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Synthesis and Anti-Influenza Virus Activity of 7-*O*-Alkylated Derivatives Related to Zanamivir

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Abstract—A series of 7-alkyl ether derivatives related to zanamivir were synthesized using direct alkylation of the C-7 alcohol of sialic acid. Alkyl ether moiety of less than 12 carbons in length showed low nanomolar inhibitory activity against influenza A virus sialidase. Furthermore, their moiety improved influenza A virus plaque reduction activity compared to zanamivir. However, removal of the 8,9-diol of the 7-*O*-alkyl derivatives resulted in loss of antiviral potency. This result suggests that 8,9-diol must play an important role in binding with both influenza A and B virus sialidases.

Influenza virus sialidase promotes virus release from infected cells and facilitates virus spread within the respiratory tract.[1] Several potential specific inhibitors of this enzyme have been developed and two (zanamivir **1**[2] and oseltamivir phosphate **2**[3]) have been approved for human use. Zanamivir **1** is delivered by inhalation because of its low oral bioavailability, small volume of distribution, and rapid renal elimination, whereas oseltamivir phosphate **2**, an ethyl ester prodrug of GS4071, is administered orally. In the search for further clinical candidates, a number of reports have also documented other potent sialidase inhibitors.[4]

In an accompanying paper,[5] we report that the synthesis of the 7-modified analogues related to zanamivir using a chemoenzymatic reaction and the replacement of the 7-OH moiety by small lipophilic groups (F, N, OMe, OEt) resulted in the retaining of excellent inhibitory activity against influenza A virus sialidase. As part of our study of the structure–activity relationship (SAR) of this series, we are interested in the corresponding analogues possessing alkyl ether further homologated at the C-7 position in order to investigate structural features important for binding within this region of the sialidase active site. These compounds could possess modified physicochemical properties which could make them more suitable for systemic delivery. However, we have found that the chemoenzymatic reaction of 4-alkoxy-4-deoxy-ManNAc, which possesses alkyl ether of more than three carbons in length, with sodium pyruvate in the presence of sialic acid aldolase does not proceed. Therefore, in our attempts to find a synthetic method to 7-modified sialic acids substituted by alkyl ether, we focused our attention on a chemical approach towards them using direct alkylation of the C-7 alcohol of sialic acid analogues.

Herein we report the synthesis of a series of alkyl ether **3** at the C-7 position of zanamivir which could not be prepared using a chemoenzymatic reaction, and furthermore, their 8,9-dideoxy derivatives **4** and biological activities (Fig. 1).

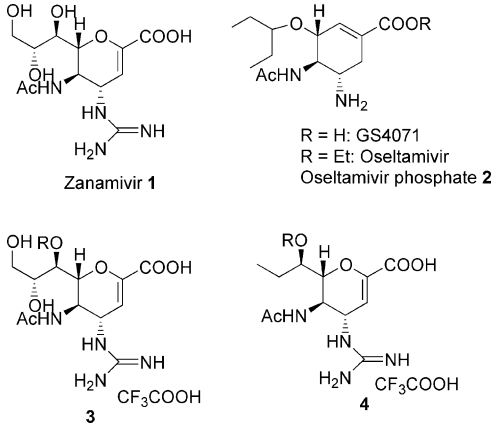

Figure 1.

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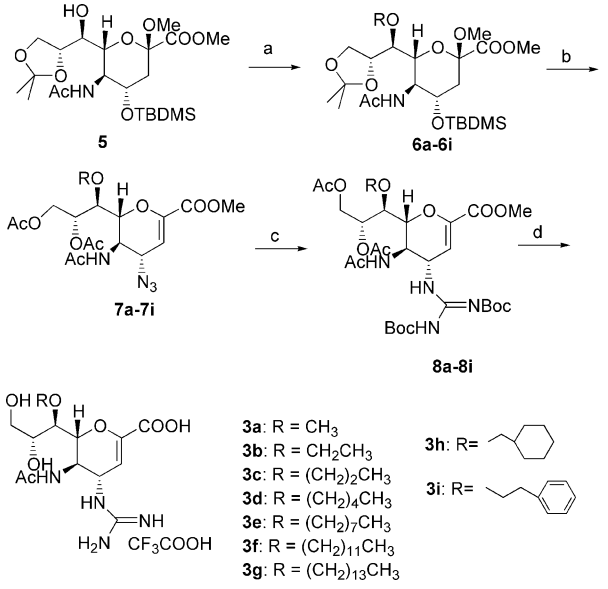
Chemistry

