Carbohydrate Research, 100 (1982) 235–245
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

SYNTHESIS AND BIOLOGICAL ACTIVITY OF *O*-(*N*-ACETYL-β-MURAMOYL-L-ALANYL-D-ISOGLUTAMINE)-(1→6)-2-ACYLAMINO-2-DEOXY-D-GLUCOSES*

AKIRA HASEGAWA, MASAHIKO OZAKI, YASUHIKO GOH, MAKOTO KISO,
Department of Agricultural Chemistry, Gifu University, Kakamigahara, Gifu 504 (Japan)
AND ICHIRO AZUMA
Institute of Immunological Science, Hokkaido University, Sapporo 060 (Japan)

(Received July 16th, 1981; accepted for publication, August 6th, 1981)

ABSTRACT

Benzoylation of benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside, benzyl 2-deoxy-2-(DL-3-hydroxytetradecanoylamino)-4,6-*O*-isopropylidene-α-D-glucopyranoside, and benzyl 2-deoxy-4,6-*O*-isopropylidene-2-octadecanoylamino-β-D-glucopyranoside, with subsequent hydrolysis of the 4,6-*O*-isopropylidene group, gave the corresponding 3-*O*-benzoyl derivatives (**4**, **5**, and **7**). Hydrogenation of benzyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside, followed by chlorination, gave a product that was treated with mercuric actate to yield 2-acetamido-1,4,6-tri-*O*-acetyl-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-β-D-glucopyranose (**11**). Treatment of **11** with ferric chloride afforded the oxazoline derivative, which was condensed with **4**, **5**, and **7** to give the (1→6)-β-linked disaccharide derivatives **13**, **15**, and **17**. Hydrolysis of the methyl ester group in the compounds derived from **13**, **15**, and **17** by 4-*O*-acetylation gave the corresponding free acids, which were coupled with L-alanyl-D-isoglutamine benzyl ester, to yield the dipeptide derivatives **19–21** in excellent yields. Hydrolysis of **19–21**, followed by hydrogenation, gave the respective *O*-(*N*-acetyl-β-muramoyl-L-alanyl-D-isoglutamine)-(1→6)-2-acylamino-2-deoxy-D-glucoses in good yields. The immunoadjuvant activity of these compounds was examined in guinea-pigs.

INTRODUCTION

Our interest in the synthesis of the various, carbohydrate analogs of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP), which is the minimal, immunoadjuvant-active structure[2] capable of (*a*) replacing whole bacterial cell in complete Freund's adjuvant, for increasing levels of antibodies against a given antigen, and (*b*) inducing a delayed type of hypersensitivity, has been outlined[3]. The synthetic, repeating di-

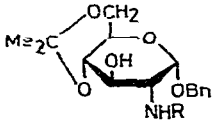
*Studies on Immunoadjuvant Active Compounds, Part XVI, For Part XV, see ref. 1.

0008-6215/82/0000–0000/$ 02.75,

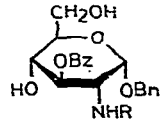
saccharide–dipeptide units of the bacterial cell-wall peptidoglycan, namely, β-MDP-(1→4)-GlcNAc[4,5] and β-GlcNAc-(1→4)-MDP[5–7], showed[1] strong activity, comparable to that of MDP, whereas β-Glc-(1→4)-MDP[8] and β-6-NAcGlc-(1→4)-MDP[1] showed very little activity, indicating that the presence of the 2-acetamido-2-deoxy-D-glucose residue is important for activity of the repeating units. On the other hand, introduction of lipophilic character to MDP[9–11] and its carbohydrate analogs[12] does not enhance the adjuvant activity, but increases the antitumor and anti-infection activities. We now report on the synthesis of *O*-(*N*-acetyl-β-muramoyl-L-alanyl-D-isoglutamine)-(1→6)-2-acetamido-2-deoxy-D-glucopyranose [a positional isomer of one of the repeating units, namely β-MDP-(1→4)-GlcNAc] and lipophilic analogs, in which the amino group of the 2-amino-2-deoxy-D-glucose residue is acylated by DL-3-hydroxytetradecanoic acid (related to the lipid A constituent of the bacterial lipopolysaccharide) or octadecanoic acid, and their immunoadjuvant activities.

RESULTS AND DISCUSSION

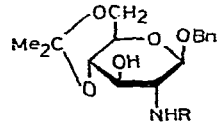
Benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside[13] (**1**), benzyl 2-deoxy-2-(DL-3-hydroxytetradecanoylamino)-4,6-*O*-isopropylidene-α-D-glucopyranoside (**3**, prepared by *N*-acylation of **2**[11] with dicyclohexylcarbodiimide and *N*-hydroxysuccinimide as the activating agents), and benzyl 2-deoxy-4,6-*O*-isopropylidene-2-octadecanoylamino-β-D-glucopyranoside[11] (**6**) were used for the preparation of the glycosyl acceptors. Benzoylation of **1**, **3**, and **6**, and subsequent hydrolysis of the 4,6-*O*-isopropylidene group with 70% aqueous acetic acid at 40–45°, gave the corresponding 3-*O*-benzoyl derivatives (**4**, **5**, and **7**) in good yields. As the glycosyl donor, 2-methyl-{4,6-di-*O*-acetyl-1,2-dideoxy-3-*O*-[D-1-(methoxycarbonyl)-



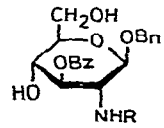
1 R = Ac
2 R = H
3 R = $-COCH_2CH(CH_2)_{10}CH_3$ (OH)
Bn = $PhCH_2$



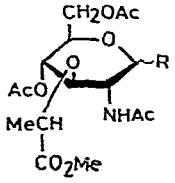
4 R = Ac
5 R = $-COCH_2CH(CH_2)_{10}CH_3$ (OBz)



