Electronic Supplementary Material (ESI) for Chemical Science.

Synthesis of Conjugation-ready Zwitterionic Oligosaccharides by Chemoselective Thioglycoside Activation

Supporting Information: Experimental Procedures

Benjamin Schumann,[a,b] Rajan Pragani,[a,c] Chakkumkal Anish,[a] Claney L. Pereira*[,a] and Peter H. Seeberger*[,a,b]

[a]Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany

[b]Freie Universität Berlin, Arnimallee 22, 14195 Berlin, Germany

[c]Present address: National Center for Advancing Translational Sciences, National Institutes of Health, 9800 Medical Center Drive, Rockville, MD 20850, USA

*e-Mail adresses:

peter.seeberger@mpikg.mpg.de

claneylebev.pereira@mpikg.mpg.de

General Experimental Details

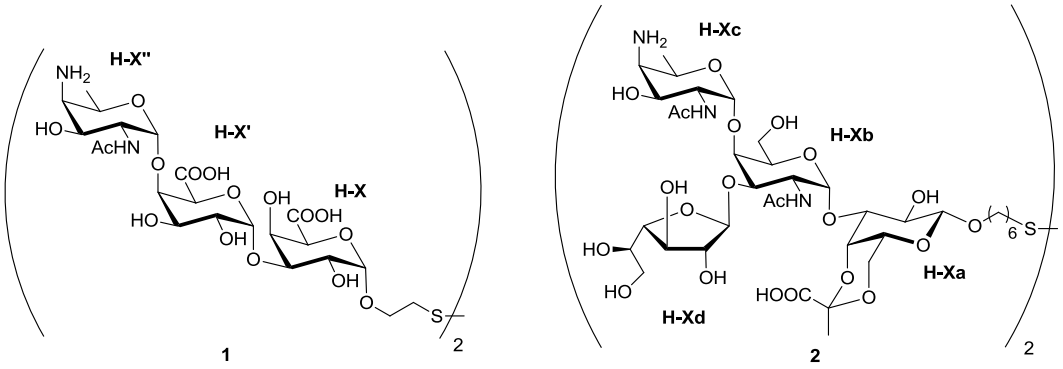
Commercial grade solvents and reagents were used unless stated otherwise. Dry solvents were obtained from a Waters Dry Solvent System. Solvents for chromatography were of technical grade and distilled under reduced pressure prior to use. Sensitive reactions were carried out in heat-dried glassware and under an argon atmosphere. Analytical thin layer chromatography (t.l.c.) was performed on Kieselgel 60 F254 glass plates pre-coated with silica gel (0.25 mm thickness). Spots were visualized with sugar stain (0.1% (v/v) 3-methoxyphenol, 2.5% (v/v) sulfuric acid in EtOH) or CAM stain (5% (w/v) ammonium molybdate, 1% (w/v) cerium(II) sulfate and 10% (v/v) sulfuric acid in water) dipping solutions. Flash chromatography was performed on Fluka Kieselgel 60 (230-400 mesh). Automated flash chromatography was carried out with a Biotage flash purification system using Fluka high-purity Kieselgel 60 (230-400 mesh). Solvents were removed under reduced pressure using a rotary evaporator and high vacuum (<1 mbar). Freeze-drying of aqueous solutions was performed using a Christ Alpha 2-4 LD Lyophilizer.

^{1}H, ^{13}C and two-dimensional NMR spectra were measured with a Varian 400-MR spectrometer or a Varian 600 spectrometer at 296 K. 1D HH-NOESY measurements were performed at Freie Universität Berlin, NMR Core Facility, with a Bruker AMX500 spectrometer at 296 K. Chemical shifts (δ) are reported in parts per million (ppm) relative to the respective residual solvent peaks ($CDCl_3$: δ 7.26 in ^{1}H and 77.16 in ^{13}C NMR; acetone-D_6: δ 2.05 in ^{1}H and 29.84 in ^{13}C NMR; CD_3OD: δ 3.31 in ^{1}H and 49.00 in ^{13}C NMR; D_2O: δ 4.79 in ^{1}H NMR). Two-dimensional NMR experiments (HH-COSY, CH-HSQC, CH-HMBC) were performed to assign peaks in ^{1}H and ^{13}C spectra. The following abbreviations are used to indicate peak multiplicities: *s* singlet; *d* doublet; *dd* doublet of doublets; *t* triplet; *dt* doublet of triplets; *m* multiplet; *br s* broad singlet. Coupling constants (*J*) are reported in Hertz (Hz). Optical rotation (OR) measurements were carried out with a Schmidt&Haensch UniPol L1000 polarimeter at λ = 589 nm and a concentration (c) expressed in g/100 mL in the solvent noted in parentheses. High resolution mass spectrometry by electrospray ionization (ESI-HRMS) was performed at Freie Universität Berlin, Mass Spectrometry Core Facility, with an Agilent 6210 ESI-TOF mass spectrometer. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) high resolution mass spectra were recorded on a Bruker Daltonics

Autoflex Speed spectrometer. Infrared (IR) spectra were measured with a Perkin Elmer 100 FTIR spectrometer.

When handling small quantities (<5 mg) of thioether-containing compounds for analytical measurements, a drop of dimethyl sulfide (0.1% v/v) was added to the solution to avoid sulfoxide formation under the influence of air.

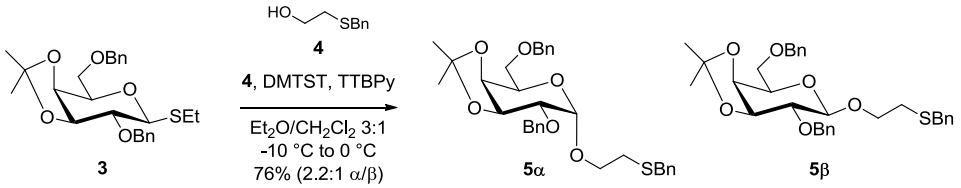
Monosaccharide residues are labeled in ^{1}H NMR spectra according to the following scheme:



Chemoselective Thioglycoside Activation using DMTST

Typically, thioglycoside (0.20 mmol) and thioether-containing alcohol (0.3 mmol) were co-evaporated twice with dry toluene and kept for 1 h under high vacuum. The mixture was dissolved in dry CH_2Cl_2 or a mixture of dry CH_2Cl_2 and Et_2O (4 mL), and 2,4,6-tri-*tert*-butylpyridine (TTBPy) and activated 3 Å molecular sieves were added. The solution was stirred for 30 min at room temperature and cooled to the indicated temperature. The mixture was treated with DMTST[1] and stirred until t.l.c. indicated complete consumption of the thioglycoside. The reaction was then diluted with CH_2Cl_2 (10 mL) and quenched by addition of 10% aq. $Na_2S_2O_3$ (10 mL) and sat. aq. $NaHCO_3$ (10 mL). After separation, the aqueous fraction was extracted with CH_2Cl_2 (3x10 mL). The organic fractions were pooled, dried over Na_2SO_4 and concentrated. The product was purified by flash chromatography using the indicated solvents.

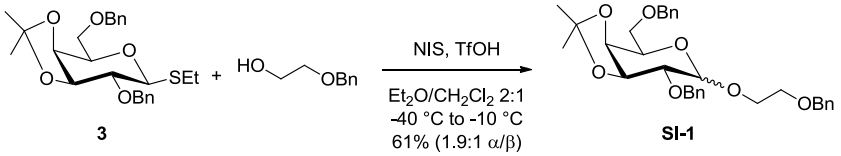
2,6-Di-*O*-benzyl-3,4-*O*-isopropylidene-α-D-galactopyranosyl-(1→1)-2-(benzylthio)ethanol (5α) and 2,6-Di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→1)-2-(benzylthio)ethanol (5β)



Thioglycoside **3**[2] (98 mg, 0.221 mmol) was glycosylated with alcohol **4**[3] (56 mg, 0.331 mmol) using DMTST (85 mg, 0.331 mmol) and TTBPy (109 mg; 0.441 mmol) in Et_2O (3.3 mL) and CH_2Cl_2 (1.1 mL) from -10 °C to 0 °C for 1 h. Flash chromatography was performed (EtOAc/hexanes 0:1 to 1:3) to give thioethers **5α** (63 mg, 0.114 mmol, 52%) and **5β** (29 mg, 0.052 mmol, 24%). Analytical data for **5α**: Clear oil; R_f (EtOAc/hexanes 1:2) = 0.77; $[\alpha]_D^{20}$ = +75.4° (c = 2.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$,) δ 7.39 – 7.19 (m, 15H, arom.), 4.80 (m, 2H, H-1, A of AB, $PhCH_2$), 4.70 (d, *J* = 12.5 Hz, 1H, B of AB, $PhCH_2$), 4.65 (d, *J* = 12.1 Hz, 1H, A of AB, $PhCH_2$), 4.55 (d, *J* = 12.1 Hz, 1H, B of AB, $PhCH_2$), 4.35 (dd, *J* = 7.8, 5.5 Hz, 1H, H-3), 4.29 (dt, *J* = 5.1, 2.5 Hz, 1H, H-5), 4.21 (dd, *J* = 5.5, 2.5 Hz, 1H, H-4), 3.81 (dt, *J* = 10.2, 6.9 Hz, 1H, A of AB, O-C*H*$_2$-CH_2), 3.78 – 3.68 (m, 4H, $PhCH_2$, H-6), 3.57 (dt, *J* = 10.2, 6.9 Hz, 1H, B of AB, O-C*H*$_2$-CH_2), 3.53 (dd, *J* = 7.9, 3.5 Hz, 1H, H-2), 2.71 – 2.58 (m, 2H, CH_2-C*H*$_2$-S), 1.40 (s, 3H, *i*Pr-CH_3), 1.34 (s, 3H, *i*Pr-CH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.4, 138.3, 129.1, 128.6, 128.42, 128.41, 128.0, 127.8, 127.67, 127.66, 127.1, 109.2, 97.3, 76.6, 76.0, 73.8, 73.5, 72.4, 69.6, 67.9, 66.9, 36.5, 30.3, 28.2, 26.5. IR (thin film) 3063, 3029, 2985, 2916, 2869, 2340, 1953, 1812, 1576, 1495, 1453, 1380, 1370, 1313, 1243, 1218, 1165, 1099, 1074, 1044, 1028, 906, 873, 845, 791, 737, 698 cm^{-1}; HRMS (ESI) calcd for $C_{32}H_{38}O_6S$ $(M+Na)^+$ 573.2287 found 573.2288 *m*/*z*. Analytical data for **5β**: Clear oil; R_f (EtOAc/hexanes 1:2) = 0.63. $[\alpha]_D^{20}$ = +16.4° (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 – 7.20 (m, 15H, arom.), 4.84 (d, *J* = 11.7 Hz, A of AB, 1H, $PhCH_2$), 4.79 (d, *J* = 11.7 Hz, B of AB, 1H, $PhCH_2$), 4.64 (d, *J* = 11.9 Hz, A of AB, 1H, $PhCH_2$), 4.57 (d, *J* = 11.9 Hz, B of AB, 1H, $PhCH_2$), 4.30 (d, *J* = 8.0 Hz, 1H, H-1), 4.15 (m, 2H, H-3, H-4), 4.01 (m, 1H, A of AB, O-C*H*$_2$-CH_2), 3.90 (ddd, *J* = 5.3, 1.5, 6.9 Hz, 1H, H-5), 3.83 – 3.72 (m, 4H, H-6, $PhCH_2$), 3.66 (m, 1H, B of AB, O-C*H*$_2$-CH_2), 3.42 – 3.36 (m, 1H, H-2), 2.79 – 2.65 (m, 2H, CH_2-C*H*$_2$-S), 1.37 (s, 3H, *i*PrCH$_3$), 1.34 (s, 3H, *i*Pr-CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.5, 138.4, 138.3, 129.0, 128.6, 128.5, 128.3, 128.3, 127.82, 127.78, 127.7, 127.2, 110.12, 110.05, 103.1, 79.6, 79.1, 73.9, 73.8, 73.7, 72.4, 69.6, 69.1, 36.6, 30.9, 27.9, 26.5. IR (thin

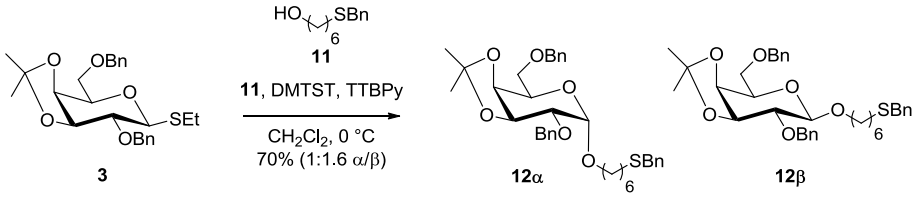
film) 3063, 3030, 2986, 2924, 2870, 1602, 1496, 1454, 1397, 1242, 1219, 1161, 1097, 1079, 1045, 872, 801, 738, 698 cm^{-1}; HRMS (ESI) calcd for $C_{32}H_{38}O_6S$ $(M+Na)^+$ 573.2287 found 573.2286 *m*/*z*.

Control Reaction: Activation of Thioglycoside 3 with NIS/TfOH



Thioglycoside **3** (65 mg, 0.146 mmol) and monobenzyl ethylene glycol (33 mg, 0.219 mmol) were co-evaporated with dry toluene (2x10 mL) and kept for 1 h under high vacuum. The mixture was dissolved in dry Et_2O (2.1 mL) and dry CH_2Cl_2 (0.7 mL) and activated molecular sieves (3 Å-AW) were added. The solution was stirred for 30 min at room temperature and cooled to -40 °C. The mixture was treated with NIS (49 mg, 0.219 mmol) and triflic acid (1.7 µL, 0.019 mmol) and slowly warmed to -10 °C over a period of 2 h, when t.l.c. indicated complete conversion of thioglycoside **3**. The reaction was diluted with CH_2Cl_2 (10 mL), quenched by adddition of Et_3N (0.2 mL), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 1:3) to give glycosides **SI-1** as an inseparable 1.9:1 (α/β) mixture of both isomers (48 mg, 0.090 mmol, 61%) as a clear oil. R_f (EtOAc/hexanes 1:3) = 0.47; $[\alpha]_D^{20}$ = +51.8° (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.21 (m, 15H), 4.89 (dd, *J* = 9.5, 7.7 Hz, 1.00H), 4.84 – 4.76 (m, 1H), 4.75 – 4.68 (m, 0.65H), 4.64 (d, *J* = 7.0 Hz, 0.35H), 4.61 (d, *J* = 7.2 Hz, 0.65H), 4.58 – 4.50 (m, 2H), 4.42 – 4.34 (m, 1H), 4.32 (m, 0.65H), 4.18 (dd, *J* = 5.6, 2.5 Hz, 0.65H), 4.15 (dd, *J* = 3.4, 1.2 Hz, 0.65H), 4.12 – 4.04 (m, 0.35H), 3.94 – 3.89 (m, 0.35H), 3.89 – 3.84 (m, 0.35H), 3.81 – 3.77 (m, 0.65H), 3.75 – 3.65 (m, 3H), 3.53 (dd, *J* = 7.8, 3.5 Hz, 0.65H), 3.45 – 3.39 (m, 0.35H), 1.39 (s, 1.95H), 1.37 (s, 1.05H), 1.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.6, 138.49, 138.48, 138.47, 138.43, 138.37, 133.1, 129.83, 129.80, 128.52, 128.47, 128.44, 128.43, 128.37, 128.34, 128.28, 128.0, 127.9, 127.79, 127.78, 127.76, 127.71, 127.69, 127.67, 127.65, 127.64, 127.57, 110.14, 110.13, 110.0, 109.2, 103.3, 97.4, 79.7, 79.1, 76.6, 76.0, 73.96, 73.94, 73.73, 73.70, 73.5, 73.4, 73.2, 72.4, 72.3, 69.7, 69.5, 69.4, 69.0, 67.5, 66.7, 28.3, 28.0, 26.53, 26.51; IR (thin film) 3031, 2986, 2931, 2968, 1721, 1497, 1454, 1380, 1370, 1243, 1219, 1166, 1100, 1074, 1044, 873, 737, 698 cm^{-1}; HRMS (ESI) calcd for $C_{32}H_{38}O_7$ $(M+Na)^+$ 557.2515 found 557.2489 *m*/*z*.

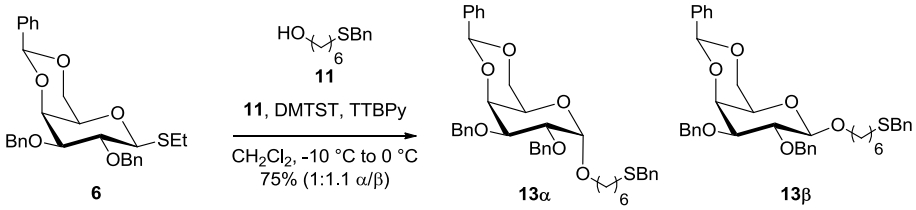
2,6-Di-*O*-benzyl-3,4-*O*-isopropylidene-α-D-galactopyranosyl-(1→1)-6-(benzylthio)hexanol (12α) and 2,6-Di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→1)-6-(benzylthio)hexanol (12β)



Thioglycoside **3** (95 mg, 0.214 mmol) was glycosylated with alcohol **11**[4] (72 mg, 0.321 mmol) using DMTST (83 mg, 0.321 mmol) and TTBPy (79 mg; 0.321 mmol) in CH_2Cl_2 (4.3 mL) at 0 °C for 1.5 h. Flash chromatography was performed (EtOAc/hexanes 0:1 to 1:4) to give thioethers **12α** (35 mg, 0.058 mmol, 27%) and **12β** (56 mg, 0.092 mmol, 43%). Analytical data for **12α**: Clear oil; R_f (EtOAc/hexanes 1:4) = 0.60; $[\alpha]_D^{20}$ = +54.6° (c = 0.2, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.20 (m, 15H, arom.), 4.78 (m, 2H, H-1, A of AB, $PhCH_2$), 4.69 (d, *J* = 12.6 Hz, 1H, B of AB, $PhCH_2$), 4.64 (d, *J* = 12.1 Hz, 1H, A of AB, $PhCH_2$), 4.53 (d, *J* = 12.1 Hz, 1H, B of AB, $PhCH_2$), 4.33 (dd, *J* = 7.8, 5.4 Hz, 1H, H-3), 4.23 – 4.11 (m, 2H, H-4, H-5), 3.77 – 3.61 (m, 5H, H-6, $PhCH_2$, A of AB, O-C*H*$_2$-CH_2), 3.51 (dd, *J* = 7.8, 3.5 Hz, 1H, H-2), 3.38 (dt, *J* = 9.8, 6.6 Hz, 1H, B of AB, O-C*H*$_2$-CH_2), 2.43 – 2.36 (m, 2H, CH_2-C*H*$_2$-S), 1.65 – 1.50 (m, 4H, aliph.), 1.44 – 1.28 (m, 10H, aliph, *i*Pr-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.8, 138.6, 138.5, 129.0, 128.6, 128.48, 128.45, 128.0, 127.8, 127.71, 127.67, 127.0, 109.2, 97.2, 76.1, 74.0, 73.6, 72.4, 69.8, 68.4, 66.8, 36.5, 31.5, 29.4, 29.3, 28.8, 28.3, 26.6, 25.9; IR (thin film) 3029, 2929, 1496, 1454, 1380, 1243, 1219, 1167, 1102, 1073, 1044, 874, 735, 698 cm^{-1}; HRMS (ESI) calcd for $C_{36}H_{46}O_6S$ $(M+Na)^+$ 629.2913 found 629.2889 *m*/*z*. Analytical data for **12β:** Clear oil; R_f (EtOAc/hexanes 1:4) = 0.27; $[\alpha]_D^{20}$ = +12.6° (c = 0.33, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.18 (m, 15H, arom.), 4.84 (d, *J* = 11.7 Hz, 1H, A of AB, $PhCH_2$), 4.78 (d, *J* = 11.7 Hz, 1H, B of AB, $PhCH_2$), 4.64 (d, *J* = 11.9 Hz, 1H, A of AB, $PhCH_2$), 4.56 (d, *J* = 11.9 Hz, 1H, B of AB, $PhCH_2$), 4.29 (d, *J* = 8.1 Hz, 1H, H-1), 4.16 – 4.11 (m, 2H, H-3, H-4 or H-5), 3.97 – 3.87 (m, 2H, H-4 or H-5, A of AB, H-6), 3.82 – 3.76 (m, 2H, B of AB, H-6, A of AB, O-C*H*$_2$-CH_2), 3.69 (s, 2H, $PhCH_2$), 3.55 – 3.45 (m, 1H, B of AB, O-C*H*$_2$-CH_2), 3.43 – 3.34 (m, 1H, H-2), 2.40 (t, *J* = 7.2 Hz, 2H, CH_2-C*H*$_2$-S), 1.69 – 1.49 (m, 4H, aliph.), 1.41 – 1.31 (m, 10H, aliph., *i*Pr-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.8, 138.5, 138.4, 128.9, 128.54, 128.49, 128.3, 128.2, 127.8, 127.7, 127.6, 127.0, 110.0, 103.1, 79.8, 79.2, 74.0, 73.73, 73.71, 72.4, 69.9, 69.7, 36.5, 31.5, 29.7, 29.3,

28.8, 27.9, 26.5, 25.9; IR (thin film) 2934, 1454, 1370, 1219, 1076, 1045, 872, 737, 697 cm^{-1}; HRMS (ESI) calcd for $C_{36}H_{46}O_6S$ $(M+Na)^+$ 629.2913 found 629.2990 *m*/*z*.

