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Water-Soluble Cholesteryl-Containing Phosphorothioate Monogalactosides: Synthesis, Properties, and Use in Lowering Blood Cholesterol by Directing Plasma Lipoproteins to the Liver

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The synthesis of several monogalactoside-terminated phosphorothiolated cholesteryl derivatives is described. Monogalactosyl derivatives are coupled by phosphorothiolation to cholesterol by using ethylene glycol units as hydrophilic spacer moieties. The resulting compounds are easily soluble in water. Upon addition of such solutions to human serum (to 2 mM final concentration) the compounds are readily incorporated into lipoproteins. Isolated low-density lipoprotein (LDL) and high-density lipoprotein (HDL), preloaded with the compounds, are rapidly cleared from the circulation by the liver. The hepatic association is blocked by *N*-acetylgalactosamine, which indicates that galactose-specific recognition sites are responsible for the increased liver uptake. The plasma clearance and hepatic uptake of LDL loaded with the compounds is substantially higher (about 2-fold) than clearance and uptake of HDL containing the compounds. The selectivity of the effects of monogalactoside-terminated phosphorothiolated cholesteryl derivatives on the in vivo behavior of LDL as compared to that of HDL indicates that these compounds might be used to lower specifically LDL levels in patients with a high LDL-cholesterol level.

Introduction

High plasma levels of low-density lipoprotein (LDL) cholesterol are correlated with an increased occurrence of atherosclerosis.[1,2] LDL is the major vehicle for the transport of cholesterol in the circulation. A smaller part of the circulating cholesterol is transported by high-density lipoproteins (HDL), the level of which is inversely correlated with atherosclerotic disease.[1] Removal of LDL from plasma occurs mainly via specific LDL (apo B,E) receptors in the liver.[3,4] The importance of these receptors is evident from the congenital disease familial hypercholesterolemia (FH). The homozygous form of the disease is characterized by the lack of functional LDL receptors.[5] As a result, LDL levels in the circulation are 6-8 times above normal and myocardial infarction usually occurs before the age of 20. In heterozygotes, LDL levels are 2-3 times the normal levels. These individuals are still at a much greater risk of myocardial infarction.[5]

Therapies of FH are aimed at lowering the levels of circulating LDL. Current drug therapies of heterozygous FH consist of the administration of inhibitors of HMG-CoA reductase (the rate-limiting enzyme in the synthesis of cholesterol) and/or bile acid sequestrants. These treatments induce an increase in the number of active LDL receptors in liver, which results in lower levels of circulating LDL.[5-8] Homozygotes do not respond appreciably to these therapies.[9]

In our studies, we examined the potential to induce removal of circulating LDL via alternative receptor mechanisms, in particular via hepatic galactose-specific receptors. Two different types of galactose-specific hepatic receptors have been described, specific for parenchymal and Kupffer cells, respectively. The receptor on parenchymal cells is the classical asialoglycoprotein receptor originally described by Ashwell and Morell.[10] The receptor on Kupffer cells was characterized more recently.[11] It specifically recognizes particles larger than 10-15 nm that have exposed galactose residues.[12] In previous papers[13,14]

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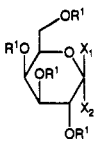
we demonstrated that incorporation of a cholesterol derivative with three terminal galactose residues (tris-gal-chol) into LDL induces galactose-specific uptake of the atherogenic particle by the liver. However the hepatic uptake of HDL, which has a protective function for atherosclerosis,[1] is increased to the same extent by the incorporation of tris-gal-chol.[15,16] Uptake of tris-gal-chol-loaded LDL proceeds via galactose receptors on Kupffer cells,[14] whereas tris-gal-chol-loaded HDL is taken up via the asialoglycoprotein receptors on parenchymal cells.[15] It has been shown earlier[17] that the latter receptor binds molecules with a triantennary terminal galactose structure with much higher affinity than molecules with bis- or monoantennary galactose residues. To reduce the galactose-specific uptake of HDL (and hopefully retain that of LDL), we have synthesized cholesterol derivatives with a single galactose residue. To assure the water-solubility of the compounds, the galactose and cholesterol moieties were connected by a hydrophilic spacer consisting of a number of ethylene glycol units and a negatively charged phosphate group. A phosphorothioate group was used to increase the stability against hydrolytic enzymes.[18] In addition, the negatively charged phosphate might lead to a preferred interaction of the compounds with the positively charged LDL particle,[19] as has been reported for other negatively charged molecules.[20]

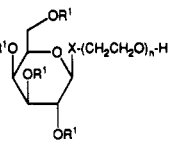
In the present paper we describe the synthesis and some properties of these compounds. We further present data on the effects of incorporation of the compounds into LDL and HDL on the fate of these lipoproteins after intravenous injection into rats.

Results and Discussion

Synthesis. The partially protected galactopyranoside **2a**, a key intermediate in the synthetic route to the target compound **8a**, was easily accessible[21,22] by a silver triflate promoted glycosylation[23] of bromide **1a**[24] with ethylene glycol in the presence of 2,6-di-*tert*-butylpyridine. Homogeneous **2a** was obtained in a 89% yield after workup and purification by short-column chromatography. Apart from **2a** also a small amount (6%) of a byproduct derived from the reaction of **2a** with **1a** could be isolated. The β-configuration of the glycosidic bond in the desired compound **2a** was unambiguously ascertained by ^1H and ^{13}C NMR spectroscopy.



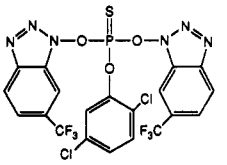
1a. R^1 = Benzoyl, X_1 = H, X_2 = Br
b. R^1 = Acetyl, X_1 = SH, X_2 = H



2a. R^1 = Benzoyl, X = O, n = 1
b. R^1 = Acetyl, X = S, n = 1
c. R^1 = Acetyl, X = S, n = 3

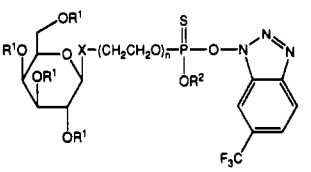
The corresponding thio derivative **2b** was prepared by treatment of the partially protected thiogalactopyranose **1b**[25,26] with 2-iodoethanol in the presence of *N,N*-diisopropylethylamine. Subsequent isolation and purification gave compound **2b** in 87% yield. In a similar fashion, **2c** was obtained in a high yield by reaction of the same thiogalactoside with the monoiodo derivative of triethylene glycol.[27] The presence of the required β-glycosidic linkage in **2b** as well as in **2c** was confirmed by the characteristic large coupling constant between H-1 and H-2 (J = 9–10 Hz)[28] and by the expected ^{13}C NMR chemical shift of the C-1 atom[29] at 84.2 and 83.2 ppm, respectively.

