SYNTHESIS OF THE LINEAR B TYPE 2 TRISACCHARIDE Galα3Galβ4GlcNAcβOTMSEt, AND COUPLING OF THE CORRESPONDING 2-CARBOXYETHYL β-THIOGLYCOSIDE TO SEPHAROSE

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SYNTHESIS OF THE LINEAR B TYPE 2 TRISACCHARIDE Galα3Galβ4GlcNAcβOTMSEt, AND COUPLING OF THE CORRESPONDING 2-CARBOXYETHYL β-THIOGLYCOSIDE TO SEPHAROSE

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ABSTRACT

The synthesis of the Linear B type 2 trisaccharide (Galα3Galβ4GlcNAc-βOTMSEt) and the corresponding 2-carboxyethyl β-thioglycoside is described, as well as coupling of the latter to Sepharose.

Key Words: Glycoside synthesis; Thioglycoside; Glycoconjugate; Xenotransplantation

INTRODUCTION

The lack of donor organs for transplantation has over the last decade increased the interest in xenotransplantation. Use of organs from pigs would dramatically increase the number of patients that could be treated by transplantation. A major obstacle for xenotransplantation is the initial interaction with natural antibodies present in the host, leading to hyperacute rejection of the graft, often within minutes.[1]

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The specificity for isolated antibodies has been studied,[2,3] and the strongest binding was observed for glycoconjugates processing terminal αGal residues, including the B-disaccharide (Galα3Gal) and the Linear B type 2 trisaccharide (Galα-3Galβ4GlcNAc). Soluble B and B type 2 saccharides have shown the ability to inhibit the pig to human antibody determined rejection.[4] Other antigens have been identified,[5] including the Thomsen–Friedenreich (Galα3GalNAc) and the P^k antigen (Galα4Gal β4Glc). However, the major target for xenoreactive antibodies is believed to be the Galα3Gal epitope.

Here we present the synthesis of the 2-(trimethylsilyl)ethyl glycoside of the Linear B type 2 trisaccharide (Syntheses of the trisaccharide and the related pentasaccharide have previously been reported in Ref. [6]), as well as the synthesis and coupling of the corresponding 2-carboxyethyl β-thioglycoside to Sepharose. Both are useful products for further studies on the xenorejection mechanisms and the development of strategies to overcome the barriers involved with xenotransplantation.

RESULTS AND DISCUSSION

Planning the synthesis, it was important to us that the strategy would allow modifications at the reducing end of the trisaccharide late in the synthetic sequence. Such a strategy would be very useful for synthesis of a variety of glycoconjugates. Use of O-TMSEt-glycosides provides such flexibility.[7] It is a stable anomeric protecting group that easily and in few steps allows transformation into a variety of glycoconjugates (e.g., glycolipids, -proteins, and -polymers).[8] The present synthesis of conjugate **14** is an example of the synthetic use of an O-TMSEt glycoside.

The benzylidene protected TMSEt-galactopyranoside **2**[7] was glycosylated with the donor **1**[9] using N-iodosuccinimide and triflic acid as the promoting system (Scheme 1), leaving a mixture of disaccharides. It was difficult to isolate the desired product at this stage, so the mixture was O-benzoylated to ease the separation. After benzoylation the desired compound **3** was isolated in an overall yield of 40% from the mixture. No attempt was made to isolate and characterize any of the other products (regio- and stereoisomers). The benzyl groups and the benzylidene acetal were removed by palladium on carbon catalyzed hydrogenolysis, and the product was O-acetylated in a pyridine/acetic anhydride mixture to give **4** in 98% yield. Compound **4** was quantitatively converted to the anomeric acetate **5** (α/β 1:25).[7] The thioglycoside **6** was obtained in 74% yield by treating **5** with trimethylthiomethylsilane and boron trifluoride etherate.[10] Glycosylation of **7**[7] with **6** (1.37 eq.) using an N-iodosuccinimide/triflic acid mixture as promoter left recovered acceptor (30%) and the trisaccharide **8** in 91% yield, based on recovered acceptor. The trisaccharide **8** was deprotected using a four-step procedure, omitting purification of any of the intermediates. The trisaccharide was hydrogenated to remove the benzyl groups followed by de-O-acetylation under Zemplén conditions (MeOH/MeONa). The phthalimido group was removed with hydrazine monohydrate in refluxing methanol. The liberated amino group was acetylated in a mixture of methanol and acetic anhydride to give **9** in 50% overall yield after purification.

