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A Practical Synthesis of Zanamivir Phosphonate Congeners with Potent Anti-influenza Activity

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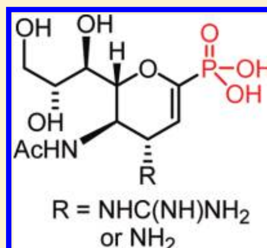
Supporting Information

ABSTRACT: Two phosphonate compounds **1a** (4-amino-1-phosphono-DANA) and **1b** (phosphono-zanamivir) are synthesized and shown more potent than zanamivir against the neuraminidases of avian and human influenza viruses, including the oseltamivir-resistant strains. For the first time, the practical synthesis of these phosphonate compounds is realized by conversion of sialic acid to peracetylated phosphono-DANA diethyl ester (**5**) as a key intermediate in three steps by a novel approach. In comparison with zanamivir, the high affinity of **1a** and **1b** can be partly attributable to the strong electrostatic interactions of their phosphonate groups with the three arginine residues (Arg118, Arg292, and Arg371) in the active site of neuraminidases. These phosphonates are nontoxic to the human 293T cells; they protect cells from influenza virus infection with EC_{50} values in low-nanomolar range, including the wild-type WSN (H1N1), the 2009 pandemic (H1N1), the oseltamivir-resistant H274Y (H1N1), RG14 (H5N1), and Udorn (H3N2) influenza strains.



These phosphonate compounds, prepared from sialic acid in >10% yields, are more potent than zanamivir against the neuraminidases of avian and human influenza viruses, including the oseltamivir-resistant strains.

■ INTRODUCTION

Outbreaks of influenza continue to cause widespread morbidity and mortality worldwide. The most effective therapies for the treatment of influenza infections involve administration of Relenza (zanamivir, ZA in Figure 1) and Tamiflu,[1] whereas vaccines are often utilized for prevention of influenza virus infections. Tamiflu is a prodrug that is readily hydrolyzed by hepatic esterases to give the corresponding oseltamivir carboxylic acid (OC) as the active inhibitor to interact with three arginine residues (Arg118, Arg292, and Arg371) in the active site of viral neuraminidase (NA).[2] Both ZA and OC are designed to have (oxa)cyclohexene scaffolds to mimic the intermediate of oxonium-like geometry in the enzymatic cleavage of *N*-acetylneuraminic acid (Neu5Ac),[1,2] the outmost saccharide on the cell surface glycoprotein for binding with the active site of viral NA.

The phosphonate group is generally used as a bioisostere of carboxylate in drug design.[3] In comparison with carboxylic acid, the phosphonic acid has higher acidity and stronger electrostatic interactions with the guanidine group.[3a,4] Indeed, we have demonstrated that the oseltamivir phosphonate congeners,[5] such as tamiphosphor and guanidino-tamiphosphor (Figure 1),[5a] are more potent than OC to inhibit the NAs of H1N1 and H5N1 viruses, including the clinically relevant OC resistant H274Y mutant. The enhanced affinity of tamiphosphor and guanidino-tamiphosphor is attributable to the strong electrostatic interactions of the phosphonate group with the three arginine residues (Arg118, Arg292 and Arg371) in NA. Tamiphosphor and guanidino-tamiphosphor are also more effective than oseltamivir in protecting mice against lethal challenge with H1N1 human influenza virus.[5c] As a continuing study, we report herein the synthesis of zanamivir phosphonate congeners and their remarkable inhibitory activities against influenza viruses.

■ RESULTS AND DISCUSSION

Chemical Synthesis. The synthesis of 4-amino-1-phosphono-DANA (**1a**) and phosphono-zanamivir (**1b**) has been pursued previously but remains an unsolved problem. In an approach, the enzymatic aldol reaction of 2-*N*-acetylmannosamine (ManNAc) with pyruvate or phosphoenolpyruvate (PEP) has been carried out to give Neu5Ac (sialic acid).[6a] However, acetylphosphonate is not a substrate of Neu5Ac aldolase and fails to act as a PEP surrogate to react with ManNAc to provide the phosphonate congener of Neu5Ac.[6b] Alternatively, both Chan and Whitesides' teams have independently synthesized the dimethyl ester of Neu5Ac phosphonate congener using an indium-mediated addition reaction of dimethyl (3-bromopropen-2-yl)phosphonate to ManNAc, followed by ozonolysis of the double bond to reveal the keto group.[7a−c] Unfortunately, the Neu5Ac phosphonate is unstable under either slightly acidic or basic conditions, giving a lactone product by elimination of the phosphonate group.[7b,8] For

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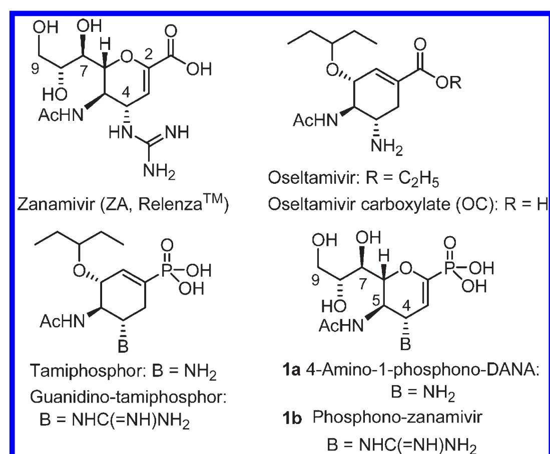


Figure 1. Compounds possessing inhibitory activities against the neuraminidases of influenza viruses.

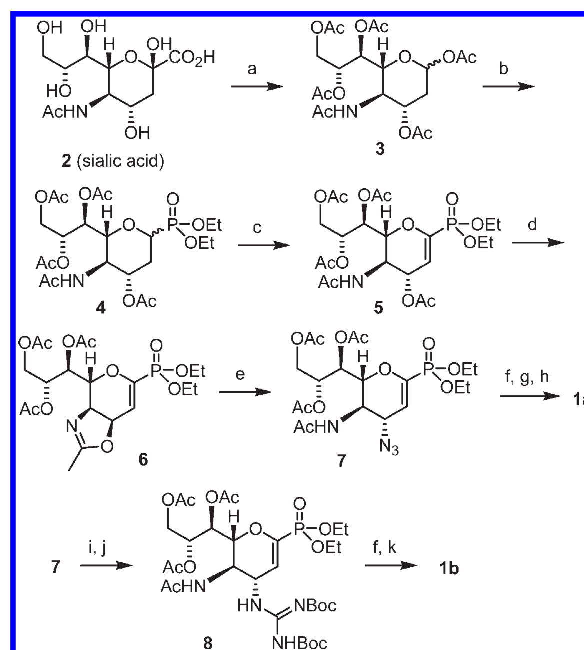
this reason, Neu5Ac phosphonate may not be an ideal intermediate for elaboration to the desired phosphonates **1a** and **1b**.

