2-(Trimethylsilyl)ethyl Glycosides.# Synthesis of the Asialo-GM_1-Tetrasaccharide, Spacer Glycoside, and BSA and Sepharose Glycoconjugates

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The tri- and tetra-saccharide glycosides β-D-GalNAc-(1-4)-β-D-Gal-(1-4)-β-D-Glc-1-OTMSEt and β-D-Gal-(1-3)-β-D-GalNAc-(1-4)-β-D-Gal-(1-4)-β-D-Glc-1-OTMSEt (asialo-GM_1) have been synthesized by sequential glycosylations of a suitably protected 2-(trimethylsilyl)ethyl (TMSEt) lactoside with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl chloride and 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl bromide. The tetrasaccharide glycoside was transformed into the corresponding hemiacetal sugar as well as the 1-chloro sugar. The latter was used for glycosylation of 2-bromoethanol. The resulting glycoside was used to alkylate methyl 3-mercaptopropionate and the resulting spacer glycoside was used for the preparation of the title glycoconjugates.

Asialo GM_1-ceramide [β-D-Gal-(1-3)-β-D-GalNAc-(1-4)-β-D-Gal-(1-4)-β-D-Glc-Cer], as well as the di- and tri-saccharide glycolipids lactosyl ceramide [β-D-Gal-(1-4)-β-D-Glc-Cer] and β-D-GalNAc-(1-4)-β-D-Gal-(1-4)-β-D-Glc-Cer have been reported to be receptors for various bacteria.[1] Furthermore, expression of asialo GM_1 by a limited number of mammalian cells has been suggested to be associated with early events in the activation of resting cells.[2] We report the synthesis of the tri- and tetra-saccharide glycosides **6** and **9** as well as the asialo GM_1 tetrasaccharide **10** and its BSA and Sepharose conjugates **15** and **16**. Syntheses of asialo GM_1-tetrasaccharides have been reported.[3]

The synthetic scheme is based on the use of the 2-(trimethylsilyl)ethyl (TMSEt) group[4,5] for the anomeric protection/deprotection/activation sequence needed for the preparation of glycoconjugates. A similar route was used by Hasegawa and coworkers in their synthesis of GM_{1b} ganglioside.[6] These reports add hydrazinolysis (e.g. **3**→**5**) of phthalimido groups to the list of reactions that do not affect TMSEt glycosides.[4]

The partly benzylated TMSEt lactoside **1**[4] was glycosylated with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl chloride[7] under silver triflate catalysis to give **3** (60%) and **4** (9%) after separation on a silica column. The trisaccharide **3** was de-phthaloylated with hydrazine hydrate, the liberated amino and hydroxy groups were acetylated, and the *O*-acetyl groups were removed by methanolic sodium methoxide to give **5** (94%) in one sequence of reactions without purification of intermediates. Hydrogenolysis of the benzyl groups of **5** and chromatographic purification gave the TMSEt glycoside **6** (94%). Benzylidene protection of OH-4 and OH-6 of **5** gave the glycosyl acceptor **7**[6] (96%), having only one free hydroxy group (OH-3). Glycosylation of **7** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide under silver triflate catalysis gave a mixture of the desired tetrasaccharide glycoside and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose, that could not be easily separated. The mixture was hydrogenolyzed to remove benzyl groups and the product was acetylated and purified by chromatography, which gave pure **8** (74%). The *O*-acetyl groups of **8** were removed with methanolic sodium methoxide to give, after chromatography, the TMSEt glycoside **9** (91%). Treatment of **9** with dichloromethane/trifluoroacetic acid[4] at room temperature for 20 min, followed by chromatographic purification, gave the tetrasaccharide **10** (90%). Compounds **6**, **9**, and **10** are water soluble compounds of interest as *inter alia* inhibitors of bacterial and antibody adhesion.

