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SYNTHESIS OF 6-(5-CHOLESTEN-3β-YLOXY)HEXYL 4-*O*-(6-DEOXY-β-D-GALACTOPYRANOSYL)-1-THIO-β-D-GLUCOPYRANOSIDE AND DERIVATIVES THEREOF FOR *in vivo* LIPOSOME STUDIES

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ABSTRACT

6-(5-Cholesten-3β-yloxy)hexyl 4-*O*-(6-deoxy- and 3,6-anhydro-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside were prepared from benzyl 4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside, and incorporated into phospholipid vesicles. When injected intravenously into rats, these disaccharide-modified liposomes were found to be taken up mainly by liver cells.

INTRODUCTION

Saccharide determinants of glycolipids and glycoproteins present at the cell surface are known to participate in intercellular recognition-processes[1]. For example, mammalian hepatic-receptors are specific for (terminal) D-galactosyl groups of desialylated serum glycoproteins[2], whereas, in avian liver, the receptors recognize terminal 2-acetamido-2-deoxy-D-glucose[3]. Alveolar macrophages have been shown to bind glycoproteins and synthetic glycoconjugates that have a D-mannosyl, 2-acetamido-2-deoxy-D-glucosyl, or D-glucosyl group in the exposed, nonreducing position[4]. The uptake of some lysosomal enzymes by fibroblasts in culture involves recognition of D-mannose 6-phosphate on the enzyme molecules[5,6]. Such receptor recognition and uptake of saccharide derivatives have been extended to liposomes containing glycoproteins and glycolipids. For example, asialofetuin-entrapped liposomes containing bleomycin were taken up by the parenchymal cells of the liver[7].

In addition to natural glycolipids and glycoproteins, synthetic glycolipids of low molecular weight are also effective for the introduction of saccharide determinants onto the surface of liposomes. Thus, the β-D-galactosylated phosphatidyl-2-aminoethanol (PE) prepared from *p*-aminophenyl β-D-galactopyranoside, PE, and glutaraldehyde could be incorporated into lipid vesicles[8]. These modified liposomes were rapidly taken up by the hepatocytes[8].

In an effort to improve the selectivity, stability, and other *in vivo* characteristics of liposomes, we have synthesized a series of glycolipids, consisting of various saccharides coupled to cholesterol *via* a spacer arm, for incorporation into lipo-

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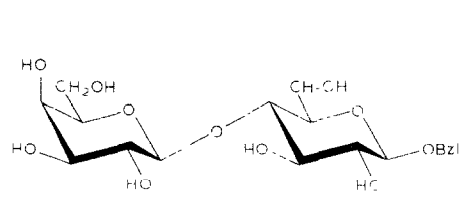
somes[9,10], and the *in vivo* behavior of liposomes containing some of these glycolipids has been reported[11–15]. As an extension of these studies, we now describe the synthesis and evaluation of 6-(5-cholesten-3β-yloxy)hexyl 4-*O*-(6-deoxy-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside and some of its derivatives, for clarification of some *in vivo*, tissue-distribution results.

CHEMISTRY

Acetalation of benzyl 4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside[16] (benzyl β-lactoside, **1**) with benzaldehyde, followed by acetylation gave crystalline **2**, which was treated with *N*-bromosuccinimide[17] (NBS) in refluxing carbon tetrachloride–tetrachloroethane to yield two products. The unreacted starting-material and the products were separated by column chromatography, and the more mobile component was identified by its 300-MHz, n.m.r. spectrum as the expected product, **3** (see the Experimental section). The major component had a lower chromatographic mobility, and was assigned structure **4**. This assignment was verified by successive transformation of **4** into **5**, **5** into **7**, and **7** into **8**. The formation of **4** from **2** indicated the susceptibility of the benzyl group to attack[17] by NBS.