In another approach, Vasella and co-workers have produced a phosphonate congener of Neu5Ac2en (DANA).[9] Starting from sialic acid, their synthetic method requires a lengthy procedure (20 steps) and many chromatographic separations to give a low yield (<1%) of phosphono-DANA.

In this study, we devised a concise and efficient route to prepare peracetylated phosphono-DANA diethyl ester (**5**) as a key intermediate leading to the novel zanamivir congeners **1a** and **1b** (Scheme 1). Based on the method recently described by Gervay-Hague and co-workers,[10] sialic acid (**2**) was treated with acetic anhydride in pyridine to afford the peracetylation product, which was heated at 100 °C to induce decarboxylation to furnish compound **3**. Using trimethylsilyl diethyl phosphite as an appropriate nucleophile, the substitution reaction of **3** was carried out by the promotion of trimethylsilyl trifluoromethylsulfonate (TMSOTf) to give the phosphonate compound **4** as a mixture of α and β anomers (2:3). These anomers were separated by chromatography and characterized by NMR spectral analyses. For synthetic purpose, the anomeric mixture **4** without further separation was treated with *N*-bromosuccinimide (NBS) under photochemical conditions to give an α-bromination compound, which was subsequently treated with pyridine to give the conjugated phosphonate **5**. The radical bromination was effectively conducted in CH_2Cl_2 solution, presumably facilitated by the synergistic captodative effect[11] of the alkoxy and phosphonate groups. In comparison with Vasella's method,[9] the preparation of phosphono-DANA **5** is much simplified to three steps, and only one chromatographic purification is required to give 25% overall yield (from sialic acid) by our present method.

Compound **5** was converted into the oxazoline **6** in the presence of acetic anhydride, acetic acid, and concentrated H_2SO_4. The regio- and stereoselective ring-opening reaction of oxazoline **6** with azidotrimethylsilane was carried out to afford the azido compound **7** as the pivotal intermediate leading to the target phosphonates **1a** and **1b**. Thus, phosphonate diester **7** was treated with bromotrimethylsilane to remove both ethyl groups, followed by saponification and azide reduction, to afford 4-amino-1- phosphono-DANA (**1a**). To introduce a guanidino substituent, the azido group in compound **7** was first reduced to amine and then reacted with 1,3-bis(*tert*-butoxycarbonyl)-2-methylthiopseudourea in the presence of $HgCl_2$ and Et_3N to

Scheme 1. Synthesis of Zanamivir Phosphonate Congeners 1a and 1b[a]



[a] Reagents and conditions: (a) Ac_2O, py, RT, 12 h, then 100 °C, 5 h, 50%; (b) $P(OEt)_2OTMS$, TMSOTf, 0 °C to RT, 24 h, 62%; (c) NBS, CH_2Cl_2, $h\nu$, 6 h, then pyridine, 50 °C, 2 h, 75%; (d) concd H_2SO_4, Ac_2O, AcOH, RT, 48 h, 80%; (e) $TMSN_3$, *t*-BuOH, 80 °C, 24 h, 87%; (f) TMSBr, CH_2Cl_2, 0 °C, 24 h; (g) MeONa, MeOH, RT, 1 h; (h) H_2, Lindlar catalyst, MeOH, RT, 3 h, 71% (3 steps); (i) H_2, Lindlar catalyst, EtOH, RT, 5 h; (j) MeS−C(=NBoc)NHBoc, $HgCl_2$, Et_3N, CH_2Cl_2, RT, 12 h, 83% (2 steps); (k) MeONa, MeOH, RT, 1 h, 76% (2 steps); py = pyridine; TMS = trimethylsilyl; OTf = trifluorosulfonate; NBS = *N*-bromosucccinimide; and Boc = *tert*-butoxycarbonyl.

give compound **8**. Thus, phosphono-zanamivir (**1b**) was obtained from phosphonate **8** in an one-pot operation comprising three consecutive reactions: solvolysis of the phosphonate ester with bromotrimethylsilane, removal of the *tert*-butoxycarbonyl (Boc) groups on workup with methanol (presumably rendered by the in situ generated acid), and deacetylation with sodium methoxide. For the first time, 4-amino-1-phosphono-DANA (**1a**) and phosphono-zanamivir (**1b**) were prepared in reasonable quantities for evaluation of their anti-influenza activities.

Enzyme and Cell-Based Assays. The fluorescence assays for **1a** and **1b** were measured against the neuraminidase of several influenza strains using 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MUNANA) as the substrate and the influenza associated neuraminidases as the enzyme sources. Table 1 shows the IC_{50} values of compounds at concentrations for 50% inhibition of NA activities and the relative NA inhibitory potencies of these compounds. Though the phosphonate analogs of sialic acid are weak sialidase inhibitors with IC_{50} values in the submillimolar range,[7c,9] the phosphonates **1a** and **1b** mimicking the intermediate of oxonium-like geometry in the enzyme-catalyzed hydrolysis are very effective NA inhibitors with IC_{50} values in the nanomolar range. Both **1a** and **1b** are more potent than zanamivir against all five neuraminidases. These two phosphonate compounds are also significantly more active in inhibiting the oseltamivir-resistant neuraminidase mutant from WSN 274Y that has a tyrosine

Table 1. Neuraminidase Inhibition, Anti-influenza, and Cytotoxicity

bioassay[a]	measurement	1a	1b	zanamivir	oseltamivir acid
WSN (H1N1)	IC_{50} (nM)[b]	0.6 ± 0.1	1.0 ± 0.6	5.3 ± 2.9	3.1 ± 1.6
	EC_{50} (nM)[c]	1.6 ± 0.4	2.2 ± 1.0	22 ± 10	14 ± 4.0
WSN 274Y (H1N1)	IC_{50} (nM)[b]	0.5 ± 0.0	0.3 ± 0.2	2.0 ± 0.9	559 ± 90
	EC_{50} (nM)[c]	32 ± 7.1	24 ± 9.0	361 ± 231	28 000 ± 14 000
pandemic (H1N1)	IC_{50} (nM)[b]	0.8 ± 0.2	0.7 ± 0.1	3.7 ± 0.9	1.5 ± 0.4
	EC_{50} (nM)[c]	25 ± 7.1	65 ± 53	314 ± 66	120 ± 62
RG14 (H5N1)	IC_{50} (nM)[b]	0.8 ± 0.4	0.6 ± 0.4	3.5 ± 0.7	0.6 ± 0.0
	EC_{50} (nM)[c]	990 ± 20	2000 ± 800	15 000 ± 4600	1000 ± 670
Udorn (H3N2)	IC_{50} (nM)[b]	5.2 ± 1.8	4.4 ± 1.1	32 ± 8.2	3.2 ± 0.0
	EC_{50} (nM)[c]	33 ± 21	28 ± 25	36 ± 16	2.6 ± 1.8
293T cell	CC_{50} (nM)[d]	>30 000	>50 000	>100 000	>100 000