For the synthesis of **13** (Scheme 2), the trisaccharide **8** was de-O-benzylated by hydrogenolysis, then O-acetylated in a pyridine/acetic anhydride (1:1) mixture to give
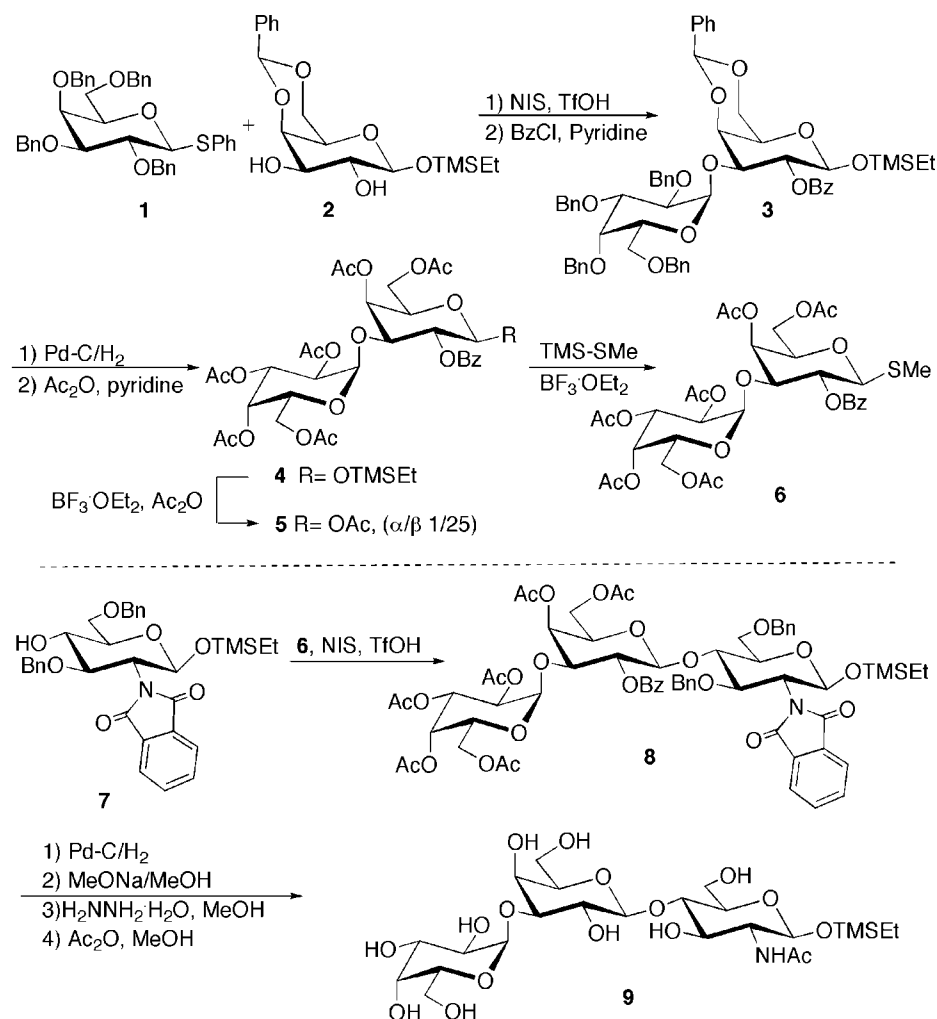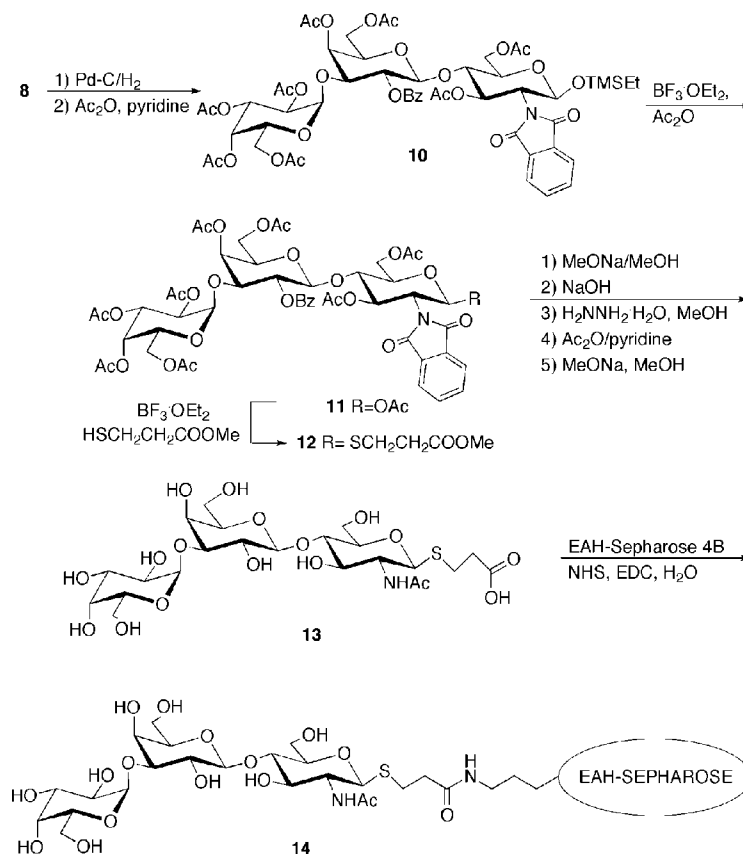
Scheme 1.

