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Synthesis of a novel series of 4-*O*-phosphono-D-glucosamine derivatives (lipid A subunit analogs) carrying the *C*-branched 2-tetradecylhexadecanoyl group

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ABSTRACT

A novel series of 4-*O*-phosphono-D-glucosamine derivatives (lipid A subunit analogs) carrying the 3-*O*-linked or 2-*N*-linked *C*-branched 2-tetradecylhexadecanoyl group have been synthesized starting from benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside and benzyl 2-amino-6-*O*-benzyloxymethyl-2-deoxy-4-*O*-(diphenoxyphosphinyl)-β-D-glucopyranoside, respectively. Some of the new compounds were potent inducers of phagocytic activity in macrophages and showed antiviral activity against vaccinia virus.

INTRODUCTION

Lipid A (refs. 1,2), which is the hydrophobic component of bacterial lipopolysaccharide (LPS), exhibits not only toxicities but also many beneficial immunopharmacological activities. In order to elucidate the molecular requirements for manifestation of the biological activities a variety of 4-*O*-phosphono-D-glucosamine derivatives related to the nonreducing-sugar part of lipid A have been synthesized[3].

Among the synthetic compounds, GLA-59 and GLA-60, which have a 3-hydroxytetradecanoyl and a 3-tetradecanoyloxytetradecanoyl group (total of three acyl chains) occupying positions 2 and 3 of the glucosamine skeleton, had strong immunostimulatory activities[4] but no pyrogenicity. On the contrary, lipid X (ref. 5) and GLA-46, both carrying two 3-hydroxytetradecanoyl groups, did not show any activity[6,7]. In addition, the activity of GLA-47, which corresponds to the nonreducing-sugar subunit of natural lipid A and carries two 3-tetradecanoyloxytetradecanoyl groups (total of four acyl chains), was much weaker than that of GLA-59 or GLA-60. These results indicate that three fatty acyl chains, including one 3-acyloxyacyl group, are the required features for the expression of significant biological activities. Recently, it has been reported[8] that tissue toxicity is reduced after hydrolysis of the 3-acyloxyacyl group by human neutrophils.

We report here the synthesis of a novel series of 4-*O*-phosphono-D-glucosamine

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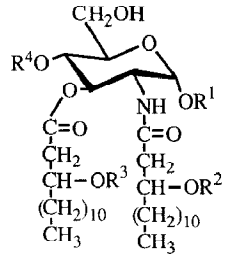


	R^1	R^2	R^3	R^4
Lipid X	Ⓟ	H	H	H
GLA-46	H	H	H	Ⓟ
GLA-47	H	C_{14}	C_{14}	Ⓟ
GLA-59	H	C_{14}	H	Ⓟ
GLA-60	H	H	C_{14}	Ⓟ

Ⓟ = $(HO)_2P(O)$

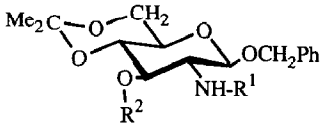
C_n = $CH_3(CH_2)_{n-2}C(O)$

derivatives (lipid A subunit analogs, **19–24** and **33–35**) containing the alkyl-branched 2-tetradecylhexadecanoyl group, which structurally resembles the ester-branched 3-acyloxyacyl group but is resistant to the hydrolytic enzymes encountered *in vivo*.

RESULTS AND DISCUSSION

Treatment of benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside[3] (**1**) with 2,2,2-trichloroethoxycarbonyl chloride gave the 2-(2,2,2-trichloroethoxycarbonyl)amino derivative (**2**) in 77% yield. Compound **2** was esterified with 2-tetradecylhexadecanoic acid[9] in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) and 4-dimethylaminopyridine (DMAP) to afford **3**. Hydrolytic removal of the isopropylidene group with aqueous acetic acid gave **4**, which after treatment with benzyl chloromethyl ether and 1,1,3,3-tetramethylurea in dichloromethane gave **5**. Phosphorylation of **5** at O-4 with diphenyl phosphorochloridate, and cleavage of the *N*-(2,2,2-trichloroethoxycarbonyl) group with zinc dust in acetic acid at 60° gave the useful intermediate **6** in 82% yield. Compound **6** was *N*-acylated to afford **7–12** in high yields. The benzyloxy and benzyloxymethoxy groups of **7–12** were simultaneously hydrogenolyzed over 10% palladium-on-carbon, giving **13–18**. The phenoxy groups were then cleaved by hydrogenolysis in the presence of platinum catalyst to obtain the desired compounds **19–24**.

