

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

Joseph C. M^cAuliffe, David Rabuka and Ole Hindsgaul*

The Burnham Institute, 10901 North Torrey Pines Road, San Diego, CA, USA, 92037

Supplementary Material

Materials and Methods

General

Chemicals and solvents were obtained from Aldrich Chemical Co. (Milwaukee, WI) and were used as received. Carbodiimide resin was obtained from Novabiochem (San Diego, CA). Molecular sieves (Aldrich) were held at 160°C for 48 h and cooled under vacuum immediately before use. Thin-layer chromatography was performed on silica gel 60 glass-backed plates obtained from Merck (Germany). Plates were developed using the specified solvents and developed by heating after treatment with 10% H₂SO₄ in ethanol. Flash chromatography was performed with silica gel (230-400 mesh) obtained from Fluka (Switzerland). ¹H-NMR spectra were acquired on a Varian 300 MHz INOVA NMR spectrometer. Mass spectra were obtained using either a PE Biosystems Voyager DE-RP MALDI-TOF mass spectrometer or a Thermo Finnigan TSQ Quantum triple quadrupole mass spectrometer.

Experimental for compounds 3-13:

Compound 3

A solution of the 2-chloro compound **2** (2.0 g, 3.93 mmol) in toluene (6 mL) was added to a well stirred slurry of methyl glycolate (2.0 mL, 26 mmol) and silver zeolite (3.6 g) in toluene (10 mL) at 0°C. After stirring at room temperature for 24 h, the mixture was filtered and concentrated to give a yellow oil. Purification of the crude product on a silica gel column eluted with toluene/acetone (3:1) gave the α -sialoside **3** in 79% yield. ¹H NMR (300 MHz, CDCl₃): δ 5.35 (ddd, $J_{7,8}$ = 8.7 Hz, $J_{8,9}$ = 2.7, 5.4 Hz, H-8); 5.28 (dd, $J_{6,7}$ = 1.8 Hz, H-7); 5.17 (d, J_{NH} = 9.6 Hz, NH); 4.90 (ddd, $J_{3,4}$ = 4.5, 12.3 Hz, $J_{4,5}$ = 10.2 Hz, H-4); 4.31, 4.16 (AB pattern, J_{gem} = 16.5 Hz, OCH₂CO); 4.23 (dd, $J_{9,9}$ = 12.6 Hz, H-9); 4.04 (dd, H-9); 4.01 (t, $J_{5,6}$ = 10.5 Hz, H-5); 3.94 (dd, H-6); 3.77, 3.71 (2s, 6H, CO₂Me); 2.68 (dd, $J_{3,3}$ = 12.6 Hz, H-3_{ax}); 2.11, 2.02, 2.01, 1.85 (4s, 15H, COMe); 1.99 (t, H-3_{eq}). ¹³C NMR (75 MHz, CDCl₃): δ 171.0, 170.6, 170.2, 170.1, 170.0, 169.6, 167.5 (CH₃CO, glycolyl C=O, C-1); 98.1 (C-2); 72.5, 68.8, 68.1, 67.0 (C-4, -6, -7, -8); 62.3, 61.8 (glycolyl CH₂, C-9); 53.0, 52.0, 49.2 (CO₂CH₃, C-5); 37.5 (C-3); 23.2, 21.1, 20.9, 20.8, 20.7 (CH₃CO). Found: m/z 586.19; calc: 586.17 [M+Na]⁺.

Compound 4

The protected sialoside **3** (180 mg, 0.31 mmol) was treated with 1M NaOH in 50% aqueous methanol (4 mL) over 2 h. The solution was then treated with Amberlite IR-120, H⁺ acid resin, filtered and concentrated to give the diacid **4** (105 mg, 93%). ¹H NMR (300 MHz, d₇-DMF): δ 8.07 (d, NH); 4.35, 4.24 (AB pattern, J_{gem} = 16.1 Hz, OCH₂CO); 2.75 (dd, $J_{3,3}$ = 12.6 Hz, $J_{3,4}$ = 4.5 Hz, H-3_{ax}); 1.97 (s, 3H, COMe); 1.72 (dd, $J_{3,4}$ = 10.9 Hz, H-3_{eq}). Found: m/z 366.0; calc: 366.0 [M-H]⁻.