Treatment of **8** with 1,1-dichloromethyl methyl ether/zinc chloride[5] furnished the corresponding chloride **11** (95%), which was used, without purification, as the glycosyl donor in silver triflate mediated glycosylation of 2-bromoethanol, to give the 2-bromoethyl glycoside[8] **12** (72%). Compound **12** was treated with methyl 3-mercaptopropionate/cesium carbonate, which gave the glycoside **13** (89%) after chromatographic purification. Deacetylation and chromatography as above gave the spacer-arm glycoside **14** (85%), which was used for coupling to bovine serum albumin and aminated Sepharose, essentially as described.[9] According to sulfur combustion analysis, the asialo-GM_1-BSA conjugate **15** contained 12 mol of hapten per mol and the asialo-GM_1-Sepharose conjugate **16** contained 38 μmol of hapten

Part 6. For part 5, see Ref. 4.

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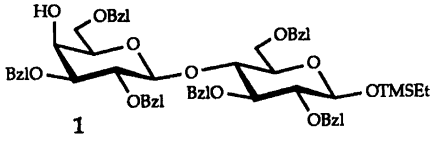
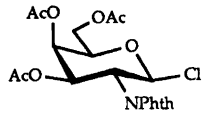


1



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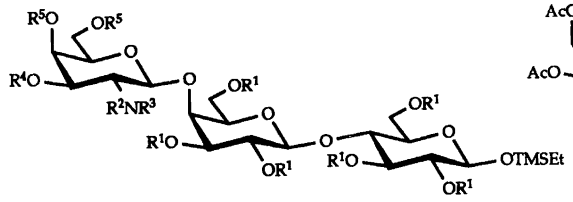
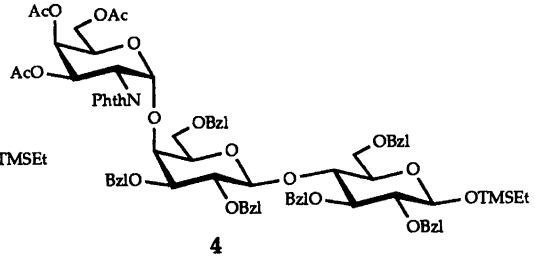


3 R^1=Bzl; R^2,R^3=Phth; R^4=R^5=Ac
5 R^1=Bzl; R^2,=R^4=R^5=H; R^3=Ac
6 R^1=R^2 =R^4=R^5=H; R^3=Ac
7 R^1= Bzl; R^2,=R^4= H; R^3=Ac; R^5=CHPh



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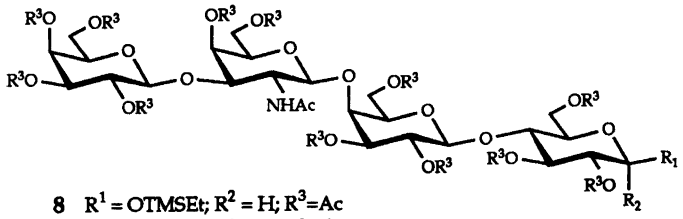

8 R^1 = OTMSEt; R^2 = H; R^3=Ac
9 R^1 = OTMSEt; R^2 = H; R^3=H
10 R^1,R^2 = H,OH; R^3 = H
11 R^1 =H; R^2 = Cl; R^3=Ac
12 R^1=OCH_2CH_2Br; R^2=H; R^3 = Ac
13 R^1=$OCH_2CH_2SCH_2CH_2COOCH_3$; R^2=H; R^3 = Ac
14 R^1=$OCH_2CH_2SCH_2CH_2COOCH_3$; R^2=H; R^3 = H
15 R^1=$OCH_2CH_2SCH_2CH_2CO)_n$BSA,;R^2=H; R^3 = H
16 R^1=$OCH_2CH_2SCH_2CH_2CO)_n$Sepharose; R^2=H; R^3 = H

per gram. Conjugates **15** and **16** are useful *inter alia* as antigen and affinity chromatography gel, respectively, for the preparation and purification of monoclonal antibodies with asialo-GM_1 specificity.