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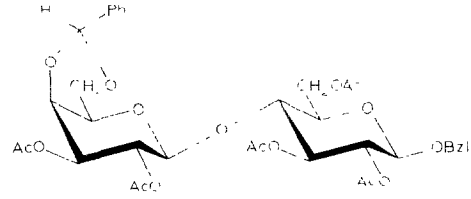

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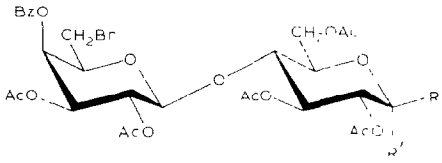
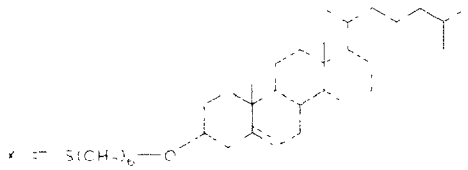
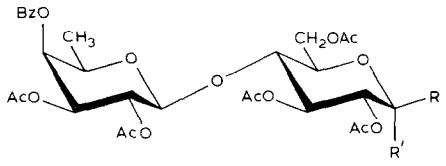


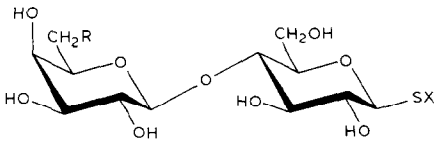


3 R = OBzl, R′ = H
4 R = R′ = H, OH
5 R = R′ = H, OAc
6 R = H, R′ = Br
7 R = SH, R′ = H
8 R = X, R = H

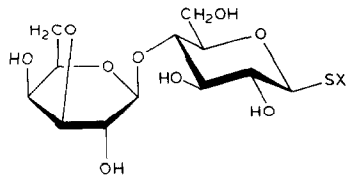
Treatment of **8** with sodium methoxide in methanol–oxolane gave two products, which were separated by column chromatography. The less mobile component was identified as the 6′-bromo-6′-deoxy derivative, **15**. The other product was the 3′,6′-anhydro-β-lactoside, **16**, formed by intramolecular, nucleophilic displacement of the 6′-bromine atom by the 3′-hydroxyl group. Under controlled, deblocking conditions, only **15** was isolated in good yield. The synthesis of 6-(5-cholesten-



9 R, R′ = H, OAc
10 R = H, R′ = Br
11 R = SH, R′ = H
12 R = X, R′ = H



13 R = OH
14 R = H
15 R = Br



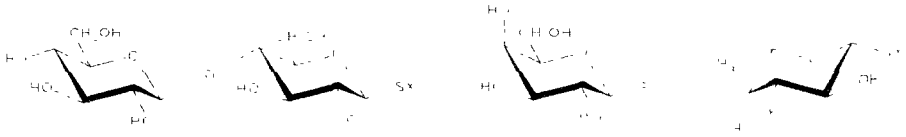
16

3-β-yloxy)hexyl 6′-deoxylactoside (**14**) was accomplished in essentially the same way as for **15** (see the Experimental section). The *in vivo* behavior of liposomes containing **14, 16**, and other glycolipids will be discussed next.

BIOLOGICAL RESULTS AND DISCUSSION

Previously, we reported the synthesis of a series of glycolipids[9,10] with 6-(5-cholesten-3β-yloxy)-1-thiohexyl as the aglycon and the following carbohydrate groups, which occur naturally in membrane oligosaccharides[18], as the sugar determinants: D-glucosyl, D-galactosyl, D-mannosyl, 2-acetamido-2-deoxy-D-glucosyl, 2-acetamido-2-deoxy-D-galactosyl, L-fucosyl, L-arabinosyl, D-xylosyl, and *N*-acetylneuraminic-2-yl acid. These glycolipids can be incorporated into liposome bilayers, to provide specific, saccharide determinants on the surface. To evaluate their usefulness in selective delivery *in vivo*, we investigated the tissue distribution of these modified liposomes in rats. Liposomes were prepared from purified 1,2-distearoyl-*sn*-glycerol-3-yl phosphocholine[19] (DSPC) and a glycolipid (14 mol%), with $^{51}CrO_4^{2-}$ as an internal marker. The modified liposomes were injected intravenously into rats, and the radioactivity in each tissue was determined after dissection. The tissue distribution of liposomes in rats was found to vary with different glycolipids (data not shown); however, no clear and useful direction of liposomes to target organs and tissues was observed. In each case, the liposomes were removed from the blood stream primarily by the liver and spleen, as expected for intravenously injected liposomes[20,21].

The tissue distribution of the β-lactoside[10] **13** and the β-cellobioside **17** *in*



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vivo revealed some interesting aspects. Liposomes containing these two disaccharides were rapidly taken up by liver, as compared to control liposomes or liposomes containing the β-D-galactoside **18** or the β-L-fucoside **19** (Table I). For example, two hours after intravenous injection of liposomes containing **13** and **17**, 76–77% of the label was found in the liver, whereas, for control liposomes or liposomes containing **18** or **19**, only 15–19% of the label was found in the liver. These data may suggest more effective recognition of the terminal D-galactose and D-glucose of the two disaccharides by hepatocytes, as they are more exposed above the liposomal membrane for interactions with the cell-surface receptors.

