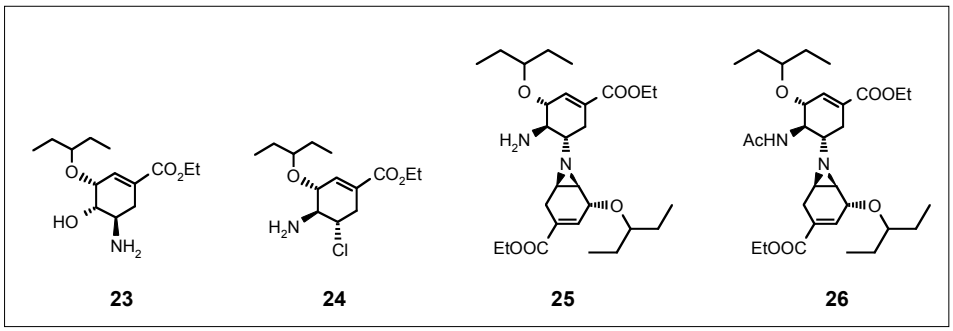
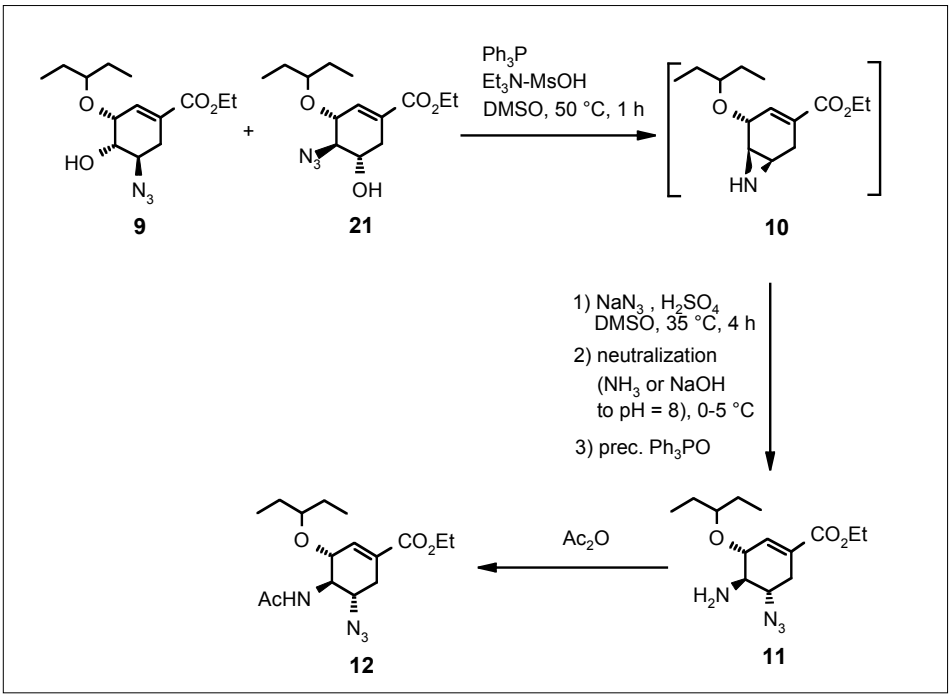


Fig. 2. By-products of the aziridine formation



Scheme 8. Optimized synthesis of acetamidoazide **12** from the azidoalcohols **9** and **21**

operations. Besides the impact on process safety, higher yields of aminoazide **11** were reached due to less thermal burden on aziridine **10**.

Reaction calorimetric measurements for the ring opening showed that the heat released for the addition of sodium azide can be neglected. Aziridine ring opening and formation of aminoazide **11** proceeded with the addition of sulfuric acid solution and was controlled by the feed rate. Formation of acetamidoazide **12** was then performed by dosing acetic anhydride to the solution of aminoazide **11** in dibutylether between 0–25 °C. According to reaction calorimetry heat evolution was fully controlled by dosage. The only impurity detected in the isolated material **12** at a level above 1% was the acetylated dimer **26**. Lab experiments revealed that up to 3% of **26** in the acetamidoazide **12** were acceptable for the manufacture of oseltamivir phosphate (**1**).

