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Synthesis of the saccharide moiety of galactosylgloboside (SSEA-3) and its conjugation to bovine serum albumin and Sepharose

Ulf Nilsson, Göran Magnusson *

Organic Chemistry 2, Chemical Center, The Lund Institute of Technology, University of Lund, P. O. Box 124, S-221 00 Lund, Sweden

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Abstract

The pentasaccharide glycoside corresponding to galactosylgloboside (SSEA-3), β-D-Gal*p*-(1 → 3)-β-D-Gal*p*NAc-(1 → 3)-α-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-1-$OCH_2CH_2Si(CH_3)_3$ (**4**), was synthesized via glycosylation (87%) of 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-[2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**2**) with the glycosyl donor methyl 4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1-thio-β-D-galactopyranoside (**1**), followed by removal of protecting groups. Compound **4** was transformed into the spacer glycoside β-D-Gal*p*-(1 → 3)-β-D-Gal*p*NAc-(1 → 3)-α-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-1-SCH_2CH_2COOH (**10**), which was coupled to bovine serum albumin (BSA), and Sepharose beads, to give the corresponding neoglycoprotein (**11**, 6 mol of saccharide/mol of BSA) and glycosylated Sepharose (**12**, 2.7 μmol of saccharide/mL of sedimented beads), respectively. An improved synthesis of a protected globotetraoside β-D-Gal*p*NAc-(1 → 3)-α-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-1-$OCH_2CH_2SiMe_3$ is also reported.

Keywords: Galactosylgloboside; Thioglycoside donor; 2-(Trimethylsilyl)ethyl glycoside; BSA-conjugate; Sepharose-conjugate

1. Introduction

Galactosylgloboside [also called stage-specific embryonic antigen-3 (SSEA-3), globopentaosyl ceramide, and IV^3GalGb_4Cer] is present in human [1] and green monkey [2] kidney tissue as well as in human teratocarcinoma cells [3]. The amount of

* Corresponding author.

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galactosylgloboside on cell surfaces varies during embryogenesis [4], hence the acronym SSEA-3. Monoclonal antibodies raised against murine morula stage embryos [3] or an octasaccharidic glycosyl ceramide containing the galactosylgloboside structure [5] recognized galactosylgloboside. The heavy- and light-chain sequences of the latter antibodies have been determined [6].

