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THE SYNTHESIS OF GLYCOLIPIDS CONTAINING A HYDROPHILIC SPACER-GROUP*

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ABSTRACT

Cholesterol-containing glycolipids incorporating a new hydrophilic spacer-group, 8-amino-3,6-dioxaoctanoic acid, were synthesized. This spacer group eliminates many of the problems inherent in the use of hydrophobic or charged, spacer arms.

INTRODUCTION

Synthetic, cholesterol-containing glycolipids having structure **1** have been synthesized and incorporated into small unilamellar liposomes[1]. The resultant liposomes are then rendered agglutinable by the appropriate lectin[1]. These cholesterol derivatives appear to bind in the membrane much like cholesterol itself. For example, they are distributed evenly on both sides of the bilayer, they decrease the permeability of the liposomes to small, polar molecules, and they exert a condensing effect on the phospholipids in the liposomes[1]. In addition, these glycolipids can also be incorporated into erythrocytes by an exchange process from the liposomes. However, they could not be functionally incorporated into erythrocytes, no matter how long the spacer group was[2]. For example, bovine erythrocytes, which are not susceptible to agglutination by concanavalin A, were not rendered so by incorporation of α-D-mannosyl-containing glycolipids of type **1**. It was reasoned that this lack of success was due to the hydrophobic spacer arms used. Relatively great distances of the sugar residue from the membrane might have to be achieved, which would require the hydrocarbon spacer-arms to be fully extended. The strong hydrophobic interactions of the hydrocarbon chains would unfortunately tend to limit the effective distances achieved. Since a major goal of our studies is to functionally incorporate synthetic glycolipids into cells in an attempt to understand the role(s) of cell surface in recognition phenomena, a more satisfactory spacer-group chemistry was sought. An uncharged

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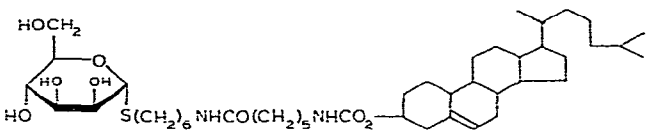
hydrophilic spacer was required that could be conveniently polymerized to generate discrete chain lengths. We report herein the synthesis of such a spacer, 8-trifluoroacetylamino-3,6-dioxaoctanoic acid, and the synthesis of cholesterol-containing, β-D-galactosyl glycolipids derived from it. As anticipated, these glycolipids can be successfully incorporated into cells, suggesting that hydrophilic spacer-arms are indicated when relatively long distances from the cholesterol to the sugar molecule is of importance[2].

RESULTS AND DISCUSSION

The hydrophilic spacer-group was easily synthesized from the commercially available 8-chloro-3,6-dioxaoctan-1-ol (**2**) in five steps. The chloride **2** was converted into the iodide **3** by heating under reflux **2** with sodium iodide in ethyl methyl ketone. The iodide **3** was then transformed into the phthalimido derivative **4** by treating it with potassium phthalimide. Oxidation of this compound with the Jones reagent led to the formation of **5**, which was converted into the *N*-trifluoroacetyl derivative **6**. Although **5**, having an amino terminal group protected as the phthalimido derivative, is suitable for incorporation into the desired glycolipid derivatives, the ease of removal of the trifluoroacetyl group of **6** makes this the precursor of choice.

A suitable D-galactose derivative was available in 2-aminoethyl 1-thio-β-D-galactopyranoside (**10**). Although prepared previously by the condensation of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose (**7**) with ethylene imine followed by



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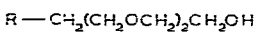
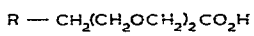
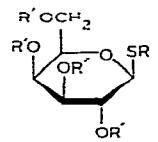


