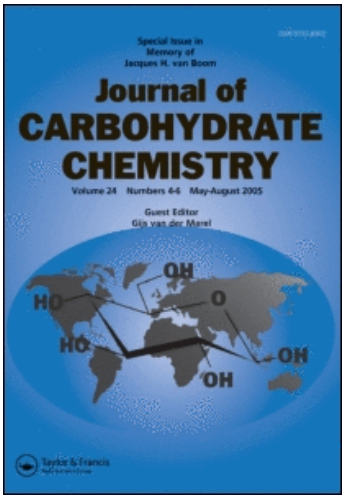

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CYCLODEXTRIN—A CARRIER OR SYNTHON? SYNTHESIS OF PER-*O*-METHYL-β-CYCLODEXTRIN-GM_3

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ABSTRACT

Cyclodextrins are currently under investigation for their utility as drug delivery agents. One property of the parent cyclic oligosaccharides which requires further modification for advanced drug delivery applications is that of site-directing capability. We have utilized β-cyclodextrin as a starting material for the synthesis of the GM_3 trisaccharide and have conjugated this targeting ligand to a derivative of the parent β-cyclodextrin. Synthesis of this cyclodextrin derivative, namely 18, is presented.

INTRODUCTION

Pharmaceutical applications for the family of cyclic oligosaccharides collectively knowns as the cyclodextrins (CyDs) have been contemplated for some time.[1] Their ability to form inclusion complexes has been exploited for altering the chemical and physical properties of guest (drug) molecules including solubility, *in vivo* stability, reduction of toxicity and irritancy, etc. In order for these drug carriers to function as advanced drug delivery systems, their ability to deliver drugs to a targeted site requires optimization.[2]

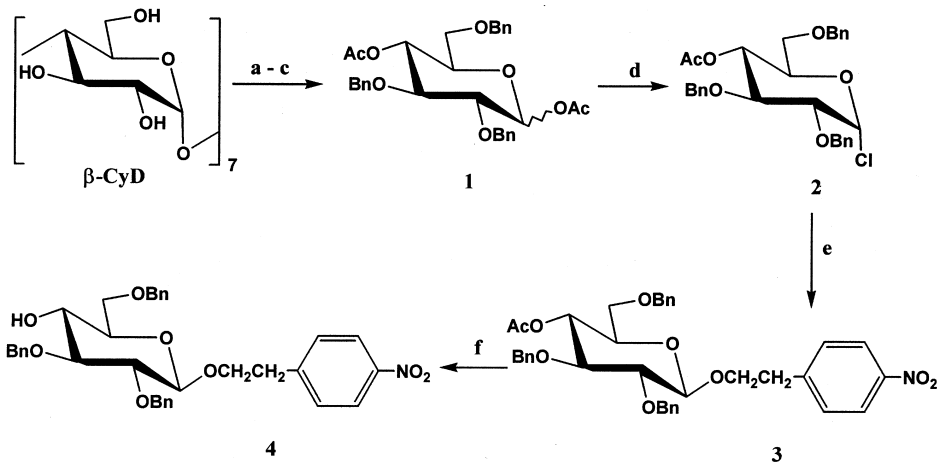
Along with a relatively hydrophobic interior cavity for the molecular encapsulation of guest molecules, the architecture of CyDs is such that two faces are apparent. The wider opening of the cyclic oligosaccharide is populated with secondary hydroxyl groups while the opposite face houses an array of primary

hydroxyls. These faces are separated by a distance of at least 7.9 Å.[3] An immediate consequence of this spatial arrangement is that the two faces can be exploited in a regioselective manner to provide both a region for drug complexation/conjugation and a region for arming with targeting ligands. Furthermore, the symmetric nature of the CyD skeleton allows for the installation of the latter in a multivalent fashion.[4]

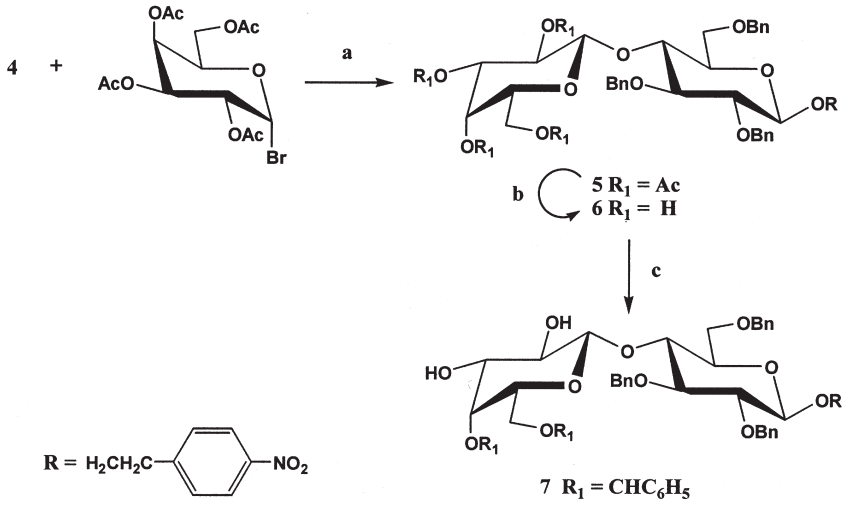
The ganglioside GM_3 is present on normal melanocytes. However, due to altered levels of expression in melanoma, it can be recognized by murine cytotoxic T cells and suppressor T cells as a tumor-associated antigen.[5] The synthesis of this antigen has been reported by several groups.[6] We report herein, the synthesis of this epitope from cyclodextrin, and the conjugation of this trisaccharide as a potential targeting ligand, to an amino-activated β-CyD derivative.

RESULTS AND DISCUSSION

The cyclodextrin skeleton is well suited for providing rapid access to intermediates for further elaboration into the lacto-series backbone in the sense that the glycosidic linkage at the 4-hydroxyl position can be viewed as a protecting group. Thus, as previously reported by Sato et al.,[7] glucose synthon **1** can be readily obtained by controlled acid hydrolysis of the fully benzylated β-CyD followed by subsequent acetylation of the anomeric and 4-hydroxyl moieties as outlined in Scheme 1. Anomeric activation as the chloride **2** is then accomplished by treatment with HCl saturated ether.[8] Reaction of **2** with *p*-nitrophenethyl alcohol using freshly prepared silver-sieves as the promoter[9] provided PNPE-glucose derivative **3** in 84 % yield as the β-glycoside ($J_{1,2}$ = 7.5 Hz). De-*O*-acetylation of **3** afforded glycosyl acceptor **4**.

