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Synthesis of *O*-Glycolyl-Linked Neuraminic Acids through a Spirocyclic Intermediate

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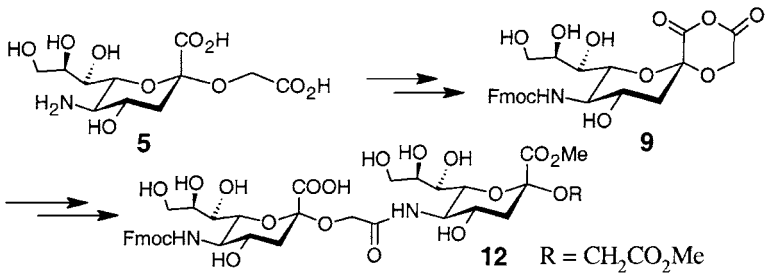
ABSTRACT



The neuraminic acid derivative 5 is readily converted in several steps to the neuraminic acid dimer 12, linked through the hydroxyl of a 5-*N*-glycolyl group in an α-2,5 glycosidic linkage. The sequence is shown to proceed through a spirocyclic intermediate 9 by in situ NMR experiments. Similar derivatives of *N*-glycolylneuraminic acid (Neu5Gc), including polymers, have been identified from marine sources, including starfish and sea urchins, often as sulfated derivatives and are thought to mediate sperm–egg recognition.

O-Glycolyl-linked oligosialic acids are novel carbohydrate polymers initially identified as components of glycoproteins associated with the egg jelly coat of sea urchins.[1] The repeating unit **1** (Figure 1) consists of a 5-*N*-glycolyl-neuraminic acid unit (Neu5Gc) linked to adjacent sugars through the glycolyl hydroxyl in an α-2,5 glycosidic linkage. Subsequent studies have suggested that 9-O-sulfated Neu5Gc oligomers are involved in sperm−egg recognition in a number of sea urchin species.[2] Smaller oligomers, including the dimer and trimer, are produced by the starfish *Asterias rubens* as their 8-O-methylated derivatives.[3]

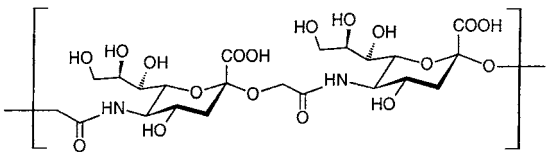


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Figure 1. Repeating unit of poly-*N*-glycolylneuraminic acid.

Despite the unique structures of these molecules, little effort has been directed toward their chemical synthesis. This challenge led us to examine methods for the assembly of *O*-glycolyl-linked sialic acids that could be readily extended to the synthesis of oligomers. Rather than employing a glycosylation-based approach for the assembly of the *O*-glycolyl-linkage, we adapted a strategy first developed by Gervay et al. for the synthesis of neuraminic acid oligomers linked through the C-1 carboxylate group.[4]

(1) Kitazume, S.; Kitajima, K.; Inoue, S.; Troy, F. A.; Cho, J.-W.; Lennarz, W. J.; Inoue, Y. *J. Biol. Chem.* **1994**, *269*, 22712−22718. (b) Karamanos, N. K.; Manouras, A.; Anagnostides, S.;. Makatsori, E.; Tsegenidis, T.; Antonopoulos, C. A. *Biochimie* **1996**, *78*, 171−182. (c) Kitazume, S.; Kitajima, K.; Inoue, S.; Haslam, S. M.; Morris, H. R.; Dell, A.; Lennarz, W. J.; Inoue, Y. *J. Biol. Chem.* **1996**, *271*, 6694−6701.

(2) Kitazume-Kawaguchi, S.; Inoue, S.; Inoue, Y.; Lennarz, W. J. *Proc. Natl. Acad. Sci.* **1997**, *94*, 3650−3655.

(3) (a) Bergwerff, A. A.; Hulleman, S. H. D.; Kamerling, J. P.; Vliegenthart, J. F. G.; Shaw, L.; Reuter, G.; Schauer, R. In *Polysialic Acid*; Roth, J., Rutishauser, U., Troy, F., II, Eds.; Birkhaeuser: Basel, 1993; pp 201−272. (b) Kitazume-Kawaguchi, S. *Trends Glycosci. Glycotechnol.* **1998**, *10*, 383−392.