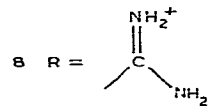
2 R = Cl
3 R = I
4 R = $C_6H_4(CO)_2N$



5 R = $C_6H_4(CO)_2N$
6 R = CF_3CONH

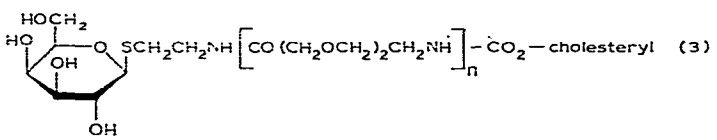


7 R = H, R = Ac



8 R = Br⁻, R′ = Ac

9 R = $-CH_2CH_2NHCOCF_3$, R′ = Ac
10 R = $-CH_2CH_2NH_2$, R′ = H



11 n = 1
12 n = 2

deprotection[3], an alternative synthesis was sought in order to avoid the use of the unstable and highly toxic ethylene imine[3]. Such an end was realized by alkylation of **7**, generated *in situ* by reduction of the corresponding thiouronium salt **8**, with 1-iodo-2-(trifluoroacetylamino)ethane, to generate **9** under the conditions developed previously for the synthesis of 6-aminohexyl 1-thio-β-D-galactopyranoside[4]. Deprotection yielded the desired **10** in high yield.

Since the starting material for the synthesis was the well characterized *S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranosyl)thiouronium bromide[4] (**8**), and since the reductive conditions employed were known not to cause anomerization prior to alkylation for closely related compounds, the resultant aminoethyl thiogalactoside was expected to be in the β-D configuration[4]. Examination of the n.m.r. spectrum of **10**, or of its fully protected precursor **9**, revealed the signals assigned to the anomeric proton as doublets with the relatively large coupling constant (J 9–10 Hz) expected of the β-D configuration[5].

The aminoethyl 1-thio-β-D-galactopyranoside (**10**) was coupled to the *N*-protected amino acid spacer (**5** or **6**) in the presence of 2-ethoxy-*N*-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ) and, following deprotection, the free amino compound was coupled to cholesterol chloroformate to form one of the desired synthetic glycolipids, or coupled to a second spacer such as **6** to lengthen further the chain and give **11** and **12**, respectively. The amino acid polymerization steps may be repeated to provide even greater distances between the cholesterol and sugar residues. In addition, the compounds could be prepared in a radioactive form by D-galactose oxidase oxidation of the β-D-galactoside followed by sodium boro[^{3}H]hydride reduction.

As mentioned earlier, synthetic glycolipids with hydrophilic spacer-groups can be functionally incorporated into cells[2]. For example, the α-D-mannosyl congener of **12** could be exchanged into bovine erythrocytes[2]. These cells, which are not normally agglutinable by concanavalin, became agglutinable at a concentration of 4 μg/mL of the lectin[2]. It was not possible to achieve functional incorporation using hydrophobic spacer arms based on 6-aminohexanoic acid[2].

EXPERIMENTAL

General. — 2-[2-(2-Chloroethoxy)ethoxy]ethanol, D-galactose, potassium phthalimide, trifluoroacetic anhydride, 2-aminoethanol, and triethylamine were obtained from the Aldrich Chem. Co., Milwaukee, WI 53233; cholesterol chloroformate and catalase from Sigma Chemical Co., St. Louis, MO 63178; D-galactose oxidase from Worthington Biochemical Corp., Freehold, NJ 07728; *S*-ethyltrifluorothioacetate and 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) from the Pierce Chem. Co., Rockford, IL 61105; and Silica Gel 60 and hydrazine hydrate from MCB Reagents, E. Merck, Darmstadt. All solvents were of the highest purity available. *N*,*N*-Dimethylformamide was distilled over calcium hydride prior to its use. N.m.r. spectra were recorded with a Perkin–Elmer R-24A spectrometer. Microanalyses were performed by the Galbraith Laboratories Inc., Knoxville, TN 37821.

8-Iodo-3,6-dioxaoctane-1-ol (**3**). — Sodium iodide (46.72 g, 0.325 mol) was dissolved in reagent grade butanone (350 mL) by stirring at room temperature. 8-Chloro-3,6-dioxaoctan-1-ol (**2**, 42.16 g, 0.25 mol) was added and the solution boiled under reflux for 19 h. The mixture was filtered to remove salts, and the precipitate washed with butanone (2 × 50 mL). The filtrates were combined, evaporated under diminished pressure, and the residue dissolved in dichloromethane (150 mL). The solution was washed with a solution of sodium hydrogensulfite (20 g in 50 mL of water), followed by a mixture of 0.5M sodium hydrogencarbonate (20 mL) and saturated sodium chloride (50 mL), and finally with saturated sodium chloride solution (3 × 100 mL), dried (magnesium sulfate), and evaporated under diminished pressure to give a yellowish oil (61.4 g). This product could be further purified by Kugelrohr distillation to yield a liquid (58.3 g) $b.p._{\cdot 0.05\,mm}$ 105–125°.

