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A method for the synthesis of an oseltamivir PET tracer

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Abstract—A protocol applicable for the synthesis of an oseltamivir positron emission tomography (PET) tracer was developed. Acetylation of amine **3** with CH_3COCl, followed by deprotection and aqueous workup, produced oseltamivir **4** from **3** within 10 min. The obtained **4** was sufficiently pure for PET studies. This method can be extended to PET tracer synthesis using $CH_3{}^{11}COCl$.

Oseltamivir phosphate (Tamiflu®, **1**: Fig. 1), invented by the scientists of Gilead Science[1] and licensed to Roche in 1996, is the first orally active antiviral drug for the treatment of influenza types A and B. This drug potently inhibits the influenza virus neuraminidase by binding to the active site of the enzyme as a transition state mimic of sialic acid hydrolysis, a critical molecular level event for the influenza life cycle. As a result, release and spread of the virus from infected cells are prevented. Tamiflu is approved in many countries worldwide for the treatment and prevention of influenza. Because the active site of neuraminidase is highly conserved in all virus subtypes, Tamiflu is thought to be consistently effective for treating otherwise fatal avian influenza (H5N1 type virus), a possible cause of a potential influenza pandemic.[2] According to the World Health Organization, stockpiling of Tamiflu is currently the only way to guard against this possible pandemic. Some countries are stockpiling or intending to stockpile enough Tamiflu to treat 20–40% of their population.

There are two main concerns related to Tamiflu. One is its manufacturing process. The current process utilizes naturally occurring shikimic acid as the starting material,[3] which is not ideal to cover the worldwide demand of Tamiflu. Therefore, the development of a more efficient synthetic route is under intensive investigation. To date, five groups, including ours, have independently reported alternative synthetic routes.[4]

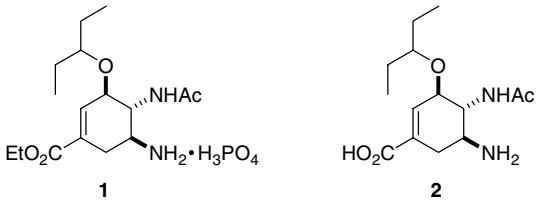

Figure 1.

The other concern is the possible side effects of Tamiflu on the nervous system. Tamiflu is considered to be a safe drug without severe side effects. Recently in Japan, however, where 80% of the world supply of Tamiflu is consumed, it was reported that Tamiflu might cause abnormal behavior (such as hallucinations and impulsive behavior). According to the Ministry of Health, Labor, and Welfare of Japan, 211 cases of abnormal behavior that are believed to be associated with the use of Tamiflu have been reported, of which eight resulted in deaths. It is noteworthy that in most cases the patients were adolescents or teenagers. More comprehensive statistical investigations are now underway to clarify the relationship between the abnormal behavior and Tamiflu administration. Meanwhile, very recent findings of neuroexcitatory effects in animal models, such as insects and mammals,[5] have demonstrated that Tamiflu and its carboxylate metabolite (Ro64-0802: **2**)

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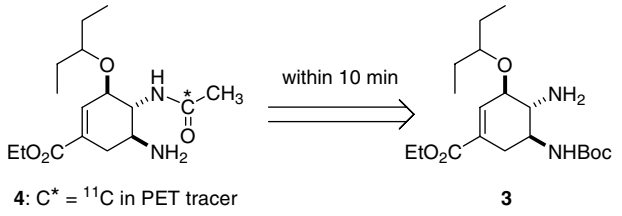
could indeed affect nervous function. Tamiflu (oseltamivir) passes through the blood brain barrier in rats, but its penetration into the brain is limited by an active efflux transporter, P-glycoprotein, at the blood–brain barrier, thereby attenuating the neuroexcitatory effects of Tamiflu under normal conditions.[6] Therefore, clarification of the pharmacologic effects of Tamiflu on the human nervous system is an urgent task. We hypothesized that PET studies might be an effective way to more precisely examine the distribution of oseltamivir in the brains of higher animals. In this communication, we describe a method applicable for the synthesis of an oseltamivir PET tracer.