The final reduction of acetamidoazide **12** to give oseltamivir (**1**) can be accomplished by using various methods and reagents. During lab development the originally preferred heterogeneous catalytic hydrogenation proved to be problematic with respect to the functionalities in the molecule. Lindlar catalyst, Raney Nickel, Pd/C *etc.* led to an unfavorable impurity profile, *e.g.* due to double bond migration to the '1,6'-position (**27**) or reduction of the double bond (**28**) (Scheme 9). Once formed both impurities **27** and **28** are difficult to remove by the final crystallization of the phosphate salt **1**.

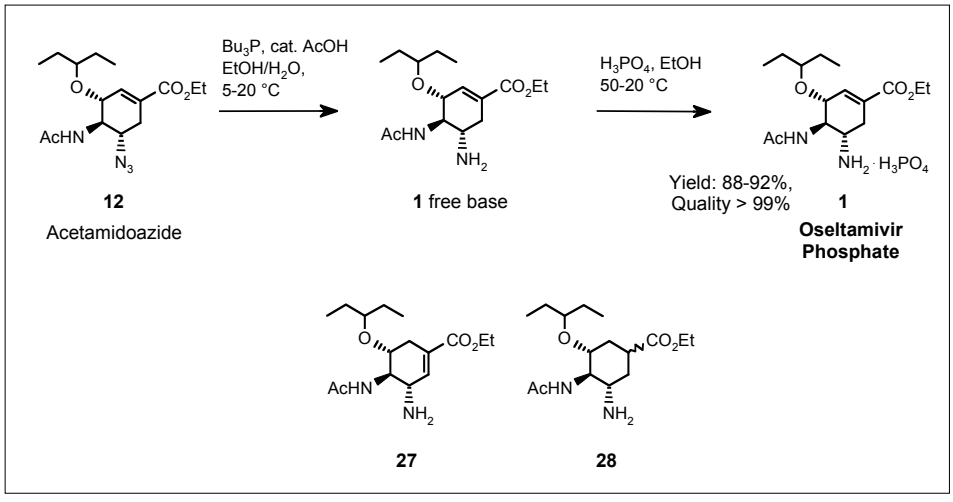
To avoid all these problems a Staudinger type phosphine reduction was developed using commercially available tri-n-butylphosphine.[8] By adding a slight excess of tri-n-butylphosphine to an aqueous ethanolic solution of **12** at 5 °C a very selective and smooth conversion to the free base of **1** was obtained. Subsequently the reaction mixture was warmed to room temperature in order to complete the reduction. After removal of water by azeotropic distillation the reaction mixture was used without further processing in the phosphate salt formation. A solution of *ortho*-phosphoric acid in ethanol was added at 50 °C followed by seed crystals to induce crystallization of oseltamivir phosphate **1**. After cooling to room temperature **1** was isolated, washed and dried. By this process oseltamivir phosphate (**1**) was obtained in a yield of 88–92% related to acetamidoazide **12** and in a very high quality (≥99% assay). The crystallization of **1** from ethanol proved to be very efficient in removing both the N-acetylated aziridine dimer **26**, the main impurity remaining of the azide steps and tri-n-butylphosphine oxide, the by-product of the Staudinger reaction.

5. The Search for Alternative Approaches

5.1. *Questions for Synthesis & Process Research*

As described above, the investigations performed finally led to the currently used technical process for the production of oseltamivir phosphate (**1**) starting from shikimic acid (**15**). However, for quite some time during the early development stage two most important questions remained unanswered: a) would it be possible to finally acquire tonnes of shikimic acid to start large-scale production of the API and b) would it be possible to find strong partners to perform the azide steps on large scale in a safe and efficient way. Therefore, Synthesis & Process Research embarked early on the investigation on shikimic acid independent approaches as well as on the exploration of azide-free transformations of the key epoxide **8**.

Since the results of these investigations are well documented in an earlier CHIMIA publication[4] we summarize here the final results for the sake of completeness.



Scheme 9. Selective azide reduction of the acetamidoazide **12** to oseltamivir (**1**) (free base) and phosphate salt formation

5.2. *Azide-Free Transformations*

Although the safe use of azide chemistry on an industrial scale is well established, 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 of the epoxide **8** to **1**.[7] To this end, a suitable non-azide nitrogen nucleophile and appropriate conditions had to be found compatible with the functional groups present and the pronounced tendency of such highly functionalized cyclohexene derivatives towards aromatization.

