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SYNTHESIS OF 2-DEOXY-4-*O*-PHOSPHONO-3-*O*-TETRADECANOYL-2-[(3*R*)- AND (3*S*)-3-TETRADECANOYLOXYTETRADECANAMIDO]-D-GLUCOSE: A DIASTEREOISOMERIC PAIR OF 4-*O*-PHOSPHONO-D-GLUCOSAMINE DERIVATIVES (GLA-27) RELATED TO BACTERIAL LIPID A

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ABSTRACT

The diastereoisomeric, 4-*O*-phosphono-D-glucosamine derivatives named in the title have been synthesized, starting from benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside and (3*RS*)-3-hydroxytetradecanoic acid.

INTRODUCTION

In the course of a synthetic approach[1–3] designed to clarify the molecular requirements for manifestation of the biological activities of lipid A, which is the endotoxic principle of the bacterial lipopolysaccharide[4–11], it was found that a 4-*O*-phosphono-D-glucosamine derivative named GLA-27 (which corresponds to compound **15** in ref. 2) showed some distinct biological activities, such as *Limulus* amebocyte-lysate gelation, interferon- and tumor necrosis factor (TNF)-induction, and mitogenic and polyclonal B cell-activation activities[12,13]. This compound, however, did not exhibit significant pyrogenic activity, in contrast to lipid A, suggesting that it might be possible to disassociate the desired biological activities from the unwanted toxicity. Recent studies[14] on the structure–activity relationship of a variety of monosaccharide analogs related to GLA-27 showed that presence of the 2-*N*-(3-tetradecanoyloxytetradecanoyl), 3-*O*-tetradecanoyl, and 4-*O*-phosphono groups is essential for activity.

The lipid A of many Gram-negative bacteria, *e.g. Salmonella* species and *Escherichia coli,* has amide-bound (3*R*)-3-hydroxytetradecanoic acid[15,16] as a common and prominent constituent, but little is known about the relationship between its absolute configuration and the activity. It thus seemed important to elucidate the biological influence of the asymmetric carbon atom (C-3) of the 3-hydroxytetradecanoyl group. We now describe the synthesis of the title diastereoisomeric pair (GLA-27-*R* and GLA-27-*S*), which respectively carry the amide-bound (3*R*)- and (3*S*)-3-tetradecanoyloxytetradecanoyl group at N-2 of the D-glucosamine backbone.

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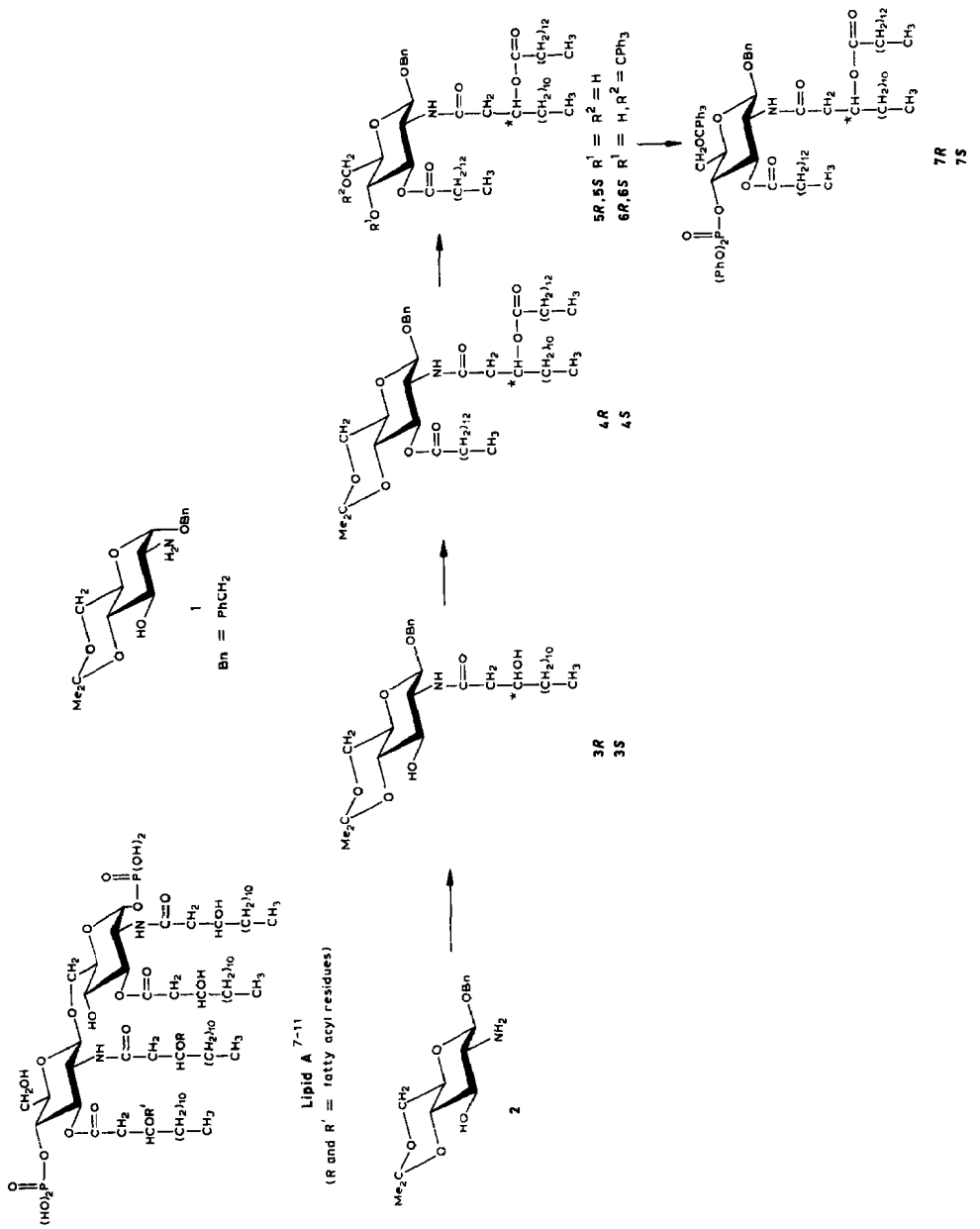


RESULTS AND DISCUSSION

The use of optically pure (3*R*)- and (3*S*)-3-hydroxytetradecanoic acid may provide the shortest route to the title compounds, but the chemical synthesis[17] of the two enantiomers in the laboratory has so far not proved facile. The (*R*) isomer

is readily obtainable by optical resolution with dehydroabietylamine[18] as described by Demary *et al.*[19], and the (*S*) isomer may be prepared from the mother liquor left after separation of dehydroabietylammonium (3*R*)-3-hydroxytetradecanoate. However, the optical purity (~80%) of the (*S*) isomer thus obtained was deemed unsatisfactory for our purposes.