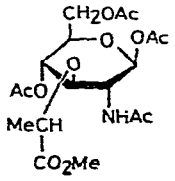
6 R = $-CO(CH_2)_{16}CH_3$



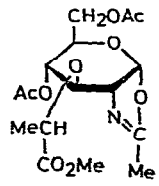
7 R = $-CO(CH_2)_{16}CH_3$



8 R = OBn
9 R = OH
10 R = Cl



11

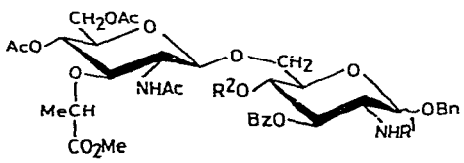


12

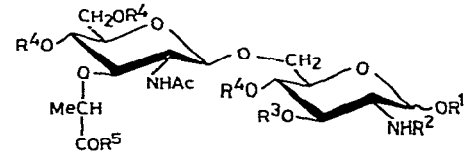
ethyl]-α-D-glucopyrano}-[2,1-*d*]-2-oxazoline (**12**) was synthesized. Hydrogenolysis of benzyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside[14] (**8**) with 10% Pd–C catalyst afforded compound **9** as an anomeric mixture in 95% yield. Treatment of **9** with acetyl chloride in dichloroethane gave the α-glycosyl chloride **10**, which was not purified but treated immediately with mercuric acetate in acetic acid, to give 2-acetamido-1,4,6-tri-*O*-acetyl-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-β-D-glucopyranose (**11**). The β configuration of **11** was established by n.m.r. spectroscopy. Treatment of **11** with anhydrous ferric chloride in dichloromethane[15] then gave the oxazoline **12** in excellent yield.

When toluene-*p*-sulfonic acid was added to a mixture of the oxazoline **12** and the acceptor (**4**, **5**, and **7**) in dichloroethane at 50–60°, the disaccharide derivatives **13**, **15**, and **17** were obtained in yields of 86, 82, and 84%, respectively, and converted into the corresponding 4-acetates (**14**, **16**, and **18**). The n.m.r. spectra of **14**, **16**, and **18** contained the H-3, H-4, and H-4′ signals as triplets at δ 4.87–5.47, consistent with the structures of the (1→6)-β-linked disaccharide derivatives. Saponification of **14**, **16**, and **18** with 0.1M aqueous potassium hydroxide in 1,4-dioxane gave the corresponding free acids.

Coupling of the acids with L-alanyl-D-isoglutamine benzyl ester[16], using dicyclohexylcarbodiimide and *N*-hydroxysuccinimide (DCC–HOSu) as the activating agents in 1,4-dioxane, afforded the corresponding lactoyl-dipeptide derivatives (**19**–**21**) in good yields. Hydrolysis of the *O*-acyl and benzyl ester groups in **19**–**21** with sodium methoxide in methanol gave crystalline **22**–**24**, respectively, in good yields. Hydrogenolysis of the benzyl group in **22**–**24** in the presence of 10% Pd–C catalyst at 40° yielded the *C*-(*N*-acetyl-β-muramoyl-L-alanyl-D-isoglutamine)-(1→6)-2-acylamino-2-deoxy-D-glucopyranoses (**25**–**27**) as amorphous products.



13 R^1 = Ac, R^2 = H
14 R^1 = R^2 = Ac
15 R^1 = $-COCH_2CH(CH_2)_{10}CH_3$, R^2 = H
(OBz)
16 R^1 = $-COCH_2CH(CH_2)_{10}CH_3$, R^2 = Ac
(OBz)
17 R^1 = $-CO(CH_2)_{16}CH_3$, R^2 = H
18 R^1 = $-CO(CH_2)_{16}CH_3$, R^2 = Ac



19 R^1 = Bn, R^2 = R^4 = Ac, R^3 = Bz, R^5 = a
20 R^1 = Bn, R^2 = $-COCH_2CH(CH_2)_{10}CH_3$, R^3 = Bz, R^4 = Ac, R^5 = a
(OBz)
21 R^1 = Bn, R^2 = $-CO(CH_2)_{16}CH_3$, R^3 = Bz, R^4 = Ac, R^5 = a
22 R^1 = Bn, R^2 = Ac, R^3 = R^4 = H, R^5 = b
23 R^1 = Bn, R^2 = $-COCH_2CH(CH_2)_{10}CH_3$, R^3 = R^4 = H, R^5 = b
(OH)
24 R^1 = Bn, R^2 = $-CO(CH_2)_{16}CH_3$, R^3 = R^4 = H, R^5 = b
25 R^1 = R^3 = R^4 = H, R^2 = Ac, R^5 = b
26 R^1 = R^3 = R^4 = H, R^2 = $-COCH_2CH(CH_2)_{10}CH_3$, R^5 = b
(OH)
27 R^1 = R^3 = R^4 = H, R^2 = $-CO(CH_2)_{16}CH_3$, R^5 = b

a = L-Ala-D-isoGln-OBn
b = L-Ala-D-isoGln

TABLE I

ADJUVANT ACTIVITY OF *O*-(*N*-ACETYL-β-MURAMOYL-L-ALANYL-D-ISOGLUTAMINE)-(1→6)-2-ACYLAMINO-2-DEOXY-D-GLUCOSES ON THE INDUCTION OF DELAYED-TYPE HYPERSENSITIVITY TO ABA–*N*-ACETYL-TYROSINE IN GUINEA-PIGS

Compounds[a]	*Skin reaction with ABA-BSA*[b] *(100 μg) (diam. in mm ±SE)*[c] *at*	
	24 h	*48 h*
25	21.3 ±1.1	13.8 ±0.8
26	(7.8 ±1.5)	0
27	(10.8 ±1.8)	(5.1 ±1.4)
β-MDP-(1→4)-GlcNAc[d]	19.9 ±1.1	20.5 ±1.3
β-GlcNAc-(1→4)-MDP[d]	20.2 ±1.0	19.9 ±1.5
MDP	21.0 ±1.8	19.0 ±2.0
Control[e]	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; the values in parentheses indicate the size of the erythema. [d]See ref. 1. [e]ABA–*N*-acetyltyrosine in Freund's incomplete adjuvant.

