Carbohydrate Research, 167 (1987) 317–324
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

New synthetic immunomodulators combining a 4-*O*-phosphono-D-glucosamine derivative related to bacterial lipid A with 1-deoxy-*N*-acetylmuramoyl dipeptide analogs*

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(Received December 22nd, 1986; accepted for publication in revised form, March 30th, 1987)

Endotoxic lipopolysaccharide (LPS)[2] and peptidoglycan[3] are major components of bacterial cell walls, and both substances display a variety of immunopharmacological activities. In the course of investigations on the structure–activity relationships of lipid A[4] and *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP)[3], which respectively carry most of the endotoxic activity of LPS and the adjuvant activity of peptidoglycan, we demonstrated[5] that 2-deoxy-4-*O*-phosphono-3-*O*-tetradecanoyl-2-[(3*R*)- and (3*S*)-3-tetradecanoyloxytetradecanoylamino]-D-glucopyranose (GLA-27-*R* and GLA-27-*S*)[6] can elicit several kinds of beneficial biological activity of endotoxin without any significant toxicity.

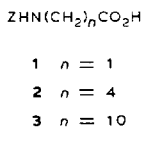
In the biological experiments[5b], GLA-27-*S* had stronger mediator-inducing activities than GLA-27-*R*. On the other hand, the B cell activation was strong with GLA-27-*R*, and weak with GLA-27-*S*. In addition, some lipophilic analogs[7] of *N*-[2-*O*-(2-acetamido-1,5-anhydro-2,3-dideoxy-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (1-deoxy-MDP methyl ester) exhibited strong immunoadjuvant, as well as anti-infection, activities.

Based on these findings, we have now designed a new type of immunomodulator (**19**–**21**) by combining GLA-27-*R* with 1-deoxy-MDP methyl ester through acylamido spacer-arms.

Compound **4** was treated with *N*-(benzyloxycarbonyl)glycine (**1**) in the presence of dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP), to give *N*-{2-*O*-[2-acetamido-1,5-anhydro-6-*O*-(*N*-benzyloxycarbonylglycyl)-2,3-dideoxy-D-glucitol-3-yl]-D-lactoyl}-L-alanyl-D-isoglutamine methyl ester (**5**) in 74% yield. In the same manner, the primary hydroxyl group (O-6) of **4** was

*Studies on Immunoadjuvant-Active Compounds. Part 36, For Part 35, see ref. 1. Presented at the XIIIth International Carbohydrate Symposium, Ithaca, August, 10–15, 1986.

0008-6215/87/$ 03.50



1 $n = 1$
2 $n = 4$
3 $n = 10$

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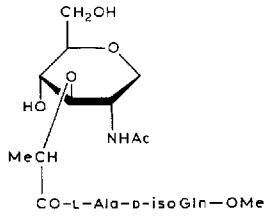

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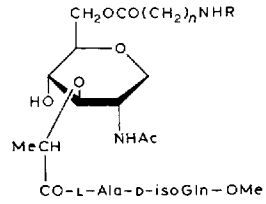
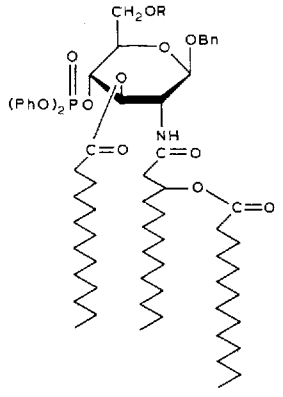
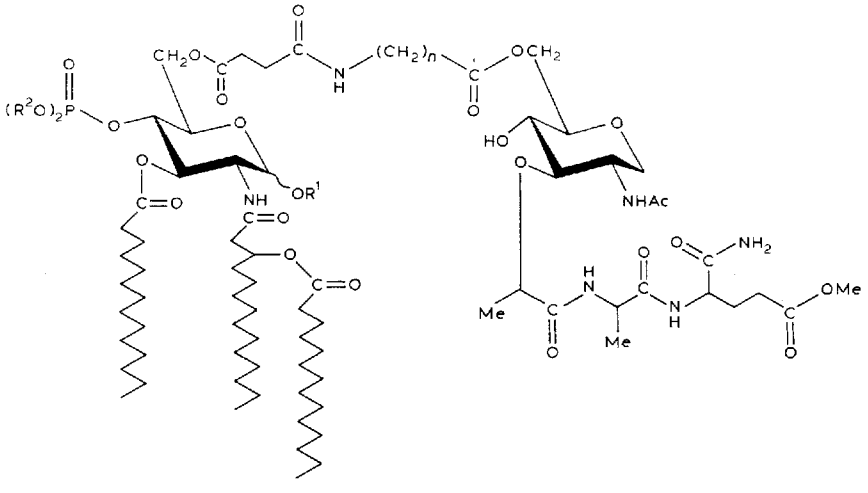


