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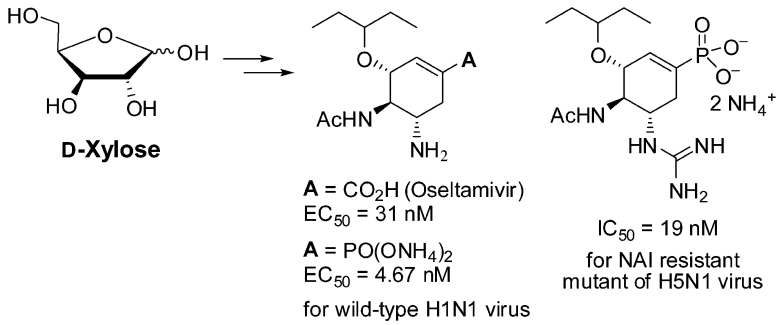

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Synthesis of Tamiflu and its Phosphonate Congeners Possessing Potent Anti-Influenza Activity

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Influenza remains a major health problem for humans and animals.[1] At present, four drugs are approved for influenza prophylaxis and treatment:[2] amantadine and rimantadine act as the M2 ion channel blockers, whereas Tamiflu (the phosphate salt of oseltamivir ethyl ester) and Relenza (zanamivir) inhibit the activity of neuraminidase (NA). The NA inhibitors (NAIs) are designed to have (oxa)cyclohexene scaffolds to mimic the oxonium transition-state in the enzymatic cleavage of sialic acid.[3] Tamiflu (**1**, shown in Scheme 1) is an orally administrated anti-influenza drug.[4] On hydrolysis by hepatic esterases, the active carboxylate, oseltamivir (**2**, also known as GS4071), is exposed to interact with three arginine residues (Arg118, Arg292, and Arg371) in the active site of NA.[3]

The phosphonate group is generally used as a bioisostere of carboxylate in drug design.[5] In comparison with the carboxylate−guanidinium ion pair, a phosphonate ion exhibits stronger electrostatic interactions with the guanidinium ion. Our preliminary molecular docking experiments (Figure 1) using the known N1 crystal structure (PDB code: 2HU4)[3c] reveal that the putative phosphonate inhibitor **3a** indeed binds strongly with the triarginine residues of NA, in addition to other interactions exerted by the C_3-pentyloxy, C_4-acetamido, and C_5-amino groups in the binding pocket similar to the NA−oseltamivir complex. Because the previously reported methods[4] for the synthesis of oseltamivir/Tamiflu are not amenable to exchange of the C-1 carboxyl group to a phosphonate group, we thus explored a novel approach to the synthesis of both oseltamivir/Tamiflu and the phosphonate congeners using D-xylose as an appropriate chiral precursor (Scheme 1).

In brief, our present synthetic method is straightforward to culminate in an enantioselective synthesis of Tamiflu, oseltamivir, the phosphonate congener, and the guanidine analogues with reasonably high yields (5.2−13.5%). An intramolecular Horner−Wadsworth−Emmons reaction was carried out to furnish the cyclohexene carboxylate **8a** and phosphonate **8b**. On treatment with diphenylphosphoryl azide according to Mitsunobu's method,[6] the hydroxyl group in **8a**/**8b** was successfully substituted by an azido group with the inversed configuration. The hazardous reagent of sodium azide was avoided in this procedure. This synthetic scheme allows late functionalization, which makes it attractive from a medicinal chemistry point of view.

The greater potencies of the phosphonate congeners, **3** (namely Tamiphosphor) versus oseltamivir **2** and guanidine **13b** versus **13a**, were observed in the wild-type neuraminidases of H1N1 and H5N1 influenza viruses (Table 1). Both compounds **3** and **2** are significantly less potent toward the NAI resistant mutants of H274Y[7] than the wild-type enzymes. Nevertheless, the phosphonate compound **13b** is an effective inhibitor that inhibits both mutant enzymes at low nM concentrations. Compounds **14a** and **14b**, which lack the

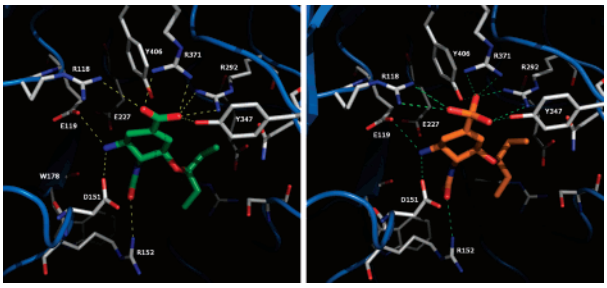

Figure 1. Molecular models of oseltamivir **2** (left panel) and the phosphonate compound **3a** (right panel) in the active site of influenza virus neuraminidase (N1 subtype). The complex of the phosphonate compound **3a** has more extensive hydrogen bonding interactions (8 pairs ligand−NA H-bonds) with key residues in the NA active site than the oseltamivir-NA complex (6 pairs ligand−NA H-bonds)