[a] Influenza viruses A/WSN/1933 (H1N1), H274Y neuraminidase mutant from A/WSN/1933 (H1N1), A/California/7/2009 (pandemic H1N1), A/Vietnam/1194/2004 RG14 (H5N1), and A/Udorn/307/1972 (H3N2) were used as bioassay materials for neuraminidase inhibition and anti-influenza assays. [b] A fluorescent substrate, 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MUNANA), was used to determine the IC_{50} values that are compound concentrations causing 50% inhibition of different influenza neuraminidase enzymes. [c] The anti-influenza activities against different influenza strains were measured as EC_{50} values that are the compound concentrations for 50% protection of the cytopathic effects due to the infection by different influenza strains. [d] The highest concentration used without noticeable toxic effects in the assay of cytotoxicity on 293T cells.

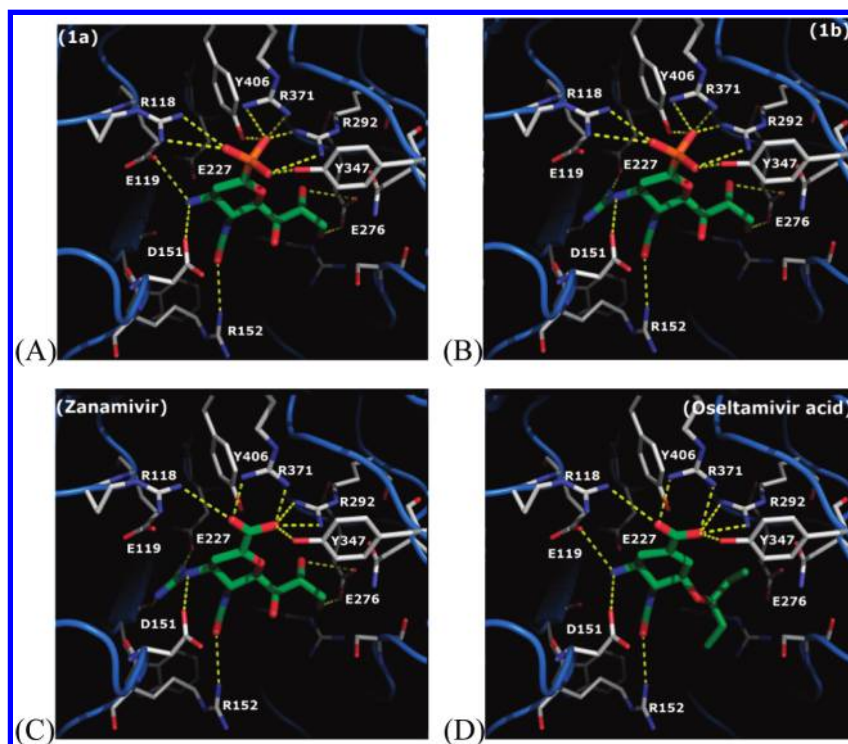


Figure 2. Molecular models of phosphonates **1a** (A) and **1b** (B), compared with zanamivir (C) and oseltamivir acid (D), in the active site of influenza virus neuraminidase (N1 subtype). The complex of the phosphonate compound has more extensive hydrogen-bonding interactions with key residues in the neuraminidase active site.

residue replacing the parental histidine at the 274 position of the neuraminidase.

The anti-influenza activities of these compounds were measured against five influenza strains for abilities to protect the influenza infection-mediated cytopathic effects. The anti-influenza activities were determined as EC_{50} values that are the concentrations for 50% protection of the infection-mediated cytopathic effects. Table 1 shows that **1a** and **1b** have greater anti-influenza activities against the H1N1 influenza viruses, such as the WSN and the 2009 pandemic H1N1 strains. Their anti-influenza activities are particularly noticeable against the oseltamivir-resistant WSN (H1N1) virus, which carries 274Y mutation in NA. The superior anti-influenza activities of **1a** and **1b** for this mutant neuraminidase may impact our option for treating this prevailing influenza strain. Phosphonates **1a** and **1b** are also comparable to zanamivir and oseltamivir as anti-influenza agents against the RG14 (H5N1) and the Udorn (H3N2) influenza strains. These potent anti-influenza agents **1a** and **1b**

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are nontoxic to the human 293T cells at the highest testing concentrations (>30 μM).

Molecular Modeling. Using the known N1 crystal structure (PDB code: 2HU4),[12] our molecular docking experiments reveal that phosphonates **1a** and **1b** bind strongly with the three arginine residues (Arg118, Arg292, and Arg371) of NA (Figure 2). The C_4-amino (or guanidino), C_5-acetamido, and C_7−C_9 glyceryl moieties of **1a** (or **1b**) also exhibit substantial interactions with NA. The phosphonate complex has more extensive hydrogen-bonding interactions with key residues in the NA active site than the corresponding carboxylate (4-amino-DANA or ZA). The high affinity of **1a** and **1b** can be partly attributable to the strong electrostatic interactions of their phosphonate groups with the triarginine residues in the NA active site.

CONCLUSION

Our study provides a practical synthesis of novel phosphonate compounds **1a** and **1b**, which are found to possess potent activity against human and avian influenza viruses and the emerging H274Y mutant strain. There are methods to improve the pharmacokinetic and pharmacodynamic properties of phosphonate drugs.[4c] For example, the phosphonate group in **1a** and **1b** can be elaborated to phosphonate monoester that still retains a negative charge to act as the carboxylate isostere under physiological conditions.[4f,5c] This strategy has been shown in our previous study by employing the monoester derivatives of tamiphosphor and guanidino-tamiphosphor as effective drugs in treating mice having influenza.[5c]

It is noted that 4-amino-1-phosphono-DANA (**1a**) bearing a C_4-amino substituent still exhibits the NA inhibition and anti-influenza activities comparable to phosphono-zanamivir (**1b**) having a C_4-guanidino group, even though 4-amino-DANA has lower NA inhibitory activity than zanamivir by 2−3 orders of magnitude.[13] In view of the low oral bioavailability of zanamivir, caused partly by high polarity of its guanidinium group, compound **1a** having a less basic amino group in lieu of the guanidino group may increase the lipophilicity to benefit bioavailability for the drug development.

