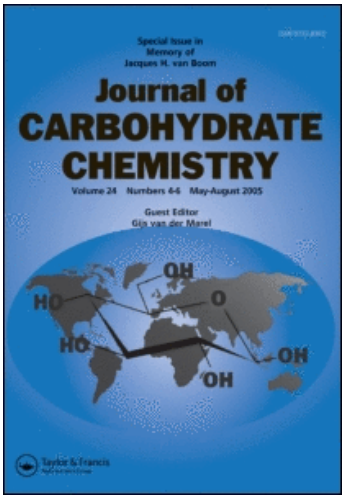

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Synthesis of 1,5-Anhydro- 2-deoxy- 4-*O*-phosphono-3-*O*-tetradecanoyl-2-[(3*R*)- and (3*S*)-3-Tetradecanoyloxytetradecanamido]-D-glucitol (GLA-40) Related to Bacterial Lipid A

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SYNTHESIS OF 1,5-ANHYDRO-2-DEOXY-4-O-PHOSPHONO-3-O-TETRADECANOYL-2-[(3R)- AND (3S)-3-tetradecanoyloxytetradecanamido]-D-GLUCITOL (GLA-40) RELATED TO BACTERIAL LIPID A

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ABSTRACT

The diastereoisomeric pair of a biologically active, lipid A-subunit analogs named in the title have been synthesized, starting from 2-amino-1,5-anhydro-2-deoxy-4,6-O-isopropylidene-D-glucitol and optically active 3-hydroxytetradecanoic acid.

INTRODUCTION

In our continuing, synthetic efforts[1] to elucidate the molecular requirements for manifestation of the biological activities of lipid A, which is the endotoxic principle of the bacterial lipopolysaccharide[2], and to obtain new sources of nontoxic, biological-response modifiers (BRM), it was demonstrated[3] that a 4-O-phosphono-D-glucosamine derivative named GLA-27 and its homologs expressed several biological activities (B cell activation activities, and interferon- and tumor necrosis factor-induction activities etc.) similar to those of lipid A.

(3R)-3-Hydroxytetradecanoic acid is a prominent constituent of bacterial lipid A. A recent study[4] on the biological influence of the asymmetric center (C-3) of the 3-hydroxytetradecanoyl group in GLA-27 has shown that the (S)-isomer (GLA-27-S) exhibited much

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stronger mediator-inducing activities than the (R)-isomer (GLA-27-R), but B cell activation activities were strongly expressed by GLA-27-R and weakly expressed by GLA-27-S. We here describe the synthesis of the title diastereoisomeric pair (GLA-40-R and GLA-40-S), which respectively carry the amide-bound (3R)- and (3S)-3-tetradecanoyloxytetradecanoyl group at N-2 of the 2-amino-1,5-anhydro-2-deoxy-D-glucitol backbone.

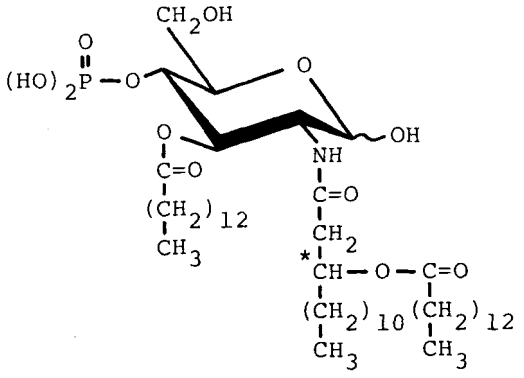
RESULTS AND DISCUSSION

N-Deacetylation of 2-acetamido-1,5-anhydro-2-deoxy-4,6-O-isopropylidene-D-glucitol[5] with barium hydroxide gave crystalline 2 in high yield. Treatment of 2 with (3R)- or (3S)-3-hydroxytetradecanoic acid[1] in the presence of dicyclohexylcarbodiimide gave the corresponding 1,5-anhydro-2-deoxy-2-[(3R)- or -(3S)-3-hydroxytetradecanamido]-4,6-O-isopropylidene-D-glucitol (3R or 3S), in which the optical purity of 3-hydroxytetradecanoic acid was >95% (for R) and ca. 80% (for S), respectively.

