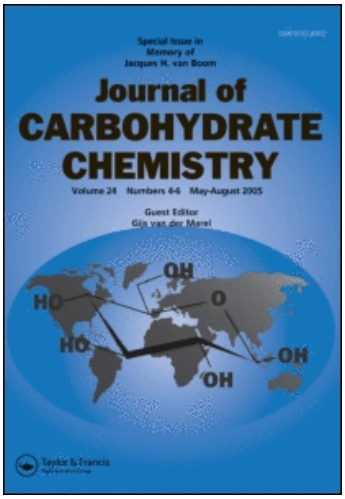

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Nonreducing-Sugar Subunit Analogs of Bacterial Lipid a Carrying the Ester-Bound (3*R*)-3-(Acyloxy)Tetradecanoyl Group

Makoto Kiso[a]; Yuji Ogawa[a]; Yushun Fujishima[a]; Minoru Fujita[a]; Shinji Tanaka[a]; Akira Hasegawa[a]
[a] Department of Agricultural Chemistry, Gifu University Yanagido, Gifu, Japan

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NONREDUCING-SUGAR SUBUNIT ANALOGS OF BACTERIAL LIPID A CARRYING THE ESTER-BOUND (3R)-3-(ACYLOXY)TETRADECANOYL GROUP

Makoto Kiso, Yuji Ogawa, Yushun Fujishima, Minoru Fujita, Shinji Tanaka, and Akira Hasegawa

Department of Agricultural Chemistry, Gifu University
Yanagido, Gifu 501-11, Japan

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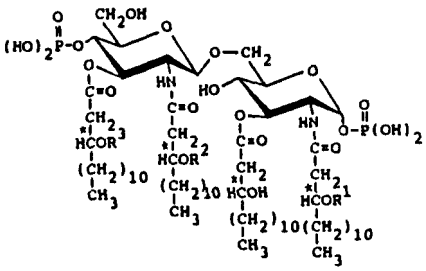
ABSTRACT

In order to elucidate further the relationship between the composition of the fatty acyl groups in the nonreducing-sugar subunit of bacterial lipid A and its biological activity, 3-O-[(3R)-3-(acyloxy)tetradecanoyl]-2-deoxy-2-[(3R)-3-hydroxytetradecanamido]-4-O-phosphono-D-glucose [GLA-63(R,R) and GLA-64(R,R)], and 3-O-[(3R)-3-(acyloxy)tetradecanoyl]-2-deoxy-4-O-phosphono-2-tetradecanamido-D-glucose [GLA-67(R), GLA-68(R) and GLA-69(R)] have been synthesized. Benzyl 2-[(3R)-3-(benzyloxymethoxy)tetradecanamido]-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranoside (5) and benzyl 2-deoxy-4,6-O-isopropylidene-2-tetradecanamido-β-D-glucopyranoside (6) were each esterified with (3R)-3-dodecanoyloxytetradecanoic acid (1), (3R)-3-tetradecanoyloxytetradecanoic acid (2) or (3R)-3-hexadecanoyloxytetradecanoic acid (3), to give 7-11, which were then transformed, by the sequence of deisopropylidenation, 6-O-tritylation and 4-O-phosphorylation, into a series of desired compounds.

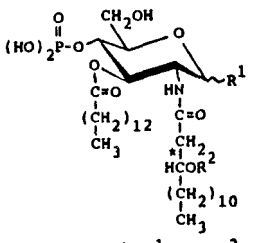
INTRODUCTION

In the course of our investigation[1-3] on the relationship between the chemical structure and the biological activity of lipid A[4] and related compounds, it has been revealed[5,6] that a series of 4-O-phosphono-D-glucosamine derivatives named GLA-27,[1b] GLA-40,[2] GLA-59[3a] and GLA-60[3a] (see FIG. 1), elicit some distinct and beneficial

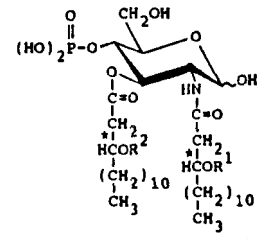


Bacterial Lipid A

	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}



	R^1	R^2
GLA-27	OH	C_{14}
GLA-40	H	C_{14}
GLA-57(R)	OH	C_{12}
GLA-58(R)	OH	C_{16}



	R^1	R^2
GLA-59	C_{14}	H
GLA-60	H	C_{14}
GLA-61(R,R)	C_{12}	H
GLA-62(R,R)	C_{16}	H

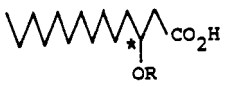
$C_n = CH_3(CH_2)_{n-2}CO$

FIG. 1

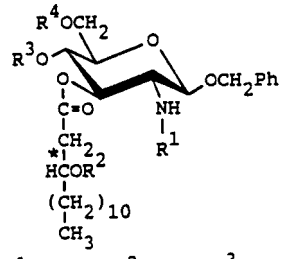
biological activities of bacterial endotoxin. GLA-60 is generally more active than others, and for GLA-27 and GLA-40 dissociation of the activity depending on the stereospecificity between the R and S configuration of the 3-hydroxytetradecanoyl group was observed.[5c,7] All these compounds are, however, nontoxic and nonpyrogenic relative to the parent lipid A. These results indicate that the activity is critically affected by the composition of both the amide- and ester-bound fatty acyl groups.[8]