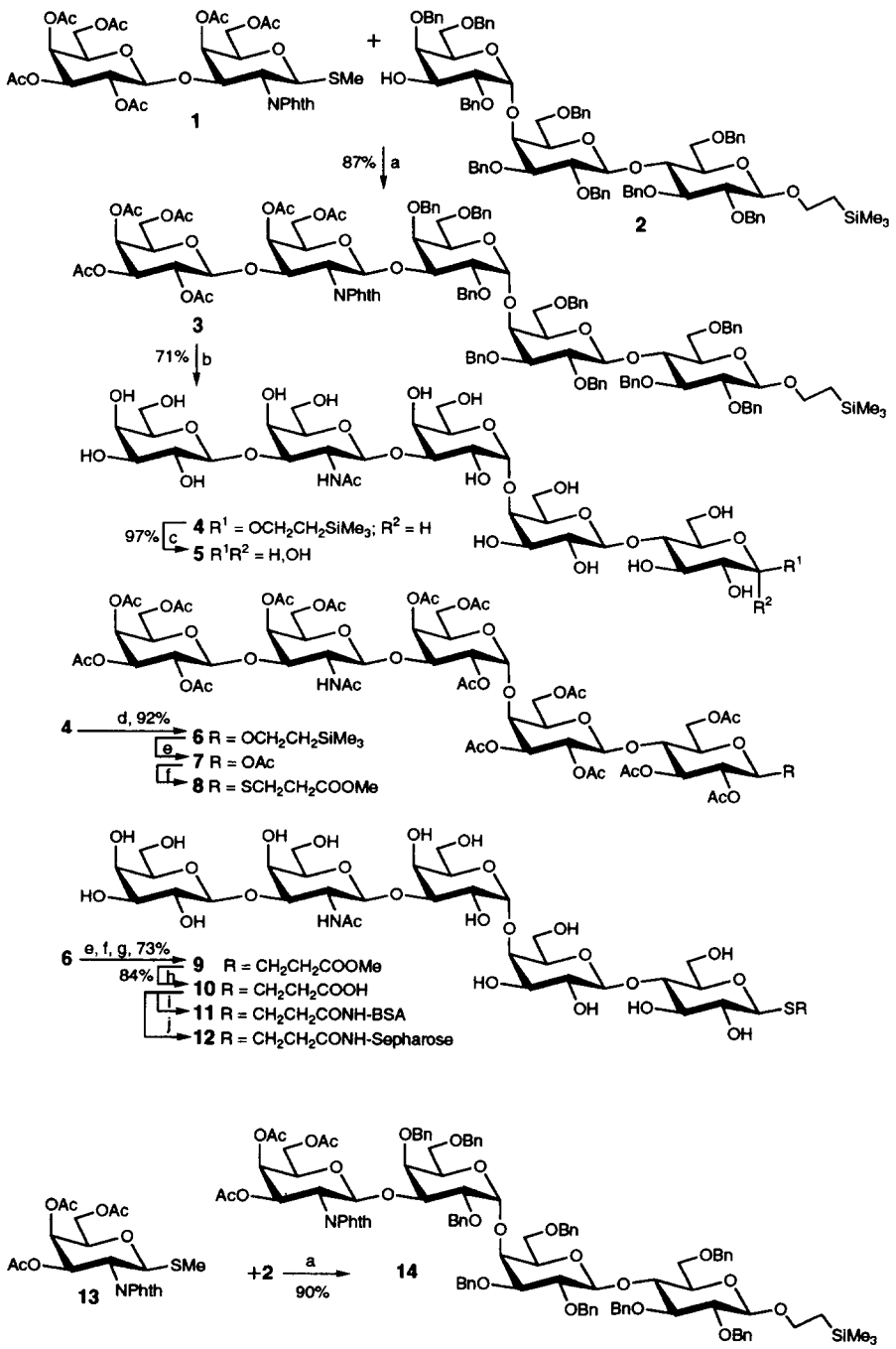
A synthesis of galactosylgloboside has been reported [7], as well as the corresponding sialylated and sulfated galactosylglobosides [8]. We now report an improved synthetic route to the galactosylglobotetraose saccharide ($GalGb_4$), as well as the preparation of the corresponding neoglycoprotein ($GalGb_4$–BSA) and Sepharose conjugate ($GalGb_4$–Sepharose).

The glycosyl donor **1** (obtained in $> 60\%$ yield from the monosaccharide building blocks [9]) was used in the glycosylation of the known [10] acceptor **2**. Thus, a mixture of **1** and **2** was treated in acetonitrile with *N*-iodosuccinimide and trifluoromethanesulfonic acid (NIS/TfOH) [11], which gave the desired pentasaccharide **3** in 87% yield after chromatographic purification. When triethylsilyl trifluoromethanesulfonate (TESOTf) was used instead of TfOH, the reaction was rather unpredictable and the yield varied. Thus, in a typical experiment, compound **3** was obtained in 49% yield together with 50% of silylated **2**. Similar problems were encountered by Ogawa et al. [7], who used the trichloroacetimidate corresponding to **1** and obtained the desired pentasaccharide in only 22% yield, together with 44% of a trichloroacetamide (rearranged trichloroacetimidate). Another example of a less satisfactory glycosylation of **2** is found in our preparation [10] of the globotetraoside **14**, using the β-glycosyl chloride corresponding to **13** in dichloromethane, which gave only a 35% yield (α/β 15:85) and a substantial amount of the phthalimidogalactal. In contrast, treatment of **2** and **13** [12] with NIS/TfOH in acetonitrile, as above, gave **14** in 90% yield as described in the Experimental section. The improved yield of the glycosylation may be due to the use of acetonitrile as solvent (nitrile effect) [13] (Scheme 1).

The pentasaccharide **3** was transformed into the glycoside **4** in one pot by sequential treatment with hydrogen/Pd-on-carbon, hydrazine hydrate, acetic anhydride/pyridine, and *O*-deacetylation in methanolic sodium methoxide. Compound **4** was obtained in 71% overall yield after chromatography. Treatment of **4** with trifluoroacetic acid/dichloromethane [14] gave the reducing pentasaccharide **5** in 97% yield after purification by gel filtration. Acetylation of **4** with acetic anhydride/pyridine gave the fully acetylated glycoside **6** in 92% yield after chromatography. Treatment of **6** with boron trifluoride etherate/acetic anhydride [14] gave a quantitative yield of the anomeric acetate **7** (β/α 96:4), which was used without further purification in the following reactions.