The fully protected precursors **6a–c** of **8a–c** were assembled by a well-established[30,31] one-pot, two-step phosphorylation procedure. Thus phosphorylation of **2a** with the bifunctional reagent **3**, prepared in situ from



3

2,5-dichlorophenyl phosphorodichloridothioate[32] and 1-hydroxy-6-(trifluoromethyl)benzotriazole,[33] gave the putative intermediate **4a**. Condensation of **4a** with the



4a. R^1 = Benzoyl, R^2 = 2,5-$Cl_2C_6H_3$, X = O, n = 1
b. R^1 = Acetyl, R^2 = 2,5-$Cl_2C_6H_3$, X = S, n = 1
c. R^1 = Acetyl, R^2 = 2,5-$Cl_2C_6H_3$, X = S, n = 3

cholesteryl derivative **5**[34,35] proceeded smoothly, as gauged by TLC analysis, within 2 h at ambient temperature. After workup and purification, **6a** was obtained in a good yield

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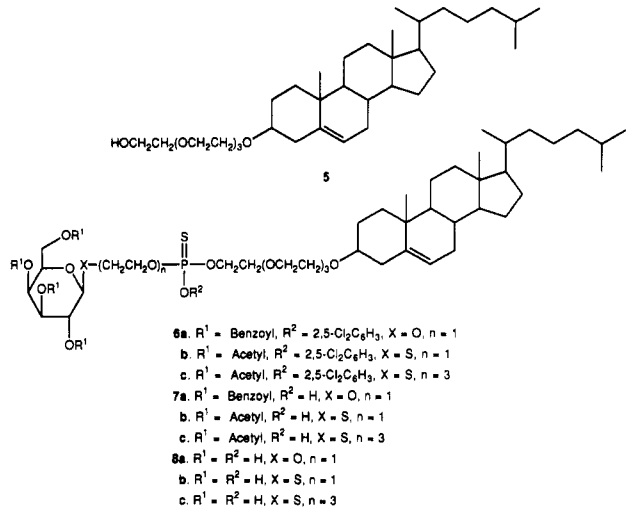

as a mixture of diastereomers due to the chiral phosphorus atom. The anomeric thio analogues **6b** and **6c** were prepared in a similar fashion as described above for **6a** and they could be isolated in a 79% and 81% yield, respectively. In this respect it is of interest to note that any attempt to separate the diastereomers of the individual fully protected derivatives **6a–c** was not successful.

Complete deblocking of the fully protected compounds **6a–c** was performed in two steps. Firstly, oximate ion promoted[36] hydrolysis of the 2,5-dichlorophenyl (R^2) group gave the corresponding chiral phosphorothioate derivatives **7a–c**. Subsequently, the benzoyl (R^1) groups from **7a** and acetyl (R^1) groups from **7b** and **7c** were removed by ammonolysis. Purification of the crude products by gel filtration and their conversion into the respective sodium salts by cation exchange furnished **8a–c** in a yield of 44–51% (based on **6a–c**). NMR spectroscopic data (^1H, ^{13}C, and ^{31}P) were in full accordance with the proposed structures of **8a–c**.

Interaction with Lipoproteins in Human Serum. The compounds were readily soluble in water. Stock solutions of 10 mM in phosphate-buffered saline (PBS) were made, which were optically clear. Upon dialysis, no loss of material to the outer compartment was observed, which indicates that the compounds form micelles with a MW of at least 10 kDa.

The interaction of the compounds **8a–c** with serum lipoproteins was studied by mixing these stock solutions with human serum to final concentrations of 2 mM. To separate very low density lipoprotein (VLDL), LDL, and HDL from the remainder of the serum proteins, the mixtures were subjected to density-gradient ultracentrifugation in KBr/NaCl solutions.[37] As observed previously with tris-gal-chol,[14] it was found that the LDL fraction isolated from serum mixed with any of the three compounds had a distinctly higher buoyant density (peak density at 1.060 g/mL) than LDL from the control serum (peak density at 1.035 g/mL). The density distribution of the HDL fraction was not markedly affected. The fractions were assayed for cholesterol and total carbohydrate. Table I shows that all lipoproteins have taken up compounds **8a–c** since they contain more carbohydrate when isolated from the serum with added compound. The distribution over the density fractions is almost similar for each of the three compounds. The occurrence of part of these compounds in the $d > 1.21$ g/mL fraction indicates that not all material is bound to the lipoproteins but is either bound to serum albumin or not bound at all. If it is assumed that an LDL particle contains 1785 molecules of cholesterol (free and esterified) and an HDL particle 45,[38] it can be calculated from these data that on average 730 molecules of **8a–c** are associated with an LDL particle and 64 molecules with an HDL particle. Taking into account that the diameter of an LDL particle is ca. 2.5 times the diameter of an HDL particle, it can be calculated that under these conditions an LDL particle contains about twice as much of the compounds per unit of surface area than an HDL particle. This is considerably higher than calculated earlier for tris-gal-chol,[14] but experiments at that time were done with a 0.85 mM final concentration of glycolipid.

Table I. Results of Determination of the Association of **8a**, **8b**, and **8c** with the Lipoproteins in Human Serum[a]

		cholesterol, μg	carbohydrate, μg	ratio carbohydrate/cholesterol
serum + PBS	VLDL	77	40	
	LDL	1327	340	0.26
	HDL	433	80	0.18
	LPDS	101	1330	
	total	1939	1790	0.92
serum + **8a**	VLDL	104	100	
	LDL	1048	480	0.45 (0.19)
	HDL	627	500	0.80 (0.62)
	LPDS	70	1710	
	total	1850	2790	1.51
serum + **8b**	VLDL	108	80	
	LDL	1161	580	0.50 (0.24)
	HDL	500	460	0.92 (0.74)
	LPDS	85	1590	
	total	1885	2710	1.44
serum + **8c**	VLDL	101	100	
	LDL	1296	520	0.40 (0.14)
	HDL	484	390	0.81 (0.63)
	LPDS	66	1720	
	total	1947	2730	1.40

[a] Aliquots of 1.6 mL of human serum were mixed with 0.4 mL of 10 mM solutions of compounds **8a**, **8b**, or **8c** in phosphate-buffered saline (PBS), or with 0.4 mL of PBS, and incubated for 10 min at room temperature. The mixtures were then subjected to density-gradient centrifugation.[37] The VLDL ($d < 1.006$ g/mL), LDL ($1.006 < d < 1.063$ g/mL), HDL ($1.063 < d < 1.210$ g/mL) and LPDS (lipoprotein-deficient serum; $d > 1.210$ g/mL) fractions were collected, dialyzed against PBS, and assayed for cholesterol and carbohydrate. The results are expressed as μg of total cholesterol and μg of total carbohydrate per density fraction. The data in parentheses represent the carbohydrate/cholesterol ratios (μg/μg) of the two major cholesterol carriers LDL and HDL in serum + compounds compared to the lipoproteins in serum + PBS.

We considered the possibility that the exposure of the serum to high salt concentrations during ultracentrifugation might interfere with the binding of compounds **8a–c** to the lipoproteins. For that reason we isolated HDL from serum by immunoabsorption. This was done by applying either 1 mL of serum alone or 1 mL of serum incubated with compounds **8a–c** on a column of protein G substituted Sepharose, which was preloaded with a goat anti-human apo-A1 antiserum. After washing with PBS (to remove the unbound material), the absorbed material (immunoglobulins and HDL) was eluted with 0.1 M glycine hydrochloride, pH 2.7. The eluate was assayed for cholesterol and carbohydrate. It was found that in the HDL from serum incubated with compounds **8a–c**, the carbohydrate contents had increased on average by 0.47 mg of carbohydrate/mg cholesterol. This is somewhat, but not much, lower than the data obtained after ultracentrifugation (cf. Table I, increase of about 0.60 mg carbohydrate/mg cholesterol).

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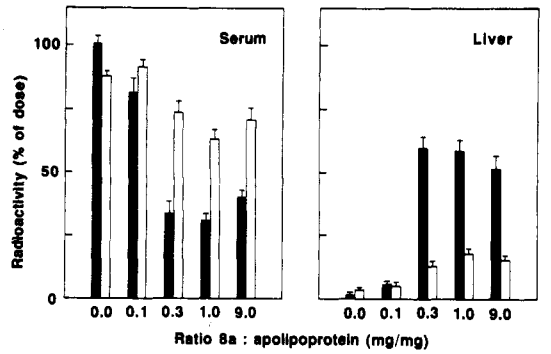

Figure 1. Effect of **8a** on the serum decay and liver association of [^{125}I]LDL and [^{125}I]HDL. Compound **8a** was mixed with radiolabeled LDL (■) or HDL (□) at the indicated ratios, expressed as mg of compound/mg of apolipoprotein. To convert to μg of carbohydrate/μg of cholesterol, the ratios should be multiplied by 0.45 for LDL and 0.06 for HDL (based on lipoproteins compositions reported before[38]). After 10 min at room temperature, the mixtures were injected into rats at a dose of 50 μg of apoprotein/kg of body weight. At 5 min after injection, the trichloroacetic acid precipitable radioactivities in the serum (left-hand panel) and the radioactivities associated with the liver (right-hand panel) were determined. Values are means ± SEM of 3 or 4 rats.