Experimental

Melting points are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. ^1H and ^{13}C NMR spectra were recorded with a Varian XL-300 instrument. Chemical shifts are given in ppm downfield from the signal for Me_4Si, with reference to internal $CHCl_3$ (7.26 ppm). 1,4-Dioxane was used as an internal reference (67.4 ppm) in ^{13}C NMR experiments in D_2O. Thin layer chromatography was performed on Kieselgel 60 F254 plates (Merck). Column chromatography was performed on SiO_2 (Matrex LC-gel; 60A, 35–70 MY, Grace). The glycoside syntheses were performed under dry nitrogen. All evaporations were conducted at or below 40 °C, under reduced pressure.

*2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl 4-*O*-[2,3,6-tri-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* **3** *and 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-[2,3,6-tri-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* **4**. TMSEt lactoside **1**[4] (1.00 g, 1.02 mmol), silver trifluoromethanesulfonate (440 mg, 1.71 mmol), and *N,N,N′,N′*-tetramethylurea (220 µl, 1.8 mmol) were dissolved in dry dichloromethane (10 ml) and activated molecular sieves (4 Å, 1 g) were added. The mixture was cooled to −30 °C under N_2 and a solution of the β-chloride **2**[7] (640 mg, 1.41 mmol) in dichloromethane (5 ml) was added dropwise. The cooling bath was removed and the mixture was left for 16 h at room temperature, then filtered (Celite) and concentrated. The residue was chromatographed (SiO_2; heptane EtOAc 6:1 → 3:1) to give **3** (856 mg, 60 %) and **4** (122 mg, 9 %). Compound **3** had: $[\alpha]_D^{22}$ +3° (*c* 1.2, $CHCl_3$) {lit.[4] $[\alpha]_D^{22}$ +3° ($CHCl_3$); lit.[6] $[\alpha]_D^{22}$ +4.1° (CH_2Cl_2)}. ^1H NMR ($CDCl_3$): δ 6.16 (dd, 1 H, H-3″), 5.56 (d, 1 H, *J* 3.6 Hz, H-4″), 5.38 (d, 1 H, *J* 8.4 Hz, H-1″), 4.36 (d, 1 H, *J* 7.8 Hz, H-1′), 4.21 (d, 1 H, *J* 7.6 Hz,

H-1), 3.01 (dd, 1 H, *J* 7.6, 9.5 Hz, H-2″), 2.21, 2.03, 1.85 (3 s, 3 H each, OAc), 1.02 (m, 2 H, CH_2Si), 0.02 (s, 9 H, $SiMe_3$). Anal. C, H, N.

Compound **4** had: $[\alpha]_D^{22}$ +85° (*c* 0.5, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 6.64 (dd, 1 H, *J* 3.0, 12.1 Hz, H-3″), 5.53 (br d, 1 H, H-4″), 5.30 (d, 1 H, *J* 3.7 Hz, H-1″), 4.31 (d, 1 H, *J* 7.8 Hz, H-1′), 4.29 (d, 1 H, *J* 7.4 Hz, H-1). 2.12, 1.85, 1.84 (3 s, 3 H each, OAc), 1.00 (m, 2 H, CH_2Si), 0.01 (s, 9 H, $SiMe_3$).

*2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl 4-*O*-[2,3,6-tri-*O*-benzyl-4-*O*-(2-acetamido-2-deoxy-β-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* **5**. Compound **3** (3.50 g, 2.95 mmol) was dissolved in ethanol (50 ml), hydrazine hydrate (5 ml) was added, and the mixture was stirred at 85 °C for 75 min and then co-concentrated with ethanol (5×25 ml). The residue was acetylated with acetic anhydride/pyridine (30 ml, 1:1) for 15 h at room temperature and the mixture was co-concentrated with toluene. The residue was deacetylated with methanolic sodium methoxide (0.05 M) for 4 h and the mixture was neutralized with Duolite (H^+) resin, filtered and concentrated. The residue was chromatographed (SiO_2; $CHCl_3$/MeOH 30:1 → 20:1) to give **5** (2.78 g, 94 %); $[\alpha]_D^{22}$ +11° (*c* 0.7, $CHCl_3$) {lit.[6] $[\alpha]_D^{22}$ +58.3° (CH_2Cl_2)}. ^1H NMR ($CDCl_3$): δ 4.65 (d, 1 H, *J* 7.8 Hz, H-1″), 4.42 (d, 1 H, *J* 7.5 Hz, H-1′), 4.38 (d, 1 H, *J* 7.6 Hz, H-1), 1.49 (s, 3 H, NHAc), 1.05 (m, 2 H, CH_2Si), 0.03 (s, 9 H, $SiMe_3$). Anal. C, H, N.