Compound 5

The protected sialoside **3** (849 mg, 1.5 mmol) was treated with 2M NaOH (15 mL) at 95°C over 12 h and was followed by adjustment of the solution to pH 7 with Amberlite IR-120, H⁺ acid resin. Purification of the mixture on Sephadex LH-20 gave the amino acid **5** (449 mg, 89%). ¹H NMR (300 MHz, D₂O): δ 4.12, 3.83 (2d, AB pattern, $J_{\text{gem}} = 15.2$ Hz, OCH₂CO); 3.12 (t, $J_{4,5} = 10.2$ Hz, H-5); 2.75 (dd, $J_{3,3} = 12.1$ Hz, $J_{3,4} = 5.1$ Hz, H-3_{ax}); 1.77 (t, $J_{3,4} = 11.8$ Hz, H-3_{eq}). ¹³C NMR (75 MHz, D₂O): δ 175.1, 171.6, (glycolyl C=O, C-1); 98.0 (C-2); 72.3, 69.7, 68.2, 65.9 (C-4, -6, -7, -8); 60.9, 60.3 (glycolyl CH₂, C-9); 50.2 (C-5); 37.5 (C-3). Found: m/z 324.08; calc: 324.09 [M-H⁺].

Compound 6

The amino acid **5** (812 mg, 2.0 mmol) in H₂O/dioxane (20 mL, 1:1) was treated with K₂CO₃ (640 mg, 4.6 mmol) and a solution of Fmoc-Cl (1.2 g, 4.6 mmol) in dioxane (10 mL) at 5°C and stirred overnight. The reaction mixture was then concentrated and partitioned between H₂O and ether. The aqueous phase was then adjusted to pH 3 with Amberlite IR-120, H⁺ acid resin and applied to a short column of C-18 reverse phase silica. Elution with MeOH/H₂O gave the diacid **6** (550 mg) as an oil in average yield (51%). ¹H NMR (300 MHz, CD₃OD): δ 7.22-7.81 (m, 8H, Ar H); 4.35, 4.19 (AB pattern, $J_{\text{gem}} = 16.2$ Hz, OCH₂CO); 4.35 (m, 2H, CHCH₂O); 4.21 (bt, CHCH₂O); 3.52-3.86 (m, 7H, H-4,5,6,7,8,9,9); 2.80 (dd, $J_{3,3} = 12.6$ Hz, $J_{3,4} = 4.8$ Hz, H-3_{ax}); 1.76 (t, $J_{3,4} = 12.1$ Hz, H-3_{eq}).

Compound 7

The amino acid **5** (412 mg, 1.2 mmol) in MeOH (80 mL) was treated with 2M HCl in ether (10 mL) overnight at 4°C. The solution was adjusted to pH 7 with Amberlite IRA-400, OH⁻ base resin and concentrated to give an oil. Purification of this oil on silica gel gave the *bis*-methyl ester **7** in good yield (300 mg, 71%). ¹H NMR (300 MHz, CD₃OD): δ 4.37, 4.30 (AB pattern, 2H, OCH₂CO); 3.80, 3.71 (2s, 6H, CO₂Me); 3.63-3.88 (m, 5H, H-7,8,9,9); 3.40-3.51 (m, 2H, H-4,6); 2.82 (t, $J_{4,5} = J_{5,6} = 10$ Hz, H-5); 2.69 (dd, $J_{3,3} = 12.5$ Hz, $J_{3,4} = 4.6$ Hz, H-3_{ax}); 1.77 (t, $J_{3,4} = 12$ Hz, H-3_{eq}).