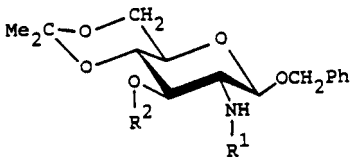
The amide-bound (acyloxy)acyl group in lipid A varies[9] with the bacterial species such as Salmonella minnesota, Proteus mirabilis and Escherichia coli as shown in FIG. 1. On the other hand, the ester-bound (acyloxy)acyl group of these lipids is the only (3R)-3-tetradecanoyloxytetradecanoyl (C_{14}-O-C_{14}) group found in the nonreducing-sugar subunit. While the foregoing GLA-27, GLA-40 and GLA-59 carry the amide-bound C_{14}-O-C_{14} group at N-2 of 2-amino-2-deoxy-D-glucose (D-glucosamine) or 2-amino-1,5-anhydro-2-deoxy-D-glucitol, GLA-60 carries the ester-bound C_{14}-O-C_{14} group at O-3 of the D-glucosamine backbone.

In a preceding paper,[3b] we have reported the synthesis of some optically active homologs of GLA-27 and GLA-59 [GLA-57(R), GLA-58(R), GLA-61(R,R) and GLA-62(R,R)] in order to elucidate the biological



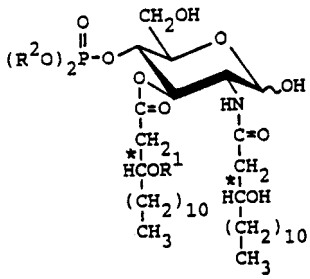
1 R = C_{12}
2 R = C_{14}
3 R = C_{16}



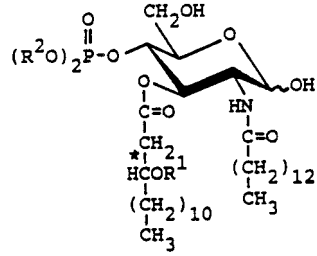


	R^1	R^2
4	H	H
5	C_{14}-OBom	H
6	C_{14}	H
7	C_{14}-OBom	C_{14}-O-C_{12}
8	C_{14}-OBom	C_{14}-O-C_{16}
9	C_{14}	C_{14}-O-C_{12}
10	C_{14}	C_{14}-O-C_{14}
11	C_{14}	C_{14}-O-C_{16}

	R^1	R^2	R^3	R^4
12	C_{14}-OBom	C_{12}	H	H
13	C_{14}-OBom	C_{12}	H	Ph_3C
14	C_{14}-OBom	C_{12}	$(PhO)_2PO$	Ph_3C
15	C_{14}-OBom	C_{16}	H	H
16	C_{14}-OBom	C_{16}	H	Ph_3C
17	C_{14}-OBom	C_{16}	$(PhO)_2PO$	Ph_3C
18	C_{14}	C_{12}	H	H
19	C_{14}	C_{12}	H	Ph_3C
20	C_{14}	C_{12}	$(PhO)_2PO$	H
21	C_{14}	C_{14}	H	H
22	C_{14}	C_{14}	H	Ph_3C
23	C_{14}	C_{14}	$(PhO)_2PO$	H
24	C_{14}	C_{16}	H	H
25	C_{14}	C_{16}	H	Ph_3C
26	C_{14}	C_{16}	$(PhO)_2PO$	H



	R^1	R^2
27	C_{12}	Ph
28	C_{16}	Ph
GLA-63(R,R)	C_{12}	H
GLA-64(R,R)	C_{16}	H



	R^1	R^2
29	C_{12}	Ph
30	C_{14}	Ph
31	C_{16}	Ph
GLA-67(R)	C_{12}	H
GLA-68(R)	C_{14}	H
GLA-69(R)	C_{16}	H

C_{14}-O-C_n = $CH_3(CH_2)_{10}$*$CHCH_2CO$ (O-C_n)

C_{14}-OBom = $CH_3(CH_2)_{10}$*$CHCH_2CO$ (OCH_2OCH_2Ph)

FIG. 2

influence of the amide-bound (3R)-3-dodecanoyloxy- or (3R)-3-hexadecanoyloxy-tetradecanoyl group (C_{14}-O-C_{12} or C_{14}-O-C_{16}). We now describe the synthesis of some homologs of GLA-60 [GLA-63(R,R), GLA-64(R,R), GLA-67(R), GLA-68(R) and GLA-69(R); see FIG. 2] which respectively carry the ester-bound C_{14}-O-C_{12}, C_{14}-O-C_{14} or C_{14}-O-C_{16} group.

