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SYNTHESIS OF 2,6-DI(ACYLAMINO)-2,6-DIDEOXY-3-*O*-(D-2-PROPANOYL-L-ALANYL-D-ISOGLUTAMINE)-D-GLUCOPYRANOSES*

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ABSTRACT

Five 2,6-di(acylamino)-2,6-dideoxy-3-*O*-(D-2-propanoyl-L-alanyl-D-isoglutamine)-D-glucopyranoses (lipophilic, muramoyl dipeptide analogs) were synthesized from benzyl 2-(benzyloxycarbonylamino)-3-*O*-(D-1-carboxyethyl)-2-deoxy-5,6-*O*-isopropylidene-β-D-glucopyranoside (**1**). Methanesulfonylation of **3**, derived from the methyl ester of **1** by *O*-deisopropylidenation, gave the 6-methanesulfonate (**4**). (Tetrahydropyran-2-yl)ation of **4** gave benzyl 2-(benzyloxycarbonylamino)-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-6-*O*-(methylsulfonyl)-5-*O*-(tetrahydropyran-2-yl)-β-D-glucofuranoside, which was treated with sodium azide to give the corresponding 6-azido derivative (**6**). Condensation of benzyl 6-amino-2-(benzyloxycarbonylamino)-2,6-dideoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-5-*O*-(tetrahydropyran-2-yl)-β-D-glucofuranoside, derived from **6** by reduction, with the activated esters of octanoic, hexadecanoic, and eicosanoic acid gave the corresponding 6-*N*-fatty acyl derivatives (**8–10**). Coupling of the 2-amino derivatives, obtained from compounds **8**, **9**, and **10** by catalytic reduction, with the activated esters of the fatty acids, gave the 2,6-(diacylamino)-2,6-dideoxy derivatives (**11–15**). Condensation of the acids, formed from **11–15** by de-esterification, with the benzyl ester of L-alanyl-D-isoglutamine, and subsequent hydrolysis, afforded benzyl 2,6-di(acylamino)-2,6-dideoxy-3-*O*-(D-2-propanoyl-L-alanyl-D-isoglutamine benzyl ester)-β-D-glucofuranosides. Hydrogenation of the dipeptide derivatives thus obtained gave the five lipophilic analogs of 6-amino-6-deoxymuramoyl dipeptide, respectively, in good yields.

INTRODUCTION

In the course of an investigation[2] on the relationship between the immunoadjuvant activity and the structure of the carbohydrate moiety in *N*-acetylmuramoyl-L-alanyl-D-isoglutamine[3] (MDP), which is the minimal, immunoadjuvant-active component of bacterial cell-wall peptidoglycan, we demonstrated that not only is the restricted configuration of the sugar moiety important for the activity[2] but also that chemical modifications[4–6] of the functional groups in the carbohydrate moiety

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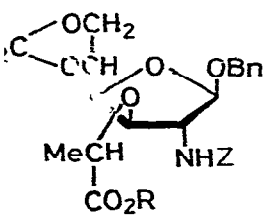
produce various, important effects on the manifestation of activity. Recently, it has been shown that lipophilic derivatives[7,8] of MDP bearing the lipid moiety at C-6 of the sugar skeleton, or at the end of the peptide chain, have strong antitumor and anti-infection activities that are not found for MDP itself. In addition, we have also observed that introduction[4b,9,10] of lipophilic character at C-2 in muramoyl-L-alanyl-D-isoglutamine, or at C-6 in *N*-acetyl-6-amino-6-deoxymuramoyl-L-alanyl-D-isoglutamine, causes potent antitumor activity based on the immune reaction, as well as strong, immunoadjuvant activities.

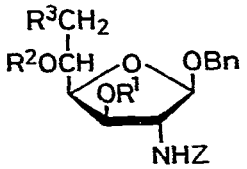
In view of these facts, we now describe the synthesis of some 2,6-(diacylamino)-2,6-dideoxy-3-*O*-(D-2-propanoyl-L-alanyl-D-isoglutamine)-D-glucopyranoses.

RESULTS AND DISCUSSION

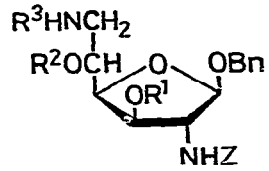
Treatment of benzyl 2-(benzyloxycarbonylamino)-3-*O*-(D-1-carboxyethyl)-2-deoxy-5,6-*O*-isopropylidene-β-D-glucofuranoside[11] (**1**) with diazomethane gave crystalline **2** in good yield; this was hydrolyzed by heating with 60% aqueous acetic acid, to afford **3**. Selective mesylation, with methanesulfonyl chloride in pyridine at −10°, of the primary hydroxyl group on C-6 in **3** afforded, in 89% yield, **4**, which on (tetrahydropyran-2-yl)ation, gave **5**, quantitatively. Displacement of the mesyloxy group in **5** with sodium azide in dry *N*,*N*-dimethylformamide for 15 h at 80° gave



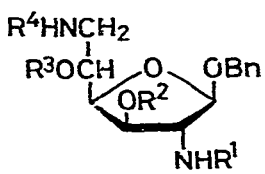
1 R = H
2 R = Me
Bn = $PhCH_2$
Z = $PhCH_2OCO$