Compounds 8 and 10

a) The diacid **4** (30.5 mg, 80 μmol) in d₇-DMF (750 μL) was transferred to an NMR tube and the ¹H NMR spectrum acquired. The glycolyl CH₂ protons were represented by two doublets at δ 4.35 and 4.24 with a geminal coupling constant of 16.1 Hz. Addition of diisopropylcarbodiimide (DIC) (15 μL, 100 μmol) resulted in the appearance of a new signal at δ 4.94 in place of the AB pattern observed for the glycolyl protons before addition of the carbodiimide. This transition was also seen when carbodiimide resin (Novabiochem) was used in place of DIC and was attributed to the formation of the cyclic anhydride **8**. Attempts to isolate this material were not successful. ¹H NMR (300 MHz, d₇-DMF): δ 8.13 (d, NH); 4.94 (s, 2H, OCH₂CO); 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}).

Addition of benzylamine (12 μL, 110 μmol) to the NMR tube resulted in the rapid disappearance (2-3 minutes) of the signal at δ 4.94 and the appearance of an AB pattern with doublets at δ 4.37, 4.23. Purification of the new compound over silica gel gave the benzyl amide **10** (28 mg, 72%). ¹H NMR for compound **10** (300 MHz, CD₃OD): δ 7.19-7.27 (m, 5H, ArH); 4.42 (s, 2H, PhCH₂); 4.37, 4.23 (AB pattern, $J_{\text{gem}} = 15.1$ Hz, OCH₂CO); 3.59-3.98 (m, 7H, H-4,5,6,7,8,9,9); 2.74 (bdd, H-3_{ax}); 2.00

(s, 3H, COMe); 1.74 (bt, H-3_{eq}). ¹³C NMR (75 MHz, CD₃OD): δ 175.1 (C-1); 172.3 (glycolyl C=O); 139.6 (ArC); 129.6, 128.7, 128.3 (ArCH); 101.4 (C-2); 74.8, 72.6, 69.7, 69.0 (C-4, -6, -7, -8); 64.3, 63.7 (glycolyl CH₂, C-8); 54.1 (C-5); 43.8 (ArCH₂NH); 40.8 (C-3); 22.8 (CH₃CO). HMBC long range ¹H/¹³C correlation NMR; Signal at δ 172.3 (glycolyl CONH) correlates to signals at δ 4.37 and 4.23 (glycolyl CH₂), and δ 4.42 (benzyl CH₂). Signal at δ 175.1 (C-1) correlates to signal at 1.74 (H-3_{eq}). Found: m/z 479.1; calc: 479.2 [M+Na]⁺.

- b) A slurry of **4** (5.0 mg, 13.6 μmol) in ACN (1 mL) was treated with *N*-(3-dimethylaminopropyl)-*N*'-ethyl carbodiimide (EDAC) (2.8 mg, 15 μmol). Aliquots (10 μL) of the reaction mixture were removed before and after addition of the carbodiimide and were diluted into MeOH (990 μL) prior to infusion into an electrospray mass spectrometer operating in negative mode. The sample before EDAC treatment gave a signal at m/z 366.1, corresponding to [M-H]⁺ for the diacid **4**. Carbodiimide treatment 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**.

Compounds 9 and 11

The diacid **6** (25 mg, 45.7 μmol) in d₇-DMF (750 μL) was transferred to an NMR tube and the ¹H NMR spectrum acquired. Addition of DIC (9 μL, 57 μmol) to the tube resulted in the disappearance of two doublets at δ 4.19 and 4.35 and the appearance of a singlet at δ 4.93 integrating for two protons over 5 minutes. As before, this transition was also seen when carbodiimide resin (Novabiochem) was used in place of DIC and was attributed to the formation of the cyclic anhydride **9**. ¹H NMR (300 MHz, d₇-DMF): δ 7.27-7.98 (m, 9H, ArH, NH); 4.93 (s, 2H, OCH₂CO); 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}).

