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Synthesis of a Serine-Based Neuraminic Acid *C*-Glycoside

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Cell-surface carbohydrates are classified by the nature of their linkages to the protein as either *N*-linked or *O*-linked. *O*- and *N*-glycans are involved in a number of important biological functions. These activities can be lost on glycoprotein catabolism when these glycan linkages are enzymatically hydrolyzed. The design and synthesis of novel *C*-linked glycans should provide catabolically stable glycoproteins useful for understanding and regulating important biological processes. Our efforts are currently directed toward the synthesis of *C*-glycosides of ulosonic acids. This paper describes the first synthesis of a serine-based neuraminic acid *C*-glycoside. The protecting group chemistry required for both carbohydrate and peptide syntheses complicates this approach. Different protecting group strategies were investigated for use in the samarium diiodide mediated *C*-glycosylation reaction. The key elements of our synthetic approach involve the following: (i) the substitution of homoserine for serine in the *C*-glycosylation reaction to introduce a carbon in place of the *O*-glycosidic oxygen, (ii) the use of benzyloxycarbonyl as a homoserine protecting group, compatible with samarium diiodide mediated *C*-glycosylation reaction, and (iii) the reduction of the carbonyl group in homoserine early in the synthesis to improve *C*-glycosylation yield and to avoid lactone formation. Using this combined approach, we prepared 4-*O*-acetyl-4-[2-*C*-(1-methyl 5-acetamido 4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*erythro*-L-*manno*-nononate)]-2*S*-(benzyloxycarbonyl)amino-1-carboxylic acid **(1)**, which will be used in peptide synthesis to prepare glycopeptides containing catabolically stable *C*-linked neuraminic acid.

Introduction

Glycoproteins are major components on the surface of mammalian cells. Many carry *O*-linked oligosaccharides (*O*-glycans), which are conjugated through serine or threonine residues. Others carry *N*-linked oligosaccharides (*N*-glycans), conjugated through an asparagine residue. The recognition of these *O*- and *N*-glycoconjugates plays a key role in the transmission of biological information at the cellular level.[1−3] Their numerous biological functions include roles in cellular recognition, adhesion, cell-growth regulation, cancer cell metastasis, and inflammation. Cell-surface glycans also serve as attachment sites for infectious bacteria, viruses, and toxins, resulting in pathogenesis.[4,5] Anomalies in cell-surface carbohydrates are often closely associated with cell transformation, malignancy, and other various pathological conditions, including immunodeficiency syndromes, cancer, and inflammation.[6] The synthesis of structurally defined, catabolically stable glycoconjugates should provide the opportunity to probe and intervene in critical biological processes. A carbon-linked analogue (*C*-glycopeptide), in which the native *O*-glycosidic linkage has been replaced by the enzymatically and chemically more resistant *C*-glycosidic linkage, should show enhanced stability in vivo and may have applications in modern medicine for the control of bacterial and viral diseases, cancer therapy, and treating inflammatory processes.[7−9]

Neuraminic acids may be the single most important monosaccharide components of glycoconjugates, since they occupy the nonreducing terminal positions, act as ligands for many glycoprotein receptors, and are often the first saccharide lost in glycan catabolism. Neuraminic acid residues in cell membranes and glycoproteins are also involved in many physiologic and pathophysiologic phenomena.[10,11] Of particular interest to our laboratory are mucins, important in cell protection and lubrication.[12,13] Mucins are rich in neuraminic acid containing glycans attached to serine or threonine. The synthesis

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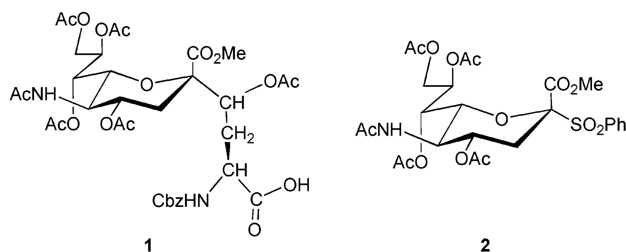


FIGURE 1. Serine-based *C*-glycoside **1** and neuraminic acid-based glycosylation donor **2**.