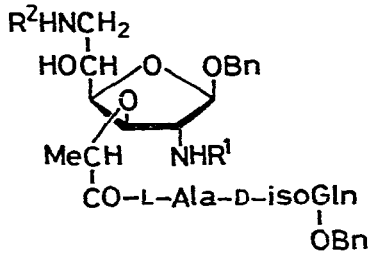


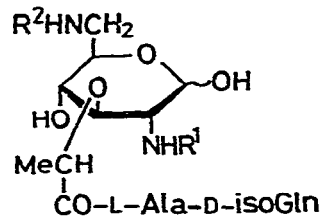
3 R^1 = a, R^2 = H, R^3 = OH
4 R^1 = a, R^2 = H, R^3 = OMs
5 R^1 = a, R^2 = Thp, R^3 = OMs
6 R^1 = a, R^2 = Thp, R^3 = N_3
a = $-CH(Me)CO_2Me$
Thp = tetrahydropyran-2-yl



7 R^1 = a, R^2 = Thp, R^3 = H
8 R^1 = a, R^2 = Thp, R^3 = b
9 R^1 = a, R^2 = Thp, R^2 = c
10 R^1 = a, R^2 = Thp, R^3 = d
b = octanoyl
c = hexadecanoyl
d = eicosanoyl







11 R^1 = R^4 = b, R^2 = a, R^3 = Thp
12 R^1 = R^4 = c, R^2 = a, R^3 = Thp
13 R^1 = R^4 = d, R^2 = a, R^3 = Thp
14 R^1 = b, R^2 = a, R^3 = Thp, R^4 = d
15 R^1 = d, R^2 = a, R^3 = Thp, R^4 = b

16 R^1 = R^2 = b
17 R^1 = R^2 = c
18 R^1 = R^2 = d
19 R^1 = b, R^2 = d
20 R^1 = d, R^2 = b

21 R^1 = R^2 = b
22 R^1 = R^2 = c
23 R^1 = R^2 = d
24 R^1 = b, R^2 = d
25 R^1 = d, R^2 = b

benzyl 6-azido-2-(benzyloxycarbonylamino)-2,6-dideoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-5-*O*-(tetrahydropyran-2-yl)-β-D-glucofuranoside (**6**) in 87% yield. Reduction of the azide function in compound **6** in ethanol with Raney nickel (W-1) gave the desired 6-amino compound **7**, which was used for the next reaction without purification.

Compound **7** was treated with *N*-(octanoyloxy)succinimide in dry 1,4-dioxane for 5 h at room temperature, affording benzyl 2-(benzyloxycarbonylamino)-2,6-dideoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-6-(octanoylamino)-5-*O*-(tetrahydropyran-2-yl)-β-D-glucofuranoside (**8**) in 80% yield. When treated with *N*-(hexadecanoyloxy)succinimide, or *N*-(eicosanoyloxy)succinimide, according to the procedure just described, compound **7** gave the 6-*N*-fatty acyl derivatives **9** and **10** in excellent yield. Selective hydrogenolysis of the benzyloxycarbonyl group in compounds **8–10** with hydrogen in the presence of 10% Pd–C catalyst in ethanol, and subsequent condensation with the activated esters of octanoic, hexadecanoic, and eicosanoic acid as already described, yielded the expected benzyl 2,6-di(acylamino)-2,6-dideoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-β-D-glucofuranosides **11–15** in good yield. Saponification of **11** with 0.1M aqueous potassium hydroxide in 1,4-dioxane gave the free acid, which was used for the next reaction without purification. Coupling of the acid with L-alanyl-D-isoglutamine benzyl ester, using dicyclohexylcarbodiimide and *N*-hydroxysuccinimide as the activating agents, and subsequent hydrolysis of the tetrahydropyran-2-yl group under mildly acidic conditions (80% aqueous acetic acid) gave benzyl 2,6-dideoxy-2,6-di(octanoylamino)-3-*O*-(D-2-propanoyl-L-alanyl-D-isoglutamine benzyl ester)-β-D-glucofuranoside (**16**). In the same way, coupling of the acids derived from compounds **12–15**, by hydrolysis, with the L-alanyl-D-isoglutamine derivatives, and subsequent removal of the tetrahydropyran-2-yl group, yielded the corresponding dipeptides **17–20** in good yields.

Hydrogenolysis of the benzyl groups in compound **16**, in methanol–acetic acid, with hydrogen in the presence of Pd–C catalyst gave 2,6-dideoxy-2,6-di(octanoylamino)-3-*O*-(D-2-propanoyl-L-alanyl-D-isoglutamine)-D-glucopyranose (**21**) in good yield. By essentially the same procedure, compounds **17–20** yielded the corresponding 2,6-di(acylamino)-2,6-dideoxy-3-*O*-(D-2-propanoyl-L-alanyl-D-isoglutamine)-D-glucopyranoses (**22–25**) in good yields.

EXPERIMENTAL

General methods. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Specific rotations were determined with a Union PM-201 polarimeter, and i.r. spectra were recorded with a Jasco IRA-1 spectrophotometer. N.m.r. spectra were recorded at 60 and 90 MHz with Hitachi R-24-BS and R-22 spectrometers. Preparative chromatography was performed on silica gel (Waco Co.; 300 mesh) with the solvent systems specified. Evaporations were conducted *in vacuo*.