*2-(Trimethylsilyl)ethyl 4-*O*-[4-*O*-(2-acetamido-2-deoxy-β-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* **6**. Compound **5** (230 mg, 0.194 mmol) was dissolved in acetic acid (10 ml), palladium-on-carbon (5 %, 100 mg) was added, and the mixture was stirred under hydrogen (1 atm) for 2 days, then filtered (Celite) and concentrated. The residue was chromatographed (SiO_2; $CHCl_3$/MeOH/H_2O 10:5:1) to give **6** (118 mg, 94 %); $[\alpha]_D^{22}$ −5° (*c* 0.5, H_2O). ^1H NMR (D_2O): δ 4.58 (d, 1 H, *J* 8.3 Hz, H-1″), 4.46 (d, 1 H, *J* 8.1 Hz, H-1′), 4.24 (d, 1 H, *J* 8.0 Hz, H-1), 3.35 (dd, 1 H, *J* 7.8, 9.8 Hz, H-2″), 3.22 (br t, 1 H, H-2), 2.01 (s, 3 H, NHAc), 0.97 (m, 2 H, CH_2Si), −0.01 (s, 9 H, $SiMe_3$). ^{13}C NMR (D_2O): δ 175.8, 104.0, 103.6, 102.4, 79.5, 77.1, 73.7, 73.4, 72.0, 69.2, 68.8, 67.8, 67.7, 67.4, 66.4, 62.0, 61.6, 60.0, 53.7, 23.4, 18.6, −1.5.

*2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-[2,3,6-tri-*O*-benzyl-4-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* **7**. Compound **5** (2.73 g, 2.30 mmol), α,α-dimethoxytoluene (0.75 ml, 5 mmol), and *p*-toluenesulfonic acid (ca. 30 mg) were dissolved in dry acetonitrile (25 ml). The mixture was stirred at room temperature for 18 h, then triethylamine (1 ml) was added and the solvent was removed. The residue was chromatographed (SiO_2; heptane/EtOAc 1:1→1:2) to give **7** (2.8 g, 96 %); $[\alpha]_D^{22}$ +26° (*c* 1.1, $CHCl_3$) {lit.[6] $[\alpha]_D$ +24° (CH_2Cl_2)}. ^1H NMR ($CDCl_3$): δ 5.60 (s, 1 H, PhCH), 4.51 (d, 1 H, *J* 8.3 Hz, H-1″), 4.40, 4.38 (2 d, 1 H each, *J* 7.6, 7.6 Hz, H-1,1′), 1.62 (s, 3 H, NHAc), 1.12 (m, 2 H, CH_2Si), 0.03 (s, 9 H, $SiMe_3$). Anal. C, H, N.