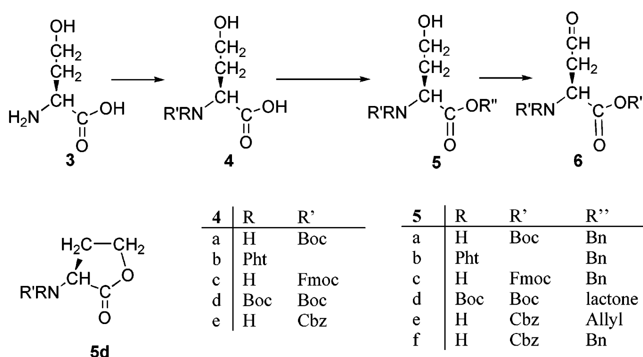
of serine-based *C*-glycosides has only been carried out in a few laboratories and has relied on cross-metathesis of oxazolidine silyl enol ether,[14–16] or the Ramberg–Bäcklund rearrangement.[17] It is difficult to apply these methodologies to the synthesis of serine-based neuraminic acid *C*-glycosides for several reasons: (1) The carboxyl group at C2 of neuraminic acid sterically restricts glycoside formation. (2) The carboxyl function attached at C2 electronically disfavors oxonium ion formation, an intermediate for almost all glycosylation reactions. (3) The lack of a substituent at C3 precludes an assisting and/or directing effect of an adjacent functional group resulting in poor stereochemical control. Using a method for the preparation of *C*-glycosides of neuraminic acids with SmI_2, pioneered in our laboratory,[18] we set out to synthesize a serine-based *C*-glycoside of neuraminic acid. This stable linkage should contribute significantly to understanding biological recognition and might serve to enhance or suppress biological events at the molecular level.

Results and Discussion

To prepare a *C*-glycosidic linkage, it was first necessary to synthesize a neuraminic acid donor **2**[19] and a serine-based acceptor **6**. Homoserine was chosen as the substitute of serine since during the *C*-glycosylation process its additional carbon is required to replace the interglycosidic *O*-linkage. Our strategy to prepare the acceptor involved the orthogonal protection of amino and carboxyl groups in homoserine and the oxidation of its hydroxyl group to an aldehyde acceptor for *C*-glycosylation.

The initial synthesis of aldehyde acceptor **6** is shown in Scheme 1. Multiple amino protecting groups (Fmoc, Pht, Boc, Cbz)[20–25] and carboxylic acid protecting groups (Bn, allyl) were examined in the *C*-glycosylation reaction. The resulting acceptors (**6a**–**c**) did not undergo *C*-

SCHEME 1



4	R	R'
a	H	Boc
b	Pht	
c	H	Fmoc
d	Boc	Boc
e	H	Cbz

5	R	R'	R''
a	H	Boc	Bn
b	Pht		Bn
c	H	Fmoc	Bn
d	Boc	Boc	lactone
e	H	Cbz	Allyl
f	H	Cbz	Bn

TABLE 1. ***C*-Glycosylation with 2 under SmI_2 Conditions**

entry	R	R′	R″	yield (%)[a] 7	yield (%)[a] 8
6a	H	Boc	Bn	ND	ND
6b	Pht	Bn	ND	ND	
6c	H	Fmoc	Bn	ND	ND
6e	H	Cbz	Allyl	38	5
6f	H	Cbz	Bn	40	0

[a] ND = not detected.

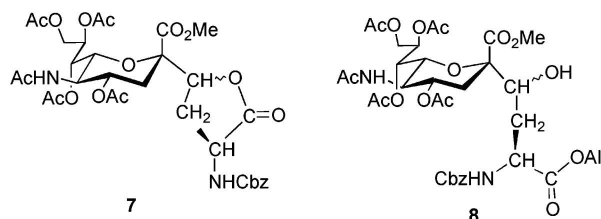


FIGURE 2. *C*-glycosylation products.

glycosylation with glycosyl donor **2** using SmI_2 (Table 1). Failure in all of these syntheses was attributed to the high reactivity of SmI_2, resulting in many side reactions, including deprotection,[26] condensation and reduction of the aldehyde acceptor, and reduction of Neu5Ac sulfone **2**. Cbz-protected homoserine **4e** was then converted to the corresponding allyl ester **5e** and benzyl ester **5f**, respectively (Scheme 1). Both **5e** and **5f** were smoothly oxidized, using Dess–Martin periodinane, to aldehyde acceptors **6e** and **6f**.[27] The *C*-glycosylation reaction of these two acceptors afforded *C*-glycoside **7** with concomitant loss of carboxyl protection and subsequent lactonization. Only a small amount of the desired allyl protected homoserine *C*-glycoside was obtained (**8**). This intramolecular cyclization product is favorable due to the formation of a stable, five-membered ring, commonly observed in homoserine-based syntheses[28] (Figure 2)

Lactone **7** could be opened by using acetic acid and water as reaction media, affording the unprotected serine-based neuraminic acid *C*-glycoside.[29,30] Attempts

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SCHEME 2[a]

[a] Reagents: (i) TBDMSCl, pyridine, (ii) $LiBH_4$, THF, (iii) BnBr, 1.2 equiv of NaH, DMF, 0 °C.