Benzyl 2-(benzyloxycarbonylamino)-2-deoxy-5,6-O-isopropylidene-3-O-[D-*1-*

(methoxycarbonyl)ethyl]-β-D-glucofuranoside (**2**). — To a solution of benzyl 2-(benzyoxycarbonylamino)-3-*O*-(D-1-carboxyethyl)-2-deoxy-5,6-*O*-isopropylidene-β-D-glucofuranoside[11] (**1**; 4.5 g) in ether (50 mL) was added an ether solution of diazomethane, and the mixture was kept at room temperature; after 1 h, the excess of the reagent was decomposed by adding acetic acid. The mixture was evaporated, to give a crystalline mass. Recrystallization from ether–hexane afforded **2** (4.1 g, 89%) as needles, m.p. 80°, $[\alpha]_D^{25}$ −29.8° (*c* 1.0, chloroform); ν_{max}^{Nujol} 3300 (NH), 1735 and 1140 (ester), 1690 and 1540 (amide), 840 (Me_2C), and 760 and 695 cm^{-1} (phenyl); n.m.r. data at 60 MHz (in chloroform-*d*): δ 1.31, 1.37 (2 s, 6 H, Me_2C), 1.38 (d, 3 H, $J_{Me,CH}$ 7.0 Hz, MeCH), 3.65 (s, 3 H, MeO), 4.46, 4.75 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 4.99 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 5.09 (s, 2 H, CO_2CH_2Ph), 5.48 (d, 1 H, $J_{2,NH}$ 7.5 Hz, NH), and 7.27 (s, 10 H, 2 Ph).

Anal. Calc. for $C_{28}H_{35}NO_9$: C, 63.50; H, 6.66; N, 2.64. Found: C, 63.38; H, 6.73; N, 2.71.

Benzyl 2-(benzyloxycarbonylamino)-2-deoxy-3-O-[D-1-(methoxycarbonyl)ethyl]-β-D-glucofuranoside (**3**). — A solution of **2** (4.0 g) in 60% aqueous acetic acid (50 mL) was heated for 2 h at 45°, and then evaporated. The residue crystallized from ethanol–ether, to give **3** (3.2 g, 86%) as needles, m.p. 93°, $[\alpha]_D^{25}$ −80° (*c* 1.0, chloroform); ν_{max}^{Nujol} 3400–3280 (OH, NH), 1730 and 1240 (ester), 1690 and 1540 (amide), and 740 and 690 cm^{-1} (phenyl); n.m.r. data at 60 MHz (in chloroform-*d*): δ 1.41 (d, 3 H, $J_{Me,CH}$ 7.0 Hz, MeCH), 3.10 (m, 2 H, 2 OH), 3.64 (s, 3 H, MeO), 4.42, 4.72 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 4.95 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.08 (s, 2 H, CO_2CH_2Ph), 5.90 (d, 1 H, $J_{2,NH}$ 7.0 Hz, NH), and 7.29 (s, 10 H, 2 Ph).

Anal. Calc. for $C_{25}H_{31}NO_9$: C, 61.34; H, 6.38; N, 2.86. Found: C, 61.28; H, 6.37; N, 2.79.

Benzyl 2-(benzyloxycarbonylamino)-2-deoxy-6-O-mesyl-3-O-[D-1-(methoxycarbonyl)ethyl]-β-D-glucofuranoside (**4**). — To a cooled solution of **3** (3.0 g) in dry pyridine (20 mL) was added methanesulfonyl chloride (850 mg) at −20°, and the mixture was kept for 5 h at −10°. The mixture was evaporated, the residue extracted with chloroform, and the extract successively washed with 2M hydrochloric acid, 5% sodium carbonate, and water, dried (sodium sulfate), and evaporated to a syrup which was purified by chromatography on a column of silica gel (80 g) with 100:1 chloroform–methanol. Compound **4** was obtained as needles; after recrystallization from ether–hexane, 3.1 g (89%), m.p. 65°, $[\alpha]_D^{25}$ −56° (*c* 1.0, chloroform); ν_{max}^{Nujol} 3500 (OH), 3280 (NH), 1760 (ester), 1695 and 1545 (amide), 1165 (SO_2), and 735 and 690 cm^{-1} (phenyl); n.m.r. data at 60 MHz (in chloroform-*d*): δ 1.44 (d, 3 H, $J_{Me,CH}$ 7.0 Hz, MeCH), 2.97 (s, 3 H, MeS), 3.66 (s, 3 H, MeO), 4.46, 4.75 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 5.00 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.12 (s, 2 H, CO_2CH_2Ph), 5.63 (d, 1 H, $J_{2,NH}$ 8.0 Hz, NH), and 7.30 and 7.33 (2 s, 10 H, 2 Ph).

Anal. Calc. for $C_{26}H_{33}NO_{11}S$: C, 55.01; H, 5.86; N, 2.47. Found: C, 55.13; H, 5.86; N, 2.52.

Benzyl 2-(benzyloxycarbonylamino)-2-deoxy-6-O-mesyl-3-O-[D-1-(methoxycarbonyl)ethyl]-5-O-(tetrahydropyran-2-yl)-β-D-glucofuranoside (**5**). — To a stirred

solution of **4** (3.0 g) in dry 1,4-dioxane (30 mL) were added dihydropyran (1.5 g) and *p*-toluenesulfonic acid monohydrate (30 mg). The mixture was stirred for 3 h at room temperature, treated with Amberlite IR-45 (OH^-) ion-exchange resin to remove the acid, and evaporated to a syrup which was chromatographed on a column of silica gel (60 g) with chloroform, and then with 150:1 chloroform–methanol. The latter eluate gave compound **5** (3.3 g, 96%) as a syrup, $[\alpha]_D^{25}$ −28° (*c* 1.0, chloroform); ν_{max}^{film} 3320 (NH), 1750 and 1240 (ester), 1700 and 1520 (amide), 1170 (SO_2), and 750 and 690 cm^{-1} (phenyl); n.m.r. data at 60 MHz (in chloroform-*d*): δ 1.34, 1.38 (2 d, 3 H, $J_{Me,CH}$ 7.0 Hz, MeCH), 2.84, 2.97 (2 s, 3 H, MeS), 3.60 (s, 3 H, MeO), 4.94 (s, 1 H, H-1), 5.05 (s, 2 H, CO_2CH_2Ph), 5.63 (d, 1 H, $J_{2,NH}$ 7.0 Hz, NH), and 7.22 and 7.24 (2 s, 10 H, 2 Ph).

