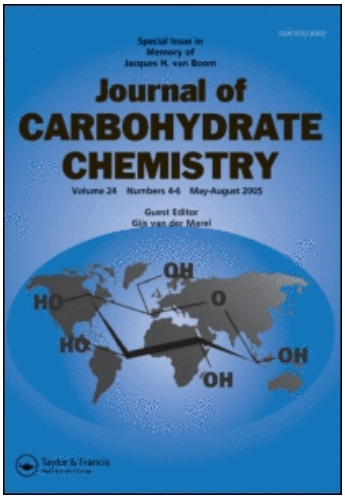

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Chemical Combination of Biologically Active Derivatives ofN-Acetylmuramoyl Dipeptide and Lipid-A, and Their Biological Activities

Akira Hasegawa[a]; Eiji Seki[a]; Yushun Fujishima[a]; Koji Kigawa[a]; Makoto Kiso[a]; Hideharu Ishida[b]; Ichiro Azuma[b]

[a] Department of Agricultural Chemistry, Gifu University, Gifu, Japan [b] Institute of Immunological Science, Hokkaido University, Sapporo, Japan

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CHEMICAL COMBINATION OF BIOLOGICALLY ACTIVE DERIVATIVES OF N-ACETYLMURAMOYL DIPEPTIDE AND LIPID-A, AND THEIR BIOLOGICAL ACTIVITIES*

Akira Hasegawa, Eiji Seki, Yushun Fujishima, Koji Kigawa, Makoto Kiso, Hideharu Ishida**, and Ichiro Azuma**

Department of Agricultural Chemistry, Gifu University, Gifu 501-11, Japan
**Institute of Immunological Science, Hokkaido University, Sapporo 060, Japan

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ABSTRACT

Chemical coupling of biologically active derivatives of N-acetylmuramoyl dipeptide with a derivative related to the nonreducing subunit of lipid A was carried out using acyl groups as a spacer. The products exhibited efficient antitumor activity, as well as strong, immunoadjuvant activity.

INTRODUCTION

In our continuing efforts to elucidate the relationships between the biological activities of N-acetylmuramoyl-L-alanyl-D-isoglutamine and the structure of the carbohydrate moiety, and to obtain glycopeptide adjuvants, we have demonstrated that the introduction[2] of a longer chain, fatty acyl group at C-6 of the sugar moiety in 1-deoxy-

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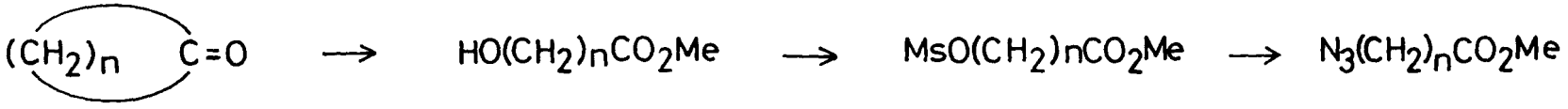
MDP, or replacement[3,4] of the hydroxyl group at C-1 of the sugar skeleton in MDP by acylthio groups causes potent antitumor and anti-infection activities, based on the immune reaction, that are not found for MDP itself, as well as strong, immunoadjuvant activity. Furthermore, these compounds did not show pyrogenic activity,[5] which is one of the side effects of MDP, at a dose of 75μg/kg in rabbits.

In the course of our investigation on the structure-activity relationships of lipid A, which is a unique, hydrophobic component of the endotoxic, bacterial lipopolysaccharide, we have found that 2-deoxy-4-O-phosphoryl-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanoylamino]-D-glucopyranose (GLA-27)[6-9], the nonreducing subunit analog of lipid A, exhibited several kinds of endotoxin biological activity. However, lethal toxicity and pyrogenic activity were about three orders of magnitude weaker than those of lipid A.

In view of these facts, we have designed a new type of immunomodulator, by coupling the nontoxic 1-deoxy-MDP and lipid A analogs, each with different immunological activity, using acyl groups as a spacer.

RESULTS AND DISCUSSION

Treatment of cyclohexanone or cyclododecanone with m-chloroperbenzoic acid in the presence of sodium hydrogen carbonate, and subsequent cleavage of the lactones thus obtained by methanol and concd sulfuric acid, gave methyl 6-hydroxyhexanoate (4) and methyl 12-hydroxy dodecanoate (5) in good yields, respectively. Methylation of 16-hydroxyhexadecanoic acid (3) with diazomethane afforded 6. When reacted with methanesulfonyl chloride in pyridine, compounds (4-6) gave the corresponding O-mesyl derivatives (7-9), which readily exchanged the mesyloxy group on being heated at 80-85°C in N,N-dimethylformamide, to form the azidofatty acid methyl esters (10-12) in good yields. Compounds (10-12) were used as the spacers for coupling of 1-deoxy-MDP derivative with GLA-27 derivative. De-esterification of compound 10 with 1M aqueous potassium hydroxide in 1,4-dioxane gave the acid; this was coupled with N-[2-O-(2-acetamido-1,5-anhydro-2,3-dideoxy-D-glucitol 3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester[2](13), using di-