*2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-{2,3,6-tri-*O*-acetyl-4-*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]-β-*D*-galactopyranosyl}-β-*D*-glucopyranoside* **8**. Compound **7** (2.5 g, 1.96 mmol), silver trifluoromethanesulfonate (900 mg, 3.5 mmol), and *N,N,N′,N′*-tetramethylurea (470 mg, 4.00 mmol) were dissolved in dry dichloromethane (50 ml) and activated molecular sieves (4 Å, 2 g) were added. The mixture was cooled to −50 °C under N_2 and a solution of α-acetobromogalactose (1.24 g, 3 mmol) in dichloromethane (10 ml) was added dropwise. The cooling bath was removed and the mixture was left for 22 h at room temperature and filtered (Celite). The filtrate was washed with saturated aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4) and concentrated. The residue was chromatographed (SiO_2; heptane/EtOAc 3:1→5:2) to give a mixture of 2,3,4,6-tetra-*O*-acetyl-α,β-*D*-galactopyranose and the expected tetrasaccharide. The mixture (3.82 g) was dissolved in acetic acid (75 ml), palladium-on-carbon (5 %, 250 mg) was added, and the mixture was stirred under hydrogen (1 atm) for 48 h, then filtered (Celite) and concentrated. The residue was acetylated with acetic anhydride/pyridine (25 ml, 1:1) for 16 h at 60 °C an the mixture was concentrated. The residue was chromatographed (SiO_2; toluene/EtOH 20:1→10:1) to give **8** (2.0 g, 74 %); $[\alpha]_D^{22}$ +1° (*c* 0.6, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 6.85 (d, 1 H, NH), 5.37, 5.32 (2 d, 1 H each, *J* 3.2, 2.6 Hz, H-4″, 4‴), 5.16 (dd, 1 H, *J* 7.6, 10.3 Hz, H-2′), 5.15 (dd, 1 H, *J* 3.3, 9.4 Hz, H-3), 5.10 (dd, 1 H, *J* 7.7, 10.3 Hz, H-2‴), 5.00 (d, 1 H, *J* 7.4 Hz, H-1″), 4.98 (dd, 1 H, *J* 3.4, 11.0 Hz, H-3″), 4.95 (dd, 1 H, *J* 3.4, 10.4 Hz, H-3‴), 4.90 (dd, 1 H, *J* 8.0, 9.7 Hz, H-2), 4.83 (dd, 1 H, *J* 2.7, 10.5 Hz, H-3′), 4.55 (d, 1 H, *J* 7.8 Hz, H-1‴), 4.45 (d, 1 H, *J* 7.9 Hz, H-1), 4.39 (d, 1 H, *J* 7.6 Hz, H-1′), 3.05 (br d, 1 H, H-2″), 2.14–1.96 (10 s, 39 H, Ac), 0.8–1.0 (m, 2 H, CH_2Si), 0.00 (s, 9 H, $SiMe_3$). ^{13}C NMR ($CDCl_3$): δ 172.1–169.2 (C=O), 100.9, 100.2, 100.0, 98.1, 75.8, 73.5, 72.8, 72.7, 72.4, 72.0, 71.5, 71.1, 70.8, 70.6, 69.0, 67.4, 66.9, 62.8, 62.5, 62.1, 61.0, 29.6, 23.5–20.5, 17.8, −1.5. Found: C 49.3; H 6.2; N 1.1. Calc. for $C_{55}H_{81}NO_{33}Si$: C 50.3; H 6.2; N 1.1.

*2-(Trimethylsilyl)ethyl 4-*O*-{4-*O*-[2-acetamido-2-deoxy-3-*O*-(β-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-galactopyranosyl}-β-*D*-glucopyranoside* **9**. Compound **8** (300 mg, 0.22 mmol) was dissolved in methanolic sodium methoxide (10 ml, 0.05 M) and the mixture was stirred for 10 h at room temperature, then neutralized with Duolite (H^+) resin, filtered, and concentrated. The residue was chromatographed (SiO_2; $CHCl_3$/MeOH/H_2O 10:5:1) to give **9** (168 mg, 91 %); $[\alpha]_D^{22}$ −15° (*c* 0.6, MeOH). ^1H NMR (D_2O): δ 4.63 (d, 1 H, *J* 8.4 Hz, H-1″), 4.45 (d, 1 H, *J* 8.05 Hz, H-1′), 4.38 (d, 2 H, *J* 7.6 Hz, H-1, 1‴), 3.35 (dd, 1 H, *J* 7.5, 9.1 Hz, H-2), 3.22 (br dd, 1 H, H-2″), 1.99 (s, 3 H, NHAc),

0.95 (m, 2 H, CH_2Si), 0.02 (s, 9 H, $SiMe_3$). ^{13}C NMR (D_2O): δ 175.7, 105.7, 103.8, 103.2, 102.2, 80.6, 79.3, 76.9, 75.8, 75.6, 75.4, 75.3, 75.25, 73.6, 73.3, 71.9, 71.5, 69.4, 69.2, 68.9, 61.9, 61.5, 60.9, 52.4, 23.3, 18.4, −1.6.

