A New Linker for Solid-Phase Synthesis of Heparan Sulfate Precursors by Sequential Assembly of Monosaccharide Building Blocks

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Supporting Information

General Methods
All anhydrous reactions were performed in flame-dried or oven-dried glassware under a positive pressure of dry argon. Air or moisture-sensitive reagents and anhydrous solvents were transferred with oven-dried syringes or cannulae. Purification of compounds was performed on a Biotage SP4 automated flash chromatography system, Biotage AB, Uppsala, Sweden or by conventional flash chromatography using Merck silica gel 60 (63-200 mesh). Size exclusion chromatography was performed on Sephadex LG-20 (Sigma-Aldrich). Pooled glycoside containing fractions were lyophilized on an ALPHA-2-4 LSC freeze-dryer from Christ, Osterode, Germany. All solution phase reactions were monitored using analytical thin layer chromatography (TLC) with 0.2 mm pre-coated silica gel aluminum plates 60 F254 (E. Merck). Components were visualized by illumination with a short-wavelength (254 nm) ultra-violet light and/or by charring with vanillin, ceric ammonium molybdate, potassium permanganate, or phosphomolybdate staining solution. All solvents used for anhydrous reactions were distilled. Tetrahydrofuran (THF) was distilled from sodium/benzophenone under Argon. Dichloromethane and acetonitrile were distilled from calcium hydride. Methanol was distilled from calcium sulfate. N,N-dimethylformamide (DMF) was stored over activated molecular sieves 4Å under argon.

$^1$H (500 MHz) and $^{13}$C NMR (125 MHz) spectra were recorded at ambient temperature on a Bruker Avance 500 spectrometer. Deuterated chloroform (CDCl$_3$), methanol (CD$_3$OD), or water (D$_2$O) were used as NMR solvents, unless otherwise stated. Chemical shifts are reported in ppm downfield from TMS and corrected using the solvent residual peak or TMS as an internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and br, broad. Low resolution mass spectrometry (LRMS) was performed LCT Time of Flight Premier XE mass spectrometer equipped with electrospray source (Waters) with a pump rate of 5 µL/min using electrospray ionization (ESI) or a Voyager DE-Pro matrix-assisted desorption ionization-time of flight (MALDI-TOF), (Applied Biosystem, Foster City, CA) mass spectrometer operated in the reflectron/positive-ion mode with DHB in MeOH as the MALDI matrix. High resolution mass spectrometry (HRMS) data was acquired on LCT Time of Flight Premier XE mass spectrometer. Samples in CH$_2$Cl$_2$/MeOH 1:1 were mixed with Agilent ES tuning mix for internal calibration, and infused into the mass spectrometer at 5 µL/min. Microwave irradiation was performed on Biotage Initiator monomode oven, Biotage AB, Uppsala, Sweden.
Procedure A (preparative cleavage from the resin)
Preparative cleavage of the product from the resin was performed according to Roussel, F., Takhi, M., Schmidt R. R., J. Org. Chem. 2001, 66, 8540.
To a dry Schlenk flask under Argon, dry resin loaded compound and NaOMe in MeOH (pH=8)/CH2Cl2 (1:4) solution were added. The resin was shaken for 2 hours, then filtered and the filtrate was neutralized with Amberlite IR-120 ion acid exchange resin. The cleavage procedure was repeated 2 times and the filtrates were combined and concentrated.

Procedure B (analytical cleavage from the resin)
To a 0.2-0.5 mL Biotage microwave reaction flask, 3-5 mg of resin loaded compound was added. Then NaOMe in MeOH (pH=8)/CH2Cl2 (1:4) solution was added and the sealed reaction vessel was heated in microwave at 55ºC for 5 minutes. The crude reaction mixture was analyzed by TLC and MALDI-TOF MS.

Procedure C (Bu2SnO mediated cleavage from the resin)
To a 0.2-0.5 mL Biotage microwave reaction flask, 3-5mg of resin loaded compound was added. Then 1 eq Bu2SnO (solution in MeOH) was added and the sealed reaction vessel was heated in microwave at 120ºC for 10 minutes. The crude reaction mixture was analyzed by TLC and MALDI-TOF MS.

N-Benzyl-5-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentan-1-amine (2)
To a solution of 5-(benzylamino)pentan-1-ol (1) (5.00 g, 25.9 mmol) in dry DMF (25 mL) at 0°C, imidazole (3.52 g, 51.7 mmol) and dimethylthexylsilyl chloride (6.09 mL, 31.0 mmol) were added respectively. The reaction mixture was stirred overnight, then diluted with ether and washed with a saturated solution of ammonium chloride and water. The organic layer was then dried over MgSO 4 and concentrated. Flash column chromatography (10% EtOAc/Toluene) afforded compound 2 as a clear oil (6.6 g, 80%).

\[ \delta (\text{CDCl}_3) \]