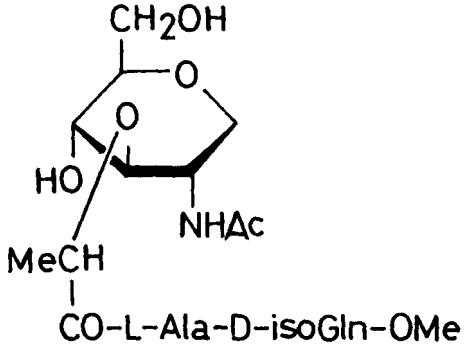


1 n = 5
2 n = 11
3 $HO(CH_2)_{15}CO_2H$

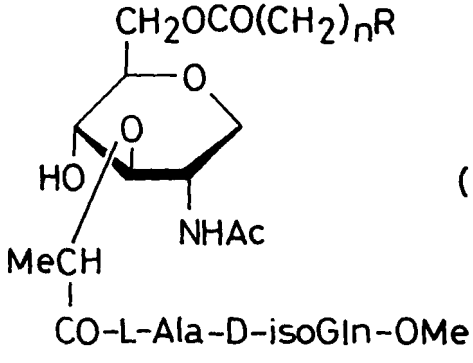
4 n = 5
5 n = 11
6 n = 15

7 n = 5
8 n = 11
9 n = 15

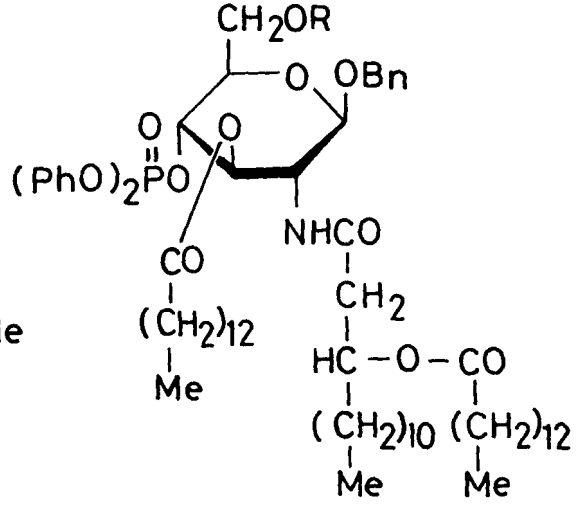
10 n = 5
11 n = 11
12 n = 15



13

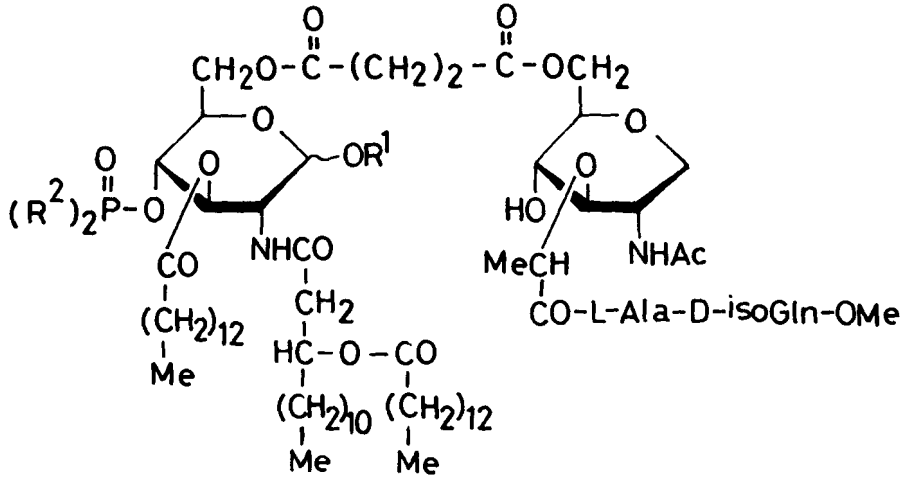


14 n = 5, R = N_3
15 n = 11, R = N_3
16 n = 15, R = N_3
17 n = 5, R = NH_2
18 n = 11, R = NH_2
19 n = 15, R = NH_2

