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Ring Opening of Sialyllactones with Glycine Esters: Synthesis of Selectively Protected Glycinyl-NeuAc Saccharopeptides§

Jacquelyn Gervay,* P.S. Ramamoorthy, and Nellie N. Mamuya

Department of Chemistry, The University of Arizona, Tucson, Arizona, 85721

Abstract: Two different classes of sialyllactones were prepared as potential substrates for ring opening reactions with naturally occurring amino acids. The sialyllactones underwent reaction with glycine ethyl ester hydrochloride salt to afford selectively protected glycine-NeuAc adducts. The reactions could be performed on NeuAc derivatives capable of serving as glycosylation donors. These compounds represent a new class of saccharopeptides, composed of sugar amino acids and naturally occurring amino acids. © 1997 Elsevier Science Ltd.

N-Acetyl neuraminic (NeuAc) (**1**) acid is an important component of cell surface glycoconjugates involved in cellular recognition processes.[1] Synthesis of small molecule analogs of NeuAc is an active area of research in a number of laboratories, particularly in the context of making neuraminidase inhibitors.[2] In our laboratories, we are exploring the possibility of preparing NeuAc analogs that can be incorporated into peptide bond forming protocols.[3] In the past few years, several papers have appeared in the literature describing syntheses of sugar amino acid equivalents and their incorporation into saccharopeptides.[4] Classical peptide-bond forming reactions are typically employed in coupling sugar amino acids to each other and to naturally occurring amino acids, but there are far fewer examples of the latter coupling. In this report, we describe an efficient route to selectively protected glycine-NeuAc saccharopeptides via ring opening of sialyllactones.

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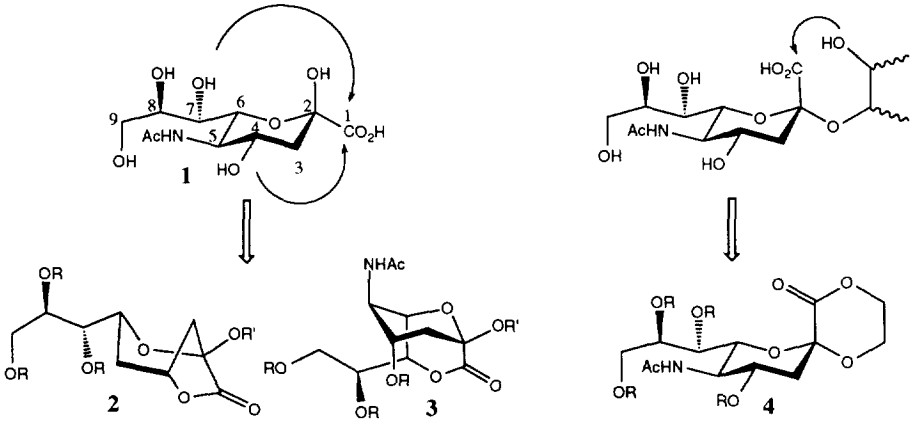
§ Affectionately dedicated to Professor Samuel J. Danishefsky for leading by example in his relentless pursuit of excellence.

Sialylglycoconjugates are susceptible to intramolecular esterification due to the proximity of the anomeric carboxylic acid to neighboring hydroxyl groups. When the carboxylic acid is in the equatorial position, cyclization occurs either at the C-4 or C-7 hydroxyl whereas the axial carboxylic acid can only undergo spirocyclization (Figure 1). Sialyllactones are naturally occurring compounds,[5] and they are frequently reported as byproducts in syntheses of NeuAc containing molecules.[6] It occurred to us that sialyllactones could serve as useful synthetic intermediates, providing a unique opportunity to achieve selective deprotection of strategic hydroxyl groups present on the NeuAc molecule. For example, ring opening reactions of sialyllactones **2-4** would provide routes to NeuAc analogs having the C-4, the C-7, and the glycosidic hydroxyls selectively unprotected. Since some of the most potent neuraminidase inhibitors known require C-4 manipulation,[2] we initially concentrated our efforts on the synthesis of 1,4-sialyllactones and their subsequent ring opening reactions.