The immunoadjuvant activities of compounds **25–27** on the induction of the delayed type of hypersensitivity to *N*-acetyl-L-tyrosine-3-azobenzene-4′-arsonate (ABA–*N*-acetyltyrosine) in guinea-pigs were examined[17] (see Table I). Compound **25** showed strong activity, comparable to that of MDP or β-MDP-(1→4)-GlcNAc (one of the repeating disaccharide–dipeptide units of the bacterial cell-wall peptidoglycan) at 24 h, but slightly weaker at 48 h, indicating that the position of the glycosidic linkage between MDP and 2-acetamido-2-deoxy-D-glucose is unimportant for activity. However, the lipophilic analogs **26** and **27** showed negligible activity, indicating that the position of introduction of lipophilicity into the molecule is critical for activity, as 6-*O*-acyl-[9,10], 6-acylamino-[12], and 2-acylamino-MDP[11] analogs had strong immunoadjuvant activity.

EXPERIMENTAL

General methods. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Specific rotations were determined with a Union MP-201 polarimeter, and i.r. spectra were recorded with a Jasco IR-1 spectrophotometer. N.m.r. spectra were recorded at 90 MHz with a Hitachi R-22 spectrometer, and the n.m.r. data were confirmed by use of decoupling techniques. Preparative chromatography was performed on silica gel (Waco Co.; 300 mesh) with the solvent systems specified. Evaporations were conducted *in vacuo*.

Benzyl 2-deoxy-2-(DL-*3-hydroxytetradecanoylamino*)*-4,6-*O*-isopropylidene-*α-D-*glucopyranoside* (**3**). — To a solution of DL-3-hydroxytetradecanoic acid (790 mg) in

dry 1,4-dioxane (10 mL) were added *N*-hydroxysuccinimide (450 mg) and dicyclohexylcarbodiimide (800 mg). The mixture was stirred for 1 h at room temperature, and then the 1,3-dicyclohexylurea formed was removed by filtration. Benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside[11] (**2**; 1.0 g) was added to the filtrate, and the mixture was stirred overnight at room temperature and then evaporated. The residue was purified by chromatography on a column of silica gel (50 g) with (*a*) chloroform and (*b*) 100:1 chloroform–methanol. Eluant (*b*) afforded syrupy **3** (1.25 g, 72%), $[\alpha]_D^{25}$ +109° (*c* 0.43, methanol).

Anal. Calc. for $C_{30}H_{49}NO_7$: C, 67.26; H, 9.22; N, 2.61. Found: C, 67.01; H, 9.55; N, 2.48.

*Benzyl 2-acetamido-3-*O*-benzoyl-2-deoxy-α-*D*-glucopyranoside* (**4**). — Compound **1**[13] (150 mg) was benzoylated with benzoyl chloride (0.1 mL) in pyridine (3 mL), in the usual way. A solution of the resulting 3-benzoate in 70% aqueous acetic acid (5 mL) was heated for 1 h at 40–45° and then evaporated, and the residue was crystallized from ether, to give **4** (145 mg, 82%) as needles, m.p. 89–91°, $[\alpha]_D^{25}$ +138° (*c* 0.2, chloroform); ν_{max}^{Nujol} 3380 (OH), 3260 (NH), 1720 and 1265 (ester), 1640 and 1540 (amide), and 720 cm^{-1} (phenyl).

Anal. Calc. for $C_{22}H_{25}NO_7$: C, 63.60; H, 6.07; N, 3.37. Found: C, 63.58; H, 5.95; N, 3.29.

*Benzyl 3-*O*-benzoyl-2-(*DL*-3-benzoyloxytetradecanoylamino)-2-deoxy-α-*D*-glucopyranoside* (**5**). — *O*-Benzoylation of **3** (213 mg) with benzoyl chloride (0.2 mL) in pyridine (3 mL), in the usual way, gave the 3-benzoate, which was dissolved in 70% aqueous acetic acid (10 mL). The mixture was heated for 1 h at 40–45° and then evaporated, and the residue was crystallized from ether, to give **5** (216 mg, 77%) as needles, m.p. 131–133°, $[\alpha]_D^{25}$ +113° (*c* 0.24, chloroform); ν_{max}^{Nujol} 3400–3300 (OH), 3260 (NH), 1720 and 1270 (ester), 1640 and 1540 (amide), and 730, 710, and 700 cm^{-1} (phenyl).

Anal. Calc. for $C_{41}H_{53}NO_9$: C, 69.96; H, 7.59; N, 1.99. Found: C, 70.02; H, 7.63; N, 2.15.

*Benzyl 3-*O*-benzoyl-2-deoxy-2-octadecanoylamino-β-*D*-glucopyranoside* (**7**). — Benzyl 2-deoxy-4,6-*O*-isopropylidene-2-octadecanoylamino-β-D-glucopyranoside[11] (**6**, 400 mg) was treated with benzoyl chloride (0.5 mL)–pyridine (5 mL) for 1 h at room temperature. The 4,6-*O*-isopropylidene group in the resulting 3-benzoate was hydrolyzed by heating for 1 h at 40–45° in 70% aqueous acetic acid (10 mL). The product was crystallized from ether, to give **7** as needles (347 mg, 78%), m.p. 124–126°, $[\alpha]_D^{25}$ +2.5° (*c* 0.2, chloroform); ν_{max}^{Nujol} 3400 (OH), 3235 (NH), 1725 and 1260 (ester), 1660 and 1545 (amide), and 735, 700, and 700 cm^{-1} (phenyl).

Anal. Calc. for $C_{38}H_{57}NO_7$: C, 71.33; H, 8.98; N, 2.19. Found: C, 71.28; H, 8.99; N, 2.18.