The choice of mercaptopropionic acid as spacer aglycon deserves some comment. A chemically pure oligosaccharide obtained by organic synthesis represents a substantial investment. Therefore, it is important that ensuing chemical reactions, leading to functional derivatives (such as various neoglycoconjugates [15]), proceed in high yield and stereoselectivity. Glycosides carrying a mercaptopropionic acid aglycon [16] (or other mercaptoalkanoic acids) are easily prepared and thioglycosides are normally more stable in the aqueous environment employed with most applications of glycoconjugates.

The crude acetate **7** (containing 4% of the corresponding α-acetate) was treated with



Scheme 1. (a) NIS, TfOH, MeCN, CH_2Cl_2, −50°C. (b) H_2, Pd/C, AcOH, then $H_2NNH_2 \cdot H_2O$, EtOH, then Ac_2O, pyridine, then MeONa, MeOH. (c) CF_3COOH, CH_2Cl_2. (d) Ac_2O, pyridine. (e) $BF_3 \cdot Et_2O$, Ac_2O, CH_2Cl_2. (f) $HSCH_2CH_2COOMe$, $BF_3 \cdot Et_2O$, CH_2Cl_2. (g) MeONa, MeOH. (h) NaOH, H_2O. (i) BSA, TSTU, Et_3N. (j) EAH–Sepharose, *N*-hydroxysuccinimide, $EtN{=}C{=}N(CH_2)_3NMe_2$.

methyl mercaptopropionate and boron trifluoride etherate to give **8** (88%, contaminated by 3% of the unreactive **7**α). Compound **8** was deacetylated to give **9** (73% overall yield from **6**) after removal of **5** by chromatography on silica gel. Hydrolysis of the methyl ester group in **9** gave the spacer glycoside **10** in quantitative yield. Attempted glycosylation of mercaptopropionic acid itself gave the acetylated derivative of **10** in only ~ 15% yield, in contrast to successful glycosylations with lower saccharides [16,17].

Coupling of compound **10** to bovine serum albumin (BSA) was performed using *N*,*N*,*N*′,*N*′-tetramethyl(succinimido)uronium tetrafluoroborate [18] as the promoter, to give the corresponding conjugate **11**. Gel electrophoresis of **11** showed a major band corresponding to a molecular mass of 73–75 kDa, as compared to the band for unconjugated BSA (67 kDa). In a similar coupling reaction, aminohexyl-Sepharose was conjugated with **10**, using *N*-hydroxysuccinimide/1-(3-dimethylaminopropyl)-3-ethylcarbodiimide as promoter, to give the Sepharose conjugate **12**. Sulfur combustion analysis of **11** and **12** showed the saccharide contents to be 6 mol/mol of BSA and 2.7 μmol/mL of sedimented Sepharose beads, respectively. The calculated molecular mass of a BSA conjugate carrying six saccharide residues is ~ 73 kDa, which is in good agreement with the gel electrophoresis data.

2. Experimental

General methods.—Melting points are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. The ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian XL-300 or a Bruker ARX 500 spectrometer. 1,4-Dioxane was used as internal reference (67.4 ppm) in ^{13}C-NMR experiments in D_2O. Concentrations were made using rotary evaporation with bath temperature at or below 40°C. Anhydrous Na_2SO_4 was used as drying agent for the organic extracts in the work-up procedures. TLC was performed on Kieselgel 60 F_{254} plates (Merck). Column chromatography was performed using SiO_2 (Matrex LC-gel; 60 A, 35-70 MY, Grace). BSA and EAH-Sepharose were obtained from Sigma and Pharmacia, respectively.

*2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-benzyl-4-*O*-{2,4,6-tri-*O*-benzyl-3-*O*-[4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-α-*D*-galactopyranosyl}-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**3**).—Methyl 4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1-thio-β-D-galactopyranoside [9] (**1**; 210.3 mg, 0.28 mmol), 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-[2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside [10] (**2**; 282.1 mg, 0.20 mmol), and acid-washed molecular sieves AW-300 (0.2 g) were stirred in dry CH_2Cl_2 (1.4 mL) and dry MeCN (4.1 mL) under Ar for 75 min. The solution was cooled to −50°C and a solution of *N*-iodosuccinimide (90 mg, 0.40 mmol) and trifluoromethanesulfonic acid (13.6 μL, 0.06 mmol) in dry MeCN (0.6 mL) was added. After 1 h, *N*,*N*-diisopropylethylamine (1 mL) was added and the mixture was filtered and concentrated. Flash chromatography of the residue (2:1 heptane–EtOAc) gave **3** (367.7 mg, 87%); $[\alpha]_D^{25}$ +10.3° (*c* 1, $CHCl_3$). ^{1}H-NMR data (500 MHz, $CDCl_3$): δ 5.31 (br d, 1 H, *J* 2.9 Hz, H-4‴), 5.27 (dd, 1 H, *J* 1.1, 3.4 Hz, H-4⁗), 5.23 (d, 1 H, *J* 8.2 Hz, H-1‴), 5.03 (d, 1 H, *J* 10.9 Hz, PhCH_2), 4.98 (d, 1 H, *J* 11.4 Hz, PhCH_2),

