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Solid-Phase Chemoenzymatic Synthesis of *C*-Sialosides

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Abstract: The chemoenzymatic regioselective acylation of Neu5Ac followed by SmI_2-mediated *C*-glycosylation on a solid support is described for five *C*-glycosides. This method should facilitate the construction of combinatorial libraries of inhibitors of neuraminidase activity and hemagglutinin interaction as potential antiviral agents.

Glycoconjugates play important roles in regulating biological events.[1] Synthesis of structurally defined glycoconjugates, while demanding, can provide an array of novel molecules to investigate biological phenomena or treat diseases. The breadth of glycoconjugate structures make combinatorial strategies ideally suited for the preparation of libraries for screening.[2−6] Significant progress has been made in combinatorial synthesis of oligosaccharides and glycomimetics.[7−9] Glycoconjugates containing *N*-acetylneuraminic acid (Neu5Ac) can inhibit neuraminidase, a glycoprotein on the surface of the influenza virus that plays an important role in viral infection.[10,11] A number of neuraminidase inhibitors are currently in use for treating influenza virus.[12]

We reported the solution-phase stereoselective synthesis of neuraminic acid-based α-*C*-glycosides using SmI_2[13,14] that showed potent neuraminidase inhibitory activity.[15] These *C*-glycosides are substrate-based neuraminidase inhibitors that might also target hemagglutinin, another important influenza surface glycoprotein, providing a second point of inhibition in the viral infection pathway that in combination with neuraminidase inhibition might lead to the synergistic prevention of viral resistance. Here, we describe a chemoenzymatic regioselective acylation of Neu5Ac followed by SmI_2-catalyzed *C*-glycoside synthesis on solid supports that will facilitate the construction of a combinatorial library for screening as inhibitors of neuraminidase activity and hemagglutinin interaction. While SmI_2 is widely used in organic synthesis,[16−18] few reports describe the use of SmI_2 in solid-phase synthesis, and these are limited to the cleavage of product from support[19−22] or cyclization reactions.[21]

Our chemoenzymatic strategy begins with the attachment of neuraminic acid to a solid support through an appropriate linker followed by activation for use as a *C*-glycosylation donor. We reasoned that the C9 hydroxyl group of Neu5Ac was a logical point of attachment. Since chemical coupling of **3** through the C9 hydroxyl would require extensive blocking and deblocking steps, we examined an enzymatic route. We first screened a collection of proteases and lipases[23] for enzyme-catalyzed transesterification.[24] Subtilisin Carlsberg (from *Bacillus licheniformis*) was the most reactive enzyme on **3**. We next examined whether free, immobilized, or hexane-solubilized[25] subtilisin was capable of directly coupling **3** to the 2,2,2-trifluoroethyl ester of carboxyl-TentaGel resin. Attachment yields were low using free subtilisin in pyridine, only improving slightly with ion-paired subtilisin dissolved in hexane,[25] probably attributable to steric hindrance.

To overcome this limitation, we altered our strategy to enzymatically generate a derivative of **3** containing an amino group as a linker. To that end, *N*-Boc-L-phenylalanine was selected. As depicted in Scheme 1, *N*-Boc-L-Phe (**1**) was converted to trifluoroethyl ester (**2**) to afford an activated ester donor necessary for subtilisin-catalyzed transesterification of Neu5Ac (**3**). Compound **3** was converted to its corresponding methyl ester (**4**) under acidic conditions.[26] Transesterification of **2** with **4** gave the acylated neuraminic acid derivative **5**. The regioselective acylation at C9 of Neu5Ac was confirmed by the downfield shift of H-9, from 3.80 to 4.46 ppm, in the ^{1}H NMR spectrum. The *N*-Boc protecting group in **5** was removed quantitatively with TFA affording **6**, which contains a free amino group useful for coupling to the solid support.

