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SYNTHESIS OF NONREDUCING-SUGAR SUBUNIT ANALOGS OF BACTERIAL LIPID A CARRYING AN AMIDE-BOUND (3*R*)-3-ACYLOXY-TETRADECANOYL GROUP

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ABSTRACT

Two types of optically active, 4-*O*-phosphono-D-glucosamine derivatives related to the nonreducing-sugar subunit of bacterial lipid A, one being 2-[(3*R*)-3-acyloxytetradecanamido]-2-deoxy-4-*O*-phosphono-3-*O*-tetradecanoyl-D-glucose (GLA-27 type; GLA-57 and GLA-58), and the other 2-[(3*R*)-3-acyloxytetradecanamido]-2-deoxy-3-*O*-[(3*R*)-3-hydroxytetradecanoyl]-4-*O*-phosphono-D-glucose (GLA-59 type; GLA-61 and GLA-62), have been synthesized. The amino group of benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside was first acylated with the (3*R*)-3-dodecanoyloxytetradecanoyl or (3*R*)-3-hexadecanoyloxytetradecanoyl group, and then the remaining hydroxyl group was esterified with the tetradecanoyl or (3*R*)-3-(benzyloxymethoxy)tetradecanoyl group, respectively. The resulting protected intermediates were each converted, by the sequence of *O*-deisopropylidenation, 6-*O*-tritylation, and 4-*O*-phosphorylation, into the desired compounds.

INTRODUCTION

Lipid A has been proved[1] to be the active center responsible for most of the endotoxic activity of bacterial lipopolysaccharide (LPS).

Since discovery that a 4-*O*-phosphono-D-glucosamine derivative named[2] GLA-27, which has a structure analogous to that of the nonreducing-sugar subunit of bacterial lipid A, can express 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, a variety of analogs have been synthesized[3] and their biological activities tested[4]. 2-Deoxy-3-*O*-(3-hydroxytetradecanoyl)-4-*O*-phosphono-2-(3-tetradecanoyloxytetradecanamido)-D-glucose (GLA-59)[3c] has also been found[5] as an immunological active molecule similar to GLA-27. It is of interest that the two compounds have a common, amide-bound tetradecanoyloxytetradecanoyl group at N-2 of the D-glucosamine moiety.

It has been revealed that the amide-bound acyloxyacyl group in lipid A varies

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with the bacterial species[6]. The lipid A of *Salmonella minnesota*[7] has both a dodecanoyloxy-, and a hexadecanoyloxy-tetradecanoyl group, whereas that of the *Proteus mirabilis* Re mutant[8] has the tetradecanoyloxy- and hexadecanoyloxy-tetradecanoyl groups. In the *Escherichia coli* lipid A, however, only the dodecanoyloxytetradecanoyl group has been characterized[9]. In addition, a D-glucosamine-derived phospholipid named[10] lipid Y, found in certain *E. coli* mutants, also carries the amide-bound hexadecanoyloxytetradecanoyl group.

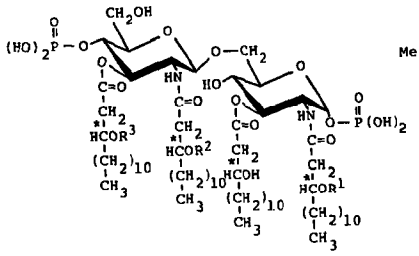
We now describe the synthesis of some nonreducing-sugar subunit analogs of bacterial lipid A which carry the amide-bound dodecanoyloxy- and hexadecanoyloxy-tetradecanoyl group at N-2 of the D-glucosamine backbone.

RESULTS AND DISCUSSION

(3*R*)-3-Dodecanoyloxytetradecanoic acid (**2**) and (3*R*)-3-hexadecanoyloxytetradecanoic acid (**3**) were prepared *via* the phenacyl ester of (3*R*)-3-hydroxytetradecanoic acid as previously described[3c]. These (3*R*)-3-acyloxytetradecanoic acids were each treated[2b] with benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**1**), in the presence of 3-(3-dimethylamino)propyl-1-ethylcarbodiimide hydrochloride (WSC) in dichloromethane, to give the corresponding benzyl 2-(acyloxytetradecanamido)-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**5** or **6**, respectively). Esterification of the remaining 3-hydroxyl group with tetradecanoic and (3*R*)-3-(benzyloxymethoxy)tetradecanoic acid in the presence of WSC (or dicyclohexylcarbodiimide, DCC) and 4-(dimethylamino)pyridine (DMAP), gave **7, 8, 9,** and **10**, from which **11, 14, 17,** and **20** were obtained by hydrolytic removal of the isopropylidene group. After 6-*O*-tritylation, the diphenylphosphono group was introduced at O-4 in the usual manner[2b,3c], and the product was purified by chromatography.

