One-Pot Synthesis of a Pentasaccharide with Antibiotic Activity

Ping Wang, Heeseob Lee, Minoru Fukuda and Peter H. Seeberger*

Glycobiology Program, Cancer Research Center, Burnham Institute for Medical Research, La Jolla, CA 92037, USA

Experimental Section

General Information. All chemicals used were reagent grade and used as supplied except where noted. All reactions were performed in oven-dried glassware under an inert atmosphere (nitrogen or argon) unless noted otherwise. Reagent grade dichloromethane (CH₂Cl₂), tetrahydrofuran (THF), diethyl ether (Et₂O) and toluene (PhMe) were passed through activated neutral alumina column prior to use. Reagent grade N,N-dimethylformamide (DMF) and methanol (MeOH) were dried over activated molecular sieves prior to use. Pyridine, triethylamine and acetonitrile were distilled over CaH₂ prior to use. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F₂₅₄ plates (0.25mm). Compounds were visualized by UV irradiation or dipping the plate in a cerium sulfate-ammonium molybdate (CAM) solution or phosphomolybdic acid (PMA) or sulforic acid ethanol solution, or spraying with Bial’s reagent (orcinol in acidic ethanol). Flash column chromatography was carried out using forced flow of the indicated solvent on Kieselgel 60 (230-400 mesh).

¹H, and ¹³C NMR spectra were recorded in CDCl₃ with chemical shifts referenced to internal standards CDCl₃ (7.26 ppm ¹H, 77.0 ppm ¹³C) unless otherwise stated. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; brs, broad singlet for ¹H NMR data. Signals were assigned by means of DEPT, ¹H-¹H COSY and ¹H-¹³C HSQC spectra. High-resolution mass spectral (HRMS) analyses were performed by The Scripps Research Institute.
Phenyl 2,3,6-tri-O-benzoyl-1-thio-ß-D-galactopyranoside (9)

Compound 8 (2.00 g, 3.52 mmol) was treated with acetic acid (70%) at 60°C for 4 h until TLC indicated that the reaction was complete. The reaction mixture was concentrated in vacuo and the remaining residue was diluted with EtOAc, washed with aqueous NaHCO₃ solution, dried, filtered and concentrated. The residue was dissolved in pyridine and benzoyl chloride was added to the stirred solution at -30°C. The reaction was quenched by the addition of methanol after 1 h, diluted with EtOAc, washed with HCl (1M), dried over NaSO₄ and concentrated. The residue was purified by flash chromatography on silica gel (petroleum ether : EtOAc = 5:1 → 3:1) to give 9 (1.54 g, 75%) as a white solid. Rf 0.33 (petroleum ether : AcOEt = 4:1); ¹H NMR (300 MHz, CDCl₃): δ 8.07-7.96 (m, 6 H, ArH), 7.52-7.33 (m, 11 H, ArH), 7.20-7.17 (m, 3 H, ArH), 5.84 (t, J = 9.9 Hz, 1 H), 2.48 (d, J = 8.4 Hz, 1 H), 5.01 (d, J =10.5 Hz, 1 H), 4.70-4.66 (m, 2 H), 4.43 (s, 1 H), 4.16 (t, J = 5.79 Hz, 1 H), 2.80-2.60 ( br, 1 H, OH); ¹³C NMR (75 MHz, CDCl₃): δ 166.6, 166.0, 165.6, 133.7, 133. 68, 133.5, 133.46, 133.0, 132.5, 130.3, 130.1, 130.06, 130.0, 129.96, 129.8, 129.6, 129.1, 129.07, 128.7, 128.6, 128.1, 87.1, 75.4, 68.1, 67.8, 63.6; ESI-HRMS (m/z) Calcd for C₃₃H₂₈O₈SNa: 607.1403; Found: 607.1394.