To clarify the foregoing observations, the 6′-deoxy-β-lactoside **14** and the 3′,6′-anhydro-β-lactoside **16** (see Chemistry) were also incorporated into liposomes for *in vivo*, tissue-distribution studies. Surprisingly, these modified liposomes were also taken up rapidly by liver cells (see Table I; 2 h after intravenous injection, 81 and 65% of the label was respectively found in the liver). It is noteworthy that **16** also enhanced the uptake of liposomes by spleen. Because the (terminal) saccharide groups, 6-deoxy-D-galactosyl and 3,6-anhydro-D-galactosyl, are those of unnatural sugars, the enhanced uptake by the liver of liposomes modified with the disaccharides (see Table I) is unlikely to have been caused by an improved receptor-recognition; instead, they may affect the *in vivo* results indirectly by influencing the physical properties of liposomes, *e.g.*, vesicle size, charge, or aggregation. These data clearly indicate that, in addition to receptor-mediated, cel-

TABLE I

TISSUE DISTRIBUTION[a] (%) OF [^{51}Cr]CHROMATE AT TWO HOURS AFTER INTRAVENOUS INJECTION

Tissue	*Control*	**19**	**18**	**17**	**13**	**14**	**16**
Blood	56.32	40.76	47.90	6.71	5.75	7.42	11.09
	±1.45	±0.45	±1.38	±1.98	±0.47	±1.71	±3.32
Liver	14.71	15.59	18.79	75.68	76.72	81.36	65.31
	±1.60	±3.15	±1.68	±2.08	±2.90	±4.08	±5.25
Spleen	2.13	2.96	1.50	1.78	0.35	1.34	7.07
	±0.21	±0.55	±0.15	±1.06	±0.04	±0.72	±3.48
Kidney	2.18	1.46	1.85	0.39	0.71	0.66	0.63
Small intestine	4.19	4.16	2.36	0.85	1.30	1.26	1.85
Large intestine	0.48	0.46	0.25	0.14	0.16	0.18	0.18
Stomach	0.34	0.31	0.26	0.11	0.21	0.20	0.25
Lung	0.85	0.88	0.92	0.19	0.19	0.26	0.34

[a]Each set of values is an average from 2 experiments (3 rats each), as % of injected radioactivity. Values for blood, liver, and spleen include the standard deviation.

lular recognition and uptake, the alteration of liposome surface-characteristics by hydrophilic, saccharide derivatives may profoundly affect the tissue-distribution of liposomes *in vivo*.

EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover Unimelt apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 241 polarimeter. Thin-layer chromatography (t.l.c.) was performed on silica gel GF_{254} (Analtech) plates, and the spots were detected with a ceric sulfate (1%)–sulfuric acid (10%) spray. Column chromatography was conducted on silica gel 60 (70–230 mesh, ASTM). N.m.r. spectra at 300 MHz were recorded for solutions in chloroform-*d* (unless stated otherwise), with tetramethylsilane as the internal standard. Conventional processing consisted of drying organic solutions with anhydrous sodium sulfate, filtering, and evaporating the filtrate under diminished pressure.

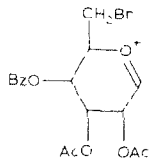
Experiments in vivo. — Liposome compositions (molar ratios) were: control liposomes, Chol–DSPC–PG (phosphatidylglycerol) (10:9:1); glycolipid-containing liposomes, glycolipid–Chol–DSPC–PG (3:7:9:1). Labeled liposomes containing sodium [^{51}Cr]chromate (New England Nuclear) were prepared as reported[12], by sonication to slight opalescence and removal of unincorporated chromate by gel filtration on Sephadex G-50 (Pharmacia). The liposome suspension containing 5 mg of lipid and 4×10^5 c.p.m. of ^{51}Cr (0.45 mL per rat) was injected into the tail vein of Wistar rats (170–200 g; Charles River Laboratories). After 2 h, the rats were killed with ether, and dissected. The radioactivity in the individual organs was determined with a gamma counter. Total blood-radioactivity was calculated from the radioactivity of a sample, assuming a blood pool of 7.3 mL per 100 g of body weight.

*Benzyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-*D*-galacto-pyranosyl)-β-*D*-glucopyranoside* (**2**). — A mixture of **1** (10 g), anhydrous zinc chloride (15 g), and benzaldehyde (50 mL) was stirred for 2 d at room temperature. The clear solution was diluted with ethyl ether–petroleum ether, and the solvents decanted. This process was repeated a few times, to give a solid which was treated with acetic anhydride in pyridine overnight at room temperature. The mixture was poured into ice–water, and the crystals that formed on stirring were filtered off, and washed generously with water. Recrystallization from methanol gave **2** (13 g, 77%); m.p. 209°, $[\alpha]_D^{27}$ +10° (*c* 1.0, chloroform); *m/z* 730 (M^+), 688 ($M^+ - CH_2{=}C{=}O$), and 623 ($M^+ - OCH_2Ph$).

Anal. Calc. for $C_{36}H_{42}O_{16}$: C, 59.17; H, 5.79. Found: C, 58.77; H, 5.79.

*Benzyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3-di-*O*-acetyl-4-*O*-benzoyl-6-bromo-6-deoxy-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**3**) *and 2,3,6-tri-*O*-acetyl-4-*O*-(2,3-di-*O*-acetyl-4-*O*-benzoyl-6-bromo-6-deoxy-β-*D*-galactopyranosyl)-*D*-glucopyranose* (**4**). — A mixture of **2** (12 g, 16.4 mmol), NBS (3.2 g, 18 mmol), and

barium carbonate (12 g) in carbon tetrachloride (300 mL) and tetrachloroethane (50 mL) was boiled, with stirring, under reflux for 3.5 h. The mixture was cooled and filtered, and the filtrate was evaporated to a syrup which was partitioned between ethyl ether and water. The ethereal layer was dried, and evaporated *in vacuo* to a syrup that was placed on a column of silica gel and eluted with 4:1 (v/v) chloroform–ethyl acetate. Compound **3** (R_F 0.34, 3.0 g) was eluted first, followed by the starting material **2** (R_F 0.15, 0.86 g), and then **4** (R_F 0.07, 4.64 g). The combined yield of **3** and **4** was 78%. Compound **4** had $[\alpha]_D^{27}$ +37° (*c* 1.35, chloroform); *m/z* 701, 703 ($M^+ - OH$), 658, 660 ($M^+ - HOAc$), 641, 643 ($M^+ - OH - HOAc$), and 413, 415 (i).



i

Compound **3** had $[\alpha]_D^{27}$ +0.6° (*c* 1.46, chloroform); n.m.r. ($CDCl_3$): δ 4.53 $J_{1,2}$ 8.0 Hz, H-1), 5.02 (2 d, $J_{2,3}$ 9.0 Hz, H-2), 5.25 (t, $J_{3,4}$ 9.0 Hz, H-3), 3.91 (t, $J_{4,5}$ 9.0 Hz, H-4), 3.66 (o, H-5), 4.58 (2 d, $J_{6e,5}$ 2.0, $J_{6a,6e}$ 12.0 Hz, H-6*e*), 4.14 (2 d, $J_{6a,5}$ 5.0 Hz, H-6*a*), 4.59 (d, $J_{1',2'}$ 8.0 Hz, H-1'), 5.21 (2 d, $J_{2',3'}$ 10.0 Hz, H-2'), 5.09 (2 d, $J_{3',4'}$ 3.0 Hz, H-3'), 5.79 (d, H-4'), 3.93 (H-5'), 3.38 (2 H-6'), and 2.17, 2.05, 2.03, 2.02, and 1.94 (5 s, 5 OAc); *m/z* 808, 810 (M^+), 701, 703 ($M^+ - OCH_2Ph$), and 413, 415 (*i*).

Anal. Calc. for $C_{36}H_{41}BrO_{16}$: C, 53.41; H, 5.16; Br, 9.87. Found: C, 53.08; H, 5.08; Br, 9.66.