Hydrolytic removal of the trityl group gave the protected synthetic intermediates **13, 16, 19**, and **22**, from which **23, 24, 25**, and **26** were obtained in nearly quantitative yield by hydrogenolytic removal of the benzyl group in the presence of palladium catalyst. The pair **23** and **24**, or **25** and **26**, showed ^{1}H-n.m.r. spectra quite similar to that of the corresponding precursor of GLA-27[2b] or GLA-59[3c], respectively, except for minor differences in the number of methylene protons and the chemical shift of the hydroxyl protons. In all these compounds, the α-D-pyranose form preponderates in the equilibrium mixture in chloroform-*d*, as previously described for a series of homologous compounds. Finally, the phenyl groups were cleaved by hydrogenolysis in the presence of platinum catalyst, to afford the desired GLA-57(*R*), GLA-58(*R*), GLA-61(*R,R*) and GLA-62(*R,R*) as colorless powders which gave a positive test with the specific spray-reagent[11] for the phosphate group. It may be noted that, like GLA-27(*R*), these compounds are essentially insoluble in most of the organic solvents.

The structures of GLA-57(*R*) and GLA-61(*R,R*) are closely related to those of the nonreducing-sugar subunit of the lipid A of *S. minnesota* and *E. coli*, whereas



Bacterial lipid A

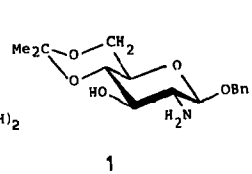
	R^1	R^2	R^3
S. minnesota	C_{16}	C_{12}	C_{14}
P. mirabilis	C_{16}	C_{14}	C_{14}
E. coli	H	C_{12}	C_{14}

$C_{12} = CH_3(CH_2)_{10}CO$

$C_{14} = CH_3(CH_2)_{12}CO$

$C_{16} = CH_3(CH_2)_{14}CO$

* *(R)*-configuration



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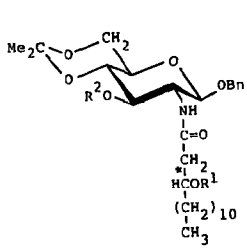
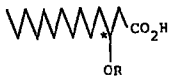
Bn = $PhCH_2$





2 R = C_{12}

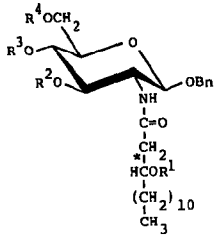
3 R = C_{16}

4 R = Bom

Bom = $PhCH_2OCH_2$

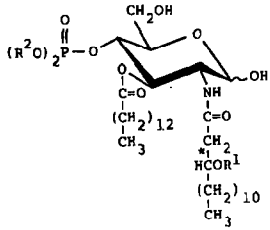
	R^1	R^2
5	C_{12}	H
6	C_{16}	H
7	C_{12}	C_{14}
8	C_{16}	C_{14}
9	C_{12}	C_{14}-OBom
10	C_{16}	C_{14}-OBom

C_{14}-OBom = $CH_3(CH_2)_{10}\overset{*}{C}HCH_2CO$ (OBom)

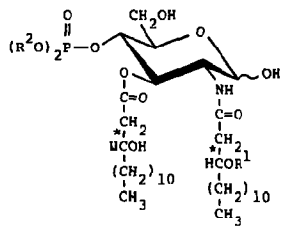


	R^1	R^2	R^3	R^4
11	C_{12}	C_{14}	H	H
12	C_{12}	C_{14}	H	Tr
13	C_{12}	C_{14}	$(PhO)_2PO$	H
14	C_{16}	C_{14}	H	H
15	C_{16}	C_{14}	H	Tr
16	C_{16}	C_{14}	$(PhO)_2PO$	H
17	C_{12}	C_{14}-OBom	H	H
18	C_{12}	C_{14}-OBom	H	Tr
19	C_{12}	C_{14}-OBom	$(PhO)_2PO$	H
20	C_{16}	C_{14}-OBom	H	H
21	C_{16}	C_{14}-OBom	H	Tr
22	C_{16}	C_{14}-OBom	$(PhO)_2PO$	H

Tr = Ph_3C



	R^1	R^2
23	C_{12}	Ph
24	C_{16}	Ph
GLA-57*(R)*	C_{12}	H
GLA-58*(R)*	C_{16}	H
[GLA-27*(R)*	C_{14}	H]



	R^1	R^2
25	C_{12}	Ph
26	C_{16}	Ph
GLA-61*(R,R)*	C_{12}	H
GLA-62*(R,R)*	C_{16}	H
[GLA-59*(R,R)*	C_{14}	H]

GLA-58(*R*) and GLA-62(*R,R*) have partial structures similar to lipid Y. Furthermore, the importance of the chain length of the C_{14} *O*-acyl group will be clarified by comparing the biological activities of these compounds with those of GLA-27(*R*) and GLA-59(*R,R*), which carry the amide-bound tetradecanoyloxytetradecanoyl (C_{14}–*O*-C_{14}) group at N-2 of the D-glucosamine backbone.