Effects of Mono-Gal-Chol on the Serum Decay and Liver Association of LDL and HDL. To study the effectiveness of compounds **8a–c**, experiments were done to study the effect of loading human serum lipoproteins with these compounds on the subsequent in vivo behavior of the particles. Intravenously injected native human LDL and HDL disappeared very slowly from the circulation of the rat (half-life > 4 h). Only 1.7 ± 0.7% and 3.5 ± 0.4% of the injected dose was found in the liver at 5 min after injection of [^{125}I]LDL or [^{125}I]HDL, respectively (means ± SEM of three rats). To study the effect of **8a** on the metabolic behavior of [^{125}I]LDL and [^{125}I]HDL, a solution of **8a** in PBS was mixed at various ratios with the two lipoproteins. The mixtures were subsequently injected into rats, and at 5 min after injection the amounts of radioactivity in the serum and in the liver were determined. Figure 1 shows that the incorporation of **8a** leads to a reduction in the levels of labeled LDL and HDL in the serum, caused by an increase in the hepatic association. The magnitude of the effect depends on the mixing ratios. The **8a**-stimulated association of LDL to the liver was maximal at a ratio of 0.3 mg of **8a**/mg apolipoprotein. At this ratio about 60% of the dose became associated with the liver. The stimulation of the association of HDL to the liver was maximal at a ratio of 1.0 mg of **8a**/mg of apolipoprotein. However, the effects of HDL were smaller than those observed with LDL. Only up to about 20% of the injected dose became associated with the liver after 5 min, even at high degrees of loading with **8a**.

To study the mechanism of the liver association, the animals were preinjected with *N*-acetylgalactosamine. Table II shows that the **8a**-stimulated liver association of LDL and HDL was almost completely inhibited by the preinjection. Preinjection with the same dose of *N*-acetylglucosamine had no significant effect. This indicates that galactose-specific recognition sites in the liver were responsible for the association.

Full details on the in vivo fate of lipoproteins containing compound **8a** will be published elsewhere. The effects of compounds **8b** and **8c** on the metabolism of LDL and HDL were studied less extensively. Preliminary experiments indicated, however, that the effects of these compounds were very similar to thse of **8a**.

Table II. Effects of *N*-Acetylgalactosamine and *N*-Acetylglucosamine on **8a**-Stimulated Liver Association of [^{125}I]LDL and [^{125}I]HDL[a]

	% dose in serum and liver			
	8a/LDL		**8a**/HDL	
pretreatment	liver	serum	liver	serum
controls	60.1 ± 4.4	33.8 ± 5.0	18.4 ± 2.6	63.8 ± 3.5
N-acetyl-galactos-amine	5.1 ± 0.9*	92.2 ± 8.6*	5.8 ± 1.9*	78.8 ± 3.0*
N-acetyl-glucos-amine	61.6 ± 3.0[b]	29.3 ± 3.5[b]	22.2 ± 3.3[b]	61.1 ± 2.4[b]

[a] Compound **8a** was mixed with radiolabeled LDL or HDL at ratios of 0.3 and 1.0 mg/mg of apolipoprotein, respectively. After 10 min at room temperature, the mixtures were injected into rats at a dose of 50 μg of apolipoprotein/kg of body weight. The rats had been preinjected, 1 min prior to injection of the labeled mixtures, with *N*-acetylgalactosamine or *N*-acetylglucosamine, both at a dose of 400 mg/kg. Controls were preinjected with phosphate-buffered saline (PBS) as solvent. After 5 min, the trichloroacetic acid precipitable radioactivity in the serum and the radioactivity associated with the liver were determined. Differences with respect to the controls were tested for significance by Wilcoxon's two-sample test.[44] Values are means ± SEM of 3 or 4 rats. *$P < 0.05$. [b] Not significant.

Concluding Remarks

The three monogalactoside-terminated phosphorothiolated cholesteryl derivatives that had been synthesized were soluble in aqueous solutions, which facilitated their application in biological systems. Upon addition to human serum, the compounds incorporated into lipoproteins. Incorporation of the compounds into LDL and HDL resulted in the rapid removal of the particles from the circulation, which was due to galactose-specific uptake by the liver. The plasma clearance and hepatic uptake of the complexes with LDL was, however, substantially higher than clearance and uptake of HDL loaded with the same compounds. Although LDL particles contained about twice as much of the compounds per unit of surface area than HDL particles under our conditions, the difference in uptake was probably due to differences in the receptors involved. Preliminary data indicated that, as with tris-gal-chol,[16] incorporation of the compounds into LDL induces uptake by Kupffer cells, whereas incorporation into HDL leads to uptake by parenchymal cells. It had been shown earlier that the galactose receptor on parenchymal cells had a relatively low affinity for monogalactoside-terminated ligands.[17]

The selectivity of the effects of **8a–c** on the in vivo behavior of LDL and HDL warrants further studies with these compounds. An attractive potential application of the compounds is their use for specific reduction of levels of circulating LDL in patients with high LDL-cholesterol levels.

Experimental Section

Chemicals and Solvents. Penta-*O*-benzoyl-α/β-D-galactopyranose was prepared by acylation with benzoyl chloride according to Ness et al.[24] 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-galactopyranose (**1b**) was prepared according to Cerny et al.[25] and Frgala et al.[26] Acetic anhydride and benzoyl chloride from Baker, sodium iodide and 2-[2-(2-chloroethoxy)ethoxy]ethanol from Janssen, and 2,6-di-*tert*-butylpyridine and *syn*-pyridine-2-carboxaldoxime from Aldrich were used without further purification. Phosphorus tribromide from Janssen was distilled before use. Silver triflate from Fluka was activated by heating at 100 °C under vacuum and exclusion of light during 1 h. Ethylene glycol and tetraethylene glycol both from Janssen were dried with $MgSO_4$ and distilled under vacuum. 2-Chloroethanol from Janssen was dried with, then distilled from, $MgSO_4$ in the presence of a

little Na_2CO_3 to remove traces of acid. Diisopropylethylamine (DIPEA) from Merck was distilled from KOH (5 g/L) and stored over 4-Å molecular sieves. N^1,N^1,N^3,N^3-Tetramethylguanidine (TMG) from Aldrich was distilled under reduced pressure and stored over 4-Å molecular sieves. Dioxane, pyridine, and acetonitrile were dried by heating under reflux with CaH_2 (5 g/L) for 16 h, then distilled and stored over molecular sieves (5 Å, 4 Å, and 4 Å, respectively). Pyridine for phosphorylation reactions was redistilled from *p*-toluenesulfonyl chloride (60 g/L) and KOH (25 g/L) and stored over 4-Å molecular sieves. Dioxane used for phosphorylation reactions was redistilled from $LiAlH_4$ (5 g/L) and stored over 5-Å molecular sieves. Toluene and dichloromethane were dried by heating under reflux with P_2O_5 (30 g/L) during 2 h and distilled. Toluene was stored over sodium wire. Dichloromethane (CH_2Cl_2) was stored over basic alumina (5 g/L). Acetone and butanone or methyl ethyl ketone were dried with, then distilled from and stored over, K_2CO_3. 1-Hydroxy-6-(trifluoromethyl)benzotriazole was prepared according to the method of König and Geiger[33] and dried in vacuo (P_2O_5) for 70 h at 50 °C. Triethylammonium bicarbonate (TEAB) buffer (2 M) was prepared by passing a stream of CO_2 gas through a cooled (ice-water bath) solution of triethylamine (825 mL) in deionized water (2175 mL) until saturation (pH 7.0). Cation-exchange resin (sodium form) was obtained by passing a solution of NaOH (2 M, 100 mL) through a column packed with cation-exchange resin (Dowex 50W X8, 100–200 mesh; Fluka, H^+ form, 1.5 × 10 cm) followed by washing of the column with deionized water until pH 7. Evaporations were carried out under reduced pressure (15 or 0.5 mmHg) at bath temperatures below 40 °C. Protein G substituted Sepharose was obtained from Pharmacia and used as described in the information leaflet. Polyclonal anti-human apo-A1 antiserum, raised in a goat, was a generous gift from Dr. L. M. Havekes (Gaubius Institute TNO).

