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Inhibition of Neuraminidase with Neuraminic Acid *C*-Glycosides

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Abstract—Neuraminic (sialic) acid based α-*C*-glycosides have been synthesized and their inhibitory activity towards bacterial neuraminidase (sialidase) was examined. While some *C*-glycosides were found to be potent inhibitors (K_i 15–30 μM) of this neuraminidase, others afforded no measurable activity. The structure–activity relationship of these *C*-glycosides is discussed in the context of other previously reported sialidase inhibitors.

The interaction between cell surface carbohydrates and their protein receptors are implicated in many important biological events.[1–4] Synthetic carbohydrate derivatives are potentially useful tools to study cellular interactions, the biosynthesis of glycoproteins, the catabolism of glycoconjugates,[5,6] and the mechanism of their enzymatic processing.[7] Inhibitors of enzymes, such as neuraminidases, involved in these processes may represent potent antiviral,[8–11] antibacterial[12,13] and anticancer agents.[14–16] Neuraminidases are hydrolases, obtained from diverse species and tissues, that bind to sialic acid present on the outer layer of cell membranes and result in its catabolism.[17] This de-sialation has dramatic consequence for infection, adhesion and recognition events.

Approaches aimed at the discovery of neuraminidase inhibitors are primarily based upon the knowledge of substrate structure, enzymatic mechanism, transition state analogues[18] and the enzyme's active site.[19] The exploration of a new class of sialidase inhibitors, such as *C*-glycosides[20] and the investigation of their structure–activity relationship (SAR) represents an attractive approach to control events of crucial importance to glycobiology and immunology. *C*-glycosides are catabolically stable analogues that have been used as receptor ligands[21] and glycosidase inhibitors.[22] In the current study, a series of neuraminic acid based α-*C*-glycosides are examined as potential inhibitors of the bacterial neuraminidase from *Clostridium perfringens*.

Recently, our laboratory applied a method for diastereocontrolled synthesis of α-*C*-glycosides of *N*-acetylneuraminic acid (5-acetamido-3, 5-dideoxy-D-glycero-D-galacto-2-nonulosonic acid, Neu5Ac), using samarium iodide under Barbier conditions,[23] which had been previously used to prepare *C*-glycosides of hexoses.[24–26] Through a simple, high-yielding reaction, this same chemistry could be used to couple different ketones or aldehydes with peracetylated sialic acid sulfone and form α-*C*-glycosides of Neu5Ac (Scheme 1).[27–30]

Five compounds prepared using this chemistry, ranging from simple *C*-glycosides to *C*-disaccharides, were selected to undertake a study of their activity as sialidase inhibitors (Table 1). All of the compounds prepared (Entries **1**–**5**) contained hydroxyl group at the *C*-glycoside bridge, which posed stereochemical concerns. This hydroxyl group, while improving the hydrophilicity of the *C*-bridge, also adds steric bulk that might affect the conformation and the neuraminidase binding of these compounds. However, all attempts to remove this bridge hydroxyl group were unsuccessful.[31,32]

Following deprotection and complete characterization,[33] the five *C*-glycosides (Table 1) were tested for their ability to inhibit bacterial neuraminidase. The enzyme assay was performed according to Potier's method[34] using MU-Neu5Ac [2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid] as the substrate (Scheme 2).[35] The K_m value of *C. perfringens* sialidase acting on MU-Neu5Ac was determined to be 0.1 mM, consistent with reported previous values.[36]

Each of the five *C*-glycosides was added at the three different concentrations to the buffer employed in the

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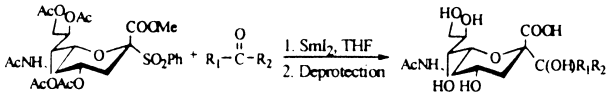
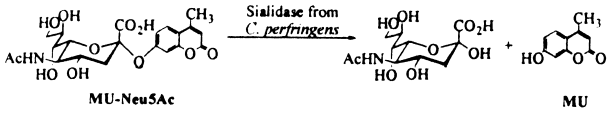


Scheme 1.