EXPERIMENTAL

General methods. — The instrumental and chromatographic procedures employed were those previously given[2b]. ^{1}H-N.m.r. spectra were recorded at 270 MHz. For details of the reaction procedures employed, see also, ref. 3c.

(3R)-3-Dodecanoyloxytetradecanoic acid (**2**) *and (3R)-3-hexadecanoyloxytetradecanoic acid* (**3**). — To a cooled, stirred solution of (3*R*)-3-hydroxytetradecanoic acid phenacyl ester[3c] (2 g) in pyridine (21 mL) were added a catalytic amount of DMAP and dodecanoyl or hexadecanoyl chloride (1.2 mol equiv.) in dry dichloromethane; the mixture was stirred overnight at room temperature. Methanol was added, and the mixture was processed as described[3c] for the preparation of (3*R*)-3-tetradecanoyloxytetradecanoic acid, to give the corresponding phenacyl ester of (3*R*)-3-dodecanoyloxytetradecanoic acid (2.87 g, 95.3%); m.p. 35.5°, $[\alpha]_D$ +1.3° (*c* 0.7, chloroform); ν_{max}^{film} 1740, 1730, 1710 (C=O), 780–670 (Ph), and complete loss of the peak at 3600–3200 cm^{-1} (OH); and of (3*R*)-3-hexadecanoyloxytetradecanoic acid (3.3 g, 99.4%); m.p. 38.5–40°, $[\alpha]_D$ +1° (*c* 0.9, chloroform).

Finally, the phenacyl esters of the (3*R*)-3-acyloxytetradecanoic acids (2 g) were each treated with zinc dust (3 g) in acetic acid (10 mL) at 50°. The resulting, free acids were each purified by chromatography on a column of silica gel (Wakogel C-200) with dichloromethane, to give **2** or **3** as a syrup in nearly quantitative yield.

Compound **2** had $[\alpha]_D$ −1° (*c* 0.9, chloroform); i.r. data ν_{max}^{film} 3700–2500 (CO_2H), 1750, 1720 (C=O), and complete loss of the peaks at 780–670 cm^{-1} (Ph).

Anal. Calc. for $C_{26}H_{50}O_4$ (426.66): C, 73.19; H, 11.81. Found: C, 73.42; H, 11.67.

Compound **3** had $[\alpha]_D$ −1° (*c* 1, chloroform); i.r. data similar to those of **2**.

Anal. Calc. for $C_{30}H_{58}O_4$ (482.76): C, 74.63; H, 12.11. Found: C, 74.78; H, 12.23.

Benzyl 2-deoxy-2-[(3R)-3-dodecanoyloxytetradecanamido]-4,6-O-isopropylidene-β-D-glucopyranoside (**5**) *and benzyl 2-deoxy-2-[(3R)-3-hexadecanoyloxytetradecanamido]-4,6-O-isopropylidene-β-D-glucopyranoside* (**6**). — To a solution of **1** (0.65 g) in dry dichloromethane (5 mL) were added **2** (0.9 g) and WSC (0.62 g); the mixture was stirred at room temperature. After completion of the reaction (t.l.c., 2:1 ethyl acetate–hexane), the mixture was evaporated to a residue that was chromatographed on a column of silica gel (Wakogel C-200) with 400:1 dichloromethane–methanol, to afford **5** (1.36 g; 90%), which was lyophilized from 1,4-dioxane solution; m.p. 63–64°, $[\alpha]_D$ −47° (*c* 1, chloroform); ν_{max}^{film} 3500, 3375,

3300 (OH, NH), 1730 (ester), 1660, 1550, 1530 (amide), 860 (CMe_2), and 740–680 cm^{-1} (Ph).

Anal. Calc. for $C_{42}H_{71}NO_8$ (718.00): C, 70.25; H, 9.97; N, 1.95. Found: C, 70.39; H, 10.14; N, 1.81.