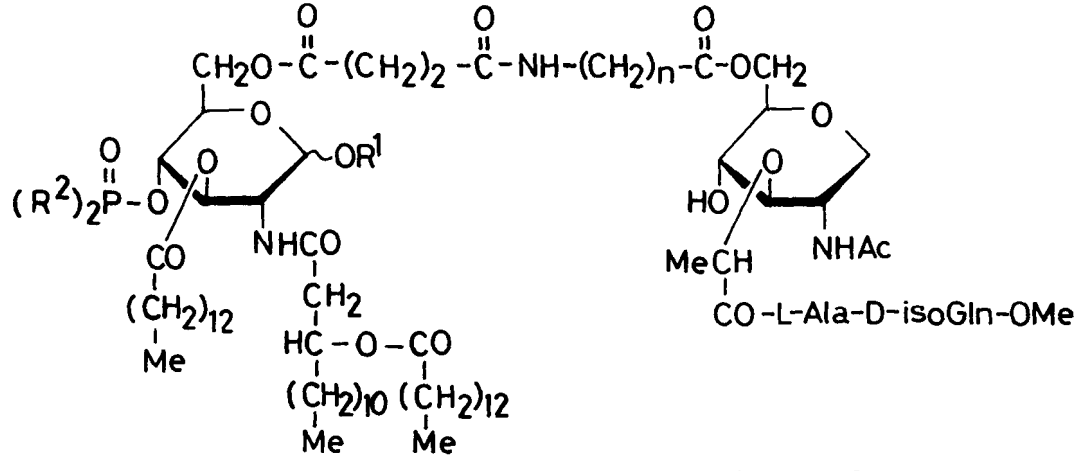


20 R = H
21 R = $CO(CH_2)_2CO_2H$

SCHEME 1



22 R^1 = Benzyl, R^2 = OPh
23 R^1 = H, R^2 = OPh
24 R^1 = H, R^2 = OH



25 R^1=Benzyl, R^2=OPh, n=5
26 R^1=Benzyl, R^2=OPh, n=11
27 R^1=Benzyl, R^2=OPh, n=15
28 R^1=H, R^2=OPh, n=5
29 R^1=H, R^2=OPh, n=11
30 R^1=H, R^2=OPh, n=15
31 R^1=H, R^2=OH, n=5
32 R^1=H, R^2=OH, n=11
33 R^1=H, R^2=OH, n=15

SCHEME 2

cyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine as the activating agents, to afford N-{2-O-[2-acetamido-1,5-anhydro-6-O-(6-azidohexanoyl)-2,3-dideoxy-D-glucitol-3-yl]-D-lactoyl}-L-alanyl-D-isoglutamine methyl ester (14) in 65% yield. In the same way, condensation of 13 with the acids, derived from 11 and 12 by saponification, respectively gave the corresponding 6-O-acyl derivatives (15 and 16) in good yields. Reduction of the azide group in compounds 14-16 with hydrogen in the presence of 10% Pd-C catalyst in methanol-acetic acid gave the corresponding amino-derivatives (17-19) in 70-81% yields, respectively.

On the other hand, treatment of benzyl 2-deoxy-4-O-diphenylphosphoryl-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanoylamino]-D-glucopyranose[6] (20) with succinic anhydride in 1,2-dichloroethane in the presence of small amount of pyridine at 70°C, afforded the 6-O-succinoyl derivative (21) in 96% yield. Condensation of 13 with 21 in dry 1,4-dioxane in the presence of DCC and 4-dimethylaminopyridine at room temperature yielded the desired 6-[{benzyl 2-deoxy-4-O-diphenylphosphoryl-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranoside-6-oyl}-succinoyl]-1-deoxy-MDP methyl ester (22) in 78% yield. In a similar way, reaction of compound 21 with 17-19 in N,N-dimethylformamide yielded the corresponding, coupling products (25-27), respectively. Hydrogenolytic removal of the benzyl group in compounds (22, 25-27) with Pd-black catalyst gave 23, 28-30 in good yields. Finally, the phenyl groups in compounds (23, 28-30) (in ethyl acetate-hexane-methanol) were removed by hydrogenation with platinum black as catalyst, to afford the desired products (24, 31-33) in almost quantitative yields, respectively.

Immunoadjuvant activities of the compounds (24, 31-33) thus obtained, on 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[10](see Table 1). All of the compounds except 24, showed strong activity, comparable to that of MDP at a dose of 100 μg or 10 μg, although the molecular weights of the compounds (31-33) are about four times of that of MDP, indicating that the introduction of lipid A analog as a lipophilic character at C-6 of the carbohydrate moiety in 1-deoxy-MDP methyl ester is favorable for the activity. Moreover, the compounds 31-33 displayed antitumor and anti-

TABLE 1

Adjuvant Activities of 1-Deoxy-MDP-Lipid-A Analogs on Induction of Delayed-type of Hypersensitivity to ABA-N-Acetyltyrosine in Guinea-pigs

Compounds	Dose (μg)	Skin Reaction with ABA-BSA[a] (50 μg) (diam. in mm ± SE)[b] at	
		24 h	48 h
24	100	16.8 ± 0.6	15.5 ± 0.6
	10	(3.5 ± 2.0)	(7.8 ± 3.2)
31	100	20.3 ± 1.5	17.5 ± 0.7
	10	15.5 ± 1.0	11.5 ± 3.1
32	100	19.8 ± 0.3	16.5 ± 0.4
	10	14.3 ± 0.6	13.8 ± 0.6
33	100	19.8 ± 0.2	16.8 ± 0.3
	10	17.8 ± 0.5	14.3 ± 1.8
MDP	100	17.3 ± 0.5	15.3 ± 0.7
	10	14.8 ± 0.9	17.0 ± 0.8
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.

infection activities[5b], promising results suggesting further development.