4.98 (dd, 1 H, *J* 7.8, 10.3 Hz, H-2''''), 4.91 (d, 1 H, *J* 11.1 Hz, PhCH_2), 4.80 (d, 1 H, *J* 3.6 Hz, H-1''), 4.79 (dd, 1 H, *J* 3.5, 10.3 Hz, H-3''''), 4.69 (d, 1 H, *J* 11.1 Hz, PhCH_2), 4.67 (dd, 1 H, *J* 3.2, 11.2 Hz, H-3'''), 4.59 (dd, 1 H, *J* 8.2, 11.2 Hz, H-2'''), 4.46 (d, 1 H, *J* 7.6 Hz, H-1'), 4.42 (d, 1 H, *J* 7.8 Hz, H-1''''), 4.37 (d, 1 H, *J* 7.7 Hz, H-1), 4.16 (dd, 1 H, *J* 2.9, 10.3 Hz, H-3''), 3.88 (t, 1 H, *J* 9.2 Hz, H-3), 3.78 (dd, 1 H, *J* 3.6, 10.3 Hz, H-2''), 2.12, 2.08, 2.06, 1.93, 1.88, 1.47 (6 s, 3 H each, Ac), 1.03 (m, 2 H, CH_2Si), 0.03 (s, 9 H, $SiMe_3$). Anal. Calcd for $C_{118}H_{133}NO_{33}Si$: C, 66.8; H, 6.3; N, 0.7. Found: C, 66.6; H, 6.5; N, 0.6.

2-(Trimethylsilyl)ethyl 4-O-{4-O-[3-O-(2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranosyl}-β-D-glucopyranoside (**4**).—Compound **3** (500.4 mg, 0.235 mmol) was hydrogenated over Pd/C (10%, 375 mg) in AcOH (25 mL) for 7 h, then filtered through Celite and concentrated. The residue was treated with $H_2NNH_2 \cdot H_2O$ (150 μL) in EtOH (11 mL) at 80°C for 2.5 h, concentrated and co-concentrated with three portions of EtOH and one portion of toluene to remove excess of $H_2NNH_2 \cdot H_2O$. Acetylation with Ac_2O (7.5 mL) and pyridine (7.5 mL) overnight followed by *O*-deacetylation (0.08 M MeONa in MeOH, 20 mL) for 2 h gave crude **4**. Flash chromatography (65:35:5 CH_2Cl_2–MeOH–H_2O) furnished pure **4** (162.2 mg, 71%), according to TLC analysis and ^{1}H- and ^{13}C-NMR; $[\alpha]_D^{25}$ +52° (*c* 1, H_2O). ^{1}H-NMR data (500 MHz, D_2O): δ 4.85 (d, 1 H, *J* 3.9 Hz, H-1''), 4.62 (d, 1 H, *J* 8.5 Hz, H-1'''), 4.45 (d, 1 H, *J* 7.8 Hz, H-1'), 4.43 (d, 1 H, *J* 8.1 Hz, H-1), 4.38 (d, 1 H, *J* 7.7 Hz, H-1''''), 4.31 (br t, 1 H, *J* 6.5 Hz, H-5''), 4.19 (br d, 1 H, *J* 2.9 Hz, H-4''), 4.11 (d, 1 H, *J* 3.1 Hz, H-4'''), 3.51 (dd, 1 H, *J* 7.8, 10.2 Hz, H-2'), 3.46 (dd, 1 H, *J* 7.7, 9.9 Hz, H-2''''), 3.22 (br t, 1 H, *J* 8.4 Hz, H-2), 1.96 (s, 3 H, Ac), 1.01 (dt, 1 H, *J* 5.5, 12.9 Hz, CH_2Si), 0.91 (dt, 1 H, *J* 5.3, 12.9 Hz, CH_2Si), −0.04 (s, 9 H, $SiMe_3$). ^{13}C-NMR data (500 MHz, D_2O): δ 176.2, 105.9, 104.4, 104.1, 102.5, 101.6, 80.7, 79.9, 79.8, 78.4, 76.6, 76.1, 75.9, 75.8, 75.7, 74.1, 73.6, 73.3, 72.1, 71.8, 71.4, 70.1, 69.7, 69.5, 69.1, 68.8, 62.14, 62.09, 61.5, 61.4, 61.2, 52.6, 23.4, 18.7, −1.4. Mass spectrum: Calcd for $C_{37}H_{68}NO_{26}Si$ (M + 1): *m/z* 970.3799. Found: *m/z* 970.3785.