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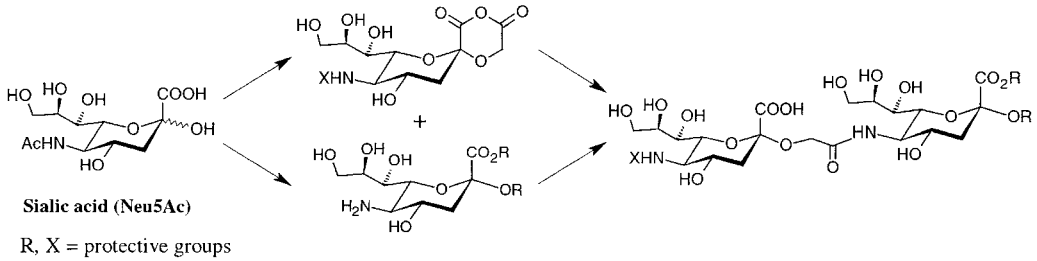
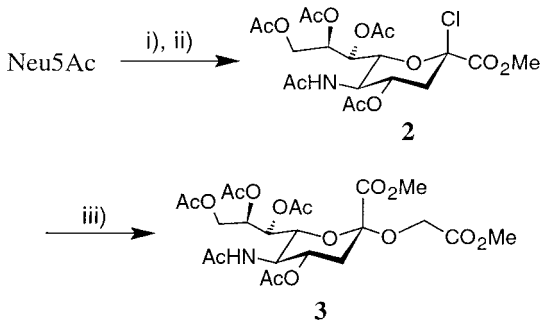
Scheme 1. Strategy for the Assembly of the Neu5Gc-α2,5-$O_{glycolyl}$-Neu5Gc Linkage



A similar approach was recently applied by Ren et al. and led to the synthesis of a Neu5Gcα2,5-$O_{glycolyl}$Neu5Gc dimer.[5] We now describe the synthesis of a novel spirocyclic sialoside intermediate **9**, which undergoes a regioselective ring opening with a minimally protected neuraminic acid derivative to give an α-2,5-linked Neu5Gc dimer set up for further extension (Scheme 1).

A direct route to pure α-glycolyl sialosides was crucial to our approach. Previous studies have shown that high yields of simple aliphatic α-sialosides could be obtained from treatment of sialyl donors with lower alcohols in the presence of insoluble promoters such as silver zeolite.[6] In our hands, conversion of *N*-acetylneuraminic acid to the 2-chloro derivative **2**,[7] followed by treatment with methyl glycolate and silver zeolite in toluene, gave the α-glycolyl glycoside **3** in 69% overall yield. Stereoselectivity was judged as being better than 15:1 in favor of the α-anomer (Scheme 2).

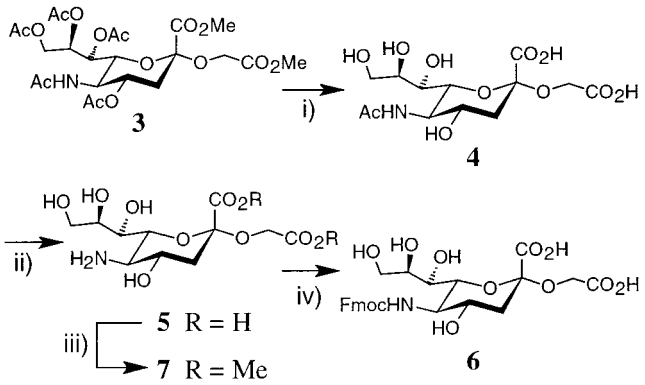
Scheme 2[a]



[a] Reaction conditions: (i) MeOH, H^+. (ii) AcOH, AcCl. (iii) Me glycolate, Ag zeolite, toluene, rt, 30 h, 69% over three steps.

We next directed our attention to the synthesis of a suitably protected intermediate that could act as both acceptor and donor. Thus, treatment of compound **3** with 1 M NaOH in aqueous methanol over 2 h, followed by acid resin, gave the diacid **4** in near quantitative yield. Further treatment of compound **4** with 2 M NaOH at 95 °C gave the amino acid **5** (89%) following neutralization with acid resin and purification on Sephadex LH-20. The amino acid **5** was subsequently converted to both the *N*-Fmoc **6** and methyl ester **7** derivatives (Scheme 3).

Scheme 3[a]



[a] Reaction conditions: (i) NaOH, MeOH/H_2O then H^+ resin, 93%. (ii) 2 M NaOH, 95 °C, 10 h then H^+ resin, 89%. (iii) HCl/MeOH (1:99), 12 h, 71%. (iv) Fmoc-Cl, $NaHCO_3$, dioxane/H_2O, then H^+ resin, 51%.