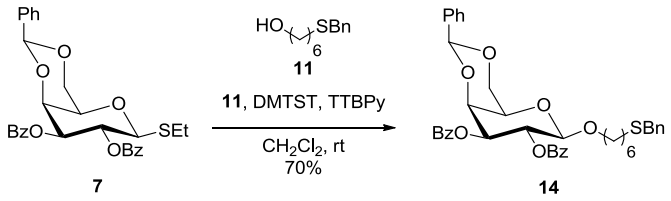
2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→1)-6-(benzylthio)hexanol (13α) and 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→1)-6-(benzylthio)hexanol (13β)



Thioglycoside **6**[5] (110 mg, 0.223 mmol) was glycosylated with alcohol **11** (75 mg, 0.335 mmol) using DMTST (87 mg, 0.335 mmol) and TTBPy (110 mg; 0.447 mmol) in CH_2Cl_2 (4.5 mL) from -10 °C to 0 °C for 1.5 h. Flash chromatography was performed twice (EtOAc/hexanes 1:6, then EtOAc/toluene 1:10) to give thioethers **13α** (52 mg, 0.080 mmol, 35%) and **13β** (58 mg, 0.089 mmol, 40%,). Analytical data for **13α**: White foam; R_f (EtOAc/hexanes 1:4) = 0.5; $[\alpha]_D^{20}$ = +84.3° (c = 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 – 7.50 (m, 2H, arom.), 7.44 – 7.22 (m, 18H, arom.), 5.47 (s, 1H, PhCH), 4.89 (d, *J* = 3.5 Hz, 1H, H-1), 4.88 – 4.78 (m, 2H, $PhCH_2$), 4.74 (d, *J* = 12.2 Hz, 1H, B of AB, $PhCH_2$), 4.66 (d, *J* = 12.0 Hz, 1H, B of AB, $PhCH_2$), 4.22 – 4.17 (m, 2H, H-4, A of AB, H-6), 4.06 (dd, *J* = 10.1, 3.5 Hz, 1H, H-2), 4.03 – 3.96 (m, 2H, H-3, B of AB, H-6), 3.70 (s, 2H, $PhCH_2$), 3.65 – 3.57 (m, 2H, H-5, A of AB, O-C*H*$_2$-CH_2), 3.49 – 3.40 (m, 1H, B of AB, O-C*H*$_2$-CH_2), 2.41 (t, *J* = 5.6 Hz, 2H, CH_2-C*H*$_2$-S), 1.62 – 1.51 (m, 4H, aliph.), 1.40 – 1.26 (m, 4H, aliph.); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.1, 139.0, 138.8, 138.1, 129.0, 128.6, 128.43, 128.42, 128.2, 128.0, 127.8, 127.7, 127.6, 127.0, 126.5, 101.2, 98.3, 76.3, 75.9, 75.0, 73.7, 72.3, 69.7, 68.5, 62.8, 36.54, 31.53, 29.5, 29.3, 28. 8, 26.0; IR (thin film) 3030, 2922, 2858, 1496, 1454, 1400, 1364, 1340, 1247, 1100, 1049, 1028, 796, 739, 697 cm^{-1}; HRMS (ESI) calcd for $C_{40}H_{46}O_6S$ $(M+Na)^+$ 677.2912 found 677.2905 *m*/*z*. Analytical data for **13β:** Clear oil; R_f (EtOAc/hexanes 1:4) = 0.29; $[\alpha]_D^{20}$ = +41.1° (c = 0.33, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.52 (m, 2H, arom.), 7.42 – 7.21 (m, 18H, arom.), 5.50 (s, 1H, PhCH), 4.92 (d, *J* = 10.9 Hz, 1H, A of AB, $PhCH_2$), 4.81 – 4.74 (m, 3H, $PhCH_2$), 4.37 (d, *J* = 7.8 Hz, 1H, H-1), 4.30 (dd, *J* = 12.3, 1.5 Hz, 1H, A of AB, H-6), 4.11 (dd, *J* = 3.7, 0.8 Hz, 1H, H-4), 4.01 (dd, *J* = 12.3, 1.8 Hz, 1H, B of AB, H-6), 3.96 (dt, *J* = 9.4, 6.4 Hz, 1H, A of AB, O-C*H*$_2$-CH_2), 3.83 (dd, *J* = 9.7, 7.7 Hz, 1H, H-2), 3.68 (s, 2H, $PhCH_2$), 3.55 (dd, *J* = 9.7, 3.7 Hz, 1H,

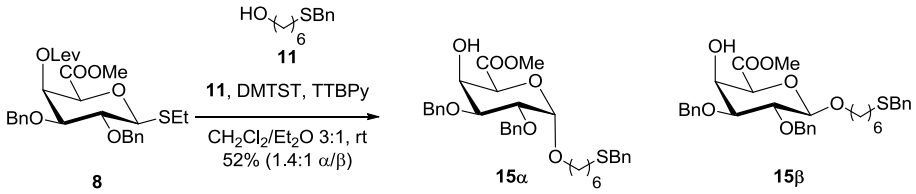
H-3), 3.49 (dt, *J* = 9.4, 6.8 Hz, 1H, B of AB, O-C*H*$_2$-CH$_2$), 3.31 (d, *J* = 1.0 Hz, 1H, H-5), 2.43 – 2.35 (t, *J* = 5.2 Hz, 2H, CH$_2$-C*H*$_2$-S), 1.71 – 1.59 (m, 2H, aliph.), 1.58 – 1.50 (m, 2H, aliph.), 1.44 – 1.30 (m, 4H, aliph.); ^{13}C NMR (100 MHz, CDCl$_3$) δ 139.1, 138.8, 138.7, 138.1, 129.02, 128.97, 128.6, 128.5, 128.41, 128.25, 128.1, 127.9, 127.8, 127.7, 127.0, 126.7, 103.9, 101.5, 79.5, 78.6, 75.4, 74.23, 72.17, 70.0, 69.5, 66.6, 36.5, 31.5, 29.8, 29.3, 28.9, 25.9; IR (thin film) 3031, 2925, 2858, 1496, 1454, 1365, 1178, 1101, 1057, 1028, 734, 698 cm^{-1}; HRMS (ESI) calcd for C$_{40}$H$_{46}$O$_6$S (M+Na)$^+$ 677.2912 found 677.2905 *m*/*z*.

2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→1)-6-(benzylthio)hexanol (14)



Thioglycoside **7**[6] (100 mg, 0.192 mmol) was glycosylated with alcohol **11** (65 mg, 0.288 mmol) using DMTST (99 mg, 0.384 mmol) and TTBPy (57 mg; 0.231 mmol) in CH$_2$Cl$_2$ (3.2 mL) at room temperature for 3 h. Flash chromatography was performed (EtOAc/CH$_2$Cl$_2$/hexanes 1:1:3 to 1:1:2) to give thioether **14** (92 mg, 0.134 mmol, 70%) as a clear oil, which solidified upon standing. R$_f$ (EtOAc/CH$_2$Cl$_2$/hexanes 1:1:2) = 0.66; [α]$_D^{20}$ = +100.2° (c = 1.0, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.15 – 7.89 (m, 4H, arom.), 7.58 – 7.43 (m, 4H, arom.), 7.43 – 7.19 (m, 12H, arom.), 5.87 (dd, *J* = 10.4, 8.0 Hz, 1H, H-2), 5.55 (s, 1H, PhCH), 5.38 (dd, *J* = 10.4, 3.6 Hz, 1H, H-3), 4.73 (d, *J* = 8.0 Hz, 1H, H-1), 4.62 – 4.56 (m, 1H, H-4), 4.41 (dd, *J* = 12.4, 1.4 Hz, 1H, A of AB, H-6), 4.14 (dd, *J* = 12.4, 1.7 Hz, 1H, B of AB, H-6), 3.96 (dt, *J* = 9.6, 6.1 Hz, 1H, A of AB, O-C*H*$_2$-CH$_2$), 3.69 – 3.62 (m, 3H, H-5, S-CH$_2$-Ph), 3.51 (dt, *J* = 9.6, 6.7 Hz, 1H, B of AB, O-C*H*$_2$-CH$_2$), 2.26 (t, *J* = 7.4 Hz, 2H, CH$_2$-C*H*$_2$-S), 1.59 – 1.41 (m, 2H, aliph.), 1.31 (m, 2H, aliph.), 1.17 (m, 4H, aliph.); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.4, 165.3, 138.8, 137.7, 133.5, 133.1, 130.1, 129.9, 129.8, 129.3, 129.0, 128.9, 128.5, 128.5, 128.4, 128.2, 127.0, 126.4, 101.4, 101.0, 73.8, 72.9, 69.6, 69.3, 69.1, 66.6, 36.4, 31.4, 29.4, 29.1, 28.6, 25.6; IR (thin film) 2933, 2857, 1723, 1602, 1451, 1368, 1315, 1275, 1179, 1110, 1097, 1000, 737, 709 cm^{-1}; HRMS (ESI) calcd for C$_{40}$H$_{42}$O$_8$S (M+Na)$^+$ 705.2498 found 705.2476 *m*/*z*.

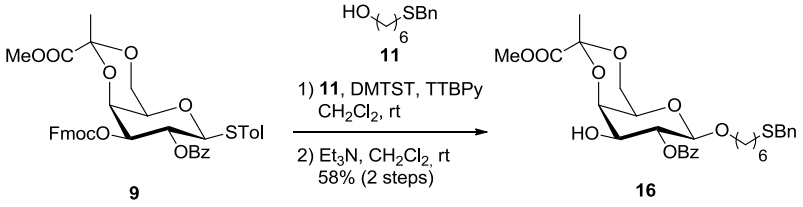
Methyl (2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosid)uronate-(1→1)-6-(benzylthio)hexanol (15α) and Methyl (2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosid)uronate-(1→1)-6-(benzylthio)hexanol (15β)



Thioglycoside **8** (87 mg, 0.164 mmol; *see* below) was glycosylated with alcohol **11** (85 mg, 0.379 mmol) using DMTST (63.5 mg, 0.246 mmol) and TTBPy (97 mg; 0.392 mmol) in Et_2O (2.4 mL) and CH_2Cl_2 (0.8 mL) at room temperature for 8 h. Flash chromatography was performed (EtOAc/CH_2Cl_2/hexanes 0:0:1 to 2:1:1) to give the corresponding glycosides as an inseparable 1.4:1 (α/β) mixture of both isomers as a clear oil (60 mg). The compound mixture in CH_2Cl_2 (2.2 mL) was then treated with a mixture of glacial acetic acid (0.14 mL, 2.393 mmol) and pyridine (0.2 mL, 2.411 mmol), and subsequently with hydrazine hydrate (5.9 µL, 0.121 mmol) at room temperature. The reaction was stirred for 2 h at that temperature, quenched by addition of acetone (0.2 mL) and concentrated. The residue was dissolved in CH_2Cl_2 (20 mL) and washed with 1 N HCl (10 mL) and sat. aq. $NaHCO_3$ (10 mL). The combined aqueous fractions were extracted with CH_2Cl_2 (2x10 mL), the combined organic fractions were dried over Na_2SO_4 and concentrated. Flash chromatography was performed (EtOAc/hexanes 1:2) to give alcohols **15α** (29 mg, 0.049 mmol, 30% over two steps) and **15β** (22 mg, 0.037 mmol, 22% over two steps). Analytical data for **15α**: Clear oil; R_f (EtOAc/hexanes 1:1) = 0.57; $[\alpha]_D^{20}$ = +47.1° (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.18 (m, 15H, arom.), 4.87 (d, *J* = 3.5 Hz, 1H, H-1), 4.83 – 4.77 (m, 2H, $PhCH_2$), 4.72 (d, *J* = 11.4 Hz, 1H, B of AB, $PhCH_2$), 4.62 (d, *J* = 12.1 Hz, 1H, B of AB, $PhCH_2$), 4.39 (m, 2H, H-4, H-5), 3.95 (dd, *J* = 9.8, 3.0 Hz, 1H, H-3), 3.86 (dd, *J* = 9.8, 3.5 Hz, 1H, H-2), 3.81 (s, 3H, $COOCH_3$), 3.70 (s, 2H, $PhCH_2$), 3.63 (dt, *J* = 9.7, 6.9 Hz, 1H, A of AB, O-C*H*$_2$-CH_2), 3.42 (dt, *J* = 9.7, 6.9 Hz, 1H, B of AB, O-C*H*$_2$-CH_2), 2.52 (br s, 1H, OH), 2.40 (t, *J* = 7.3 Hz, 2H, CH_2-C*H*$_2$-S), 1.57 (m 4H, aliph.), 1.45 – 1.22 (m, 4H, aliph.); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 138.7, 138.4, 138.0, 128.9, 128.7, 128.58, 128.56, 128.18, 128.11, 128.07, 128.0, 127.9, 127.0, 97.7, 77.0, 75.3, 73.6, 73.0, 69.9, 68.9, 52.7, 36.5, 31.4, 29.3, 29.2, 28.7, 25.8; IR (thin film) 2929, 1764, 1454, 1208, 1101, 1028, 738, 699 cm^{-1}; HRMS (ESI) calcd for $C_{34}H_{42}O_7S$ $(M+Na)^+$ 617.2548 found 617.2542 *m*/*z*. Analytical data for **15β**: Clear oil; R_f (EtOAc/hexanes 1:1) = 0.63; $[\alpha]_D^{20}$ = -7.1°

(c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.19 (m, 15H, arom.), 4.90 (d, *J* = 11.1 Hz, 1H, A of AB, $PhCH_2$), 4.77 – 4.67 (m, 3H, B of AB, $PhCH_2$), 4.34 (d, *J* = 7.7 Hz, 1H, H-1), 4.31 (dd, *J* = 3.5, 1.4 Hz, 1H, H-4), 4.04 (d, *J* = 1.4 Hz, 1H, H-5), 4.00 (dt, *J* = 9.4, 6.4 Hz, 1H, A of AB, O-C*H*$_2$-CH_2), 3.82 (s, 3H, $COOCH_3$), 3.69 – 3.63 (m, 3H, $PhCH_2$, H-2), 3.55 (dd, *J* = 9.3, 3.4 Hz, 1H, H-3), 3.49 (dt, *J* = 9.4, 6.8 Hz, 1H, B of AB, O-C*H*$_2$-CH_2), 2.47 – 2.34 (t, *J* = 7.6 Hz, 2H, CH_2-C*H*$_2$-S), 1.62 (d, *J* = 6.2 Hz, 2H, aliph.), 1.52 (dd, *J* = 14.1, 6.8 Hz, 2H, aliph.), 1.45 – 1.28 (m, 4H, aliph.); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7, 138.8, 138.6, 137.7, 129.0, 128.7, 128.6, 128.5, 128.20, 128.17, 128.0, 127.8, 127.0, 103.5, 79.8, 78.4, 75.3, 73.8, 72.7, 70.2, 68.1, 52.7, 36.5, 31.5, 29.7, 29.3, 28.8, 25.9; IR (thin film) 2930, 1765, 1454, 1211, 1099, 1040, 738, 698 cm^{-1}; HRMS (ESI) calcd for $C_{34}H_{42}O_7S$ $(M+Na)^+$ found 617.2548 found 617.2547 *m*/*z*.

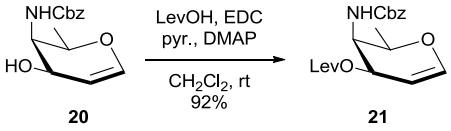
2-*O*-Benzoyl-4,6-[1-(*R*)-(methoxycarbonyl)-ehylidene]-β-D-galactopyranosyl-(1→1)-6-(benzylthio)hexanol (16)



Thioglycoside **9**[7] (200 mg, 0.287 mmol) was glycosylated with alcohol **11** (129 mg, 0.574 mmol) using DMTST (148 mg, 0.574 mmol) and TTBPy (74.7 mg, 0.301 mmol) in CH_2Cl_2 (5.7 mL) at room temperature for 20 h. Flash chromatography was performed (EtOAc/hexanes 1:3 to 1:1) to give the crude glycoside, which contained excess alcohol **11**. The material was then dissolved in CH_2Cl_2 (5.2 mL) and treated with Et_3N (0.3 mL, 2.15 mmol) at room temperature. The mixture was stirred for 1.5 h at that temperature, diluted with toluene (10 mL) and concentrated. The crude product was purified by flash chromatography (EtOAc/hexanes 1:2 to 1:1) to give alcohol **16** (96 mg, 0.167 mmol, 58% over two steps) as a clear oil. R_f (EtOAc/hexanes 1:2) = 0.18; $[\alpha]_D^{20}$ = -8.7° (c = 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 – 7.89 (m, 2H, arom.), 7.73 – 7.12 (m, 8H, arom.), 5.32 (dd, *J* = 10.0, 8.0 Hz, 1H, H-2), 4.50 (d, *J* = 8.0 Hz, 1H, H-1), 4.18 (dd, *J* = 3.8, 0.9 Hz, 1H, H-4), 4.08 (ddd, *J* = 32.6, 12.8, 1.7 Hz, 2H, H-6), 3.90 (dt, *J* = 9.7, 6.1 Hz, 1H, A of AB, O-C*H*$_2$-CH_2), 3.84 – 3.78 (m, 4H, $COOCH_3$, H-3), 3.62 (s, 2H, $PhCH_2$), 3.42 (m, 2H, B of AB, O-C*H*$_2$-CH_2, H-5) 2.43 (br s, 1H, OH), 2.23 (t, *J* = 7.3 Hz, 2H, CH_2-C*H*$_2$-S), 1.63 (s, 3H, CH_3), 1.53 – 1.38 (m, 2H, aliph.), 1.38 – 1.07 (m, 6H, aliph.); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 166.2, 138.7, 133.2, 130.1, 129.9, 128.9,

128.5, 128.44, 128.42, 126.9, 101.0, 98.9, 72.7, 71.6, 71.4, 69.9, 65.8, 65.2, 52.9, 36.3, 31.3, 29.3, 29.0, 28.6, 25.9, 25.6; IR (thin film) 2935, 1726, 1452, 1373, 1270, 1206, 1178, 1121, 1087, 983, 711 cm^{-1}; HRMS (ESI) calcd for $C_{30}H_{38}O_9S$ $(M+Na)^+$ 597.2134 found 597.2119 *m/z.*

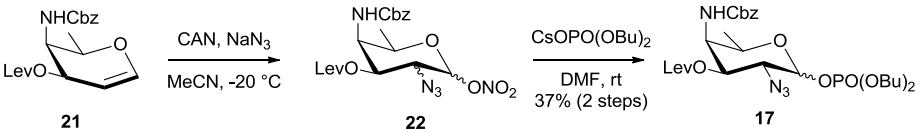
4-*O*-(Benzyloxycarbonyl)amino-3-*O*-levulinoyl-4,6-dideoxy-D-galactal (21)



20 **21**

To a stirred solution of alcohol **20**[8] (1.64 g, 6.21 mmol) in CH_2Cl_2 (40 mL) were added at 0 °C pyridine (0.5 mL, 6.23 mmol), levulinic acid (0.96 mL, 9.32 mmol), DMAP (0.15 g, 1.24 mmol) and EDC (1.2 mL, 6.83 mmol). The mixture was warmed to room temperature and stirred at that temperature. After 3 h, 0.5 equiv. levulinic acid and 0.5 equiv. EDC were added to drive the reaction to completion. After 5 h, the mixture was diluted with 100 mL CH_2Cl_2 and washed with water (50 mL), sat. aq. NH_4Cl (50 mL), sat. aq. $NaHCO_3$ (50 mL) and brine (50 mL). The organic fraction was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 1:1) to give ester **21** (2.07 g, 5.73 mmol, 92%) as a clear oil. R_f (EtOAc/hexanes 3:7) = 0.40; $[\alpha]_D^{20}$ = +1.2° (c = 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.28 (m, 5H, arom.), 6.41 (dd, *J* = 6.3, 1.8 Hz, 1H, H-1), 5.50 (d, *J* = 5.5 Hz, 1H, H-3), 5.17 (d, *J* = 12.2 Hz, 1H, A of AB, $PhCH_2$), 5.07 (d, *J* = 12.3 Hz, 1H, B of AB, $PhCH_2$), 4.99 (d, *J* = 10.0 Hz, 1H, NH), 4.65 (dt, *J* = 6.3, 1.7 Hz, 1H, H-2), 4.20 (m, 2H, H-4, H-5), 2.87 – 2.69 (m, 1H, Lev-CH_2), 2.68 – 2.47 (m, 2H, Lev-CH_2), 2.35 (dt, *J* = 17.1, 6.2 Hz, 1H, Lev-CH_2), 2.15 (s, 3H, Lev-CH_3), 1.29 (d, *J* = 6.5 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.7, 172.3, 157.0, 146.3, 136.6, 128.6, 128.3, 128.1, 99.6, 76.8, 72.9, 67.0, 66.5, 48.5, 38.0, 29.9, 28.0, 16.7; HRMS (ESI) calcd for $C_{19}H_{23}NO_6$ $(M+Na)^+$ 384.1423 found 384.1415 *m/z*.

Dibutyl [2-azido-4-(benzyloxycarbonyl)amino-3-*O*-levulinoyl-2,4,6-trideoxy-D-galactopyranosyl] phosphate (22)



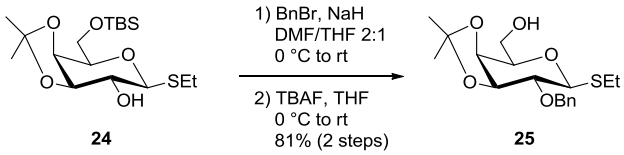
21 **22** **17**

To a stirred solution of galactal **21** (3.17 g, 8.77 mmol) in dry MeCN (44 mL) were added at -25°C ceric ammonium nitrate (14.42 g, 26.3 mmol) and sodium azide (0.86 g, 13.15 mmol). The reaction was stirred vigorously between -25 °C and -20 °C for 6 h. The mixture was

diluted with cold Et_2O (50 mL), washed with cold water (3x30 mL), dried over Na_2SO_4 and concentrated. The residue was filtered through a plug of silica gel (EtOAc/hexanes/Et_3N 1:1:0.01) to give crude glycosyl nitrate **22** as a 4:1 *galacto*/*talo* mixture (2.0 g) as a slightly yellow oil.