RESULTS AND DISCUSSION

(3R)-3-(Acyloxy)tetradecanoic acids (1-3) have been prepared *via* the phenacyl esters of (3R)-3-hydroxytetradecanoic acid as described previously.[3] The 3-O-esterifications of benzyl 2-[(3R)-3-(benzyloxymethoxy)tetradecanamido]-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranoside (5)[3] with 1 and 3, and of benzyl 2-deoxy-4,6-O-isopropylidene-2-tetradecanamido-β-D-glucopyranoside (6)[1a] with 1-3 were accomplished by use of 3-[(dimethylamino)propyl]-1-ethylcarbodiimide hydrochloride (WSC) and a catalytic amount of 4-dimethylaminopyridine (DMAP). The resulting compounds 7-11 were each converted, by hydrolytic removal of the isopropylidene group, to 12, 15, 18, 21 and 24. The primary hydroxyl groups of 12 and 15 were tritylated, to give 13 and 16, and then the diphenylphosphono group was introduced at O-4 by treatment with diphenyl phosphorochloridate in a mixture of pyridine-dichloromethane-DMAP, to afford 14 and 17, respectively. Similarly, compounds 18, 21 and 24 were each tritylated at O-6 and then phosphorylated at O-4. The products were, after a brief purification, treated with tetrafluoroboric acid to give 20, 23 and 26 in high yields, respectively.

Hydrogenolytic deprotections of the benzyl, benzyloxymethoxy and trityl groups from 14 and 17, or of the benzyl group from 20, 23 and 26 in the presence of palladium catalyst, gave 27-31, quantitatively.

In their ^{1}H NMR spectra, the anomeric protons respectively appeared as narrow doublets or near singlets at δ 5.27 (for 27), 5.29 (for 28 and 29), 5.28 (for 30) and 5.30 (for 31), and four axial protons (H-2~5) were definitely assigned. This result indicates

that the α-D-pyranose form of all these compounds preponderates in the equilibrium mixture in chloroform-d.

The phenyl groups were finally cleaved by hydrogenolysis in the presence of platinum catalyst, to afford the desired GLA-63(R,R), GLA-64(R,R), GLA-67(R), GLA-68(R) and GLA-69(R) as colorless powders, which were clearly positive to the specific spray-reagent[10] for the phosphono group.

These subunit analogs of lipid A are classified into the two subhomologous series, one being GLA-63(R,R), GLA-60(R,R) and GLA-64(R,R), and the other, GLA-67(R), GLA-68(R) and GLA-69(R). Since it has already been found[8,11] that GLA-57 is active as GLA-27, while GLA-58 is almost inactive, the importance of the chain-length of the C_{14}-O-(acyl) group, or the biological influence of the hydroxyl group at C-3 of the 3-hydroxytetradecanoyl moiety might become more clear by comparing biological activities in each series. The comprehensive investigation on the structure-activity relationships of the nonreducing-sugar subunit of lipid A may also contribute toward the elucidation of the action mechanism[12-14] of bacterial endotoxin against the immunocompetent cells such as B lymphocytes, macrophages and neutrophils.

EXPERIMENTAL

General Procedures. Melting points were determined with a Yanagimoto melting-point apparatus and are uncorrected. Specific rotations were determined with a Union PM-201 polarimeter, and IR spectra were recorded with a Jasco IRA-1 or IR-100 spectrophotometer. ^{1}H NMR spectra were recorded at 60 and 270 MHz with Hitachi R-24B and JEOL JNM-GX270 spectrometers, respectively. Preparative chromatography was performed on silica gel (Wako Co., 200 or 300 mesh) with the solvent systems (v/v) specified.

Benzyl 2-Deoxy-4,6-O-isopropylidene-2-tetradecanamido-β-D-glucopyranoside (6). To a solution of 4[1b] (0.6 g) in dichloromethane (4 mL) were added tetradecanoic acid (0.44 g) and WSC (0.6 g), and the mixture was stirred for 4.5 h at room temperature

and processed in the usual manner. The product was purified by chromatography on a column of silica gel with 400:1 dichloromethane-methanol, to give 6[1a] (0.95 g; 94.2%): mp 117-119 °C, $[\alpha]_D$ -67° (c 0.5, chloroform); IR (Nujol) 3600-3100 (OH, NH), and 1640 and 1545 cm^{-1} (amide); ^1H NMR (60 MHz, $CDCl_3$) δ 0.88 (near t, 3H, Me), 1.0-1.75 (m, 28H, $-CH_2-$ and CMe_2), 1.95-2.4 (m, 2H, $-COCH_2-$), and 7.25 (s, 5H, Ph).

Anal. Calcd for $C_{30}H_{49}NO_6$ (519.72): C, 69.33; H, 9.50; N, 2.70. Found: C, 69.52; H, 9.38; N, 2.65.