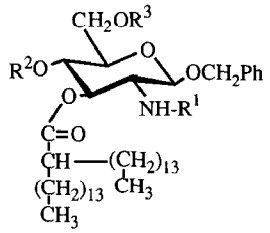
Compounds **33–35** were obtained by using benzyl 2-amino-6-*O*-benzyloxymethyl-2-deoxy-4-*O*-(diphenoxyphosphinyl)-β-D-glucopyranoside[10] (**25**) as a starting ma-



	R^1	R^2
1	H	H
2	TCEC	H
3	TCEC	TH

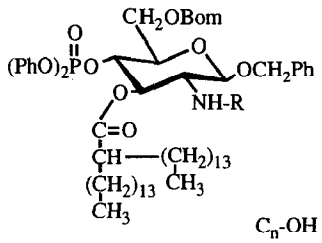
TCEC = $CCl_3CH_2OC(O)$

TH = $CH_3(CH_2)_{13}CHC(O)$ with $CH_3(CH_2)_{13}$



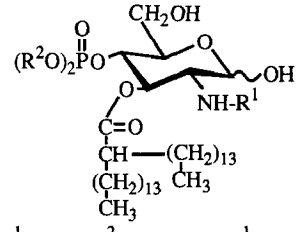
	R^1	R^2	R^3
4	TCEC	H	H
5	TCEC	H	Bom
6	H	$(PhO)_2P(O)$	Bom

Bom = $PhCH_2OCH_2$



	R
7	C_{12}
8	C_{14}
9	C_{16}
10	C_{12}-OH
11	C_{14}-OH
12	C_{16}-OH

C_n-OH = $CH_3(CH_2)_{n-4}CHCH_2C(O)$ with OH



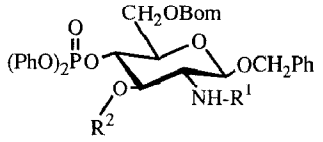
	R^1	R^2		R^1	R^2
13	C_{12}	Ph	**19**	C_{12}	H
14	C_{14}	Ph	**20**	C_{14}	H
15	C_{16}	Ph	**21**	C_{16}	H
16	C_{12}-OH	Ph	**22**	C_{12}-OH	H
17	C_{14}-OH	Ph	**23**	C_{14}-OH	H
18	C_{16}-OH	Ph	**24**	C_{16}-OH	H

terial. The amino group of **25** was first acylated with 2-tetradecylhexadecanoic acid, and then the remaining hydroxyl group was esterified with (3*RS*)-3-(benzyloxymethoxy)-dodecanoic acid[3d], (3*RS*)-3-(benzyloxymethoxy)tetradecanoic acid[3d], and (3*RS*)-3-(benzyloxymethoxy)hexadecanoic acid[3d], respectively, to give the corresponding **27**–**29**. The resulting compounds were each converted into the desired analogs by hydrogenolytic removal of the benzyl, benzyloxymethyl, and phenyl groups according to procedures detailed previously[3].

Compounds **22**, **23**, and **34**, obtained as just described, induced the phagocytic activity of peritoneal macrophages as strongly as GLA-59 and GLA-60. Antiviral activity against vaccinia virus was expressed by compounds **22**, **23**, **33**, and **34**, especially compound **22**, which showed higher activity than that of GLA-60. These results suggest that the naturally occurring, ester-branched 3-acyloxyacyl group can be replaced by alkyl-branched acyl groups with retention of the biological activities of lipid A.

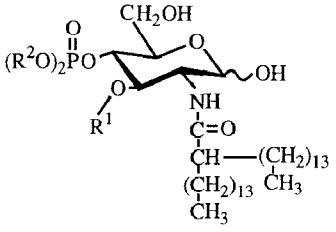
EXPERIMENTAL

General methods. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. All evaporations were conducted *in vacuo*. Preparative chromatography was performed on silica gel (Wako Co., 200 mesh) with the solvent systems specified. Specific rotations were determined with a Union



	R^1	R^2
25	H	H
26	TH	H
27	TH	C_{12}-OBom
28	TH	C_{14}-OBom
29	TH	C_{16}-OBom

C_n-OBom = $CH_3(CH_2)_{n-4}CHCH_2C(O)$
$PhCH_2OCH_2O$



	R^1	R^2
30	C_{12}-OH	Ph
31	C_{14}-OH	Ph
32	C_{16}-OH	Ph
33	C_{12}-OH	H
34	C_{14}-OH	H
35	C_{16}-OH	H

PM-201 polarimeter, and i.r. spectra were recorded with a Jasco A-100 spectrophotometer. ^{1}H-N.m.r. spectra were obtained with a Jeol JNM-GX 270 spectrometer.

