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SYNTHESIS OF THE OPTICALLY ACTIVE 4-*O*-PHOSPHONO-D-GLUCOSAMINE DERIVATIVES RELATED TO THE NONREDUCING-SUGAR SUBUNIT OF BACTERIAL LIPID A

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ABSTRACT

The optically active lipid A-subunit homologs named GLA-46, GLA-47, GLA-59, and GLA-60 have been synthesized stepwise by successive acylation at N-2 and O-3 of benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside with the 3-*O*-(benzyloxy)methyl or 3-*O*-tetradecanoyl derivative of optically active 3-hydroxytetradecanoic acid, and phosphorylation at O-4 of the D-glucosamine residue.

INTRODUCTION

In a series of investigations[1] on the relationship between the molecular structure and the biological activity of the nonreducing-sugar subunit analogs of lipid A, which has been proved to be the active center of the biological activities of bacterial endotoxins[2], it was demonstrated[1c–g] that several kinds of biological activity of endotoxin can be expressed by some 4-*O*-phosphono-D-glucosamine derivatives, such as GLA-27 (refs. 1a and 3) and GLA-40 (ref. 4). Among the synthetic analogs, however, 2-deoxy-2-(3-hydroxytetradecanamido)-3-*O*-(3-hydroxytetradecanoyl)-4-*O*-phosphono-D-glucose (GLA-46) did not show detectable biological activity[1e,g], although the acyl groups bound at N-2 and O-3 are the same[5] as those in lipid X. 2-Deoxy-4-*O*-phosphono-2-(3-tetradecanoyloxytetradecanamido)-3-*O*-(3-tetradecanoyloxytetradecanoyl)-D-glucose (GLA-47) exhibited strong *Limulus* amebocyte-lysate gelation activity[1e,g]. In addition, a recent investigation[6] on the biological effect of the chiral center (C-3) of the 3-hydroxytetradecanoyl group in GLA-27 showed that the (*S*) isomer (GLA-27-*S*) had stronger mediator-inducing activities than the (*R*) isomer (GLA-27-*R*). On the other hand, the B cell activation was strong with GLA-27-*R* and weak with GLA-27-*S*. These results suggest that the biological activities can be selectively elicited by modifying the molecular structures of lipid A and its subunit analogs.

We describe here the synthesis of some optically active, 4-*O*-phosphono-D-glucosamine derivatives, which have two asymmetric carbon atoms in the lipophilic

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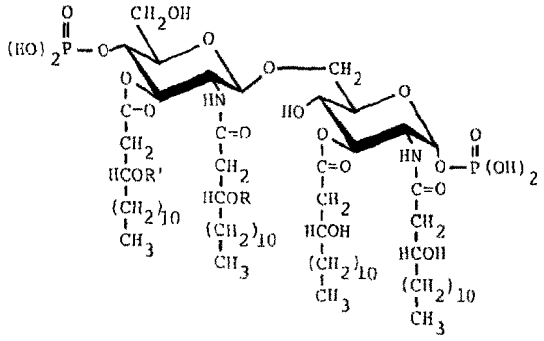
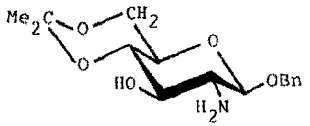


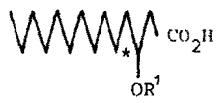
E. coli Lipid A

(R = lauroyl, R^1 = myristoyl)



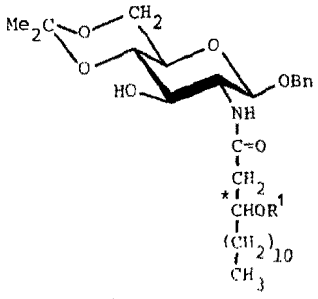
1

Bn = $PhCH_2$

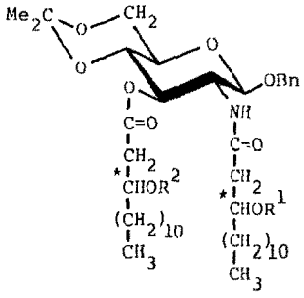


2*R*,2*S* R = Bom
3*R*,3*S* R = Myr

Bom = $PhCH_2OCH_2$
Myr = $CH_3(CH_2)_{12}CO$



4*R*,4*S* R^1 = Bom
5*R*,5*S* R^1 = Myr



	R^1	R^2
6*R*	Bom	Bom
7*R*	Myr	Myr
8*R*,8*S*	Myr	Bom
9*R*,9*S*	Bom	Myr

part, related to the nonreducing-sugar subunit of the bacterial lipid A (refs. 7 and 8).

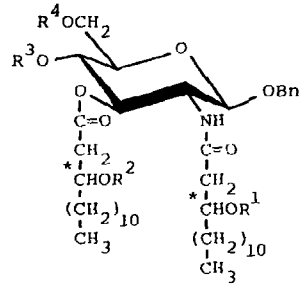
RESULTS AND DISCUSSION

Previously[3], we described a synthesis of the diastereoisomeric pair of GLA-27 by separating the corresponding, protected intermediates on a column of silica gel. This procedure is, however, not suitable for the synthesis of derivatives carrying the 3-hydroxytetradecanoyl group at both N-2 and O-3 of the D-glucosamine residue, because of the complexity of separating the four diastereomers. (*R*)-3-Hydroxytetradecanoic acid (>95% optically pure) is readily obtainable by optical resolution with dehydroabietylamine[9], and the (*S*) isomer (~80% optically pure) can be prepared from the mother liquor left after separation of dehydroabietylammonium (*R*)-3-hydroxytetradecanoate as described previously[3]. Therefore, in the present study, we first prepared two types of 3-*O*-protected 3-hydroxytetradecanoic acid, one being 3-(benzyloxymethoxy)tetradecanoic acid (**2R** and **2S**), and the other, 3-tetradecanoyloxytetradecanoic acid (**3R** and **3S**), and then condensed them with benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside[3] (**1**).

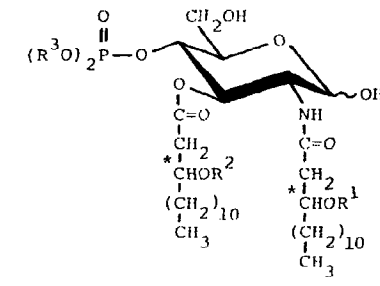
The 3-*O*-protected 3-hydroxytetradecanoic acids **2R, 2S, 3R**, and **3S** were prepared by the following successive reactions: (*1*) esterification of (3*R*)- or (3*S*)-3-hydroxytetradecanoic acid with phenacyl bromide, (*2*) benzyloxymethylation or tetradecanoylation of the OH-3 group, and (*3*) removal of the phenacyl group from the protected esters. All steps proceeded in >90% yield.