To crude glycosyl nitrate **22** (2.0 g) was added at room temperature a solution of cesium dibutyl phosphate (2.21 g, 6.45 mmol) in dry DMF (28 mL). The mixture was stirred at that temperature for 4.5 h, diluted with EtOAc (100 mL) and poured into water (100 mL). The organic phase was washed with water (5x50 mL) and the combined aqueous fractions were extracted with EtOAc (50 mL). The combined organic fractions were dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 45:55 to 50:50) to give glycosyl phosphate **17** (1.84 g, 3.00 mmol, 37%, 1:10 α/β) as a clear oil. R_f (EtOAc/hexanes 4:1) = 0.50 (β-anomer) & 0.72 (α-anomer). Analytical data for the β-anomer: $[\alpha]_D^{20}$ = +10.8° (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.28 (m, 5H, arom.), 5.15 (d, *J* = 12.1 Hz, 2H, NH, A of AB, $PhCH_2$), 5.10 – 5.03 (d, *J* = 12.3 Hz, 1H, B of AB, $PhCH_2$), 4.98 (t, *J* = 8.6 Hz, 1H, H-1), 4.74 (dd, *J* = 10.7, 3.9 Hz, 1H, H-3), 4.22 – 4.00 (m, 5H, H-4, PO-C*H*$_2$-CH_2), 3.91 – 3.79 (m, 1H, H-5), 3.61 (dd, *J* = 10.7, 8.2 Hz, 1H, H-2), 2.87 – 2.72 (m, 1H, Lev-CH_2), 2.72 – 2.51 (m, 2H, Lev-CH_2), 2.41 (ddd, *J* = 13.1, 9.4, 5.5 Hz, 1H, Lev-CH_2), 2.21 – 2.11 (s, 3H, Lev-CH_3), 1.79 – 1.56 (m, 4H, aliph.), 1.53 – 1.31 (m, 4H, aliph.), 1.30 – 1.19 (s, 3H, H-6), 0.92 (t, *J* = 7.4 Hz, 6H, aliph.); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.4, 172.0, 156.6, 136.4, 128.7, 128.4, 128.1, 97.83, 97.77, 73.3, 70.6, 68.3, 68.22, 68.16, 68.1, 67.3, 61.5, 61.4, 51.8, 38.0, 32.3, 32.24, 32.21, 32.17, 29.89, 27.91, 18.71, 18.70, 16.5, 13.67, 13.65; IR (thin film) 3658, 2981, 2115, 1720, 1462, 1382, 1252, 1153, 1073, 1030, 957, 820, 755 cm^{-1}; HRMS (ESI) calcd for $C_{27}H_{41}N_4O_{10}P$ $(M+Na)^+$ 635.2458 found 635.2422 *m*/*z*.

Ethyl 2-*O*-benzyl-3,4-isopropylidene-1-thio-β-D-galactopyranoside (25)



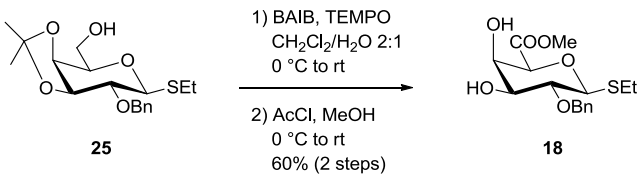
24 **25**

To a stirred solution of alcohol **24**[9] (45.7 g, 121 mmol) in DMF (150 mL) and THF (75 mL) were added at 0 °C first portionwise sodium hydride (60% (w/w), 7.24 g, 181 mmol) and then benzyl bromide (17.2 mL, 145 mmol). The mixture was stirred for 1 h at 0 °C, slowly warmed to room temperature and stirred for 16 h at that temperature. The reaction was quenched

at 0 °C with sat. aq. NH_4Cl (20 mL), diluted with water (200 mL) and EtOAc (150 mL) and stirred for 15 min at 0 °C. After separation, the organic phase was washed with water (5x100 mL) and the combined aqueous fractions were extracted with EtOAc (2x100 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated to give the crude benzyl ether (61 g) as a yellow oil.

To a stirred solution of the crude benzyl ether (61 g) in THF (370 mL) was added at 0 °C tetrabutylammonium fluoride (1 M in THF, 166 mL, 166 mmol). The mixture was warmed to room temperature and stirred for 1 h at that temperature. The reaction was diluted with sat. aq. $NaHCO_3$ (200 mL) and EtOAc (100 mL). After separation, the aqueous phase was extracted with EtOAc (3x100 mL), the combined organic fractions were dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 1:3 to 1:1) to give alcohol **25** (35 g, 98 mmol, 81% over two steps) as a clear oil. R_f (EtOAc/hexanes 1:2) = 0.25; $[\alpha]_D^{20}$ = +1.1° (c = 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.38 (m, 2H, arom.), 7.37 – 7.23 (m, 3H, arom.), 4.84 (d, *J* = 11.4 Hz, 1H, A of AB, $PhCH_2$), 4.76 (d, *J* = 11.4 Hz, 1H, B of AB, $PhCH_2$), 4.43 (d, *J* = 9.6 Hz, 1H, H-1), 4.25 (dd, *J* = 13.1, 7.2 Hz, 1H, H-3), 4.19 (dd, *J* = 5.7, 2.0 Hz, 1H, H-4), 3.94 (dd, *J* = 11.1, 6.9 Hz, 1H, A of AB, H-6), 3.84 – 3.73 (m, 2H, H-5, B of AB, H-6), 3.46 (dd, *J* = 9.6, 5.3 Hz, 1H, H-2), 2.72 (m, 2H, S-C*H*$_2$-CH_3), 2.22 (s, 1H, OH), 1.43 (s, 3H, *i*Pr-CH_3), 1.35 (s, 3H, *i*Pr-CH_3), 1.33 – 1.26 (m, 3H, S-CH_2-C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.9, 128.41, 128.37, 127.9, 110.3, 83.9, 79.8, 79.1, 76.8, 74.1, 73.6, 62.7, 27.9, 26.5, 24.8, 15.1; IR (thin film) 3234, 2980, 2934, 1455, 1381, 1368, 1245, 1217, 1079, 1037, 875, 743, 697 cm^{-1}; HRMS (ESI) calcd for $C_{18}H_{26}O_5S$ $(M+Na)^+$ 377.1398 found 377.1416 *m*/*z*.

Methyl (ethyl 1-thio-β-D-galactopyranosid)uronate (18)

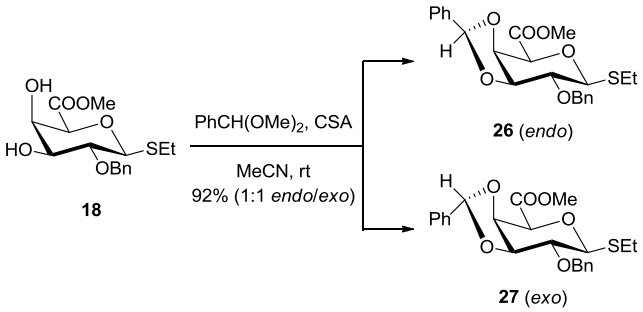


To a vigorously stirred solution of alcohol **25** (6.0 g, 16.9 mmol) in CH_2Cl_2 (50 mL) and H_2O (25 mL) were added at 0 °C TEMPO (0.53 g, 3.4 mmol) and BAIB (10.9 g, 33.9 mmol). The mixture was warmed to room temperature and stirred for 1 h at that temperature. The reaction was quenched with 10% aq. $Na_2S_2O_3$ (10 mL) and diluted with EtOAc (30 mL). After separation, the organic phase was washed with 10% $Na_2S_2O_3$ (4x20 mL). The aqueous phase

was extracted with EtOAc (2x20 mL), the combined organic fractions were dried over Na_2SO_4 and concentrated to give the crude acid (7.92 g) as a yellow oil.

To a stirred solution of acetyl chloride (6.04 mL, 85 mmol) in MeOH (300 mL) was added dropwise at 0 °C a solution of the crude acid (7.92 g) in MeOH (40 mL). The mixture was warmed to room temperature and stirred for 2 h at that temperature. The reaction was quenched at 0 °C with sat. aq. $NaHCO_3$ (30 mL) and neutralized to pH 7 with solid $NaHCO_3$. The volatiles were evaporated and the mixture was diluted with EtOAc (70 mL). After separation, the aqueous phase was extracted with EtOAc (5x50 mL). The combined organic fractions were dried over Na_2SO_4 and concentrated. Flash chromatography was performed (EtOAc/hexanes 2:3 to 1:1 to 1:0) to give the crude product, which was crystallized in MeOH at -20 °C (5 mL/g crude product) to give diol **18** (3.47 g, 10.1 mmol, 60% over two steps) as a white solid. R_f (EtOAc) = 0.51; $[\alpha]_D^{20}$ = -32.0° (c = 0.50, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.46 – 7.40 (m, 2H, arom.), 7.34 – 7.23 (m, 3H, arom.), 4.84 – 4.77 (m, 2H, $PhCH_2$), 4.49 (d, *J* = 9.7 Hz, 1H, H-1), 4.26 (s, 1H, H-5), 4.18 (dd, *J* = 3.4, 1.3 Hz, 1H, H-4), 3.77 (s, 3H, $COOCH_3$), 3.70 (dd, *J* = 9.2, 3.5 Hz, 1H, H-3), 3.52 (t, *J* = 9.4 Hz, 1H, H-2), 2.88 – 2.66 (m, 2H, S-C*H*$_2$-CH_3), 1.30 (t, *J* = 7.4 Hz, 3H, S-CH_2-C*H*$_3$); ^{13}C NMR (100 MHz, CD_3OD) δ 170.5, 139.9, 129.3, 129.1, 128.6, 86.1, 79.6, 78.7, 76.3, 75.7, 71.9, 52.6, 25.6, 15.5. IR (thin film) 3461, 2929, 1744, 1440, 1350, 1267, 1216, 1101, 1029, 836, 741, 699 cm^{-1}; HRMS (ESI) calcd for $C_{16}H_{22}O_6S$ $(M+Na)^+$ 365.1034 found 365.1058 *m*/*z*.

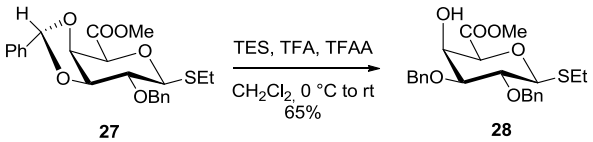
Methyl (ethyl 2-*O*-benzyl-3,4-*O*-*endo*-benzylidene-1-thio-β-D-galactopyranosid)uronate (26) and Methyl (ethyl 2-*O*-benzyl-3,4-*O*-*exo*-benzylidene-1-thio-β-D-galactopyranosid)uronate (27)



To a stirred solution of diol **18** (2.99 g, 8.73 mmol) in dry acetonitrile (29 mL) were added at room temperature benzaldehyde dimethyl acetal (6.57 mL, 43.6 mmol) and DL-camphorsulfonic acid (0.51 g, 2.18 mmol). The mixture was stirred at room temperature for

5 h and the reaction was quenched by addition of Et_3N (0.35 mL). The mixture was concentrated and the residue was filtered through a short plug of silica gel (EtOAc/hexanes/Et_3N 1:8:0.02 to 1:1:0.02) to give benzylidene acetals **26** (*endo*) and **27** (*exo*) (3.46 g, 8.03 mmol, 92%) as a 1:1 mixture. The isomers were separated by selective crystallization of *exo*-isomer **27** from EtOAc/hexanes and chromatographic separation of the mother liquor (Biotage, flat gradient of 10% to 40% EtOAc in hexanes + 0.5% Et_3N). Analytical data for **26**: Clear oil; R_f (EtOAc/hexanes 1:2) = 0.40; $[\alpha]_D^{20}$ = -59.3° (c = 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$,) δ 7.37 (m, 10H, arom.), 5.93 (s, 1H, PhCH), 4.69 (dd, *J* = 11.5 Hz, 2H, $PhCH_2$), 4.64 – 4.61 (dd, *J* = 6.4 Hz, 2.5 Hz, 1H, H-4), 4.58 (d, *J* = 8.6 Hz, 1H, H-1), 4.46 (m, 2H, H-3, H-5), 3.81 (s, 3H, $COOCH_3$) 3.61 (dd, *J* = 8.6, 5.7 Hz, 1H, H-2), 2.88 – 2.60 (m, 2H, S-C*H*$_2$-CH_3), 1.30 (t, *J* = 7.4 Hz, 3H, S-CH_2-C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 137.5, 136.8, 129.5, 128.4, 128.39, 128.35, 127.9, 127.1, 104.9, 84.1, 78.6, 78.1, 75.7, 75.1, 73.3, 52.6, 24.8, 14.9; IR (thin film) 2928, 2874, 1767, 1737, 1455, 1438, 1267, 1216, 1150, 1091, 1076, 1028, 756, 698 cm^{-1}; HRMS (ESI) calcd for $C_{23}H_{26}O_6S$ $(M+Na)^+$ 453.1348 found 453.1352 *m*/*z*. Analytical data for **27**: White foam; R_f (EtOAc/hexanes 1:2) = 0.50; $[\alpha]_D^{20}$ = -42.0° (c = 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.22 (m, 10H, arom.), 6.06 (s, 1H, PhCH), 4.91 – 4.76 (dd, 2H, *J* = 15.2, 11.5 Hz, $PhCH_2$), 4.71 – 4.55 (m, 3H, H-1, H-3, H-4), 4.37 (d, *J* = 1.8 Hz, 1H, H-5), 3.79 (s, 3H, $COOCH_3$), 3.69 (dd, *J* = 8.4, 5.1 Hz, 1H, H-2), 2.93 – 2.59 (m, 2H, S-C*H*$_2$-CH_3), 1.33 (t, *J* = 7.4 Hz, 3H, S-CH_2-C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 138.0, 137.3, 129.4, 128.5, 128.5, 128.1, 126.4, 104.0, 83.7, 79.3, 75.6, 75.5, 74.3, 73.4, 52.6, 25.1, 15.0; IR (thin film) 2903, 2873, 1765, 1738, 1497, 1455, 1438, 1344, 1267, 1213, 1140, 1098, 1075, 1028, 1002, 969, 920, 758, 737, 697 cm^{-1}; HRMS (ESI) calcd for $C_{23}H_{26}O_6S$ $(M+Na)^+$ 453.1348 found 453.1338 *m*/*z.*

Methyl (ethyl 2,3-*O*-benzyl-1-thio-β-D-galactopyranosyl)uronate (28)

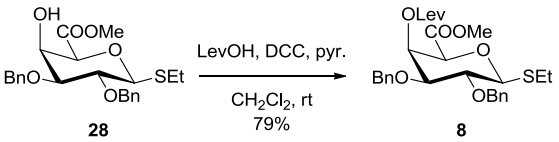


27 **28**

To a stirred solution of acetal **27** (206 mg, 0.48 mmol) and triethylsilane (2.29 mL, 14.35 mmol) in CH_2Cl_2 (7 mL) over activated molecular sieves (3 Å-AW) were added at 0 °C trifluoroacetic anhydride (20 µL, 0.14 mmol) and then trifluoroacetic acid (0.22 mL, 2.87 mmol). The reaction was warmed to room temperature and stirred for 5 h at that

temperature. The mixture was diluted with EtOAc (10 mL), quenched with Et_3N (0.4 mL) at 0 °C and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 1:1) to give alcohol **28** (134 mg, 0.31 mmol, 65%) as a white foam. R_f (EtOAc/hexanes 2:3) = 0.47; $[\alpha]_D^{20}$ = -14.0° (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.27 (m, 10H, arom.), 4.89 (d, *J* = 10.3 Hz, 1H, A of AB, $PhCH_2$), 4.81 – 4.67 (m, 3H, $PhCH_2$), 4.43 (d, *J* = 9.6 Hz, 1H, H-1), 4.39 (d, *J* = 0.9 Hz, 1H, H-4), 4.06 (s, 1H, H-5), 3.82 (s, 3H, $COOCH_3$), 3.71 (t, *J* = 9.3 Hz, 1H, H-2), 3.61 (dd, *J* = 8.9, 3.3 Hz, 1H, H-3) 2.96 – 2.67 (m, 2H, S-C*H*$_2$-CH_3), 2.52 (d, *J* = 1.8 Hz, 1H, OH), 1.32 (t, *J* = 7.4 Hz, 3H, S-CH_2-C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 138.1, 137.5, 128.7, 128.51, 128.50, 128.3, 128.1, 128.0, 85.2, 81.7, 77.4, 77.1, 76.0, 72.4, 68.1, 52.8, 25.0, 15.1; IR (thin film) 3492, 2927, 2870, 1764, 1736, 1497, 1454, 1351, 1266, 1210, 1128, 1098, 1029, 903, 844, 741, 698 cm^{-1}; HRMS (ESI) calcd for $C_{23}H_{28}O_6S$ $(M+Na)^+$ 455.1504 found 455.1511 *m*/*z*.

Methyl (ethyl 2,3-*O*-benzyl-4-*O*-levulinoyl-1-thio-β-D-galactopyranosyl)uronate (8)

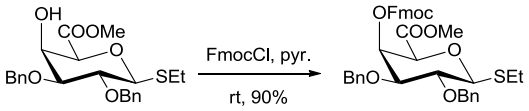


28 **8**

To a stirred solution of alcohol **28** (94 mg, 0.217 mmol) in CH_2Cl_2 (1.9 mL) were added at room temperature levulinic acid (386 mg, 3.26 mmol), DCC (673 mg, 3.26 mmol) and pyridine (0.26 mL, 3.26 mmol). The mixture was stirred at that temperature for 35 h, diluted with CH_2Cl_2 (5 mL) and filtered through Celite. The mixture was concentrated, the residue was dissolved in a minimal volume of CH_2Cl_2 (1-3 mL) and filtered through cotton wool. The same procedure was repeated 3 times. The residue was purified by flash chromatography (EtOAc/toluene 1:1) to give ester **8** (91 mg, 0.171 mmol, 79%) as a slightly yellow oil. R_f (EtOAc/toluene 2:3) = 0.57; $[\alpha]_D^{20}$ = +24.2° (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.28 (m, 10H, arom.), 5.84 (dd, *J* = 3.3, 1.3 Hz, 1H, H-4), 4.83 (d, *J* = 10.2 Hz, 1H, A of AB, $PhCH_2$), 4.79 – 4.74 (m, 2H, B of AB, $PhCH_2$, A of AB, $PhCH_2$), 4.53 (d, *J* = 11.3 Hz, 1H, B of AB, $PhCH_2$), 4.48 (d, *J* = 9.4 Hz, 1H, H-1), 4.16 (d, *J* = 1.3 Hz, 1H, H-5), 3.79 (s, 3H, $COOCH_3$), 3.64 (m, 2H, H-2, H-3), 2.87 – 2.59 (m, 6H, Lev-CH_2, S-C*H*$_2$-CH_3), 2.16 (s, 3H, Lev-CH_3), 1.33 (t, *J* = 7.4 Hz, 3H, S-CH_2-C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.1, 171.8, 167.2, 138.1, 137.6, 128.50, 128.46, 128.4, 128.3, 128.0, 127.9, 85.4, 80.4, 77.3, 76.0, 75.9, 72.0, 68.1, 52.8, 38.1, 29.9, 28.1, 25.1, 15.1; IR (thin film) 2968, 2868, 1746, 1720, 1497, 1454, 1363, 1264, 1213,

1156, 1124, 1104, 1028, 988, 736, 699 cm^{-1}; HRMS (ESI) calcd for $C_{28}H_{34}O_8S$ $(M+Na)^+$ 553.1872 found 553.1872 *m*/*z*.

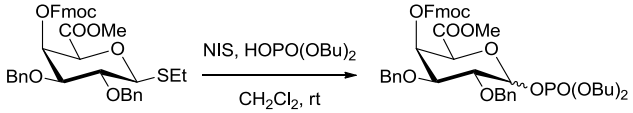
Methyl (ethyl 2,3-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranosyl)uronate (29)



28 **29**

To a stirred solution of alcohol **28** (160 mg, 0.370 mmol) in pyridine (1.2 mL) was added at 0 °C FmocCl (383 mg, 1.48 mmol). The mixture was warmed to room temperature and stirred for 3 h at that temperature. The mixture was diluted with EtOAc (50 mL) and washed with 1 N HCl (2x30 mL) and sat. aq. $NaHCO_3$ (30 mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 1:2) to give carbonate **29** (217 mg, 0.331 mmol, 90%) as a white foam. R_f (EtOAc/hexanes 1:4) = 0.35; $[\alpha]_D^{20}$ = -0.3° (c = 3.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 7.6, 0.8 Hz, 2H, arom.), 7.66 – 7.58 (m, 2H, arom.), 7.44 – 7.27 (m, 11H, arom.), 7.24 – 7.19 (m, 3H, arom.), 5.72 (dd, *J* = 3.1, 1.3 Hz, 1H, H-4), 4.85 (dt, *J* = 10.2, 8.7 Hz, 3H, $PhCH_2$), 4.61 (d, *J* = 11.4 Hz, 1H, $PhCH_2$), 4.51 (d, *J* = 9.2 Hz, 1H, H-1), 4.42 – 4.32 (m, 2H, Fmoc), 4.28 – 4.18 (m, 2H, H-5, Fmoc), 3.81 – 3.69 (m, 5H, $COOCH_3$, H-2, H-3), 2.92 – 2.72 (m, 2H, S-C*H*$_2$-CH_3), 1.36 (t, *J* = 7.4 Hz, 3H, S-CH_2-C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 154.8, 143.6, 143.3, 141.4, 138.0, 137.5, 128.44, 128.42, 128.39, 128.0, 127.93, 127.87, 127.2, 125.5, 125.3, 120.1, 120.0, 99.5, 85.5, 80.5, 76.1, 75.8, 72.2, 72.0, 70.4, 52.8, 46.6, 25.1, 15.1; IR (thin film) 2953, 1752, 1451, 1386, 1257, 1108, 1028, 741, 699 cm^{-1}; HRMS (ESI) calcd for $C_{38}H_{38}O_8S$ $(M+Na)^+$ 677.2185 found 677.2167 *m*/*z*.

