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New acyclic analogues of lipid A: synthesis of 4-phosphonoxybutyl and 3-phosphonoxypropyl glycosides of 2-amino-2-deoxy-D-glucose

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ABSTRACT

Several analogues of lipid A have been synthesized, in which the reducing monosaccharide moiety of the parent molecule has been replaced by an acyclic spacer. The new compounds show high endotoxic activity and are able to protect neutropenic mice against pseudomonas infection, two properties characteristic of LPS-like molecules.

INTRODUCTION

Most of the biological properties of lipopolysaccharides (LPS) have been shown to reside in the lipid A moiety[1]. In addition to "beneficial properties" (activation of macrophage/monocytes, B cell mitogenicity, enhancement of host resistance against bacterial or viral infection and tumor)[1,2], most lipid A analogues unfortunately show severe toxic side effects, including the well-known and often fatal "endotoxic shock", which severely hampers potential clinical use.

Chemical research in the field of lipid A has developed rapidly following the total synthesis of the *Escherichia coli* lipid A[3]. A large number of lipid A analogues have since been synthesized and their biological activity examined[4]. Although separation of immunostimulatory and toxic effects could be achieved to a large extent in certain cases, immunostimulatory lipid A-like molecules completely devoid of toxicity have not yet been described. The possibility of designing non-toxic lipid A analogues relies on the hypothesis that different parts of the lipid A molecule are responsible for toxic and immunostimulatory effects. The identification of these structural elements requires systematic chemical modification of the original structure and assessment of corresponding biological activities.

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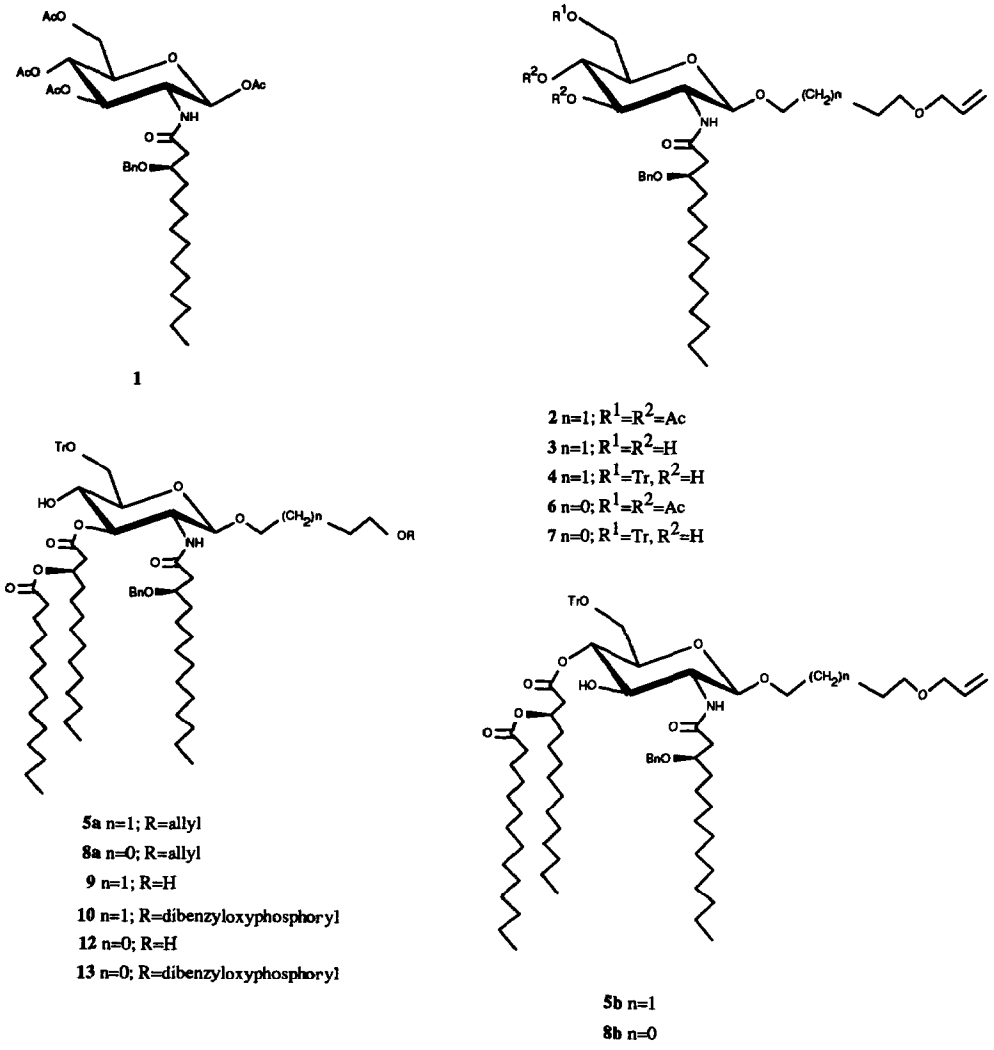
The complexity of the lipid A molecule clearly renders such studies very difficult and therefore our initial aim was to identify a simple structure compatible with high LPS-like activity and which would be amenable to systematic modification. For obvious reasons, in such studies, the molecule used as a starting point should exhibit high biological activity. Examination of the literature suggests that, in the lipid A area, highest biological activities correspond to analogues possessing an intact disaccharide backbone linked to a variable number of suitably located long-chain acyl groups and phosphates[5]. In contrast, monosaccharide analogues are considerably less active. In this work, starting from a disaccharide lead structure we synthesized a series of lipid A analogues in which the reducing sugar unit was replaced by acyclic spacers. We wished primarily to examine the role of the phosphate groups (location and number) with regard to biological activity. The role of the spacer was also studied briefly. Finally, we compared two acylation patterns of the remaining glucosamine unit which had proved to be beneficial with regard to biological activity in the monosaccharide series of lipid A analogues[4a,6]. These patterns are 2-*N*-[(*R*)-3-hydroxytetradecanoyl]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl] and 2-*N*-[(*R*)-3-hydroxytetradecanoyl]-3,4-di-*O*-[(*R*)-3-hydroxytetradecanoyl].

RESULTS AND DISCUSSION

Synthesis.

2-N-[(R)-3-hydroxytetradecanoyl]-3-O-[(R)-3-tetradecanoyloxytetradecanoyl]glucosamine derivatives.—Our synthesis commenced (Scheme 1) with the fully acylated glucosamine derivative **1** which was obtained from the known, 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose[7], using the mixed anhydride derived from (*R*)-3-benzyloxymyristoic acid[8] and isobutyl chloroformate. Compound **1** was glycosylated with 4-allyloxybutanol, using Kiso and Anderson's conditions[9] ($FeCl_3$, Drierite, CH_2Cl_2), to afford the acetylated glycoside **2**. Removal of the ester groups (NaOMe–MeOH) afforded **3** which was tritylated using standard conditions to give **4**. Treatment of **4** with (*R*)-3-tetradecanoyloxytetradecanoic acid[10], 1,3-dicyclohexylcarbodiimide (DCC), and 4-(dimethylamino)pyridine (DMAP) gave **5a** and **5b**, *O*-acylated at C-3 and C-4, respectively, which could be separated by column chromatography. The desired key intermediate **5a** was isolated, and the minor isomer **5b** was deacylated and recycled to afford more **5a**; **5a** was converted into the desired phosphorylated compound **11** as shown in Schemes 1 and 2. The allyl protecting group was first isomerized [$(PPh_3)_3Rh(I)Cl$ (Wilkinson's catalyst), diazabicyclooctane (DABCO), ethanol] to the corresponding propenyl derivative, obtained as a 1:1 mixture of *cis* and *trans* isomers as indicated by proton magnetic resonance (^1H NMR). The propenyl group could be selectively cleaved using aqueous trifluoroacetic acid in THF (under these conditions, the trityl group was unaffected). Selective phosphorylation of the primary hydroxyl group (dibenzyl phosphorochloridate, pyridine, benzene) was followed by removal of the trityl

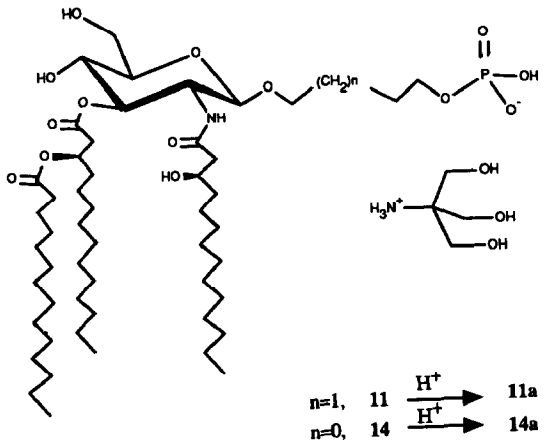


Scheme 1.

group (HCOOH–ether). Finally, hydrogenolysis of the benzyl groups (10% Pd–C), and addition of one equivalent of tris(hydroxymethyl)aminomethane (TRIS) afforded our first target molecule **11** (Scheme 2).

Using 3-allyloxypropanol instead of 4-allyloxybutanol, the same reaction sequence led to intermediate **8a** (Scheme 1), which was converted into the primary phosphate **14** (Schemes 1 and 2).