*2-Acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[*D*-1-(methoxycarbonyl)ethyl]-*D*-glucopyranose* (**9**). — To a solution of benzyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside[14] (**8**, 1.44 g) in methanol (100 mL), acetic acid (2 mL), and water (10 mL) was added 10% Pd–C catalyst (500 mg),

and hydrogen was bubbled through for 6.5 h while the solution was stirred at 30–35°; t.l.c. then showed the hydrogenation to be complete. The catalyst was removed by filtration, the filtrate was evaporated, and recrystallization of the residue from ethanol–ether gave **9** (1.11 g, 95%) as needles, m.p. 153–155°, $[\alpha]_D^{25}$ +76° (*c* 0.2, chloroform; equil.), ν_{max}^{Nujol} 3320 and 3170 (OH, NH), 1760, 1730, and 1230 (ester), and 1645 and 1560 cm^{-1} (amide); n.m.r. data (chloroform-*d*): δ 1.36 (d, 3 H, *J* 7.0 Hz, *Me*CH), 2.03, 2.05, and 2.10 (3 s, 9 H, AcN, 2 AcO), 3.77 (s, 3 H, MeO), 5.05 (t, 1 H, $J_{3,4} = J_{4,5} = 9.2$ Hz, H-4), and 5.58 (d, $J_{1,2}$ 2.5 Hz, H-1α).

Anal. Calc. for $C_{16}H_{25}NO_{10}$: C, 49.10; H, 6.44; N, 3.58. Found: C, 49.03; H, 6.58; N, 3.41.

2-Acetamido-1,4,6-tri-O-acetyl-2-deoxy-3-O-[D-1-(methoxycarbonyl)ethyl]-β-D-glucopyranose (**11**). — To a suspension of **9** (3.0 g) in 1,2-dichloroethane (6 mL) was added acetyl chloride (6 mL), the mixture was stirred for 22 h at room temperature, and then chloroform (50 mL) was added. The mixture was successively washed with ice–water and M sodium carbonate, dried (sodium sulfate), and evaporated, to give the glycosyl chloride **10**. To a stirred solution of **10** in acetic acid (10 mL) was added mercuric acetate (2.6 g), and the mixture was stirred for 1 h at room temperature. Chloroform (100 mL) was added, the mixture was filtered, and the residue was washed with chloroform. The filtrate and washings were combined, washed with water, M sodium carbonate, and water, dried (sodium sulfate), and evaporated. Recrystallization of the residue from ether gave **11** (1.95 g, 59%) as needles, m.p. 173°, $[\alpha]_D^{25}$ +18° (*c* 0.5, chloroform); ν_{max}^{Nujol} 3320 (NH), 1760 and 1230 (ester), and 1660 and 1540 cm^{-1} (amide); n.m.r. data (chloroform-*d*): δ 1.35 (d, 3 H, *J* 7.8 Hz, *Me*CH), 2.01, 2.09, 2.13, and 2.14 (4 s, 12 H, AcN, 3 AcO), 3.79 (s, 3 H, MeO), 5.10 (t, 1 H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4), 5.65 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1), and 6.85 (d, 1 H, $J_{2,NH}$ 7.0 Hz, NH).

Anal. Calc. for $C_{18}H_{27}NO_{11}$: C, 49.88; H, 6.28; N, 3.23. Found: C, 49.71; H, 6.36; N, 3.24.

2-Methyl-{4,6-di-O-acetyl-1,2-dideoxy-3-O-[D-1-(methoxycarbonyl]ethyl]-α-D-glucopyrano}-[2,1-d]-2-oxazoline (**12**). — To a stirred solution of **11** (300 mg) in dry dichloromethane (25 mL) was added ferric chloride (300 mg), and the mixture was stirred for 1.5 h at room temperature; t.l.c. then showed the reaction to be complete. The mixture was washed with water, dried (sodium sulfate), and evaporated. The product was purified by chromatography on a column of silica gel (20 g) with (*a*) chloroform and (*b*) 100:1 chloroform–methanol. Eluant (*b*) afforded **12** (243 mg, 94%) as a syrup, $[\alpha]_D^{25}$ +43.8° (*c* 3.7, chloroform); ν_{max}^{film} 1740 and 1230 (ester), and 1660 cm^{-1} (C=N); n.m.r. data (chloroform-*d*): δ 1.43 (d, 3 H, *J* 7.2 Hz, *Me*CH), 2.03, 2.08 (2 s, 9 H, 2 AcO, MeC), 3.73 (s, 3 H, MeO), 4.53 (q, 1 H, *J* 7.2 Hz, MeC*H*), 4.77 (near d, 1 H, $J_{4,5}$ 9.0 Hz, H-4), and 6.01 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1).

Anal. Calc. for $C_{16}H_{23}NO_9$: C, 51.47; H, 6.21; N, 3.75. Found: C, 51.28; H, 6.34; N, 3.61.

Benzyl 2-acetamido-6-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-[D-1-(methoxycarbonyl)ethyl]-β-D-glucopyranosyl}-3-O-benzoyl-2-deoxy-α-D-glucopyranoside

(**13**). — To a stirred solution of **4** (140 mg) and **12** (260 mg) in dry 1,2-dichloroethane (3 mL) was added toluene-*p*-sulfonic acid monohydrate (10 mg), and the mixture was stirred for 30 h at 50–60°. The acid was then removed by stirring for 5 min with Amberlite IR-410 (OH^-) resin. After filtration, the solution was evaporated to a syrup which was crystallized from ethanol, to give **13** as needles (230 mg, 86%), m.p. 242–245°, $[\alpha]_D^{25}$ +100° (*c* 0.5, chloroform); ν_{max}^{Nujol} 3400–3300 (OH, NH), 1750, 1720, and 1280–1240 (ester), 1660 and 1550 (amide), and 720 cm^{-1} (phenyl); n.m.r. data (chloroform-*d*): δ 1.31 (d, 3 H, *J* 7.2 Hz, *Me*CH), 1.77, 1.98, 2.02, and 2.08 (4 s, 12 H, 2 AcN, 2 AcO), 3.70 (s, 3 H, MeO), 4.44, 4.72 (2 d, 2 H, J_{gem} 12.0 Hz, PhCH_2), 4.87, 4.97 (2 t, 2 H, $J_{2,3} = J_{3,4} = J_{3',4'} = J_{4',5'} = 8.0$ Hz, H-3,4'), 4.91 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.87 (d, 1 H, $J_{2,NH}$ 9.0 Hz, NH), 6.78 (d, 1 H, $J_{2',NH}$ 6.0 Hz, NH), 7.27 (s, 5 H, Ph), and 7.23–8.02 (m, 5 H, Ph).