*Benzyl 2-deoxy-4,6-*O*-isopropylidene-2-(2,2,2-trichloroethoxycarbonyl)amino-β-*D*-glucopyranoside* (**2**). — To a solution of benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside[3] (**1**, 2 g) in dichloromethane (50 mL) were added saturated sodium hydrogencarbonate (50 mL) and 2,2,2-trichloroethoxycarbonyl chloride (2.73 g). The mixture was vigorously stirred for 2 h at room temperature. The organic layer was washed with water, dried (sodium sulfate), and concentrated. The residual syrup was chromatographed on a column of silica gel with 150:1 dichloromethane–methanol to give **2** (2.4 g, 77%), which was crystallized from ether–hexane, m.p. 157–159°; $[\alpha]_D$ −54.9° (*c* 1.0, CH_2Cl_2); ν_{max} 3450 (OH), 3350 (NH), 2930 and 2850 (CH), 1720 and 1550 (amide), 860 (Me_2C), and 740–700 cm^{-1} (CCl, Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 1.43, 1.52 (2 s, 6 H, Me_2C), 2.88 (br. s, 1 H, O*H*), 3.28 (m, 1 H, H-5), 3.45 (m, 1 H, H-2), 3.60 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 3.83 (t, 1 H, J_{gem} = $J_{5,6a}$ = 10.5 Hz, H-6a), 3.91 (m, 1 H, H-4), 3.96 (dd, 1 H, J_{gem} 10.5, $J_{5,6b}$ 5.5 Hz, H-6b), 4.58, 4.89 (2 d, 2 H, J_{gem} 12 Hz, PhCH_2), 4.6–4.75 (m, 3 H, CCl_3CH_2 and H-1), 5.15 (m, 1 H, N*H*), and 7.32 (m, 5 H, Ph-H).

Anal. Calc. for $C_{19}H_{24}Cl_3NO_7$ (484.77): C, 47.08; H, 4.99; N, 2.89. Found: C, 47.21; H, 5.10; N, 2.66.

*Benzyl 2-deoxy-4,6-*O*-isopropylidene-3-*O*-(2-tetradecylhexadecanoyl)-2-(2,2,2-trichloroethoxycarbonyl)amino-β-*D*-glucopyranoside* (**3**). — To a solution of **2** (2 g) in 1,2-dichloroethane (40 mL) were added 2-tetradecylhexadecanoic acid (2.4 g), 1-(3-

dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC; 1.57 g), and a catalytic amount of 4-dimethylaminopyridine (DMAP). The mixture was stirred overnight at room temperature, then concentrated. The residue was chromatographed on a column of silica gel with dichloromethane to give **3** (3.8 g, 97%) as a syrup, $[\alpha]_D$ −23.9° (*c* 1.0, CH_2Cl_2); ν_{max} 3350 (NH), 2950 and 2850 (CH), 1730 (carbonyl), 1550 (NH), 860 (Me_2C), and 760–700 cm^{-1} (CCl, Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (t, 6 H, Me), 1.05–1.75 (m, 52 H, CH_2), 1.33, 1.46 (2 s, 6 H, Me_2C), 2.34 (m, 1 H, C*H*CO), 3.48 (m, 1 H, H-5), 3.65–3.95 (m, 3 H, H-2, 4, 6a), 3.99 (dd, 1 H, J_{gem} 10.6, $J_{5,6b}$ 5.5 Hz, H-6b), 4.41, 4.54, 4.87, 4.93 (4 d, 4 H, J_{gem} 12 Hz, CCl_3CH_2 and $PhCH_2$), 4.55 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 5.24 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 5.64 (m, 1 H, N*H*), and 7.28 (m, 5 H, Ph-H).

Anal. Calc. for $C_{49}H_{82}Cl_3NO_8$ (919.56): C, 64.00; H, 8.99; N, 1.52. Found: C, 63.82; H, 9.13; N, 1.31.