4-O-{4-O-[2-acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-galactopyranosyl}-α,β-D-glucopyranose **10**. Compound **9** (15 mg, 0.0186 mmol) was dissolved in dry dichloromethane (60 μl), trifluoroacetic acid (130 μl) was added under N_2. The mixture was stirred at room temperature for 20 min, *n*-propyl acetate (0.7 ml) was added, and the mixture was co-concentrated with toluene (5×0.5 ml).[5] The residue was chromatographed (SiO_2; CH_2Cl_2/MeOH/H_2O 10:10:1) to give **10** (12 mg, 90%); $[\alpha]_D^{22}$ −1.2° (*c* 1.1, H_2O). ^1H NMR (D_2O): δ 5.20 (d, 0.4 H, *J* 3.6 Hz, H-1α), 4.69 (br d, 0.6 H, H-1β), 4.65 (d, 1 H, *J* 8.3 Hz, H-1″), 4.43 (d, 1 H, *J* 7.5 Hz, H-1′), 4.40 (d, 1 H, *J* 7.8 Hz, H-1‴), 3.39 (br t, 1 H, H-2), 3.25 (br t, 1 H, H-2″), 2.02 (s, 3 H, NHAc). ^{13}C NMR (D_2O): δ 175.7, 105.6, 103.8, 103.7, 103.2, 96.6, 92.6, 80.6, 79.3, 79.2, 76.9, 75.8, 75.6, 75.3, 75.2, 75.1, 74.6, 73.3, 72.2, 71.9, 71.4, 70.9, 69.4, 68.9, 61.8, 61.6, 60.9, 60.8, 60.7, 52.4, 23.3, 12.95, 12.93.

2-Bromoethyl 2,3,6-tri-O-acetyl-4-O-{2,3,6-tri-O-acetyl-4-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]-β-D-galactopyranosyl}-β-D-glucopyranoside **12**. Compound **8** (310 mg, 0.236 mmol) was dissolved in dry chloroform (5 ml), 1,1-dichloromethyl methyl ether (105 μl, 1.18 mmol) and freshly fused zinc chloride (10 mg) were added, and the mixture was stirred under N_2 for 18 h. Dichloromethane (50 ml) was added and the mixture was washed with saturated aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4) and concentrated to give crude **11** (275 mg, 95%); ^1H NMR ($CDCl_3$): δ 6.81 (d, 1 H, *J* 6.6 Hz, NH), 6.23 (d, 1 H, *J* 3.9 Hz, H-1), 5.50 (dt, 1 H, *J* 9.7 Hz, H-3), 5.38 (dd, 1 H, H-4″), 5.34 (br d, 1 H, H-4‴), 5.03 (d, 1 H, *J* 8.1 Hz, H-1″), 4.56 (d, 1 H, *J* 7.9 Hz, H-1‴), 4.42 (d, 1 H, *J* 7.6 Hz, H-1′), 2.14–1.82 (11 s, 39 H, Ac). The crude chloride **11** (265 mg, 0.216 mmol) was dissolved in dry dichloromethane (2 ml) and the solution was added to a cooled (−30°C) mixture of 2-bromoethanol (71 μl, 1 mmol), silver trifluoromethanesulfonate (129 mg, 0.5 mmol), dichloromethane (4 ml) and molecular sieves (4 Å, 0.5 g) under N_2. The mixture was stirred at −30°C for 2 h and at room temperature for 20 h. 2-Bromoethanol (71 μl, 1 mmol) and silver trifluoromethanesulfonate (129 mg, 0.5 mmol) were added and the stirring was continued for 16 h. The mixture was filtered (Celite), diluted with dichloromethane (50 ml), washed with saturated aqueous sodium hydrogencarbonate and water, dried (Na_2SO_4) and concentrated. The residue was chromatographed (SiO_2; toluene/EtOH 20:1) to give **12** (201 mg, 71%); $[\alpha]_D^{22}$ +3.7° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 6.60 (d, 1 H, *J* 6.6 Hz, NH), 5.37, 5.33 (2 br d, 1 H each, *J* 3.2, 3.4 Hz, H-4″, 4‴), 5.02 (d, 1 H, *J* 8.3 Hz, H-1″), 4.61 (d, 1 H, *J* 7.8 Hz, H-1‴), 4.51 (d, 1 H, *J* 7.6 Hz, H-1′), 4.40 (d, 1 H, *J* 7.8 Hz, H-1), 3.45 (m, 2 H, CH_2Br), 2.14–1.96 (10 s, 39 H, Ac). ^{13}C NMR ($CDCl_3$): δ 172.1–169.3 (C=O), 100.93, 100.89, 100.2, 98.2, 75.6, 73.6, 72.9, 72.8, 72.4, 72.1, 72.0, 71.1, 70.8, 70.6, 69.8, 69.6, 69.0, 68.9, 66.8, 62.8, 62.5, 61.9, 61.6, 61.0, 55.6, 30.5, 29.9, 23.6, 20.9–20.5. Found: C 48.6; H 5.5; N 1.1. Calc. for $C_{52}H_{72}BrNO_{33}$: C 47.4; H 5.5; N 1.1.