Table 1. Inhibitory Activities against Wild-Type and Mutant Influenza Virus Neuraminidases

	neuraminidase inhibition, IC_{50} (nM)			
compd	Wt (WSN)[a]	Mut (WSN)[b]	Wt (Hanoi)[c]	Mut (Hanoi)[d]
2	5.90 (± 0.62)	295 (± 31)	62.9 (± 5.7)	971 (± 54)
3[e]	0.30 (± 0.05)	526 (± 44)	13.3 (± 1.0)	1210 (± 490)
13a	4.10 (± 0.51)	252 (± 31)	160 (± 32)	1150 (± 380)
13b[e]	0.12 (± 0.02)	7.39 (± 0.67)	1.82 (± 0.11)	19.5 (± 1.4)
14a	36700	ND[f]	ND[f]	ND[f]
14b[e]	3200	ND[f]	ND[f]	ND[f]

[a] NA from influenza virus A/WSN/1933 (H1N1). [b] NA (H274Y) from influenza virus A/WSN/1933 (H1N1). [c] NA from influenza virus A/Hanoi/30408/2005 (H5N1). [d] NA (H274Y) from influenza virus A/Hanoi/30408/2005 (H5N1). [e] As the ammonium salt depicted in Scheme 1. [f] Not determined.

Table 2. Neuraminidase Inhibition, Anti-influenza, and Cytotoxicity Activities of Oseltamivir **2**, Phosphonate Congener **3**, and the Related Analogues

compd	K_i (nM)[a]	EC_{50} (nM)[b]	CC_{50} (μM)[c]	S.I.[d]
2	2.90 (± 0.30)	31.3 (± 3.5)	>100	>3200
3[e]	0.15 (± 0.02)	4.67 (± 0.68)	74 (± 5.7)	15800
13a	2.02 (± 0.25)	5.60 (± 1.2)	>100	>17800
13b[e]	0.06 (± 0.01)	0.09 (± 0.02)	~5	~56000

[a] Neuraminidase inhibition against influenza virus A/WSN/1933 (H1N1). K_i values were determined by using Cheng−Prusoff equation.[8] [b] Concentrations of NA inhibitors for 50% protection of the cytopathogenic effects due to flu (A/WSN/1933) infection. [c] The highest concentration used is 100 μM in the assay of cytotoxicity on MDCK cells. [d] Selectivity index, the ratio of CC_{50} to EC_{50}. [e] As the ammonium salt depicted in Scheme 1.

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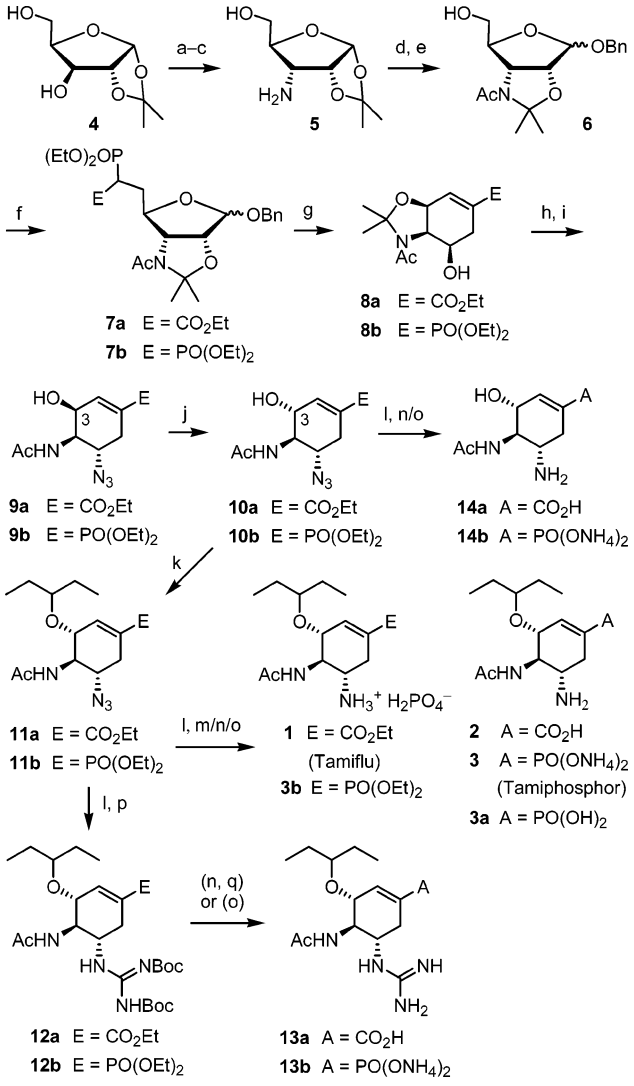
Scheme 1. Synthesis of Tamiflu **1**, Oseltamivir **2**, the Guanidine Analogue **13a**, and the Phosphonate Congeners **3**, **3b**, and **13b**[a]