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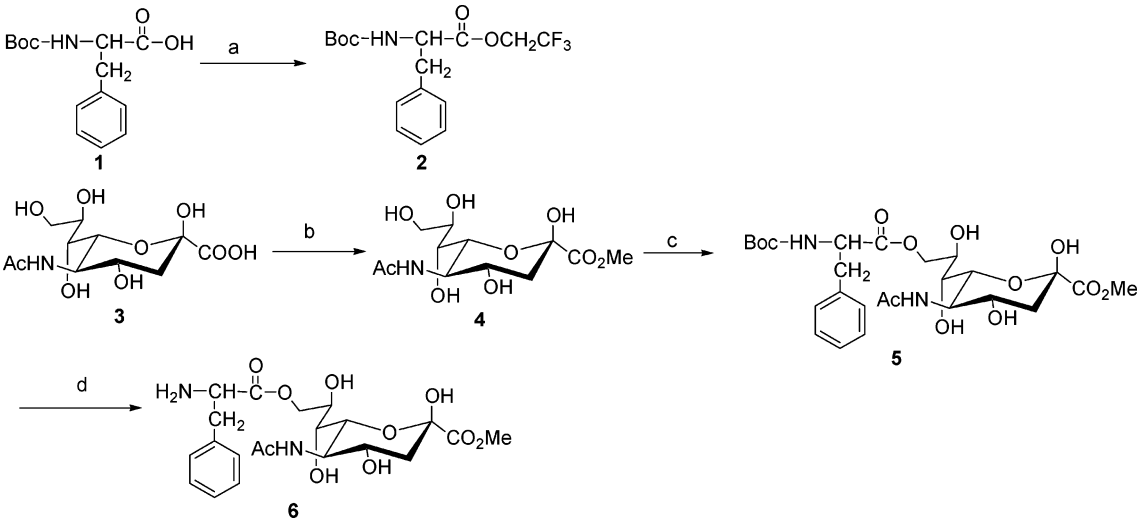
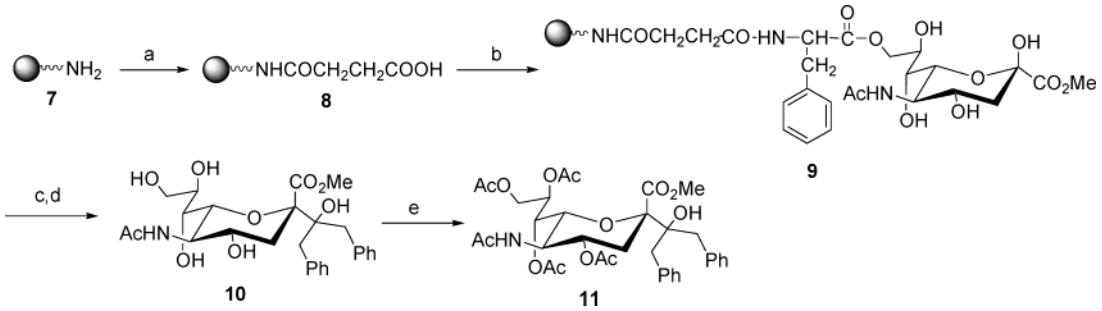
SCHEME 1. Synthesis of Linker-Bound Neuraminic Acid Methyl Ester[a]



[a] Key: (a) DCC, DMAP, CF_3CH_2OH, CH_2OH, rt; (b) Amberlite H^+ resin, MeOH; (c) subtilisin Carlsberg ChiroCLEC-BL, **4**, pyridine, rt; (d) CF_3COOH/H_2O (2:1).

SCHEME 2. *C*-Glycoside Synthesis on the CPG[a]



[a] Key: (a) succinic anhydride, toluene, reflux; (b) (i) DDC, HOBt, Et_3N, **6** (coupling repeated three times), (ii) DCC, HOBt, $CH_3CH_2CH_2NH_2$; (c) AcCl, MeOH, AcOH; (d) (i) aldehyde or ketone, SmI_2, THF, (ii) NaOMe, MeOH; (e) Ac_2O, pyridine.

Carboxyl TentaGel resin is a commonly used support in solid-phase synthesis.[27,28] However, SmI_2-mediated *C*-glycosylation on the TentaGel quickly turned the resin brown, and no product was obtained. We concluded that the failure of *C*-glycosylation on beads might be due to the relatively high hydrophobicity of the TentaGel resin, making it tightly bind the SmI_2 reagent, preventing the desired reaction from taking place. This conclusion is supported by a 100% increase in mass observed for the resin following *C*-glycosylation.

To overcome the problem of a hydrophobic solid phase interfering with the reaction, we used functionalized controlled pore glass (CPG) beads (**7**) as the solid support.[29] Aminopropyl-CPG (Glass-$SiOCH_2CH_2CH_2NH_2$, 120−200 mesh, 0.2 mmol/g loading capacity) was converted to a carboxyl-CPG support (**8**) by treating with succinic anhydride in toluene under reflux conditions. Full conversion was confirmed using amino staining reagents, diisopropylethylamine (DIPEA), and trinitrobenzensulfonic acid (TNBSA) (Scheme 2).