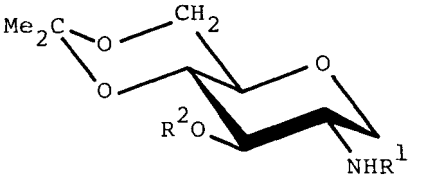
The remaining secondary hydroxyl groups of 3R or 3S were simultaneously esterified with tetradecanoyl chloride in pyridine, to give 4R or 4S, respectively, which was then treated with 80% aqueous acetic acid to hydrolyze the isopropylidene group. The resulting 5R or 5S was crystallized from ethanol-ether, to afford the optically pure samples of diastereoisomeric, 1,5-anhydro-2-deoxy-3-O-tetradecanoyl-2-[(3R)- and -(3S)-3-tetradecanoyloxytetradecanamido]-D-glucitol. The primary hydroxyl group of 5R or 5S was tritylated, and then the diphenylphosphono group was introduced at O-4 as described[1] previously, to give 7R or 7S, respectively.

Hydrogenolytic removal of the trityl group of 7R or 7S in the presence of palladium catalyst gave 8R or 8S in near quantitative yield, respectively. Finally, the phenyl groups were cleaved by hydrogenolysis in the presence of pre-reduced, Adams' platinum catalyst, to afford the desired, title compounds 9R (GLA-40-R) and 9S (GLA-40-S) as colorless powders.

The comparison of their biological activities may provide use-



GLA-27



1 R^1 = Ac , R^2 = H

2 R^1 = R^2 = H

3R,3S

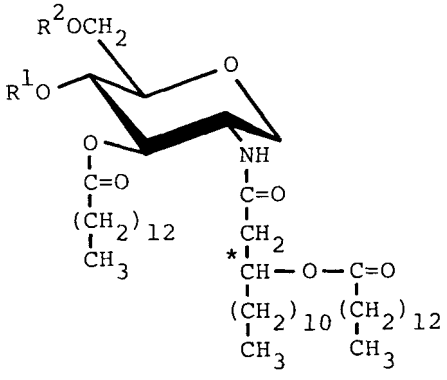
R^1 = $COCH_2\overset{*}{C}H(CH_2)_{10}CH_3$ (OH)

R^2 = H

4R,4S

R^1 = $COCH_2\overset{*}{C}H(CH_2)_{10}CH_3$ ($OCO(CH_2)_{12}CH_3$)

R^2 = $CO(CH_2)_{12}CH_3$

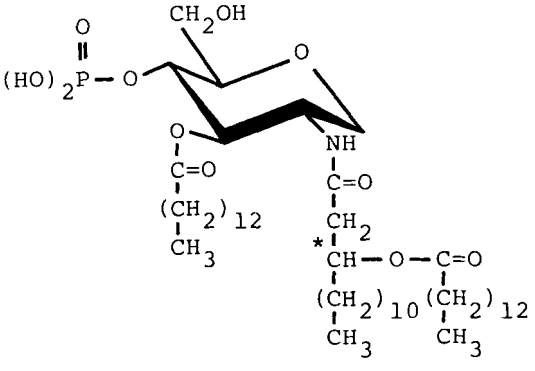


5R,5S R^1 = R^2 = H

6R,6S R^1 = H , R^2 = CPh_3

7R,7S R^1 = $P(OPh)_2$ (=O) , R^2 = CPh_3

8R,8S R^1 = $P(OPh)_2$ (=O) , R^2 = H



9R (GLA-40-R)

9S (GLA-40-S)

ful informations about the biological importance of the chirality in the 3-hydroxytetradecanoyl group, as well as of the hydroxyl group at C-1 of the sugar backbone.

EXPERIMENTAL

General Procedures. See ref. 1b.

2-Amino-1,5-anhydro-2-deoxy-4,6-O-isopropylidene-D-glucitol (2). A mixture of 2-acetamido-1,5-anhydro-2-deoxy-4,6-O-isopropylidene-D-glucitol[5] (8.62 g), water (100 ml) and barium hydroxide octahydrate (17.5 g) was stirred for 24 h under reflux. Methanol was added, and the precipitates were filtered off through Celite, and washed with a small amount of ethanol. The filtrate and washings were combined, and concentrated; the resulting precipitates were again removed by filtration. The solvents were evaporated, and the residue was chromatographed on a column of silica gel (Wakogel C-200) with 50:1 chloroform-methanol. The product crystallized from ether, to give 2 (5.82 g; 82%); mp 132-138°, $[\alpha]_D$ -7.8° (c 0.9, chloroform); IR (Nujol): 3390, 3310, 1600 (NH_2), 3170 (OH), and 850 cm^{-1} (CMe_2); NMR data (90 MHz, $CDCl_3$): δ 1.43, 1.50 (2 s, 6 H, CMe_2), 2.25-2.45 (broad s, 3 H, NH_2 and OH), and 2.7-4.1 (m, 8 H, ring protons).