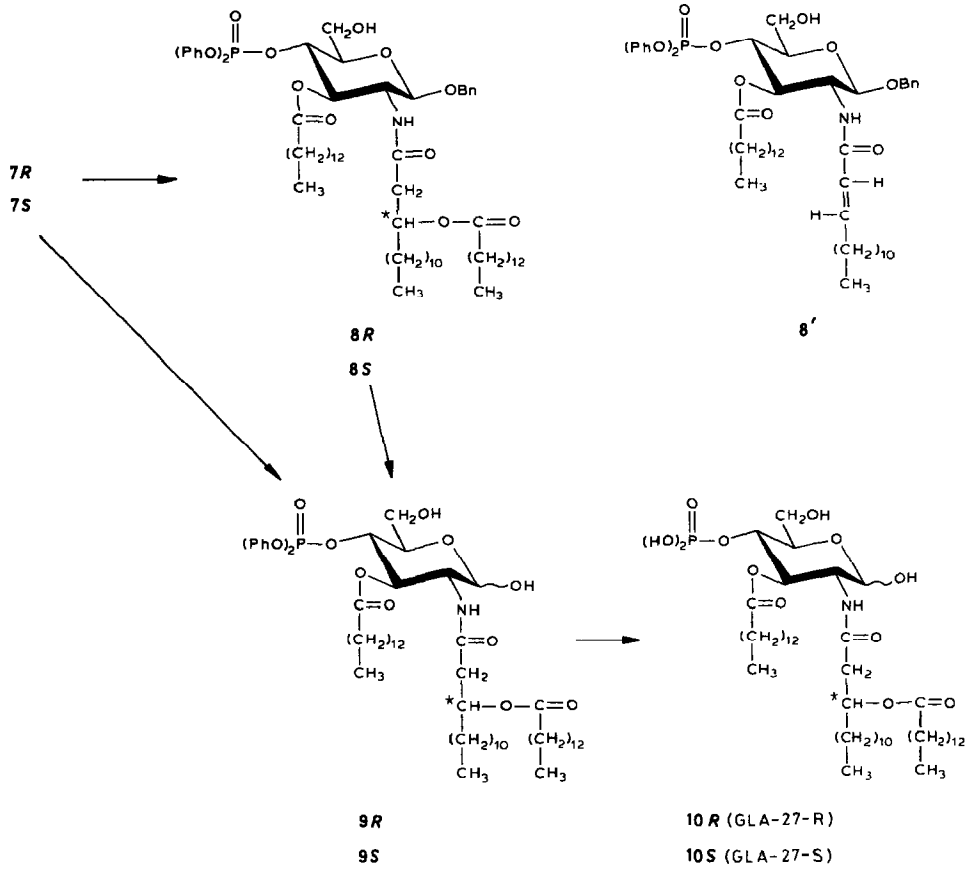
As described in a previous paper[1b], we have shown that both 2-deoxy-2-[(3*R*)- and (3*S*)-3-hydroxytetradecanamido]-D-glucose, which constitute a diastereoisomeric pair of the fundamental, monosaccharide skeleton of lipid A, could be prepared from the corresponding, protected intermediates purified by usual chromatography on a column of silica gel. As a result of our continued investigation, it has been found that the diastereoisomers **3*R*** and **3*S*** could also be simply separated by similar chromatographic resolution on silica gel. This procedure, therefore, seemed the most efficient for synthesis of the title compounds.

A variety of analogs of 4-*O*-phosphono-D-glucosamine has been synthesized by starting[1h,3] from benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-α- and -β-D-glucopyranoside (**1** and **2**). Treatment of **2** with (3*RS*)-3-hydroxytetradecanoic acid in the presence of dicyclohexylcarbodiimide (DCC) gave a mixture of benzyl 2-deoxy-2-[(3*R*)- and (3*S*)-3-hydroxytetradecanamido]-4,6-*O*-isopropylidene-β-D-glucopyranoside (**3*R*** and **3*S***) that showed different mobilities in t.l.c. [R_F 0.48 (**3*R***) and 0.46 (**3*S***) with 10:1 chloroform–methanol], and were readily separated by chromatography on a column of silica gel, conveniently under medium pressure; **3*R*** was eluted slightly faster than **3*S*** with 100:1 chloroform–methanol. The use of 5:1 ethyl acetate–hexane (R_F 0.35 (**3*R***) and 0.26 (**3*S***)] as the eluant gives better separation. Identification of **3*R*** and **3*S*** regarding the absolute configuration of the 3-hydroxytetradecanoyl group was achieved (*a*) directly by preparing **3*R*** from **2** and optically pure (3*R*)-3-hydroxytetradecanoic acid, and (*b*) indirectly by converting the diastereoisomers (**3*R*** and **3*S***) into the known benzyl 2-[(3*R*)- and (3*S*)-3-acetoxytetradecanamido]-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside[1b], respectively.

The remaining hydroxyl groups of **3*R*** and **3*S*** were simultaneously esterified with tetradecanoyl chloride in pyridine, to give crystalline **4*R*** and **4*S***, respectively, which were then treated with aqueous acetic acid to remove the 4,6-*O*-isopropylidene group. The resulting **5*R*** and **5*S*** were each tritylated, and then the diphenylphosphoryl group was introduced[1f,g,2,3,20] at the 4-hydroxyl group, to afford **7*R*** and **7*S***, respectively, in high yield.

It was found that, when hydrolytic removal of the trityl group of **7*R*** was conducted with aqueous acetic acid at 50°, benzyl 2-deoxy-4-*O*-(*O*,*O*-diphenylphosphono)-3-*O*-tetradecanoyl-2-[(*E*)-tetradec-2-enoylamido]-β-D-glucopyranoside (**8′**) was formed as a by-product (besides **8*R***). However, diethylaluminum chloride[21] or tetrafluoroboric acid[22], instead of acetic acid, caused selective detritylation, to give **8*R*** and **8*S*** in almost quantitative yields. These protected synthetic intermediates should be useful for further chemical modifications at C-6 and also at C-1.

Hydrogenolytic removal of the benzyl group of **8*R*** and **8*S***, or simultaneous



deprotection of both the benzyl and the trityl group of **7*R*** and **7*S*** in the presence of palladium catalyst gave **9*R*** and **9*S***, quantitatively. In their n.m.r. spectra, the anomeric protons appeared as doublets ($J_{1,2}$ 3–4 Hz after D_2O treatment) at δ 5.24 and 5.22, respectively, and the four axial protons (H-2–5) having large coupling constants ($J_{2,3}$ 10.6, $J_{3,4} = J_{4,5}$ = 9.5–10 Hz), were definitively assigned. This result indicated that the α-D-pyranose form of **9*R*** and **9*S*** preponderates in the equilibrium mixture in chloroform-*d*. Finally, the phenyl groups were cleaved by hydrogenolysis in the presence of pre-reduced Adams' platinum catalyst, to afford the desired, title compounds **10*R*** (GLA-27-R) and **10*S*** (GLA-27-S) as colorless powders. It may be noteworthy that the two compounds exhibit extremely different solubilities in organic solvents, particularly chloroform, in which **10*R*** is essentially insoluble.

The structure of GLA-27 is closely related to that of the nonreducing-sugar subunit of lipid A, whereas lipid X[23,24], which has been considered to activate both B-lymphocytes[25] and macrophages[26], corresponds to the reducing-sugar subunit. If it is true that both 4-*O*- and 1-*O*-phosphono-D-glucosamine derivatives can exhibit several biological activities related to those of lipid A, this would be of great

interest in the elucidation of the structural requirements for expression of the biological activity. The present study, expected to make a contribution to comprehensive understanding in this field, may provide new sources of nonpyrogenic, biological-response modifiers (BRM).

EXPERIMENTAL

General methods. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Specific rotations were determined with a Union PM-201 polarimeter, and i.r. spectra were recorded with a Jasco IRA-1 spectrophotometer. ^{1}H-N.m.r. spectra were recorded at 60, 90, and 270 MHz with Hitachi R-24B, R-22, and JEOL JNM-GX270 spectrometers, respectively. T.l.c. was performed on silica gel 60 (Merck, aluminum sheets), and column chromatography on silica gel (Wako Co.; 200 or 300 mesh) was accomplished with the solvent systems (v/v) specified.

*Benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-α-*D*-glucopyranoside* (**1**) *and benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-*D*-glucopyranoside* (**2**). — A mixture of benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside[27] or benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside[28] (10 g of each), water (150 mL), and barium hydroxide octahydrate (18 g) was stirred overnight under reflux, cooled, extracted with chloroform, and the extracts were combined, washed with water, dried, and evaporated. The residue crystallized from ether–hexane, to give **1** (8 g; 91%) and **2** 8.5 g; 97%), respectively.

Compound **1** had m.p. 137–138°, $[\alpha]_D$ +122° (*c* 0.9, chloroform); ν_{max}^{Nujol} 3700–3000 (OH, NH_2), 1590 (NH_2), 860 (CMe_2), and 770–700 cm^{-1} (Ph); n.m.r. data (60 MHz, $CDCl_3$): δ 1.41, 1.49 (2 s, 6 H, CMe_2), 4.40, 4.70 (2 d, 2 H, J_{gem} 12 Hz, C*H*$_2$Ph), 4.83 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), and 7.36 (s, 5 H, Ph).