Benzyl 2-[(3R)-3-(Benzyloxymethoxy)tetradecanamido]-2-deoxy-3-O-dodecanoyloxytetradecanoyl-4,6-O-isopropylidene-β-D-glucopyranoside (7) and Benzyl 2-[(3R)-3-(Benzyloxymethoxy)tetradecanamido]-2-deoxy-3-O-hexadecanoyloxytetradecanoyl-4,6-O-isopropylidene-β-D-glucopyranoside (8). To a solution of 5[3a] (0.65 g) in dichloromethane (5 mL) were added 1[3b] (0.42 g), WSC (0.28 g) and a catalytic amount of DMAP, and the mixture was stirred for 5 h at room temperature, the reaction being monitored by TLC (1:1 hexane-ethyl acetate). The product was purified by chromatography on a column of silica gel with 3:1 hexane-ethyl acetate, to give 7 in 80% yield, which crystallized from ether: mp 77.5-79 °C, $[\alpha]_D$ -23° (c 0.4, dichloromethane); IR (Nujol) 3500-3300 (NH), 1750, 1730 (ester), 1670, 1540 (amide), 860 (Me_2C), and 780-690 cm^{-1} (Ph); ^1H NMR data were quite similar to those of the corresponding intermediate of GLA-60(R,R)[3a] except for the number of methylene protons.

Anal. Calcd for $C_{64}H_{105}NO_{11}$ (1064.49): C, 72.21; H, 9.94; N, 1.32. Found: C, 72.39; H, 10.13; N, 1.36.

Compound 8 was obtained by treatment of 5 (0.65 g) with (3R)-3-hexadecanoyloxytetradecanoic acid[3b] (3; 0.475 g) as described for 7, and crystallized from ether: mp 71-72 °C, $[\alpha]_D$ -22° (c 0.7, dichloromethane); IR (Nujol) 3500-3300 (NH), 1750, 1730 (ester), 1670, 1540 (amide), 860 (Me_2C), and 780-690 cm^{-1} (Ph); ^1H NMR data were similar to those of 7 except for the number of methylene protons.

Anal. Calcd for $C_{68}H_{113}NO_{11}$ (1120.58): C, 72.88; H, 10.16; N, 1.25. Found: C, 73.04; H, 9.08; N, 1.13.

Benzyl 2-[(3R)-3-(Benzyloxymethoxy)tetradecanamido]-2-deoxy-4-O-diphenylphosphono-3-O-[(3R)-3-dodecanoyloxytetradecanoyl]-6-O-trityl-β-D-glucopyranoside (14). Hydrolytic removal of the isopropylidene group from 7, as previously described,[1b] gave 12, quantitatively: IR (film) 3700-3100 (OH, NH), 1730 (ester), 1650, 1550 (amide), and complete loss of the peak at 860 cm^{-1} (Me_2C).

A solution of 12 (0.42 g) in pyridine (5 mL) was stirred at 90 °C, and then trityl chloride (0.23 g) was added; stirring was continued for 6 h at 90 °C. The mixture was cooled, methanol was added to decompose excess reagent, and then concentrated. After extractive processing, the product was purified by chromatography on a column of silica gel with 300:1 dichloromethane-methanol to afford 13 (0.42 g; 82%): $[\alpha]_D$ -13° (c 0.9, chloroform); IR (film) 3500 (OH), 3280 (NH), 3100-3040 (Ph), 1740 (ester), 1650, 1550 (amide) and 740-680 cm^{-1} (Ph); ^1H NMR (270 MHz, $CDCl_3$) δ 0.88 (near t, 9H, Me), 1.0-1.9 (m, 58H, $-CH_2-$), 2.1-2.6 (m, 6H, $-COCH_2-$), 3.0 (d, 1H, $J_{4,OH}$ = 3.3 Hz, OH-4), 3.2-3.5 (m, 3H, H-5,6,6'), 3.68 (m, 1H, H-4), 3.92 (m, 1H, H-3 of C_{14}-OBom), 4.10 (near q, 1H, H-2), 4.27 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 4.4-4.95 (m, 7H, $-OCH_2O-$, $C\underline{H}_2Ph$ and H-3), 5.12 (m, 1H, H-3 of C_{14}-O-C_{12}), 6.09 (d, 1H, J = 9.2 Hz, NH), and 6.9-7.7 (m, 25H, Ph).

A mixture of compound 13 (0.4 g), diphenyl phosphorochloridate (0.25 g) and DMAP (69 mg) in 4:3 dichloromethane-pyridine (3.5 mL) was stirred overnight at room temperature, methanol was added, and the solvents were evaporated off. The product was purified by chromatography on a column of silica gel to give 14 (0.4 g; 85%) as amorphous: $[\alpha]_D$ -4.3° (c 0.9, chloroform); IR (film) 3300 (NH), 3100-3000 (Ph), 1730 (ester), 1660, 1530 (amide), 940 (P-O-Ph), and 770-660 cm^{-1} (Ph); ^1H NMR (270 MHz, $CDCl_3$) δ 4.5-5.0 (m, 8H, H-1,4, $-OCH_2O-$ and $C\underline{H}_2Ph$), 5.23 (near t, 1H, H-3), and 6.8-7.5 (m, 35H, Ph).