Compounds **2R, 2S, 3R**, and **3S** thus obtained were each treated with **1** in the presence of dicyclohexylcarbodiimide (DCC), to give the corresponding benzyl 2-(acylamido)-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosides (**4R, 4S, 5R**, and **5S**) in high yields, and these were esterified at O-3 of the sugar by treatment with **2R, 2S, 3R**, or **3S** in the presence of DCC and 4-(dimethylamino)pyridine (DMAP). The resulting **6R, 7R, 8R, 8S, 9R**, and **9S** were treated with aqueous acetic acid, to give **10R, 13R, 16R, 16S, 19R**, and **19S**, and the primary hydroxyl group at C-6 was tritylated. Introduction of the diphenylphosphono group at O-4 of **11R, 14R, 17R, 17S, 20R**, and **20S**, and successive hydrolytic removal of the trityl group with tetrafluoroboric acid[10] respectively afforded the useful synthetic intermediates **12R, 15R, 18R, 18S, 21R**, and **21S** in high yields.

Both the benzyl and the benzyloxymethyl groups were removed by hydrogenolysis with palladium-black catalyst, and the product was carefully purified by chromatography, to give **22R, 23R, 24R, 24S, 25R**, and **25S**, whose optical purity was judged to be >99% pure, based on n.m.r. analysis. Finally, the phenyl groups were cleaved by hydrogenolysis in the presence of pre-reduced Adams's platinum catalyst, to afford the desired compounds GLA-46 (*R,R*), GLA-47 (*R,R*), GLA-59 (*R,R*), GLA-59 (*S,S*), GLA-60 (*R,R*), and GLA-60 (*S,S*), respectively, as colorless powders.



	R^1	R^2	R^3	R^4
10*R*	Bom	Bom	H	H
11*R*	Bom	Bom	H	Tr
12*R*	Bom	Bom	$(PhO)_2PO$	H
13*R*	Myr	Myr	H	H
14*R*	Myr	Myr	H	Tr
15*R*	Myr	Myr	$(PhO)_2PO$	H
16*R*,16*S*	Myr	Bom	H	H
17*R*,17*S*	Myr	Bom	H	Tr
18*R*,18*S*	Myr	Bom	$(PhO)_2PO$	H
19*R*,19*S*	Bom	Myr	H	H
20*R*,20*S*	Bom	Myr	H	Tr
21*R*,21*S*	Bom	Myr	$(PhO)_2PO$	H



	R^1	R^2	R^3
22*R*	H	H	Ph
23*R*	Myr	Myr	Ph
24*R*, 24*S*	Myr	H	Ph
25*R*, 25*S*	H	Myr	Ph
GLA-46 *(R,R)*	H	H	H
GLA-47 *(R,R)*	Myr	Myr	H
GLA-59 *(R,R)*	Myr	H	H
GLA-59 *(S,S)*	Myr	H	H
GLA-60 *(R,R)*	H	Myr	H
GLA-60 *(S,S)*	H	Myr	H

Tr = Ph_3C

EXPERIMENTAL

General methods. — See ref. 3.

Preparation of (3R)- and (3S)-3-(benzyloxymethoxy)tetradecanoic acid (**2R** *and* **2S**), *and (3R)- and (3S)-3-tetradecanoyloxytetradecanoic acid* (**3R** *and* **3S**). — To a solution of (3*R*)- or (3*S*)-3-hydroxytetradecanoic acid[3] [2 g; optical purity >95% for (*R*) and ~80% for (*S*)] in ethyl acetate (100 mL) were added triethylamine (1.36 mL) and phenacyl bromide (1.95 g), and the mixture was stirred for 6 h at room temperature. The resulting precipitate was filtered off, and washed with ethyl acetate. The filtrate and washings were combined, and evaporated to a syrup that was chromatographed on a column of silica gel (Wakogel C-200) with 500:1 dichloromethane–methanol, to give the corresponding phenacyl esters in 90–95% yield. The (*R*) isomer had m.p. 72–73°, $[\alpha]_D$ −5.2° (*c* 1.0, chloroform).

To a cooled solution of each phenacyl ester (2.3 g) in 1:1 (v/v) dichloromethane–diisopropylethylamine (18.4 mL) was added dropwise benzyloxymethyl chloride (3.71 mL), and the mixture was stirred at room temperature. After completion of the reaction (t.l.c., 150:1 dichloromethane–methanol), methanol was added, and the mixture was evaporated *in vacuo*. The residue, after extractive processing, was chromatographed on a column of silica gel (Wakogel C-300) with 15:1 hexane–ethyl acetate, to afford (3*R*)- or (3*S*)-3-(benzyloxymethoxy)tetra-

decanoic acid phenacyl ester in nearly quantitative yield. The (*R*) isomer had m.p. 35°, $[\alpha]_D$ −7° (*c* 0.628, chloroform).

When the phenacyl ester of (3*R*)- or (3*S*)-3-hydroxytetradecanoic acid (2.5 g) in dry pyridine (27 mL) was treated with tetradecanoyl (myristoyl) chloride (2.05 g) in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP), the corresponding 3-tetradecanoyloxytetradecanoic acid phenacyl ester was obtained in ~90% yield. The (*R*) isomer had m.p. 37.5–38°, $[\alpha]_D$ +0.8° (*c* 0.863, chloroform).

Finally, the phenacyl esters of each 3-*O*-protected 3-hydroxytetradecanoic acid (3 g) in acetic acid (15 mL) was treated at 50° with zinc dust (4.3 g) added portionwise. The product was purified by chromatography on a column of silica gel (Wakogel C-300) with 10:1 hexane–ethyl acetate or dichloromethane as the eluant, to give **2R** and **2S**, or **3R** and **3S**, in almost quantitative yield; these compounds were lyophilized from 1,4-dioxane solutions, and stored in a refrigerator.

Compound **2R** was a syrup, $[\alpha]_D$ −6.7° (*c* 0.924, chloroform); ν_{max}^{film} 3600–2400 (CO_2H), 1720 (C=O), 1050 (ether), and 770–690 cm^{-1} (Ph).

Anal. Calc. for $C_{22}H_{36}O_4$ (364.51): C, 72.49; H, 9.96. Found: C, 72.30; H, 10.12.

Compound **2S** was a syrup, $[\alpha]_D$ +4.0° (*c* 1.34, chloroform); ν_{max}^{film} 3600–2400 (CO_2H), 1720 (C=O), 1050 (ether), and 770–690 cm^{-1} (Ph).

Anal. Calc. for $C_{22}H_{36}O_4$ (364.51): C, 72.49; H, 9.96. Found: C, 72.36; H, 9.89.

Compound **3R** had m.p. 38.5–40°, $[\alpha]_D$ −0.93° (*c* 1.40, chloroform); ν_{max}^{Nujol} 3600–2400 (CO_2H), and 1740 and 1715 cm^{-1} (C=O).

Anal. Calc. for $C_{28}H_{54}O_4$ (454.71): C, 73.95; H, 11.97. Found: C, 73.84; H, 12.00.

Compound **3S** had $[\alpha]_D$ +0.56° (*c* 0.924, chloroform); ν_{max}^{Nujol} 3600–2400 (CO_2H), and 1740 and 1710 cm^{-1} (C=O).

Anal. Calc. for $C_{28}H_{54}O_4$ (454.71): C, 73.95; H, 11.97. Found: C, 74.15; H, 11.88.