The syntheses of 4-guanidino-Neu5Ac2en derivatives **3** are shown in Schemes 1 and 2. The alcohol at the C-7 position of **5**[6] could be successfully alkylated with a variety of $(RO)_2SO_2$[7] in the presence of NaH (2.0 equiv) in DMF to afford the corresponding alkyl ethers **6a–6i** in moderate yield. The treatment of **6a–6i** with Ac_2O, AcOH, and H_2SO_4 (10:10:1, v/v) followed by introduction of N_3 at the C-4 position via oxazoline gave **7a–7i**, respectively.[8] Guanidine formation with *N*, *N*′-bis (*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxyamidine provided **8a–8i**. Subsequent basic hydrolysis and treatment of CF_3COOH–CH_2Cl_2 afforded the targets **3a–3i**. As an extension of the series of **3**, we modified the terminal of the alkyl groups at the C-7 position. Treatment of **5** with $(BnOCH_2CH_2O)_2SO_2$ in the presence of NaH and successive removal of the benzyl group with Pd/C under H_2 atmosphere provided **9**. Compound **9** was converted to **10** under the same conditions as those described above. The terminal alcohol of **10** was tosylated, followed by introduction of azide gave **11**. Reduction of the azide of **11** gave the amine **12** and its subsequent acetylation provided **13**. Compounds **10**, **11**, **12**, and **13** were converted to **3j**, **3k**, **3l**, and **3m**, respectively, under the same conditions as those described above.

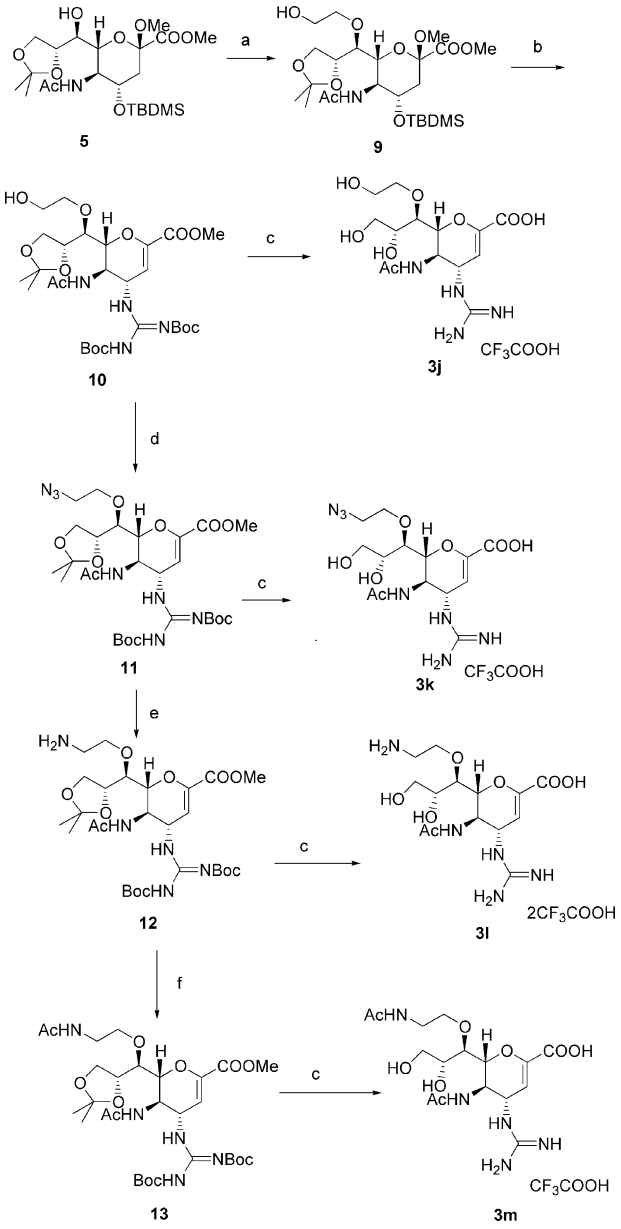
The synthesis of 8,9-dideoxy derivatives **4** is shown in Scheme 3. Compounds **6a–6e** were converted to thiocarbonate **14a–14e** after replacement of TBDMS ether by the acetyloxy group at the C-4 position. Subsequent treatment of **14a–14e** with $P(OMe)_3$[9] as a solvent, followed by hydrogenation of compounds **15a–15e** afforded the corresponding alkyl ethers **16a–16e**, respectively. Compounds **4a–4e** were prepared from **16a–16e** under the same conditions as those described above.



