

The Taming of the Flu? Influenza and Oseltamivir Phosphate

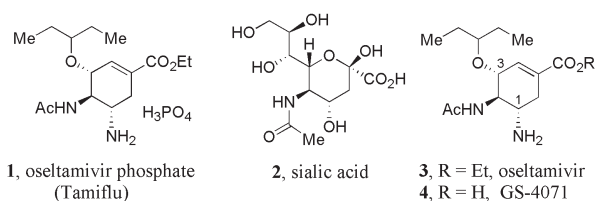
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Introduction

Each year new strains of the influenza virus evolve and spread globally, typically infecting around 20% of the world population and killing several hundreds of thousands of people. Most affected are the very young, the elderly and those with chronic medical conditions. Occasionally an influenza strain will arise that is so virulent, the death toll climbs into the millions. Examples of such pandemics include the Spanish Flu (1918-1920), which is estimated to have killed between 20 to 100 million people, the Asian Flu (1958-1959) with a death toll of around 1 million, and the Hong Kong Flu (1968-1969) which killed around $\frac{3}{4}$ of a million people.¹

Vaccines provide some protection against influenza, although the efficacy can vary from year to year. Their effectiveness can be as high as 85% when new strains of the virus are similar to the strains used to prepare the vaccine. If new strains differ significantly from those of the vaccine, vaccine effectiveness is reduced.² Antiviral drugs, the *neuraminidase inhibitors*, provide another line of defence against influenza by slowing the spread of the virus within the body.³ Tamiflu (oseltamivir phosphate; **1**) is currently the only such orally active inhibitor available and therefore is the best known of these inhibitors. This article describes the design, synthesis and the use of Tamiflu in the treatment of influenza.



Disabling a Master of Disguise

The influenza virus is able to *reshuffle* its genetic material over time.⁴ This can occur through mutation or through the reassortment of viral RNA when two different viruses occupy the same cell. Reassortment can involve viruses from different species. A genetic analysis of the 2009 H1N1 *swine* flu virus has detected the presence of swine, avian and human influenza genes.⁵ The reshuffling of viral genes can result in new surface antigens, which allow the virus to re-infect organisms that are immune to earlier strains of the virus. The influenza virus has two main types of glycoprotein antigens on its surface; hemagglutinin (H), which is involved in binding of the virus to target cells, and neuraminidase (N) that is involved in the release of viral progeny from the infected cell. There are nine known subtypes of N and sixteen of H, and they are referred to in the official naming of influenza viruses. For example, the current swine flu is an H1N1 influen-

za virus, whereas the Hong Kong flu of 1968-69 was an H3N2 virus.¹

The structure and function of neuraminidase was elucidated early in the 1990's and provided a viable target for drug design.³ Neuraminidase cleaves sialic acid (**2**) residues that bind new viruses to the host cell, releasing them to infect new cells. Inhibitors based on the structure of substrate **2** of neuraminidase were explored and eventually resulted in the synthesis of the analogue oseltamivir (**3**).

Tamiflu Development

Oseltamivir (**3**), developed at Gilead Sciences in the 1990's, was brought to market by Roche in 1999 as the corresponding phosphate **1** and named Tamiflu.^{6,7} It is considered to be the only effective treatment for avian flu and has been used more recently to treat those infected with the 2009 H1N1 swine flu.

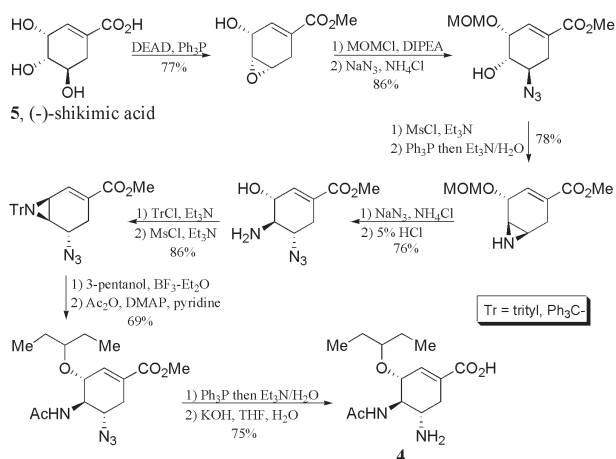
The development of **3** followed structure-based drug design using sialic acid (**2**) as a template.³ The replacement of the sugar-like core of **2** with a cyclohexene ring introduces greater metabolic stability. The 3-oxypentyl side chain of **2** was identified as providing the optimum fit into the active site of neuraminidase. In itself, **3** is actually a pro-drug as the C5 ester has a much higher oral bioavailability than carboxylic acid moiety of **4** (GS-4071), which is the active component once inside the body.