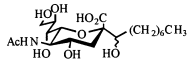
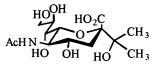
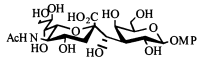
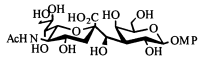
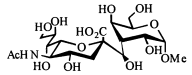
Scheme 2.

enzymatic assay to investigate their inhibition of sialidase activity. Relative fluorescence was recorded by using the same procedure as the K_m measurement.[35] The results were fit using Microsoft Excel and Cleland's Kin computer programs to different inhibition models. The best fit was achieved with the competitive inhibition model. The K_i value was determined using a Lineweaver–Burk double reciprocal plot (Table 1). Data on the neuraminidase activity (Table 1) show that *C*-glycosides having hydrophobic substituents at 2- position of neuraminic acid (Entries **1** and **2**) display the highest level of neuraminidase inhibitory activity. Hydrophilic *C*-glycosides, which more closely resemble the structure of the natural neuraminidase substrate, Neu5Ac (2→3) Gal, show considerably less activity as inhibitors. (Entries **3** and **4**) The two galactose containing *C*-glycosides (Entries **3** and **4**) have identical structure with the exception of their different configuration at the bridge hydroxyl methylene groups and anomeric protection and showed similar inhibitory effect. This result suggests that presence of the bridge hydroxyl group probably does not adversely impact *C*-glycoside binding to neuraminidase. A change in the configuration of the connection between the two saccharide units at the 3′ position, converting the galactose residue to gulose, afforded a profound change, with this *C*-glycoside having no measurement neuraminidase inhibitory activity (Entry **5**). Lectin affinity chromatography[37–39] of 2,6 specific *Sambucus nigra* agglutinin, SNA lectin and 2,3 specific *Maackia amurensis* agglutinin, MAA lectin was used to confirm the structural similarity between the natural *O*-glycoside and *C*-glycoside analogue **3**–**5**. As expected neither compound bound to SNA lectin, while both bound to MAA lectin.

A number of different neuraminidase inhibitors have been reported.[18] Extensive studies by several laboratories[19,40–45] have been undertaken on 2-deoxy-2, 3-didehydro-*N*-acetylneuraminic acid transition state analogueues as neuraminidase inhibitors. Studies on these derivatives indicate the importance of the carboxylate, and the acetamido (NH and methyl) groups in Neu5Ac.[40] Replacement of the glycerol side chain of Neu5Ac with carboxamide groups[42] and the preparation of bicyclo derivatives also afforded potent neuraminidase inhibitors.[43,45] In addition, these studies investigated the space surrounding the 4-position of the Neu5Ac residue. One of these agents, the 4-guanidino derivative, Zanamivir is undergoing clinical evaluation as a potential agent for the treatment of influenza.[46] These agents generally exhibit μM K_i values against various neuraminidases using the same fluorescent substrate used in the current study.

In addition to transition state analogues, inhibitors based on product structure have also been investigated. The aminocyclic Neu5Ac analogue[47] and an analogue formed through the replacement of the Neu5Ac carboxyl group with a phosphonate group[48] showed activity comparable to the transition state inhibitors. A carbocyclic Neu5Ac analogue, however, was not a neuraminidase inhibitor.[49] Inhibitors based on substrate structure include both thioglycosides displaying mM inhibition constants[50] and *S*-disaccharides displaying

Table 1. Inhibition constants of *C*-glycosides

Entries	Structures	FABHRMS	K_i values (μM)
1		$[M+Na^+]^+$ for $C_{19}H_{35}O_9N$ Calcd: 444.4766 Found: 444.4763	16
2		$[M+Na^+]^+$ for $C_{14}H_{25}O_9N$ Calcd: 374.3426 Found: 374.3422	29
3		$[M-H]^-$ for $C_{25}H_{37}O_{15}N$ Calcd: 590.2088 Found 590.2086	110
4		$[M-H]^-$ for $C_{25}H_{37}O_{15}N$ Calcd: 590.2088 Found: 590.2085	229
5		$[M-H]^-$ for $C_{19}H_{33}O_{14}N$ Calcd: 498.4601 Found: 498.4598	>>1000