EXPERIMENTAL

General Procedures. 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 (Waco Co.; 200 mesh) with the solvent systems specified. Specific rotations were determined with a Union PM-201 polarimeter at 25°C, and IR spectra were recorded with a Jasco A-100 spectrometer. NMR spectra were recorded with a Jeol JMN-GX 270 spectrometer.

Metyl 6-hydroxyhexanoate (4). To a solution of cyclohexanone (4.0 g) in dichloromethane (45 mL) was added, with stirring, m-chloroperbenzoic acid (14 g) and sodium hydrogen carbonate (6.8 g) at 0°C: the mixture was stirred overnight at room temperature, and extracted with chloroform. The extract was washed with water, dried (sodium sulfate), and concentrated. The residue was dissolved in methanol (8.2 mL), and a solution of methanol (8.3 mL) and concd sulfuric acid (0.83 mL) was added to the mixture; it was then heated for 20 min at 60°C, cooled, neutralized with 10% aqueous sodium hydroxide, and evaporated. The residue was extracted with ethyl acetate; the extract washed with water, dried (sodium sulfate), and concentrated to a syrup, which was purified by chromatography on a column of silica gel (250 g) with chloroform, to give 4 (6.4 g; 72%) as a syrup; IR (film): 3430 (OH), 1740 and 1260 (ester).

Anal. Calcd for $C_7H_{14}O_3$: 57.51; H, 9.65. Found: C, 57.55; H, 9.60.

Methy 12-hydroxydodecanoate (5). Compound 5 was obtained as a syrup, according to the procedure described for 4; in almost quantitative yield, IR (film): 3450 (OH), and 1740 and 1260 cm^{-1} (ester).

Anal. Calcd for $C_{13}H_{26}O_3$: C, 67.79; H, 11.38. Found: C, 67.98; H, 11.52.

Methyl 16-hydroxyhexadecanoate (6). 16-Hydroxyhexadecanoic acid (1.0 g) was treated with large excess of diazomethane in ether, to give 6, quantitatively; mp 54°; IR (Nujol): 3400 (OH), and 1740 and 1260 cm^{-1} (ester).

Anal. Calcd for $C_{17}H_{34}O_3$: C, 71.28; H, 11.96. Found: C, 71.28; H, 11.81.

Methyl 6-O-mesyl-hexanoate (7). To an ice-cooled solution of 4 (2.15 g) in dry pyridine (20 mL) was added methanesulfonyl chloride (2.54 g), and the mixture was kept for 5 h at 0°C. The mixture was concentrated, the residue extracted with chloroform, and the extract successively washed with 2M hydrochloric acid, M sodium carbonate, and water, dried (sodium sulfate), and concentrated to a syrup, which was purified by chromatography on a column of silica gel (100 g) with

chloroform, to afford 7 as a syrup in 90% yield; IR (film): 1740 and 1260 (ester), and 1360 and 1180 cm^{-1} (mesyl).

Anal. Calcd for $C_8H_{16}O_5S$: C, 42.84; H, 7.19. Found: C, 42.66; H, 7.24.

Methyl 12-O-mesyl-dodecanoate (8). Compound 8 was obtained as a syrup in 73% yield, as described for 7; IR (film): 2930 and 2850 (Me, methylene), 1735 and 1260 (ester), and 1360 and 1180 cm^{-1} (mesyl).

Anal. Calcd for $C_{14}H_{28}O_5S$: C, 54.52; H, 9.15. Found: C, 54.63; H, 9.08.

Mesyl 16-O-mesyl-hexadecanoate (9). Compound 9 was obtained as crystals in 70% yield; mp 60°; IR (KBr): 2930 and 2850 (Me, methylene), 1740 and 1260 (ester), and 1360 and 1180 cm^{-1} (mesyl).

Anal. Calcd for $C_{18}H_{36}O_5S$: C, 59.31; H, 9.95. Found: C, 59.25; H, 9.78.

Methyl 6-azido-hexanoate (10). To a solution of 7 (1.3 g) in dry N,N-dimethylformamide (DMF; 10 mL) was added sodium azide (2.6 g), and the mixture was heated, with stirring, for 20 h at 80-85°C. It was then cooled, the salts were filtered off, and the filtrate was concentrated to a syrup which was extracted with chloroform. The extract was successively washed with 2M hydrochloric acid, M sodium carbonate, and water, dried (sodium sulfate), and concentrated. The residue was chromatographed on a column of silica gel (100 g) with chloroform, to give 10 (745 mg, 76%) as a syrup; IR (film): 2930 and 2860 (Me, methylene), 2100 (azide), and 1740 and 1260 cm^{-1} (ester).

Anal. Calcd for $C_7H_{13}N_3O_2$: C, 49.11; H, 7.65; N, 24.55. Found: C, 49.23; H, 7.69; N, 24.25.

Methyl 12-azido-dodecanoate (11). Compound 11 was obtained as a syrup in 91% yield, according to the procedure described for 10; IR (film): 2930 and 2850 (Me, methylene), 2100 (azide), and 1740 and 1260 cm^{-1} (ester).