Benzyl 2-[(3R)- and (3S)-3-(benzyloxymethoxy)tetradecanamido]-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranoside (**4R** *and* **4S**). — To a solution of benzyl 4,6-*O*-isopropylidene-β-D-glucopyranoside (**1**) (1.6 g) in dry dichloromethane (12 mL) were added **2R** or **2S** (1.86 g) and DCC (1.62 g), and the mixture was stirred at room temperature. After completion of the reaction (t.l.c., 2:1 ethyl acetate–hexane), the dicyclohexylurea was filtered off, and washed with dichloromethane. The filtrate and washings were combined, and evaporated to a residue that was chromatographed on a column of silica gel (Wakogel C-200) with dichloromethane, to give **4R** (2.72 g; 80.2%) and **4S** (2.71 g; 84%), respectively, which were lyophilized from 1,4-dioxane solution.

Compound **4R** had m.p. 109–110°, $[\alpha]_D$ −56.5° (*c* 0.66, chloroform); ν_{max}^{Nujol} 3450 (OH), 3280 (NH), 1650, 1560 (amide), 860 (CMe_2), and 760–690 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (t, 3 H, CH_3), 1.0–1.7 (m, 20 H, $-CH_2-$), 1.44, 1.52 (2 s, 6 H, CCH_3), 2.35, 2.44 (2 dd, 2 H, J_{gem} 15, $J_{2',3'}$ 4 and 6–7 Hz,

–COCH_2–), 3.21 (m, 1 H, H-5), 3.55 (m, 1 H, H-2), 3.59 (t, 1 H, $J_{2,3} = J_{3,4}$ 9–10 Hz, H-3), 3.75–4.0 [m, 4 H, H-4, H-6*a*,*e*, and H-3 of the 3-(benzyloxymethoxy)-tetradecanoyl group], 4.4–4.9 (m, 6 H, –OCH_2O– and CH_2Ph), 6.43 (d, 1 H, N*H*), and 7.2–7.4 (m, 10 H, Ph).

Anal. Calc. for $C_{38}H_{57}NO_8$ (655.84): C, 69.59; H, 8.76; N, 2.14. Found: C, 69.72; H, 8.87; N, 2.09.

Compound **4S** had m.p. 67–70°, $[\alpha]_D$ −49.7° (*c* 0.561, chloroform); ν_{max}^{film} 3450 (OH), 3300 (NH), 1650, 1560 (amide), 860 (CMe_2), and 760–690 cm^{-1} (Ph); ^{1}H-n.m.r. data were similar to those of **4R**, with minor differences in the chemical shifts.

Anal. Calc. for $C_{38}H_{57}NO_8$ (655.84): C, 69.59; H, 8.76; N, 2.14. Found: C, 69.80; H, 8.91; N, 2.05.

*Benzyl 2-deoxy-4,6-O-isopropylidene-2-[(3R)- and (3S)-3-tetradecanoyloxy tetradecanamido]-β-*D*-glucopyranoside* (**5R** *and* **5S**). — To a solution of **1** (2 g) in dry dichloromethane (20 mL) were added **3R** or **3S** (3 g) and DCC (2.7 g). The mixture was stirred for 4.5 h at room temperature, and processed as described for **4R** or **4S**, to give **5R** (2.2 g; 91%) and **5S** (2.15 g; 89%), respectively.

Compound **5R** had m.p. 66–70°, $[\alpha]_D$ −49.3° (*c* 1.127, chloroform); ν_{max}^{Nujol} 3600–3100 (OH, NH), 1720 (ester), 1660, 1530 (amide), 860 (CMe_2), and 760–690 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (t, 6 H, CH_3), 1.0–1.45, 1.45–1.65 (m, 42 H, –CH_2–), 1.43, 1.52 (2 s, 6 H, CCH_3), 2.2–2.3, 2.35–2.5 (m, 4 H, –COCH_2–), 3.30 (m, 1 H, $J_{4,5} = J_{5,6a}$ = 9.5 Hz, H-5), 3.55 (m, 1 H, H-2), 3.60 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 3.82 (~t, 1 H, $J_{6a,6e}$ 10.6 Hz, H-6*a*), 3.88 (t, 1 H, H-4), 3.94 (dd, 1 H, H-6*e*), 4.57, 4.86 (2 d, 2 H, CH_2Ph), 4.70 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.07 (m, 1 H, H-3 of the 3-tetradecanoyloxytetradecanoyl group), 6.18 (d, 1 H, N*H*), and 7.32 (s, 5 H, Ph).

Anal. Calc. for $C_{44}H_{75}NO_8$ (746.05): C, 70.83; H, 10.13; N, 1.88. Found: C, 70.68; H, 9.99; N, 1.82.

Compound **5S** had m.p. 79–82°, $[\alpha]_D$ −44.9° (*c* 1.20, chloroform); ν_{max}^{Nujol} 3480 (OH), 3340 (NH), 1710 (ester), 1650, 1540 (amide), and 740–690 cm^{-1} (Ph); ^{1}H-n.m.r. data were similar to those of **5R**, with minor differences in the chemical shifts, such as, *e.g.*, δ 3.47 (m, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 9.2, $J_{2,NH}$ 6.6 Hz, H-2), 3.93 (t, 1 H, $J_{3,4} = J_{4,5}$ 9–9.6 Hz, H-4), 4.76 (d, 1 H, H-1), and 6.05 (d, 1 H, N*H*).

Anal. Calc. for $C_{44}H_{75}NO_8$ (746.05): C, 70.83; H, 10.13; N, 1.88. Found: C, 70.60; H, 10.23; N, 1.79.

*Benzyl 2-[(3R)-3-(benzyloxymethoxy)tetradecanamido]-3-O-[(3R)-3-(benzyloxymethoxy)tetradecanoyl]-2-deoxy-4,6-O-isopropylidene-β-*D*-glucopyranoside* (**6R**). — To a solution of **4R** (0.75 g) in dichloromethane (6 mL) were added **2R** (0.417 g), DCC (0.471 g), and DMAP (71 mg), and the mixture was stirred for 5 h at room temperature. The dicyclohexylurea was filtered off, and washed with dichloromethane. The filtrate and washings were combined, and evaporated to a syrup that was chromatographed on a column of silica gel (Wakogel C-300) with 10:1 hexane–ethyl acetate, to afford **6R** (1.13 g; 98.6%), which was lyophilized from a solution in 1,4-dioxane; m.p. 56–62°, $[\alpha]_D$ −22.4° (*c* 1.193, chloroform); ν_{max}^{Nujol} 3350 (NH), 1740 (ester), 1660, 1530 (amide), 860 (CMe_2), and 760–690 cm^{-1}

(Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (~t, 6 H, CH_3), 1.0–1.8 (m, 40 H, $-CH_2-$), 1.32, 1.42 (2 s, 6 H, CCH_3), 2.2–2.7 (m, 4 H, $-COCH_2-$), 3.88, 4.03 [2 m, 2 H, H-3 of the 3-(benzyloxymethoxy)tetradecanoyl group], 4.45–4.85 (10 d, 10 H, $-OCH_2O-$ and CH_2Ph), 4.99 (~t, 1 H, $J_{2,3}$ 10 Hz, H-3), and 7.2–7.4 (m, 15 H, Ph).