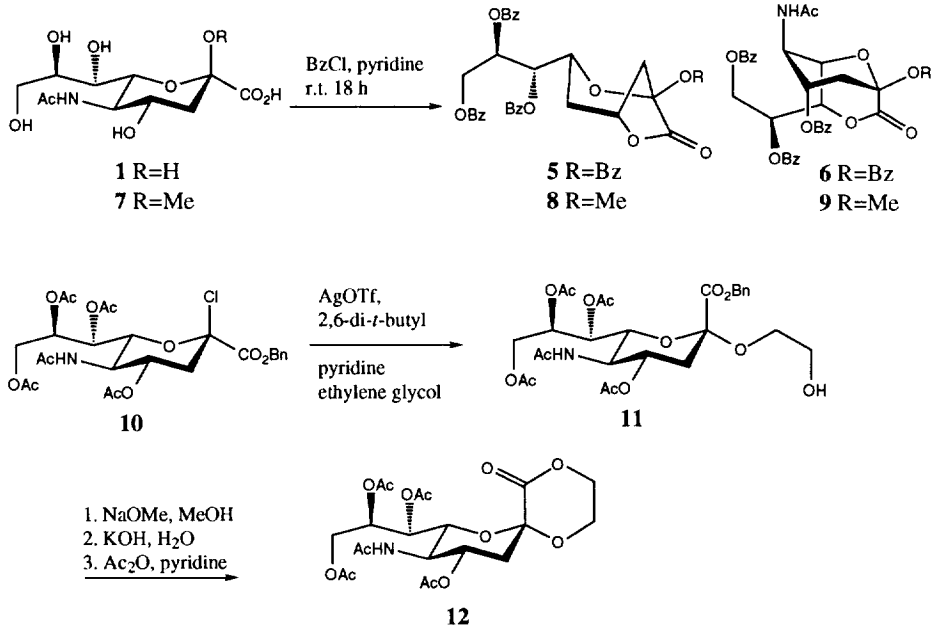
Figure 1



Our synthetic design included having the option to perform glycosylation reactions after having formed the amino acid-NeuAc adduct. Therefore, the anomeric benzoyl 1,4-lactone (**5**) was targeted in addition to the methyl glycoside analog (**8**). Syntheses of the sialyllactones are outlined in Scheme 1. NeuAc (**1**) was reacted with benzoyl chloride in pyridine according to the procedure of Sato et al. to provide a 1:1.3 ratio of the 1,4- and 1,7-sialyllactones, **5** and **6** respectively.[7] Interestingly, under the same reaction conditions the β-methyl glycoside of NeuAc (**7**)[8] preferentially provided the 1,4-lactone in a 3.6:1 ratio of **8**:**9** in 62% yield.[9]

In order to prepare the spirocyclic lactone **12**, the anomeric chloride of peracetylated NeuAc benzyl ester (**10**)[10] (1 eq.) was activated with silver triflate (1.2 eq.) in the presence of ethylene glycol (4 eq.) and 2,6-di-*tert*-butyl pyridine (2.0 eq.) to give **11**. The benzyl ester facilitates the observed α-selectivity by stabilizing the oxonium intermediate through anchimeric assistance. The axial carbonium ion is less stable and more reactive than the equatorial anomer due to the reverse anomeric effect, and equatorial attack preferentially occurs.[10] Spontaneous lactonization often occurs in sialylglycoconjugates structurally related to **11**,[6] but that was not the case under our reaction conditions. In order to effect lactonization, the α-glycoside **11** was deprotected to form the carboxylic acid which underwent spirocyclization upon reacetylation providing **12** in 47% yield.