Chromatography. Thin-layer chromatography (TLC) was carried out with silica F_{254} preformed 0.1-mm-thick layers on a plastic backing (Schleicher and Schüll DC Fertigfolien F1500 LS254) in the following mobile phases: A, ether/petroleum ether 40–60 °C, 2:1 (v/v); B, CH_2Cl_2/CH_3OH, 96:4 (v/v); C, CH_2Cl_2/CH_3OH, 92:8 (v/v); D, CH_2Cl_2; E, CH_2Cl_2/acetone, 98:2 (v/v); F, CH_2Cl_2/acetone, 96:4 (v/v), G, CH_2Cl_2/acetone, 95:5 (v/v); H, CH_2Cl_2/CH_3OH, 4:1 (v/v); I, CH_2Cl_2/CH_3OH, 7:3 (v/v). Spots were visualized either under UV (254 or 356 nm) light or by spraying with sulfuric acid/methanol (1:4, v/v) and charring at 140 °C for a few minutes. Preparative column chromatography was performed on silica (230–400 mesh ASTM, Merck) suspended in CH_2Cl_2. For gel filtration Sephadex LH-20 from Pharmacia, suspended in CH_2Cl_2/CH_3OH (2:1, v/v), was used. Column fractions of these runs were analyzed by TLC.

Instruments and Analyses. Elemental analyses were done for compounds **1a**, **2a–c**, **5**, **6a–c**, and **8a–c**. Optical rotations were determined at room temperature with a Perkin-Elmer 141 polarimeter. ^{31}P and ^{13}C NMR spectra were measured at 80.7 and 50.1 MHz, respectively, with a JEOL JNM-FX 200 spectrometer equipped with a PG 200 computer operating in the Fourier transform mode. ^1H NMR spectra were measured at 200 MHz, with the above mentioned spectrometer, or at 300 MHz, with a Bruker WM-300 spectrometer equipped with an ASPECT-2000 computer operating in the Fourier transform mode. ^{31}P Chemical shifts are given in ppm (δ) relative to 85% phosphoric acid as external standard and ^1H and ^{13}C chemical shifts in ppm (δ) relative to tetramethylsilane as internal standard.

Syntheses. 2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl Bromide (1a). To a stirred and cooled (ice-water bath) solution of penta-*O*-benzoyl-D-galactopyranose (α/β-mixture, 3.5 g, 5.0 mmol) and PBr_3 (3.0 mL) in Ac_2O (10.0 mL) was added dropwise water (4.4 mL) during a period of 30 min and the mixture was stirred for 1 h at 0 °C. TLC (A) showed complete conversion. The reaction mixture was diluted with cold CH_2Cl_2 (50 mL) and washed with cold water (2 × 50 mL), cold 10% $NaHCO_3$ solution (2 × 50 mL), and cold water (2 × 50 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness. The residual oil was purified on a column of silica (30 g) by elution with a 0–2% gradient of acetone in CH_2Cl_2. The appropriate fractions were pooled and evaporated to a white glass. **1**: yield 5.56 g (84%); R_f 0.61 (A); $[\alpha]^{20}_D$ +219.7° (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 8.13–7.22 (m, 20 H, H aromatic, benzoyl), 6.98 (d, 1 H, H-1, $J_{1,2}$ = 4.0 Hz, α-configuration), 6.13 (dd, 1 H, H-4, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.2 Hz), 6.06 (dd, 1 H, H-3, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.4 Hz), 5.67 (dd, 1 H, H-2, $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ = 10.4 Hz), 4.93 (dd, 1 H, H-5, $J_{5,6}$ = 6.9 Hz, $J_{5,6'}$ = 6.2 Hz), 4.65 (dd, 1 H, H-6, $J_{5,6}$ = 6.8 Hz, $J_{6,6'}$ = 11.5 Hz), 4.47 (dd, 1 H, H-6', $J_{5,6'}$ = 6.1 Hz, $J_{6,6'}$ = 11.5 Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 165.5, 165.2, and 165.1 (4 × C=O, benzoyl), 133.5–128.1 (C aromatic, benzoyl), 88.4 (C-1, α-configuration), 71.8 (C-5), 68.9, 68.5, and 68.1 (C-2, C-3 and C-4), 61.6 (C-6). Anal. ($C_{34}H_{27}BrO_9$) C, H.