Anal. Calc. for $C_{60}H_{91}NO_{11}$ (1002.33): C, 71.89; H, 9.15; N, 1.40. Found: C, 71.68; H, 9.13; N, 1.43.

*Benzyl 2-deoxy-4,6-*O*-isopropylidene-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-*D*-glucopyranoside* (**7R**). — To a solution of **5R** (1.35 g) in dichloromethane (9 mL) were added **3R** (0.82 g), DCC (0.75 g), and DMAP (0.105 g), and the mixture was stirred at room temperature. After completion of the reaction (t.l.c., 1:1 hexane–ethyl acetate), the mixture was processed as described for **6R**, to give **7R** (1.73 g; 81%); m.p. 64–65°, $[\alpha]_D$ −23.1° (*c* 0.955, chloroform); ν_{max}^{Nujol} 3360 (NH), 1745, 1730 (ester), 1660, 1640 (amide), 860 (CMe_2), and 780–690 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (~t, 12 H, CH_3), 1.0–1.4, 1.4–1.85 (m, 84 H, $-CH_2-$), 1.36, 1.47 (2 s, 6 H, CCH_3), 2.1–2.7 (m, 8 H, $-COCH_2-$), 5.04, 5.15 (2 m, 2 H, H-3 of the 3-tetradecanoyloxytetradecanoyl group), and 5.06 (t, 1 H, H-3).

Anal. Calc. for $C_{72}H_{127}NO_{11}$ (1182.74): C, 73.11; H, 10.82; N, 1.18. Found: C, 73.38; H, 11.00; N, 1.24.

*Benzyl 3-*O*-[(3*R*)-3-(benzyloxymethoxy)tetradecanoyl]-2-deoxy-4,6-*O*-isopropylidene-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-β-*D*-glucopyranoside* (**8R**) *and benzyl 3-*O*-[(3*S*)-3-(benzyloxymethoxy)tetradecanoyl]-2-deoxy-4,6-*O*-isopropylidene-2-[(3*S*)-3-tetradecanoyloxytetradecanamido]-β-*D*-glucopyranoside* (**8S**). — Compound **5R** (0.75 g) in dichloromethane (6 mL) was esterified with **2R** (0.37 g) in the presence of DCC (0.44 g) and DMAP (71 mg) as described for **6R** and **7R**, to give **8R** in nearly quantitative yield; m.p. 71–72°, $[\alpha]_D$ −22° (*c* 0.91, chloroform); ν_{max}^{Nujol} 3350 (NH), 1730 (ester), 1655, 1520 (amide), 855 (CMe_2), and 760–680 cm^{-1} (Ph); ^{1}H-n.m.r. data: δ 0.88 (t, 9 H, CH_3), 1.0–1.8 (m, 62 H, $-CH_2-$), 2.15–2.7 (m, 6 H, $-COCH_2-$), 3.9–4.07 [m, 3 H, H-2, H-6*e*, and H-3 of the 3-(benzyloxymethoxy)tetradecanoyl group], 5.03 (m, 1 H, H-3 of the 3-tetradecanoyloxytetradecanoyl group), 5.07 (t, 1 H, H-3), and 7.2–7.4 (m, 10 H, Ph).

Anal. Calc. for $C_{66}H_{109}NO_{11}$ (1092.54): C, 72.55; H, 10.06; N, 1.28. Found: C, 72.76; H, 10.20; N, 1.31.

Treatment of **5S** with **2S**, as just described for **8R**, gave **8S** in nearly quantitative yield; m.p. 38–40°, $[\alpha]_D$ −32.1° (*c* 1.126, chloroform); ν_{max}^{film} 3300 (NH), 1740 (ester), 1660, 1560 (amide), 860 (CMe_2), and 760–690 cm^{-1} (Ph); ^{1}H-n.m.r. data were similar to those of **8R**, except for minor differences in the splitting patterns at δ 2.1–2.7 ($-COCH_2-$) and 4.5–4.9 ($-OCH_2O-$ and CH_2Ph).

*Benzyl 2-[(3*R*)-3-(benzyloxymethoxy)tetradecanamido]-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-*D*-glucopyranoside* (**9R**) *and benzyl 2-[(3*S*)-3-(benzyloxymethoxy)tetradecanamido]-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(3*S*)-3-tetradecanoyloxytetradecanoyl]-β-*D*-glucopyranoside* (**9S**). — To a solution of compound **4R** (0.7 g) in dichloromethane (6 mL) were added

3R (0.49 g), DCC (0.44 g), and DMAP (66 mg). The mixture was stirred for 5 h at room temperature, and processed as described for **7R**, to give in 92% yield **9R**, which crystallized from ethanol; m.p. 70–72°, $[\alpha]_D$ −24.6° (*c* 1.21, chloroform); ν_{max}^{Nujol} 1745, 1730 (ester), 1670, 1530 (amide), and 760–690 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (~t, 9 H, CH_3), 1.0–1.75 (m, 62 H, $-CH_2-$), 1.36, 1.46 (2 s, 6 H, CCH_3), 2.2–2.7 (m, 6 H, $-COCH_2-$), 3.84–3.96 [m, 2 H, H-6*e* and H-3 of the 3-(benzyloxymethoxy)tetradecanoyl group], 4.97 (~t, 1 H, $J_{2,3} = J_{3,4}$ ~10 Hz, H-3), and 5.16 (m, 1 H, H-3 of the 3-tetradecanoyloxytetradecanoyl group).

Anal. Calc. for $C_{66}H_{109}NO_{11}$ (1092.54): C, 72.55; H, 10.06; N, 1.28. Found: C, 72.31; H, 10.17; N, 1.26.

Compound **9S** was obtained in almost quantitative yield; m.p. 39–40°, $[\alpha]_D$ −30.1° (*c* 1.18, chloroform); ν_{max}^{film} 3300 (NH), 1750 (ester), 1660, 1540 (amide), 860 (CMe_2), and 760–690 cm^{-1} (Ph); ^{1}H-n.m.r. data were similar to those of **9R**, with minor differences in the splitting patterns at δ 2.2–2.7 ($-COCH_2-$) and 4.3–4.9 ($-OCH_2O-$ and CH_2Ph), and H-3 was observed at δ 5.0 as a triplet ($J_{2,3} = J_{3,4}$ 9–10 Hz).

Anal. Calc. for $C_{66}H_{109}NO_{11}$ (1092.54): C, 72.55; H, 10.06; N, 1.28. Found: C, 72.29; H, 9.90; N, 1.22.

Benzyl 2-[(3R)-3-(benzyloxymethoxy)tetradecanamido]-3-O-[(3R)-3-(benzyloxymethoxy)tetradecanoyl]-2-deoxy-4-O-(diphenylphosphono)-β-D-glucopyranoside (**12R**). — A mixture of **6R** (0.8 g), acetic acid (8 mL), and water (0.6 mL) was stirred for 3 h at 45°, and then evaporated. The residue was chromatographed on a column of silica gel (Wakogel C-200) with 100:1 dichloromethane–methanol, to give **10R** in 91% yield; m.p. 95–96°, $[\alpha]_D$ −42.1° (*c* 0.847, chloroform); ν_{max}^{Nujol} 3450 (OH), 3300 (NH), 1740 (ester), 1660, 1560 (amide), 760–690 (Ph), and complete loss of the peak at 860 cm^{-1} (CMe_2).