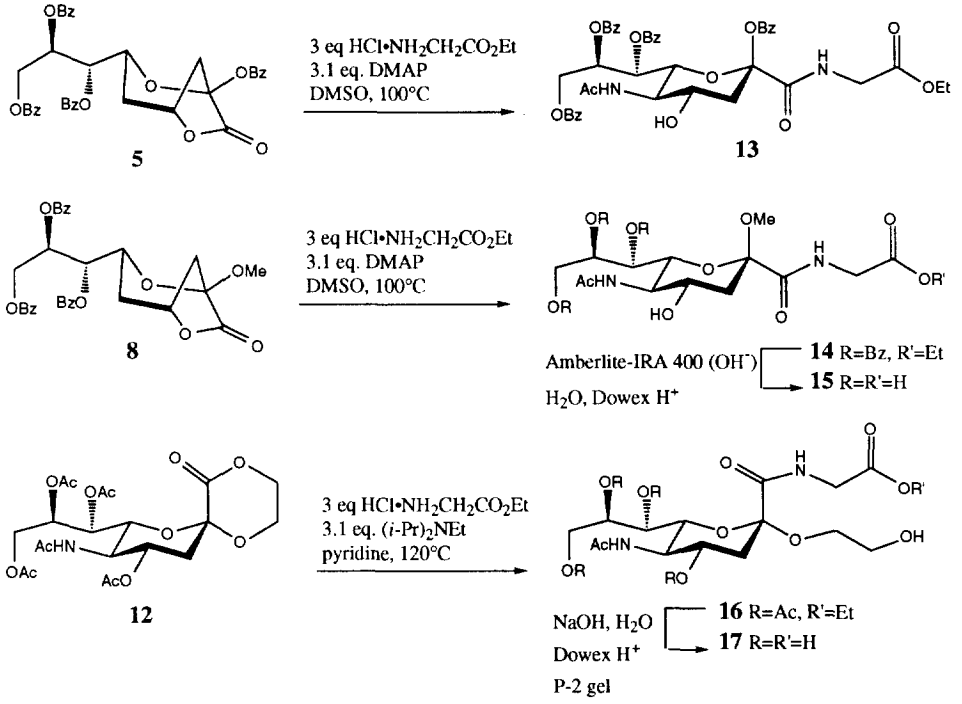
Scheme 1



Ring opening of the 1,4-lactone **5** with glycine ethyl ester hydrochloride salt (3.0 eq.), in the presence of DMAP (3.1 eq.) proceeded slowly (Scheme 2). After several days of reaction in deuterated DMSO at 100°C, NMR showed complete disappearance of starting material and **13** was obtained in 26% yield after purification. Reaction of **8** proceeded similarly to give **14** in 34% isolated yield. The yields of these reactions were disappointingly low, suggesting that the product

was undergoing decomposition and/or polymerization. Better results were realized by performing the reaction for a shorter time; and although starting material remained, it could be recycled. For example, the spirocyclic lactone **12** was subjected to ring opening in pyridine (120°C) to give the glycine-NeuAc adduct **16** in 50% yield after 48 hrs.[11] Deprotection of **14** and **16** afforded saccharopeptides **15** and **17**, respectively. These compounds did not inhibit clostridial sialidase at 0.2mM concentrations.[12]

Scheme 2



The slow rates of these reactions may be a consequence of steric congestion, since the lactone carbonyl is a neopentyl-like center and/or poor nucleophilicity of the amine functionality.[13] As a consequence, it may be difficult to introduce more sterically demanding amino acids in this fashion. Nonetheless the glycine component may serve to link NeuAc to other amino acids through peptide bond formation with the NeuAc-glycinyl acid functionality. These reactions offer routes into a new class of compounds, composed of sugar amino acids and naturally occurring amino acids, that may be useful in combinatorial approaches to drug development. In addition to providing selectively protected adducts, a new type of sialyl donor (**13**) has been prepared and its glycosylation is currently under investigation in our laboratories.

Experimental Section:

Methyl (methyl-5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-β-D-manno-2-nonulopyranosid)oate **(7a)**: *N*-acetylneuraminic acid (**1**) (1 g, 3.23 mmol) was dissolved in 150 mL of distilled methanol. Dowex 50 (H^+) resin (2.0 g) was added to the solution, and the resulting mixture was refluxed with stirring under argon for 48 hours. The Dowex 50 (H^+) resin was filtered and washed with cold methanol. The filtrate and washings from the resin was evaporated *in vacuo* to give a yellow syrup. The crude product (903 mg, 83%) contained a mixture of α- and β-anomers. Recrystallization from diethyl ether:methanol (3:1) afforded pure white crystals of **7a** (540 mg, 55% yield).

Data for **7a**: ^1H NMR (250MHz, D_2O) δ 1.74 (dd, J = 13.0, 11.3 Hz, H_{3ax}), 2.00 (s, Ac), 2.35 (dd, J = 13.2, 4.77 Hz, H_{3eq}), 3.23 (s, Me), 3.57 (d, J = 9.4 Hz, H_7), 3.62 (dd, J = 12.0, 5.8 Hz, H_9), 3.82 (s, Me), 3.79-3.77 (m, $H_{9'}$), 3.85-3.82 (m, H6, H_8), 3.86 (d, J = 8.7 Hz, H_5), 3.98 (dd, J = 11.0, 4.3 Hz, H_4).

Methyl-5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-β-D-manno-2-nonulopyranosidic acid **(7)**: Compound **7a** (500 mg, 1.48 mmol) was dissolved in 15 mL of 0.06N NaOH. The mixture was stirred at room temperature for 3 hours, then neutralized with Dowex 50 (H^+) resin and lyophilized to give **7** as a white powder (432 mg, 90%).

Data for **7**: ^1H NMR (250MHz, D_2O) δ 1.69 (dd, J = 13.0, 11.2 Hz, H_{3ax}), 1.99 (s, Ac), 2.32 (dd, J = 13.2, 4.8 Hz, H_{3eq}), 3.21 (s, Me), 3.52 (d, J = 9.5 Hz, H_7), 3.60 (dd, J = 11.9, 5.7 Hz, H_9), 3.79 (dd, J = 12.2, 5.7 Hz, $H_{9'}$), 3.82-3.76 (m, H_6, H_8) 3.88 (d, J = 10.2 Hz, H_5), 3.97 (dd, J = 11.3, 5.0 Hz, H_4).