Addition of benzylamine (6 μL, 55 mmol) to the NMR tube resulted in the disappearance over 2-3 minutes of the singlet at δ 4.93 and the appearance of an AB pattern with doublets centered at δ 4.34 and 4.12, as expected for the benzyl amide **11**. Purification of the new compound over silica gel gave the benzyl amide **10** (21 mg, 69%) ¹H NMR (300 MHz, d₇-DMF): δ 7.20-7.96 (m, 13H, ArH); 4.34, 4.12 (AB pattern, *J*_{gem} = 15.4 Hz, OCH₂CO); 4.22-4.45 (m, 5H); 3.50-3.96 (m, 7H, H-4,5,6,7,8,9,9); 2.95 (dd, H-3_{ax}); 1.59 (t, H-3_{eq}). ¹³C NMR (75 MHz, CD₃OD): δ 172.1 (glycolyl C=O); 170.9 (C-1); 159.2 (Fmoc C=O); 145.0, 144.7, 141.8, 141.7, 140.6 (ArC); 129.9-120.7 (ArCH); 101.2 (C-2); 74.2, 72.6, 70.5, 68.8 (C-4, -6, -7, -8); 67.2, 64.8, 64.5 (Fmoc CH₂O, glycolyl CH₂, C-8); 55.6 (C-5); 47.7 (FmocCH₂); 42.7 (C-3). HMBC long range ¹H/¹³C correlation NMR; Signal at δ 172.1 (glycolyl CONH) correlates to signals at δ 4.34 and 4.12 (glycolyl CH₂), and δ 4.42 (benzyl CH₂). Found: m/z 659.4; calc: 659.4 [M+Na]⁺.

Compound 12

The diacid **6** (302 mg, 0.55 mmol) and the amine **7** (185 mg, 0.52 mmol) in NMP (10 mL) were treated with DIEA (300 μL, 1.72 mmol) and pyBOP (300 mg, 0.58 mmol) with stirring. TLC after 3 h indicated incomplete reaction and thus extra DIEA (100 μL) and pyBOP (100 mg) were added. The reaction mixture was diluted with water (20 mL) and applied to C-18 reverse phase silica. Elution with MeOH gave an oil that was subsequently purified on a column of silica gel to give the disialoside **12** as a colorless oil (230 mg, 57%). ¹H NMR (300 MHz, CD₃OD): δ 7.26-7.80 (m, 8H, ArH); 4.41,

4.31 (AB pattern, $J_{\text{gem}} = 16.5$ Hz, $\text{OCH}_2\text{CO}_2\text{Me}$); 4.32, 4.16 (AB pattern, $J_{\text{gem}} = 16.4$ Hz, OCH_2CONH); 3.81, 3.70 (2s, 6H, CO_2Me); 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}). ^{13}C NMR (75 MHz, CD_3OD): δ 171.0, 170.7, 169.9 (C-1, C1', glycolyl C=O), 157.45 (Fmoc C=O); 143.9, 143.6, 140.7, 140.6 (ArC); 127.5, 127.1, 125.3, 120.1 (ArCH); 99.3, 97.7 (C-2, -2'); 72.6, 72.3, 71.8, 70.7, 69.0, 69.9, 67.8, 67.3, 66.2, 65.7, 63.7, 63.4, 62.8, 60.6, 54.0, 52.5, 51.5, 51.4, 48.6, 46.6. Found: m/z 905.3; calc 905.4 $[\text{M}+\text{Na}]^+$.

Compound 13

The diacid **6** (185 mg, 0.34 mmol) in DMF (10 mL) was treated with pyBOP (203 mg, 0.39 mmol) and DIEA (150 μL) over 10 min followed by the amine **7** (115mg, 0.33 mmol). TLC (ACN/AcOH/ H_2O , 4:1:1) after 15 min showed a new higher rf compound and the absence of the majority of the starting materials. Methyl iodide (100 μL , 1.6 mmol) and additional DIEA (150 μL) were added resulting in the slow conversion to a new compound (TLC). Isolation of this material on C18 reverse phase silica was followed by purification over silica (DCM/MeOH, 1:9) and gave the disialoside **13** (105 mg, 35%). ^1H NMR (300 MHz, CD_3OD): δ 7.24-7.80 (m, 8H, ArH); 4.39, 4.31 (AB pattern, $J_{\text{gem}} = 16.2$ Hz, $\text{OCH}_2\text{CO}_2\text{Me}$); 4.35, 4.05 (AB pattern, $J_{\text{gem}} = 15.8$ Hz, OCH_2CONH); 3.83, 3.81 (2s, 6H, CO_2Me); 3.70 (s, 3H, glycolyl CO_2Me); 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; calc 919.5 $[\text{M}+\text{Na}]^+$.