1,2,3,6-Tetra-O-acetyl-4-O-(2,3-di-O-acetyl-4-O-benzoyl-6-bromo-6-deoxy-β-D-galactopyranosyl)-D-glucopyranose (**5**). — Compound **4** (2.5 g) was acetylated with acetic anhydride (10 mL) in pyridine (10 mL), and processed in the usual way, to give **5** (2.1 g, 79%); $[\alpha]_D^{27}$ +45° (*c* 1.03, chloroform); n.m.r. ($CDCl_3$): δ 6.32 (d, $J_{1,2}$ 4.0 Hz, H-1α; α:β, ~11:9), 5.72 (d, $J_{1,2}$ 8.0 Hz, H-1β), 5.55 (t, *J* 9.5 Hz, H-3α), 5.34 (t, *J* 9.0 Hz, H-3β), 4.60 (d, $J_{1',2'}$ 8.0 Hz, H-1'), and 5.80 (d, $J_{4',3'}$ 3.5 Hz, H-4'); *m/z* 760, 762 (M^+), 700, 702 ($M^+ - HOAc$), 640, 642 ($M^+ - 2\ HOAc$), and 413, 415 (i).

Anal. Calc. for $C_{31}H_{37}BrO_{17}$: C, 48.89; H, 4.90. Found: C, 48.60; H, 4.93.

2,3,6-Tri-O-acetyl-4-O-(2,3-di-O-acetyl-4-O-benzoyl-6-bromo-6-deoxy-β-D-galactopyranosyl)-1-thio-β-D-glucopyranose (**7**). — A solution of **5** (1.0 g) in dry dichloromethane (0.5 mL) was treated with 30–32% hydrobromic acid in glacial acetic acid (10 mL) for 1.5 h at 0–5°, poured into ice–water, and the mixture immediately extracted with dichloromethane. The extract was successively washed with ice-cold aq. sodium hydrogencarbonate solution and water, dried, and evaporated, to give **6** as a syrup [1.0 g; R_F 0.5 in 2:1 (v/v) $CHCl_3$–EtOAc]; n.m.r. ($CDCl_3$): δ 6.57 (d, $J_{1,2}$ 4.0 Hz, H-1), 1.93, 2.05, 2.07, 2.11, and 2.15 (5 s, 5 OAc).

A mixture of **6** (900 mg) and thiourea (135 mg) in dry acetone (5 mL) was

boiled under reflux for 10 h. The solution was cooled, and evaporated *in vacuo* to a syrup which was partitioned between ethyl ether (10 mL) and water (10 mL). The aqueous layer was washed with ethyl ether (3 × 10 mL), and boiled with potassium metabisulfite ($K_2S_2O_5$; 257 mg) and chloroform (15 mL) under reflux for 2 h. The organic layer was dried, and evaporated, to give **7** (527 mg). An analytical sample had $[\alpha]_D^{27}$ +26.5° (*c* 2.07, chloroform); n.m.r. ($CDCl_3$): δ 4.56 (t, *J* 9.5 Hz, H-1), 4.95 (t, *J* 9.5 Hz, H-2), 5.29 (t, H-3), 3.90 (t, H-4), 4.53 (2 d, $J_{6e,5}$ 2.0, $J_{6e,6a}$ 12.0 Hz, H-6*e*), 4.13 (2 d, $J_{6a,5}$ 5.0 Hz, H-6*a*), 4.59 (d, $J_{1',2'}$ 8.0 Hz, H-1'), 5.23 (2 d, $J_{2',3'}$ 10.0 Hz, H-2'), 5.10 (2 d, $J_{3',4'}$ 3.5 Hz, H-3'), 5.80 (d, H-4'), and 2.16, 2.10, 2.06, 2.05, and 1.95 (5 s, 5 OAc); *m/z* 701, 703 (M^+ − SH), 674, 676 (M^+ − HOAc), 641, 643 (M^+ − SH −HOAc), and 413, 415 (i).

Anal. Calc. for $C_{29}H_{35}BrO_{15}S$: C, 47.35; H, 4.80. Found: C, 47.06; H, 4.90.