Anal. Calc. for $C_9H_{17}NO_4$ (203.23): C, 53.19; H, 8.43; N, 6.89. Found: C, 53.50; H, 8.21; N, 6.80.

1,5-Anhydro-2-deoxy-2-[(3R)-3-hydroxytetradecanamido]-4,6-O-isopropylidene-D-glucitol (3R) and 1,5-anhydro-2-deoxy-2-[(3S)-3-hydroxytetradecanamido]-4,6-O-isopropylidene-D-glucitol (3S). To a solution of 2 (0.5 g) in dry 1,4-dioxane (15 ml) were added, portionwise, 1.08 g of (3R)- or (3S)-3-hydroxytetradecanoic acid [optical purity; >95% (R) and ca. 80% (S), respectively][1] and dicyclohexylcarbodiimide (DCC; 1.26 g), and the mixture was stirred overnight at room temperature. DCC-urea was removed by filtration, and washed with a small amount of 1,4-dioxane. The filtrate and washings were combined, and concentrated to a residue that was chromatographed on a column of silica gel (Wakogel C-200) with 100:1 and then 50:1 chloroform-methanol, to give a syrup of 3R (0.91 g; 86%) or 3S

(0.87 g; 82%), which was lyophilized from 1,4-dioxane solution.

Compound 3R had mp 62-65°, $[\alpha]_D$ -17.3° (c 0.648, chloroform); IR (KBr): 3700-3100 (OH, NH), 1620, 1550 (amide), and 840 cm^{-1} (CMe_2); NMR data (270 MHz, $CDCl_3$): δ 0.88 (t, 3 H, Me), 1.0-2.0 (m, 20 H, $-CH_2-$), 1.43, 1.48 (2 s, 6 H, CMe_2), 2.29, 2.44 (2 dd, 2 H, J_{gem} 15, $J_{2',3'}$ 9 and 3 Hz, $-COCH_2-$), 3.10-4.20 (m, 10 H, ring protons, H-3' and OH), and 6.09 (broad s, 1 H, NH).

Compound 3S had mp 101-108°, $[\alpha]_D$ -3.7° (c 0.6, chloroform); IR (KBr): 3650-3100 (OH, NH), 1640, 1540 (amide), and 850 cm^{-1} (CMe_2); NMR data were very similar to those of 3R.

1,5-Anhydro-2-deoxy-4,6-O-isopropylidene-3-O-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-D-glucitol (4R) and 1,5-anhydro-2-deoxy-4,6-O-isopropylidene-3-O-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]-D-glucitol (4S). To a cooled solution of 3R or 3S (0.63 g) in dry pyridine (3 ml) containing 4-dimethylaminopyridine (0.27 g) was added myristoyl chloride (1.08 g) dissolved in dry dichloromethane (3 ml), and the mixture was stirred overnight at room temperature. Methanol was added, and then the mixture was concentrated. After extractive processing, the product was purified by chromatography on a column of silica gel (Wakogel C-200) with 200:1 chloroform-methanol, to give a syrup of 4R (1.13 g; 91%) or 4S (1.04 g; 83%), which was then lyophilized from 1,4-dioxane solution.

Compound 4R had mp 43-45°, $[\alpha]_D$ -6.3° (c 0.768, chloroform); IR (KBr): 3350 (NH), 1720, 1730 (ester), 1660, 1520 (amide), and 850 cm^{-1} (CMe_2); NMR data (270 MHz, $CDCl_3$): δ 0.88 (t, 9 H, Me), 1.1-1.75 (m, 64 H, $-CH_2-$), 1.37, 1.47 (2 s, 6 H, CMe_2), 2.2-2.5 (m, 6 H, $-COCH_2-$), 3.11 (~t, 1 H, $J_{gem} \simeq J_{1a,2}$ 12-13 Hz, H-1a), 3.24 (m, 1 H, $J_{4,5} \simeq J_{5,6a}$ 9-10, $J_{5,6e}$ 5 Hz, H-5), 3.70 (t, 1 H, $J_{3,4} \simeq J_{4,5}$ 9.5 Hz, H-4), 3.71 (t, 1 H, H-6a), 3.91 (dd, 1 H, H-6e), 4.0-4.25 (m, 2 H, H-1e and H-2), 4.88 (t, 1 H, $J_{2,3} \simeq J_{3,4}$ 9.5 Hz, H-3), 5.10 (m, 1 H, H-3 of the 3-tetradecanoyloxytetradecanoyl group), and 6.00 (d, 1 H, J 7 Hz, NH).