5 $n = 1$, R = Z
6 $n = 4$, R = Z
7 $n = 10$, R = Z
8 $n = 1$, R = H
9 $n = 4$, R = H
10 $n = 10$, R = H



11 R = H
12 R = $CO(CH_2)_2CO_2H$



	n	R^1	R^2
13	1	Bn(β)	Ph
14	4	Bn(β)	Ph
15	10	Bn(β)	Ph
16	1	H	Ph
17	4	H	Ph
18	10	H	Ph
19	1	H	H
20	4	H	H
21	10	H	H

acylated with 5-(benzyloxycarbonylamino)pentanoic acid[8] (**2**) and 11-(benzyloxycarbonylamino)undecanoic acid[9] (**3**), respectively, to afford the corresponding 6-*O*-aminoacyl derivatives (**6** and **7**) in good yields. Hydrogenolytic removal of the benzyloxycarbonyl group from compounds **5–7** was conducted in methanol in the presence of 10% Pd–C catalyst to give **8–10** in quantitative yields, respectively.

Treatment of compound **11** (ref. 6) with succinic anhydride and a small amount of pyridine in 1,2-dichloroethane at 85° afforded the succinic ester **12** (94%), which was coupled with the 6-*O*-(aminoacyl)-1-deoxy-MDP derivatives **8–10** in 1:1 dry 1,4-dioxane–*N*,*N*-dimethylformamide (DMF) in the presence of DCC and DMAP at room temperature, to give a series of coupling products **13–15**. In the ^{1}H-n.m.r. spectra, the resonances characteristic of both the protected GLA-27-*R* and 1-deoxy-MDP moieties were clearly observed, indicating the desired structures.

Hydrogenolytic removal of the benzyl group of **13–15** with Pd-black catalyst gave **16–18** in good yields. Finally, the phenyl groups were removed by hydrogenation with Adams' platinum catalyst in methanol–ethyl acetate–hexane, to afford the desired products (**19–21**) in almost quantitative yields.

Immunoadjuvant activities of the compounds (**19–21**) thus obtained, on the induction of the delayed type of hypersensitivity to *N*-acetyl-L-tyrosine-3-azobenzene-4′-arsonic acid (ABA-*N*-acetyltyrosine) in guinea-pigs were examined (see Table I) as described previously[10]. All of the compounds showed stronger activity than that of the similar compounds[1] containing GLA-27-*RS* and different spacer-arms. Preliminary studies indicate that all of these immunomodulators also exhibit stronger colony stimulation factor (CSF)-induction activity than that of GLA-27 alone[11].

TABLE I

ADJUVANT ACTIVITY OF NEW SYNTHETIC IMMUNOMODULATORS ON THE INDUCTION OF DELAYED-TYPE OF HYPERSENSITIVITY TO ABA-*N*-ACETYLTYROSINE IN GUINEA PIGS

Compound	*Dose (μg)*	*Skin reaction with ABA-BSA[a] (50 μg) (diam. in mm ± SE)[b]*	
		24 h	*48 h*
19	100	20.5 ±0.7	15.8 ±0.3
	10	15.5 ±1.9	(8.5 ±2.0)
20	100	19.8 ±0.7	15.3 ±0.5
	10	16.0 ±0.7	(2.3 ±1.5)
21	100	21.5 ±0.9	17.8 ±0.6
	10	20.8 ±0.5	16.0 ±0.4
MDP	10	19.3 ±0.6	16.3 ±0.5
Control[c]		0	0

[a]Azobenzenearsonate–*N*-acetyl-L-tyrosine–bovine serum albumin. [b]The data indicate the average diameter ± the standard error (SE) of the skin reaction (induration) of four guinea pigs; the values in parentheses indicate the size of erythema. [c]ABA-*N*-acetyltyrosine in Freund's incomplete adjuvant.