Anal. Calc. for $C_{16}H_{23}NO_5$ (309.35): C, 62.12; H, 7.49; N, 4.53. Found: C, 62.08; H, 7.48; N, 4.42.

Compound **2** had m.p. 127.5–130°, $[\alpha]_D$ −95.5° (*c* 1.03, chloroform); ν_{max}^{KBr} 3600–3000 (OH, NH_2), 1580 (NH_2), 860 (CMe_2), and 750–690 cm^{-1} (Ph); n.m.r. data (60 MHz, $CDCl_3$): δ 1.43, 1.50 (2 s, 6 H, CMe_2), 4.33 (d, 1 H, J_{gem} 12 Hz, C*H*$_2$Ph), and 7.30 (s, 5 H, Ph).

Anal. Calc. for $C_{16}H_{23}NO_5$ (309.35): C, 62.12; H, 7.49; N, 4.53. Found: C, 62.34; H, 7.36; N, 4.39.

*Benzyl 2-deoxy-2-[(3R)-3-hydroxytetradecanamido]-4,6-*O*-isopropylidene-β-*D*-glucopyranoside* (**3R**) *and benzyl 2-deoxy-2-[(3S)-3-hydroxytetradecanamido]-4,6-*O*-isopropylidene-β-*D*-glucopyranoside* (**3S**). — To a solution of **2** (3 g) in dry dichloromethane (45 mL) were added (3*RS*)-3-hydroxytetradecanoic acid (2.37 g) and dicyclohexylcarbodiimide (DCC; 4 g), and the mixture was stirred for 1.5 h at room temperature. More (3*RS*)-3-hydroxytetradecanoic acid (0.5 g) was added, and stirring was continued for 1.5 h. DCC-urea was removed by filtration, and washed with a small amount of chloroform. The filtrate and washings were

combined, and evaporated to a residue which was chromatographed on a column of silica gel (Wakogel C-300) with 100:1 chloroform–methanol, to give **3*R*** (2.2 g), **3*R*** + **3*S*** (1.0 g), and **3*S*** (1.9 g) (total yield, 98%). The diastereoisomeric mixture is almost completely separable by re-chromatography. Compounds **3*R*** and **3*S*** were dried *in vacuo*, to give colorless solids.

Compound **3*R***: t.l.c., R_F 0.48 (10:1 chloroform–methanol), 0.35 (5:1 ethyl acetate–hexane); m.p. 99–102°, $[\alpha]_D$ −70° (*c* 0.664, chloroform); ν_{max}^{KBr} 3600–3100 (OH, NH), 1650, 1550 (amide), 860 (CMe_2), and 760–690 cm^{-1} (Ph); n.m.r. data (270 MHz, $CDCl_3$): δ 0.88 (t, 3 H, Me), 1.15–1.60 (m, 20 H, -CH_2-), 1.39, 1.50 (2 s, 6 H, CMe_2), 2.21, 2.30 (2 dd, 2 H, J_{gem} 15, $J_{2a',3'}$ 8.8, $J_{2b',3'}$ 3.3 Hz, -$COCH_2$-), 3.12 (m, 1 H, $J_{4,5}$ 9.5, $J_{5,6a}$ 9.9, $J_{5,6b}$ 5.5 Hz, H-5), 3.4 (~q, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 9.2, $J_{2,NH}$ 7 Hz, H-2), 3.58 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, H-3), 3.81 (~t, 1 H, J_{gem} 10.6, $J_{5,6a}$ 9.9 Hz, H-6a), 3.92 (m, 1 H, H-3'), 3.94 (dd, 1 H, J_{gem} 10.6, $J_{5,6b}$ 5.5 Hz, H-6b), 4.02 (~t, 1 H, $J_{3,4}$ 9.2, $J_{4,5}$ 9.5 Hz, H-4), 4.55, 4.86 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), 4.85 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 6.55 (d, 1 H, *J* 7 Hz, NH), and 7.25–7.45 (m, 5 H, Ph).

Anal. Calc. for $C_{30}H_{49}NO_7$ (535.73): C, 67.26; H, 9.22; N, 2.61. Found: C, 67.31; H, 9.18; N, 2.56.

Compound **3*S***: t.l.c., R_F 0.46 (10:1 chloroform–methanol), 0.26 (5:1 ethyl acetate–hexane); m.p. 131–131.5°, $[\alpha]_D$ −47.5° (*c* 0.569, chloroform); ν_{max}^{KBr} 3600–3100 (OH, NH), 1650, 1550 (amide), 860 (CMe_2), and 760–690 cm^{-1} (Ph); n.m.r. data (270 MHz, $CDCl_3$): δ 0.88 (t, 3 H, Me), 1.15–1.65 (m, 20 H, -CH_2-), 1.42, 1.51 (2 s, 6 H, CMe_2), 2.21, 2.35 (2 dd, 2 H, J_{gem} 15, $J_{2a',3'}$ 9.2, $J_{2b',3'}$ 2.7 Hz, -$COCH_2$-), 3.26 (m, 1 H, $J_{4,5}$ 9.5, $J_{5,6a}$ 9.9, $J_{5,6b}$ 5.5 Hz, H-5), 3.61 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9 Hz, H-3), 3.65–4.0 (m, 6 H, H-2,4,5,6a,b,3'), 4.54, 4.84 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), 4.60 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 6.59 (broad d, 1 H, NH), and 7.2–7.45 (m, 5 H, Ph); compound **3*S*** exhibits an extreme tendency to gel in $CDCl_3$, to give some unexpected peaks different from those of **3*R***.

Anal. Calc. for $C_{30}H_{49}NO_7$ (535.73): C, 67.26; H, 9.22; N, 2.61. Found: C, 67.36; H, 9.34; N, 2.73.

Identification of **3R** *and* **3S**. — *(a) Direct proof.* (3*R*)-3-Hydroxytetradecanoic acid was prepared by a slight modification of the optical-resolution procedure described by Gottstein and Cheney[18], and Demary *et al.*[19], as follows.

To a solution of dehydroabietylamine (Tokyo Kasei's CP; 5.9 g) in petroleum ether (150 mL) was added (3*RS*)-3-hydroxytetradecanoic acid (5.0 g) at 20°. After a short time, crystallization of the salt started, and diethyl ether (150 mL) was immediately added; the mixture was kept for 1 h at 20°, and the fine, colorless crystals were filtered off, washed with 1:1 petroleum ether–diethyl ether, and dried, to afford (*R*)-acid-rich dehydroabietylammonium salt (4.89 g; m.p. 117–120°). This salt was dissolved in methanol (70 mL), and then 1:1 petroleum ether–diethyl ether (80 mL) was added; the mixture was kept in a refrigerator at 0–4°, to give colorless plates (0.94 g; m.p. 128–129°) which were filtered off. The filtrate was evaporated to a solid which gave, from solution in the minimum volume

of methanol, colorless needles (1.6 g; m.p. 127–128°). The third crystallization from methanol gave similar needles (1.2 g; m.p. 126–127°). By treatment with sodium carbonate, the first crop of crystals (m.p. 128–129°) gave almost pure (3*R*)-3-hydroxytetradecanoic acid {0.4 g; $[\alpha]_D$ −15.5° (*c* 0.68, chloroform); lit.[15] −16° (*c* 2, chloroform)}. The second and third crops of crystals (2.8 g; m.p. 126–128°) were combined and treated with sodium carbonate, to afford ~95% optically pure (3*R*)-3-hydroxytetradecanoic acid {1.2 g; $[\alpha]_D$ −14.4° (*c* 1, chloroform)}.

The mother liquor obtained by filtration of the (*R*)-acid-rich dehydroabietylammonium salt (m.p. 117–120°) was concentrated, and the concentrate kept overnight at 0–4°, to give additional crystals (1.59 g; m.p. 101–106°). The filtrate gave ~80% optically pure (3*S*)-3-hydroxytetradecanoic acid {1.3 g; $[\alpha]_D$ +9.4° (*c* 0.557, chloroform)}.

The authentic sample of **3*R*** was prepared from **2** and optically pure (3*R*)-3-hydroxytetradecanoic acid as just described, and this sample showed exactly the same R_F value and other physical properties, including spectral data, as those found for the chromatographically purified **3*R***.