The 4-phosphate derivative **17** was prepared in 3 steps starting from the intermediate **5a** as shown in Scheme 3. Phosphorylation of the free hydroxyl group in **5a** (BuLi–dibenzyl phosphorochloridate–THF)[11] afforded **15** in moderate yield. First attempts to remove the allyl group in **15**, using Wilkinson's catalyst in



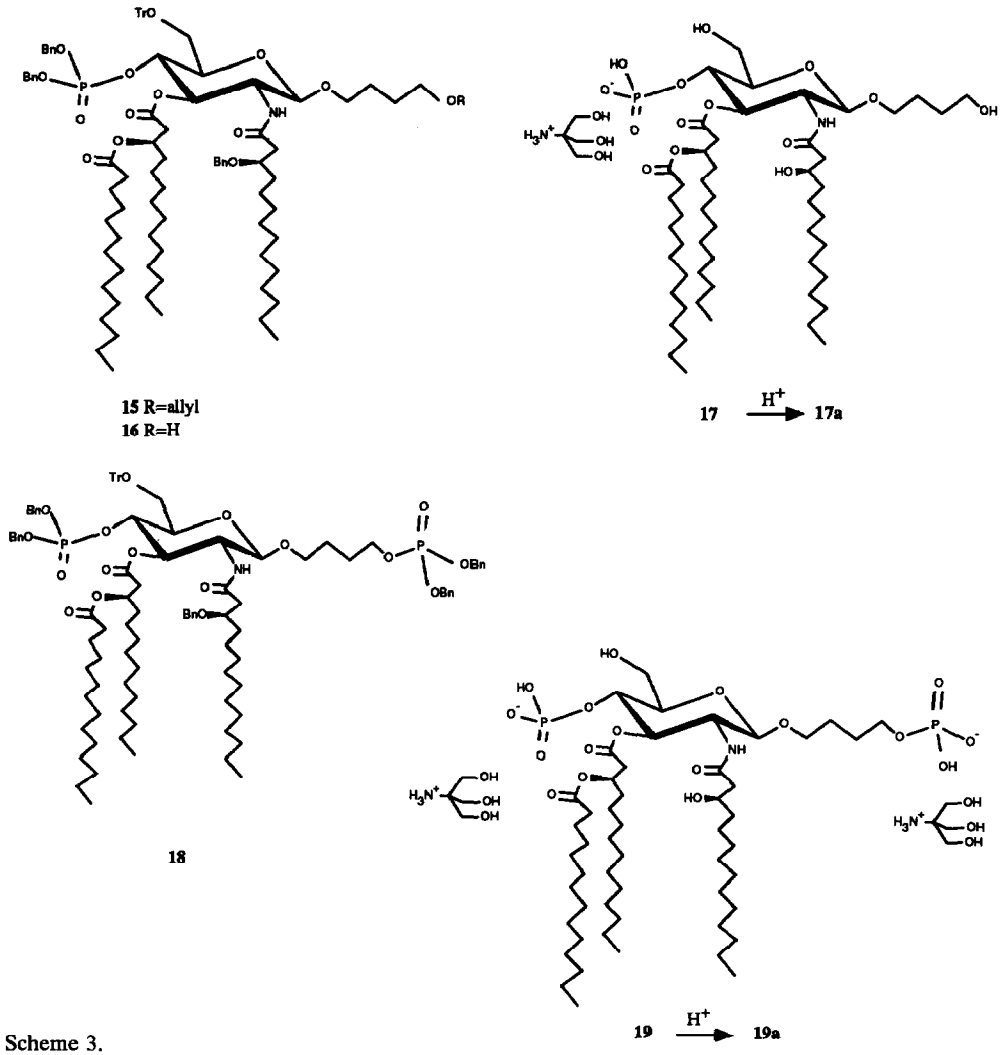
Scheme 2.

ethanol, led predominantly to cleavage of the phosphate group. The desired deprotection could be carried out very efficiently using 1,5-cyclooctadienbis(methyldiphenylphosphine)iridium hexafluorophosphate [Ir(COD)PF_6] as a catalyst[12]. Thus, the allyl ether **15** was treated under an argon atmosphere with a catalytic amount of the activated catalyst, to afford the corresponding propenyl derivative as a pure *trans* isomer. Hydrolysis of the propenyl group as above using aqueous trifluoroacetic acid in THF followed by trityl cleavage (HCOOH–ether), hydrogenolysis of the benzyl groups, and addition of one equivalent of TRIS afforded **17**.

Our first attempts to prepare the protected bisphosphate derivative **18** from **10** were unsuccessful. Due to the low reactivity of the 4-hydroxyl group in **10**, harsh phosphorylation conditions (BuLi–dibenzyl phosphorochloridate–THF) are necessary, which are not compatible with the phosphate ester already present in the molecule. Better results were obtained starting from the 4-phosphate derivative **16** and employing milder conditions to introduce the primary phosphate group (dibenzyl phosphorochloridate–pyridine–benzene). Cleavage of the trityl group in **18** (HCOOH–ether), hydrogenolysis of the benzyl groups, and addition of TRIS yielded the bisphosphate derivative **19**.

2-N-[(R)-3-hydroxytetradecanoyl]-3,4-di-O-[(R)-3-hydroxytetradecanoyl]glucosamine derivatives. Starting from the trityl derivative **4**, the monophosphate **23** (Scheme 4) was prepared. Thus, treatment of **4** with (*R*)-3-benzyloxytetradecanoic acid and DCC in the presence of DMAP as catalyst gave the triacylated glucosamine derivative **20**. Removal of the allyl protecting group [Ir(COD)PF_6] followed by phosphorylation (dibenzyl phosphorochloridate–pyridine–benzene) yielded **22**. Cleavage of the trityl ether, hydrogenolysis of the benzyl groups, and addition of TRIS afforded the target molecule **23**.

Since the above TRIS salts are not crystalline, slight inaccuracy in the stoichiometry free acid–TRIS is unavoidable. For perfect characterization, analytical

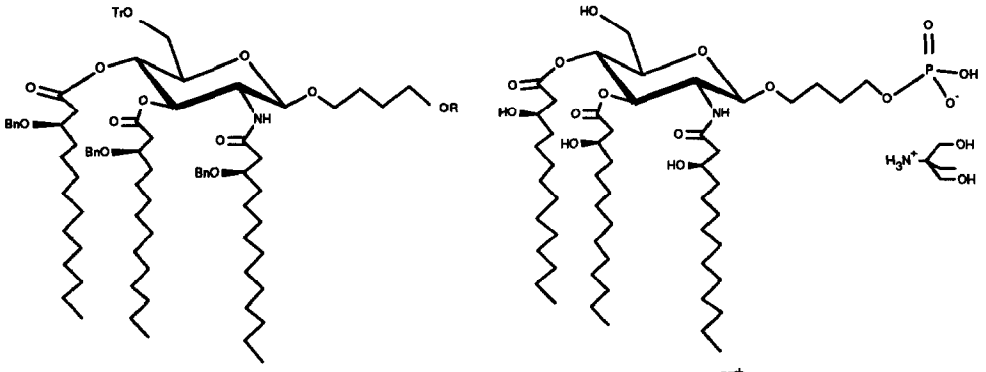


Scheme 3.

samples of the corresponding free acids were prepared for which correct elemental analyses were obtained in all cases.

Biological activity.

Compounds **11, 14, 17, 19,** and **23** were assessed in several assays commonly used for evaluation of lipid A analogues. Compounds **11–19** protected neutropenic mice against pseudomonas infection[13] and induced endotoxic shock in galactosamine-sensitized mice[14], a characteristic property of LPS-like substances. Compared to our standard 2-deoxy-2-[(*R*)-3-hydroxytetradecanamido]-3,4-di-*O*-[(*R*)-3-hydroxytetradecanoyl]-α-D-glucopyranose 1-phosphate[4a], however, the new compounds showed an unfavourable therapeutic ratio. Compound **23**, surprisingly, was



20 R=allyl
21 R=H
22 R=dibenzyloxyphosphoryl

Scheme 4.

only weakly active in the above biological assays. This contrasts with our findings using monosaccharide lipid A analogues, where 2,3,4-*N*,*O*-acylated glucosamine derivatives showed a better biological profile[4a] than 2,3-*N*,*O*-acylated derivatives. The effect of the new compounds on B cell activation has been reported elsewhere[15].

The present work constitutes a first step towards the design of structurally simple lipid A analogues. It shows that replacement of one of the monosaccharide units in the lipid A structure can be effected while maintaining biological activity. Based on the present work, further studies aimed at synthesizing even simpler molecules with lipid A-like activity are underway and the corresponding results will be reported in due course[16].

EXPERIMENTAL

General methods.—^{1}H NMR spectra (internal Me_4Si) were recorded with a Bruker WM-250 (250 MHz) spectrometer. Fast atom bombardment (FAB) mass spectra were recorded with a VG 70-SE spectrometer. Optical rotation measurements were conducted on a Perkin–Elmer 141 polarimeter. Melting points are uncorrected. Thin layer chromatography (TLC) was performed using Silica Gel 60 F_{254} plates (Merck) and column chromatography was done on Merck–Lichroprep columns. Evaporations and concentrations were carried out in vacuo below 40°C unless otherwise specified.