EXPERIMENTAL

General methods. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. 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 PM-201 polarimeter at 25°, and i.r. spectra were recorded with a Jasco A-100 spectrophotometer. ^{1}H-N.m.r. spectra were recorded at 270 MHz with a Jeol JNM-GX 270 spectrometer.

N-{*2*-O-[*2-Acetamido-1,5-anhydro-6*-O-*(*N*-benzyloxycarbonylglycyl)-2,3-dideoxy*-D-*glucitol-3-yl*]-D-*lactoyl*}-L-*alanyl*-D-*isoglutamine methyl ester* (**5**). — To a solution of compound **4** (100 mg) in 2:1 1,4-dioxane–DMF (3 mL) were successively added DMAP (5 mg), compound **1** (51.2 mg), and DCC (84 mg), and the mixture was stirred for 8 h at 0°. The mixture was evaporated to a syrup which was chromatographed on a column of silica gel (2 g) with 100:1 and 25:1 chloroform–methanol. The latter eluate afforded **5** (102.7 mg, 74%), which crystallized from ether, m.p. 226.5°, $[\alpha]_D$ +13.5° (*c* 1, 2:1 chloroform–methanol); ν_{max}^{KBr} 3380 (OH), 3240 (NH), 1740, 1200 (ester), 1640, 1530 (amide), and 800–650 cm^{-1} (phenyl); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): δ 1.37 (d, 3 H, *J* 6.6 Hz, C*H*$_3$CH), 1.39 (d, 3 H, *J* 6.9 Hz, C*H*$_3$CH), 1.96 (s, 3 H, C*H*$_3$CO), 3.69 (s, 3 H, C*H*$_3$O), 5.12 (s, 2 H, C*H*$_2$Ph), and 7.35 (s, 5 H, Ph–H).

Anal. Calc. for $C_{30}H_{43}N_5O_{13}$: C, 52.86; H, 6.36; N, 10.27. Found: C, 52.58; H, 6.42; N, 10.15.

N-{*2*-O-[*2-Acetamido-1,5-anhydro-6*-O-*(5-benzyloxycarbonylaminopentanoyl and 11-benzyloxycarbonylaminoundecanoyl)-2,3-dideoxy*-D-*glucitol-3-yl*]-D-*lactoyl*}-L-*alanyl*-D-*isoglutamine methyl ester* (**6** *and* **7**). — Compounds **6** and **7** were prepared by 6-*O*-acylation of **4** with **2** or **3**, as described for **5**, to give **6** (80%) and **7** (70%), respectively.

Compound **6** had m.p. 197.0°, $[\alpha]_D$ +16.3° (*c* 1, 1:1 chloroform–methanol); ν_{max}^{KBr} 3410 (OH), 3280 (NH), 2970, 2870 (CH_2 and Me), 1730, 1230 (ester), 1690, 1550 (amide), and 800–700 cm^{-1} (phenyl); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): δ 1.52–1.78 (m, 4 H, 2 C*H*$_2$), 1.94 (s, 3 H, C*H*$_3$CO), 3.06 (t, 1 H, $J_{1a,1e} \approx J_{1a,2}$ = 11 Hz, H-1*a* of glucitol), 3.12–3.23 (m, 2 H, C*H*$_2$NHZ), 4.09 (dd, 1 H, $J_{1e,2}$ 5 Hz, H-1*e* of glucitol), 5.08 (s, 2 H, C*H*$_2$Ph), 7.30–7.37 (s, 5 H, Ph–H), and others similar to those of **5**.

Anal. Calc. for $C_{33}H_{49}N_5O_{13}$: C, 54.76; H, 6.82; N, 9.68. Found: C, 54.63; H, 6.76; N, 9.57.