(b) Indirect proof. The chromatographically separated **3*R*** and **3*S*** were each treated with 90% aqueous acetic acid at 45°, and the products were acetylated with acetic anhydride–pyridine. The n.m.r. spectra of these acetylation products were identical with those of the previously reported benzyl 2-[(3*R*)- and (3*S*)-3-acetoxytetradecanamido]-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside[1b], respectively.

*Benzyl 2-deoxy-4,6-*O*-isopropylidene-3-*O*-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-*D*-glucopyranoside* (**4R**) *and benzyl 2-deoxy-4,6-*O*-isopropylidene-3-*O*-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]-β-*D*-glucopyranoside* (**4S**). — To a solution of **3*R*** (0.83 g) or **3*S*** (0.86 g) in 2:1 v/v dichloromethane–pyridine (9 mL) containing 4-dimethylaminopyridine (0.2 g) was added myristoyl chloride (2.4 mol equiv.), and the mixture was stirred overnight at room temperature. Methanol was added, and then the mixture was evaporated. The syrupy residue was dissolved in chloroform, and the solution was successively washed with ice-cold 2M hydrochloric acid and water, dried, and evaporated. The product was purified by chromatography on a column of silica gel, with chloroform as the eluant, and crystallized from ether–hexane to give **4*R*** (1.18 g; 80%) or **4*S*** (1.25 g; 85%).

Compound **4*R*** had m.p. 86–87°, $[\alpha]_D$ −32.1° (*c* 1.08, chloroform); ν_{max}^{Nujol} 3410 (NH), 1740 (ester), 1680, 1520 (amide), 860 (CMe_2), and 760–650 cm^{-1} (Ph); n.m.r. data (270 MHz, $CDCl_3$): δ 0.8–1.0 (~t, 9 H, Me), 1.1–1.7 (m, 64 H, -CH_2-), 1.35, 1.47 (2 s, 6 H, CMe_2), 2.15–2.5 (m, 6 H, -$COCH_2$-), 3.35 (m, 1 H, $J_{4,5}$ 9.5, $J_{5,6a}$ 10, $J_{5,6b}$ 5.5 Hz, H-5), 3.73 (~t, 1 H, $J_{3,4}$ 9.9, $J_{4,5}$ 9.5 Hz, H-4), 3.82 (~t, 1 H, J_{gem} 10.6, $J_{5,6a}$ 10 Hz, H-6a), 3.97 (dd, 1 H, J_{gem} 10.6, $J_{5,6b}$ 5.5 Hz, H-6b), 4.11 (~q, 1 H, $J_{1,2}$ 8.8, $J_{2,3}$ 9.9, $J_{2,NH}$ 9.5 Hz, H-2), 4.53 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.57, 4.86 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), 5.03 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.9 Hz, H-3), 4.97–5.13 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.91 (d, 1 H, *J* 9.5 Hz, NH), and 7.2–7.4 (m, 5 H, Ph).

Anal. Calc. for $C_{58}H_{101}NO_9$ (956.45): C, 72.84; H, 10.64; N, 1.46. Found: C, 72.75; H, 10:50; N, 1.34.

Compound **4*S*** had m.p. 80–81°, $[\alpha]_D$ −34.6° (*c* 0.847, chloroform); ν_{max}^{Nujol} 3380 (NH), 1740, 1720 (ester), 1670, 1520 (amide), 860 (CMe_2), and 760–690 cm^{-1} (Ph); n.m.r. data (270 MHz, $CDCl_3$): δ 0.8–1.0 (~t, 9 H, Me), 1.1–1.7 (m, 64 H, -CH_2-), 1.35, 1.47 (2 s, 6 H, CMe_2), 2.15–2.5 (m, 6 H, -$COCH_2$-), 3.37 (m, 1 H, $J_{4,5} = J_{5,6a}$ 10, $J_{5,6b}$ 5.5 Hz, H-5), 3.74 (~t, 1 H, $J_{3,4} = J_{4,5}$ = 10 Hz, H-4), 3.82 (~t, 1 H, J_{gem} 10.6, $J_{5,6a}$ 10 Hz, H-6a), 3.96 (dd, 1 H, J_{gem} 10.6, $J_{5,6b}$ 5.5 Hz, H-6b), 4.14 (~q, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 10, $J_{2,NH}$ 9.5 Hz, H-2), 4.53 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.56, 4.86 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), 5.06 (~t, 1 H, $J_{2,3} = J_{3,4}$ = 10 Hz, H-3), 4.98–5.15 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.97 (d, 1 H, $J_{2,NH}$ 9.5 Hz, NH), and 7.2–7.4 (m, 5 H, Ph).

Anal. Calc. for $C_{58}H_{101}NO_9$ (956.45): C, 72.84; H, 10.64; N, 1.46. Found: C, 72.91; H, 10.58; N, 1.51.

*Benzyl 2-deoxy-3-*O*-tetradecanoyl-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-β-*D*-glucopyranoside* (**5*R***). — A mixture of **4*R*** (0.98 g), chloroform (6.5 mL), and acetic acid (18 mL) was stirred at 45° while water (3.5 mL) was added dropwise; stirring was continued for 2 h at 45°, the mixture was evaporated at 45°, and the residue was chromatographed on a column of silica gel (Wakogel C-300) with 400:1 chloroform–methanol. The product crystallized from ether–hexane, to give **5*R*** (0.905 g; 96%), m.p. 134–135° (145–147° from methanol–hexane), $[\alpha]_D$ −21.4° (*c* 0.729, chloroform); ν_{max}^{KBr} 3450 (OH), 3300 (NH), 1740, 1710 (ester), 1660, 1560 (amide), and 760–690 cm^{-1} (Ph); n.m.r. data (270 MHz, $CDCl_3$): δ 0.8–1.0 (~t, 9 H, Me), 1.1–1.7 (m, 64 H, -CH_2-), 2.1–2.5 (m, 6 H, -$COCH_2$-), 2.62 (broad s, 2 H, OH), 3.42 (m, 1 H, H-5), 3.72 (~t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 3.81 (dd, 1 H, J_{gem} 12, $J_{5,6a}$ 4.4 Hz, H-6a), 3.91 (dd, 1 H, J_{gem} 12, $J_{5,6b}$ 3.1 Hz, H-6b), 3.95 (m, 1 H, H-2), 4.62, 4.84 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), 4.62 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.97–5.13 (m, 2 H, H-3 and H-3 of the 3-hydroxytetradecanoyl group), 6.07 (d, 1 H, *J* 9 Hz, NH), and 7.1–7.4 (m, 5 H, Ph).

Anal. Calc. for $C_{55}H_{97}NO_9$ (916.39): C, 72.09; H, 10.67; N, 1.53. Found: C, 72.28; H, 10.55; N, 1.49.

*Benzyl 2-deoxy-3-*O*-tetradecanoyl-2-[(3*S*)-3-tetradecanoyloxytetradecanamido]-β-*D*-glucopyranoside* (**5*S***). — A solution of **4*S*** (0.75 g) in chloroform (5 mL) and acetic acid (13.5 mL) was stirred at 45° while water (4 mL) was added dropwise; stirring was continued for 5 h at 45°, and the mixture was processed as described for **5*R***, to give amorphous **5*S*** (0.662 g; 92%), which was lyophilized from a solution in 1,4-dioxane to afford a colorless powder; m.p. 151–152.5°, $[\alpha]_D$ −23° (*c* 0.746, chloroform); ν_{max}^{KBr} 3440 (OH), 3275 (NH), 1730 (ester), 1650, 1560 (amide), and 760–690 cm^{-1} (Ph); n.m.r. data (270 MHz, $CDCl_3$): δ 0.8–1.0 (~t, 9 H, Me), 1.1–1.7 (m, 6 H, -CH_2-), 2.1–2.5 (m, 6 H, -$COCH_2$-), 2.82 (broad s, 2 H, OH), 3.42 (m, 1 H, H-5), 3.73 (~t, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 3.81 (dd, 1 H, J_{gem} 12, $J_{5,6a}$ 4.4 Hz, H-6a), 3.90 (dd, 1 H, J_{gem} 12, $J_{5,6b}$ 3 Hz, H-6b), 3.99 (~q, 1 H, H-2), 4.60, 4.85 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), 4.65 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1),

4.97–5.15 (m, 2 H, H-3 and H-3 of the 3-hydroxytetradecanoyl group), 6.18 (d, 1 H, *J* 9 Hz, NH), and 7.2–7.4 (m, 5 H, Ph).

