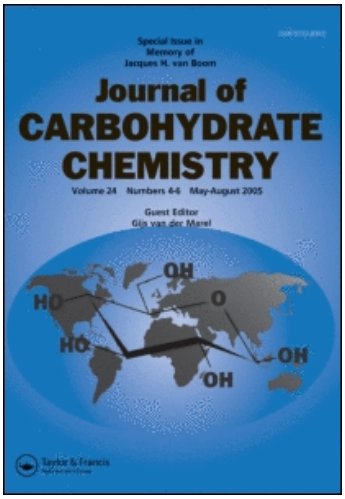

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The Chemical Modification of the C-1 Substituent of A 4-O-Phosphono-O-Glucosamine Derivative (GLA-27) Related to Bacterial Lipid A

Yuji Ogawa[a]; Yushun Fujishima[a]; Isako Konishi[a]; Makoto Kiso[a]; Akira Hasegawa[a]
[a] Department of Agricultural Chemistry, Gifu University, Gifu, Japan

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THE CHEMICAL MODIFICATION OF THE C-1 SUBSTITUENT OF A 4-O-PHOSPHONO-D-GLUCOSAMINE DERIVATIVE (GLA-27) RELATED TO BACTERIAL LIPID A

Yuji Ogawa, Yushun Fujishima, Isako Konishi,
Makoto Kiso, and Akira Hasegawa

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

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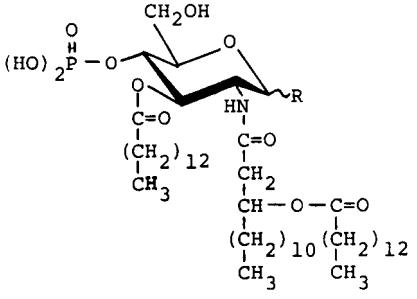
ABSTRACT

1-Thio and 1-O-phosphono derivatives (9-11 and 15) of 2-deoxy-4-O-phosphono-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-D-glucose (GLA-27-RS), which exhibits some of the beneficial biological activities of bacterial endotoxin, have been synthesized from benzyl 2-deoxy-4,6-O-isopropylidene-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside (1), and 2-deoxy-4-O-diphenylphosphono-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-D-glucopyranose (12), respectively.

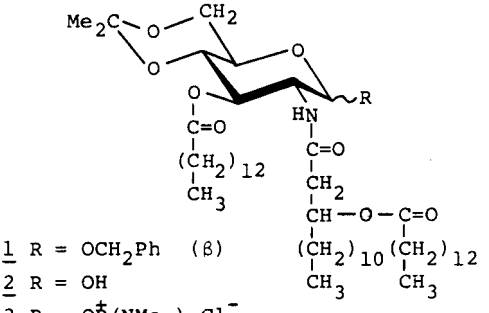
INTRODUCTION

Since the discovery of a biologically active 4-O-phosphono-D-glucosamine derivative named GLA-27[1], which is an analog of the nonreducing-sugar subunit of bacterial lipid A[2], a variety of its analogs have been synthesized[3] and their biological activities



GLA - 27 R = OH

GLA - 40 R = H

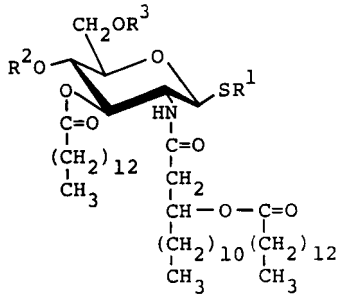


1 R = OCH_2Ph (β)

2 R = OH

3 R = $O\overset{+}{P}(NMe_2)_3Cl^-$

4 R = SAc (β)

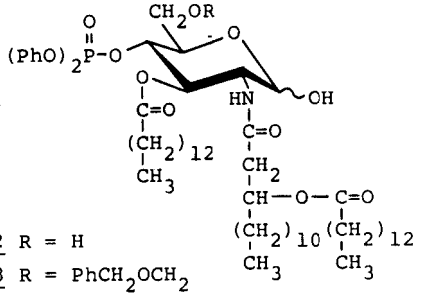


	R^1	R^2	R^3
5	AC	H	H
6	AC	H	TCEC
7	AC	$(TCEO)_2PO$	TCEC
8	Myr	$(TCEO)_2PO$	TCEC
9	AC	$(HO)_2PO$	H
10	Myr	$(HO)_2PO$	H
11	H	$(HO)_2PO$	H