Methyl-5-acetamido-7,8,9-tri-O-benzoyl-3,5-dideoxy-β-D-manno-2-nonulopyranoside-1,4 lactone, **(8)** ***and Methyl-5-acetamido-4,8,9-tri-O-benzoyl-3,5-dideoxy-β-D-2-manno-nonulopyranoside-1,7 lactone,*** **(9)**: Compound **7** (280 mg, 0.866 mmol) was diluted in 7 mL of dried pyridine and benzoyl chloride (0.78 mL, 6.76 mmol) was added dropwise to the reaction mixture over a period of 30 minutes at 0°C. The reaction mixture was stirred under nitrogen and allowed to warm to room temperature. Stirring was maintained at room temperature for 18 hours. Ice cold water was then poured into the reaction flask, and the mixture was extracted with ethyl acetate. The extraction step was repeated four times. After the fifth extraction, the organic layer was washed with brine and water, and dried over magnesium sulfate. The resulting mixture was evaporated *in vacuo* to give a yellow syrup. The residue was separated and purified by silica gel column chromatography. A solvent mixture of 7:3 (benzene : acetone) was used. The different eluents collected were evaporated *in vacuo* to give 267 mg (51%) of **8** (R_f = 0.26) and 70 mg (14%) of **9** (R_f = 0.39).

Data for **8**: ^1H NMR (250MHz, $CDCl_3$) δ1.90 (s, Me), 2.19 (dd, J = 13.1, 5.4 Hz, H_{3proR}), 3.26 (dd, J =10.1, 7.2 Hz, H_5), 3.41 (apparent t, J = 13.1 Hz, H_{3proS}), 3.42 (s, Me), 4.39 (dd, J

=12.4, 6.1 Hz, H_9), 4.69 (d, *J* =10.1 Hz, H_6), 4.85 (d, *J* =5.4 Hz, H_4), 4.87 (dd, *J* =12.4, 2.6 Hz, $H_{9'}$), 5.57 (d, *J* =7.1 Hz, H_7), 5.88-5.85 (m, H_8), 6.24 (d, *J* = 7.2 Hz, NH), 7.91-7.22 (m, phenyl H's). ^{13}C NMR (75MHz, $CDCl_3$) δ170.9, 170.1, 166.4, 165.9, 165.2, 134.1, 133.5, 133.1, 129.9, 129.6, 129.5, 129.3, 129.1, 128.7, 128.5, 128.3, 128.1, 97.4, 72.1, 69.9, 69.7, 63.1, 52.3, 51.3, 32.4, 23.1. FABMS *m/z* calcd for $C_{33}H_{31}O_{11}N$: 617.1897, found 640.1802 (M + Na).

Data for **9**: ^1H NMR (250MHz, $CDCl_3$) δ1.89 (s, Me), 2.09 (dd, *J* =7.8 Hz, H_3), 4.38 (s, H_5), 4.56 (dd, *J* =12.5, 4.1 Hz, H_9), 4.85 (s, H_6), 4.89 (dd, *J* = 12.5, 4.5 Hz, H_9), 5.37 (s, H_7), 5.56-5.53 (m, H_8), 6.32 (d, *J* = 7.8 Hz, NH), 7.90-7.06 (m, phenyl H's). ^{13}C NMR (75MHz, $CDCl_3$) δ169.6, 165.7, 165.3, 164.5, 162.2, 134.4, 133.6, 133.4, 133.1, 130.4, 130.3, 129.9, 129.8, 129.6, 129.5, 129.4, 129.0, 128.7, 128.6, 128.5, 128.5, 128.3, 128.2, 95.1, 76.1, 71.4, 71.1, 67.9, 61.8, 51.8, 48.2, 34.2, 22.9. FABMS *m/z* calcd for $C_{33}H_{31}O_{11}N$ 617.1897, found 640.1804 (M + Na).

***Benzyl (glycolyl- 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-β-D-manno-2-nonulopyranosidi)oate* (11):** $CaCO_3$ (300 mg) and AgOTf (145 mg, 0.56 mmol, 1.50 eq) were weighed into a 3-neck round bottom flask under argon and THF (5 mL) was added. To this suspension, 2,6-di-*tert*-butylpyridine (0.168 mL, 0.75 mmol, 2.00 eq) was added followed by ethylene glycol (0.079 mL, 1.42 mmol, 3.8 eq). The white slurry was cooled to -55°C and a solution of the sialyl chloride (**10**)[10] (220 mg, 0.37 mmol, 1 eq) in THF (3 mL) was added slowly over 1.5 h via syringe pump. The reaction was kept at -55°C for an additional hour and allowed to slowly warm to 0°C over 3h where it was maintained overnight. The reaction was quenched by dilution with CH_2Cl_2 and filtered. The organic layer was washed successively with saturated $NaHCO_3$ solution, H_2O and brine before being dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography (6:4 hexanes/acetone) to give 60 mg (26%) of **11** (R_f = 0.10).