EXPERIMENTAL SECTION

General. All the reagents were commercially available and used without further purification unless indicated otherwise. All solvents were anhydrous grade unless indicated otherwise. Influenza A/WSN/1933 (H1N1) virus was obtained from Dr. Shin-Ru Shih at Chang Gung University in Taiwan. The recombinant H5N1 virus NIBRG14 (A/Vietnam/1194/2004), pandemic H1N1 (A/California/07/2009), and H3N2 (A/Udorn/1972) were obtained from Dr. Jia-Tsrong Jan (The Genomics Research Center, Academia Sinica). WSN 274Y was selected with Tamiflu from Influenza A/WSN/1933 (H1N1) in our lab. All viruses were cultured in the allantoic cavities of 10 day old embryonated chicken eggs for 72 h and purified by sucrose gradient centrifugation. Madin-Darby canine kidney (MDCK) cells were obtained from American Type Culture Collection (Manassas, Va) and were grown in Dulbecco's modified Eagle's medium (DMEM, GibcoBRL) containing 10% fetal bovine serum (GibcoBRL) and penicillin-streptomycin (GibcoBRL) at 37 °C under 5% CO_2.

All nonaqueous reactions were carried out in oven-dried glassware under a slight positive pressure of argon unless otherwise noted. Reactions were magnetically stirred and monitored by thin-layer chromatography on silica gel. Flash chromatography was performed on silica gel of 60−200 μm particle size. Yields are reported for spectroscopically pure compounds. Melting points were recorded on an Electrothermal MEL-TEMP 1101D melting point apparatus and are not corrected. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE 600 spectrometer. The ^{31}P NMR spectra were recorded on Bruker AVANCE 500 spectrometer. Chemical shifts are given in δ values relative to tetramethylsilane (TMS); coupling constants *J* are given in Hz. Internal standards were $CDCl_3$ (δ_H = 7.24), MeOH-d_4 (δ_H = 3.31), or D_2O (δ_H = 4.79) for ^{1}H NMR spectra, $CDCl_3$ (δ_c = 77.0) or MeOH-d_4 (δ_c = 49.15) for ^{13}C NMR spectra, and H_3PO_4 in D_2O (δ_P = 0.00) for ^{31}P NMR spectra. The splitting patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), and dd (double of doublets). IR spectra were recorded on a Thermo Nicolet 380 FT-IR spectrometer. Optical rotations were recorded on a Perkin-Elmer Model 341 polarimeter. High-resolution ESI mass spectra were recorded on a Bruker Daltonics spectrometer.

3-Acetamido-4,6-diacetoxy-2-(1,2,3-triacetoxy)propyl-3,4,5,6-tetrahydro-2*H*-pyran (3). Under an atmosphere of nitrogen, a suspension of *N*-acetylneuraminic acid (5 g, 16.2 mmol) in pyridine (75 mL) and acetic anhydride (75 mL) was stirred at room temperature for 12 h and then heated at 100 °C for 5 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residual brownish glassy oil was dissolved in CH_2Cl_2 (150 mL) and washed successively with saturated aqueous $NaHCO_3$ (100 mL), aqueous 1 M HCl (100 mL), and brine (100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The brownish residue was purified by column chromatography on silica gel (EtOAc/hexane, 67:33 to 100:0) to afford **3** as a pale-yellow foam (3.8 g, 50%), which contained inseparable mixture of anomers (α/β = 1:5). The anomeric mixture of **3** was used in the next step without further separation. $C_{20}H_{29}NO_{12}$; TLC (EtOAc) R_f = 0.35; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.26 (0.83 H, d, *J* = 2.5 Hz, H-1β), 5.62 (0.17 H, dd, *J* = 10.3, 2.1 Hz, H-1α), 5.43 (0.17 H, ddd, *J* = 6.1, 4.4, 1.9 Hz), 5.29−5.27 (1.66 H, m), 5.22 (0.83 H, td, *J* = 10.6, 4.9 Hz), 5.17 (0.83 H, td, *J* = 6.5, 2.7 Hz), 5.11−5.07 (0.34 H, m), 5.03 (0.17 H, dd, *J* = 6.5, 2.7 Hz), 4.36 (0.17 H, dd, *J* = 12.5, 2.6 Hz), 4.31 (0.83 H, dd, *J* = 12.5, 2.8 Hz), 4.08−3.98 (2.83 H, m), 3.74 (0.17 H, dd, *J* = 10.5, 2.5 Hz), 2.17−2.15 (0.17 H, m), 2.15−2.13 (0.83 H, m), 2.11 (2.49 H, s), 2.10 (0.51 H, s), 2.09 (0.51 H, s), 2.08 (2.49 H, s), 2.07 (0.51 H, s), 2.04 (2.49 H, s), 2.03 (0.51 H, s), 2.017 (2.49 H, s), 2.013 (0.51 H, s), 2.00 (2.49 H, s), 2.00−1.98 (0.83 H, m), 1.98−1.96 (0.17 H, m), 1.88 (2.49 H, s), 1.87 (0.51 H, s); HRMS calcd for $C_{20}H_{29}NO_{12}Na$: 498.1587, found: *m*/*z* 498.1557 $[M + Na]^+$.

Diethyl [5-Acetamido-4-acetoxy-6-(1,2,3-triacetoxy)propyl-3,4,5,6-tetrahydro-2*H*-pyran-2-yl] Phosphonate (4). The anomeric mixture of **3** (2.15 g, 4.52 mmol) and diethyl trimethylsilyl phosphite (3.11 mL, 13.65 mmol) in anhydrous CH_2Cl_2 (30 mL) was treated with trimethylsilyl trifluoromethylsulfonate (TMSOTf, 1.23 mL, 6.78 mmol) at 0 °C. After 30 min, the mixture was warmed to room temperature and stirred for 24 h. The mixture was poured into ice water (20 mL), and the aqueous layer was extracted with CH_2Cl_2 (20 mL, 2×). The combined extracts were washed successively with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (acetone/EtOAc, 1:9) to afford **4** as a colorless syrup (1.55 g, 62%), which contained a mixture of the α- and β-anomers (2:3). The anomeric mixture of phosphonate **4** was used in the next step without further separation. The analytical samples of pure α- and β-anomers (**4α** and **4β**) were obtained by flash column chromatography on silica gel (EtOAc/acetone, 100:0 to 90:10).