Dibutyl [methyl (2,3-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-αβ-D-galactopyranosyl)uronate] phosphate (30)

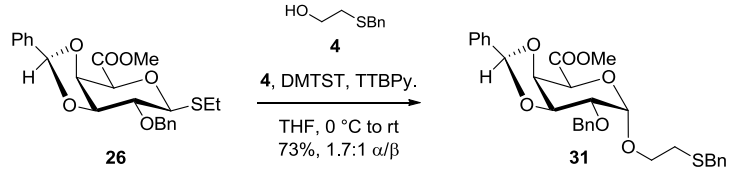


29 **30**

Thioglycoside **29** (200 mg, 0.31 mmol) was co-evaporated with dry toluene (2x30 mL), kept under high vacuum for 1 h and dissolved in dry CH_2Cl_2 (3 mL). Activated molecular sieves (3

Å-AW) were added and the solution was stirred for 15 min at room temperature. The solution was then cooled to 0 °C, treated with dibutyl phosphoric acid (128 mg, 0.61 mmol) and stirred for another 15 min. The mixture was then treated with NIS (89 mg, 0.40 mmol), warmed to room temperature and stirred for 3 h at that temperature. The reaction was diluted with CH_2Cl_2 (20 mL) and quenched with a 1:1 (v/v) mixture of 10% aq. $Na_2S_2O_3$ and sat. aq. $NaHCO_3$ (20 mL). After separation, the aqueous phase was extracted with CH_2Cl_2 (3x30 mL), the combined organic fractions were dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 1:1 to 2:1) to give glycosyl phosphate **30** (218 mg, 0.27 mmol, 89%, 10:1 α/β) as a clear oil. Analytical data for **30**α: R_f (EtOAc/hexanes 1:2) = 0.44; $[\alpha]_D^{20}$ = +61.8° (c = 3.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H, arom.), 7.59 (ddd, *J* = 8.3, 7.5, 0.8 Hz, 2H, arom.), 7.47 – 7.19 (m, 14H, arom.), 5.99 (dd, *J* = 6.9, 2.9 Hz, 1H, H-1), 5.77 (dd, *J* = 2.7, 1.7 Hz, 1H, H-4), 4.81 (m, 4H, H-5, $PhCH_2$), 4.67 (d, *J* = 11.6 Hz, 1H, $PhCH_2$), 4.37 (m, 2H, Fmoc), 4.21 (t, *J* = 7.6 Hz, 1H, Fmoc), 4.12 – 3.97 (m, 6H, P-O-CH_2, H-2, H-3), 3.76 (s, 3H, $COOCH_3$), 1.68 – 1.52 (m, 4H, aliph.), 1.45 – 1.24 (m, 4H, aliph.), 0.91 (dt, *J* = 11.5, 7.4 Hz, 6H, aliph.); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 154.6, 143.5, 143.2, 141.33, 141.25, 137.9, 137.6, 128.4, 128.3, 128.0, 127.94, 127.89, 127.85, 127.8, 127.24, 127.23, 125.4, 125.2, 120.1, 120.0, 95.7, 95.6, 77.4, 74.7, 74.4, 74.3, 73.8, 72.5, 72.3, 70.4, 70.3, 68.1, 68.0, 67.84, 67.78, 52.8, 46.6, 32.3, 32.21, 32.19, 32.1, 18.7, 18.6, 13.63, 13.61; IR (thin film) 3065, 3033, 2960, 2934, 2874, 1755, 1452, 1385, 1351, 1259, 1113, 1028, 997, 956, 910, 858, 781, 758, 741, 699 cm^{-1}; HRMS (ESI) calcd for $C_{44}H_{51}O_{12}P$ $(M+Na)^+$ 825.3015 found 825.3020 *m*/*z*.

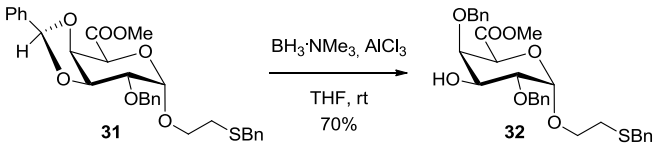
Methyl (2-*O*-benzyl-3,4-*O*-*endo*-benzylidene-αβ-D-galactopyranosyl)uronate-(1→1)-2-(benzylthio)ethanol (31)



Thioglycoside **26** (102 mg, 0.237 mmol), alcohol **4** (60 mg, 0.355 mmol) and TTBPy (117 mg, 0.474 mmol) were co-evaporated with dry toluene (3x10 mL) and kept under high vacuum for 30 min. The mixture was dissolved in THF (4.8 mL) and stirred over activated molecular sieves (3 Å) for 30 min at room temperature. The solution was cooled to 0 °C and treated with DMTST (92 mg, 0.355 mmol in 0.2 mL dry CH_2Cl_2). The reaction was warmed to room

temperature and stirred for 2 h at that temperature. The reaction was quenched with a 1:1 (v/v) mixture of MeOH and Et_3N (0.1 mL) and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes/Et_3N 0:1:0.01 to 30:70:0.01 to 45:55:0.01) to give thioether **31** (59 mg, 0.110 mmol, 46%), along with the corresponding β-isomer (35 mg, 0.065 mmol, 27%). Analytical data for **31**: Clear oil; R_f (EtOAc/hexanes 2:3) = 0.56; $[\alpha]_D^{20}$ = +25.0° (c = 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.14 (m, 15H, arom.), 5.96 (s, 1H, PhCH), 4.88 (d, *J* = 3.0 Hz, 1H, H-5), 4.86 (d, *J* = 3.6 Hz, 1H, H-1), 4.71 (d, *J* = 12.3 Hz, 1H, A of AB, $PhCH_2$), 4.64 (dd, *J* = 6.1, 3.0 Hz, 1H, H-4), 4.58 – 4.50 (m, 2H, H-3, B of AB, $PhCH_2$), 3.84 (s, 3H, $COOCH_3$), 3.82 – 3.73 (m, 3H, $PhCH_2$, A of AB, O-C*H*$_2$-CH_2), 3.67 – 3.57 (m, 2H, H-2, B of AB, O-C*H*$_2$-CH_2), 2.75 – 2.59 (m, 2H, CH_2-C*H*$_2$-S); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 138.2, 138.0, 137.4, 129.4, 129.0, 128.7, 128.49, 128.47, 128.1, 127.9, 127.3, 126.8, 104.2, 98.0, 76.1, 75.8, 75.6, 72.9, 68.5, 67.9, 52.7, 36.8, 30.6; IR (thin film) 2918, 1767, 1737, 1495, 1454, 1215, 1166, 1094, 1045, 923, 759, 698 cm^{-1}; HRMS (ESI) calcd for $C_{30}H_{32}O_7S$ $(M+Na)^+$ 559.1766 found 559.1731 *m/z*.

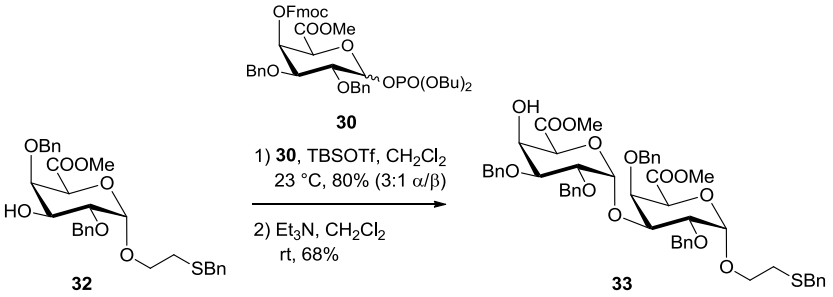
Methyl (2,4-di-*O*-benzyl-α-D-galactopyranosid)uronate-(1→1)-2-(benzylthio)ethanol (32)



To a stirred solution of acetal **31** (100 mg, 0.186 mmol) in dry THF (5.3 mL) were added at room temperature borane trimethylamine complex (57 mg, 0.745 mmol) and then aluminium chloride (149 mg, 1.12 mmol). The reaction was stirred at that temperature for 4.5 h and quenched by addition of water (10 mL) and 1 M aq. HCl (5 mL). The mixture was extracted with EtOAc (3x10 mL), the combined organic fractions were dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 2:5 to 1:1) to give alcohol **32** (70.0 mg, 0.130 mmol, 70%) as a clear oil. R_f (EtOAc/hexanes 1:1) = 0.71; $[\alpha]_D^{20}$ = +60.8° (c = 3.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.17 (m, 15H, arom.), 4.93 (d, *J* = 3.5 Hz, 1H, H-1), 4.84 (d, *J* = 11.7 Hz, 1H, A of AB, $PhCH_2$), 4.70 (d, *J* = 11.9 Hz, 1H, A of AB, $PhCH_2$), 4.66 – 4.59 (m, 2H, B of AB, $PhCH_2$), 4.53 (d, *J* = 1.6 Hz, 1H, H-5), 4.28 (dd, *J* = 3.2, 1.7 Hz, 1H, H-4), 4.22 – 4.08 (m, 1H, H-3), 3.88 (dd, *J* = 10.1, 3.5 Hz, 1H, H-2), 3.78 – 3.68 (m, 3H, $PhCH_2$, A of AB, O-C*H*$_2$-CH_2), 3.66 (s, 3H, $COOCH_3$), 3.49 (m, 1H, B of AB, O-C*H*$_2$-CH_2), 2.63 – 2.54 (m, 2H, 2H, CH_2-C*H*$_2$-S), 2.43 – 2.35 (m, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2,

138.3, 138.2, 138.0, 129.0, 128.62, 128.58, 128.4, 128.23, 128.17, 128.0, 127.8, 127.1, 97.3, 77.9, 76.7, 75.2, 73.1, 70.7, 69.8, 68.1, 52.3, 50.3, 36.6, 30.6; IR (thin film) 3506, 3029, 2918, 1762, 1496, 1454, 1344, 1211, 1152, 1106, 1060, 1026, 917, 739, 698 cm^{-1}; HRMS (ESI) calcd for $C_{30}H_{34}O_7S$ $(M+Na)^+$ 561.1923 found 561.1879 *m/z*.

Methyl (2,3-di-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-α-D-galactopyranosyl)uronate-(1→3)-methyl (2,4-di-*O*-benzyl-α-D-galactopyranosyl)uronate-(1→3)-(2-(benzylthio)ethanol (33)

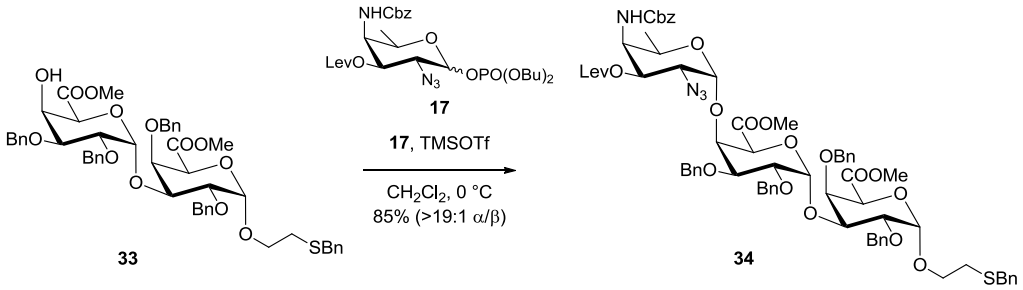


Alcohol **32** (90 mg, 0.166 mmol) and glycosyl phosphate **30** (208 mg, 0.259 mmol) were co-evaporated with dry toluene (3x10 mL) and kept under high vacuum for 1 h. The mixture was dissolved in dry CH_2Cl_2 (3.3 mL) and stirred over activated molecular sieves (3 Å-AW) for 30 min at room temperature. The solution was cooled to 0 °C and treated dropwise with TBSOTf (57 μL, 0.133 mmol, in 0.2 mL dry CH_2Cl_2). The solution was warmed to room temperature and stirred for 20 h at that temperature. The reaction was diluted with CH_2Cl_2 (10 mL) and quenched with a 1:1 (v/v) mixture of MeOH and pyridine (0.2 mL). The solution was filtered through Celite and concentrated. The crude product was filtered through a short plug of silica gel (EtOAc/hexanes 1:1) to give the intermediate disaccharide mixture (150 mg, 0.133 mmol, 80%, 3:1 α/β) as an inseparable mixture as a clear oil.

To a stirred solution of the disaccharide mixture (150 mg) in CH_2Cl_2 (2.6 mL) was added at room temperature Et_3N (1.1 mL, 7.96 mmol). The reaction was stirred for 3 h at that temperature and co-evaporated with toluene (2x10 mL). The residue was purified by flash chromatography (EtOAc/hexanes 1:6 to 2:3 to 1:1) to give alcohol **33** (62 mg, 0.068 mmol, 51%) along with the corresponding β-anomer (20 mg, 0.022 mmol, 17%). Analytical data for **33**: Clear oil; R_f (EtOAc/hexanes 1:1) = 0.36; $[\alpha]_D^{20}$ = +86.3° (c = 0.50, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.15 (m, 23H, arom.), 7.04 (dd, *J* = 6.6, 2.6 Hz, 2H, arom.), 5.28 (d, *J* = 2.4 Hz, 1H, H-1'), 5.03 (A of AB, d, *J* = 11.6 Hz, 1H, $PhCH_2$), 4.96 (d, *J* = 3.5 Hz, 1H, H-1), 4.84

(dd, *J* = 11.6, 6.4 Hz, 2H, A of AB, $PhCH_2$, H-5'), 4.75 – 4.63 (m, 3H, $PhCH_2$), 4.61 – 4.52 (m, 2H, $PhCH_2$), 4.45 (s, 1H, H-5), 4.37 (B of AB, d, *J* = 11.5 Hz, 1H, $PhCH_2$), 4.30 (s, 1H, H-4), 4.26 (dd, *J* = 10.2, 2.6 Hz, 1H, H-3), 4.19 (m, 1H, H-4'), 4.05 (dd, *J* = 10.2, 3.5 Hz, 1H, H-2), 3.98 (m, 2H, H-2', H-3'), 3.82 – 3.68 (m, 3H, A of AB, O-C*H*$_2$-CH_2, $PhCH_2$), 3.63 (s, 3H, $COOCH_3$), 3.56 – 3.47 (m, 4H, B of AB, O-C*H*$_2$-CH_2, $COOCH_3$), 2.54 (m, 2H, CH_2-C*H*$_2$-S), 2.44 (br s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 169.1, 138.7, 138.4, 138.2, 137.9, 137.7, 129.2, 128.7, 128.62, 128.56, 128.55, 128.3, 128.2, 128.1, 128.02, 127.98, 127.9, 127.7, 127.3, 127.1, 97.6, 95.8, 76.0, 75.2, 75.1, 74.6, 74.1, 72.9, 72.4, 71.0, 70.2, 68.8, 68.6, 52.4, 52.2, 36.7, 30.3; IR (thin film) 3517, 3062, 3030, 2933, 1763, 1735, 1603, 1496, 1454, 1344, 1273, 1212, 1148, 1105, 1061, 1028, 916, 805, 740, 698 cm^{-1}; HRMS (ESI) calcd for $C_{51}H_{56}O_{13}S$ $(M+Na)^+$ 931.3339 found 931.3340 *m/z*.

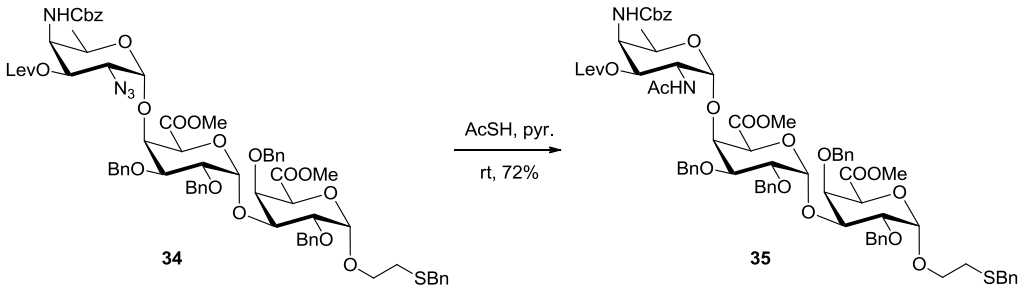
2-Azido-4-(benzyloxycarbonyl)amino-3-*O*-levulinoyl-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-methyl (2,3-di-*O*-benzyl-α-D-galactopyranosyl)uronate-(1→3)-methyl (2,4-di-*O*-benzyl-α-D-galactopyranosyl)uronate-(1→1)-2-(benzylthio)ethanol (34)



Alcohol **33** (65 mg, 0.062 mmol) and glycosyl phosphate **17** (61 mg, 0.100 mmol) were co-evaproated with dry toluene (3x10 mL) and kept under high vacuum for 30 min. The mixture was dissolved in CH_2Cl_2 (2.1 mL) and stirred over activated molecular sieves (3 Å-AW) for 1 h at room temperature. The solution was cooled to 0 °C and treated with TMSOTf (17 µL, 0.093 mmol in 0.2 mL dry CH_2Cl_2). The reaction was stirred for 3 h at that temperature and quenched with a 1:1 (v/v) mixture of MeOH and Et_3N (0.5 mL). The mixture was diluted with CH_2Cl_2 (20 mL), filtered through Celite and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 1:2 to 1:1) to give trisaccharide **34** (69 mg, 0.053 mmol, 85%) as a clear oil. R_f (EtOAc/hexanes 2:3) = 0.43; $[\alpha]_D^{20}$ = +116.6° (c = 0.50, acetone); ^{1}H NMR (400 MHz, acetone-D_6) δ 7.51 – 7.10 (m, 30H, arom.), 6.28 (d, *J* = 10.1 Hz, 1H, NH), 5.45 (d, *J* = 3.1 Hz, 1H, H-1'), 5.22 – 5.11 (m, 3H, H-3'', 2x A of AB, $PhCH_2$), 5.05 – 4.97 (m, 2H, H-1, A of AB, $PhCH_2$), 4.93 – 4.81 (m, 5H, H-1'', H-5', $PhCH_2$), 4.76 (d, *J* = 12.8 Hz, 1H, B of AB,

$PhCH_2$), 4.61 (d, *J* = 11.7 Hz, 1H, A of AB, $PhCH_2$), 4.54 (m, 2H, H-5, B of AB, $PhCH_2$), 4.49 (m, 2H, H-4, H-5''), 4.43 (d, *J* = 1.4 Hz, 1H, H-4'), 4.39 (d, *J* = 11.2 Hz, 1H, B of AB, $PhCH_2$), 4.29 (dd, *J* = 10.4, 2.8 Hz, 1H, H-3), 4.16 – 4.01 (m, 3H, H-2', H-3', H-4''), 3.91 (dd, *J* = 10.4, 3.5 Hz, 1H, H-2), 3.83 – 3.73 (m, 3H, $PhCH_2$, A of AB, O-C*H*$_2$-CH_2), 3.70 – 3.63 (m, 4H, H-2'', $COOCH_3$), 3.61 – 3.52 (m, 1H, B of AB, O-C*H*$_2$-CH_2), 3.44 (s, 3H, $COOCH_3$), 2.80 – 2.63 (m, 2H, Lev-CH_2), 2.63 – 2.45 (m, 3H, Lev-CH_2, CH_2-C*H*$_2$-S), 2.38 (m, 1H, Lev-CH_2), 2.13 (s, 3H, Lev-CH_3), 0.92 – 0.81 (m, 3H, H-6''); ^{13}C NMR (100 MHz, acetone-D_6) δ 206.0, 172.3, 169.5, 169.3, 157.8, 140.3, 139.7, 139.4, 139.0, 138.3, 129.4, 129.14, 129.11, 129.08, 129.06, 129.05, 128.7, 128.6, 128.6, 128.5, 128.4, 128.31, 128.26, 128.17, 128.15, 127.7, 127.5, 99.9, 97.9, 96.3, 77.6, 77.3, 76.2, 75.8, 75.7, 75.1, 74.6, 73.0, 72.8, 71.4, 71.0, 70.2, 69.4, 66.7, 65.8, 58.3, 53.8, 52.0, 51.8, 38.1, 36.9, 17.8, 16.6; IR (thin film) 2925, 2210, 1719, 1497, 1454, 1347, 1215, 1147, 1108, 1028, 742, 699 cm^{-1}; HRMS (ESI) calcd. for $C_{70}H_{78}N_4O_{19}S$ $(M+Na)^+$ 1333.4879 found 1333.4911 *m/z*.

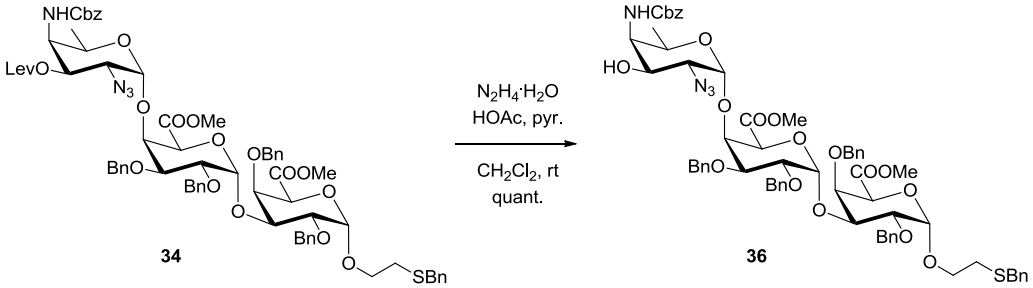
2-Acetamido-4-(benzyloxycarbonyl)amino-3-*O*-levulinoyl-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-methyl (2,3-di-*O*-benzyl-α-D-galactopyranosyl)uronate-(1→3)-methyl (2,4-di-*O*-benzyl-α-D-galactopyranosyl)uronate-(1→1)-(2-(benzylthio)ethanol (35)



To a stirred solution of azide **34** (20.0 mg, 0.015 mmol) in dry pyridine (0.4 mL) was added at room temperature thioacetic acid (0.4 mL). The reaction was stirred for 24 h at that temperature. The mixture was co-evaporated with toluene (2x5 mL) and the residue was purified by flash chromatography (EtOAc/hexanes 1:8 to 5:1) to give acetamide **35** (15 mg, 0.011 mmol, 72%) as a white foam. R_f (EtOAc/hexanes 4:1) = 0.25; $[\alpha]_D^{20}$ = +99.5° (c = 1.0, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.47 – 7.13 (m, 30H, arom.), 5.43 (d, *J* = 3.3 Hz, 1H, H-1'), 5.17 (d, *J* = 12.6 Hz, 1H, A of AB, $PhCH_2$), 5.11 (d, *J* = 11.0 Hz, 1H, A of AB, $PhCH_2$), 5.05 – 4.99 (m, 2H, H-1, B of AB, $PhCH_2$), 4.93 (dd, *J* = 6.6, 2.5 Hz, 1H, H-3''), 4.80 (m, 3H, $PhCH_2$), 4.73 – 4.65 (m, 3H, H-5, $PhCH_2$), 4.60 (d, *J* = 3.9 Hz, 1H, H-1''), 4.57 (d, *J* = 1.2 Hz, 1H, H-5'), 4.51 (d, *J* = 11.1 Hz, 1H, A of AB, $PhCH_2$), 4.48 (d, *J* = 2.4 Hz, 1H, H-4'), 4.42 – 4.33 (m, 3H, H-

5'', B of AB, $PhCH_2$), 4.25 – 4.19 (m, 2H, H-2'', H-3'), 4.16 (d, *J* = 1.9 Hz, 1H, H-4), 4.07 (dd, *J* = 10.4, 3.3 Hz, 1H, H-2'), 3.99 (dd, *J* = 3.9, 1.9 Hz, 1H, H-4''), 3.96 – 3.90 (m, 2H, H-2, H-3), 3.78 – 3.67 (m, 6H, A of AB, O-C*H*$_2$-CH_2, $COOCH_3$, $PhCH_2$), 3.59 – 3.52 (m, 1H, B of AB, O-C*H*$_2$-CH_2), 3.25 (s, 3H, $COOCH_3$), 2.75 – 2.47 (m, 4H, Lev-CH_2, CH_2-C*H*$_2$-S), 2.41 – 2.31 (m, 1H, A of AB, Lev-CH_2), 2.31 – 2.18 (m, 1H, B of AB, Lev-CH_2), 2.10 (s, 3H, Ac-CH_3), 1.94 (s, 3H, Lev-CH_3), 0.89 (d, *J* = 6.3 Hz, 3H, H-6''); ^{13}C NMR (100 MHz, CD_3OD) δ 209.2, 173.9, 173.7, 171.0, 160.0, 159.5, 140.2, 140.1, 139.8, 139.3, 139.1, 138.6, 130.3, 130.1, 129.6, 129.53, 129.49, 129.41, 129.38, 129.1, 129.0, 128.9, 128.81, 128.77, 128.4, 127.9, 100.3, 98.4, 96.8, 78.2, 77.4, 76.4, 76.3, 75.9, 75.5, 75.0, 73.8, 73.7, 71.9, 71.6, 71.1, 70.0, 67.5, 66.4, 54.1, 52.7, 52.4, 38.4, 37.5, 31.2, 29.7, 29.1, 23.1, 16.9; IR (thin film) 3444, 2927, 2112, 1763, 1724, 1660, 1497, 1455, 1349, 1263, 1111, 1028, 913, 743, 700 cm^{-1}; HRMS (ESI) calcd for $C_{72}H_{82}N_2O_{20}S$ $(M+Na)^+$ 1349.5079 found 1349.5029 *m/z*.