Scheme 1. Reagents and conditions: (a) NaH (2.0 equiv), $(RO)_2SO_2$ (1.2 equiv), DMF (30–80%); (b) (i) Ac_2O, AcOH, concd H_2SO_4 (10:10:1, v/v), (ii) NaN_3, Dowex 50W (H^+), DMF (30–80%); (c) (i) Lindlar cat., H_2, EtOH (40–80%), (ii) *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxyamidine, THF (70–90%); (d) (i) NaOH, H_2O (80–90%), (ii) CF_3COOH–CH_2Cl_2 (1:3, v/v) (60–80%).

Biological Activities

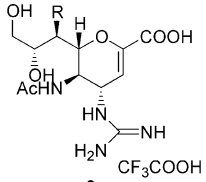
The influenza A virus sialidase inhibitory[10] and plaque reduction activities[11] of compounds **3** are summarized in Table 1. In an accompanying paper, we demonstrated that 7-modified derivatives related to zanamivir possessing relatively small lipophilic substituents such as F,



Scheme 2. Reagents and conditions: (a) (i) NaH (2.0 equiv), $(BnOCH_2CH_2O)_2SO_2$ (1.2 equiv), DMF (50%), (ii) Pd/C, H_2, EtOH (80%); (b) (i) Ac_2O, AcOH, concd H_2SO_4 (10:10:1, v/v) (40%), (ii) NaN_3, Dowex 50W (H^+), DMF (80%), (iii) Lindlar cat., H_2, EtOH (70%), (iv) *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxyamidine, THF (90%); (v) NaOMe, MeOH (85%), (vi) acetone dimethyl acetal, cat. TsOH (70%); (c) (i) NaOH, H_2O (80–90%), (ii) CF_3COOH–CH_2Cl_2 (1:3, v/v) (60–70%); (d) (i) TsCl, Pyridine, CH_2Cl_2 (90%), (ii) NaN_3, DMF (70%); (e) Lindlar cat., H_2, EtOH (70%); (f) Ac_2O, pyridine (90%).

N_3, OMe, and OEt groups showed similar sialidase inhibitory activity to zanamivir. In this paper, we investigated the extensive SAR of a variety of alkyl ether analogues at the C-7 position related to zanamivir. Linear alkyl ether chains of less than 12 carbons in length exhibited similar inhibitory activity to zanamivir against influenza A virus sialidase, regardless of the carbon chain length of the alkyl ether. These compounds, **3a**, **3b**, **3c**, **3d**, **3e**, and **3f**, showed a pronounced improvement in activity against influenza A virus plaque reduction assay compared to zanamivir. However, the moiety of linear chains of more than 12 carbons in length (**3g**) resulted in a slight decrease in sialidase inhibitory and plaque reduction activities, probably because of unfavorable steric and electrostatic interaction within the enzyme binding site. Addition of a terminal OH, N_3, NH_2, or NHAc did not significantly affect the binding as these compounds, **3j**, **3k**, **3l**, and **3m**, had a similar potency to **3b**. Removal of the 8,9-diol of the C-7 alkyl ether derivatives resulted in similar activity against influenza A virus sialidase to zanamivir