α-Anomer ***4α***. $C_{22}H_{36}NO_{13}P$; colorless foam; TLC (EtOAc/acetone, 9:1) R_f = 0.25; $[\alpha]_D^{20}$ = +39.4 (*c* = 4.6, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.30 (1 H, dd, *J* = 5.7, 1.7 Hz), 5.24 (1 H, d, *J* = 9.9 Hz, NH), 5.18 (1 H, td, *J* = 6.6, 2.5 Hz), 4.98 (1 H, td, *J* = 10.6, 5.0 Hz), 4.40 (1 H, dd, *J* = 12.3, 5.0 Hz), 4.22−4.09 (5 H, m), 3.97 (1 H, q, *J* = 10.1 Hz), 3.74 (1 H, td, *J* = 12.5, 2.4 Hz), 3.62 (1 H, dd, *J* = 10.3, 2.0 Hz), 2.27

(1 H, dd, J = 12.8, 4.9 Hz), 2.09 (3 H, s), 2.05 (3 H, s), 2.02 (3 H, s), 2.01 (3 H, s), 1.98−1.92 (1 H, m), 1.87 (3 H, s), 1.35−1.31 (6 H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9 (C), 170.5 (C), 170.3 (C), 170.2 (C), 170.1 (C), 79.0 (CH, d, $^3J_{c\text{-}p}$ = 17.3 Hz), 71.8 (CH, d, $^1J_{c\text{-}p}$ = 174.6 Hz, C-1), 71.6 (CH, d, $^3J_{c\text{-}p}$ = 20.9 Hz), 71.0 (CH), 67.9 (CH), 63.4 (CH_2, d, $^2J_{c\text{-}p}$ = 6.9 Hz, $POCH_2$), 62.8 (CH_2, d, $^2J_{c\text{-}p}$ = 6.2 Hz, $POCH_2$), 62.2 (CH_2, C-8), 49.6 (CH, C-4), 31.3 (CH_2, C-2), 23.1 (CH_3), 20.9 (CH_3), 20.8 (CH_3), 20.7 (CH_3, 2 ×), 16.5 (CH_3, d, $^3J_{c\text{-}p}$ = 5.4 Hz, $POCH_2\underline{C}H_3$), 16.3 (CH_3, d, $^3J_{c\text{-}p}$ = 5.4 Hz, $POCH_2\underline{C}H_3$); ^{31}P NMR (202 MHz, $CDCl_3$)δ 18.5; HRMS calcd for $C_{22}H_{35}NO_{13}P$: 552.1846, found: m/z 552.1921 [M − H]$^-$.

β-Anomer ***4β***. $C_{22}H_{36}NO_{13}P$; colorless foam; TLC (EtOAc/acetone, 9:1) R_f = 0.28; $[\alpha]_D^{20}$ = −40.1 (c = 3.0, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.45 (1 H, d, J = 10.1 Hz, NH), 5.35 (1 H, dd, J = 7.3, 2.3 Hz), 5.32 (1 H, td, J = 15.0, 4.8 Hz), 5.21−5.18 (1 H, m), 4.45 (1 H, d, J = 10.0 Hz), 4.33 (1 H, dd, J = 12.4, 2.8 Hz), 4.30 (1 H, dd, J = 12.3, 7.1 Hz), 4.19−4.13 (2 H, m), 4.12−4.04 (4 H, m), 2.35−2.31 (1 H, m), 2.11 (3 H, s), 2.08 (3 H, s), 2.017 (3 H, s), 2.011 (3 H, s), 2.09−2.03 (1 H, m), 1.88 (3 H, s), 1.34 (3 H, t, J = 7.0 Hz), 1.33 (3 H, t, J = 7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8 (C), 170.6 (C), 170.2 (C), 170.1 (C), 169.8 (C), 74.0 (CH), 69.7 (CH), 69.5 (CH), 67.9 (CH, d, $^1J_{c\text{-}p}$ = 157.2 Hz, C-1), 67.7 (CH), 63.0 (CH_2, d, $^2J_{c\text{-}p}$ = 7.2 Hz, $POCH_2$), 62.7 (CH_2, d, $^2J_{c\text{-}p}$ = 6.6 Hz, $POCH_2$), 62.0 (CH_2, C-8), 49.0 (CH, C-4), 29.5 (CH_2, d, $^2J_{c\text{-}p}$ = 3.2 Hz, C-2), 23.1 (CH_3), 21.0 (CH_3), 20.9 (CH_3), 20.7 (CH_3, 2 ×), 16.2 (CH_3, d, $^3J_{c\text{-}p}$ = 5.1 Hz, $POCH_2\underline{C}H_3$), 16.3 (CH_3, d, $^3J_{c\text{-}p}$ = 5.1 Hz, $POCH_2\underline{C}H_3$); ^{31}P NMR (202 MHz, $CDCl_3$) δ 21.4; HRMS calcd for $C_{22}H_{35}NO_{13}P$: 552.1846, found: m/z 552.1879 [M − H]$^-$.

Diethyl [5-Acetamido-4-acetoxy-6-(1,2,3-triacetoxy)prozpyl-4,5,6-trihydropyran-2-yl] Phosphonate (5). The anomeric mixture of phosphonate **4** (1.1 g, 2 mmol) and N-bromosuccinimide (885 mg, 5 mmol) in anhydrous CH_2Cl_2 (20 mL) was heated to reflux under irradiation from a 100 W tungsten lamp. The progress of reaction was monitored by TLC. Upon completion (∼6 h) the mixture was cooled to room temperature, and the precipitate succinimide was filtered off. The filtrate was evaporated under reduced pressure to give a crude 2-bromo derivative as yellow syrup, which was used in the next step without further purification.

A solution of the above-prepared bromine compound in anhydrous pyridine (10 mL) was stirred at 50 °C for 2 h. The solution was concentrated under reduced pressure, and the brown residue was purified by column chromatography on silica gel (EtOAc/acetone, 100:0 to 90:10) to afford conjugated phosphonate **5** as colorless foam (827 mg, 75% for two steps). $C_{22}H_{34}NO_{13}P$; TLC (EtOAc/acetone, 9:1) R_f = 0.28; $[\alpha]_D^{20}$ = +43.8 (c = 0.59, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.74 (1 H, dd, J = 10.7, 2.2 Hz), 5.54 (1 H, d, J = 8.2 Hz, NH), 5.42−5.40 (2 H, m), 5.26 (1 H, td, J = 6.4, 2.9 Hz), 4.39−4.34 (2 H, m), 4.29 (1 H, q, J = 9.1 Hz), 4.17−4.09 (5 H, m), 2.09 (3 H, s), 2.05 (3 H, s), 2.04 (3 H, s), 2.02 (3 H, s), 1.91 (3 H, s), 1.35 (3 H, t, J = 7.0 Hz), 1.31 (3 H, t, J = 7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8 (C), 170.4 (C), 170.3 (C), 169.8 (C), 169.7 (C), 147.8 (C, d, $^1J_{c\text{-}p}$ = 225 Hz, C-1), 113.0 (CH, d, $^2J_{c\text{-}p}$ = 22.8 Hz, C-2), 76.5 (CH, d, $^3J_{c\text{-}p}$ = 9.3 Hz), 69.9 (CH), 68.4 (CH, d, $^3J_{c\text{-}p}$ = 15.2 Hz), 67.2 (CH), 63.2 (CH_2, d, $^2J_{c\text{-}p}$ = 5.4 Hz, $POCH_2$), 63.0 (CH_2, d, $^2J_{c\text{-}p}$ = 5.7 Hz, $POCH_2$), 61.8 (CH_2, C-8), 46.4 (CH, C-4), 23.0 (CH_3), 20.78 (CH_3), 20.73 (CH_3), 20.63 (CH_3), 20.60 (CH_3), 16.16 (CH_3, d, $^3J_{c\text{-}p}$ = 4.8 Hz, $POCH_2CH_3$), 16.12 (CH_3, d, $^3J_{c\text{-}p}$ = 4.8 Hz, $POCH_2\underline{C}H_3$); ^{31}P NMR (202 MHz, $CDCl_3$) δ 6.4; HRMS calcd for $C_{22}H_{33}NO_{13}P$: 550.1690, found: m/z 550.1684 [M − H]$^-$.