TCEC = CCl_3CH_2OCO

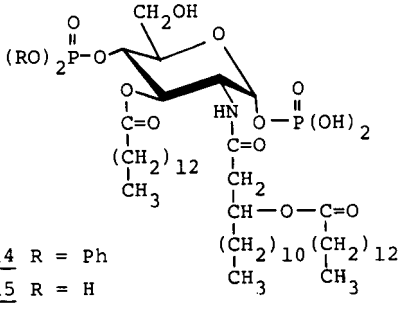
TCE = CCl_3CH_2

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



12 R = H

13 R = $PhCH_2OCH_2$



14 R = Ph

15 R = H

investigated.[4] In this course, 1,5-anhydro-2-deoxy-4-O-phosphono-3-O-tetradecanoyl-2-[(3R) and (3S)-3-tetradecanoyloxytetradecanamido]-D-glucitol (GLA-40)[3c] has been found[4,5] as an immunologically active molecule similar to GLA-27, suggesting that the chemical modification of the C-1 substituent might be possible with retention of the beneficial biological activities. We have also found that 1-thio analogs[6] of N-acetylmuramoyl dipeptide (MDP)[7] exhibit potent immunopharmacological activities showing the effectiveness of the introduction of the sulfur atom at the C-1 position of the D-glucosamine skeleton. On the other hand, the toxic effect of the phosphoric group at the C-1 position of the natural lipid A has also been noted.[8] We now describe the synthesis of the 1-thio and 1-O-phosphono derivatives (9-11 and 15) of GLA-27-RS as part of a study to elucidate the relationship between C-1 substitution and the biological activity.

RESULTS AND DISCUSSION

Hydrogenolytic removal of the benzyl group of benzyl-2-deoxy-4,6-O-isopropylidene-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside[1a] (1) in the presence of palladium-on-carbon catalyst in ethanol gave 2 in quantitative yield. A solution of 2 in dichloromethane was stirred with carbon tetrachloride and hexamethylphosphorous triamide at -60 °C, to give phosphonium salt 3, which was then treated with potassium thioacetate at -60 °C, to afford 1-S-acetyl-2-deoxy-4,6-O-isopropylidene-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-1-thio-β-D-glucopyranose (4) in 52% yield (based on 2). In the ^{1}H NMR spectrum of 4, the 1-S-acetyl protons appeared as a three proton singlet at δ 2.34 and the anomeric proton as a doublet ($J_{1,2}$ = 10.5 Hz) at δ 5.09, showing that the configuration of the SAc group at the C-1 position is β. Hydrolytic removal of the 4,6-O-isopropylidene group of 4 with tetrafluoroboric acid in acetone gave 5, which was treated with 2,2,2-trichloroethoxycarbonyl chloride in pyridine at -60 °C. The resulting 6-O-(2,2,2-trichloroethoxycarbonyl) derivative 6 was then phosphorylated at O-4 with bis(2,2,2-trichloroethyl)-phosphorochloridate, to afford 1-S-acetyl-4-O-[bis(2,2,2-trichloro-

ethyl)phosphono]-2-deoxy-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxy tetradecanamido]-1-thio-6-O-(2,2,2-trichloroethoxycarbonyl)-β-D-glucopyranose (7) in 68% yield.

For the synthesis of the 1-S-tetradecanoyl derivative, 1-S-acetyl group in 7 was selectively solvolyzed with a slight excess of sodium methoxide in 1:1 ethanol-dichloromethane at -30°C. The reaction mixture was deionized with a cation-exchange resin and then treated with tetradecanoic acid in the presence of dicyclohexylcarbodiimide (DCC) in dichloromethane to give 8 in 66% yield.

Simultaneous deprotection of the 2,2,2-trichloroethoxycarbonyl and bis(2,2,2-trichloroethyl)phosphono groups in 7 and 8, in the presence of zinc dust in acetic acid, afforded the desired 1-S-acetyl-2-deoxy-4-O-phosphono-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-1-thio-β-D-glucopyranose (9) and its 1-S-tetradecanoyl derivative (10) in 76 and 61% yield, respectively. Selective solvolysis of the 1-S-acetyl group in 9 afforded 2-deoxy-4-O-phosphono-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-1-thio-β-D-glucopyranose (11) in 90% yield.

