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Tamiflu Synthesis

Symmetry-Based Design for the Chemoenzymatic Synthesis of Oseltamivir (Tamiflu) from Ethyl Benzoate**

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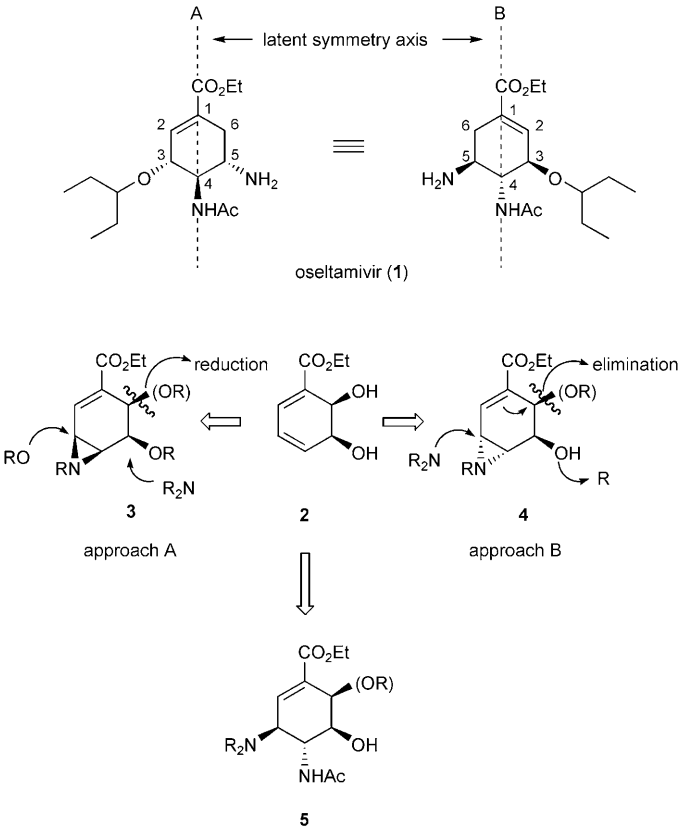
The possibility of a major influenza pandemic (especially the avian H5N1 influenza) continues to be a serious health concern. The development of effective antiviral medicines is hampered by the exceptionally high mutation rates of the influenza virus and, for the research effort to be successful, any new drugs must target the molecular mechanisms specific to the proliferation of the virus. The mechanism of infection involves the protein neuraminidase (NA), which is essential to viral replication. It is responsible for the glycosidic cleavage of sialic acid from a glycoprotein of a host cell in a process that liberates the virion from the infected cell.[1] Of the compounds that have been found to be effective as inhibitors of NA, by mimicking the oxonium intermediate of sialic acid glycolysis, oseltamivir (**1**), as its phosphate, or Tamiflu appears to be superior. It is orally active and serves as a prodrug, the active form of which is the corresponding carboxylic acid. It also has a superior bioavailability and is active at nanomolar levels.[2]

Although oseltamivir is not a particularly complex molecule, its practical synthesis on a scale large enough to guard against an influenza pandemic presents a formidable challenge. To date many ingenious syntheses have been developed and published,[3] several of which have the potential for optimization on a scale large enough to meet the requirements of a commercial synthesis.[4] Herein we report a flexible symmetry-based synthesis of oseltamivir, starting from a *cis*-dihydrodiol which is derived by enzymatic dihydroxylation of ethyl benzoate.

Three chemoenzymatic approaches to oseltamivir, starting from the *cis*-dihydrodiol derived from bromobenzene, were reported in 2008: those of Hudlicky,[5] Fang,[3p] and Banwell.[3o] Although the *cis* diol derived from bromobenzene is a commercially available material, any synthesis originating from this compound will require a palladium-catalyzed carbonylation step to introduce the ester functionality, usually at an advanced stage of the synthesis. The *cis*-diol **2**, derived from ethyl benzoate, already contains this functionality and offers several other advantages in the flexibility of the approach.