Anal. Calcd for $C_{92}H_{124}NO_{14}P$ (1498.97): C, 73.72; H, 8.34; N, 0.93. Found: C, 73.51; H, 8.25; N, 0.89.

Benzyl 2-[(3R)-3-(Benzyloxymethoxy)tetradecanamido]-2-deoxy-4-O-diphenylphosphono-3-O-[(3R)-3-hexadecanoyloxytetradecanoyl]-6-O-trityl-β-D-glucopyranoside (17). Compound 15 was prepared by deisopropylidenation from 8: mp 104-106 °C, $[\alpha]_D$ -14° (c 0.5, chloro-

form). 6-O-Tritylation of 15 (0.7 g) was conducted as described for 13, to afford amorphous 16 (0.68 g; 80%): $[\alpha]_D$ -10° (c 1, chloroform); ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.0 (d, 1H, $J_{4,OH}$ = 3.6 Hz, OH-4), 3.2-3.5 (m, 3H, H-5,6,6'), 3.68 (m, 1H, $J_{3,4} = J_{4,5}$ = 9.2 Hz, H-4), 3.92 (m, 1H, H-3 of C_{14}-OBom), 4.11 (near q, 1H, H-2), 4.28 (d, 1H, J = 8.4 Hz, H-1), and 7.1-7.6 (m, 25H, Ph).

Compound 16 (0.6 g) was treated with diphenyl phosphorochloridate as described for 14 to give 17 in 85% yield: $[\alpha]_D$ -3° (c 1, chloroform); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (near t, 9H, Me), 1.0-1.8 (m, 66H, $-CH_2-$), 2.1-2.4 (m, 6H, $-COCH_2-$), 3.36 (dd, 1H, J_{gem} = 10, $J_{5,6}$ = 6.2 Hz, H-6), 3.4-3.6 (m, 2H, H-5,6'), 3.9-4.05 (m, 2H, H-2 and H-3 of C_{14}-OBom), 4.5-5.0 (m, 8H, H-1,4, $-OCH_2O-$ and $C\underline{H}_2Ph$), 5.11 (m, 1H, H-3 of C_{14}-O-C_{12}), 5.23 (near t, 1H, H-3), 6.24 (d, J = 8.8 Hz, NH), and 6.8-7.5 (m, 35H, Ph).

Anal. Calcd for $C_{96}H_{132}NO_{14}P$ (1555.08): C, 74.15; H, 8.56; N, 0.90. Found: C, 74.40; H, 8.39; N, 0.92.

Benzyl 2-Deoxy-4-O-diphenylphosphono-2-tetradecanamido-3-O-[(3R)-3-dodecanoyloxytetradecanoyl]-β-D-glucopyranoside (20). To a solution of 6 (0.65 g) in dichloromethane (5 mL) were added 1 (0.42 g), dicyclohexylcarbodiimide (DCC, 0.28 g) and a catalytic amount of DMAP, and the mixture was processed as described for 7. The resulting 9 was treated with tetrafluoroboric acid[15] in acetone, to give 18 (64% in two steps): $[\alpha]_D$ -21° (c 1, dichloromethane); IR (film) 3480, 3300 (OH, NH), 1740 (ester), and 1670 and 1560 cm^{-1} (amide); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (t, 9H, Me), 1.0-1.7 (m, 60H, $-CH_2-$), 2.0-2.65 (m, 6H, $-COCH_2-$), 3.44 (m, 1H, H-5), 3.70 (near t, 1H, J = 9.2-9.5 Hz, H-4), 3.78-3.95 (2dd, 2H, H-6,6'), 4.02 (near q, 1H, H-2), 4.57, 4.83 (2d, 2H, $C\underline{H}_2Ph$), 4.60 (d, 1H, J = 8.4 Hz, H-1), 5.03 (t, 1H, H-3), 5.12 (m, 1H, H-3 of C_{14}-O-C_{12}), 6.06 (d, 1H, NH), and 7.28 (near s, 5H, Ph).

Tritylation of 18 gave 19 {$[\alpha]_D$ -21° (c 1.6, dichloromethane)}, which was then converted to 20 by the usual way[1b]: $[\alpha]_D$ -19° (c 0.4, dichloromethane); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (t, 9H, Me), 1.0-1.7 (m, 60H, $-CH_2-$), 2.0-2.4 (m, 6H, $-COCH_2-$), 3.1 (broad s, 1H, OH), 3.3-3.8 (m, 4H, H-2,5,6,6'), 4.59, 4.87 (2d, 2H, J_{gem} = 12 Hz,

$C\underline{H}_2Ph$), 4.73 (q, 1H, $J_{3,4} = J_{4,5} = J_{4,P} = 9.5$ Hz, H-4), 5.03 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1), 5.12 (m, 1H, H-3 of C_{14}-O-C_{12}), 5.57 (near t, 1H, H-3), 5.85 (d, 1H, NH), and 7.0-7.4 (m, 15H, Ph).