2-Hydroxyethyl 2',3',4',6'-Tetra-*O*-benzoyl-β-D-galactopyranoside (2a). A mixture of acetonitrile (2.5 mL), ethylene glycol (0.31 g, 5.0 mmol), and 4-Å molecular sieves (0.5 g) was stirred for 10 min under an N_2 atmosphere at room temperature. Then a solution of bromide **1a** (0.66 g, 1.0 mmol), which had been dried by repeated coevaporation with toluene (2 × 5 mL), in CH_2Cl_2 (5 mL) was added and the mixture was cooled (−30 °C) in a dry ice/ethanol bath. To the cooled mixture was added, under exclusion of light and moisture, a solution of activated silver triflate (257 mg, 1.0 mmol) in toluene (5 mL) and 2,6-di-*tert*-butylpyridine (0.225 mL, 1.0 mmol). After stirring for 2 h at −30 °C, the mixture was slowly warmed up and stirred for 16 h at 20 °C in the dark. TLC (B) showed complete conversion into a major product tentatively identified as **2a** and a minor product. The mixture was filtered over a pad of Celite, washed with CH_2Cl_2, and evaporated to dryness. The residue was taken up in CH_2Cl_2 (100 mL) and washed with 0.1 N HCl solution (25 mL), H_2O (25 mL), 10% $NaHCO_3$ solution (25 mL), and H_2O (25 mL), respectively. The organic layer was dried over $MgSO_4$, filtered, and concentrated to a syrup. Separation of the products was achieved by chromatography of the mixture on a column of silica (7 g) eluted with a 0–3% gradient of acetone in CH_2Cl_2. Fractions containing either one of these products were pooled and evaporated to a pure colorless oil. **2a**: yield 582 mg (89%); R_f 0.16 (A), 0.59 (B); $[\alpha]^{20}_D$ +105.1° (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 8.11–7.18 (m, 20 H, H aromatic, benzoyl), 6.06 (d, 1 H, H-4', $J_{3',4'}$ = 3.3 Hz), 5.86 (dd, 1 H, H-2', $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 10.5 Hz), 5.70 (dd, 1 H, H-3', $J_{2',3'}$ = 10.5 Hz, $J_{3',4'}$ = 3.4 Hz), 4.95 (d, 1 H,H-1', $J_{1',2'}$ = 8.0 Hz), 4.66 (dd, 1 H, H-6', $J_{5',6'}$ = 6.9 Hz, $J_{6',6''}$ = 11.3 Hz), 4.52 (dd, 1 H, H-6'', $J_{5',6''}$ = 5.9 Hz, $J_{6',6''}$ = 11.3 Hz), 4.43 (dd, 1 H, H-5', $J_{5',6''}$ = 6.5 Hz, $J_{5',6''}$ = 6.1 Hz), 3.97 (dt, 1 H, OCH-a1, $J_{a1,b}$ = 4.6 Hz, $J_{a1,a2}$ = 10.9 Hz), 3.86 (dt, 1 H, OCH-a2, $J_{a2,b}$ = 4.5 Hz, $J_{a1,a2}$ = 10.8 Hz), 3.73 (t, 2 H, HOC*H*-b, $J_{a1,b} = J_{a2,b}$ = 4.5 Hz), 2.6 (bs, 1 H, CH_2OH); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 165.8 and 165.3 (4 × C=O, benzoyl), 133.4–128.0 (C aromatic, benzoyl), 101.8 (C-1', β-configuration), 72.4 (OCH_2), 71.4 and 71.3 (C-3' and C-5'), 69.7 (C-2'), 68.0 (C-4'), 62.1 (C-6'), 61.5 (CH_2OH). Anal. ($C_{36}H_{32}O_{11}$) C, H. Minor product: **1,2-bis[(2',3',4',6'-tetra-*O*-benzoyl-β-D-galactopyranosyl)oxy]ethane**; yield 73 mg (6%); R_f 0.36 (A); $[\alpha]^{20}_D$ +78.0° (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 8.11–7.18 (m, 40 H, H aromatic, benzoyl), 5.88 (d, 2 H, H-4', $J_{3',4'}$ = 3.2 Hz), 5.75 (dd, 2 H, H-2', $J_{1',2'}$ = 7.9 Hz, $J_{2',3'}$ = 10.4 Hz), 5.54 (dd, 2 H, H-3', $J_{2',3'}$ = 10.4 Hz, $J_{3',4'}$ = 3.4 Hz), 4.82 (d, 2 H, H-1', $J_{1',2'}$ = 7.9 Hz), 4.62 (dd, 2 H, H-6', $J_{5',6'}$ = 6.7 Hz, $J_{6',6''}$ = 11.4 Hz), 4.35 (dd, 2 H, H-6'', $J_{5',6''}$ = 6.6 Hz, $J_{6',6''}$ = 11.4 Hz), 3.97 (m, 4 H, 2 × OCH_2), 3.89 (t, 2 H, H-5', $J_{5',6'} = J_{5',6''}$ = 6.5 Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 165.8, 165.5, 165.4, and 165.2 (4 × C=O, benzoyl), 133.5–128.2 (C aromatic, benzoyl), 101.0 (C-1', β-configuration), 71.3 and 71.0 (C-3' and C-5'), 70.0 (C-2'), 68.7 (2 × OCH_2), 67.9 (C-4'), 61.8 (C-6').

2-Hydroxyethyl 2',3',4',6'-Tetra-*O*-acetyl-1'-thio-β-D-galactopyranoside (2b). A solution of 2-chloroethanol (0.45 mL, 7.5 mmol) and NaI (3.75 g, 25.0 mmol) in 50 mL butanone was refluxed for 16 h. After being cooled the suspension was filtered and the precipitate washed with butanone (10 mL). The filtrate was added dropwise over 15 min to a solution of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose (**1b**, 1.82 g, 5.0 mmol) in butanone (10 mL), followed by the addition of DIPEA (0.85 mL, 5.0 mmol). TLC (B) of the mixture after 1 h at room temperature showed the complete conversion of starting material. The mixture was diluted with 2 volumes of water and repeatedly extracted with CH_2Cl_2 (4 × 25 mL). The combined CH_2Cl_2 extracts were washed with 5% aqueous NaOH (25 mL), 5% aqueous H_2SO_4 (25 mL), and H_2O (25 mL), successively, dried over $MgSO_4$, filtered, and evaporated to dryness. The crude oil was chromatographically purified on a silica column (20 g) with a 0–4% gradient of acetone in CH_2Cl_2. The appropriate fractions were collected and con-

centrated to give a light yellowish oil. **2b**: yield 1.77 g (87%); $[\alpha]^{20}_D$ 13.8 (*c* 1, $CHCl_3$); R_f 0.41 (B), 0.57 (C); ^1H NMR (200 MHz, $CDCl_3$): δ 5.44 (dd, 1 H, H-4, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ = 0.8 Hz), 5.27 (dd, 1 H, H-2, $J_{1,2}$ = 9.7 Hz, $J_{2,3}$ = 10.0 Hz), 5.07 (dd, 1 H, H-3, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.2 Hz), 4.57 (d, 1 H, H-1, $J_{1,2}$ = 9.7 Hz), 4.16–4.03 (m, 3 H, H-5, H-6′, and H-6″), 3.79 (bt, 2 H, HOC*H*-b), 3.12 (bs, 1 H, OH), 3.00 (dt, 1 H, SCH-a_1, $J_{a1,b}$ = 5.4 Hz, $J_{a1,a2}$ = 14.4 Hz), 2.78 (dt, 1 H, SCH-a_2, $J_{a2,b}$ = 5.9 Hz, $J_{a1,a2}$ = 14.4 Hz), 2.17, 2.09, 2.07, and 1.99 (4 × s, 12 H, 4 × CH_3 acetyl); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 170.1, 169.9, 169.7, and 169.3 (4 × C=O, acetyl), 84.2 (C-1), 74.3, 71.4, 67.0, and 66.8 (C-2, C-3, C-4, and C-5), 61.8 (CH_2OH), 61.4 (C-6), 34.3 (SCH_2), 20.5, 20.3, and 20.2 (4 × CH_3, acetyl). Anal. ($C_{16}H_{24}O_{10}S$) C, H, S.

8-Hydroxy-3,6-dioxaoctyl 2′,3′,4′,6′-Tetra-*O*-acetyl-1′-thio-β-D-galactopyranoside (2c). In the same way as described above a refluxed solution of 2-(chloroethoxy)ethanol (0.58 mL, 4.0 mmol) and NaI (1.88 g, 12.5 mmol) in 25 mL of butanone was, after cooling, filtration, and washing, added dropwise to a solution of **1b** (0.91 g, 2.5 mmol) in butanone (5 mL), followed by the addition of DIPEA (0.43 mL, 2.5 mmol). Workup and purification on a silica column (10 g) by elution with a 0–10% gradient of acetone in CH_2Cl_2 as described above gave a light yellowish oil. **2c**: yield 1.20 g (90%); R_f 0.28 (B), 0.49 (C); $[\alpha]^{20}_D$ −7.1° (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 5.42 (d, 1 H, H-4, $J_{3,4}$ = 3.2 Hz), 5.20 (t, 1 H, H-2, $J_{1,2}$ = $J_{2,3}$ = 9.5 Hz), 5.07 (dd, 1 H, H-3, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 3.0 Hz), 4.69 (d, 1 H, H-1, $J_{1,2}$ = 9.5 Hz), 4.16–4.04 (m, 3 H, H-5, H-6′, and H-6″), 3.73–3.58 (m, 10 H, 5 × OCH_2), 3.21 (bs, 1 H, OH), 3.00 (dt, 1 H, SCH-a1, $J_{a1,b}$ = 6.8 Hz, $J_{a1,a2}$ = 13.6 Hz), 2.78 (dt, 1 H, SCH-a2, $J_{a2,b}$ = 6.6 Hz, $J_{a1,a2}$ = 13.6 Hz), 2.17, 2.16, 2.05, and 1.98 (4 × s, 12 H, 4 × CH_3 acetyl); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 169.8, 169.7, 169.4, and 169.0 (4 × C=O, acetyl), 83.2 (C-1), 73.7, 71.3, 66.9, and 66.8 (C-2, C-3, C-4 and C-5), 72.0–69.7 (5 × CH_2O), 61.0 (CH_2OH and C-6), 28.7 (SCH_2), 20.2 and 20.1 (4 × CH_3, acetyl). Anal. ($C_{20}H_{32}O_{12}S$) C, H, S.