SCHEME 3[a]

[a] Reagents: (i) dihydropyran, PPTS, CH_2Cl_2, (ii) TBAF, THF, (iii) Dess–Martin periodinane, CH_2Cl_2, (iv) **2**, SmI_2, THF, (v) Ac_2O, pyridine, (vi) PPTS, EtOH, 50 °C, (vii) $RuCl_3$, $NaIO_4$, CCl_4, CH_3CN, H_2O, 0 °C.

to protect the resulting free amino acid with FmocCl[31,32] only afforded the *N*-Fmoc protected, lactonization product.

It was clear that the protection of the amino group and the presence of an α-COOH group in homoserine complicated samarium-based *C*-glycosylation. Reduction of the carboxyl group at the beginning of the synthesis and its subsequent oxidation following *C*-glycosylation was next examined to avoid lactonization and improve the *C*-glycosylation yield. The hydroxyl group in Cbz-L-homoserine allyl ester **5e** was protected as the silyl ether **9**,[33] and the allyl ether was reduced to afford alcohol **10**[34] (Scheme 2). Surprisingly, benzylation of the newly generated hydroxyl group in **10** with benzyl bromide and sodium hydride in DMF at 0 °C afforded aziridine **11** as a major product. Reducing the reaction temperature and the amount of NaH failed to prevent the formation of the undesired aziridine. Benzylation under acidic conditions with silver oxide and benzyl bromide, however, prevented the formation of aziridine **11** but afforded the desired **12** in very low yield.[35]

Failure of benzyl protection led us to examine tetrahydropyranyl ether (THP) protection, which is easy to install, stable to most nonacidic reagents, and easy to remove. THP protection of **10** went smoothly[36] and afforded **13** in 83% yield (Scheme 3). Two isomers were generated in a ratio of 3:2, and all subsequent synthetic steps resulted in a mixture of diastereomeric products. Quantitative tetrabutylammonium fluoride (TBAF) removal of silyl ether afforded **14**, which was readily oxidized to the *C*-glycosylation aldehyde acceptor **15**. *C*-Glycosylation of **15** afforded α-*C*-glycoside **16** in 45% isolated yield. It is important to note that this yield is higher than that obtained in the previous *C*-glycosylation reactions (Table 1). Acetylation of the newly generated bridge hydroxyl group afforded fully protected *C*-glycoside **17**. Removal of THP with pyridinium *p*-toluensulfonate (PPTS), followed by oxidation of the resulting hydroxyl group, generated our target, L-homoserine based *C*-glycoside **1**.[37,38] Temperature control and the amount of the catalyst were critical in the $RuCl_3$-based oxidation step to prevent lactonization.

Experimental Section

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 360 or 400 MHz and carbon-13 (^{13}C NMR) spectra were recorded at 400 MHz in $CDCl_3$. Chemical Shifts are reported in δ units, parts per million (ppm) downfield from tetramethylsilane (TMS). Mass spectra were obtained in the electrospray-ionization (ESI) mode. Unless otherwise noted, reactions were monitored by thin-layer chromatography on aluminum sheets, Silica Gel 60 F254. The spots were detected under short-wavelength UV light (254 nm) or by dipping the plates into Von's reagent (1.0 g of ceric ammonium sulfate and 24.0 g of ammonium molybdate in 31 mL of sulfuric acid and 470 mL of water), followed by heating. Flash chromatography was performed with 200–400 mesh Silica Gel 60 under nitrogen pressure.

l-2-[(Benzyloxycarbonyl)amino]-4-hydroxybutyric Acid (4e). Benzyl chloroformate (2.58 mL, 1.5 mmol) was added to a solution of **3** (1.44 g, 12.0 mmol) in 1 N $NaHCO_3$ (50 mL). The reaction mixture was stirred at room temperature for 24 h and then extracted with ether (2 × 40 mL). The aqueous phase was ice cooled, carefully acidified to pH 2–3 with 3 N HC1, and extracted with EtOAc (4 × 40 mL). The extract was dried over anhyd Na_2SO_4, filtered, and evaporated. The resulting oil (2.65 g, 89%) was directly used in the next step