μM K_i values.[51] The current study shows that simple *C*-glycosides **1** and **2** display μM K_i values. Furthermore, the more hydrophobic the aglycones afford greater neuraminidase inhibitory activity. This is similar to reports of the potent inhibitory activity of the octadecyl glycoside of Neu5Ac.[52] In a previous study in which *C*-glycosides were prepared by replacement of the C-2 hydroxyl group with a hydroxylmethylene group, only very weak inhibitory effects towards *Vibrio cholerae* sialidase were observed.[53] This might be due to the high hydrophilicity of aglycone substitution, consistent with the current study. Studies on *S*-linked Neu5Ac (2→6) Glc and Gal demonstrated the importance of the C-4 configuration of the hexose residue, as well as the conformation about the *S*-glycosidic linkage on the measured K_i.[51,52] The current study shows that configuration at C-3 of the 2,3 linked Neu5Ac *C*-disaccharides **4** and **5** profoundly influences the observed K_i while the perturbation of the conformation about the *C*-glycosidic linkage **3** and **4** appears to have little effect on the K_i. Studies using liposomes,[54] polymerized liposomes,[20] polymers[55] and dendrimers[56] of Neu5Ac derivatives showed that multivalency can markedly enhance ligand interaction and biological effects. Future studies will examine whether improvements in *C*-glycoside neuraminidase inhibitory activity can be obtained by multivalency.

In summary, the present study shows that certain Neu5Ac *C*-glycosides are potent neuraminidase inhibitors displaying K_i values comparable to those of the transition state analogue Neu5Ac-2-ene (5-acetamido-2, 6-anhydro-3, 5-dideoxy-D-glycero-D-galacto-non-2-enonic acid), which is currently in clinical trials as an anti-influenza agent.[40,41,57] A neuraminidase based on Neu5Ac *C*-glycosides mimetic of the natural *O*-glycoside substrate may take advantage of both tightly binding and resistance enzyme cleavage. This property may lead to longer retention time in vivo and higher inhibitory effects affording a new class of antibacterial and antiviral agents.

