

Replication Cycle of the Influenza Virus and Recent Syntheses of Neuraminidase Inhibitor Oseltamivir (Tamiflu™)

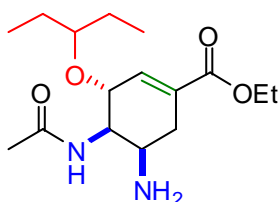
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ABSTRACT

Oseltamivir (Tamiflu™)



Influenza continues to be a major health concern worldwide, because of the annual flu season as well as the constant threat of pandemic. Oseltamivir (Tamiflu) a neuraminidase inhibitor has proven to be effective for the prophylaxis and treatment of influenza. Herein, the replication cycle of the influenza virus is discussed as well as Tamiflu's mode of action as a neuraminidase inhibitor. Finally, three recent concise syntheses of the drug are presented and compared to current production methods.

Influenza is one of the oldest and most well studied viruses. The first reliably recorded influenza pandemic occurred in 1580 in Asia and eventually spread throughout the globe¹. The 1918 pandemic or "Spanish Flu", the most severe flu pandemic to date, was responsible for approximately 20 million deaths worldwide.² It is estimated that if a pandemic of this magnitude were to occur today it could result in more than 100 million deaths worldwide. The emergence of the H5N1 virus or "Bird Flu" in 1997 raised further concern of a potential worldwide pandemic and launched additional research into developing antiviral agents to fight influenza. Increased emphasis was also placed on ramping up the production of anti-influenza medications, such as Tamiflu™.³ According to the World Health Organization, approximately 500,000 people are

hospitalized and 36,000 die each year as a result of the influenza virus in the U.S. alone. These figures along with the more recent appearance of the highly virulent H1N1 virus or "Swine Flu" exemplify the perpetual need for efficient synthesis of anti-influenza therapeutics. Herein a brief survey of the influenza replication cycle, as well as recent syntheses of oseltamivir (Tamiflu) will be discussed.

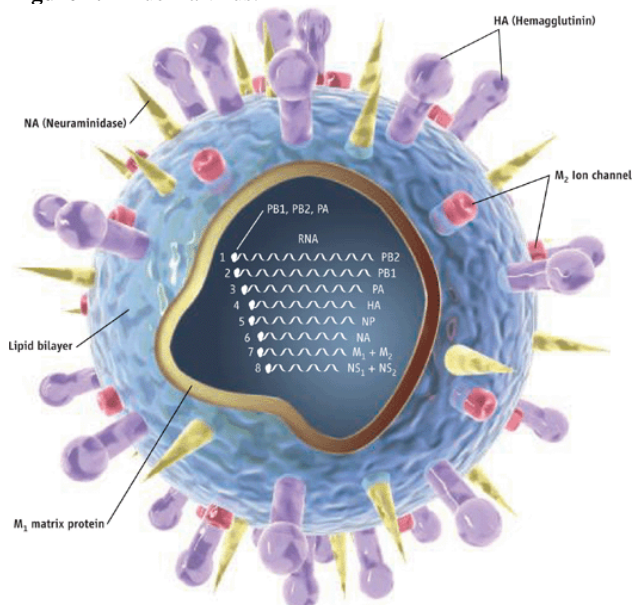
The influenza virus is pleomorphic, typically having a spherical or elongated shape. It contains three surface proteins: hemagglutinin (HA), neuraminidase (NA), and the M2 proton ion channel (Figure 1). The virus membrane consists of a lipid bilayer derived from the host cell, and a structural M1 matrix protein just beneath the lipid bilayer. Inside the virus lies the ribonucleoprotein (RNP) complex consisting of eight negative sense single stranded RNAs, the polymerase proteins (PB1, PB2, PA), and the nucleoprotein (NP).