Anal. Calc. for $C_{55}H_{97}NO_9$ (916.39): C, 72.09; H, 10.67; N, 1.53. Found: C, 72.21; H, 10.54; N, 1.63.

*Benzyl 2-deoxy-3-*O*-tetradecanoyl-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-6-*O*-trityl-β-*D*-glucopyranoside* (**6*R***). — A solution of **4*R*** (0.915 g) in dry pyridine (8 mL) was stirred at 90°, and then trityl chloride (0.43 g) was added; stirring was continued for 3 h at 90°, the mixture was cooled, and methanol was added in order to decompose the excess of the reagent; then, it was evaporated, and a solution of the residual syrup in chloroform was successively washed with ice-cold 2M hydrochloric acid, water, 10% sodium carbonate, and water, dried, and evaporated. The residue was chromatographed on a column of silica gel (Wakogel C-200) with chloroform, to give the title compound **6*R***, which crystallized from ethanol (0.99 g; 86%); m.p. 92–93.5°, $[\alpha]_D$ −25.5° (*c* 0.721, chloroform); ν_{max}^{KBr} 3500 (OH), 3300 (NH), 3150–3000 (Ph), 1740 (ester), 1660, 1560 (amide), and 790–690 cm^{-1} (Ph); n.m.r. data (90 MHz, $CDCl_3$): δ 0.8–1.0 (~t, 9 H, Me), 1.1–1.7 (m, 64 H, -CH_2-), 2.1–2.5 (m, 6 H, -$COCH_2$-), 2.7 (broad s, 1 H, OH), 4.63, 4.90 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), 4.54 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 5.95 (d, 1 H, *J* 9 Hz, NH), and 7.1–7.55 (m, 20 H, Ph).

Anal. Calc. for $C_{74}H_{111}NO_9$ (1158.71): C, 76.71; H, 9.65; N, 1.21. Found: C, 76.83; H, 9.57; N, 1.18.

*Benzyl 2-deoxy-3-*O*-tetradecanoyl-2-[(3*S*)-3-tetradecanoyloxytetradecanamido]-6-*O*-trityl-β-*D*-glucopyranoside* (**6*S***). — A solution of **5*S*** (0.661 g) in dry pyridine (5 mL) was stirred at 90°, while trityl chloride (0.31 g) was added, and the mixture was processed as described for **6*R***. The title compound crystallized from ethanol; m.p. 84–84.5°, $[\alpha]_D$ −25° (*c* 1.19, chloroform); ν_{max}^{KBr} 3470 (OH), 3290 (NH), 3150–3000 (Ph), 1740 (ester), 1650, 1560 (amide), and 790–690 cm^{-1} (Ph); n.m.r. data (90 MHz, $CDCl_3$): δ 0.8–1.0 (~t, 9 H, Me), 1.1–1.7 (m, 64 H, -CH_2-), 2.1–2.5 (m, 6 H, -$COCH_2$-), 4.54 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.63, 4.90 (2 d, 2 H, CH_2Ph), 6.1 (d, 1 H, NH), and 7.1–7.6 (m, 20 H, Ph).

Anal. Calc. for $C_{74}H_{111}NO_9$ (1158.71): C, 76.71; H, 9.65; N, 1.21. Found: C, 76.86; H, 9.72; N, 1.30.

*Benzyl 2-deoxy-4-*O*-(*O*,*O*-diphenylphosphono)-3-*O*-tetradecanoyl-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-6-*O*-trityl-β-*D*-glucopyranoside* (**7R**). — A mixture of **6*R*** (6.1 g), 4-dimethylaminopyridine (0.85 g) and 2:1 dichloromethane–pyridine (15 mL) was stirred at 0°, while diphenyl phosphorochloridate (3.3 g) was added; stirring was continued overnight at room temperature. Methanol was added, and then the mixture was evaporated to a syrup that was taken up in chloroform. The solution was successively washed with ice-cold 2M hydrochloric acid, water, 10% sodium carbonate, and water, dried, and evaporated to a syrup that was chromatographed on a column of silica gel (Wakogel C-300) with chloroform. Further impurities were removed by chromatography on a column of silica gel with 7:1 hexane–ethyl acetate as the eluant, and the resulting, amorphous

7*R* (5.6 g; 76.5%) was lyophilized from 1,4-dioxane solution to give a colorless powder; m.p. 61.5–63°, $[\alpha]_D$ −15.9° (*c* 1.2, chloroform); ν_{max}^{film} 3280 (NH), 1740 (ester), 1650, 1560 (amide), 950 (P-O-Ph), and 780–680 cm^{-1} (Ph); n.m.r. data (270 MHz, $CDCl_3$): δ 0.8–1.0 (~t, 9 H, Me), 1.0–1.75 (m, 64 H, -CH_2-), 1.98–2.5 (m, 6 H, -$COCH_2$-), 3.42 (dd, 1 H, J_{gem} 10.6, $J_{5,6a}$ 6.2 Hz, H-6a), 3.55–3.67 (m, 2 H, H-5,6b), 4.10 (~q, 1 H, *J* 8–10 Hz, H-2), 4.62 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.74 (~q, 1 H, $J_{3,4} \simeq J_{4,5} \simeq J_{4,P}$ = 9–10 Hz, H-4), 4.73, 4.97 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), 5.04 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.24 (~t, 1 H, *J* 10.6, 9.2 Hz, H-3), 5.83 (d, 1 H, *J* 8.8 Hz, NH), and 6.8–7.6 (m, 30 H, Ph).

Anal. Calc. for $C_{86}H_{120}NO_{12}P$ (1390.87): C, 74.27; H, 8.70; N, 1.01. Found: C, 74.42; H, 8.86; N, 1.10.

*Benzyl 2-deoxy-4-*O*-(*O,O*-diphenylphosphono)-3-*O*-tetradecanoyl-2-[(3*S*)-3-tetradecanoyloxytetradecanamido]-6-*O*-trityl-β-*D*-glucopyranoside* (**7S**). — Compound **6*S*** (6.24 g) was treated with diphenyl phosphorochloridate (3.82 g) in 2:1 dichloromethane–pyridine (15 mL) containing 4-dimethylaminopyridine (0.99 g) as just described for **7*R***. Chromatography on columns of silica gel with chloroform, and then with 7:1 hexane–ethyl acetate gave compound **7*S***, which was lyophilized from 1,4-dioxane solution to afford a colorless powder (6 g; 80%); m.p. 64.5–65°, $[\alpha]_D$ −10° (*c* 1.5, chloroform); ν_{max}^{film} 3280 (NH), 1740 (ester), 1650, 1560 (amide), 950 (P-O-Ph), and 780–680 cm^{-1} (Ph); n.m.r. data (270 MHz, $CDCl_3$): δ 0.8–1.0 (~t, 9 H, Me), 1.0–1.75 (m, 64 H, -CH_2-), 1.98–2.5 (m, 6 H, -$COCH_2$-), 3.42 (dd, 1 H, J_{gem} 10.6, $J_{5,6a}$ 6.2 Hz, H-6a), 3.55–3.75 (m, 2 H, H-5,6b), 4.14 (~q, 1 H, *J* 8–10 Hz, H-2), 4.62 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.75 (~q, 1 H, $J_{3,4} \simeq J_{4,5} \simeq J_{4,P}$ = 9–10 Hz, H-4), 4.72, 4.97 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), 5.05 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.27 (~t, 1 H, *J* 10.6, 9.2 Hz, H-3), 5.87 (d, 1 H, *J* 8.8 Hz, NH), and 6.8–7.6 (m, 30 H, Ph).

Anal. Calc. for $C_{86}H_{120}NO_{12}P$ (1390.87): C, 74.27; H, 8.70; N, 1.01. Found: C, 74.38; H, 8.64; N, 1.12.