Anal. Calc. for $C_{38}H_{48}N_2O_{16}$: C, 57.86; H, 6.13; N, 3.55. Found: C, 57.75; H, 6.16; N, 3.49.

To a solution of **13** (190 mg) in dry pyridine (5 mL) was added acetic anhydride (2 mL), the mixture was kept for 4 h at room temperature and then evaporated, and the residue was extracted with chloroform. The extract was successively washed with 2M hydrochloric acid, M sodium carbonate, and water, dried (sodium sulfate), and evaporated. Recrystallization of the residue from ether afforded the 4-acetate **14** (196 mg, 98%) as needles, m.p. 239–242°, $[\alpha]_D^{25}$ +56° (*c* 0.2, chloroform); ν_{max}^{Nujol} 3300 and 3270 (NH), 1740, 1720, 1280–1230 (ester), 1650 and 1540 (amide), and 730, 710, and 690 cm^{-1} (phenyl); n.m.r. data (chloroform-*d*): δ 1.31 (d, 3 H, *J* 7.0 Hz, *Me*CH), 1.78, 1.90, 1.97, 2.04, and 2.08 (5 s, 15 H, 2 AcN, 3 AcO), 3.71 (s, 3 H, MeO), 4.50, 4.78 (2 d, 2 H, J_{gem} 12.0 Hz, PhCH_2), 4.96 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.98, 5.20, and 5.46 (3 t, 3 H, $J_{2,3} = J_{3,4} = J_{4,5} = J_{3',4'} = J_{4',5'} = 9.0$ Hz, H-3,4,4'), 5.92 (d, 1 H, $J_{2,NH}$ 9.0 Hz, NH), 6.46 (d, 1 H, $J_{2',NH}$ 7.0 Hz, NH), 7.31 (s, 5 H, Ph), and 7.26–7.98 (m, 5 H, Ph).

Anal. Calc. for $C_{40}H_{50}N_2O_{17}$: C, 57.82; H, 6.07; N, 3.37. Found: C, 57.63; H, 6.15; N, 3.42.

*Benzyl 6-*O*-{2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[*D*-1-(methoxycarbonyl)-ethyl]-β-*D*-glucopyranosyl}-3-*O*-benzoyl-2-(*DL*-3-benzoyloxytetradecanoylamino)-2-deoxy-α-*D*-glucopyranoside* (**15**). — To a stirred solution of **5** (120 mg) and **12** (142 mg) in dry 1,2-dichloroethane (3 mL) was added toluene-*p*-sulfonic acid monohydrate (10 mg), and the mixture was stirred for 30 h at 60°; it was then processed as already described. The product was crystallized from ether, to give **15** (150 mg, 82%) as needles, m.p. 192–194°, $[\alpha]_D^{25}$ +86.5° (*c* 0.5, chloroform); ν_{max}^{Nujol} 3400 (OH), 3270 (NH), 1750, 1730, 1720, and 1290–1240 (ester), 1660 and 1560 (amide), and 720 and 710 cm^{-1} (phenyl); n.m.r. data (chloroform-*d*): δ 0.87 (t, 3 H, *J* 5.6 Hz, *Me*CH_2), 1.02–1.25 (m, 20 H, 10 methylene), 1.31 (d, 3 H, *J* 7.0 Hz, *Me*CH), 1.98, 2.02, and 2.07 (3 s, 9 H, AcN, 2 AcO), 3.69 (s, 3 H, MeO), 4.28, 4.56 (2 d, 2 H, J_{gem} 12.0 Hz, PhCH_2), 4.80 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.96, 5.32 (2 t, 2 H, $J_{2,3} = J_{3,4} = J_{3',4'} = J_{4',5'} = 9.0$ Hz, H-3,4'), 6.01 (d, 1 H, $J_{2,NH}$ 9.0 Hz, NH), 6.33 (d, 1 H, $J_{2',NH}$ 6.0 Hz, NH), 7.21 (s, 5 H, Ph), and 7.20–8.03 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{57}H_{76}N_2O_{18}$: C, 63.55; H, 7.11; N, 2.60. Found: C, 63.49; H, 7.15; N, 2.62.

Acetylation of **15** (85 mg) with acetic anhydride–pyridine, as already described, gave the 4-acetate **16** (80 mg, 91%) as needles, m.p. 199–200°, $[\alpha]_D^{25}$ +63° (*c* 0.2, chloroform); ν_{max}^{Nujol} 3260 (NH), 1740, 1720, and 1280–1220 (ester), 1650 and 1540 (amide), and 720–700 cm^{-1} (phenyl); n.m.r. data (chloroform-*d*): δ 0.87 (t, 3 H, *J* 5.8 Hz, *Me*CH_2), 1.03–1.12 (m, 20 H, 10 methylene), 1.30 (d, 3 H, *J* 7.0 Hz, *Me*CH), 1.89, 1.96, 2.04, and 2.07 (4 s, 12 H, AcN, 3 AcO), 3.71 (s, 3 H, MeO), 4.30, 4.64 (2 d, 2 H, J_{gem} 11.0 Hz, PhCH_2), 4.85 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.87, 5.18, and 5.47 (3 t, 3 H, $J_{2,3} = J_{3,4} = J_{4,5} = J_{3',4'} = J_{4',5'} = 9.0$ Hz, H-3,4,4'), 6.02 (d, 1 H, $J_{2,NH}$ 9.0 Hz, NH), 6.30 (d, 1 H, $J_{2',NH}$ 6.0 Hz, NH), 7.24 (s, 5 H, Ph), and 7.22–7.98 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{59}H_{78}N_2O_{19}$: C, 63.31; H, 7.02; N, 2.50. Found: C, 63.25; H, 7.13; N, 2.42.