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<td>H NMR</td>
<td>7.30-7.15 (5H, m), 3.73 (2H, s), 3.52 (2H, t, J=6.5 Hz), 2.58 (2H, t, J=6.8 Hz), 1.62-1.51 (1H, m), 1.51-1.41 (4H, m), 1.36-1.24 (2H, m), 0.83 (3H, s), 0.82 (3H, s), 0.78 (6H, s), 0.02 (6H, s); 13C NMR (125 MHz, CDCl3) δ 140.58, 128.35, 128.08, 126.83, 62.79, 54.08, 49.46, 34.18, 32.69, 29.88, 25.12, 23.62, 20.36, 18.48, -3.39; HRMS (ESI): Calcd for C20H37NOSi [M+H]+ 336.2723, found 336.2708.</td>
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4-(Hydroxymethyl)benzyl 2,2,2-trichloroethyl carbonate (4)
To 1,4-benzene-dimethanol (1.93 g, 14.0 mmol) in dry CH3CN (50 mL) and pyridine (2.25 mL, 28.0 mmol), a solution of 2,2,2-trichloroethoxycarbonyl-chloride (1.88 mL, 14.0 mmol) in CH3CN (125 mL) was added dropwise over a period of 3 hours. After 4 hours the reaction was diluted with EtOAc and washed with saturated solution of CuSO4 and water. The organic layer was then dried over MgSO4 and concentrated. Flash column chromatography (30% EtOAc/toluene) afforded 4 as a clear oil (2.23 g, 53%).

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<td>H NMR</td>
<td>7.46-7.33 (4H, m), 5.24 (2H, s), 4.77 (2H, s), 4.71 (2H, s); 13C NMR (125 MHz, CDCl3) δ 153.96, 141.63, 133.88, 128.79, 127.18, 94.35, 76.88, 70.44, 64.91; HRMS (ESI): Calcd for C11H11Cl3O4 [M+Na]+ 334.9621, found 334.9604.</td>
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4-(((2,2,2-Trichloroethoxy)carbonyloxy)methyl)benzyl N-benzyl N-(5-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentyl) carbamate (6)

To a solution of 4-(hydroxymethyl)benzyl 2,2,2-trichloroethyl carbonate (4) (4.57 g, 14.6 mmol) in CH₂Cl₂ (100 mL) at 0°C, pyridine (2.35 mL, 29.2 mmol) and 4-nitrophenyl chloroformate (3.52 g, 17.5 mmol) were added. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The solution was then concentrated and the resulting crystals were washed several times with hexanes to remove pyridine. Next, the dried solid was dissolved in DMF and N-benzyl-5-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentan-1-amine (2) (5.87 g, 17.5 mmol) and DIPEA (3.31 mL, 19.0 mmol) were added at 0°C. The reaction mixture was stirred overnight, then diluted with diethyl ether and washed with saturated solution of NH₄Cl. The organic extract was dried over MgSO₄ and concentrated. Flash column chromatography (gradient of 10 to 50% EtOAc/hexanes) gave the title product in 79% yield as a colorless oil.

1H NMR (500 MHz, CDCl₃) δ 7.50-7.10 (9H, m), 5.31-5.12 (4H, m), 4.79 (2H, s), 4.55-4.47 (2H, m), 3.61-3.50 (2H, m), 3.34-3.16 (2H, m), 1.68-1.41 (5H, m), 1.38-1.22 (2H, m), 0.89 (3H, s), 0.88 (3H, s), 0.85 (6H, s), 0.08 (6H, s); 13C NMR (125 MHz, CDCl₃) δ 156.59, 156.02, 153.90, 137.87, 137.59, 134.09, 128.59, 128.51, 127.99, 127.68, 127.24, 127.06, 94.32, 76.84, 70.33, 66.60, 62.63, 50.48, 50.19, 47.33, 46.25, 34.16, 32.42, 27.90, 27.49, 25.09, 23.13, 20.34, 18.48, -3.41; HRMS (ESI): Calcd for C₃₂H₄₆Cl₃NO₆Si [M+Na]+ 696,2057, found 696,1990.

4-(Hydroxymethyl)benzyl N-benzyl N-((5-(2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentyl) carbamate (7)

To a solution of 4-(((2,2,2-trichloroethoxy)carbonyloxy)methyl)benzyl benzyl(5-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentyl)carbamate (5) (6.16 g, 9.12 mmol) in 150 mL of AcOH/THF (1:10) at 0ºC was added freshly activated Zn dust (1.79 g, 27.4 mmol). The reaction mixture was stirred for 4 hours, diluted with EtOAc and the residual zinc was filtered off. The filtrate was concentrated in vacuo and after flash column chromatography (gradient 10 to 25% EtOAc/hexanes), compound 6 was obtained as an oil (>95%).

1H NMR (500 MHz, CDCl₃) δ 7.45-7.10 (9H, m), 5.24-5.07 (2H, m), 4.75-4.63 (2H, m), 4.56-4.41 (2H, m), 3.61-3.44 (2H, m), 3.33-3.10 (2H, m), 1.71 (1H, s), 1.66-1.38 (5H, m), 1.36-1.15 (2H, m), 0.88 (3H, s), 0.86 (3H, s), 0.83 (6H, s), 0.06 (6H, s); 13C NMR (125 MHz, CDCl₃) δ 137.93, 128.50, 128.06, 127.79, 127.23, 127.05, 66.85, 65.06, 62.60, 50.18, 47.21, 46.24, 34.17, 32.43, 27.91, 27.52, 25.11, 23.12, 20.34, 18.48, -3.41; HRMS (ESI): Calcd for C₂₉H₄₅NO₄Si [M+Na]+ 522.3016, found 522.2964.

