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Note

The synthesis of 16-mercaptohexadecanyl glycosides for biosensor applications

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Abstract

The P^k trisaccharide and the central disaccharide element of asialo GM_1 activated as their trichloroacetimidates were each used to glycosylate 16-(*p*-toluensulfonyloxy)hexadecanol **1**. Displacement of the tosyl group by thiocyanate followed by sodium borohydride reduction and saponification afforded oligosaccharide 16-mercaptohexadecanyl glycosides that were isolated as the corresponding disulfides **6** and **17** unless oxygen was rigorously excluded from the solvents used for work-up. Dithiothreitol reduction of disulfides and subsequent isolation under an inert atmosphere with degassed solvents gave the thiols **7** and **18**. Chemisorption of ω-glycosyl alkanethiols and alkanethiols onto gold electrodes produces self-assembled monolayers that can act as amperometric biosensors for the detection of proteins that bind to the immobilized oligosaccharide epitope.

Keywords: 16-mercaptohexadecanyl glycosides; P^k trisaccharide; Asialo GM_1; Asialo GM_2; Amperometric biosensors

Carbohydrates serve as attachment sites for toxins, bacteria, and viruses, and can, therefore, be employed for the detection of those agents in physiological samples. However, the effective design of a rapid and sensitive assay requires the coupling of the protein–sugar association process to a change in a physical quantity such as an electrical current. Amperometric biosensors [1] utilize the electron transfer properties of self-assembled monolayers (SAMs) created by chemisorption of alkanethiols and ω-functionalized alkanethiols mixtures from ethanol solution onto a gold surface [2–4]. If the chain length of the alkanethiol and ω-functionalized alkanethiols are matched in the biosensor, the ligand does not perturb the dielectric monolayer until a specific binding event occurs between it and a protein receptor. Two recognition systems that involve the interaction of bacteria or their toxins with glycolipids were chosen for development of amperometric biosensors. The disaccharide β-D-Gal*p*NAc-(1→4)-β-D-Gal*p*, a fragment of some human glycosphingolipids (GM_1, GM_2, for instance), represents the minimal structural element recognized by adhesins, proteins with sugar binding sites, located on filamentous structures of *Pseudomonas aeruginosa* and *Candida albicans*, a dimorphic yeast [5–7]. The P^k trisaccharide, the carbohydrate portion of the glycosphingolipid Gb_3, is a receptor for toxins [8–12] secreted by pathogenic bacteria such as *Shigella dysenteriae* [8]

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and certain strains of uropathogenic *Eshericia coli*. Covalent attachment of a neoglycoprotein to gold surfaces via thiols has been reported for biosensor detection of P-fimbriated *E. coli* bacteria [13].

The syntheses of 16-mercaptohexadecanyl glycosides of the disaccharide β-D-Gal*p*NAc-(1→4)-β-D-Gal*p* and P^k trisaccharide α-D-Gal*p*-(1→4)-β-D-Gal*p*-β-D-(1→4)-Glc*p* are reported by a method that is general and represents one possibility for the fabrication of a carbohydrate-based biosensor by immobilizing carbohydrate-ligands on the surface of gold electrodes [3]. The scheme uses an aglycone prepared from commercially available 1,16-dihydroxyhexadecane. Controlled tosylation gave the monotosylated diol **1** in 42% yield, and following O-glycosylation of this tether, displacement of the tosyl group by thiocyanate introduced a latent thiol function. Reduction of the thiocyanate with sodium borohydride gave a thiol and simultaneously hydrolyzed, partially or completely, the ester-protecting groups of the carbohydrate. Manipulation in solvents without exclusion of dissolved oxygen resulted in almost quantitative oxidation of thiol to disulfide. Thiols were prepared immediately prior to use by reduction under controlled conditions. In fact, either thiol or disulfide are known to undergo chemisorption to gold surfaces [2–4,14].

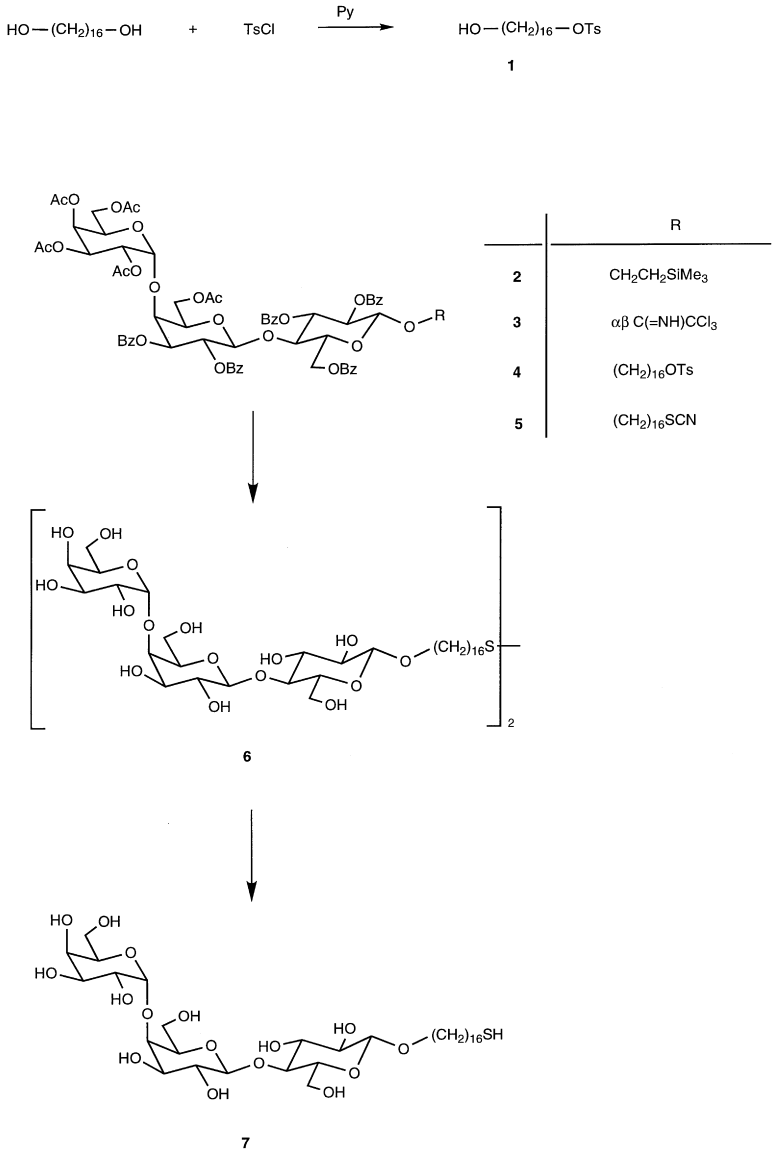
Trisaccharide **2**, prepared according to published procedures [15], was converted into an anomeric mixture of trichloroacetimidates **3** and reacted with alcohol **1** in CH_2Cl_2 in the presence of a catalytic amount of trimethylsilyl trifluoromethanesulfonate. The tosyloxy group of the trisaccharide glycoside **4** was displaced by thiocyanate to provide the protected trisaccharide glycoside **5**. Reduction of thiocyanate by the action of sodium borohydride [16] followed by saponification of acyl groups gave the trisaccharide ligand **6**. The disulfide **6** was converted to the thiol **7** by reduction with dithiothreitol (DTT) under argon followed by reverse-phase high-performance liquid chromatography (HPLC).