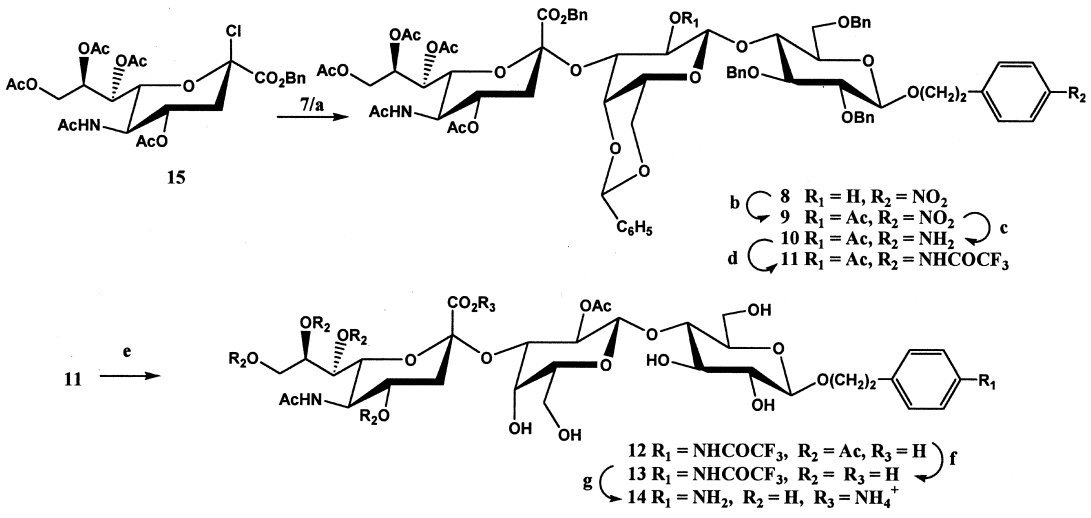


Scheme 1.



Scheme 2.

Glycosidation of **4** with acetobromogalactose in the presence of silver salts (Scheme 2) yielded the fully blocked lactose-PNPE derivative **5** in good yield (75%). Compound **5** was subjected to de-*O*-esterification to give tetrol **6** (83%). Simultaneous protection of the 4′- and 6′-hydroxy groups was subsequently realized by reaction with α,α′-dimethoxytoluene and catalytic *p*-toluenesulfonic acid to provide the desired sialyl acceptor as the 2′,3′-diol **7** (90% yield). Introduction of this diol to sialic acid donor **15**[10] (1.68 equiv) in the presence of silver salts (Scheme 3) led to a crude mixture containing starting diol **7** along with sialic acid



Scheme 3.

by-products (Neu5Ac-2-OH and 2,3-dehydro-Neu5Ac) and the desired sialoside **8**. Unfortunately, trisaccharide **8** could not be cleanly separated from this complex mixture, and column chromatography over silica gel at this stage only allowed for the separation of the Neu5Ac-2-OH derivative from the remainder of the mixture. Partial separation of diol **7** from the remaining mixture was accomplished by crystallization from chloroform leaving a mixture consisting mainly of the 2,3-dehydro-NeuNAc and **8**. This mixture was then acetylated using 2:1 pyridine-acetic anhydride, and the resulting product was readily separated to furnish the desired sialoside **9** (29% overall yield from **7**). Homonuclear decoupling experiments provided confirmation of several of the proton NMR resonances in the spectrum of this trisaccharide, and in particular, the downfield shift of H-2′ to δ 5.15 ppm which confirmed the regioselectivity of the glycosidation reaction. Next, the nitro moiety of **9** was converted into the *p*-NTFA derivative by the two step sequence involving initial treatment with $Zn/CuSO_4$ in tetrahydrofuran-acetic acid-water,[11] to give the free amine **10**. Protection of this functionality as the *p*-NTFA derivative was accomplished with pyridine-trifluoracetic anhydride to provide the desired trisaccharide **11** (90% yield from the fully protected nitro derivative **9**). Although the ^{1}H NMR spectrum of **11** was nearly identical to that of the nitro derivative **9**, the two proton doublet at δ 8.0 ppm normally associated with the 2-(*p*-nitrophenyl)ethyl derivatives had now shifted upfield. As well, the ^{19}F NMR spectrum of **11** displayed a fluorine singlet at δ -75.84 ppm.

Sequential deprotection of **11** was initiated by hydrogenation over wet 5% palladium-on-carbon containing a small amount of acetic acid to give intermediate **12**. Subsequent reaction of **12** with sodium methoxide in methanol revealed that de-*O*-acetylation of the 2′ hydroxyl was somewhat sluggish, however, prolonged treatment provided two lower R_f compounds. Neutralization of this reaction mixture followed by subsequent separation by column chromatography over silica gel gave the totally deblocked trisaccharide **13** (62%) followed by the ninhydrin positive amino compound **14** (12%). From the observed sluggish de-*O*-esterification of the acetylated hydroxyl group at C-2 of the galactose moiety, it appears that the topology of the trisaccharide is such that the sialic acid moiety has reduced steric accessibility to this region. The α-stereochemistry of the glycosylation reaction was firmly established by the fact that the signal for H-3″e of the sialic acid residue was located at δ 2.74 ppm,[12] and the remaining spectral data was consistent with the proposed structure. The amino derivative 14 was also available by treatment of **13** with ammonium hydroxide. The conjugation of a galactose ligand to mono-6-amino-6-deoxy-β-CyD using the isothiocyanate coupling strategy has been reported to proceed in reasonable yield.[13] In order to investigate the efficiency of conjugation of GM_3 analog **14** with a CyD carrier, the known mono-6-amino-per-*O*-methyl CyD **16**[14] was synthesized. Reaction with thiophosgene provided isothiocyanate **17** in quantitative yield (Scheme 4). Finally, conjugation of **17** with **14** in pyridine provided **18** in low (23%) yield.

