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SYNTHESIS AND IMMUNOADJUVANT ACTIVITIES OF THE REPEATING, DISACCHARIDE-DIPEPTIDE UNIT OF THE BACTERIAL, CELL-WALL PEPTIDOGLYCAN AND OF SOME CARBOHYDRATE ANALOGS*

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ABSTRACT

The repeating disaccharide-dipeptide units of the bacterial, cell-wall peptidoglycan, one being *O*-(*N*-acetyl-β-muramoyl-L-alanyl-D-isoglutamine)-(1→4)-2-acetamido-2-deoxy-D-glucose, and the other, *O*-(2-acetamido-2-deoxy-β-D-glucosyl)-(1→4)-*N*-acetyl-muramoyl-L-alanyl-D-isoglutamine, have been synthesized. Some carbohydrate analogs, such as *O*-(*N*-acetyl-β-muramoyl-L-alanyl-D-isoglutamine)-(1→4)-*N*-acetylmuramoyl-L-alanyl-D-isoglutamine, *O*-β-D-glucosyl-(1→4)-*N*-acetylmuramoyl-L-alanyl-D-isoglutamine, and *O*-(6-acetamido-6-deoxy-β-D-glucosyl)-(1→4)-*N*-acetylmuramoyl-L-alanyl-D-isoglutamine, were also synthesized. Their immunoadjuvant activities were examined in guinea-pigs.

INTRODUCTION

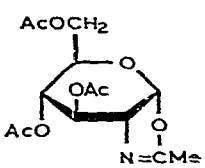
The fundamental structure of the bacterial, cell-wall peptidoglycans (some of them having potent, immunostimulant activities) generally consists of alternating, β-(1→4)-linked pyranosides of 2-acetamido-2-deoxy-D-glucose (GlcNAc) and *N*-acetylmuramic acid (MurNAc) joined to peptide. The discovery[4] of a simple, dipeptide derivative of muramic acid, namely, *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP), which is the minimal structure necessary for immunoadjuvant activity, has stimulated recent work on the synthetic MDP analogs[5]. In continuation of our interest in clarifying the relationship between the structure of the carbohydrate moiety in, and the activity of, MDP, we have further examined the influence, on the adjuvant activity of MDP, of a longer carbohydrate moiety[2,3,6]. For similar purposes, Kusumoto *et al.*[7] and Durette *et al.*[8] synthesized the repeating disaccharide-dipeptide units of the peptidoglycan by using the Koenigs–Knorr reaction and the Lemieux method, respectively.

*Studies on Immonoadjuvant Active Compounds, Part XV. For Part XIV, see ref. 1. For preliminary reports on part of this work, see refs. 2 and 3.

The use of oxazoline derivatives is a well established method for introducing β-linked GlcNAc units into synthetic oligosaccharides. In this connection, it has been found that, when the coupling of allyl 2-acetamido-3,6-di-*O*-(2-butenyl)[9]- and -3,6-di-*O*-benzyl[10]-2-deoxy-β-D-glucopyranoside with 2-methyl-(4-*O*-acetyl-3,6-di-*O*-benzyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline[11] was conducted in 1,2-dichloroethane containing a 10–20 mmolar proportion of *p*-toluenesulfonic acid, the desired β-(1→4)-linked disaccharides could be isolated crystalline in 30–40% yield. We now report the synthesis of β-MDP-(1→4)-GlcNAc (**29**), β-GlcNAc-(1→4)-MDP (**30**), and β-MDP-(1→4)-MDP (**31**) by the improved oxazoline procedure just described, and of some carbohydrate analogs in which the GlcNAc moiety was replaced by D-glucose (giving **42**) and 6-acetamido-6-deoxy-D-glucose (giving **43**) by the Koenigs–Knorr reaction. Their immunoadjuvant activities are also described.

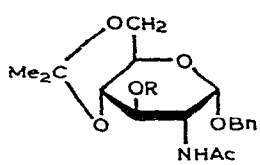
RESULTS AND DISCUSSION

The most familiar oxazoline, namely, 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**1**) was used as the glycosyl donor for the synthesis of **29**, **30**, and **31**. As acceptors, we employed benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside[12] (**5**) and benzyl 2-acetamido-6-*O*-benzyl-3-*O*-(2-butenyl)-2-deoxy-α-D-glucopyranoside (**9**), which were readily prepared, stepwise,

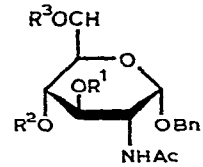


1

Ac = MeCO



2 R = H
3 R = Bn
4 R = Bue
Bn = $PhCH_2$
Bue = $MeCH{=}CHCH_2$



5 $R^1 = R^3 = Bn$, $R^2 = H$
6 $R^1 = Bue$, $R^2 = R^3 = H$
7 $R^1 = Bue$, $R^2 = H$, $R^3 = Bz$
8 $R^1 = Bue$, $R^2 = Thp$, $R^3 = H$
9 $R^1 = Bue$, $R^2 = H$, $R^3 = Bn$
10 $R^1 = Bue$, $R^2 = Ac$, $R^3 = Br$
Bz = PhCO
Thp = tetrahydropyran-2-yl

by benzylation or crotylation of benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside[13] (**2**), hydrolytic removal of the isopropylidene group, and selective, or stepwise (**6**–**9**), benzylation of the primary hydroxyl group on C-6.

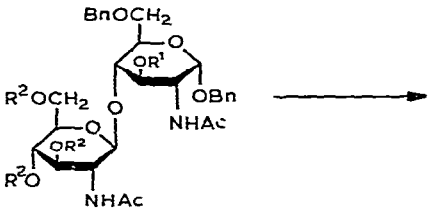
The coupling of **1** with the acceptor **5** or **9** was achieved in 1,2-dichloroethane solution containing *p*-toluenesulfonic acid as the catalyst, to give the protected chitobioside derivatives (**11** and **12**) as fine needles. The stereochemistry of the glycosyla-

tions was established by examination of the 400-MHz, n.m.r. spectra, and in comparison with studies[9,10] using the same glycosylation procedure.