Linker containing Neu5Ac **6** was coupled to carboxyl-CPG **8** in the presence of DDC and HOBt to afford CPG-bound Neu5Ac derivative **9** (Scheme 2). A small amount of beads was withdrawn and cleaved in NaOMe/methanol, and the resulting hydrolysis product was compared by TLC with authentic Neu5Ac methyl ester (**4**). The amount of **4** released, determined by colorimetric assay, demonstrated that 83% of the CPG functional groups carried **6**. The remaining free carboxyl groups in **9** were capped with propylamine under the same coupling conditions. The neuraminic acid chloride donor was then prepared on the solid support and *C*-glycosylation carried out with 5 equiv of dibenzyl ketone under SmI_2-mediated coupling in THF. Beads containing product were washed, *C*-glycoside was cleaved from the CPG beads under basic conditions, and hydrolysis product was neutralized and filtered to remove the beads. The combined filtrates were evaporated under reduced pressure and passed through a G-25 spin column to remove salt contaminants. The yield of *C*-glycoside **10** was 61% on the basis of the solid-phase loading capacity and its structure confirmed by NMR and HRMS. To assign the stereochemistry of the new glycosidic linkage, **10** was acetylated to give **11** and the glycosidic linkage determined, as α, on the basis of empirical rules:[30] (1) the chemical shift of H-4 at 4.69 ppm (in the β-anomer H-4 should resonate at >5.0 ppm) and

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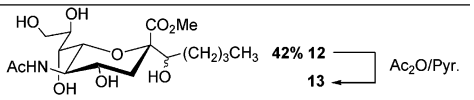
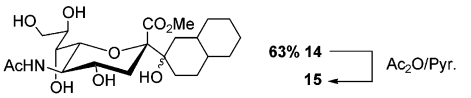
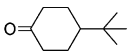
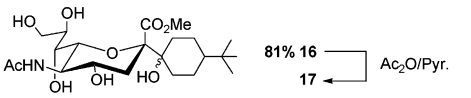
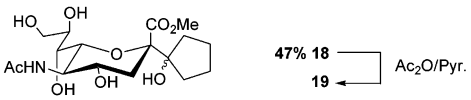
TABLE 1. α-*C*-glycosides Synthesized on Solid Support

Electrophiles	Products
$OHC(CH_2)_3CH_3$	 42% 12 / 13 — Ac_2O/Pyr.
	 63% 14 / 15 — Ac_2O/Pyr.
	 81% 16 / 17 — Ac_2O/Pyr.
	 47% 18 / 19 — Ac_2O/Pyr.

[a] Yields were determined based on the solid-phase loading capacity.

(2) the chemical shift difference between the two H9 protons of Neu5Ac [$\Delta(\delta$H9a-δH9b)] is 0.16 ppm (the β anomer should show a $\Delta\delta = 0.6-0.8$ ppm).

The coupling reaction of four additional aldehydes and ketones on solid support afforded recovered yields ranging from 42 to 81% (Table 1). α-*C*-glycosides **12** and **14** were mixtures of *R*/*S* (1:1) diastereomers at the newly formed hydroxymethylene bridge stereocenter.

This new methodology for the solid-phase synthesis of *C*-glycosides will now be applied to selected ketones and aldehydes, containing a variety of functionally groups and pharmacophores, to generate a small library for testing as inhibitors of neuraminidase activity and hemagglutinin binding to Neu5Ac containing glycoconjugates.

Experimental Section

Colorimetric Assay of Neu5Ac. A series of tubes were prepared, in triplicate, containing 5, 10, 20, 30, and 40 nmol of Neu5Ac. The volume of each tube was adjusted to 200 μL with water. Control tubes that contained 200 μL of water were also prepared. A 200 μL aliquot of Bial reagent (0.2 g of orcinol, 81.4 mL of concentrated HCl, 2 mL (1%) of ferric chloride and adjust with water to 100 mL) was added to each tube, and the tubes were mixed by vortexing and covered with glass marble balls. The tubes were heated at 100 °C for 15 min and cooled by immersiion in water at room temperature. Isoamyl alcohol (1 mL) was added to each tube, and the tubes were cooled in an ice bath for 5 min. The upper organic phase was transferred from each tube to a 1 mL cuvette with a Pasteur pipet, and the absorbance was measured at 570 nm with the control as reference. A standard curve was prepared as a plot of absorbance as a function of Neu5Ac concentration using the average of triplicates. The concentration of neuraminic acid in the sample was calculated from this standard curve.