Anal. Calcd for $C_{13}H_{25}N_3O_2$: C, 61.15; H, 9.87; N, 16.45. Found: C, 61.33; H, 10.05; N, 16.18.

Methyl 16-azido-hexadecanoate (12). Compound 12 was obtained as crystals in 83% yield, as described for 10; mp 48°; IR (KBr): 2930 and 2850 (Me, methylene), 2100 (azide), and 1740 and 1260 cm^{-1} (ester).

Anal. Calcd for $C_{17}H_{33}N_3O_2$: C, 65.56; H, 10.68; N, 13.49. Found: C, 65.49; H, 10.65; N, 13.21.

N-{2-O-[2-Acetamido-1,5-anhydro-6-O-(6-azido-hexanoyl)-2,3-dideoxy-D-glucitol-3-yl]-D-lactoyl}-L-alanyl-D-isoglutamine methyl ester (14). To a solution of 10 (70 mg) in 1,4-dioxane (1 mL) was added 1M potassium hydroxide (1 mL), and the mixture was stirred for 30 min at room temperature and treated with Amberlite IR-120 (H^+) resin to remove the base. The resin was filtered off, and washed with methanol; the filtrate and washings were combined, and concentrated to give the acid, which was used for the next reaction without purification. To a solution of N-[2-O-(2-acetamido-1,5-anhydro-2,3-dideoxy-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester[2] (13; 200 mg) in 1,4-dioxane (1 mL) and DMF (1 mL) were added the acid obtained above, dicyclohexylcarbodiimide (DCC; 168 mg) and 4-dimethylaminopyridine (100 mg), and the mixture was stirred for 24 h at room temperature, and then concentrated. The residue was chromatographed on a silica gel plate (Kieselgel 60F-254) with 5:1 chloroform-methanol, to afford 14 (167 m 65%) as crystals; mp 139-141°, $[\alpha]_D$ + 11.8° (c 1.6, 1:1 chloroform-methanol); IR (KBr): 3270 (OH, NH), 2940 and 2850 (Me, methylene), 2100 (azide), 1740 and 1250 (ester), and 1660, 1550, and 1535 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 1.22-1.30 (m, 8H, $4CH_2$), 1.37 (d, 3H, $J_{Me,CH}$ 6.5 Hz, MeCH), 1.42 (d, 3H, $J_{Me,CH}$ 6.9 Hz, MeCH), 1.96 (s, 3H, AcN), and 3.70 (s, 3H, MeO).

Anal. Calcd for $C_{26}H_{43}N_7O_{11}$: C, 49.60; H, 6.89; N, 15.57. Found: C, 49.41; H, 6.98; N, 15.42.

N-{2-O-[2-Acetamido-1,5-anhydro-6-O-(12-azido-dodecanoyl)-2,3-dideoxy-D-glucitol-3-yl]-D-lactoyl}-L-alanyl-D-isoglutamine methyl ester (15). Compound 15 was obtained as crystals in 71% yield, by condensation of 13 with the acid derived from 11, according to the procedure described for 14; mp 185-186.5°, $[\alpha]_D$ + 11.6° (c 1.3, 1:1 chloroform-methanol); IR (KBr): δ 3400, 3280 (OH, NH), 2940 and 2850 (Me, methylene), 2100 (azide), 1740, 1720, 1260, and 1230 (ester), and 1640 and 1550 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 1.27 (s, 20H, $10CH_2$), 1.37 (d, 3H, $J_{Me,CH}$ 7.0 Hz, MeCH), 1.41 (d, 3H, $J_{Me,CH}$ 7.5 Hz, MeCH), 1.94 (s, 3H, AcN), and 3.69 (s, 3H, MeO).

Anal. Calcd for $C_{32}H_{55}N_7O_{11}$: C, 53.84; H, 7.77; N, 13.74. Found: C, 53.65; H, 7.86; N, 13.51.

N-{2-O-[2-Acetamido-1,5-anhydro-6-O-(16-azido-hexadecanoyl)-2,3-dideoxy-D-glucitol-3-yl]-D-lactoyl}-L-alanyl-D-isoglutamine methyl

ester (16). Compound 16 was obtained as crystals in 64% yield, by coupling of 13 with the acid derived from 12, according to the method described for 14; mp 200-202°, $[\alpha]_D$ + 13.7° (c 1.1, 1:1 chloroform-methanol); IR (KBr): 3400, 3280 (OH, NH), 2930 and 2850 (Me, methylene), 2100 (azide), 1740 and 1250-1230 (ester), and 1660, 1650, 1550, and 1540 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 1.27 (s, 28H, $14CH_2$), 1.37 (d, 3H, $J_{Me,CH}$ 7.0 Hz, MeCH), 1.42 (d, 3H, $J_{Me,CH}$ 7.2 Hz, MeCH), 1.97 (s, 3H, AcN), and 3.69 (d, 3H, MeO).