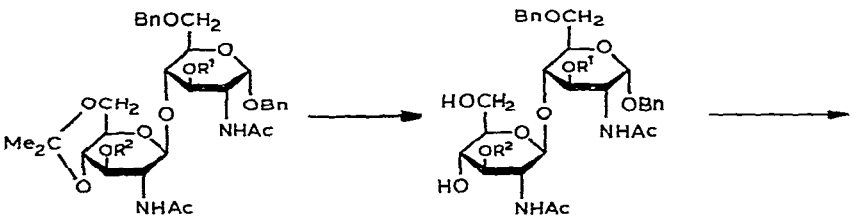
O-Deacetylation of **11** and **12**, and subsequent 4′,6′-*O*-isopropylidenation gave **15** and **16** in almost quantitative yields. Compound **15** was condensed with L-2-chloropropanoic acid in the presence of sodium hydride, to give the β-MurNAc-(1→4)-GlcNAc derivative **17**. On the other hand, benzylation of **16**, and removal of the 2-butenyl group with potassium *tert*-butoxide in dimethyl sulfoxide[14], afforded **19**, which was then condensed with L-2-chloropropanoic acid as just described, to give the β-GlcNAc-(1→4)-MurNAc derivative **20**. For the synthesis of the β-MurNAc-(1→4)-MurNAc derivative **22**, the 2-butenyl group in **16** was removed first, to afford **21**, which was then condensed with L-2-chloropropanoic acid.

1 + 5 ⟶ 11

1 + 9 ⟶ 12



11 R^1 = Bn, R^2 = Ac
12 R^1 = Bue, R^2 = Ac
13 R^1 = Bn, R^2 = H
14 R^1 = Bue, R^2 = H

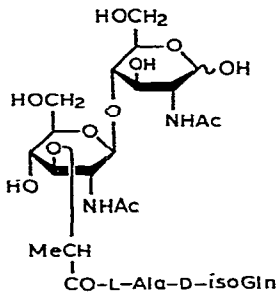


15 R^1 = Bn, R^2 = H
16 R^1 = Bue, R^2 = H
17 R^1 = Bn, R^2 = a
18 R^1 = Bue, R^2 = Bn
19 R^1 = H, R^2 = Bn
20 R^1 = a, R^2 = Bn
21 R^1 = R^2 = H
22 R^1 = R^2 = a
23 R^1 = Bn, R^2 = b
24 R^1 = b, R^2 = Bn
25 R^1 = R^2 = b

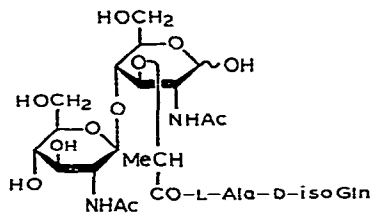
26 R^1 = Bn, R^2 = b
27 R^1 = b, R^2 = Bn
28 R^1 = R^2 = b

a = -CH(Me)CO_2H
b = -CH(Me)CO—L-Ala—D-isoGln-OBn

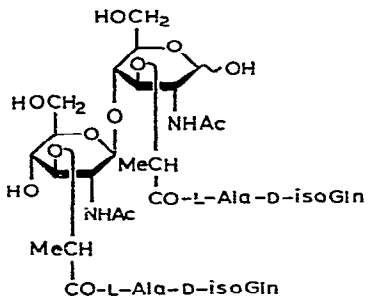
Couplings of **17**, **20**, and **22** with L-alanyl-D-isoglutamine benzyl ester were conducted with dicyclohexylcarbodiimide and *N*-hydroxysuccinimide as the activating agents, to give the corresponding lactoyl-dipeptide derivatives **23**, **24**, and **25**, respectively. Hydrolytic removal of the 4′,6′-*O*-isopropylidene group gave **26**, **27**, and **28**, which were hydrogenolyzed in the presence of 10% palladium–carbon (Pd–C) catalyst to afford the desired disaccharide-dipeptide units (**29** and **30**) and their analog **31**, as amorphous materials.



29



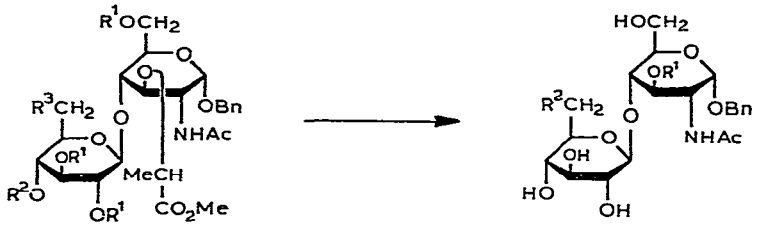
30



31

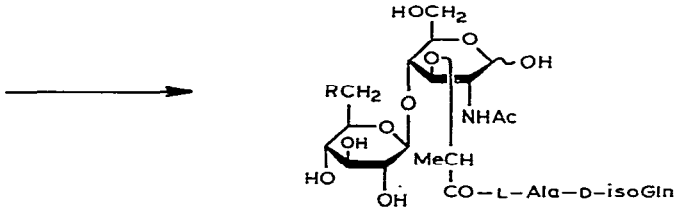
The synthesis of the carbohydrate analogs, **42** and **43**, was accomplished as follows. Compound **38**, prepared by the alkaline hydrolysis of[15] **32**, was coupled with L-alanyl-D-isoglutamine benzyl ester as already described, to give amorphous **40**. The desired β-Glc-(1→4)-MurNAc (**42**) was readily prepared by hydrogenolysis of **40** in the presence of 10% Pd–C catalyst. On the other hand, 4′,6′-*O*-isopropylidenation of **32**, and subsequent perbenzoylation of the product gave benzyl 2-acetamido-6-*O*-benzoyl-2-deoxy-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside, which was then treated with 60% aqueous acetic acid, to afford crystalline **33**. The selective mesylation of 6′-OH, and treatment of the ester with sodium azide in *N*,*N*-dimethylformamide,

followed by acetylation, gave **35**. Mild hydrogenolysis of **35**, acetylation of the product, and purification of the acetate on a column of silica gel, afforded amorphous **36**. *O*-Deacetylation of **36**, and alkaline hydrolysis of the muramic ester gave **39**, which was coupled with L-alanyl-D-isoglutamine benzyl ester to afford **41**. Hydrogenolytic removal of the benzyl groups gave the desired 6′-acetamido analog **43**.