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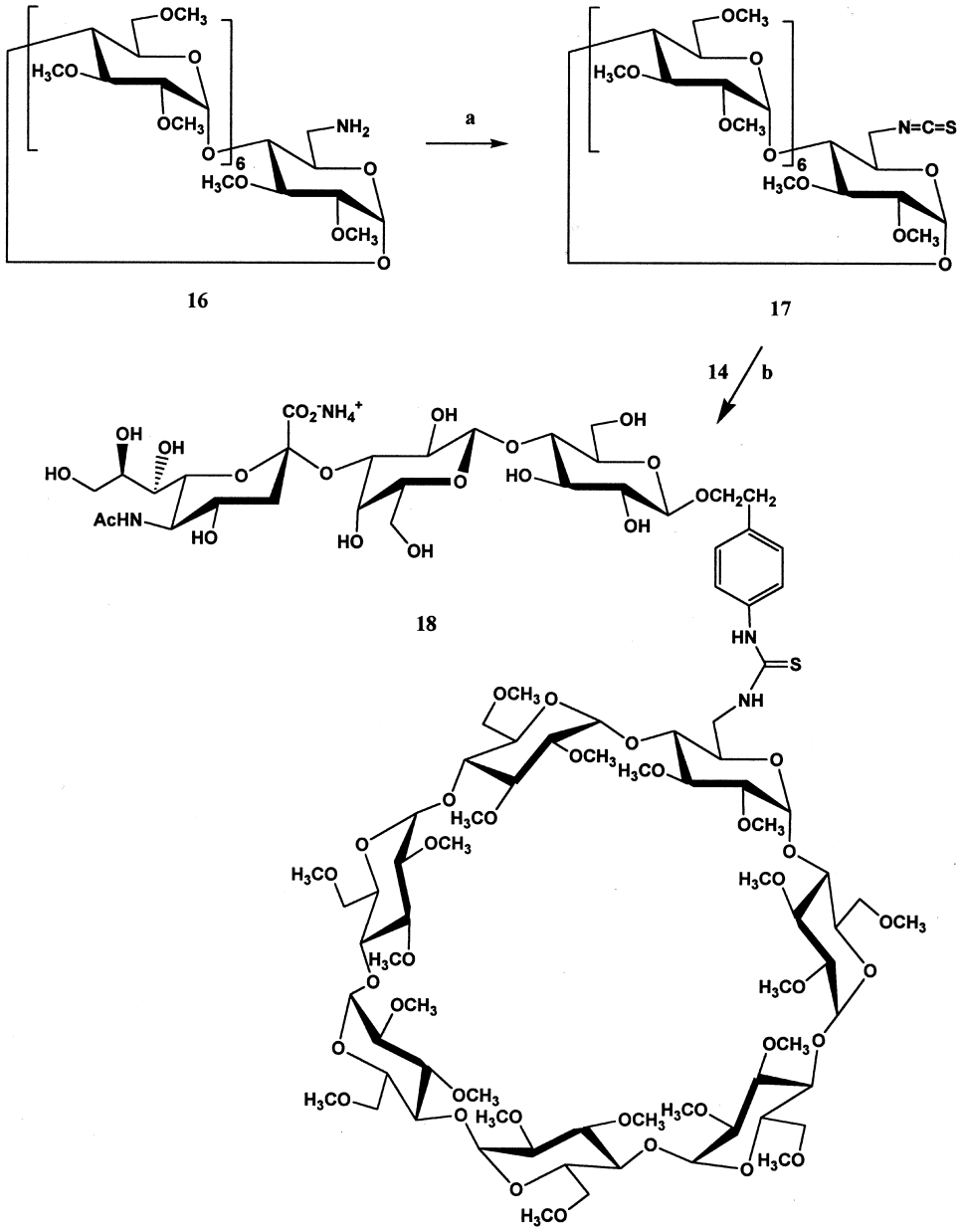

Scheme 4.

CONCLUSION

The synthesis of a cyclodextrin derivative armed with a targeting ligand, namely **18**, has been accomplished, albeit in low yield. The parent cyclic oligosaccharide has served as a convenient starting material to gain entry into the lactose

backbone ganglioside series. Work is currently underway to prepare the heptavalent GM_3-CyD.

EXPERIMENTAL

General Methods. NMR spectra were recorded on a Bruker AM 300 spectrometer (^{1}H: 300 MHz; ^{19}F: 282 MHz; ^{13}C: 75 MHz) or Varian Unity 500 spectrometer in $CDCl_3$ or D_2O solution unless otherwise stated. Chemical shifts in $CDCl_3$ solutions are reported in parts per million downfield from TMS, or in the case of D_2O solutions, using HOD set at δ 4.82 (25 °C) unless otherwise specified. ^{13}C NMR spectral assignments were aided by the J-MOD technique.[15] ^{19}F NMR spectra are reported in parts per million using external C_6F_6 (set at 0.0 ppm). Fast atom bombardment (FAB) mass spectra were obtained with a Kratos AE1 MS9 mass spectrometer while Electrospray (ES) mass spectra were obtained with a Micromass VG ZabSpec TOF instrument in the negative ion mode. Reactions were monitored by thin-layer chromatography (TLC) on Kieselgel 60 F_{254} (Merck) and visualization was accomplished by charring with 5% methanolic sulfuric acid. Column chromatography was performed using Merck 9385 silica gel (40 - 63μ). Cyclodextrin was dried *in vacuo* over P_2O_5 at 60 °C prior to use. Chloroform was distilled from P_2O_5 and pyridine was distilled from CaH_2 and stored over molecular sieves 3 Å. Activated powdered molecular sieves 4 Å were obtained from Aldrich Chemical Company and were dried *in vacuo* at 100 °C.

2-(*p*-Nitrophenyl)ethyl 2,3,6-Tri-*O*-benzyl-β-D-glucopyranoside **(4).** A solution of crude 2,3,6-tri-*O*-benzyl-α-D-glucopyranose[7] (3.15 g, 7 mmol) in 2:1 pyridine-acetic anhydride (75 mL) was stirred at room temperature for 48 h. The reaction mixture was then concentrated and the residue was subjected to column chromatography over silica gel using 7:1 hexane-ethyl acetate to provide 2.82 g (68 %) of 1,4-di-*O*-acetyl-2,3,6-tri-*O*-benzyl-α/β-D-glucopyranoside **1**: R_f 0.29 (3:1 hexane-ethyl acetate); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38 - 7.22 (m, 15H, aromatic), 6.34 (d, 0.5H, $J_{1,2}$ = 3.9 Hz, H-1δ), 5.63 (d, 0.5H, $J_{1,2}$ = 7.8 Hz, H-1β), 5.11 (t, 0.5H, J = 10.3 Hz, H-4α), 5.10 (t, 0.5H, J = 10.3 Hz, H-4β), 4.87 (d, 0.5H, J = 11.7 Hz, OC*H*$_2$C$_6$H$_5$), 4.80 (d, 0.5H, J = 11.7 Hz, OC*H*$_2$C$_6$H$_5$), 4.76 (d, 0.5H, J = 11.2 Hz, OC*H*$_2$C$_6$H$_5$), 4.72 (d, 0.5H, J = 11.7 Hz, OC*H*$_2$C$_6$H$_5$), 4.68 (d, 0.5H, J = 11.2 Hz, OC*H*$_2$C$_6$H$_5$), 4.63 (m, 1.5H, OC*H*$_2$C$_6$H$_5$), 4.51 (d, 0.5H, J = 11.7 Hz, OC*H*$_2$C$_6$H$_5$), 4.50 (d, 0.5H, J = 12.2 Hz, OC*H*$_2$C$_6$H$_5$), 4.47 (d, 0.5H, J = 12.2 Hz, OC*H*$_2$C$_6$H$_5$), 4.46 (d, 0.5H, J = 12.2 Hz, OC*H*$_2$C$_6$H$_5$), 3.93 (dt, 0.5H, $J_{5,6}$ = 4.4 Hz, H-5), 3.86 (t, 0.5H, J = 9.5 Hz, H-3α), 3.71 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2α), 3.66 (t, 0.5H, J = 9.0 Hz, H-3β), 3.65 - 3.60 (m, 1H), 3.56 – 3.43 (m, 2H), 2.17 (s, 1.5H, $COCH_3$), 2.05 (s, 1.5H, $COCH_3$), 1.84 (s, 1.5H, $COCH_3$), 1.82 (s, 1.5H, $COCH_3$).