Treatment of 2-deoxy-4-O-diphenylphosphno-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-D-glucopyranose[1a] (12), which is a synthetic intermediate of GLA-27, with benzyl chloromethyl ether in the presence of N,N-diisopropylethylamine in dichloromethane at room temperature gave the 6-O-benzyloxymethyl derivative 13 in 61% yield. The anomeric hydroxyl group was phosphorylated[9] by treatment with butyllithium and dibenzyl phosphorochloridate in THF at -70°C, and then the dibenzylphosphono group was immediately subjected to hydrogenolysis with palladium black catalyst to afford 14. Finally, hydrogenolytic deprotection of the diphenyl group in 14 in the presence of pre-reduced, Adams' platinum catalyst, afforded the desired 2-deoxy-1,4-di-O-phosphono-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-D-glucopyranose (15) in 90% yield.

Comparison of the biological activities of 9-11 and 15 may provide useful information about the biological importance of the hydrophilic-lypophilic balance at the C-1 position of the sugar skeleton.

EXPERIMENTAL

General procedures. Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Specific rotations were determined with a Union PM-201 polarimeter, and IR spectra were recorded with a Jasco A-100 spectorophotometer. ^{1}H NMR spectra were recorded with a Jeol JNM-GX 270 spectrometer. Preparative chromatography on silica gel (Wako Co.: 200 mesh) was accomplished with the solvent systems specified. Concentrations and evaporations were conducted in vacuo.

2-Deoxy-4,6-O-isopropylidene-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-D-glucopyranose (2). To a solution of benzyl 2-deoxy-4,6-O-isopropylidene-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside[1a] (1, 5.2 g) in ethanol (100 mL) was added 10% palladium-on-carbon catalyst (1 g) that had been pre-activated and washed well with methanol; the mixture was stirred overnight in a hydrogen atmosphere. The catalyst was filtered off, washed with benzene, and the filtrate and washings were combined, and concentrated. The residue was chromatographed on a column of silica gel with 200:1 dichloromethane-methanol to give 2 (4.5 g, 98%): mp 120-122°, $[\alpha]_D$ -2° (c 1.1, chloroform); IR (KBr) 3360 (OH, NH), 1740 (ester), 1650, 1530 (amide), and 860 cm^{-1} (Me_2C); ^{1}H NMR ($CDCl_3$) δ 0.8-1.0 (t, 9H, Me), 1.0-1.7 (m, 64H, $-CH_2-$), 1.36, 1.47 (2s, 6H, Me_2C), 2.0-2.5 (m, 6H, $-COCH_2-$), 3.35 (m, 1H, H-5), and complete loss of the peaks at 7.2-7.4 (m, 5H, ph).

Anal. Calcd for $C_{51}H_{95}NO_9$ (866.31): C, 70.71; H, 11.05; N, 1.62. Found: C, 70.67; H, 11.04; N, 1.51.

1-S-Acetyl-2-deoxy-4,6-O-isopropylidene-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-1-thio-β-D-glucopyranose (4). A mixture of 2 (1.2 g), carbon tetrachloride (0.9 g) and dichloromethane (30 mL) was stirred at -60°C, and then a solution of hexamethylphosphorous triamide (0.62 g) in dichloromethane (15 mL) was added; the mixture was stirred for 3 h at -60°C. Potassium thioacetate (0.87 g) was added, and stirring was continued overnight at room temperature. The mixture was extracted with chloroform, and the extract was washed with ice-cold 2M hydrochloric acid and water, dried, and concentrated. The residual syrup was chromatographed on a

column of silica gel with 8:1 hexane-ethyl acetate, to give the title compound 4 (0.65 g, 52%), which crystallized from methanol: mp 77-79°, $[\alpha]_D$ -2.4° (c 1.1, chloroform); IR (KBr) 3400 (NH), 1750 (ester), 1700 (SAc), 1660, 1550 (amide), and 860 cm^{-1} (Me_2C); ^1H NMR ($CDCl_3$) δ 0.8-1.0 (t, 9H, Me), 1.1-1.7 (m, 64H, $-CH_2-$), 1.36, 1.46 (2s, 6H, Me_2C), 2.2-2.5 (m, 6H, $-COCH_2-$), 2.34 (s, 3H, SAc), 3.49 (m, 1H, H-5), 3.74 (m, 2H, H-4,6a), 3.94 (dd, 1H, J_{gem} = 10.5, $J_{5,6b}$ = 5.1 Hz, H-6b), 4.30 (near q, 1H, $J_{1,2} = J_{2,3} = J_{2,NH}$ = 9.8-10.5 Hz, H-2), 4.97-5.10 (m, 2H, H-3, and H-3 of the tetradecanoyloxytetradecanoyl group), 5.13, 5.15 (2d, 1H, $J_{1,2}$ = 10.5 Hz, H-1), and 5.95, 6.05 (2d, 1H, J = 9.8 Hz, NH).