10 in 92% yield. Conversion of the TMSEt glycoside **10** to the β-acetate **11** was done in 92% yield.[7] The β-acetate **11** was used as donor in the glycosylation of methyl 3-mercapto propionate with boron trifluoride etherate as the promoter. The glycosylation gave **12** in 65% yield. Compound **12** was deprotected and *N*-acetylated in five steps. First, compound **12** was de-*O*-acylated under Zemplén conditions (MeOH/MeONa). Second, the methyl ester was hydrolyzed using aqueous sodium hydroxide. The phthalimido group was removed with hydrazine monohydrate in refluxing methanol, and the crude product was then acetylated in a mixture of acetic anhydride and pyridine, followed by de-*O*-acetylation to give **13** in 41% overall yield. The conjugate **13** was covalently coupled to EAH Sepharose 4B by mixing the two in the presence of *N*-hydroxysuccinimide (NHS) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide

Scheme 2.

hydrochloride (EDC).[11] The sulfur contents of EAH−Sepharose and the glycocon-
jugate **14** were obtained by elemental analysis. The difference corresponded to 0.62
μmol of **13** per mL of sedimented gel. Together with compound **9**, the gel with
covalently linked **13** is useful for studies of xenorejection mechanisms, including
detection and isolation of antibodies against the transplanted graft.

EXPERIMENTAL

General Methods.   Melting points are uncorrected. NMR spectra were recorded on a
300 or a 400 MHz instrument. ^{1}H NMR spectral assignments were based on COSY, a
double resonance method. Reactions were conducted at ambient temperature unless
otherwise specified. Concentrations were carried out using rotary evaporation with
bath temperatures at or below 40°C. Anhydrous Na_2SO_4 was used as the drying agent
for the organic extracts in the workup procedures. TLC was performed on kiselgel 60
F_{254} plates (Merck). Chromatography was performed on SiO_2 (Matrex LC-gel: 60A,

35–70 MY, Grace) using the flash technique.[12] Compounds submitted for elemental analysis were dried under high vacuum (0.01 mm Hg) at room temperature for 24 h and stored under argon. Compounds **1**,[9] **2**[7] and **7**[7] were synthesized as described.

2-(Trimethylsilyl)ethyl 2-*O*-benzoyl-4,6-di-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside (3). A mixture of 2-(trimethylsilyl)ethyl 4,6-di-*O*-benzylidene-β-D-galactopyranoside (**2**) (5.52 g, 15 mmol), phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**1**) (10.4 g, 16.5 mmol) and 4 Å molecular sieves (5 g) in dichloromethane (150 mL) was cooled to −30°C under nitrogen. Over 50 min a solution of *N*-iodosuccinimide (3.73 g, 16.6 mmol) and trifluoromethanesulfonic acid (0.15 mL, 1.1 mmol) in dichloromethane/diethyl ether (1:1, 150 mL) was added to the stirred reaction mixture. After complete addition, the reaction mixture was stirred for another 20 min at −30°C. The mixture was filtered through celite and washed with a saturated aqueous solution of $NaHCO_3$ and a 15% aqueous sodium bisulfite solution. The organic layer was dried and concentrated. The residue was chromatographed (EtOAc/heptane 1:3) to give a mixture of disaccharides (10 g). The disaccharide mixture was *O*-benzoylated in a mixture of pyridine and benzoyl chloride at 80°C for 140 min. The resulting mixture was cooled to ambient temperature. Ethyl acetate and water were added, and the organic layer was washed with water, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl. The isolated organic layer was dried and concentrated. The residue was chromatographed (toluene/EtOAc/MeOH 10:1:0.1), followed by crystallization from heptane−ethanol. Recrystallization from heptane/ethanol gave **3** (5.99 g, 40%); mp. 106–109°C (methanol−water); $[\alpha]_D^{22}$ +75° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.12–8.17 (2 H, Ar), 8.03–8.08 (2 H, Ar), 7.09–7.67 (26 H, Ar), 5.69 (dd, 1 H, *J* 8.1 Hz, 10.1 Hz, H-2), 5.48 (s, 1 H, Ph*CH*), 5.14 (d, 1 H, *J* 3.5 Hz, H-1′), 4.82 (d, 1 H, *J* 11.5 Hz, Bn), 4.61 (d, 1 H, *J* 11.6 Hz, Bn), 4.60 (d, 1 H, *J* 8.0 Hz, H-1), 4.54 (d, 1 H, *J* 11.7 Hz, Bn), 4.28–4.49 (m, 6 H), 3.97–4.09 (m, 4 H, H-2′, H-3, H-6, −O*CH*$_{2a}$- CH_2Si−), 3.93 (bt, 1 H, *J* 6.5 Hz, H-5′), 3.72 (dd, 1 H, *J* 2.9 Hz, 10.1 Hz, H-3′), 3.52−3.61 (m, 1 H, −O*CH*$_{2b}$$CH_2$Si−), 3.46 (bd, 1 H, *J* 2.8 Hz, H-4′), 3.40 (bs, 1 H, H-5), 3.33 (dd, 1 H, *J* 6.6 Hz, 9.4 Hz, H-6′), 3.25 (dd, 1 H, *J* 6.0 Hz, 9.4 Hz, H-6′), 0.82−0.99 (m, 2 H, −CH_2TMS), −0.06 (s, 9 H, −SiMe$_3$); [13]C NMR ($CDCl_3$): δ 165.4, 126.9−139.23 (Ar), 101.5, 101.2, 94.9, 79.0, 76.2, 75.5, 75.0, 74.6, 73.7, 73.6, 72.5, 72.3, 70.9, 70.3, 69.7, 69.6, 67.1, 67.0, 18.3, −1.0; HRMS calcd for $C_{59}H_{66}O_{12}SiNa$ (M + Na): 1017.4221, found 1017.4226.