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without further purification. ^{1}H NMR ($CDCl_3$): 1.83–1.94 (m, 1H), 2.08–2.14 (m, 1H), 2.83 (br s, 1H), 3.68–3.71 (m, 2H), 4.37–4.41 (m, 1H), 5.07 (s, 2H), 6.58 (br s, 1H), 7.26–7.39 (m, 5H), 11.1 (br s, 1H). HRESIMS: calcd for $C_{12}H_{16}NO_5$ [M + H]$^+$ 254.1035, found *m/z* 254.1028 [M + H]$^+$. Anal. Calcd for $C_{12}H_{16}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.76; H, 5.90; N, 5.45.

Allyl (2*S*)-2-(Benzyloxycarbonyl)amino Butanoate (5e). NaOH (419 mg, 10.5 mmol) in water (5 mL) was added to a solution of **4e** (2.65 g, 10.5 mmol) in ethanol (30 mL). The mixture was stirred at room temperature for 24 h and evaporated to dryness. Dry residue (1.04 g, 3.5 mmol) was dissolved in 10 mL of DMF and allyl bromide (746 μL, 5.25 mmol) was added. After the reaction mixture was stirred in the dark for 48 h at room temperature, it was extracted by EtOAc and water. The organic phase was dried over anhyd Na_2SO_4 and evaporated under reduced pressure. The product (2.95 g, 96%) was obtained from silica gel chromatography, using solvent gradient (100% hexane to 60% hexane in EtOAc). ^{1}H NMR ($CDCl_3$): 1.71–1.81 (m, 1H), 2.08–2.22 (m, 1H), 3.13 (t, 1H, J = 6.48 Hz), 3.59–3.76 (m, 2H), 4.51–4.58 (m, 1H), 4.62 (d, 2H, J = 6.8 Hz), 5.10 (s, 2H), 5.22–5.35 (m, 2H), 5.82–5.93 (m, 1H), 7.33 (m, 5H). ^{13}C NMR ($CDCl_3$): 172.1, 156.6, 136.0, 131.3, 128.4, 128.1, 128.0 118.8, 67.05, 65.98, 58.6, 51.3, 35.2. HRESIMS: calcd for $C_{15}H_{20}NO_5$ [M + H]$^+$ 294.1353, found *m/z* 294.1341 [M + H]$^+$.

Benzyl (2*S*)-2-(Benzyloxycarbonyl)amino Butanoate (5f). *N*-Cbz-L-homoserine benzyl ester was obtained by the same procedure as described for **5e** except benzyl bromide was added instead of allyl bromide. ^{1}H NMR ($CDCl_3$): 1.67–1.75 (m, 1H), 2.13–2.20 (m, 1H), 2.75 (br s, 1H), 3.60–3.75 (m, 2H), 4.56–4.62 (m, 1H), 5.12 (d, 2H, J = 10.4 Hz), 5.18 (s, 2H), 5.67 (d, 1H, J = 7.5 Hz), 7.32–7.38 (m, 10H). HRESIMS: calcd for $C_{19}H_{21}NO_5Na$ [M + Na]$^+$ 366.1315, found *m/z* 366.1317 [M + Na]$^+$.

Allyl (2*S*)-2-*N*-(Benzyloxycarbonyl)amino-3-formyl Propionate (6e). Dess–Martin periodinane (191.2 mg, 0.45 mmol) was added to a solution of **5e** (120 mg, 0.41 mmol) in CH_2Cl_2 solution. The reaction mixture was stirred for 2 h at room temperature and evaporated to dryness. The residue was suspended in diethyl ether (20 mL), extracted with aqueous $NaHCO_3$/$Na_2S_2O_3$ solution (100 mL of saturated $NaHCO_3$ containing 25 g of $Na_2S_2O_3$) and saturated brine, and dried over anhyd Na_2SO_4. The organic layer was evaporated and passed through a silica gel column to obtain **6e** with 86% yield. ^{1}H NMR ($CDCl_3$): 2.95 (dd, 1H, J = 5.1 Hz, 18.5 Hz), 3.04 (dd, 1H, J = 5.1 Hz, 18.5 Hz), 4.62–4.75 (m, 3H), 5.11 (s, 2H), 5.23–5.27 (m, 2H), 5.82–5.87 (m, 2H), 7.29–7.37 (m, 5H), 9.68 (s, 1H). ^{13}C NMR ($CDCl_3$): 199.0, 170.3, 155.8, 136.0, 131.2, 128.4, 128.1, 128.0, 118.9, 67.0, 66.3, 49.0, 45.6. HRESIMS: calcd for $C_{15}H_{18}NO_5Na$ [M + H]$^+$ 292.1185, found *m/z* 292.1187 [M + H]$^+$.

