Synthesis of a C - Glycoside Analog of sTn: an HIV and Tumor Associated Antigen

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Experimental Section

General Methods: Nuclear magnetic resonance (\(^1\)H NMR) spectra were recorded at 25°C, in deuterated chloroform. Chemical shifts (δ) were recorded in ppm and coupling constants (J) in Hz, relative to tetramethylsilane as the internal standard. Thin-layer chromatography (TLC) was performed using E. Merck plates of silica gel 60 with fluorescent indicator. Visualization was effected by spraying the plates with Von’s reagent (1.0 g of ceric ammonium sulfate and 24.1 g of ammonium molybdate in 31 ml of sulfuric acid and 470 ml of water) followed by heating. Flash chromatography was conducted with silica gel (230-430 mesh, E. Merck).

6-Deoxy-1,2,3,4 - di-O-isopropylidene α-D-galacto-heptopyranosyl (5)

To a solution of 4 (544 mg, 2 mmole) in 70 ml of anhydrous MeOH at 0°C, and under argon atmosphere, NaBH₄ (76 mg, 2 mmole) was added. The mixture was stirred at 0°C until disappearance of the starting material monitored by TLC. Ethyl acetate was then added and the mixture washed with water, dried over anhydrous Na₂SO₄, and concentrated by rotary evaporation. Flash chromatography of the residue gave pure 5 (550 mg, quantitative yield). \(^1\)H NMR (500 MHz, CDCl₃) δ 5.53 (d, 1H, J = 5 Hz, H-1), 4.14 (dd, 1H, J = 2 Hz, H = 8 Hz, H-3), 4.31 (dd, 1H, H-2), 4.14 (dd, 1H, J = 2 Hz, H-4), 4.01 (dd, 1H, J = 9 Hz, H-5), 3.81 - 3.78 (m, 2H, H-7a & H-7b), 2.01 - 1.94 (m, 1H, H-6a), 1.79 - 1.73 (m, 1H, H-6b), 1.54, 1.47, 1.35, and 1.34 (4s, 4 × 3H, isopropylidene CH₃); HRMS calcd for C₁₃H₁₈O₇Na [M+Na] + found 297.1235.

6-Deoxy-D-galacto-heptopyranosyl (6)

Compound 5 (4.2 g, 15.33 mmole) was suspended in 250 ml water. Amberlite IR-120 (H⁺) ion-exchange resin (20 g previously washed with water at 80°C) was added and the suspension was heated at 70°C. After 3 h, the deprotection was complete, the reaction mixture was cooled to room temperature and the resin removed by filtration. The filtrate was concentrated by rotary evaporation and flash chromatography to afford 870 mg of pure compound 6 (4.2 g, 15.33 mmole). \(^1\)H NMR (500 MHz, CDCl₃) δ 5.18 (d, 1H, J = 3.5 Hz, H-10), 4.52 (d, 1H, J = 8 Hz, H-1B).

3, 4, 7-Tri-O-acetyl-1,5-anhydro-2,6-dideoxy-D-lyxo-hept-1-enitol (7)

To a suspension of 6-deoxy-D-galacto-heptopyranosyl 6 (190 mg, 0.98 mmole) in 3 ml acetic anhydride, 100 μl of 30% HBr/acetic acid was added under argon atmosphere and the reaction mixture cooled with an ice-water bath was stirred overnight, during which time the suspended syrup went into solution. The solution was then treated with an additional 8 ml of 30% HBr/acetic acid and stirred for 7 h at 0°C. Excess HBr was neutralized by adding anhydrous sodium acetate (2.66 g). The reaction mixture was then added to a suspension of pulverized Cs₂CO₃-5H₂O (315 mg) and zinc dust (12.6 g) in a solution of water (10 ml) and acetic acid (10 ml) containing sodium acetate trihydrate. The resultant reaction mixture was stirred vigorously using mechanical stirrer for 5 h with ice water cooling, filtered, and the collected solid was washed first with ethyl acetate followed by water. The organic soluble product was washed successively with saturated aqueous NaHCO₃ and brine, and dried over anhydrous Na₂SO₄. The organic layer was concentrated to provide colorless syrup that was purified by flash chromatography on silica gel to afford 7 in quantitative yield. \(^1\)H NMR (500 MHz, CDCl₃) δ 4.77 (dd, 1H, J = 6 Hz, H-6), 6.55 (m, 1H, J = 1.5 Hz), 5.35 (dd, 1H, J = 4.5 Hz, H-4), 4.67 (dd, 1H, J = 7 Hz, J = 2 Hz, H-2), 4.26 - 4.12 (m, 3H, H-5, H-7a & H-7b), 2.15, 2.06, 2.02 (3S, 3 × 3H, 3 OAc), 2.04 - 1.98 (m, 1H, H-6a), 1.89 - 1.82 (m, 1H, H-6b); HRMS calcd for C₁₁H₁₀O₂Na [M+Na] + 309.095, found 309.0948.