2-(Trimethylsilyl)ethyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranoside (4). Compound **3** (1.77 g, 1.78 mmol) was dissolved in acetic acid (50 mL) and hydrogenolyzed (Pd−C/H$_2$ 0.23 mPa) for 32 h. The reaction mixture was filtered through celite. Pyridine (1 mL) was added and the organic layer was concentrated. The residue was *O*-acetylated in acetic anhydride/pyridine (1:1, 50 mL). After 19 h the reaction mixture was repeatedly co-evaporated with toluene. The residue was chromatographed (toluene/EtOAc 5:2) to give **4** (1.39 g, 98%); $[\alpha]_D^{22}$ +102° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.05–8.10 (2 H, Ar), 7.44–7.63 (3 H, Ar), 5.52 (dd, 1 H, *J* 7.9 Hz, 10.1 Hz, H-2), 5.42 (bd, 1 H, *J* 3.0 Hz, H-4), 5.20−5.28 (m, 2 H, H-1′, H-2′), 5.06 (dd, 1 H, *J* 3.2 Hz, 10.3 Hz, H-3′), 4.92 (dd, 1 H, *J* 1.5 Hz, 3.3 Hz, H-4′), 4.63 (d, 1 H, *J* 7.9 Hz, H-1), 4.14−4.27 (m, 2 H),

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3.96–4.08 (m, 3 H, H-3, H-5′, –OCH_{2a}CH$_2$TMS), 3.90 (bt, 1 H, J 6.7 Hz, H-5), 3.75–3.88 (m, 2 H), 3.54–3.63 (m, 1 H, –OCH_{2b}CH$_2$TMS), 2.20. 2.085, 2.08, 2.055, 1.925, 1.90 (6 × s,18 H, 6 × Ac); ^{13}C NMR (CDCl$_3$): δ 170.9, 170.8, 170.43, 170.42, 169.8, 165.4, 133.9, 130.2, 129.8, 129.0, 101.4, 94.0, 74.1, 71.1, 70.7, 68.2, 68.0, 67.4, 67.0, 66.8, 65.5, 61.9, 61.5, 21.3, 21.2, 21.1, 21.0, 20.95, 18.4, −1.1; HRMS calcd for C$_{36}$H$_{50}$O$_{18}$-SiNa (M+Na): 821.2664, found 821.2665.

1,4,6-Tri-*O*-acetyl-2-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranose (5). To a solution of **4** (1.3 g, 1.63 mmol) in dry toluene (8.2 mL) were added acetic anhydride (2.3 mL, 24.4 mmol) and boron trifluoride etherate (0.16 mL, 1.3 mmol). The reaction mixture was stirred at ambient temperature. After 1 h saturated aqueous NaHCO$_3$ was added, and the organic layer was isolated, dried and concentrated to give **5** (quant) as a mixture of anomers (α/β 1:25). A sample of the β-anomer was obtained by chromatography (heptane/EtOAc 1:1): [α]$_D^{22}$+125° (*c* 1.0, CHCl$_3$); ^{1}H NMR (CDCl$_3$): δ 8.02–8.07 (m, 2 H, Ar), 7.44–7.64 (3 H, Ar), 5.89 (d, 1 H, J 8.2 Hz, H-1), 5.65 (dd, 1 H, J 8.3 Hz, 10.1 Hz, H-2), 5.46 (bd, 1 H, J 2.3 Hz, H-4), 5.19–5.26 (m, 2 H, H-1′ and H-2′), 5.04 (dd, 1 H, J 3.5 Hz, 10.5 Hz, H-3′), 4.87 (dd, 1 H, J 1.4 Hz, 3.4 Hz, H-4′), 4.11–4.25 (m, 3 H, H-3, H-6), 4.06 (bt, 1 H, J 6.9 Hz, H-5), 3.98 (bdt, 1 H, J 1.3 Hz, 6.5 Hz, H-5′), 3.83 (dd, 1 H, J 6.4 Hz, 11.3 Hz, H-6′), 3.78 (dd, 1 H, J 6.9 Hz, 11.2 Hz, H-6′), 2.22, 2.09, 2.08, 2.07, 2.05, 1.96, 1.91 (7 × s, 21 H, 7 × Ac); ^{13}C NMR (CDCl$_3$): δ 170.9, 170.7, 170.44, 170.4, 170.2, 169.9, 169.6, 165.3, 134.3, 130.2, 129.2, 129.1, 93.9, 92.6, 73.7, 72.4, 69.5, 68.0, 67.3, 67.0, 66.9, 65.2, 61.73, 61.7, 21.24, 21.2, 21.16, 21.1, 21.0, 20.96; HRMS calcd for C$_{33}$H$_{40}$O$_{19}$Na (M+Na): 763.2061, found 763.2067.

Methyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-1-thio-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galacto-pyranosyl)-β-D-galactopyranoside (6). To a stirred solution of **5** (1.23 g, 1.6 mmol) and trimethylthiomethylsilane (0.7 mL, 4.8 mmol) in dichloromethane (10 mL) was added boron trifluoride etherate (0.25 mL, 2.1 mmol). After 6 h dichloromethane was added, and the mixture was washed with a saturated aqueous solution of NaHCO$_3$, dried and concentrated. Crystallization from saturated aqueous methyl *tert*-butyl ether gave **6** (998 mg, 74%); mp 88–92°C; [α]$_D^{27}$+ 117° (*c* 0.7, CHCl$_3$); ^{1}H NMR (CDCl$_3$): δ 8.05–8.10 (2 H, Ar), 7.45–7.64 (3 H, Ar), 5.60 (t, 1 H, J 9.6 Hz, H-2), 5.47 (d, 1 H, J 3.1 Hz, H-4), 5.20–5.26 (m, 2 H, H-1′, H-2′), 5.06 (dd, 1 H, J 3.4 Hz, 10.8 Hz, H-3′), 4.91 (dd, 1 H, J 1.5 Hz, 3.2 Hz, H-4′), 4.53 (d, 1 H, J 9.8 Hz, H-1), 4.18 (d, 2 H, J 7.2 Hz, H-6), 4.10 (dd, 1 H, J 3.1, 10.0, H-3), 4.03 (bt, 1 H, J 6.4 Hz, H-5′), 3.96 (t, 1 H, J 7.0 Hz, H-5), 3.84 (dd, 1 H, J 6.9 Hz, 11.1 Hz, H-6), 3.77 (dd, 1 H, J 6.5 Hz, 11.1 Hz, H-6), 3.22 (s, CH$_3$O in cryst. solv.), 2.24, 2.21, 2.085, 2.08, 2.05, 2.035, 1.905 (21 H, 6 × Ac and –SMe); ^{13}C NMR (CDCl$_3$): δ 170.7, 170.4, 170.3, 169.9, 165.6, 134.1, 130.2, 129.5, 129.1, 94.0, 83.9, 75.1, 68.0, 67.3, 67.0, 66.9, 65.8, 62.1, 61.6, 21.2, 21.1, 21.0, 20.95, 11.7; HRMS calcd for C$_{32}$H$_{40}$O$_{17}$SNa (M+Na): 751.1884, found 751.1902.

2-(Trimethylsilyl)ethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-4-*O*-[4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyrano-syl]-β-D-glucopyranoside (8). To a cooled (−30°C) mixture of **6** (900 mg, 1.10 mmol), 2-(trimethylsilyl)ethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyra-

noside (**7**) (890 mg, 1.51 mmol) and molecular sieves (4 Å, 350 mg) in dichloromethane (13 mL) was added over 5 min a solution of *N*-iodosuccinimide (270 mg, 1.2 mmol) and trifluoromethanesulfonic acid (10 mL, 0.11 mmol) in dichloromethane-diethyl ether (1:1, 14 mL). After 40 min the reaction mixture was filtered through celite, washed with a saturated aqueous solution of $NaHCO_3$ followed by washing with 15% aqueous sodium bisulfite. The organic layer was dried and concentrated. The residue was chromatographed twice (EtOAc/toluene 1:2 and 1:2 → 2:3) to give recovered acceptor **7** (264 mg) and **8** (1.28 g, 91%); $[\alpha]_D^{22}$ +77° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.05–8.10 (2 H, Ar), 7.34–7.71 (12 H, Ar), 6.86–7.08 (5 H, Ar), 5.53 (dd, 1 H, *J* 8.1 Hz, 10.2 Hz, H-2′), 5.32 (bd, 1 H, *J* 3.1 Hz, H-4′), 5.17–5.24 (m, 2 H, H-1″, H-2″), 5.00–5.06 (m, 1 H, H-3″), 5.02 (d, 1 H, *J* 8.4 Hz, H-1), 4.90 (dd, 1 H, *J* 1.4 Hz, 3.2 Hz, H-4″), 4.86 (d, 1 H, *J* 12.3 Hz, Bn), 4.85 (d, 1 H, *J* 8.0 Hz, H-1′), 4.78 (d, 1 H, *J* 12.1 Hz, Bn), 4.50 (d, 1 H, *J* 12.3 Hz, Bn), 4.45 (d, 1 H, *J* 12.1 Hz, Bn), 4.25 (dd, 1 H, *J* 8.5 Hz, 10.7 Hz, H-3), 3.95–4.17 (m, 5 H, incl. H-2, H-5″), 3.69–3.92 (m, 6 H, incl. H-3′), 3.63 (bd, 1 H, *J* 9.8 Hz), 3.33–3.42 (m, 2 H, −O*CH*$_{2b}$-CH$_2$TMS), 2.10, 2.06, 2.055, 2.05, 1.90, 1.87 (18 H, 6 × Ac), 0.62–0.80 (m, 2 H, −*CH*$_2$TMS), −0.18 (s, 9 H, −SiMe$_3$); ^{13}C NMR ($CDCl_3$): δ 170.8, 170.75, 170.5, 170.4, 170.3, 169.9, 165.1, 123.7–139.0 (Ar), 100.8, 98.2, 94.4, 78.2, 77.3, 75.1, 74.8, 74.6, 74.0, 71.4, 71.0, 68.1, 67.9, 67.3, 67.2, 67.1, 66.9, 65.5, 61.5, 61.4, 56.2, 21.2, 21.1, 21.02, 20.99, 20.95, 18.1, −1.1; HRMS calcd for $C_{64}H_{75}O_{24}NSiNa$ (M + Na): 1292.4346, found 1292.4323.