4-((Benzyl(5-hydroxypentyl)carbamoyloxy)methyl)benzyl benzoate (8)

To a cooled solution (0ºC) of 7 (0.958 g, 1.91 mmol) in dry CH₂Cl₂ (6 mL), pyridine (0.46 mL, 5.73 mmol) and benzoyl chloride (0.33 mL, 2.87 mmol) were added and the solution stirred overnight. The mixture was diluted with EtOAc and washed with 1M HCl, saturated solution of NaHCO₃ and water. The organic phase was dried over anhydrous MgSO₄, filtered and concentrated. The residue was purified by column chromatography (1:1 CH₂Cl₂/Hexane) to afford the benzoylated intermediate (1.09 g, 95%).

1H NMR (500 MHz, CDCl₃) δ 8.10-8.09 (2H, d, J = 7.6Hz, aromatic), 7.57-7.24 (12H, m, aromatic), 5.38 (2H, s, CH₂), 5.21 (2H, s, CH₂), 4.52 (2H, s, CH₂), 3.57 (2H, s, CH₂), 1.67 - 1.50 (5H, m, CH₂, TDS), 1.32 (2H, m, CH₂), 0.91 (3H, s), 0.90 (3H, s), 0.86
HF-pyridine (5 mL) was added to a cooled solution (0°C) of the intermediate (1.3 g, 2.15 mmol) in dry THF (5 mL). The mixture was stirred overnight then diluted with EtOAc and NaHCO₃ (solid) was added. The reaction mixture was then filtered and washed with saturated solution of NaHCO₃ and water. The organic phase was dried over anhydrous MgSO₄, filtered and concentrated. The residue was purified by column chromatography (3:7 EtOAc/hexanes) to afford 8 (0.675 g, 68%).

Resin bound 4-(Hydroxymethyl)benzyl N-benzyl N-(5-((2,3-dimethylbutan-2-yl)dimethylsilyloxy)pentyl) carbamate (9-SP)

To a dry Schlenk flask 500mg of carboxypolystyrene resin (2.19 mmol/g capacity) was added under Argon. The resin was swollen with CH₂Cl₂ for 10 minutes then washed 3 times with CH₂Cl₂. To this resin in 23 mL of CH₂Cl₂, 7 (0.028 g, 0.110 mmol) dissolved in 2 mL of CH₂Cl₂, DIC (0.034 g, 0.548 mmol), and DMAP (0.002 g, 0.022 mmol) were added and the reaction mixture shaken overnight. After complete reaction of the linker with the solid support (determined by TLC), the reaction solution was filtered off and the resulting resin was washed with 3 cycles of CH₂Cl₂, MeOH and CH₂Cl₂. Finally, the resin was washed with 3 times THF and dried under vacuum. Capping of the unreacted carboxylate groups was performed according to Roussel, F., Takhi, M., Schmidt R. R., J. Org. Chem. 2001, 66, 8540.

Resin bound 4-(Hydroxymethyl)benzyl N-benzyl N-(5-hydroxypentyl) carbamate (10-SP)

Dry resin (0.50 g) was transferred to a Teflon reaction vessel and swollen with THF (5 mL). Equal volume (5 mL) of HF pyridine (Note: this reagent is extremely toxic!) was added and the reaction mixture was shaken for 3 hours. The reaction mixture was filtered off and the resulting resin was washed with 3 cycles of CH₂Cl₂, MeOH and CH₂Cl₂. Finally, the resin was washed with 3 times THF and dried under vacuum.
Resin bound 4-(Hydroxymethyl)benzyl N-benzyl N-(5-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyloxy)pentyl) carbamate (12-SP)

To a dry schlenk flask under Argon, resin loaded with the linker (0.10 g, 0.2 mmol/g), donor 11 (0.146 g, 0.22 mmol) and CH2Cl2 (1.5 mL) were added respectively. The resin was shaken for 15 minutes, and the reaction mixture was cooled to 0ºC. TMSOTf (5 μL, 0.022 mmol) was added and the reaction mixture was allowed to warm to room temperature. After 1 hour, the reaction solution was filtered off, washed with 3 times with CH2Cl2 and the glycosylation cycle was repeated. The resin was then washed with 3 cycles of CH2Cl2 then MeOH, 3 times with CH2Cl2 and dried under vacuum.