[a] Reagents and reaction conditions: (a) Me_3CCOCl, pyridine, 0 °C, 8 h; 89%. (b) PDC, Ac_2O, reflux, 1.5 h; $HONH_2{-}HCl$, pyridine, 60 °C, 24 h; 82%. (c) $LiAlH_4$, THF, 0 °C, then reflux 1.5 h; 88%. (d) Ac_2O, pyridine, 25 °C, 3 h; HCl/1,4-dioxane (4 M), BnOH, toluene, 0−25 °C, 24 h; 85%. (e) 2,2′-dimethoxypropane, toluene, catalyst *p*-TsOH, 80 °C, 4 h; 90%. (f) Tf_2O, pyridine, CH_2Cl_2, −15 °C, 2 h; $EtO_2CCH_2PO(OEt)_2$ or $H_2C[PO(OEt)_2]_2$, NaH, catalyst 15-crown-5, DMF, 25 °C, 24 h; 80% for **7a** and 73% for **7b**. (g) H_2, Pd/C, EtOH, 25 °C, 24 h; NaH, THF, 25 °C, 1 h, 83% for **8a**; or NaOEt, EtOH, 25 °C, 5 h, 80% for **8b**. (h) $(PhO)_2PON_3$, (*i*-Pr)N═C═N(*i*-Pr), PPh_3, THF, 25 °C, 48 h. (i) HCl, EtOH, reflux, 1 h; 83% for **9a** and 74% for **9b**. (j) Tf_2O, pyridine, CH_2Cl_2, −15 to −10 °C, 2 h; KNO_2, 18-crown-6, DMF, 40 °C, 24 h; 70% for **10a** and 71% for **10b**. (k) $Cl_3CC(═NH)OCHEt_2$, CF_3SO_3H, CH_2Cl_2, 25 °C, 24 h; 78% for **11a** and 82% for **11b**. (l) H_2, Lindlar catalyst, EtOH, 25 °C, 16 h; 85% for **3b**. (m) H_3PO_4, EtOH, 40 °C, 1 h; 91% for **1**. (n) KOH, THF/H_2O, 0−25 °C, 1 h; 88% for **2** and 81% for **14a**. (o) TMSBr, $CHCl_3$, 25 °C, 24 h; aqueous NH_4HCO_3, lyophilization; 85% for **3** (as the ammonium salt), 72% for **13b** and 75% for **14b**. (p) *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea, $HgCl_2$, Et_3N, DMF, 0−25 °C, 10−16 h; 78% for **12a** and 58% for **12b**. (q) TFA, CH_2Cl_2, 0 °C, 1 h; 88% for **13a**.

pentyloxy group at the C-3 hydroxyl position in comparison with **2** and **3**, showed inferior NAI activity.

Consistent with our expectation, phosphonate **3** is a potent NA inhibitor and antiflu agent against influenza H1N1 virus with K_i and EC_{50} values of 0.15 and 4.67 nM (Table 2). In comparison, phosphonate **3** is more active than oseltamivir by 19- and 7-folds, respectively, in the NA inhibition and antiflu assays. The phosphonate **3** was further evaluated at multiple concentrations to determine the growth inhibition on the host MDCK cells. The deduced CC_{50} value of phosphonate **3** was 74 μM. The phosphonate **3**, showing a high selectivity index of greater than 15800, is thus a potent antiviral agent against H1N1 virus with no toxicity to the host MDCK cells. By replacing the amino group in **3** with a guanidino group, the phosphonate **13b** exhibits an enhanced NA inhibition (K_i = 0.06 nM) and antiflu activity (EC_{50} = 0.09 nM). By analogy to the previous reports,[3] the guanidinium group may exert strong electrostatic interactions with the residues of Glu119, Asp151, and Glu227.

Before a safe and effective vaccine is available to protect the possible pandemic avian flu, neuraminidase inhibitors are the only therapy we have. The recent reports on the drug resistant avian flu infections and the side effects in children receiving Tamiflu treatments suggest that new chemical identities for neuraminidase inhibitors are needed for our battle against the threat of the pandemic flu. The phosphonate congeners described in this study are significantly more potent than the carboxylate congeners against the wild-type neuraminidases of H1N1 and H5N1. In addition, compound **13b** is an effective inhibitor at 19 nM for the H274Y mutant of a H5N1 neuraminidase. Because the high polarity of phosphonate and guanidinium groups may cause a problem of orally bioavailability, further investigation of formulation and use of prodrugs, for example, acyloxymethyl- and arylphosphonate esters,[9] may eventually solve the problem in the drug development.

Supporting Information Available: Complete ref 3a and ref 7, experimental section, computer modeling of neuraminidase inhibition, ^1H, ^{13}C, and ^{31}P NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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