8-Phthalimido-3,6-dioxaoctan-1-ol (**4**). — A 500-mL, three neck flask, fitted with a thermometer and nitrogen blanket was charged with **3** (39 g, 0.15 mol), dry *N*,*N*-dimethylformamide (150 mL, dried over and distilled from calcium hydride, and stored over 3A molecular sieves), and potassium phthalimide (30 g, 0.165 mol). The mixture was stirred under nitrogen for 18 h at 95°, at which time the mixture was allowed to cool and the solvent removed *in vacuo*. The residue was dissolved in water (60 mL), and the solution extracted with dichloromethane in a continuous extraction apparatus for 18 h. The organic extract was dried (magnesium sulfate), filtered, and the solvent removed under diminished pressure to leave **4** as an amber-colored oil (54.3 g) which was submitted to Kugelrohr distillation yielding an oil (38.2 g), $b.p._{\cdot 0.05-0.1\,mm}$ 155–195°. This material (~20 g) in dichloromethane (75 mL) was further purified by chromatography on a column of silica gel (300 g, wet packed in dichloromethane in a column 42-cm high and 4.5-cm in diameter). The column was developed with 4:3 (v/v) dichloromethane–chloroform (3.5 L), and 1:19 (v/v) methanol–chloroform (2 L). Compound **4** was eluted with the latter mixture. Evaporation gave a colorless oil (16.7 g) which solidified on storage. Drying under high vacuum gave a white solid, m.p. 55–57°: n.m.r. ($CDCl_3$): δ 7.8 (d, 4 H, *J* 2 Hz, Ar-H), 3.87 (t, 2 H, *J* 4 Hz, N-CH_2), 3.65 and 3.6 (s, 10 H, CH_2O), and 3.0 (broad, 1 H-OH).

Anal. Calc. for $C_{14}H_{17}NO_5$: C, 60.20; H, 6.15; N, 5.02. Found: C, 60.57; H, 6.13; N, 5.06.

8-Phthalimido-3,6-dioxaoctanoic acid (**5**). — A solution of **4** (1.4 g, 5 mmol) in acetone (75 mL) was stirred as 2.67M Jones reagent [3.0 mL, 8 mmol, prepared by dissolving chromic trioxide (26.72 g) in conc. sulfuric acid (23 mL) and diluting to a volume of 100 mL] was added dropwise within ~15 min. The solution was stirred for ~30 min, then 2-propanol (a few drops) was added. Water (50 mL) was added to dissolve the precipitated chromium salts, and the acetone removed by evaporation under diminished pressure. The aqueous mixture was transferred to a separatory funnel, sat. sodium chloride solution (25 mL) added, and the solution was extracted with dichloromethane (7 × 25 mL). The combined dichloromethane extracts were dried (magnesium sulfate), filtered, and evaporated to dryness leaving a solid (1.4 g).

which was crystallized from ethyl acetate–hexane to give white crystals (1.1 g), m.p. 105–109°; n.m.r. ($CDCl_3$): δ 8.50 (s, J 1 H, CO_2H), 7.77 (d, 4 H, J 2 Hz, Ar-H), and 3.72 (s, 6 H, CH_2).

8-Trifluoroacetylamino-3,6-dioxaoctanoic acid (**6**). — A solution of **5** (4.0 g, 13.64 mmol) in a solution of hydrazine hydrate (1.82 g, 68.4 mmol, Eastman) in methanol (50 mL) was boiled under reflux for 3 h, at which time t.l.c. analysis revealed no starting material. The solvent was removed *in vacuo* and high vacuum applied to remove the excess of hydrazine. The residue was dissolved in water (25 mL), the pH adjusted to 2.8 with conc. hydrochloric acid, and the resultant suspension kept overnight in a refrigerator. The precipitate was filtered off, washed, and discarded. The combined filtrates were evaporated under reduced pressure to give the amine hydrochloride salt (3.74 g).