Compound **1** (1.5 g, 2.8 mmol) was dissolved in hydrogen chloride saturated ethyl ether, and the reaction was stirred at room temperature and monitored by TLC (3:1 hexane-ethyl acetate). Upon completion of the reaction, argon was passed through the solution for 30 min. The organic solution was then diluted with ether and washed with water, saturated aqueous sodium hydrogen carbonate, brine,

dried (Na_2SO_4), filtered and concentrated. Column chromatography over silica gel using 4:1 hexane-ethyl acetate gave 860 mg (60%) of 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl chloride **2**: R_f 0.37 (3:1 hexane-ethyl acetate); ^{1}H NMR ($CDCl_3$, 500 MHz)δ 7.38 - 7.22 (m, 15H, aromatic), 6.03 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 5.13 (t, 1H, J = 10.3 Hz, H-4α or β), 5.11 (t, 1H, J = 10.3 Hz, H-4α or β), 4.88 (d, 1H, J = 11.7 Hz, OC*H*$_2$C$_6$H$_5$), 4.73 (d, 1H, J = 11.7 Hz, OC*H*$_2$C$_6$H$_5$), 4.68 (d, 1H, J = 11.7 Hz, OC*H*$_2$C$_6$H$_5$), 4.65 (d, 1H, J = 11.7 Hz, OC*H*$_2$C$_6$H$_5$), 4.51 (d, 1H, J = 11.7 Hz, OC*H*$_2$C$_6$H$_5$), 4.47 (d, 1H, J = 11.7 Hz, OC*H*$_2$C$_6$H$_5$), 3.93 (dt, 1H, $J_{5,6}$ = 3.9 Hz, H-5), 3.96 (t, 1H, J = 9.5 Hz, H-3), 3.76 (dd, 1H, H-2), 3.51 (dd, 1H, $J_{6a,6b}$ = 11.0 Hz, H-6a), 3.46 (dd, 1H, H-6b), 1.84 (s, 3H, $COCH_3$).

To a stirred suspension of dry powdered activated molecular sieves 4 Å (3.1 g) and toluene was added dropwise, a solution of silver trifluoromethanesulfonate (2.6 g, 10 mmol) in toluene (25 mL), and the mixture was stirred in the dark at ambient temperature for 5 h. The solid was allowed to settle and then decanted. To the suspension of Ag-sieves was added *p*-nitrophenethyl alcohol (1.1 g, 6.6 mmol) and the reaction stirred for an additional 30 min. At this time, the suspension was cooled to 0 °C under argon, and a solution of chloride **2** (850 mg, 1.7 mmol) in dry chloroform (17 mL) was added dropwise. The mixture was allowed to warm to ambient temperature and the reaction progress was monitored by TLC (3:1 hexane-ethyl acetate). After 20 h, the mixture was filtered through Celite and the pad washed several times with chloroform. The combined organic filtrate was washed with saturated aqueous sodium bicarbonate, brine, dried (Na_2SO_4), filtered and concentrated. Column chromatography over silica gel using 4:1 hexane-ethyl acetate gave 900 mg (84%) of 2-(*p*-nitrophenyl)ethyl 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **3**: R_f 0.14 (4:1 hexane-ethyl acetate); $[\alpha]_D$ -4.5° (*c* 0.25, chloroform); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.05 (d, 2H, J = 9.0 Hz, aromatic), 7.4 - 7.1 (m, 17H, aromatic), 4.96 (bt, 1H, J = 9.0 Hz, H-4), 4.78 (d, 1H, J = 12.0 Hz, OC*H*$_2$C$_6$H$_5$), 4.65 (d, 1H, J = 11.5 Hz, OC*H*$_2$C$_6$H$_5$), 4.59 (bd, 2H, J = 12.0 Hz, OC*H*$_2$C$_6$H$_5$), 4.51 (s, 2H, OC*H*$_2$C$_6$H$_5$), 4.42 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.24 (dt, 1H, J_d = 9.5 Hz, J_t = 6.0 Hz, OCH_2), 3.80 (dt, 1H, J_t = 7.0 Hz, OCH_2), 3.58 (t, 1H, J = 9.0 Hz, H-3), 3.56 - 3.52 (m, 3H, H-5, H-6a, H-6b), 3.44 (dd, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 3.04 (bt, 2H, OCH_2CH_2), 1.83 (s, 3H, $COCH_3$).

A solution of **3** (890 mg, 1.39 mmol) in methanolic sodium methoxide was stirred at room temperature overnight. The reaction mixture was then deionized with IR 120 (H^+) resin, filtered and concentrated. The residue was taken up in chloroform and washed with aqueous saturated sodium bicarbonate solution, brine, dried (Na_2SO_4), filtered and concentrated to give 830 mg (quantitative) of **4** as a foam: R_f 0.37 (20:10:1 hexane-ethyl acetate-ethanol); $[\alpha]_D$ -9.5° (*c* 0.5, chloroform); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.05 (d, 2H, J = 9.0 Hz, aromatic), 7.4 - 7.1 (m, 17H, aromatic), 4.90 (d, 1H, J = 11.5 Hz, OC*H*$_2$C$_6$H$_5$), 4.70 (d, 1H, J = 11.5 Hz, OC*H*$_2$C$_6$H$_5$), 4.66 (d, 1H, J = 12.0 Hz, OC*H*$_2$C$_6$H$_5$), 4.60 (d, 1H, J = 11.5 Hz, OC*H*$_2$C$_6$H$_5$), 4.59 (d, 1H, J = 12.0 Hz, OC*H*$_2$C$_6$H$_5$), 4.55 (d, 1H, J = 11.5 Hz, OC*H*$_2$C$_6$H$_5$), 4.41 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.23 (dt, 1H, J_d = 9.5 Hz, J_t = 6.0 Hz, OCH_2), 3.79 (dt, 1H, J_t = 6.5 Hz, OCH_2), 3.77 (dd, 1H, $J_{6a,6b}$ = 10.5 Hz, $J_{5,6a}$ = 3.5 Hz, H-6a), 3.65 (dd, 1H, $J_{5,6b}$ = 5.0 Hz, H-6b), 3.58 (td, 1H, J_t = 9.0 Hz,