Compound **7** had m.p. 192°, $[\alpha]_D$ +15.5° (*c* 1, 1:1 chloroform–methanol); ν_{max}^{KBr} 3410 (OH), 3280 (NH), 2950, 2860 (CH_2 and Me), 1730, 1230 (ester), 1650, 1550 (amide), and 700 cm^{-1} (phenyl); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): δ 1.27–1.67 (m, 16 H, 8 C*H*$_2$), 1.40 (d, 3 H, *J* 7.0 Hz, C*H*$_3$CH), 1.95 (s, 3 H, C*H*$_3$CO), 3.06 (t, 1 H, $J_{1a,1e} \approx J_{1a,2}$ = 11 Hz, H-1*a* of glucitol), 3.15 (m, 2 H, C*H*$_2$NHZ), 3.68 (s, 3 H, C*H*$_3$O), 5.08 (s, 2 H, C*H*$_2$Ph), and 7.34 (s, 5 H, Ph–H).

Anal. Calc. for $C_{39}H_{61}N_5O_{13}$: C, 57.98; H, 7.61; N, 8.67. Found: C, 57.86; H, 7.63; N, 8.61.

N-{2-O-[*2-Acetamido-1,5-anhydro-2,3-dideoxy-6-O-glycyl*-D-*glucitol-3-yl*]-D-*lactoyl*}-L-*alanyl*-D-*isoglutamine methyl ester* (**8**). — To a solution of **5** (203 mg) in methanol (10 mL) was added 10% Pd–C catalyst (100 mg), and hydrogen was bubbled through for 10 min while the solution was stirred at room emperature. The catalyst was filtered off, and washed with 1:1 dichloromethane–methanol. The filtrate and washings were combined, and evaporated to a syrup, which crystallized from ether to afford **8** (165.8 mg, quant.), m.p. 124.5–127.0°, $[\alpha]_D$ +16.1° (*c* 0.73, 1:1 dichloromethane–methanol); ν_{max}^{KBr} 3600–3180 (broad, OH and NH), 2950, 2875 (CH_2 and Me), 1740 (ester), and 1670, 1560 cm^{-1} (amide): ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): δ 1.37 (d, 3 H, *J* 6.96 Hz, CH_3CH), 1.40 (d, 3 H, *J* 6.96 Hz, CH_3CH), 1.96 (s, 3 H, CH_3CO), 2.41–2.47 (m, 2 H, $COCH_2NH_2$), 3.07 (t, 1 H, $J_{1a,1e} \approx J_{1a,2}$ = 11 Hz, H-1*a* of glucitol), and 3.69 (s, 3 H, CH_3O).

Anal. Calc. for $C_{22}H_{37}N_5O_{11}$: C, 48.26; H, 6.81; N, 12.79. Found: C, 48.31; H, 6.77; N, 12.74.

N-{2-O-[*2-Acetamido-6-O-(5-aminopentanoyl and 11-aminoundecanoyl)-1,5-anhydro-2,3-dideoxy*-D-*glucitol-3-yl*]-D-*lactoyl*}-L-*alanyl*-D-*isoglutamine methyl ester* (**9** *and* **10**). — Other 6-*O*-aminoacyl derivatives (**9** and **10**) were obtained (by hydrogenolytic removal of the benzyloxycarbonyl group from **6** or **7**, respectively) in nearly quantitative yield.

Compound **9** had m.p. 102–104°, $[\alpha]_D$ +18.0° (*c* 0.92, 1:2 chloroform–methanol); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): δ 1.37 (d, 3 H, *J* 6.6 Hz, CH_3CH), 1.40 (d, 3 H, *J* 7.3 Hz, CH_3CH), 1.63–1.86 (m, 4 H, 2 CH_2), and others similar to those of **8**, with minor differences in the chemical shifts.

Anal. Calc. for $C_{25}H_{43}N_5O_{11}$: C, 50.93; H, 7.35; N, 11.88. Found: C, 51.03; H, 7.47; N, 11.90.

Compound **10** had m.p. 167–168°, $[\alpha]_D$ +17.6° (*c* 1.12, 1:2 chloroform–methanol); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): δ 1.21–1.30, 1.49–1.72 (m, 16 H, 8 CH_2), 1.38 (d, 3 H, *J* 6.6 Hz, CH_3CH), 1.41 (d, 3 H, *J* 6.9 Hz, CH_3CH), 2.65–2.75 (m, 2 H, CH_2NH_2), 4.30 (q, 1 H, *J* 7.3 Hz, CH_3CH), and other peaks similar to those of **8**, with minor differences in the chemical shifts.