Trifluoroethyl [2-[(1,1-Dimethylethoxy)carbonylamino]-3-phenyl]propanoate (2). Compound **2** was synthesized by mixing *N*-(*tert*-butoxycarbonyl)phenylalanine **1** (4.68 g, 17.6 mmol) with dimethylaminopyridine (DMAP, 2.15 g, 17.6 mmol), trifluoroethanol (192 mL, 26.4 mmol), and DCC (1.09 g, 21.1 mmol) in dry methylene chloride (50 mL) at 0 °C. After 2 h, the reaction medium was warmed to room temperature and stirred overnight. The solvent was evaporated under reduced pressure and separated by a silica gel chromatography (eluting with 95 vol % hexane in ethyl acetate, followed by 80 vol % hexane in ethyl acetate) to give compound **2** in 90% yield (5.50 g): ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.24−7.33 (m, 3H, Ar), 7.13−7.15 (m, 2H, Ar), 4.91 (d, 1H, J = 7.2 Hz, NH), 4.66 (m, 1H), 4.49−5.56 (m, 1H), 4.38−4.49 (m, 1H), 3.04−3.17 (m, 2H), 1.45 (s, 9H, t-Boc); ^{19}F NMR (300 MHz, $CDCl_3$) δ −73.97 (t, CH_2CF_3); FABMS 370 $[M + Na]^+$.

Methyl 5-Acetamido-3,5-dideoxy-9-*O*-[2-[(1,1-dimethylethoxy)carbonylamino]-3-phenyl]propanoyl-D-erythro-L-*manno*-nononate (5). ChiroCLEC-BL (100 mg, Altus Biologics, Inc.) and 4 Å molecular sieves (500 mg) were added to a solution of **2** (510 mg, 1.47 mmol) and **4** (480 mg, 1.49 mmol) in pyridine (20 mL). The solution was allowed to stand at room temperature for 7 days and then filtered to remove enzyme and molecular sieves. After the solvent was evaporated under reduced pressure, the residue was subjected to silica gel chromatography and eluted with methylene chloride to 90% methylene chloride in methanol to give compound **5** (689 mg, 81%): ^{1}H NMR (400 MHz, D_2O) δ 7.23−7.32 (m, 5H, Ar), 4.39−4.46 (m, 2H), 4.28 (dd, 1H, J = 7.6, 11.4 Hz), 4.01−4.10 (m, 2H), 3.94−3.97 (m, 1H), 3.80−3.84 (m, 4H, COO*Me*), 3.56 (dd, 1H, J = 1.28, 9.18 Hz), 3.19 (dd, 1H, J = 4.6, 13.8 Hz), 2.90 (dd, 1H, J = 9.5, 13.8 Hz), 2.24 (dd, 1 H, J = 4.8, 12.8 Hz), 2.02 (s, 3H, NHCOCH_3), 1.89 (t, 1H, J = 12.8 Hz), 1.33 (s, 9H, *t*-Boc); ^{13}C NMR (400 MHz, D_2O) δ 175.2, 173.8, 171.9, 158.0, 138.6, 130.5, 129.6, 127.9, 96.8, 80.8, 74.3, 72.1, 70.4, 69.5, 68.4, 67.9, 58.0, 54.5, 53.3, 40.9, 38.9, 28.8, 22.8; ESI-MS 571 $[M + H]^+$, 593 $[M + Na]^+$; HRMS calcd for $C_{26}H_{38}N_2O_{12}Na$ $[M + Na]^+$ 593.2322, found *m*/*z* 593.2327 $[M + Na]^+$.

Methyl 5-Acetamido-3,5-dideoxy-9-*O*-[2-[(1,1-dimethylethoxy)carbonylamino]-3-phenyl]propanoyl-D-*erythro*-L-*manno*-nononate (6). Compound **5** (370 mg, 0.65 mmol) was dissolved in 50% trifluoroacetic acid (10 mL). After 20 min, the solution was evaporated under reduced pressure. Toluene was added three times to coevaporate the trifluoroacetic acid, affording compound **6** (301 mg) without purification: ^{1}H NMR (400 MHz, D_2O) δ 7.22−7.37 (m, 5H, Ar), 4.33−4.43 (m, 3H) 4.01−4.10 (m, 2H), 3.72−4.03 (m, 4H), 3.61 (s, 1H, COO*Me*) 3.57 (t, 1H, J = 9.36 Hz), 3.31 (dd, 1H, J = 5.4, 14.4 Hz), 3.14 (dd, 1H, J = 7.9, 14.4 Hz), 2.24 (dd, 1 H, J = 5.04, 12.9 Hz), 1.88 (s, 3H, NHCOCH_3), 1.80 (t, 1H, J = 12.6 Hz); ESI-MS 471 $[M + H]^+$; HRMS calcd for $C_{21}H_{30}N_2O_{10}$ $[M + H]^+$ 471.1979, found *m*/*z* 471.1974 $[M + H]^+$.