Data for **11**: ^1H NMR (250 MHz, $CDCl_3$) δ 1.85 (s, 3H, NAc), 1.98 (m, H_{3ax}), 2.00 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.11 (s, 6H, OAc), 2.62 (dd, *J* = 12.8, 4.6 Hz, H_{3eq}), 3.36 (m, 1H), 3.64 (br s, 2H), 3.78 (m, 1H), 4.07 (m, 3H), 4.30 (dd, *J* = 12.4, 2.4 Hz, 1H), 4.83 (m, 1H), 5.20 (s, 2H), 5.3 (m, 4H), 7.35 (s, 5H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 170.8, 170.7, 170.3, 170.2, 170.1, 167.7, 134.7, 128.7, 128.6, 128.4, 98.7, 72.7, 68.9, 68.7, 67.9, 67.2, 66.6, 62.4, 61.5, 49.2, 37.9, 23.1, 21.1, 20.8, 20.7. IR ($CDCl_3$, cm^{-1}) 3356, 2961, 1747, 1666, 1549, 1456, 1371, 1226, 1128, 1072, 1041. FABMS *m/z* calcd for $C_{28}H_{37}NO_{14}$: 611.2214, found 612.2322 ($M+H^+$).

***Glycolyl-5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-β-D-manno-2-nonulopyranosid)spirocyclic lactone* (12):** Compound **11** (35 mg, 0.057 mmol, 1 eq.) was dissolved in absolute MeOH (2 mL) and NaOMe (40 mg) was added. After stirring for 3h,

the reaction was concentrated to remove the methanol. The residue was dissolved in H_2O (3 mL) and KOH (15 mg) was added. After stirring for an additional 1h, the reaction was carefully acidified with Dowex-50 H^+ resin and filtered. The filtrate was concentrated and pyridine (8 mL) was added followed by acetic anhydride (1.5 mL). The reaction was stirred overnight before being azeotroped with toluene. EtOAc was added and the reaction was cooled to 0°C and MeOH was added. After stirring for ~2h, the reaction was acidified with Dowex-50 H^+ resin and filtered. The filtrate was concentrated and the residue was purified by flash column (7:3, benzene/acetone) to give 14 mg (47%) of **12** (Rf = 0.25).

Data for **12**: $[\alpha]_D$ = -70.0 (0.2, CH_2Cl_2). ^1H NMR (250 MHz, $CDCl_3$) δ 1.87 (t, *J* = 13.3 Hz, H_{3ax}), 1.89 (s, 3H, NAc), 2.02 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.39 (dd, *J* =13.5, 5.8 Hz, H_{3eq}), 3.82 (m, 1H, lactone H), 4.01 (m, 2H, H_6, H_9), 4.23 (m, 2H, H_5, H_9), 4.45 (m, 3H, lactone H's), 5.34 (m, 3H, NH, H_7, H_8), 5.54 (m, 1H, H_4). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 170.8, 170.5, 170.3, 170.2, 169.7, 164.2, 95.9, 72.3, 69.7, 68.5, 67.8, 66.6, 62.4, 58.4, 49.0, 37.8, 23.1, 20.8, 20.7. FABMS *m/z* calcd for $C_{21}H_{29}O_{13}N$: 503.1639, found 504.1712 ($M+H^+$).

***Ethylglycinyl-(Benzoyl-5-acetamido-7,8,9-tri-O-benzoyl-3,5-dideoxy-ß-D-manno-2-nonulopyranosid)amide* (13).** The 1,4 lactone (**5**) (200 mg, 0.282 mmol) was dissolved in 10 mL of dimethyl sulfoxide-d_6 and glycine ethyl ester hydrochloride (139 mg, 0.865 mmol) and DMAP (116 mg, 0.950 mmol) were added to the solution. The reaction mixture was allowed to stir at 100 °C for one week under argon. The reaction was monitored by ^1H NMR on a AM 250 Bruker spectrophotometer. After seven days the product was poured into distilled water and extracted with ethyl acetate. The ethyl acetate extract was evaporated *in vacuo* to a dark brown syrup. The syrupy residue was purified by column chromatography (ethyl acetate:methanol) stepwise elution from 100:0 to 95:5. The first eluate washed away the impurities and remaining starting material. The second eluate was evaporated in vacuo to give 59 mg (26%) of **13** (R_f=0.36).