32 $R^1 = R^2 = H$, $R^3 = OH$
33 $R^1 = Bz$, $R^2 = H$, $R^3 = OH$
34 $R^1 = Bz$, $R^2 = H$, $R^3 = OMs$
35 $R^1 = Bz$, $R^2 = Ac$, $R^3 = N_3$
36 $R^1 = Bz$, $R^2 = Ac$, $R^3 = NHAc$
37 $R^1 = R^2 = H$, $R^3 = NHAc$
Ms = $MeSO_2$

38 $R^1 = a$, $R^2 = OH$
39 $R^1 = a$, $R^2 = NHAc$
40 $R^1 = b$, $R^2 = OH$
41 $R^1 = b$, $R^2 = NHAc$



42 R = OH
43 R = NHAc

The immunoadjuvant activities of the synthetic MDP analogs 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 as previously described[16] (see Table I). Both of the synthetic disaccharide-dipeptide units, **29** and **30**, exhibited potent activity, but the difference in the activity, in comparison with that of MDP, could not be regarded as significant, even at lower doses (see Exp. 1). (Tsujimoto *et al.*[17] reported that **30** was more active than MDP itself at lower doses.) Among the analogs, compound **31** showed strong activity (see Exp. 2), in contrast to **42** and **43**, which were almost inactive. These results suggest that MDP is certainly

TABLE I

ADJUVANT ACTIVITY OF THE REPEATING DISACCHARIDE-DIPEPTIDE UNITS OF THE BACTERIAL, CELL-WALL PEPTIDOGLYCAN, AND OF SOME CARBOHYDRATE ANALOGS, ON THE INDUCTION OF DELAYED-TYPE HYPERSENSITIVITY TO ABA-*N*-ACETYLTYROSINE IN GUINEA-PIGS

Compounds	*Dose (μg)*	*Skin reaction with ABA-BSA[a] (100 μg) (diam. in mm ± SE)[b] at*	
		24 h	*48 h*
Exp. 1[c]			
29	140	20.4 ±1.1	22.4 ±1.3
	14	20.9 ±1.5	21.2 ±1.1
30	140	20.4 ±1.0	26.0 ±1.5
	14	21.6 ±1.2	19.0 ±1.3
MDP	100	22.2 ±0.8	23.0 ±1.8
	10	21.4 ±1.6	18.5 ±3.4
Exp. 2			
31	100	20.3 ±0.6	20.0 ±1.2
MDP	100	20.3 ±0.5	17.8 ±0.7
Exp. 3			
42	100	(9.2 ±1.8)	(5.8 ±1.7)
43	100	(12.7 ±3.3)	(7.9 ±2.3)
MDP	100	21.0 ±1.8	19.0 ±2.0
Control[d]		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 the erythema. [c]The doses of **29** and **30** were determined as based on their molecular weights. [d]ABA-*N*-acetyltyrosine in Freund's incomplete adjuvant.

the minimum, adjuvant-active structure of the bacterial, cell-wall peptidoglycan, and that the acetamido group on C-2 of the longer carbohydrate moiety is necessary for manifestation of the 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 PM-201 polarimeter, and i.r. spectra were recorded with a Jasco IRA-1 spectrophotometer. N.m.r. spectra were recorded at 90 and 400 MHz with Hitachi R-22 and Bruker WH-400 spectrometers, respectively. F.d.–mass spectra were recorded with a Hitachi M-80 spectrometer. Preparative chromatography was performed on silica gel (Waco Co.; 300 mesh) with the solvent system specified. Evaporations were conducted *in vacuo*.

Benzyl 2-acetamido-3-O-(2-butenyl)-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranoside (**4**). — To a stirred solution of **2** (5 g) in *N*,*N*-dimethylformamide (25 mL)

kept at 0° were added powdered barium oxide (10 g), barium hydroxide octahydrate (3.5 g), and 1-bromo-2-butene (crotyl bromide) (3.3 g). The mixture was stirred for 36 h at room temperature, water was added, and stirring was continued for an additional 1 h. The suspended barium salts were filtered off with Celite, and washed with chloroform, and the filtrate and washings were combined and evaporated. The residue was dissolved in chloroform, washed with water, dried, and evaporated to a syrup which was chromatographed on a column of silica gel (100 g) with (*a*) chloroform and (*b*) 100:1 chloroform–methanol. Eluant (*b*) gave compound **4** (4.41 g, 76.4%), m.p. 102–104° (dec.), $[\alpha]_D$ +125.4° (*c* 1, chloroform); n.m.r. data (in $CDCl_3$): δ 1.41 and 1.50 (2 s, 6 H, Me_2C), 1.6–1.8 (m, 3 H, CH_3-CH=CH-), 1.94 (s, 3 H, MeCO), 4.90 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.4–5.75 (m, 3 H, -CH=CH- and NH), and 7.3 (s, 5 H, Ph).

Anal. Calc. for $C_{22}H_{31}NO_6$: C, 65.16; H, 7.71; N, 3.45. Found: C, 65.23; H, 7.66; N, 3.24.

Benzyl 2-acetamido-6-O-benzoyl-3-O-(2-butenyl)-2-deoxy-α-D-glucopyranoside (**7**). — A solution of **4** (35 g) in 60% aqueous acetic acid (50 mL) was heated for 3 h at 50–60°, cooled, and evaporated, and the residue crystallized from ethyl acetate to give **6** (30.4 g, 96.4%) as needles, m.p. 157°, $[\alpha]_D$ +150.2° (*c* 1, methanol). To a stirred solution of **6** (10 g) in dry pyridine (60 mL), kept at −20°, was added benzoyl chloride (3.85 mL). The mixture was stirred for 1 h at this temperature, and then water was added. Pyridine was removed by evaporation, the residue was extracted with chloroform, and the extract was successively washed with 2M hydrochloric acid, M sodium carbonate, and water, dried, and evaporated. The residue crystallized from ethyl acetate–hexane, to give needles of **7** (10.6 g, 82%), m.p. 169–170°, $[\alpha]_D$ +70.4° (*c* 0.73, chloroform); ν_{max}^{Nujol} 3480 (OH), 3280 (NH), 1710 (C=O), 1640 and 1550 (amide), and 710 cm^{-1} (Ph).

Anal. Calc. for $C_{26}H_{31}NO_7$: C, 66.51; H, 6.66; N, 2.98. Found: C, 66.39; H, 6.61; N, 3.00.

Benzyl 2-acetamido-3-O-(2-butenyl)-2-deoxy-4-O-(tetrahydropyran-2-yl)-α-D-glucopyranoside (**8**). — To a stirred solution of **7** (10 g) in abs. 1,4-dioxane (50 mL) were added dihydropyran (10 mL) and *p*-toluenesulfonic acid monohydrate (120 mg). The mixture was stirred for 3 h at room temperature, treated with Amberlite IR-410 (OH^-) resin to remove the acid, and evaporated. Treatment of the residue with methanolic sodium methoxide at room temperature, followed by extractive processing, gave a mixture of diastereoisomers due to the tetrahydropyran-2-yl group. Chromatography of the product on silica gel with (*a*) 100:1 and (*b*) 50:1 chloroform–methanol gave two kinds of crystalline compounds, **8a** (5.71 g, 59.6%) {m.p. 112–113° (dec.), $[\alpha]_D$ +133.4° (*c* 1, chloroform); ν_{max}^{Nujol} 3400 (OH), 3260 (NH), 1640 and 1540 (amide), and disappearance of the band at 1710 cm^{-1} (C=O); n.m.r. data (in $CDCl_3$): loss of benzoyl-*H* and appearance of tetrahydropyranyl-*H* at δ 1.1–2.0}, and **8b** (3.06 g, 32%) {m.p. 129–130°, $[\alpha]_D$ +88.6° (*c* 0.74, chloroform); ν_{max}^{Nujol} 3500 (OH), 3200 (NH), and 1630 and 1560 cm^{-1} (amide); n.m.r. data (in $CDCl_3$): similar to those of **8a**}.