The ^{1}H NMR spectrum of the diacid **4** in DMF-d_7 contained two doublets at δ 4.24 and 4.35 with a coupling constant of 16.1 Hz corresponding to the glycolyl methylene protons. Upon addition of diisopropylcarbodiimide (DIC) (1.2 equiv) to the NMR tube, these signals were replaced by a singlet at δ 4.94, attributed to the formation of a spirocyclic anhydride **8** (Scheme 4). A similar experiment was performed in acetonitrile (ACN) and monitored by electrospray MS in the negative mode. Addition of *N*-(3-dimethylaminopropyl)-*N*′-ethyl carbodiimide (EDAC) to a solution of **4** (*m*/*z* 367.1) in ACN resulted in the appearance of new signals at *m*/*z* 348.1 and 384.0 corresponding to the expected values for $[M - H^+]^-$ and $[M + Cl^-]^-$ of the anhydride **8**.

The anhydride **8** was also observed following treatment of **4** with a resin-bound carbodiimide (NovaBiochem) and showed no signs of decomposition after standing at room temperature for 30 min. Addition of benzylamine (1.2 equiv)

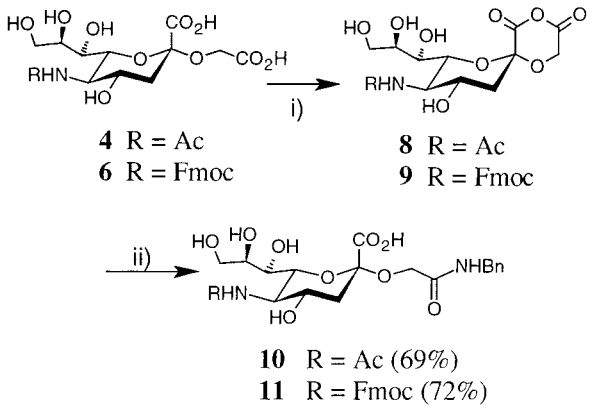
(4) (a) Gervay, J.; Flaherty, T. M.; Nguyen, C. *Tetrahedron Lett.* **1997**, *38*, 1493−1496. (b) Ramamoorthy, P. S.; Gervay, J. *J. Org. Chem.* **1997**, *62*, 7801−7805.

(5) Ren, C.-T.; Chen, C.-S.; Wu, S.-H. *J. Org. Chem.* **2002**, *67*, 1376−1379.

(6) Ossowski, P.; Garegg, P. J. *Acta Chem. Scand.* **1983**, *B 37*, 249−250.

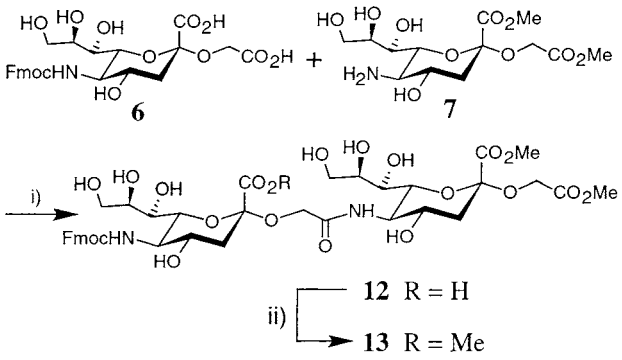
(7) Roy, R.; Laferreire, C. *Can. J. Chem.* **1990**, *68*, 2045−2054.

Scheme 4[a]



[a] Reaction conditions: (i) DIC, DMF. (ii) $BnNH_2$ (1.2 equiv), 5 min.

Scheme 5[a]



[a] Reaction conditions: (i) pyBOP, DIEA, NMP, 3 h, 57%. (ii) MeI, DIEA, DMF, 24 h, 62%

rapidly (ca. 2−3 min) produced the benzyl amide **10**, derived from attack at the less hindered glycolyl carbonyl. This assignment was confirmed by an examination of long-range carbon−proton coupling through the acquisition an HMBC spectrum. The glycolyl carbonyl signal at δ 172.3 correlated to the glycolyl methylene protons at δ 4.37 and 4.23, as well as the benzyl methylene protons at δ 4.42.

Repetition of this sequence with the *N*-Fmoc diacid **6** resulted in a similar outcome, producing a compound upon treatment with carbodiimide resin that was identified as the spirocyclic anhydride **9** on the basis of NMR spectra.[8] Treatment of the anhydride **9** with a slight excess of benzylamine gave the benzyl amide **11** in good yield in under 5 min. Selectivity for the desired regioisomer was judged as being 8:1 or better from the HMBC NMR spectrum. In this case, the signal at δ 172.1 (glycolyl *C*ONH) was found to correlate to signals at δ 4.34 and 4.12 (glycolyl CH_2) and δ 4.42 (benzyl CH_2).