$J_{4,OH}$ = 2.0 Hz, H-4), 3.46 (ddd, 1H, H-5), 3.40 (dd, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 3.38 (t, 1H, J_t = 9.0 Hz, H-3), 3.04 (bt, 2H, OCH_2CH_2).

Anal. Calcd for $C_{35}H_{37}O_8N$ (599.66): C, 70.10; H, 6.22; N, 2.34. Found: C, 69.79; H, 6.11; N, 2.33.

2-(*p*-Nitrophenyl)ethyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **(5).** A mixture of **4** (2.38 g, 3.97 mmol), silver carbonate (5.47 g, 19.9 mmol), catalytic silver trifluoromethanesulfonate (51 mg, 0.2 mmol), and powdered Drierite (10 g) in dry dichloromethane (25 mL) was cooled to -70 °C under argon. To the reaction mixture was added dropwise, a solution of acetobromogalactose (3.27 g, 7.9 mmol) in dry toluene (10 mL), and the mixture was allowed to slowly warm to -10 °C and stirred overnight at this temperature. The reaction mixture was then filtered through Celite and concentrated. Column chromatography over silica gel using 2:1 hexane-ethyl acetate yielded 2.77 g (75%) of **5**: R_f 0.11 (2:1 hexane-ethyl acetate); $[\alpha]_D$ -1.3° (*c* 0.25, chloroform); ^{1}H NMR δ 8.05 (d, 2H, J = 9.0 Hz, aromatic), 7.40 - 7.10 (m, 15H, aromatic), 7.10 (m, 2H, aromatic), 5.25 (dd, 1H, $J_{3',4'}$ = 3.5 Hz, $J_{4',5'}$ = 1.0 Hz, H-4′), 5.10 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{1',2'}$ = 8.0 Hz, H-2'), 4.91 (d, 1H, J = 11.0 Hz, $OCH_2C_6H_5$), 4.81 (dd, 1H, H-3'), 4.78 (d, 1H, J = 11.0 Hz, $OCH_2C_6H_5$), 4.74 (d, 1H, J = 12.0 Hz, $OCH_2C_6H_5$), 4.61 (s, 2H, $OCH_2C_6H_5$), 4.60 (d, 1H, $J_{1',2'}$ or $J_{1,2}$ = 8.0 Hz, H-1′ or H-1), 4.47 (d, 1H, J = 12.0 Hz, $OCH_2C_6H_5$), 4.38 (d, 1H, $J_{1,2}$ or $J_{1',2'}$ = 8.0 Hz, H-1 or H-1′), 4.21 (dt, 1H, J_d = 9.5 Hz, J_t = 6.0 Hz, OCH_2), 3.99 (dd, 1H, $J_{6'a,6'b}$= 11.0 Hz, $J_{5',6a'}$ = 7.5 Hz, H-6'a), 3.93 (t, 1H, J = 9.5 Hz, H-4), 3.84 (dd, 1H, $J_{5',6b'}$ = 6.0 Hz, H-6'b), 3.77 (dt, 1H, J_t = 6.0 Hz, OCH_2), 3.72 - 3.69 (m, 2H, H-6a and H-6b), 3.56 (t, 1H, J_t = 9.0 Hz, H-3), 3.52 (bdt, 1H, $J_{4',5'}$ = 1.0 Hz, H-5'), 3.38 (dd, 1H, H-2), 3.37 (dt, 1H, J_d = 9.0 Hz, J_t = 2.5 Hz, H-5), 3.07 - 3.01 (m, 2H, OCH_2CH_2), 2.09 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$).

Anal. Calcd for $C_{49}H_{55}O_{17}N$ (929.35): C, 63.27; H, 5.96; N, 1.51. Found: C, 62.88; H, 5.90; N, 1.48.

2-(*p*-Nitrophenyl)ethyl (4,6-*O*-Benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **(7).** To a solution of **5** (2.65 g, 2.85 mmol) in dry methanol (20 mL) was added a solution of sodium metal (61 mg, 2.6 mmol) in dry methanol (7 mL) and the solution was stirred for 18 h at room temperature. The mixture was then neutralized with IR 120 (H^+) resin, filtered and the solvents were removed to give 2.1 g (quantitative) of 2-(*p*-nitrophenyl)ethyl (β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **6** which was used directly in the next step without further purification or characterization: R_f 0.18 (5:5:1 hexane-ethyl acetate-ethanol).

A solution of tetrol **6** (2.1 g, 2.78 mmol), benzaldehyde dimethyl acetal (740 mg, 4.87 mmol), and *p*-toluenesulphonic acid monohydrate (27 mg, 0.14 mmol) in dry acetonitrile (30 mL) was stirred at room temperature overnight. The reaction was quenched with an excess of triethylamine, and the reaction mixture was then concentrated. Column chromatography over silica gel using chloroform then 100:1 chloroform-methanol as eluant gave 1.96 g (83%) of **7**: R_f 0.22 (30:1 chloroform-