These efforts 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 of **8** to **29** and the subsequent Pd/C-catalyzed deallylation to **30**, but also for the short, selective and effective introduction of the second amino function present in **31** followed by the selective N-acetylation under acidic conditions followed by deallylation of **32** and phosphate salt formation (Scheme 10). This new and efficient reaction sequence finally led to the drug substance **1** in good overall yield.

The reaction cascade developed for the direct conversion of **30** to **31** with concomitant introduction of the second amino function shown in Scheme 11 represents an efficient sequence allowing for the combination of six chemical steps without the isolation of intermediates. This so-called 'domino sequence' exemplifies a potentially very economical way of working and constitutes a new, straightforward and practical conversion of an aminoalcohol into a vicinal diamine employing readily available and safe reagents. As an interesting variation of the 'allylamine' route of the epoxide **8** to **1** a closely related process has been established and developed.[9]

5.3. *Shikimic Acid Independent Approaches*

5.3.1. *The Need for Shikimic Acid Independent Approaches*

Due to the pace of development of oseltamivir phosphate (**1**) and especially due to the initial uncertainty regarding the commercial tonne-scale availability of the starting material shikimic acid (**15**), the intensive search for shikimic acid independent routes to the API was mandatory and was therefore pushed forward in parallel to the technical development of the routes described above.

The main synthetic challenges regarding the target molecule **1** are

a) the efficient induction of three stereogenic centers,
b) the regioselective introduction of the 1,2-double bond,
c) the introduction of the two amino functions and
d) the formation of the 3-pentyl ether side chain.

From the various proposals and concepts collected from Roche chemists *via* a global request, not surprisingly Diels-Alder approaches were prevalent followed by a large variation of further proposals.

5.3.2. *Diels-Alder Approaches*

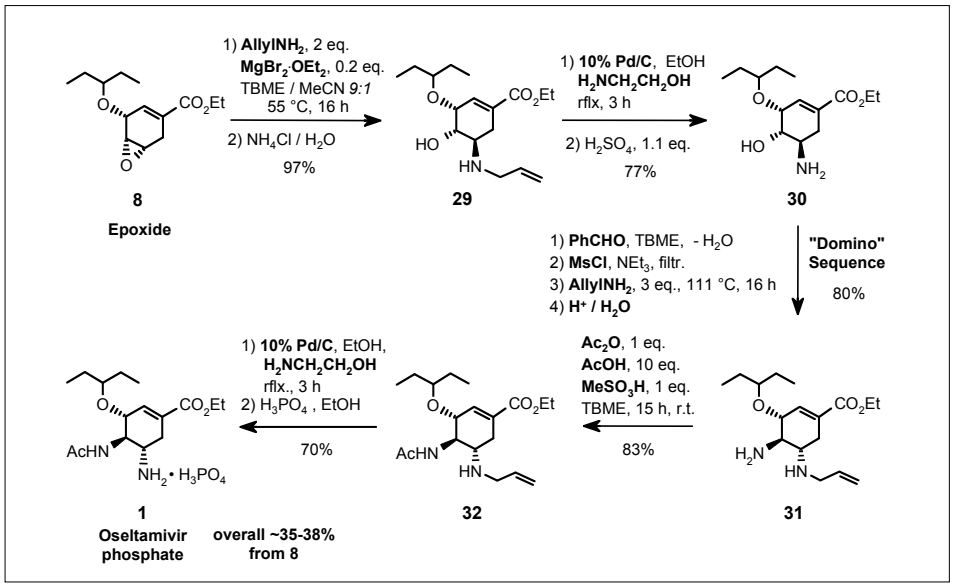
From a large number of Diels-Alder concepts tested[4] the furan-ethyl acrylate concept shown in Scheme 12 finally led to the first shikimic acid independent access to oseltamivir phosphate (**1**). The synthesis started with the Zn-chloride promoted Diels-Alder reaction of cheap furane and ethyl acrylate leading preferentially to the desired exo-isomer **33**, the product of thermodynamic control. Enzymatic resolution then allowed access to the *R*-isomer (–)-**33** in about 20% yield (not optimized).

Addition of diphenylphosphoryl azide in a [3+2] fashion to (–)-**33** followed by thermal nitrogen extrusion and trans-esterification at phosphorus delivered – unexpectedly[4] – the endo-isomer of aziridine **34** which smoothly underwent eliminative ring opening to form **35** followed by direct O-mesylation and regio- and stereoselective introduction of the 3-pentyl-ether side chain under Lewis-acid catalysis to provide **36**. Acid-catalyzed N–P-bond cleavage then led to **37** isolated as the hydrochloride. The specific configuration of this intermediate finally allowed for an azide-free transformation to the drug substance **1** in analogy to the processes shown in the Schemes 10 and 11.