3-Allyloxypropanol and 4-allyloxybutanol.—Sodium hydride (55% in oil; 8.7 g, 200 mmol) was washed 3 times with hexane and suspended in DMF. To the stirred suspension, 1,3-propanediol (30.4 g, 400 mmol) was added slowly, under Ar, while maintaining the temperature below 30°C. Stirring was continued for 2 h and allyl bromide (24.2 g, 200 mmol) was added at such a rate that the temperature did not

exceed 30°C. Stirring was continued for 4 h, then water (100 mL) was added, and the aqueous solution was extracted with petroleum ether (2 × 50 mL). The organic phase, containing all the 1,3-bis-allyloxypropane formed during the reaction was discarded. The aqueous layer was then extracted with ether (3 × 100 mL). The organic solution containing the desired 3-allyloxypropanol and some DMF was washed with water (2 × 50 mL), dried, and concentrated under reduced pressure to afford crude 3-allyloxypropanol[17] (6.6 g, still containing 3% of DMF as shown by NMR), which was used without further purification for the next step. ^{1}H NMR ($CDCl_3$): δ 1.80 (m, 2 H, *J* 6 Hz, $OCH_2CH_2CH_2O$), 2.56 (br s, 1 H, OH), 3.62 (t, 2 H, *J* 6 Hz, $OCH_2CH_2CH_2OCH_2CH{=}CH_2$), 3.78 (br t, 2 H, $OCH_2CH_2CH_2OH$), 4.0 (m, 2 H, $OCH_2CH{=}CH_2$), 5.06–5.46 (2 H, $OCH_2CH{=}CH_2$), 5.90 (m, 1 H, $OCH_2CH{=}CH_2$).

In the same way, from 1,4-butanediol (36 g), 4-allyloxybutanol (16.6 g) was obtained; bp 70–75°C (1 mmHg); lit.[18] bp 99–104°C (19.5 mmHg). ^{1}H NMR ($CDCl_3$): δ 1.50–1.90 (4 H, 2 OCH_2CH_2), 2.58 (br s, 1 H, OH), 3.5 (t, 2 H, *J* 6 Hz, $CH_2CH_2OCH_2CH{=}CH_2$), 3.66 (br t, 2 H, CH_2CH_2OH), 4.0 (m, 2 H, $OCH_2CH{=}CH_2$), 5.0–5.50 (2 H, $OCH_2CH{=}CH_2$), 5.96 (m, 1 H, $OCH_2CH{=}CH_2$).

1,3,4,6-Tetra-O-acetyl-2-[(R)-3-benzyloxytetradecanamido]-2-deoxy-β-D-glucopyranose (**1**).—A solution of (*R*)-3-benzyloxytetradecanoic acid (13.36 g, 40 mmol) and Et_3N (4.04 g, 5.56 mL, 40 mmol) in CH_2Cl_2 (40 mL) was added dropwise to a cold (−30°C) solution of isobutyl chloroformate (5.46 g, 40 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred for 1 h at −30°C and 1 h at 20°C, and a solution of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose (11.32 g, 32.6 mmol) in CH_2Cl_2 (30 mL) was added dropwise while maintaining the temperature below 25°C. Stirring was continued for 16 h, and the mixture was then concentrated to ca. 50 mL at which point a crystalline precipitate had formed. 1:1 Toluene–hexane (300 mL) was added and the solution was washed with water. The organic layer was dried and evaporated under vacuum, and the residue was dissolved in CH_2Cl_2 and applied to the top of a short column of silica gel (diameter × height = 10 × 5 cm). A fast-migrating yellow band was first removed using CH_2Cl_2 as eluant. The desired compound along with slower migrating impurities was eluted using 9:1 CH_2Cl_2–ether. The crystalline residue obtained upon evaporation of the solvent was partially dissolved by refluxing in boiling ether (100 mL) for 1 h. The mixture was stored at 5°C overnight to afford pure **1** as a crystalline powder (15.22 g, 70%); mp 147–148°C; $[\alpha]_D^{20}$ 5.1° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3 H, *J* 6.5 Hz, CH_3), 1.15–1.38 (m, 18 H, 9 alkyl CH_2), 1.40–1.64 (m, 2 H, $BnOCHCH_2CH_2$), 1.99, 2.01, 2.02, 2.07 (4 s, 4 × 3 H, 4 CH_3CO), 2.26–2.48 (m, 2 H, $BnOCHCH_2CO$), 3.66–3.79 (m, 2 H, CHOBn and H-5), 4.12 (dd, $J_{6a,6b}$ 12, $J_{5,6a}$ 2.5 Hz, H-6a), 4.21 (m, 1 H, H-2), 4.29 (dd, $J_{5,6b}$ 4 Hz, H-6b), 4.47 and 4.62 (ABq, 2 H, J_{gem} 12 Hz, $PhCH_2$), 5.03–5.18 (m, 2 H, H-3, 4), 5.58 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 6.42 (d, 1 H, *J* 9.5 Hz, NH), 7.30–7.45 (m, 5 H, 5 Ph-H); FABMS: *m/z* 664 (MH^+). Anal. Calcd for $C_{35}H_{53}NO_{11}$: C, 63.33; H, 8.05; N, 2.11; Found: C, 63.47; H, 8.09; N, 2.03.

4-Allyloxybutyl 3,4,6-tri-O-acetyl-2-[(R)-3-benzyloxytetradecanamido]-2-deoxy-β-D-glucopyranoside (**2**).—A suspension of powdered dry $CaSO_4$ (13.0 g, 95.5 mmol) in dry CH_2Cl_2 (130 mL) containing $FeCl_3$ (8.12 g, 50 mmol) and **1** (21.54 g, 32.5 mmol) was stirred for 5 min at 20°C under Ar. 4-Allyloxybutanol (8.45 g, 65 mmol) was added and stirring was continued for 24 h. The mixture was slowly poured into satd aq $NaHCO_3$ and extracted with ether. The organic extract was dried and concentrated under vacuum and the residue thus obtained was chromatographed over silica gel (eluant: 4:1 then 1:1 toluene–EtOAc) to afford pure **2**. The compound was dissolved in CH_2Cl_2 and obtained as a crystalline powder (15.6 g, 65%) upon evaporation of the solvent; mp 81.5–85°C; $[\alpha]_D^{20}$ − 5° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 3 H, *J* 6.5 Hz, CH_3), 1.15–1.38 (m, 18 H, 9 alkyl CH_2), 1.40–1.70 (m, 6 H, BnOCHCH_2CH$_2$ and 2 OCH$_2$CH_2), 1.97, 2.01, 2.07 (3 s, 3 × 3 H, 3 CH_3CO), 2.30–2.50 (m, 2 H, BnOCHCH_2CO), 3.32–3.45 (m, 3 H, C*H*OBn and OCH$_2$CH$_2$CH$_2$CH_2O), 3.63 (m, 1 H, H-5), 3.67–3.88 (m, 3 H, H-2 and OCH_2CH$_2$CH$_2$CH$_2$O), 3.94 (m, 2 H, OCH_2CH=CH$_2$), 4.10 (dd, $J_{6a,6b}$ 12, $J_{5,6a}$ 2.5 Hz, H-6a), 4.26 (dd, $J_{5,6b}$ 4 Hz, H-6b), 4.47 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.48 and 4.60 (ABq, 2 H, J_{gem} 12 Hz, PhCH_2), 5.03 (t, $J_{3,4} = J_{4,5} = 10$ Hz, H-4), 5.12–5.32 (3 H, H-3 and OCH$_2$CH=CH_2), 5.90 (m, 1 H, OCH$_2$C*H*=CH$_2$), 6.51 (d, 1 H, *J* 8 Hz, NH), 7.28–7.43 (m, 5 H, 5 Ph-H). Anal. Calcd for $C_{40}H_{63}NO_{11}$: C, 65.46; H, 8.65; N, 1.91; Found: C, 65.56; H, 8.60; N, 1.88.

3-Allyloxypropyl 3,4,6-tri-O-acetyl-2-[(R)-3-benzyloxytetradecanamido]-2-deoxy-β-D-glucopyranoside (**6**).—From **1** (6.00 g, 9.0 mmol), using the same conditions as for the preparation of **2**, 5.26 g (80%) of **6** were obtained; mp 83–85°C; $[\alpha]_D^{20}$ − 7.3° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 3 H, *J* 6.5 Hz, CH_3), 1.15–1.40 (m, 18 H, 9 alkyl CH_2), 1.40–1.70 (m, 2 H, BnOCHCH_2CH$_2$), 1.80 (m, 2 H, OCH$_2$CH_2CH$_2$O), 1.98, 2.02, 2.09 (3 s, 3 × 3 H, 3 CH_3CO), 2.30–2.50 (m, 2 H, BnOCHCH_2CO), 3.35–3.56 (m, 3 H, C*H*OBn and OCH$_2$CH$_2$CH_2O), 3.61 (m, 1 H, H-5), 3.68–3.91 (m, 3 H, H-2 and OCH_2CH$_2$CH$_2$O), 3.93 (m, 2 H, OCH_2CH=CH$_2$), 4.10 (dd. $J_{6a,6b}$ 12, $J_{5,6a}$ 2.5 Hz, H-6a), 4.26 (dd, $J_{5,6b}$ 4 Hz, H-6b), 4.42 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.47 and 4.60 (ABq, 2 H, J_{gem} 12 Hz, PhCH_2), 5.25 (t, $J_{3,4} = J_{4,5} = 10$ Hz, H-4), 5.12–5.33 (3 H, H-3 and OCH$_2$CH=CH_2), 5.90 (m, 1 H, OCH$_2$C*H*=CH$_2$), 6.50 (d, 1 H, *J* 8 Hz, NH), 7.25–7.45 (m, 5 H, 5 Ph-H). Anal. Calcd for $C_{39}H_{61}NO_{11}$: C, 65.07; H, 8.54; N, 1.95; Found: C, 65.20; H, 8.50; N, 1.90.