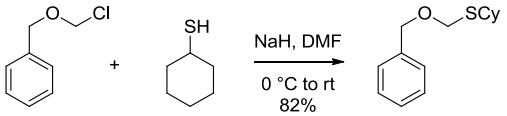
2-Azido-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-methyl (2,3-di-*O*-benzyl-α-D-galactopyranosyl)uronate-(1→3)-methyl (2,4-di-*O*-benzyl-α-D-galactopyranosyl)uronate-(1→1)-2-(benzylthio)ethanol (36)



To a stirred solution of Lev ester **34** (30 mg, 0.023 mmol) in CH_2Cl_2 (1.0 mL) was added at room temperature first a mixture of pyridine (56 µL, 0.692 mmol) and acetic acid (37 µL, 0.646 mmol), and then hydrazine hydrate (2 µL, 0.041 mmol). The mixture was stirred for 4 h at room temperature, diluted with EtOAc (2 mL), quenched with acetone (0.1 mL) and poured into water (15 mL). The aqueous phase was extracted with EtOAc (4x10 mL), the combined organic extracts were dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 1:2 to 2:3) to give alcohol **36** (28 mg, 0.023 mmol, quant.) as a clear oil. R_f (EtOAc/hexanes 1:1) = 0.34; $[\alpha]_D^{20}$ = +122.0° (c = 0.36, acetone); ^{1}H NMR (400 MHz, acetone-D_6) δ 7.52 – 7.08 (m, 30H, arom.), 6.14 (d, *J* = 10.1 Hz, 1H, NH), 5.48 (d, *J* = 2.8 Hz, 1H, H-1'), 5.15 (d, *J* = 11.2 Hz, 1H, A of AB, $PhCH_2$), 5.10 (d, *J* = 12.6 Hz, 1H, A of AB, $PhCH_2$), 5.05 – 4.99 (m, 2H, H-1, B of AB, $PhCH_2$), 4.91 (d, *J* = 11.7

Hz, 3H, $PhCH_2$), 4.82 – 4.73 (m, 3H, H-1'', H-5', B of AB, $PhCH_2$), 4.60 (d, *J* = 11.7 Hz, 1H, A of AB, $PhCH_2$), 4.54 (m, 2H, H-5, B of AB, $PhCH_2$), 4.49 (s, 1H, H-4), 4.44-4.35 (m, 4H, H-4', H-5'', $PhCH_2$), 4.29 (dd, *J* = 10.4, 2.8 Hz, 1H, H-3), 4.16 – 4.09 (m, 1H, H-3'), 4.08 – 4.02 (dd, *J* = 2.6, 5.8 Hz, 1H, H-2'), 3.96 (dd, *J* = 10.1, 2.7 Hz, 1H, H-4''), 3.91 (dd, *J* = 10.4, 3.5 Hz, 1H, H-2), 3.82 – 3.74 (m, 3H, $PhCH_2$, A of AB, O-C*H*$_2$-CH_2), 3.68 (s, 3H, $COOCH_3$), 3.56 (m, 1H, B of AB, O-C*H*$_2$-CH_2), 3.43 (s, 3H, $COOCH_3$), 3.38 (dd, *J* = 11.3, 3.9 Hz, 1H, H-2''), 2.59 (dd, *J* = 7.4, 5.3 Hz, 2H, CH_2-C*H*$_2$-S), 0.86 (d, *J* = 6.4 Hz, 3H, H-6''); ^{13}C NMR (100 MHz, acetone-D_6) δ 169.6, 169.4, 158.3, 140.4, 139.9, 139.8, 139.5, 139.3, 138.3, 130.0, 129.3, 129.2, 129.13, 129.12, 129.08, 128.8, 128.7, 128.6, 128.53, 128.45, 128.4, 128.3, 128.2, 127.8, 127.6, 100.2, 97.9, 96.3, 77.4, 77.1, 76.4, 75.8, 75.2, 74.5, 72.8, 71.5, 71.2, 69.5, 67.6, 66.70, 66.66, 61.2, 57.2, 52.1, 51.8, 37.0, 30.8, 16.9; IR (thin film) 3363, 3031, 2929, 2111, 1764, 1728, 1522, 1497, 1455, 1347, 1263, 1107, 1028, 915, 741, 699 cm^{-1}; HRMS (ESI) calcd. for $C_{65}H_{72}N_4O_{17}S$ $(M+Na)^+$ 1235.4511 found 1235.4539 *m/z*.

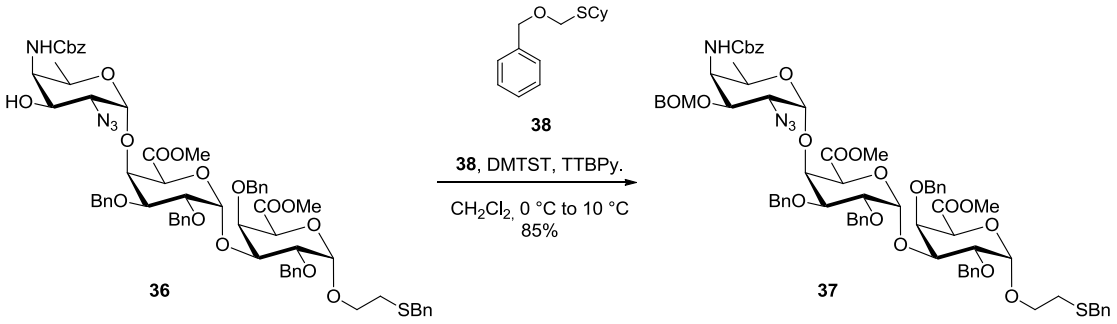
Benzyloxymethyl cyclohexyl sulfide (38)



38

To a stirred solution of cyclohexanethiol (1.46 mL, 11.9 mmol) in DMF (36 mL) was added at 0 °C sodium hydride (0.338 g, 14.1 mmol). The mixture was treated with benzyloxymethyl chloride (2.0 mL, 75% (w/w), 10.8 mmol) and warmed to room temperature. The reaction was stirred at that temperature for 16 h, quenched at 0 °C with 1 M aq. NaOH (20 mL) and diluted with water (100 mL) and hexanes (70 mL). After separation, the aqueous phase was extracted with hexanes (3x70 mL), the combined organic fractions were dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 1:50) to give *S*,*O*-acetal **38** (2.1 g, 8.9 mmol, 82%) as a slightly yellow oil. R_f (EtOAc/hexanes 1:10) = 0.80; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.27 (m, 5H, arom.), 4.77 (s, 2H, O-CH_2-S), 4.63 (s, 2H, O-CH_2Ph), 2.93 – 2.80 (m, 1H, S-CH), 2.07 – 1.98 (m, 2H, aliph.), 1.83 – 1.70 (m, 2H, aliph.), 1.61 (dd, *J* = 10.1, 4.0 Hz, 1H, aliph.), 1.47 – 1.20 (m, 5H, aliph.); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.8, 128.6, 128.3, 127.9, 71.8, 69.6, 43.3, 34.2, 26.3, 25.9; IR (thin film) 2928, 2852, 1497, 1449, 1310, 1265, 1061, 1028, 739, 697 cm^{-1}; HRMS (ESI) calcd for $C_{14}H_{20}OS$ $(M+Na)^+$ 259.1133 found 259.1132 *m/z*.

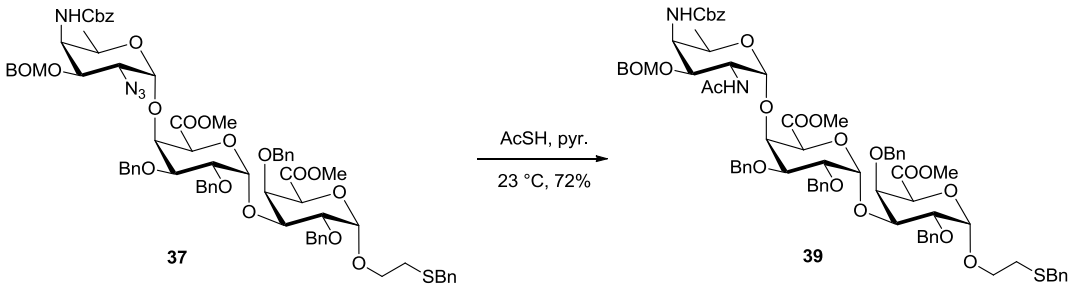
2-Azido-4-(benzyloxycarbonyl)amino-3-*O*-benzyloxymethyl-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-methyl (2,3-di-*O*-benzyl-α-D-galactopyranosyl)uronate-(1→3)-methyl (2,4-di-*O*-benzyl-α-D-galactopyranosyl)uronate-(1→1)-2-(benzylthio)ethanol (37)



Alcohol **36** (49 mg, 0.040 mmol), *S*,*O*-acetal **38** (180 mg, 0.81 mmol) and TTBPy (400 mg, 1.62 mmol) were co-evaporated with dry toluene (3x10 mL). The mixture was dissolved in dry CH_2Cl_2 (2.0 mL) and stirred over activated molecular sieves (3 Å) for 30 min at room temperature. The mixture was cooled to 0 °C and DMTST (24 mg, 0.60 mmol in 0.3 mL CH_2Cl_2) was added dropwise over a period of 1.5 h, while the reaction temperature was kept below 10 °C. The reaction was stirred for another 45 min, quenched by addition of a 10:1 (v/v) mixture of MeOH and Et_3N (0.5 mL) and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 1:10 to 1:2) to give acetal **37** (46 mg, 0.034 mmol, 85%) as a clear oil. R_f (EtOAc/hexanes 2:3) = 0.58; $[\alpha]_D^{20}$ = +127.0° (c = 0.27, acetone); ^{1}H NMR (400 MHz, acetone-D_6) δ 7.54 – 7.03 (m, 35H, arom.), 6.24 (d, *J* = 9.5 Hz, 1H, NH), 5.48 (d, *J* = 2.8 Hz, 1H, H-1'), 5.15 (d, *J* = 11.3 Hz, 1H, A of AB, $PhCH_2$), 5.08 (s, 2H, $PhCH_2$), 5.03 (d, *J* = 3.5 Hz, 1H, H-1), 4.98 (d, *J* = 7.2 Hz, 1H, A of AB, BnO-CH_2-O), 4.87 – 4.80 (m, 5H, H-1'', $PhCH_2$, H-5'), 4.78 – 4.71 (m, 3H, B of AB, BnO-CH_2-O, $PhCH_2$), 4.66 (d, *J* = 11.7 Hz, 1H, B of AB, $PhCH_2$), 4.61 (d, *J* = 11.7 Hz, 1H, A of AB, $PhCH_2$), 4.54 (m, 2H, H-5, B of AB, $PhCH_2$), 4.49 (m, 1H, H-4), 4.42 (s, 1H, H-4'), 4.40 – 4.34 (m, 2H, H-5'', B of AB, $PhCH_2$), 4.30 (dd, *J* = 10.4, 2.8 Hz, 1H, H-3), 4.15 (d, *J* = 9.4 Hz, 2H, H-3'', H-4''), 4.05 (m, 2H, H-2',H-3'), 3.91 (dd, *J* = 10.4, 3.5 Hz, 1H, H-2), 3.84 – 3.73 (m, 3H, $PhCH_2$, A of AB, O-C*H*$_2$-CH_2), 3.68 (s, 3H, $COOCH_3$), 3.60 – 3.54 (m, 2H, H-2'', B of AB, O-C*H*$_2$-CH_2), 3.44 (s, 3H, $COOCH_3$), 2.59 (dd, *J* = 7.5, 5.2 Hz, 2H, CH_2-C*H*$_2$-S), 0.88 (d, *J* = 6.3 Hz, 3H, H-6''); ^{13}C NMR (100 MHz, acetone-D_6) δ 169.6, 169.4, 158.0, 140.4, 139.9, 139.8, 139.5, 139.20, 139.16, 138.4, 130.0, 129.4, 129.2, 129.14, 129.11, 129.0, 128.8, 128.7, 128.6, 128.5, 128.38, 128.36, 128.3, 128.2, 127.8, 127.6, 100.0, 98.0, 96.3, 93.1, 77.53, 77.48, 77.0, 76.3, 75.9, 75.8, 75.3, 74.5, 73.0, 72.9, 72.1, 71.5, 71.2, 70.4, 69.5, 66.7, 66.4, 60.2, 53.7, 52.1, 51.9, 37.0, 30.9, 16.9; IR (thin film) 2925, 2110, 1765, 1727, 1497,

1455, 1345, 1238, 1106, 1039, 915, 739, 698 cm^{-1}; HRMS (ESI) calcd for $C_{73}H_{80}N_4O_{18}S$ $(M+Na)^+$ 1355.5086 found 1355.5071 *m/z*.

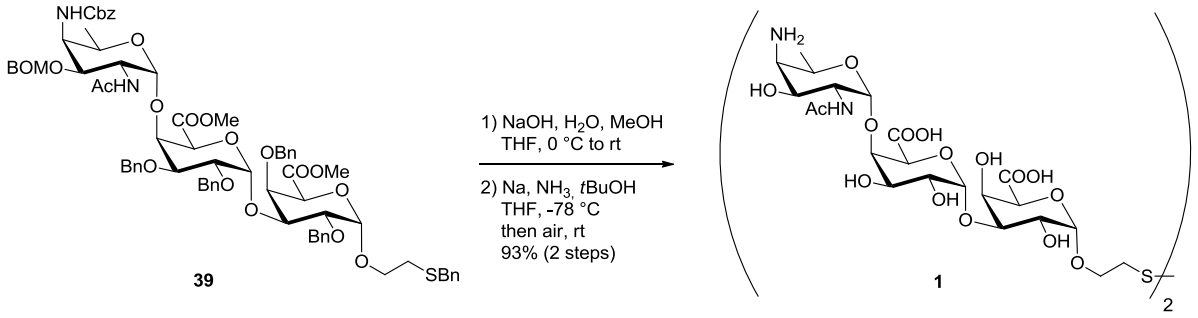
2-Acetamido-4-(benzyloxycarbonyl)amino-3-*O*-benzyloxymethyl-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-methyl (2,3-di-*O*-benzyl-α-D-galactopyranosyl)uronate-(1→3)-methyl (2,4-di-*O*-benzyl-α-D-galactopyranosyl)uronate-(1→1)-2-(benzylthio)ethanol (39)



To a stirred solution of azide **37** (29 mg, 0.022 mmol) in dry pyridine (0.35 mL) was added at 0 °C thioacetic acid (0.35 mL). The mixture was warmed to room temperature and stirred for 24 h at that temperature. The solution was co-evaporated with toluene (2x5 mL) and the residue was purified by flash chromatography (EtOAc/hexanes 1:10 to acetone/hexanes 1:7 to 1:5 to 1:3) to give acetamide **39** (21 mg, 0.016 mmol, 72%) as a white foam. R_f (acetone/hexanes 2:3) = 0.25; $[\alpha]_D^{20}$ = +104.7° (c = 0.36, acetone); ^{1}H NMR (600 MHz, acetone-D_6) δ 7.48 (d, *J* = 7.0 Hz, 2H, arom.), 7.42 – 7.18 (m, 31H, arom.), 7.16 – 7.08 (m, 2H, arom.), 6.49 (d, *J* = 10.3 Hz, 1H, NH), 5.93 (d, *J* = 9.5 Hz, 1H, NH), 5.52 (d, *J* = 2.9 Hz, 1H, H-1'), 5.15 (d, *J* = 11.2 Hz, 1H, A of AB, $PhCH_2$), 5.09 (s, 2H, $PhCH_2$), 5.05 (d, *J* = 3.5 Hz, 1H, H-1), 4.92 – 4.80 (m, 4H, $PhCH_2$, A of AB, BnO-CH_2-O), 4.77 (d, *J* = 12.8 Hz, 2H, $PhCH_2$, H-5'), 4.71 – 4.56 (m, 5H, H-1'', $PhCH_2$, B of AB, Bn-O-CH_2-O), 4.51 (d, *J* = 11.4 Hz, 2H, H-5, B of AB, $PhCH_2$), 4.48 (m, 1H, H-4), 4.43 – 4.33 (m, 2H, H-4', H-5'', B of AB, $PhCH_2$), 4.29 (dd, *J* = 10.4, 2.8 Hz, 1H, H-3), 4.24 – 4.15 (m, 1H, H-2''), 4.14 – 4.01 (m, 3H, H-2', H-3', H-4''), 3.91 (dd, *J* = 10.3, 3.5 Hz, 1H, H-2), 3.86 (dd, *J* = 11.4, 4.2 Hz, 1H, H-3''), 3.83 – 3.72 (m, 3H, $PhCH_2$, A of AB, O-C*H*$_2$-CH_2), 3.69 (s, 3H, $COOCH_3$), 3.62 – 3.54 (m, 1H, B of AB, O-C*H*$_2$-CH_2), 3.33 (s, 3H, $COOCH_3$), 2.60 (dd, *J* = 8.1, 4.7 Hz, 2H, CH_2-C*H*$_2$-S), 1.81 (s, 3H, Ac-CH_3), 0.92 (d, *J* = 6.3 Hz, 3H, H-6''); ^{13}C NMR (100 MHz, acetone-D_6) δ 170.0, 169.6, 169.2, 158.1, 140.4, 140.0, 139.8, 139.6, 139.4, 139.2, 130.0, 129.3, 129.18, 129.15, 128.9, 128.7, 128.6, 128.5, 128.4, 128.32, 128.28, 127.9, 127.6, 99.9, 97.9, 96.7, 93.6, 77.6, 77.1, 76.0, 75.8, 75.6, 74.4, 73.4, 73.1, 72.9, 71.6, 71.1, 69.8, 69.49, 66.47, 54.0, 52.1, 51.9, 49.2, 37.0, 30.9, 23.5, 17.2; IR (thin film) 3030, 2933, 1764,

1718, 1670, 1520, 1455, 1368, 1248, 1107, 1043, 916, 740, 699 cm^{-1}; HRMS (ESI) calcd for $C_{75}H_{84}N_2O_{19}S$ $(M+Na)^+$ 1371.5281 found 1371.5314 *m/z*.

2,2'-Dithiobis[2-acetamido-4-amino-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-α-D-galactopyranosyluronate-(1→3)-α-D-galactopyranosyluronate-(1→1)-1-ethanol] (1)

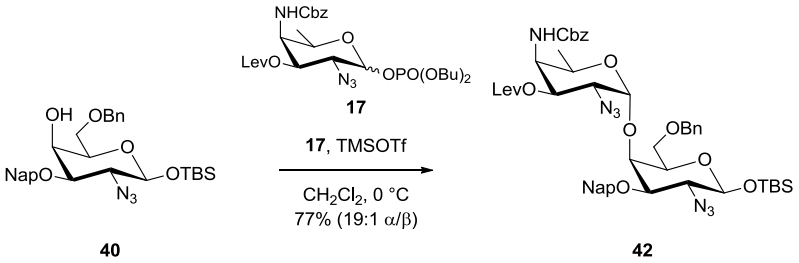


To a stirred solution of ester **39** (18 mg, 13.3 µmol) in THF (2.0 mL) and MeOH (0.75 mL) was added at 0 °C a 1 M aq. solution of NaOH (0.8 mL, 0.800 mmol). The reaction was slowly warmed to room temperature and stirred for 16 h. The reaction was diluted with water (5 mL), acidified to pH 4 with 0.5 M aq. $NaHSO_4$, and poured into EtOAc (5 mL). After separation, the aqueous fraction was extracted with EtOAc (8x10 mL), the combined organic fractions were dried over Na_2SO_4 and concentrated to give the intermediate diacid as a white foam.