Anal. Calc. for $C_{31}H_{55}N_5O_{11}$: C, 55.26; H, 8.23; N, 10.39. Found: C, 55.39; H, 8.09; N, 10.27.

Benzyl 6-O-(3-carboxypropanoyl)-2-deoxy-4-O-(diphenoxyphosphinyl)-3-O-tetradecanoyl-2-[*(3*R*)-3-tetradecanoyloxytetradecanoylamino*]-*β*-D-*glucopyranoside* (**12**). — To a solution of **11** (301 mg) in 1,2-dichloroethane (3.5 mL) and pyridine (0.6 mL) was added succinic anhydride (785 mg), and the mixture was refluxed for 3 h, and evaporated. The residue was extracted with chloroform, and the extract was washed with water, dried (sodium sulfate), and evaporated to a syrup which was chromatographed on a column of silica gel (3 g) with 500:1 dichloromethane–methanol, to afford **12** (308 mg, 94%) which was lyophilized from 1,4-dioxane solution; m.p. 82–85°, $[\alpha]_D$ −18.5° (*c* 1.3, 100:1 chloroform–methanol); ν_{max}^{KBr} 3300

(NH), 2940, 2860 (CH_2 and Me), 2660 (CO_2H), 1750, 1220 (ester), 1660, 1550 (amide), 960 (P–O–Ph), and 780–650 cm^{-1} (phenyl); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (t, 9 H, J 6.4 Hz, 3 CH_3), 0.90–1.55 (m, 64 H, 32 CH_2), 2.05–2.47 (m, 6 H, 3 CH_2CO), 2.59 (m, 4 H, $CO(CH_2)_2CO_2H$), 4.57, 4.85 (2 dd, 2 H, J_{gem} 11.9 Hz, CH_2Ph), 4.71 (d, 1 H, $J_{1,2}$ 7.93 Hz, H-1), 5.01 (m, 1 H, H-3 of the 3-tetradecanoyloxytetradecanoyl group), 5.38 (t, 1 H, $J_{3,4}$ 10 Hz, H-3), and 7.12–7.33 (m, 15 H, 3 Ph–H).

Anal. Calc. for $C_{71}H_{110}NO_{15}P$: C, 68.30; H, 8.88; N, 1.12. Found: C, 68.49; H, 8.76; N, 1.07.

6-O-[{Benzyl 2-deoxy-4-O-(diphenoxyphosphinyl)-3-O-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranoside-6-yl}-2-(succinylamino)ethanoyl]-1-deoxy-MDP methyl ester (**13**). — To a solution of **8** (163.1 mg) in 1:1 1,4-dioxane–DMF (2 mL) were added, with stirring, DMAP (10 mg), compound **12** (447.2 mg), and DCC (147.6 mg), and the mixture was stirred for 72 h at room temperature, the course of the reaction being monitored by t.l.c. The precipitates were filtered off, and washed with 5:1 chloroform–methanol. The filtrate and washings were combined, and evaporated to a syrup, which was chromatographed on a plate of silica gel (Kieselgel 60 F_{254}), to afford **13** (81.2 mg, 15.3%) which crystallized from ether; m.p. 168.5–172°, $[\alpha]_D$ −4.2° (*c* 0.81, 1:1 chloroform–methanol); ν_{max}^{KBr} 3400 (OH), 3270 (NH), 2940, 2860 (CH_2 and Me), 1740 (ester), 1660, 1540 (amide), 950 (P–O–Ph), and 800–650 cm^{-1} (phenyl); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): GLA-27 unit: δ 0.88 (t, 9 H, J 6.4 Hz, 3 CH_3), 4.60, 4.85 (2 dd, 2 H, J_{gem} 12.1 Hz, CH_2Ph), 5.04 (m, 1 H, H-3 of the 3-tetradecanoyloxytetradecanoyl group), 5.35 (t, 1 H, $J_{2,3} = J_{3,4} = 10$ Hz, H-3), and 7.12–7.29 (m, 15 H, 3 Ph–H); 1-deoxy-MDP unit: 1.96 (s, 3 H, CH_3CO), and 3.68 (s, 3 H, CH_3O); spacer unit: 2.62 [m, 4 H, $CO(CH_2)_2CO$].