Benzyl 2-acetamido-6-O-benzyl-3-O-(2-butenyl)-2-deoxy-α-D-glucopyranoside (**9**). — To a solution of **8** (**8a** or **8b**) (5 g) in *N,N*-dimethylformamide (30 mL) kept at 0° were added powdered barium oxide (10 g), barium hydroxide octahydrate (3.53 g), and benzyl bromide (3.33 mL). The mixture was stirred for 18 h at room temperature, and then water was added. The mixture was filtered, and the solid was washed with chloroform. The filtrate and washings were combined, washed with water, dried, and evaporated to afford a residue (6 g) which was treated with 70% aqueous acetic acid (10 mL) for 4 h at 40–50°. The mixture was cooled and evaporated, and the residue crystallized from ethyl acetate–hexane, to give **9** (4.41 g, 86.4%), m.p. 147–148°, $[\alpha]_D$ +100.8° (*c* 1, chloroform); ν_{max}^{Nujol} 3560–3460 (OH), 3280 (NH), 1650 and 1550 (amide), and 730 and 690 cm^{-1} (Ph). The structure of **9** was further confirmed by its conversion into the 4-*O*-acetyl derivative **10**.

Anal. Calc. for $C_{26}H_{33}NO_6$: C, 68.55; H, 7.30; N, 3.08. Found: C, 68.51; H, 7.25; N, 2.96.

Benzyl 2-acetamido-4-O-acetyl-6-O-benzyl-3-O-(2-butenyl)-2-deoxy-α-D-glucopyranoside (**10**). — Compound **9** (320 mg) was acetylated with acetic anhydride (0.2 mL) in dry pyridine (2 mL). The mixture was evaporated, and the residue crystallized from ethyl acetate–hexane, to afford **10** as needles, m.p. 134–135°, $[\alpha]_D$ +70.6° (*c* 0.32, chloroform); ν_{max}^{Nujol} 3300 (NH), 1740 (C=O), and 1640 and 1540 cm^{-1} (amide); n.m.r. data (in $CDCl_3$): δ 1.6–1.8 (m, 3 H, CH_3-CH=CH-), 1.94 and 1.96 (2 s, 6 H, MeCO), 4.94 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.04 (t, 1 H, $J_{3,4} \simeq J_{4,5}$ = 10 Hz, H-4), and 7.27 and 7.30 (2 s, 10 H, 2 Ph).

Benzyl 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside (**11**). — To a stirred solution of[12] **5** (1.5 g) in 1,2-dichloroethane (9 mL) were added *p*-toluenesulfonic acid (34 mg) and oxazoline **1** (3 g) dissolved in 1,2-dichloroethane (11 mL), and the mixture was stirred for 20 h at the reflux temperature. When the oxazoline had almost disappeared, the mixture was extracted with chloroform in the usual way, and the organic layer was successively washed with 10% sodium carbonate and water, dried, and evaporated, to give a residue which was chromatographed on a column of silica gel (150 g) with (*a*) chloroform and (*b*) 100:1 chloroform–methanol. The disaccharide fraction obtained from eluant (*b*) crystallized from hot ethanol, to give **11** (760 mg; 30.4%, based on **5**) as needles, m.p. 249–250°, $[\alpha]_D$ +63.5° (*c* 1, chloroform); ν_{max}^{Nujol} 3300 and 3240 (NH), 1740 (C=O), 1650, 1640 and 1530 (amide), and 735, 725, and 690 cm^{-1} (Ph); n.m.r. data at 400 MHz (in $CDCl_3$): δ 1.69, 1.72, 1.93, 2.00, and 2.02 (5 s, 15 H, MeCO), 4.92 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 4.15 (ddd, 1 H, $J_{2,3}$ 10.5, $J_{2,NH}$ 9.0 Hz, H-2), 3.57 (dd, 1 H, $J_{3,4}$ 8.7 Hz, H-3), 3.95 (dd, 1 H, $J_{4,5}$ 10.0 Hz, H-4), 3.67 (ddd, 1 H, $J_{5,6}$ 2.6, 2.1 Hz, H-5), 3.44 and 3.61 (2 dd, 2 H, J_{gem} 10.8 Hz, H-6), 5.09 (d, 1 H, NH), 4.35 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1'), 4.02 (ddd, 1 H, $J_{2',3'}$ 10.5, $J_{2',NH'}$ 9.8 Hz, H-2'), 4.83 (dd, 1 H, $J_{3',4'}$ 9.2 Hz, H-3'), 4.99 (dd, 1 H, $J_{4',5'}$ 10.0 Hz, H-4'), 3.50 (ddd, 1 H, $J_{5',6'}$ 4.6, 2.3 Hz, H-5'), 3.93 and 4.18 (2 dd, 2 H, J_{gem} 12.3 Hz, H-6'), 4.59 (d, 1 H, NH'), 4.37, 4.44, 4.51, 4.64, 4.89, and 4.91 (6 d, 6 H, J_{gem} 12.1–12.6 Hz, CH_2-Ph), and 7.2–7.6 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{43}H_{52}N_2O_{14}$: C, 62.91; H, 6.39; N, 3.41. Found: C, 63.13; H, 6.36; N, 3.29.