Anal. Calc. for $C_{51}H_{95}NO_8$ (850.28): C, 72.04; H, 11.26; N, 1.65. Found: C, 72.30; H, 11.46; N, 1.59.

Compound 4S had mp 58-60°, $[\alpha]_D$ -7.4° (c 0.95, chloroform); IR (KBr): 3280 (NH), 1730 (ester), 1650, 1520 (amide), and 850 cm^{-1} (CMe_2); NMR data (270 MHz, $CDCl_3$) were similar to those of 4R except for minor differences in the chemical shift.

Anal. Calc. for $C_{51}H_{95}NO_8$ (850.28): C, 72.04; H, 11.26; N, 1.65. Found: C, 72.28; H, 11.39; N, 1.55.

1,5-Anhydro-2-deoxy-3-O-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-D-glucitol (5R) and 1,5-anhydro-2-deoxy-3-O-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]-D-glucitol (5S). A mixture of 4R (0.65 g) or 4S (0.94 g), 80% acetic acid (20-30 ml) and chloroform (5 ml) was stirred for 5-6 h at 45°; the reaction being monitored by t.l.c. (50:1 chloroform-methanol). The mixture was concentrated at 45°, and the residue was chromatographed on a column of silica gel (Wakogel C-200) with 400:1 chloroform-methanol. The product crystallized from ethanol-ether or ethanol only, to give 5R (0.51 g; 83%) or 5S (0.63 g; 70%), respectively.

Compound 5R had mp 106-107°, $[\alpha]_D$ -1.2° (c 0.528, chloroform); IR (KBr): 3450 (OH), 3300 (NH), 1730 (ester), and 1650 and 1560 cm^{-1} (amide); NMR data (270 MHz, $CDCl_3$): δ 0.88 (t, 9 H, Me), 1.1-1.45, 1.45-1.75 (m, 58 H + 6 H, $-CH_2-$), 1.75-2.2 (broad s, 2 H, OH), 2.2-2.5 (m, 6 H, $-COCH_2-$), 3.12 (t, 1 H, $J_{gem} \simeq J_{1a,2}$ 10-11 Hz, H-1a), 3.31 (m, 1 H, $J_{4,5}$ 9.5 Hz, H-5), 3.75 (t, 1 H, $J_{3,4} \simeq J_{4,5}$ 9.2-9.5 Hz, H-4), 3.80 (dd, 1 H, J_{gem} 11.7, $J_{5,6}$ 4.4 Hz, H-6), 3.90 (dd, 1 H, $J_{5,6'}$ 3-4 Hz, H-6'), 4.07 (m, 1 H, H-2), 4.11 (dd, 1 H, J_{gem} 10.6 $J_{1e,2}$ 5.1 Hz, H-1e), 4.82 (~t, 1 H, H-3), 5.09 (m, 1 H, H-3 of the 3-tetradecanoyloxytetradecanoyl group), and 6.05 (d, 1 H, J 7.3 Hz, NH).

Anal. Calc. for $C_{48}H_{91}NO_8$ (810.22): C, 71.15; H, 11.32; N, 1.73. Found: C, 71.33; H, 11.24; N, 1.77.

Compound 5S had mp 90.5-91.5°, $[\alpha]_D$ -1.8° (c 0.758, chloroform); IR and NMR data were similar to those of 5R.

Anal. Calc. for $C_{48}H_{91}NO_8$ (810.22): C, 71.15; H, 11.32; N, 1.73. Found: C, 71.29; H, 11.28; N, 1.73.