2,5-Dichlorophenyl Bis[6-(trifluoromethyl)benzotriazolyl] Phosphorothioate (3). A solution of 2,5-dichlorophenyl phosphorodichloridothioate[32] (2.16 g, 7.3 mmol) in anhydrous dioxane (7.0 mL) was added dropwise to a stirred solution of dry 1-hydroxy-6-(trifluoromethyl)benzotriazole[33] (3.01 g, 14.8 mmol) and anhydrous pyridine (1.2 mL, 15.0 mmol) in anhydrous dioxane (30 mL) at room temperature. The reaction mixture was stirred for 1 h at room temperature and the precipitated pyridinium hydrochloric acid salt was removed by filtration under anhydrous conditions to give a stock solution (0.2 M) of **3** in dioxane, which could be stored for several weeks at −20 °C. ^{31}P NMR (external D_2O lock) δ 65.4.

11-(5-Cholesten-3β-yloxy)-3,6,9-trioxaundecan-1-ol (5). A solution of cholesteryl *p*-toluenesulfonate[34] (2.70 g, 5.0 mmol) in dioxane (40 mL) and tetraethylene glycol (45 mL) was refluxed during 2 h. TLC (B or C) showed complete conversion. The mixture was evaporated under reduced pressure. The residual oil was taken up in ether (100 mL) and washed with an aqueous 10% $NaHCO_3$ solution (50 mL) and H_2O (50 mL). The organic layer was dried by using $MgSO_4$, filtered, and evaporated. The crude oil was purified on a silica column (50 g) eluted with a 0–5% gradient of CH_3OH in CH_2Cl_2. The appropriate fractions were collected and concentrated to a colorless oil. **5**: yield 2.55 g (91%); R_f 0.30 (B), 0.47 (C); $[\alpha]^{20}_D$ −18.3° (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 5.25 (m, 1 H, chol H-6), 3.59–3.48 (m, 16 H, 8 × CH_2O), 3.20 (m, 2 H, chol H-3 and O-H), 2.50–0.60 (remaining chol protons) with 0.91 (s, 3 H, CH_3-19), 0.83 (d, 3 H, CH_3-21, $J_{20,21}$ = 6.3 Hz), 0.78 (d, 6 H, CH_3-26 and CH_3-27, $J_{25,26}$ = $J_{25,27}$ = 6.6 Hz), 0.60 (s, 3 H, CH_3-18); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 140.7 (C-5), 121.3 (C-6), 79.3 (C-3), 72.4–61.4 (8 × CH_2O), 56.6 (C-14), 56.0 (C-17), 50.0 (C-9), 42.1 (C-13), 39.6, 39.3, and 38.8 (C-4, C-12 and C-24), 37.1, 36.7, and 36.0 (C-1, C-10, and C-12), 35.6 (C-20), 31.8 (C-7), 31.7 (C-8), 28.2 and 28.1 (C-2 and C-16), 27.8 (C-25), 24.1 (C-15), 23.7 (C-23), 22.7 and 22.4 (C-26 and C-27), 20.9 (C-11), 19.2 (C-19), 18.6 (C-21), 11.7 (C-18). Anal. ($C_{35}H_{62}O_5$) C, H.

General Procedure for the Synthesis of the Fully Protected Phosphorothiolated Galactopyranosylcholesteryl Derivatives 6a–c. A solution of phosphorylating agent **3** in dioxane (0.2 M, 5.75 mL, 1.15 mmol) was added individually to compound **2a** (641 mg, 1.0 mmol), **2b** (408 mg, 1.0 mmol), or **2c** (497 mg, 1.0 mmol), which had been dried by repeated coevaporation with pyridine (2 × 10 mL). After 15 min TLC (D) indicated complete conversion into intermediate **4a**, **4b**, and **4c**, respectively (all R_f 0). To this mixture was added under exclusion of moisture a solution of **5** (0.70 g, 1.25 mmol), which had been dried by repeated coevaporation (2 × 10 mL), in anhydrous pyridine (5 mL). After stirring for 1 h at 20 °C, TLC (D or E) showed the absence of **4a**, **4b**, and **4c**. The reaction mixture was diluted with CH_2Cl_2 (75 mL) and washed with TEAB buffer (1 M, 50 mL) and H_2O (50 mL). The organic layer was dried over $MgSO_4$, filtered, concentrated to a small volume and coevaporated twice with toluene to dryness. The oily residue was purified on a silica column (20 g) by elution with a 0–5% gradient of acetone in CH_2Cl_2. The fractions containing pure **6a**, **6b**, and **6c**, respectively, were concentrated to colorless glasses.

[(2′,3′,4′,6′-Tetra-*O*-benzoyl-β-D-galactopyranosyl)oxy]-ethyl 11-(5-Cholesten-3β-yloxy)-3,6,9-trioxaundecanyl 2,5-dichlorophenyl phosphorothioate (6a): yield 1.10 g (77%); R_f 0.30 (D), 0.47 (E), 0.60 (F); ^{31}P NMR (CH_2Cl_2, mixture of diastereomers) δ 63.51, 63.46; ^1H NMR (300 MHz, $CDCl_3$, mixture of diastereomers) δ 8.12–7.08 (m, 23 H, H aromatic, benzoyl and 2,5-dichlorophenyl), 6.01 (d, 1 H, H-4′, $J_{3',4'}$ = 3.4 Hz), 5.82 (dd, 1 H, H-2′, $J_{1',2'}$ = 7.9 Hz, $J_{2',3'}$ = 10.4 Hz), 5.60 (dd, 1 H, H-3′, $J_{2',3'}$ = 10.4 Hz, $J_{3',4'}$ = 3.4 Hz), 5.33 (m, 1 H, chol H-6), 4.98 and 4.96 (2 × d, 1 H, H-1′, $J_{1',2'}$ = 7.9 Hz), 4.71–3.62 (m, 23 H, H-5′, H-6′, H-6″ and 10 × CH_2O), 3.17 (m, 1 H, chol H-3), 2.49–0.68 (remaining chol protons) with 0.99 (s, 3 H, CH_3-19), 0.92 (d, 3 H, CH_3-21, $J_{20,21}$ = 6.3 Hz), 0.86 (d, 6 H, CH_3-26 and CH_3-27, $J_{25,26}$ = $J_{25,27}$ = 6.6 Hz), 0.68 (s, 3 H, CH_3-18); $^{13}C\{^1H\}$ NMR ($CDCl_3$, mixture of diastereomers) δ 165.9, 165.4, and 165.2 (4 × C=O, benzoyl), 140.7 (C-5), 133.5–122.3 (C aromatic, benzoyl, and 2,5-dichlorophenyl), 121.4 (C6), 101.3 (C-1′), 79.4 (C-3), 70.9–67.2 (10 × CH_2O), 71.7, 71.5, 69.6, and 68.2 (C-2′, C-3′, C-4′, and C-5′), 61.9 (C-6′), 56.7 (C-14), 56.1 (C-17), 50.1 (C-9), 42.2 (C-13), 39.7, 39.4, and 39.0 (C-4, C-12 and C-24), 37.1, 36.8, and 36.1 (C-1, C-10, and C-22), 35.7 (C-20), 31.8 (C-7), 31.7 (C-8), 28.3 and 28.1 (C-2 and C-16), 27.9 (C-25), 24.2 (C-15), 23.7 (C-23), 22.7 and 22.5 (C-26 and C-27), 20.9 (C-11), 19.3 (C-19), 18.6 (C-21), 11.8 (C-18). Anal. ($C_{77}H_{95}Cl_2O_{17}PS$) C, H, P, S.