N\(^{\text{\cancel{N}}}\)-(Benzoxycarbonyl)-O-3,4,7-tri-O-acetyl-2-azido-2,6-dideoxy-α-D-galacto-heptopyranosyl-L-serine benzyl ester (9)

A suspension of N\(^{\text{\cancel{N}}}\)-Z-Ser-OBn (534 mg, 1.82 mmole) and glycolosyl bromide 8 (661 mg, 1.64 mmole) and activated powdered 4Å molecular sieves (1.5 g) in a 1:1 mixture of dry CHCl₃/benzene (30 ml : 30 ml) was stirred for 1 h at room temperature under dry argon atmosphere. The mixture was cooled to ~40°C and AgClO₄ (341 mg, 1.66 mmole) was added. The reaction was slowly brought to room temperature over 1 h and stirred overnight. After this time, TLC analysis confirmed the completion of the reaction. The reaction mixture was diluted with methylene chloride, filtered through celite, washed with water, and dried over anhydrous Na₂SO₄. The organic layer was concentrated by rotary evaporation and flash column chromatography afforded the pure 650 mg of a anomeric product 9 and 55 mg of α anomeric mixture of 6 (300 mg, quantitative yield). \(^1\)H NMR (500 MHz, CDCl₃) δ 7.40 - 7.30 (m, 10H, 2 × C₆H₅), 5.72 (d, 1H, J = 8.5 Hz, NH), 5.29 (d, 1H, J = 2.5 Hz, H-5), 5.25(dd, 1H, J = 3 Hz, J = 11 Hz, H-3), 5.22 (s, 2H, CH₂Ph), 5.126 (s, 2H, CH₂Ph), 4.84 (d, 1H, J = 3.5 Hz, H-1), 4.60 (bm, 1H, H-a), 4.14 (m, 1H, H-7a), 4.06-3.90 (m, 4H, H-5, H-7b, H-βa, & H-βb), 3.55 (dd, 1H, J = 3.5 Hz, J = 10.5 Hz, H-2), 2.14, 2.05, 1.98 (3s, 3 × 3H, 3Ac), 1.77 (m, 1H, H-6a), 1.68 (m, 1H, H-6b); HRMS calcd for C₂₀H₂₃NO₄BrLi [M+Li] + 679.2227, found 679.2216. β Isomer \(^1\)H NMR (500 MHz, CDCl₃) δ 7.40-7.30 (m, 10H, 2 × C₆H₅), 5.81 (d, 1H, J = 8 Hz, NH-α), 5.235 (s, 2H, CH₂Ph), 5.21 (d, 1H, J = 3 Hz, H-4), 5.21 (s, 2H, CH₂Ph), 5.74(dd, 1H, J = 3.5 Hz, J = 11 Hz, H-3), 4.42 (dd, 1H, J = 3 Hz, J = 10 Hz, Hβa), 4.28 (d, 1H, J = 8 Hz, H-1), 4.14-4.07 (m, 2H, H-7a & H-7b), 3.91 (dd, 1H, H-8b). 3.64-3.59 (m, 2H, H-5 &H-2), 2.171, 2.05, 2.04 (3s, 3 × 3H, 3Ac), 1.86 (m, 1H, H-6a), 1.75 (m, 1H, H-6b).
Aβ-(Benzyloxycarbonyl)-O-(2-acetamido-3,4,7-tri-O-acetyl-2,6-dideoxy-α-D-galacto-heptopyranosyl)-L-serine benzyl ester (10)

To a solution of 9 (600 mg) in 35 ml of dry pyridine at 0°C, dry thiocacetic acid (35 ml) was slowly added and the reaction was allowed to warm to room temperature while stirring. After 18 h, toluene was added and the mixture was concentrated. The residue was concentrated by rotary evaporation. Pyridine and thiocacetic acid were removed from the residue by multiple co-evaporation with toluene. Flash chromatography of the residue gave pure 10 (565 mg, 92% yield). 1H NMR (500 MHz, CDCl3), δ 7.40 – 7.30 (m, 10H, 2 x C6H5), 5.67 (d, 1H, J = 9 Hz, NHAc), 5.22-5.18 (m, 6H, NH Neu5Ac, H-4, 2 x benzylic CH2), 5.13 (s, 2H, CH 2Ph), 5.08 (dd, 1H, J = 3 Hz, J = 11 Hz, H-3), 4.71 (d, 1H, J = 3.5 Hz, H-1), 4.62 (bm, 1H, H-a), 4.49 (d(d, 1H, H-2), 4.42 (bd, 1H, H-5), 4.10 (dd, 1H, J = 3.5 Hz, H-βa), 3.83 (dd, 1H, J < 1 Hz, H-βb), 2.65 (dd, J = 8.5 Hz, J = 18 Hz, H-6a), 2.39 (dd, 1H, J = 4 Hz, H-6b), 2.10, 1.99 & 1.89 (3s, 3 x 3H, 3 Ac); HRMS calcd for C33H41N2O13 [M+H]+ 629.0024, found 629.2449