4-(Hydroxymethyl)benzyl N-benzyl N-(5-(3,4,6-tri-O-benzyl-α-D-mannopyranosyloxy)pentyl) carbamate (12)

Compound 12 (10mg, 79% yield) was obtained by preparative cleavage from 80mg of resin 12-SP according to Procedure A. 1H and 13C NMR spectra for this compound were recorded at 50°C to resolve spectra of rotamers. 1H NMR (500 MHz, CDCl3) δ 7.38-7.00 (24H, m), 5.14-5.02 (2H, m), 4.80-4.65 (2H, m), 4.64-4.50 (5H, m), 4.49-4.34 (4H, m), 3.95-3.84 (1H, m), 3.82-3.70 (2H, m), 3.70-3.57 (3H, m), 3.57-3.42 (1H, m), 3.35-3.02 (3H, m), 2.75-1.65 (2H, br s), 1.60-1.30 (4H, m), 1.30-1.00 (2H, m); 13C NMR (125 MHz, CDCl3) δ 156.52, 141.00, 138.48, 138.41, 138.11, 137.98, 136.19, 128.51, 128.45, 128.28, 128.09, 127.90, 127.77, 127.58, 127.49, 127.28, 126.98, 99.27, 80.35, 75.06, 74.59, 73.50, 71.99, 71.30, 68.54, 67.42, 66.96, 64.85, 50.54, 46.39, 28.99, 27.66, 23.32; HRMS (ESI): Calcd for C48H55NO9Na [M+Na]+ 812.3774, found 812.3718.

5-Aminopentyl α-D-mannopyranoside (13)

To a solution of 12 (40 mg, 0.05 mmol) in MeOH (2 mL) with 10% formic acid, palladium black (40mg) was added and the reaction mixture was stirred overnight under H2 atmosphere. The reaction mixture was then filtered over Celite, concentrated and the resulting residue was purified by Sephadex column chromatography to afford 13 in quantitative yield. 1H NMR (500 MHz, CDCl3) δ 8.39 (1H, br s), 4.74 (1H, s), 3.82 (1H, dd, J=4.5, 8.9 Hz), 3.80-3.63 (4H, m), 3.60 (1H, dd, J=9.5, 9.5 Hz), 3.55-3.42 (2H, m), 2.99-2.88 (2H, m), 1.76-1.58 (4H, m), 1.56-1.42 (2H, m); 13C NMR (125 MHz, CDCl3) δ 168.54, 101.71, 74.84, 72.79, 72.35, 72.31, 68.81, 68.20, 63.11, 40.81, 30.19, 28.50, 24.48; HRMS (ESI): Calcd for C11H23NO6 [M+Na]+ 288.1423, found 288.1424.

4-(Phenylcarboxymethyl)benzyl N-benzyl N-(5-(3-O-benzyl-2-O-benzoyl-4-O-levulinoyl-6-O-tert-butylidiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (15)

Idopyranosyl donor 14 (0.106 g, 0.132 mmol), linker acceptor 8 (0.050 g, 0.108 mmol), and activated molecular sieves were suspended in dry CH2Cl2 (1 mL) for 1h at room temperature under argon and then cooled to -20ºC. N-iodosuccinimide (NIS) (0.030 g, 0.132 mmol) and trifluoromethanesulfonic acid (TfOH) (2.83 μL, 0.03 mmol) were added and the reaction mixture was allowed to warm to room temperature. After 3h the reaction mixture was quenched with saturated solution of NaHCO3 and solid Na2S2O3. The mixture was filtered and washed with saturated solution of NaHCO3, H2O and brine.
The organic layer was dried over anhydrous MgSO₄, filtered and concentrated. The residue was purified by column chromatography (gradient of 95/5 to 50/50 Hexane/EtOAc) to afford 15 (0.100 g, 80%).

$$^1$$H NMR (500 MHz, CDCl₃) δ 8.08 - 7.14 (34H, m, aromatic), 5.35 (2H, ps), 5.19 - 1.8 (3H, m, H-2, CH₂), 5.09 (1H, ps, H-4), 4.97 (1H, ps, H-1), 4.81 (1H, d, J = 11.8Hz, CH₂Ph), 4.72 (1H, d, J = 11.8Hz, CH₂Ph), 4.48 - 4.41 (m, 3H, m, H-5, CH₂), 3.91 (1H, s, (CH₃)₃C); 13C NMR (125 MHz, CDCl₃) δ 205.73, 171.96, 166.35, 165.23, 156.60, 156.03 (qC), 137.90, 137.01, 136.88, 135.58, 135.54, 133.14, 133.01, 130.04, 129.76, 129.69, 129.66, 129.06, 128.50, 128.38, 128.34, 128.26, 128.19, 127.99, 127.70, 127.65, 127.55, 127.42, 127.20, 127.06, 97.90 (C-1), 73.28 (C-3), 72.13 (CH₂Ph), 68.29 (C-2), 67.80, 67.54 (C-4), 66.72, 66.35 (C-5), 62.66 (C-6), 50.51, 50.20, 47.20, 46.20, 37.76, 29.59, 29.07, 27.98, 27.86, 27.52, 26.72, 23.37, 19.10; HRMS (ESI): Calcd for C₆₉H₇₅NO₁₃SiNa [M+Na]⁺ 1176.4906, found 1176.4854: $\alpha$D$^20$ = -1.07º (c = 0.8).