6-(5-Cholesten-3β-yloxy)hexyl 2,3,6-tri-O-acetyl-4-O-(2,3-di-O-acetyl-4-O-benzoyl-6-bromo-6-deoxy-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (**8**). — A solution of **7** (410 mg) and 6-(5-cholesten-3β-yloxy)-1-iodohexane (330 mg) in dry dichloromethane (5 mL) containing triethylamine (80 μL) was kept under nitrogen overnight at room temperature. It was concentrated to a small volume, and the concentrate was placed on a column of silica gel and eluted with 3:97 EtOAc–$CHCl_3$. The desired fractions were combined, and evaporated *in vacuo*, to give **8** (300 mg, 45%); m.p. 85–88° (MeOH), $[\alpha]_D^{27}$ −2.4° (*c* 1.1, chloroform); n.m.r. ($CDCl_3$): δ 4.47 (d, $J_{1,2}$ 10.0 Hz, H-1), 4.97 (t, $J_{2,3}$ 10.0 Hz, H-2), 5.27 (t, $J_{3,4}$ 10.0 Hz, H-3), 3.85 (t, $J_{4,5}$ 10.0 Hz, H-4), 3.78 (o, H-5), 4.51 (2 d, $J_{6e,5}$ 2.0 Hz, $J_{6e,6a}$ 12.0 Hz, H-6*e*), 4.11 (2 d, $J_{6a,5}$ 5.0 Hz, H-6*a*), 4.57 (d, $J_{1',2'}$ 8.0 Hz, H-1'), 5.21 (2 d, $J_{2',3'}$ 10.0 Hz, H-2'), 5.07 (2 d, $J_{3',4'}$ 3.5 Hz, H-3'), 5.78 (d, H-4'), 3.93 (m, H-5'), 3.36 (2 H-6'), 5.34 (d, *J* 5.0 Hz, olefinic), 3.44 (t, *J* 6.5 Hz, CCH_2O), 3.11 (m, ), 2.65 (q, SCH_2C), 0.99 (s, CH_3-19), 0.90 (d, *J* 6.0 Hz, CH_3-21), 0.86 and 0.84 (CH_3-26 and CH_3-27), 0.67 (s, CH_3-18), 8.10–8.07 and 7.68–7.50 (aromatic), and 2.13, 2.06, 2.04, 2.03, and 1.94 (5 s, 5 OAc); *m/z* 701, 703 (M^+ − aglycon), 641, 643 (M^+ − aglycon − HOAc), and 413, 415 (i).

Anal. Calc. for $C_{62}H_{91}BrO_{16}S$: C, 61.83; H, 7.62; Br, 6.63; S, 2.66. Found: C, 61.61; H, 7.64; Br, 7.06; S, 2.83.

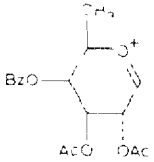
6-(5-Cholesten-3β-yloxy)hexyl 4-O-(6-bromo-6-deoxy-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (**15**) *and the 4-O-(3,6-anhydro-β-D-galactopyranosyl) analog* (**16**). — A solution of **8** (100 mg) and sodium methoxide (5 mg) in 2:1 (v/v) methanol–oxolane (3 mL) was kept overnight at room temperature. More of the mixture of solvents was added, the solution was de-ionized with AG-50W X4 (H^+) resin, the suspension filtered, and the filtrate evaporated *in vacuo* to a crystalline mass that consisted of two compounds. These were separated by column chromatography on silica gel, with 19:1 (v/v) chloroform–methanol as the eluant. The more mobile compound was **16** (30 mg); m.p. 183–186°, $[\alpha]_D^{27}$ −24.4°

(*c* 1.23, oxolane); R_f 0.3 (9:1 $CHCl_3$–MeOH). The other product was identified as **15** (22 mg); m.p. 147–148° (dec.), $[\alpha]_D^{27}$ −15.4° (*c* 1.03, oxolane); R_f 0.2.

Anal. Calc. for $C_{45}H_{57}BrO_{10}S \cdot H_2O$: C, 59.52; H, 8.77. Found: C, 59.98; H, 8.78.

Only **15** was isolated when a solution of **8** (100 mg) and sodium methoxide (3 mg) in 2:1 (v/v) methanol–oxolane (3 mL) was kept for 2 h at room temperature.

1,2,3,6-Tetra-O-acetyl-4-O-(2,3-di-O-acetyl-4-O-benzoyl-6-deoxy-β-D-galactopyranosyl)-D-glucopyranose (**9**). — A solution of **3** (3.0 g) in methanol (25 mL) was mixed with 10% palladium-on-charcoal (3.0 g) and barium carbonate (1.0 g), and hydrogenolyzed until compound **3** had disappeared. The catalyst was filtered off, and the filtrate was evaporated *in vacuo*, to give **4**; this was acetylated with acetic anhydride–pyridine, to yield **5**, which was dissolved in ethyl acetate (30 mL) and hydrogenated in the presence of Raney nickel and triethylamine (1.2 mL), the progress of the reaction being monitored by t.l.c. The catalyst was filtered off, and the filtrate was evaporated to a syrup (1.8 g; 67% from **3**) which was triturated with ice–water, to give **9**; m.p. 91–94°, $[\alpha]_D^{27}$ +70.7° (*c* 1.1, chloroform); n.m.r. ($CDCl_3$): δ 6.30 (d, $J_{1,2}$ 3.5 Hz, H-1α) and 5.72 (d, $J_{1,2}$ 8.0 Hz, H-1β); *m/z* 622 (M^+ − HOAc), 563 (M^+ − HOAc − OAc), and 335 (ii)