[(2′,3′,4′,6′-Tetra-*O*-acetyl-1′-thio-β-D-galactopyranosyl)-oxy]ethyl 11-(5-Cholesten-3β-yloxy)-3,6,9-trioxaundecanyl 2,5-Dichlorophenyl Phosphorothioate (6b): yield 0.97 g (81%); R_f 0.27 (E), 0.43 (F); ^{31}P NMR (CH_2Cl_2, mixture of diastereomers) δ 63.19, 62.92; ^1H NMR ($CDCl_3$, mixture of diastereomers): δ 7.46–7.16 (m, 3 H, H aromatic, 2,5-dichlorophenyl), 5.44 (d, 1 H, H-4′, $J_{3',4'}$ = 3.0 Hz), 5.32 (m, 1 H, chol H-6), 5.21 (t, 1 H, H-2′, $J_{1',2'}$ = $J_{2',3'}$ = 10.0 Hz), 5.05 (dt, 1 H, H-3′, $J_{2',3'}$ = 10.0 Hz, $J_{3',4'}$ = J_{PH} = 2.9 Hz), 4.55 and 4.52 (2 × d, 1 H, H-1′, $J_{1',2'}$ = 9.9 Hz), 4.38–3.63 (m, 21 H, H-5′, H-6′, H-6″ and 9 × CH_2O), 3.12 (m, 2 H, chol H-3 and SCH-a), 2.95 (m, 1 H, SCH-b), 2.16, 2.08, 2.04, and 1.99 (4 × s, 12 H, 4 × CH_3 acetyl), 2.35–0.67 (remaining chol protons) with 0.99 (s, 3 H, CH_3-19), 0.91 (d, 3 H, CH_3-21, $J_{20,21}$ = 6.3 Hz), 0.86 (d, 6 H, CH_3-26 and CH_3-27, $J_{25,26}$ = $J_{25,27}$ = 6.6 Hz), 0.67 (s, 3 H, CH_3-18); $^{13}C\{^1H\}$ NMR ($CDCl_3$, mixture of diastereomers): δ 170.2, 170.1, 169.9, and 169.5 (4 × C=O, acetyl), 140.8 (C-5), 132.7–122.8 (C aromatic, 2,5-dichlorophenyl), 121.4 (C-6), 84.0 (C-1′), 79.3 (C-3), 74.5, 71.6, 67.0 and 66.9 (C-2′, C-3′, C-4′, and C-5′), 70.8–67.2 (9 × CH_2O), 61.2 (C-6′), 56.6 (C-14), 56.0 (C-17), 50.0 (C-9), 42.2 (C-13), 39.7, 39.4, and 38.9 (C-4, C-12, and C-24), 37.1, 36.7, and 36.1 (C-1, C-10, and C-22), 35.7 (C-20), 31.8 (C-7), 31.7 (C-8), 29.7 (d, J_{CP} = 7.3 Hz, SCH_2), 28.2 (C-2 and C-16), 27.9 (C-25), 24.2 (C-15), 23.7 (C-23), 22.7 and 22.5 (C-26 and C-27), 20.9 (C-11), 20.7, 20.6, and 20.5 (4 × CH_3, acetyl), 19.3 (C-19), 18.6 (C-21), 11.7 (C-18). Anal. ($C_{57}H_{87}Cl_2O_{16}PS_2$) C, H, P, S.

[(2′,3′,4′,6′-Tetra-*O*-acetyl-1′-thio-β-D-galactopyranosyl)-oxy]-3,6-dioxaoctyl 11-(5-Cholesten-3β-yloxy)-3,6,9-trioxaundecanyl 2,5-Dichlorophenyl Phosphorothioate (6c): yield 1.01 g (79%); R_f 0.19 (E), 0.26 (F); ^{31}P NMR (CH_2Cl_2, mixture of diastereomers) δ 63.52 (bs); ^1H NMR ($CDCl_3$, mixture of diastereomers) δ 7.48–7.13 (m, 3 H, H aromatic, 2,5-dichlorophenyl), 5.43 (d, 1 H, H-4′, $J_{3',4'}$ = 2.8 Hz), 5.31 (m, 1 H, chol H-6), 5.23 (t, 1 H, H-2′, $J_{1',2'}$ = $J_{2',3'}$ = 10.0 Hz), 5.04 (dd, 1 H, H-3′, $J_{2',3'}$ = 10.0 Hz, $J_{3',4'}$ = 3.1 Hz), 4.59 (d, 1 H, H-1′, $J_{1',2'}$ = 9.8 Hz), 4.14–3.62 (m, 29 H, H-5′, H-6′, H-6″ and 13 × CH_2O), 3.15 (m, 1 H, chol H-3), 2.92 (m, 1 H, SCH-a), 2.81 (m, 1 H, SCH-b), 2.13, 2.06, 2.03, and 1.97 (4 × s, 12 H, 4 × CH_3 acetyl), 2.39–0.65 (remaining chol protons) with 0.99 (s, 3 H, CH_3-19), 0.91 (d, 3 H, CH_3-21, $J_{20,21}$ = 6.3 Hz), 0.86 (d, 6 H, CH_3-26 and CH_3-27, $J_{25,26}$ = $J_{25,27}$ = 6.6

Hz), 0.67 (s, 3 H, CH_3-18); $^{13}C\{^1H\}$ NMR ($CDCl_3$, mixture of diastereomers) δ 169.8, 169.7, 169.5, and 169.1 (4 × C=O, acetyl), 140.5 (C-5), 132.3–122.5 (C aromatic, 2,5-dichlorophenyl), 121.2 (C-6), 83.7 (C-1'), 79.0 (C-3), 74.0, 71.5, and 66.9 (C-2', C-3', C-4', and C-5'), 70.8–67.8 (13 × CH_2O), 61.1 (C-6'), 56.4 (C-14), 55.8 (C-17), 49.8 (C-9), 41.9 (C-13), 39.4, 39.2, and 38.7 (C-4, C-12, and C-24), 36.9, 36.5, and 35.8 (C-1, C-10, and C-22), 35.4 (C-20), 31.6 (C-7), 31.5 (C-8), 29.1 (SCH_2), 28.0 and 27.9 (C-2 and C-16), 27.6 (C-25), 23.9 (C-15), 23.5 (C-23), 22.5 and 22.2 (C-26 and C-27), 20.7 (C-11), 20.4 and 20.3 (4 × CH_3, acetyl), 19.0 (C-19), 18.4 (C-21), 11.5 (C-18). Anal. ($C_{61}H_{95}Cl_2O_{18}PS_2$) C, H, P, S.

General Procedure for Deblocking of the Fully Protected Phosphorothioylated Galactopyranosylcholesteryl Derivatives 6a–c to 8a–c. To a solution of one of the fully protected compounds **6a–c** (0.5 mmol) in anhydrous pyridine (10 mL) was added *syn*-pyridine-2-carboxaldoxime (702 mg, 5.75 mmol) and TMG (575 mg, 5.0 mmol). After standing for 8 h at 20 °C TLC (G) showed full conversion of starting material into a product with lower R_f value (**7a–c**) and the solvent mixture was concentrated. The residual oil was taken up in a mixture of NH_4OH (14.8 M, 25 mL) and CH_3OH (50 mL) and kept for 3 h at 20 °C. TLC (H or I) showed fully deacylation and the solvent mixture was evaporated under reduced pressure. The residue was redissolved in CH_2Cl_2/CH_3OH (2:1, v/v) and applied to a Sephadex LH-20 column, equilibrated and eluted with the same solvent mixture. The appropriate fractions were collected and concentrated to afford pure unprotected galactopyranosylcholesteryl derivatives **8a–c** in the ammonium form. The derivatives were converted into the sodium form by passing through a column (1.5 × 10 cm) of Dowex 50W cation-exchange resin (Na^+ form) and the resulting fractions containing **8a**, **8b**, and **8c** were respectively pooled, concentrated, and lyophilized from H_2O/dioxane.