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Figure 1. Influenza virus.⁴



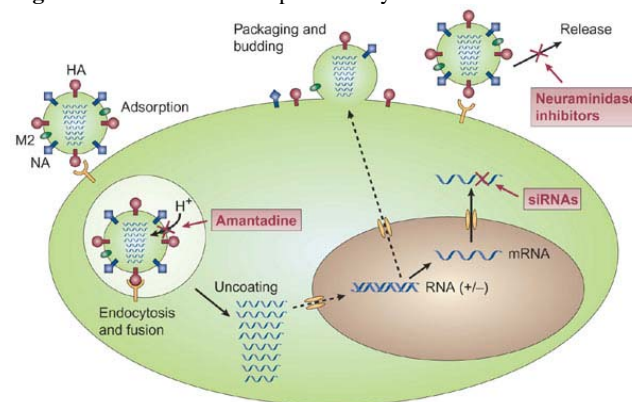
Binding of the HA to sialic acid residues on the membrane of the host cell initiates the replication cycle (Figure 2). The virus next undergoes endocytosis and is encapsulated in an endosome. At this point the M2 proton channel facilitates an influx of H^+ into the virus. The resultant drop in pH effects both the fusion of the virus to the endosomal wall and the release of viral RNPs into the host cell. Once in the cell, RNPs are transported through the nuclear membrane into the nucleus where they undergo transcription and translation forming new viral proteins. Due to a lack of viral RNA transcription checking mechanism, mutations are relatively frequent. The new viral proteins are transported out of the nucleus and assemble near the cell membrane, and undergo budding. The new virus is now formed but is still attached to the host cell via the binding of the HA to the sialic acid residues. In order for the virus to be liberated the NA must cleave the sialic acid residues. Now free from the host cell the virus is able to freely move throughout the host and infect new cells.⁵

Although the influenza virus has been known for thousands of years, relatively few remedies exist. Vaccinations have become a major weapon in preventing infection and are believed to reduce confirmed cases of the flu by 70-90% in healthy adults. However, vaccines have no effect on subjects who have already contracted

(4) From [Kaiser, J. *Science* **2006**, *312*, 380.] Reprinted with permission from AAAS.

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Figure 2. Influenza virus replication cycle.⁶



the virus, and due to the rapid mutations of influenza new vaccines must be made every year taking 4-5 months to produce. This limits their effectiveness in preventing and controlling a possible influenza pandemic.

Anti-viral medications are another method for the treatment of influenza, but until recently amantadine⁷ and rimantadine were the only two FDA approved anti-influenza drugs. These drugs work by blocking the M2 proton channel, preventing release of the RNPs into the host cell and therefore stopping viral replication.⁸ Although these drugs have been shown to be effective, they carry a number of side effects and have given rise to drug resistant viruses with high frequency.⁹

Another target for the treatment of influenza has been the NA protein. Inhibition of this protein prevents the release of new virions into the host controlling both the duration and the spread of the infection. NA inhibitors were seen as attractive targets because the NA is essential and specific for the lifecycle of the virus.¹⁰ The first NA inhibitors DANA (2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid) and the *N*-trifluoroacetyl analogue FANA showed good activity *in vitro* but did not prove useful in animal studies. Although these compounds found no clinical use, they served as leads for the development of currently marketed NA inhibitors: zanamivir (Relenza),¹¹ and oseltamivir (Tamiflu).¹² All four of these compounds were designed according to the 'transition state analogue' principle.¹³ Researchers

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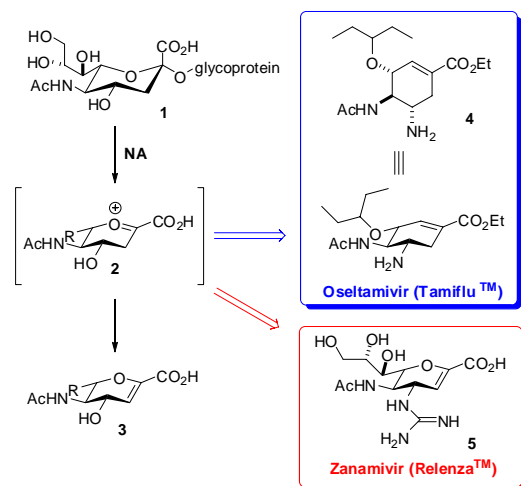
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attempted to mimic the transition state of the sialic acid cleavage by NA. Cleavage of the sialic acid residue **1** generates oxocarbenium ion **2**, which proceeds to dihydropyran **3** (Scheme 1).