There are two synthetic strategies to build the β-Gal*p*NAc(1→4)Gal*p* disaccharide linkage. One involves glycosylation of 4-O-unprotected galactose derivatives with various *N*-acetyl and *N*-phthaloyl galactosamine donors [17]. Another route is selective glycosylation of a galactose 3,4-diol derivative **10** by a 2-azido-2-deoxy-galactopyranosyl bromide derivative **11**. According to Paulsen [18] and Sinäy [19], the reaction promoted by heterogeneous activators leads mainly to a β-(1→4) glycosidic linkage. This method has some advantages, since it avoids using a comparatively expensive galactosamine starting material, and the absence of a protecting group at O-3 of the acceptor avoids steric and electronic influences on the reactivity of the 4-hydroxyl group.

Acetonation of 2-(trimethylsilyl)ethyl galactopyranoside followed by benzylation of **8** [15] gave **9** and then the 3,4-diol acceptor **10** [20,21] after acetal hydrolysis. Glycosylation of **10** by glycosyl bromide **11** [22,23] in dichloromethane in the presence of silver silicate on alumina [24] as the promoter provided disaccharide **12** in 74% yield. The site of galactosylation was confirmed after reduction of the azido function and removal of benzyl groups followed by per-acetylation to give the acetamido derivative **13**. Comparison of the ^1H NMR spectra of disaccharides **12** and **13** shows that, after acetylation, the signal of H-3 had shifted downfield by more than 1 ppm from the 3.68–3.57 ppm area to 4.91 ppm, whereas the chemical shift of H-4 did not change significantly.

The 2-(trimethylsilyl)ethyl glycoside **13** was converted into the trichloroacetimidate **14**. Glycosylation of **1** by **14** gave the disaccharide glycoside **15**. Nucleophilic displacement of the tosyloxy group by thiocyanate was carried out as described for the synthesis of **5**. Reduction of thiocyanate **16** accompanied by deacetylation afforded the disaccharide **17**. This disulfide was reduced to the thiol **18** with DTT and isolated by HPLC.

The synthetic scheme compares favourably with other procedures used to generate substituted alkanethiols [2,3,25]. However, ω-glycosyl alkanethiols exhibit a strong propensity for oxidation to the corresponding disulfides and any isolation protocol that employed solvents that had not been degassed resulted in virtually quantitative yields of the disulfides **6** and **17**. In order to monitor the oxidation state of the sulfur, ^1H NMR of the -CH_2S- group was diagonstic. Methanol-d_4 was the preferred solvent since D_2O solution gave only broad signals, presumably owing to the formation of micelles. The chemical shift of the -CH_2SH methylene protons were observed at δ 2.48 ppm, while the those of the corresponding protons in disulfide derivatives -CH_2S-SCH_2- had δ 2.68 ppm. This was particularly useful since mass spectral measurements in the commonly employed Cleland's matrix (dithiothreitol:dithioerythitol, 5:1) reduces any disulfide to thiol. Electrospray mass

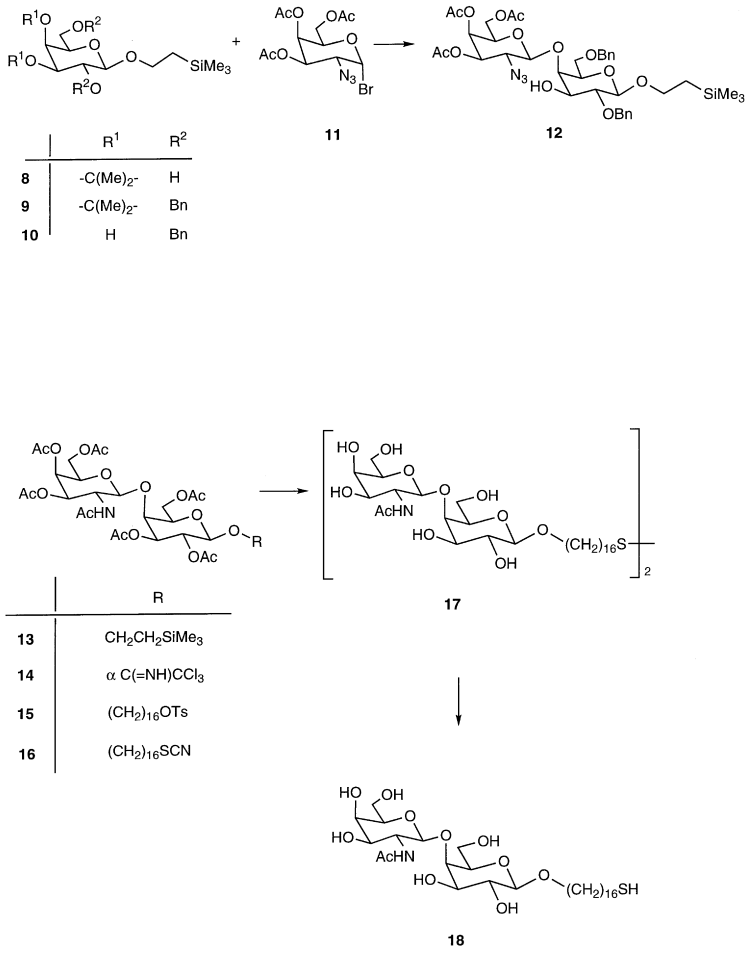


spectra were recorded to observe the disulfides. The deacylated ω-glycosyl alkanethiols **7** and **18** or their disulfide derivatives exhibited rather poor solubility characteristics and were poorly soluble in water and ethanol. Warm methanol was the most effective protic solvent.

Amperometric biosensors, prepared by incorporation of these glycoconjugates into SAMs, are able to detect verotoxin binding to the P^k trisaccharide **6**, and pilin protein or pilin peptide binding to the asialo GM_1 disaccharide **17** [1b].