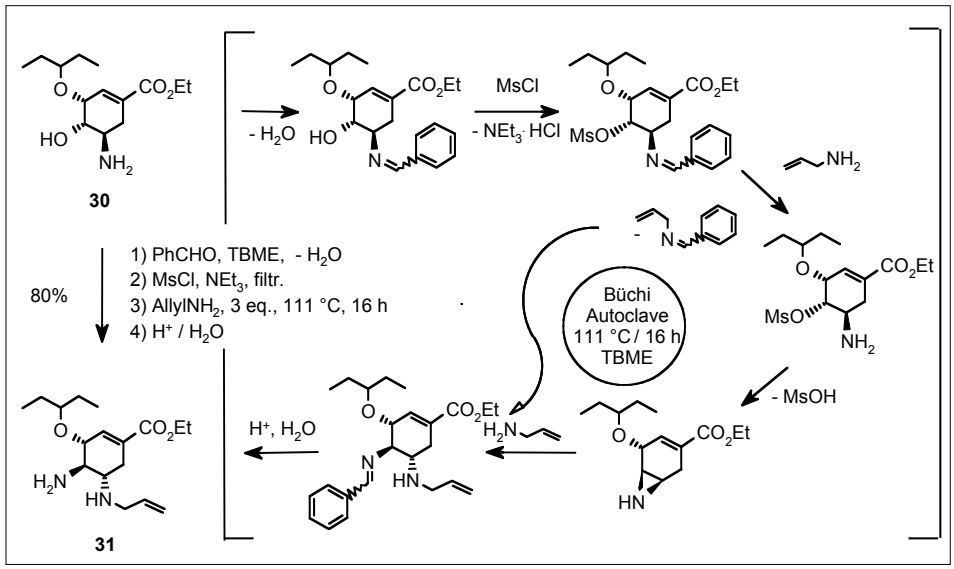
5.3.3. *Aromatic Ring Transformations: The Desymmetrization Concept*

Taking advantage of a desymmetrization protocol over racemate cleavage regarding effectiveness, the *meso*-approach shown in Scheme 13 is based on the enzymatic hydrolysis of the all-*cis meso*-diester **41** to the optically active mono-acid **42.**[4] Starting from cheap dimethoxy phenol **38** etherification with 3-pentyl mesylate, dibromination and ethoxycarbonylation gave the symmetrically substituted isophthalic diester **38** which was hydrogenated over Ru-Al_2O_3 providing exclusively the all-*cis meso*-diester **40**.

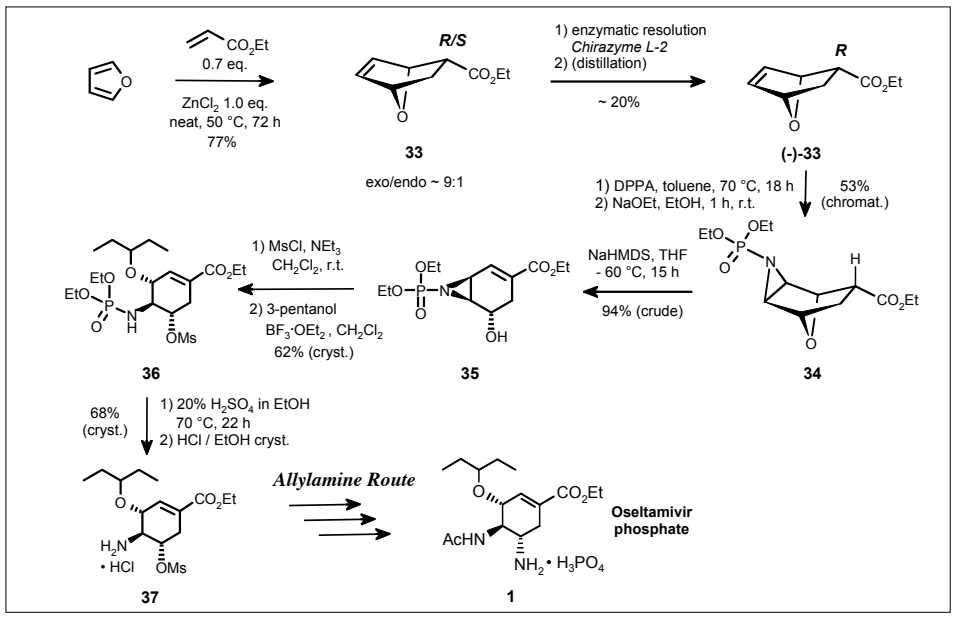
After selective O-demethylation the resulting *meso*-diester **41** was enzymatically hydrolyzed using pig liver esterase (PLE) affording the optically active mono-acid **42** in almost quantitative yield and with high optical purity (*ee*: 96–98%). The conversion of the β-hydroxy-acid **42** into the drug substance **1** was straightforward and started with Yamada-Curtius degradation of **42** allowing the introduction of the 5-amino-functionality by formation of the oxazolidinone **43**. In an efficient 'one-pot' procedure the N-Boc-protected oxazolidinone was treated with catalytic amounts of sodium hydride in refluxing toluene triggering the effective and selective formation