Anal. Calc. for $C_{31}H_{41}NO_{12}S$: C, 57.13; H, 6.34; N, 2.15. Found: C, 57.22; H, 6.35; N, 2.09.

Benzyl 6-azido-2-(benzyloxycarbonylamino)-2,6-dideoxy-3-O-[D-*1-(methoxycarbonyl)ethyl*]*-5-O-(tetrahydropyran-2-yl)-β-*D*-glucofuranoside* (**6**). — To a solution of **5** (3.0 g) in dry *N*,*N*-dimethylformamide (20 mL) was added sodium azide (2.4 g), and the mixture was heated, with stirring, for 15 h at 80°, evaporated, and the residue extracted with chloroform. The extract was washed with water, dried (sodium sulfate), and evaporated to a syrup which was purified by chromatography on a column of silica gel (60 g) with 200:1 chloroform–methanol, to afford compound **6** (2.4 g, 87%) as a syrup, $[\alpha]_D^{20}$ −24° (*c* 1.1, chloroform); ν_{max}^{film} 3290 (NH), 2080 (azide), 1720 and 1240 (ester), 1700 and 1520 (amide), and 730 and 690 cm^{-1} (phenyl); n.m.r. data at 60 MHz (in chloroform-*d*): δ 1.36 (d, 3 H, $J_{Me,CH}$ 7.0 Hz, MeCH), 3.62 (s, 3 H, MeO), 4.43, 4.73 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 4.99 (s, 1 H, H-1), 5.00 (s, 2 H, CO_2CH_2Ph), 5.61 (d, 1 H, $J_{2,NH}$ 7.0 Hz, NH), and 7.27 and 7.30 (2 s, 10 H, 2 Ph).

Anal. Calc. for $C_{30}H_{38}N_4O_9$: C, 60.19; H, 6.40; N, 9.36. Found: C, 60.25; H, 6.39; N, 9.29.

Benzyl 2-(benzyloxycarbonylamino)-2,6-dideoxy-3-O-[D-*1-(methoxycarbonyl)-ethyl*]*-6-(octanoylamino)-5-O-(tetrahydropyran-2-yl)-β-*D*-glucofuranoside* (**8**). — To a solution of **6** (410 mg) in ethanol (25 mL) was added Raney Ni (W-1; 500 mg), and the mixture was stirred for 1.5 h at room temperature. The catalyst was filtered off, and washed with methanol, and the filtrate and washings were combined, and evaporated to a syrup which was used for the next reaction without purification. To a solution of the amino compound **7** in dry 1,4-dioxane (5 mL) was added *N*-(octanoyloxy)-succinimide (230 mg), and the mixture was stirred at room temperature while the progress of the reaction was monitored by t.l.c.; after 5 h, the starting material was no longer detectable. The mixture was evaporated to a syrup which was chromatographed on a column of silica gel (30 g) with chloroform and then 100:1 chloroform–methanol. The latter eluate gave 385 mg (80%) of **8** as a syrup, $[\alpha]_D^{25}$ −29° (*c* 1.2, chloroform); ν_{max}^{film} 3280 (NH), 2900 and 2830 (Me, methylene), 1740 and 1240 (ester), 1700, 1650, and 1530 (amide), and 750 and 690 cm^{-1} (phenyl); n.m.r. data at 90 MHz (in chloroform-*d*): δ 0.87 (near t, 3 H, J_{Me,CH_2} 5.4 Hz, $MeCH_2$), 1.23 (m, 10 H, 5

methylene), 1.49 (d, 3 H, $J_{Me,CH}$ 7.0 Hz, MeCH), 1.54 (m, 6 H, tetrahydropyranyl methylene), 2.02 (m, 2 H, $COCH_2$), 3.60, 3.63 (2 s, 3 H, MeO), 5.05 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 5.08 (s, 2 H, CO_2CH_2Ph), and 7.21–7.30 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{38}H_{54}N_2O_{10}$: C, 65.31; H, 7.79; N, 4.01. Found: C, 65.23; H, 7.61; N, 3.99.

Benzyl 2-(benzyloxycarbonylamino)-2,6-dideoxy-6-(hexadecanoylamino)-3-O-[D-1-(methoxycarbonyl)ethyl]-5-O-(tetrahydropyran-2-yl)-β-D-glucofuranoside (**9**). — Reduction of **6** (220 mg) with Raney Ni (W-1; 400 mg) in ethanol (20 mL), with processing as already described, gave the amino compound **7**. Coupling of the amine with *N*-(hexadecanoyloxy)succinimide (190 mg) in dry 1,4-dioxane (5 mL), as described for **8**, afforded **9** (290 mg, 97%) as a syrup, $[\alpha]_D^{25}$ −27° (*c* 0.3, chloroform); ν_{max}^{film} 3280 (NH), 2900 and 2830 (Me, methylene), 1730 and 1240 (ester), 1700, 1640, and 1520 (amide), and 730 and 690 cm^{-1} (phenyl); n.m.r. data at 90 MHz (in chloroform-*d*): δ 0.88 (near t, 3 H, J_{Me,CH_2} 5.4 Hz, $MeCH_2$), 1.25 (26 H, 13 methylene), 1.40 (d, 3 H, $J_{Me,CH}$ 7.0 Hz, MeCH), 1.55 (m, 6 H, tetrahydropyranyl methylene), 2.05 (m, 2 H, CH_2CO), 3.64 (s, 3 H, MeO), 4.51, 4.81 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 5.10 (s, 3 H, H-1, CO_2CH_2Ph), 5.69 (d, 1 H, $J_{2,NH}$ 6.5 Hz, NH), 6.34 (near t, 1 H, $J_{6,NH}$ 6.0 Hz, NH), and 7.30 (s, 10 H, 2 Ph).