Anal. Calcd for $C_{36}H_{63}N_7O_{11}$: C, 56.16; H, 8.25; N, 12.73. Found: C, 56.02; H, 8.25; N, 12.58.

N-{2-O-[2-Acetamido-6-O-(6-aminohexanoyl)-1,5-anhydro-2,3-dideoxy-D-glucitol-3-yl]-D-lactoyl}-L-alanyl-D-isoglutamine methyl ester (17). Compound 14 (115 mg) was dissolved in methanol (15 mL) and two drops of acetic acid; 10% Pd-C catalyst (100 mg) was added, and hydrogen was bubbled through for 10 h while the solution was stirred at room temperature. The catalyst was filtered off, and the filtrate was concentrated to a syrup which was chromatographed on a column of silica gel (10 g) with (a) 50:1, (b) 30:1, and (c) 15:1 chloroform-methanol. Eluant (c) gave 17 (77 mg, 70%) as crystals; mp 109-113° dec., $[\alpha]_D$ + 8.3° (c 0.6, 1:1 chloroform-methanol); IR (KBr): 3300 (OH, NH), 2940 and 2850 (Me, methylene), 1730 and 1250 (ester), and 1660 and 1550 cm^{-1} (amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 1.25-1.34 (m, 8H, $4CH_2$), 1.37, 1.41 (2d, 6H, $J_{Me,CH}$ 6.8 Hz, 2MeCH), 1.96 (s, 3H, AcN), and 3.70 (s, 3H, MeO).

Anal. Calcd for $C_{26}H_{45}N_5O_{11}$: C, 51.72; H, 7.51; N, 11.60. Found: C, 51.53; H, 7.79; N, 11.40.

Other 6-O-aminoacyl derivatives (18 and 19) of 1-deoxy-MDP methyl ester respectively prepared from 15 and 16, according to the procedure described for 17, and showed similar IR and NMR spectra, which were consistent with the structures assigned.

Compound 18 was obtained as crystals in 81% yield; mp 110-113°, $[\alpha]_D$ + 17.0° (c 0.8, 1:1 chloroform-methanol).

Anal. Calcd for $C_{32}H_{57}N_5O_{11}$: C, 55.88; H, 8.35; N, 10.18. Found: C, 55.74; H, 8.43; N, 10.00.

Compound 19 was obtained as crystals in 76% yield; mp 170-172° dec., $[\alpha]_D$ + 1.5° (c 0.3, 1:1 chloroform-methanol).

Anal. Calcd for $C_{36}H_{65}N_5O_{11}$: C, 58.12; H, 8.92; N, 9.42. Found: C, 58.33; H, 9.14; N, 9.38.

Benzyl 6-O-(3-carboxypropanoyl)-2-deoxy-4-O-diphenylphosphoryl-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranoside (21). To a solution of 20[6] (900 mg) in 1,2-dichloroethane (10 mL) and pyridine (0.17 mL) was added, and the mixture was refluxed for 2 h, and concentrated. The residue was extracted with chloroform, the extract washed with water, dried (sodium sulfate), and concentrated to a syrup, which was purified by chromatography on a column of silica gel (50 g) with 200:1 chloroform-methanol to afford 21 (940 mg, 96%) as an amorphous mass; mp 75-78°, $[\alpha]_D$ - 17.5° (c 2.2, chloroform); IR (KBr): 3275 (NH), 2660 (COOH), 1740 and 1240 (ester), 1710 (C=O), 1660, 1640, and 1560 (amide), 960 (P-O-Ph), and 780-650 cm^{-1} (phenyl); NMR ($CDCl_3$): δ 0.88 (t, 9H, J_{Me,CH_2} 6.6 Hz, $3MeCH_2$), 1.11-1.30 (m, 64H, $32CH_2$), 2.05-2.47 (m, 8H, $4CH_2$), 2.60 (m, 4H, succinoyl CH_2), 3.69 (m, 1H, H-5), 4.12 (m, 1H, H-2), and 7.12-7.33 (m, 15H, 3Ph).

Anal. Calcd for $C_{71}H_{110}NO_{15}P$: C, 68.30; H, 8.88; N, 1.12. Found: C, 68.38; H, 8.91; N, 1.15.

6-O-[{Benzyl 2-deoxy-4-diphenylphosphoryl-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranoside-6-oyl}-succinoyl]-1-deoxy-MDP methyl ester (22). To a solution of 13 (150 mg) in DMF (2.5 mL) and 1,4-dioxane (2 mL) were added, with stirring, compound 21 (460 mg), DCC (160 mg), and 4-dimethylaminopyridine (90 mg), and the mixture was stirred for 24 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 concentrated to a syrup, which was chromatographed on a column of silica gel (20 g) with 100:1 and then 10:1 chloroform-methanol. The latter eluate afforded 22 (351 mg, 78%) as a syrup; IR (film): 3600-3300 (OH, NH), 2940 and 2850 (Me, methylene), 1740 and 1260 (ester), 1660 and 1550 (amide), 960 (P-O-Ph), and 800-680 cm^{-1} (phenyl); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.89 (t, 9H, J_{Me,CH_2} 6.4 Hz, $3MeCH_2$), 1.13-1.31 (m, 70H, $35CH_2$), 1.39 (d, 3H, $J_{Me,CH}$ 6.6 Hz, MeCH), 1.42 (d, 3H, $J_{Me,CH}$ 7.0 Hz, MeCH), 1.96 (s, 3H, AcN), 2.63 (m, 4H, succinoyl CH_2), 3.01 (t, 1H, $J_{1a,1e} = J_{1a,2}$ = 10.7 Hz, H-1a), 3.68 (s, 3H, MeO), 5.44 (m, 1H, H-3'), and 7.12-7.38 (m, 15H, 3Ph).