1. Experimental

General methods.—Optical rotations were measured on a Perkin-Elmer 241 polarimeter in a 1 dm cell at ambient temperature (22 ± 2 °C). Analytical thin-layer chromatography (TLC) was performed on Silica Gel 60-F_{254} (Merck) with detection by quenching of fluorescence and/or by charring with 10% H_2SO_4 in ethanol solution followed by heating at 180 °C. Millex-HV (0.45 μM) filter units were from Millipore (Missisuaga, ON). Column chromatography was performed on Silica Gel 60 (Merck, 40–60 μm), and solvents were distilled prior to use. Sep-Pak C_{18} reverse-phase cartridges (Waters, Missisuaga, ON) were conditioned prior to use by washing with MeOH (10 mL) and water (20 mL). ^{1}H NMR spectra were recorded at 360 MHz (Bruker WM-360) or at 500 MHz (Varian Unity 500) in $CDCl_3$ (referenced to residual $CHCl_3$ at 7.24 ppm), CD_3OD (referenced to residual CD_2HOD at 3.3 ppm), or D_2O (referenced to



internal or external acetone at 2.225 ppm). Mass spectrometric analysis was performed by positive mode electrospray ionization on a Micromass ZabSpec Hybrid Sector-TOF. The liquid carrier was infused into the electrospray source by means of a Harvard syringe pump at a flow rate of 10 μL/min. The sample solution was introduced via a 1 μL loop injector. Prepurified nitrogen was used as a spray pneumatic aid and filtered nitrogen as the bath gas, heated at ca. 80 °C. For low resolution, the mass spectra were acquired by magnet scan at a rate of 10 s/decade. For exact mass measurements, the spectra were obtained by voltage scan at a rate of 10 s/decade. Data acquisition and processing was achieved by using the OPUS software package on a Digital Alpha station with VMS operating system. All commercial reagents were used as supplied and solvents were distilled from appropriate desiccants prior to use [26].

*16-(*p-*Toluenesulfonyloxy)hexadecanol* (**1**).—Tosyl chloride (0.8 g, 4.21 mmol) was added to a solution of 1,16-dihydroxyhexadecane (1.1 g, 4.25 mmol) in dry pyridine (10 mL). After 2 h the mixture was concentrated, diluted with acetone (20 mL), silica gel (5 g) was added and acetone was removed in vacuum. The solid was slurred onto a silica gel column and eluted with pentane–ethyl acetate (2:1) to yield **1** (748 mg, 42%), m.p. 58 °C. ^{1}H NMR: δ_H 7.78 and 7.32 (2d, 4 H, J = 8.1 Hz, arom.), 3.99 (t, 2H, ^3J = 6.6 Hz, CH_2OTs), 3.62 (t, 2 H, ^3J = 6.5 Hz, CH_2OH), 2.43 (s, 3 H, CH_3), 1.63–1.50 (m, 2 H, CH_2CH$_2$OTs), 1.32–1.20 (m, 26 H, 13 CH_2). Anal calcd for $C_{23}H_{40}SO_4$ (412.63): C, 66.95; H, 9.77; S, 7.77. Found: C, 66.96; H, 9.90; S, 7.73.

*16-(*p-*Toluenesulfonyloxy)hexadecanyl 4-*O-*[6-*O-*acetyl-2,3-di-*O-*benzoyl-4-*O-*(2,3,4,6-tetra-*O-*acetyl-*α-D-*galactopyranosyl)-*β-D-*galactopyranosyl]-2,3,6-tri-*O-*benzoyl-*β-D-*glucopyranoside* (**4**).—Trifluoroacetic acid (2 mL) was added to a solution of trisaccharide **2** [15] (445 mg, 0.33 mmol) in CH_2Cl_2 (4 mL). After 45 min, EtOAc (2 mL) and toluene (2 mL) were added and the mixture was concentrated, co-evaporated with toluene three times,

and dried in vacuum. A mixture of the residue with Cl_3CCN (1 mL) in CH_2Cl_2 (5 mL) was cooled at 0 °C and DBU (40 μL) was added. After 30 min the mixture was concentrated and chromatographed on silica gel with toluene–acetone (3:1) to give an α/β mixture of imidates **3** (396 mg, 86%). ^{1}H NMR: δ_H 8.59 (β-NH), 8.53 (α-NH).

The imidate mixture **3** (100 mg, 72 μmol), monotosylated diol **1** (36 mg, 87 μmol), and 4Å molecular sieves (200 mg) in dry CH_2Cl_2 (4 mL) were stirred for 1 h. Then TMSOTf (8 μL, 40 μmol) was added. After 2 h, triethylamine (0.1 mL) was added and the solids were removed by filtration. The filtrate was concentrated and dried in vacuum. Chromatography of the residue on silica gel with pentane–ethyl acetate (3:2) gave **4** (84 mg, 71%), $[\alpha]_D$ + 75.2° (*c*. 0.9; $CHCl_3$). ^{1}H NMR: δ_H 8.02–7.16 (m, 29 H, arom.), 5.73 (t, 1 H, $J_{2,3} \simeq J_{3,4} = 9.2$ Hz, H-3), 5.61 (dd, 1 H, $J_{1',2'} = 7.8$ Hz, $J_{2',3'} = 10.8$ Hz, H-2′), 5.45 (dd, 1 H, $J_{3'',4''} = 3.2$ Hz, $J_{4'',5''} = 1.1$ Hz, H-4″), 5.33 (dd, 1 H, $J_{1,2} = 7.8$ Hz, H-2), 5.28 (dd, 1 H, $J_{2'',3''} = 11.1$ Hz, H-3), 5.08 (dd, 1 H, $J_{1'',2''} = 3.6$ Hz, H-2″), 5.02 (dd, 1 H, $J_{3',4'} = 2.8$ Hz, H-3′), 3.92 (d, 1 H, H-1″), 4.80 (d, 1 H, H-1′), 4.64 (d, 1 H, H-1), 4.59 (dd, 1 H, $J_{6a,6b} = 12.0$ Hz, $J_{5,6a} = 2.0$ Hz, H-6a), 4.44 (dd, 1 H, $J_{5,6b} = 4.7$ Hz, H-6b), 4.41 (broad t, 1 H, H-5″), 4.35 (t, 1 H, $J_{3',4'} \simeq J_{4',5'} = 9.4$ Hz, H-4′), 4.12 (d, 1 H, H-4′), 4.00 (t, 2 H, $^3J = 6.5$ Hz, CH_2OTs), 3.83 (ddd, 1 H, H-5), 3.80–3.70 (m, 3 H, H-6″a, CH_2O, H-6′a), 3.66 (dd, 1 H, $J_{6'a,6'b} = 11.1$ Hz, $J_{5',6'b} = 7.4$ Hz, H-6′b), 3.54 (dd, 1 H, $J_{5'',6''b} = 5.7$ Hz, $J_{6''a,6''b} = 10.8$ Hz, H-6″b), 3.49 (broad t, 1 H, H-5′), 3.38 (dt, 1 H, $^2J = 9.7$ Hz, $^3J = 6.8$ Hz, CH_2O), 2.42 (s, 3 H, Me), 2.02, 1.97, 1.95, 1.92, 1.91 (5s, 15 H, 5 Ac), 1.60 (p, 2 H, $^3J = 7.3$ Hz, CH_2CH_2OTs), 1.40–0.86 (m, 26 H, 13 CH_2). Anal. calcd for $C_{86}H_{100}SO_{29}$ (1629.79) C, 63.38; H, 6.18; S, 1.97. Found: C, 63.42; H, 6.12; S, 2.00.