Anal. Calc. for $C_{46}H_{70}N_2O_{10}$: C, 68.12; H, 8.70; N, 3.45. Found: C, 68.32; H, 8.73; N, 3.41.

Benzyl 2-(benzyloxycarbonylamino)-2,6-dideoxy-6-(eicosanoylamino)-3-O-[D-1-(methoxycarbonyl)ethyl]-5-O-(tetrahydropyran-2-yl)-β-D-glucofuranoside (**10**). — Reduction of **6** (350 mg) with Raney Ni (500 mg) in ethanol (20 mL), as described for **8**, gave the amino compound, which was condensed with *N*-(eicosanoyloxy)succinimide (350 mg) in dry 1,4-dioxane (10 mL) as already described, to yield **10** (460 mg, 91%) as a syrup, $[\alpha]_D^{25}$ −29° (*c* 1.2, chloroform); ν_{max}^{film} 3300 and 3230 (NH), 2900 and 2830 (Me, methylene), 1750 and 1240 (ester), 1710, 1650, and 1520 (amide), and 730 and 690 cm^{-1} (phenyl); n.m.r. data at 90 MHz (in chloroform-*d*): δ 0.87 (near t, 3 H, J_{Me,CH_2} 5.4 Hz, $MeCH_2$), 1.25 (34 H, 17 methylene), 1.41 (d, 3 H, $J_{Me,CH}$ 7.0 Hz, MeCH), 1.52 (m, 6 H, tetrahydropyranyl methylene), 2.10 (m, 2 H, CH_2CO), 3.60 (s, 3 H, MeO), 4.49, 4.73 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 5.05 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 5.07 (s, 2 H, CO_2CH_2Ph), and 7.22 and 7.26 (2 s, 10 H, 2 Ph).

Anal. Calc. for $C_{50}H_{78}N_2O_{10}$: C, 69.25; H, 9.07; N, 3.23. Found: C, 69.08; H, 9.25; N, 3.35.

Benzyl 2,6-dideoxy-2,6-di(octanoylamino)-3-O-[D-1-(methoxycarbonyl)ethyl]-5-O-(tetrahydropyran-2-yl)-β-D-glucofuranoside (**11**). — Compound **8** (240 mg) was dissolved in ethanol (15 mL), 10% Pd–C catalyst (100 mg) was added, and hydrogen was bubbled through while the mixture was stirred for 1 h at room temperature, the course of the reaction being monitored by t.l.c. The catalyst was removed by filtration, and the filtrate was evaporated below 40°, to give the syrupy, free amino compound which was used for the next reaction. To a solution of the 2-amino derivative in dry 1,4-dioxane (5 mL) was added *N*-(octanoyloxy)succinimide (140 mg), and the

mixture was stirred for 8 h at room temperature, and evaporated to a syrup which was purified by chromatography on a column of silica gel (20 g) with chloroform and then 100:1 chloroform–methanol. The latter eluate afforded 220 mg (84%) of **11** as a syrup, $[\alpha]_D^{25}$ −31.5° (*c* 0.24, chloroform); ν_{max}^{film} 3300 (NH), 2920 and 2830 (Me, methylene), 1750 and 1210 (ester), 1650 and 1540 (amide), and 740 and 700 cm^{-1} (phenyl).

Anal. Calc. for $C_{38}H_{62}N_2O_9$: C, 66.06; H, 9.05; N, 4.06. Found: C, 66.25; H, 9.31; N, 3.87.

Benzyl 2,6-dideoxy-2,6-di(hexadecanoylamino)-3-O-[D-1-(methoxycarbonyl)-ethyl]-5-O-(tetrahydropyran-2-yl)-β-D-glucofuranoside (**12**). — Compound **9** (130 mg) was hydrogenated in the presence of 10% Pd–C catalyst (70 mg) in ethanol (10 mL), as described in the preparation of **11**, to give the 2-amino compound. The amine was coupled with *N*-(hexadecanoyloxy)succinimide (200 mg) as already described, to give **12** (125 mg, 85%) as a syrup, $[\alpha]_D^{25}$ −22° (*c* 0.12, chloroform): ν_{max}^{film} 3300 (NH), 2920 and 2830 (Me, methylene), 1750 and 1210 (ester), 1650, 1630 and 1545 (amide), and 730 and 700 cm^{-1} (phenyl).

Anal. Calc. for $C_{54}H_{94}N_2O_9$: C, 70.85; H, 10.35; N, 3.06. Found: C, 70.84; H, 10.66; N, 2.79.

Benzyl 2,6-dideoxy-2,6-di(eicosanoylamino)-3-O-[D-1-(methoxycarbonyl)ethyl]-5-O-(tetrahydropyran-2-yl)-β-D-glucofuranoside (**13**). — Selective hydrogenolysis of the benzyloxycarbonyl group in **10** (210 mg) in ethanol (10 mL) with hydrogen in the presence of 10% Pd–C catalyst (100 mg), and subsequent coupling with *N*-(eicosanoyloxy)succinimide (160 mg) in dry 1,4-dioxane (10 mL), by the procedure just described, afforded **13** (230 mg, 92%) as a syrup, $[\alpha]_D^{25}$ −26° (*c* 0.7, chloroform); ν_{max}^{film} 3260 (NH), 2900 and 2830 (Me, methylene), 1750 and 1210 (ester), 1650 and 1550 (amide), and 730 and 700 cm^{-1} (phenyl).