Benzyl 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-6-O-benzyl-3-O-(2-butenyl)-2-deoxy-α-D-glucopyranoside (**12**). — The coupling of **9** (1 g) with oxazoline **1** (2 g) was accomplished by the procedure just described for the preparation of **11**. In this reaction, however, the oxazoline disappeared almost completely after 10 h, and the products were chromatographed on a column of silica gel (100 g) with (*a*) chloroform, (*b*) 200:1, and (*c*) 100:1 chloroform–methanol. The disaccharide fraction obtained from eluant (*c*) crystallized from hot ethanol, to afford **12** (622 mg; 36.1%, based on **9**) as needles, m.p. 248–250°, $[\alpha]_D$ +41.0° (*c* 0.5, chloroform); ν_{max}^{Nujol} 3300 and 3240 (NH), 1740 (C=O), 1650, 1640 and 1530 (amide), and 735, 720, and 690 cm^{-1} (Ph); n.m.r. data (in $CDCl_3$): δ 1.5–1.8 (m, 3 H, CH_3-CH=CH-), 1.72, 1.91, 1.98, 2.20, and 2.03 (5 s, 15 H, 5 MeCO), 4.4 (d, 1 H, $J_{1',2'}$ 9 Hz, H-1'), 4.92 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), and 7.2–7.6 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{40}H_{52}N_2O_{14}$: C, 61.21; H, 6.68; N, 3.57. Found: C, 61.45; H, 6.81; N, 3.33.

Benzyl 2-acetamido-4-O-(2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside (**15**). — Compound **11** (400 mg) was treated with methanolic sodium methoxide to give amorphous **13** (333 mg), m.p. 260–262° (dec.), $[\alpha]_D$ +63.2° (*c* 0.5, methanol) {lit.[8a] m.p. 252–256° (dec.), $[\alpha]_D$ +72° (*c* 1.2, methanol)}; ν_{max}^{Nujol} 3600–3100 (OH and NH), 1640 and 1530 (amide), 730, 725, and 690 (Ph), and loss of 1740 cm^{-1} (C=O). To a stirred solution of **13** (300 mg) in dry *N*,*N*-dimethylformamide (3 mL) were added *p*-toluenesulfonic acid monohydrate (15 mg) and 2,2-dimethoxypropane (1 mL). The mixture was stirred for 2 h at 50°, cooled, and treated with Amberlite IRA-410 (OH^-) ion-exchange resin to remove the acid. The resin was filtered off, and the filtrate was evaporated to a syrup which was chromatographed on a column of silica gel with (*a*) chloroform, and (*b*) 100:1 chloroform–methanol. The product obtained from eluant (*b*) crystallized from ethanol, to give **15** (260 mg, 82%) as needles, m.p. 251–252°, $[\alpha]_D$ +60.4° (*c* 0.5, chloroform); ν_{max}^{Nujol} 3440 (OH), 3300 and 3250 (NH), 1640 and 1530 (amide), 850 (Me_2C), and 730 and 690 cm^{-1} (Ph).

Anal. Calc. for $C_{40}H_{50}N_2O_{11}$: C, 65.38; H, 6.86; N, 3.81. Found: C, 65.19; H, 7.04; N, 3.69.

Benzyl 2-acetamido-4-O-(2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-6-O-benzyl-3-O-(2-butenyl)-2-deoxy-α-D-glucopyranoside (**16**). — *O*-Deacetylation of **12** (600 mg) with methanolic sodium methoxide gave amorphous **14** (467 mg), m.p. 270–271° (dec.), $[\alpha]_D$ +69.6° (*c* 0.5, methanol); ν_{max}^{Nujol} 3600–3100 (OH and NH), 1650 and 1550 (amide), 730, 725, and 690 (Ph), and disappearance of 1740 cm^{-1} (C=O). Isopropylidenation of **14** (400 mg) by the procedure just described for the preparation of **15** afforded amorphous **16** (384 mg), m.p. 244–245° (dec.), $[\alpha]_D$ +40.5° (*c* 0.326, chloroform); ν_{max}^{Nujol} 3440 (OH), 3280 and 3260 (NH), 1650 and 1530 (amide), 850 (Me_2C), and 730, 725, and 690 cm^{-1} (Ph).

Anal. Calc. for $C_{37}H_{50}N_2O_{11}$: C, 63.59; H, 7.21; N, 4.01. Found: C, 63.72; H, 7.20; N, 4.14.

Benzyl 2-acetamido-4-O-[2-acetamido-3-O-(D-1-carboxyethyl)-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl]-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside (**17**). — To a stirred solution of **15** (200 mg) in dry 1,4-dioxane (3 mL) was added the sodium hydride reagent (26 mg), and the mixture was stirred for 1.5 h at 95°, and then cooled to 65°. L-2-Chloropropanoic acid (39 mg) was added to the stirred mixture, and the mixture was stirred for 3 h at 65°, and then processed as previously described[13]. Compound **17** (200 mg, 91%) crystallized from ethyl acetate (needles), m.p. 232–233°, $[\alpha]_D$ +75° (*c* 0.4, chloroform); ν_{max}^{Nujol} 3500–2700 (CO_2H), 3300 (NH), 1720 (C=O), 1650 and 1530 (amide), 850 (Me_2C), and 730, 725, and 690 cm^{-1} (Ph); n.m.r. data (in $CDCl_3$): δ 1.2–1.5 (m, 9 H, Me_2C and MeCH), 1.78 and 1.90 (2 s, 6 H, 2 MeCO), 5.35 and 5.85 (2 d, 2 H, 2 NH), 6.8–7.2 (very broad s, 1 H, CO_2H), and 7.2–7.5 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{43}H_{54}N_2O_{13}$: C, 64.00; H, 6.75; N, 3.47. Found: C, 64.28; H, 6.55; N, 3.34.

Benzyl 2-acetamido-4-O-(2-acetamido-3-O-benzyl-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-6-O-benzyl-3-O-(2-butenyl)-2-deoxy-α-D-glucopyranoside (**18**). — To a stirred solution of **16** (200 mg) in *N*,*N*-dimethylformamide (2 mL) were added powdered barium oxide (500 mg), barium hydroxide octahydrate (140 mg), and benzyl bromide (0.1 mL). The mixture was stirred for 18 h at room temperature, and then processed as described for the preparation of **9**. The product, **18** (181 mg, 80%), was obtained as needles from hot ethanol, m.p. 219–220°, $[\alpha]_D$ +61.5° (*c* 1, chloroform); ν_{max}^{KBr} 3260 (NH), 1650 and 1540 (amide), 860 (Me_2C), and 740 and 700 cm^{-1} (Ph).

Anal. Calc. for $C_{44}H_{56}N_2O_{11}$: C, 66.98; H, 7.16; N, 3.55. Found: C, 67.21; H, 7.91; N, 3.35.