16-(Thiocyano)hexadecanyl 4-O-[6-O-acetyl-2,3-di-O-benzoyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (**5**).—A solution of tosylate **4** (84 mg, 51 μmol) and KSCN (50 mg, 0.5 mmol) in DMF (2 mL) was stirred at 80 °C for 2 h. The mixture was concentrated, dissolved in CH_2Cl_2 (30 mL), washed with water, and concentrated again. Chromatography of the residue on silica gel with pentane–ethyl acetate (3:2) gave **5** (67.4 mg, 86%), $[\alpha]_D$ + 81.2° (*c*. 0.6; $CHCl_3$). IR 2153.6 ($\nu_{c\text{-}scn}$). ^{1}H NMR: δ_H 8.02–7.16 (m, 25 H, arom.), 5.73 (t, 1 H, $J_{2,3} \simeq J_{3,4} = 9.2$ Hz, H-3), 5.61 (dd, 1 H, $J_{1',2'} = 7.8$ Hz, $J_{2',3'} = 10.8$ Hz, H-2′), 5.45 (dd, 1 H, $J_{3'',4''} = 3.2$ Hz, $J_{4'',5''} = 1.1$ Hz, H-4″), 5.33 (dd, 1 H, $J_{1,2} = 7.8$ Hz, H-2), 5.28 (dd, 1 H, $J_{2'',3''} = 11.1$ Hz, H-3), 5.08 (dd, 1 H, $J'_{1'',2''} = 3.6$ Hz, H-2″), 5.02 (dd, 1 H, $J_{3',4'} = 2.8$ Hz, H-3′), 3.92 (d, 1 H, H-1″), 4.80 (d, 1 H, H-1′), 4.64 (d, 1 H, H-1), 4.59 (dd, 1 H, $J_{6a,6b} = 12.0$ Hz, $J_{5,6a} = 2.0$ Hz, H-6a), 4.44 (dd, 1 H, $J_{5,6b} = 4.7$ Hz, H-6b), 4.41 (broad t, 1 H, H-5″), 4.35 (t, 1 H, $J_{3',4'} \simeq J_{4',5'}$ 9.4 Hz, H-4′), 4.12 (d, 1 H, H-4′), 3.83 (ddd, 1 H, H-5), 3.80–3.70 (m, 3H, H-6″a, CH_2O, H-6′a), 3.66 (dd, 1 H, $J_{6'a,6'b} = 11.1$ Hz, $J_{5',6'b} = 7.4$ Hz, H-6′b), 3.54 (dd, 1 H, $J_{5'',6''b} = 5.7$ Hz, $J_{6''a,6''b} = 10.8$ Hz, H-6″b), 3.49 (broad t, 1 H, H-5′), 3.38 (dt, 1 H, $^2J = 9.7$ Hz, $^3J = 6.8$ Hz, CH_2O), 2.91 (t, 2 H, $^3J = 7.3$ Hz, CH_2SCN), 2.02, 1.97, 1.95, 1.92, 1.91 (5s, 15 H, 5 Ac), 1.79 (p, 2 H, $^3J = 7.3$ Hz, CH_2CH_2SCN), 1.40–0.86 (m, 26 H, 13 CH_2). Anal. calcd for $C_{80}H_{93}$ SNO_{26} (1516.65): C, 63.35; H, 6.18; N, 0.92; S, 2.11. Found: C, 63.41; H, 5.82; N, 0.91; S, 2.70.

Bis{16-[4-O-[4-O-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranosyloxy]hexadecanyl} disulfide (**6**).—To a solution of **5** (60 mg, 39 μmol) in dry MeOH (4 mL) solid sodium borohydride (~40 mg) was added under argon. After stirring for 2 h at 45 °C, the mixture was concentrated and dissolved under gentle reflux in a solution of NaOH (50 mg) in water (10 mL). After stirring overnight at 45 °C, the mixture was neutralized with Dowex resin 50×8-400 (H^+ form) and then applied to a Sep-Pak (C-18) cartridge (Waters). The cartridge was washed with 20, 40, 60, and 80% solution of MeOH in water, then with pure MeOH. The methanol fractions containing sugar were concentrated to give **6** (24.4 mg, 81%), $[\alpha]_D$ + 43.5° (*c*. 0.8; H_2O). ^{1}H NMR (CD_3OD, 45 °C): δ_H 4.95 (d, 1 H, $J_{1'',2''} = 3.8$ Hz, H-1″), 4.42 (m, X of ABX system, 1 H, H-1′), 4.27 (d, 1 H, $J_{1,2} = 7.8$ Hz, H-1), 4.24 (ddd, 1 H, $J_{4'',5''} = 1.2$ Hz, $J_{5'',6''a} = 5.0$ Hz, $J_{5'',6''b}$ 6.6 Hz, H-5″), 3.98 (broad d, 1 H, $J_{4',5'} = 1.2$ Hz, H-4′), 3.91 (dd, 1 H, $J_{3'',4''} = 3.2$ Hz, H-4″), 3.90–3.66 (m 10 H, H-6a, H-6b, H-5′, H-6′a, H-6′b, H-2″, H-3″, H-6″a, H-6″b, CH_2O), 3.58–3.49 (m, 5 H, H-3, H-4, H-2′, H-3′, CH_2O), 3.38 (dt, 1 H, $J_{5,6a} = J_{5,6b} = 9.5$ Hz, $J_{5,4} = 3.8$ Hz, H-5), 3.23 (dd, 1 H, $J_{2,3} = 9.0$ Hz, H-2), 2.68 (t, 2 H, $^3J = 7.3$ Hz, CH_2S-SCH_2), 1.67 (p, 2 H, $^3J = 7.0$ Hz, CH_2CH_2S), 1.61 (p, $^3J = 6.9$ Hz, CH_2CH_2O), 1.42–1.26 (m, 24 H, 12 CH_2). Electrospray MS: 1541.8 (calcd for $C_{68}H_{126}S_2O_{32}Na$ 1541.8).