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methanol); $[\alpha]_D$ +1° (*c* 1, chloroform); ^{1}H NMR δ 8.05 (d, 2H, J = 9.0 Hz, aromatic), 7.50 - 7.30 (m, 20H, aromatic), 7.10 (m, 2H, aromatic), 5.45 (s, 1H, benzylidene), 4.99 (d, 1H, J = 11.0 Hz, OC*H*$_2$C$_6$H$_5$), 4.90 (d, 1H, J = 11.0 Hz, OC*H*$_2$C$_6$H$_5$), 4.71 (d, 1H, J = 12.0 Hz, OC*H*$_2$C$_6$H$_5$), 4.65 (d, 1H, J = 12.0 Hz, OC*H*$_2$C$_6$H$_5$), 4.61 (d, 1H, J = 12.0 Hz, OC*H*$_2$C$_6$H$_5$), 4.58 (d, 1H, J = 12.0 Hz, OC*H*$_2$C$_6$H$_5$), 4.55 (d, 1H, $J_{1',2'}$ or $J_{1,2}$ = 7.5 Hz, H-1′ or H-1), 4.40 (d, 1H, $J_{1,2}$ or $J_{1',2'}$ = 7.5 Hz, H-1 or H-1′), 4.22 (dt, 1H, J_d = 9.5 Hz, J_t = 6.0 Hz, OCH$_2$), 4.05 (dd, 1H, $J_{6'a,6'b}$ = 12.0 Hz, $J_{5',6a'}$ = 1.0 Hz, H-6′a), 4.04 - 4.01 (m, 2H), 4.02 (t, 1H, J = 9.0 Hz, H-4), 3.79 (dt, 1H, J_t = 6.0 Hz, OCH$_2$), 3.78 (dd, 1H, $J_{5',6b'}$ = 2.0 Hz, H-6′b), 3.76 (m, 1H), 3.68 (t, 1H, J = 9.0 Hz, H-3), 3.62 (bm, 1H), 3.50-3.44 (m, 3H), 3.42 (dd, 1H, H-2), 3.04 (m, 2H, OCH$_2$C*H*$_2$), 2.88 (bs, 1H, OH), 2.41 (d, 1H, J_d = 9.0 Hz, OH).

Anal. Calcd for $C_{48}H_{51}O_{13}N$ (849.34): C, 67.82; H, 6.05; N, 1.65. Found: C, 67.58; H, 5.93; N, 1.68.

2-(*p*-Nitrophenyl)ethyl (Benzyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **(9).** A mixture of diol **7** (1.9 g, 2.24 mmol), silver trifluoromethanesulfonate (1.1 g 4.28 mmol), 2,6-di-*t*-butylpyridine (945 mg, 4.94 mmol), powdered Drierite (3 g), and anhydrous tetrahydrofuran (12 mL) was strirred at room temperature under argon atmosphere for 8 h to ensure dryness. The reaction mixture was then cooled to -35 °C under argon, and a solution of chloride **15**[10] (2.2 g, 3.37 mmol) in dry toluene (4 mL) was then added dropwise. Upon completion of the addition, the reaction mixture was stirred for an additional 30 min at this temperature and then at 0 °C overnight. The reaction mixture was then diluted with chloroform and filtered through Celite. The organic filtrate was washed with saturated aqueous sodium bicarbonate, water, dried (Na_2SO_4), filtered, and concentrated. The resulting yellowish foam was then purified by column chromatography over silica gel using 20:10:1 hexane-ethyl acetate-ethanol as eluant to give crude 2-(*p*-nitrophenyl)ethyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **8**, contaminated with diol **7** and unsaturated Neu5NAc.

The crude **8** (1.2 g) was treated with 2:1 pyridine-acetic anhydride (24 mL) at room temperature for 48 h and the mixture was then concentrated. After coevaporation with toluene, the resulting yellowish foam was purified by column chromatography over silica gel using 95:5 chloroform-methanol to provide 940 mg (29% overall yield from **7**) as a white solid after lyophilization from benzene: R_f 0.36 (20:1 chloroform-methanol); $[\alpha]_D$ +15.8° (*c* 0.5, chloroform); ^{1}H NMR δ 8.00 (d, 2H, J = 9.0 Hz, aromatic), 7.40 - 7.05 (m, 27H, aromatic), 5.58 (ddd, 1H, $J_{7'',8''}$ = 9.0 Hz, $J_{8'',9''b}$ = 6.0 Hz, $J_{8'',9''a}$ = 2.5 Hz, H-8″), 5.36 (dd, 1H, $J_{6'',7''}$ = 2.5 Hz, H-7″), 5.15 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, $J_{1',2'}$ = 8.0 Hz, H-2′), 5.13 (d, 1H, J = 12.0 Hz, OC*H*$_2$C$_6$H$_5$), 5.05 (d, 1H, J = 12.0 Hz, OC*H*$_2$C$_6$H$_5$), 5.04 (d, 1H, J = 12.0 Hz, OC*H*$_2$C$_6$H$_5$), 5.03 (d, 1H, J = 10.5 Hz, NH), 4.82 (s, 1H, benzylidene), 4.80 (d, 1H,

H-1′), 4.80 (m, 1H, H-4″), 4.65-4.55 (m, 4H, OC*H*$_2$C$_6$H$_5$), 4.38 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.35 (dd, 1H, $J_{9''a,9''b}$ = 12.5 Hz, H-9″a), 4.29 (dd, 1H, $J_{3',4'}$ = 3.5 Hz, H-3′), 4.19 (dt, 1H, J_d = 9.5 Hz, J_t = 6.0 Hz, OCH$_2$), 4.07 (q, 1H, J = 10.5 Hz, H-5″), 3.97 (dd, 1H, H-9″b), 3.90 (dd, 1H, $J_{6'a,6'b}$ = 12.5 Hz, $J_{5',6a'} < 1.0$ Hz, H-6′a), 3.87 (dd, 1H, $J_{6a,6b}$ = 11.0 Hz, $J_{5,6a}$ = 1.5 Hz, H-6a), 3.84 (dd, 1H, H-6″), 3.83 (t, 1H, J = 9.5 Hz, H-4), 3.78 (dt, 1H, J_t = 7.0 Hz, OCH$_2$), 3.72 (dd, 1H, $J_{5,6b}$ = 5.5 Hz, H-6b), 3.63 (t, 1H, J = 9.0 Hz, H-3), 3.52 (dd, 1H, $J_{5',6b'}$ = 1.0 Hz, H-6′b), 3.49 (ddd, 1H, H-5), 3.40 (d, 1H, H-4′), 3.36 (dd, 1H, H-2), 3.05 (bs, 1H, H-5′), 3.04-3.00 (m, 2H, OCH$_2$C*H*$_2$), 2.73 (dd, 1H, $J_{3''e,3''a}$ = 12.5 Hz, $J_{3''e,4''}$ = 4.5 Hz, H-3″e), 2.20 (s, 3H, COCH$_3$), 2.12 (s, 3H, COCH$_3$), 2.06 (s, 3H, COCH$_3$), 2.01 (s, 3H, COCH$_3$), 2.00 (s, 3H, COCH$_3$), 1.88 (s, 3H, COCH$_3$), 1.80 (t, 1H, H-3″a).

Anal. Calcd for $C_{76}H_{84}O_{26}N_2$ (1440.53): C, 63.31; H, 5.88; N, 1.94. Found: C, 63.00; H, 5.77; N, 1.94.