Anal. Calcd for $C_{65}H_{102}NO_{12}P$ (1120.46): C, 69.67; H, 9.18; N, 1.25. Found: C, 69.43; H, 9.27; N, 1.24.

Benzyl 2-Deoxy-4-O-diphenylphosphono-2-tetradecanamido-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (23). Esterification of 6 (0.8 g) with 2[3a] (0.7 g) in the presence of DCC (0.44 g) and a catalytic amount of DMAP as described for 20, to give 10 (70-80%), and hydrolytic removal of the isopropylidene group afforded 21: $[\alpha]_D$ -21° (c 0.4, chloroform); ^1H NMR (270 MHz, $CDCl_3$) δ 0.88 (t, 9H, Me), 1.0-1.7 (m, 64H, $-CH_2-$), 2.0-2.65 (m, 6H, $-COCH_2-$), 3.43 (m, 1H, H-5), 4.02 (near q, 1H J = 9 Hz, H-2), 4.59 (d, 1H, J = 8.4 Hz, H-1), 4.59, 4.84 (2d, 1H, $C\underline{H}_2Ph$), 5.00 (dd, 1H, J = 10.6 and 8.8 Hz, H-3), 5.10 (m, 1H, H-3 of C_{14}-O-C_{14}), 5.87 (d, 1H, J = 9.2 Hz, NH), and 7.27 (near s, 5H, Ph).

Tritylation of 21, to give 22 {$[\alpha]_D$ -21° (c 1, dichloromethane)}, from which the title compound 23 was obtained (59% in three steps): mp 99-102 °C, $[\alpha]_D$ -22° (c 1, dichloromethane); IR (film) 3480, 3300 (OH, NH), 1750 (ester), 1660, 1580 (amide), 965 (P-O-Ph), and 760 cm^{-1} (Ph).

Anal. Calcd for $C_{67}H_{106}NO_{12}P$ (1148.57): C, 70.07; H, 9.30; N, 1.22. Found: C, 69.86; H, 9.28; N, 1.13.

Benzyl 2-Deoxy-4-O-diphenylphosphono-3-O-[(3R)-3-hexadecanoyloxytetradecanoyl]-2-tetradecanamido-β-D-glucopyranoside (26). Compound 24 was prepared via 11 from 6 by the same sequence described for 20 and 23 in 64% yield; mp 127-129 °C, $[\alpha]_D$ -20° (c 0.9, chloroform); IR (film) 3480, 3300 (OH, NH), 1740 (ester), and 1660 and 1550 cm^{-1} (amide).

Tritylation of 24 gave 25 in 80% yield {mp 113-116 °C, $[\alpha]_D$ -23° (c 0.9, chloroform)}, from which the title compound 26 was obtained as previously described: mp 103-107 °C, $[\alpha]_D$ -22° (c 0.9, chloroform); IR (film) 3500, 3280 (OH, NH), 1740 (ester), 1650, 1560 (amide), and 970 cm^{-1} (P-O-Ph).

Anal. Calcd for $C_{69}H_{110}NO_{12}P$ (1176.56): C, 70.43; H, 9.42; N, 1.19. Found: C, 70.71; H, 9.24; N, 1.16.

2-Deoxy-4-O-diphenylphosphono-3-O-[(3R)-3-dodecanoyloxytetradecanoyl]-2-[(3R)-3-hydroxytetradecanamido]-D-glucose (27) and 2-Deoxy-4-O-diphenylphosphono-3-O-[(3R)-3-hexadecanoyloxytetradecanoyl]-2-[(3R)-3-hydroxytetradecanamido]-D-glucose (28). To a solution of 14 (0.35 g) in 1:1:1 ethanol-methanol-benzene (30 mL) was added palladium-black catalyst (0.5 g), and the mixture was stirred for 2 days in a hydrogen atmosphere. The catalyst was filtered off, and washed with chloroform. The filtrate and washings were combined, and concentrated to a syrup that was chromatographed on a column of silica gel with 50:1 dichloromethane-methanol, to give 27 (0.22 g, 90%): mp 121-122 °C, $[\alpha]_D$ +0.7° (c 0.6, chloroform); IR (KBr) 3600-3150 (OH, NH), 3050 (Ph), 1740 (ester), 1640, 1540 (amide), 960 (P-O-Ph), and 770-680 (Ph); ^{1}H NMR for the α-anomer (270 MHz, $CDCl_3$) δ 0.88 (near t, 9H, Me), 1.0-1.6 (m, 58H, $-CH_2-$), 2.0-2.5 (m, 6H, $-COCH_2-$), 4.70 (q, 1H, $J_{3,4} = J_{4,5} = J_{4,P} = 9.5$ Hz, H-4), 5.10 (m, 1H, H-3 of C_{14}-O-C_{12}), 5.27 (broad s, 1H, H-1), and 7.1-7.4 (m, 10H, Ph).