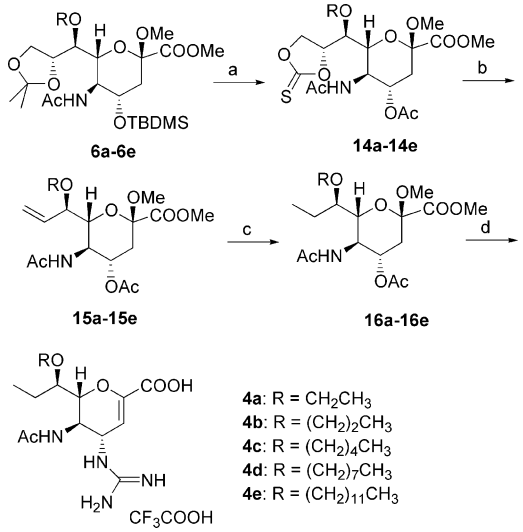
Table 1. Sialidase inhibitory and plaque reduction activities of compounds **3** [IC_{50} (ng/mL)]



3

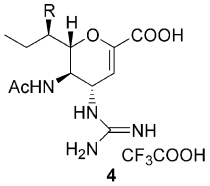
	R	Sialidase inhibitory assay	Plaque reduction assay
		A/PR/8/34	A/Yamagata/32/89
Zanamivir	OH	5.1–10.2 (1.0)[a]	1.9–20 (1.0)[a]
3a	O—	6.1 (1.2)	3.0 (0.15)
3b	O	26.4 (2.6)	0.7 (0.14)
3c	O	30.6 (3.0)	1.5 (0.35)
3d	O	14.3 (2.6)	2.0 (0.23)
3e	O	20.2 (2.0)	1.8 (0.42)
3f	O	49.6 (4.9)	2.2 (0.51)
3g	O	262 (26)	11.0 (5.8)
3h	O	36.6 (3.6)	3.0 (1.6)
3i	O Ph	26.3 (2.6)	1.9 (1.0)
3j	O OH	10.3 (1.4)	1.8 (0.21)
3k	O N_3	14.8 (2.0)	1.5 (0.13)
3l	O NH_2	47.7 (6.5)	7.5 (0.63)
3m	O NHAc	9.8 (1.4)	2.4 (0.2)

[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 5.1–10.2 and 1.9–20 ng/mL, respectively.



Scheme 3. Reagents and conditions: (a) (i) TBAF, THF (70–80%), (ii) Ac_2O, pyridine (90%), (iii) $SCCl_2$, DMAP, CH_2Cl_2 (60–70%); (b) $P(OMe)_3$ (60–70%); (c) Pd/C, H_2 MeOH (80%); (d) (i) Ac_2O, AcOH, concd H_2SO_4 (10:10:1, v/v) (50–60%), (ii) NaN_3, Dowex 50W (H^+), DMF (40–80%), (iii) Lindlar cat., H_2, EtOH (40–80%), (iv) *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxyamidine, THF (70–90%), (v) NaOH, H_2O (80–90%), (vi) CF_3COOH–CH_2Cl_2 (1:3, v/v) (60–80%).

Table 2. Sialidase inhibitory and plaque reduction activities of compounds **4** [IC_{50} (ng/mL)]



4

	R	Sialidase inhibitory assay	Plaque reduction assay
		A/PR/8/34	A/Yamagata/32/89
Zanamivir		5.4–10.2 (1.0)[a]	1.9–12 (1.0)[a]
4a	O	35.1 (1.9)	16.0 (8.4)
4b	O	14.1 (1.9)	9.0 (0.75)
4c	O	9.1 (1.7)	3.8 (1.3)
4d	O	16.6 (2.3)	40.0 (3.3)
4e	O	28.4 (5.3)	> 50 (> 5.9)