Benzyl 2-acetamido-4-O-(2-acetamido-3-O-benzyl-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-6-O-benzyl-2-deoxy-α-D-glucopyranoside (**19**). — A mixture of **18** (200 mg) and potassium *tert*-butoxide (288 mg) in dry dimethyl sulfoxide (5 mL) was stirred for 4 h at 50°. Cold water was added to the mixture, and the product was extracted with chloroform. The chloroform layer was washed with water, dried, and evaporated to a syrup which was chromatographed on a column of silica gel with (*a*) chloroform, (*b*) 100:1, and (*c*) 50:1 chloroform–methanol. The syrup obtained from eluant (*c*) crystallized from ethyl acetate to afford **19** (142 mg, 76.2%) as needles, m.p. 204–206°, $[\alpha]_D$ +84.3° (*c* 0.312, chloroform); ν_{max}^{KBr} 3420 (OH), 3260 (NH), 1670 and 1550 (amide), 850 (Me_2C), and 735 and 695 cm^{-1} (Ph); n.m.r. data (in $CDCl_3$) showed the disappearance of the 2-butenyl group.

Anal. Calc. for $C_{40}H_{50}N_2O_{11}$: C, 65.38; H, 6.86; N, 3.81. Found: C, 65.47; H, 6.68; N, 3.92.

Benzyl 2-acetamido-4-O-(2-acetamido-3-O-benzyl-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-6-O-benzyl-3-O-(D-1-carboxyethyl)-α-D-glucopyranoside (**20**). — The introduction of the D-1-carboxyethyl group into **19** (150 mg) was conducted

as described for the preparation of **17**. Extractive processing, and chromatography on a column of silica gel with 50:1 chloroform–methanol, afforded a syrup, which crystallized from ethyl acetate–hexane to give needles of **20** (132 mg, 80%), m.p. 136–138°, $[\alpha]_D$ +59.1° (*c* 0.45, chloroform); ν_{max}^{KBr} 3500–2700 (CO_2H), 3280 (NH), 1720 (C=O), 1650 and 1540 (amide), 850 (Me_2C), and 740 and 695 cm^{-1} (Ph); n.m.r. data (in $CDCl_3$): δ 1.3 (d, 3 H, *J* 7 Hz, MeCH), 1.47 (near s, 6 H, Me_2C), 1.73 and 1.95 (2 s, 6 H, MeCO), and 7.1–7.4 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{43}H_{54}N_2O_{13}$: C, 64.00; H, 6.75; N, 3.47. Found: C, 63.83; H, 6.74; N, 3.51.

*Benzyl 2-acetamido-4-O-(2-acetamido-2-deoxy-4,6-O-isopropylidene-β-*D*-glucopyranosyl)-6-O-benzyl-2-deoxy-α-*D*-glucopyranoside* (**21**). — A mixture of **16** (500 mg) and potassium *tert*-butoxide (643 mg) in dry dimethyl sulfoxide (10 mL) was stirred for 8 h at 50°, and processed as described for the preparation of **19**. Chromatography on a column of silica gel with 20:1 chloroform–methanol gave amorphous **21** (398 mg, 86%), m.p. 191–192° (dec.), $[\alpha]_D$ +50° (*c* 0.512, chloroform); ν_{max}^{Nujol} 3600–3340 (OH), 3260 (NH), 1650 and 1550 (amide), 850 (Me_2C), and 740 and 695 cm^{-1} (Ph).

Anal. Calc. for $C_{33}H_{44}N_2O_{11}$: C, 61.47; H, 6.88; N, 4.35. Found: C, 61.41; H, 6.72; N, 4.41.

*Benzyl 2-acetamido-4-O-[2-acetamido-3-O-(*D*-1-carboxyethyl)-2-deoxy-4,6-O-isopropylidene-β-*D*-glucopyranosyl]-6-O-benzyl-3-O-(*D*-1-carboxyethyl)-2-deoxy-α-*D*-glucopyranoside* (**22**). — Carboxyethylation of both OH-3 and -3′ of **21** (200 mg) was simultaneously achieved by the procedure employed for the preparation of **17** and **20**. The product was purified by chromatography on a column of silica gel with (*a*) chloroform, (*b*) 100:1, and (*c*) 50:1 chloroform–methanol. Eluant (*c*) gave compound **22** (213 mg, 87%), which crystallized from ether, m.p. 193–194°, $[\alpha]_D$ +171.7° (*c* 0.22, chloroform); ν_{max}^{Nujol} 3600–2300 (CO_2H), 3460 and 3300 (NH), 1730 and 1710 (C=O), 1660–1520 (amide), 850 (Me_2C), and 730 and 690 cm^{-1} (Ph); n.m.r. data (in CD_3OD + $CDCl_3$): δ 1.35 and 1.39 (2 d, 6 H, *J* 7 Hz, MeCH), 1.40 and 1.50 (2 s, 6 H, Me_2C), 1.96 (near s, 6 H, 2 MeCO), and 7.27 and 7.36 (2 s, 10 H, 2 Ph).

Anal. Calc. for $C_{39}H_{52}N_2O_{15}$: C, 59.38; H, 6.65; N, 3.55. Found: C, 59.65; H, 6.59; N, 3.28.

*Benzyl 2-acetamido-4-O-[2-acetamido-2-deoxy-4,6-O-isopropylidene-3-O-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine benzyl ester)-β-*D*-glucopyranosyl]-3,6-di-O-benzyl-2-deoxy-α-*D*-glucopyranoside* (**23**). — To a stirred solution of **17** (90 mg) in dry 1,4-dioxane (2 mL) were added *N*-hydroxysuccinimide (HOSu) (26 mg) and dicyclohexylcarbodiimide (DCC) (46 mg), the mixture was stirred for 2.5 h at room temperature, and then triethylamine (15 mg) and L-alanyl-D-isoglutamine benzyl ester trifluoroacetate (56 mg) dissolved in dry 1,4-dioxane (1 mL) were added. The mixture was stirred for 2 h at room temperature, and evaporated. The residue was chromatographed on a column of silica gel with (*a*) chloroform, (*b*) 100:1, and (*c*) 50:1 chloroform–methanol. Eluant (*c*) gave amorphous **23** (107 mg, 88%), m.p. 256–258° (dec.), $[\alpha]_D$ +49.4° (*c* 0.5, chloroform); n.m.r. data (in $CDCl_3$ + CD_3OD): δ 1.2–

1.6 (m, 12 H, MeCH and Me_2C), 1.86 and 1.93 (2 s, 6 H, 2 MeCO), 1.9–2.6 (m, 4 H, $-CH_2CH_2-$), and 7.2–7.45 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{58}H_{73}N_5O_{16}$: C, 63.54; H, 6.71; N, 6.39. Found: C, 63.13; H, 6.88; N, 6.11.