Anal. Calcd for $C_{91}H_{142}N_5O_{14}P$: C, 63.51; H, 8.32; N, 4.07. Found: C, 63.48; H, 8.41; N, 4.12.

6-O-[{Benzyl 2-deoxy-4-O-diphenylphosphoryl-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranoside-6-O-oyl}-6-(succinoylamino)hexanoyl]-1-deoxy-MDP methyl ester (25). Compound 25 was obtained as a syrup in 55% yield, by condensation of 17 with 21, according to the procedure described for 22; $[\alpha]_D$ - 2.4° (c 0.7, 1:1 chloroform-methanol); IR (film): 3300 (OH, NH), 2940 and 2850 (Me, methylene), 1740 and 1250 (ester), 1660 and 1550 (amide), 960 (P-O-Ph), and 780-690 (Ph); NMR ($CDCl_3$): δ 0.88 (t, 9H, J_{Me,CH_2} 6.5 Hz, $3MeCH_2$), 1.98 (3, 3H, AcN), 3.67 (s, 3H, MeO), 5.41 (m,1H, H-3'), and 7.12-7.35 (m, 15H, 3Ph).

Anal. Calcd for $C_{95}H_{153}N_6O_{25}P$: C, 63.03; H, 8.52; N, 4.64. Found: C, 62.88; H, 8.63; N, 4.51.

Other coupling compounds (26 and 27) were synthesized by condensation of 18 or 19 with 21, as described for 22.

Compound 26 was obtained as a syrup in 65% yield; $[\alpha]_D$ - 1.7° (c 0.4, 1:1 chloroform-methanol); IR (film): 3300 (OH, NH), 2940 and 2850 (Me, methylene), 1740 and 1260 (ester), 1660 and 1550 (amide), 960 (P-O-Ph), and 780-690 cm^{-1} (phenyl).

Anal. Calcd for $C_{101}H_{165}N_6O_{25}P$: C, 64.04; H, 8.78; N, 4.44. Found: C, 63.89; H, 8.80; N, 4.38.

Compound 27 was obtained as a syrup in 52% yield; $[\alpha]_D$ + 2.7° (c 0.6, 1:1 chloroform-methanol); IR (film): 3300 (OH, NH), 2930 and 2850 (Me, methylene), 1740 and 1250 (ester), 1660 and 1550 (amide), 960 (P-O-Ph), and 770-690 cm^{-1} (phenyl).

Anal. Calcd for $C_{105}H_{173}N_6O_{25}P$: C, 64.66; H, 8.94; N, 4.31. Found: C, 64.49; H, 8.98; N, 4.20.

6-O-[{2-Deoxy-4-diphenylphosphoryl-3-O-tetradecanoly-2-[(3RS)-3-tetradecanoyloxytetradecanoylamino]-D-glucopyranose-6-oyl}-succinoyl]-1-deoxy-MDP methyl ester (23). A solution of 22 (106 mg) in hexane (1 mL), ethyl acetate (2 mL), and methanol (3 mL) was hydrogenated in the presence of Pd-black (60 mg) for 24 h at room temperature; at this time, t.l.c. showed the reaction to be complete. The suspension was filtered, and the filtrate was concentrated to a syrup which was purified by chromatography on a column of silica gel (15 g) with 100:1 and 20:1 chloroform-methanol. The latter eluate gave 23 (79 mg, 78%) as crystals; mp 104-108°, $[\alpha]_D$ + 16° (c 0.8, 1:1 chloroform-methanol); IR (KBr): 3600-3300 (OH, NH), 2940 and 2850 (Me, methylene), 1740

and 1250 (ester), 1660 and 1540 (amide), 960 (P-O-Ph), and 780-690 cm^{-1} (phenyl); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.89 (t, 9H, J_{Me,CH_2} 6.6 Hz, $3MeCH_2$), 1.13-1.27 (m, 64H, $32CH_2$), 1.39 (d, 3H, $J_{Me,CH}$ 6.6 Hz, MeCH), 1.41 (d, 3H, $J_{Me,CH}$ 6.9 Hz, MeCH), 1.96 (s, 3H, AcN), 2.62 (s, 4H, succinoyl CH_2), 3.09 (t, 1H, $J_{1a,1e} = J_{1a,2}$ = 10.5 Hz, H-1a), 3.69 (s, 3H, MeO), 5.12 (m, 1H, C-3 proton of the 3-hydroxytetradecanoic acid residue), 5.51 (t, 1H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3 of GLA-27 moiety), and 7.12-7.40 (m, 10H, 2Ph).