2-(*p*-Trifluoroacetamidophenyl)ethyl (Benzyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **(11).** A solution of **9** (930 mg, 0.65 mmol) in 15:2:0.6 tetrahydrofuran-acetic acid-water was cooled to 0 °C and zinc powder (2 g) was then added in one portion. To the rapidly stirred mixture was added 4 mL of aqueous copper sulfate solution (100 mg $CuSO_4 \cdot 5H_2O$ per 1 mL H_2O) and the mixture was stirred at room temperature for 1 h. After dilution with chloroform, the reaction was filtered through Celite and the organic filtrate was washed with saturated aqueous sodium bicarbonate, dried (Na_2SO_4), filtered, and concentrated. Finally, the residue was coevaporated with toluene to give crude 2-(*p*-aminophenyl)ethyl (benzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **10**: R_f 0.26 (20:1 chloroform-methanol, visualization with ninhydrin spray).

The crude amine **10** was dissolved in anhydrous dichloromethane (10 mL) containing anhydrous pyridine (525 μL, 6.5 mmol) and the reaction mixture was cooled to -20 °C under argon. To the reaction was added trifluoroacetic anhydride (305 μL, 2.17 mmol) and the reaction mixture was stirred at this temperature for an additional 10 min. The mixture was diluted with dichloromethane and then washed with saturated aqueous sodium bicarbonate solution, dried (Na_2SO_4), filtered, and concentrated. The resulting compound was purified by column chromatography over silica gel using 20:10:1 then 15:10:1 hexane-ethyl acetate-ethanol as eluant to give 880 mg (90%) of **11** as a white solid after lyophilization from benzene: R_f 0.1 15:10:1 (hexane-ethyl acetate-ethanol); $[\alpha]_D$ +1.7° (*c* 0.4, chloroform); ^{1}H NMR δ 7.70 (bs, 1H, NHTFA), 7.40 - 7.10 (m, 29H, aromatic), 5.57 (ddd, 1H, $J_{7'',8''}$ = 9.0 Hz, $J_{8'',9''b}$ = 6.0 Hz, $J_{8'',9''a}$ = 2.5 Hz, H-8″), 5.35 (dd, 1H, $J_{6'',7''}$ = 2.5 Hz, H-7″), 5.14 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{1',2'}$ = 8.0 Hz, H-2′), 5.11 (d, 1H, J = 11.5 Hz, OC*H*$_2$C$_6$H$_5$), 5.04 (d, 1H, J = 11.5 Hz, OC*H*$_2$C$_6$H$_5$), 5.02 (d, 1H, J = 11.5 Hz, OC*H*$_2$C$_6$H$_5$), 5.02 (d, 1H, J = 10.5 Hz, NH), 4.82 (s, 1H, benzylidene), 4.80 (d, 1H, J = 11.5 Hz, OC*H*$_2$C$_6$H$_5$), 4.79 (d, 1H, H-1′), 4.79 (m, 1H,

H-4″), 4.62 (d, 1H, J = 12.0 Hz, OC*H*$_2$C$_6$H$_5$), 4.61 (d, 1H, J = 11.5 Hz, OC*H*$_2$C$_6$H$_5$), 4.59 (d, 1H, J = 12.0 Hz, OC*H*$_2$C$_6$H$_5$), 4.53 (d, 1H, J = 11.5 Hz, OC*H*$_2$C$_6$H$_5$), 4.37 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.36 (dd, 1H, $J_{9''a,9''b}$ = 12.5 Hz, H-9″a), 4.29 (dd, 1H, $J_{3',4'}$ = 3.5 Hz, H-3′), 4.16 (dt, 1H, J_d = 9.5 Hz, J_t = 6.0 Hz, OCH$_2$), 4.06 (q, 1H, J = 10.5 Hz, H-5″), 3.97 (dd, 1H, H-9″b), 3.90 (dd, 1H, $J_{6'a,6'b}$ = 12.0 Hz, $J_{5',6'a'} < 1.0$ Hz, H-6′a), 3.87 (dd, 1H, $J_{6a,6b}$ = 11.0 Hz, $J_{5,6a}$ = 1.5 Hz, H-6a), 3.83 (dd, 1H, H-6″), 3.82 (t, 1H, J = 9.5 Hz, H-4), 3.73 (dt, 1H, J_t = 7.0 Hz, OCH$_2$), 3.72 (dd, 1H, $J_{5,6b}$ = 5.5 Hz, H-6b), 3.62 (t, 1H, J = 9.0 Hz, H-3), 3.52 (dd, 1H, $J_{6'b,6'a}$ = 12.0 Hz, $J_{5',6b'}$ = 1.0 Hz, H-6′b), 3.46 (ddd, 1H, H-5), 3.41 (d, 1H, H-4′), 3.35 (dd, 1H, H-2), 3.05 (bs, 1H, H-5′), 2.92 (t, 2H, OCH$_2$C*H*$_2$), 2.73 (dd, 1H, $J_{3''e,3''a}$ = 12.5 Hz, $J_{3''e,4''}$ = 4.5 Hz, H-3″e), 2.20 (s, 3H, COCH$_3$), 2.11 (s, 3H, COCH$_3$), 2.05 (s, 3H, COCH$_3$), 2.00 (s, 6H, COCH$_3$), 1.87 (s, 3H, COCH$_3$), 1.79 (t, 1H, H-3″a); ^{19}F NMR -75.84 (s).

Anal. Calcd for $C_{78}H_{85}O_{25}N_2F_3$ (1506.54): C, 62.13; H, 5.69; N, 1.86. Found: C, 61.78; H, 5.53; N, 1.88.

2-(*p*-Trifluoroacetamidophenyl)ethyl (5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic)-(2→3)-(β-D-galactopyranosyl)-(1→4)-β-D-gluco-pyranoside **(13).** To a mixture of **11** (840 mg, 0.58 mmol), methanol (50 mL), and 5% palladium-on-carbon was added acetic acid (500 μL), and the mixture was hydrogenated at 5 psi, room temperature for 20 h. The reaction mixture was then filtered through Celite and concentrated to give 538 mg of 2-(*p*-trifluoroacetamidophenyl)ethyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(2-*O*-acetyl-4,6-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside **12** which was homogeneous by TLC, R_f 0.39 (65:35:3 chloroform-methanol-water).