Anal. Calcd for $C_{53}H_{97}NO_9S$ (924.42): C, 68.86; H, 10.58; N, 1.52. Found: C, 69.08; H, 10.45; N, 1.38.

1-S-Acetyl-2-deoxy-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-1-thio-β-D-glucopyranose (5). To a suspension of 4 (1.2 g) in acetone (50 mL) was added tetrafluoroboric acid (42% in water; 0.56 mL), and the mixture was vigorously stirred for 30 min at room temperature. Triethylamine (0.38 mL) was added, and then the solvent was evaporated. The residue was chromatographed on a column of silica gel with 200:1 dichloromethane-methanol. The product crystallized from ethanol, to afford 5 (1.1 g, 98%): mp 107°, $[\alpha]_D$ +7.8° (c 1.1, chloroform); IR (KBr) 3600 (OH), 3300 (NH), 1730 (ester), 1700 (SAc), and 1660, 1550 cm^{-1} (amide); ^1H NMR ($CDCl_3$) δ 0.8-1.0 (t, 9H, Me), 1.1-1.7 (m, 64H, $-CH_2-$), 2.1-2.8 (m, 8H, $-COCH_2-$, and OH), 2.36 (s, 3H, SAc), 3.60 (m, 1H, H-5), 3.73-3.94 (m, 3H, H-4,6), 4.23 (near q, 1H, $J_{1,2} = J_{3,2} = J_{2,NH}$ = 9.8-10.6 Hz, H-2), 5.06 (m, 2H, H-3, and H-3 of the tetradecanoyloxytetradecanoyl group), 5.20, 5.22 (2d, 1H, $J_{1,2}$ = 10.6 Hz, H-1), and 6.29, 6.35 (2d, 1H, J = 9.8 Hz NH).

Anal. Calcd for $C_{50}H_{93}NO_9S$ (884.35): C,67.91; H, 10.60; N, 1.58. Found: C, 67.96; H, 1.56; N, 1.53.

1-S-Acetyl-2-deoxy-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-1-thio-6-O-(2,2,2-trichloroethoxycarbonyl)-β-D-glucopyranose (6). A solution of 5 (1.4 g) in 1:1 dichloromthane-pyridine (25 mL) was stirred at -60°C, while 2,2,2-trichloroethoxycarbonyl chloride (0.5 g) was added; stirring was continued for 15 min at -60°C. Water was added, and then the mixture was concentrated. The residue was chromatographed on a column of a silica gel

with 8:1 hexane-ethyl acetate to give 6 (0.9 g, 54%) as a syrup, which was dissolved in 1,4-dioxane and the dioxane was removed by lyophilization leaving 6 as a solid: mp 87-88°, $[\alpha]_D$ +0.8° (c 0.9, chloroform); IR (KBr) 3500 (OH), 3360 (NH), 1750, 1740 (ester), 1700 (SAc), 1650, 1550 (amide), and 780-720 cm^{-1} (CCl_3); ^1H NMR ($CDCl_3$) δ 0.8-1.0 (t, 9H, Me), 1.1-1.6 (m, 64H, $-CH_2-$), 2.1-2.5 (m, 6H, $-COCH_2-$), 2.35 (s, 3H, SAc), 3.03 (broad s, 1H, OH), 3.60-3.85 (m, 2H, H-4,5), 4.25 (m, 1H, H-2), 4.51 (m, 2H, H-6), 4.77 (m, 2H, CCl_3CH_2-), 5.01-5.13 (m, 2H, H-3, and H-3 of the tetradecanoyloxytetradecanoyl group), 5.20, 5.22 (2d, 1H, $J_{1,2}$ = 10.6 Hz, H-1), and 6.10-6.30 (m, 1H, NH).

Anal. Calcd for $C_{53}H_{94}NO_{11}Cl_3S$ (1059.76): C, 60.07; H, 8.94; N, 1.32. Found: C, 60.17; H, 9.06; N, 1.44.