To a stirred solution of liquid ammonia (10 mL) was added at -78 °C a solution of the crude diacid in THF (2.0 mL). The mixture was treated with *t*BuOH (0.8 mL) and lumps of freshly cut sodium (90 mg) were added until a deeply blue color persisted. The reaction was stirred at -78 °C for 45 min and quenched by addition of solid NH_4OAc (300 mg). The solution was warmed to room temperature under a stream of argon and co-evaporated with MeOH (2x10 mL) and water (2x5 mL). The residue was left exposed to air for 16 h, purified by size exclusion chromatography (Sephadex G-25, MeOH/5 mM aq. NH_4OAc 1:10) and lyophilized repeatedly to give disulfide **1** (7.6 mg, 6.2 µmol, 93% over two steps) as a white solid. $[\alpha]_D^{20}$ = +80.3° (c = 0.10, H_2O); ^1H NMR (600 MHz, D_2O) δ 5.32 (d, *J* = 3.8 Hz, 1H, H-1'), 5.12 (d, *J* = 3.8 Hz, 1H, H-1), 5.05 (d, *J* = 3.8 Hz, 1H, H-1''), 4.83 (d, *J* = 5.3 Hz, 1H, H-5''), 4.66 (s, 1H, H-5'), 4.59 (s, 1H, H-4), 4.48 (s, 1H, H-4'), 4.45 (d, *J* = 2.5 Hz, 1H, H-5), 4.26 (d, *J* = 11.1 Hz, 1H, H-3''), 4.21 (dd, *J* = 10.6, 3.0 Hz, 1H, H-3'), 4.11 (m, 3H, H-2'', H-3, A of AB, O-CH_2-CH_2), 4.03 – 3.96 (m, 2H, H-2, H-2'), 3.96 – 3.89 (m, 1H, B of AB, O-CH_2-CH_2), 3.67 (s, 1H, H-4''), 3.11 – 3.01 (m,

2H, CH_2-C*H*$_2$-S-S), 2.20 (s, 3H, Ac-CH_3), 1.35 (d, *J* = 6.6 Hz, 3H, H-6'');
HRMS (MALDI) calcd for $C_{44}H_{70}N_4O_{32}S_2$ $(M-H)^-$ 1229.3330 found 1229.3342 *m*/*z*.

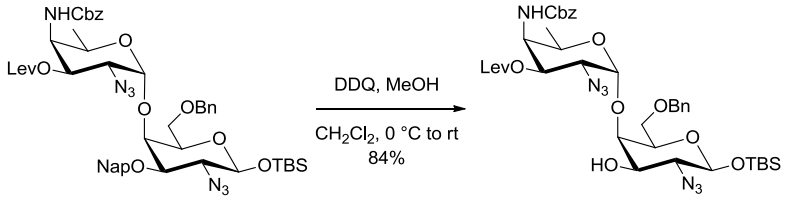
tert-Butyldimethylsilyl 2-azido-4-(benzyloxycarbonyl)amino-3-_O_-levulinoyl-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-2-azido-6-_O_-benzyl-2-deoxy-3-_O_-naphthyl-β-D-galactopyranoside (42)



Alcohol **40**[7] (40 mg, 0.073 mmol) and glycosyl phosphate **17** (67 mg, 0.109 mmol) were co-evaporated with dry toluene (3x5 mL) and kept under high vacuum for 48 h. The mixture was dissolved in dry CH_2Cl_2 (3.7 mL) and stirred over activated molecular sieves (3 Å-AW) for 30 min at room temperature. The solution was cooled to 0 °C and treated dropwise with TBSOTf (25 µL, 0.109 mmol). The reaction was stirred for 1.5 h at that temperature and quenched by addition of a 1:1 (v/v) mixture of MeOH and Et_3N. The mixture was diluted with CH_2Cl_2 (20 mL), filtered through Celite and concentrated. The crude product was purified by flash chromatography (EtOAc/hexanes 1:4 to 1:2) to give disaccharide **42** (53 mg, 0.056 mmol, 77%) as a clear oil. R_f (EtOAc/hexanes 1:2) = 0.63; $[\alpha]_D^{20}$ = +66.1° (c = 5.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 7.6, 2.9 Hz, 4H, arom.), 7.63 – 7.46 (m, 3H, arom.), 7.43 – 7.28 (m, 10H, arom.), 5.30 (dd, *J* = 11.3, 3.8 Hz, 1H, H-3c), 5.14 (d, *J* = 12.2 Hz, 1H, A of AB, $PhCH_2$), 5.03 (d, *J* = 12.3 Hz, 1H, B of AB, $PhCH_2$), 4.96 – 4.89 (m, 3H, H-1c, A of AB, CH_2Ph, $NapCH_2$), 4.84 (d, *J* = 12.5 Hz, 1H, B of AB, $NapCH_2$), 4.73 – 4.64 (m, 1H, H-5c), 4.51 (d, *J* = 1.4 Hz, 2H, $PhCH_2$), 4.42 (d, *J* = 7.5 Hz, 1H, H-1b), 4.17 (dd, *J* = 9.5, 2.1 Hz, 1H, H-4c), 4.11 (d, *J* = 2.9 Hz, 1H, H-4b), 3.92 (t, *J* = 9.1 Hz, 1H, H-6b), 3.67 (dd, *J* = 10.7, 7.5 Hz, 1H, H-2b), 3.55 (dd, *J* = 9.1, 5.6 Hz, 1H, H-6b), 3.44 (dd, *J* = 8.9, 5.7 Hz, 1H, H-5b), 3.31 (dd, *J* = 11.3, 3.9 Hz, 1H, H-2c), 3.18 (dd, *J* = 10.7, 3.0 Hz, 1H, H-3b), 2.91 – 2.56 (m, 3H, Lev-CH_2), 2.46 (m, 1H, Lev-CH_2), 2.18 (d, *J* = 4.5 Hz, 3H, Lev-CH_3), 0.92 (s, 9H, TBS), 0.84 (d, *J* = 6.4 Hz, 3H, H-6c), 0.12 (d, *J* = 0.4 Hz, 6H, TBS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.6, 172.2, 156.7, 137.6, 136.5, 135.1, 133.3, 133.2, 128.7, 128.6, 128.42, 128.38, 128.11, 128.09, 128.07, 128.0, 127.8, 126.6, 126.3, 126.1, 125.7, 98.9, 97.8, 78.1, 77.4, 73.6, 72.9, 72.8, 70.2, 67.1, 65.5, 64.9, 58.0, 52.9, 38.1,

29.9, 28.1, 25.8, 25.7, 18.1, 16.4, -4.1, -5.0; IR (thin film) 3346, 2931, 2858, 2109, 1718, 1603, 1510, 1408, 1349, 1253, 1146, 1076, 1040, 895, 839, 784, 752, 698 cm^{-1}; HRMS (ESI) calcd for $C_{49}H_{61}N_7O_{11}Si$ $(M+Na)^+$ 974.4096 found 974.4051 *m*/*z*.

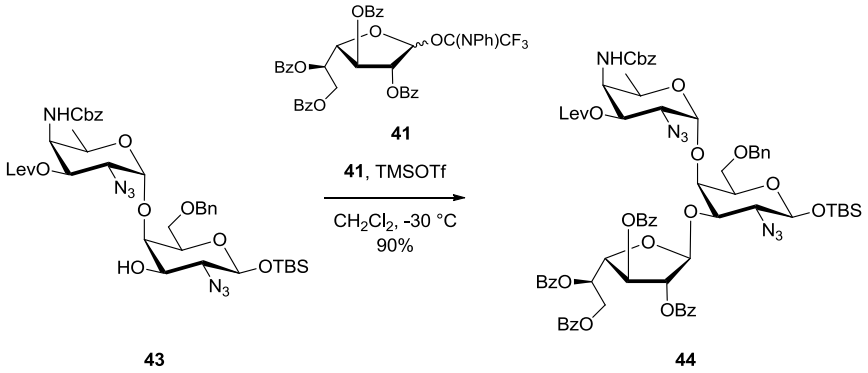
tert-Butyldimethylsilyl 2-azido-4-(benzyloxycarbonyl)amino-3-_O_-levulinoyl-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-2-azido-6-_O_-benzyl-2-deoxy-β-D-galactopyranoside (43)



42 **43**

To a stirred solution of naphthyl ether **42** (270 mg, 0.28 mmol) in a CH_2Cl_2 (5.1 ml) and MeOH (0.6 mL) was added at 0 °C DDQ (193 mg, 0.85 mmol). The mixture was slowly warmed to room temperature and stirred for 12 h at that temperature. The reaction was diluted with Et_2O (20 mL), quenched by addition of a 1:1 (v/v) mixture of sat. aq. $NaHCO_3$ and 10% aq. $Na_2S_2O_3$ (10 mL) and stirred vigorously for 15 min. After separation, the aqueous layer was extracted with Et_2O (3x20 mL). The combined organic fractions were dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 1:8 to 1:1) to give alcohol **43** (230 mg, 0.24 mmol, 84%) as a clear oil. R_f (EtOAc/hexanes 1:2) = 0.31; $[\alpha]_D^{20}$ = +66.4° (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.28 (m, 10H, arom.), 5.24 (dd, *J* = 11.2, 3.7 Hz, 1H, H-3c), 5.16 (d, *J* = 12.2 Hz, 1H, A of AB, $PhCH_2$), 5.06 (d, *J* = 12.3 Hz, 1H, B of AB, $PhCH_2$), 5.00 (d, *J* = 9.4 Hz, 1H, NH), 4.90 (d, *J* = 4.0 Hz, 1H, H-1c), 4.65 (dd, *J* = 13.0, 6.5 Hz, 1H, H-5c), 4.53 (s, 2H, $PhCH_2$), 4.48 (d, *J* = 7.1 Hz, 1H, H-1b), 4.26 (dd, *J* = 9.5, 2.2 Hz, 1H, H-4c), 3.97 (d, *J* = 1.7 Hz, 1H, H-4b), 3.92 (t, *J* = 10.4 Hz, 1H, A of AB, H-6b), 3.59 (m, 2H, H-5b, B of AB, H-6b), 3.51 (dd, *J* = 11.2, 4.0 Hz, 1H, H-2c), 3.42 – 3.33 (m, 2H, H-2b, H-3b), 3.00 – 2.53 (m, 4H, Lev-CH_2, OH), 2.51 – 2.39 (m, 1H, Lev-CH_2), 2.18 (s, 3H, Lev-CH_3), 1.16 (d, *J* = 6.5 Hz, 3H, H-6c), 0.93 (s, 9H, TBS), 0.14 (s, 6H, TBS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.5, 172.2, 156.7, 137.9, 136.4, 129.72, 128.71, 128.6, 128.4, 128.1, 127.9, 127.8, 99.3, 97.7, 77.3, 73.5, 73.3, 71.7, 70.8, 67.6, 67.2, 67.0, 65.6, 58.3, 52.7, 38.0, 29.9, 28.1, 25.8, 18.1, 16.4, -4.1, -5.0; IR (thin film) 3355, 2930, 2858, 2111, 1719, 1719, 1524, 1456, 1363, 1253, 1179, 1253, 1145, 1116, 1076, 840, 784, 751, 699, 676 cm^{-1}; HRMS (ESI) calcd for $C_{38}H_{53}N_7O_{11}Si$ $(M+Na)^+$ 834.3470 found 834.3439 *m*/*z*.

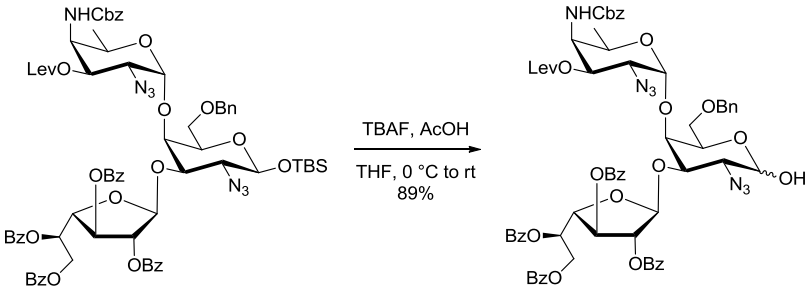
***tert*-Butyldimethylsilyl 2-Azido-4-(benzyloxycarbonyl)amino-3-*O*-levulinoyl-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-[2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)]-2-azido-6-*O*-benzyl-2-deoxy-β-D-galactopyranoside (44)**



Alcohol **43** (181 mg, 0.22 mmol) and imidate **41**[7] (250 mg, 0.33 mmol) were co-evaporated with dry toluene (3x20 mL) and kept under high vacuum for 16 h. The mixture was dissolved in dry CH_2Cl_2 (11 mL) and stirred over activated molecular sieves (3 Å-AW) for 1 h at room temperature. The solution was cooled to -30 °C and treated dropwise with TMSOTf (10 µL, 0.055 mmol in 0.2 mL dry CH_2Cl_2). The reaction was stirred for 1.5 h at that temperature, quenched with a 1:1 (v/v) mixture of EtOH and Et_3N (0.5 mL) and diluted with CH_2Cl_2 (20 mL). The mixture was filtered through Celite and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 1:3 to 2:3) to give trisaccharide **44** (280 mg, 0.20 mmol, 90%) as a clear oil; R_f (EtOAc/hexanes 2:3) = 0.59; $[\alpha]_D^{20}$ = +46.5° (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 7.2 Hz, 2H, arom.), 8.00 (dd, *J* = 13.7, 7.2 Hz, 4H, arom.), 7.89 – 7.82 (m, 2H, arom.), 7.58 – 7.45 (m, 5H, arom.), 7.42 – 7.27 (m, 17H, arom.), 6.02 (dt, *J* = 6.4, 4.5 Hz, 1H, H-5d), 5.63 (m, 3H, H-1d, H-2d, H-3d), 5.29 – 5.13 (m, 2H, H-3c, A of AB, $PhCH_2$), 5.06 (d, *J* = 12.3 Hz, 1H, B of AB, $PhCH_2$), 4.94 (d, *J* = 3.6 Hz, 1H, H-1c), 4.87 – 4.69 (m, 4H, H-4d, NH, H-6d), 4.63 – 4.44 (m, 4H, H-5c, $PhCH_2$, H-1b), 4.18 (d, *J* = 7.1 Hz, 1H, H-4c), 4.03 (d, *J* = 2.1 Hz, 1H, H-4b), 3.85 (dd, *J* = 10.4, 10.4 Hz, 1H, H-6b), 3.71 – 3.51 (m, 4H, H-2b, H-3b, H-5b, H-6b), 3.10 (dd, *J* = 11.2, 3.6 Hz, 1H, H-2c), 2.92 – 2.52 (m, 3H, Lev-CH_2), 2.50 – 2.37 (m, 1H, Lev-CH_2), 2.18 (s, 3H, Lev-CH_3), 1.18 (d, *J* = 6.4 Hz, 3H, H-6c), 0.91 (s, 9H, TBS), 0.13 (s, 6H, TBS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.6, 172.2, 166.3, 165.9, 165.8, 156.7, 137.9, 136.6, 133.8, 133.5, 133.3, 130.1, 130.0, 129.94, 129.89, 129.7, 129.5, 129.1, 129.0, 128.8, 128.7, 128.62, 128.58, 128.5, 128.4, 128.2, 128.0, 127.9, 106.3, 98.6, 98.0, 82.1, 81.7, 78.2, 77.4, 76.2, 75.0, 73.6, 70.7, 70.6, 68.1, 67.2, 65.3, 65.1, 63.5, 58.6, 52.9, 38.1, 30.0, 29.9, 28.1, 25.8, 18.1, 16.7, -4.0, -5.0; IR (thin film) 3419, 2930, 2858, 2113, 1725, 1602, 1585,

1507, 1492, 1316, 1264, 1178, 1109, 1096, 1070, 1027, 840, 785, 711 cm^{-1}; HRMS (ESI) calcd for $C_{72}H_{79}N_7O_{20}Si$ $(M+Na)^+$ 1412.5047 found 1412.5050 *m*/*z*.

2-Azido-4-(benzyloxycarbonyl)amino-3-*O*-levulinoyl-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-[2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)]-2-azido-6-*O*-benzyl-2-deoxy-D-galactopyranose (45)

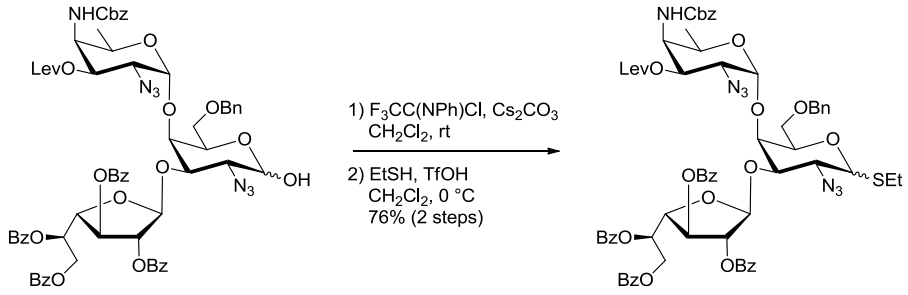


44 **45**

To a stirred solution of silyl ether **44** (280 mg, 0.20 mmol) in THF (10 mL) were added at 0 °C acetic acid (115 µL, 2.014 mmol) and tetrabutylammonium fluoride (1 M in THF, 2.0 mL, 2.0 mmol). The reaction was slowly warmed to room temperature and stirred for 2 h at that temperature. The mixture was diluted with Et_2O (50 mL) and washed with water (3x30 mL). The combined aqueous fractions were extracted with Et_2O (2x20 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated. The residue was filtered through a short plug of silica gel (EtOAc/hexanes 1:2 to 1:1) to give lactol **45** (229 mg, 0.18 mmol, 89%, 3:2 α/β) as a clear oil. R_f (EtOAc/hexanes 2:3) = 0.20; $[\alpha]_D^{20}$ = +69.2° (c = 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (dd, *J* = 11.0, 4.0 Hz, 2H), 8.03 – 7.93 (m, 5H), 7.90 – 7.84 (m, 2H), 7.55 – 7.42 (m, 5H), 7.42 – 7.27 (m, 22H), 6.08 – 5.83 (m, 1.5H), 5.74 – 5.57 (m, 3H), 5.37 (t, *J* = 3.0 Hz, 0.6H), 5.26 – 5.02 (m, 4H), 4.98 – 4.65 (m, 6H), 4.65 – 4.43 (m, 4.4H), 4.26 (dd, *J* = 12.2, 4.9 Hz, 1.4H), 4.20 – 4.10 (m, 2H), 4.04 (d, *J* = 2.2 Hz, 0.4H), 4.00 (d, *J* = 7.1 Hz, 0.4H), 3.84 – 3.57 (m, 5H), 3.22 (dd, *J* = 11.2, 3.7 Hz, 0.6H), 3.13 (dd, *J* = 11.2, 3.7 Hz, 0.4H), 2.89 – 2.74 (m, 1H), 2.72 – 2.51 (m, 2H), 2.49 – 2.36 (m, 1H), 2.21 – 2.13 (m, 3H), 1.18 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.7, 172.1, 166.3, 166.2, 165.9, 165.79, 165.78, 165.6, 165.0, 156.7, 138.0, 137.7, 137.6, 136.5, 133.83, 133.76, 133.6, 133.52, 133.49, 133.4, 130.1, 130.04, 129.98, 129.92, 129.90, 129.85, 129.8, 129.59, 129.57, 129.38, 129.35, 129.1, 128.99, 128.95, 128.9, 128.8, 128.72, 128.68, 128.6, 128.5, 128.4, 128.33, 128.30, 128.25, 128.14, 128.07, 128.0, 125.4, 107.0, 106.3, 98.8, 98.4, 97.0, 92.1, 82.09, 82.08, 81.99, 81.95, 81.9, 81.8, 81.4, 78.1, 77.7, 77.4, 76.6, 75.2, 74.6, 73.62, 73.55, 70.6, 70.5, 69.8, 67.2, 65.3,

63.3, 60.5, 58.9, 52.8, 38.0, 29.9, 28.1, 21.6, 16.7; IR (thin film) 3426, 2935, 2111, 1722, 1602, 1505, 1452, 1316, 1264, 1778, 1109, 1070, 1027, 711 cm^{-1}; HRMS (ESI) calcd for $C_{66}H_{65}N_7O_{20}$ $(M+Na)^+$ 1298.4182 found 1298.4198 *m*/*z*.

Ethyl 2-azido-4-(benzyloxycarbonyl)amino-3-*O*-levulinoyl-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-[2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)]-2-azido-6-*O*-benzyl-2-deoxy-1-thio-D-galactopyranoside (46)



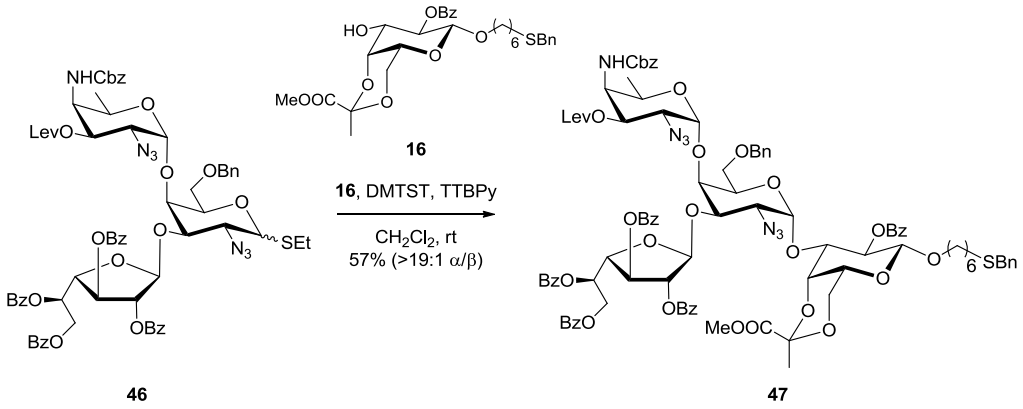
45 **46**

To a stirred solution of alcohol **45** (115 mg, 0.090 mmol) in CH_2Cl_2 (4.5 mL) were added at room temperature 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (47 mg, 0.225 mmol) and cesium carbonate (73 mg, 0.225 mmol). The reaction was stirred for 1.5 h, diluted with hexanes/0.5% Et_3N (20 mL) and filtered through basic Celite. The mixture was concentrated and the residue was filtered through a short plug of silica gel (EtOAc/hexanes/Et_3N 1:10:0.05 to 1:2:0.15) to give the crude imidate (125 mg) as a clear oil.