Anal. Calc. for $C_{62}H_{110}N_2O_9$: C, 73.19; H, 9.91; N, 2.75. Found: C, 72.85; H, 10.35; N, 2.51.

Benzyl 2,6-dideoxy-6-(eicosanoylamino)-3-O-[D-1-(methoxycarbonyl)ethyl]-2-(octanoylamino)-5-O-(tetrahydropyran-2-yl)-β-D-glucofuranoside (**14**). — Coupling of the amino compound obtained from **10** (240 mg), by selective hydrogenolysis as just described, with *N*-(octanoyloxy)succinimide (140 mg) in dry 1,4-dioxane (5 mL) as already described, afforded **14** (200 mg, 84%) as a syrup, $[\alpha]_D^{25}$ −36.5° (*c* 0.35, chloroform); ν_{max}^{film} 3260 (NH), 2900 and 2830 (Me, methylene), 1750 and 1210 (ester), 1640 and 1550 (amide), and 735 and 700 cm^{-1} (phenyl).

Anal. Calc. for $C_{50}H_{86}N_2O_9$: C, 69.89; H, 10.09; N, 3.26. Found: C, 69.59; H, 10.34; N, 3.01.

Benzyl 2,6-dideoxy-2-(eicosanoylamino)-3-O-[D-1-(methoxycarbonyl)ethyl]-6-(octanoylamino)-5-O-(tetrahydropyran-2-yl)-β-D-glucofuranoside (**15**). — Coupling of the amino compound obtained from **8** (230 mg), by hydrogenolysis, with *N*-(eicosanoyloxy)succinimide (230 mg) in dry 1,4-dioxane (5 mL), as already described, gave **15** (250 mg, 88%) as a syrup, $[\alpha]_D^{25}$ −28° (*c* 0.4, chloroform); ν_{max}^{film} 3280 (NH),

2900 and 2830 (Me, methylene), 1740 and 1210 (ester), 1650 and 1540 (amide), and 750 and 690 cm^{-1} (phenyl).

Anal. Calc. for $C_{50}H_{86}N_2O_9$: C, 69.89; H, 10.09; N, 3.26. Found: C, 69.68; H, 10.25; N, 3.19.

Benzyl 2,6-dideoxy-2,6-di(octanoylamino)-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine benzyl ester)-β-D-glucofuranoside (**16**). — To a solution of **11** (100 mg) in 1,4-dioxane (20 mL) was added 0.1M potassium hydroxide (2.2 mL), and the solution was stirred for 1 h at room temperature, and then treated with Amberlite IR-120B (H^+) ion-exchange resin to remove the base; the resin was filtered off, and washed with methanol. The filtrate and washings were combined, and evaporated, to afford the free acid, which was used for the next reaction without purification. To a solution of the acid in dry 1,4-dioxane (5 mL) were added, with stirring, *N*-hydroxysuccinimide (HOSu) (25 mg) and dicyclohexylcarbodiimide (DCC) (45 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 benzyl ester trifluoroacetate (95 mg) and triethylamine (0.06 mL) were added to the mixture, and it was stirred for 8 h at room temperature, and then evaporated. The residue was chromatographed on a column of silica gel (20 g) with chloroform, and then with 50:1 chloroform–methanol. The latter eluate afforded the coupling product, which was dissolved in 80% aqueous acetic acid (25 mL). The solution was heated for 2 h at 45°, and evaporated to a syrup which was purified by chromatography on a column of silica gel (20 g) with chloroform and then 30:1 chloroform–methanol. From the latter eluate, compound **16** (66 mg, 52%) was obtained as a syrup, $[\alpha]_D^{25}$ −54° (*c* 0.6, chloroform); ν_{max}^{film} 3240 (OH, NH), 2929 and 2830 (Me, methylene), 1720 and 1210 (ester), 1640 and 1530 (amide), and 750 and 690 cm^{-1} (phenyl); n.m.r. data at 90 MHz (in 1:1 chloroform-*d*–methanol-d_4): δ 0.88 (near t, 6 H, J_{Me,CH_2} 5.2 Hz, 2 $MeCH_2$), 1.33 (d, 3 H, $J_{Me,CH}$ 6.4 Hz, MeCH), 4.54, 5.78 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 5.00 (s, 1 H, H-1), 5.09 (s, 2 H, CO_2CH_2Ph), and 7.27 and 7.30 (2 s, 10 H, 2 Ph).

Anal. Calc. for $C_{47}H_{71}N_5O_{11}$: C, 63.99; H, 8.11; N, 7.94. Found: C, 63.65; H, 8.29; N, 7.71.