1-S-Acetyl-4-O-[bis(2,2,2-trichloroethyl)phosphono]-2-deoxy-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-1-thio-6-O-(2,2,2-trichloroethoxycarbonyl)-β-D-glucopyranose (7). A mixture of 6 (0.99 g), 4-dimethylaminopyridine (0.234 g) and 1:1 dichloromethane-pyridine (2 mL) was stirred at 0°C, while bis(2,2,2-trichloroethyl) phosphorochloridate (1.5 g) was added; stirring was continued overnight at room temperature. The mixture was extracted with chloroform, and the extract was washed with ice-cold 2M hydrochloric acid and water, dried, and concentrated to a syrup, which was chromatographed on a column of a silica gel with 8:1 hexane-ethyl acetate. The product 7 (0.9 g, 68%) was crystallized from methanol: mp 63-65°, $[\alpha]_D$ +6.7° (c 1.16, chloroform); IR (KBr) 3350(NH), 1750 (ester), 1710 (SAc), 1660, 1550 (amide), 910 (P-O-CH_2), and 780-720 cm^{-1} (CCl_3); ^1H NMR ($CDCl_3$) δ 0.8-1.0 (t, 9H, Me), 1.1-1.8 (m, 64H, $-CH_2-$), 2.2-2.5 (m, 6H, $-COCH_2-$), 2.36 (s, 3H, SAc), 3.91 (d, 1H, J = 9.9 Hz, H-5), 4.2-4.35 (m, 2H, H-2,6a), 4.47 (dd, 1H, J_{gem} = 10.5, $J_{5,6b}$ = 3.8 Hz, H-6b), 4.63 [m, 5H, H-4, and $(CCl_3CH_2O)_2P$], 4.76 (m, 2H, CCl_3CH_2OC), 5.02 (m, 2H, H-3, and H-3 of the tetradecanoyloxytetradecanoyl group), 5.20, 5.21 (2d, 1H, $J_{1,2}$ = 10.5 Hz, H-1), and 5.95, 6.04 (2d, 1H, J = 9.6 Hz, NH).

Anal. Calcd for $C_{57}H_{97}NO_{14}Cl_3PS$ (1402.54): C, 48.81; H, 6.97; N, 1.00. Found: C, 48.72; H, 6.83; N, 0.88.

4-O-[Bis(2,2,2-trichloroethyl)phosphono]-2-deoxy-3-O-tetradecanoyl-1-S-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-1-thio-6-O-(2,2,2-trichloroethoxycarbonyl)-β-D-glucopyranose (8). To a solution of 7 (0.1 g) in 1:1 ethanol-dichloromethane (10 mL) was added sodium methoxide (5 mg) at -30°C, and the mixture was stirred for 30 min at the same temperature and then treated with Amberlite IR 120 (H^+) resin to deionize the solution. The resin was filtered off, washed with chloroform, and the filtrate and washings were combined, and concentrated. The resulting 4-O-[bis(2,2,2-trichloroethyl)phosphono]-2-deoxy-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-1-thio-6-O-(2,2,2-trichloroethoxycarbonyl)-β-D-glucopyranose was treated with tetradecanoic acid (0.08 g) in the presence of dicyclohexylcarbodiimide (DCC; 0.15 g) in dichloromethane (7 mL). The mixture was stirred for 30 min at room temperature, and then concentrated. The residue was chromatographed on a column of silica gel with dichloromethane, to afford 8 (0.74 g, 66%) as a syrup: $[\alpha]_D$ +5.1° (c 0.74, chloroform); IR (film) 3300 (NH), 1770, 1740 (ester), 1710 (thioester), 1660, 1550 (amide), 910 (P-O-CH_2), and 780-720 cm^{-1} (CCl_3); ^1H NMR ($CDCl_3$) δ 0.8-1.0(m, 12H, Me), 1.1-1.8(m, 86H, -CH_2-), 2.2-2.5(m, 8H, -$COCH_2$-), 3.85 (d, 1H, J = 9.9 Hz, H-5), 4.15-4.36 (m, 3H, H-2,6), 4.53-4.66 (m, 7H, H-4, and CCl_3CH_2-), 5.02 (m, 1H, H-3 of the tetradecanoyloxytetradecanoyl group), 5.19 (d, 1H, $J_{1,2}$ = 10.6 Hz, H-1), 5.25 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 8.8 Hz, H-3), and 6.00, 6.06 (2d, 1H, J = 9.5 Hz, NH).

