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SYNTHESIS OF (*N*-ACETYL-1-*O*-ACYLMURAMOYL)-L-ALANYL-D-ISOGLUTAMINES, AND THEIR IMMUNOADJUVANT ACTIVITIES*

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ABSTRACT

1-*O*-Acyl derivatives of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP) have been synthesized from 2-acetamido-1-*O*-benzoyl-4,6-*O*-isopropylidene-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranose. Their immunoadjuvant activities were examined in guinea-pigs.

INTRODUCTION

Recently, it has been shown that lipophilic derivatives[2,3] of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP; the minimal structure[4] required for the immunoadjuvant activity of bacterial, cell-wall peptidoglycans) bearing the lipid moiety at C-6 of the sugar skeleton, or at the end of the peptide chain, have strong antitumor and anti-infection activities that are not found for MDP itself. In addition, it has also been observed that introduction[5–7] of lipophilic character at C-2 in muramoyl-L-alanyl-D-isoglutamine, at C-6 in *N*-acetyl-6-amino-6-deoxymuramoyl dipeptide, or at C-1 in *N*-acetyl-1-thiomuramoyl dipeptide, causes potent antitumor activity based on the immune reaction, as well as strong, immunoadjuvant activities. However, the lipophilic analogs[8–10] at both C-2 and C-6 in 6-amino-6-deoxymuramoyl dipeptide, or at C-2 in 6-*O*-[2-acetamido-2-deoxy-3-*O*-(D-2-propanoyl-L-alanyl-D-isoglutamine)-β-D-glucopyranosyl]-2-amino-2-deoxy-D-glucopyranose showed negligible activity, indicating that the position of introduction of lipophilicity into the molecule is critical for activity. In view of these facts, we now describe the synthesis of some 1-*O*-acyl-MDP derivatives, and their immunoadjuvant activities.

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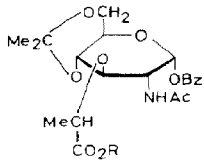
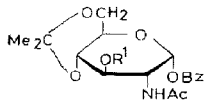
RESULTS AND DISCUSSION

De-esterification of 2-acetamido-1-*O*-benzoyl-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranose[7] (**1**) with 0.2M aqueous potassium hydroxide in 1,4-dioxane gave crystalline **2**; this was coupled with the methyl ester of L-alanyl-D-isoglutamine, using dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (HOSu) as the activating agents, to afford *N*-[2-*O*-(2-acetamido-1-*O*-benzoyl-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (**3**) in quantitative yield. On treatment with sodium methoxide in methanol at 0°, compound **3** gave **4** as a syrup in good yield.

On the other hand, *O*-debenzoylation of **1** with sodium methoxide in methanol, and subsequent saponification, gave 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-4,6-*O*-isopropylidene-D-glucopyranose, which was used for the next reaction without purification. In the same way, coupling of the acid with the benzyl ester of L-alanyl-D-isoglutamine gave *N*-[2-*O*-(2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine benzyl ester (**5**). Acetylation of compounds **4** and **5** with acetic anhydride gave the



1
2



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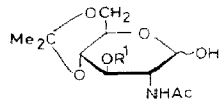
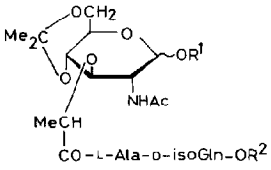


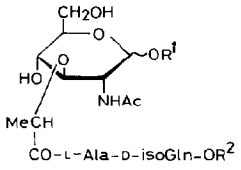
4
5

corresponding 1-*O*-acetyl derivatives (**6** and **9**). When treated with decanoyl chloride or hexadecanoyl chloride in pyridine and dichloromethane at 0°, compounds **4** and **5** afforded the corresponding 1-*O*-(fatty acyl) derivatives (**7**, **8**, **10**, and **11**), respectively. Hydrolysis of the isopropylidene group in compounds **6**–**8** under mildly acidic conditions respectively gave the desired *N*-[2-*O*-(2-acetamido-1-*O*-acyl-2,3-dideoxy-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl esters (**12**–**14**) in quantitative yields.