4-(Hydroxymethyl)benzyl N-benzyl N-(5-(3-O-benzyl-2-O-benzoyl-4-O-levulinoyl-6-O-tert-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (16)

A solution of 15 (0.020 g) in dry MeOH (0.14 mL) and dibutyltin oxide (0.004 g, 0.017 mmol) was heated under MW irradiation at 120ºC for 40 min. LC-MS analysis of the crude mixture showed the following product distribution: product 16: 70.5%; compound with additionally cleaved 2-OBz group: 16.4%, compound with additionally cleaved Lev group: 5.3%; starting material: 7.8%.

$$^1$$H NMR (500 MHz, CDCl₃) δ 8.07 - 7.15 (m, 24H, aromatic), 5.15 (3H, H-2, CH₂), 5.07 (1H, ps, H-4), 4.96 - 4.87 (1H, m, H-1), 4.80 - 4.78 (1H, m), 4.72 - 4.64 (3H, m), 4.48 - 4.39 (3H, m), 3.89 (1H, s, H-3), 3.81 - 3.75 (2H, m, H-6), 3.70 - 3.63 (1H, m), 3.37 - 3.28 (1H, m), 3.21 - 3.12 (2H, m), 2.54 - 2.53 (2H, m, CH₂(Lev)), 2.43 - 2.41 (2H, m, CH₂(Lev)), 2.03 (3H, s, (CH₃)₃C); 13C NMR (126 MHz, CDCl₃) δ 205.78, 171.98, 165.30, 156.67, 156.09, 140.90, 137.91, 136.17, 135.59, 135.56, 133.38, 133.15, 129.78, 129.72, 128.50, 128.41, 128.21, 128.09, 127.72, 127.67, 127.57, 127.44, 127.30, 127.10, 97.89 (C-1), 73.26 (C-3), 72.13 (CH₂Ph), 68.28 (C-2), 67.80, 67.54 (C-4), 66.92, 66.36 (C-5), 62.66 (C-6), 50.51, 50.20, 47.20, 46.20, 37.76, 29.59, 29.07, 27.98, 27.86, 27.52, 26.72, 23.37, 19.10; HRMS (ESI): Calcd for C₆₀H₇₅NO₁₃SiNa [M+Na]⁺ 1072.4644, found 1072.4539.

4-(Phenylcarboxymethyl)benzyl N-benzyl N-(5-(3-O-benzyl-2-O-benzoyl-4-O-levulinoyl-α-L-idopyranosyloxy)uronate)pentyl) carbamate (17)

To a cooled solution (0ºC) of 15 (0.076g, 0.065 mmol) in dry THF (3 mL), HF-pyridine (0.3 mL) was added and the solution stirred overnight. The mixture was diluted with EtOAc and NaHCO₃ (solid) was added, filtered and washed with saturated solution of NaHCO₃ and water. The organic phase was dried over anhydrous MgSO₄, filtered and concentrated. The crude was used in the next step without purification. To a solution of the crude intermediate in acetonitrile/water (1/1 v/v, 1 mL), TEMPO (2 mg, 0.013 mmol) and BAIB (46 mg, 0.143 mmol) were added and the reaction mixture was stirred for 4h. The reaction mixture was quenched by the addition of 1M Na₂SO₃ (0.7 mL). The layers were separated and the aqueous layer was acidified with 1M HCl, and extracted 3 times with CH₂Cl₂. The combined organic layers were dried over anhydrous MgSO₄ and...
concentrated. The residue was purified by flash column chromatography (gradient of 80/19/1 to 99/0/1 EtOAc/Hexane/HOAc) to obtain compound 20 as an oil (51 mg, 84%). 

\[ \text{1H NMR (500 MHz, CDCl}_3 \delta 8.07 - 7.13 (m, 24H, aromatic), 5.35 - 5.32 (3H, m, H-4, CH}_2 \text{ linker), 5.18 - 5.09 (4H, m, H-1, H-2, CH}_2 \text{ linker), 4.97 - 4.94 (1H, m, H-5), 4.81 - 4.44 (6H, m), 3.91 (1H, s, H-3), 3.74 - 3.71 (1H, m), 3.52 - 3.46 (1H, m), 3.21 - 3.15 (2H, m), 2.67 - 2.62 (2H, m), 2.51 - 2.42 (2H, m), 2.09 (3H, s), 1.63 - 1.49 (4H, m), 1.32 - 1.27 (2H, m); \]

\[ \text{13C NMR (126 MHz, CDCl}_3 \delta 206.70, 171.52, 170.10, 166.44, 165.14, 156.68 (C carbamato), 156.18 (C carbamato), 137.75, 137.39, 136.75, 135.73, 133.56, 133.06, 130.01, 129.79, 129.32, 128.52, 128.44, 128.36, 128.27, 128.04, 127.76, 127.48, 127.27, 127.08, 98.46 (C-1), 72.28 (C-3), 72.14 (CH}_2 \text{Ph), 68.72, 67.85 (C-4), 66.86, 66.76, 66.38 (C-2), 65.71 (C-5), 50.53, 50.22, 47.11, 46.15, 37.76, 29.55, 28.96, 27.88, 27.32, 23.29; \]

\[ \text{LRMS (ESI): Calcd for C}_{53}\text{H}_{55}\text{NO}_{14}\text{Na} [M+Na]^+ 952.36, found 952.32; } \]

\[ \alpha_{D}^{20} = +2.9^\circ (c = 1.15). \]