To a stirred solution of the crude imidate (125 mg) in CH_2Cl_2 (4.3 mL) over activated molecular sieves (3 Å-AW) was added ethanethiol (7 µL, 0.095 mmol) and the mixture was stirred for 30 min at room temperature. The solution was cooled to 0 °C and treated with trifluoromethanesulfonic acid (1.5 µL, 0.017 mmol). The reaction was stirred for 1.5 h at that temperature, quenched with Et_3N (0.05 mL) and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 1:8 to 1:5) to give thioglycoside **46** (90 mg, 0.068 mmol, 76% over two steps, 1:1 α/β) as a clear oil. R_f (EtOAc/hexanes 1:2) = 0.44-0.59; $[\alpha]_D^{20}$ = +74.3° (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, acetone-D_6) δ 8.18 – 8.03 (m, 4H), 8.01 – 7.96 (m, 2H), 7.95 – 7.89 (m, 2H), 7.65 – 7.56 (m, 4H), 7.52 – 7.23 (m, 18H), 6.24 – 6.16 (m, 1H), 6.09 – 6.01 (m, 1H), 5.91 (d, *J* = 1.5 Hz, 0.5H, H-1d), 5.85 (d, *J* = 1.3 Hz, 0.5H, H-1d), 5.83 – 5.73 (m, 2H), 5.65 (d, *J* = 5.5 Hz, 1H), 5.32 – 5.24 (m, 1H), 5.22 – 5.17 (m, 1H, H-1c), 5.16 – 5.09 (m, 2H), 5.05 (d, *J* = 12.5 Hz, 0.5H), 4.89 – 4.69 (m, 3H), 4.60 – 4.48 (m, 3H), 4.43 – 4.31 (m, 2H), 4.23 (ddd, *J* = 10.1, 4.1, 2.1 Hz, 1H), 4.16 (dd, *J* = 10.7, 2.5 Hz, 0.5H), 4.05 (dd, *J* =

10.2, 2.6 Hz, 0.5H), 3.98 – 3.83 (m, 3H), 3.74 – 3.64 (m, 1H), 2.81 – 2.47 (m, 5H), 2.44 – 2.29 (m, 1H), 2.13 (s, 3H), 1.40 – 1.18 (m, 6H); ^{13}C NMR (100 MHz, acetone-D_6) δ 206.1, 172.40, 172.35, 166.5, 166.31, 166.29, 166.2, 166.00, 165.96, 157.8, 139.3, 139.2, 138.3, 134.48, 134.45, 134.35, 134.32, 134.29, 134.13, 134.10, 130.7, 130.58, 130.56, 130.54, 130.49, 130.47, 130.3, 130.18, 130.16, 130.1, 129.54, 129.52, 129.47, 129.43, 129.41, 129.32, 129.29, 129.14, 129.12, 128.8, 128.72, 128.65, 128.5, 128.42, 128.36, 108.1 (C-1d), 107.8 (C-1d), 99.74 (C-1c), 99.65 (C-1c), 85.1, 83.2, 82.9, 82.7, 82.2, 82.0, 80.4, 78.9, 78.8, 77.9, 77.4, 77.3, 76.4, 73.7, 71.7, 71.6, 71.3, 70.9, 69.1, 68.7, 66.9, 66.1, 64.3, 63.9, 61.1, 59.6, 53.9, 38.2, 28.8, 28.7, 24.41, 24.37, 17.24, 17.19, 15.7, 15.1; IR (thin film) 3365, 2927, 2111, 1724, 1585, 1504, 1452, 1316, 1264, 1178, 1109, 1097, 1027, 975, 740, 711 cm^{-1}; HRMS (ESI) calcd for $C_{68}H_{69}N_7O_{19}S$ $(M+Na)^+$ 1342.4267 found 1342.4253 *m*/*z*.

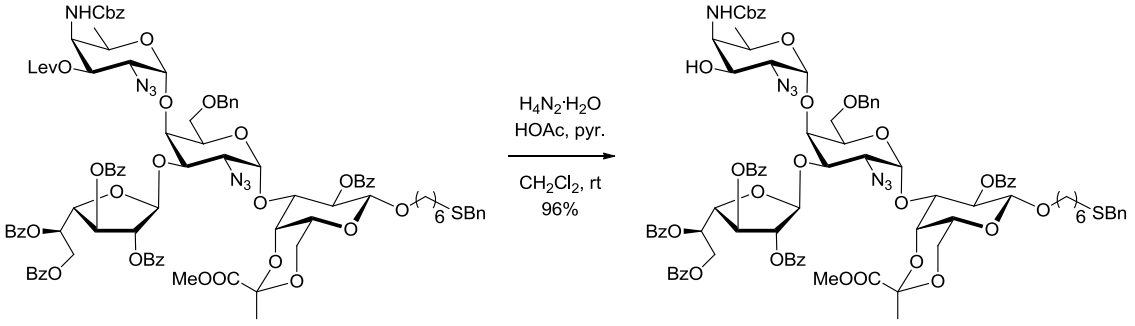
2-Azido-4-(benzyloxycarbonyl)amino-3-*O*-levulinoyl-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-[2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)]-2-azido-6-*O*-benzyl-2-deoxy-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-[1-(*R*)-(methoxycarbonyl)-ethylidene]-β-D-galactopyranosyl-(1→1)-6-(benzylthio)-1-hexanol (47)



Thioglycoside **46** (88 mg, 0.067 mmol) and alcohol **16** (77 mg, 0.133 mmol) were glycosylated using DMTST (34 mg, 0.167 mmol) and TTBPy (116 mg, 0.467 mmol) in CH_2Cl_2 (3.3 mL) at room temperature for 14 h. 0.5 equiv. of DMTST were added and stirring was continued for 6 h. The reaction was quenched with a 1:1 (v/v) mixture of sat. aq. $NaHCO_3$ and 10% aq. $Na_2S_2O_3$ (10 mL), and diluted with CH_2Cl_2 (10 mL). After separation, the aqueous layer was extracted with CH_2Cl_2 (5x10 mL), the combined organic fractions were dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (EtOAc/toluene 0:1 to 1:4 to 1:3) to obtain a mixture of tetrasaccharide **47** and excess acceptor **16**. The residue was subjected to size exclusion chromatography (Sephadex LH-20, MeOH/CH_2Cl_2 1:1) to give

tetrasaccharide **47** (70 mg, 57%) as a white foam. R_f (EtOAc/toluene 1:2) = 0.46; $[\alpha]_D^{20}$ = +40.0° (c = 0.74, acetone); ^{1}H NMR (600 MHz, acetone-D_6) δ 8.13 (d, *J* = 7.9 Hz, 2H, arom.), 8.09 (d, *J* = 7.1 Hz, 2H, arom.), 8.05 (d, *J* = 7.7 Hz, 2H, arom.), 7.98 (d, *J* = 7.3 Hz, 2H, arom.), 7.86 (d, *J* = 7.7 Hz, 2H, arom.), 7.62 (m, 5H, arom.), 7.53 – 7.25 (m, 25H, arom.), 6.19 (d, *J* = 10.0 Hz, 1H, NH), 6.00 (dd, *J* = 4.1, 3.2 Hz, 1H, H-5d), 5.76 (s, 1H, H-1d), 5.74 (s, 1H, H-2d), 5.71 – 5.68 (m, 1H, H-3d), 5.53 (dd, *J* = 5.2 Hz, 6.4 Hz, 1H, H-2a), 5.45 (s, 1H, H-1b), 5.25 (dd, *J* = 11.4, 3.9 Hz, 1H, H-3c), 5.15 (d, *J* = 12.5 Hz, 1H, A of AB, $PhCH_2$), 5.06 – 5.02 (m, 2H, H-1c, B of AB, $PhCH_2$), 4.95 (s, 1H, H-4d), 4.80 – 4.74 (m, 1H, A of AB, H-6d), 4.72 (m, 1H, H-5c), 4.63 (m, 2H, H-1a, B of AB, H-6d), 4.50 (d, *J* = 3.6 Hz, 1H, H-4a), 4.42 (dd, *J* = 8.4 Hz, 2H, $PhCH_2$), 4.24 – 4.17 (m, 2H, H-3a, H-4c), 4.14 (d, *J* = 10.8 Hz, 1H, H-3b), 4.10 (s, 3H, H-4b, H-5b, A of AB, H-6a), 3.99 (d, *J* = 12.3 Hz, 1H, B of AB, H-6a), 3.88 – 3.80 (m, 2H, A of AB, O-C*H*$_2$-CH_2, H-2b), 3.80 – 3.73 (m, 5H, $COOCH_3$, A of AB, H-6b, H-2c), 3.63 (m, 3H, S-C*H*$_2$-Ph, B of AB, H-6b), 3.58 (s, 1H, H-5a), 3.46 (dt, *J* = 10.0, 6.4 Hz, 1H, B of AB, O-C*H*$_2$-CH_2), 2.74 (m, 1H, Lev-CH_2), 2.66 (m, 1H, Lev-CH_2), 2.50 (dt, *J* = 17.0, 7.4 Hz, 1H, Lev-CH_2), 2.43 – 2.33 (m, 1H, Lev-CH_2), 2.22 (t, *J* = 7.3 Hz, 2H, CH_2-C*H*$_2$-S), 2.11 (s, 3H, Lev-CH_3), 1.51 (s, 3H, pyruv.-CH_3), 1.47 – 1.37 (m, 3H, aliph.), 1.25 (m, 5H, aliph., H-6c), 1.19 – 1.11 (m, 3H, aliph.); ^{13}C NMR (150 MHz, acetone-D_6) δ 206.1, 172.4, 171.1, 166.5, 166.3, 166.2, 165.8, 165.7, 157.8, 140.0, 138.3, 134.4, 134.33, 134.25, 134.1, 134.00, 130.8, 130.7, 130.6, 130.5, 130.4, 130.3, 130.2, 130.1, 129.7, 129.6, 129.5, 129.42, 129.37, 129.3, 129.2, 129.1, 128.74, 128.69, 128.4, 128.3, 127.5, 108.5 (C-1d), 101.8 (C-1a), 99.6, 99.5 (C-1c), 94.4 (C-1b), 82.6, 82.0, 78.6, 75.9, 73.73, 73.71, 73.6, 71.7, 71.3, 70.9, 69.9, 67.4, 66.9, 66.5, 66.0, 64.3, 60.0, 59.3, 53.9, 52.8, 38.2, 36.5, 31.7, 26.3, 26.2, 26.1, 17.2; IR (thin film) 2111, 1726, 1452, 1266, 1110, 711 cm^{-1}; HRMS (ESI) calcd for $C_{96}H_{101}N_7O_{28}S$ $(M+Na)^+$ 1854.6307 found 1854.6344 *m*/*z*.

2-Azido-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-[2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)]-2-azido-6-*O*-benzyl-2-deoxy-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-[1-(*R*)-(methoxycarbonyl)-ethylidene]-β-D-galactopyranosyl-(1→1)-6-(benzylthio)-1-hexanol (48)

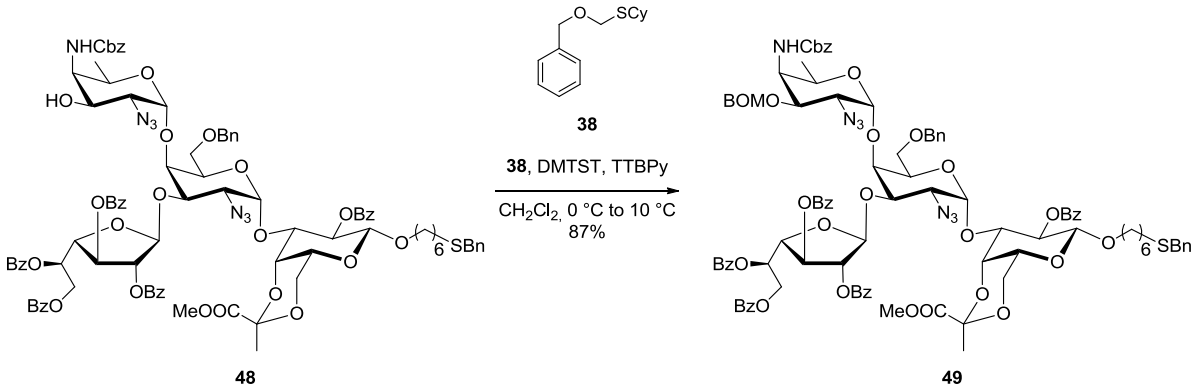


47 48

To a stirred solution of Lev ester **47** (49 mg, 0.027 mmol) in CH_2Cl_2 (2.7 mL) was added at room temperature first a mixture of pyridine (65 µL, 0.802 mmol) and acetic acid (43 µL, 0.749 mmol), and then hydrazine hydrate (2.3 µL, 0.045 mmol). The mixture was stirred for 1.5 h at room temperature, quenched with acetone (0.1 mL) and subjected to size exclusion chromatography (Sephadex LH-20, MeOH/CH_2Cl_2 1:1) to give alcohol **48** (45 mg, 0.026 mmol, 96%) as a white foam. R_f (EtOAc/toluene 2:3) = 0.31; $[\alpha]_D^{20}$ = +98.9° (c = 0.27, acetone); ^{1}H NMR (600 MHz, acetone-D_6) δ 8.13 (d, *J* = 7.7 Hz, 2H, arom.), 8.09 (d, *J* = 7.2 Hz, 2H, arom.), 8.03 (d, *J* = 7.7 Hz, 2H, arom.), 7.98 (d, *J* = 7.6 Hz, 2H, arom.), 7.87 (d, *J* = 7.7 Hz, 2H, arom.), 7.62 (m, 4H, arom.), 7.54 – 7.25 (m, 26H, arom.), 6.05 (d, *J* = 9.9 Hz, 1H, NH), 5.98 (dd, *J* = 9.3, 5.3 Hz, 1H, H-5d), 5.76 – 5.71 (m, 2H, H-1d, H-2d), 5.70 (dd, *J* = 5.9, 3.2 Hz, 1H, H-3d), 5.57 – 5.49 (dd, *J* = 6.8, 5.2 Hz, 1H, H-2a), 5.45 (s, 1H, H-1b), 5.12 (d, *J* = 12.6 Hz, 1H, A of AB, $PhCH_2$), 5.04 (d, *J* = 12.6 Hz, 1H, B of AB, $PhCH_2$), 4.94 (m, 2H, H-1c, H-4d), 4.74 (dd, *J* = 11.9, 3.9 Hz, 1H, A of AB, H-6d), 4.66 – 4.56 (m, 3H, H-1a, H-5c, B of AB, H-6d), 4.51 (d, *J* = 3.7 Hz, 1H, H-4a), 4.47 – 4.39 (m, 2H, $PhCH_2$), 4.34 – 4.26 (m, 1H, H-3c), 4.19 (dd, *J* = 10.0, 3.7 Hz, 1H, H-3a), 4.14 – 4.03 (m, 5H, H-3b, H-4b, H-5b, A of AB, H-6a, H-4c), 3.99 (d, *J* = 12.8 Hz, 1H, B of AB, H-6a), 3.85 – 3.74 (m, 6H, A of AB, O-C*H*$_2$-CH_2, H-2b, A of AB, H-6b, $COOCH_3$), 3.70 – 3.62 (m, 3H, S-C*H*$_2$-Ph, B of AB, H-6b), 3.58 (s, 1H, H-5a), 3.52 – 3.43 (m, 2H, H-2c, B of AB, O-C*H*$_2$-CH_2), 2.22 (t, *J* = 7.4 Hz, 2H, CH_2-C*H*$_2$-S), 1.51 (s, 3H, pyruv.-CH_3), 1.44 (m, 2H, aliph.), 1.34 – 1.19 (m, 6H, aliph., H-6c), 1.15 (m, 3H, aliph.); ^{13}C NMR (150 MHz, acetone-D_6) δ 171.1, 166.4, 166.3, 165.8, 165.7, 158.3, 140.0, 138.3, 134.4, 134.3, 134.2, 134.1, 130.73, 130.65, 130.6, 130.49, 130.46, 130.4, 130.3, 130.2, 130.1, 129.7, 129.6, 129.5, 129.37, 129.35, 129.23, 129.16, 129.14, 128.70, 128.67, 128.4, 128.2, 127.5, 108.2 (C-1d), 101.8 (C-1a), 99.5

(C-1c), 94.1 (C-1b), 82.5, 81.7, 78.5, 76.0, 73.6, 71.6, 71.3, 69.9, 67.4, 66.8, 66.5, 66.1, 64.2, 62.0, 60.1, 57.3, 52.8, 36.5, 31.7, 30.4, 29.8, 29.1, 26.2, 26.1, 17.4; IR (thin film) 2936, 2326, 2163, 2111, 1728, 1602, 1452, 1266, 1109, 1071, 1029, 985, 711 cm^{-1}; HRMS (ESI) calcd for $C_{91}H_{95}N_7O_{26}S$ $(M+Na)^+$ 1756.5940 found 1756.5895 *m*/*z*.

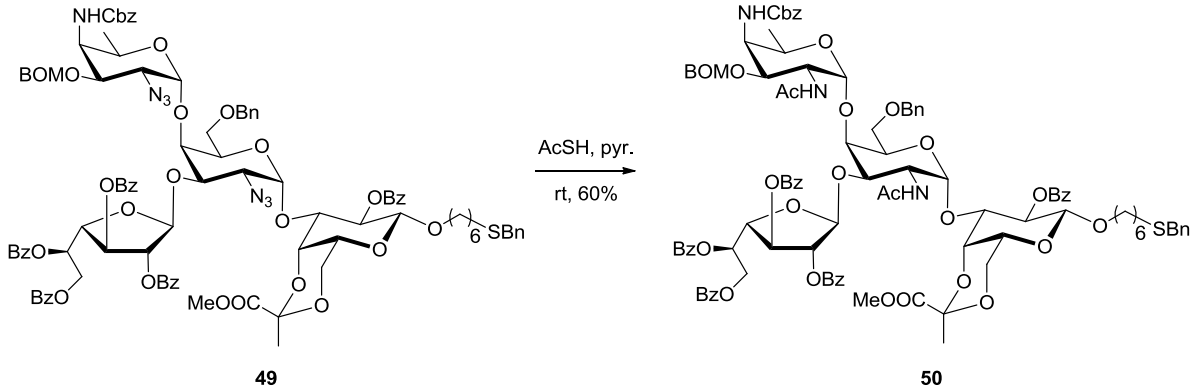
2-Azido-4-(benzyloxycarbonyl)amino-3-*O*-benzyloxymethyl-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-[2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)]-2-azido-6-*O*-benzyl-2-deoxy-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-[1-(*R*)-(methoxycarbonyl)-ethylidene]-β-D-galactopyranosyl-(1→1)-6-(benzylthio)-1-hexanol (49)



Alcohol **48** (23 mg, 0.013 mmol), *S*,*O*-acetal **38** (95 mg, 0.401 mmol) and TTBPy (132 mg, 0.535 mmol) were co-evaproated with dry toluene (3x10 mL). The mixture was dissolved in CH_2Cl_2 (2.0 mL) and stirred over activated molecular sieves (3 Å) for 30 min at room temperature. The mixture was cooled to 0 °C and DMTST (16 mg, 0.401 mmol in 0.3 mL CH_2Cl_2) was added dropwise over a period of 1.5 h, while the reaction temperature was kept below 10 °C. The reaction was stirred for another 45 min, quenched by addition of a 10:1 (v/v) mixture of MeOH and Et_3N (0.5 mL) and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes 0:1 to 1:1) to give acetal **49** (22 mg, 0.012 mmol, 87%) as a white foam. R_f (EtOAc/hexanes 2:3) = 0.46; $[\alpha]_D^{20}$ = +76.5° (c = 0.53, acetone); ^{1}H NMR (400 MHz, acetone-D_6) δ 8.16 – 8.07 (m, 4H, arom.), 8.04 (d, *J* = 7.3 Hz, 2H, arom.), 8.01 – 7.96 (d, *J* = 7.6 Hz, 2H, arom.), 7.86 (d, *J* = 8.3 Hz, 2H, arom.), 7.70 – 7.55 (m, 5H, arom.), 7.52 – 7.24 (m, 30H, arom.), 6.09 (d, *J* = 10.1 Hz, 1H, NH), 5.98 (dt, *J* = 7.4, 3.8 Hz, 1H, H-5d), 5.76 – 5.72 (m, 2H, H-1d, H-2d), 5.70 (dd, *J* = 5.8, 3.2 Hz, 1H, H-3d), 5.54 (dd, *J* = 10.0, 8.1 Hz, 1H, H-2a), 5.46 (s, 1H, H-1b), 5.13 – 5.06 (m, 2H, $PhCH_2$), 5.01 (dd, *J* = 8.1, 5.5 Hz, 2H, H-1c, A of AB, BnO-CH_2-O), 4.95 (dd, *J* = 5.8, 3.0 Hz, 1H, H-4d), 4.75 (m, 3H, A of AB, H-6d, A of AB, $PhCH_2$, B of AB, BnO-CH_2-O), 4.69 – 4.54 (m, 4H, H-1a, H-5c, B of AB, H-6d, B of AB, $PhCH_2$), 4.52 (d, *J* =

3.6 Hz, 1H, H-4a), 4.43 (s, 2H, $PhCH_2$), 4.35 (dd, *J* = 11.0, 4.2 Hz, 1H, H-3c), 4.30 (d, *J* = 10.0 Hz, 1H, H-4c), 4.20 (dd, *J* = 10.1, 3.8 Hz, 1H, H-3a), 4.15 – 4.03 (m, 4H, H-3b, H-4b, H-5b, A of AB, H-6a), 3.99 (d, *J* = 12.6 Hz, 1H, B of AB, H-6a), 3.88 – 3.76 (m, 3H, A of AB, O-C*H*$_2$-CH_2, H-2b, A of AB, H-6b), 3.74 (s, 3H, $COOCH_3$), 3.72 – 3.65 (m, 2H, H-2c, B of AB, H-6b), 3.64 (s, 2H, CH_2-Ph), 3.57 (s, 1H, H-5a), 3.46 (dt, *J* = 10.0, 6.5 Hz, 1H, B of AB, O-C*H*$_2$-CH_2), 2.23 (t, *J* = 7.3 Hz, 2H, CH_2-C*H*$_2$-S), 1.51 (s, 3H, pyruv-CH_3), 1.49 – 1.37 (m, 2H, aliph.), 1.35 – 1.20 (m, 6H, H-6c, aliph.), 1.19 – 1.12 (m, 3H, aliph.); ^{13}C NMR (100 MHz, acetone-D_6) δ 171.2, 166.5, 166.3, 165.9, 165.7, 157.9, 140.0, 139.4, 139.2, 138.4, 134.4, 134.34, 134.27, 131.1, 130.8, 130.7, 130.64, 130.57, 130.5, 130.4, 130.3, 130.2, 130.1, 129.72, 129.65, 129.5, 129.43, 129.39, 129.24, 129.18, 129.16, 128.9, 128.7, 128.41, 128.37, 128.3, 127.5, 108.6 (C-1d), 101.9 (C-1a), 99.7, 99.5 (C-1c), 94.2 (C-1b), 92.9, 82.6, 81.8, 78.6, 76.1, 73.67, 73.65, 73.62, 72.5, 71.7, 71.4, 71.1, 70.3, 69.9, 69.2, 67.4, 66.8, 66.6, 66.5, 66.1, 64.2, 61.1, 60.1, 53.6, 52.8, 36.5, 31.8, 30.1, 29.9, 29.7, 26.2, 26.1, 17.4; IR (thin film) 2940, 2111, 1728, 1452, 1264, 1110, 1042, 711 cm^{-1}; HRMS (ESI) calcd for $C_{99}H_{103}N_7O_{27}S$ $(M+Na)^+$ 1876.6520 found 1876.6628 *m*/*z*.