Anal. Calcd for $C_{84}H_{136}N_5O_{24}P$: C, 61.86; H, 8.41; N, 4.29. Found: C, 61.59; H, 8.35; N, 4.24.

Other 1-deoxy-MDP-GLA-27 derivatives (28-30) were respectively prepared by hydrogenation of the compounds (25-27), according to the procedure described for 23, and exhibited similar IR and NMR spectra, which were consistent with the structures assigned.

Compound 28 was obtained as a syrup in 72% yield; $[\alpha]_D$ + 2.7° (c 0.6, 1:1 chloroform-methanol).

Anal. Calcd for $C_{88}H_{147}N_6O_{25}P$: C, 61.45; H, 8.61; N, 4.89. Found: C, 61.38; H, 8.75; N, 4.76.

Compound 29 was obtained as a syrup in 79% yield; $[\alpha]_D$ + 8.1° (c 0.9, 1:1 chloroform-methanol).

Anal. Calcd for $C_{94}H_{159}N_6O_{25}P$: C, 62.58; H, 8.88; N, 4.66. Found: C, 62.41; H, 8.96; N, 4.65.

Compound 30 was obtained as a syrup in 88% yield; $[\alpha]_D$ + 4.1° (c 0.9, 1:1 chloroform-methanol).

Anal. Calcd for $C_{98}H_{167}N_6O_{25}P$: C, 63.27; H, 9.09; N, 4.52. Found: C, 63.18; H, 9.13; N, 4.35.

6-O-[{2-Deoxy-4-O-phosphoryl-3-O-tetradecanoyl-2-[(3RS)-3-tetradecanoyloxytetradecanoylamino]-D-glucopyranose-6-oyl}-succinoyl]-1-deoxy-MDP methyl ester (24). Compound 23 (79 mg) was dissolved in hexane (1 mL), ethyl acetate (1 mL), and methanol (3 mL); platinum black (50 mg) was added, and hydrogen was bubbled through while the mixture was stirred overnight at room temperature; at that time, the reaction was complete. After removal of the catalyst by filtration, the filtrate was concentrated to give 24 in quantitative yield, which showed a single spot in t.l.c.; mp 175-177°, $[\alpha]_D$ + 15.3° (c 1.3, 1:1 chloroform-methanol); IR (KBr): 3500-3300 (OH, NH), 2940 and 2850 (Me, methylene), 1740 and 1250 (ester), and 1660 and 1550 cm^{-1}

(amide); NMR (1:1 $CDCl_3$-CD_3OD): δ 0.89 (t, 9H, J_{Me,CH_2} 6.6 Hz, $3MeCH_2$), 1.23-1.28 (m, 64H, $32CH_2$), 1.39 (d, 3H, $J_{Me,CH}$ 6.2 Hz, MeCH), 1.42 (d, 3H, $J_{Me,CH}$ 7.0 Hz, MeCH), 1.96 (s, 3H, AcN), 2.71 (m, 4H, succinoyl CH_2), 3.69 (s, 3H, MeO), 5.12 (m, 1H, C-3 proton of the 3-hydroxytetradecanoic acid residue), and 5.36 (t, 1H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3 of GLA-27 moiety).

Anal. Calcd for $C_{72}H_{128}N_5O_{24}P$: C, 58.48; H, 8.73; N, 4.74. Found: C, 58.21; H, 8.91; N, 4.73.

Other 1-deoxy-MDP-GLA-27 analogs (31-33) were respectively prepared by hydrogenation of the compounds (28-30), using platinum black as catalyst, according to the method described for 24, and showed similar IR and NMR spectra, which were consistent with the structures assigned.

Compound 31 was obtained as an amorphous mass in quantitative yield; $[\alpha]_D$ + 4.1° (c 0.9, 1:1 chloroform-methanol).

Anal. Calcd for $C_{76}H_{139}N_6O_{25}P$: C, 58.22; H, 8.94; N, 5.36. Found: C, 58.05; H, 9.13; N, 5.09.

Compound 32 was obtained as crystals in quantitative yield; mp 103-105°, $[\alpha]_D$ + 10.5° (c 0.6, 1:1 chloroform-methanol).

Anal. Calcd for $C_{82}H_{151}N_6O_{25}P$: C, 59.62; H, 9.21; N, 5.09. Found: C, 59.52; H, 9.36; N, 5.11.

Compound 33 was obtained as crystals in quantitative yield; mp 133-135°, $[\alpha]_D$ + 9.0° (c 0.7, 1:1 chloroform-methanol).

Anal. Calcd for $C_{86}H_{159}N_6O_{25}P$: C, 60.47; H, 9.38; N, 4.92. Found: C, 60.33; H, 9.45; N, 4.89.

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