O-Deisopropylidenation of compounds **9**–**11** by mild, acid hydrolysis, and subsequent, hydrogenolytic removal of the benzyl group (in methanol–1,4-dioxane, with hydrogen in the presence of 10% Pd-C catalyst at room temperature) yielded the *N*-[2-*O*-(2-acetamido-1-*O*-acyl-2,3-dideoxy-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamines (**18**–**20**).

The immunoadjuvant activities of the compounds thus obtained on the in-





6 R^1 = Ac, R^2 = Me
7 R^1 = Cap, R^2 = Me
8 R^1 = Pal, R^2 = Me
9 R^1 = Ac, R^2 = Bn
10 R^1 = Cap, R^2 = Bn
11 R^1 = Pal, R^2 = Bn
Cap = decanoyl
Pal = hexadecanoyl

12 R^1 = Ac, R^2 = Me
13 R^1 = Cap, R^2 = Me
14 R^1 = Pal, R^2 = Me
15 R^1 = Ac, R^2 = Bn
16 R^1 = Cap, R^2 = Bn
17 R^1 = Pal, R^2 = Bn
18 R^1 = Ac, R^2 = H
19 R^1 = Cap, R^2 = H
20 R^1 = Pal, R^2 = H

duction of the delayed type of hypersensitivity to *N*-acetyl-L-tyrosine-3-azobenzene-4′-arsonate (ABA-*N*-acetyltyrosine) in guinea-pigs were examined[10] (see Table I). All of the compounds showed strong activity, almost comparable to that of MDP, indicating that the introduction of lipophilicity at C-1 in MDP does not diminish the activity.

EXPERIMENTAL

General methods. — Melting points were determined with a Yanagimoto

TABLE I

ADJUVANT ACTIVITY OF *N*-ACETYL-1-*O*-ACYLMURAMOYL-L-ALANYL-D-ISOGLUTAMINES ON THE INDUCTION OF DELAYED-TYPE HYPERSENSITIVITY TO ABA-*N*-ACETYLTYROSINE IN GUINEA-PIGS

Compounds[a]	*Skin reaction with ABA-BSA*[b] *(100 μg) (diam. in mm + SE)*[c] *at*	
	24 h	*48 h*
12	21.6 ±0.9	19.3 ±1.5
13	22.9 ±0.9	19.5 ±0.8
18	23.3 ±0.8	20.5 ±0.8
19	25.0 ±0.8	23.0 ±0.6
20	22.2 ±0.8	17.6 ±0.7
MDP	23.9 ±0.6	23.8 ±0.5
Control[d]	0	0

[a]Dose: 100 μg. [b]Azobenzenearsonate-*N*-acetyl-L-tyrosine-bovine serum albumin. [c]The data indicate the average diameter ± the standard error (SE) of the skin reaction (induration) of four guinea-pigs. [d]ABA-*N*-acetyltyrosine in Freund's incomplete adjuvant.

micro melting-point apparatus and are uncorrected. Specific rotations were determined with a Union PM-201 polarimeter, and i.r. spectra were recorded with a Jasco IRA-1 spectrometer. N.m.r. spectra were recorded at 90 MHz with a Hitachi R-22 spectrometer. Preparative chromatography was performed on silica gel (Waco Co.; 300 mesh) with the solvent systems specified. Evaporations were conducted *in vacuo*.

2-Acetamido-1-O-benzoyl-3-O-(D-1-carboxyethyl)-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranose (**2**). — To a cooled solution of 2-acetamido-1-*O*-benzoyl-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranose[7] (**1**; 450 mg) in 1,4-dioxane (10 mL) was added 0.2M potassium hydroxide (10 mL); the mixture was stirred for 3 min at room temperature, and then treated with Amberlite IR-120 (H^+) resin, and the resin was filtered off, and washed with methanol. The filtrate and washings were combined, and evaporated to a syrup which was extracted with chloroform. The extract was successively washed with 2M hydrochloric acid and water, dried (sodium sulfate), and evaporated to give a crystalline product. Recrystallization from ether–hexane gave needles (285 mg, 66%); m.p. 159°, $[\alpha]_D$ +133° (*c* 0.5, methanol); ν_{max}^{Nujol} 3230 (NH), 2750–2600 (OH), 1725 and 1255 (ester), 1700 (C=O), 1620 and 1550 (amide), 850 (Me_2C), and 700 and 680 cm^{-1} (phenyl).

