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Synthesis and Anti-Influenza Evaluation of Orally Active Bicyclic Ether Derivatives Related to Zanamivir

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Abstract—We synthesized bicyclic ether sialidase inhibitors such as tetrahydro-furan-2-yl, tetrahydro-pyran-2-yl, and oxepan-2-yl derivatives related to zanamivir. These compounds substituted by diol at the C-3′ and C-4′ positions resulted in the retention of low nanomolar inhibitory activities against not only influenza A virus sialidase but also influenza A virus in cell culture. Compound **11a** in particular showed comparable efficacy in vivo relative to that of oseltamivir phosphate.

Sialidase is one of two glycoproteins expressed on the influenza virus surface and catalyzes the cleavage of sialic acid residues from glycoproteins, glycolipids and oligosaccharides. It has been shown that sialidase is required for release of newly produced virions from infected cells and that it facilitates the movement of the virus through the mucus of the respiratory tract.[1] The catalytic activity of sialidase is essential for influenza virus replication and infectivity. Accordingly, inhibitors of this enzyme are of interest as potential anti-influenza agents. Indeed, the effectiveness of sialidase inhibitors as anti-influenza agents has been demonstrated both in animal models and in human clinical trials by several research groups[2] and highlighted by recent approval for human use of zanamivir[3] and oseltamivir phosphate[4] (Fig. 1). Zanamivir is delivered by inhalation because of its low oral bioavailability whereas oseltamivir phosphate is administered orally. As part of our study of the structure activity relationships of 4-guanidino-7-substituted Neu5Ac2en derivatives, we reported that the appropriate alkyl ether derivatives at the C-7 position of 4-guanidino-Neu5Ac2en had more potent activity against influenza virus replication compared to zanamivir and that the multivalent sialidase inhibitor **3** (Fig. 1) carrying 4-guanidino-Neu5Ac2en analogues via a linker of alkyl ether at the C-7 position was much more effective than zanamivir in the mouse/influenza virus infection model by intranasal administration.[5] However, compound **2** and the polyvalent sialidase inhibitor **3** were less effective than oseltamivir phosphate (data not shown) when delivered systemically to the mouse. There have been no reports of orally active derivatives related to zanamivir. Therefore, in order to develop an orally bioavailable sialidase inhibitor, we designed a newly synthesized sialidase inhibitor possessing cyclic ether moieties such as tetrahydro-furan-2-yl, tetrahydro-pyran-2-yl, and oxepan-2-yl groups in place of the glycerol side chain of zanamivir. We postulated that these compounds would possess modified physicochemical properties which could make them more suitable for systemic delivery.

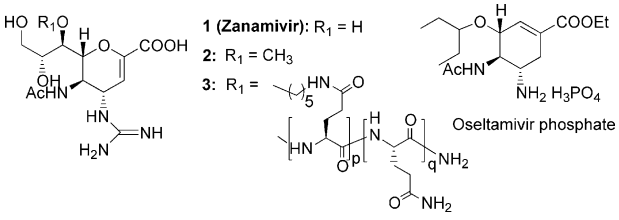

Figure 1.

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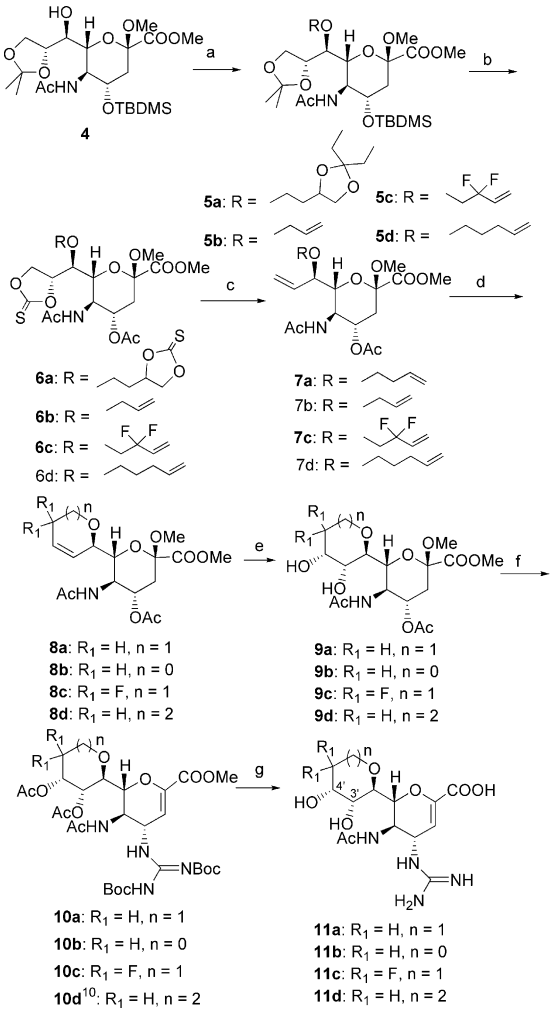
Herein we report the synthesis of tetrahydro-furan-2-yl, tetrahydro-pyran-2-yl and oxepan-2-yl derivatives related to zanamivir and their biological activities.