16-(Mercapto)hexadecanyl 4-O-[4-O-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**7**).—Dithiothreitol (10 mg, 70 μmol) was added to a solution of **6** (10.8 mg, 14 μmol) in degassed water (5 mL) and the pH was adjusted to 8 with a sat. solution of NH_4HCO_3. After stirring for 24 h under argon, the mixture was heated to dissolve the precipitated residue, filtered through a Millex-HV 0.45 μm membrane filter (Millipore), and chromatographed on a C-18 reverse phase HPLC column with a step gradient of 20, 40, 60, 80, and 100% aqueous MeOH. The methanol fractions containing sugar were concentrated to give faster moving **7** (2 mg, 18.5%), $[\alpha]_D$ +40.1° (*c*. 0.5; MeOH). ^{1}H NMR (CD_3OD, 45 °C): δ_H 4.95 (d, 1 H, $J_{1'',2''}$ = 3.8 Hz, H-1″), 4.42 (m, X of ABX system, 1 H, H-1′), 4.27 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1), 4.24 (ddd, 1 H, $J_{4'',5''}$ = 1.2 Hz, $J_{5'',6''a}$ = 5.0 Hz, $J_{5'',6''b}$ = 6.6 Hz, H-5″), 3.98 (broad d, 1 H, $J_{4',5'}$ = 1.2 Hz, H-4′), 3.91 (dd, 1 H, $J_{3'',4''}$ = 3.2 Hz, H-4″), 3.90–3.66 (m 10 H, H-6a, H-6b, H-5′, H-6′a, H-6′b, H-2″, H-3″, H-6″a, H-6″b, CH_2O), 3.58–3.49 (m, 5H, H-3, H-4, H-2′, H-3′, CH_2O), 3.38 (dt, 1 H, $J_{5,6a}$ = $J_{5,6b}$ = 9.5 Hz, $J_{5,4}$ = 3.8 Hz, H-5), 3.23 (dd, 1 H, $J_{2,3}$ = 9.0 Hz, H-2), 2.48 (t, 2 H, ^3J = 7.2 Hz, CH_2S), 2.02 (s, 3 H, Ac), 1.65–1.53 (m, 4 H, CH_2CH_2S, CH_2CH_2O), 1.42–1.28 (m, 24 H, 12 CH_2). Electrospray MS:783.3823 (calcd. for $C_{34}H_{64}SO_{16}Na$ 783.3812). A slower moving fraction of disulfide **6** (2.1 mg, 19.4%) was also recovered. Due to the poor solubility of **6** and **7** in water, most of the material was retained on the Millex 0.45 μm filter and recovered by washing with MeOH to provide a mixture of **6** and **7** (1:3 by NMR, 6.7 mg, 62%).

2-(Trimethylsilyl)ethyl 3,4-O-isopropylidene-β-D-galactopyranoside (**8**).—A solution of trimethylsilylethyl β-D-galactopyranoside [15] (4.86 g, 17.3 mmol) and 2,2-dimethoxypropane (2 mL) in acetone (30 mL) in the presence of *p*-toluenesulfonic acid (50 mg) was stirred for 4 h. Then triethylamine (1 mL) was added, the mixture was concentrated and co-evaporated with toluene. Chromatography of the residue on silica gel with pentane–ethyl acetate (7:3) gave **8** (3.63 g, 63%), m.p. 93–94 °C, $[\alpha]_D$ +6.3° (*c*. 1.9; $CHCl_3$) (ref. 20 m.p. 88–89.5 °C, $[\alpha]_D$ +9.6°) ^{1}H NMR: δ_H 4.18 (d, 1 H, $J_{1,2}$ = 8.3 Hz, H-1), 4.14 (dd, 1 H, $J_{3,4}$ = 5.5 Hz, $J_{4,5}$ = 2.1 Hz, H-4), 4.08 (dd, 1 H, $J_{2,3}$ = 7.4 Hz, H-3), 4.01–3.95 (m, 2 H, H-6a, CH_2O), 3.86–3.83 (m, 2 H, H-5, H-6b), 3.54–3.49 (m, 2 H, H-2, CH_2O), 1.50, 1.05 (2s, 6 H, isopropylidene), 0.99 (m, 2 H, CH_2Si), 0.0035 (s, 9 H, $SiMe_3$). Anal. calcd. for $C_{14}H_{28}SiO_6$ (320.45): C, 52.47; H, 8.81. Found: C, 52.30; H, 9.08.

2-(Trimethylsilyl)ethyl 2,6-di-O-benzyl-3,4-O-isopropylidene-β-D-galactopyranoside (**9**).—A mixture of **8** (550 mg, 1.71 mmol) and sodium hydride (80%, 130 mg, 4.3 mmol) in DMF (4 mL) was stirred for 0.5 h, then benzyl bromide (0.61 mL, 5.1 mmol) was added dropwise. After 16 h MeOH (1 mL) was added, the mixture was diluted with CH_2Cl_2 (100 mL), and the organic solution was washed with water and concentrated. Chromatography of the residue on silica gel in pentane-ethyl acetate (6:1) gave **9** (700 mg, 82%), m.p. 93–94 °C, $[\alpha]_D$ +19.2° (*c*. 1.6; $CHCl_3$) (ref. 21 $[31\alpha]_D$ +25.4°). ^{1}H NMR: δ_H 7.39–7.23 (m, 10 H, arom.), 4.83 and 4.78 (2d, 2 H, ^2J = 11.7 Hz, CH_2Ph), 4.62 and 4.54 (2d, 2 H, ^2J = 11.8 Hz, CH_2Ph), 4.29 (d, 1 H, $J_{1,2}$ = 8.1 Hz, H-1), 4.12–4.11 (m, 2 H, H-3, H-4), 4.05–3.97 (m, 1 H, CH_2O), 3.89 (ddd, 1 H, $J_{4,5}$ = 1.3 Hz, $J_{5,6a}$ = 5.2 Hz, $J_{5,6b}$ = 6.9 Hz, H-5), 3.79 (dd, 1 H, $J_{6a,6b}$ = 10.1 Hz, H-6a), 3.76 (dd, 1 H, H-6b), 3.6–3.52 (m, 1 H, CH_2O), 3.35 (m, 1 H, H-2), 1.33 and 1.3 (2s, 6 H, isopropylidene), 1.03 (m, 2 H, CH_2Si), 0.002 (s, 9 H, $SiMe_3$). Anal. calcd for $C_{28}H_{40}SiO_6$ (500.70): C, 67.17; H, 8.05. Found: C, 67.07; H, 8.22.

2-(Trimethylsilyl)ethyl 2,6-di-O-benzyl-β-D-galactopyranoside (**10**).—A solution of **9** (630 mg, 1.26 mmol) in aqueous 80% acetic acid (5 mL) was stirred at 60 °C for 6 h. The mixture was concentrated, co-evaporated with toluene, and chromatographed on silica gel in pentane–ethyl acetate (2:1) to yield **10** (550 mg, 93%), m.p. 61–62 °C, $[\alpha]_D$ +2.8° (*c*. 3.1; $CHCl_3$) (ref. 21. $[\alpha]_D$ +7.5°). ^{1}H NMR: δ_H 7.26–7.35 (m, 10 H, arom.), 4.96 (d, 1 H, ^2J = 11.5 Hz, CH_2Ph), 4.67 (d, 1 H, ^2J = 11.5 Hz, CH_2Ph), 4.57 (s, 2 H, CH_2Ph), 4.36 (d, 1 H, $J_{1,2}$ = 7.6 Hz, H-1), 4.02 (m, 1 H, CH_2O), 4.96 (dd, 1 H, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 0.9 Hz, H-4), 3.78 (dd, 1 H, $J_{5,6a}$ = 5.7 Hz, $J_{6a,6b}$ = 10.0 Hz, H-6a), 3.74 (dd, 1 H, $J_{5,6b}$ = 5.7 Hz, H-6b), 3.61–3.55 (m, 3 H, H-3, H-5, CH_2O), 3.46 (dd, 1 H, $J_{2,3}$ 9.4 Hz, H-2), 1.02 (m, 2 H, CH_2Si), 0.014 (s, 9 H, $SiMe_3$). Anal. calcd for $C_{25}H_{36}SiO_6$ (470.63): C, 65.19; H, 7.88. Found: C, 65.14; H, 7.88.