Anal. Calc. for $C_{31}H_{37}NO_9$: C, 57.66; H, 6.22; N, 3.20. Found: C, 57.60; H, 6.15; N, 3.09.

N-*[2-O-(2-Acetamido-1-O-benzoyl-2,3-dideoxy-4,6-O-isopropylidene-α-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl*-D-*isoglutamine methyl ester* (**3**). — To a solution of **2** (510 mg) in dry 1,4-dioxane (5 mL) were added, with stirring, *N*-hydroxysuccinimide (HOSu) (144 mg) and dicyclohexylcarbodiimide (DCC) (375 mg), and the mixture was stirred for 30 min at room temperature; at that time, the starting material had been converted into the activated ester. L-Alanyl-D-isoglutamine methyl ester trifluoroacetate (380 mg) and triethylamine (170 mg) were added to the mixture, and it was stirred for 20 min at room temperature; the precipitates were filtered off, and washed with dry 1,4-dioxane. The filtrate and washings were combined, and evaporated to a syrup which was extracted with chloroform. The extract was washed with water, dried (sodium sulfate), and evaporated. The residue was purified by chromatography on a column of silica gel (10 g) with 30:1 chloroform–methanol, to give compound **3** (780 mg, quantitative) as an amorphous mass; $[\alpha]_D$ +132° (*c* 2.3, methanol); ν_{max}^{KBr} 3400–3200 (NH), 1720 and 1260 (ester), 1650 and 1550 (amide), 840 (Me_2C), and 705 cm^{-1} (phenyl); n.m.r. data (in chloroform-*d*): δ 1.31–1.35 (m, 12 H, Me_2C, 2 MeC), 1.87 (s, 3 H, AcN), 3.59 (s, 3 H, MeO), and 6.34 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1).

Anal. Calc. for $C_{30}H_{45}N_4O_{12}$: C, 55.36; H, 6.50; N, 8.61. Found: C, 55.12; H, 6.68; N, 8.51.

N-*[2-O-(2-Acetamido-2,3-dideoxy-4,6-O-isopropylidene-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester* (**4**). — To an ice-cooled solution of **3** (320 mg) in methanol (5 mL) was added sodium methoxide (10 mg), and the

mixture was kept for 15 min at 0°, and then treated with Amberlite IRC-50 (H^+) resin. The product was purified by chromatography on a column of silica gel (10 g) with chloroform and then 15:1 chloroform–methanol. The latter eluate gave **4** (220 mg, 81%) as a syrup; $[\alpha]_D$ +27° (*c* 0.4, methanol); ν_{max}^{film} 3450–3100 (OH, NH) 1720 and 1240 (ester), 1650 and 1530 (amide), and 840 cm^{-1} (Me_2C).

Anal. Calc. for $C_{23}H_{38}N_4O_{11}$: C, 50.54; H, 7.01; N, 10.25. Found: C, 50.15; H, 7.20; N, 10.11.

N-*[2-O-(2-Acetamido-2,3-dideoxy-4,6-O-isopropylidene-*D*-glucopyranose-3-yl)-*D*-lactoyl]-*L*-alanyl-*D*-isoglutamine benzyl ester* (**5**). — To an ice-cooled solution of **1** (2.7 g) in methanol (20 mL) was added sodium methoxide (30 mg), and the mixture was kept for 10 min at room temperature; at that time, the *O*-benzoyl group in **1** was completely hydrolyzed off. 0.2M Potassium hydroxide (50 mL) was added to the mixture, and it was kept for 30 min at room temperature, and then treated with Amberlite IRC-50 (H^+) resin to remove the base; the resin was filtered off, and washed with water. The filtrate and washings were combined, and evaporated at 30°. To a stirred solution of the residue in dry 1,4-dioxane (30 mL) and *N,N*-dimethylformamide (10 mL) were added HOSu (1.4 g) and DCC (2.5 g); the stirring was continued for 30 min at room temperature. 1,3-Dicyclohexylurea formed was filtered off, and washed with dry 1,4-dioxane (10 mL). The filtrate and washings were combined, L-alanyl-D-isoglutamine benzyl ester trifluoroacetate (1.4 g) and triethylamine (700 mg) were added to the solution, and it was stirred for 30 min at room temperature, and evaporated. The residue was chromatographed on a column of silica gel (50 g) with (*a*) chloroform, (*b*) 100:1, (*c*) 40:1, and (*d*) 20:1 chloroform–methanol. Eluant (*d*) afforded **5** (1.5 g, 42%) as an amorphous mass; ν_{max}^{KBr} 3400–3280 (OH, NH), 1730 and 1270 (ester), 1650 and 1530 (amide), 850 (Me_2C), and 750 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{29}H_{42}N_4O_{11}$: C, 55.94; H, 6.80; N, 9.00. Found: C, 55.83; H, 6.80; N, 8.90.