Compound **6** (1.44 g; 93.5%) was obtained by treatment of **1** (0.64 g) with **3** (1.2 g) as described for **5**; m.p. 71–72°, $[\alpha]_D$ −48° (*c* 0.9, chloroform); ν_{max}^{film} 3600–3200 (OH, NH), 1730 (ester), 1660, 1530 (amide), 850 (CMe_2), and 760–680 cm^{-1} (Ph).

Anal. Calc. for $C_{46}H_{79}NO_8$ (774.10): C, 71.37; H, 10.29; N, 1.81. Found: C, 71.18; H, 10.35; N, 1.77.

Benzyl 2-deoxy-2-[(3R)-3-dodecanoyloxytetradecanamido]-4,6-O-isopropylidene-3-O-tetradecanoyl-β-D-glucopyranoside (**7**) *and benzyl 2-deoxy-2-[(3R)-3-hexadecanoyloxytetradecanamido]-4,6-O-isopropylidene-3-O-tetradecanoyl-β-D-glucopyranoside* (**8**). — To a solution of **5** (0.55 g) in dry dichloromethane (6 mL) were added tetradecanoic acid (0.21 g), WSC (0.294 g), and a catalytic amount of DMAP; the mixture was stirred at room temperature. After completion of the reaction (t.l.c., 80:1 dichloromethane–methanol), the mixture was processed as described for **5**. The product was purified by chromatography on a column of silica gel (Wakogel C-200) with 500:1 dichloromethane–methanol, to give **7** (0.69 g; 97%), which was lyophilized from 1,4-dioxane solution; m.p. 86–88°, $[\alpha]_D$ −34° (*c* 0.9, chloroform); ν_{max}^{film} 3350 (NH), 1740 (ester), 1660, 1550, 1530 (amide), 860 (CMe_2), and complete loss of the peak at 3500 cm^{-1} (OH).

Anal. Calc. for $C_{56}H_{97}NO_9$ (928.35): C, 72.45; H, 10.53; N, 1.51. Found: C, 72.31; H, 10.40; N, 1.49.

Compound **8** (0.74 g, 97%) was obtained by treatment of **6** (0.6 g) with tetradecanoic acid (0.212 g) in the presence of WSC (0.297 g) and DMAP (10 mg) as described for **7**; m.p. 88–89°, $[\alpha]_D$ −32° (*c* 1, chloroform); ν_{max}^{film} 3400 (NH), 1740 (ester), 1680, 1520 (amide), 860 (CMe_2), and complete loss of the peak at 3500 cm^{-1} (OH).

Anal. Calc. for $C_{60}H_{105}NO_9$ (984.45): C, 73.20; H, 10.75; N, 1.42. Found: C, 73.46; H, 10.78; N, 1.33.

Benzyl 3-O-[(3R)-3-(benzyloxymethoxy)tetradecanoyl]-2-deoxy-2-[(3R)-3-dodecanoyloxytetradecanamido]-4,6-O-isopropylidene-β-D-glucopyranoside (**9**). — Compound **5** (0.55 g) in dichloromethane (6 mL) was treated with **4** (ref. 3c) (0.334 g) in the presence of WSC (0.294 g) and DMAP (9 mg), and the mixture was processed as described for **7** and **8**. The product was purified by chromatography on a column of silica gel (Wakogel C-200) with 500:1 dichloromethane–methanol, to give **9** (0.8 g, 98.2%); m.p. 54–56°, $[\alpha]_D$ −22° (*c* 1, chloroform); ν_{max}^{film} 3320 (NH), 1740, 1730 (ester), 1660, 1550, 1530 (amide), 860 (CMe_2), and 760–680 cm^{-1} (Ph).

Anal. Calc. for $C_{64}H_{105}NO_{11}$ (1064.49): C, 72.21; H, 9.94; N, 1.32. Found: C, 72.03; H, 9.81; N, 1.29.

Benzyl 3-O-[(3R)-3-(benzyloxymethoxy)tetradecanoyl]-2-deoxy-2-[(3R)-3-hexadecanoyloxytetradecanamido]-4,6-O-isopropylidene-β-D-glucopyranoside (**10**).

— Compound **10** (0.79 g, 97.7%) was obtained by treatment of **6** (0.56 g) with **4** (0.314 g) in the presence of WSC (0.277 g) and DMAP (9 mg) as described for **7**, **8**, and **9**; m.p. 61–63°, $[\alpha]_D$ −21° (*c* 1, chloroform); ν_{max}^{film} 3340 (NH), 1740 (ester), 1660, 1540 (amide), 860 (CMe_2), and 760–690 cm^{-1} (Ph).