Anal. Calc. for $C_{93}H_{145}N_6O_{25}P$: C, 62.82; H, 8.22; N, 4.73. Found: C, 63.01; H, 8.29; N, 4.82.

6-O-[{Benzyl 2-deoxy-4-O-(diphenoxyphosphinyl)-3-O-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranoside-6-yl}-5-(succinylamino)pentanoyl and -11-(succinylamino)undecanoyl]-1-deoxy-MDP methyl ester (**14** *and* **15**). — Compound **12** was coupled with **9** or **10**, as described for **13**, to give **14** (55.6%) and **15** (20.5%), respectively.

Compound **14** had m.p. 194°, $[\alpha]_D$ −2.4° (*c* 1, 2:1 chloroform–methanol); ν_{max}^{KBr} 3320 (NH), 2930, 2860 (CH_2 and Me), 1740 (ester), 1660, 1545 (amide), 950 (P–O–Ph), and 780–650 cm^{-1} (phenyl); ^{1}H-n.m.r. data were consistent with the structures assigned.

Anal. Calc. for $C_{96}H_{151}N_6O_{25}P$: C, 63.35; H, 8.36; N, 4.62. Found: C, 63.47; H, 8.23; N, 4.59.

Compound **15** had m.p. 187–189°, $[\alpha]_D$ −6.1° (*c* 1, 2:1 chloroform–methanol); i.r. and ^{1}H-n.m.r. data were consistent with the structures assigned.

Anal. Calc. for $C_{102}H_{163}N_6O_{25}P$: C, 64.33; H, 8.63; N, 4.41. Found: C, 64.48; H, 8.99; N, 4.50.

6-O-[{2-Deoxy-4-O-(diphenoxyphosphinyl)-3-O-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanoylamino]-D-glucopyranose-6-yl}-2-(succinylamino)-ethanoyl]-1-deoxy-MDP methyl ester (**16**). — A solution of **13** (64.6 mg) in 4:2:1 methanol–ethyl acetate–hexane (3.5 mL) was stirred in a hydrogen atmosphere in the presence of Pd-black catalyst (60 mg) for 24 h at room temperature, the course of the reaction being monitored by t.l.c. with aniline hydrogenphthalate[12] as a spray-reagent. The catalyst was filtered off, and washed with 1:1 chloroform–methanol. The filtrate and washings were combined, and evaporated to a syrup which was purified by chromatography on a column of silica gel (1.5 g) with 20:1 dichloromethane–methanol to afford **16** (59.7 mg, quant.); $[\alpha]_D$ +14.4° (*c* 1.2, 1:1:1 chloroform–methanol–ethyl acetate): ^{1}H-n.m.r. data: GLA-27 unit: δ 0.89 (t, 9 H, *J* 6.4 Hz, 3 CH_3), 5.51 (t, 1 H, $J_{2,3} = J_{3,4} = 10$ Hz, H-3), 7.13–7.37 (m, 10 H, 2 Ph–H), and complete loss of the benzyl protons; 1-deoxy-MDP unit: 1.96 (s, 3 H, CH_3CO), 3.10 (t, 1 H, $J_{1a,1e} \approx J_{1a,2} = 11$ Hz, H-1*a*), and 3.69 (s, 3 H, CH_3O); spacer unit: 2.62 [m, 4 H, CO(CH_2)$_2$CO].

Anal. Calc. for $C_{86}H_{139}N_6O_{25}P$: C, 61.19; H, 8.30; N, 4.98. Found: C, 61.31; H, 8.24; N, 5.09.

6-O-[{2-Deoxy-4-O-(diphenoxyphosphinyl)-3-O-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanoylamino]-D-glucopyranose-6-yl}-5-(succinylamino)pentanoyl and -11-(succinylamino)undecanoyl]-1-deoxy-MDP methyl ester (**17** *and* **18**). — *O*-Debenzylation of **14** and **15**, as described for **16**, gave **17** (70%) and **18** (59%), respectively, as syrups.