N-*[2-O-(2-Acetamido-1-O-acetyl-2,3-dideoxy-4,6-O-isopropylidene-*D*-glucopyranose-3-yl)-*D*-lactoyl]-*L*-alanyl-*D*-isoglutamine methyl ester* (**6**). — Compound **4** (220 mg) was acetylated at room temperature with acetic anhydride (3 mL)–pyridine (3 mL), and the product was purified by chromatography on a column of silica gel (10 g) with 15:1 chloroform–methanol as the eluant. The acetate (**6**) was obtained as a syrup (230 mg, quantitative); $[\alpha]_D$ +52.3° (*c* 0.5, methanol); ν_{max}^{film} 3370–3240 (NH), 1750, 1730, 1240, and 1220 (ester), 1650 and 1530 (amide), and 850 cm^{-1} (Me_2C); n.m.r. data (in chloroform-*d*): δ 1.30–1.51 (m, 12 H, Me_2C, 2 MeC), 1.95 (s, 3 H, AcN), 2.13 (s, 3 H, AcO), 5.75 (d, $J_{1,2}$ 8.0 Hz, H-1β), and 6.15 (d, $J_{1,2}$ 3.8 Hz, H-1α); anomeric ratio (α:β) was estimated at ~7:1 from the ratio of the intensity of H-1α and of H-1β.

Anal. Calc. for $C_{25}H_{40}N_4O_{12}$: C, 51.01; H, 6.85; N, 9.52. Found: C, 50.83; H, 7.02; N, 9.48.

N-*[2-O-(2-Acetamido-1-O-decanoyl-2,3-dideoxy-4,6-O-isopropylidene-*D*-glucopyranose-3-yl)-D-lactoyl]-*L*-alanyl-*D*-isoglutamine methyl ester* (**7**). — To a

stirred solution of **4** (240 mg) in pyridine (2 mL) and dichloromethane (4 mL) was added a solution of decanoyl chloride (200 mg) in dichloromethane (4 mL) at 0°, and the mixture was stirred for 5 h at 0°; methanol (1 mL) was added to the mixture, the solution evaporated, and the residue extracted with chloroform. The resulting residue was chromatographed on a column of silica gel (10 g) with (*a*) 70:1 and (*b*) 20:1 chloroform–methanol. The eluate obtained with (*b*) gave **7** (160 mg, 52%) as a syrup; $[\alpha]_D$ +29.1° (*c* 0.3, methanol); ν_{max}^{film} 3260 (NH), 2900 and 2830 (Me, methylene), 1740 and 1260 (ester), 1650 and 1540 (amide), and 860 cm^{-1} (Me_2C); n.m.r. data (in chloroform-*d*): δ 0.87 (near t, 3 H, J_{MeCH_2} 5.5 Hz, $MeCH_2$), 1.25–1.50 (m, 26 H, Me_2C, 2 MeC, 7 CH_2), 1.94 (s, 3 H, AcN), 3.67 (s, 3 H, MeO), 5.70 (d, $J_{1,2}$ 9.0 Hz, H-1β), and 6.13 (d, $J_{1,2}$ 3.8 Hz, H-1α); the anomeric ratio (α:β), estimated as just described, was ~4:1.

Anal. Calc. for $C_{33}H_{56}N_4O_{15}$: C, 56.55; H, 8.05; N, 7.99. Found: C, 56.41; H, 8.01; N, 7.80.