*Benzyl 6-{2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[*D*-1-(methoxycarbonyl)-ethyl]-β-*D*-glucopyranosyl}-3-*O*-benzoyl-2-deoxy-2-octadecanoylamino-β-*D*-glucopyranoside* (**17**). — Coupling of **7** (140 mg) with the oxazoline **12** (200 mg) in dry 1,2-dichloromethane (5 mL) in the presence of toluene-*p*-sulfonic acid monohydrate (15 mg), as described for the preparation of **13**, yielded **17** (187 mg, 84%) as needles, m.p. 227–230°, $[\alpha]_D^{25}$ +2° (*c* 0.3, chloroform): ν_{max}^{Nujol} 3430, 3350, and 3270 (OH, NH), 1750, 1730, 1280, and 1250 (ester), 1660 and 1540 (amide), and 740, 720, and 700 cm^{-1} (phenyl); n.m.r. data (2:1 chloroform-*d*–methanol-d_4): δ 0.88 (t, 3 H, *J* 5.4 Hz, *Me*CH_2), 1.05–1.26 (m, 30 H, 15 methylene), 1.30 (d, 3 H, *J* 7.0 Hz, *Me*CH), 1.98, 2.06, and 2.12 (3 s, 9 H, AcN, 2 AcO), 3.71 (s, 3 H, MeO), 4.98, 5.20 (2 t, 2 H, $J_{2,3} = J_{3,4} = J_{3',4'} = J_{4',5'} = 8.8$ Hz, H-3,4'), 7.28 (s, 5 H, Ph), and 7.26–8.04 (m, 5 H, Ph).

Anal. Calc. for $C_{54}H_{80}N_2O_{16}$: C, 64.01; H, 7.96; N, 2.77. Found: C, 64.23; H, 7.79; N, 2.75.

Acetylation of **17** (320 mg) with acetic anhydride–pyridine, as already described, gave the 4-acetate **18** (318 mg, 96%) as needles, m.p. 208–209°, $[\alpha]_D^{25}$ −10° (*c* 0.2, chloroform); ν_{max}^{Nujol} 3260 (NH), 1750, 1270, and 1240 (ester), 1650 and 1540 (amide), and 730, 710, and 700 cm^{-1} (phenyl); n.m.r. data (chloroform-*d*): δ 0.88 (t, 3 H, *J* 6.0 Hz, *Me*CH_2), 1.07–1.30 (m, 30 H, 15 methylene), 1.31 (d, 3 H, *J* 7.0 Hz, *Me*CH), 1.90, 1.98, 2.04, and 2.10 (4 s, 12 H, AcN, 3 AcO), 3.70 (s, 3 H, MeO), 4.98, 5.12, and 5.42 (3 t, 3 H, $J_{2,3} = J_{3,4} = J_{4,5} = J_{3',4'} = J_{4',5'} = 9.0$ Hz, H-3,4,4'), 5.90 (d, 1 H, $J_{2,NH}$ 9.0 Hz, NH), 6.73 (d, 1 H, $J_{2',NH}$ 6.0 Hz, NH), 7.27 (s, 5 H, Ph), and 7.24–7.98 (m, 5 H, Ph).

Anal. Calc. for $C_{56}H_{82}N_2O_{17}$: C, 63.74; H, 7.83; N, 2.65. Found: C, 63.68; H, 7.85; N, 2.59.

*Benzyl 2-acetamido-6-*O*-[2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine benzyl ester)-β-*D*-glucopyranosyl-4-*O*-acetyl-3-*O*-benzoyl-2-deoxy-α-*D*-glucopyranoside* (**19**). — To a solution of **14** (220 mg) in 1,4-dioxane (3 mL) was added 0.1M potassium hydroxide (4 mL), and the solution was stirred

for 5 min at room temperature, and then treated with Amberlite IR-120 (H^+) resin to remove the base. The resin was filtered off and washed with methanol, and the filtrate and washings were combined and evaporated. A solution of the resulting, crystalline, free acid in dry 1,4-dioxane (4 mL) was stirred with *N*-hydroxysuccimide (HOSu, 46 mg) and dicyclohexylcarbodiimide (DCC, 82 mg) for 3 h at room temperature; at that time, the starting material had been converted into the activated ester. L-Alanyl-D-isoglutamine benzyl ester trifluoroacetate[16] (143 mg) and triethylamine (0.1 mL) were then added to the mixture, which was stirred for 20 h at room temperature and then evaporated. The residue was chromatographed on a column of silica gel (20 g) with (*a*) chloroform, (*b*) 100:1, and (*c*) 50:1 chloroform–methanol. Eluant (*c*) afforded **19** (266 mg, 91%) as crystals, m.p. 249–252°, $[\alpha]_D^{25}$ +46.5° (*c* 0.2, chloroform); ν_{max}^{Nujol} 3260 (NH), 1750, 1720, and 1280–1230 (ester), 1670, 1660, 1645, 1550, and 1530 (amide), and 730, 720, and 700 cm^{-1} (phenyl).

Anal. Calc. for $C_{54}H_{67}N_5O_{20}$: C, 58.63; H, 6.11; N, 6.33. Found: C, 58.55; H, 6.13; N, 6.24.