Benzyl 2-deoxy-3-O-(2-tetradecylhexadecanoyl)-2-(2,2,2-trichloroethoxycarbonyl)amino-β-D-glucopyranoside (**4**). — A solution of **3** (3.7 g) in aqueous 80% acetic acid (120 mL) was stirred for 3 h at 50°, and then concentrated to a syrup, which was chromatographed on a column of silica gel with 100:1 dichloromethane–methanol to give **4** (3.5 g, quant.). The compound was crystallized from hexane, m.p. 73–74°, $[\alpha]_D$ −17.2° (*c* 1.0, CH_2Cl_2); ν_{max} 3350 (OH, NH), 2950 and 2860 (CH), 1720 (carbonyl), 1540 (NH), and 740–700 cm^{-1} (CCl, Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (t, 6 H, Me), 1.0–1.65 (m, 52 H, CH_2), 2.38 (m, 1 H, C*H*CO), 2.60 (br. s, 2 H, O*H*), 3.42 (m, 1 H, H-5), 3.6–3.8 (m, 2 H, H-2, 4), 3.84 (dd, 1 H, J_{gem} 12, $J_{5,6a}$ 4.5 Hz, H-6a), 3.94 (dd, 1 H, J_{gem} 12, $J_{5,6b}$ 3.3 Hz, H-6b), 4.51, 4.63, 4.83, 4.88 (4 d, 4 H, J_{gem} 12 Hz, CCl_3CH_2 and $PhCH_2$), 4.60 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.04 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 5.3 (m, 1 H, N*H*), and 7.31 (m, 5 H, Ph-H).

Anal. Calc. for $C_{46}H_{78}Cl_3NO_8$ (879.50): C, 62.82; H, 8.94; N, 1.59. Found: C, 63.01; H, 9.09; N, 1.62.

Benzyl 6-O-benzyloxymethyl-2-deoxy-3-O-(2-tetradecylhexadecanoyl)-2-(2,2,2-trichloroethoxycarbonyl)amino-β-D-glucopyranoside (**5**). — To a cooled mixture of **4** (3.5 g), 1,1,3,3-tetramethylurea (0.9 g), and dichloromethane (100 mL) was added benzyl chloromethyl ether (0.9 g); stirring was continued overnight at room temperature. Methanol (10 mL) was added and the solvents were evaporated. The residue was extracted with dichloromethane, washed with 2M hydrochloric acid and water, dried (sodium sulfate), and concentrated. The residual syrup was chromatographed on a column of silica gel with dichloromethane to afford syrupy **5** (2.2 g, 52%), $[\alpha]_D$ −14.0° (*c* 1.0, CH_2Cl_2); ν_{max} 3500 (OH), 3350 (NH), 2950 and 2860 (CH), 1730 (carbonyl), 1550 (NH), and 740–700 cm^{-1} (CCl, Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (t, 6 H, Me), 1.05–1.70 (m, 52 H, CH_2), 2.37 (m, 1 H, C*H*CO), 2.66 (br. s, 1 H, O*H*), 3.47 (m, 1 H, H-5), 3.75 (m, 2 H, H-2, 4), 3.93 (d, 2 H, $J_{5,6}$ 3.7 Hz, H-6), 4.45–4.9 (m, 9 H, H-1, OCH_2O, $PhCH_2$, and CCl_3CH_2), 5.0 (m, 1 H, N*H*), 5.04 (t, 1 H, $J_{2,3} = J_{3,4}$ = 10.8 Hz, H-3), and 7.2–7.4 (m, 10 H, Ph-H).

Anal. Calc. for $C_{54}H_{86}Cl_3NO_9$ (999.65): C, 64.88; H, 8.67; N, 1.40. Found: C, 64.62; H, 8.79; N, 1.69.