N-*[2-O-(2-Acetamido-2,3-dideoxy-1-O-hexadecanoyl-4,6-O-isopropylidene-*D-*glucopyranose-3-yl)-*D-*lactoyl]-*L-*alanyl-*D-*isoglutamine methyl ester* (**8**). — Coupling of **4** (133 mg) with hexadecanoyl chloride (67 mg) in pyridine (1 mL) and dichloromethane (3 mL), as described for **7**, gave **8** (100 mg, 52%) as a syrup; $[\alpha]_D$ +30.0° (*c* 0.42, methanol); ν_{max}^{film} 3250 (NH), 2900 and 2830 (Me, methylene), 1740 and 1260 (ester), 1650 and 1530 (amide), and 860 cm^{-1} (Me_2C); n.m.r. data (in chloroform-*d*): δ 0.89 (near t, 3 H, J_{MeCH_2} 6.0 Hz, $MeCH_2$), 1.20–1.53 (m, 38 H, Me_2C, 2 MeC, 13 CH_2), 1.92, 1.93 (2 s, AcN), 3.68 (s, 3 H, MeO), 5.70 (d, $J_{1,2}$ 8.5 Hz, H-1β), and 6.14 (d, $J_{1,2}$ 3.8 Hz, H-1β); anomeric ratio (α:β) estimated as described for **6**, was ~7:3.

Anal. Calc. for $C_{50}H_{88}N_4O_{15}$: C, 59.67; H, 8.75; N, 7.14. Found: C, 59.63; H, 8.91; N, 7.15.

N-*[2-O-(2-Acetamido-1-O-acetyl-2,3-dideoxy-4,6-O-isopropylidene-*D-*glucopyranose-3-yl)-*D-*lactoyl]-*L-*alanyl-*D-*isoglutamine benzyl ester* (**9**). — Compound **5** (100 mg) was acetylated at room temperature with acetic anhydride (0.5 mL)–pyridine (1 mL) as described for **6**, to give **9** (110 mg, quantitative); $[\alpha]_D$ +29.1° (*c* 0.2, chloroform); ν_{max}^{film} 3350–3230 (NH), 1730 and 1220 (ester), 1640 and 1530 (amide), 850 (Me_2C), and 750 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{41}H_{60}N_4O_{17}$: C, 56.02; H, 6.66; N, 8.43. Found: C, 55.90; H, 6.85; N, 8.43.

N-*[2-O-(2-Acetamido-1-O-decanoyl-2,3-dideoxy-4,6-O-isopropylidene-*D-*glucopyranose-3-yl)-*D-*lactoyl]-*L-*alanyl-*D-*isoglutamine benzyl ester* (**10**). — Coupling of **5** (150 mg) with decanoyl chloride (150 mg) in pyridine (1 mL) and dichloromethane (3 mL), according to the procedure already described, gave **10** (98 mg, 51%) as a syrup; $[\alpha]_D$ +23.4° (*c* 0.3, chloroform); ν_{max}^{film} 3300–3230 (NH), 2920 and 2830 (Me, methylene), 1730 and 1260 (ester), 1640 and 1530 (amide), 850 (Me_2C), and 750 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{50}H_{68}N_4O_{15}$: C, 60.29; H, 7.79; N, 7.21. Found: C, 60.18; H, 7.93; N, 7.20.

N-*[2-O-(2-Acetamido-2,3-dideoxy-1-O-hexadecanoyl-4,6-O-isopropylidene-*D-*glucopyranose-3-yl)-*D-*lactoyl]-*L-*alanyl-*D-*isoglutamine benzyl ester* (**11**). — Coupling of **5** (150 mg) with hexadecanoyl chloride (150 mg) in pyridine (1 mL) and dichloromethane (4 mL), as described for **7**, yielded **11** (111 mg, 52%) as a syrup; $[\alpha]_D$ +29.2° (*c* 1.1, chloroform); ν_{max}^{film} 3360–3230 (NH), 2900 and 2830 (Me, methylene), 1730 and 1260 (ester), 1650 and 1530 (amide), 850 (Me_2C), and 750 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{45}H_{72}N_4O_{12}$: C, 62.77; H, 8.43; N, 6.50. Found: C, 62.59; H, 8.50; N, 6.44.