Anal. Calc. for $C_{68}H_{113}NO_{11}$ (1120.58): C, 72.88; H, 10.16; N, 1.25. Found: C, 73.14; H, 9.99; N, 1.17.

Benzyl 2-deoxy-4-O-(diphenylphosphono-2)-[(3R)-3-dodecanoyloxytetradecanamido]-4,6-O-isopropylidene-3-O-tetradecanoyl-β-D-glucopyranoside (**13**). — *O*-Deisopropylidenation of **7** (0.65 g) was performed by treatment with 95% acetic acid (16 mL) as described previously[2b], to give **11** in 82% yield; m.p. 144–146°, $[\alpha]_D$ −24° (*c* 0.8, chloroform).

A solution of **11** (0.48 g) in dry pyridine (7 mL) was stirred at 90°, and then trityl chloride (0.3 g) was added; stirring was continued for 6 h at 90°, the mixture was cooled, methanol was added, and the solvents were evaporated. After extractive processing, the product was purified by chromatography on a column of silica gel (Wakogel C-200) with 500:1 dichloromethane–methanol, to afford **12** (0.61 g; quantitative); m.p. 100–101°, $[\alpha]_D$ −27° (*c* 0.9, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (~t, 9 H, CH_3), 1.0–1.4, 1.4–1.7 (m, 54 H + 6 H, $-CH_2-$), 2.15–2.5 (m, 6 H, $-COCH_2-$), 2.63 (d, 1 H, *J* 3.7 Hz, O*H*), 3.3–3.5 (m, 3 H, H-5,6,6′), 3.75 (m, 1 H, H-4), 4.05 (~q, 1 H, H-2), 4.5 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.63, 4.88 (2 d, 2 H, J_{gem} 12.1 Hz, CH_2Ph), 4.93 (dd, 1 H, $J_{2,3}$ 9, $J_{3,4}$ 10 Hz, H-3), 5.04 (m, 1 H, H-3 of C_{14}–O–C_{12}), 5.81 (d, 1 H, *J* 9.2 Hz, N*H*), and 7.1–7.6 (m, 20 H, Ph–H).

The introduction of the diphenylphosphono group at O-4 of **12** (0.58 g) was accomplished with diphenyl phosphorochloridate (0.217 g) and DMAP (94 mg) in 2:1 dichloromethane–pyridine as described in ref. 2b. The product was purified by chromatography on a column of silica gel (Wakogel C-300) with 5:1 hexane–ethyl acetate, to give benzyl 2-deoxy-4-*O*-(diphenylphosphono)-2-[(3*R*)-3-dodecanoyloxytetradecanamido]-3-*O*-tetradecanoyl-6-*O*-trityl-β-D-glucopyranoside (0.52 g; 74.4%), which was then treated with tetrafluoroboric acid[12] in acetone, to afford **13** in almost quantitative yield; m.p. 85.5–86°, $[\alpha]_D$ −32° (*c* 0.9, chloroform); ν_{max}^{film} 3500, 3300 (OH, NH), 3150–3000 (Ph), 1740 (ester), 1650, 1560 (amide), 960 (P–O–Ph), and 780–680 cm^{-1} (Ph).

Anal. Calc. for $C_{65}H_{102}NO_{12}P$ (1120.46); C, 69.67; H, 9.18; N, 1.25. Found: C, 69.83; H, 9.20; N, 1.14.

Benzyl 2-deoxy-4-O-(diphenylphosphono)-2-[(3R)-3-hexadecanoyloxytetradecanamido]-4,6-O-isopropylidene-3-O-tetradecanoyl-β-D-glucopyranoside (**16**). — A mixture of **8** (0.67 g) and 95% acetic acid (16 mL) was processed as described for **11**, to give **14** in almost quantitative yield; m.p. 135–137°, $[\alpha]_D$ −22° (*c* 0.8, chloroform); ν_{max}^{film} 3500, 3300 (OH, NH), 1740, 1705 (ester), 1660, 1570 (amide), 750–680 (Ph), and complete loss of the peak at 860 cm^{-1} (CMe_2).

Tritylation of the primary hydroxyl group of **14** (0.56 g) was conducted with trityl chloride (0.3 g) in pyridine (8 mL) as described for **12**, to afford **15** (0.7 g;

99.4%); m.p. 74–76°, $[\alpha]_D$ −25° (*c* 1, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (t, 9 H, C*H*$_3$), 1.0–1.7 (m, 68 H, –C*H*$_2$–), 2.15–2.5 (m, 6 H, –COC*H*$_2$–), 2.61 (d, 1 H, $J_{4,OH}$ 3.7 Hz, O*H*), 3.3–3.5 (m, 3 H, H-5,6,6'), 3.75 (m, 1 H, H-4), 4.05 (~q, 1 H, *J* ~9 Hz, H-2), 4.50 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.63, 4.88 (2 d, 2 H, C*H*$_2$Ph), 4.93 (~t, 1 H, H-3), 5.04 (m, 1 H, H-3 of C_{14}–O–C_{16}), 5.75 (d, 1 H, *J* 9.2 Hz, N*H*), and 7.1–7.55 (m, 20 H, Ph–H).