2-Acetamido-4-(benzyloxycarbonyl)amino-3-*O*-benzyloxymethyl-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-[2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)]-2-cetamido-6-*O*-benzyl-2-deoxy-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-[1-(*R*)-(methoxycarbonyl)-ethylidene]-β-D-galactopyranosyl-(1→1)-6-(benzylthio)-1-hexanol (50)

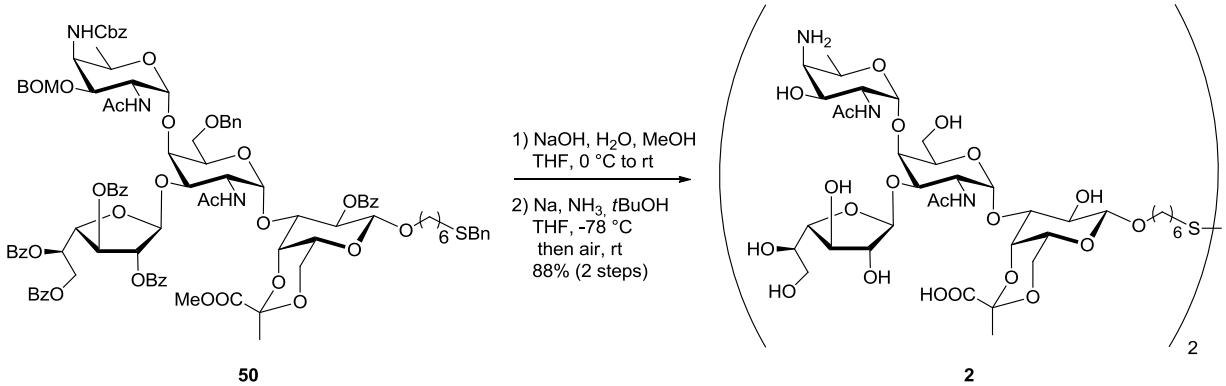


49 **50**

To a stirred solution of diazide **49** (21 mg, 11.3 µmol) in dry pyridine (0.22 mL) was added at 0 °C thioacetic acid (0.2 mL). The mixture was warmed to room temperature and stirred for 48 h at that temperature. The solution was co-evaporated with toluene (2x5 mL) and the residue was purified by flash chromatography (EtOAc/hexanes 1:2 to acetone/hexanes 1:3 to 1:2 to 3:4) to give diacetamide **50** (13 mg, 6.8 µmol, 60%) as a white foam. R_f

(acetone/hexanes 1:2) = 0.29; $[\alpha]_D^{20}$ = +56.4° (c = 0.25, acetone); ^{1}H NMR (400 MHz, acetone-D_6) δ 8.11 – 8.08 (m, 2H, arom.), 8.06 – 7.92 (m, 8H, arom.) 7.68 – 7.54 (m, 4H, arom.), 7.52 – 7.27 (m, 31H, arom.), 6.86 (d, *J* = 9.8 Hz, 1H, NH), 6.71 (d, *J* = 7.2 Hz, 1H, NH), 5.92 (m, 2H, NH, H-5d), 5.71 – 5.64 (m, 3H, H-1d, H-2d, H-3d), 5.41 (dd, *J* = 10.2, 8.1 Hz, 1H, H-2a), 5.10 (d, *J* = 3.9 Hz, 3H, H-1b, $PhCH_2$), 5.06 – 5.01 (m, 1H, H-1c), 4.98 (dd, *J* = 7.1, 3.4 Hz, 1H, H-4d), 4.89 (d, *J* = 6.9 Hz, 1H, A of AB, BnO-CH_2-O), 4.79 – 4.49 (m, 8H, H-1a, H-2b, H-5c, H-6d, B of AB, BnO-CH_2-O, $PhCH_2$), 4.46 – 4.35 (m, 3H, H-4a, $PhCH_2$), 4.07 (m, 7H, H-2c, H-3a, H-4b, H-4c, H-5b, H-6a), 3.90 – 3.78 (m, 3H, H-3c, H-3b, A of AB, O-CH_2-CH_2), 3.77 (s, 3H, $COOCH_3$), 3.70 – 3.58 (m, 3H, $PhCH_2$, A of AB, H-6b), 3.54 (s, 1H, H-5a), 3.51 – 3.39 (m, 2H, B of AB, O-CH_2-CH_2, B of AB, H-6b), 2.21 (t, *J* = 6.4 Hz, 2H, CH_2-CH_2-S), 1.83 (s, 3H, Ac-CH_3), 1.80 (s, 3H, Ac-CH_3), 1.49 (s, 3H, pyruv.-CH_3), 1.47 – 1.37 (m, 2H, aliph.), 1.31 – 1.09 (m, 9H, H-6c, aliph.); ^{13}C NMR (100 MHz, acetone-D_6) δ 171.2, 170.2, 170.1, 166.5, 166.2, 166.0, 165.6, 140.0, 139.6, 139.54, 138.51, 134.46, 134.3, 134.2, 134.1, 130.82, 130.81, 130.78, 130.7, 130.6, 130.5, 130.4, 130.1, 129.7, 129.6, 129.47, 129.45, 129.4, 129.22, 129.19, 129.15, 128.7, 128.64, 128.58, 128.4, 128.3, 128.21, 128.18, 127.6, 109.1 (C-1d), 101.9 (C-1a), 99.6, 99.5 (C-1b), 94.5 (C-1c), 93.8, 82.9, 80.9, 78.6, 76.6, 73.6, 73.2, 71.6, 71.5, 71.1, 70.03, 69.98, 67.7, 66.7, 66.5, 66.3, 66.0, 64.2, 54.1, 53.0, 50.6, 49.1, 36.5, 32.3, 31.8, 30.6, 30.3, 30.1, 29.9, 29.1, 26.4, 26.2, 23.3, 23.2, 17.7; IR (thin film) 2928, 1726, 1683, 1509, 1452, 1267, 1111, 1071, 1047, 711 cm^{-1}; HRMS (ESI) calcd for $C_{103}H_{111}N_3O_{29}S$ $(M+Na)^+$ 1908.6921 found 1908.6906 *m*/*z*.

6,6'-dithiobis[2-*N*-Acetyl-4-amino-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-[β-D-galactofuranosyl-(1→3)]-2-*N*-acetyl-2-deoxy-α-D-galactopyranosyl-(1→3)-4,6-O-[1-(*R*)-(carboxy)-ethylidene]-β-D-galactopyranoside-(1→1)-1-hexanol] (2)

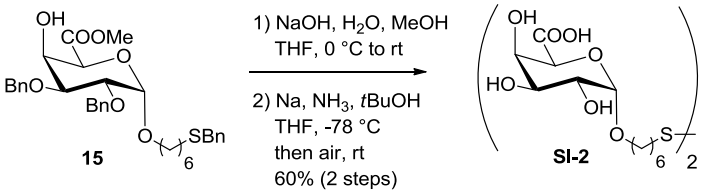


50 **2**

To a stirred solution of ester **50** (12 mg, 6.4 µmol) in THF (1.5 mL) and MeOH (0.75 mL) was added at 0 °C a 1 M aq. solution of NaOH (0.8 mL). The reaction was slowly warmed to room temperature and stirred for 16 h at that temperature. The mixture was diluted with water (5 mL), acidified to pH 4 with 0.5 M aq. $NaHSO_4$, and poured into EtOAc (5 mL). After separation, the aqueous fraction was extracted with EtOAc (8x10 mL), the combined organic fractions were dried over Na_2SO_4 and concentrated to give the intermediate acid as a white foam.

To a stirring solution of liquid ammonia (8 mL) was added at -78 °C a solution of the crude acid in THF (2.0 mL). The mixture was treated with *t*BuOH (0.8 mL) and lumps of freshly cut sodium (65 mg) were added until a deeply blue color persisted. The reaction was stirred at -78 °C for 45 min and quenched by addition of solid NH_4OAc (200 mg). The solution was warmed to room temperature under a stream of argon and co-evaporated with MeOH (2x10 mL) and water (2x5 mL). The residue was left exposed to air for 16 h, purified by size exclusion chromatography (Sephadex G-25, MeOH/5 mM aq. NH_4OAc 4:6) and lyophilized repeatedly to give disulfide **2** (5.1 mg, 2.8 µmol, 88%) as a white solid, containing approx. 10% of the corresponding thiol. $[\alpha]_D^{20}$ = +60.6° (c = 0.14, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.42 (d, *J* = 3.7 Hz, 1H, H-1b), 5.18 (d, *J* = 3.4 Hz, 1H, H-1d), 5.10 (d, *J* = 3.8 Hz, 1H, H-1c), 4.77 (d, *J* = 8.1 Hz, 1H, H-5c), 4.70 (dd, *J* = 11.2, 3.5 Hz, 1H, H-2b), 4.59 (d, *J* = 7.9 Hz, 1H, H-1a), 4.55 (d, *J* = 4.0 Hz, 1H, H-4a), 4.41 (dd, *J* = 11.4, 4.3 Hz, 1H, H-3c), 4.32 (dd, *J* = 7.5, 5.5 Hz, 1H, H-5b), 4.26 (d, *J* = 2.2 Hz, 1H, H-4d or H-3d), 4.22 (dd, *J* = 11.1, 2.4 Hz, 1H, H-3b), 4.18 – 4.08 (m, 5H, H-2c, H-2d, H-4d or H-3d, H-4b, A of AB, H-6a), 4.04 (m, 2H, B of AB, H-6a, A of AB, O-C*H*$_2$-CH_2), 3.92 – 3.86 (m, 2H, H-3a, H-5d), 3.87 – 3.72 (m, 6H, H-2a, H-6b, H-6d, B of AB, O-C*H*$_2$-CH_2), 3.67 (s, 1H, H-5a), 3.55 (s, 1H, H-4c), 3.02 (dd, *J* = 9.1, 6.8 Hz, 0.2H, CH_2-C*H*$_2$-SH), 2.88 (t, *J* = 7.2 Hz, 1.9H, CH_2-C*H*$_2$-S-S), 2.18 (s, 3H, Ac-CH_3), 2.16 (s, 3H, Ac-CH_3), 1.85 – 1.73 (m, 4H, aliph.), 1.56 (s, 3H, pyruv.-CH_3), 1.55 – 1.50 (m, 4H, aliph.), 1.39 (d, *J* = 6.7 Hz, 3H, H-6c); HRMS (MALDI) calcd for $C_{74}H_{124}N_6O_{42}S_2$ $(M+Na)^+$ 1855.7085 found 1855.7010 *m*/*z*.

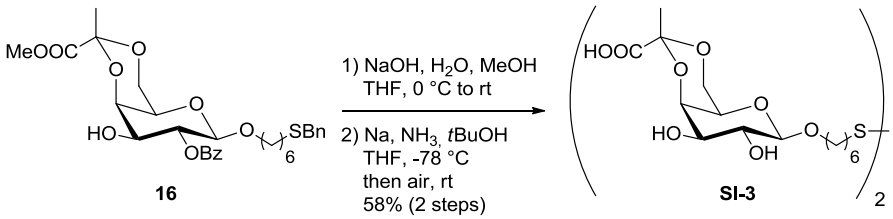
6,6'-Dithiobis[α-D-galactopyranosyluronate-(1→1)-1-hexanol] (SI-2)



To a stirred solution of ester **15** (10 mg, 0.017 mmol) in THF (1.0 mL) and MeOH (0.5 mL) was added at 0 °C a 1 M solution of NaOH in water (0.8 mL). The reaction was slowly warmed to room temperature and stirred for 16 h at that temperature. The reaction was diluted with EtOAc (5 mL) and water (5 mL) and acidified to pH 4 with 0.5 M aq. $NaHSO_4$. After separation, the aqueous fraction was extracted with EtOAc (8x5 mL), the combined organic fractions were dried over Na_2SO_4 and concentrated to give the intermediate acid as a white foam.

To a stirred solution of liquid ammonia (8 mL) was added at -78 °C a solution of the crude acid in THF (2 mL). The mixture was treated with *t*BuOH (0.4 mL) and lumps of freshly cut sodium (45 mg) were added until a deeply blue color persisted. The reaction was stirred at -78 °C for 45 min and quenched by addition of solid NH_4OAc (100 mg). The solution was warmed to room temperature under a stream of argon and co-evaporated with MeOH (2x10 mL) and water (2x5 mL). The residue was left exposed to air for 16 h, purified by size exclusion chromatography (Sephadex G-25, 9:1 MeOH/5 mM aq. NH_4OAc) and lyophilized repeatedly to give disulfide **9** (3.1 mg, 5.1 µmol, 60% over two steps) as a white solid. $[\alpha]_D^{20}$ = +29.8° (c = 0.29, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.09 (d, *J* = 3.7 Hz, 1H, H-1), 4.47 – 4.33 (m, 2H, H-4, H-5), 4.04 (dd, *J* = 10.1, 3.1 Hz, 1H, H-3), 3.97 (dd, *J* = 10.1, 3.7 Hz, 1H, H-2), 3.90 – 3.76 (m, 1H, A of AB, O-C*H*$_2$-CH$_2$), 3.74 – 3.63 (m, 1H, B of AB, O-C*H*$_2$-CH$_2$), 2.91 (t, *J* = 7.2 Hz, 2H, CH$_2$-C*H*$_2$-S), 1.91 – 1.68 (m, 4H, aliph.), 1.67 – 1.46 (m, 4H, aliph.); HRMS (MALDI) calcd for $C_{24}H_{42}O_{14}S_2$ $(M-H)^-$ 617.1938 found 617.1954 *m*/*z*.

6,6'-dithiobis[4,6-O-[1-(*R*)-(carboxy)-ethylidene]-β-D-galactopyranoside-(1→1)-1-hexanol] (SI-3)



To a stirred solution of ester **16** (30 mg, 0.052 mmol) in THF (0.6 mL) and MeOH (0.3 mL) was added at 0 °C a 1 M solution of NaOH in water (0.6 mL). The reaction was slowly warmed to room temperature and stirred for 16 h at that temperature. The reaction was diluted with MeOH (2 mL), neutralized with Amberlite 120 (H^+), filtered and concentrated to give the intermediate acid as a white foam.

To a stirred solution of liquid ammonia (8 mL) was added at -78 °C a solution of the crude acid in THF (2 mL). The mixture was treated with *t*BuOH (0.6 mL) and lumps of freshly cut sodium (80 mg) were added until a deeply blue color persisted. The reaction was stirred at -78 °C for 45 min and quenched by addition MeOH (2 mL) and solid NH_4OAc (100 mg). The solution was warmed to room temperature under a stream of argon and co-evaporated with MeOH (2x5 mL) and water (2x5 mL). The residue was left exposed to air for 16 h, purified by size exclusion chromatography (Sephadex G-25, 4:1 MeOH/5 mM aq. NH_4OAc) and solid phase extraction (Chromafix C18 cartridge, Macherey-Nagel, Düren, Germany) and lyophilized repeatedly to give disulfide **SI-3** (11 mg, 0.030 mmol, 58% over two steps) as a white solid, containing approx. 10% of the corresponding thiol. $[\alpha]_D^{20}$ = -22.3° (c = 0.44, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 4.53 (d, *J* = 7.8 Hz, 1H, H-1), 4.26 (d, *J* = 3.7 Hz, 1H, H-4), 4.13 (d, *J* = 12.0 Hz, 1H, A of AB, H-6), 4.08 – 3.96 (m, 2H, B of AB, H-6, A of AB, O-C*H*$_2$-CH$_2$), 3.83 – 3.63 (m, 4H, H-2, H-3, H-5, B of AB, O-C*H*$_2$-CH$_2$), 2.88 (t, *J* = 7.2 Hz, 1.65H, CH$_2$-C*H*$_2$-S-S), 2.66 (t, *J* = 7.3 Hz, 0.35H, CH$_2$-C*H*$_2$-SH), 1.92 – 1.69 (m, 4H, aliph.), 1.60 – 1.46 (m, 7H, pyruv.-CH$_3$, aliph.). HRMS (MALDI) calcd for $C_{30}H_{50}O_{16}S_2$ $(M-H)^-$ 729.2462 found 729.2480 *m*/*z*.

Antisera and polysaccharides

Rabbit *S. pneumoniae* serotype 1 typing serum and Sp1 polysaccharide were obtained from SSI Diagnostica (Hillerod, Denmark). Rabbit anti-*B. fragilis* antiserum and PS A1 polysaccharide were a kind gift by Prof. Dennis Kasper, Harvard Medical School.

Preparation of Glycan Microarray Slides

Maleimide-functionalized glycan array slides were prepared as reported previously.[10] Disulfide-containing glycans (1 mM or 0.5 mM in phosphate-buffered saline (PBS)) were reduced using 1.0 equivalent of tris(2-carboxyethyl)phosphine (TCEP) and spotted onto the functionalized microarray slides using an automatic piezoelectric arraying robot (Scienion, Berlin, Germany) at 0.2 nL per spot. Polysaccharides were spotted onto the same slides at a concentration of 0.05 mg/mL in PBS. Slides were then incubated in a humid chamber for 24 h at room temperature and quenched in a 0.2% (v/v) solution of 2-mercaptoethanol in PBS for 1 h at room temperature. The slides were washed with water (3x) and MeOH (3x), dried and stored under argon until use.

Glycan Array Binding Experiments

Glycan-functionalized slides were blocked using blocking solution (1% (w/v) bovine serum albumin (BSA) in PBS) for 1 h at room temperature. Slides were washed with water (3x) and MeOH (3x) and dried. A 64-well gasket (FlexWell 64, Grace Bio-Labs, Bend, US) was appended and antisera were applied in the depicted dilutions. The slides were incubated for 16 h at 4 °C, washed with washing buffer (0.1% (v/v) Tween 20 in PBS, 3x) and incubated with secondary antibody (goat anti-rabbit-FITC conjugate (abcam, Cambridge, UK), 1:200 in blocking solution) for 2 h at room temperature. The slides were washed with washing buffer (3x) and water (3x) and dried by centrifugation in a 50 mL tube. Fluorescence readout was performed using an Axon GenePix 4300A microarray scanner and GenePix Pro 7 software (both MDS, Sunnyvale, US).

Glycoconjugate Synthesis

To a solution of BSA (0.25 mg, 3.8 nmol) in 0.1 M sodium phosphate buffer (NaPi) pH 7.4 (1 mL) was added at room temperature a solution of *N*-succinimidyl-3-

(bromoacetamido)propionate (SBAP) (81 µg, 263 nmol) in DMF (10 µL). The mixture was left for 1 h at that temperature and concentrated using membrane filtration (Amicon 0.5 mL Ultra centrifuge membrane, 10 kDa cut-off, Millipore, Billerica, US). The protein solution was diluted with water and concentrated again. This process was repeated three times and the solution was diluted with water to 0.25 mL. An analytical sample was taken and the protein solution was treated with 750 mM NaPi pH 7.4 (33 µL). PS A1 repeating unit disulfide **2** (418 µg, 460 nmol resp. to the monomer) in 100 µL 0.1 M NaPi pH 7.4 was treated at room temperature with a 100 mM aqueous solution of TCEP (4.6 µL, 460 nmol), left for 1 h at that temperature under an argon atmosphere and added to the solution of the activated protein. The mixture was left at room temperature for 4 h and at 4 °C for 16 h. The glycoconjugate was purified using membrane filtration (see above). Another analytical sample was taken. The purified glycoconjugate in 0.1 M NaPi pH 7.4 (0.5 mL) was then treated at room temperature with L-cysteine (100 µg, 826 nmol) in water (0.1 mL). The mixture was left for 2 h at that temperature and purified by membrane filtration. Glycan loading was assessed by MALDI-TOF-MS and SDS-PAGE.

References

1. (a) Fügedi, P.; Garegg, P. J. *Carbohydr. Res.* **1986,** *149* (1), C9-C12. (b) Ravenscroft, M.; Roberts, R. M. G.; Tillett, J. G. *J Chem. Soc. Perkin Trans. 2* **1982,** (12), 1569-1572.

2. Halkes, K. M.; Lefeber, D. J.; Fransen, C. T.; Kamerling, J. P.; Vliegenthart, J. F. *Carbohydr. Res.* **1998,** *308* (3-4), 329-38.

3. Salvatore, R. N.; Smith, R. A.; Nischwitz, A. K.; Gavin, T. *Tetrahedron Lett.* **2005,** *46* (51), 8931-8935.

4. 28: (a) Saferni, O.L.; Clark, R.N.; Smart, B.E. *J. Chem. Soc. C.* **1966**, 645-648**.**

5. Scanlan, E. M.; Mackeen, M. M.; Wormald, M. R.; Davis, B. G. *J. Am. Chem. Soc.* **2010**, *132* (21), 7238-7239.

6. Nilsson, S.; Lönn, H.; Norberg, T. *Glycoconjugate J.* **1991**, *8*, 9-15.

7. Pragani, R.; Seeberger, P. H. *J. Am. Chem. Soc.* **2011**, *133* (1), 102-107.

8. Pragani, R.; Stallforth, P.; Seeberger, P. H. *Org. Lett.* **2010**, *12* (7), 1624-1627.

9. Marinier, A.; Martel, A.; Bachand, C.; Plamondon, S.; Turmel, B.; Daris, J. P.; Banville, J.; Lapointe, P.; Ouellet, C.; Dextraze, P.; Menard, M.; Wright, J. J.; Alford, J.; Lee, D.; Stanley, P.; Nair, X.; Todderud, G.; Tramposch, K. M. *Bioorg. Med. Chem.* **2001**, *9* (6), 1395-1427.

10. T. Horlacher, M. A. Oberli, D. B. Werz, L. Kröck, S. Bufali, R. Mishra, J. Sobek, K. Simons, M. Hirashima, T. Niki, P. H. Seeberger, *ChemBioChem*, 2010, **11**, 1563.