Chemistry

The synthesis of the tetrahydro-furan-2-yl, tetrahydro-pyran-2-yl, and oxepan-2-yl derivatives substituted by diol at the C-3′ and C-4′ positions is illustrated in Scheme 1. Compound **4**[5b] was alkylated with toluene-4-sulfonic acid 2-(2,2-diethyl-[1,3]dioxolan-4-yl)-ethyl ester,[6] allyl iodide, trifluoro-methanesulfonic acid 2,2-difluoro-but-3-enyl ester,[7] or 5-iodo-pent-1-ene in the presence of NaH in DMF to give the corresponding compound **5a, 5b**, **5c**, or **5d**, in moderate yield. The removal of the ketals with 80% acetic acid after protection of the 4-OH group by the acetyl group, followed by the formation of the thiocarbonates with thiophosgene and dimethylaminopyridine provided compounds **6a**–**6d**, which were then reduced to give the corresponding di-terminal olefines **7a**–**7d**. Subsequently, ring-closing metathesis (RCM) reactions with Grubbs' catalyst could be successively accomplished to afford the corresponding cyclic compounds **8a**–**8d**. This is the first example of application of the RCM reaction for sialic acid modifications. Oxidation of compounds **8a**–**8d** with OsO_4 and NMO provided the diols **9a**–**9d**, respectively, as single diastereomers. The (3′*R*, 4′*R*)-configurations of the diols of compounds **9a** and **9b** were determined by X-ray crystallographic analysis[8] of compounds **11a** and **11b**, respectively.



Scheme 1. Reagents and conditions: (a) alkylation reagents (2.0 equiv), NaH (2.0 equiv), DMF (50–70%); (b) (i) TBAF, THF (70–80%), (ii) Ac_2O, pyridine (90–100%), (iii) AcOH–H_2O (4:1) (70–80%), (iv) thiophosgene (1.2–2.4 equiv), dimethylaminopyridine (2.4–5.0 equiv), CH_2Cl_2 (80–90%); (c) $P(OCH_3)_3$, 120 °C (60–90%); (d) benzylidene-bis (tricyclo-hexylphosphine) dichlororuthenium (0.01 equiv), CH_2Cl_2 (50–100%); (e) OsO_4 (0.01 equiv), *N*-methylmorphorine *N*-oxide (1.2 equiv), acetone–H_2O (50–90%); (f) (i) Ac_2O, AcOH, cat. H_2SO_4 (10:10:1, v/v) (80–90%), (ii) NaN_3, Dowex 50W (H^+), *t*-BuOH (70–80%), (iii) Lindlar cat., H_2, EtOH (70–80%), (iv) 1*H*-pyrazol-1-[*N*,*N*′-bis(*tert*-butoxycarbonyl)] carboxamidine, THF (95%); (g) (i) CF_3COOH–CH_2Cl_2, (ii) aq NaOH, (90–100%).