Benzyl (2S)-2-*N*-(Benzyloxycarbonyl)amino-3-formyl Propionate (6f). Compound **6f** was obtained by using the same method as described for **6e**. ^{1}H NMR ($CDCl_3$): 3.02–3.20 (ddd, 2H), 4.69–4.71 (m, 1H), 5.12 (s, 2H), 5.18 (d, 2H), 5.68 (d, 1H, J = 7.6 Hz), 7.31–7.39 (m, 10H), 9.71 (s, 1H). ^{13}C NMR ($CDCl_3$): 199.0, 170.5, 155.9, 136.0, 135.0, 128.6, 128.5, 128.3, 128.2, 128.1, 67.7, 67.2, 49.2, 45.8.

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-[L-2-[(benzyloxycarbonyl)amino]-4-butyrolactone]-D-*erythro*-L-*manno*-nononate (7). Neu5Ac phenyl sulfone (**2**) and 1.2 equiv of electrophile (**6e** or **6f**) were dried together under high vacuum for 4 h. SmI_2 (6 equiv freshly prepared from Sm and 1,2-diiodoethane, 0.1 M in THF) was added in one portion at room temperature with vigorous stirring. After 8 h, the reaction mixture was diluted with ether and extracted with 1 N HCl, saturated $Na_2S_2O_3$, $NaHCO_3$, and brine. The organic layer was dried over anhyd Na_2SO_4. The filtrate was concentrated under reduced pressure and purified on a silica gel column with EtOAc as fluent. The *C*-glycoside **7** and **8** were obtained as oils in 38% and 5% yield from acceptor **6e**, respectively. *C*-Glycosylation of acceptor **6f** by donor **2** afforded **7** in 40% yield. ^{1}H NMR ($CDCl_3$): 1.89, 1.94, 2.03, 2.09, 2.13, 2.17 (7 × s, 21H), 2.2–2.35 (m, 3H), 2.43 (1H, dd), 3.80 (s, 3H), 3.89–4.09 (m, 2H), 4.22–4.34 (m, 2H), 4.55 (t, 1H), 4.71 (dd, 1H), 4.87–4.96 (ddd, 1H), 5.11–5.27, (m, 3H), 5.20–5.41 (m, 2H), 6.00 (d, 1H), 7.32–7.37 (m, 5H). ^{13}C NMR ($CDCl_3$): 173.2, 171.1, 171.0, 170.3, 170.2, 170.0, 169.4, 156.3, 136.1, 128.5, 128.2, 79.7, 72.9, 69.0, 68.4, 67.6, 67.3, 62.8, 53.2, 50.1, 49.8, 33.9, 30.9, 28.3, 23.2, 21.0, 20.9, 20.8, 20.7, 20.6. HRESIMS: calcd for $C_{32}H_{46}N_2O_{16}Na$ [M + Na]$^+$ 731.2276, found *m/z* 731.2283 [M + Na]$^+$.

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-[2*S*-allyl-2-(benzyloxycarbonyl)amino butanoate)]-D-*erythro*-L-*manno*-nononate (8). ^{1}H NMR ($CDCl_3$): 1.94–2.24 (m), 2.76–2.82 (m, 1H), 3.72–3.81 (m, 5H), 4.02–4.37 (m, 3H), 4.48–4.75 (m, 3H), 4.87–4.95 (m, 2H), 5.06–5.25 (m, 3H), 5.32–5.41 (m, 2H), 5.88–6.02 (m, 2H), 7.29–7.37 (m, 5H). ESIMS: $C_{35}H_{46}N_2O_{17}$ 767 [M + H]$^+$, 789 [M + Na]$^+$.