In order to obtain the free base, a solution of the salt in water (20 mL) was applied to a column of Bio Rex 1-X8 ion-exchange resin (OH^-; 50-mL bed volume, 1.4 meq/mL of resin). The product was eluted with 2M acetic acid (400 mL) and the solvent removed under diminished pressure to give an amorphous solid (2.15 g). The solid was treated with trifluoracetic anhydride (20 mL, Aldrich). It dissolved within ~15 min with stirring, and the resultant solution was kept for 2 h at ambient temperature. The excess of anhydride was removed under reduced pressure, and the residue taken up in water and kept for 1 h. The solvent was evaporated to give a liquid (3.18 g) which was purified by Kugelrohr distillation resulting in a colorless viscous liquid (2.8 g): $b.p._{0.05\,mm}$ 150–150°; n.m.r. ($CDCl_3$): δ 10.3 (s, 1 H, $-CO_2H$), 8.33 (broad, 1 H, NH), 4.20 (s, 2 H, $-CH_2CO_2-$), 3.73 (s, 4 H, $-CH_2O$), and 3.67 (s, 2 H, $-CH_2-N$).

Anal. Calc. for $C_8H_{12}F_3NO_5$: C, 37.07; H, 4.68; N, 5.40. Found: C, 37.27; H, 4.73; N, 5.47.

1-Iodo-2-(trifluoroacetylamino)ethane. — 2-Aminoethanol (6.11 g, 0.1 mol) was dissolved in dichloromethane (25 mL) and stirred as *S*-ethyl trifluorothioacetate (17.4 g, 0.11 mol) was added dropwise within ~30 min. The mixture was stirred vigorously for 30 min. The solvent was boiled off (in a fume hood), and several portions of dichloromethane were added and evaporated in a nitrogen stream to remove the last of the ethane thiol. The remainder of the solvent was removed *in vacuo*, leaving a yellowish, partially crystalline oil, which was purified by Kugelrohr distillation (90–100°, 0.1 mm Hg) yielding 15.72 g of 2-(trifluoroacetylamino)ethanol; n.m.r. (CD_3COCD_3): δ 8.37 (s, br, 1 H, NH), 4.07 (s, br, 1 H, OH), and 3.87–3.33 (m, 4 H, CH_2).

A solution of this compound in dry pyridine (50 mL distilled in the presence of barium oxide) was cooled in an ice-salt bath with stirring, and treated with *p*-toluenesulfonyl chloride (38 g, 0.2 mol). After 1 h, the solution was transferred to a refrigerator (4°) and kept overnight. The suspension was poured into ice–water (2 L) and mixed vigorously. After a few minutes of stirring in the cold room, the product solidified and was collected on a Büchner funnel, washed several times with water, and dried *in vacuo* to leave 26.8 g of an off-white solid. This was added to a

solution of sodium iodide (22.5 g, 0.150 mol) in butanone (175 mL) and boiled under reflux for 3 h. The suspension was filtered and the precipitate washed with acetone. The combined filtrates were concentrated *in vacuo*. The resultant residue was partitioned between ethyl ether (50 mL) and sodium hydrogensulfite solution (20 g in 50 g of water). The aqueous phase was washed once with ether (25 mL), and the ether extracts were combined and washed with M sodium hydrogencarbonate (25 mL), sat. sodium chloride solution (25 mL), and dried (magnesium sulfate containing 1 g of charcoal). Filtration followed by evaporation left 21.8 g of a white solid. Crystallization from hexane–ethyl acetate gave white crystals, m.p. 65–66°; n.m.r. ($CDCl_3$): δ 7.3 (s, br, 1 H, NH), 3.67 (t, 2 H, J 6 Hz, $-CH_2N$), and 3.27 (t, 2 H, J 6 Hz, $-CH_2I$).

Anal. Calc. for $C_4H_5F_3INO$: C, 17.99; H, 1.89; N, 5.24. Found: C, 18.30; H, 1.94; N, 5.19.