4-O-{4-O-[3-O-(2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranosyl}-β-D-glucopyranose (**5**).—Compound **4** (10.2 mg, 10.5 mmol) was dissolved in CH_2Cl_2 (70 μL) and CF_3COOH (70 μL) under N_2. After 22 min, *n*-propyl acetate (0.95 mL) and toluene (2 mL) were added and the mixture was concentrated. Gel filtration (Sephadex G25) gave pure **5** (8.9 mg, 97%), according to TLC analysis and ^{1}H- and ^{13}C-NMR; $[\alpha]_D^{25}$ +67° (*c* 0.3, H_2O). ^{1}H-NMR data (300 MHz, D_2O): δ 5.20 (d, 1 H, *J* 3.7 Hz, H-1α), 4.89 (d, 1 H, *J* 3.9 Hz, H-1''), 4.66 (d, 1 H, *J* 8.1 Hz, H-1'''), 4.63 (d, 1 H, *J* 7.8 Hz, H-1β), 4.49 (d, 1 H, *J* 7.7 Hz, H-1'), 4.42 (d, 1 H, *J* 7.6 Hz, H-1''''), 4.36 (br t, 1 H, *J* 6.4 Hz, H-5''), 2.00 (s, 3 H, Ac). ^{13}C-NMR data (300 MHz, D_2O): δ 177.9, 107.5, 106.0, 105.7, 103.1, 98.5, 94.6, 82.3, 81.6, 81.5, 79.99, 79.95, 78.2, 77.7, 77.6, 77.4, 77.3, 77.2, 76.7, 75.3, 75.2, 7.9, 74.2, 74.0, 73.6, 73.3, 73.0, 72.9, 71.7, 71.3, 70.7, 70.4, 68.8, 68.3, 65.2, 63.7, 63.3, 63.13, 63.05, 62.8, 62.7, 54.2, 25.0. Mass spectrum: Calcd for $C_{32}H_{55}NNaO_{26}Si$ (M + Na): *m/z* 892.2910. Found: *m/z* 892.2885.

2-(Trimethylsilyl)ethyl 4-O-(4-O-{3-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranosyl]-2,4,6-tri-O-acetyl-

α-D-galactopyranosyl}-2,3,6-tri-O-acetyl-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**6**).—Compound **4** (40.8 mg, 0.0434 mmol) was stirred in Ac_2O (1.5 mL) and pyridine (1.5 mL) for 23 h, then concentrated. Flash chromatography (10:1 toluene–EtOH) of the residue gave **6** (61.5 mg, 92%), $[\alpha]_D^{25}$ +48° (*c* 1, $CHCl_3$). ^{1}H-NMR data (500 MHz, $CDCl_3$): δ 5.65 (d, 1 H, *J* 7.1 Hz, NH), 5.59 (dd, 1 H, *J* 0.8, 3.3 Hz, H-4″), 5.42 (br d, 1 H, *J* 3.3 Hz, H-4‴), 5.34 (dd, 1 H, *J* 0.9, 3.4 Hz, H-4⁗), 5.19 (dd, 1 H, *J* 3.6, 10.5 Hz, H-2″), 5.18 (d, 1 H, *J* 8.0 Hz, H-1‴), 5.18 (t, 1 H, *J* 9.4 Hz, H-3), 5.13 (dd, 1 H, *J* 7.8, 10.4 Hz, H-2⁗), 5.10 (dd, 1 H, *J* 7.7, 10.9 Hz, H-2′), 4.96 (dd, 1 H, *J* 3.4, 10.4 Hz, H-3⁗), 4.92 (d, 1 H, *J* 3.6 Hz, H-1″), 4.87 (dd, 1 H, *J* 8.0, 9.4 Hz, H-2), 4.75 (dd, 1 H, *J* 2.6, 10.9 Hz, H-3′), 4.68 (dd, 1 H, *J* 3.3, 10.8 Hz, H-3‴), 4.61 (d, 1 H, *J* 7.8 Hz, H-1⁗), 4.52 (d, 1 H, *J* 7.7 Hz, H-1′), 4.49 (d, 1 H, *J* 7.9 Hz, H-1), 4.00 (br d, 1 H, *J* 2.6 Hz, H-4′), 3.78 (t, 1 H, *J* 9.3 Hz, H-4), 3.22 (dt, 1 H, *J* 8.0, 10.8 Hz, H-2‴), 2.13, 2.11, 2.10, 2.09, 2.08, 2.07, 2.062, 2.056, 2.05, 2.04, 2.03, 1.96, 1.93 (13 s, 48 H, Ac), 0.89 (m, 2 H, CH_2Si), 0.00 (s, 9 H, $SiMe_3$). Anal. Calcd for $C_{67}H_{97}NO_{41}Si$: C, 50.3; H, 6.1; N, 0.9. Found: C, 50.6; H, 6.1; N, 0.7.