Syntheses of Oseltamivir (3)

Many syntheses of **3** have been developed since the 1990's,^{3,6-15} too many to fully describe in this article. While all of the syntheses contain fascinating chemistry, only a few are presented and discussed in detail below.

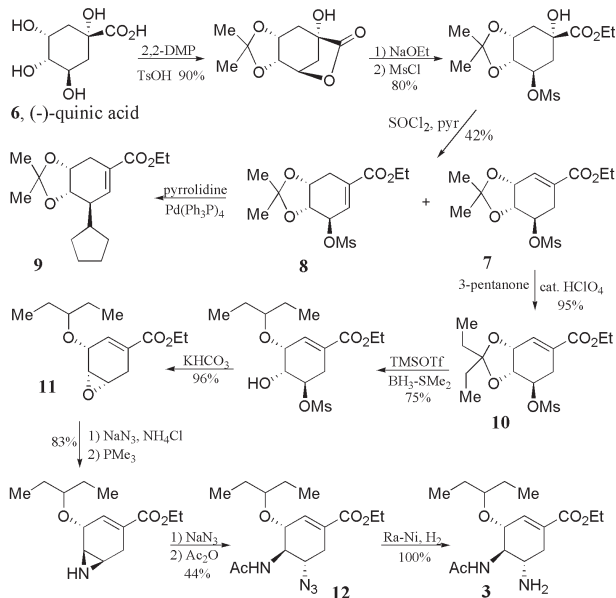
Choice of Starting Material

A number of starting materials have been used in the synthesis of **3** based upon their structural similarity to the drug, their availability and, of course, their cost. The first synthesis of **3**, used (-)-shikimic acid (**5**) (Scheme 1) as the starting material⁶ due to the similarity of its structure to the target, its useful functionality, its ready availability and its low cost. Initially, acid **5** was obtained by extraction of the Chinese star anise, but when large scale syntheses were developed there was concern that the supply of star anise might be limiting. Other sources of **5** were sought and the extraction of ginkgo leaves was investigated. Additionally, other starting materials were also explored as shown in Schemes 2-4. Eventually, a bacterial fermentation, using a genetically engineered strain of *E. coli*, was developed and it now provides the majority of **5** required for the synthesis of **3** by drug companies such as Roche.¹⁶



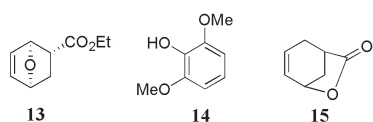
Scheme 1. The first synthesis of oseltamivir, see refs. 3 and 6.

(-)-Quinic acid (**6**) (Scheme 2) is similar in structure to **5** but initially was more abundant and much cheaper. However, its conversion into **3** involves a greater number of steps. Gilead used **6** in the first process route to **3** as shown in Scheme 2, although Roche subsequently reverted to the use of **5** in their first process route.³



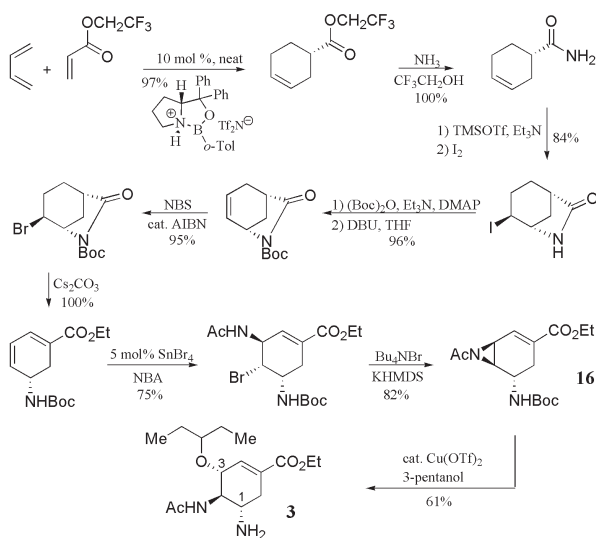
Scheme 2. The first process route to oseltamivir (**3**); see ref. 6.

Oseltamivir has also been synthesised from other starting materials, e.g. **13-15**, partially driven by early predictions of shortages of acid **5**. Abrecht *et al.* have described two novel approaches.⁸ The first utilised a Diels-Alder cycloaddition followed by enzymatic resolution to provide the oxabicycloheptene **13** for further elaboration into **3**. The second approach involved selective functionalization, reductive dearomatization and desymmetrization of phenolic diether **14**. This starting material was also used by Zutter *et al.* to synthesise **3**.⁹



In 2006, Corey *et al.* also used a Diels-Alder approach (Scheme 3) to develop a relatively short synthesis of **3** with good yield (~30%) over 12 steps.¹⁰ The use of inex-

pensive and abundant starting materials, complete enantio-, regio- and diastereocontrol, plus the avoidance of explosive azide intermediates make this approach elegant. Corey's group did not patent this method but left it in the open literature for anyone to use.