*2-(Trifluoroacetylamino)ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-*D*-galactopyranoside* (**9**). — A solution of the thiouronium bromide[4] **8** (3.65 g, 7.5 mmol) in distilled water (10 mL) (some warming necessary) was stirred into a mixture of potassium carbonate (1.2 g, 8.7 mmol). Sodium hydrogen sulfite (2.74 g, 14.4 mmol) was added in a single portion. Immediately thereafter a solution of 1-iodo-2-(trifluoroacetylamino)ethane (2.20 g, 8.25 mmol) in acetone (6 mL) was added. Small portions of acetone and water were used to wash in the residual reagents. The suspension was stirred vigorously for 30 min, two phases being apparent. Water (15 mL) and chloroform (25 mL) were added, the phases separated, and the aqueous phase was extracted with chloroform (15 mL). The combined chloroform extracts were washed twice with 25-mL portions of M hydrochloric acid, twice with 25-mL portions of water, and dried (1 g of magnesium sulfate). Filtration followed by evaporation of the solvent left a colorless, amorphous residue (4.10 g). This was purified by preparative h.p.l.c. chromatography using a Waters Associates Prep 500 system equipped with a silica gel column, elution with 1 : 19 (v/v) acetone–dichloromethane, and a flow rate of 50 mL/min to give a major component, isolated after evaporation as an amorphous solid (2.69 g): t.l.c. (silica gel): R_F 0.14 (99 : 1, v/v chloroform–methanol) and 0.28 (19 : 1, v/v dichloromethane–acetone); n.m.r. ($CDCl_3$): δ 7.23 (br, 1 H, NH), 5.52, 4.97 (m, 3 H, H-2, -3, and -4), 5.1 (s, 3 H, H-6 and -5), 4.53 (d, 1 H, J 10 Hz, H-1), 3.57 (t, 3 H, J 5 Hz, CH_2N), 3.07–2.77 (m, 2 H, SCH_2C), 2.17, 2.07, 2.03, and 2.00 (closely spaced singlets, 12 H, $OCOCH_3$).

*2-Aminoethyl 1-thio-β-*D*-galactopyranoside* (**10**). — Compound **9** (2.45 g, 5.12 mmol) was dissolved in dry methanol (20 mL, distilled from sodium methoxide), and treated with a solution of sodium methoxide (120 mg of sodium in 10 mL of methanol). The reaction was allowed to proceed for 60 min at ambient temperature [t.l.c. (19 : 1, v/v, chloroform–methanol, silica gel) indicated that the starting material disappeared within 5 min]. The still alkaline solution was neutralized (pH test paper) by the addition of Dowex 50 W-X8 (H^+), cation-exchange resin, (20 mL), with thorough mixing for 5–10 min. The suspension was filtered, the resin washed with several portions of methanol, and the solvent evaporated *in vacuo* to leave a solid

crystalline residue (1.82 g). This material was used without further purification, by dissolving it in 1:1 (v/v) ethanol–water (50 mL) and treating the solution with 15–20 mL of BioRad AG 1-X8 (1.5 meq./mL, OH^-, 50–20 mesh) anion-exchange resin. The suspension was shaken vigorously, the progress of the hydrolysis being monitored by t.l.c. (silica gel, 3:2:1, v/v, ethyl acetate–acetic acid–water); the starting material disappeared after 20 min and a new, ninhydrin-positive spot appeared. Shaking was continued for a total of 2 h to insure complete hydrolysis. The resin was removed by filtration, and washed with several portions of ethanol–water and water. The combined filtrates were concentrated *in vacuo* to leave 1.04 g (yield 76%) of a white, crystalline residue, which was recrystallized from ethanol (1 g from ~100 mL) to yield 0.889 g of white needles, m.p. 155–156.5° (after drying at 5 μm Hg for 18 h at 8°) (lit[3] m.p. 130–131°); n.m.r. (D_2O): δ 4.67 (d, *J* 10 Hz, H-1), 3.98–3.28 (m, 6 H, H-2, -3, -4, -5, and -6), and 2.85 (s, 4 H, SCH_2CH_2N).