2-(Methoxycarbonyl)ethyl 4-O-(4-O-{3-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranosyl]-2,4,6-tri-O-acetyl-α-D-galactopyranosyl}-2,3,6-tri-O-acetyl-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-1-thio-β-D-glucopyranoside (**8**).—Compound **6** (58.4 mg, 0.0365 mmol) was treated with $BF_3 \cdot Et_2O$ (47 μL, 0.37 mmol) and Ac_2O (152 μL, 1.66 mmol) in dry CH_2Cl_2 (1.9 mL) for 45 min under Ar, then diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated to give a quantitative yield of crude **7** (α/β 4:96), which was used without further purification in the synthesis of **8**. ^{1}H-NMR data (300 MHz, $CDCl_3$): δ 6.22 (d, 1 H, *J* 3.6 Hz, H-1α), 5.67 (d, 1 H, *J* 7.8 Hz, H-1β). Crude **7** was treated with methyl 3-mercaptopropionate (15.8 μL, 0.0146 mmol) and $BF_3 \cdot Et_2O$ [19] (17.5 μL, 0.22 mmol) in dry CH_2Cl_2 (0.3 mL) for 90 min, diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated. Flash chromatography (9:1 toluene–EtOH) gave a mixture of **8** and unreacted α anomer of the acetate **7** (52.3 mg; **8**/**7** 97:3). ^{1}H-NMR data (300 MHz, $CDCl_3$): δ 5.64 (d, 1 H, *J* 7.1 Hz, NH), 5.59 (dd, 1 H, *J* 0.6, 3.4 Hz, H-4″), 5.42 (d, 1 H, *J* 3.4 Hz, H-4‴), 5.34 (dd, 1 H, *J* 3.4 Hz, H-4⁗), 4.75 (dd, 1 H, *J* 2.4, 10.9 Hz, H-3′), 4.67 (dd, 1 H, *J* 3.4, 11.1 Hz, H-3‴), 4.61 (d, 1 H, *J* 7.8 Hz, H-1⁗), 4.52 (d, 1 H, *J* 10.3 Hz, H-1), 4.51 (d, 1 H, *J* 7.4 Hz, H-1′), 3.69 (s, 3 H, OMe), 3.22 (dt, 1 H, *J* 7.6, 10.9 Hz, H-2‴), 2.89 (m, 2 H, SCH_2), 2.67 (br t, 2 H, *J* 7.9 Hz, CH_2CO), 2.14 2.11, 2.10, 2.08, 2.07, 2.063, 2.056, 2.05, 2.04, 1.96, 1.93 (11 s, 48 H, Ac).

2-(Methoxycarbonyl)ethyl 4-O-{4-O-[3-O-(2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranosyl}-1-thio-β-D-glucopyranoside (**9**).—Compound **8**, containing ca. 3% of **7**α (52.3 mg) was treated with methanolic NaOMe (1.2 mL, 13 mM) for 85 min, and the mixture was neutralized with Duolite C26 (H^+) resin, filtered, and concentrated. Flash chromatography (5:5:1 CH_2Cl_2–MeOH–H_2O) of the residue gave **9** (25.9 mg, 73% overall yield from **6**), $[\alpha]_D^{25}$ +72° (*c* 1.1, H_2O). ^{1}H-NMR data (300 MHz, D_2O): δ 4.88 (d, 1 H, *J* 3.7 Hz, H-1″), 4.66 (d, 1 H, *J* 8.3 Hz, H-1‴), 4.55 (d, 1 H, *J* 10.0 Hz, H-1), 4.48 (d, 1 H, *J* 7.6 Hz, H-1′), 4.42 (d, 1 H, *J* 7.6 Hz, H-1⁗), 4.35 (br t, 1 H, *J* 6.4 Hz, H-5″), 4.22 (br s, 1 H, H-4″), 4.15 (br d, 1 H, *J* 2.7 Hz, H-4‴), 3.69 (s, 3 H, OMe), 3.32 (br t,