Benzyl 2-amino-6-O-benzyloxymethyl-2-deoxy-4-O-(diphenoxyphosphinyl)-3-O-

*(2-tetradecylhexadecanoyl)-β-*D*-glucopyranoside* (**6**). — To a cooled solution of **5** (2.1 g) and DMAP (256 mg) in pyridine (10 mL) were added diphenyl phosphorochloridate (1.1 g) and dichloromethane (5 mL). The mixture was stirred overnight at room temperature. Methanol (5 mL) was added, and the solution was then concentrated. The residue was dissolved in acetic acid (100 mL), and zinc dust (16 g) was added. The mixture was vigorously stirred for 3 h at 60° then filtered, and the solid was washed with dichloromethane. The filtrate and washings were combined and concentrated to a syrup, which was chromatographed on a column of silica gel with 300:1 dichloromethane–methanol to give **6** (1.8 g, 82%). The compound was crystallized from methanol, m.p. 41–43°; $[\alpha]_D$ −12.5° (*c* 1.2, CH_2Cl_2); ν_{max} 2950 and 2860 (CH), 1750 (ester), 1600 (amine), 960 (P–O–Ph), and 780–700 cm^{-1} (Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (t, 6 H, Me), 1.0–1.65 (m, 52 H, CH_2), 2.35 (m, 3 H, C*H*CO and NH_2), 2.95 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2), 3.6–3.9 (m, 3 H, H-5, 6), 4.36 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.5–4.95 (m, 7 H, OCH_2O, PhCH_2, and H-4), 5.20 (t, 1 H, $J_{2,3} = J_{3,4}$ = 10 Hz, H-3), and 7.1–7.4 (m, 20 H, Ph-H).

Anal. Calc. for $C_{63}H_{94}NO_{10}P$ (1056.41): C, 71.63; H, 8.97; N, 1.33. Found: C, 71.84; H, 9.11; N, 1.51.

*Benzyl 6-*O*-benzyloxymethyl-2-deoxy-4-*O*-(diphenoxyphosphinyl)-2-dodecanamido-3-*O*-(2-tetradecylhexadecanoyl)-β-*D*-glucopyranoside* (**7**). — To a solution of **6** (200 mg) in dichloromethane (5 mL) were added dodecanoic acid (50 mg) and WSC (60 mg). The mixture was stirred overnight at room temperature and directly chromatographed on a column of silica gel with 300:1 dichloromethane–methanol to give **7** (200 mg, 85%) as a syrup, $[\alpha]_D$ −13.4° (*c* 1.0, CH_2Cl_2); ν_{max} 3300 (NH), 2950 and 2860 (CH), 1740 (ester), 1650 and 1550 (amide), 960 (P–O–Ph), and 760–700 cm^{-1} (Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (t, 9 H, Me), 1.0–1.65 (m, 70 H, CH_2), 2.07, 2.30 (2 m, 3 H, C*H*CO and CH_2CO), 3.72, 3.88 (2 m, 3 H, H-5, 6), 4.98 (q, 1 H, $J_{1,2} = J_{2,3} = J_{2,NH}$ = 8.8 Hz, H-2), 4.45–4.95 (m, 8 H, H-1, 4, OCH_2O, and PhCH_2), 5.45 (t, 1 H, $J_{2,3} = J_{3,4}$ = 8.8 Hz, H-3), 5.55 (d, 1 H, $J_{2,NH}$ 8.8 Hz, N*H*), and 7.05–7.4 (m, 20 H, Ph-H).

Anal. Calc. for $C_{75}H_{116}NO_{11}P$ (1238.72): C, 72.72; H, 9.44; N, 1.13. Found: C, 72.83; H, 9.23; N, 1.20.

*Other benzyl 2-acylamino-6-*O*-benzyloxymethyl-2-deoxy-4-*O*-(diphenoxyphosphinyl)-3-*O*-(2-tetradecylhexadecanoyl)-β-*D*-glucopyranosides* (**8–12**). — Compound **6** was respectively esterified with tetradecanoic acid, hexadecanoic acid, (3*RS*)-3-hydroxydodecanoic acid, (3*RS*)-3-hydroxytetradecanoic acid, and (3*RS*)-3-hydroxyhexadecanoic acid to afford **8–12**, as described for **7**. These compounds were crystallized from ethanol. I.r. and ^{1}H-n.m.r. data were consistent with the structures assigned; other physical data are recorded in Table I.