Synthesis and Characterization of Carboxylated CPG (8). AMP-CPG (**7**, 1 g, 0.2 mmol, Fluka) was suspended in toluene (10 mL), and succinic anhydride (0.1 g,) was added. The reaction mixture was brought to reflux for 24 h. The resulting resin was washed with methylene chloride, 1 N HCl, H_2O, MeOH, acetone, and DCM (10 mL × 4) to afford carboxylated resin **8**. The pH value is 5 when suspended small portion of **8** in water. A small portion (approximately 20 mg) of beads **8** was transferred separately to 2 mL glass vials. DMF (2 drops), TNBSA (1 drop), and DIPEA (2 drops) were added to resin suspension. The vials were shaken gently, and solvent was removed from the vials using a glass pipet. DMF was added to wash each set of beads two times. Aminopropyl-derivatized glass beads were stained red, and beads **7** remained unchanged (white), indicating the full conversion of primary amino end groups in purchased beads to carbonyl end groups in **8**.

Attachment of Neuraminic Acid to Solid Support (9). Beads **8** were dried under vacuum before starting the reaction. Beads (1 g, 0.2 mmol) were suspended in DMF (7 mL) and stirred for 1 h under argon, after which time DCC (206 mg, 1 mmol) was added. In a separate flask, **6** (188 mg, 0.4 mmol) was dissolved in DMF (0.5 mL), and HOBt (135 mg, 1 mmol) and triethylamine (92 μL, 0.6 mmol) were added. The mixture was stirred until the HOBt dissolved. This mixture was transferred to bead suspension at 0 °C, and the reaction was stirred at 0 °C for 2 h. The reaction was warmed to room temperature and stirred overnight. The beads were washed four times (10 mL × 4) with DMF, DCM, MeOH, acetone, and DCM. The resulting beads were vacuum-dried, and the above procedure was repeated two more times. The residual carboxyl groups were blocked using the same immobilization procedure except that at this time **6** was replaced with 1 mmol of propylamine and 1 mmol of DCC and reacted for 5 h. The beads **9** were washed and dried.

Chlorination of 9. CPG-bound **9** (1 g) was added to acetyl chloride (5 mL), MeOH (0.2 mL), and acetic acid (0.4 mL). The mixture was stirred at room temperature for 2 d, washed with DCM, acetone, MeOH, and DCM, and dried to generate *C*-glycosylation donor **9**.

Solid-Phase Synthesis of *C*-Glycosides. CPG-bound *C*-glycosylation donor **10** (500 mg, 0.1 mmol) and electrophile (0.5 mmol) were added to a 50 mL round-bottom flask equipped with magnetic stir bar and dried under high vacuum for 6 h. Freshly prepared SmI_2 catalyst (10 mL) was added, and the reaction mixture was stirred overnight at room temperature. The beads were recovered and washed four times (10 mL × 4) with THF, saturated aq $Na_2S_2O_3$, aq $NaHCO_3$, water, acetone, MeOH, and DCM to obtain beads with off-white color. After the beads were dried under vacuum, they were suspended in 7 mL of dry methanol and NaOMe (catalytic amount) was added. The reaction mixture was stirred at room temperature overnight. The resulting mixture was washed extensively with methanol. The solvent washes were combined and evaporated to dryness. The residue was passed through a small G-25 spin column to remove salt. Acetylation of C-glycoside was undertaken to permit the full assignment of its NMR spectrum.

Acetylation of *C*-Glycoside. To a solution of *C*-glycoside (40 μmol) in pyridine (350 μL) was added acetic anhydride (150 μL, 1.5 mmol). The solution was stirred overnight at room temperature and evaporated to dryness. The residual pyridine and acetic anhydride were removed by coevaporation with toluene, and the residue was purified by silica gel chromatography.