Allyl-(2*S*)-2-(benzyloxycarbonyl)amino-4-[(*tert*-butyldimethylsilyl)oxy]butanoate (9). *tert*-Butyldimethylsilyl chloride (1.21 g, 2.43 mmol) was added to a solution of **5f** (476 mg, 1.62 mmol) in pyridine (5 mL). The reaction mixture was stirred at 0 °C for 4 h and evaporated under reduced pressure. Purification of the residue by silica gel chromatography (80% hexanes in EtOAc) yielded **9** as an oil (560 mg, 85%). ^{1}H NMR ($CDCl_3$): 0.041 (s, 3H), 0.037 (s, 3H), 0.88 (s, 9H), 1.95–2.13 (m, 2H), 3.69–3.73 (m, 2H), 4.62 (d, 1H, J = 5.6 Hz), 4,78 (m, 2H), 5.11 (d, 2H), 5.23 (d, 1H, J = 10.4 Hz), 5.32 (d, 1H, J = 16.8 Hz), 5.82–5.96 (m, 2H), 7.29–7.35 (m, 5H). ^{13}C NMR ($CDCl_3$): 171.9, 155.9, 136.4, 131.6, 128.3, 127.8, 127.7, 118.4, 66.7, 65.6, 59.8, 52.5, 33.7, 25.7, 22.5, 5.73, 5.75. ESIMS: 408 [M + H]$^+$, 430 [M + Na]$^+$. HRESIMS: calcd for $C_{21}H_{33}NO_5NaSi$ [M + Na]$^+$ 430.2026, found *m/z* 430.2007 [M + Na]$^+$. Anal. Calcd for $C_{21}H_{33}NO_5Si$: C, 61.88; H, 8.16; N, 3.44. Found: C, 61.80; H, 8.23; N, 3.47.

2*S*-2-(Benzyloxycarbonyl)amino-4-[(*tert*-butyldimethylsilyl)oxy]butan-1-ol (10). $LiBH_4$ (180 μL in THF, 0.35 mmol) was added, at 0 °C, to a solution of **9** (120 mg, 0.29 mmol) in THF (2 mL). After being stirred for 1 h at 0 °C and 6 h at room temperature, the reaction was quenched with saturated aq $NaHCO_3$. The aqueous phase was extracted with EtOAc and the combined organic phases were washed with brine, dried over anhyd Na_2SO_4, and concentrated to dryness under reduced pressure. The crude product was purified by silica gel chromatography (33% hexanes in EtOAc) to give compound **10** in 81% yield. ^{1}H NMR ($CDCl_3$): 0.037 (s, 3H), 0.041 (s, 3H), 0.91 (s, 9H), 1.69–1.95 (m, 2H), 3.40 (br s, 1H), 3.60–3.71 (m, 4H), 3.82–3.91 (m, 1H), 5.12 (s, 2H), 5.65 (s, 1H), 7.29–7.35 (m, 5H). ^{13}C NMR ($CDCl_3$): 156.9, 136.5, 128.5, 128.0, 127.9, 66.7, 65.3, 59.9, 51.8, 33.7, 25.7, 18.1, −5.57, −5.60. HRESIMS: calcd for $C_{18}H_{31}NO_4NaSi$ [M + Na]$^+$ 376.1920, found *m/z* 376.1940 [M + Na]$^+$.

2*S*-2-(Benzyloxycarbonyl)amino-4-[(*tert*-butyldimethylsilyl)oxy]-(1-tetrahydro-2*H*-pyran-2-yloxy)butanol (13). A solution of **10** (133 mg, 0.38 mmol) and dihydropyran (51.5 μL, 0.57 mmol) in dry CH_2Cl_2 (5 mL) containing pyridinium *p*-toluenesulfonate (PPTS, 9.5 mg, 0.038 mmol) was stirred for 7 h at room temperature. Then the solution was diluted with CH_2Cl_2 and washed with brine to remove the catalyst. After evaporation of the organic solvent, silica gel chromatography separation (90% hexanes in EtOAc) gave 165 mg of THP ether **13** (83%). ^{1}H NMR ($CDCl_3$): 0.04 (s, 3H), 0.03 (s, 3H), 0.88 (s, 9H), 1.48–1.90 (m, 8H), 3.45–3.55 (m, 2H), 3.68–3.91 (m, 4H), 3.91–3.98 (m, 1H), 4.54–4.58 (m, 1H), 5.09 (s, 2H), 5.30 (d, 0.4H, J = 7.2 Hz), 5.49 (d, 0.6H, J = 7.2 Hz), 7.27–7.35 (m, 5H). HRESIMS: calcd for $C_{23}H_{39}NO_5NaSi$ [M + Na]$^+$ 460.2495, found *m/z* 460.2480 [M + Na]$^+$.