Benzyl 2-acetamido-4-O-(2-acetamido-3-O-benzyl-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-6-O-benzyl-2-deoxy-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine benzyl ester)-α-D-glucopyranoside (**24**). — Coupling of **20** (45 mg) with L-alanyl-D-isoglutamine benzyl ester trifluoroacetate (26 mg) in dry 1,4-dioxane (1.5 mL) by using HOSu (10 mg), DCC (18 mg), and triethylamine (8 mg) was performed as described in the previous section. Chromatography on a column of silica gel with 30:1 chloroform–methanol afforded amorphous **24** (37.5 mg, 61.5%), m.p. 206–208° (dec.), $[\alpha]_D$ +40.5° (*c* 0.375, chloroform); n.m.r. data (in $CDCl_3$): δ 1.2–1.35 (m, 6 H, 2 MeCH), 1.44 and 1.49 (2 s, 6 H, Me_2C), 1.75 and 1.90 (2 s, 6 H, MeCO), 1.9–2.5 (m, 4 H, $-CH_2CH_2-$), and 7.17–7.45 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{58}H_{73}N_5O_{16}$: C, 63.54; H, 6.71; N, 6.39. Found: C, 63.75; H, 6.82; N, 6.31.

Benzyl 2-acetamido-4-O-[2-acetamido-2-deoxy-4,6-O-isopropylidene-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine benzyl ester)-β-D-glucopyranosyl]-6-O-benzyl-2-deoxy-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine benzyl ester)-α-D-glucopyranoside (**25**). — To a stirred solution of **22** (75 mg) in dry oxolane (3 mL) were added HOSu (32 mg) and DCC (57 mg), the mixture was stirred for 30 min at room temperature, and then L-alanyl-D-isoglutamine benzyl ester trifluoroacetate (120 mg) and triethylamine (0.1 mL) were added. The mixture was stirred for 6 h at room temperature, the 1,3-dicyclohexylurea formed was filtered off, and the filtrate was evaporated. The residue was chromatographed on a column of silica gel with (*a*) chloroform, (*b*) 50:1, and (*c*) 20:1 chloroform–methanol. Eluant (*c*) gave crystalline **25** (110 mg, 84.6%), m.p. 183–184°, $[\alpha]_D$ +33.7° (*c* 0.52, 1:1 chloroform–methanol); ν_{max}^{Nujol} 3650–3100 (NH), 1735 C=O), 1650 and 1530 (amide), 850 (Me_2C), and 730, 720 and 690 cm^{-1} (Ph); n.m.r. data (in $CDCl_3$ + CD_3OD): δ 1.2–1.6 (m, 18 H, MeCH and Me_2C), 1.93 (near s, 6 H, 2 MeCO), 1.7–2.6 (m, 8 H, $-CH_2CH_2-$), and 7.2–7.4 (m, 20 H, 4 Ph); f.d.–m.s.: *m/z* 1390 $[(M + Na)^+]$ and 1406 $[(M + K)^+]$.

2-Acetamido-4-O-[2-acetamido-2-deoxy-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine)-β-D-glucopyranosyl]-2-deoxy-D-glucopyranose (**29**). — A solution of **23** (100 mg) in 60% aqueous acetic acid (2 mL) was heated for 1 h at 50°, and then evaporated at 45°. The residue was coevaporated with benzene–ethanol to give amorphous **26** in quantitative yield, m.p. 247–248° (dec.), $[\alpha]_D$ +57.7° (*c* 0.3, methanol); ν_{max}^{KBr} showed loss of the band at 850 cm^{-1} (Me_2C). To a solution of **26** (50 mg) in 10:1 methanol–acetic acid was added 10% Pd–C catalyst (50 mg), and hydrogen was bubbled through for 5 h while the solution was stirred at 20–25°. Water was added to the mixture, and hydrogen was bubbled through the mixture for 3 h. The catalyst was filtered off, and successively washed with methanol and water. The filtrate and washings were combined, and evaporated at 40°, to give amorphous **29** (60 mg), $[\alpha]_D$ +10.7° (*c* 1, water; equil.); ν_{max}^{KBr} showed loss of the

bands at 740 and 695 cm^{-1} (Ph); n.m.r. data (in D_2O): δ 1.48 and 1.55 (2 d, 6 H, *J* 7 Hz, 2 MeCH), 2.1 and 2.15 (2 s, 6 H, 2 MeCO), 1.9–2.5 (m, 4 H, $-CH_2CH_2-$), and complete disappearance of the phenyl protons.

Anal. Calc. for $C_{27}H_{45}N_5O_{16}$: C, 46.61; H, 6.52; N, 10.07. Found: C, 47.02; H, 6.26; N, 9.77.

*2-Acetamido-4-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-2-deoxy-3-*O*-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine)-*D*-glucopyranose* (**30**). — Treatment of **24** (33 mg) with 60% aqueous acetic acid (2 mL) as described in the previous section gave amorphous **27** (quantitative), m.p. 232–234° (dec.), $[\alpha]_D$ +31.2° (*c* 0.17, methanol); ν_{max}^{KBr} showed loss of the band at 850 cm^{-1} (Me_2C). To a solution of **27** (29 mg) in 1 : 1 methanol–benzene was added 10% Pd–C catalyst (20 mg), and hydrogen was bubbled through for 3 h at 20–25°. The catalyst was filtered off, and washed with methanol. The filtrate and washings were combined, and evaporated. The residue was dissolved in 50 : 1 water–acetic acid (10 mL), and hydrogen was again bubbled through the mixture for 3 h at 20–25°. The mixture was processed as just described, to give amorphous **30** (20 mg), $[\alpha]_D$ −2° (*c* 1, water; equil.) {lit.[7] $[\alpha]_D$ +0.6° (*c* 1, water; equil.)} ν_{max}^{KBr} showed loss of the bands at 730 and 690 cm^{-1} (Ph); n.m.r. data (in D_2O): δ 1.5–1.57 (2 d, 6 H, *J* 7 Hz, 2 MeCH), 2.05 (near s, 6 H, 2 MeCO), 1.9–2.5 (m, 4 H, $-CH_2CH_2-$), and complete loss of the phenyl protons.

Anal. Calc. for $C_{27}H_{45}N_5O_{16}$: C, 46.61; H, 6.52; N, 10.07. Found: C, 47.10; H, 6.17; N, 10.38.