The high diasteroselectivity of osmylation could be explained in terms of the most preferable conformation (A) of compound **8a** based on molecular orbital calculation (3-21G* basis set) as shown in Figure 2. Thus, the oxygen of the tetrahydropyran moiety is orientated away from the oxygen of the dihydropyran moiety to avoid electrostatic repulsion and one side of the cyclic olefin is covered by the acetamide group. Therefore, osmylation occurred from the sterically more accessible face, the π-face opposite to that of the preexisting acetamide group. Compounds **9a**–**9d** were converted to compounds **11a**–**11d** under the same conditions as that described previously.[5] The synthesis of the tetrahydro-furan-2-yl, tetrahydro-pyran-2-yl, and oxepan-2-yl derivatives substituted by diol at the C-4′ and C-5′ positions is illustrated in Scheme 2. Removal of the acetonide of compound **12**, followed by treatment of compound **13** with sodium periodate gave the aldehyde **14**. Subsequently, alkylation of compound **14** was performed with allyltrimethylsilane in the presence of $TiCl_4$ to provide the diastereomixture of the homoallyl alcohols (**15a**/**15b** = 2:1) which could be separated using a silica gel column. The (*S*)-configuration of the C-7 alcohol of compound **15b** was determined by X-ray crystallographic analysis of **19c**. Alkylation of the alcohols of compounds **15a** and **15b** with allyl trichloroacetimidate and TfOH, followed by ring closing metathesis reaction

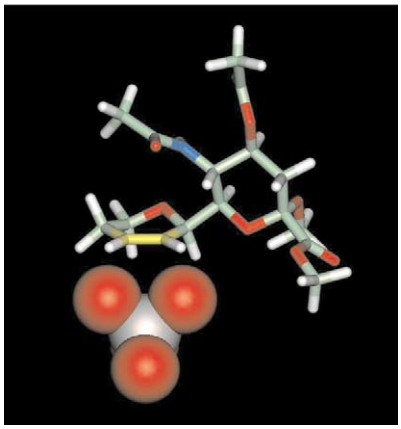
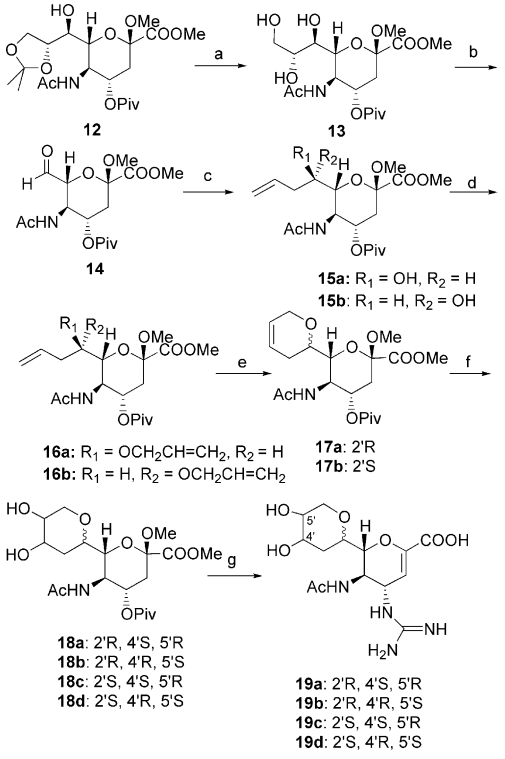
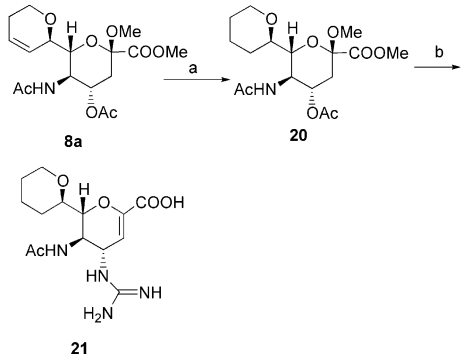


Figure 2. The preferred conformation (A) of compound **8a** calculated using Macromodel.

of compounds **16a** and **16b** provided compounds **17a** and **17b**, respectively, in good yield. Osmium oxidation of **17a** and **17b** each furnished mixtures of two *cis* diols (4′*S*, 5′*R*:4′*R*, 5′*S* = 2:1), which were separated using a silica gel column regardless of the C-2′ stereochemistry. Compounds **18a**, **18b**, **18c**, and **18d** were thus converted to the target compounds **19a**, **19b**, **19c**, and **19d**, respectively. The absolute configurations of the diols of compounds **19b** and **19c** were determined by their X-ray crystallographic analysis.[8] The olefin of compound **8a** was reduced with Pd/C under H_2 atmosphere to afford the tetrahydrofuran derivatives **20**, which yielded compound **21** as shown in Scheme 3.