PET imaging techniques allow for visualization of the distribution of a radioactive probe molecule under physiologic conditions in a living whole body system. A PET tracer containing short-lived positron-emitting radionuclides (such as ^{11}C) spontaneously decomposes through β^+-decay, producing a positron and a stable nucleus containing the same atomic mass (e.g., ^{11}B from ^{11}C). The generated positron then collides with an electron of a nearby molecule resulting in a pair of annihilation γ-ray emissions. PET imaging is created by detecting this annihilation γ-ray.[7] The following three main factors should be considered in designing a PET tracer: (1) the availability of the labeling precursor with short-lived positron-emitting radio-nuclides; (2) the timing of the introduction of short-lived positron-emitting radio-nuclides to the tracer molecule; (3) the ease of purification of the final compound to eliminate contaminating radioactive side products. For a high signal-to-noise ratio in PET studies, the short-lived positron-emitting radionuclides should be introduced to the target compound through a clean conversion at a late stage of synthesis.

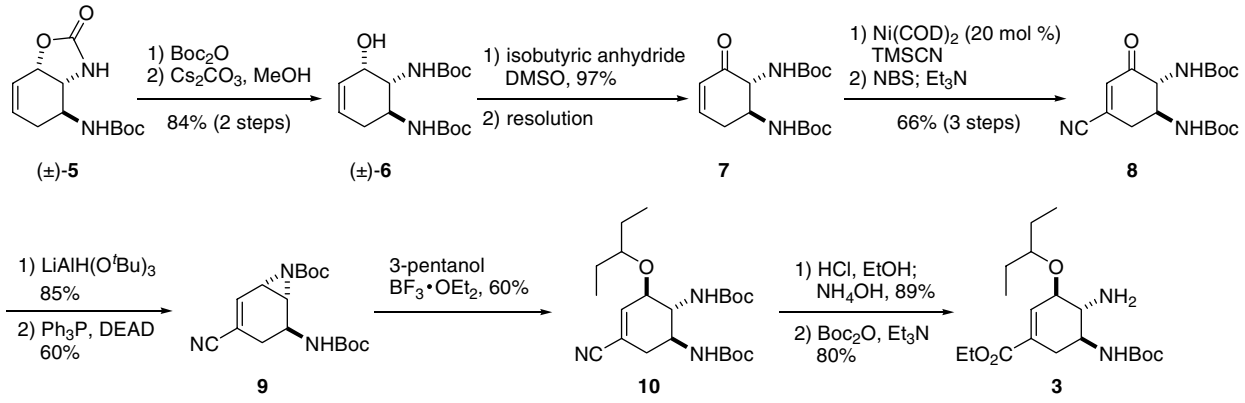


Scheme 1. Design of Tamiflu PET tracer and its synthetic plan.

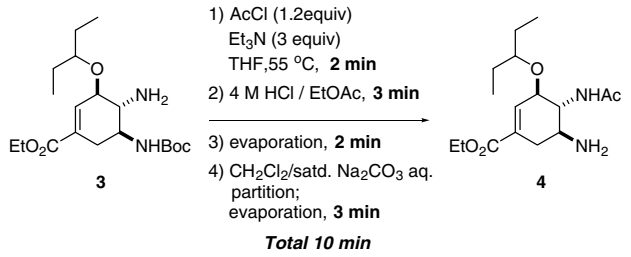
We hypothesized that labeling the acetamide part of **1** (Tamiflu) or **4** (oseltamivir) would fulfill these requirements (Scheme 1). Based on our previous synthesis,[4c] the acetyl group can be introduced in high yield to the late stage-intermediate **3**. In addition, there are several reports on the synthesis and use of $CH_3{}^{11}COCl$ in PET studies.[8] A possible side product, $CH_3{}^{11}CO_2H$, can be easily extracted or evaporated out from the target tracer molecule. Because the half-life of ^{11}C is 20.3 min, conditions should be optimized to complete the synthesis and purification of the tracer **4** from **3** within approximately 10 min. The precursor **3** would be synthesized by modifying our third-generation synthetic route of Tamiflu.[4e]