Data for **13**: $[\alpha]_D$ +0.08 (c 2.0x10^3, $CHCl_3$); ^1H NMR (250MHz, $CDCl_3$) δ 1.27 (t, *J* = 7.1 Hz 3H, CH_3), 1.98 (s, 3H, Ac), 3.82 (dd, *J* =17.9, 8.0 Hz, H_5), 4.12 (apparent d, *J* =5.8Hz, 2H, glycine CH_2) , 4.23-4.17 (m, H_4, CH_2), 4.46 (dd, *J* = 12.3, 7.2 Hz, H_9), 4.58 (d, *J* = 2.7 Hz, H_6), 4.86 (dd, *J* = 9.8, 2.7 Hz, H_7), 4.93 (dd, *J* = 12.3, 2.8 Hz, $H_{9'}$), 6.01-5.95 (m, H_8 & NH), 8.08-7.38 (m, 15 phenyl H's). ^{13}C NMR (75MHz, $CDCl_3$) δ 171.9, 169.8, 169.4, 166.3, 166.1, 165.3, 161.6, 145.1, 133.6, 133.5, 133.2, 133.1, 129.9, 129.9, 129.7, 129.6, 129.3, 128.9, 128.8, 128.5, 128.2, 109.9, 70.1, 69.0, 66.7, 63.5, 61.3, 60.3, 51.0, 41.2, 29.6, 23.0, 20.9. FABMS *m/z* calcd for $C_{43}H_{42}O_{14}N_2$ 810.2636, found 833.2543 (M + Na).

***Ethylglycinyl-(Methyl-5-acetamido-7,8,9-tri-O-benzoyl-3,5-dideoxy-ß-D-manno-2-nonulopyranosid)amide* (14):** The 1,4 lactone **8** (100 mg, 0.16 mmol) was dissolved in 5

mL of dimethyl sulfoxide-d_6 and glycine ethyl ester hydrochloride (33 mg, 0.243 mmol) and dimethylaminopyridine (30 mg, 0.248 mmol) were added to the solution. The reaction mixture was allowed to stir at 100°C for 9 days under argon when NMR showed quantitative conversion. The product was poured into distilled water and extracted with ethyl acetate. The ethyl acetate extract was evaporated in vacuo to a dark brown syrup. The residue was separated and purified by silica gel column chromatography using ethyl acetate:methanol gradient (100:0 to 95:5) as eluent to give 40 mg (34%) of **14** (R_f=0.32).

Data for **14**: $[\alpha]_D$ +0.09° (c 2.6, $CHCl_3$). ^1H NMR (250MHz, $CDCl_3$) δ1.27 (t, J = 7.2 Hz 3H, CH_3), 1.57 (apparent t, J =12.4 Hz, H_{3ax}), 1.98 (s, 3H, Ac), 2.56 (dd, J =13.3, 4.7 Hz, H_{3eq}), 3.07 (s, 3H, OMe), 3.56 (dd, J =18.6, 10.0Hz, H_5), 4.06 (apparent t, J =4.6 Hz, 2H, glycine CH_2) , 4.21 (d, J =4.7 Hz, H_4), 4.21 (q, J =7.1 Hz, 2H, CH_2), 4.33 (d, J = 4.3 Hz, H_6), 4.51 (dd, J = 12.7, 5.2 Hz, H9), 5.01 (dd, J = 12.7, 2.1 Hz, H9'), 5.81 (d, J = 5.2 Hz, H_8), 5.81 (s, H_7), 5.86 (d, J = 10.0 Hz, NH), 8.10-7.23 (m, 15 phenyl H's). ^{13}C NMR (75MHz, $CDCl_3$) δ171.7, 169.3, 168.0, 166.2, 166.0, 165.6, 133.8, 133.69, 133.3, 130.2, 129.8, 129.7, 129.4, 129.2, 128.7, 128.6, 128.4, 100.0, 70.8, 70.5, 69.5, 67.1, 62.9, 61.6, 54.0, 51.1, 41.2, 40.2, 29.7, 23.5, 14.2. FABMS *m/z* calcd for $C_{37}H_{40}O_{13}N_2$ 720.2530, found: 743.2412 (M + Na).

***Glycinyl-(Methyl-5-acetamido-3,5-dideoxy-β-D-manno-2-nonulopyranosid) amide*(15):** To a solution of **14** (40 mg, 0.07 mmol) in anhydrous MeOH (20 mL), Amberlite IRA 400 (OH^-) ion exchange resin was added. The mixture was stirred at room temperature for 4 h. The resin was filtered and washed with methanol. The filtrate and washings were combined and evaporated *in vacuo* to give a yellow solid. The material was taken up in 0.06N NaOH (5 mL) and stirred at room temperature for 3 hours, after which the pH of the mixture was adjusted to 4.0 with Dowex-H^+ resin. The Dowex resin was filtered off, and distilled water (25 mL) was then poured into the reaction flask, and the mixture was extracted with ethyl acetate (25mL x 5). The aqueous layer was collected and lyophilized overnight to a white powder (12 mg, 45% yield).