Scheme 2. Reagents and conditions: (a) AcOH–H_2O (4:1) (70%); (b) $NaIO_4$, acetone–H_2O (90%); (c) allyltrimethylsilane (2.0 equiv), $TiCl_4$ (1.5 equiv) (77% as a mixture of *R* and *S* diastereomers); (d) allyl 2,2,2-trichloroacetimidate (10 equiv), TfOH (1.5 equiv), CH_2Cl_2–cyclohexane (20–25%); (e) benzylidene-bis(tricyclohexylphosphine)dichlororuthenium (0.01 equiv), CH_2Cl_2 (80%); (f) OsO_4 (0.01 equiv), *N*-methylmorphorine *N*-oxide (1.2 equiv), acetone–H_2O (70–80% as a mixture of 4′*S*, 5′*R*-diol and 4′*R*, 5′*S*-diol); (g) (i) Ac_2O, AcOH, cat. H_2SO_4 (10:10:1, v/v) (80–90%), (ii) NaN_3, Dowex 50W (H^+), *t*-BuOH (70–80%), (iii) Lindlar cat., H_2, EtOH (70–80%), (iv) 1*H*-pyrazol-1-[*N*,*N*′-bis(*tert*-butoxycarbonyl)] carboxamidine, THF (95%), (v) CF_3COOH–CH_2Cl_2, (vi) aq NaOH, (90–100%).

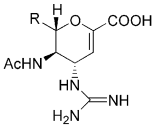


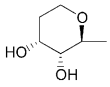
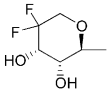
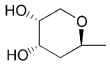
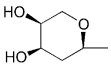
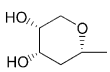
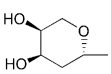
Scheme 3. Reagents and conditions: (a) Pd/C, H_2, EtOH (84%); (b) same conditions as those described in Scheme 1f and g.

Biological Activities

The influenza A virus sialidase inhibitory and plaque reduction activities[9] of a range of the bicyclic sialidase inhibitors are summarized in Table 1. Tetrahydro-furan-2-yl, tetrahydro-pyran-2-yl, and oxepan-2-yl derivatives substituted by diol at the C-3′ and C-4′ positions, **11a**, **11b**, **11c**, and **11d**, exhibited comparable inhibitory

Table 1. Sialidase inhibitory and plaque reduction activities of bicyclic sialidase inhibitors related to zanamivir IC_{50} (ng/mL)[b]



Compd	R	Sialidase inhibitory assay	Plaque reduction assay
		A/PR/8/34	A/Yamagata/32/89
Zanamivir		0.6–12.0 (1.0)[a]	0.9–6.6 (1.0)[a]
11a		10.3 (0.94)	0.6 (0.46)
11b		6.1 (1.22)	1.4 (0.82)
11c		10.7 (0.88)	1.4 (0.53)
11d		12.4 (1.12)	2.2 (1.47)
21		240 (4.00)	> 100
19a		2.45 (22.2)	> 100
19b		998 (90.7)	> 100
19c		370 (30.8)	> 100
19d		3600 (300)	> 100

[a]Since IC_{50} values varied depending on the experiments, the relative potencies of the compounds to zanamivir are shown in the parentheses based on the IC_{50} values of zanamivir as a reference. IC_{50} values of zanamivir in enzyme inhibition and plaque reduction were 0.6–12.0 ng/mL and 0.9–6.6 ng/mL, respectively.
[b]ND, not determined.

activities against influenza A virus sialidase to that of zanamivir. Compound **11a** showed slightly increased activity (2-fold) in a plaque reduction assay relative to zanamivir. However, removal of the diol at the C-3′ and C-4′ positions resulted in reduced inhibitory activity (**21**). Movement of the diol to the C-4′ and C-5′ positions from the C-3′ and C-4′ positions, respectively, showed a significant loss of enzyme inhibitory activity (**11a** vs **19a**). Compounds **19a** and **19c** possessing the diol of the 4′*S* and 5′*R* configurations were relatively more potent than compounds **19b** and **19d** possessing the diol of the 4′*R* and 5′*S* configurations. This result suggests the position and the stereochemistry of the diol of the tetrahydro-pyran side chain must play an important role in binding affinity with a virus sialidase.