4-Allyloxybutyl 2-[(R)-3-benzyloxytetradecanamido]-2-deoxy-β-D-glucopyranoside (**3**).—Compound **2** (10.0 g, 13.6 mmol) was dissolved in dry MeOH (55 mL) and a solution of NaOMe in MeOH (0.1 M, 5.5 mL) was added. The mixture was stirred for 1 h, AcOH (0.033 g, 0.55 mmol) was added, and the MeOH was evaporated. The residue was partitioned between water and CH_2Cl_2, and the organic phase was dried ($MgSO_4$) and evaporated to dryness to afford **3** as an amorphous solid (8.25 g, 99%); $[\alpha]_D^{20}$ −28.9° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.89 (t, 3 H, *J* 6.5 Hz, CH_3), 1.20–1.42 (m, 18 H, 9 alkyl CH_2), 1.45–1.70 (m, 6 H, BnOCHCH_2CH$_2$ and 2 OCH$_2$CH_2), 2.39 (t, 1 H, *J* 6.5 Hz, OH), 2.35–2.55 (m, 2 H, BnOCHCH_2CO),

3.12 (br s, 1 H, OH), 3.30–3.65 (7 H), 3.73–3.92 (4 H), 3.94 (m, 2 H, $OCH_2CH{=}CH_2$), 4.34 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.52 and 4.57 (ABq, 2 H, J_{gem} 11.5 Hz, $PhCH_2$), 5.18 (dm, 1 H, J_{cis} 10 Hz, $OCH_2CH{=}CH_{2a}H_{2b}$), 5.26 (dm, 1 H, J_{trans} 19 Hz, $OCH_2CH{=}CH_{2a}H_{2b}$), 5.39 (br d, 1 H, J 2.5 Hz, OH), 5.90 (m, 1 H, $OCH_2CH{=}CH_2$), 6.51 (d, 1 H, J 8 Hz, NH), 7.28–7.43 (m, 5 H, 5 Ph-H). FABMS.: m/z 608 (MH^+), 630 (MNa^+). Anal. Calcd for $C_{34}H_{57}NO_8$: C, 67.19; H, 9.45; N, 2.30; Found: C, 67.05; H, 9.37; N, 2.29.

4-Allyloxybutyl 2-[(R)-3-benzyloxytetradecanamido]-2-deoxy-3-O-[(R)-3-tetradecanoyloxytetradecanoyl]-6-O-trityl-β-D-glucopyranoside (**5a**).—A mixture of **3** (8.25 g, 13.6 mmol) and trityl chloride (7.25 g, 26 mmol) in pyridine (50 mL) was stirred for 24 h at room temperature. MeOH (11 mL) was then added and stirring was continued overnight. The mixture was partitioned between ether and brine, and the organic phase was washed successively with 1M HCl, satd aq $NaHCO_3$, and water, dried, and concentrated under reduced pressure. The residue was purified by chromatography under reduced pressure through a short silica gel column (diameter × height = 9 × 12 cm). The column was first washed with toluene (1.5 L) which was discarded, then the tritylated compound was eluted using 1:1 EtOAc–toluene. Evaporation of the solvents afforded the desired trityl derivative **4** (10.9 g, 94%) which was directly used for the subsequent step; ^1H NMR ($CDCl_3$): δ 0.88 (t, 3 H, J 6.5 Hz, CH_3), 1.18–1.42 (m, 18 H, 9 alkyl CH_2), 1.47–1.73 (m, 6 H, $BnOCHCH_2CH_2$ and 2 OCH_2CH_2), 2.35–2.55 (m, 2 H, $BnOCHCH_2CO$), 2.78 (br s, 1 H, OH), 3.30–3.65 (7 H), 3.75–3.89 (4 H), 3.92 (m, 2 H, $OCH_2CH{=}CH_2$), 4.34 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.52 and 4.57 (ABq, 2 H, J_{gem} 11.5 Hz, $PhCH_2$), 5.18 (dm, 1 H, J_{cis} 10 Hz, $OCH_2CH{=}CH_{2a}H_{2b}$), 5.22 (br s, 1 H, OH), 5.24 (dm, 1 H, J_{trans} 19 Hz, $OCH_2CH{=}CH_{2a}H_{2b}$), 5.88 (m, 1 H, $OCH_2CH{=}CH_2$), 6.81 (d, 1 H, J 8 Hz, NH), 7.15–7.53 (m, 20 H, 20 Ph-H).

A mixture of the trityl derivative **4** (4.37 g, 5.1 mmol), (*R*)-3-tetradecanoyloxytetradecanoic acid (2.36 g, 5.2 mmol), 1,3-dicyclohexylcarbodiimide (DCC, 1.07 g, 5.2 mmol), and 4-dimethylaminopyridine (DMAP, 0.020 g) in dry CH_2Cl_2 (10 mL) was stirred at 20°C. After 2 h, more acid (0.76 g, 1.68 mmol) and DCC (0.345 g, 1.68 mmol) were added and stirring was continued for 2 h. The precipitated dicyclohexylurea was filtered off, the CH_2Cl_2 evaporated under reduced pressure, and the residue chromatographed over silica gel (eluant: 7:1 toluene–EtOAc) to give 3 fractions from which two compounds homogeneous in TLC (4:1 toluene–EtOAc) were obtained. Fraction I (1.3 g, R_f 0.9), a gum, was not further characterized. Fraction II (3.2 g, R_f 0.56), a gum, was the desired 3-*O*-acylated compound **5a**. Fraction III (1.5 g, R_f 0.47), a gum, was the 4-*O*-acylated isomer **5b**, which was deacylated using the same conditions as for the preparation of **3**. Acylation of the recovered **4** (0.81 g) and chromatography as above afforded more **5a**; yield for combined runs: 4.0 g (60%); $[\alpha]_D^{20}$ −15.4° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (m, 9 H, 3 CH_3), 1.15–1.42 (m, 56 H, 28 alkyl CH_2), 1.42–1.75 (m, 10 H, $BnOCHCH_2CH_2$, $MyrOCHCH_2CH_2$, CH_2CH_2COO, and 2 OCH_2CH_2), 2.25 (t, 2 H, J 7.5 Hz, CH_2CH_2COO), 2.39 (m, 2 H, $BnOCHCH_2CO$ or

MyrOCHCH_2CO), 2.46 (dd, 1 H, J_{gem} 15, J 4.5 Hz, MyrOCHCH_aH$_b$CO or BnOCHCH_aH$_b$CO), 2.57 (dd, 1 H, J 8 Hz, MyrOCHCH$_a$$H_b$CO or BnOCHCH$_a$$H_b$CO), 3.08 (d, 1 H, J 3 Hz, OH), 3.28–3.47 (6 H), 3.60–4.02 (6 H), 4.17 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.50 and 4.62 (ABq, 2 H, J_{gem} 11.5 Hz, PhCH_2), 4.89 (dd, 1 H, $J_{2,3\text{ or }3,4}$ 9, $J_{3,4\text{ or }2,3}$ 10.5 Hz, H-3), 5.12 (m, 1 H, MyrOCH), 5.15 (dm, 1 H, J_{cis} 10 Hz, OCH$_2$CH=CH_{2a}H$_{2b}$), 5.24 (dm, 1 H, J_{trans} 19 Hz, OCH$_2$CH=CH$_{2a}$$H_{2b}$), 5.88 (m, 1 H, OCH$_2CH$=CH$_2$), 6.37 (d, 1 H, J 8 Hz, NH), 7.15–7.53 (m, 20 H, 20 Ph–H). Anal. Calcd for $C_{81}H_{123}NO_{11}$: C, 75.60; H, 9.63; N, 1.09; Found: C, 75.90; H, 10.00; N, 1.20.

3-Allyloxypropyl 2-[(R)*-3-benzyloxytetradecanamido]-2-deoxy-6-*O*-trityl-β-*D*-glucopyranoside* (**7**).—From **6** (5.39 g), using the same conditions as for the preparation of **3** and **4**, 5.74 g (91%) of **7** were obtained; $[\alpha]_D^{20}$ −31.9° (*c* 1, $CHCl_3$); ^{1}H NMR (1:1 $CDCl_3$–CD_3OD 1:1): δ 0.89 (t, 3 H, J 6.5 Hz, CH_3), 1.20–1.40 (18 H, 9 alkyl CH_2), 1.45–1.75 (m, 2 H, BnOCHCH_2CH$_2$), 1.85 (m, 2 H, OCH$_2$CH_2CH$_2$O), 2.35–2.60 (m, 2 H, BnOCHCH_2CO), 2.72 (br s, 1 H, OH), 3.30–3,69 (9 H), 3.75–3.79 (4 H), 4.34 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.53 and 4.59 (ABq, 2 H, J_{gem} 11.5 Hz, PhCH_2), 5.15 (dm, 1 H, J_{cis} 10 Hz, OCH$_2$CH=CH_{2a}H$_{2b}$), 5.19 (br s, 1 H, OH), 5.24 (dm, 1 H, J_{trans} 19 Hz, OCH$_2$CH=CH$_{2a}$$H_{2b}$), 5.88 (m, 1 H, OCH$_2CH$=CH$_2$), 6.76 (d, 1 H, J 8 Hz, NH), (7.15 m, 20 H, 20 Ph-H). Anal. Calcd for $C_{52}H_{69}NO_8$: C, 74.70; H, 8.32; N, 1.68; Found: C, 74.50; H, 8.10; N, 1.80.