*2-Deoxy-4-*O*-(diphenoxyphosphinyl)-2-dodecanamido-3-*O*-(2-tetradecylhexadecanoyl)-*D*-glucopyranose* (**13**). — A mixture of **7** (80 mg), 10% palladium-on-carbon (80 mg), and ethanol (30 mL) was stirred overnight at room temperature in a hydrogen atmosphere. The catalyst was filtered off and washed with ethanol. The filtrate and washings were combined, and concentrated. The residue was chromatographed on a column of silica gel with 50:1 dichloromethane–methanol to give **13** (40 mg, 60%),

TABLE I

Some physical properties of the products

Compd. no.	*Yield (%)*	*M.p. (°C)*	$[\alpha]_D$ (°) (c, *in* CH_2Cl_2)	*Molecular formula*	*Composition found, (calc.) (%)*		
					C	*H*	*N*
8	67	44–45	−16.6 (1.0)	$C_{77}H_{120}NO_{11}P$	73.20 (73.01)	9.63 (9.55)	0.95 (1.11)
9	61	49–51	−17.1 (1.0)	$C_{79}H_{124}NO_{11}P$	73.31 (73.28)	9.50 (9.65)	1.21 (1.08)
10	80	36–37	−16.1 (0.8)	$C_{75}H_{116}NO_{12}P$	72.01 (71.80)	9.13 (9.32)	1.26 (1.12)
11	80	47–48	−14.8 (1.1)	$C_{77}H_{120}NO_{12}P$	72.00 (72.10)	9.26 (9.43)	0.88 (1.09)
12	77	50–51	−16.0 (1.0)	$C_{79}H_{124}NO_{12}P$	72.50 (72.39)	9.71 (9.53)	1.13 (1.07)
14	82	61–62	+8.9 (0.6)	$C_{62}H_{106}NO_{10}P$	70.23 (70.49)	10.30 (10.11)	1.08 (1.33)
15	75	63–64	+8.3 (0.5)	$C_{64}H_{110}NO_{10}P$	70.90 (70.88)	10.45 (10.22)	1.22 (1.29)
16	80	51–52	+9.1 (0.7)	$C_{60}H_{102}NO_{11}P$	68.88 (69.00)	10.01 (9.84)	1.29 (1.34)
17	77	57–58	+9.5 (0.7)	$C_{62}H_{106}NO_{11}P$	69.60 (69.43)	9.90 (9.96)	1.51 (1.31)
18	88	60–61	+7.0 (0.6)	$C_{64}H_{110}NO_{11}P$	70.01 (69.85)	10.22 (10.07)	1.20 (1.27)
28	50	–	−11.7 (1.0)	$C_{85}H_{128}NO_{13}P$	72.65 (72.77)	9.47 (9.20)	0.80 (1.00)
29	37	–	−6.3 (1.0)	$C_{87}H_{132}NO_{13}P$	73.00 (73.02)	9.47 (9.30)	0.88 (0.98)
30	55	–	+7.8 (0.9)	$C_{60}H_{102}NO_{11}P$	69.20 (69.00)	9.57 (9.84)	1.20 (1.34)
31	50	–	+7.6 (0.6)	$C_{62}H_{106}NO_{11}P$	69.60 (69.43)	9.90 (9.96)	1.25 (1.31)
32	59	–	+7.6 (0.9)	$C_{64}H_{110}NO_{11}P$	69.78 (69.85)	9.99 (10.07)	1.35 (1.27)

which was lyophilized from a 1,4-dioxane solution, m.p. 44–45°; $[\alpha]_D$ +8.3° (*c* 0.4, CH_2Cl_2); ν_{max} 3400 (OH, NH), 2950 and 2860 (CH), 1760 (ester), 1660 and 1550 (amide), 980 (P–O–Ph), and 780–700 cm^{-1} (Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (t, 9 H, Me), 1.0–1.65 (m, 70 H, CH_2), 2.05–2.35 (m, 3 H, C*H*CO and CH_2CO), 3.63 (br. s, 2 H, H-6), 4.00 (d, 1 H, $J_{4,5}$ 10 Hz, H-5), 4.26 (m, 1 H, $J_{1,2}$ 3.3, $J_{2,3}$ 9.5, $J_{2,NH}$ 8.8 Hz, H-2), 4.76 (q, 1 H, $J_{3,4} = J_{4,5} = J_{4,P}$ = 9.5 Hz, H-4), 5.28 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 5.51 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 5.95 (d, 1 H, $J_{2,NH}$ 8.8 Hz, N*H*), and 7.1–7.4 (m, 10 H, Ph-H).

Anal. Calc. for $C_{60}H_{102}NO_{10}P$ (1028.44): C, 70.07; H, 10.00; N, 1.36. Found: C, 69.81; H, 10.20; N, 1.19.