2-(Trimethylsilyl)ethyl 2-acetamido-2-deoxy-1-thio-4-*O*-(3-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (9). Compound **8** (194 mg, 0.153 mmol) was dissolved in AcOH and hydrogenolyzed (Pd−C/H$_2$ 1 atm) overnight. The catalyst was filtered off and the mixture concentrated, followed by co-evaporation with methanol/toluene. The residue was de-*O*-acetylated using Zemplén conditions. After another 12 h the mixture was neutralized with Duolite C436 (H$^+$), filtered and concentrated. The residue was redissolved in MeOH (10 mL) and hydrazine monohydrate (0.50 mL, 10.3 mmol) was added. The mixture was heated to reflux. After 24 h additional hydrazine monohydrate (0.50 mL, 10.3 mmol) was added and the reflux was continued. After 12 h the mixture was concentrated and co-evaporated with EtOH twice. The residue was dissolved in MeOH (10 mL) and acetic anhydride (1.0 mL). After stirring for 12 h, the mixture was concentrated and co-evaporated with toluene/MeOH. The residue was chromatographed (CH_2Cl_2/MeOH/H_2O 10:5:1) to give **9** (49 mg, 50%); $[\alpha]_D^{23}$ +58° (*c* 1.0, H_2O); ^1H NMR (D_2O): δ 4.99 (d, 1 H, *J* 3.9 Hz, H-1″), 4.41 (d, 1 H, *J* 7.8 Hz, H-1 or H-1′), 4.39 (d, 1 H, *J* 7.8 Hz, H-1 or H-1′), 4.01–4.07 (m, 2 H), 3.77–3.94 (m, 4 H), 3.47–3.73 (m, 13 H), 3.39–3.46 (m, 1 H), 1.87 (s, 3 H, −NH*Ac*), 0.78–0.88 (m, 1 H, −*CH*$_{2a}$TMS), 0.66–0.76 (m, 1 H, −*CH*$_{2b}$TMS), −0.15 (s, 9 H, −Si-Me$_3$); ^{13}C NMR (D_2O): δ 174.7, 103.1, 100.6, 95.7, 78.9, 77.5, 75.4, 75.1, 73.2, 71.2, 69.9, 69.4, 68.7, 68.5, 65.1, 61.3, 61.2, 60.5, 55.4, 22.6, 17.4, −2.1; HRMS calcd for $C_{25}H_{47}O_{16}NSiNa$ (M + Na): 668.2562, found 668.2542.

2-(Trimethylsilyl)ethyl 3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (10). Compound **8** (1.24 g, 0.98 mmol) was hydrogenolyzed (Pd−C/H$_2$, 1 atm) for 22 h in glacial acetic acid (40 mL). The mixture was

filtered through celite. Pyridine (0.5 mL) was added, and the mixture was concentrated with toluene. The residue was *O*-acetylated in acetic anhydride/pyridine (1:1, 60 mL). After 19 h the mixture was concentrated and co-evaporated with toluene. Crystallization from methanol gave **10** (968 mg, 84%), mp 156–159°C; $[\alpha]_D^{20}$ +65° (*c* 1.0, CHCl$_3$); ^{1}H NMR (CDCl$_3$): δ 8.07–8.13 (2 H, Ar), 7.68–7.88 (4 H, Ar), 7.43–7.63 (3 H, Ar), 5.73 (dd, 1 H, *J* 8.7, 10.6 Hz, H-3), 5.44 (dd, 1 H, *J* 8.0, 10.2 Hz, H-2′), 5.36 (d, 1 H, *J* 2.8 Hz, H-4′), 5.31, (d, 1 H, *J* 8.5 Hz, H-1), 5.15–5.23 (m, 2 H, H-1″, H-2″), 4.95 (dd, 1 H, *J* 3.4 Hz, 10.6 Hz, H-3″), 4.81 (dd, 1 H, *J* 1.4 Hz, 3.2 Hz, H-4″), 4.65 (d, 1 H, *J* 7.7 Hz, H-1′), 4.31 (dd, 1 H, *J* 2.0 Hz, 11.6 Hz, H-6), 4.03–4.25 (m, 4 H, incl. H-2, H-6), 4.01 (dd, 1 H, *J* 3.2 Hz, 10.2 Hz, H-3′), 3.96 (bt, 1 H, *J* 6.9 Hz, H-5″), 3.73–3.90 (m, 5 H, H-4, H-5′, H-6″, −O*CH*$_{2a}$CH$_2$TMS), 3.65–3.73 (m, 1 H, H-5), 3.45 (dt, 1 H, *J* 6.7 Hz,).8 Hz, −O*CH*$_{2b}$CH$_2$TMS), 2.17, 2.06, 2.05, 2.02, 1.99, 1.92, 1.87 (7 × s, 24 H, 8 × Ac), 0.64–0.83 (m, 2 H, −*CH*$_2$TMS), −0.18 (s, 9 H, −SiMe$_3$); ^{13}C NMR (CDCl$_3$): δ 170.8, 170.64, 170.57, 170.4, 170.3, 170.2, 169.7, 164.8, 134.2, 130.4, 129.2, 129.0, 123.9, 101.3, 97.7, 93.9, 76.9, 73.8, 72.8, 71.6, 71.1, 70.8, 68.0, 67.7, 67.2, 67.0, 66.9, 64.9, 62.6, 61.7, 61.4, 55.4, 21.22, 21.2, 21.1, 21.08, 21.0, 20.9, 18.1, −1.2; HRMS calcd for C$_{54}$H$_{67}$O$_{26}$NSi (M+Na): 1196.3618, found 1196.3624.