Having obtained favorable results in the above model systems, we turned our attention to the synthesis of *O*-glycolyl-linked sialyl dimers. Treatment of the diacid **6** with a slight excess of a coupling reagent was followed after 2 min with a solution of the amine **7**. Best results were obtained with the coupling agent pyBOP and diisopropylethylamine (DIEA) in *N*-methylpyrrolidone (NMP). Solid-phase extraction followed by chromatography on silica gel gave the dimer **12** in 57% yield. In all instances the desired product was formed swiftly with little evidence of the unwanted regioisomer.[9]

In this case, the complexity of the HMBC NMR spectrum of compound **12** did not allow unambiguous structural assignment, so this compound was further converted to the methyl ester **13** with methyl iodide and DIEA. The ^{1}H NMR spectrum of compound **13** exhibited singlets at δ 3.83 and 3.81, corresponding to methoxyl groups at C-1 and C-1′, in addition to a singlet at δ 3.70 arising from the glycolyl methyl ester functionality at C-2. This evidence, combined with the outcome of the model experiments and comparison to published spectra of similar compounds,[3a,5] led us to conclude that the ring opening of the spirocyclic anhydride **9** occurred preferentially through attack at the less hindered glycolyl carbonyl functionality.

In conclusion, we have successfully demonstrated the formation of the naturally occurring Neu5Gcα2,5-$O_{glycolyl}$-Neu5Gc linkage by an efficient procedure that should be amenable to solid-phase synthesis and the construction of higher oligomers.[10] In addition, the spirocyclic sialosides **8** and **9** derived from the amino acid **5** could serve as versatile reagents for the formation of sialoconjugates under very mild conditions.

Acknowledgment. This work was supported in part by a grant from the National Institutes of Health.

Supporting Information Available: Experimental and analytical details for compounds **3**−**13**, including NMR and mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) ^{1}H NMR for compound **8** ^{1}H NMR (300 MHz, DMF-d_7): δ 8.13 (d, N*H*); 4.94 (s, 2H, OCH_2CO); 2.58 (dd, $J_{3,3}$ = 12 Hz, $J_{3,4}$ = 5 Hz, H-3$_{ax}$); 1.97 (s, 3H, COMe); 1.72 (dd, $J_{3,4}$ = 10 Hz, H-3$_{eq}$). For compound **9** (300 MHz, DMF-d_7): δ 7.27−7.98 (m, 9H, Ar*H*, N*H*); 4.93 (s, 2H, OCH_2CO); 4.24−4.36 (m, 3H); 3.40−3.95 (m, 7H); 2.59 (dd, $J_{3,3}$ = 13.4 Hz, $J_{3,4}$ = 5.4 Hz, H-3$_{ax}$); 1.81 (dd, $J_{3,4}$ = 11.2 Hz, H-3$_{eq}$).

(9) Analytical data for compound **12** ^{1}H NMR (300 MHz, CD_3OD): δ 7.26−7.80 (m, 8H, Ar*H*); 4.41, 4.31 (AB pattern, J_{gem} = 16.5 Hz, OCH_2-CO_2Me); 4.32, 4.16 (AB pattern, J_{gem} = 16.4 Hz, OCH_2CONH); 3.81, 3.70 (2s, 6H, CO_2*Me*); 3.48−3.94 (m, 12H); 3.10−3.24 (m, 2H), 2.76, 2.84 (2dd, 2H-3$_{ax}$); 1.74 (bt, 2H, H-3$_{eq}$). Found, *m*/*z* 905.3; calcd, 905.4 [M + Na]$^+$. For compound **13** ^{1}H NMR (300 MHz, CD_3OD): δ 7.24−7.80 (m, 8H, Ar*H*); 4.39, 4.31 (AB pattern, J_{gem} = 16.2 Hz, OCH_2CO_2Me); 4.35, 4.05 (AB pattern, J_{gem} = 15.8 Hz, OCH_2CONH); 3.83, 3.81 (2s, 6H, CO_2*Me*); 3.70 (s, 3H, glycolyl CO_2*Me*); 3.45−3.90 (m, 14H); 2.68, 2.76 (2dd, 2H-3$_{ax}$); 1.75, 1.92 (2dd, 2H, H-3$_{eq}$). Found, *m*/*z* 919.2; calcd, 919.5 [M + Na]$^+$.

(10) Szabo, L.; Smith, B. L.; McReynolds, K. D.; Parrill, A. L.; Morris, E. R.; Gervay, J. *J. Org. Chem.* **1998**, *63*, 1074−1078.