Methyl 5-acetamido-3,5-dideoxy-2-*C*-(hydroxybisbenzyl)-D-*erythro*-L-*manno*-nononate (10): ^{1}H NMR (400 MHz, MD_3OD) δ 7.31−7.11 (m, 10H, Ar), 3.87−3.47 (m, 7H), 3.78 (1s, 3H, $COOCH_3$), 2.50 (dd, 1H), 2.01 (s, 3H, $NHCOCH_3$), 1.98 (t, 1H); ^{13}C NMR (400 MHz, MD_3OD) δ 175.4, 174.7, 174.0, 139.2, 139.0, 135.8, 132.4, 132.2, 130.7, 129.6, 128.7, 128.6, 127.9, 127.1, 127.0, 88.2, 79.1, 75.6, 75.6, 73.8, 73.5, 72.9, 70.3, 69.2, 68.9, 68.6, 64.7, 64.5, 54.4, 53.5, 52.9, 52.4, 42.9, 41.6, 41.4, 37.6, 36.0, 30.7, 22.6; $[\alpha]^{20}_D$ −0.162 (*c* 0.5, MeOH); HRMS calcd for $C_{27}H_{35}NO_9Na$ [M + Na]$^+$ 540.2210, found *m/z* 540.2234 [M + Na]$^+$.

Methyl 5-acetamido-4,7,8,9,tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-(hydroxybisbenzyl)-D-*erythro*-L-*manno*-nononate (11): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.16 (m, 10H, Ar), 5.35−5.27 (m, 2H, NH), 5.11 (m, 1H), 4.69 (m, 1H, *J* = 4.5, 7.4, 12.6 Hz), 4.26 (dd, 1H, *J* = 2.1, 12.7 Hz), 4.17−4.08 (m, 3H), 3.66 (s, 3H, $COOCH_3$), 3.19 (0.5 ABq, 1H, CH_2Ph), 2.98 (s, 1H, OH), 2.95 (ABq, 2H,CH_2Ph), 2.70 (0.5 ABq, 1H, CH_2Ph), 2.43 (dd, 1H, *J* = 4.5, 12.7 Hz), 2.24, 2.19, 2.05, 1.99, 1.87 (5s, 5 × 3H, $COCH_3$), 1.79, (t, *J* = 12.7, 12.7 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.1, 170.7, 170.2, 170.1, 169.9, 136.9, 136.8, 131.4, 131.3, 127.9, 127.8, 126.5, 126.3, 86.6, 77.6, 73.4, 70.4, 68.4, 67.7, 62.5, 52.4, 49.3, 40.9, 40.6, 33.3, 23.2, 21.3, 20.9, 20.9, 20.7, 14.1; $[\alpha]^{20}_D$ −5.5 (*c* 0.9, $CHCl_3$); HRMS calcd for $C_{35}H_{43}NO_{13}Na$ [M + Na]$^+$ 708.2632, found *m/z* 708.2632 [M + Na]$^+$.

Methyl 5-acetamido-3,5-dideoxy-2-*C*-(hydroxypentyl)-D-*erythro*-L-*manno*-nononate (12): ^{1}H NMR (500 MHz, MD_3OD) (*R*/*S* = 1:1) δ 3.86−3.38 (m, 14H), 3.78 (2s, 6H, $COOCH_3$), 2.50 (dd, 1H, *J* = 4.5 Hz and *J* = 13.5 Hz), 2.42 (dd, 1H, *J* = 4.5 and *J* = 13.25), 2.02 (t, 1H, *J* = 11.5 Hz), 2.00 (s, 6H, $NHCOCH_3$), 1.75 (t, 1H, *J* = 11 Hz), 1.65 (m, 2H), 1.48−1.20 (m, 12H), 0.9 (m, 6H); ^{13}C NMR (500 MHz, MD_3OD) (*R*/*S* = 1:1) δ 174.8, 174.4, 85.5, 84.7, 76.6, 76.1, 75.8, 75.5, 72.8, 72.5, 70.4, 70.2, 69.2, 64.7, 64.7, 54.2, 54.1, 53.1, 53.1, 49.9, 38.0, 35.7, 32.0, 31.5, 30.8, 29.6, 29.5, 23.5, 23.5, 22.7, 22.7, 14.8; ESI-MS 394.1 [M + H]$^+$, 416.1 [M + Na]$^+$; HRMS calcd for $C_{17}H_{31}NO_9Na$ [M + Na]$^+$ 416.4184, found *m/z* 416.1888 [M + Na]$^+$.