A solution of **10R** (0.6 g) in dry pyridine (6 mL) was stirred at 90° with trityl chloride (0.35 g), and stirring was continued for 5 h at 90°. The mixture was then cooled, methanol was added and evaporated, and the product was purified by chromatography on a column of silica gel (Wakogel C-200) with 500:1 dichloromethane–methanol, to give **11R** (0.66 g; 88%); m.p. 72–75°, $[\alpha]_D$ −33.7° (*c* 1.062, chloroform), ν_{max}^{Nujol} 3450 (OH), 3300 (NH), 1740 (ester), 1660, 1550 (amide), and 790–690 cm^{-1} (Ph).

Phosphorylation of **11R** (0.6 g) in dry 2:1 dichloromethane–pyridine (1.5 mL) with diphenyl phosphorochloridate (0.4 g) and DMAP (0.12 g) was accomplished by the method described in ref. 3, to give benzyl 2-[(3*R*)-3-(benzyloxymethoxy)-tetradecanamido-3-*O*-[(3*R*)-3-(benzyloxymethoxy)tetradecanoyl]-2-deoxy-4-*O*-(diphenylphosphono)-6-*O*-trityl-β-D-glucopyranoside in 75% yield [a mixture of **10R** and **11R** (~20%) was recovered], which was then treated, after chromatographic purification, with tetrafluoroboric acid in acetonitrile[10], to give **12R** in nearly quantitative yield; m.p. 87–88°, $[\alpha]_D$ −16.9° (*c* 0.629, chloroform), ν_{max}^{film} 3500 (OH), 3270 (NH), 3350–3000 (Ph), 1750 (ester), 1650, 1560 (amide), 960 (P–O–Ph), and 790–680 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 3.24 (~d, 1 H, $J_{4,5}$ 9.5 Hz,

H-5), 3.59, 3.70 (2 dd, 2 H, J_{gem} ~13 Hz, H-6,6'), 4.4–4.9 (m, 12 H, H-1, H-4, –OC*H*$_2$O– and C*H*$_2$Ph), 5.42 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 9.2 Hz, H-3), and 7.05–7.4 (m, 25 H, Ph).

Anal. Calc. for $C_{69}H_{96}NO_{14}P$ (1194.45): C, 69.38; H, 8.10; N, 1.17. Found: C, 69.61; H, 7.98; N, 1.12.

Benzyl 2-deoxy-4-O-(diphenylphosphono)-2-[(3R)-3-tetradecanoyloxytetradecanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (**15R**). — A mixture of **7R** (1.36 g), acetic acid (18 mL), and water (0.45 mL) was treated in the same manner, to give **13R** in 80% yield; m.p. 101–101.5°, $[\alpha]_D$ −16.8° (*c* 0.92, chloroform); ν_{max}^{Nujol} 3600–3200 (OH, NH), 1730 (ester), 1660, 1550 (amide), 760–690 (Ph), and complete loss of the peak at 860 cm^{-1} (CMe_2).

Compound **13R** (0.87 g) was treated with trityl chloride (0.425 g) in pyridine (10 mL) for 3.5 h, as described for **11R**, to give **14R** (1.01 g; 95.7%) {m.p. 93–97°, $[\alpha]_D$ −19.4° (*c* 1.322, chloroform)}, which was then phosphorylated with diphenyl phosphorochloridate as just described. The product was treated with tetrafluoroboric acid, to afford **15R** in 71% yield (2 steps); m.p. 92–93°, $[\alpha]_D$ −17.5° (*c* 1.10, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ3.08 (very broad t, 1 H, O*H*), 3.48 (~d, 1 H, $J_{4,5}$ ~10 Hz, H-5), 3.5–3.8 (m, 3 H, H-2,6,6'), 4.72 (q, 1 H, $J_{3,4} = J_{4,5} = J_{4,P}$ = 9–10 Hz, H-4), 5.50 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.56 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 9.2 Hz, H-3), and 7.1–7.4 (m, 15 H, Ph).

Anal. Calc. for $C_{81}H_{132}NO_{14}P$ (1374.86): C, 70.76; H, 9.68; N, 1.02. Found: C, 70.55; H, 9.76; N, 1.00.

Benzyl 3-O-[(3R)-3-(benzyloxymethoxy)tetradecanoyl]-2-deoxy-4-O-(diphenylphosphono)-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside (**18R**) *and benzyl 3-O-[(3S)-3-(benzyloxymethoxy)tetradecanoyl]-2-deoxy-4-O-(diphenylphosphono)-2-[(3S)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside* (**18S**). — Compound **8R** (0.87 g) was treated with 90% aqueous acetic acid (11 mL) at 45° as described for **10R** and **13R**, and the product crystallized from ether, to give **16R** (0.73 g; 87%); m.p. 100–101.5°, $[\alpha]_D$ −35.9° (*c* 0.754, chloroform); ν_{max}^{Nujol} 3480 (OH), 3280 (NH), 1730 (ester), 1660, 1570 (amide), 770–690 (Ph), and complete loss of the peak at 855 cm^{-1} (CMe_2). Tritylation of **16R** (0.68 g), and chromatographic purification of the product as described for **11R** and **14R**, gave **17R** {$[\alpha]_D$ −31.2° (*c* 0.902, chloroform)} in 93% yield.

4-*O*-Phosphorylation and detritylation of **17R** afforded, in 2 steps, **18R** (68%) {m.p. 69.5–70.5°, $[\alpha]_D$ −14.5° (*c* 0.724, chloroform)}; ν_{max}^{film} 3500 (OH), 3275 (NH), 3150–3000 (Ph), 1740 (ester), 1650, 1560 (amide), 960 (P–O–Ph), and 780–680 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 3.11 (broad s, 1 H, O*H*), 3.47 (~d, 1 H, $J_{4,5}$ 9.5 Hz, H-5), 4.72 (q, 1 H, $J_{3,4} = J_{4,5} = J_{4,P}$ = 9.5 Hz, H-4), 4.97 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), and 7.05–7.4 (m, 20 H, Ph).

Anal. Calc. for $C_{75}H_{114}NO_{14}P$ (1284.65): C, 70.12; H, 8.94; N, 1.09. Found: C, 69.83; H, 9.06; N, 1.14.

Hydrolytic removal of the isopropylidene group from **8S** was achieved as for **6R**, to give **16S** {m.p. 94–96°, $[\alpha]_D$ −14° (*c* 1.213, chloroform)}; ν_{max}^{film} 3460 (OH),

3300 (NH), 1740 (ester), 1660, 1560 (amide), and 770–700 cm^{-1} (Ph); tritylation of the primary hydroxyl group afforded **17S** {m.p. 70–72°, $[\alpha]_D$ −17° (*c* 0.87, chloroform)}. Introduction of the diphenylphosphono group at O-4, and detritylation, gave **18S** (66%, in 2 steps); m.p. 67–70°, $[\alpha]_D$ −16.3° (*c* 1.02, chloroform); ν_{max}^{film} 3480 (OH), 3300 (NH), 3150–3000 (Ph), 1740 (ester), 1650, 1550 (amide), 960 (P–O–Ph), and 780–680 cm^{-1} (Ph).