*Other 2-acylamino-2-deoxy-4-*O*-(diphenoxyphosphinyl)-3-*O*-(2-tetradecylhexadecanoyl)-*D*-glucopyranoses* (**14–18**). — These compounds were prepared by hydrogenolysis of **8–12**, according to the method described for **13**. I.r. and ^{1}H-n.m.r. data were consistent with the structures assigned; other physical data are recorded in Table I.

2-Deoxy-2-dodecanamido-4-O-phosphono-3-O-(2-tetradecylhexadecanoyl)-D-glucopyranose (**19**). — To a solution of **13** (40 mg) in ethanol (30 mL) was added Adams' platinum catalyst (40 mg), and the mixture was stirred overnight in a hydrogen atmosphere. The catalyst was filtered off, and washed with ethanol. The filtrate and washings were combined and concentrated to afford **19** (30 mg, 88%), which was lyophilized from a 1,4-dioxane suspension. It gave a positive test for the phosphono group with the molybdenum spray reagent of Dittmer and Lester[11], m.p. 140–141°; $[\alpha]_D$ +20.5° (*c* 0.1, 1:1 CH_2Cl_2–MeOH); ν_{max} 3350 (OH, NH), 2940 and 2860 (CH), 1730 (ester), and 1650 and 1550 cm^{-1} (amide).

Anal. Calc. for $C_{48}H_{94}NO_{10}P$ (876.25): C, 65.80; H, 10.81; N, 1.60. Found: C, 66.01; H, 10.93; N, 1.78.

Other 2-acylamino-2-deoxy-4-O-phosphono-3-O-(2-tetradecylhexadecanoyl)-D-glucopyranoses (**20–24**). — Compounds **14–18** were hydrogenolyzed to yield **20–24**, as described for **19**. The i.r. data were similar to those of **19**; other physical data are recorded in Table II.

Benzyl 6-O-benzyloxymethyl-2-deoxy-4-O-(diphenoxyphosphinyl)-2-(2-tetradecylhexadecanamido)-β-D-glucopyranoside (**26**). — To a solution of benzyl 2-amino-6-*O*-benzyloxymethyl-2-deoxy-4-*O*-(diphenoxyphosphinyl)-β-D-glucopyranoside[10](**25**, 1.2 g) in 1,2-dichloroethane (40 mL) were added 2-tetradecylhexadecanoic acid (1.3 g) and WSC (0.7 g). The mixture was stirred overnight at room temperature and concentrated. The residue was chromatographed on a column of silica gel with 300:1 dichloromethane–methanol to give **26** (1 g, 50%) as a syrup, $[\alpha]_D$ −11.8° (*c* 0.9, CH_2Cl_2); ν_{max} 3400 (OH, NH), 2940 and 2860 (CH), 1650 and 1550 (amide), 960 (P–O–Ph), and

TABLE II

Compounds for which $[\alpha]_D$ was determined in dichloromethane–methanol

Compd. no.	*Yield (%)*	*M.p. (°C)*	$[\alpha]_D$ *(°)* (c, *in 1:1* CH_2Cl_2*-MeOH)*	*Molecular formula*	*Composition found, (calc.) (%)*		
					C	*H*	*N*
20	86	159–161	+21.3 (0.1)	$C_{50}H_{98}NO_{10}P$	66.43 (66.41)	10.80 (10.92)	1.49 (1.55)
21	95	174–175	+23.3 (0.1)	$C_{52}H_{102}NO_{10}P$	70.10 (66.99)	11.25 (11.03)	1.63 (1.50)
22	90	162–163	+18.0 (0.1)	$C_{48}H_{94}NO_{11}P$	64.57 (64.62)	10.49 (10.62)	1.49 (1.57)
23	93	164–165	+17.0 (0.1)	$C_{50}H_{98}NO_{11}P$	65.20 (65.26)	10.99 (10.73)	1.28 (1.52)
24	88	170–172	+12.5 (0.1)	$C_{52}H_{102}NO_{11}P$	65.90 (65.86)	11.10 (10.84)	1.20 (1.48)
33	86	115–116	+10.1 (0.1)	$C_{48}H_{94}NO_{11}P$	64.80 (64.62)	10.80 (10.62)	1.33 (1.57)
34	80	121–122	+12.5 (0.1)	$C_{50}H_{98}NO_{11}P$	65.30 (65.26)	10.91 (10.73)	1.55 (1.52)
35	85	122–123	+13.3 (80.1)	$C_{52}H_{102}NO_{11}P$	65.97 (65.86)	11.01 (10.84)	1.20 (1.48)