Anal. Calcd for $C_{69}H_{121}NO_{14}Cl_9PS$ (1570.87): C, 52.76; H, 7.76; N, 0.89. Found: C, 52.85; H, 7.70; N, 0.94.

1-S-Acetyl-2-deoxy-4-O-phosphono-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-1-thio-β-D-glucopyranose (9). To a solution of compound 7 (0.2 g) in acetic acid (5 mL) was added zinc dust (1 g), and the mixture was stirred overnight at room temperature. The suspension was filtered off, washed with chloroform, and the filtrate and washings were combined, and concentrated. The residual syrup was chromatographed on a column of silica gel with dichloromethane, to give the title compound 9 (0.1 g, 76%), which was dissolved in 1,4-dioxane and the dioxane was removed by lyophilization leaving 9 as a solid. It gave positive test for phosphate group using the phosphomolybdate spray reagent[10]: mp 194-195°, $[\alpha]_D$ +10.4°

(c 1.0, chloroform); IR (KBr) 3700-3000 (OH, NH), 1740 (ester), 1700 (SAc), 1660, 1540 (amide), and complete loss of the peaks at 910 ($P-O-CH_2$) and 780-720 cm^{-1} (CCl_3).

Anal. Calcd for $C_{50}H_{94}NO_{12}PS$ (964.33): C, 62.28; H, 9.82; N, 1.45. Found: C, 62.47; H, 9.70; N, 1.41.

Since the ^1H NMR spectrum of 9 showed some complexity (broadening, overlapping, etc), the phosphoric group at O-4 was esterified with diazomethane in ether solution, to give the corresponding 1-S-acetyl-2-deoxy-4-O-dimethylphosphono-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-1-thio-β-D-glucopyranose in quantitative yield: ^1H NMR ($CDCl_3$) δ 0.8-1.0 (t, 9H, Me), 1.1-1.8 (m, 64H, $-CH_2-$), 2.15-2.5 (m, 6H, $-COCH_2-$), 2.34 (s, 3H, SAc), 3.55-3.80 (m, 3H, H-5,6), 3.70, 3.73, 3.74, 3.78 [2d, 6H, $P-(OMe)_2$], 4.26 (q, 1H, H-2), 4.52 (q, 1H, H-4), 5.03 (m, 1H, H-3 of the tetradecanoyloxytetradecanoyl group), 5.15 (m, 2H, H-1,3), and 5.81, 5.89 (2d, 1H, NH).

2-Deoxy-4-O-phosphono-3-O-tetradecanoyl-1-S-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-1-thio-β-D-glucopyranose (10). Compound 10 was obtained in 61% yield as an amorphous material, according to the procedure described for 9, $[\alpha]_D$ +10.0° (c 0.2, chloroform); IR (film) 3600-3300 (OH, NH), 1740 (ester), 1720 (thioester), 1660, 1540 (amide), and complete loss of the peaks at 780-720 cm^{-1} (CCl_3).

Anal. Calcd for $C_{62}H_{118}NO_{12}PS$ (1132.65): C, 65.75; H, 10.50; N, 1.24. Found: C, 65.87; H, 10.37; N, 1.34.

2-Deoxy-4-O-phosphono-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-1-thio-β-D-glucopyranose (11). A solution of 9 (0.07 g) in 1:1 ethanol-dichloromethane (14 mL) was stirred at -30°C, and sodium methoxide (5 mg) was added; stirring was continued for 30 min at -30°C, and then Amberlite IR 120 (H^+) resin was added in order to deionize the solution. The resin was filtered off, and washed with chloroform. The filtrate and washings were combined, and concentrated to afford compound 11 (0.06 g, 90%) as a syrup, which was dissolved in 1,4-dioxane and the dioxane was removed by lyophilization leaving 11 as a solid: mp 206-208°, $[\alpha]_D$ +12.5° (c 0.8, chloroform); IR (KBr) 3700-3100 (OH, NH), 1740, 1720 (ester), and 1660, 1540 cm^{-1} (amide); ^1H NMR ($CDCl_3$) δ 0.8-1.0 (t, 9H, Me),

1.1-1.8 (m, 64H, $-CH_2-$), 2.1-2.5 (m, 6H, $-COCH_2-$), and complete loss of the peak at 2.34 (s, 3H, SAc).

Anal. Calcd for $C_{48}H_{92}NO_{11}PS$ (922.29): C, 62.51; H, 10.05; N, 1.52. Found: C, 62.63; H, 9.97; N, 1.49.