Benzyl 2-[(3R)-3-(benzyloxymethoxy)tetradecanamido]-2-deoxy-4-O-(diphenylphosphono)-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (**21R**) *and benzyl 2-[(3S)-3-(benzyloxymethoxy)tetradecanamido]-2-deoxy-4-O-(diphenylphosphono)-3-O-[(3S)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside* (**21S**). — Hydrolysis of the isopropylidene group from **9R** (0.7 g) was achieved in almost quantitative yield, to give **19R** {m.p. 115–116°, $[\alpha]_D$ −17.6° (*c* 0.981, chloroform)}, which was then tritylated. The resulting **20R** {m.p. 85–86°, $[\alpha]_D$ −19.1° (*c* 1.03, chloroform)} was treated with 5 mol. equiv. of diphenyl phosphorochloridate in pyridine, and the mixture was processed as described previously, to afford **21R** (70% in 3 steps); m.p. 82.5–83°, $[\alpha]_D$ −20.7° (*c* 0.637, chloroform); ν_{max}^{film} 3460 (OH), 3280 (NH), 3150–3000 (Ph), 1740 (ester), 1650, 1560 (amide), 960 (P–O–Ph), and 780–680 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 3.23 (~d, 1 H, $J_{4,5}$ 9.5 Hz, H-5), 3.58, 3.69 (2 dd, 2 H, J_{gem} 13 Hz, H-6,6'), 4.68 (~q, 1 H, $J_{3,4} = J_{4,5} = J_{4,P}$ 9–10 Hz, H-4), and 7.1–7.4 (m, 20 H, Ph).

Anal. Calc. for $C_{75}H_{114}NO_{14}P$ (1284.65): C, 70.12; H, 8.94; N, 1.09. Found: C, 70.39; H, 9.10; N, 1.08.

Compound **19S** was obtained from **9S**; m.p. 83–84°, $[\alpha]_D$ −21.4° (*c* 1.14, chloroform), and was converted into **21S** as previously decribed; m.p. 57–59°, $[\alpha]_D$ −23.6° (*c* 1.05, chloroform); ν_{max}^{film} 3470 (OH), 3300 (NH), 3150–3000 (Ph), 1750 (ester), 1650, 1560 (amide), 960 (P–O–Ph), and 780–680 cm^{-1} (Ph).

2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-3-O-[(3R)-3-hydroxytetradecanoyl]-4-O-phosphono-D-glucose [*GLA-46*(R,R)]. — To a solution of **12R** (0.154 g) in methanol (10 mL) was added palladium-black catalyst (80 mg), 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 residue that was chromatographed on a column of silica gel (Wakogel C-200) with 50:1 dichloromethane–methanol, to give **22R** (0.103 g; 92.5%); m.p. 88–89°, $[\alpha]_D$ −9.6° (*c* 1.04, chloroform); ν_{max}^{film} 3600–3100 (OH, NH), 1740 (ester), 1640, 1540 (amide), 960 (P–O–Ph), and 800–690 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$) for the α anomer: δ 0.8–0.95 (m, 6 H, CH_3), 1.0–1.5 (m, 40 H, $-CH_2-$), 2.05–2.35 (m, 4 H, $-COCH_2-$), 3.5–4.2 (m, 9 H, H-5,6,6', O*H*, and H-3 of the 3-hydroxytetradecanoyl group), 4.27 (m, 1 H, $J_{2,3} = J_{2,NH}$ = 9–10, $J_{1,2}$ 3.3 Hz, H-2), 4.85 (q, 1 H, $J_{3,4} = J_{4,5} = J_{4,P}$ = 9.2 Hz, H-4), 5.43 (~s, 1 H, H-1), 5.51 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9–10 Hz, H-3), 6.60 (d, 1 H, *J* 8.8 Hz, N*H*), and 7.05–7.4 (m, 10 H, Ph).

Anal. Calc. for $C_{46}H_{74}NO_{12}P$ (864.04): C, 63.94; H, 8.63; N, 1.62. Found: C, 63.72; H, 8.69; N, 1.58.

Finally, the phenyl groups of **22R** (0.1 g) were removed by hydrogenolysis in

the presence of pre-reduced Adams platinum catalyst (40 mg) in ethanol (12 mL) as reported previously[3], to give quantitatively the title compound GLA-46(*R,R*), which was lyophilized from 1,4-dioxane solution; m.p. 153–155°, $[\alpha]_D$ +8.5° (*c* 0.694, 3:1 chloroform–methanol); it was positive to the specific spray-reagent[11] for the phosphono group; ν_{max}^{KBr} 3680–2500 (OH, NH, and CH), 1730 (ester), 1650, 1560 (amide), and complete loss of the peaks at 960 (P–O–Ph) and 800–690 cm^{-1} (Ph).

Anal. Calc. for $C_{34}H_{66}NO_{12}P$ (711.86): C, 57.36; H, 9.35; N, 1.97. Found: C, 57.67; H, 9.55; N, 2.02.

*2-Deoxy-4-*O*-phosphono-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-*D*-glucose* [*GLA-47*(R,R)]. — Hydrogenolytic removal of the benzyl group from **15R** (0.11 g) was conducted as described for **22R**, to afford **23R** in nearly quantitative yield; m.p. 68–70°, $[\alpha]_D$ +4.2° (*c* 0.622, chloroform); ν_{max}^{film} 3600–3150 (OH, NH), 1740 (ester), 1660, 1540 (amide), 960 (P–O–Ph), and 800–670 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$) for a mixture of $\alpha:\beta$ = ~2:1: δ 0.75–0.95 (m, 12 H, CH_3), 1.0–1.7 (m, 84 H, $-CH_2-$), 2.1–2.5 (m, 8 H, $-COCH_2-$), 4.65–4.83 [2 q, 1 H, H-4(α,β)], 5.26 (β), 5.46 (α) [2 dd, 1 H, H-3(α,β)], 5.33 (d, ~2/3 H, H-1α), 6.29, 6.83 (2 d, 1 H, *J* 8.1 and 6.2 Hz, respectively, N*H*α,β), and 7.05–7.4 (m, 10 H, Ph).

Anal. Calc. for $C_{74}H_{126}NO_{14}P$ (1284.99): C, 69.16; H, 9.90; N, 1.90. Found: C, 69.35; H, 9.84; N, 1.12.

The phenyl groups of **23R** (0.6 g) were hydrogenolyzed in methanol by a way similar to that described for GLA-46(*R,R*), to give GLA-47(*R,R*); m.p. 152–153°, $[\alpha]_D$ +14° (*c* 0.52, 3:1 chloroform–methanol); ν_{max}^{KBr} 3680–2500 (OH, NH, and CH), 1740 (ester), 1660, 1560 (amide), and complete loss of the peaks at 960 (P–O–Ph) and 800–670 cm^{-1} (Ph).