Anal. Calcd for $C_{58}H_{96}NO_{13}P$ (1046.37): C, 66.58; H, 9.25; N, 1.34. Found: C, 66.34; H, 9.38; N, 1.34.

Compound 28 was obtained from 17 in nearly quantitative yield as described for 27: mp 117-118 °C, $[\alpha]_D$ +0.7° (c 0.6, chloroform); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (near t, 9H, Me), 1.0-1.7 (m, 66H, $-CH_2-$), 2.05-2.45 (m, 6H, $-COCH_2-$), 4.02 (near d, 1H, $J_{4,5} = 10.3$ Hz, H-5), 4.22 (m, 1H, H-2), 4.71 (q, 1H, J = 9.5 Hz, H-4), 5.10 (m, 1H, H-3 of C_{14}-O-C_{16}), 5.29 (broad s, 1H, H-1), 5.49 (t, 1H, J = 10 Hz, H-3), 6.52 (d, 1H, J = 8.8 Hz, NH), and 7.0-7.5 (m, 10H, Ph).

Anal. Calcd for $C_{62}H_{104}NO_{13}P$ (1102.48): C, 67.55; H, 9.51; N, 1.27. Found: C, 67.28; H, 9.66; N, 1.31.

2-Deoxy-4-O-diphenylphosphono-3-O-[(3R)-3-dodecanoyloxytetradecanoyl]-2-tetradecanamido-D-glucose (29), 2-Deoxy-4-O-diphenylphosphono-2-tetradecanamido-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-D-glucose (30) and 2-Deoxy-4-O-diphenylphosphono-3-O-[(3R)-3-hexadecanoyloxytetradecanoyl]-2-tetradecanamido-D-glucose (31). Compounds 20, 23 and 26 were each hydrogenolyzed in the presence of

palladium catalyst as described for 27 and 28, to afford 29, 30 and 31 in nearly quantitative yields, respectively.

Compound 29 had $[\alpha]_D$ +5.4° (c 0.37, dichloromethane): ^{1}H NMR data for the α anomer (270 MHz, $CDCl_3$) δ 0.88 (t, 9H, Me), 1.0-1.7 (m, 60H, $-CH_2-$), 2.05-2.45 (m, 6H, $-COCH_2-$), 4.02 (near d, 1H, J = 10.6 Hz, H-5), 4.23 (m, 1H, H-2), 4.73 (near q, 1H, J = 9.5 Hz, H-4), 5.12 (m, 1H, H-3 of C_{14}-O-C_{12}), 5.29 (narrow d, 1H, J = 3 Hz, H-1), 5.48 (t, 1H, J = 10 Hz, H-3), 6.11 (d, 1H, J = 8.8 Hz, NH), and 7.1-7.4 (m, 10H, Ph).

Anal. Calc. for $C_{58}H_{96}NO_{12}P$ (1030.37): C, 67.61; H, 9.39; N, 1.36. Found: C, 67.90; H, 9.53; N, 1.28.

Compound 30 had mp 80-83 °C, $[\alpha]_D$ +9.3° (c 0.8, dichloromethane): ^{1}H NMR data for the α anomer (270 MHz, $CDCl_3$) δ 0.88 (t, 9H, Me), 1.0-1.7 (m, 64H, $-CH_2-$), 2.05-2.45 (m, 6H, $-COCH_2-$), 3.61 (near s, 2H, H-6,6'), 4.01 (near d, 1H, J = 10 Hz, H-5), 4.22 (m, 1H, H-2), 4.73 (q, 1H, $J_{3,4} = J_{4,5} = J_{4,P}$ = 9.5 Hz, H-4), 5.12 (m, 1H, H-3 of C_{14}-O-C_{14}), 5.28 (near s, 1H, H-1), 5.48 (t, 1H, J = 10 Hz, H-3), 6.10 (d, 1H, J = 8.4 Hz, NH), and 7.1-7.45 (m, 10H, Ph).

Anal. Calcd for $C_{60}H_{100}NO_{12}P$ (1058.44): C, 68.09; H, 9.52; N, 1.32. Found: C, 68.35; H, 9.37; N, 1.24.

Compound 31 had mp 88-91 °C, $[\alpha]_D$ +9.6° (c 1.5, dichloromethane): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (t, 9H, Me), 1.0-1.7 (m, 68H, $-CH_2-$), 2.05-2.45 (m, 6H, $-COCH_2-$), 3.61 (near s, 2H, H-6,6'), 4.01 (near d, 1H, J = 10 Hz, H-5), 4.23 (m, 1H, H-2), 4.74 (q, 1H, J = 9.5 Hz, H-4), 5.12 (m, 1H, H-3 of C_{14}-O-C_{16}), 5.30 (near s, 1H, H-1), 5.49 (near t, 1H, J = 9-10 Hz, H-3), 6.07 (d, 1H, J = 8.4 Hz, NH), and 7.1-7.45 (m, 10H, Ph).