3-Allyloxypropyl-2-[(R)*-3-benzyloxytetradecanamido]-2-deoxy-3-*O*-[*(R)*-3-tetradecanoyloxytetradecanoyl]-6-*O*-trityl-β-*D*-glucopyranoside* (**8a**).—From **7** (4.11 9, 4.92 mmol), using the same conditions as for the preparation of **5a**, the 3-*O*-acylated material **8a** was obtained [gum, 2.76 g, R_f 0.51 (4:1 toluene–EtOAc)]. The undesired 4-*O*-acylated isomer **8b** (1.32 g, R_f 0.45) was deacylated and reacylated to afford more **8a**; yield for combined runs: 3.28 g (52%); $[\alpha]_D^{20}$ −14.2° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 9 H, 3 CH_3), 1.15–1.42 (m, 56 H, 28 alkyl CH_2), 1.42–1.75 (m, 6 H, BnOCHCH_2CH$_2$, MyrOCHCH_2CH$_2$, and CH_2CH$_2$COO), 1.82 (m, 2 H, OCH$_2$CH_2CH$_2$O), 2.25 (t, 2 H, J 7.5 Hz, CH$_2$CH_2COO), 2.39 (m, 2 H, BnOCHCH_2CO or MyrOCHCH_2CO), 2.46 (dd, 1 H, J_{gem} 15, J 4.5 Hz, MyrOCHCH_aH$_b$CO or BnOCHCH_aH$_b$CO), 2.57 (dd, 1 H, J 8 Hz MyrOCHCH$_a$$H_b$CO or BnOCHCH$_a$$H_b$CO), 3.08 (d, 1 H, J 3 Hz, OH), 3.28–3.58 (6 H), 3.62–4.02 (6 H), 4.14 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.49 and 4.62 (ABq, 2 H, J_{gem} 11.5 Hz, PhCH_2), 4.87 (dd, 1 H, $J_{2,3\text{ or }3,4}$ 9, $J_{3,4\text{ or }2,3}$ 10.5 Hz, H-3), 5.12 (m, 1 H, MyrOCH), 5.14 (dm, 1 H, J_{cis} 10 Hz, OCH$_2$CH=CH_{2a}H$_{2b}$), 5.23 (dm, 1 H, J_{trans} 19 Hz, OCH$_2$CH=CH$_{2a}$$H_{2b}$), 5.88 (m, 1 H, OCH$_2CH$=CH$_2$), 6.33 (d, 1 H, J 8 Hz, NH), 7.15–7.55 (m, 20 H, 20 Ph-H). Anal. Calcd for $C_{80}H_{121}NO_{11}$: C, 75.49; H, 9.58; N, 1.10; Found: C, 75.50; H, 9.20; N, 1.10.

4-Hydroxybutyl 2[(R)*-3-benzyloxytetradecanamido]-2-deoxy-3-*O*-[*(R)*-3-tetradecanoyloxytetradecanoyl]-6-*O*-trityl-β-*D* glucopyranoside* (**9**).—Compound **5a** (4.0 g, 3.1 mmol) was dissolved in EtOH (60 mL), Wilkinson's catalyst (0.63 g, 0.68 mmol) and DABCO (0.216 g, 1.92 mmol) were added, and the mixture was refluxed for 3 h. The solvent was evaporated and the residue was chromatographed over silica gel

(eluant: 9:1 toluene–EtOAc) to afford 4-propenyloxybutyl 2-[(*R*)-3-benzyloxytetradecanamido]-2-deoxy-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-6-*O*-trityl-β-D-glucopyranoside (2.93 g). This was dissolved in THF (70 mL) and 9 mL of aq 50% CF_3CO_2H was added. The mixture was stirred for 2 h and more CF_3CO_2H in water (10 mL) was added. After 2 h, the reaction was complete. The mixture was diluted with ether and extracted successively with satd aq $NaHCO_3$, then water. The organic layer was dried and the solvent evaporated. Chromatography of the residue over silica gel (eluant: 4:1 toluene–EtOAc) afforded pure **9** (1.67 g, 43% from **5a**); $[\alpha]_D^{20}$ −16.7° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 9 H, 3 CH_3), 1.11–1.41 (m, 56 H, 28 alkyl CH_2), 1.41–1.75 (m, 10 H, BnOCHCH_2CH_2, MyrOCHCH_2CH_2, CH_2CH_2COO, and 2 OCH_2CH_2), 1.92 (br m, 1 H, CH_2O*H*), 2.25 (t, 2 H, *J* 7.5 Hz, CH_2CH_2COO), 2.39 (m, 2 H, BnOCHCH_2CO or MyrOCHCH_2CO), 2.46 (dd, 1 H, J_{gem} 15, *J* 4.5 Hz, MyrOCHCH_aH$_b$CO or BnOCHCH_aH$_b$CO), 2.57 (dd, 1 H, *J* 8 Hz, MyrOCHCH$_a$$H_b$CO or BnOCHCH$_a$$H_b$CO), 3.10 (d, 1 H, *J* 3 Hz, CHO*H*), 3.28–3.47 (4 H), 3.56 (br m, 2 H, CH_2CH_2OH), 3.70 (m 1 H, H-5), 3.77–3.90 (2 H), 3.98 (m, 1 H, H-2), 4.19 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.52 and 4.62 (ABq, 2 H, J_{gem} 11.5 Hz, PhCH_2) 4.89 (dd, 1 H, $J_{2,3\text{ or }3,4}$ 9, $J_{3,4\text{ or }2,3}$ 10.5 Hz, H-3), 5.13 (m, 1 H, MyrOC*H*), 6.31 (d, 1 H, *J* 8 Hz, NH), 7.15–7.53 (m, 20 H, 20 Ph-H). Anal. Calcd for $C_{78}H_{118}NO_{11}$: C, 75.20; H, 9.55; N, 1.12. Found: C, 75.10; H, 9.20; N, 1.20.

3-Hydroxypropyl 2-[(R)*-3-benzyloxytetradecanamido]-2-deoxy-3-*O*-[*(R)*-3-tetradecanoyloxytetradecanoyl]-6-*O*-trityl-β-D-glucopyranoside* (**12**).—Using similar conditions as for the preparation of **9**, the allyl derivative **8a** (3.20 g, 2.51 mmol) was converted into **12** (1.54 g, 50% from **8a**); $[\alpha]_D^{20}$ −16° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 9 H, 3 CH_3), 1.05–1.42 (m, 56 H, 28 alkyl CH_2), 1.41–1.65 (m, 6 H, BnOCHCH_2CH_2, MyrOCHCH_2CH_2, and CH_2CH_2COO), 1.74 (m, 1 H, OCH$_2$CH_2CH$_2$O), 2.25 (t, 2 H, *J* 7.5 Hz, CH$_2$CH_2COO), 2.39 (m, 3 H, BnOCHCH_2CO or MyrOCHCH_2CO + overlapping CH$_2$O*H*), 2.46 (dd, 1 H, J_{gem} 15, *J* 4.5 Hz, MyrOCHCH_aH$_b$CO or BnOCHCH_aH$_b$CO), 2.57 (dd, 1 H, *J* 8 Hz, MyrOCHCH$_a$$H_b$CO or BnOCHCH$_a$$H_b$CO), 3.12 (d, 1 H, *J* 3 Hz, CHO*H*), 3.28–3.47 (3 H), 3.53 (m, 1 H), 3.60–3.76 (m, 3H), 3.82 (m, 1 H, C*H*OBn), 3.88–4.08 (m, 2 H), 4.13 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.51 and 4.64 (ABq, 2 H, J_{gem} 11.5 Hz, PhCH_2), 4.84 (dd, 1 H, $J_{2,3\text{ or }3,4}$ 9, $J_{3,4\text{ or }2,3}$ 10.5 Hz, H-3), 5.13 (m, 1 H, MyrOC*H*), 6.40 (d, 1 H, *J* 8 Hz, NH), 7.17–7.57 (m, 20 H, 20 Ph-H). Anal. Calcd for $C_{77}H_{117}NO_{11}$: C, 75.02; H, 9.57; N, 1.14. Found: C, 74.52; H, 9.53; N, 1.25.