Phenyl (3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-1-thio-ß-D-galactopyranoside (12)

A solution of glycosyl bromide 10 (600 mg, 1.52 mmol), alcohol 9 (979 mg, 1.67 mmol), and sym-collidine (0.185 mL, 1.40 mmol) in anhydrous CH₂Cl₂ (10 mL) containing 4Å MS was stirred at -30°C for 30 min. Silver triflate (AgOTf) (771 mg, 3.0 mmol) was
added and the solution was stirred for 2 h at the -30 °C under an atmosphere of nitrogen before the reaction was quenched with triethylamine. The mixture was filtered to remove the molecularsieves and was washed with CH₂Cl₂. The filtrate was concentrated under vacuum. The crude product was purified by silica gel column chromatography (petroleum ether : EtOAc = 3:1) to afford 12 (1.04 g, 76%) as a white foam. R_f = 0.29 (petroleum ether : EtOAc = 3:1); ^1H NMR (300 MHz, CDCl₃): δ 8.09 (d, J = 8.1 Hz, 2 H), 7.97-7.94 (m, 4 H), 7.61-7.50 (m, 7 H), 7.39-7.26 (m, 7 H), 5.60 (t, J = 9.9 Hz, 1 H), 5.45-5.35 (m, 2 H), 5.03-4.94 (m, 32 H), 4.80-4.74 (m, 1 H), 4.48 (d, J = 2.4 Hz, 1 H), 4.22 (t, J = 6.6 Hz, 1 H), 4.04 (s, 1 H), 4.00 (t, J = 2.4 Hz). 3.63-3.64 (m, 2 H), 2.11, 2.10, 1.97 (3 s, 9 H); ^13C NMR (75 MHz, CDCl₃): δ 170.7, 170.0, 169.9, 166.3, 166.1, 165.2, 133.9, 133.7, 133.6, 133.5, 131.4, 130.2, 129.9, 129.8, 129.5, 129.0, 128.9, 128.74, 128.66, 128.6, 99.3, 86.2, 76.5, 75.0, 71.8, 68.8, 68.1, 67.6, 63.0, 62.5, 61.2, 20.93, 20.90, 20.8; ESI-HRMS (m/z) Calcd for C₄₅H₄₃N₃O₁₅SNa: 920.2313; Found: 920.2308.

O-(3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-D-galactopyranose (13)

To a solution of 12 (500 mg, 0.56 mmol) in THF and water (8 mL, 4:1) at 0 °C was slowly added NBS (149 mg, 0.84 mmol). After stirring at room temperature for 30 min, the solvent was evaporated. The crude product was purified by silica gel lash column chromatography (petroleum ether: EtOAc = 3:1) to give an α, β mixture of 13 (406 mg, 90%) as a colorless oil. R_f 0.25 (petroleum ether: AcOEt = 2:1). ^1H NMR (300 MHz, CDCl₃): δ 8.05-7.92 (m, 6 H), 7.60-7.30 (m, 9 H), 5.87-5.84 (m, 1 H), 5.78(d, J = 2.4 Hz, 1 H), 5.70-5.67 (m, 1 H), 5.55 (t, J = 7.2 Hz, 1 H), 5.08 (d, J = 3.0 Hz, 1 H), 5.05-4.95 (m, 1 H), 4.60-4.40 (m, 4 H), 4.38-4.36 (m, 1 H), 4.20-4.18 (m, 1 H), 3.88-3.84 (m, 1 H), 3.61-3.58 (m, 1 H), 3.46-3.43 (m, 1 H), 2.08, 2.03, 1.89 (3 s, 9 H); ^13C NMR (75 MHz,
CDCl₃): δ 170.7, 170.6, 170.14, 170.08, 166.3, 166.2, 166.1, 166.0, 134.7, 133.9, 133.5, 130.10, 130.06, 130.01, 129.93, 129.88, 129.85, 129.7, 129.4, 129.3, 129.2, 128.88, 128.83, 128.80, 128.75, 128.72, 128.70, 128.67, 128.6, 123.9, 98.9, 91.1, 71.3, 70.1, 69.3, 68.5, 68.3, 68.0, 62.5, 62.1, 61.4, 20.9, 20.8, 20.76, 20.74, 20.66; ESI-HRMS (m/z) Calcd for C₃₀H₃₀N₅O₁₆Na: 828.2228; Found: 828.2221.