Diethyl [4-(1,2,3-Triacetoxy)propyl-2-methyl-3a,7a-dihydro-*4H*-pyrano[3,4-*d*]oxazol-6-yl] Phosphonate (6). To a solution of phosphonate **5** (550 mg, 1 mmol) in a mixture of acetic acid (2 mL) and acetic anhydride (2 mL) was treated with concd H_2SO_4 (0.2 mL). The mixture was stirred for 48 h at room temperature, poured into cold (0 °C) saturated aqueous $NaHCO_3$ (pH 9), and stirred for 30 min before extraction with EtOAc (30 mL, 5×). The combined extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residual oil was purified by column chromatography on silica gel (acetone/EtOAc, 1:9) to afford **6** as pale-yellow syrup (394 mg, 80% for two steps). $C_{20}H_{30}NO_{11}P$; TLC (EtOAc/acetone, 9:1) R_f = 0.30; $[\alpha]_D^{20}$ = −11.6 (c = 0.50, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.20 (1 H, dd, J = 10.3, 4.0 Hz), 5.58 (1 H, ddd, J = 6.6, 2.9, 1.1 Hz), 5.38 (1 H, td, J = 7.7, 2.5 Hz), 4.71 (1 H, ddd, J = 8.6, 4.0, 2.0 Hz), 4.40 (1 H, dd, J = 12.4, 2.5 Hz), 4.19 (1 H, dd, J = 12.5, 5.9 Hz), 4.18−4.07 (4 H, m), 3.93 (1 H, td, J = 9.2, 0.6 Hz), 3.34 (1 H, dd, J = 10.1, 2.7 Hz), 2.11 (3 H, s), 2.04 (3 H, s), 2.03 (3 H, s), 1.98 (3 H, s), 1.34 (3 H, t, J = 7.0 Hz), 1.32 (3 H, t, J = 7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5 (C), 169.7 (C), 169.4 (C), 167.2 (C, N = CCH_3), 150.1 (C, d, $^1J_{c\text{-}p}$ = 225 Hz, C-1), 111.9 (CH, d, $^2J_{c\text{-}p}$ = 23.4 Hz, C-2), 76.1 (CH, d, $^3J_{c\text{-}p}$ = 6.3 Hz), 71.2 (CH, d, $^3J_{c\text{-}p}$ = 15.3 Hz), 69.6 (CH), 68.8 (CH), 63.1 (CH_2, d, $^2J_{c\text{-}p}$ = 5.9 Hz, $POCH_2$), 62.9 (CH_2, d, $^2J_{c\text{-}p}$ = 5.7 Hz, $POCH_2$), 61.8 (CH, C-4), 61.6 (CH_2, C-8), 20.7 (CH_3), 20.6 (CH_3), 20.5 (CH_3), 16.2 (CH_3, d, $^3J_{c\text{-}p}$ = 5.1 Hz, $POCH_2CH_3$), 16.1 (CH_3, d, $^3J_{c\text{-}p}$ = 5.1 Hz, $POCH_2CH_3$), 14.0 (CH_3, N=CCH_3); ^{31}P NMR (202 MHz, $CDCl_3$) δ 6.4; HRMS calcd for $C_{20}H_{29}NO_{11}P$: 490.1478, found: m/z 490.1374 [M − H]$^-$.

Diethyl [5-Acetamido-4-azido-6-(1,2,3-triacetoxy)propyl-4,5,6-trihydropyran-2-yl] Phosphonate (7). To a solution of oxazoline **6** (393 mg, 0.8 mmol) in t-BuOH (10 mL) was treated with azidotrimethylsilane (0.53 mL, 4 mmol) at 80 °C for 24 h. The solution was poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc (30 mL, 3×). The combined extracts were dried over $MgSO_4$, filtered, and concentrated to afford the azido compound **7** as a colorless syrup (371 mg, 87%), which was practically pure to be used in the next step. An analytical sample was obtained by flash column chromatography on silica gel (10% acetone in EtOAc). $C_{20}H_{31}N_4O_{11}P$; TLC (EtOAc/acetone, 9:1) R_f = 0.30; $[\alpha]_D^{20}$ = +82.7 (c = 0.58, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.75 (1 H, dd, J = 10.3, 2.4 Hz), 5.73 (1 H, d, J = 8.6 Hz), 5.38 (1 H, dt, J = 7.1, 1.5 Hz), 5.26 (1 H, ddd, J = 8.5, 5.8, 2.6 Hz), 4.53−4.50 (2 H, m), 4.36 (1 H, dd, J = 12.5, 2.6 Hz), 4.17−4.08 (5 H, m), 3.67 (1 H, q, J = 9.2 Hz), 2.10 (3 H, s), 2.05 (3 H, s), 2.02 (3 H, s), 1.99 (3 H, s), 1.34 (3 H, t, J = 7.1 Hz), 1.32 (3 H, t, J = 7.1 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8 (C), 170.5 (C), 170.1 (C), 169.7 (C), 147.7 (C, d, $^1J_{c\text{-}p}$ = 224 Hz, C-1), 112.4 (CH, d, $^2J_{c\text{-}p}$ = 22.9 Hz, C-2), 75.9 (CH, d, $^3J_{c\text{-}p}$ = 9.2 Hz), 69.7 (CH), 67.3 (CH), 63.5 (CH_2, d, $^2J_{c\text{-}p}$ = 5.7 Hz, $POCH_2$), 63.3 (CH_2, d, $^2J_{c\text{-}p}$ = 5.9 Hz, $POCH_2$), 61.9 (CH_2, C-8), 57.8 (CH, d, $^3J_{c\text{-}p}$ = 14.7 Hz), 48.5 (CH, C-4), 23.2 (CH_3), 20.8 (CH_3), 20.77 (CH_3), 20.71 (CH_3), 16.27 (CH_3, d, $^3J_{c\text{-}p}$ = 5.7 Hz, $POCH_2CH_3$), 16.23 (CH_3, d, $^3J_{c\text{-}p}$ = 5.7 Hz, $POCH_2\underline{C}H_3$); ^{31}P NMR (202 MHz, $CDCl_3$) δ 6.2; HRMS calcd for $C_{20}H_{30}N_4O_{11}P$: 533.1649, found: m/z 533.1540 [M − H]$^-$.