1,5-Anhydro-2-deoxy-3-O-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-6-O-trityl-D-glucitol (6R) and 1,5-anhydro-2-

deoxy-3-O-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]-6-O-trityl-D-glucitol (6S). A solution of 4R or 4S (0.5 g) in dry pyridine (10 ml) was stirred at 90°, and then trityl chloride (0.23 g) was added; stirring was continued for 3 h at 90°, the mixture was cooled, and methanol was added in order to decompose the excess of the reagent; then it was concentrated. After extractive processing, the product was purified by chromatography on a column of silica gel (Wakogel C-200) with 500:1 chloroform-methanol, and then lyophilized from 1,4-dioxane solution, to give 6R (0.52 g; 80%) and 6S (0.5 g; 77%), respectively.

Compound 6R had mp 39-40°, $[\alpha]_D$ -5.3° (c 0.378, chloroform); IR (film): 3480 (OH), 3280 (NH), 1730, 1715 (ester), 1650, 1575 (amide), and 715 cm^{-1} (Ph); NMR data (270 MHz, $CDCl_3$): δ 2.69 (broad s, 1 H, OH), 3.39 (m, 2 H, H-6,6'), 5.98 (d, 1 H, NH), 7.2-7.5 (m, 15 H, Ph), and other signals were similar to those of 5R.

Compound 6S was a syrup, $[\alpha]_D$ -7.1° (c 0.34, chloroform); IR (film): 3700-3200 (OH, NH), 1730 (ester), 1660, 1550 (amide), and 700 cm^{-1} (Ph); NMR data (270 MHz, $CDCl_3$) were similar to those of 6R except for δ 2.73 (~s, 1 H, OH) and 6.21 (d, 1 H, NH).

1,5-Anhydro-2-deoxy-4-O-diphenylphosphono-3-O-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-6-O-trityl-D-glucitol (7R) and 1,5-anhydro-2-deoxy-4-O-diphenylphsphono-3-O-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]-6-O-trityl-D-glucitol (7S). A mixture of 6R or 6S (0.45 g), 4-dimethylaminopyridine (78 mg) and dry pyridine (3 ml) was stirred at 0°, while diphenyl phosphorochloridate (0.345 g) dissolved in dry dichloromethane (3 ml) was added; stirring was continued overnight at room temperature. Methanol was added, and then the mixture was concentrated to a syrup. After extractive processing and chromatographic purification as previously described[1], the product was lyophilized from 1,4-dioxane solution, to give 7R (0.44 g; 80%) and 7S (0.43 g; 78%), respectively.

Compound 7R had mp 52-53°, $[\alpha]_D$ +6.5° (c 1.08, chloroform); IR (Nujol): 3280 (NH), 1730 (ester), 1640, 1550 (amide), 950 (P-O-Ph), and 700 cm^{-1} (Ph); NMR data (270 MHz, $CDCl_3$): δ 4.78 (q, 1 H, $J_{3,4} \simeq$

$J_{4,5} \simeq J_{4,p}$ 9.2 Hz, H-4), and 6.85-7.55 (m, 25 H, Ph).

Compound 7S was a syrup, $[\alpha]_D$ +6° (c 0.313, chloroform); IR and NMR data were similar to those of 7R.

1,5-Anhydro-2-deoxy-4-O-diphenylphosphono-3-O-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-D-glucitol (8R) and 1,5-anhydro-2-deoxy-4-O-diphenylphosphono-3-O-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]-D-glucitol (8S). To a solution of 7R or 7S (0.35 g) in 2:1 ethanol-methanol (30 ml) was added palladium-black catalyst prepared from palladium chloride (0.1 g), and the mixture was stirred in a hydrogen atmosphere. After completion of the reaction (t.l.c., 30:1 chloroform-methanol), the mixture was treated in the usual way. Chromatographic purification (Wakogel C-200) with 200:1 chloroform-methanol afforded a syrup of 8R or 8S in near quantitative yield.

Compound 8R crystallized from ethanol-ether, mp 67-69°, $[\alpha]_D$ -11.3° (c 0.566, chloroform); IR (KBr): 3380, 3230 (OH, NH), 1720 (ester), 1640, 1550 (amide), 950 (P-O-Ph), and 710 and 680 cm^{-1} (Ph); NMR data (270 MHz, $CDCl_3$): δ 3.15 (~t, 1 H, $J_{gem} \simeq J_{1a,2}$ 12-13 Hz, H-1a), 3.34 (~d, 1 H, $J_{4,5}$ 8.8 Hz, H-5), 3.57 (dd, 1 H, J_{gem} 13 Hz, H-6), 3.69 (~d, 1 H, H-6'), 4.75 (q, 1 H, $J_{3,4} \simeq J_{4,5} \simeq J_{4,p}$ 9.2-9.5 Hz, H-4), 5.07 (m, 1 H, H-3 of the 3-tetradecanoyloxytetradecanoyl group), 5.16 (t, 1 H, $J_{2,3} \simeq J_{3,4}$ 9-10 Hz, H-3), and 7.05-7.4 (m, 10 H, Ph).