1-O-(3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-D-galactopyranosyl Trichloroacetimidate (2)

A suspension of 13 (350 mg, 0.43 mmol), K₂CO₃ (180 mg), and NCCl₃ (0.20 mL) in CH₂Cl₂ (5 mL) was stirred at ambient temperature overnight. The mixture was filtered and washed with CH₂Cl₂. The filtrate was concentrated in vacuo and the crude product was purified by silica gel column flash chromatography (petroleum ether: EtOAc = 4:1) to afford 2 (321 mg, 78%) as a white foam. ¹H NMR (300 MHz, CDCl₃): δ 8.67 (s, 1 H, NH), 8.04-7.95 (m, 6 H), 7.58-7.34 (m, 9 H), 6.85(d, J = 3.6 Hz, 1 H), 6.00-5.84 (m, 2 H), 5.58 (t, J = 9.9 Hz, 1 H), 5.12 (d, J = 3.6 Hz, 1 H), 5.04 (d, J = 9.9 Hz, 1 H), 4.75-4.69 (m, 4 H), 4.40-4.36 (m, 1 H), 3.93-3.87 (m, 1 H), 3.64-3.60 (m, 1 H), 3.52-3.28 (m, 1 H), 2.10, 2.03, 1.89 (3 s, 9 H, OAc).

Ethyl 2-deoxy-6-pivaloyl-2-phthalimido-1-thio-β-D-glucopyranoside (3)

To a solution of 14 (1.02 g, 2.87 mmol) in anhydrous pyridine (5 mL) was added pivaloyl chloride (0.42 mL, 3.41 mmol) at 0 °C. The reaction was stirred at 0 °C for 3 h, concentrated and the residue was dissolved in EtOAc (60 mL) and washed twice with
water (10 mL). The organic layer was dried over Na₂SO₄ and evaporated at reduced pressure to afford 3 as white syrup (1.03 g, 82%). Rf 0.50 (petroleum ether : EtOAc = 2:1); ¹H NMR (300 MHz, CDCl₃): δ 7.82-7.78 (m, 2 H), 7.74-7.69 (m, 2 H), 5.31(d, J = 10.8 Hz, 1 H), 4.41-4.31 (m, 3 H), 4.16 (t, J = 10.2 Hz, 1 H), 3.89-3.85 (br, s, 1 H), 3.70-3.65 (br, s, 2 H), 3.40 (t, J = 7.5 Hz, 1 H), 2.73-2.57 (m, 2 H), 1.27-1.14 (m, 12 H); ¹³C NMR (75 MHz, CDCl₃): δ 179.4, 168.5, 168.3, 134.4, 132.03, 132.01, 124.2, 123.5, 81.2, 78.1, 72.6, 71.9, 64.0, 60.7, 55.7, 39.0, 27.3, 24.3, 21.2, 15.2, 14.3; ESI-HRMS (m/z) Calcd for C₂₁H₂₇NO₇SNa: 460.1406; Found: 460.1394.

Octyl 2-acetamido-2-deoxy-α-D-galactopyranoside (16)

A solution of 15 (3.00 g, 13.58 mmol) in octanol (20 mL) was added BF₃•OEt₂ (1.62 mL, 12.9 mmol). The reaction was stirred at 80 °C overnight. The reaction was quenched with HCl (con) before the solution was concentrated under vacuum to give a residue that was purified by silica gel flash column chromatography (CH₂Cl₂ : MeOH = 9:1) to afford 16 (3.53 g, 78%) as a white foam. Rf 0.30 (CH₂Cl₂ : MeOH = 9:1);

Octyl 2-azido-2-deoxy-α-D-galactopyranoside (17)

A solution of 16 (1.46 g, 4.38 mmol) in sodium hydroxide (1 M, 30 mL) was stirred at 100 °C overnight. The reaction was quenched with HCl (1 M) before the solution was concentrated under vacuum to give a residue that was treated with K₂CO₃ (960 mg, 6.95 mmol), CuSO₄ (7 mg, 0.04 mmol) in MeOH (15 mL) and water (10 mL). A solution of triflic azide (0.4 M, 5 mL) in CH₂Cl₂ was then added dropwise. The reaction was stirred
at room temperature for 12 h and concentrated. After addition of CH₂Cl₂ the solution was washed with water. The organic phase was dried and concentrated. The residue was purified by silica gel flash column chromatography (CH₂Cl₂ : MeOH = 10:1) to afford 17 (1.04 g, 75%) as a white foam. Rf = 0.29 (petroleum ether : EtOAc = 15:1); ¹H NMR (300 MHz, CDCl₃): δ 4.94 (d, J = 3.6 Hz, 1 H), 4.46-4.40 (m, 1 H), 4.20-4.12 (m, 1 H), 4.10-4.02 (m, 1 H), 4.00-3.95 (m, 2 H), 4.30-4.05 (m, 5 H), 3.70-3.64 (m, 1 H), 3.49-3.43 (m, 2 H), 1.65-1.58 (m, 2 H), 1.40-1.20 (m, 10 H), 0.88 (t, J = 3.6, 3 H); ESI-HRMS (m/z) Calcd for C₁₄H₂₇N₃O₅Na: 340.1849; Found: 340.1838.

Octyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside (18)

Compound 17 (850 mg, 2.68 mmol) was treated with TsOH•H₂O (20 mg) and benzaldehyde dimethylacetal (0.6 mL, 4.00 mmol) at room temperature overnight. The reaction mixture were concentrated under vacuum to give a residue that was purified by flash chromatography on silica gel (petroleum ether : EtOAc = 7:1 → 5:1) to afford 18 (869 mg, 80%) as a white solid. Rf,0.33 (petroleum ether : AcOEt = 5:1); ¹H NMR (300 MHz, CDCl₃): δ 7.50-7.47 (m, 2 H), 7.38-7.36 (m, 3 H), 5.31 (d, J = 10.8 Hz, 1 H), 4.41-4.31 (m, 3 H), 4.16 (t, J = 10.2 Hz, 1 H), 5.57 (d, J = 3.9 Hz, 1 H), 4.99 (d, J = 3.6 Hz, 1 H), 4.29-4.24 (m, 2 H), 4.18 (d, J = 10.8 Hz, 1 H), 4.08 (d, J = 12.0 Hz, 1 H), 3.73-3.67 (m, 2 H), 3.55-3.48 (m, 2 H), 2.55-2.50 (br, 1 H, OH), 1.61 (t, J = 6.6 Hz, 2 H), 1.40-1.22 (m, 10 H), 0.90-0.86 (m, 3 H); ¹³C NMR (75 MHz, CDCl₃): δ 137.5, 129.5, 128.5, 126.4, 101.5, 98.8, 75.8, 69.5, 68.8, 67.6, 62.9, 60.9, 32.0, 29.6, 29.5, 29.4, 26.3, 22.8, 14.3; ESI-HRMS (m/z) Calcd for C₂₁H₃₁N₃O₅Na: 428.2162; Found: 428.2149.

Octyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→3)-4,6-O-benzylidene-2-azido-2-deoxy-α-D-galactopyranoside (20)
To a solution of compound 18 (405 mg, 1.00 mmol), 19 (740 mg, 1.5 mmol) in anhydrous CH₂Cl₂ (10 mL) containing 4Å MS was added TMSOTf (10 µL) at -78 °C for and stirred for 2 h at the same temperature. Another portion of TMSOTf (20 µL) was added and the solution was stirred for an additional 2 h at room temperature before the reaction was quenched by the addition of Et₃N. The mixture was filtered and washed with CH₂Cl₂. The filtrate was concentrated to give a residue that was purified by silica gel flash column chromatography (petroleum ether : EtOAc = 3:1 ) to afford 20 (611 mg, 83%) as a white foam. R<sub>f</sub> = 0.29 (petroleum ether : EtOAc = 3: 1); <sup>1</sup>H NMR (300 MHz, CDCl₃): δ 7.56-7.53 (m, 2 H), 7.39-7.36 (m, 3 H), 5.57 (s, 1 H), 5.42 (d, J = 3.3 Hz, 1 H), 5.33-5.27 (m, 1 H), 5.06-5.01 (m, 2 H), 4.80 (d, J = 7.8 Hz, 1 H), 4.39 (d, J = 3.0 Hz, 1 H), 4.30-4.05 (m, 5 H), 3.96 (t, J = 6.3 Hz, 1 H), 3.84-3.80 (m, 1 H), 3.74-3.69 (m, 2 H), 3.56-3.51 (m, 1 H), 2.17, 2.07, 2.05, 1.99 (4 s, 12 H), 1.65-1.58 (m, 2 H), 1.40-1.20 (m, 10 H), 0.88 (t, J = 3.6, 3 H) ; <sup>13</sup>C NMR (75 MHz, CDCl₃): δ 170.79, 170.76, 170.6, 169.9, 138.1, 129.3, 128.6, 126.6, 102.9, 101.1, 99.0, 76.34, 76.31, 71.5, 71.3, 69.6, 69.2, 69.1, 67.4, 63.5, 61.8, 59.3, 32.2, 29.85, 29.76, 29.6, 29.5, 26.5, 23.1, 21.15, 21.12, 21.0, 14.5; ESI-HRMS (m/z) Calcd for C₃₅H₄₉N₃O₁₄Na: 758.3113; Found: 758.3091.

Octyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→3)-2-azido-2-deoxy-α-D-galactopyranoside (4)

A solution of compound 20 (500 mg, 0.68 mmol) in acetic acid (70%, 10 mL) was stirred at 60 °C for 3 h. The solution was concentrated to give a residue that was purified by
silica gel flash column chromatography (petroleum ether : EtOAc = 1:1) to afford 4 (343 mg, 78%) as a white solid. $R_f = 0.20$ (petroleum ether : EtOAc = 1:1); $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 5.42 (s, 1 H), 5.26 (t, $J = 9.9$ Hz, 1 H), 5.08-5.02 (m, 1 H), 4.96 (d, $J = 2.1$ Hz, 1 H), 4.75 (d, $J = 2.1$ Hz, 1 H), 4.20-4.05 (m, 5 H), 3.90-3.80 (m, 3 H), 3.75-3.68 (m, 1 H), 3.62-3.58 (m, 1 H), 3.55-3.45 (m, 1 H), 3.15-3.05 (br, 1 H, OH), 2.90-2.80 (br, 1 H, OH), 2.18, 2.10, 2.08, 1.99 (4 s, 12 H), 1.65-1.58 (m, 2 H), 1.40-1.20 (m, 10 H), 0.88 (t, $J = 3.6$, 3 H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 170.6, 170.3, 170.2, 169.9, 102.0, 98.2, 78.4, 71.2, 70.8, 69.5, 69.4, 68.6, 67.2, 61.7, 58.7, 31.9, 29.5, 29.4, 29.3, 26.2, 22.7, 20.73, 20.69, 20.6, 14.2; ESI-HRMS ($m/z$) Calcd for C$_{28}$H$_{45}$N$_3$O$_{14}$Na: 670.2800; Found: 670.2794.

Octyl (3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1 → 4)-(2,3,6-tri-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-2-deoxy-6-O-pivaloyl-2-phthalimido-β-D-glucopyranosyl- (1 → 6)-[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 3)]-2-azido-2-deoxy-α-D-galactopyranoside (6)

A suspension of 2 (150 mg, 0.158 mmol), 3 (62 mg, 0.142 mmol) and 4Å molecular sieves in anhydrous CH$_2$Cl$_2$ (5 mL) was stirred at -70°C for 30 min before TMSOTf (2 µL) was added slowly. After stirring for 1 h at -70°C, the mixture was warmed to -50°C. A solution of 4 (140 mg, 0.213 mmol) in anhydrous CH$_2$Cl$_2$ (1.0 mL) and NIS (100 mg) was added and stirred for 20 min before TfOH (30 uL) was added slowly. The mixture was stirred at -50°C for 30 min, then warmed to -10°C. After stirring at -50°C for 1 h,
the reaction was quenched by the addition of Et₃N. The mixture was filtered, the filtrates were concentrated and the remaining residue was purified by silica gel flash column chromatography (petroleum ether : EtOAc = 1:1) to afford 20 (162 mg, 63%) as a white foam. Rf = 0.23 (petroleum ether : EtOAc = 1:1); ¹H NMR (300 MHz, CDCl₃): δ 8.02-7.65 (m, 10 H), 7.60-7.30 (m, 9 H), 5.80-5.70 (m, 1 H), 5.55-5.50 (m, 1 H), 5.32-5.25 (m, 3 H), 5.22-5.18 (m, 1 H), 5.02 (t, J = 9.6 Hz, 1 H), 4.98-4.80 (m, 2 H), 4.798-4.65 (m, 1 H), 4.60-4.42 (m, 4 H), 4.38-4.30 (m, 2 H), 4.26-4.22 (m, 1 H), 4.20-3.90 (m, 8 H), 3.85-3.80 (m, 2 H), 3.76-3.70 (m, 2 H), 3.65-3.55 (m, 4 H), 3.45-3.40 (m, 1 H), 3.58-3.52 (m, 1 H), 3.00-2.95 (m, 1 H), 2.60-2.58 (br, 1 H, OH), 2.14, 2.07, 2.06, 2.05, 2.01, 1.98, 1.96 (7 s, 21 H, OAc), 1.80 (s, 2 H), 1.40-1.20 (m, 10 H), 1.10-1.08 (m, 9 H), 0.88 (t, J = 3.6, 3 H); ¹³C NMR (75 MHz, CDCl₃): δ 177.3, 170.5, 170.4, 170.2, 170.1, 169.8, 169.7, 169.5, 166.2, 165.9, 165.1, 133.9, 133.6, 133.4, 131.9, 129.94, 129.87, 129.7, 129.1, 128.62, 128.58, 128.5, 128.4, 123.4, 102.1, 101.4, 99.2, 98.4, 97.8, 83.3, 78.2, 76.0, 73.5, 73.1, 72.3, 71.1, 71.0, 70.7, 69.8, 69.1, 68.7, 68.5, 68.2, 68.0, 67.96, 67.9, 67.7, 66.9, 62.0, 61.4, 61.1, 58.2, 55.8, 38.7, 36.6, 31.84, 31.80, 29.7, 29.6, 29.4, 29.23, 29.21, 27.14, 27.11, 26.9, 26.1, 22.7, 22.6, 20.69, 20.67, 20.65, 20.63, 20.61, 20.60, 20.58, 20.56, 14.15, 14.1; ESI-HRMS (m/z) Calcd for C₈₆H₁₀₃N₇O₃₆Na: 1832.6342; Found: 1832.6312.