4-Dibenzyloxyphosphoryloxybutyl 2-[(R)*-3-benzyloxytetradecanamido]-2-deoxy-3-*O*-[*(R)*-3-tetradecanoyloxytetradecanoyl]-6-*O*-trityl-β-D-glucopyranoside* (**10**).—Compound **9** (0.93 g, 0.75 mmol) was dissolved in dry benzene (10 mL). Pyridine (0.79 g, 10 mmol) and 1 M dibenzyl phosphorochloridate in benzene[19] (2.4 mL) were added. The mixture was stirred for 1.5 h and MeOH (1 mL) was added. Stirring was continued for 10 min, the solvent was evaporated, and the residue was chromatographed over silica gel (eluant: 2:1 toluene–EtOAc) to afford **10** (0.708 g, 63%); $[\alpha]_D^{20}$ −13.4° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 9 H, 3 × CH_3),

1.11–1.41 (m, 56 H, 28 alkyl CH_2), 1.41–1.75 (m, 10 H, BnOCHCH_2CH$_2$, MyrOCHCH_2CH$_2$, CH_2CH$_2$COO, and 2 OCH$_2$CH_2), 2.25 (t, 2 H, *J* 7.5 Hz, CH$_2$CH_2COO), 2.39 (m, 2 H, BnOCHCH_2CO or MyrOCHCH_2CO), 2.46 (dd, 1 H, J_{gem} 15, *J* 4.5 Hz, MyrOCHCH_aH$_b$CO or BnOCHCH_aH$_b$CO), 2.57 (dd, 1 H, *J* 8 Hz, MyrOCHCH$_a$$H_b$CO or BnOCHCH$_a$$H_b$CO), 3.10 (br s, 1 H, OH), 3.26–3.46 (4 H), 3.62–3.83 (3 H), 3.86–4.02 (2 H), 4.16 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.47 and 4.58 (ABq, 2 H, J_{gem} 11.5 Hz, PhCH_2, 4.90 (dd, 1 H, $J_{2,3\text{ or }3,4}$ 9, $J_{3,4\text{ or }2,3}$ 10.5 Hz, H-3), 5.00 (d, 4 H, $J_{H,P}$ 8 Hz, 2 POCH_2Ph), 5.13 (m, 1 H, MyrOC*H*), 6.47 (d, 1 H, *J* 8 Hz, NH), 7.15–7.53 (m, 30 H, 30 Ph-H). Anal. Calcd for $C_{92}H_{132}NO_{14}P$: C, 73.32; H, 8.83; P, 2.06; Found: C, 73.58; H, 8.96; P, 2.18.

3-Dibenzyloxyphosphoryloxypropyl 2-[(R)*-3-benzyloxytetradecanamido]-2-deoxy-3-*O*-[*(R)*-3-tetradecanoyloxytetradecanoyl]-6-*O*-trityl-β-*D*-glucopyranoside* (**13**).—Treatment of **12** (0.80 g, 0.65 mmol) as described for the preparation of **10** afforded **13** (0.768 g, 70%); $[\alpha]_D^{20}$ −2.8° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 9 H, 3 CH_3), 1.11–1.41 (m, 56 H, 28 alkyl CH_2), 1.41–1.75 (m, 8 H, BnOCHCH_2CH$_2$, MyrOCHCH_2CH$_2$, CH_2CH$_2$COO, and OCH$_2$CH_2CH$_2$O), 2.25 (t, 2 H, *J* 7.5 Hz, CH$_2$CH_2COO), 2.30–2.66 (m, 4 H, BnOCHCH_2CO and MyrOCHCH_2CO), 3.07 (d, 1 H, *J* 3 Hz, OH), 3.25–3.45 (4 H), 3.60–3.78 (m, 2 H), 3.80–3.94 (m, 2 H), 4.05 (m, 1 H, H-2), 4.20 (m, 2 H), 4.50 (s, 2 H, PhCH_2), 4.85 (dd, 1 H, $J_{2,3\text{ or }3,4}$ 9, $J_{3,4\text{ or }2,3}$ 10.5 Hz, H-3), 4.99 (d, 1 H, $J_{H,P}$ 8 Hz, POCH_2Ph), 5.13 (m, 1 H, MyrOC*H*), 7.05 (d, 1 H, *J* 8 Hz, NH),7.15–7.53 (m, 30 H, 30 Ph-H). Anal. Calcd for $C_{91}H_{130}NO_{14}P$: C, 73.21; H, 8.78; P, 2.07; Found: C, 73.10; H, 8.82; P, 2.01.

4-Phosphonoxybutyl 2-deoxy-2-[(R)*-3-hydroxytetradecanamido]-3-*O*-[*(R)*-3-tetradecanoyloxytetradecanoyl]-β-*D*-glucopyranoside mono TRIS salt* (**11**).—The trityl derivative **10** (0.200 g, 0.13 mmol) was dissolved in ether (20 mL), HCO_2H (16 mL) was added, and the mixture was stirred for 15 min at 20°C. The resulting solution was carefully neutralized by addition of water and solid $NaHCO_3$, then washed with water, and dried, and the residue was purified by chromatography over silica gel (eluant: 1:1 toluene–EtOAc). The residue (0.147 g) was dissolved in 4:1 THF–water (10 mL). Palladium (10% on charcoal, 0.130 g) was added and the mixture was hydrogenated for 15 min. The catalyst was filtered off over Celite, TRIS (0.015 g, 0.12 mmol) was added, and the solution was lyophilized. In order to eliminate the remaining colloidal particles of carbon, the lyophilizate was dissolved in 8:1 THF–water, and filtered through a short Sephadex LH 20 column (2 × 5 cm), eluting with the same solvent. The filtrate was lyophilized, and the resulting powder was washed with pentane, filtered, and dried to give pure **11** (0.116 g, 80%). For analytical purposes, a sample of the corresponding free acid **11a** was obtained by Bligh–Dyer extraction[20]: the TRIS salt (0.030 g) was dissolved in a mixture of $CHCl_3$ (15 mL), MeOH (30 mL), and water (12 mL). Upon addition of $CHCl_3$ and 0.1 M HCl (15 mL each), two clear phases were obtained. The organic (lower) phase was evaporated to afford **11a**; $[\alpha]_D^{20}$ −10.4° (*c* 0.5, 1:1 $CHCl_3$–MeOH); ^{1}H NMR ($CDCl_3$–CD_3OD + 1 drop of CF_3CO_2D): δ 0.88 (m, 9 H, 3

CH_3), 1.10–1.70 (66 H, 28 alkyl CH_2, HOCHCH_2CH$_2$, MyrOCHCH_2CH$_2$, CH_2CH$_2$COO, and 2 OCH$_2$CH_2), 2.10–2.31 (4 H, CH$_2$CH_2COO and HOCHCH_2CO or MyrOCHCH_2CO), 2.45 (dd, 1 H, J_{gem} 15, J 4.5 Hz, MyrOCHCH_aH$_b$CO or HOCHCH_aH$_b$CO), 2.56 (dd, 1 H, J 8 Hz, MyrOCHCH$_a$$H_b$CO or HOCHCH$_a$$H_b$CO), 4.41 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.88 (dd, 1 H, $J_{2,3\text{ or }3,4}$ 9, $J_{3,4\text{ or }2,3}$ 10.5 Hz, H-3). Anal. Calcd for $C_{52}H_{100}NO_{14}P$: C, 62.81; H, 10.14; P, 3.12; Found: C, 62.31; H, 10.11; P 3.59.

3-Phosphonoxypropyl 2-deoxy-2-[(R)*-3-hydroxytetradecanamido]-3-*O*-[*(R)*-3-tetradecanoyloxytetradecanoyl]-β-*D*-glucopyranoside monoTRIS salt* (**14**).—From the trityl derivative **13** (0.713 g, 0.48 mmol), using the same conditions as for the preparation of **11**, the monoTRIS salt **14** (0.415 g, 79%) was obtained. The corresponding free acid **14a** was obtained by Bligh–Dyer extraction as described above; $[\alpha]_D^{20}$ −14.4° (*c* 0.5, 1:1 $CHCl_3$–MeOH); ^{1}H NMR ($CDCl_3$–CD_3OD 9:1 + 1 drop of CF_3CO_2D): δ 0.88 (m, 9 H, 3 CH_3), 1.10–1.60 (62 H, 28 alkyl CH_2, HOCHCH_2CH$_2$, MyrOCHCH_2CH$_2$, and CH_2CH$_2$COO), 1.79 (m, 2 H, OCH$_2$CH_2CH$_2$O), 2.08–2.30 (4 H, CH$_2$CH_2COO and HOCHCH_2CO or MyrOCHCH_2CO), 2.43 (dd, 1 H, J_{gem} 15, J 5 Hz, MyrOCHCH_aH$_b$CO or HOCHCH_aH$_b$CO), 2.67 (dd, 1 H, J 7 Hz, MyrOCHCH$_a$$H_b$CO or HOCHCH$_a$$H_b$CO), 4.39 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.83 (dd, 1 H, $J_{2,3\text{ or }3,4}$ 9, $J_{3,4\text{ or }2,3}$ 10.5 Hz, H-3), 5.04 (m, 1 H, MyrOCH); FABMS: *m/z* 980 (MH^+). Anal. Calcd for $C_{51}H_{98}NO_{14}P$: C, 62.49; H, 10.08; P, 3.16; Found: C, 62.00; H, 10.15; P. 3.69.