Methyl 5-acetamido-4,7,8,9,tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-(hydroxypentyl)-D-*erythro*-L-*manno*-nononate (13): ^{1}H NMR (500 MHz, $CDCl_3$) (*R*/*S* = 1:1) δ 5.46 (m, 2H), 5.42 (dt, 1H, *J* = 2.5, 8 Hz), 5.38 (dt, 1H, *J* = 2.5, 8 Hz), 5.14 (m, 2H, NH), 4.80 (m, 2H), 4.38 (dd, 1H, *J* = 4.5, 12.5 Hz), 4.34 (dd, 1H, *J* = 4.5, 12.5 Hz), 4.21−4.00 (m, 6H), 3.80 (2s, 6H, OCH_3), 2.53 (dd, 1H, *J* = 4.5, 14.2), 2.45 (dd, 1H, *J* = 4.5, 12.5 Hz), 2.37 (dd, 1H, *J* = 4.5, 12.5), 2.18, 2.16, 2.15, 2.14, 2.13, 2.09, 2.06, 2.03, 1.91, 1.87 (10s, 30H, $COCH_3$), 1.85 (m, 1H), 1.70 (m, 2H), 1.50−1.20 (m, 12H), 0.9 (m, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) (*R*/*S* = 1:1) δ 171.4, 171.32, 171.30, 171.2, 171.14, 171.12, 75.7, 73.5, 73.4, 71.9, 71.8, 70.3, 70.0, 69.2, 68.9, 68.8, 68.0, 67.9, 63.1, 62.99, 62.97, 52.5, 49.7, 49.6, 34.7, 33.5, 32.0, 30.6, 28.6, 28.4, 28.2, 23.4, 23.3, 22.7, 22.6, 21.4, 21.13, 21.0, 20.9, 15.8; ESI-MS 561.2 [M + H]$^+$; HRMS calcd for $C_{25}H_{39}NO_{13}Na$ [M + Na]$^+$ 584.5651, found *m/z* 584.2328 [M + Na]$^+$.

Methyl 5-acetamido-3,5-dideoxy-2-*C*-(hydroxydecahydro-2-naphthalaneyl)-D-*erythro*-L-*manno*-nononate (14): ^{1}H NMR (500 MHz, MD_3OD) (*R*/*S* = 1:1) δ 3.82 (2s, 6H, $COOCH_3$), 3.85−3.40 (m, 14H), 2.53 (m, 2H), 2.01 (s, 6H, $NHCOCH_3$), 1.99 (m, 2H), 1.95−0.8 (m, 34H, decahydro-2-naphthalene); ^{13}C NMR (500 MHz, MD_3OD) (*R*/*S* = 1:1) δ 174.9, 166.8, 87.9, 79.7, 79.4, 76.9, 75.8, 73.0, 72.9, 70.5, 69.6, 64.7, 64.6, 54.1, 53.2, 47.5, 36.6, 36.0, 31.8, 31.6, 28.4, 28.0, 26.6, 26.1, 22.7, 21.9; ESI-MS 460.3 [M + H]$^+$, 482.2 [M + Na]$^+$; HRMS calcd for $C_{22}H_{38}NO_9$ [M + H]$^+$ 460.5385, found *m/z* 460.2556 [M + H]$^+$.

Methyl 5-acetamido-4,7,8,9,tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-(hydroxydecahydro-2-naphthalanyl)-D-*erythro*-L-*manno*-nononate (15): ^{1}H NMR (500 MHz, $CDCl_3$) (*R*/*S* = 1:1) δ 5.08 (m, 2H), 5.31 (dd, 2H, *J* = 2, 8 Hz), 5.14 (m, 2H, NH), 4.76 (m, 2H), 4.33 (m, 2H), 4.14−3.98 (m, 6H), 3.80 (s, 6H, OCH_3), 2.50 (dd, 2H, *J* = 4.5, 12.5 Hz), 2.16, 2.12, 2.05, 2.04 (s, 4 × 6H, $COCH_3$), 1.92 (t, 2H, *J* = 12.5 Hz), 1,8−1.14 (m, 32 H, decahydro-2-naphthalene); ^{13}C NMR (300 MHz, $CDCl_3$) (*R*/*S* = 1:1) δ 171.3, 171.0, 170.9, 170.7, 170.4, 170.2, 73.5, 73.4, 70.6, 69.0, 68.9, 68.0, 62.9, 62.7, 52.6, 52.5, 49.8, 35.1, 33.2, 32.9, 30.7, 30.5, 30.2, 29.9, 27.3, 26.9, 26.7, 26.2, 25.1, 25.0, 23.4, 21.4, 21.1, 21.0, 20.9, 20.0, 14.3, 13.3, 12.2; ESI-MS 628.3 [M + H]$^+$, 650.2 [M + Na]$^+$; HRMS calcd for $C_{30}H_{45}NO_{13}Na$ [M +Na]$^+$ 650.6662, found *m/z* 650.2790 [M + Na]$^+$.