6-O-Benzyloxymethyl-2-deoxy-4-O-diphenylphosphono-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-D-glucopyranose (13). A mixture of 2-deoxy-4-O-diphenylphosphono-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-D-glucopyranose 12[1a] (0.1 g), N,N-diisopropylethylamine (0.2 mL), and dichloromethane (1 mL) was stirred at 0°C, while benzyl chloromethyl ether (0.1 g) was added; stirring was continued for 5 h at room temperature. The mixture was extracted with chloroform, and the extract was washed with ice-cold 2M hydrochloric acid and water, dried, and concentrated. The residue was chromatographed on a column of silica gel with 300:1 dichloromethane-methanol to afford 13 (0.07 g, 61%) as a syrup; $[\alpha]_D$ +13.5° (c 0.8, chloroform); IR (film) 3440 (OH), 3300 (NH), 1740 (ester), 1660, 1540 (amide), 960 (P-O-Ph), and 780-680 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) for the α anomer, δ 0.8-1.0 (t, 9H, Me), 1.0-1.75 (m, 64H, $-CH_2-$), 2.05-2.5 (m, 6H, $-COCH_2-$), 3.5-3.8 (m, 3H, H-5,6), 4.1-4.3 (m, 2H, H-2, and OH), 4.5-5.0 (m, 5H, H-4, $-OCH_2-$, and $-CH_2Ph$), 5.10 (m, 1H, H-3 of the tetradecanoyloxytetradecanoyl group), 5.21, 5.26 (2d, 0.7H, $J_{1,2}$ = 2.9 Hz, H-1), 5.47, 5.48 (2t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 6.04, 6.13 (2d, 1H, J = 8.6 Hz, NH), and 7.0-7.5 (m, 15H, Ph).

Anal. Calcd for $C_{68}H_{108}NO_{13}P$ (1178.57): C, 69.30; H, 9.24; N, 1.19. Found: C, 69.45; H, 9.40; N, 1.28.

2-Deoxy-4-O-diphenylphosphono-1-O-phosphono-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-D-glucopyranose (14). To a solution of 13 (0.116 g) in THF (5 mL), cooled to -60°C, was added butyllithium (1.6M in hexane; 0.077 mL), and after 4 min, a solution of dibenzyl phosphorochloridate (0.044 g) in THF (2 mL) was added; stirring was continued for 20 min at the same temperature. Palladium black catalyst, prepared from palladium chloride (0.05 g), was added and the mixture was stirred for 20 min in a hydrogen atmosphere. The catalyst was filtered off, and washed with dichloromethane. The filtrate and washings were combined, and directly chromatographed on a column of silica gel with 20:1 dichloromethane-methanol, to give

amorphous 14 (0.098 g, 88%), which gave positive test for phosphate group using the phosphomolybdate spray reagent: $[\alpha]_D$ +25.2° (c 0.32, chloroform); IR (film) 3400 (OH, NH), 1740, 1720 (ester), 1660, 1550 (amide), 960 (P-O-Ph), and 780-680 cm^{-1} (Ph).

Anal. Calcd for $C_{60}H_{101}NO_{15}P_2$ (1138.40): C, 63.30; H, 8.94; N, 1.23. Found: C, 63.41; H, 8.88; N, 1.34.

2-Deoxy-1,4-di-O-phosphono-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanamido]-D-glucopyranose (15). Platinum dioxide (0.05 g) was suspended in ethanol (5 mL), and hydrogen was bubbled through the suspension for 15 min. The resulting precipitate was filtered off, washed with ethanol and added to a solution of 14 (0.075 g) in 2:1 ethanol-methanol (15 mL). The mixture was stirred overnight in a hydrogen atmosphere. The catalyst was filtered off, and washed with 1:1 chloroform-methanol; the filtrate and washings were combined, and concentrated. The product 15 was suspended in 1,4-dioxane and the dioxane was removed by lyophilization to leave colorless powder (0.067 g, 90%): mp 159-161°, $[\alpha]_D$ +31.0° (c 0.67, chloroform); IR (KBr) 3400 (OH, NH), 1730 (ester), 1650, 1550 (amide), and complete loss of the peaks at 960 (P-O-Ph) and 780-680 cm^{-1} (Ph).

Anal. Calcd for $C_{48}H_{93}NO_{15}P_2$ (986.21): C, 58.46; H, 9.50; N, 1.42. Found: C, 58.64; H, 9.37; N, 1.38.

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