**Resin bound 4-(Hydroxymethyl)benzyl N-benzyl N-(5-(3-O-benzyl-2-O-benzoyl-4-O-levulinoyl-6-O-tert-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (18-SP)**

To a dry schlenk flask under Argon, resin loaded with the linker (0.50 g, 0.2 mmol/g), donor 14 (0.440 g, 0.550 mmol) and CH2Cl2 (10 mL) were added respectively. The resin was shaken for 15 minutes, then NIS (0.160 g, 0.715 mmol) was added and the reaction mixture was cooled to -20ºC. Triflic acid (0.002 μL, 0.015 mmol) was added and the reaction mixture was allowed to warm to room temperature. After 3 hours, the reaction solution was filtered off and resin was washed with 3 cycles of CH2Cl2 then MeOH and 3 times with CH2Cl2.

**4-(Acetoxyethyl)benzyl N-benzyl N-(5-(2,4-di-O-acetyl-3-O-benzyl-6-O-tert-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (19)**

Compound 19 was obtained in 83% overall yield by preparative cleavage (Procedure A) and purification from 18-SP. Next, the intermediate (0.012 g, 0.014 mmol) was taken up in CH2Cl2 (1.5 mL) and treated with pyridine (0.1 mL, 1.3 mmol), acetic anhydride (0.1 mL, 1.0 mmol) and catalytic amount of DMAP. After 12 hours, the reaction mixture was diluted with CH2Cl2 and successively washed with saturated solution of CuSO4 and H2O. The organic phase was then dried over anhydrous MgSO4, filtered and concentrated. Preparative TLC (30% EtOAc/hexanes) afforded compound 19 as a white solid (0.013 g, 96% yield).

\[ \text{1H NMR (500 MHz, CDCl}_3 \delta 7.70-7.10 (24H, m), 5.21-5.11 (2H, m), 5.08 (2H, s), 5.04-4.95 (1H, m), 4.94-4.88 (1H, m), 4.81-4.71 (3H, m), 4.70-4.64 (1H, m), 4.53-4.41 (2H, m), 4.39-4.31 (1H, m), 3.79-3.70 (3H, m), 3.70-3.60 (1H, m), 3.40-3.26 (1H, m), 3.26-3.08 (2H, m), 2.09 (3H, s), 2.04 (3H, s), 1.97 (3H, s), 1.72-1.42 (4H, m), 1.37-1.17 (2H, m), 1.02 (9H, s); \]

\[ \text{13C NMR (125 MHz, CDCl}_3 \delta 170.85, 170.10, 169.55, 156.62, 156.07, 157.87, 136.89, 135.56, 133.16, 133.07, 129.78, 129.72, 128.53, 128.37, 128.24, 127.98, 127.72, 127.67, 127.63, 127.50, 127.31, 127.27, 127.08, 97.82, 72.69, 71.97, 67.97, 67.76, 67.21, 66.74, 66.03, 65.98, 62.31, 50.52, 50.21, 47.22, 46.21, 29.69, 29.05, 26.72, 23.37, 20.99, 20.86, 19.11; \]

\[ \text{HRMS (ESI): Calcd for C}_{53}\text{H}_{61}\text{NO}_{8}\text{SiNa} [M+Na]^+ 870.4013, found 870.3979. \]
4-(Hydroxymethyl)benzyl N-benzyl N-(5-(3-O-benzyl-2-O-benzoyl-4-O-levulinoyl-6-O-tert-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (20)

Compound 20 was obtained according to cleavage Procedure C. MALDI-TOF MS analysis showed complete retention of all protecting groups. MALDI-TOF MS: Calcd for C_{62}H_{71}NO_{12}SiNa [M+Na]^+ 1072.46, found 1072.70.

![Figure 1 Maldi-Tof MS for glycoconjugate 20](image)

Resin bound 4-(Hydroxymethyl)benzyl N-benzyl N-(5-(3-O-benzyl-2-O-benzoyl-6-O-tert-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (22-SP)

Compound 22-SP was prepared according to a modified procedure of Roussel, F., Takhi, M., Schmidt R. R., J. Org. Chem. 2001, 66, 8540.

In a dry Schlenk flask purged with Argon, 18-SP (0.100 g) was swollen in CH_{2}Cl_{2} (1.5 mL). After 15 minutes of shaking, MeOH (10% by volume) and NH_{2}NH_{2} HOAc (2 equivalents) were added and the agitation was continued for 2 hours. The resin was then filtered washed with CH_{2}Cl_{2} and the cycle was repeated 2 more times. The resin was then filtered, washed with 3 cycles of CH_{2}Cl_{2} then MeOH and 3 times with CH_{2}Cl_{2}. Finally, the resin 22-SP was dried under vacuum.