Scheme 1. Transition state mimics, zanamivir and oseltamivir



Both zanamivir **5** and oseltamivir **4** are effective in their ability to mimic the oxocarbenium intermediate and are valuable drugs for prophylaxis and treatment of influenza. In particular, oseltamivir has been shown to shorten illness duration by 1-3 days as well as reduce the risk of lower respiratory tract complications, particularly bronchitis and pneumonia.¹⁴ Oseltamivir is preferred over zanamivir due to its higher bioavailability and longer half-life.¹⁵

Roche's current process synthesis for oseltamivir centers around naturally occurring (-)-shikimic acid **6**.¹⁶ In three steps, they arrive at acetal **7**. A reductive acetal opening generates epoxide **8**. Epoxide opening with sodium azide followed by treatment with triphenylphosphine yields aziridine **9**. The final four steps deliver oseltamivir phosphate in good yield. Although this route allows for the synthesis of Tamiflu on ton scale the synthesis suffers from two main drawbacks: the use of potentially explosive azides in two different steps, and the availability and cost of pure shikimic acid in large quantities.^{16c} To avoid these shortcomings, multiple syntheses of Tamiflu have recently appeared in the literature. As a recent review¹⁷ has been published on this topic, only the more recent syntheses are covered.¹⁸

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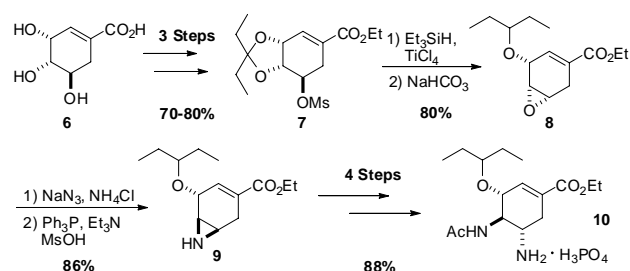
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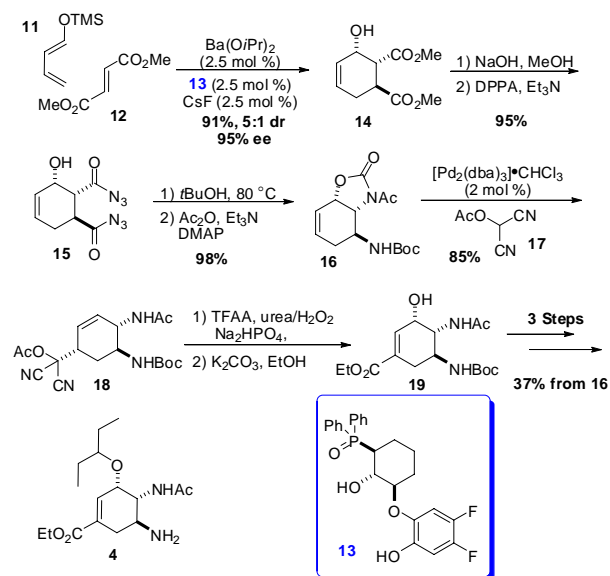
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Scheme 2. Roche's Process Synthesis.



Shibasaki and Kanai's synthesis¹⁹ of Tamiflu centers around a barium catalyzed asymmetric Diels-Alder reaction to assemble the core carbocycle (Scheme 3). Treatment of siloxy diene **11** with catalytic Ba(OiPr)₂ in the presence of methyl fumarate generates the desired cyclohexene diester in good yields. It was found that multidentate ligand **13** provides high enantioselectivity and moderate endo/exo selectivity. The Diels-Alder reaction was carried out on 58g scale with no change in reactivity or selectivity. In four steps including formation

Scheme 3. Shibasaki and Kanai's synthesis



of a potentially explosive diacyl azide, they arrive at oxazolidinone **16**. Utilizing malononitrile **17** as an acyl anion equivalent, a palladium catalyzed allylic substitution reaction affords **18**. Epoxidation and treatment with K₂CO₃ in EtOH converts the

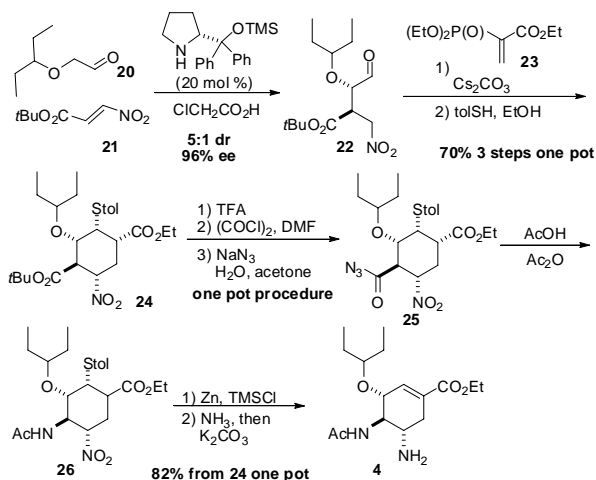
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acetoxydicyanomethyl unit into the desired ester followed by epoxide opening yielding **19**. Three steps including deprotection and installation of the 3-pentyloxy group affords oseltamivir. Although this synthesis addresses the use of shikimic acid as a starting material it still requires the formation of a potentially explosive diacyl azide intermediate.