Benzyl 2,6-dideoxy-2,6-di(hexadecanoylamino)-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine benzyl ester)-β-D-glucofuranoside (**17**). — Hydrolysis of **12** (50 mg) with 0.1M potassium hydroxide (0.8 mL) in 1,4-dioxane (5 mL), with processing as already described, gave the free acid. Coupling of the acid with the L-alanyl-D-isoglutamine derivative (35 mg) in dry 1,4-dioxane (3 mL) by using HOSu (10 mg), DCC (20 mg), and triethylamine (0.03 mL) as described for **16**, and subsequent hydrolysis by heating with 80% aqueous acetic acid (10 mL) for 2 h at 45°, gave **17** (56 mg, 93%) as a syrup, $[\alpha]_D^{22}$ −35° (*c* 0.5, chloroform); ν_{max}^{film} 3300 (OH, NH), 2920 and 2840 (Me, methylene), 1720 and 1220 (ester), 1660 and 1540 (amide), and 720 and 700 cm^{-1} (phenyl); n.m.r. data at 90 MHz (in 1:1 chloroform-*d*–methanol-d_4): δ 0.87 (t, 6 H, J_{Me,CH_2} 5.2 Hz, 2 $MeCH_2$), 1.35 (d, 3 H, $J_{Me,CH}$ 7.0 Hz, MeCH),

4.45, 4.78 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 5.00 (s, 1 H, H-1), 5.08 (s, 2 H, CO_2CH_2Ph), and 7.30 (s, 10 H, 2 Ph).

Anal. Calc. for $C_{63}H_{103}N_5O_{11}$: C, 68.38; H, 9.38; N, 6.33. Found: C, 68.00; H, 9.52; N, 6.19.

*Benzyl 2,6-dideoxy-2,6-di(eicosanoylamino)-3-*O*-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine benzyl ester)-β-*D*-glucofuranoside* (**18**). — Hydrolysis of **13** (110 mg) with 0.1M potassium hydroxide (1.6 mL) in 1,4-dioxane (10 mL), as already described, gave the free acid, which was condensed with the peptide (70 mg) in dry 1,4-dioxane (5 mL) by using HOSu (20 mg), DCC (40 mg), and triethylamine (0.06 mL), as described in the preparation of **16**, to give the dipeptide derivative. The coupling product was hydrolyzed by heating with 80% aqueous acetic acid (20 mL) for 2 h at 45°, as already described, to give **18** (63 mg) as a syrup, $[\alpha]_D^{25}$ −29° (*c* 0.5, chloroform); ν_{max}^{film} 3350 and 3230 (OH, NH), 2900 and 2820 (Me, methylene), 1730 and 1230 (ester), 1640 and 1530 (amide), and 730 and 680 cm^{-1} (phenyl); n.m.r. data at 90 MHz (in chloroform-*d*): δ 0.88 (near t, 6 H, J_{Me,CH_2} 5.2 Hz, 2 $MeCH_2$), 1.36 (d, 3 H, $J_{Me,CH}$ 7.0 Hz, MeCH), 4.56, 4.80 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 5.00 (s, 1 H, H-1), 5.09 (s, 2 H, CO_2CH_2Ph), 6.10, 6.54, 6.98 (3 H, 3 NH), and 7.27 and 7.30 (2 s, 10 H, 2 Ph).

Anal. Calc. for $C_{71}H_{119}N_5O_{11}$: C, 69.97; H, 9.84; N, 5.75. Found: C, 69.80; H, 10.15; N, 5.56.

*Benzyl 2,6-dideoxy-6-(eicosanoylamino)-2-(octanoylamino)-3-*O*-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine benzyl ester)-β-*D*-glucofuranoside* (**19**). — Hydrolysis of **14** (80 mg) with 0.1M potassium hydroxide (1.4 mL) in 1,4-dioxane (10 mL), coupling of the acid formed with the dipeptide (60 mg) in dry 1,4-dioxane (5 mL) by using HOSu (20 mg), DCC (30 mg), and triethylamine (0.03 mL), and subsequent hydrolysis of the product by heating with 80% aqueous acetic acid (20 mL) at 45°, as described in the preparation of **16**, afforded compound **19** (47 mg, 48%) as a syrup, $[\alpha]_D^{25}$ −31.5° (*c* 0.4, chloroform); ν_{max}^{film} 3300 (OH, NH), 2920 and 2830 (Me, methylene), 1740 and 1220 (ester), 1660 and 1540 (amide), and 760, 720, and 700 cm^{-1} (phenyl); n.m.r. data at 90 MHz (in 1:1 chloroform-*d*–methanol-d_4): δ 0.88 (near t, 6 H, J_{Me,CH_2} 5.2 Hz, 2 $MeCH_2$), 1.40 (d, 3 H, $J_{Me,CH}$ 7.2 Hz, MeCH), 5.00 (s, 1 H, H-1), 5.10 (s, 2 H, CO_2CH_2Ph), and 7.30 (s, 10 H, 2 Ph).

Anal. Calc. for $C_{59}H_{95}N_5O_{11}$: C, 67.46; H, 9.12; N, 6.67. Found: C, 67.24; H, 9.50; N, 6.51.

*Benzyl 2,6-dideoxy-2-(eicosanoylamino)-6-(octanoylamino)-3-*O*-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine benzyl ester)-β-*D*-glucofuranoside* (**20**). — Hydrolysis of **15** (150 mg) with 0.1M potassium hydroxide (2.7 mL) in 1,4-dioxane (20 mL), coupling of the acid with the dipeptide (110 mg) by using HOSu (30 mg), DCC (55 mg), and triethylamine (0.06 mL), and subsequent hydrolysis of the product by heating with 80% aqueous acetic acid (30 mL), as already described, yielded **20** (145 mg, 79%) as a syrup, $[\alpha]_D^{25}$ −30° (*c* 1.45, chloroform); ν_{max}^{film} 3300 (OH, NH), 2920 and 2850 (Me, methylene), 1730 and 1220 (ester), 1660 and 1540 (amide), and 760 and 700 cm^{-1} (phenyl); n.m.r. data at 90 MHz (in 1:1 chloroform-*d*–methanol-d_4): δ 0.88 (near t,

6 H, J_{Me,CH_2} 5.2 Hz, 2 $MeCH_2$), 1.43 (d, 2 H, $J_{Me,CH}$ 7.0 Hz, MeCH), 4.44, 4.76 (2 d, 2 H, J_{gem} 12.0 Hz, benzyl methylene), 5.02 (s, 1 H, H-1), 5.08 (s, 2 H, CO_2CH_2-Ph), and 7.30 (s, 10 H, 2 Ph).