4-(Hydroxymethyl)benzyl N-benzyl N-(5-(3-O-benzyl-2-O-benzoyl-6-O-tert-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (22)

Compound 22 was obtained according to cleavage Procedure C. MALDI-TOF MS analysis showed complete cleavage of the levulinyl protecting group. MALDI-TOF MS: Calcd for C_{57}H_{65}NO_{10}SiNa [M+Na]^+ 974.43, found 975.25.
Resin bound 4-(Hydroxymethyl)benzyl N-benzyl N-(5-(3-O-benzyl-2-O-benzoyl-4-O-(2-azido-3-O-benzyl-6-O-benzoyl-2-deoxy-4-O-levulinoyl-α-D-glucopyranosyl)-6-O-tert-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (23-SP) and 4-(Hydroxymethyl)benzyl N-benzyl N-(5-(3-O-benzyl-4-O-(2-azido-3-O-benzyl-2-deoxy-α-D-glucopyranosyl)-6-O-tert-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (23)

To a dry Schlenk flask under Argon, 22-SP (0.5 g, 0.1 mmol), donor 21 (0.320 g, 0.5 mmol) and CH$_2$Cl$_2$ (5 mL) were added respectively. The resin was shaken for 15 minutes and then the reaction mixture was cooled to -40°C. TMSOTf (5 μL, 0.025 mmol) was added and the reaction mixture was allowed to warm to room temperature. After 2 hour, the reaction solution was filtered off, the resin was washed 3 times with dry CH$_2$Cl$_2$ and the glycosylation cycle was repeated 4 times. Progress of the glycosylation reaction was monitored by cleavage of a sample aliquot followed by LC-MS analysis. After 5 cycle, LC-MS spectrum indicated 82% conversion of the monosaccharide to the disaccharide derivative. The resin was then washed with 3 cycles of CH$_2$Cl$_2$ then MeOH, 3 times with CH$_2$Cl$_2$ and dried under vacuum.
Figure 3 LC-MS data for conversion of monosaccharide 22-SP (average retention time=4.39 min) to the disaccharide 23-SP (average retention time=4.81 min) derivative

Resin bound 4-(Hydroxymethyl)benzyl N-benzyl N-(5-(3-O-benzyl-2-O-benzoyl-4-O-(2-azido-3-O-benzyl-6-O-benzoyl-2-deoxy-α-D-glucopyranosyl)-6-O-tert-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (24-SP)

Compound 24-SP was prepared according to a modified procedure of Roussel, F., Takhi, M., Schmidt R. R., J. Org. Chem. 2001, 66, 8540.

In a dry Schlenk flask purged with Argon, 23-SP (0.100 g) was swollen in CH$_2$Cl$_2$ (1.5 mL). After 15 minutes of shaking, MeOH (10% by volume) and NH$_2$NH$_2$ HOAc (2 equivalents) were added and the agitation was continued for 2 hours. The resin was then filtered washed with CH$_2$Cl$_2$ and the cycle was repeated 2 more times. The resin was then filtered, washed with 3 cycles of CH$_2$Cl$_2$ then MeOH and 3 times with CH$_2$Cl$_2$. Finally, the resin 24-SP was dried under vacuum.

4-(Acetoxymethyl)benzyl N-benzyl N-(5-(2-O-acetyl-3-O-benzyl-4-O-(4,6-O-di-acetyl-2-azido-3-O-benzyl-2-deoxy-α-D-glucopyranosyl)-6-O-tert-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (24)

Compound 24 was obtained from 23-SP in 45% overall yield after preparative cleavage according to Procedure A and purification. The intermediate (6mg, 0.005 mmol) was
taken up in CH₂Cl₂ (1.5 mL) and treated with pyridine (0.1 mL, 1.3 mmol), acetic anhydride (0.1 mL, 1.0 mmol), and a catalytic amount of DMAP. After 12 hours, the reaction mixture was diluted with CH₂Cl₂ and successively washed with saturated solution of CuSO₄ and H₂O. The organic phase was then dried over anhydrous MgSO₄, filtered and concentrated. Preparative TLC (30% EtOAc/hexanes) afforded compound 24 as a white solid (6 mg, 93% yield).

1H NMR (500 MHz, CDCl₃) δ 7.77-7.10 (25H, m), 5.22-5.11 (2H, m), 5.08 (2H, s), 5.01 (1H, dd, J=9.7 Hz), 4.96-4.92 (1H, m), 4.91 (1H, d, J=3.6 Hz), 4.87-4.82 (1H, m), 4.80 (1H, d, J=11.7 Hz), 4.76 (1H, d, J=11.1 Hz), 4.65 (1H, d, J=11.8 Hz), 4.55 (1H, d, J=11.0 Hz), 4.50-4.42 (2H, m), 4.28-4.23 (1H, m), 3.99 (1H, dd, J=4.5, 12.4 Hz), 3.92-3.79 (5H, m), 3.78-3.72 (1H, m), 3.69-3.59 (3H, m), 3.40 (1H, dd, J=3.6, 10.2 Hz), 3.36-3.26 (1H, m), 3.26-3.10 (2H, m), 2.09 (6H, s), 1.94 (3H, s), 1.88 (3H, s), 1.70-1.45 (4H, m), 1.35-1.20 (2H, m), 1.04 (9H, s); 13C NMR (125 MHz, CDCl₃) δ 170.84, 170.49, 170.05, 169.27, 156.60, 156.06, 137.75, 137.38, 136.89, 135.62, 135.46, 133.14, 132.97, 129.89, 129.83, 128.53, 128.35, 127.99, 127.79, 127.64, 127.35, 127.28, 127.09, 97.84, 95.91, 77.99, 74.94, 72.25, 72.06, 71.44, 69.71, 68.51, 67.87, 67.59, 66.74, 65.97, 63.34, 63.03, 61.70, 50.51, 50.19, 47.19, 46.19, 29.69, 29.08, 26.79, 23.40, 20.99, 20.82, 20.66, 20.59, 19.14.; HRMS (ESI): Calcd for C₇₁H₈₄N₄O₁₇SiNa [M+Na]+ 1315.5498, found 1315.5516.