Anal. Calc. for $C_8H_{17}NO_5S$: C, 40.15; H, 7.17; N, 5.85. Found: C, 40.38; H, 7.50; N, 5.74.

*2-[8-(N-Cholesterylcarbamoylamino)-3,6-dioxaoctanamino]ethyl 1-thio-β-*D*-galactopyranoside* (**11**) *from* **6**. — Compound **10** (219 mg, 1 mmol) was coupled to 8-trifluoroacetamido-3,6-dioxaoctanoic acid (**6**, 263 mg, 1.1 mmol) in the presence of EEDQ (272 mg, 1.1 mmol) and 1:1 (v/v) methanol–ethanol (2 mL). The solution was stirred overnight at room temperature (disappearance of the ninhydrin-positive material). After removal of the solvent, the amorphous product was purified on silica gel (10 g) developed with a step gradient of 19:1, 9:1, and 17:3 (v/v) chloroform–methanol. Standard Bio-Rex 1 (OH^-) procedure removed the trifluoroacetyl group from the product (448 mg). The amine which resulted (290 mg, 0.75 mmol) was neither purified nor characterized, but was dissolved in *N*,*N*-dimethylformamide (5 mL, distilled from calcium hydride), and a slight excess of triethylamine was added (80 mg, 0.8 mmol), followed by cholesterol chloroformate (387 mg, 0.86 mmol). The resultant suspension was stirred overnight at ambient temperature at which time the ninhydrin-positive material had disappeared. The solvent was removed by rotary evaporation under high vacuum (1 mm) to leave a yellowish, amorphous residue that was treated with water (20 mL). A white precipitate formed that was collected by filtration. The solid was dried by repeated additions and evaporations of ethyl alcohol followed by drying *in vacuo*. The compound was purified by chromatography on Silica Gel 60 (30 g) wet-packed into a 125-mL cylindrical separatory funnel (height of silica 5 cm). The column was developed with a step gradient of 19:1 (300 mL) (discard), followed by 9:1 (v/v) chloroform–methanol (300 mL) (30-mL fractions collected). The product emerged in the latter eluate and was isolated as a white powder (217 mg), which was crystallized from dichloromethane (15 mL) and petroleum ether, white crystals (198 mg); m.s. (field desorption): *m/e* 796 (M^+).

Anal. Calc. for $C_{42}H_{72}N_2O_{10}S$: C, 63.29; H, 9.10; N, 3.51. Found: C, 62.97; H, 9.51; N, 3.43.

From **5**. Compound **10** (478 mg, 2 mmol) and **5** (646 mg, 2.2 mmol) were coupled in the presence of EEDQ (593 mg, 2.4 mmol) in 1:1 (v/v) ethanol–methanol

(4 mL) for 24 h at ambient temperature, at which time t.l.c. (silica gel, 8:2:1, v/v, ethyl acetate–acetic acid–water) revealed that all starting **10** had disappeared. The solvent was removed *in vacuo* and the product purified by chromatography on silica gel with 8:2:1 (v/v) ethyl acetate–acetic acid–water. Two major u.v. absorbing bands were observed. The fast-moving product was discarded and the second, slow-moving product was collected, and the solvent removed under reduced pressure. Alternately, larger quantities of purified product were readily available by applying the crude product mixture to a C-18 column in a Waters Associates Prep 500 system using a solvent system of 2:3 (v/v) methanol–water and a flow rate of 100 mL/min. The major (u.v. positive) compound was collected (728 mg); n.m.r. (CD_3OD): δ 7.77 (s, 4 H, Ar-H), 4.5–3.4 (m), and 2.87 (t, 2 H, J 6 Hz, S-CH_2-C).

The phthalimido group was removed by lyophilizing the solvent (728 mg, 1.42 mmol), dissolving the resultant oil in methanol (10 mL) and treating with hydrazine hydrate (141 mg of $NH_2NH_2 \cdot H_2O$, 2.84 mmol). The resultant mixture was kept for 3 h at ambient temperature, when t.l.c. (silica gel, 8:2:1, v/v, ethyl acetate–acetic acid–water) revealed that the starting material had disappeared. The solvent was removed under reduced pressure, and the residue dissolved in water and lyophilized. The amorphous residue was taken up in distilled water (25 mL) and the pH adjusted to 2.0 by the addition of hydrochloric acid, giving a white precipitate. The suspension was kept for 20 min, the phthalhydrazide removed by filtration and washed with several portions of water, and the filtrates were combined. Lyophilization gave the amine hydrochloride salt as an amorphous residue (0.79 g).