Diethyl {5-Acetamido-4-[*N*^{2},*N*^{3}-bis(*tert*-butoxycarbonyl)]guanidino-6-(1,2,3-triacetoxy)propyl-4,5,6-trihydropyran-2-yl} Phosphonate (8). A solution of azide **7** (350 mg, 0.71 mmol) in ethanol (25 mL) was hydrogenated with Lindlar catalyst (30 mg) under an atmosphere of hydrogen. The mixture was stirred for 5 h at room temperature, filtered through a pad of Celite, and washed with ethanol. The filtrate was concentrated under reduced pressure to give colorless foam (278 mg). The crude amine product was dissolved in anhydrous CH_2Cl_2 (30 mL) and treated with 1,3-bis($tert$-butoxycarbonyl)-2-methylthiopseudourea (247 mg, 0.85 mmol) and Et_3N (230 μL, 1.7 mmol). The mixture was cooled to 0 °C, and $HgCl_2$ (231 mg, 0.85 mmol) was added slowly. The suspension was warmed to room temperature and stirred for 12 h, after which the mixture was diluted with EtOAc and filtered through a pad of Celite. The filtrate was concentrated and purified by flash column chromatography (EtOAc) to afford guanidine **8** (442 mg, 83% yield) as a colorless foam. TLC (EtOAc) R_f = 0.45; $[\alpha]_D^{20}$ = +18.5 (c = 0.88, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.32 (1 H, s), 8.48 (1 H, d, J = 8.5 Hz), 6.12 (1 H, d, J = 8.5 Hz), 5.71 (1 H, dd,

J = 10.3, 2.0 Hz), 5.35 (1 H, d, J = 6.6 Hz), 5.23 (1 H, td, J = 6.5, 2.7 Hz), 5.10−5.06 (1 H, m), 4.37 (1 H, dd, J = 12.5, 2.8 Hz), 4.25−4.20 (2 H, m), 4.19−4.12 (2 H, m), 4.12−4.05 (3 H, m), 2.09 (3 H, s), 2.06 (3 H, s), 2.02 (3 H, s), 1.85 (3 H, s), 1.46 (9 H, s), 1.45 (9 H, s), 1.36 (3 H, t, J = 7.1 Hz), 1.31 (3 H, t, J = 7.1 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.0 (C), 170.5 (C), 170.1 (C), 169.8 (C), 162.7 (C), 157.2 (C), 152.6 (C), 147.4 (C, d, $^1J_{c\text{-}p}$ = 224 Hz, C-1), 114.2 (CH, d, $^2J_{c\text{-}p}$ = 23.3 Hz, C-2), 83.9 (C), 79.8 (C), 77.9 (CH, d, $^3J_{c\text{-}p}$ = 9.3 Hz), 70.1 (CH), 67.4 (CH), 63.4 (CH_2, d, $^2J_{c\text{-}p}$ = 5.7 Hz, $POCH_2$), 63.0 (CH_2, d, $^2J_{c\text{-}p}$ = 5.7 Hz, $POCH_2$), 62.1 (CH_2, C-8), 49.0 (CH, d, $^3J_{c\text{-}p}$ = 15.2 Hz), 48.1 (CH, C-4), 28.2 (CH_3, 3×), 28.0 (CH_3, 3×), 23.1 (CH_3), 20.9 (CH_3), 20.8 (CH_3), 20.7 (CH_3), 16.29 (CH_3, $POCH_2CH_3$), 16.25 (CH_3, $POCH_2CH_3$); ^{31}P NMR (202 MHz, $CDCl_3$) δ 6.4; HRMS calcd for $C_{31}H_{50}N_4O_{15}P$: 749.3010, found: m/z 749.3172 $[M - H]^-$.

[5-Acetamido-4-amino-6-(1,2,3-hydroxy)propyl-4,5,6-trihydropyran-2-yl] Phosphonic acid (1a). A solution of diethyl phosphonate **7** (80 mg, 0.15 mmol) in anhydrous CH_2Cl_2 (4 mL) at 0 °C was treated with bromotrimethylsilane (0.12 mL, 0.87 mmol). After stirring for 24 h at 0 °C, MeOH (2 mL) was added, and the mixture was concentrated under reduced pressure. The residue was dissolved in anhydrous MeOH (5 mL) and treated with sodium methoxide (5.4 M solution in MeOH, 0.9 mL, 4.86 mmol). After stirring for 1 h at room temperature, the mixture was filtered through Dowex 50W × 8 (H^+ form) and then concentrated under reduced pressure. The residue was dissolved in MeOH (5 mL) and subjected to hydrogenation (1 atm) in the presence of Lindlar catalyst (20 mg) at room temperature. After 3 h, the mixture was filtered through a pad of Celite and rinsed with MeOH. The filtrate was concentrated, and the residual solids were washed with Et_2O (3 × 10 mL) to afford the phosphonate **1a** (35 mg, 71%). $C_{10}H_{19}N_2O_8P$; solid, mp 108−110 °C (dec.); $[\alpha]_D^{20}$ = −73.6 (c = 0.70, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.43 (1 H, dd, J = 9.5, 2.0 Hz), 4.32−4.28 (2 H, m), 4.12 (1 H, dt, J = 9.3, 2.8 Hz), 3.85 (1 H, ddd, J = 9.1, 6.2, 2.5 Hz), 3.81 (1 H, dd, J = 11.8, 2.7 Hz), 3.62 (1 H, br d, J = 9.5 Hz), 3.59 (1 H, dd, J = 11.9, 6.2 Hz), 2.01 (3 H, s); ^{13}C NMR (150 MHz, D_2O) δ 174.7 (C), 153.7 (C, d, $^1J_{c\text{-}p}$ = 211.3 Hz, C-1), 103.3 (CH, d, $^2J_{c\text{-}p}$ = 22.8 Hz, C-2), 75.3 (CH, d, $^3J_{c\text{-}p}$ = 8.1 Hz, C-5), 69.6 (CH), 67.6 (CH), 62.9 (CH_2, C-8), 49.8 (CH, d, $^3J_{c\text{-}p}$ = 13.5 Hz, C-3), 45.8 (CH, C-4), 22.1 (CH_3); ^{31}P NMR (202 MHz, D_2O) δ 2.5. HRMS calcd for $C_{10}H_{18}N_2O_8P$: 325.0801, found: m/z 325.0920 $[M - H]^-$.