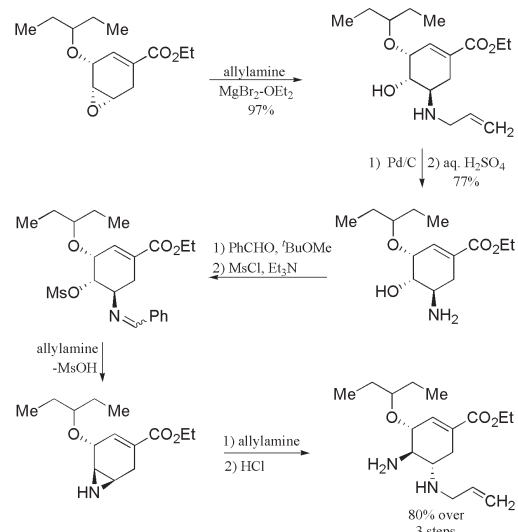
Scheme 3. The Corey approach to Oseltamivir - see ref. 10.

In 2008, Trost and Zhang published¹¹ what was, at the time, the shortest synthesis of **3**. It involved use of the commercially available lactone **15** and required just eight steps to give **3** in an overall yield of 30%. In 2009, Nie *et al.* also published an eight step synthesis (47% yield) using acid **5**, as the starting material.¹²

Azide-Free Approaches

The early syntheses of **3** depicted in Schemes 1 and 2 utilized azide chemistry to incorporate amine groups via the opening of aziridines and/or epoxides. The hazardous and toxic nature of azides, particularly at an industrial scale of operation, provided the opportunity for improvement through the development of alternative reagents.

The first azide-free synthesis of **3** (Scheme 4) was developed by Karpf and Trussardi¹³ and used the magnesium bromide etherate-catalysed addition of allylamine, followed by Pd catalysed deallylation to provide the desired amines. *t*-Butylamine and diallylamine were used in a similar fashion by the Roche group in Colorado.¹⁴



Scheme 4. An azide-free approach to Oseltamivir - see ref. 13.

Syntheses from starting materials other than acids **5** or **6** provide more options for the introduction of the amine functionalities of **3**. Iodolactonization was used to introduce the first amine functionality via the Corey *et al.* methodology of Scheme 3.¹⁰ This enabled regioselective ring opening of the *N*-acetyl aziridine **16** with 3-pentanol allowing simultaneous placement of the necessary amine functionality at C2 and introduction of the desired 3-oxy-pentanyl ether at C3. Other researchers have used a similar approach in establishing the C2 and C3 functionality.¹¹

Process Route Considerations

Syntheses developed by drug companies, *e.g.* as shown in Scheme 2, favour reactions that are amenable to large scale preparations. Reactions that are strongly exothermic, produce viscous mixtures and/or suspended solids, use hazardous reagents, volatile solvents and expensive reagents have to be avoided where possible.¹⁷ Reaction products that can be used subsequently without purification or which are readily purified by crystallisation are preferred over those requiring chromatography. Gilead's first process route (Scheme 2) used many readily available and inexpensive reagents and crystallization was used to purify compounds at key points during the synthesis, *e.g.* compounds **7**, **11** and **12**. When compound **7** proved difficult to separate from by-product **8** using fractional crystallization, **8** was removed by selective conversion to the pyrrolidinium compound **9**. Introduction of the diethyl ketal moiety of compound **10** was not made until late in the synthesis because such ketals do not readily crystallize.

How Effective is Tamiflu at Treating Influenza?

The effectiveness of Tamiflu depends on its use. Prophylactic use of Tamiflu in placebo-controlled double-blind studies has been shown to have a 74 to 82% efficacy in preventing infection.¹⁸ No significant side effects were observed.

The use of Tamiflu to treat those infected with influenza is most effective when treatment starts within two days of the first symptoms. Treatment reduces symptoms and shortens the duration of the infection by one to two days.

Conclusion

Tamiflu provides a mildly effective treatment for influenza, but it would be overstating its usefulness to claim that it has *tamed the flu*. However, drug synthesis is as much about the journey as the destination. The new methodologies and novel approaches developed by chemists to synthesise oseltamivir (**3**) also serve to advance the field of synthetic organic chemistry, providing new tools for the synthesis of tomorrow's drug molecules. The development of anti-viral drugs has always challenged medicinal chemists, and Tamiflu is but one step in the direction of new, more effective influenza drugs.

References

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