Resin bound 4-(Hydroxymethyl)benzyl N-benzyl N-(5-(3-O-benzyl-2-O-benzoyl-4-O-2-azido-3-O-benzyl-4-O-(3-O-benzyl-2-O-benzoyl-6-O-tert-butyldiphenylsilyl-4-O-levulinoyl-α-D-idopyranosyl)-6-O-benzoyl-2-deoxy-α-D-glucopyranosyl)-6-O-tert-butyldiphenylsilyl-α-L-idopyranosyloxy)pentyl) carbamate (26-SP)

To a dry Schlenk flask purged with Argon, 24-SP (0.15 g, 0.03 mmol), donor 25 (0.128 g, 0.15 mmol) and CH₂Cl₂ (2 mL) were added. The resin was shaken for 15 minutes and then the reaction mixture was cooled to -40ºC. TMSOTf (2 μL, 0.007 mmol) was added and the reaction mixture was allowed to warm to room temperature. After 2 hours, the reaction solution was filtered off, the resin was washed 3 times with dry CH₂Cl₂ and the glycosylation was repeated 3 times under the same conditions. Progress of the glycosylation reaction was monitored by analytical cleavage followed by LC-MS analysis. After cycle 4, LC-MS spectrum indicated 53% conversion of the disaccharide to the trisaccharide derivative. The resin was then washed with 3 cycles of CH₂Cl₂ then MeOH, 3 times with CH₂Cl₂ and dried under vacuum.
Figure 4 LC-MS data for conversion of disaccharide 23-SP (average retention time=4.39 min) to trisaccharide 26-SP (average retention time=6.68 min) derivative

4-(Acetoxymethyl)benzyl N-benzyl N-(5-(2-O-acetyl-3-O-benzyl-4-O-(6-O-acetyl-2-azido-3-O-benzyl-4-O-(2,4-di-O-acetyl-3-O-benzyl-6-O-tert-butyldiphenylsilyl-α-L-idopyranosyl)-2-deoxy-α-D-glucopyranosyl)-6-O-tert-butyldiphenylsilyl-α-L-idopyranosylxy)pentyl) carbamate (27)

Compound 27 was obtained in 10% overall yield by preparative cleavage (Procedure A) and purification from 26-SP. Next, the intermediate (4mg, 0.002 mmol) was taken up in CH2Cl2 (1.5 mL) and treated with pyridine (0.1 mL, 1.3 mmol), acetic anhydride (0.1 mL, 1.0 mmol), and catalytic amount of DMAP. After 12 hours, the reaction mixture was diluted with CH2Cl2 and successively washed with saturated solution of CuSO4 and H2O. The organic phase was then dried over anhydrous MgSO4, filtered and concentrated. Preparative TLC (30% EtOAc/hexanes) afforded compound 27 as a white solid (4mg, 95% yield).

1H NMR (500 MHz, CDCl3) δ 7.74-7.09 (44H, m), 5.21-5.11 (2H, m), 5.08 (2H, s), 5.02-4.98 (2H, m), 4.95-4.81 (5H, m), 4.79-4.60 (4H, m), 4.55-4.41 (4H, m), 4.22-4.00 (4H, m), 3.94-3.80 (3H, m), 3.80-3.73 (3H, m), 3.73-3.57 (5H, m), 3.37-3.08 (4H, m), 2.09 (3H, s), 2.03 (3H, s), 1.98 (3H, s), 1.92 (3H, s), 1.90 (3H, s), 1.70-1.40 (4H, m), 1.35-1.20 (2H, m), 1.06 (9H, s), 1.01 (9H, s); 13C NMR shift values taken from HSQC δ 135.56, 135.51, 129.85, 129.69, 128.32, 127.90, 127.85, 127.82, 97.90, 97.77, 96.81, 79.16, 75.40, 75.33, 74.30, 73.23, 72.79, 72.48, 72.34, 69.79, 68.95, 68.51, 68.42, 67.78, 67.55,
66.69, 66.63, 66.57, 65.88, 63.84, 63.74, 63.36, 62.40, 62.16, 61.82, 61.87, 50.33, 47.16, 46.07, 29.57, 29.19, 26.94, 26.87, 23.00, 21.17, 21.12, 21.01; HRMS (ESI): Calcd for C_{102}H_{120}N_{4}O_{23}Si_{2}Na [M+Na]^+ 1847.7780, found 1847.7733.
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COSY spectrum of compound 27
HSQC spectrum of compound 27