Oseltamivir contains a latent symmetry axis[6] through C1 and C4, a feature that can be exploited in the design of flexible synthetic routes starting from either of the two diastereomeric aziridines **3** or **4**, respectively. The concept of latent symmetry has been applied to the enantiodivergent syntheses of pinitol,[6a] pancratistatin,[7] carbohydrates,[8] and trihydroxyheliotridanes.[9] Its application in the synthesis of oseltamivir is shown in Scheme 1.

The two representations of oseltamivir shown are identical structures; however, if the substituents at C3, C4, and C5 are not specifically defined then the configurations of these three carbon atoms represent an "enantiomeric switch" which would be controlled by the translocation of the double bond. We can exploit this latent symmetry by designing an approach in which the order and the site of introduction of either the nitrogen or oxygen atom can be interchanged. In approach A



Scheme 1. Symmetry-based design for oseltamivir from *cis*-dihydrodiol via diastereomeric vinylaziridines.

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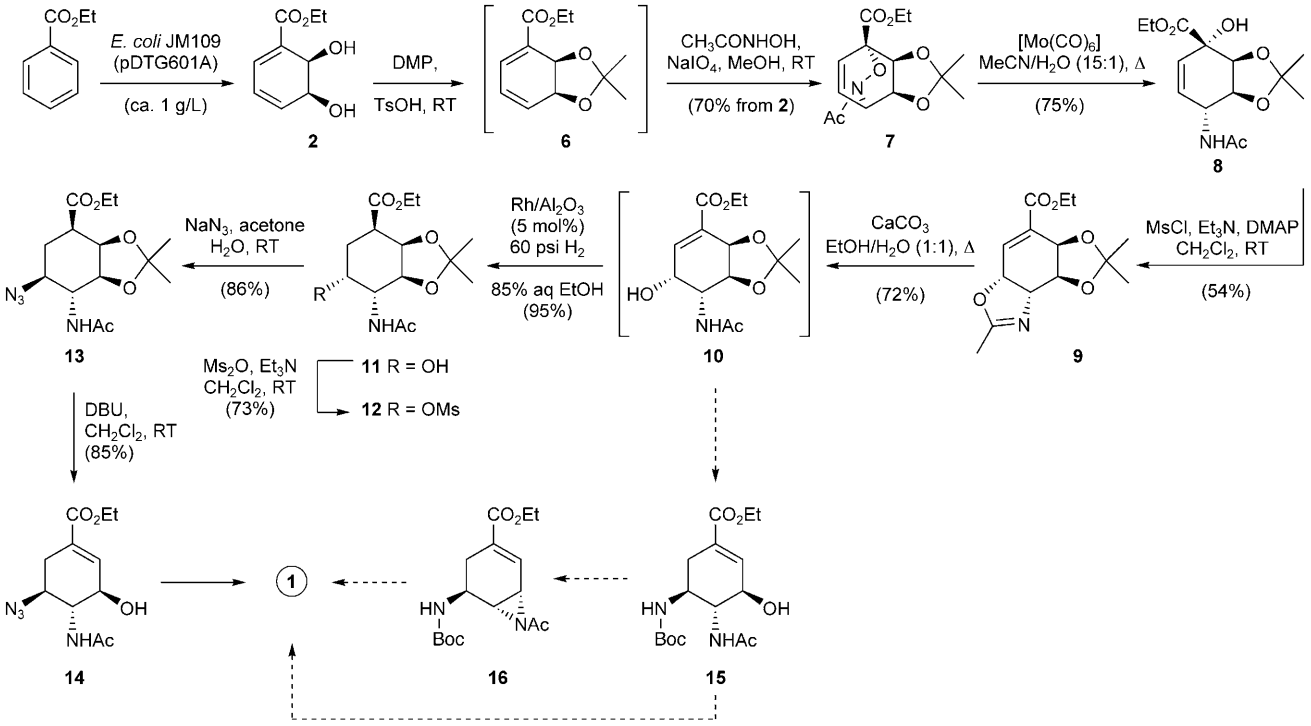
the ether functionality is introduced to the activated allylic position in **3**, with subsequent inversion at C5 with a nitrogen nucleophile, and removal of the C6 hydroxy group by reduction. In this approach the acrylate double bond remains in its original position. Conversely, the nitrogen functionality is introduced in aziridine **4**, a subsequent "symmetry switch" of the double bond with concomitant elimination of the C6 hydroxy group, and final alkylation of the C5 hydroxy group. Approach A has already been tested in our first-generation attempt from the diol derived from bromobenzene. Approach B relies on the synthesis of the *N*-acyl *anti*-aziridine **4**, which upon ring-opening would provide the allylic nitrogen substituent and necessitate the translocation of the double bond by hydrogenation and final base-catalyzed elimination of the C6 hydroxy group. We have tested this approach by preparing **4** (R = Ts), and we have converted this material into an advanced oseltamivir intermediate wherein we encountered problems with the removal of the *N*-tosyl group. As the synthesis of **4** (R = Ac) was not trivial we chose an alternate and more convenient route to **5** (the putative product of opening of *N*-acetyl **4** with a nitrogen nucleophile) to validate the symmetry-based synthetic route shown in approach B. This strategy has an additional advantage in that the C6 hydroxy group need not be removed by reduction, but rather by elimination upon the translocation of the olefin and the creation of the allylic alcohol.