The key intermediate **3** was synthesized as shown in Scheme 2. The known racemic carbamate **5**, which was rapidly synthesized through Diels–Alder reaction and the Curtius rearrangement as key steps,[4e] was converted to **6** via two straightforward steps. After oxidizing **6** to enone **7** under the modified Swern conditions, enantiomers were separated using chiral preparative HPLC [Daicel, Chiralpak AD-H, 2-propanol/hexane 1:20, flow 1.0 mL/min: t_R 15.2 min (undesired) and 17.0 min (desired)].[4c] A Ni-catalyzed 1,4-addition of TMSCN followed by bromination and elimination of HBr produced β-cyano enone **8** in 66% yield in three steps. Stereoselective reduction of **8** followed by Mitsunobu reaction produced aziridine **9**, to which the 3-pentyloxy group was introduced in the presence of $BF_3{\cdot}OEt_2$, producing di-Boc protected **10**. Ethanolysis of the nitrile under acidic conditions proceeded with concomitant Boc cleavage, and the subsequent regioselective mono-Boc protection of the less hindered amino group produced the key intermediate **3** in high yield.

Having obtained intermediate **3**, the conditions were then optimized for the conversion of **3** to **4** to fulfill the requirements of PET tracer synthesis (Scheme 3).[9] Amine **3** was treated with acetyl chloride (1.2 equiv) and triethylamine (3 equiv) in hot (55 °C) THF for 2 min. This reaction hardly proceeded at ambient temperature, whereas *N*,*N*′-diacetylation proceeded in the presence of an excess (5 equiv) amount of acetyl chloride. After completion of the acetylation, 4 M HCl in ethyl acetate was added to the same reaction vessel to



Scheme 2. Synthesis of key intermediate **3**.



Scheme 3. Rapid synthesis of oseltamivir.

cleave the Boc group. This deprotection was completed in 3 min. Evaporation of the solvents (2 min), partition of the residue in CH_2Cl_2/saturated Na_2CO_3 aq biphasic system, phase separation, and evaporation of CH_2Cl_2 (3 min) produced **4** in an analytically pure form. Therefore, sufficiently pure **4** for PET studies was obtained from **3** in 10 min (20-mg scale).[10] Yield of the two-step conversion was 82% after silica gel column chromatography.

In summary, we developed a procedure that can be applied to the synthesis of a Tamiflu PET tracer. The selection of an appropriate precursor (**3**) for introduction of an unstable nucleus and careful optimization of the reaction conditions were key. Synthesis of the PET tracer using this method and studies of its application to PET imaging of [^{11}C]Tamiflu in higher animals are currently ongoing in our group.

Acknowledgments

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9. Unlabeled CH_3COCl was used in this study.
10. *Experimental details:* Acetyl chloride (4.6 μL, 0.0645 mmol, 1.2 equiv) was added to a solution of **3** (19.9 mg, 0.0537 mmol) and triethylamine (22.5 μL, 0.161 mmol, 3.0 equiv) in THF (0.4 mL) at 55 °C. After 2 min, the reaction vessel was removed from the heating bath, and 4 M hydrogen chloride in ethyl acetate (1.5 mL) was added in one portion. After 3 min, the reaction mixture was concentrated under vacuum. A saturated aqueous solution of sodium hydrogen carbonate (1 mL) was then added to the residue. The aqueous layer was extracted with CH_2Cl_2 (2× 2 mL) using a Chem Elute extraction column (available from Varian). The solvent was evaporated under reduced pressure, producing analytically pure **4**. The isolation of **4** was completed in 3 min. The residue was passed through silica gel column chromatograph (CH_2Cl_2/MeOH = 7:1) to afford **4** (13.8 mg, 82%) as a colorless oil.