*Benzyl 2-deoxy-4-*O*-(*O,O*-diphenylphosphono)-3-*O*-tetradecanoyl-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-β-*D*-glucopyranoside* (**8R**). — *(a) With aq. acetic acid.* A solution of **7*R*** (0.8 g) in acetic acid (10 mL) was stirred at 50°, while water (0.53 mL) was added dropwise. After completion of the reaction (t.l.c., 25:1 chloroform–methanol), the mixture was evaporated at 45° to a syrup that was chromatographed on a column of silica gel (Wakogel C-200) with 400:1 chloroform–methanol as the eluant, to give the title compound **8*R*** (0.49 g; 75%) and by-product **8′** (0.1 g; 15%). Compound **8*R*** crystallized from methanol; m.p. 84–84.5°, $[\alpha]_D$ −30.3° (*c* 1.40, chloroform); ν_{max}^{KBr} 3480 (OH), 3300 (NH), 1730 (ester), 1660, 1540 (amide), 960 (P-O-Ph), and 780–690 cm^{-1} (Ph); n.m.r. data (270 MHz, $CDCl_3$): δ 0.8–1.0 (~t, 9 H, Me), 1.1–1.7 (m, 64 H, -CH_2-), 1.99–2.48 (m, 6 H, -$COCH_2$-), 3.46 (~d, 1 H, $J_{4,5}$ 10, $J_{5,6a}$ 3, $J_{5,6b}$ 1.5 Hz, H-5), 3.60 (dd, 1 H, J_{gem} 13, $J_{5,6a}$ 3 Hz, H-6a), 3.73 (dd, 1 H, J_{gem} 13, $J_{5,6b}$ 1.5 Hz, H-6b), 3.93 (~q, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10, $J_{2,NH}$ 8.8 Hz, H-2), 4.60, 4.86 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), 4.74 (~q, 1 H, $J_{3,4} \simeq J_{4,5} \simeq J_{4,P}$ = 10 Hz, H-4), 4.75 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.99 (m, 1 H,

H-3 of the 3-hydroxytetradecanoyl group), 5.45 (~t, 1 H, $J_{2,3} \simeq J_{3,4}$ = 10 Hz, H-3), 5.89 (d, 1 H, *J* 8.8 Hz, NH), and 7.1–7.45 (m, 15 H, Ph).

Anal. Calc. for $C_{67}H_{106}NO_{12}P$ (1148.57): C, 70.07; H, 9.30; N, 1.22. Found: C, 70.25; H, 9.17; N, 1.18.

(b) With diethylaluminum chloride. A stirred solution of **7*R*** (0.834 g) in dichloromethane (10 mL) was cooled to 0°, and diethylaluminum chloride (Aldrich; 1.67 mL; 1.8M solution in toluene) in dichloromethane (5 mL) was added. The mixture was stirred for 5 min at 0° and carefully treated with IRA-45 (OH^-) ion-exchange resin accompanied by the addition of methanol (30 mL) under cooling. The resin was filtered off, and the filtrate was evaporated to a syrup that was chromatographed on a column of silica gel (Wakogel C-300) with 5:1 hexane–ethyl acetate. The product crystallized from methanol, to give needles of **8*R*** in 86% yield without the formation of **8′**. All of the physical properties and spectral data were identical with those of **8*R*** prepared by method (*a*).

*Benzyl 2-deoxy-4-*O*-(*O*,*O*-diphenylphosphono)-3-*O*-tetradecanoyl-2-[(*E*)-tetradec-2-enoylamido]-β-*D*-glucopyranoside* (**8′**). — The title compound was obtained as a by-product in the course of the preparation of **8*R*** as already described. It was a syrup, $[\alpha]_D$ −25° (*c* 0.924, chloroform); ν_{max}^{film} 3480 (OH), 3275 (NH), 1740 (ester), 1660, 1630, 1590, 1560 (C=C, amide), 960 (P-O-Ph), and 780–650 cm^{-1} (Ph); n.m.r. data (270 MHz, $CDCl_3$): δ 0.8–1.0 (~t, 6 H, Me), 1.0–1.6 (m, 40 H, $-CH_2-$), 1.9–2.3 (m, 4 H, $=CHCH_2-$, $-COCH_2-$), 3.47 (~d, 1 H, $J_{4,5}$ 9.5 Hz, H-5), 3.63 (dd, 1 H, J_{gem} 13, $J_{5,6a}$ 3.3 Hz, H-6a), 3.75 (~d, 1 H, J_{gem} 13 Hz, H-6b), 4.14 (~q, 1 H, H-2), 4.55–4.95 (m, 4 H, H-1,4, CH_2Ph), 5.40 (~t, 1 H, *J* ~10 Hz, H-3), 5.65 (~d, 1 H, J_{trans} 15.4, $-COCH=CH-$), 6.81 (m, 1 H, J_{trans} 15.4, $J_{vicinal}$ 7 Hz, $-COCH=CHCH_2-$), and 7.1–7.45 (m, 15 H, Ph).

Anal. Calc. for $C_{53}H_{78}NO_{10}P$ (920.18): C, 69.18; H, 8.54; N, 1.52. Found: C, 69.38; H, 8.36; N, 1.41.

*Benzyl 2-deoxy-4-*O*-(*O*,*O*-diphenylphosphono)-3-*O*-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]-β-*D*-glucopyranoside* (**8S**). — To a suspension of **7*S*** (0.333 g) in acetonitrile (6.7 mL) was added tetrafluoroboric acid (42% in water; 0.05 mL), and the mixture was stirred vigorously for 15 min at room temperature. Triethylamine (0.033 mL) was added, and the solvent was removed by evaporation. The residue was chromatographed on a column of silica gel with 5:2 hexane–ethyl acetate. The product crystallized from methanol, to afford needles of **8*S*** in 90% yield; m.p. 97–98.5°, $[\alpha]_D$ −29.5° (*c* 1.03, chloroform); ν_{max}^{KBr} 3480 (OH), 3300 (NH), 1730 (ester), 1660, 1540 (amide), 960 (P-O-Ph), and 780–690 cm^- (Ph); n.m.r. data (270 MHz, $CDCl_3$): δ 0.8–1.0 (~t, 9 H, Me), 1.0–1.7 (m, 64 H, $-CH_2-$), 2.0–2.5 (m, 6 H, $-COCH_2-$), 3.46 (~d, 1 H, $J_{4,5}$ 10 Hz, H-5), 3.61 (dd, 1 H, J_{gem} 13, $J_{5,6a}$ 3 Hz, H-6a), 3.73 (dd, 1 H, J_{gem} 13, $J_{5,6b}$ 1.5 Hz, H-6b), 3.96 (~q, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10, $J_{2,NH}$ 8.4 Hz, H-2), 4.60, 4.86 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), 4.75 (~q, 1 H, $J_{3,4} \simeq J_{4,5} \simeq J_{4,P}$ = 10 Hz, H-4), 4.74 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 5.03 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.47 (~t, 1 H, $J_{2,3} \simeq J_{3,4}$ = 10 Hz, H-3), 5.97 (d, 1 H, *J* 8.4 Hz, NH), and 7.1–7.45 (m, 15 H, Ph).

Anal. Calc. for $C_{67}H_{106}NO_{12}P$ (1148.57): C, 70.07; H, 9.30; N, 1.22. Found: C, 70.23; H, 9.20; N, 1.19.