The introduction of the diphenylphosphono group at O-4 of **15** (0.7 g) was performed by the procedure described for **13**, to give benzyl 2-deoxy-4-*O*-(diphenylphosphono)-2-[(3*R*)-3-hexadecanoyloxytetradecanamido]-3-*O*-tetradecanoyl-6-*O*-trityl-β-D-glucopyranoside (0.76 g; 91%), which was then treated with aqueous acetic acid. The resulting **16** (nearly quantitative) was purified by chromatography on a column of silica gel (Wakogel C-300) with 5:2 hexane–ethyl acetate; m.p. 83–85°, $[\alpha]_D$ −31° (*c* 1, chloroform); i.r. data similar to those of **13**.

Anal. Calc. for $C_{69}H_{110}NO_{12}P$ (1176.56): C, 70.43; H, 9.42; N, 1.19. Found: C, 70.62; H, 9.27; N, 1.04.

*Benzyl 3-*O*-[(3*R*)-3-(benzyloxymethoxy)tetradecanoyl]-2-deoxy-4-*O*-(diphenylphosphono)-2-[(3*R*)-3-dodecanoyloxytetradecanamido]-β-D-glucopyranoside* (**19**). — Compound **9** (0.77 g) was treated with aqueous acetic acid as described for **11** and **14**, to give **17** (0.7 g, 94.5%); m.p. 96–98°, $[\alpha]_D$ −38° (*c* 0.9, chloroform). The 6-*O*-trityl derivative **18** (0.75 g; 91%) was obtained from **17** (0.67 g) in the usual way; m.p. 84–86°, $[\alpha]_D$ −33° (*c* 1, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (~t, 9 H, C*H*$_3$), 1.0–1.7 (m, 58 H, –C*H*$_2$–), 2.15–2.65 (m, 4 H, H-4,5,6,6'), 3.9–4.1 (m, 2 H, H-2, and H-3 of C_{14}-OBom), 4.44 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.45–4.95 (6 d, 6 H, –OC*H*$_2$O– and C*H*$_2$Ph), 4.85 (dd, 1 H, H-3), 5.05 (m, 1 H, H-3 of C_{14}–O–C_{12}), 5.71 (d, 1 H, N*H*), and 7.1–7.55 (m, 25 H, Ph–H).

4-*O*-Phosphorylation of **18** (0.72 g), followed by detritylation, afforded compound **19** (62% in 2 steps); m.p. 68–71°, $[\alpha]_D$ −16° (*c* 1, chloroform); ν_{max}^{film} 3500, 3280 (OH, NH), 3150–3000 (Ph), 1740 (ester), 1650, 1560 (amide), 960 (P–O–Ph), and 780–680 cm^{-1} (Ph).

Anal. Calc. for $C_{73}H_{110}NO_{14}P$ (1256.60): C, 69.77; H, 8.82; N, 1.11. Found: C, 70.05; H, 8.73; N, 1.00.

*Benzyl 3-*O*-[(3*R*)-3-(benzyloxymethoxy)tetradecanoyl]-2-deoxy-4-*O*-(diphenylphosphono)-2-[(3*R*)-3-hexadecanoyloxytetradecanamido]-β-D-glucopyranoside* (**22**). — Compound **20** (0.66 g, 91.3%) was obtained from **10** (0.75 g) as described for **17**; m.p. 97–97.5°, $[\alpha]_D$ −38° (*c* 0.8, chloroform); i.r. data similar to those of **17**.

The 6-*O*-tritylation of **20** (0.57 g) gave **21** (0.69 g, 99%); m.p. 79–81.5°, $[\alpha]_D$ −30° (*c* 1, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (t, 9 H, C*H*$_3$), 1.0–1.7 (m, 66 H, –C*H*$_2$–), 2.1–2.7 (m, 6 H, –COC*H*$_2$–), 3.08 (~s, 1 H, O*H*), 3.2–3.5 (m, 4 H, H-4,5,6,6'), 3.9–4.1 (m, 2 H, H-2, and H-3 of C_{14}–O–C_{16}), 5.73 (d, 1 H, *J* 8.8, N*H*), and 7.1–7.55 (m, 25 H, Ph–H).