2-(2-Methoxycarbonylethylthio)ethyl 2,3,6-tri-O-acetyl-4-O-{2,3,6-tri-O-acetyl-4-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]-β-D-galactopyranosyl}-β-D-glucopyranoside **13**. Compound **12** (185 mg, 0.142 mmol) was dissolved in *N,N*-dimethylformamide (3 ml), methyl 3-mercaptopropionate (66 μl, 0.6 mmol) and cesium carbonate (65 mg, 0.2 mmol) were added, and the mixture was stirred at room temperature for 2 h, then diluted with dichloromethane (50 ml), washed with water, dried (Na_2SO_4), and concentrated. The residue was chromatographed (SiO_2; toluene/EtOH 25:1→5:1) to give **13** (169 mg, 89%); $[\alpha]_D^{22}$ +4.3° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 6.51 (d, 1 H, *J* 6.3 Hz, NH), 5.37, 5.33 (2 br d, 1 H each, *J* 3.2, 3.3 Hz, H-4″, 4‴), 5.02 (d, 1 H, *J* 8.1 Hz, H-1″), 4.56 (d, 1 H, *J* 7.8 Hz, H-1‴), 4.48 (d, 1 H, *J* 7.9 Hz, H-1′), 4.40 (d, 1 H, *J* 7.8 Hz, H-1), 3.69 (s, 3 H, $COOCH_3$), 2.79 (t, 2 H, *J* 7.2 Hz, SCH_2), 2.70 (br t, 2 H, *J* 7.6 Hz, CH_2S), 2.59 (br t, 2 H, *J* 7.4 Hz, SCH_2CH_2CO), 2.14–1.96 (10 s, 39 H, Ac). ^{13}C NMR ($CDCl_3$): δ 172.0–169.3 (C=O), 100.9, 100.7, 100.3, 98.1, 75.6, 73.5, 72.9, 72.8, 72.6, 72.4, 72.1, 71.3, 71.1, 70.9, 70.6, 69.6, 69.0, 68.9, 66.9, 62.8, 62.5, 62.0, 61.0, 55.6, 51.8, 34.7, 31.4, 27.3, 23.6, 20.9–20.5. Anal. C, H, N.