2-Deoxy-4-O-(O,O-diphenylphosphono)-3-O-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-D-glucose (**9R**). — *(a) From* **7*R***. To a solution of **7*R*** (0.8 g) in 1:1 ethanol–methanol (60 mL) was added palladium-black catalyst prepared from palladium chloride (0.4 g), and the mixture was stirred overnight in a hydrogen atmosphere. The catalyst was filtered off, and washed with methanol. The filtrate and washings were combined, and evaporated to a syrup that was chromatographed on a column of silica gel (Wakogel C-200) with 150:1 chloroform–methanol, to give amorphous **9*R*** (0.48 g; 92%); $[\alpha]_D$ +3.2° (*c* 1.24, chloroform); ν_{max}^{film} 3600–3200 (OH, NH), 1740 (ester), 1650, 1570 (amide), 960 (P-O-Ph), and 800–650 cm^{-1} (Ph); n.m.r. data for the α anomer (270 MHz, $CDCl_3$): δ 0.8–1.0 (~t, 9 H, Me), 1.0–1.7 (m, 64 H, $-CH_2-$), 2.0–2.5 (m, 6 H, $-COCH_2-$), 3.38 (broad s, 1 H, OH-6; disappeared on treatment with D_2O), 3.5–3.7 (m, 2 H, H-6), 4.01 (~d, 1 H, $J_{4,5}$ ~10 Hz, H-5), 4.19 (d, 1 H, $J_{1,OH}$ 3–4 Hz, OH-1), 4.23 (m, 1 H, $J_{1,2}$ 3–4, $J_{2,3}$ 10.6, $J_{2,NH}$ 9.2 Hz, H-2; partially overlapping with OH-1), 4.75 (m, 1 H, $J_{3,4} \simeq J_{4,5} \simeq J_{4,P}$ 9.5 Hz, H-4), 5.09 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.24 (~t, 1 H, $J_{1,2} \simeq J_{1,OH}$ = 3–4 Hz, H-1; changed to doublet on treatment with D_2O), 5.48 (~t, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9.5 Hz, H-3), 6.10 (d, 1 H, *J* 9.2 Hz, NH), and 7.1–7.45 (m, 10 H, Ph).

Anal. Calc. for $C_{60}H_{100}NO_{12}P$ (1058.44): C, 68.09; H, 9.52; N, 1.32. Found: C, 67.84; H, 9.36; N, 1.23.

(b) From **8*R***. To a solution of **8*R*** (0.25 g) in methanol (20 mL) was added 10% palladium-on-carbon catalyst that had been pre-activated and washed well with ethanol; the mixture was stirred overnight in a hydrogen atmosphere. The catalyst was filtered off, and washed with hot methanol, and the filtrate and washings were combined, and evaporated. The residual syrup was chromatographed on a column of silica gel (Wakogel C-200) with 200:1 chloroform–methanol, to give **9*R*** (0.21 g; 93%), which was identical with that obtained from **7*R***.

2-Deoxy-4-O-(O,O-diphenylphosphono)-3-O-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]-D-glucose (**9S**). — Compound **9*S*** was prepared in 95% yield from **8*S*** by the procedure just described for **9*R***; amorphous; $[\alpha]_D$ +2.3° (*c* 0.738, chloroform); ν_{max}^{film} 3600–3200 (OH, NH), 1740 (ester), 1660, 1550 (amide), 960 (P-O-Ph), and 800–650 cm^{-1} (Ph); n.m.r. data for the α anomer (270 MHz, $CDCl_3$): δ 0.8–1.0 (~t, 9 H, Me), 1.0–1.7 (m, 64 H, $-CH_2-$), 1.98–2.5 (m, 6 H, $-COCH_2-$), 3.31 (broad t, 1 H, *J* 7 Hz, OH-6), 3.5–3.7 (m, 2 H, H-6), 4.02 (~d, 1 H, $J_{4,5}$ ~10 Hz, H-5), 4.19 (d, 1 H, *J* 3–4 Hz, OH-1), 4.21 (m, 1 H, H-2; partly overlapping with OH-1), 4.75 (m, 1 H, $J_{3,4} \simeq J_{4,5} \simeq J_{4,P} \simeq$ 9.5 Hz, H-4), 5.10 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.22 (~t, 1 H, $J_{1,2} \simeq J_{1,OH}$ 3–4 Hz, H-1), 5.49 (~t, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9.5 Hz, H-3), 6.16 (d, 1 H, *J* 8.8 Hz, NH), and 7.1–7.45 (m, 10 H, Ph).

Anal. Calc. for $C_{60}H_{100}NO_{12}P$ (1058.44): C, 68.09; H, 9.52; N, 1.32. Found: C, 68.27; H, 9.68; N, 1.41.

*2-Deoxy-4-*O*-phosphono-3-*O*-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]*-D-*glucose* (**10R**; *GLA-27*-R) *and 2-deoxy-4-*O*-phosphono-3-*O*-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]*-D-*glucose* (**10S**; *GLA-27*-S). — Platinum dioxide (0.1 g) wa suspended in ethanol, and hydrogen was bubbled through for 15 min, while the solution was stirred at room temperature. The resulting precipitate was filtered off, washed with ethanol, and added to a solution of **9*R*** (0.14 g) or **9*S*** (0.17 g) in methanol (20 mL). Hydrogen was gently bubbled through for 1 h, with stirring, and the mixture was further stirred overnight under hydrogen. The product partly gave a white precipitate that was completely dissolved by gentle heating, and then the catalyst was filtered off, and washed well with hot methanol; the filtrate and washings were combined, and evaporated. The resulting, amorphous **10*R*** or **10*S*** gave a positive test with the specific spray-reagent[29] for the phosphono group; it was homogeneously suspended in 1,4-dioxane (sonication often necessary), and lyophilized, to give a colorless, fine powder.

Compound **10*R*** (GLA-27-*R*; 0.11 g, 95%) was essentially insoluble in chloroform, ether, methanol, and other usual organic solvents; m.p. 142–143°, $[\alpha]_D$ +20.6° (*c* 0.102, dimethyl sulfoxide); ν_{max}^{KBr} 3600–3200 (OH, NH), 1740 (ester), 1640, 1560 (amide), and complete loss of the peaks at 960 (P-O-Ph) and 800–650 cm^{-1} (Ph).

Anal. Calc. for $C_{48}H_{92}NO_{12}P$ (906.24): C, 63.62; H, 10.23; N, 1.55. Found: C, 63.35; H, 10.42; N, 1.38.

Compound **10*S*** (GLA-27-*S*; 0.14 g; 96%) was soluble in organic solvents, particularly chloroform; m.p. 130–131°, $[\alpha]_D$ +13° (*c* 0.797, chloroform); ν_{max}^{KBr} 3600–3200 (OH, NH), 1730 (ester), 1650, 1550 (amide), and complete loss of the peaks at 960 (P-O-Ph) and 800–650 cm^{-1} (Ph); n.m.r. data (270 MHz, $CDCl_3$ containing five drops of methanol-d_4): δ 0.8–1.0 (~t, 9 H, Me), 1.0–1.4, 1.4–1.7 (m, 64 H, $-CH_2-$), 2.2–2.5 (m, 6 H, $-COCH_2-$), 3.5–4.4 (m, 5 H, H-2,4,5,6a,b), 5.0–5.2 (m, 2 H, H-1, H-3 of the 3-hydroxytetradecanoyl group), 5.52–5.5 (m, 1 H, H-3), and complete loss of the peaks at 7.1–7.45.

Anal. Calc. for $C_{48}H_{92}NO_{12}P$ (906.24): C, 63.62; H, 10.23; N, 1.55. Found: C, 63.33; H, 10.02; N, 1.41.

Because the n.m.r. spectrum of **10*S***, just described, showed some complexity (broadening, overlapping, *etc.*), the phosphoric group at O-4 was esterified with diazomethane in ether solution, to give the corresponding 2-deoxy-4-*O*-(*O*,*O*-dimethylphosphono)-3-*O*-tetradecanoyl-2-[(3*S*)-3-tetradecanoyloxytetradecanamido]-D-glucose in quantitative yield; n.m.r. data (270 MHz, $CDCl_3$): δ 0.8–1.0 (~t, 9 H, Me), 1.0–1.7 (m, 64 H, $-CH_2-$), 2.2–2.5 (m, 6 H, $-COCH_2-$), 3.74, 3.75, 3.78, 3.79 [2 d, 6 H, P-$(OMe)_2$; these methyl groups are magnetically non-equivalent, and each signal appeared, coupled with phosphorus, as a doublet. Similar splitting of methyl signals was observed also[24a] for the 1-phosphate of lipid X], 4.03 (~d, 1 H, $J_{4,5}$ 9–10 Hz, H-5), 4.20 (m, 1 H, H-2), 4.46 (~q, 1 H, $J_{3,4} \simeq J_{4,5} \simeq J_{4,P}$ = 9.5 Hz, H-4), 5.10 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.22 (d, 1 H, $J_{1,2}$ 3 Hz, H-1), 5.36 (~t, 1 H, *J* ~10 Hz, H-3), and 6.14 (d, 1 H, *J* 9 Hz, NH).