Compound **21** (0.68 g) was converted, by the sequence described for **13**, **16**, and **19**, into **22** (66% in 2 steps); m.p. 68–69°, $[\alpha]_D$ −15° (*c* 1, chloroform); i.r. data similar to those of **19**.

Anal. Calc. for $C_{77}H_{118}NO_{14}P$ (1312.70): C, 70.45; H, 9.06; N, 1.07. Found: C, 70.73; H, 9.18; N, 0.96.

2-Deoxy-2-[(3R)-3-dodecanoyloxytetradecanamido]-4-O-phosphono-3-O-tetradecanoyl-D-glucose [GLA-57(R)] and 2-deoxy-2-[(3R)-3-hexadecanoyloxytetradecanamido]-4-O-phosphono-3-O-tetradecanoyl-D-glucose [GLA-58(R)]. — Compound **13** (0.23 g) was dissolved in methanol–ethanol–toluene (10 mL), and hydrogenolyzed in the presence of 10% palladium–carbon catalyst (0.1 g). The catalyst was filtered off, and washed with the same solvent. The filtrate and washings were combined, and evaporated to a residue which was chromatographed on a column of silica gel (Wakogel C-300) with 2:1 ethyl acetate–hexane, to give **23** in almost quantitative yield; m.p. 95–96°, $[\alpha]_D$ +4.3° (*c* 1.7, chloroform); ^{1}H-n.m.r. data for the α anomer ($CDCl_3$): δ 0.88 (t, 9 H, CH_3), 1.0–1.7 (m, 60 H, $-CH_2-$), 3.28 (~t, 1 H, OH-6), 3.5–3.7 (m, 2 H, H-6,6'), 3.78 (d, 1 H, $J_{1,OH}$ ~3 Hz, OH-1), 4.00 (~d, 1 H, $J_{4,5}$ 9.9 Hz, H-5), 4.24 (m, 1 H, H-2), 4.76 (~q, 1 H, $J_{3,4} = J_{4,5} = J_{4,P}$ = 9–10 Hz, H-4), 5.09 (m, 1 H, H-3 of C_{14}–O–C_{12}), 5.28 (~t, 1 H, *J* 3–4 Hz, H-1), 5.48 (~t, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9.5 Hz, H-3), 6.07 (d, 1 H, *J* 9.2 Hz, N*H*), and 7.1–7.45 (m, 10 H, Ph–H).

Compound **24** was obtained from **16** (0.21 g) as described for **23**; m.p. 92.5–93.5°, $[\alpha]_D$ +4.2° (*c* 1.6, chloroform); ^{1}H-n.m.r. data for the α anomer ($CDCl_3$): δ 0.88 (t, 9 H, CH_3), 1.0–1.7 (m, 68 H, $-CH_2-$), 2.0–2.5 (m, 6 H, $-COCH_2-$), 3.29 (~t, 1 H, OH-6), 3.5–3.7 (m, 2 H, H-6,6'), 3.86 (~s, 1 H, OH-1), 4.01 (~d, 1 H, $J_{4,5}$ 9.9 Hz, H-5), 4.23 (m, 1 H, 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 C_{14}–O–C_{16}), 5.27 (t, 1 H, $J_{1,2} = J_{1,OH}$ = 3–4 Hz, H-1), 5.48 (~t, 1 H, H-3), 6.08 (d, 1 H, *J* 9.2 Hz, N*H*), and 7.1–7.45 (m, 10 H, Ph–H).

The phenyl groups of **23** (0.16 g) and **24** (0.15 g) were removed by hydrogenolysis in the presence of pre-reduced, Adams' platinum catalyst (50 mg) in ethanol–methanol as described[2b] for the preparation of GLA-27, and the products were lyophilized from suspensions in 1,4-dioxane, to give colorless, fine powders. Because, like GLA-27(*R*), both compounds were essentially insoluble in the usual organic solvents, their $[\alpha]_D$ values could not be measured accurately.

GLA-57(*R*) had m.p. 162–163.5°; ν_{max}^{KBr} 3700–2500 (OH, NH), 1740, 1720 (ester), 1640, 1560 (amide), and complete loss of the peaks at 960 (P–O–Ph) and 780–680 cm^{-1} (Ph).

Anal. Calc. for $C_{46}H_{88}NO_{12}P$ (878.15): C, 62.91; H, 10.10; N, 1.60. Found: C, 63.25; H, 9.86; N, 1.39.

GLA-58(*R*) had m.p. 158–161°; ν_{max}^{KBr} 3700–2500 (OH, NH), 1740 (ester), 1640, 1560 (amide), and complete loss of the peaks at 960 (P–O–Ph) and 780–680 cm^{-1} (Ph).