1 H, *J* 9.0 Hz, H-2), 2.96 (m, 2 H, SCH_2), 2.76 (t, 2 H, *J* 6.9 Hz, CH_2CO), 2.00 (s, 3 H, Ac).

2-Carboxyethyl 4-O-{4-O-[3-O-(2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranosyl}-1-thio-β-D-glucopyranoside (**10**).—Compound **9** (16.7 mg, 0.0172 mmol) was treated with aqueous NaOH (1 mL, 20 mM) for 5 h 40 min and the mixture was neutralized with Duolite C26 (H^+) resin, filtered, and concentrated to give **10** (13.9 mg, 84%), $[\alpha]_D^{25}$ +50° (*c* 0.7, H_2O). ^{1}H-NMR data (300 MHz, D_2O): δ 4.89 (d, 1 H, *J* 3.6 Hz, H-1″), 4.66 (d, 1 H, *J* 8.1 Hz, H-1‴), 4.56 (d, 1 H, *J* 9.9 Hz, H-1), 4.49 (d, 1 H, *J* 7.7 Hz, H-1′), 4.44 (d, 1 H, *J* 7.5 Hz, H-1⁗), 4.37 (br t, 1 H, *J* 6.4 Hz, H-5″), 4.23 (br d, 1 H, *J* 2.2 Hz, H-4″), 4.16 (br d, 1 H, *J* 2.5 Hz, H-4‴), 2.93 (m, 2 H, SCH_2), 2.58 (t, 2 H, *J* 6.9 Hz, CH_2CO), 2.01 (s, 3 H, Ac).

Galactosylgloboside–BSA conjugate (**11**).—Compound **10** (7.7 mg, 0.008 mmol) and *N,N,N′,N′*-tetramethyl(succinimido)uronium tetrafluoroborate [18] (TSTU; 3.76 mg, 0.0126 mmol) were dissolved in 1,4-dioxane/water (0.22 mL, 4:1) and triethylamine (1.1 μL, 0.008 mmol) was added. After 1 h the solution was added to BSA (14.9 mg, 0.23 μmol) in sodium borate buffer (6 mL, 0.1 M, pH 8.5) and the mixture was left for 2.5 h at room temperature and then dialysed for 72 h against distilled water (4 × 1 L). Freeze-drying gave **11** (15.4 mg). Differential combustion analysis of BSA and **11** showed the sulfur content due to the saccharide unit to be 0.1%. This amount corresponds to a degree of binding (number of pentasaccharide molecules per BSA) of 6. SDS-PAGE showed a major band with a molecular mass of 73–75 kDa (BSA showed a different mobility and had a molecular mass of ~ 67 kDa).

Galactosylgloboside–Sepharose affinity beads (**12**).—To a solution of compound **10** (13.9 mg, 14.4 μmol), *N*-hydroxysuccinimide (12 mg, 0.1 mmol), and EAH-Sepharose (15 mL sedimented volume; washed with 0.3 L distilled water on a glass filter funnel) in distilled water (10 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (20 mg, 0.1 mmol). After 17.5 h the Sepharose beads were washed on a glass filter funnel with distilled water (0.3 L divided in small portions) to give **12**. Combustion analysis of EAH-Sepharose and **12** showed the sulfur content to be 0.067 and 0.093%, respectively. The difference corresponds to 2.7 μmol of pentasaccharide molecules per mL of sedimented beads.