A solution of **12** (538 mg) in dry methanol containing sodium metal (46 mg, 2 mmol) was stirred at room temperature and the reaction was monitored by TLC (R_f 0.15 and 0.12 (6:1.2:1 ethyl acetate-methanol-water). The reaction mixture was neutralized with acetic acid, filtered and concentrated. Column chromatography over silica gel using first 10:2:1 then 6:1.2:1 ethyl acetate-methanol-water as solvent gave pure **13** (305 mg, 62%) followed by **14** (51 mg, 12%). Data for **13**: R_f 0.15 (6:1.2:1 ethyl acetate-methanol-water); $[\alpha]_D$ +0.9° (*c* 1, methanol); ^{1}H NMR (D_2O, HOD) δ 7.45 - 7.35 (m, 4H, aromatic), 4.50 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.46 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.12 (dt, 1H, J_d = 10.0 Hz, J_t = 6.5 Hz, OCH$_2$), 4.09 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, $J_{3',4'}$ = 3.5 Hz, H-3′), 3.97 - 3.51 (m), 3.53 (dd, 1H, H-2′), 3.27 (t, 1H, J = 9.0 Hz, H-2), 2.95 (t, 2H, OCH$_2$C*H*$_2$), 2.74 (dd, 1H, $J_{3''e,3''a}$ = 12.5 Hz, $J_{3''e,4''}$ = 4.5 Hz, H-3″e), 2.00 (s, 3H, NHCO*C*H$_3$), 1.78 (t, 1H, H-3″a); ^{19}F NMR δ -75.70 (s); ^{13}C NMR δ 175.72 (NH*C*OCH$_3$), 174.57 (C-1), 138.18 (aromatic C), 133.64 (aromatic C), 130.46 (2 × aromatic C), 123.12 (2 × aromatic C), 103.35 (C-1), 102.78 (C-1′), 100.51 (C-2″), 78.76 (C-4), 76.18 (C-3′), 75.84 (C-5′), 75.43 (C-5), 75.04 (C-3), 73.57 (C-6″), 73.46 (C-2), 72.45 (C-8″), 71.27 (OCH$_2$), 70.05 (C-2′), 69.02 (C-4″), 68.81 (C-7″), 68.17 (C-4′), 63.28 (C-9″), 61.69 (C-6′), 60.75 (C-6), 52.39 (C-5″), 40.33 (C-3″), 35.41 (*C*H$_2$C$_6$H$_4$), 22.75 (NHCO*C*H$_3$). Some of the ^{13}C assignments re-

main tentative. FAB-MS, Calcd for $C_{33}H_{47}O_{20}N_2F_3$: 848.73, (negative ion, TEA matrix). Found: 847.4 (M-1).

N-{4-[2-{(Ammonium 5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-(β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyloxy)}ethyl]phenyl}-*N*′-[6^A-deoxyheptakis(2,3-di-*O*-methyl)-6^B,6^C,6^D,6^E,6^F,6^G-hexa-*O*-methyl-β-cyclodextrin-6^A-yl]thiourea **(18).** Compound **13** (24 mg) was dissolved in ammonium hydroxide solution (5 mL) and the reaction was stirred at room temperature overnight. Removal of solvents and column chromatography over silica gel using 65:35:4.5 chloroform-methanol-water furnished 2-(*p*-aminophenyl)ethyl (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-(β-D-galacto-pyranosyl)-(1→4)-β-D-glucopyranoside **14** (16 mg, 76%): R_f 0.06 (65:35:4.5 chloroform-methanol-water, ninhydrin positive); ^{1}H NMR (D_2O, HOD) δ 7.35 - 6.85 (m, 4H, aromatic), 4.52 (d, 1H, J = 8.0 Hz, H-1′ or H-1), 4.48 (d, 1H, J = 8.0 Hz, H-1 or H-1′), 4.11 (dt, 1H, obscured by H-3′, OCH_2), 4.11 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, $J_{3',4'}$ = 3.5 Hz, H-3′), 3.99 - 3.53 (m), 3.27 (t, 2H, J = 9.0 Hz), 2.85 (t, 2H, J = 7.0 Hz, OCH_2CH_2), 2.75 (dd, 1H, $J_{3''e,3''a}$ = 12.5 Hz, $J_{3''e,4''}$ = 4.5 Hz, H-3″e), 2.02 (s, 3H, $NHCOCH_3$), 1.78 (t, 1H, H-3″a).

To a stirred mixture of **16**[13] (21 mg, 15 μmol) in chloroform (1 mL) and saturated aqueous hydrogen carbonate (1 mL), was added thiophosgene (7 mg, 60 μmol) in chloroform (0.6 mL) and the reaction stirred at room temperature for 30 min. The layers were then separated and the organic solution was dried (Na_2SO_4), filtered, and concentrated to give crude 6^A-isothiocyanato-6^A-deoxyheptakis(2,3-di-*O*-methyl)-6^B,6^C,6^D,6^E,6^F,6^G-hexa-*O*-methyl-β-cyclodextrin **17**: R_f **16** 0.5; R_f **17** 0.72 (9:1 chloroform-methanol).

To a mixture of crude **17** (~21 mg) and **14** (9 mg, 12 μmol) in methanol (750 μL) was added pyridine (500 μL) and the reaction was stirred at room temperature for 48 h. The reaction mixture was concentrated and purified by column chromatography using first 65:35 chloroform-methanol to elute unreacted **17** followed by 65:35:6 chloroform-methanol water to give 6 mg (23%) **18** and finally unreacted **14**. Lyophilization of **18** from water gave a solid: R_f 0.46 (5:4:1 chloroform-methanol-0.2% aqueous calcium chloride); ^{1}H NMR (D_2O, HOD) δ 7.51 (br s, 2H, aromatic), 7.40 (br d, 2H, aromatic), 5.67 (br s, 2H), 5.5 – 5.25 (m, 7H, H-1 cyclodextrin), 4.60 (d, 1H, $J_{1,2}$ or $J_{1',2'}$ = 7.8 Hz, H-1 or H-1′ GM_3), 4.56 (dd, 1H, J = 8.0 Hz, J = 3.9 Hz), 4.3 – 3.3 (m, 132 H), 3.08 (t, 2H, J = 7.0 Hz), 3.02 (t, 2H, J = 7.0 Hz), 2.84 (dd, 1H, $J_{3e'',3a''}$ = 12.5 Hz, $J_{3e'',4''}$ = 4.9 Hz, H-3e NeuNAc), 2.11 (s, 3H, $COCH_3$), 1.88 (t, 1H, H-3a NeuNAc). ES-MS (negative ion), Calcd for $C_{94}H_{154}O_{52}N_3S$ ((M-1)-18): 2190.3. Found: 2189.9.

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