Data for **15**:$[\alpha]_D$ -0.16° (c $1.3x10^3$, H_2O). ^1H NMR(500MHz, D_2O) δ1.54 (apparent t, J = 12.6 Hz, H_{3ax}), 1.89 (s, 3H, Ac), 2.20 (dd, J =12.6, 5.0 Hz, H_{3eq}), 3.07 (s, 3H, OMe), 3.45 (d, J =9.5 Hz, H_7), 3.51 (dd, J =12.1, 5.6 Hz, H_9) , 3.67 (dd, J =12.1, 1.9 Hz, H9'), 3.73-3.70 (m, H_8), 3.76 (apparent d, J = 8.1 Hz, 2H, glycine CH_2), 3.90-3.86 (m, H_4, H_5, H_6). ^{13}C NMR (62.5MHz, D_2O) δ171.5, 169.5, 168.1, 95.9, 67.3, 66.4, 64.6, 63.2, 60.0, 48.5, 47.2, 37.6, 36.2, 18.7. FABMS *m/z* calcd for $C_{14}H_{24}O_{10}N_2$ 380.1431, found 381.1508 (M + H^+).

***Ethylglycinly(glycolyl-5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-di-deoxy-β-D-manno-2-nonulopyranosid)amide*(16):** The spirolactone **12** (50 mg, 0.1 mmol, 1 eq.) was dissolved in pyridine (3 mL) under argon and glycine ethyl ester hydrochloride (56 mg, 0.4 mmol, 4 eq.) and diisopropylethylamine (150 µL, 0.86 mmol, 8.6 eq.) were added. The reaction was

heated to 120°C (oil bath temperature) and kept at this temperature for 72h. At the end of this time, TLC indicated that s.m. was present along with a lower R_f spot. The reaction was diluted with CH_2Cl_2 and washed with H_2O. It was then washed with brine, dried with $MgSO_4$ and concentrated. The residue was first eluted with 100% EtOAc to recover **12** (25 mg, 50%) and then with 5% MeOH/EtOAc to recover **16** (12 mg, 20%).

Data for **16**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.28 (t, J = 7.05 Hz, OCH_2CH_3), 1.86 (s, 3H, NAc), 2.00 (t, H_{3ax}), 2.01 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.34 (br s, OH), 2.49 (dd, J = 12.9, 5.25 Hz, H_{3eq}), 3.78 (s, 4H, OCH_2CH_2OH), 3.87 (m, 2H, H_6, H_9), 4.19 (m, 4H, OCH_2CH_3, H_5, H_7), 4.32 (m, 1H, H_8), 4.76 (d, J = 11.7 Hz, H_9), 5.22 (m, H_4), 5.31 (br s, NCH_2CO_2Et), 5.47 (d, J = 10.0 Hz, $NHAc$), 7.69 (t, $CONHCH_2$). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 171.9, 170.9, 170.5, 170.1, 169.9, 169.6, 168.5, 99.1, 73.6, 70.8, 69.6, 68.4, 65.9, 65.7, 63.3, 61.6, 49.1, 40.8, 35.3, 23.2, 20.9, 20.8, 14.1. HRMS (CI/methane) calcd for $C_{24}H_{38}O_{15}N_2$ *m/z* 607.63, found 607.20.

***Glycinly(glycolyl-5-acetamido-3,5-di-deoxy-β-D-manno-2-nonulopyranosid) amide*(17):** The substrate **16** (12 mg, 0.02 mmol, 1 eq.) was dissolved in MeOH (5 mL) and NaOMe (10 mg) was added. The reaction was stirred overnight before being acidified with Dowex-50 H^+ resin and filtered. The residue was dissolved in H_2O and NaOH was added. After stirring at r.t. for ~7h, the reaction was neutralized with Dowex-50 H^+ resin by filtration. The residue was purified by chromatography using P-2 gel to give 6 mg (70%) of **17** (R_f = 0.54, 7:3 n-PrOH/H_2O).

^{1}H NMR (250 MHz, D_2O) δ 1.59 (t, J = 11.75 Hz, H_{3ax}), 1.81 (s, 3H, NAc), 2.53 (dd, J = 12.75, 4.37 Hz, H_{3eq}), 3.38-3.72 (m, 13 H). ^{13}C NMR (62.5 MHz, D_2O) indicates mixture of rotamers- δ 174.9, 171.2, 170.2, 169.2, 99.4, 99.3, 73.2, 73.1, 70.9, 70.7, 67.6, 67.0, 66.9, 64.9, 62.8, 60.3, 52.7, 51.5, 51.4, 47.9, 42.9, 40.8, 38.3, 38.1, 21.9.