2-(2-Methoxycarbonylethylthio)ethyl 4-O-{4-O-[2-acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-galactopyranosyl}-β-D-glucopyranoside **14**. Compound **13** (160 mg, 0.0193 mmol) was dissolved in methanolic sodium methoxide (5 ml, 0.3 M), the mixture was stirred overnight at room temperature, then neutralized with Duolite (H^+) resin, filtered, and concentrated. The residue was chromatographed (SiO_2; $CHCl_3$/MeOH/H_2O 10:5:1) to give **14** (85 mg, 85%); $[\alpha]_D^{22}$ −7° (*c* 1, MeOH). ^1H NMR (D_2O): δ 4.65 (d, 1 H, *J* 8.4 Hz, H-1″), 4.49, 4.41, 4.40 (3 d, 1 H each, *J* 7.9, 7.6, 7.9 Hz, H-1, 1′, 1‴), 4.11, 4.06 (2 d, 1 H each, *J* 2.9, 2.7 Hz, H-4″, 4‴), 3.68 (s, 3 H, OMe), 3.37 (dd, 1 H, *J* 7.8, 9.8 Hz, H-2″), 3.27 (br t, 1 H, *J* 8.6 Hz, H-2), 2.83 (br t, 2 H, SCH_2), 2.80 (br t, 2 H, CH_2S), 2.70 (br t, 2 H, SCH_2CH_2CO), 2.00 (s, 3 H, NHAc).^{13}C NMR (D_2O): δ 176.1, 175.7, 105.7, 103.8, 103.2, 102.9, 80.6, 79.3, 79.2, 76.9, 75.8, 75.6, 75.3, 75.2, 75.1, 73.5, 73.3, 71.9, 71.5, 69.9, 69.4, 68.9, 67.6, 67.3, 67.1, 66.2, 61.8, 61.6, 61.5, 60.9, 60.8, 53.2, 52.4, 35.0, 31.6, 27.3, 23.3.

BSA-tetrasaccharide conjugate **15**. Compound **14** (50 mg, 59.4 μmol) was dissolved in ethanol (2 ml), hydrazine hydrate (85%, 0.25 ml) was added and the mixture was stirred at room temperature for 18 h, then co-concentrated

with ethanol (4×5 ml). The residue was dissolved in water (ca. 5 ml), freeze dried, and the residue was dissolved in dry dimethyl sulfoxide (1 ml). Hydrogen chloride in dioxane (4 M, 105 µl) and *tert*-butyl nitrite (18 µl, 0.15 mmol) in dry dimethyl sulfoxide (0.1 ml) were added and the mixture was stirred at room temperature for 30 min. A solution of sulfamic acid (10 mg, 0.11 mmol) in dimethyl sulfoxide (0.1 ml) was added and after 15 min, the mixture was added dropwise, with stirring, to a solution of bovine serum albumin (BSA, 60 mg, 0.92 µmol) in sodium tetraborate/potassium hydrogencarbonate buffer (2.5 ml, 0.08 M $Na_2B_2O_7$ and 0.35 M $KHCO_3$). The pH was maintained at 9.0–9.4 by additions of sodium hydroxide (1 M). The mixture was stirred at room temperature overnight, then dialyzed against distilled water for 3 days. Freeze-drying of the dialyzed material gave the BSA-conjugate **15**. Sulfur combustion analysis showed 12 tetrasaccharide hapten molecules per BSA molecule.

Sepharose–tetrasaccharide conjugate **16**. Compound **14** (20 mg, 0.024 mmol) was dissolved in ethanol (2 ml), hydrazine hydrate (85 %, 250 µl) was added and the mixture was stirred at room temperature overnight, then co-concentrated with ethanol (4×1 ml). The residue was dissolved in water (2 ml), freeze dried and the residue was dissolved in dry dimethyl sulfoxide (0.3 ml). Hydrogen chloride in dioxane (4 M, 30 µl) and *tert*-butyl nitrite (29 µl) in dry dimethyl sulfoxide (0.15 ml) were added, and the mixture was stirred at room temperature for 15 min. A solution of sulfamic acid (3 mg) in dimethyl sulfoxide (0.030 ml) was added and after 15 min, the mixture was added dropwise, with stirring, to a suspension of preswelled aminated AH Sepharose 4B (0.5 g; Pharmacia, Sweden) in sodium tetraborate/potassium hydrogencarbonate buffer (2.5 ml, 0.08 M $Na_2B_2O_7$ and 0.35 M $KHCO_3$). The pH was maintained at 9.0–9.3 by additions of sodium hydroxide (1 M). The mixture was stirred at room temperature overnight, then washed with water and two aliquots of aqueous sodium chloride (0.5 M and 1 M; the latter solution containing 0.002 % NaN_3) to give an aqueous suspension of the Sepharose conjugate **16**, which was stored at 4 °C. An analytical sample was washed with water and dried. Sulfur combustion analysis showed 38 µmol of tetrasaccharide hapten molecules per gram of conjugate.

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