SαN-C-glycoside (2a)

A solution of compounds 12 (112 mg) and 13 (183 mg) was evaporated to dryness and the resulting residue was dried for 3 h under high vacuum. A solution of freshly prepared Snl in THF (0.2 M, 24 ml) was added to the dried residue placed under argon, and the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was then diluted with ethyl acetate, washed with 10% aqueous potassium carbonate/potassium tartarate solution. The two layers were separated and the aqueous layer was twice extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na2SO4, and concentrated by rotary evaporation to yield the syrup, which was purified on a small column to yield pure compound, sTn-C-glycoside 2a (165mg, 84% yield). 1H NMR (400 MHz, CDCl3), δ 7.45–7.20 (m, aromatic protons), 5.95-5.82 (2d, NH of amino acid residue of R & S isomer), 5.72 (d, NH Gal), 5.50–5.00 (m, H-7, H-4, H-3, 2 x CH2Ph & NH Neu5Ac), 4.85-4.75 (m, H'-4, 4.72(d, J=2.9 Hz, H-1), 4.60(m, dH), 4.50(m, H-2), 4.35(m, H'-9b), 4.30-4.10(m, H-5 & H'-6), 4.10-3.90(m, H-5, H'-9a & Hb), 3.90-3.70(m, H-7 of R isomer, Hb, OCH3), 3.70-3.60 (m, H-7 of S isomer), 3.30-3.20(b, OH of S isomer), 2.95-2.85(b, OH of R isomer), 2.40-2.30 (H'-3a), 2.25-1.80 (singlets, CH3CO), 1.80-1.60 (m, H-6b, & H'-3a), 1.60-1.40(H-6a) HRMS calcd for C15H34N2O14Na [M+Na]+ 625.2437, found 625.2449

sTn-keto derivative (2b)

The hydroxy methane bridged sTn derivative 2a (2mg) was taken in a micro vial. DMSO (200 µl) was added and temperature was brought to 4°C using ice cold water. Acetic anhydride (200 µl) was added carefully and left for stirring overnight and rotary evaporated to remove DMSO and acetic anhydride. The crude mixture was purified on silica gel column to obtain a pure keto product 2b (1 mg, 50% yield). 1H NMR (500MHz, CDCl3) δ 7.40-7.30 (m, aromatic protons), 5.78 (d, 1H, J = 9.5 Hz, NH of amino acid residue), 5.65 (d, 1H, J = 8 Hz, NH Gal), 5.40-5.30(m, 2H, H'-8', H-7), 5.25-5.12 (m, 6H, NH Neu5Ac, H-4, 2 x benzylic CH2), 5.16 (d, 1H, J = 10 Hz, H-βa), 5.08 (dd, 1H, J = 3 Hz, J = 11 Hz, H-3), 4.77 (d, 1H, J = 4 Hz, H-1), 4.58 (bm, 1H, H-a), 4.51 (dd, 1H, H-2), 4.06 (bd, 1H, J = 7.5 Hz, H-5), 3.99 (d, 1H, J = 9.5 Hz, H-βa), 3.92 (d, 1H, J = 10 Hz, H-βb), 3.65 (m, 2H, H-7a & H-7b), 2.16, 2.0, 1.88 (3s, 3 x 3H, 3 Ac), 1.83-1.68 (m, 2H, H-6a & H-6b); HRMS calcd for C33H38N2O12Na [M+Na]+ 653.2322, found 653.2337.
extracted with ethyl acetate. The combined organic layers were washed with 20% NaHCO₃ solution followed by water, brine solutions and finally dried over anhydrous Na₂SO₄ and concentrated by rotary evaporation to yield the syrup, which was purified on a small column to yield 2c (10mg, 65% yield).

$^1$H NMR (400MHz, CDCl₃) δ 7.45-7.20 (m, aromatic protons), 5.79 (d, J=7.8Hz, NH amino acid residue), 5.65 (d, J=8.0Hz, NH Gal), 5.38 (m, H-8), 5.30 (m, H-7), 5.25-5.00 (m, H-4, H-3, NH Neu5Ac & 2 x CH₂Ph), 4.85-4.75 (m, H-4), 4.72 (d, J=2.9Hz, H-1), 4.64-4.40 (m, 2H & H-2), 4.30 (d, J=8.0Hz, H-6), 4.11-4.00 (m, H-5, H-9a), 3.90-3.75 (m, H-7 of R isomer & βHa), 3.75 (s, OCH₃), 3.70 (H-7 of minor S isomer), 3.30 (OH of minor S isomer), 2.85 (d, J=7.5Hz, OH of major R isomer), 2.45 (d, H-3α), 2.25-1.82 (singlets, 8xCOCH₃), 1.80 (t, H-3α), 1.69 (m, H-6b), 1.44 (m, H-6a)