N-*[2-O-(2-Acetamido-1-O-acetyl-2,3-dideoxy-*D-*glucopyranose-3-yl)-*D-*lactoyl]-*L-*alanyl-*D-*isoglutamine methyl ester* (**12**). — A solution of **6** (97 mg) in 80% aqueous acetic acid (2 mL) was heated for 2 h at 45°, the course of the reaction being monitored by t.l.c. The mixture was lyophilized, to give a hygroscopic, amorphous mass (90 mg, quantitative), which showed a single spot in t.l.c.; $[\alpha]_D$ +52.9° (*c* 0.85, methanol); ν_{max}^{film} 3500–3230 (OH, NH), 1730 and 1230 (ester), and 1650 and 1530 cm^{-1} (amide); n.m.r. data (in 1:1 chloroform-*d*–methanol-d_4): δ 1.43 (2 d, 6 H, $J_{Me,CH}$ 5.0 Hz, 2 MeC), 1.94 (s, 3 H, AcN), 2.15 (s, 3 H, AcO), and 6.08 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1).

Anal. Calc. for $C_{22}H_{36}N_4O_{12}$: C, 48.17; H, 6.62; N, 10.21. Found: C, 47.85; H, 6.97; N, 9.93.

Other *N*-[2-*O*-(2-acetamido-1-*O*-acyl-2,3-dideoxy-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl esters (**13** and **14**) and the benzyl esters (**15–17**) were prepared by hydrolysis from the corresponding, 4,6-*O*-isopropylidene derivatives (**7–11**), as described for **12**.

N-*[2-O-(2-Acetamido-1-O-decanoyl-2,3-dideoxy-*D-*glucopyranose-3-yl)-*D-*lactoyl]-*L-*alanyl-*D-*isoglutamine methyl ester* (**13**). — Compound **13** was obtained as an amorphous mass in quantitative yield; $[\alpha]_D$ +32.7° (*c* 0.52, methanol); ν_{max}^{KBr} 3350–3230 (OH, NH), 2900 and 2830 (Me, methylene), 1730, 1710, and 1240 (ester), and 1650 and 1530 cm^{-1} (amide).

Anal. Calc. for $C_{30}H_{52}N_4O_{12}$: C, 54.53; H, 7.93; N, 8.48. Found: C, 54.49; H, 8.06; N, 8.52.

N-*[2-O-(2-Acetamido-1-O-hexadecanoyl-2,3-dideoxy-*D-*glucopyranose-3-yl)-*D-*lactoyl]-*L-*alanyl-*D-*isoglutamine methyl ester* (**14**). — Compound **14** was obtained as an amorphous mass in quantitative yield; $[\alpha]_D$ +11.1° (*c* 0.44, methanol); ν_{max}^{KBr} 3360–3230 (OH, NH), 2900 and 2830 (Me, methylene), 1730, 1710, and 1250 (ester), and 1650 and 1520 cm^{-1} (amide).

Anal. Calc. for $C_{36}H_{64}N_4O_{12}$: C, 58.04; H, 8.66; N, 7.52. Found: C, 58.14; H, 8.85; N, 7.27.

N-*[2-O-(2-Acetamido-1-O-acetyl-2,3-dideoxy-*D-*glucopyranose-3-yl)-*D-*lactoyl]-*L-*alanyl-*D-*isoglutamine benzyl ester* (**15**). — Compound **15** was obtained as a syrup in quantitative yield; $[\alpha]_D$ +27.0° (*c* 0.15, chloroform); ν_{max}^{film} 3360–3230 (OH, NH), 1730, 1710, and 1240–1220 (ester), 1650 and 1530 (amide), and 740 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{28}H_{40}N_4O_{12}$: C, 53.83; H, 6.46; N, 8.97. Found: C, 53.55; H, 6.73; N, 8.65.