Octyl (2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)- (2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-[(β-D-galactopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranoside (1)
Triphenylphosphine (36 mg, 0.137 mmol) was added to 6 (85 mg, 0.046 mmol) that was dissolved in THF/H₂O (6 mL, 5:1) and the reaction was refluxed for 24 h. After concentrating the reaction mixture, the remaining residue was refluxed for 24 h with ethylenediamine (1 mL, 15 mmol) in THF/CH₃CN/EtOH (9 mL, 2:2:1). The solvents were removed and the remaining residue was stirred overnight in pyridine (2 mL) and acetic anhydride (1 mL). The mixture was concentrated and the residue was dissolved in CH₂Cl₂ (20 mL), and washed twice with water (2 mL). The organic layer was dried over Na₂SO₄ and evaporated. The crude residue was treated with sodium methoxide in methanol (1 M, 5.0 mL) overnight. The crude product was purified by silica gel flash column chromatography (i-C₃H₇OH/HOAc/H₂O = 8:1:1) to afford 1 (34 mg, 70%) as an amorphous solid. Rᵣ = 0.40 (i-C₃H₇OH/HOAc/H₂O = 6:1:1); ¹H NMR (500 MHz, MeOD): δ 4.89 (d, J = 2.0 Hz, 1 H), 4.79 (d, J = 3.0 Hz, 1 H), 4.44-4.41 (m, 3 H), 4.22-4.18 (m, 1 H), 4.15-4.12 (br, 1 H), 4.05-4.00 (m, 1 H), 3.90-3.85 (m, 4 H), 3.70-3.50 (m, 15 H), 3.48-3.40 (m, 3 H), 2.00, 1.96, 1.90 (3s, 9H), 1.62-1.58 (m, 2 H), 1.40-1.20 (m, 10 H), 0.90 (m, 3 H); ESI-HRMS (m/z) Calcd for C₄₄H₇₇N₃O₂₆Na: 1086.4693; Found: 1086.4685.

IC₅₀ Determination for alpha Cholesteryl Galactosyl Transferase²

CHLαGcT was incubated in 50mM HEPES-NaOH buffer (pH 7.5) containing 3.6 μM UDP-Glc (50,000 cpm), 0.4 mM cholesterol and 0.1% Triton X-100 with and without the synthetic pentasaccharide. After 10 min incubation, the reaction was stopped by addition of 80 mM HCl (final concentration). Ten volumes of ethyl acetate were added and mixed and, after brief centrifugation, radioactivity in the ethyl acetate (upper) layer was measured. The pentasaccharide concentration that inhibited 50% of the CHLαGcT activity was calculated by a log-linear interpolation between the three points (0.8 mM, 0.4 mM, 0.13 mM) that span the 50% value.

References
H. Lee, M. Kobayashi, P. Wang, J. Nakayama, P.H. Seeberger, M. Fukuda,