REFERENCES

1 (a) M. KISO, H. NISHIGUCHI, AND A. HASEGAWA, *Carbohydr. Res.*, 81 (1980) c13–c15; (b) M. KISO, H. NISHIGUCHI, S. MURASE, AND A. HASEGAWA, *ibid.*, 88 (1981) c5–c9; (c) M. KISO, H. NISHIGUCHI, K. NISHIHORI, A. HASEGAWA, AND I. MIURA, *ibid.*, 88 (1981) c10–c13; (d) M. KISO, K. NISHIHORI, AND A. HASEGAWA, *Agric. Biol. Chem.*, 45 (1981) 545–548; (e) M. KISO, K. NISHIHORI, A. HASEGAWA, H. OKUMURA, AND I. AZUMA, *Carbohydr. Res.*, 95 (1981) c5–c8; (f) M. KISO AND A. HASEGAWA, in L. ANDERSON AND F. M. UNGER (Eds.), *Bacterial Lipopolysaccharides: Structure, Synthesis, and Biological Activities, ACS Symp. Ser.*, 231 (1983) 277–300; (g) M. KISO AND A. HASEGAWA, in J. Y. HOMMA, S. KANEGASAKI, O. LÜDERITZ, T. SHIBA, AND O. WESTPHAL (Eds.), *Bacterial Endotoxin: Chemical, Biological and Clinical Aspects*, Verlag Chemie, Weinheim, 1984, pp. 39–49; (h) T. KITO, H. ISHIDA, Y. FUJISHIMA, S. TANAKA, M. TANAHASHI, M. KISO, AND A. HASEGAWA, *Abstr. Jpn. Carbohydr. Symp., 7th*, (1984) 92–93.
2 M. KISO, H. ISHIDA, AND A. HASEGAWA, *Agric. Biol. Chem.*, 48 (1984) 251–252.
3 M. KISO, H. ISHIDA, T. KITO, S. TANAKA, AND A. HASEGAWA, *Gifu Daigaku Nogakubu Kenkyu Hokoku*, 49 (1984) 171–181.
4 C. GALANOS, O. LÜDERITZ, E. T. RIETSCHEL, AND O. WESTPHAL, in T. W. GOODWIN (Ed.), *Biochemistry of Lipid II, Int. Rev. Biochem.*, 14 (1977) 239–335.
5 O. LÜDERITZ, C. GALANOS, V. LEHMANN, H. MAYER, E. T. RIETSCHEL, AND J. WECKESSER, *Naturwissenschaften*, 65 (1978) 578–585.
6 M. R. ROSNER, J.-Y. TANG, I. BARZILAY, AND H. G. KHORANA, *J. Biol. Chem.*, 254 (1979) 5906–5917.
7 (a) K. TAKAYAMA, N. QURESHI, AND P. MASCAGNI, *J. Biol. Chem.*, 258 (1983) 12,801–12,803; (b) N. QURESHI, K. TAKAYAMA, D. HELLER, AND C. FENSELAU, *ibid.*, 258 (1983) 12,947–12,951.
8 Z. SIDORCZYK, U. ZÄHRINGER, AND E. T. RIETSCHEL, *Eur. J. Biochem.*, 137 (1983) 15–22.
9 S. M. STRAIN, S. W. FESIK, AND I. M. ARMITAGE, *J. Biol. Chem.*, 258 (1983) 2906–2910.
10 (a) M. IMOTO, S. KUSUMOTO, T. SHIBA, H. NAOKI, T. IWASHITA, E. T. RIETSCHEL, H.-W. WOLLENWEBER, C. GALANOS, AND O. LÜDERITZ, *Tetrahedron Lett.*, (1983) 4017–4020; (b) M. IMOTO, S. KUSUMOTO, T. SHIBA, E. T. RIETSCHEL, C. GALANOS, AND O. LÜDERITZ, *ibid.*, (1985) 907–908.
11 M. IMOTO, H. YOSHIMURA, N. SAKAGUCHI, S. KUSUMOTO, AND T. SHIBA, *Tetrahedron Lett.*, (1985) 1545–1548.
12 M. MATSUURA, Y. KOJIMA, J. Y. HOMMA, Y. KUBOTA, A. YAMAMOTO, M. KISO, AND A. HASEGAWA, *FEBS Lett.*, 167 (1984) 226–230.
13 Y. KUMAZAWA, M. MATSUURA, J. Y. HOMMA, Y. NAKATSURU, M. KISO, AND A. HASEGAWA, *Eur. J. Immunol.*, 15 (1985) 199–201.
14 M. MATSUURA, A. YAMAMOTO, Y. KOJIMA, J. Y. HOMMA, M. KISO, AND A. HASEGAWA, *J. Biochem. (Tokyo)*, 98 (1985) 1229–1237.
15 M. IKAWA, J. B. KOEPFLI, S. G. MUDD, AND C. NIEMANN, *J. Am. Chem. Soc.*, 75 (1953) 1035–1038.
16 E. T. RIETSCHEL, *Eur. J. Biochem.*, 64 (1976) 423–428.
17 A. TAI, M. NAKAHATA, T. HARADA, Y. IZUMI, S. KUSUMOTO, M. INAGE, AND T. SHIBA, *Chem. Lett.*, (1981) 1125–1126.
18 W. J. GOTTSTEIN AND L. C. CHENEY, *J. Org. Chem.*, 30 (1965) 2072–2073.
19 M. DEMARY, G. PUZO, AND J. ASSELINEAU, *Nouv. J. Chim.*, 2 (1978) 373–378.
20 (a) P. SZABÓ, S. R. SARFATI, C. DIOLEZ, AND L. SZABÓ, *Carbohydr. Res.*, 111 (1983) c9–c12; (b) C. DIOLEZ, M. MONDANGE, S. R. SARFATI, L. SZABÓ, AND P. SZABÓ, *J. Chem. Soc., Perkin Trans. 1*, (1984) 275–280.
21 H. KÖSTER AND N. D. SINHA, *Tetrahedron Lett.*, (1982) 2641–2644.
22 R. ALBERT, K. DAX, R. PLESCHKO, AND A. E. STÜTZ, *Carbohydr. Res.*, 137 (1985) 282–290.
23 (a) M. NISHIJIMA AND C. R. H. RAETZ, *J. Biol. Chem.*, 254 (1979) 7837–7844, 256 (1981) 10,690–10,696; (b) M. NISHIJIMA, C. E. BULAWA, AND C. R. H. RAETZ, *J. Bacteriol.*, 145 (1981) 113–121.
24 (a) K. TAKAYAMA, N. QURESHI, P. MASCAGNI, M. A. NASHED, L. ANDERSON, AND C. R. H. RAETZ, *J. Biol. Chem.*, 258 (1983) 7379–7385; (b) K. TAKAYAMA, N. QURESHI, P. MASCAGNI, L. ANDERSON, AND C. R. H. RAETZ, *ibid.*, 258 (1983) 14,245–14,252.
25 C. R. H. RAETZ, S. PURCELL, AND K. TAKAYAMA, *Proc. Natl. Acad. Sci. U.S.A.*, 80 (1983) 4624–4628.
26 M. NISHIJIMA, F. AMANO, Y. AKAMATSU, K. AKAGAWA, T. TOKUNAGA, AND C. R. H. RAETZ, *Proc. Natl. Acad. Sci. U.S.A.*, 82 (1985) 282–286.
27 A. HASEGAWA, Y. KANEDA, Y. GOH, K. NISHIHORI, AND M. KISO, *Carbohydr. Res.*, 94 (1981) 143–163.
28 A. HASEGAWA AND H. G. FLETCHER, JR., *Carbohydr. Res.*, 29 (1973) 209–222.
29 J. C. DITTMER AND R. J. LESTER, *J. Lipid Res.*, 5 (1964) 126–127.