Anal. Calc. for $C_{50}H_{96}NO_{12}P$ (934.26): C, 64.28; H, 10.36; N, 1.50. Found: C, 64.03; H, 10.21; N, 1.36.

2-Deoxy-2-[(3R)-3-dodecanoyloxytetradecanamido]-3-O-[(3R)-3-hydroxytetradecanoyl]-4-O-phosphono-D-glucose [GLA-61(R,R)] and 2-deoxy-2-[(3R)-3-hexadecanoyloxytetradecanamido]-3-O-[(3R)-3-hydroxytetradecanoyl]-4-O-phos-

phono-D-*glucose* [*GLA-62*(R,R)]. — Compound **19** (0.2 g) was hydrogenolyzed in methanol (10 mL) in the presence of 10% palladium–carbon catalyst (0.1 g), and the mixture was processed as described for GLA-57(*R*) and GLA-58(*R*), to give **25**; m.p. 88.5–90.5°, $[\alpha]_D$ −1.5° (*c* 1.5, chloroform); ^{1}H-n.m.r. data for the α anomer ($CDCl_3$): δ 0.88 (t, 9 H, CH_3), 1.0–1.8 (m, 58 H, $-CH_2-$), 2.1–2.5 (m, 6 H, $-COCH_2-$), 3.26 (~d, 1 H, OH-3 of the 3-hydroxytetradecanoyl group), 3.38 (broad t, 1 H, OH-6), 3.5–3.7 (broad s, 2 H, H-6,6′), 3.90 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 4.04 (~d, 1 H, H-5), 4.29 (m, 1 H, H-2), 4.38 (d, 1 H, OH-1), 4.77 (~q, $J_{3,4} = J_{4,5} = J_{4,P} = \sim 9.5$ Hz, H-4), 5.08 (m, 1 H, H-3 of C_{14}–O–C_{12}), 5.24 (t, 1 H, $J_{1,OH} = J_{1,2} = 3$–4 Hz, H-1), 5.51 (~t, 1 H, $J_{3,4}$ ~10 Hz, H-3), 6.30 (d, 1 H, *J* 9.2 Hz, N*H*) and 7.1–7.4 (m, 10 H, Ph–H).

Compound **26** was obtained from **22** as just described; m.p. 87–90°, $[\alpha]_D$ −1.2° (*c* 1.3, chloroform); ^{1}H-n.m.r. data for the α anomer ($CDCl_3$): δ 0.88 (t, 9 H, CH_3), 1.0–1.7 (m, 66 H, $-CH_2-$), 2.1–2.5 (m, 6 H, $-COCH_2-$), 3.23 (~d, 1 H, OH-3 of the 3-hydroxytetradecanoyl group), 4.0–4.1 (m, 2 H, H-5 and OH-1), 4.30 (m, 1 H, H-2), 4.78 (~q, *J* 9–10 Hz, H-4), 5.08 (m, 1 H, H-3 of C_{14}–O–C_{16}), 5.25 (t, 1 H, *J* 3–4 Hz, H-1), 5.51 (~t, 1 H, H-3), 6.27 (d, 1 H, *J* 9.2 Hz, N*H*), and 7.1–7.4 (m, 10 H, Ph–H).

Hydrogenolytic removal of the phenyl groups from **25** and **26** was performed in ethanol solution, and the reaction mixture was processed as described for GLA-57(*R*) and GLA-58(*R*), to give, almost quantitatively, GLA-61(*R,R*) and GLA-62(*R,R*) as colorless, fine powders.

GLA-61(*R,R*) had m.p. 177.5–180°, $[\alpha]_D$ +11° (*c* 0.1, 1:1 dichloromethane–ethanol); ν_{max}^{KBr} 3700–2600 (OH, NH), 1740 (ester), 1640, 1560 (amide), and complete loss of the peaks at 960 (P–O–Ph) and 780–680 cm^{-1} (Ph).

Anal. Calc. for $C_{46}H_{88}NO_{13}P$ (894.15): C, 61.79; H, 9.92; N, 1.57. Found: C, 61.47; H, 10.18; N, 1.69.

GLA-62(*R,R*) had m.p. 175–178°, $[\alpha]_D$ +10° (*c* 0.14, 1:1 dichloromethane–ethanol); i.r. data (KBr), same as those for GLA-61(*R,R*).

Anal. Calc. for $C_{50}H_{96}NO_{13}P$ (950.26): C, 63.19; H, 10.18; N, 1.47. Found: C, 63.53; H, 9.99; N, 1.42.

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