1,3,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-4-*O*-(4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranose (11). To a solution of **10** (100.5 mg, 0.086 mmol) in dry toluene (1 mL) and acetic anhydride (0.125 mL) was added boron trifluoride etherate (0.016 mL, 0.127 mmol) and the mixture was stirred under argon. After 3 h a saturated aqueous solution of NaHCO$_3$ (10 mL) was added, and the mixture was extracted with ethyl acetate, dried and concentrated. The residue crystallized when standing. Recrystallization from ethanol gave **11** (87.2 mg, 92%), mp 189–193°C; $[\alpha]_D^{21}$ +87° (*c* 1.0, CDCl$_3$); ^{1}H NMR (CDCl$_3$): δ 8.07–8.12 (2 H, Ar), 7.70–7.88 (4 H, Ar), 7.43–7.63 (3 H, Ar), 6.43 (d, 1 H, *J* 8.9 Hz, H-1), 5.83 (dd, 1 H, *J* 8.4 Hz, 10.5 Hz, H-3), 5.44 (dd, 1 H, *J* 7.8 Hz, 10.1 Hz, H-2′), 5.36 (d, 1 H, *J* 2.6 Hz, H-4′), 5.15–5.23 (m, 2 H, H-1″, H-2″), 4.95 (dd, 1 H, *J* 3.4 Hz, 10.5 Hz, H-3″), 4.82 (dd, 1 H, *J* 1.3 Hz, 3.3 Hz, H-4″), 4.65 (d, 1 H, *J* 7.8 Hz, H-1′), 4.33 (dd, 1 H, *J* 9.0 Hz, 10.5 Hz, H-2), 4.24–4.29 (m, 2 H), 3.72–4.16 (m, 9 H, incl. H-3′, H-5″, H-4), 2.17, 2.07, 2.04, 2.02, 1.98, 1.96, 1.94, 1.86, 1.84 (9 × s, 27 H, 9 × Ac); ^{13}C NMR (CDCl$_3$): δ 170.9, 170.8, 170.7, 170.6, 170.3, 170.2, 170.1, 169.7, 168.9, 167.9, 164.8, 134.9, 134.3, 130.4, 129.2, 128.9, 124.2, 101.0, 94.0, 89.9, 76.1, 73.8, 73.7, 71.2, 70.9, 70.7, 68.0, 67.2, 67.0, 66.9, 65.0, 62.2, 61.6, 61.5, 54.1, 21.22, 21.2, 21.18, 21.13, 21.1, 21.07, 21.0, 20.9, 20.86; HRMS calcd for C$_{51}$H$_{57}$O$_{27}$NNa (M+Na): 1138.3016, found 1138.3005.

2-(Methoxycarbonyl)ethyl 3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-4-*O*-(4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (12). Crude **11** (5.1 mg, 0.005 mmol) was co-evaporated with dry toluene, dissolved in dry dichloromethane (0.5 mL) and methyl 3-mercaptopropionate (0.0015 mL, 0.014 mmol) was added. The reaction mixture was cooled to 0°C under argon and boron trifluoride etherate (0.003 mL, 0.028 mmol) was added. After 1 h at 0°C the mixture was stirred at ambient temperature. After 24 h dichloromethane was added and the mixture was washed with a saturated aqueous solution of NaHCO$_3$ (10 mL), dried, and concentrated, followed by co-evaporation