2*S*-2-(Benzyloxycarbonyl)amino-1-(tetrahydro-2*H*-pyran-2-yloxy)-4-butanol (14). Tetrabutylammonium fluoride (180 μL of a 1 M solution in THF, 0.35 mmol) was added to a solution of **13** (101 mg, 0.23 mmol) in THF (2 mL). The solution

was stirred at room temperature for 1 h. The reaction media was then diluted with ether and extracted with water. After the solution was washed with brine, the organic layer was dried over anhyd Na_2SO_4 and purified on a silica gel column (80% to 50% hexanes in EtOAc) to afford **14** in 94% yield. ^1H NMR ($CDCl_3$): 1.46−1.88 (m, 8H), 3.20−3.33 (m, 1H), 3.47−3.55 (m, 2H), 3.62−3.75 (m, 2H), 3.77−3.87 (m, 2H), 3.95−4.10 (m, 1H), 4.52 (t, 0.7H), 4.58 (t, 0.3H), 5.07−5.16 (m, 2H), 5.24 (d, 0.3H), 5.57 (d, 0.7H), 7.31−7.36 (m, 5H). HRESIMS: calcd for $C_{17}H_{25}NO_5Na$ [M + Na]$^+$ 346.1630, found *m*/*z* 346.1630 [M + Na]$^+$.

1-Formyl 3*S*-(Benzyloxycarbonyl)amino-4-(tetrahydro-2*H*-pyran-2-yloxy)butanol (15). Compound **14** (370 mg, 1.15 mmol) was added to a solution of the Dess−Martin reagent (583 mg, 1.37 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C, warmed to room temperature for 6 h, diluted with diethyl ether (15 mL), poured into sodium thiosulfate in saturated aq $NaHCO_3$ (20 mL), and stirred for 10 min. The organic layer was washed sequentially with saturated aqueous $NaHCO_3$, brine, and deionized water, dried with anhyd Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (90% to 40% hexanes in EtOAc) to give **15** in 82% yield. ^1H NMR ($CDCl_3$): 1.43−1.72 (m, 6H), 2.68 (d, 2H), 3.40−3.45 (m, 2H), 3.72−3.78 (m, 2H), 4.15−4.32 (m, 1H), 4.44 (t, 0.6H), 4.49 (t, 0.4H), 5.03 (s, 2H), 5.24 (d, 0.4H), 5.45 (d, 0.6H), 7.31−7.39 (m, 5H), 9.77 (s, 1H). ^{13}C NMR ($CDCl_3$): 200.5, 200.4, 155.7, 136.3, 136.2, 128.48, 128.45, 128.15, 128.09, 128.02, 99.8, 98.9, 69.2, 68.6, 66.8, 66.7, 63.0, 62.3, 30.4, 30.2, 25.2, 25.1, 19.7, 19.2. HRESIMS: calcd for $C_{17}H_{23}NO_5Na$ [M + Na]$^+$ 344.1474, found *m*/*z* 344.1476 [M + Na]$^+$. Anal. Calcd for $C_{17}H_{23}NO_5$: C, 63.54; H, 7.21; N, 4.30. Found: C, 63.25; H, 7.56; N, 4.28.

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-{1-[3S-(benzyloxy carbonyl)amino-4-(tetrahydro-2*H*-pyran-2-yloxy)-1-butanol]}-D-*erythro*-L-*manno*-nononate (16). *C*-Glycoside **16** was obtained as an oil in 45% yield from the reaction of **15** and **2**, using the general *C*-glycosylation procedure described for **7**. ^1H NMR ($CDCl_3$): 1.75−2.18 (m), 2.40−2.46 (m, 1H), 3.78 (s, 3H), 4.78−4.84 (m, 1H), 5.11 (brs, 2H), 5.34−5.44 (m, 2H), 5.59 (m, 1H), 7.31−7.40 (m, 5H). HRESIMS: calcd for $C_{37}H_{53}N_2O_{17}$ [M + H]$^+$ 797.3344, found *m*/*z* 797.3348 [M + H]$^+$.