As shown in the model of the structure of compound **11a** bound with influenza virus sialidase (Fig. 3), the carboxylate was held strongly by two arginine residues (Arg 371 and Arg 118). The guanidino group forms strong charge-charge type hydrogen bond interaction with Glu 119 and Asp 151. The diol interacts with the carboxylate of Glu 276 in a bidentate hydrogen bond donor-acceptor mode. This binding feature is not significantly different from that found in the zanamivir-sialidase complex.

Furthermore, the efficacy of orally administered compound **11a** was tested in the influenza virus infected mouse model on the basis of the survival rates for treated and infected mice relative to that for control mice. Compound **11a** was administered orally once daily for 5 days after infection. It was found that compound **11a** had similar efficacy relative to oseltamivir phosphate as shown in Table 2.

In summary, we prepared the tetrahydro-furan-2-yl, tetrahydro-pyran-2-yl, and oxepan-2-yl derivatives related to zanamivir using an RCM reaction. These bicyclic ether derivatives substituted by diol at the C-3′ and C-4′ positions showed comparable sialidase inhibitory activities relative to that of zanamivir. Furthermore, compound **11a** exhibited a similar oral efficacy in the mouse/infection model to that of oseltamivir. This is the first example of an in vivo result for derivatives related to zanamivir.

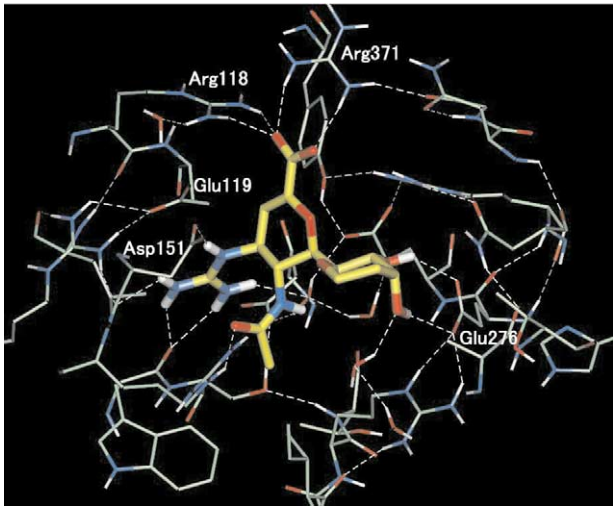


Figure 3. Model structure[11] of the complex of compound **11a** with influenza virus sialidase.

Table 2. The median survival time of compound **11a**[a] and oseltamivir phosphate[a] and the results of the Log-rank test

Compd	Median survival time[b] (days)	*p* value[c] compared with	
		Control	Oseltamivir phosphate
Control	7.5	—	—
Oseltamivir phosphate	10	<0.0001	—
11a	10	<0.0001	0.1089

Mice were infected with influenza A/PR/8/34 (H1N1) virus.
[a]Compound **11a** and oseltamivir phosphate were administered orally at doses of 10 μmol/kg once daily for 5 days after infection.
[b]The median survival times were calculated by Kaplan–Meier curve.
[c]Statistical significances of the median survival times were analyzed by the Log-rank test.

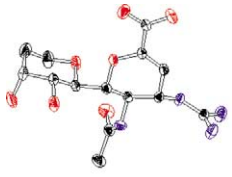
Acknowledgements

We wish to thank Dr. Mutsuo Nakajima and Ms. Reiko Kametani for performing the sialidase assay, Dr. Shuichi Miyamoto and Atsushi Kasuya for molecular modeling studies, and Mr. Youji Furukawa for performing X-ray crystallographic analysis.