with toluene. The residue was chromatographed (toluene/EtOAc 4:3) to give **12** (3.4 mg, 65%), as a crystalline compound: mp 147–150°C; $[\alpha]_D^{22} + 71°$ (c 1.0, CHCl$_3$); ^{1}H NMR (CHCl$_3$): δ 8.06–8.12 (2 H, Ar), 7.78–7.86 (2H, Ar), 7.68–7.75 (2 H, Ar), 7.55–7.63 (1 H, Ar), 7.42–7.50 (2 H, Ar), 5.75 (dd, 1 H, J 8.8 Hz, 10.2 Hz, H-3), 5.60 (dd, 1 H, J 7.8 Hz, 10.1 Hz, H-2′), 5.42 (d, 1 H, J 10.7 Hz, H-1), 5.35 (bd, 1 H, J 2.9 Hz, H-4′), 5.14–5.22 (m, 2 H, H-1″, H-2″), 4.95 bd, 1 H, J 3.3 Hz, 10.1 Hz, H-3″), 4.80 (dd, 1 H, J 1.5 Hz, 3.3 Hz, H-4″), 4.63 (d, 1 H, J 7.8 Hz, H-1′), 3.92–4.33 (m, 7 H), 3.67–3.88 (m, 5 H), 3.65 (s, 3 H, COO*Me*), 2.8 (m, 2 H, $-SCH_2-$), 2.56 (t, 2 H, CH_2CO-), 2.15, 2.05, 2.03, 2.01, 1.98, 1.92, 1.85 (7 × s, 24 H, 8 × Ac); ^{13}C NMR (CDCl$_3$): δ 171.9, 170.4, 170.38, 170.2, 170.1, 169.9, 169.8, 169.7, 169.3, 167.6, 167.3, 164.4, 134.5, 134.2, 133.8, 131.6, 131.1, 129.9, 128.8, 128.6, 123.7, 123.6, 100.9, 93.5, 81.4, 76.6, 76.3, 73.4, 71.6, 70.7, 70.3, 67.6, 67.5, 66.8, 66.6, 66.4, 64.6, 64.5, 62.3, 61.3, 61.0, 53.9, 51.7, 35.1, 25.7, 20.8, 20.7, 20.67, 20.63, 20.59, 20.5, 20.46; HRMS calcd for C$_{53}$H$_{61}$O$_{27}$NSNa (M + Na): 1198.3049, found 1198.3047.

2-Carboxyethyl 2-acetamido-2-deoxy-1-thio-4-*O*-(3-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (13). Compound **12** (57 mg, 0.048 mmol) was treated with MeOH/MeONa. After 2.5 h water (2 mL) and NaOH (2 M, 0.1 mL) were added. The reaction mixture was stirred for 6 h, neutralized with Duolite (H$^+$), filtered and concentrated. The residue was dissolved in methanol (15 mL) and hydrazine hydrate (0.25 mL, 5.1 mmol) was added. The reaction mixture was refluxed for 20 h, concentrated and co-evaporated with ethanol/toluene 3 times. The residue was acetylated in acetic anhydride/pyridine (1:1, 15 mL) overnight followed by concentration and co-evaporation with toluene. The residue was de-*O*-acetylated under Zemplén conditions. After 3 h the reaction mixture was neutralized with Duolite (H$^+$) and concentrated. The residue was chromatographed (CH$_2$Cl$_2$/MeOH/H$_2$O 14:8:1) to give **13** (12 mg, 41%), as a crystalline compound: mp 169–171°C; $[\alpha]_D^{22} + 46°$ (c 0.5, MeOH); ^{1}H NMR (D$_2$O): δ 5.03 (d, 1 H, J 3.9 Hz, H-1″), 4.55 (d, 1H, J 9.9 Hz, H-1), 4.44 (d, 1 H, J 7.7 Hz, H-1′), 4.04–4.11 (m, 2 H), 3.45–3.92 (m, 17 H), 2.86 (m, 2 H, $-SCH_2-$), 2.66 (m, 2 H, $-C(O)CH_2-$), 1.92 (s, 3 H, Ac); ^{13}C NMR (D$_2$O): δ 177.7, 177.1, 105.4, 98.1, 87.2, 81.4, 81.1, 79.9, 77.7, 76.4, 73.5, 72.3, 72.0, 71.8, 70.9, 67.5, 63.7, 62.9, 56.9, 55.0, 37.4, 28.1, 24.9; HRMS calcd for C$_{23}$H$_{38}$NO$_{17}$SNa (M − H + Na): 655.1758, found 655.1772.

Coupling of 13 to sepharose (14).[11] EAH–Sepharose 4B (2 mL) was washed with aqueous NaCl (0.5 M, 80 mL) followed by dist. water (80 mL). The prepared gel was mixed with **13** (5.2 mg, 0.0086 mmol), water (6 mL), *N*-hydroxysuccinimide (NHS, 5 mg, 0.043 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC, 8.2 mg, 0.043 mmol). The mixture was rotated for 24 h, followed by washing with water to give **14**. The sulfur contents of EAH–Sepharose (0.063%) and the conjugate **14** (0.14%) were obtained by elemental analysis. The difference corresponded to 0.62 μmol of trisaccharide **13** per mL of sedimented gel. The Sepharose conjugate **14** was suspended in aqueous NaCl (1.0%) and aqueous NaN$_3$ (0.001%).

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