Benzyl 6-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine benzyl ester)-β-D-glucopyranosyl]-4-O-acetyl-3-O-benzoyl-2-(DL-3-benzoyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (**20**). — To a solution of **16** (130 mg) in 1,4-dioxane (3 mL) was added 0.1M potassium hydroxide (2 mL), and the mixture was stirred for 5 min at room temperature and then processed as described for the preparation of **19**, to afford the free acid. The acid was coupled with the dipeptide (66 mg) in dry 1,4-dioxane (4 mL) in the presence of HOSu (21 mg), DCC (38 mg), and triethylamine (0.1 mL), as in the preparation of **19**. The product was purified by chromatography on a column of silica gel (10 g) with (*a*) chloroform and (*b*) 50:1 chloroform–methanol. Eluant (*b*) gave **20** (150 mg, 93%), m.p. 228–231°, $[\alpha]_D^{25}$ +51° (*c* 0.2, chloroform); ν_{max}^{Nujol} 3250 (NH), 1745, 1720, 1280, and 1230 (ester), 1670, 1660, 1645, and 1530 (amide), and 740–700 cm^{-1} (phenyl).

Anal. Calc. for $C_{73}H_{95}N_5O_{22}$: C, 62.87; H, 6.87; N, 5.02. Found: C, 62.91; H, 6.77; N, 4.93.

Benzyl 6-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine benzyl ester)-β-D-glucopyranosyl]-4-O-acetyl-3-O-benzoyl-2-deoxy-2-octadecanoylamino-β-D-glucopyranoside (**21**). — Compound **18** (150 mg) was hydrolysed with 0.1M potassium hydroxide (2.5 mL) in 1,4-dioxane (3 mL), as described for **14**. The resulting, free acid was coupled with the dipeptide (77 mg) in dry 1,4-dioxane (4 mL) by using HOSu (25 mg), DCC (45 mg), and triethylamine (0.1 mL), as described for **19**, to give **21** (167 mg, 88%), m.p. 235–238°, $[\alpha]_D^{25}$ −3.5° (*c* 0.4, chloroform); ν_{max}^{Nujol} 3270 (NH), 1750, 1730, 1280, and 1240 (ester), 1680, 1660, 1640, 1560, 1540, and 1530 (amide), and 730, 720, and 700 cm^{-1} (phenyl).

Anal. Calc. for $C_{70}H_{99}N_5O_{20}$: C, 63.19; H, 7.50; N, 5.26. Found: C, 63.26; H, 7.46; N, 5.21.

Benzyl 2-acetamido-6-O-[2-acetamido-2-deoxy-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine)-β-D-glucopyranosyl]-2-deoxy-α-D-glucopyranoside (**22**). — To a solution of **19** (200 mg) in methanol (20 mL) was added sodium methoxide (50 mg), and the

mixture was stirred for 10 h at room temperature, treated with Amberlite IR-120 (H^+) resin, and then evaporated. The syrupy residue was chromatographed on a column of silica gel (10 g) with (*a*) 100:1, (*b*) 10:1, and (*c*) 5:1 chloroform–methanol. Eluant (*c*) gave **22** (104 mg, 73%), m.p. 188–190°, $[\alpha]_D^{25}$ +54° (*c* 0.2, methanol); ν_{max}^{Nujol} 3400–3200 (OH, NH), 1720 (C=O), 1650 and 1560–1540 (amide), and 730 and 720 cm^{-1} (phenyl); n.m.r. data (methanol-d_4): δ 1.41, 1.42 (2 d, 6 H, *J* 6.0 Hz, 2 *Me*CH), 1.98 (s, 6 H, 2 AcN), and 7.33 (s, 5 H, Ph).

Anal. Calc. for $C_{34}H_{51}N_5O_{16}$: C, 51.97; H, 6.54; N, 9.13. Found: C, 51.69; H, 6.66; N, 9.05.

Benzyl 6-O-[2-acetamido-2-deoxy-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine)-β-D-glucopyranosyl]-2-deoxy-2-(DL-3-hydroxytetradecanoylamino)-α-D-glucopyranoside (**23**). — Hydrolysis of **20** (100 mg) with sodium methoxide (40 mg) in methanol (20 mL), as described for **19**, gave **23** (46 mg, 66%), m.p. 194–197°, $[\alpha]_D^{25}$ +40° (*c* 0.4, methanol); ν_{max}^{Nujol} 3350–3260 (OH, NH), 1720 (C=O), 1650, 1640, 1540, and 1530 (amide), and 720 and 690 cm^{-1} (phenyl); n.m.r. data (methanol-d_4): δ 0.89 (t, 3 H, *J* 5.4 Hz, *Me*CH_2), 1.40, 1.42 (2 d, 6 H, *J* 7.4 Hz, 2 *Me*CH), 1.98 (s, 3 H, AcN), and 7.33 (s, 5 H, Ph).

Anal. Calc. for $C_{46}H_{75}N_5O_{17}$: C, 56.95; H, 7.79; N, 7.22. Found: C, 56.88; H, 7.91; N, 7.15.

Benzyl 6-O-[2-acetamido-2-deoxy-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine)-β-D-glucopyranosyl]-2-deoxy-2-octadecanoylamino-β-D-glucopyranoside (**24**). — Hydrolysis of **21** (160 mg) with sodium methoxide (50 mg) in methanol (20 mL), with processing as already described, afforded **24** (85 mg, 70%), m.p. 220–223°, $[\alpha]_D^{25}$ −21° (*c* 0.2, 1:1 chloroform–methanol); ν_{max}^{Nujol} 3350–3240 (OH, NH), 1720 (C=O), 1650, 1560, and 1550 (amide), and 730 and 720 cm^{-1} (phenyl); n.m.r. data (methanol-d_4): δ 0.88 (t, 3 H, *J* 6.0 Hz, *Me*CH_2), 2.03 (s, 3 H, AcN), and 7.26 (s, 5 H, Ph).

Anal. Calc. for $C_{50}H_{83}N_5O_{16}$: C, 59.44; H, 8.28; N, 6.93. Found: C, 59.29; H, 8.32; N, 6.84.