4-Allyloxybutyl 2-[(R)*-3-benzyloxytetradecanamido]-2-deoxy-4-*O*-dibenzyloxyphosphoryl-3-*O*-[*(R)*-3-tetradecanoyloxytetradecanoyl]-6-*O*-trityl-β-*D*-glucopyranoside* (**15**).—To a cold (−78°C) solution of **5a** (1.00 g, 0.78 mmol) in THF (80 mL) were successively added a solution of butyllithium (1.6 M in hexane; 0.59 mL, 0.94 mmol) and a 1 M solution of dibenzyl phosphorochloridate in benzene (0.94 mL, 0.94 mmol). After 1 h, AcOH (0.060 g, 1 mmol) was added and the mixture was allowed to warm to room temperature. After evaporation of the solvent, the residue was purified by chromatography over silica gel (eluant: 3:1 toluene–EtOAc) to afford **15** (0.731 g, 61%); $[\alpha]_D^{20}$ −9.2° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 9 H, 3 CH_3), 1.10–1.80 (66 H, 28 alkyl CH_2, BnOCHCH_2CH$_2$, MyrOCHCH_2CH$_2$, CH_2CH$_2$COO, and 2 OCH$_2$CH_2), 2.25 (t, 2 H, J 7.5 Hz, CH$_2$CH_2COO), 2.28–2.51 (m, 4 H, BnOCHCH_2CO and MyrOCHCH_2CO), 3.28–3.97 (10 H), 4.32 (ddd, 1 H, $J_{3,4} = J_{4,5} = J_{4\text{-P}} = 9$ Hz, H-4), 4.45–4.60 (4 H), 4.60–4.74 (3 H), 5.08–5.27 (3 H, MyrOCH and OCH$_2$CH=CH_2), 5.28 (dd, 1 H, $J_{2,3\text{ or }3,4}$ 9, $J_{3,4\text{ or }2,3}$ 10.5 Hz, H-3), 5.88 (m, 1 H, OCH$_2$CH=CH$_2$), 6.43 (d, 1 H, J 8 Hz, NH), 7.05–7.53 (m, 30 H, 30 Ph-H). Anal. Calcd for $C_{95}H_{136}NO_{14}P$: C, 73.75; H, 8.86; N, 0.91; Found: C, 73.63; H, 9.04; N, 1.23.

4-Hydroxybutyl 2-[(R)*-3-benzyloxytetradecanamido]-2-deoxy-4-*O*-dibenzyloxyphosphoryl-3-*O*-[*(R)*-3-tetradecanoyloxytetradecanoyl]-6-*O*-trityl-β-*D*-glucopyranoside* (**16**).—The allyl derivative **15** (4.22 g, 2.72 mmol) was dissolved in dry THF (18 mL) under Ar. A solution of Ir(COD)PF_6 (0.025 g) in CH_2Cl_2 (2.5 mL) was added.

The catalyst was activated by allowing a stream of H_2 to pass through the mixture for 5 min, excess of H_2 was replaced by Ar, and stirring was continued for 1 h. After evaporation of the solvent, the crude propenyl derivative thus obtained was dissolved in THF (60 mL) and treated for 30 min with 30 mL of aq 50% CF_3CO_2H. The mixture was carefully neutralized with solid $NaHCO_3$, ether was added, the organic phase was washed with water and dried, and the solvent was evaporated. The residue was chromatographed over silica gel (eluant: 4:1 toluene–EtOAc) to afford **16** (3.46 g, 84%); $[\alpha]_D^{20}$ −11.2° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 9 H, 3 CH_3), 1.1–1.40 (56 H, 28 alkyl CH_2), 1.40–1.75 (10 H, BnOCHCH_2CH$_2$, MyrOCHCH_2CH$_2$, CH_2CH$_2$COO, and 2 OCH$_2$CH_2), 1.85 (br s, 1 H, OH), 2.19 (t, 2 H, *J* 7.5 Hz, CH$_2$CH_2COO), 2.28–2.50 (m, 4 H, BnOCHCH_2CO and MyrOCHCH_2CO), 3.28–4.00 (9 H), 4.32 (ddd, 1 H, $J_{3,4} = J_{4,5} = J_{4\text{-P}} = 9$ Hz, H-4), 4.45–4.74 (7 H), 5.12 (m, 1 H, MyrOC*H*), 5.24 (dd, 1 H, $J_{2,3\text{ or }3,4}$ 9, $J_{3,4\text{ or }2,3}$ 10.5 Hz, H-3), 6.33 (d, 1 H, *J* 8 Hz, NH), 7.05–7.50 (m, 30 H, 30 Ph-H); FABMS: *m/z* 1530 (MNa^+). Anal. Calcd for $C_{92}H_{132}NO_{14}P$: C, 73.32; H, 8.83; N, 0.93; Found: C, 73.17; H, 8.94; N, 1.39.

4-Hydroxybutyl 2-deoxy-2-[(R)*-3-hydroxytetradecanamido]-4-*O*-phosphono-3-*O*-[*(R)*-3-tetradecanoyloxytetradecanoyl]-β-*D*-glucopyranoside* (**17a**).—Compound **16** (0.80 g, 0.53 mmol) was dissolved in ether (24 mL) and treated with HCO_2H (15 mL). After 5 min, the reaction was worked up as described for **12** and the residue chromatographed over silica gel (eluant: EtOAc). the detritylated material thus obtained (0.375 g) was dissolved in 15 mL 4:1 THF–water, and hydrogenated for 10 min over palladium (10% on carbon). TRIS (0.031 g, 0.26 mmol) was added and the mixture was filtered. The filtrate (which still contained particles of charcoal) was lyophilized to afford the crude TRIS salt **17** (0.330 g). Bligh–Dyer extraction as described for **11** afforded **17a** (0.260 g, 49%); $[\alpha]_D^{20}$ −8.6° (*c* 0.5, 1:1 $CHCl_3$–CH_3OH); ^{1}H NMR (4:1 $CDCl_3$–CD_3OD): δ 0.88 (m, 9 H, 3 CH_3), 1.10–1.75 (66 H, 28 alkyl CH_2, HOCHCH_2CH$_2$, MyrOCHCH_2CH$_2$, CH_2CH$_2$COO, and 2 OCH$_2$CH_2), 2.10–2.40 (m, 4 H, CH$_2$CH_2COO and HOCH$_2$CH_2CO or MyrOCHCH_2), 2.54 (dd, 1 H, J_{gem} 15, *J* 4.5 Hz, MyrOCHCH_aH$_b$CO or HOCHCH_aH$_b$CO), 2.65 (dd, 1 H, *J* 8 Hz, MyrOCHCH$_a$CH_bCO or HOCHCH$_a$$H_b$CO), 3.28–4.00 (9 H), 4.32 (ddd, 1 H, $J_{3,4} = J_{4,5} = J_{4\text{-P}} = 9$ Hz, H-4), 4.54 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 5.14 (m, 2 H, MyrOC*H* and H-3). Anal. Calcd for $C_{52}H_{100}NO_{14}P$: C, 62.81; H, 10.14; P, 3.12; Found: C, 62.82; H, 10.24; P. 3.36.

4-Dibenzyloxyphosphoryloxybutyl 2-[(R)*-3-benzyloxytetradecanamido]-2-deoxy-2-4-*O*-dibenzyloxyphosphoryl-3-*O*-[*(R)*-3-tetradecanoyloxytetradecanoyl]-6-*O*-trityl-β-*D*-glucopyranoside* (**18**).—Compound **16** (1.488 g, 0.98 mmol) was dissolved in benzene (20 mL). Pyridine (1.17 mL) and 1 M dibenzyl phosphorochloridate in benzene (4 mL) were added. The mixture was stirred for 3 h at room temperature and MeOH (3 mL) was added. Stirring was continued for 30 min, the solvent was evaporated, and the residue was chromatographed over silica gel (eluant: 3:1 toluene–EtOAc) to afford **18** (1.13 g, 65%); $[\alpha]_D^{20}$ −7.4° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 9 H, 3 CH_3), 1.10–1.80 (66 H, 28 alkyl CH_2, BnOCHCH_2CH$_2$,

MyrOCHCH_2CH$_2$, CH_2CH$_2$COO, and 2 OCH$_2$CH_2), 2.18 (t, 2 H, *J* 7.5 Hz, CH$_2$CH_2COO), 2.28–2.50 (m, 4 H, BnOCHCH_2CO and MyrOCHCH_2CO), 3.25–4.05 (9 H), 4.32 (ddd, 1 H, $J_{3,4} = J_{4,5} = J_{4,P} = 9$ Hz, H-4), 4.45–4.75 (7 H), 5.0 (2 overlapping m, 4 H, 2 POCH_2Ph), 5.14 (m, 1 H, MyrOC*H*), 5.28 (dd, 1 H, $J_{2,3 \text{ or } 3,4}$ 9, $J_{3,4 \text{ or } 2,3}$ 10.5 Hz, H-3), 6.53 (d, 1 H, *J* 8 Hz, NH), 7.05–7.50 (m, 40 H, 40 Ph-H). Anal. Calcd for $C_{106}H_{145}NO_{17}P_2$: C, 72.04; H, 8.27; N, 0.79; Found: C, 71.45; H, 8.10; N, 0.77.