(β-D-Galactopyranosyloxy)ethyl 11-(5-cholesten-3β-yloxy)-3,6,9-trioxaundecanyl phosphorothioate (8a): yield 194 mg (44%); R_f 0.16 (H), 0.28 (I); ^{31}P NMR (CH_2Cl_2, mixture of diastereomers): δ 58.78, 58.54; ^1H NMR (300 MHz, $CDCl_3/CD_3OD$, mixture of diastereomers): δ 5.27 (m, 1 H, chol H-6), 4.38–3.14 (gal. protons and 10 × CH_2O), 2.30–0.60 (remaining chol protons) with 0.90 (s, 3 H, CH_3-19), 0.83 (d, 3 H, CH_3-21, $J_{20,21}$ = 6.5 Hz), 0.77 (d, 6 H, CH_3-26 and CH_3-27, $J_{25,26} = J_{25,27}$ = 6.6 Hz), 0.60 (s, 3 H, CH_3-18); $^{13}C\{^1H\}$ NMR ($CDCl_3/CD_3OD$, mixture of diastereomers) δ 141.0 (C-5), 122.3 (C-6), 104.0 (C-1'), 80.2 (C-3), 75.2, 73.8, 71.6, and 69.6 (C-2', C-3', C-4', and C-5'), 71.0–61.8 (C-6' and 10 × CH_2O), 57.2 (C-14), 56.6 (C-17), 50.7 (C-9), 42.8 (C-13), 40.2, 39.9, and 39.3 (C-4, C-12, and C-24), 37.6, 37.2, and 36.6 (C-1, C-10, and C-22), 36.2 (C-20), 32.4 (C-7 and C-8), 28.6 (C-2 and C-16), 28.4 (C-25), 24.6 (C-15), 24.2 (C-23), 23.0 and 22.7 (C-26 and C-27), 21.5 (C-11), 19.6 (C-19), 19.0 (C-21), 12.1 (C-18). Anal. ($C_{43}H_{76}NaO_{13}PS$) P.

[(1-Thio-β-D-galactopyranosyl)oxy]ethyl 11-(5-Cholesten-3β-yloxy)-3,6,9-trioxaundecanyl Phosphorothioate (8b): yield 206 mg (46%); R_f 0.18 (H), 0.31 (I); ^{31}P NMR (CH_2Cl_2, mixture of diastereomers) δ 57.75, 57.66; ^1H NMR ($CDCl_3/CD_3OD$, mixture of diastereomers) δ 5.28 (m, 1 H, chol H-6), 4.45–3.27 (gal. protons and 9 × CH_2O), 3.11 (m, 2 H, chol H-3 and SCH-a), 2.89 (m, 1 H, SCH-b), 2.31–0.61 (remaining chol protons) with 0.93 (s, 3 H, CH_3-19), 0.84 (d, 3 H, CH_3-21, $J_{20,21}$ = 6.5 Hz), 0.78 (d, 6 H, CH_3-26 and CH_3-27, $J_{25,26} = J_{25,27}$ = 6.6 Hz), 0.61 (s, 3 H, CH_3-18); $^{13}C\{^1H\}$ NMR ($CDCl_3/CD_3OD$, mixture of diastereomers) δ 141.0 (C-5), 122.3 (C-6), 85.9 (C-1'), 80.2 (C-3), 78.8, 75.0, 70.2 and 69.8 (C-2', C-3', C-4', and C-5'), 71.0–62.3 (C-6' and 9 × CH_2O), 57.2 (C-14), 56.6 (C-17), 50.7 (C-9), 42.8 (C-13), 40.2, 39.9, and 39.4 (C-4, C-12, and C-24), 37.6, 37.2, and 36.0 (C-1, C-10, and C-22), 36.2 (C-20), 32.4 (C-8), 32.3 (C-7), 29.8 (d, J_{CP} = 7.3 Hz, SCH_2), 28.7 (C-2 and C-16), 28.4 (C-25), 24.6 (C-15), 24.2 (C-23), 23.0 and 22.7 (C-26 and C-27), 21.5 (C-11), 19.6 (C-19), 19.0 (C-21), 12.1 (C-18). Anal. ($C_{43}H_{76}NaO_{12}PS_2$) P.

[(1-Thio-β-D-galactopyranosyl)oxy]-3,6-dioxaoctyl 11-(5-Cholesten-3β-yloxy)-3,6,9-trioxaundecanyl Phosphorothioate (8c): yield 490 mg (51%); R_f 0.28 (H), 0.40 (I); ^{31}P NMR (CH_2Cl_2, mixture of diastereomers) δ 58.54; ^1H NMR ($CDCl_3/CD_3OD$, mixture of diastereomers) δ 5.27 (m, 1 H, chol H-6), 4.40–3.30 (gal. protons and 13 × CH_2O), 3.15 (m, 1 H, SCH-a), 2.94–2.76 (m, 2 H, chol H-3 and SCH-b), 2.31–0.60 (remaining chol protons) with 0.92 (s, 3 H, CH_3-19), 0.84 (d, 3 H, CH_3-21, $J_{20,21}$ = 6.5 Hz), 0.78 (d, 6 H, CH_3-26 and CH_3-27, $J_{25,26} = J_{25,27}$ = 6.6 Hz), 0.60 (s, 3 H, CH_3-18); $^{13}C\{^1H\}$ NMR ($CDCl_3/CD_3OD$, mixture of diastereomers) δ 141.0 (C-5), 122.1 (C-6), 86.1 (C-1'), 80.0 (C-3), 78.7, 78.6, 74.9, and 69.9 (C-2', C-3', C-4', and C-5'), 71.2–61.9 (C-6' and 13 × CH_2O), 57.1 (C-14), 56.5 (C-17), 50.6 (C-9), 42.7 (C-13), 40.2, 39.9, and 39.3 (C-4, C-12 and C-24), 37.5, 37.2, and 36.5 (C-1, C-10, and C-22), 36.1 (C-20), 32.3 (C-7 and C-8), 29.1 (d, J_{CP} = 7.3 Hz, SCH_2), 28.5 (C-2 and C-16), 28.3 (C-25), 24.6 (C-15), 24.2 (C-23), 23.0 and 22.7 (C-26 and C-27), 19.6 (C-19), 19.0 (C-21), 12.1 (C-18). Anal. ($C_{47}H_{84}NaO_{14}PS_2$) P.

Biological Methods. Isolation of lipoproteins was done by density-gradient ultracentrifugation in KBr/NaCl solutions according to the procedure of Redgrave et al.[37] From the obtained fractions aliquots were taken for determination of cholesterol[39] and carbohydrate content.[40]

Studies with Labeled LDL in the Rat in Vivo. Human LDL and human apo E-free HDL were prepared and subsequently radioiodinated as described previously.[14,15] Mono-gal-chol **8a** was mixed at various ratios with [^{125}I]LDL or [^{125}I]HDL. After 10 min at room temperature, the mixtures were intravenously injected into male Wistar rats, anaesthesized with sodium pentobarbital. At 5 min after injection, the trichloroacetic acid precipitable radioactivity in the serum and the amount of radioactivity associated with the liver were determined as described in detail earlier.[41]

Determination of Proteins. Apolipoprotein concentrations of LDL and HDL were determined by the method of Lowry et al.[42] with bovine serum albumin as the standard. The values found for LDL were multiplied by a factor of 0.82 to correct for the higher color yield of apolipoprotein B.[43]

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