Methyl 5-acetamido-3,5-dideoxy-2-*C*-[hydroxy-4-(*tert*-butylcyclohexyl)]-D-*erythro*-L-*manno*-nononate (16): ^{1}H NMR (500 MHz, MD_3OD) δ 3.81 (s, 3H, $COOCH_3$), 3.85−3.40 (m, 7H), 2.54 (dd, 1H, *J* = 4.5, 13 Hz), 2.00 (s, 3H, $NHCOCH_3$), 2.00 (m, 1H), 1.7−1.15 (m, 9H, cyclohexane), 0.85 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (300 MHz, MD_3OD) δ 175.4, 174.7, 87.8, 75.8, 75.5, 73.1, 72.9, 70.5, 69.6, 64.6, 54.1, 53.2, 36.1, 33.4, 33.2, 32.9, 30.8, 28.0, 23.5, 23.49, 22.7; ESI-MS 462.3 [M + H]$^+$, 484.2 [M + Na]$^+$; HRMS calcd for $C_{22}H_{39}NO_9Na$ [M + Na]$^+$ 484.5354, found *m/z* 484.2521 [M + Na]$^+$.

Methyl 5-acetamido-4,7,8,9,tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-[hydroxy-4-(*tert*-butylcyclohexyl)]-D-*erythro*-L-*manno*-nononate (17): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.42 (dt, 1H, *J* = 2.5, 8 Hz), 5.30 (dd, 1H, *J* = 2.5, 8 Hz), 5.16 (bd, 1H, *J* = 10 Hz, NH), 4.76 (m, 1H), 4.32 (dd, 1H, *J* = 2.4, 12.3 Hz), 4.07 (dd, 1H, *J* = 6.2, 12.3 Hz), 3.99 (m, 2H), 3.79 (s, 3H, OCH_3), 2.57 (s, 1H, OH), 2.43 (dd, *J* = 4.5, 12.4 Hz), 2.16, 2.12, 2.05, 2.02, (4s, 4 × 3H, $COCH_3$), 1.97 (t, *J* = 12.4, 12.4 Hz), 1.87 (s, 3H, $NHCOCH_3$) 1.19−1.80 (m, 9H, cyclohexane), 0.88 (s, 9H, $C(CH_3)_3$); ESI-MS 630.3 [M + H]$^+$, 652.3 [M + Na]$^+$.

Methyl 5-acetamido-3,5-dideoxy-2-*C*-(hydroxy-cyclopentyl)-D-*erythro*-L-*manno*-nononate (18): ^{1}H NMR (500 MHz, MD_3OD) δ 3.79 (s, 3H, $COOCH_3$), 3.86−3.40 (m, 7H), 2.62 (dd, 1H, *J* = 4.5, 13 Hz), 2.00 (s, 3H, $NHCOCH_3$), 2.00 (m, 1H), 1.92−1.31 (m, 8H, cyclopentane); ^{13}C NMR (300 MHz, MD_3OD) δ 175.4, 174.7, 86.5, 86.2, 75.9, 72.9, 70.4, 69.4, 64.7, 54.2, 53.2, 37.2, 36.6, 35.4, 30.8, 24.9, 22.7; ESI-MS 392.3 [M + H]$^+$; HRMS calcd for $C_{17}H_{29}NO_9Na$ [M + Na]$^+$ 414.4025, found *m/z* 414.1730 [M + Na]$^+$.

Methyl 5-acetamido-4,7,8,9,tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-2-*C*-(hydroxycyclopenthyl)-D-*erythro*-L-*manno*-nononate (19): ^{1}H NMR (500 MHz, MD_3OD) δ 5.43 (dt, 1H, *J* = 2.5, 8 Hz), 5.32 (dd, 1H, *J* = 2.5, 8 Hz), 5.14 (bd, 1H, *J* = 10 Hz, NH), 4.76 (dt, 1H, *J* = 11, 11 Hz), 4.34 (dd, 1H, *J* = 2.5, 12.25 Hz), 4.14−4.01 (m, 3H), 3.79 (s, 3H, $COOCH_3$), 2.83 (bs, 1H, OH), 2.52 (dd, 1H, *J* = 4.5, 12.7 Hz), 2.16, 2.13, 2.05, 2.02 (4s, 4 × 3H, $COCH_3$), 1.90 (m, 1H), 1.87 (s, 3H, $COOCH_3$), 1.86−1.52 (m, 8H, cyclopentane); ESI-MS 560.3 [M + H]$^+$.

Supporting Information Available: ^{1}H NMR spectra for compounds **5**, **6**, and **10**−**19**; ^{13}C NMR spectra for compounds **5**, **10**−**16**, and **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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