4-Phosphonoxybutyl 2-deoxy-2-[(R)*-3-hydroxytetradecanamido]-4-*O*-phosphono-3-*O*-[*(R)*-3-tetradecanoyloxytetradecanoyl]-β-*D*-glucopyranoside bisTRIS salt* (**19**).—The trityl derivative **18** (1.146 g, 0.65 mmol) dissolved in ether (34 mL) was treated with HCO$_2$H (20 mL) for 5 min. After workup as described earlier, the detritylated material was purified by chromatography over silica gel (eluant: 2:1 toluene–EtOAc) (yield: 0.830 g). The product (0.780 g, 0.51 mmol) was dissolved in 4:1 water–THF (30 mL), and hydrogenated for 30 min over palladium (5% over charcoal, 0.50 g). The catalyst was filtered off, and TRIS (0.121 g, 1 mmol) was added. The solution was lyophilized, and the resulting powder was washed with ether and dried to afford **19** (0.511 g, 64% from **18**). An analytical sample of the corresponding free acid **19a** was prepared by dissolving 90 mg of the TRIS salt in 15:30:12 CHCl$_3$–MeOH–water (57 mL), and subjected to Bligh–Dyer extraction as described for **17**; $[\alpha]_D^{20}$ 0° (*c* 0.5, MeOH); ^{1}H NMR (1:1 CDCl$_3$–CD$_3$OD): δ 0.89 (m, 9 H, 3 CH$_3$), 1.15–1.81 (66 H, 28 alkyl CH$_2$, HOCHCH_2CH$_2$, MyrOCHCH_2CH$_2$, CH_2CH$_2$COO, and 2 OCH$_2$CH_2), 2.15–2.40 (m, 4 H, CH$_2$CH_2COO and HOCH$_2$CH_2CO or MyrOCHCH_2), 2.57 (dd, 1 H, J_{gem} 16, *J* 6 Hz, MyrOCHCH_aH$_b$CO or HOCHCH_aH$_b$CO), 2.69 (dd, 1 H, *J* 7.5 Hz, MyrOCHCH$_a$CH_bCO or HOCHCH$_a$$H_b$CO), 3.28–3.75 (9 H), 4.28 (ddd, 1 H, $J_{3,4} = J_{4,5} = J_{4\text{-}P} = 9$ Hz, H-4), 4.56 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 5.20 (m, 2 H, MyrOC*H* and H-3). Anal. Calcd for $C_{52}H_{101}NO_{17}P_2$: C, 58.14; H, 9.48; P, 5.77 Found: C, 57.90; H, 9.36; P, 5.74.

4-Allyloxybutyl 2-[(R)*-3-benzyloxytetradecanamido]-3,4-di-*O*-[*(R)*-3-benzyloxytetradecanoyl]-2-deoxy-6-*O*-trityl-β-*D*-glucopyranoside* (**20**).—Compound **4** (2.92 g, 3.43 mmol) was dissolved in CH$_2$Cl$_2$ (10 mL). The solution was cooled to 0°C, and (*R*)-3-benzyloxytetradecanoic acid (2.34 g, 7 mmol), DCC (1.44 g, 7 mmol), and DMAP (0.020 g) were added. The mixture was stirred for 2.5 h at 0°C, the dicyclohexylurea filtered off, the solvent evaporated, and the residue chromatographed over silica gel (eluant: 9:1 toluene–EtOAc) to afford **20** (4.26 g, 84%); $[\alpha]_D^{20}$ −10.5° (*c* 1, CHCl$_3$); ^{1}H NMR (CDCl$_3$): δ 0.88 (t, 9 H, *J* 6.5 Hz, 3 CH$_3$), 1.10–1.75 (m, 64 H, 27 alkyl CH$_2$, 3 BnOCHCH_2CH$_2$, and 2 OCH$_2$CH_2), 2.02 (dd, 1 H) and 2.15–2.50 (m, 5 H) (3 BnOCHCH_2CO), 3.14 (m, 2 H), 3.35–3.90 (9 H), 3.92 (m, 2 H, OCH_2CH=CH$_2$), 4.22–4.62 (m, 7 H, 3 PhCH_2 and H-1), 5.00–5.33 (m, 4 H, OCH$_2$CH=CH_2, H-3, and H-4), 5.89 (m, 1 H, OCH$_2$C*H*=CH$_2$), 6.22 (d, 1 H, *J* 8 Hz, NH), 7.15–7.50 (m, 30 H, 30 Ph-H). Anal. Calcd for $C_{95}H_{135}NO_{12}$: C, 76.94; H, 9.17; N, 0.94; Found: C, 76.30; H, 9.21; N, 1.15.

4-Hydroxybutyl 2-[(R)*-3-benzyloxytetradecanamido]-3,4-di-*O*-[*(R)*-3-benzyloxy-

tetradecanoyl]-2-deoxy-6-O-trityl-β-D-glucopyranoside (**21**).—The allyl derivative **20** (1.66 g, 1.12 mmol) was dissolved in THF (freshly distilled over $LiAlH_4$, 7 mL), Ar was bubbled through the solution for 5 min, and $Ir(COD)PF_6$ (0.008 g) dissolved in CH_2Cl_2 (0.8 mL) was added. Ar was again bubbled for 5 min through the resulting solution and the flask was flushed with H_2 for 1 min under vigorous stirring. Excess of H_2 was removed using a stream of Ar and the mixture was stirred for 30 min. The solvent was evaporated, the residue was dissolved in THF (30 mL), aq 50% CF_3CO_2H (12 mL) was added, and the mixture was stirred for 3 h. Workup as described for **16** and chromatography of the residue over silica gel (eluant: 4:1 toluene–EtOAc) afforded pure **21** (1.37 g, 85%); $[\alpha]_D^{20}$ 6.6° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.5 Hz, 3 CH_3), 1.10–1.75 (m, 64 H, 27 alkyl CH_2, 3 BnOCHCH_2CH$_2$, and 2 OCH$_2$CH_2), 1.85 (br s, 1 H, OH), 2.02 (dd, 1 H) and 2.15–2.50 (m, 5 H) (3 BnOCHCH_2CO), 3.15 (m, 2 H), 3.4–3.97 (9 H), 4.22–4.62 (m, 7 H, 3 PhCH_2 and H-1), 5.08 (dd, 1 H, $J_{3,4} = J_{4,5} = 9$ Hz, H-4), 5.21 (dd, 1 H, $J_{2,3} = J_{3,4} = 10$ Hz, H-3), 6.20 (d, 1 H, *J* 8 Hz, NH), 7.15–7.50 (m, 30 H, 30 Ph-H). Anal. Calcd for $C_{92}H_{131}NO_{12}$: C, 76.57; H, 9.15; N, 0.97; Found: C, 76.23; H, 9.22; N, 1.18.

4-Dibenzyloxyphosphoryloxybutyl 2-[(R)-3-benzyloxytetradecanamido]-3,4-di-O-[(R)-3-benzyloxytetradecanoyl]-2-deoxy-6-O-trityl-β-D-glucopyranoside (**22**).—The intermediate **21** (1.26 g, 0.87 mmol) dissolved in benzene (3 mL) was treated for 4 h, at room temperature, with dibenzyl phosphorochloridate (3.8 mL of a 1 M solution in benzene) in the presence of pyridine (1.75 g, 22 mmol) to afford, after purification by chromatography (silica gel, eluant: 6:1 toluene–EtOAc), **22** (0.81 g, 54%); $[\alpha]_D^{20}$ 8.2° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 3 H, *J* 6.5 Hz, CH_3), 1.10–1.75 (m, 64 H, 27 alkyl CH_2, 3 BnOCHCH_2CH$_2$, and 2 OCH$_2$CH_2), 2.02 (dd, 1 H) and 2.15–2.50 (m, 5 H) (3 BnOCHCH_2CO), 3.14 (m, 2 H), 3.3–3.05 (9 H), 4.20–4.60 (m, 7 H, 3 PhCH$_2$ and H-1), 5.08 (dd, 1 H, $J_{3,4} = J_{4,5} = 9$ Hz, H-4), 5.0 (m, 4 H, 2 POCH_2Ph), 5.07 (dd, 1 H $J_{3,4} = J_{4,5} = 9$ Hz, H-4), 5.26 (dd, 1 H, $J_{2,3} = J_{3,4} = 10$ Hz, H-3), 6.37 (d, 1 H, *J* 8 Hz, NH), 7.15–7.50 (m, 40 H, 40 Ph-H). Anal. Calcd for $C_{106}H_{144}NO_{15}P$: C, 74.75; H, 8.52; N, 0.82; Found: C, 74.51; H, 8.60; N, 0.89.

4-Phosphonoxybutyl 2-deoxy-2-[(R)-3-hydroxytetradecanamido]-3,4-di-O-[(R)-3-hydroxytetradecanoyl]-β-D-glucopyranoside monoTRIS salt. (**23**).—Compound **22** (0.876 g, 0.51 mmol) dissolved in ether (25 mL) was treated with 15 mL of HCO_2H. After 1 h, the reaction was worked up as described previously. Chromatography (3:2 toluene–EtOAc) afforded a syrup (0.589 g) which was dissolved in 4:1 THF–water (25 mL), and hydrogenated for 25 min over 10% palladium-on-charcoal (0.370 g). TRIS (0.049 g, 0.40 mmol) was added and the solution thus obtained was lyophilized. After filtration through a short Sephadex LH20 column and lyophilization, the residue was washed with ether to afford pure **23** (0.352 g, 61% from **22**). A small sample of the corresponding free acid **23a** was prepared by Bligh–Dyer extraction as described earlier; $[\alpha]_D^{20}$ −17° (*c* 0.5, 9:1 $CHCl_3$–MeOH); ^1H NMR (9:1 $CDCl_3$–CD_3OD + 1 drop of CF_3CO_2D): δ 0.88 (t, 9 H, *J* 6.5 Hz, 3

CH_3), 1.2–1.8 (m, 64 H, 27 alkyl CH_2, 3 OCHCH_2CH_2, and 2 OCH_2CH_2), 2.4–2.6 (m, 6 H, 3 HOCHCH_2CO), 4.62 (d, 1 H, J 8 Hz, H-1), 5.0 (dd, 1 H, $J_{3,4} = J_{4,5} = 9$ Hz, H-4), 5.59 (dd, 1 H, $J_{2,3} = J_{3,4} = 10$ Hz, H-3). Anal. Calcd for $C_{52}H_{100}NO_{15}P$: C, 61.82; H, 9.98; P, 3.07 Found: C, 61.84; H, 10.02; P, 3.19.

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