2-(Trimethylsilyl)ethyl 4-O-(3,4,6-tri-O-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl)-2,6-di-O-benzyl-β-D-galactopyranoside (**12**).—A mixture of **10** (4 g, 8.5 mmol), Ag-silicate on alumina (8 g), 4Å molecular sieves (10 g) in dry CH_2Cl_2 (50 mL) was stirred for 1 h, then cooled at

−15 °C, and a solution of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide **11** [22,23] (3.5 g, 8.88 mmol) in CH_2Cl_2 (20 mL) was added dropwise over 2 h. The mixture was stirred for 5 h at −15 °C and for 16 h at −15 to 0 °C. After filtration through Celite followed by concentration, the residue was chromatographed twice on silica gel with toluene–ethyl acetate (4:1) as the solvent to yield **12** (4.91 g, 74%) as a syrup, $[\alpha]_D$ −18.0° (*c*. 1.46; $CHCl_3$). ^{1}H NMR: δ_H 7.37–7.26 (m, 10 H, arom.), 5.27 (dd, 1 H, $J_{3',4'}$ = 3.3 Hz, $J_{4',5'}$ = 0.3 Hz, H-4′), 4.97 (d, 1 H, ^2J = 11.5 Hz, CH_2Ph), 4.79 (dd, 1 H, $J_{2',3'}$ = 10.7 Hz, H-3′), 4.68 (d, ^2J = 11.5 Hz, CH_2Ph), 4.39 (d, 1 H, $J_{1',2'}$ = 8.1 Hz, H-1′), 4.55 (s, 2 H, CH_2Ph), 4.38 (d, 1 H, $J_{1,2}$ = 7.3 Hz, H-1), 4.08–14.02 (m, 3 H, H-4, H-6′a, CH_2O), 3.94 (dd, 1 H, $J_{5',6'b}$ = 6.2 Hz, $J_{6'a,6'b}$ = 11.1 Hz, H-6′b), 3.68–3.57 (m, 8 H, H-2, H-2′, H-3, H-5, H-5′, H-6a, H-6b, CH_2O), 2.1, 2.03, 1.96 (3s, 9 H, 3 Ac), 1.043 (m, 2 H, CH_2Si), 0.007 (s, 9 H, $SiMe_3$). Anal. calcd for $C_{37}H_{51}N_3SiO_{13}$ (773.90): C, 57.42; H, 6.64; N, 5.43. Found: C, 57.39; H, 6.68; N, 5.35.

*2-(Trimethylsilyl)ethyl 4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside* (**13**).—A mixture of **12** (1.43 g, 1.85 mmol) and $Pd(OH)_2$/C (250 mg) in EtOH (10 mL) containing water (0.5 mL) was stirred for 3 h under hydrogen. After filtration followed by concentration, the residue was dissolved in MeOH (10 mL). Acetic anhydride (2 mL) was added and after 16 h the mixture was concentrated. A solution of the residue in acetic acid (10 mL) in the presence of 10% Pd/C (1 g) under 17.2 MPa of hydrogen was shaken at 40 °C for 24 h, then filtered, concentrated, and acetylated with Ac_2O (4 mL) in pyridine (10 mL) at 45 °C. After 4 h MeOH (1 mL) was added, and the mixture was concentrated. Chromatography of the residue on silica gel with pentane–acetone (2:1) gave **13** (1.18 g, 87%) as a foam, $[\alpha]_D$ −20.2° (c. 1.1; $CHCl_3$). ^{1}H NMR: δ_H 5.92 (dd, 1 H, $J_{2',3'}$ = 11.4 Hz, $J_{3',4'}$ = 3.4 Hz, H-3′), 5.69 (d, 1 H, $J_{NH,2'}$ = 6.9 Hz, NH), 5.36 (d, 1 H, H-4′), 5.22 (dd, 1 H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 10.4 Hz, H-2), 5.12 (d, 1 H, $J_{1',2'}$ = 8.2 Hz, H-1′), 4.91 (dd, 1 H, $J_{3,4}$ = 2.7 Hz, H-3), 4.43 (d, 1 H, H-1), 4.27 (d, 2 H, $J_{5,6a} \simeq J_{5,6b}$ = 6.1 Hz, H-6a, H-6b), 4.10 (d, 1 H, $J_{4,5}$ = 2.2 Hz, H-4), 4.02 (d, 2H, $J_{5',6'a} \simeq J_{5',6'b}$ = 6.5 Hz, H-6′a, H-6′b), 3.98 (ddd, 1 H, ^2J = 14.8 Hz, ^3J = 9.8 Hz, ^3J = 5.1 Hz, CH_2O), 3.88 (broad t, 1 H, $J_{5',6'ab}$ = 6.5 Hz, H-5′), 3.70 (broad t, 1 H, $J_{5',6'ab}$ = 6.1 Hz, H-5), 3.53 (ddd, 1 H, ^3J = 7.0 Hz, ^3J = 9.8 Hz, CH_2O), 3.32 (ddd, 1 H, H-2′), 2.10, 2.08, 2.016, 2.012, 2.008, 1.96×2 (6s, 21 H, 7 Ac), 1.01–0.83 (m, 2 H, $SiCH_2$), −0.02 (s, 9 H, $SiMe_3$). Anal. calcd for $C_{31}H_{49}NSiO_{17}$ (735.80): C, 50.60; H, 6.71; N, 1.90. Found: C, 50.88; H, 6.79; N, 1.96.