References and Notes

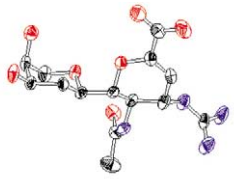
1. (a) Palese, P.; Compans, R. W. *J. Gen. Virol*. **1976**, *33*, 159. (b) Palese, P.; Jobita, K.; Ueda, M.; Compans, R. W. *Virology* **1974**, *61*, 397.
2. (a) Babu, Y. S.; Chand, P.; Bantia, S.; Kotian, P.; Dehghani, A.; El-Kattan, Y.; Lin, T. H.; Hutchison, T. L.; Elliott, A. J.; Parker, C. D.; Ananth, S. L.; Horn, L. L.; Laver, G. W.; Montgomery, J. A. *J. Med. Chem*. **2000**, *43*, 3482. (b) Maring, C. J; Giranda, V. L.; Kempf, D. J.; Stoll, V. S.; Sun, M.; Zhao, C; Gu, Y. C.; Hanessian, S.; Wang, G. T. PCT Int. Appl., WO0128996. (c) Smith, P. W.; Sollis, S. L.; Howes, P. D.; Cherry, P. C.; Starkey, I. D.; Cobley, K. N.; Weston, H.; Scicinski, J.; Merritt, A.; Whittington, A.; Wyatt, P.; Taylor, N.; Green, D.; Bethell, R.; Madar, S.; Fenton, R. J.; Morley, P. J.; Pateman, T.; Beresford, A. *J. Med. Chem*. **1998**, *41*, 787.
3. (a) von Itzstein, M.; Wu, W.-Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Phan, T. V.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418. (b) Ryan, D. M.; Ticehurst, J.; Dempsey, M. H.; Penn, C. R. *Antimicrob. Agents Chemother*. **1994**, *38*, 2270.
4. (a) Kim, C. U.; Lew, W.; Williams, M. A.; Wu, H.; Zhang, L.; Chen, X.; Escarpe, P. A.; Mendel, D. B.; Laver, W. G.; Stevens, R. C. *J. Med. Chem*. **1998**, *41*, 2451. (b) Mendel, D. B.; Tai, C. Y.; Escarpe, P. A.; Li, W.; Sidwell, R. W.;

Huffmann, J. H.; Sweet, C.; Jakeman, K. J.; Merson, J.; Lacy, S. A.; Lew, W.; Williams, M. A.; Zhang, L.; Chen, M. S.; Bischofberger, N.; Kim, C. U. *Antimicrob. Agents Chemother.* **1998**, *42*, 640.
5. (a) Honda, T.; Masuda, T.; Yoshida, S.; Arai, M.; Kobayashi, Y.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1921. (b) Honda, T.; Masuda, T.; Yoshida, S.; Arai, M.; Kaneko, S.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1925. (c) Honda, T.; Yoshida, S.; Arai, M.; Masuda, T.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1929.
6. Toluene-4-sulfonic acid 2-(2,2-diethyl-[1,3]dioxolan-4-yl)-ethyl ester was prepared as follows: 1,2,4-butanetriol was reacted with acetone dimethyl acetal in the presence of TsOH, followed by treatment of TsCl and pyridine to give the target compound.
7. Trifluoro-methanesulfonic acid 2,2-difluoro-but-3-enyl ester was prepared as follows: 2,2-difluoro-3-butenoic acid ethyl ester was treated with $LiAlH_4$ to afford the 2,2-difluoro-3-butene-1-ol, which was reacted with $(TfO)_2O$ in the presence of pyridine to give the target compound.
8. ORTEP drawings of compounds **11a**, **11b**, **19b**, and **19c**:



11a

11b



19b

19c

Crystallographic data for the structures **11a**, **11b**, **19b**, and **19c** reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication nos. CCDC 188639, 188640, 188641, 188642, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44–1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
9. The methods for sialidase inhibitory and plaque reduction assays are detailed in a previous paper.[5a]
10. The stereochemistry of the diol of **10d** was determined by ^1H NMR analysis. $J_{2',3'} = 6.0$ Hz and $J_{3',4'} = 2.5$ Hz indicated that the stereochemistry between H-2′ and H-3′ was a *trans* configuration and that between H-3′ and H-4′ was a *cis* configuration. Therefore, the configurations of the diol of **10d** were 3′*R* and 4′*R*.
11. A model of compound **11a** complexed with influenza virus sialidase was created based on the crystal structure of subtype N9 sialidase complexed with zanamivir (Varghese, J. N.; Epa, V. C.; Colman, P. M. *Protein Sci.* **1995**, *4*, 1081). The glycerol side chain of zanamivir was replaced by the 3,4-dihydroxy-tetrahydropyran-2-yl group of **11a**, and ligand conformation was optimized using the molecular mechanics program CHARMm (Accelrys Inc., San Diego, CA, USA).