N-*[2-O-(2-Acetamido-1-O-decanoyl-2,3-dideoxy-*D-*glucopyranose-3-yl)-*D-*lactoyl]-*L-*alanyl-*D-*isoglutamine benzyl ester* (**16**). — Compound **16** was obtained quantitatively as a syrup; $[\alpha]_D$ +26.5° (*c* 0.55, 1:1 chloroform–methanol); ν_{max}^{film} 3370–3220 (OH, NH), 2900 and 2830 (Me, methylene), 1730, 1720, and 1250 (ester), 1650 and 1530 (amide), and 740 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{36}H_{56}N_4O_{12}$: C, 58.68; H, 7.66; N, 7.60. Found: C, 58.49; H, 7.83; N, 7.55.

N-*[2-O-(2-Acetamido-2,3-dideoxy-1-O-hexadecanoyl-*D-*glucopyranose-3-yl)-*D-*lactoyl]-*L-*alanyl-*D-*isoglutamine benzyl ester* (**17**). — Compound **17** was obtained quantitatively as a syrup; $[\alpha]_D$ +33.3° (*c* 0.85, 1:1 chloroform–methanol); ν_{max}^{film} 3320–3230 (OH, NH), 2920 and 2830 (Me, methylene), 1730, 1720, and 1250 (ester), 1650 and 1540 (amide), and 740 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{42}H_{68}N_4O_{12}$: C, 61.44; H, 8.35; N, 6.82. Found: C, 61.32; H, 8.64; N, 6.76.

N-*[2-O-(2-Acetamido-1-O-acetyl-2,3-dideoxy-*D-*glucopyranose-3-yl)-*D-*lactoyl]-*L-*alanyl-*D-*isoglutamine* (**18**). — Compound **15** (50 mg) was dissolved in methanol (5 mL) and 1,4-dioxane (5 mL); 10% Pd–C catalyst (50 mg) was added, and hydrogen was bubbled through while the mixture was stirred for 20 min at room temperature; at that time, the reaction was complete. After removal of the catalyst by filtration, the filtrate was evaporated to give **18** (43 mg, quantitative) as an amorphous mass, which showed a single spot in t.l.c.; $[\alpha]_D$ +35.0° (*c* 0.5, methanol); ν_{max}^{KBr} 3350–3220 (OH, NH), 1720 and 1220 (ester), 1700 (C=O), and 1640 and 1520 cm^{-1} (amide).

Anal. Calc. for $C_{21}H_{34}N_4O_{12}$: C, 47.19; H, 6.41; N, 10.48. Found: C, 46.85; H, 6.63; N, 10.45.

N-*[2-O-(2-Acetamido-1-O-decanoyl-2,3-dideoxy-*D-*glucopyranose-3-yl)-*D-*lactoyl]-*L-*alanyl-*D-*isoglutamine* (**19**). — Hydrogenation of **16** (55 mg) in methanol (5 mL) and 1,4-dioxane (5 mL), in the presence of 10% Pd–C catalyst (55 mg), according to the procedure already described, gave amorphous **19** (46 mg, quantitative); $[\alpha]_D$ +31.7° (*c* 0.6, methanol); ν_{max}^{KBr} 3350–3300 (OH, NH), 2900 and 2820 (Me, methylene), 1730 and 1250 (ester), 1710 (C=O), and 1650 and 1530 cm^{-1} (amide).

Anal. Calc. for $C_{29}H_{50}N_4O_{12}$: C, 53.86; H, 7.79; N, 8.66. Found: C, 53.67; H, 7.98; N, 8.51.

N-*[2-O-(2-Acetamido-2,3-dideoxy-1-O-hexadecanoyl-*D-*glucopyranose-3-yl)-*D-*lactoyl]-*L-*alanyl-*D-*isoglutamine* (**20**). — Hydrogenation of **17** (85 mg) in methanol (10 mL) and 1,4-dioxane (10 mL) in the presence of 10% Pd–C catalyst (85 mg), as already described, gave amorphous **20** (73 mg, quantitative); $[\alpha]_D$ +24.0° (*c* 0.2, methanol); ν_{max}^{KBr} 3350–3240 (OH, NH), 2900 and 2830 (Me,

methylene), 1730 and 1260 (ester), 1710 (C=O), and 1650 and 1530 cm^{-1} (amide).

Anal. Calc. for $C_{35}H_{62}N_4O_{12}$: C, 57.52; H, 8.55; N, 7.67. Found: C, 57.39; H, 8.58; N, 7.52.

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