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References and Notes

1. Varki, A. *Glycobiology* **1993**, *3*, 97.
2. Crocker, P. R.; Feizi, T. *Curr. Opin. Struct. Biol.* **1996**, *6*, 679.
3. Varki, A. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7390.
4. Moremen, K. W.; Trimble, R. B.; Herscovics, A. *Glycobiology* **1994**, *4*, 113.
5. Kornfeld, R.; Kornfeld, S. *Annu. Rev. Biochem.* **1985**, *54*, 631.
6. Lal, A.; Pang, P.; Kalelkar, S.; Romero, P. A.; Herscovics, A.; Moremen, K. W. *Glycobiology* **1998**, *8*, 981.
7. Marshall, J. *J. Adv. Carbohydr. Chem. Biochem.* **1974**, *30*, 257.
8. Herrler, G.; Gross, H. J.; Brossmer, R. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 821.
9. Sauter, N. K.; Glick, G. D.; Crowther, R. L.; Park, S. J.; Eisen, M. B.; Skehel, J. J.; Knowles, J. R.; Wiley, D. C. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 324.
10. Toogood, P. L.; Galliker, P. K.; Glick, G. D.; Knowles, J. R. *J. Med. Chem.* **1991**, *34*, 3138.
11. Harms, G.; Reuter, G.; Corfield, A. P.; Schauer, R. *Glycoconjugate J.* **1996**, *13*, 621.
12. Swartley, J. S.; Marfin, A. A.; Edupugantis, D.; Liu, L. J.; Ceislak, P.; Perkins, B.; Wenger, J. D.; Stephans, D. S. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 271.
13. Kaijser, B.; Jodal, U. *J. Clin. Microbiol.* **1984**, *19*, 264.
14. Toyokuni, T.; Singhal, A. K. *Chem. Soc. Rev.* **1995**, 231.
15. Maclean, G. D.; Longenecker, B. M. *Can. J. Oncol.* **1994**, *4*, 249.
16. Livingston, P. O. *Cancer Biol.* **1995**, *6*, 357.
17. Colman, P. In *The Influenza Viruses*; Krug R., Ed.; Plenum: New York, 1989; pp 175–218.
18. Bamford, M. J. *J. Enz. Inhib.* **1996**, *10*, 1.
19. 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.
20. Spevak, W.; Nagy, J. O.; Charych, D. H.; Schaefer, M. E.; Gilbert, J. H.; Bednarski, M. D. *J. Am. Chem. Soc.* **1993**, *115*, 1146.
21. Arya, P.; Kutterer, K. M. K.; Qin, H.; Roby, J.; Barnes, M. L.; Kim, J. M.; Roy, R. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1127.
22. Kuribayashi, T.; Ohkawa, N.; Satoh, S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3307.
23. Molander, G.; Harris, C. *Chem. Rev.* **1996**, *96*, 307.
24. Mazeas, D.; Skrydstrup, T.; Beau, J.-M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 909.
25. Hung, S.-C.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2671.
26. Depouilly, P.; Chenede, A.; Mallet, J.-M.; Sinaÿ, P. *Bull. Soc. Chim. Fr.* **1993**, *130*, 256.
27. Vlahov, I. R.; Vlahova, P. I.; Linhardt, R. J. *J. Am. Chem. Soc.* **1997**, *119*, 1480.
28. Du, Y.; Linhardt, R. J. *Carbohydr. Res.* **1998**, *308*, 161.
29. Polat, T.; Du, Y.; Linhardt, R. J. *Synlett* **1998**, 1195.
30. Bazin, H. G.; Du, Y.; Polat, T.; Linhardt, R. J. *J. Org. Chem.* **1999**, *64*, 7254.
31. Lopez, R. M.; Hays, D. S.; Fu, G. C. *J. Am. Chem. Soc.* **1997**, *119*, 6949.
32. Myers, A. G.; Movassaghi, M.; Zheng, B. *J. Am. Chem. Soc.* **1997**, *119*, 8572.
33. Entries **1** and **2** were synthesized[28,29] containing the peracetylated methyl ester of Neu5Ac and compds. **3**–**5**[27,30] also contained a benzyl protected galactose or gulose residue. Debenzylation of Entries **3**–**5** was accomplished by adding 50 mg to 10 mL of MeOH:EtOAc:H_2O (4:4:2) containing 1 drop of 80% HOAc (aq) and 15 mg of 5% Pd/C sealed with an H_2 containing balloon with stirring for 24 h at rt. The mixture was filtered and the filtrate recovered and peracetylated with Ac_2O in Py. These peracetylated *C*-disaccharides (or Entries **1** and **2**) were deacetylated by dissolving in MeOH (36 mg/9 mL) to which catalytic amount of NaOMe was added and stirred for 19 h at rt, 1 mL of 0.2 M KOH (aq) was added and stirred 8 h at rt, neutralized with Amberlite IR-120 (H^+), filtered, and desalted on a Bio-gel P2 column. ^1H NMR (500 MHz) analyses of **1**–**5** were consistent with structure.
34. Potier, M.; Mameli, L.; Belisle, M.; Dallaire, L.; Melancon, S. B. *Anal. Biochem.* **1979**, *94*, 287.
35. Incubation mixtures contained 50 μL of 0.1 M sodium acetate buffer, pH 5.0 and 0.04 to 1.0 mM MU-Neu5Ac to determine K_m and same substrate concentration with 0.01 mM to 0.1 mM inhibitor to determine K_i. Relative fluorescence was recorded for 30 min beginning immediately after 0.5 mU neuraminidase was added (in the blank the enzyme was omitted).