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-{1-[1-acetyl-3*S*-(benzyloxycarbonyl)amino-4-(tetrahydro-2*H*-pyran-2-yloxy)-1-butanate]}-D-*erythro*-L-*manno*-nononate (17). A solution of **16** (100 mg, 0.13 mmol) in anhyd pyridine (2 mL) was reacted with acetic anhydride (0.5 mL) under nitrogen. After being stirred overnight at room temperature, the reaction mixture was quenched with methanol and evaporated under vacuum. The residue was dried by coevaporation with toluene and purified by chromatography on silica gel (50% hexanes in EtOAc) to afford **17** as a colorless oil in 95% yield. ^1H NMR ($CDCl_3$): 1.75−2.18 (m, 30H), 2.46−2.52 (m, 1H), 3.44−3.54 (m, 2H), 3.762 (m, 3H), 4.75−4.85 (m, 1H), 5.01−5.85 (m, 6H), 7.30−7.37 (m, 5H). ESIMS: 862 [M + Na]$^+$. HRESIMS: calcd for $C_{39}H_{54}N_2O_{18}Na$ [M + Na]$^+$ 861.3269, found *m*/*z* 861.3262 [M + Na]$^+$.

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-{4-[4-acetyl-2*S*-(benzyloxycarbonyl)amino-1-butanol]}-D-*erythro*-L-*manno*-nononate (18). A solution of THP ether **17** (144 mg, 0.17 mmol) and PPTS (4.3 mg, 0.017 mmol) in ethanol (4 mL) was stirred at 55 °C (bath temperature) for 5 h. The solvent was removed by evaporation in vacuo, and the residue was purified by chromatography on a silica gel column (100% to 95 vol % CH_2Cl_2 in methanol) to afford **17** (102 mg, 92%) with a diastereomer ratio of 6:4. ^1H NMR ($CDCl_3$): 1.88−2.19 (m), 2.43−2.52 (m, 1H), 2.72 (br s, 0.4 H), 2.89 (br s, 0.6H), 3.65−3.81 (m, 6H), 3.95−4.08 (m, 3H), 4.39 (d, 0.4H), 4.47 (d, 0.6H), 4.79−4.86 (m, 1H), 5.09−5.10 (d, 2H), 5.19−5.35 (m, 5H), 7.30−7.37 (m, 5H). ESIMS: 755 [M + H]$^+$, 777 [M + Na]$^+$, 793 [M + K]$^+$. HRESIMS: calcd for $C_{34}H_{46}N_2O_{17}Na$ [M + Na]$^+$ 777.2694, found *m*/*z* 777.2704 [M + Na]$^+$.

4-*O*-Acetyl-4-[2-*C*-(1-methyl 5-acetamido 4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*erythro*-L-*manno*-nononate)]-2*S*-(benzyloxycarbonyl)amino-1-carboxylic Acid (1). Sodium periodate (31 mg) and ruthenium(III) chloride hydrate (0.38 mg, 4 mol %) were added to a solution of alcohol **17** (27.5 mg, 0.04 mmol) in CCl_4 (250 μL), acetonitrile (250 μL), and water (300 μL) at 0 °C. The reaction mixture was stirred at 0 °C and changed color to yellow within 10 min. TLC indicated that there was some starting material left. Another small portion of $RuCl_3$ (0.3 mg) was added to the solution. Reaction was completed within another 0.5 h. The reaction mixture was passed through a pad of Celite and washed with EtOAc. The combined organic layer was evaporated and the residue was purified on a silica gel column (100% to 95 vol % CH_2Cl_2 in methanol) to afford **1** (13 mg, 47%). Lactone compound **7** (7 mg) was also isolated from the residue. ^1H NMR ($CDCl_3$): 1.88−2.09 (m), 2.24−2.36 (m, 1H), 2.44−2.52 (m, 1H), 3.74 (s, 1.5H), 3.78 (s, 1.5H), 3.86−3.96 (m), 4.04−4.14 (m), 4.33−4.39 (m), 4.78−4.88 (m), 5.03−5.16 (m), 5.27−5.35 (m), 7.30−7.37 (m, 5H). ESIMS: 769 [M + H]$^+$, 791 [M + Na]$^+$. HRESIMS: calcd for $C_{34}H_{44}N_2O_{18}Na$ [M + Na]$^+$ 791.2487, found *m*/*z* 791.2490 [M + Na]$^+$.

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Supporting Information Available: Spectral data for all new compounds (^1H NMR, ^{13}C NMR) and procedures for the synthesis of **5b**, **6a**, **6b**, and **6c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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