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References:

1. For leading references see: Walz, G.; Aruffo, A.; Kolanus, W.; Polley, M.J.; Phillips, M.L. Wayner, E.; Nudelman, E.; Singhal, A.K.; Hakomori, S.; Paulson, J.C. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6224. Phillips, M.L., Nudelman, E.; Gaeta, F.C.A.; Perez, M.; Singhal, A.K.; Hakomori, S.-I.; Paulson, J.C.*Science* **1990**, *250*, 1130. Finne, J. *TIBS*, **1985**, 129. Hakomori, S.-I. *Cancer Res.* **1985**, *45*, 2405. Fukushima, K.; Hirota, M.; Terasaki, P.I.;

Wakisaka, A.; Togashi, H.; Chia, D.; Suyama, N.; Fukushi, Y.; Nudelman, E.; Hakomori, S.-I. *Cancer Res.* **1984**, *44*, 5279.
2. For lead references see: Sabesan, S.; Neira, S.; Davidson, F.; Duus, J.O.; Bock, K. *J. Am. Chem. Soc.* **1995**, 116, 1616. Sabesan, S.; Neira, S.; Wasserman, Z. *Carbohydr. Res.* **1995**, *267*, 239. Bandgar, B.P.; Patil, S.V.; Zbiral, E. *Carbohydr. Res.* **1995**, *276*, 337. von Itzstein, M.; Wu, W.; 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.; Ryn, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M. and Penn, C. R. *J. Med. Chem.* **1994**, *37*, 616-624. Drigues, P.-A.; Barrere, B.; Quash, G.; Doutheau, A. *Carbohydr. Res.* **1994**, *262*, 297. Unverzagt, C. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1691. Wallimann, K.; Vasella, A. *Helv. Chim. Acta* **1990**, *73*, 1359.
3. Gervay, J.; Nguyen, C.P.; Flaherty, T.M. *J. Am. Chem. Soc.* submitted.
4. Several different terms have been used to describe these compounds including carbopeptoids, saccharopeptides, and sugar amino acids. For leading articles see: Suhara, Y.; Hildreth, J.E.K.; Ichikawa, Y. *Tetrahedron Lett.* **1996**, *37*, 1575. Suhara, Y.; Ichikawa, M.; Hildreth, J.E.K.; Ichikawa, Y. *Tetrahedron Lett.* **1996**, 2549. Muller, C.; Kitas, E.; Wessel, H.P. *J. Chem. Soc. Chem. Comm.* **1995**, 2425. Wessel, H.P.; Mitchell, C.; Lobota, C.M.; Schmid, G. *Angew. Chem. Int. Ed. Engl.* **1995**, *35*, 2712. Nicolaou, K.C.; Florke, H.; Egan, M.G.; Barth, T.; Estevez, V.A. *Tetrahedron Lett.* **1995**, *36*, 1775. von Roedern, E.G.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 687. Poitout, L.; Merrer, Y.L.; Depezay, J.-C. *Tetrahedron Lett.* **1995**, *36*, 6887. Apostolopoulos, C.D.; Couladouras, E.A.; Georgiadis, M.P. *Liebigs Ann. Chem.* **1994**, 781.
5. Dohi, T.; Nores, G.A.; Oguchi, H.; Inufusa, H.; Hakomori, S.-I. *Gangliosides and Cancer edited* by H.F. Oettgen VCH publishers, New York, NY 1989, pp. 273-282. Lifely, M.R.; Gilbert, A.S.; Moreno, C. *Carbohydr. Res.* **1984**, *134*, 229. Lifely, M.R.; Gilbert, A.S.; Moreno, C. *Carbohydr. Res.* **1981**, *94*, 193. Gross, S.J.; Williams, M.A.; McCluer, R.H. *J. of Neurochemistry* **1980**, 1351. McGuire, E.J.; Binkley, S.B. *Biochemistry* **1964**, 1248.
6. For examples see: Gervay, J.; Peterson, J.M.; Oriyama, O. Danishefsky, S.J. *J. Org. Chem.* **1993**, *58*, 5465. Danishefsky, S.J.; Koseki, K.; Griffith, D.A.; Gervay, J. Peterson, J.M.; McDonald, F.E.; Oriyama, T. *J. Am. Chem. Soc.* **1992**, *114*, 8331. Nicolaou, K.C.; Hummel, C.W.; Bockovich, N.J.; Wong, C.-H. *J. Chem. Soc. Chem. Comm.* **1991**, 870. Numata, M.; Sugimoto, M.; Shibayama, S.; Ogawa, T. *Carbohydr. Res.* **1988**, *174*, 73.
7. Sato, S.; Furuhata, K.; Ogura, H. *Chem. Pharm. Bull.* **1988**, *36*, 4678.
8. Yu, R.K.; Ledeen, R.J. *J. Biol. Chem.* **1969**, *244*, 1306.
9. Gervay, J.; Mamuya, N.M. *Tetrahedron Lett.* submitted.
10. Ratcliff, M.R.; Venot, A.P.; Abbas, Z.S.; Eur. Pat. Appl. EP 319,253 (*Chem. Abstr.* **1990**, 112, 175281a).
11. Yield based upon recovered starting material.
12. Li, Y.-Y. Dept. of Biochemistry, Tulane University School of Medicine, New Orleans, LA 70112, personal communication.
13. Recent investigations in our laboratories indicate that addition of benzhydryl amine is orders of magnitude faster than that of the glycinyl amine.

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