Anal. Calc. for $C_{60}H_{100}NO_{11}P$ (1042.39): C, 69.13; H, 9.67; N, 1.34. Found: C, 69.27; H, 9.64; N, 1.32.

Compound 8S was a syrup, $[\alpha]_D$ -9.1° (c 0.679, chloroform); IR and NMR data were similar to those of 8R.

Anal. Calc. for $C_{60}H_{100}NO_{11}P$ (1042.39): C, 69.13; H, 9.67; N, 1.34. Found: C, 68.96; H, 9.54; N, 1.29.

1,5-Anhydro-2-deoxy-4-O-phosphono-3-O-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-D-glucitol (9R; GLA-40-R) and 1,5-anhydro-2-deoxy-4-O-phosphono-3-O-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]-D-glucitol (9S; GLA-40-S). Platinum dioxide (50 mg) was suspended in ethanol, and hydrogen was bubbled through for 15 min, while the solution was stirred at room temperature. The

resulting precipitates were collected by decantation and washed twice with ethanol, and then the precipitates were added to a solution of 8R or 8S (0.1 g) in 2:1 ethanol-methanol (30 ml). Hydrogen was gently bubbled through for 1 h, with stirring, and the mixture was further stirred overnight under hydrogen. The catalyst was filtered off, and washed with ethanol-methanol; the filtrate and washings were combined, and concentrated in vacuo. The resulting 9R or 9S gave a positive test with the specific spray-reagent[6] for the phosphono group; it was homogeneously suspended in 1,4-dioxane by sonication, and lyophilized, to give colorless, fine powders of 9R (GLA-40-R) (83 mg; 97%) and 9S (GLA-40-S) (81 mg; 95%), respectively.

Compound 9R had mp 175-177°, $[\alpha]_D$ +2.6° (c 0.38, 2:1 chloroform-methanol); IR (KBr): 3680-2500 (OH, NH and CH_2), 1720 (ester), and 1620 and 1540 cm^{-1} (amide); NMR data (270 MHz, $CDCl_3$+CD_3OD): δ 0.88 (t, 9 H, Me), 1.0-1.8 (m, 64 H, $-CH_2-$), 2.15-2.5 (m, 6 H, $-COCH_2-$), and complete loss of the peaks at 7-7.4 (Ph).

Anal. Calc. for $C_{48}H_{92}NO_{11}P$ (890.20): C, 64.76; H, 10.42; N, 1.57. Found: C, 64.42; H, 10.22; N, 1.43.

Compound 9S had mp 110-112°, $[\alpha]_D$ +2.8° (c 0.36, 2:1 chloroform-methanol): IR and NMR data were similar to those of 9R.

Anal. Calc. for $C_{48}H_{92}NO_{11}P$ (890.20): C, 64.76; H, 10.42; N, 1.57. Found: C, 64.39; H, 10.26; N, 1.45.

GLA-40-S (9S) was more soluble in organic solvents than GLA-40-R (9R) similarly to the diastereoisomeric pair of GLA-27[1b]. Because of the broadening of the signals in the ^{1}H-NMR spectra of 9R and 9S, the phosphoric group at O-4 of 9S was esterified again with diazomethane in ether solution, to give the corresponding 1,5-anhydro-2-deoxy-4-O-dimethylphosphono-3-O-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]-D-glucitol in quantitative yield; NMR data (270 MHz, $CDCl_3$): δ 3.75, 3.77, 3.79, 3.81 [2 d, 6 H, $P-(OMe)_2$]; these methyl groups are magnetically non-equivalent as observed for GLA-27[1b], 4.46 (q, 1 H, $J_{3,4} \simeq J_{4,5} \simeq J_{4,p}$ 9.5 Hz, H-4), and 5.92 (d, 1 H, J 7.3 Hz, NH).

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