Hayashi's synthesis²⁰ of Tamiflu is highlighted by its operational simplicity, requiring three one-pot reaction sequences and a single chromatographic purification (Scheme 4). The first one-pot reaction establishes the cyclohexane ring. Organocatalyzed asymmetric conjugate addition of aldehyde **20** into nitroalkene **21** generates **22**. Aldehyde **22** undergoes a tandem Michael addition followed by an intramolecular Horner-Wadsworth-Emmons reaction in the presence of vinyl phosphonate **23**. Treatment with *p*-toluenethiol generates **24** and completes the first sequence. The second three step one-pot procedure forms acyl azide **25**. The final one-pot operation consists of a domino Curtius rearrangement and amide formation, reduction of the nitro group with Zn/HCl, and a base promoted retro-Michael reaction to afford Tamiflu. The authors note that the Curtius rearrangement proceeds at room temperature, which decreases the potential hazard of this reaction. This synthesis again does not rely on the use of shikimic acid but does form an acyl azide intermediate.

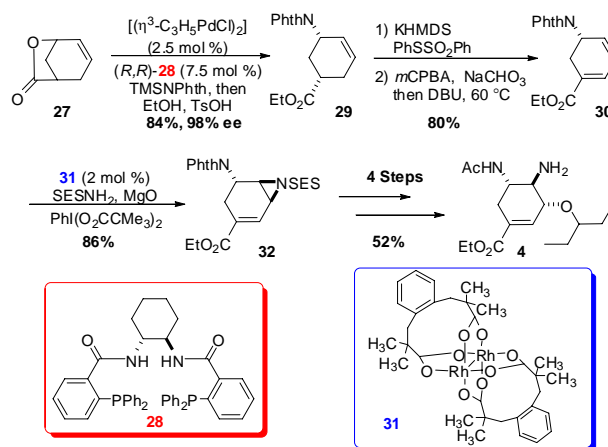
Scheme 4. Hayashi's synthesis of Tamiflu



Trost and co-workers avoid both the use of shikimic acid as a starting material as well as the formation of azide intermediates in their synthesis of Tamiflu (Scheme 5).²¹ Palladium-catalyzed asymmetric allylic alkylation of lactone **27** utilizing TMS-phthalimide as the nitrogen centered nucleophile affords the desymmetrized product.

Esterification can be carried out in the same pot to afford ester **29**. The TMS-phthalimide proved advantageous in two regards. It was necessary for the desired nucleophilic addition, and also allowed for facile acid hydrolysis. Sulfenylation of **29** followed by oxidation and thermal elimination yielded diene **30**. After screening a variety of metal catalysts rhodium proved most effective at catalyzing the desired aziridination, with SESNH_2 as the nitrene precursor, giving rise to a single regio- and diastereomer of **32**. Four more steps including nucleophilic aziridine opening, acylation, and deprotection of the nitrogens afforded Tamiflu in a short, high yielding synthesis.

Scheme 5. Trost's synthesis of Tamiflu



In conclusion, the influenza virus continues to be a major health threat as shown by the severity of past pandemics and the potential for new ones. NA inhibitors have proven to be efficient drugs for the prophylaxis and treatment of influenza. Of these NA inhibitors, Tamiflu is the drug of choice due to its high bioavailability and long half-life. For Tamiflu to be available for use during an influenza pandemic efficient large scale preparations are necessary. Three recent, concise, and scalable syntheses of Tamiflu have been presented. These syntheses benefit from the use of relatively cheap and readily available starting materials. Trost's synthesis also avoids the use of any azide intermediates therefore comparing favorably with current production methods. These syntheses along with other recent preparations of Tamiflu should allow for its synthesis in a more rapid and scalable fashion.

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