In order to obtain the free amine, the salt in water (10 mL) was neutralized with Bio-Rex 1-X8 (OH^-, 1.4 meq/mL, 2 mL) anion-exchange resin. After mixing the solution for a few minutes, the pH was alkaline, and the resin was removed by filtration through a short column of fresh resin. The column was washed with water, and the combined filtrates were lyophilized to give 0.52 g of ninhydrin-positive material; t.l.c. (silica gel, 3:2:1, v/v, ethyl acetate–acetic acid–water): R_F 0.22. This amine could be coupled to cholesteryl chloroformate by use of the aforementioned procedure, to give compound **11**, identical in all respects to that obtained by method A.

*2-[17-(*N*-Cholesterylcarbamoylamino)-3,6,12,15-tetraoxa-9-aza-10-oxo-heptadecanamino]ethyl 1-thio-β-*D*-galactopyranoside* (**12**). — This compound was synthesized from **10** (478 mg, 2 mmol) and **6** by use of a sequence of couplings (EEDQ) and deprotections (hydrazine) identical to those just described. The intermediate *N*-phthalimido-protected compounds were not characterized, but were purified by h.p.l.c. (Waters Prep 500; C-18 column) for the compound containing one spacer, and by silica gel chromatography with the standard methanol–chloroform step gradient, for the compound containing two spacers. Coupling of the deprotected amine to cholesterol chloroformate was achieved in *N*,*N*-dimethylformamide–triethylamine under conditions identical to those used earlier, and the product purified by chromatography on silica gel and elution with methanol–chloroform step gradient. The compound isolated in this fashion (322 mg) was crystallized from dichloromethane–hexane, a white solid: m.s. (field desorption): *m/e* 941 (M^+).

Anal. Calc. for $C_{48}H_{83}N_3O_{13}S$: C, 61.19; H, 8.87; N, 4.46. Found: C, 61.36; H, 8.71; N, 4.44.

*2-[8-(N-Cholesterylcarbamoylamino)-3,6-dioxaoctanamino]ethyl 1-thio-β-*D*-[6-$^3H_1$]galactopyranoside* (^3H-**11**). — The tritium-labeled 1-thiogalactosyl derivatives were synthesized *via* D-galactose oxidase-catalyzed oxidation of CH_2OH-6 to an aldehyde group, by a modification of the procedure of Maradufu *et al.*[6], followed by reduction to the alcohol with sodium borotritide. The derivative obtained by condensation of **10** with **6** (0.79 g, 1.65 mmol) was dissolved in 25mM phosphate buffer (15 mL, pH 7), catalase added to decompose hydrogen peroxide produced in the oxidation (5 mg, Sigma C-100, 25 mg protein/mL), followed by a solution of D-galactose oxidase (1.1 mL, 107 unit/mL, Worthington 1522 GAO 58P484). The mixture was incubated for 30 min, when second portions of catalase and D-galactose oxidase were added. The reaction mixture was incubated overnight at 35° in a shaking water-bath. The solvent was removed by lyophilization, and the organic compounds were extracted into methanol (5 mL). The precipitate was removed by centrifugation, and washed twice with 5-mL portions of methanol. The combined organic extracts were evaporated, and the residue dried *in vacuo*. It was dissolved in abs. ethanol (5 mL) and treated with $NaBT_4$ (3.9 mg) in ethanol (1 mL). The solution was stirred for 30 min when $NaBH_4$ (15 mg) was added to insure complete reduction. The excess of borohydride was quenched with glacial acetic acid (300 μL), the solvent removed *in vacuo*, and the residue purified on a silica gel column (10 g), developed with the methanol–chloroform system described earlier (yield 0.53 g). The *N*-trifluoroacetyl group was removed, and the resultant amine coupled with cholesterol chloroformate as described earlier to yield the radioactive glycolipid.

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