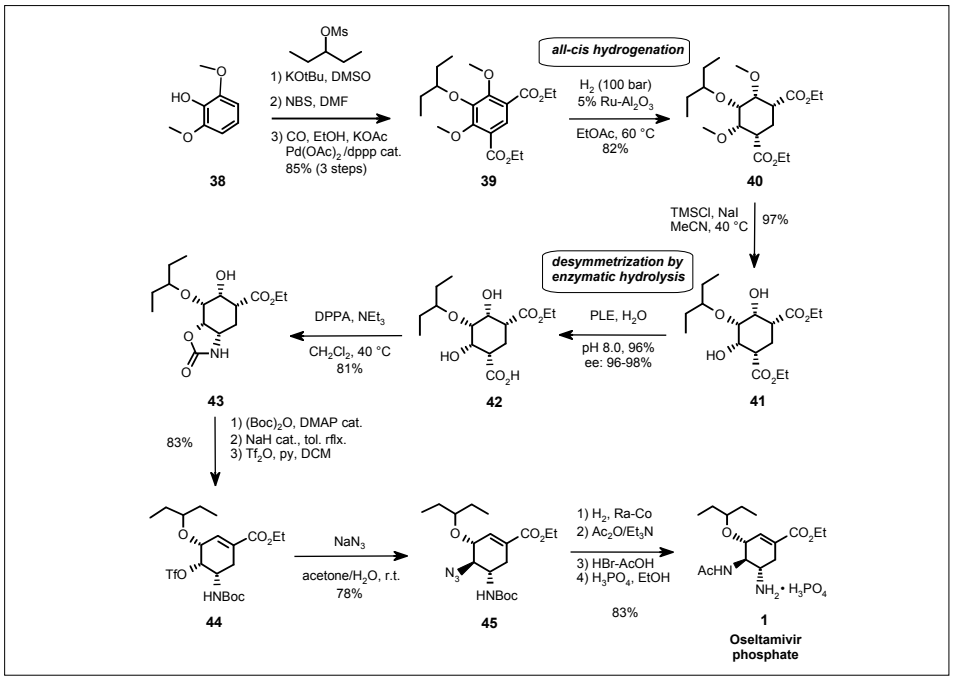
Scheme 10. Azide-free transformation of the epoxide **8** to oseltamivir phosphate (**1**)



Scheme 11. Reaction cascade combining six chemical steps but only one final work-up and isolation of **31**



Scheme 12. Furan-ethyl acrylate Diels-Alder approach to oseltamivir phosphate (**1**)



Scheme 13. Synthesis of oseltamivir phosphate (**1**) *via* desymmetrization of *meso*-diester **41**

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 **44** in an overall yield of 83%. The 4-amino functionality was finally introduced *via* S_N2-substitution of the triflate **44** 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 **40** comparing favorably with the shikimic acid based route.

Recently several academic groups embarked on shikimic acid independent syntheses of oseltamivir phosphate (**1**).[10] An elegant enantioselective Diels-Alder based route by Corey *et al.*[11] as well as syntheses applying desymmetrization and Diels-Alder concepts by Shibasaki *et al.*[12] have been published. We have to leave it up to the experienced organic chemists with manufacturing experience to evaluate the technical feasibility of these syntheses in comparison to the currently used commercial route using readily available shikimic acid (**15**) and also to the alternative routes described above.

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