Release of the hydrolysis product 4-methylumbelliferone was determined using a Kontron Instrument, SFM 25 fluorimeter ($\lambda_{ex}=360$nm, $\lambda_{em}=440$ nm). Substrate had an $\lambda_{ex}=315$ nm and an $\lambda_{em}=375$ nm, therefore exhibiting only a negligible effect on the measurement.
36. Kleineidam, R. G.; Furuhata, K.; Ogura, H.; Schauer, R. *Biol. Chem. Hoppe-Seyler* **1990**, *371*, 715.
37. Cummings, R. D. In *Methods in Enzymology*; Lennarz, W. J.; Hart, G. W., Eds.; Academic: CA, 1994; Vol. 230, pp 66–85.
38. Shibuya, N.; Goldstein, I. J.; Broekaert, W. F.; Nsimba-Lubaki, M.; Peeters, B.; Peumans, W. J. *J. Biol. Chem.* **1987**, *262*, 1596.
39. Wang, W.-C.; Cummings, R. D. *J. Biol. Chem.* **1988**, *263*, 4576.
40. Itzstein, M. V.; Dyason, J. C.; Oliver, S. W.; White, H. F.; Wu, W.; Kok, G. B.; Pegg, M. S. *J. Med. Chem.* **1996**, *39*, 388.
41. Holzer, C. T.; Itzstein, M. V.; Jin, B.; Pegg, M. S.; Stewart, W. P.; Wu, W. *Glycoconjugate J.* **1993**, *10*, 40.
42. Taylor, N. R.; Cleasby, A.; Singh, O.; Skarzynski, T.; Wonacott, A. J.; Smith, P. W.; Sollis, S. L.; Howes, P. D.; Cherry, P. C.; Bethell, R.; Colman, P.; Varghese, J. *J. Med. Chem.* **1998**, *41*, 798.
43. Jones, P. S.; Smith, P. W.; Hardy, G. W.; Upton, R. J.; Bethell, R. C. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 605.
44. Smith, P. W.; Robinson, J. Ed; Evans, D. N.; Sollis, S. L.; Howes, P. D.; Trivedi, N.; Bethell, R. C. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 601.
45. Smith, P. W.; Trivedi, N.; Howes, P. D.; Sollis, S. L.; Rahim, G.; Bethell, R. C.; Lynn, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 611.
46. Hayden, F. G.; Treanor, J. J.; Betts, R. F.; Lobo, M.; Esinhart, J. D.; Hussey, E. K. *J Am. Med. Assoc.* **1996**, *275*, 295.
47. Kudo, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *J. Antibiot.* **1992**, *45*, 954.
48. Glanzer, B. I.; Gyorgydeak, Z.; Bernet, B.; Vasella, A. *Helv. Chim. Acta* **1991**, *74*, 343.
49. Ogawa, S.; Yoshikawa, M.; Taki, T. *J. Chem. Soc., Chem. Commun.* **1992**, 406.
50. Fitz, W.; Rosenthal, P. B.; Wong, C.-H. *Bioorg. Med. Chem.* **1996**, *4*, 1349.
51. Suzuki, Y.; Sato, K.; Kiso, M.; Hasegawa, A. *Glycoconjugate J.* **1990**, *7*, 349.
52. Sabesan, S.; Neira, S.; Wasserman, Z. *Carbohydr. Res.* **1995**, *267*, 239.
53. Wallimann, V. K.; Vasella, A. *Helv. Chim. Acta* **1991**, *74*, 1520.
54. Koketsu, M.; Nitoda, T.; Sugino, H.; Juneja, L. R.; Kim, M.; Yamamoto, T.; Abe, N.; Kajimoto, T.; Wong, C.-H. *J. Med. Chem.* **1997**, *40*, 3332.
55. Choi, S.-K.; Mammen, M.; Whitesides, G. M. *Chem. Biol.* **1996**, *3*, 97.
56. Palcic, M. M.; Li, H.; Zanini, D.; Bhella, R. S.; Roy, R. *Carbohydr. Res.* **1998**, *305*, 433.
57. Calfee, D. P.; Hayden, F. G. *Drugs* **1998**, *56*, 537.