2-(Trimethylsilyl)ethyl 2,3,6-tri-O-benzyl-4-O-{2,3,6-tri-O-benzyl-4-O-[2,4,6-tri-O-benzyl-3-O-(4,6-di-O-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)α-D-galactopyranosyl]-β-D-galactopyranosyl}-β-D-glucopyranoside (**14**).—A mixture of methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside [12] (**13**; 53.3 mg, 0.11mmol), 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-[2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside [10] (**2**; 115.8 mg, 0.082 mmol), and molecular sieves AW-300 (0.1 g) was stirred in dry CH_2Cl_2 (0.6 mL) and dry MeCN (1.7 mL) under Ar for 75 min. The solution was cooled to −50°C and a solution of *N*-iodosuccinimide (37 mg, 0.16 mmol) and trifluoromethanesulfonic acid (5.6 μL, 0.025 mmol) in dry MeCN (0.25 mL) was added. After 1 h *N,N*-diisopropylethylamine (0.5 mL) was added and the mixture was filtered and concentrated. Flash chromatography of the residue (3:1 heptane–EtOAc) gave **14** (135.6 mg, 90%). Analytical data were in agreement with those previously published [10].

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References

[1] M.E. Breimer and K.-A. Karlsson, *Biochim. Biophys. Acta*, 755 (1983) 170–177; M.E. Breimer and P.-Å. Jovall, *FEBS Lett.*, 179 (1985) 165–172.
[2] J. Blomberg, M.E. Breimer, and K.-A. Karlsson, *Biochim. Biophys. Acta*, 711 (1982) 466–477.
[3] R. Kannagi, S.B. Levery, F. Ishigami, S. Hakomori, L.H. Shevinsky, B.B. Knowles, and D. Solter, *J. Biol. Chem.*, 258 (1983) 8934–8942.
[4] D. Solter and B.B. Knowles, *Proc. Natl. Acad. Sci. U.S.A.*, 75 (1978) 5565–5569; L.H. Shevinsky, B.B. Knowles, I. Damjanov, and D. Solter, *Cell*, 30 (1982) 697–705; S. Hakomori, E. Nudelman, S.B. Levery, and R. Kannagi, *J. Biol. Chem.*, 259 (1984) 4681–4685.
[5] D.M. Marcus, S. Gilbert, M. Sekine, and A. Suzuki, *Arch. Biochem. Biophys.*, 262 (1988) 620–625.
[6] J.G. Snyder, N. Weng, L. Yu-Lee, and D. M. Marcus, *Eur. J. Immunol.*, 20 (1990) 2673–2677.
[7] S. Nunomura and T. Ogawa, *Tetrahedron Lett.*, 29 (1988) 5681–5684.
[8] R. Miyawaki, H. Ishida, M. Kiso, and A. Hasegawa, *Abstracts of Papers*, XVII Int. Carbohydr. Symp., Ottawa, 1994, Abstract B1.36.
[9] M. Wilstermann and G. Magnusson, *Carbohydr. Res.*, 272 (1995) 1–7.
[10] U. Nilsson, A.K. Ray, and G. Magnusson, *Carbohydr. Res.*, 252 (1994) 117–136.
[11] G.H. Veeneman, S.H. van Leeuwen, and J.H. van Boom, *Tetrahedron Lett.*, 31 (1990) 1331–1334; P. Konradsson, D.R. Mootoo, R.E. McDevitt, and B. Fraser-Reid, *J. Chem. Soc., Chem. Commun.*, (1990) 270–272; P. Konradsson, U.S. Udodong, and B. Fraser-Reid, *Tetrahedron Lett.*, 31 (1990) 4313–4316.
[12] A. Hasegawa, T. Nagahama, H. Ohki, and M. Kiso, *J. Carbohydr. Chem.*, 11 (1992) 699–714.
[13] R. R. Schmidt, M. Behrendt, and A. Toepfer, *Synlett*, (1990) 694–696.
[14] K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmén, G. Noori, and K. Stenvall, *J. Org. Chem.*, 53 (1988) 5629–5647.
[15] G. Magnusson, A.Y. Chernyak, J. Kihlberg, and L.O. Kononov, in Y.C. Lee and R.T. Lee (Eds.), *Neoglycoconjugates: Preparation and Applications*, Academic Press, San Diego, 1994, pp 53–143.
[16] M. Elofsson, B. Walse, and J. Kihlberg, *Tetrahedron Lett.*, 32 (1991) 7613–7616.
[17] U. Nilsson, R.T. Striker, S.J. Hultgren, and G. Magnusson, submitted for publication.
[18] M. Andersson, S. Oscarsson, and L. Öberg, *Glycoconjugate. J.*, 10 (1993) 197–201.
[19] J. Dahmén, T. Frejd, G. Magnusson, and G. Noori, *Carbohydr. Res.*, 114 (1983) 328–330.