2-Acetamido-6-O-[2-acetamido-2-deoxy-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine)-β-D-glucopyranosyl]-2-deoxy-D-glucopyranose (**25**). — To a solution of **22** (100 mg) in methanol (10 mL), water (10 mL), and acetic acid (1 mL) was added 10% Pd–C catalyst (100 mg), and hydrogen was bubbled through while the mixture was stirred for 5 h at 40°; the course of the reaction was monitored by t.l.c. The catalyst was removed by filtration, and the filtrate was evaporated below 20°, to give hygroscopic, amorphous **25** (85 mg, quantitative), which showed a single spot in t.l.c.; $[\alpha]_D^{25}$ +13° (*c* 0.7, methanol; equil.); ν_{max}^{KBr} 3360–3250 (OH, NH), 1725 (C=O), and 1650 and 1550 cm^{-1} (amide).

Anal. Calc. for $C_{27}H_{45}N_5O_{16}$: C, 46.61; H, 6.52; N, 10.07. Found: C, 46.25; H, 6.89; N, 10.38.

6-O-[2-Acetamido-2-deoxy-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine)-β-D-glucopyranosyl]-2-deoxy-2-(DL-3-hydroxytetradecanoylamino)-D-glucopyranose (**26**). — Hydrogenation of **23** (40 mg), in the presence of 10% Pd–C catalyst (80 mg), in methanol (10 mL)–water (10 mL)–acetic acid (1 mL), according to the procedure

already described, gave amorphous **26** (35 mg; quantitative), $[\alpha]_D^{25}$ +7° (*c* 0.2, methanol; equil.); ν_{max}^{KBr} 3330–3250 (OH, NH), 2920 and 2840 (Me, methylene), 1720 (C=O), and 1650 and 1560 cm^{-1} (amide).

Anal. Calc. for $C_{39}H_{69}N_5O_{17}$: C, 53.23; H, 7.90; N, 7.96. Found: C, 52.95; H, 8.35; N, 7.91.

6-O-[2-Acetamido-2-deoxy-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine)-β-D-glucopyranosyl]-2-deoxy-2-octadecanoylamino-D-glucopyranose (**27**). — Hydrogenation of **24** (80 mg), in the presence of 10% Pd–C catalyst (80 mg), in methanol (10 mL)–water (10 mL)–acetic acid (1 mL), as already described, gave amorphous **27** (71 mg; quantitative), $[\alpha]_D^{25}$ +8° (*c* 0.6, methanol; equil.); ν_{max}^{KBr} 3320–3220 (OH, NH), 2920 and 2840 (Me, methylene), 1720 (C=O), and 1650, 1560, and 1550 cm^{-1} (amide).

Anal. Calc. for $C_{43}H_{77}N_5O_{16}$: C, 56.13; H, 8.44; N, 7.61. Found: C, 55.85; H, 8.71; N, 7.54.

REFERENCES

1 M. Kiso, Y. Kaneda, R. Shimizu, A. Hasegawa, and I. Azuma, *Carbohydr. Res.*, in press.
2 F. Ellouz, A. Adam, R. Ciorbaru, and E. Lederer, *Biochem. Biophys. Res. Commun.*, 59 (1974) 1317–1325; S. Kotani, Y. Watanabe, F. Kinoshita, T. Shimono, I. Morisaki, T. Shiba, S. Kusumoto, Y. Tarumi, and K. Ikenaka, *Biken J.*, 18 (1975) 105–111; C. Meiser, P. Sinaÿ, and A. Adam, *Biochem. Biophys. Res. Commun.*, 66 (1975) 1316–1322.
3 A. Hasegawa, Y. Kaneda, Y. Goh, K. Nishihori, M. Kiso, and I. Azuma, *Carbohydr. Res.*, 94 (1981) 143–163; A. Hasegawa, E. Tanahashi, Y. Goh, M. Kiso, and I. Azuma, *ibid.*, 92 (1981) 75–84.
4 P. L. Durette, E. P. Meitner, and T. Y. Shen, *Tetrahedron Lett.*, (1979) 4013–4016.
5 M. Kiso, Y. Kaneda, R. Shimizu, and A. Hasegawa, *Carbohydr. Res.*, 83 (1980) c8–c11.
6 S. Kusumoto, K. Yamamoto, and T. Shiba, *Tetrahedron Lett.*, (1978) 4407–4410.
7 P. L. Durette, E. P. Meitner, and T. Y. Shen, *Carbohydr. Res.*, 77 (1979) c1–c4.
8 A. Hasegawa, Y. Kaneda, M. Amano, M. Kiso, and I. Azuma, *Agric. Biol. Chem.*, 42 (1978) 2187–2189.
9 E. Lederer, *J. Med. Chem.*, 23 (1980) 819–825.
10 S. Kobayashi, T. Fukuda, I. Imada, M. Fujino, I. Azuma, and Y. Yamamura, *Chem. Pharm. Bull.*, 27 (1979) 3193–3196.
11 M. Kiso, Y. Goh, E. Tanahashi, A. Hasegawa, H. Okumura, and I. Azuma, *Carbohydr. Res.*, 90 (1981) c8–c11.
12 A. Hasegawa, H. Okumura, M. Kiso, I. Azuma, and Y. Yamamura, *Carbohydr. Res.*, 79 (1980) c20–c23; A. Hasegawa, H. Okumura, M. Kiso, I. Azuma, and Y. Yamamura, *Agric. Biol. Chem.*, 44 (1980) 1309–1313.
13 A. Hasegawa, K. Bito, and M. Kiso, *Gifu Daigaku Nogakubu Kenkyu Hokoku*, 40 (1977) 95–100.
14 H. M. Flowers and R. W. Jeanloz, *J. Org. Chem.*, 28 (1963) 2983–2986.
15 K. L. Matta and O. P. Bahl, *Carbohydr. Res.*, 21 (1972) 460–464.
16 S. Kusumoto, Y. Tarumi, K. Ikenaka, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 49 (1976) 533–539.
17 I. Azuma, K. Kamisango, I. Saiki, Y. Tanio, S. Kobayashi, and Y. Yamamura, *Infect. Immun.*, 29 (1980) 1193–1196.