Anal. Calcd for $C_{62}H_{104}NO_{12}P$ (1086.48): C, 68.54; H, 9.65; N, 1.29. Found: C, 68.30; H, 9.84; N, 1.27.

2-Deoxy-3-O-[(3R)-3-dodecanoyloxytetradecanoyl]-2-[(3R)-3-hydroxytetradecanamido]-4-O-phosphono-D-glucose [GLA-63(R,R)] and 2-Deoxy-3-O-[(3R)-3-hexadecanoyloxytetradecanoyl]-2-[(3R)-3-hydroxytetradecanamido]-4-O-phosphono-D-glucose [GLA-64(R,R)]. Platinum dioxide (70 mg) was 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 27 (72 mg) in methanol or 28 (70 mg) in 1:1 methanol-ethanol (20 mL), respectively. Hydrogen was gently bubbled through for 1 h, with stirring, and the mixture was further stirred overnight in a hydrogen atmosphere. The catalyst was filtered off, and washed with methanol-chloroform. The filtrate and washings were combined, and the solvents were evaporated off. The residue was dissolved in 1,4-dioxane and lyophilized, to give GLA-63(R,R) (58 mg; 93%) or GLA-64(R,R) (55 mg; 91%), respectively, as colorless fine powders, which gave positive tests with the specific spray-reagent[10] for the phosphono group.

GLA-63(R,R) had mp 156-157 °C, $[\alpha]_D$ +14° (c 0.114, dimethylsulfoxide; the value varied from +28° to -10° for the initial 10 min.); IR (KBr) 3700-3050 (OH, NH), 1710 (ester), 1640, 1550 (amide), and complete loss of the peak at 960 cm^{-1} (P-O-Ph); ^1H NMR (270 MHz, 3:2 CD_3OD-$CDCl_3$) δ 0.89 (near t, 9H, Me), 1.0-1.7 (m, 58H, $-CH_2-$), 2.2-2.75 (m, 6H, $-COCH_2-$), 5.1-5.2 (m, 2H, H-1, and H-3 of C_{14}-O-C_{12}), 5.32 (t, 1H, J = 10 Hz, H-3), and complete loss of the phenyl protons.

Anal. Calcd for $C_{46}H_{88}NO_{13}P$ (894.15): C, 61.79; H, 9.92; N, 1.57. Found: C, 62.10; H, 10.13; N, 1.36.

GLA-64(R,R) had mp 147-149 °C, $[\alpha]_D$ +25° (c 0.121, dimethylsulfoxide); IR (KBr) same as those of GLA-63(R,R).

Anal. Calcd for $C_{50}H_{96}NO_{13}P$ (950.26): C, 63.19; H, 10.18; N, 1.47. Found: C, 63.53; H, 10.39; N, 1.21.

2-Deoxy-3-O-[(3R)-3-dodecanoyloxytetradecanoyl]-4-O-phosphono-2-tetradecanamido-D-glucose [GLA-67(R)], 2-Deoxy-4-O-phosphono-2-tetradecanamido-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-D-glucose [GLA-68(R)] and 2-Deoxy-3-O-[(3R)-3-hexadecanoyloxytetradecanoyl]-4-O-phosphono-2-tetradecanamido-D-glucose [GLA-69(R)]. Compounds 29, 30 and 31 (50 mg) were each hydrogenolyzed in the presence of platinum catalyst as described for GLA-63(R,R) and GLA-64(R,R), to afford the title compounds GLA-67(R), GLA-68(R) and GLA-69(R) as colorless powders in 93-98% yields. Since these compounds were es-

sentially insoluble in usual organic solvents, their accurate $[\alpha]_D$ values could not be measured.

GLA-67(R) had mp 128-130 °C; IR (KBr) 3700-3100 (OH, NH), 1730 (ester), 1650, 1550 (amide), and complete loss of the peak at 960 cm^{-1} (P-O-Ph).

Anal. Calcd for $C_{46}H_{88}NO_{12}P$ (878.15): C, 62.91; H, 10.10; N, 1.60. Found: C, 62.64; H, 9.81; N, 1.75.

GLA-68(R) had mp 150-151 °C; IR (KBr) same as those of GLA-67(R).

Anal. Calcd for $C_{48}H_{92}NO_{12}P$ (906.24): C, 63.62; H, 10.23; N, 1.55. Found: C, 63.30; H, 10.46; N, 1.28.

GLA-69(R) had mp 140-141 °C; IR (KBr) same as those of GLA-67(R).

Anal. Calcd for $C_{50}H_{96}NO_{12}P$ (934.26): C, 64.28; H, 10.36; N, 1.50. Found: C, 64.02; H, 10.27; N, 1.70.

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