*4-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-α-D-galactopyranose trichloroacetimidate* (**14**).—A solution of **13** (1.16 g, 1.57 mmol) in CH_2Cl_2 (10 mL) and CF_3COOH (5 mL) was stirred for 15 min. Then EtOAc (5 mL) was added, the mixture was concentrated, co-evaporated with toluene (2×5 mL), and dried for 2 h under vacuum. To a solution of the residue in CH_2Cl_2 (6 mL) trichloroacetonitrile (1.15 mL) was added, the mixture was cooled to 0 °C, and DBU (0.3 mL) was added. After 1 h of stirring at room temperature, MeOH (0.5 mL) was added, and the mixture was concentrated. Chromatography of the residue on silica gel with pentane–acetone (1:1) yielded **14** (1.095 g, 89%) as a foam, $[\alpha]_D$ +51.1° (*c*. 0.9; $CHCl_3$). ^{1}H NMR: δ_H 8.62 (s, 1 H, C=N*H*), 6.48 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 5.82 (dd, 1 H, $J_{2',3'}$ = 11.3 Hz, $J_{3',4'}$ = 3.5 Hz, H-3′), 5.63 (d, 1 H, $J_{NH,2'}$ = 7.2 Hz, NH), 5.53 (dd, 1 H, $J_{2,3}$ = 10.8 Hz, H-2), 5.36 (d, 1 H, H-4′), 5.33 (dd, 1 H, $J_{3,4}$ 2.6 Hz, H-3), 5.07 (d, 1 H, $J_{1',2'}$ = 8.2 Hz, H-1′), 4.32–4.26 (m, 3 H, H-4, H-5, H-6a), 4.16 (dd, 1 H, $J_{5,6b}$ = 8.3 Hz, $J_{6a,6b}$ 12.8 Hz, H-6b), 4.04 (d, 2 H, $J_{5',6'a} \simeq J_{5',6'b}$ = 6.8 Hz, H-6′a, H-6′b), 3.90 (broad t, H-5′), 3.40 (ddd, 1 H, H-2′), 2.14, 2.12, 2.02, 2.00, 1.99, 1.98, 1.94 (7s, 21 H, 7 Ac). Anal. calcd. for $C_{28}H_{37}Cl_3N_2O_{17}$ (779.95): C, 43.12; H, 4.78; N, 3.59. Found: C, 43.40; H, 4.78; N, 3.53.

*16-(Thiocyano)hexadecanyl 4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3,6-*O*-acetyl-β-D-galactopyranoside* (**16**).—A mixture of the imidate **15** (100 mg, 0.128 mmol), monotosylated diol **1** (68 mg, 0.165 mmol), and 4Å molecular sieves (100 mg) in CH_2Cl_2 (5 mL) was stirred for 1 h. Then TMSOTf (5 μL, 64 μmol) was added. After 2 h triethylamine (1 mL) was added, and the solid was removed by filtration. The filtrate was concentrated and dried under vacuum. A solution of the residue and KSCN (70 mg, 0.72 mmol) in DMF (3 mL) was stirred at 80 °C for 2 h. The mixture was concentrated, dissolved in CH_2Cl_2 (30 mL), and the solution was washed with water and concentrated. Chromatography of the residue on silica gel with pentane–acetone (2:1) gave **16** (82 mg, 70%), $[\alpha]_D$ −15.3° (*c*. 1.8; $CHCl_3$).

^{1}H NMR: δ_H 5.91 (dd, 1 H, $J_{2',3'}$ = 11.4 Hz, $J_{3',4'}$ = 3.4 Hz, H-3′), 5.68 (d, 1 H, $J_{NH,2'}$ = 6.9 Hz, NHAc), 5.36 (d, 1 H, H-4′), 5.22 (dd, 1 H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 10.5 Hz, H-2), 5.11 (d, 1 H, $J_{1',2'}$ = 8.2 Hz, H-1′), 4.91 (dd, 1 H, $J_{3,4}$ = 2.8 Hz, H-3), 4.39 (d, 1 H, H-1), 4.28 (dd, 1 H, $J_{5,6a}$ = 5.6 Hz, $J_{6a,6b}$ = 11.7 Hz, H-6a), 4.23 (dd, 1 H, $J_{5,6b}$ = 6.4 Hz, H-6b), 4.1 (d, 1 H, H-4), 4.02 (d, 2 H, $J_{5',6ab}$ = 6.6 Hz, H-6′a, H-6′b), 3.90–3.82 (m, 2 H, H-5′, CH_2O), 3.70 (t, 1 H, H-5), 3.43 (dt, 1 H, ^3J = 7.0 Hz, ^2J = 9.7 Hz, CH_2O), 3.31 (ddd, 1 H, H-2′), 2.92 (t, 2 H, ^3J = 7.3 Hz, CH_2SCN), 2.11, 2.08, 2.04, 2.01, 2.008, 1.97, 1.96 (7s, 21 H, 7 Ac), 1.77 (p, 2 H, ^3J = 7.2 Hz, 2CH_2CH$_2$SCN), 1.56–1.20 (m, 26 H, 13 CH_2). Anal. calcd for $C_{43}H_{68}SN_2O_{17}$ (917.07): C, 56.32; H, 7.47; N, 3.05; S, 3.50. Found: C, 56.40; H, 7.59; N, 2.84; S, 3.51.

*Bis{16-[4-*O*-(2-acetamido-2-deoxy-*β*-*D*-galacto-pyranosyl)-*β*-*D*-galactopyranosyloxy]hexadecanyl} disulfide* (**17**).—A solution of **16** (373.9 mg, 0.374 mmol) and sodium borohydride (280 mg, 3.74 mmol) in dry MeOH (3 mL) was stirred under an argon atmosphere for 20 h, then the mixture was neutralized with AcOH, the solution was concentrated, taken up in water, and applied onto a Sep-Pak (C-18) cartridge (Waters). The cartridge was washed with 20, 40, 60, and 80% solution of MeOH in water, then with pure MeOH. The methanol fraction containing sugar was concentrated to give **17** (189.4 mg, 79.2%), $[\alpha]_D$ −8.3° (*c*. 0.6; H_2O). ^{1}H NMR: δ_H (CD_3OD, 45 °C): 4.64 (d, 1 H, $J_{1',2'}$ = 8.4 Hz, H-1′), 4.18 (d, 1 H, $J_{1,2}$ = 7.6 Hz, H-1), 4.02 (dd, 1 H, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ = 0.8 Hz, H-4), 3.88 (dd, 1 H, $J_{5,6a}$ = 7.6 Hz, $J_{6a,6b}$ = 11.1 Hz, H-6a), 3.85 (dd, 1 H, $J_{2',3'}$ = 10.4 Hz, H-2′), 3.84–3.79 (m, 2H, H-6′a, CH_2O), 3.75 (broad d, 1 H, $J_{3',4'}$ = 3.4 Hz, H-4′), 3.70 (dd, 1 H, $J_{5',6'b}$ = 4.3 Hz, $J_{6'a,6'b}$ = 11.3 Hz, H-6′b), 3.64–3.57 (m, 3 H, H-3, H-3′, H-6b), 3.53–3.48 (m, 3 H, H-5, H-5′, CH_2O), 3.44 (dd, 1 H, $J_{2,3}$ = 9.8 Hz, H-2), 2.68 (t, 2 H, ^3J = 7.2 Hz, CH_2S), 2.02 (s, 3 H, Ac), 1.67 (p, 2 H, ^3J = 7.2 Hz, CH_2CH$_2$S), 1.59 (p, 2 H, ^3J = 6.9 Hz, CH_2CH$_2$O), 1.42–1.28 (m, 24 H, 12 CH_2). Electrospray MS: 1299.7032 (calcd for $C_{60}H_{112}N_2S_2O_{22}Na$ 1299.7045).