Compound **17** had $[\alpha]_D$ +12.7° (*c* 1.36, 1:1 chloroform–methanol); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): GLA-27 unit: δ 0.88 (t, 9 H, *J* 6.4 Hz, 3 CH_3), 5.13 (m, 1 H, H-3 of the 3-tetradecanoyloxytetradecanoyl group), 5.50 (t, 1 H, *J* 10 Hz, H-3), and 7.10–7.39 (m, 10 H, 2 Ph–H); 1-deoxy-MDP unit: 1.98 (s, 3 H, CH_3CO) and 3.69 (s, 3 H, CH_3O); spacer unit: 2.66 [m, 4 H, CO(CH_2)$_2$CO].

Anal. Calc. for $C_{89}H_{145}N_6O_{25}P$: C, 61.79; H, 8.45; N, 4.86. Found: C, 61.97; H, 8.32; N, 4.82.

Compound **18** had $[\alpha]_D$ +13.0° (*c* 1.14, 1:1 chloroform–methanol); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): GLA-27 unit: δ 0.88 (t, 9 H, *J* 6.6 Hz, 3 CH_3), 4.73 (q, 1 H, $J_{3,4} \approx J_{4,5} \approx J_{4,P} = 10$ Hz, H-4), 5.49 (t, 1 H, *J* 10 Hz, H-3), and 7.10–7.39 (m, 10 H, 2 Ph–H); 1-deoxy-MDP unit: 1.95 (s, 3 H, CH_3CO) and 3.08 (t, 1 H, *J* 11 Hz, H-1*a*).

Anal. Calc. for $C_{95}H_{157}N_6O_{25}P$: C, 62.89; H, 8.72; N, 4.63. Found: C, 63.09; H, 8.58; N, 4.52.

6-O-[{2-Deoxy-4-O-phosphono-3-O-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanoylamino]-D-glucopyranose-6-yl}-2-(succinylamino)ethanoyl]-1-deoxy-MDP methyl ester (**19**). — Compound **16** (59.7 mg) was hydrogenolyzed in 2:1:1 methanol–ethyl acetate–hexane (4 mL) in the presence of pre-reduced Adams' platinum catalyst (50 mg). The catalyst was filtered off, and the filtrate was evaporated to give amorphous **19** (quantitative), which was lyophilized from a suspension in 1,4-dioxane, m.p. 164–165°, $[\alpha]_D$ +16.6° (*c* 0.5, 1:1 chloroform–

methanol); ν_{max}^{KBr} 3450 (OH), 3320 (NH), 2940, 2860 (CH_2 and Me), 1740 (ester), and 1660, 1550 cm^{-1} (amide); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): GLA-27 unit: δ 0.88 (t, 9 H, J 6.6 Hz, 3 CH_3), and complete loss of the phenyl protons; 1-deoxy-MDP unit: 1.95 (s, 3 H, CH_3CO) and 3.69 (s, 3 H, CH_3O); spacer unit: 2.68 [m, 4 H, $CO(CH_2)_2CO$].

Anal. Calc. for $C_{74}H_{131}N_6O_{25}P$: C, 57.87; H, 8.60; N, 5.47. Found: C, 58.03; H, 8.51; N, 5.44.

6-O-[{2-Deoxy-4-O-phosphono-3-O-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanoylamino]-D-glucopyranose-6-yl}-5-(succinylamino)pentanoyl and -11-(succinylamino)undecanoyl]-1-deoxy-MDP methyl ester (**20** *and* **21**). — Compounds **20** and **21** were respectively prepared by hydrogenation of compounds **17** and **18**, using Adams' platinum as the catalyst, according to the method described for **19**. Both compounds crystallized from ether and showed i.r. and n.m.r. spectra similar to those of **19**, indicating the desired structures.

Compound **20** had m.p. 174–175°, $[\alpha]_D$ +14.8° (*c* 1, 1:2 chloroform–methanol).

Anal. Calc. for $C_{77}H_{137}N_6O_{25}P$: C, 58.61; H, 8.75; N, 5.33. Found: C, 58.79; H, 8.91; N, 5.42.

Compound **21** had m.p. 169–170°, $[\alpha]_D$ +15.7° (*c* 0.6, 1:2 chloroform–methanol).

Anal. Calc. for $C_{83}H_{149}N_6O_{25}P$: C, 59.98; H, 9.04; N, 5.06. Found: C, 59.76; H, 9.23; N, 4.92.

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