Anal. Calc. for $C_{62}H_{118}NO_{14}P$ (1132.55): C, 65.75; H, 10.50; N, 1.24. Found: C, 65.39; H, 10.67; N, 1.18.

*2-Deoxy-3-*O*-[(3*R*)-3-hydroxytetradecanoyl]-4-*O*-phosphono-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-D-glucose* [*GLA-59*(R,R)] *and 2-deoxy-3-*O*-[(3*S*)-3-hydroxytetradecanoyl]-4-O-phosphono-2-[(3*S*)-3-tetradecanoyloxytetradecanamido]-D-glucose* [*GLA-59*(S,S)]. — Compound **18R** (0.158 g) was hydrogenolyzed in methanol as described for **22R**, and the product was purified by chromatography, to give **24R** (0.128 g; 97%); m.p. 87–88°, $[\alpha]_D$ −2.1° (*c* 1.17, chloroform); ν_{max}^{film} 3600–3150 (OH, NH), 1730 (ester), 1640, 1560 (amide), 960 (P–O–Ph), and 800–670 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$) for the α anomer: δ 0.75–0.95 (m, 9 H, CH_3), 1.0–1.7 (m, 62 H, $-CH_2-$), 2.1–2.5 (m, 6 H, $-COCH_2-$), 3.0–3.75 (m, 5 H, O*H* and H-6,6′), 3.90 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 4.05 (~d, 1 H, $J_{4,5}$ 9.5 Hz, H-5), 4.28 (m, 1 H, $J_{2,3} = J_{2,NH}$ = 9–10, $J_{1,2}$ 3.3 Hz, H-2), 4.76 (q, 1 H, $J_{3,4} = J_{4,5} = J_{4,P}$ = ~9.5 Hz, H-4), 5.09 (m, 1 H, H-3 of the 3-tetradecanoyloxytetradecanoyl group), 5.20 (d, 1 H, H-1), 5.5 (t, 1 H, *J* ~10 Hz, H-3), 6.40 (d, 1 H, *J* 9.2 Hz, N*H*), and 7.05–7.4 (m, 10 H, Ph).

Anal. Calc. for $C_{60}H_{100}NO_{13}P$ (1074.39): C, 67.07; H, 9.38; N, 1.30. Found: C, 67.32; H, 9.25; N, 1.37.

The (*S*) isomer **24S** was obtained from **18S** in nearly quantitative yield; m.p.

64.5–65°, $[\alpha]_D$ +9.5° (*c* 0.786, chloroform); ν_{max}^{film} 3600–3200 (OH, NH), 1740 (ester), 1660, 1540 (amide), 960 (P–O–Ph), and 800–680 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$) for the α anomer: δ 0.88 (~t, 9 H, CH_3), 1.0–1.7 (m, 62 H, $-CH_2-$), 2.1–2.5 (m, 6 H, $-COCH_2-$), 3.0–3.85 (m, 4 H, H-6,6′, OH and H-3 of the 3-hydroxytetradecanoyl group), 4.05 (~d, 1 H, $J_{4,5}$ ~10 Hz, H-5), 4.25 (m, 1 H, H-2), 4.77 (q, 1 H, $J_{3,4} = J_{4,5} = J_{4,P}$ 9–10 Hz, H-4), 4.93 (broad s, 1 H, O*H*), 5.05 (m, 1 H, H-3 of the 3-tetradecanoyloxytetradecanoyl group), 5.20 (broad d, 1 H, $J_{1,2}$ 3 Hz, H-1), 5.52 (~t, 1 H, H-3), 6.28 (d, 1 H, *J* 9.2 Hz, N*H*), and 7.05–7.4 (m, 10 H, Ph).

Anal. Calc. for $C_{60}H_{100}NO_{13}P$ (1074.39): C, 67.07; H, 9.38; N, 1.30. Found: C, 66.80; H, 9.27; N, 1.20.

Compounds **24R** and **24S** (0.11 g) were each hydrogenolyzed in the presence of platinum catalyst, to afford the title diastereoisomeric pair.

GLA-59(*R,R*) had m.p. 172–174°, $[\alpha]_D$ +12.8° (*c* 0.97, 3:1 chloroform–methanol); ν_{max}^{KBr} 3680–2500 (OH, NH, and CH), 1740, 1720 (ester), 1645, 1560 (amide), and complete loss of the peaks at 960 (P–O–Ph) and 800–670 cm^{-1} (Ph).

Anal. Calc. for $C_{48}H_{92}NO_{13}P$ (922.21): C, 62.51; H, 10.06; N, 1.52. Found: C, 62.85; H, 9.93; N, 1.60.

GLA-59(*S,S*) had m.p. 156–158°, $[\alpha]_D$ +17.2° (*c* 0.571, 3:1 chloroform–methanol); ν_{max}^{KBr} 3680–2500 (OH, NH, and CH), 1740, 1720 (ester), 1655, 1550 (amide), and complete loss of the peaks at 960 (P–O–Ph) and 800–680 cm^{-1} (Ph).

Anal. Calc. for $C_{48}H_{92}NO_{13}P$ (922.21): C, 62.51; H, 10.06; N, 1.52. Found: C, 62.30; H, 10.26; N, 1.35.

*2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-4-O-phosphono-3-*O-[*(3R)-3-tetradecanoyloxytetradecanoyl]-*D-*glucose* [*GLA-60*(R,R)] *and 2-deoxy-2-[(3S)-3-hydroxytetradecanamido]-4-O-phosphono-3-*O-[*(3S)-3-tetradecanoyloxytetradecanoyl]-*D-*glucose* [*GLA-60*(S,S)]. — Hydrogenolysis of **21R** (0.16 g) in methanol, as described for **22R**, afforded **25R** in nearly quantitative yield; m.p. 117–120°, $[\alpha]_D$ −2.4° (*c* 1.20, chloroform); ν_{max}^{film} 3600–3200 (OH, NH), 1740 (ester), 1640, 1540 (amide), 960 (P–O–Ph), and 800–670 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$) for a mixture of $\alpha:\beta$ = ~3:1: δ 0.88 (~t, 9 H, CH_3), 1.0–1.6 (m, 62 H, $-CH_2-$), 2.0–2.5 (m, 6 H, $-COCH_2-$), 3.45–4.1 (m, 6 H, H-5,6,6′, 2 O*H* and H-3 of the 3-hydroxytetradecanoyl group), 4.22 (m, 1 H, $J_{1,2}$ 3.3 Hz, H-2), 4.71(α), 4.75(β) (2 q, 1 H, H-4), 5.11 (m, 1 H, H-3 of the 3-tetradecanoyloxytetradecanoyl group), 5.27 (d, ~3/4 H, H-1α), 5.31(β), 5.48(α) (2 t, 1 H, H-3), 5.76 (broad s, 1 H, O*H*), 6.58(α), 7.08(β) (2 d, 1 H, N*H*), and 7.1–7.4 (m, 10 H, Ph).