780–690 cm^{-1} (Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (t, 6 H, Me), 1.0–1.65 (m, 52 H, CH_2), 1.97 (m, 1 H, CHCO), 3.37 (m, 1 H, H-5), 3.55 (br. s, 1 H, OH), 3.60–3.95 (m, 3 H, H-5, 6), 4.29 (t, 1 H, H-2), 4.45–4.95 (m, 8 H, H-1, 4, OCH_2O, and $PhCH_2$), 5.65 (d, 1 H, $J_{2,NH}$ 5.9 Hz, NH), and 7.1–7.4 (m, 20 H, Ph-H).

Anal. Calc. for $C_{63}H_{94}NO_{10}P$ (1056.41): C, 71.63; H, 8.97; N, 1.33. Found: C, 71.73; H, 9.07; N, 1.45.

Benzyl 3-O-[(3RS)-3-(benzyloxymethoxy)dodecanoyl]-6-O-benzyloxymethyl-2-deoxy-4-O-(diphenoxyphosphinyl)-2-(2-tetradecylhexadecanamido)-β-D-glucopyranoside (**27**). — A mixture of **26** (200 mg), (3*RS*)-3-(benzyloxymethoxy)dodecanoic acid (95 mg), WSC (77 mg), and a catalytic amount of DMAP in dichloromethane (10 mL) was stirred overnight at room temperature. The reaction mixture was directly chromatographed on a column of silica gel with 300:1 dichloromethane–methanol to afford **27** (120 mg, 46%) as a syrup, $[\alpha]_D$ −6.5° (*c* 1.1, CH_2Cl_2); ν_{max} 3300 (NH), 2930 and 2850 (CH), 1750 (ester), 1640 and 1540 (amide), 960 (P–O–Ph), and 760–690 cm^{-1} (Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (t, 9 H, Me), 1.0–1.6 (m, 68 H, CH_2), 1.88 (m, 1 H, CHCO), 2.15–2.55 (m, 2 H, CH_2CO), 3.60–3.91 (m, 4 H, H-5, 6, and H-3 of C_{12}-OBom), 4.19 (q, 1 H, $J_{1,2} = J_{2,3} = J_{2,NH}$ = 9.5 Hz, H-2), 4.5–4.9 (m, 12 H, H-1, 4, OCH_2O, and $PhCH_2$), 5.31 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 5.39 (d, 1 H, $J_{2,NH}$ 5.9 Hz, NH), and 7.1–7.4 (m, 20 H, Ph-H).

Anal. Calc. for $C_{83}H_{124}NO_{13}P$ (1374.87): C, 72.51; H, 9.09; N, 1.02. Found: C, 72.22; H, 9.23; N, 1.20.

Other benzyl 3-O-[(3RS)-3-(benzyloxymethoxy)acyl]-6-O-benzyloxymethyl-2-deoxy-4-O-(diphenoxyphosphinyl)-2-(2-tetradecylhexadecanamido)-β-D-glucopyranosides (**28**, **29**). — Compounds **28** and **29** were obtained by acylation of **26** with (3*RS*)-3-(benzyloxymethoxy)tetradecanoic acid and (3*RS*)-3-(benzyloxymethoxy)hexadecanoic acid, respectively, in 50% and 37% yields, according to the method described for **27**. I.r. and ^{1}H-n.m.r. data were similar to those of **27** except for the number of methylene protons detected at δ 1.0–1.6 p.p.m.; other physical data are recorded in Table I.

2-Deoxy-4-O-(diphenoxyphosphinyl)-3-O-[(3RS)-3-hydroxyacyl]-2-(2-tetradecylhexadecanamido)-D-glucopyranoses (**30**–**32**). — Compounds **30**–**32** were obtained by hydrogenolysis of **27**–**29**, according to the method described for **13**. I.r. and ^{1}H-n.m.r. data were consistent with the structures assigned; other physical data are recorded in Table I.

2-Deoxy-3-O-[(3RS)-3-hydroxyacyl]-4-O-phosphono-2-(2-tetradecylhexadecanamido)-D-glucopyranoses (**33**–**35**). — Compounds **30**–**32** were hydrogenolyzed as described for **19**, to afford the corresponding **33**–**35**. I.r. data were consistent with the structures assigned; other physical data are recorded in Table II.

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