[5-Acetamido-4-guanidino-6-(1,2,3-hydroxy)propyl-4,5,6-trihydropyran-2-yl] Phosphonic acid (1b). A solution of diethyl phosphonate **8** (130 mg, 0.17 mmol) in anhydrous CH_2Cl_2 (4 mL) was treated with bromotrimethylsilane (0.13 mL, 0.94 mmol) at 0 °C, and the reaction mixture was stirred for 24 h at 0 °C. MeOH (2 mL) was added under vigorous stirring. After 30 min, the solution was evaporated under reduced pressure, and the residue as a solution in anhydrous MeOH (5 mL) was treated with a 5.4 M solution of sodium methoxide in methanol (1 mL, 5.4 mmol). After stirring for 1 h at room temperature, the solution was filtered through Dowex 50W × 8 (H^+ form) and subjected to lyophilization. The residual pale-yellow solid was washed with Et_2O (3 × 20 mL) to afford the phosphonate **1b** (48 mg, 76%). $C_{11}H_{21}N_4O_8P$; white solid, mp 140−142 °C (dec.); $[\alpha]_D^{20}$ = +40.2 (c = 0.50, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.40 (1 H, dd, J = 9.8, 2.0 Hz), 4.38 (1 H, dt, J = 9.4, 2.8 Hz), 4.32 (1 H, br d, J = 10.6 Hz), 4.17 (1 H, br t, J = 10.0 Hz), 3.84 (1 H, ddd, J = 9.2, 6.4, 2.6 Hz), 3.82 (1 H, dd, J = 11.6, 2.6 Hz), 3.62 (1 H, br d, J = 9.3 Hz), 3.58 (1 H, dd, J = 11.7, 6.1 Hz), 1.97 (3 H, s); ^{13}C NMR (150 MHz, D_2O) δ 174.3 (C), 156.9 (C), 151.6 (C, d, $^1J_{c\text{-}p}$ = 213.1 Hz, C-1), 107.4 (CH, d, $^2J_{c\text{-}p}$ = 22.4 Hz, C-2), 75.8 (CH, d, $^3J_{c\text{-}p}$ = 9.0 Hz, C-5), 69.7 (CH), 67.8 (CH), 62.9 (CH_2, C-8), 50.8 (CH, d, $^3J_{c\text{-}p}$ = 13.9 Hz, C-3), 47.7 (CH, C-4), 21.9 (CH_3); ^{31}P NMR (202 MHz, D_2O) δ 3.6. HRMS calcd for $C_{11}H_{20}N_4O_8P$: 367.1019, found: m/z 367.1121 $[M - H]^-$.

Determination of Influenza Virus $TCID_{50}$. The $TCID_{50}$ (50% tissue culture infectious dose) was determined by serial dilution of the influenza virus stock solution onto 100 μL MDCK cells at 1 × 10^5 cells/mL in 96-well microplates. The infected cells were incubated at 37 °C under 5.0% CO_2 for 48 h and added to each wells with 100 μL per well of CellTiter 96 AQ_{ueous} non-radioactive cell proliferation assay reagent (Promega). After incubation at 37 °C for 15 min, absorbance at 490 nm was read on a plate reader. Influenza virus $TCID_{50}$ was determined using Reed−Muench method.[14]

Determination of Neuraminidase Activity by a Fluorescent Assay. The neuraminidase activity was measured using diluted allantoic fluid harvested from influenza virus infected embryonated eggs. A fluorometric assay was used to determine the NA activity with the fluorogenic substrate 2′-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid (MUNANA; Sigma). The fluorescence of the released 4-methylumbelliferone was measured in Envision plate reader (Perkin-Elmer, Wellesley, MA) using excitation and emission wavelengths of 365 and 460 nm, respectively. Neuraminidase activity was determined at 200 μM of MUNANA. Enzyme activity was expressed as the fluorescence increase during 15 min incubation at room temperature.

Determination of IC_{50} of Neuraminidase Inhibitor. Neuraminidase inhibition was determined by mixing inhibitor and neuraminidase for 10 min at room temperature, followed by the addition of 200 μM of substrate. Inhibitor IC_{50} value was determined from the dose−response curves by plotting the percent inhibition of NA activity versus inhibitor concentrations using Graph Pad Prism 4.

Determination of EC_{50} of Neuraminidase Inhibitor. The antiflu activities of neuraminidase inhibitors were measured by the EC_{50} values that were the concentrations of NA inhibitor for 50% protection of the influenza virus infection-mediated CPE (cytopathic effects). Fifty μL diluted influenza virus at 100 $TCID_{50}$ was mixed with equal volumes of NA inhibitors at varied concentrations. The mixtures were used to infect 100 μL of MDCK cells at 1 × 10^5 cells/mL in 96-wells. After 48 h incubation at 37 °C under 5.0% CO_2, the cytopathic effects (CPE) were determined with CellTiter 96 AQ_{ueous} non-radioactive cell proliferation assay reagent, as described above. Inhibitor EC_{50} value was determined by fitting the curve of percent CPE versus the concentrations of NA inhibitor using Graph Pad Prism 4.

Computer Modeling. The model of a specific compound in complex with the NA was constructed through docking this compound to the crystallographic structure of N1 neuraminidase (PDB code 2HU4). The 3D structure of compound **1a** and **1b** was built by modifying the 3D structure of zanamivir (PDB code 1NNC) with SYBYL 8.0 program (Tripos Associates). GOLD 4.0.1[15] was used to dock the compound onto the protein with flexible docking option turned on. Kollmann all atom charges[16] were assigned to the protein atoms, and Gasteiger−Hückel charges[17] were assigned to ligand atoms using the SYBYL 8.0 program. During the following docking procedure, the side-chain structure of the Asp151 and Arg156 amino acid residues remained flexible, modeled with the built-in rotamer libraries of the GOLD 4.0.1 package. Initial 1000 independent genetic algorithm cycles of computation were carried out with ligand torsion angles varying between −180 and 180°. The search efficiency was set at 200% to ensure the most exhaustive search for the docking conformational space. All other parameters were kept the same as the default settings. The docking processes were distributed to a 40-processor Linux cluster with Intel(R) Xeon(TM) CPU 3.00 GHz CPUs. The resultant ligand−protein complex structures were ranked with the GOLDSCORE scoring function to determine the top 1000 hits.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Complete ref 2a, ^{1}H, ^{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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