Anal. Calc. for $C_{60}H_{100}NO_{13}P$ (1074.39): C, 67.07; H, 9.38; N, 1.30. Found: C, 67.29; H, 9.28; N, 1.24.

The (*S*) isomer **25S** was obtained from **21S** in nearly quantitative yield; m.p. 60–62°, $[\alpha]_D$ +3.8° (*c* 1.212, chloroform); ν_{max}^{film} 3600–3200 (OH, NH), 1750, 1720 (ester), 1650, 1550 (amide), 960 (P–O–Ph), and 800–670 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$) for the α anomer: δ 0.88 (~t, 9 H, CH_3), 1.0–1.7 (m, 62 H, $-CH_2-$), 2.1–2.63 (m, 6 H, $-COCH_2-$), 3.41 (~t, 1 H, O*H*-6), 3.5–3.7 (m, 2 H, H-6,6′), 3.77

(d, 1 H, O*H*), 3.94 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 4.07 (~d, 1 H, H-5), 4.31 (m, 1 H, H-2), 4.70 (~d, 1 H, O*H*), 4.75 (q, 1 H, *J* 9.5 Hz, H-4), 4.94 (m, 1 H, H-3 of the 3-tetradecanoyloxytetradecanoyl group), 5.28 (t, 1 H, $J_{1,2} = J_{1,OH} = 3.7$ Hz, H-1), 5.57 (~t, 1 H, H-3), 6.49 (d, 1 H, N*H*), and 7.1–7.4 (m, 10 H, Ph).

Anal. Calc. for $C_{60}H_{100}NO_{13}P$ (1074.39): C, 67.07; H, 9.38; N, 1.30. Found: C, 67.19; H, 9.21; N, 1.31.

The title compounds were obtained from **25R** and **25S**, respectively, as just described, and lyophilized from 1,4-dioxane solution.

GLA-60(*R,R*) had m.p. 157–159°, $[\alpha]_D$ +13.7° (*c* 0.512, 3:1 chloroform–methanol); ν_{max}^{KBr} 3680–2500 (OH, NH, and CH), 1735, 1720 (ester), 1640, 1560 (amide), and complete loss of the peaks at 960 (P–O–Ph) and 800–670 cm^{-1} (Ph).

Anal. Calc. for $C_{48}H_{92}NO_{13}P$ (922.21): C, 62.51; H, 10.06; N, 1.52. Found: C, 62.18; H, 9.85; N, 1.43.

GLA-60(*S,S*) had m.p. 154–155°, $[\alpha]_D$ +18.4° (*c* 0.896, 3:1 chloroform–methanol); ν_{max}^{KBr} 3680–2500 (OH, NH, and CH), 1740, 1720 (ester), 1645, 1550 (amide), and complete loss of the peaks at 960 (P–O–Ph) and 800–670 cm^{-1} (Ph).

Anal. Calc. for $C_{48}H_{92}NO_{13}P$ (922.21): C, 62.51; H, 10.06; N, 1.52. Found: C, 62.77; H, 10.18; N, 1.33.

REFERENCES

1 (a) M. Kiso, H. Ishida, and A. Hasegawa, *Agric. Biol. Chem.*, 48 (1984) 251–252; (b) M. Kiso, H. Ishida, T. Kito, S. Tanaka, and A. Hasegawa, *Gifu Daigaku Nogakubu Kenkyu Hokoku*, 49 (1984) 171–181; (c) M. Matsuura, Y. Kojima, J. Y. Homma, Y. Kubota, A. Yamamoto, M. Kiso, and A. Hasegawa, *FEBS Lett.*, 167 (1984) 226–230; (d) Y. Kumazawa, M. Matsuura, J. Y. Homma, Y. Nakatsuru, M. Kiso, and A. Hasegawa, *Eur. J. Immunol.*, 15 (1985) 199–201; (e) M. Matsuura, A. Yamamoto, Y. Kojima, J. Y. Homma, M. Kiso, and A. Hasegawa, *J. Biochem. (Tokyo)*, 98 (1985) 1229–1237; (f) T. S. Taki, M. Nakano, M. Kiso, and A. Hasegawa, *Microbiol. Immunol.*, 29 (1985) 1111–1120; (g) Y. Kumazawa, M. Matsuura, T. Maruyama, J. Y. Homma, M. Kiso, and A. Hasegawa, *Eur. J. Immunol.*, 16 (1986) 1099–1103.

2 (a) C. Galanos, O. Lüderitz, E. T. Rietschel, and O. Westphal, in T. W. Goodwin (Ed.), *Biochemistry of Lipids II, Int. Rev. Biochem.*, 14 (1977) 239–335; (b) O. Lüderitz, C. Galanos, V. Lehmann, H. Mayer, E. T. Rietschel, and J. Weckesser, *Naturwissenschaften*, 65 (1978) 578–585; (c) C. Galanos, O. Lüderitz, E. T. Rietschel, O. Westphal, H. Brade, L. Brade, M. Freudenberg, U. Schade, M. Imoto, H. Yoshimura, S. Kusumoto, and T. Shiba, *Eur. J. Biochem.*, 148 (1985) 1–5.

3 M. Kiso, S. Tanaka, M. Tanahashi, Y. Fujishima, Y. Ogawa, and A. Hasegawa, *Carbohydr. Res.*, 148 (1986) 221–234.

4 M. Kiso, Y. Ogawa, S. Tanaka, H. Ishida, and A. Hasegawa, *J. Carbohydr. Chem.*, 5 (1986) 621–630.

5 (a) C. R. H. Raetz, S. Purcell, and K. Takayama, *Proc. Natl. Acad. Sci. U.S.A.*, 80 (1983) 4624–4628; (b) K. Takayama, N. Qureshi, P. Mascagni, M. A. Nashed, L. Anderson, and C. R. H. Raetz, *J. Biol. Chem.*, 258 (1983) 7379–7385.

6 M. Matsuura, Y. Kojima, J. Y. Homma, Y. Kumazawa, A. Yamamoto, M. Kiso, and A. Hasegawa, *J. Biochem. (Tokyo)*, 99 (1986) 1377–1384.

7 (a) K. Takayama, N. Qureshi, and P. Mascagni, *J. Biol. Chem.*, 258 (1983) 12,801–12,803; (b) Z. Sidorczyk, U. Zähringer, and E. T. Rietschel, *Eur. J. Biochem.*, 137 (1983) 15–22; (c) S. M. Strain, S. W. Fesik, and I. M. Armitage, *J. Biol. Chem.*, 258 (1983) 2906–2910; (d) 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.

8 M. IMOTO, H. YOSHIMURA, N. SAKAGUCHI, S. KUSUMOTO, AND T. SHIBA, *Tetrahedron Lett.*, (1985) 1545–1548.
9 M. DEMARY, G. PUZO, AND J. ASSELINEAU, *Nouv. J. Chim.*, 2 (1978) 373–378.
10 R. ALBERT, K. DAX, R. PLESCHKO, AND A. E. STÜTZ, *Carbohydr. Res.*, 137 (1985) 282–290.
11 J. C. DITTMER AND R. L. LESTER, *J. Lipid Res.*, 5 (1964) 126–127.