[a]Since IC_{50} values varied depending on experiments, the relative potencies of the compounds to zanamivir are shown in the parentheses, based on the IC_{50} value of zanamivir as a reference. IC_{50} values of zanamivir in enzyme inhibition and plaque reduction were 5.4–10.2 and 1.9–12 ng/mL, respectively.

as shown in Table 2, but relatively little effect on activity against the influenza B virus sialidase (data not shown). Evaluation of a plaque reduction assay demonstrated that 7-alkyl ether derivatives **3** related to zanamivir were more active than the 8,9-dideoxy derivatives **4**. These results suggest that 8,9-diol must play an important role in the binding affinity with sialidase of both A and B viruses. A computational study to rationalize the potent activity and high specificity of 7-alkyl ether derivatives is currently in progress.

In summary, a series of 7-alkyl ether derivatives related to zanamivir were synthesized using direct alkylation of the C-7 alcohol of sialic acid analogue. Chains of less than 12 carbon in length showed similar activity against influenza A virus sialidase. Furthermore, their moieties improved influenza A virus plaque reduction activity compared to zanamivir. However, removal of the 8,9-diol of the 7-*O*-alkyl derivatives showed loss of antiviral activity. This result suggests that 8,9-diol must play an important role in binding with both influenza A and B viruses enzymes.

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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. (c) Liu, C.; Eichelberger, M. C.; Compans, R. W.; Air, G. M. *J. Virol.* **1995**, *69*, 1099.
2. (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. (c) Woods, J. M.; Bethell, R. C.; Coates, J. A.; Healy, N.; Hiscox, S. A.; Pearson, B. A.; Ryan, D. M.; Ticehurst, J.; Tilling, J.; Walcott, S. M.; Penn, C. R. *Antimicrob. Agents Chemother.* **1993**, *37*, 1473. (d) Hayden, F. G.; Treanor, J. J.; Betts, R. F.; Lobo, M.; Esinhart, J. D.; Hussey, E. E. *JAMA* **1996**, *275*, 295.
3. (a) Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C. *J. Am. Chem. Soc.* **1997**, *119*, 681. (b) 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. (c) 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, X.; Bischofberger, N.; Kim, C. U. *Antimicrob. Agents Chemother.* **1998**, *42*, 640.
4. (a) Babu, Y. S.; Chand, P.; Montgomery, J. A. PCT Int. Appl., WO9747194. (b) 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. (c) Chand, P.; Kotian, P. L.; Dehghani, A.; El-Kattan, Y.; Lin, T.-H.; Hutchison, T. L.; Babu, Y. S.; Bantia, S.; Elliott, A. J.; Montgomery, J. A. *J. Med. Chem.* **2001**, *44*, 4379. (d) 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.
5. Honda, T.; Masuda, T.; Yoshida, S.; Arai, M.; Kobayashi, Y.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1921.
6. (a) Zbiral, E.; Phadtare, S.; Schmidt, W. *Liebigs Ann. Chem.* **1987**, 39. (b) Anazawa, K.; Furuhata, K.; Ogura, H. *Chem. Pharm. Bull.* **1988**, *36*, 4976.
7. The preparation of $(RO)_2SO_2$ is as follows: ROH was treated with $SOCl_2$ (0.5 equiv) in the presence of TEA (2.2 equiv) in CH_2Cl_2 to afford $(RO)_2SO$, which was oxidized to $(RO)_2SO_2$ by treatment of $NaIO_4$ (1.2 equiv) and cat. $RuCl_3$.
8. Kok, G. B.; Campbell, M.; Mackey, B.; von Itzstein, M. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2811.
9. Cubero, I. I.; Lopez-Espinosa, M. T.; Richardson, A. C. P.; Amlid, K. H. *Carbohydr. Res.* **1993**, *242*, 281.
10. The method for sialidase inhibitory assay is detailed in an accompanying paper; *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1921.
11. The method for plaque reduction assay is detailed in an accompanying paper; *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1921.