*16-(Mercapto)hexadecanyl 4-*O*-(2-acetamido-2-deoxy-*β*-*D*-galactopyranosyl)-*β*-*D*-galactopyrano-side* (**18**).—A solution of **17** (11.6 mg, 18 μmol) and dithiothreitol (15 mg, 5 eq.) in 7 mL of degassed water was adjusted to pH 8 with a NH_4HCO_3 solution and kept for 18 h under argon. The solution was concentrated and the residue was chromatographed on a C-18 reverse phase HPLC column using a step gradient of H_2O–MeOH (50:50–0:100) to yield the faster moving **18** (3.7 mg, 32%), $[\alpha]_D$ −8.0° (*c*. 0.3; H_2O). ^{1}H NMR: δ_H (CD_3OD): 4.63 (d, 1 H, $J_{1',2'}$ = 8.5 Hz, H-1′), 4.18 (d, 1 H, $J_{1,2}$ = 7.7 Hz, H-1), 4.01 (dd, 1 H, $J_{3,4}$ = 3.1 Hz, $J_{4,5}$ = < 1 Hz, H-4), 3.91–3.78 (m, 4 H, H-6a, H-2, H-6′a, CH_2O), 3.74 (broad d, 1 H, $J_{3',4'}$ = 3.2 Hz, H-4′), 3.70 (dd, 1 H, $J_{5',6'b}$ = 4.2 Hz, $J_{6'a,6'b}$ = 11.2 Hz, H-6′b), 3.63–3.55 (m, 3 H, H-3, H-3′, H-6b), 3.53–3.48 (m, 3 H, H-5, H-5′, CH_2O), 3.43 (dd, 1 H, $J_{2,3}$ = 9.7 Hz, H-2), 2.48 (t, 2 H, ^3J = 7.2 Hz, CH_2S), 2.02 (s, 3 H, Ac), 1.65–1.53 (m, 4 H, CH_2CH$_2$S, CH_2CH$_2$O), 1.42–1.28 (m, 24 H, 12 CH_2). Electrospray MS: 662.3553 (calcd for $C_{30}H_{57}NSO_{11}Na$ 662.3550). A slower moving component, the disulfide **17** (7.9 mg, 68%) was recovered.

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References

[1] (a) I. Willner, A. Riklin, and B. Shoham, *Adv. Mater*., 12 (1993) 912–915. (b) R.B. Lennox, R.S. Hodges, R.T. Irvin, G.D. Armstrong, D.R. Bundle, P.I. Kitov, and C. Railton, *USA P*. (1996) submitted for publication.

[2] C.D. Bain, J. Evall, and G.M. Whitesides, *J. Am. Chem. Soc*., 111 (1989) 7155–7164.

[3] K.L. Prime, and G.M. Whitesides, *Science*, 252 (1991) 1164–1167.

[4] C. Pale-Grosdemange, E.S. Simon, K.L. Prime, and G.M. Whitesides, *J. Am. Chem. Soc*., 113 (1991) 12–20.

[5] L. Yu, K.K. Lee, R.S. Hodges, W. Paranchych, and R.T. Irvin, *Infect. Immun*., 62 (1994) 5213–5219.

[6] H.C. Krivan, D.D. Roberts, and V. Ginsburg, *Proc. of the Nat. Acad. Sci. USA*, 85 (1988) 6157–6161.

[7] L. Yu, K.K. Lee, H.B. Sheth, P. Lane-Bell, G. Srivastava, O. Hindsgaul, W. Paranchych, R.S. Hodges, and R.T. Irvin, *Infect. Immun*., 62 (1994) 2843–2848.

[8] T. Waddell, S. Head, M. Petric, A. Cohen, and C. Lingwood, *Biochem. Biophys. Res. Commn.*, 152 (1988) 674–679.
[9] A. Donohue-Rolfe, M. Jacewicz, and G.T. Keusch, *Mol. Microbiol.*, 3 (1989) 1231–1236.
[10] M. Jacewicz, H. Clausen, E. Nudelman, A. Donohue-Rolfe, and G.J. Keusch, *J. Exp. Med.*, 163 (1986) 1391–1404.
[11] A. Lindberg, J.E. Brown, N. Strömberg, L.M. Westling-Ryd, J.E. Schultz, and K. Karlsson, *J. Biol. Chem.*, 262 (1987) 1779–1785.
[12] G.T. Keusch, M. Jacewicz, M. Mobassaleh, and A. Donohue-Rolfe, *Rev. Infect. Diseases*, 13 Suppl 4 (1991) S 304–310.
[13] K.G.I. Nilsson and C.-F. Mandenius, *Bio/Technol.*, 12 (1994) 1376–1378.
[14] R.G. Nuzzo and D.L. Allan, *J. Am. Chem. Soc.*, 105 (1983) 4481–4483.
[15] K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmen, G. Noori, and K. Stenvall, *J. Org. Chem.*, 53 (1988) 5629–5647.
[16] R.K. Olsen and H.R. Snyder, *J. Org. Chem.*, 30 (1965) 184–187.
[17] O.-T. Leung, S.P. Douglas, D.M. Whitfield, H.Y.S. Pang, and J.J. Krepinsky, *New J. Chem.*, 18 (1994), 349–363.
[18] H. Paulsen and M. Paal, *Carbohydr. Res.*, 137 (1985), 39–62.
[19] A. Marra and P. Sinäy, *Gazz. Chim. Ital.*, 117 (1987), 563–566.
[20] T. Murase, A. Kameyama, K.P.R. Kartha, H. Ishida, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, 8 (1989) 265–283.
[21] S. Komba, H. Ishida, M. Kiso, and A. Hasegawa, *Glyconjugate J.*, 13 (1996) 241–254.
[22] J. Broddefalk, U. Nilsson, and J. Kihlberg, *J. Carbohydr. Chem.*, 13 (1994) 129–132.
[23] R.U. Lemieux and R.M. Ratcliffe, *Can. J. Chem.*, 57 (1979) 1244–1251.
[24] H. Paulsen and O. Lockhoff, *Chem Ber.*, 114 (1981) 3102–3114.
[25] A. Noy, D.V. Vezenov, J.F. Kayyem, T.J. Meade, and C.M. Lieber, *Chem. Biol.* 4 (1997) 519–527.
[26] D.D. Perrin, W.L.F. Armarego, and D.R. Perrin, *Purification of Laboratory Chemicals*, Pergamon Press, Oxford, 1980.