Anal. Calc. for $C_{59}H_{95}N_5O_{11}$: C, 67.46; H, 9.12; N, 6.67. Found: C, 67.30; H, 9.48; N, 6.52.

*2,6-Dideoxy-2,6-di(octanoylamino)-3-*O*-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine)-*D*-glucopyranose* (**21**). — Compound **16** (60 mg) was dissolved in methanol (20 mL), 10% Pd–C catalyst (100 mg) was added, and hydrogen was bubbled through while the mixture was stirred for 30 min at room temperature. Acetic acid (2 mL) was added to the mixture, and hydrogen was again bubbled through the mixture, with stirring, for 3 h at 30°, the course of the reaction being monitored by t.l.c. The catalyst was removed by filtration, and the filtrate was evaporated below 30°, to give amorphous **21** (42 mg, 88%), which showed a single spot in t.l.c., m.p. 116–118°, $[\alpha]_D^{25}$ −4.8° (*c* 0.33, acetic acid; equil.); ν_{max}^{KBr} 3300–3230 (OH, NH), 2900 and 2830 (Me, methylene), 1700 (C=O), and 1640 and 1530 cm^{-1} (amide).

Anal. Calc. for $C_{33}H_{59}N_5O_{11}$: C, 56.47; H, 8.47; N, 9.98. Found: C, 56.12; H, 8.91; N, 9.69.

*2,6-Dideoxy-2,6-di(hexadecanoylamino)-3-*O*-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine)-*D*-glucopyranose* (**22**). — Compound **17** (40 mg) was hydrogenolyzed in the presence of 10% Pd–C catalyst (60 mg), in methanol (15 mL)–acetic acid (1 mL), as described in the preparation of **21**, to yield **22** (30 mg, 90%) as an amorphous material, m.p. 122–124°, $[\alpha]_D^{25}$ +3.2° (*c* 0.38, acetic acid; equil.); ν_{max}^{KBr} 3300–3200 (OH, NH), 2900 and 2820 (Me, methylene), 1720 (C=O), and 1640, 1620, and 1540 cm^{-1} (amide).

Anal. Calc. for $C_{49}H_{91}N_5O_{11}$: C, 63.53; H, 9.90; N, 7.56. Found: C, 63.05; H, 10.29; N, 7.41.

*2,6-Dideoxy-2,6-di(eicosanoylamino)-3-*O*-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine)-*D*-glucopyranose* (**23**). — Compound **18** (19 mg) was hydrogenolyzed in the presence of 10% Pd–C catalyst (30 mg), in methanol (10 mL)–acetic acid (1 mL), as already described, to give **23** (11 mg, 68%) as an amorphous material, m.p. 126–128°, $[\alpha]_D^{25}$ +3.2° (*c* 0.2, acetic acid; equil.); ν_{max}^{KBr} 3350 and 3230 (OH, NH), 2900 and 2820 (Me, methylene), 1720 (C=O), and 1650, 1620, and 1540 cm^{-1} (amide).

Anal. Calc. for $C_{57}H_{107}N_5O_{11}$: C, 65.92; H, 10.39; N, 6.74. Found: C, 65.73; H, 10.59; N, 6.55.

*2,6-Dideoxy-6-(eicosanoylamino)-2-(octanoylamino)-3-*O*-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine)-*D*-glucopyranose* (**24**). — Hydrogenolysis of compound **19** (40 mg) with hydrogen in the presence of 10% Pd–C catalyst (60 mg) in methanol (15 mL)–acetic acid (1 mL), according to the procedure already described, gave **24** (24 mg, 72%) as an amorphous material, m.p. 103–105°, $[\alpha]_D^{25}$ −9.0° (*c* 0.56, acetic acid; equil.); ν_{max}^{KBr} 3330 and 3240 (OH, NH), 2900 and 2820 (Me, methylene), 1700 (C=O), and 1650 and 1530 cm^{-1} (amide).

Anal. Calc. for $C_{45}H_{83}N_5O_{11}$: C, 62.11; H, 9.61; N, 8.05. Found: C, 61.88; H, 10.05; N, 7.81.

2,6-Dideoxy-2-(eicosanoylamino)-6-(octanoylamino)-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine)-D-glucopyranose (**25**). — Hydrogenolysis of **20** (132 mg) with hydrogen in the presence of 10% Pd–C catalyst (150 mg), in methanol (20 mL)–acetic acid (2 mL), according to the procedure described in the preparation of **21**, yielded **25** (95 mg, 87%) as an amorphous material, m.p. 108–109°, $[\alpha]_D^{25}$ −5.5° (*c* 0.33, acetic acid; equil.); ν_{max}^{KBr} 3350–3230 (OH, NH), 2900 and 2820 (Me, methylene), 1720 (C=O), and 1660 and 1540 cm^{-1} (amide).

Anal. Calc. for $C_{45}H_{83}N_5O_{11}$: C, 62.11; H, 9.61; N, 8.05. Found: C, 61.68; H, 9.92; N, 7.81.

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