The equivalent of the *trans*-substituted diamine derivative of type **5** became available in a short sequence through an inverse electron-demand hetero-Diels–Alder reaction of **2** with an acyl nitroso compound, subsequent reduction of the oxazine, and additional functionalization. The synthesis, outlined in Scheme 2, begins with the whole-cell fermentation of ethyl benzoate with a recombinant strain *Escherichia coli* JM109(pDTG601A)[10] to afford diol **2** in yields of approximately 1 g L^{-1} (15 L fermentor scale).[11] The diol was protected as its acetonide **6** and then reacted without purification with *N*-hydroxy acetamide in the presence of sodium periodate to afford the bicyclic oxazine **7** via an inverse electron-demand Diels–Alder cycloaddition with the acyl nitroso dienophile.[12] Reduction of the N–O bond with $[Mo(CO)_6]$ furnished the allylic alcohol **8**, which upon exposure to methanesulfonyl chloride led directly to oxazoline **9**.

Treatment of the oxazoline with calcium carbonate in refluxing aqueous ethanol furnished acetamide **10**, which was hydrogenated, without purification, to the saturated ester **11**. Conversion of the alcohol into mesylate **12**, displacement with azide to give **13**, and treatment with DBU completed the formal synthesis of oseltamivir via Fang's intermediate **14**, which is attained in ten steps (seven operations) from ethyl benzoate. The base-induced collapse of the acetonide generates the required allylic alcohol and provides for the "symmetry switch" of the double bond.

In summary, a short formal synthesis of oseltamivir from ethyl benzoate has been accomplished. It is comparable to other published routes in terms of brevity and practicality, but its symmetry-based design provides flexibility and more options for choosing the order of the introduction of the nitrogen and oxygen substituents onto the periphery of the cyclic diene diol. Ethyl benzoate, a commodity chemical, contains all of the carbon atoms of the product except for the 3-pentyl ether and is therefore a very convenient starting



Scheme 2. Synthesis. DMP = dimethoxypropane, Ts = 4-tolylsulfonyl, Ms = methanelsulfonyl, DMAP = 4-dimethylamino pyridine, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

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material for a potential large-scale synthesis of oseltamivir. Our next effort will address some additional improvements in the brevity, such as a direct introduction of Boc-protected amine at the allylic position of **10** and conversion of this material into **15** to intercept Corey's intermediate **16** or to produce oseltamivir by alkylation of the allylic alcohol. A side benefit of our investigation is also the potential for a new route to protected derivatives of 1,2-amino alcohols from conjugated dienes. We will report on additional ameliorations of this route in due course.

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