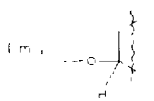


ii

Anal. Calc. for $C_{31}H_{38}O_{17}$: C, 54.55; H, 5.61. Found: C, 54.26; H, 5.43.

6-(5-Cholesten-3β-yloxy)hexyl 2,3,6-tri-O-acetyl-4-O-(2,3-di-O-acetyl-4-O-benzoyl-6-deoxy-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (**12**). — A solution of **9** (1.0 g) in dry dichloromethane (0.5 mL) was treated with 30–32% hydrobromic acid in glacial acetic acid (10 mL) for 1 h at 0–5°. The mixture was processed in the usual way, to give **10** as a syrup (1.0 g): n.m.r. ($CDCl_3$), δ 6.50 (d, $J_{1,2}$ 4.0 Hz, H-1) and 1.27 (d, *J* 6.0 Hz, CH_3).

Compound **10** (0.9 g) was converted into thiol **11** (0.63 g) as for the preparation of **7** from **6**. Condensation of **11** with 6-(5-cholesten-3β-yloxy)-1-iodohexane gave **12** in 55% yield; m.p. 85–86° (MeOH), $[\alpha]_D^{27}$ +0.6° (*c* 1.33, chloroform); n.m.r. ($CDCl_3$): δ 4.47 (d, $J_{1,2}$ 9.5 Hz, H-1), 4.98 (t, $J_{2,3}$ 9.5 Hz, H-2), 5.25 (t, $J_{3,4}$ 9.5 Hz, H-3), 3.80 (t, H-4), 3.64 (o, H-5), 4.49 (2 d, $J_{6c,5}$ 2.0, $J_{6c,6d}$ 12.0 Hz, H-6c), 4.11 (2 d, $J_{6a,5}$ 5.0 Hz, H-6a), 4.50 (d, $J_{1',2'}$ 7.5 Hz, H-1'), 5.20 (2 d, $J_{2',3'}$ 10.0 Hz, H-2'), 5.05 (2 d, $J_{3',4'}$ 3.0 Hz, H-3'), 5.46 (d, H-4'), 3.86 (q, *J* 5.5 Hz, H-5'), 1.25 (d, CH_3-6'), 5.35 (d, *J* 4.5 Hz, olefinic), 3.45 (t, *J* 6.5 Hz; CH_3CH_2O), 3.12



, 2.65 (m, SCH_2CH_2), 1.0 (s, CH_3-19), 0.91 (d, *J* 6.0 Hz, CH_3-21),

0.88 and 0.86 (CH_3-26 and CH_3-27), 0.68 (s, CH_3-18), 8.13–8.11 and 7.67–7.50 (aromatic), and 2.13, 2.07, 2.04, 2.01, and 1.94 (5 s, 5 OAc); *m/z* 1124 (M^+), 1064 (M^+ − HOAc), 623 (M^+ − aglycon), and 335 (ii).

Anal. Calc. for $C_{62}H_{92}O_{16}S$: C, 66.17; H, 8.24; S, 2.85. Found: C, 65.79; H, 8.36; S, 3.07.

6-(5-Cholesten-3β-yloxy)hexyl 4-O-(6-deoxy-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (**14**). — A solution of **12** (100 mg) and sodium methoxide (5 mg) in 1:1 (v/v) methanol–oxolane (5 mL) was kept overnight at room temperature. The mixture was processed in the usual way, to give **14** (55 mg, 76%); m.p. 170–172°, $[\alpha]_D^{27}$ −28.3° (*c* 1.2, oxolane); R_F 0.14 (9:1 $CHCl_3$–MeOH).

Anal. Calc. for $C_{45}H_{78}O_{10}S$: C, 66.63; H, 9.69; S, 3.95. Found: C, 66.87; H, 9.89; S, 4.11.

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