*2-Acetamido-4-*O*-[2-acetamido-2-deoxy-3-*O*-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine)-β-*D*-glucopyranosyl]-2-deoxy-3-*O*-(*D*-2-propanoyl-*L*-alanyl-*D*-isoglutamine)-*D*-glucopyranose* (**31**). — *O*-Deisopropylidenation of **25** with 60% aqueous acetic acid was achieved as just described for **23** or **24**, to give crystalline **28**, m.p. 208–210°, $[\alpha]_D$ +27.3° (*c* 0.3, chloroform); ν_{max}^{Nujol} showed loss of the band at 850 cm^{-1} (Me_2C); n.m.r. data (in CD_3OD): δ 1.25–1.5 (m, 12 H, 4 MeCH), 1.94 (near s, 6 H, 2 MeCO), 1.9–2.6 (m, 8 H, 2 $-CH_2CH_2-$), and 7.2–7.4 (m, 20 H, 4 Ph). To a mixture of **28** (80 mg), methanol (10 mL), acetic acid (1 mL), and water (5 mL) was added 10% Pd–C catalyst (100 mg), and hydrogen was bubbled through the mixture for 5 h while the solution was stirred at 40–45°. The catalyst was filtered off, and washed with methanol and water. The filtrate and washings were combined, and evaporated, and the residue was lyophilized, to afford an amorphous mass of **31** (54.5 mg), $[\alpha]_D$ +4.5° (*c* 0.355, methanol); n.m.r. data (in CD_3OD): δ 1.25–1.5 (m, 12 H, 4 MeCH), 1.98 (near s, 6 H, 2 MeCO), 1.8–2.6 (m, 8 H, 2 $-CH_2CH_2-$), and complete loss of the phenyl protons.

Anal. Calc. for $C_{38}H_{62}N_8O_{21} \cdot H_2O$: C, 46.34; H, 6.55; N, 11.38. Found: C, 45.93; H, 6.38; N, 11.65.

*Benzyl 2-acetamido-6-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-β-*D*-glucopyranosyl)-2-deoxy-3-*O*-[*D*-1-(methoxycarbonyl)ethyl]-α-*D*-glucopyranoside* (**33**). — 4′,6′-*O*-Isopropylidenation of[15] **32** (1.15 g) was conducted as described for the preparation of **15**. The acetal was benzoylated with benzoyl chloride (1.8 g) in dry pyridine (10 mL), and the product hydrolyzed with 60% aqueous acetic acid. Compound **33** was purified

on a column of silica gel (20 g) with chloroform–methanol, and crystallized from ethyl acetate–hexane in 86% yield, m.p. 115°, $[\alpha]_D$ +111.5° (*c* 1, chloroform); ν_{max}^{Nujol} 3600–3000 (OH and NH), 1730 (C=O), 1640 and 1550 (amide), and 700 cm^{-1} (Ph); n.m.r. data (in $CDCl_3$): δ 1.45 (d, 3 H, *J* 7 Hz, MeCH), 1.91 (s, 3 H, MeCO), 3.7 (s, 3 H, CO_2Me), and 7.1–8.1 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{46}H_{49}NO_{16}$: C, 63.37; H, 5.66; N, 1.61. Found: C, 63.29; H, 5.63; N, 1.48.

*Benzyl 2-acetamido-6-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-6-*O*-mesyl-β-*D*-glucopyranosyl)-2-deoxy-3-*O*-[*D*-1-(methoxycarbonyl)ethyl]-α-*D*-glucopyranoside* (**34**). — To a stirred solution of **33** (320 mg) in dry pyridine (5 mL), kept at −25°, was added methanesulfonyl chloride (50 mg). The mixture was stirred for 5 h, and then processed in the usual way. The product was purified by chromatography on a column of silica gel with 100:1 chloroform–methanol, to give crystalline **34** (310 mg), m.p. 114°, $[\alpha]_D$ +73° (*c* 1, chloroform); ν_{max}^{KBr} 3600–3160 (OH and NH), 1720 (C=O), 1640 and 1540 (amide), 1350 (S=O), and 700 cm^{-1} (Ph); n.m.r. data (in $CDCl_3$): δ 1.48 (d, 3 H, *J* 7 Hz, MeCH), 1.91 (s, 3 H, MeCO), 3.0 (s, 3 H, SO_2Me), 3.75 (s, 3 H, CO_2Me), and 7.1–8.1 (m, 20 H, 4 Ph).

*Benzyl 2-acetamido-4-*O*-(4-*O*-acetyl-6-*C*-azido-2,3-di-*O*-benzoyl-6-deoxy-β-*D*-glucopyranosyl)-6-*O*-benzoyl-2-deoxy-3-*O*-[*D*-1-(methoxycarbonyl)ethyl]-α-*D*-glucopyranoside* (**35**). — To a solution of the 4′-*O*-acetyl derivative (250 mg) of **34** in dry *N*,*N*-dimethylformamide (5 mL) was added sodium azide (80 mg), and the mixture was stirred for 6 h at 60°. The suspended materials were filtered off, and the filtrate was evaporated. A solution of the residue in chloroform was successively washed with 2M hydrochloric acid, 10% sodium carbonate, and water, dried, and evaporated to a syrup. Chromatography on a column of silica gel with 250:1 chloroform–methanol gave **35** (208 mg) as an amorphous material, $[\alpha]_D$ +83° (*c* 1, chloroform); ν_{max}^{film} 3320 (NH), 2090 (N_3), 1730 (C=O), and 1680 and 1520 cm^{-1} (amide); n.m.r. data (in $CDCl_3$): loss of SO_2Me (at δ 3.0).

*Benzyl 2-acetamido-4-*O*-(6-acetamido-4-*O*-acetyl-2,3-di-*O*-benzoyl-6-deoxy-β-*D*-glucopyranosyl)-6-*O*-benzoyl-2-deoxy-3-*O*-[*D*-1-(methoxycarbonyl)ethyl]-α-*D*-glucopyranoside* (**36**). — Compound **35** (208 mg) in methanol (10 mL) was hydrogenolyzed for 2 h at room temperature in the presence of 10% Pd–C catalyst (50 mg). Acetic anhydride (0.03 mL) was added, and the mixture was stirred for 2 h. The catalyst was filtered off, and the filtrate was evaporated to a syrup which was chromatographed on a column of silica gel with 100:1 chloroform–methanol. The product, **36**, crystallized from ether–hexane, m.p. 110°, $[\alpha]_D$ +93.6° (*c* 0.82, chloroform); ν_{max}^{KBr} 3350 (NH), 1730 (C=O), 1650 and 1520 (amide), and 700 cm^{-1} (Ph); n.m.r. data (in $CDCl_3$): δ 1.48 (d, 3 H, *J* 7 Hz, MeCH), 1.94, 1.98, and 2.0 (3 s, 9 H, 3 MeCO), 3.78 (s, 3 H, CO_2Me), and 7.1–8.2 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{50}H_{54}N_2O_{17}$: C, 62.88; H, 5.70; N, 2.93. Found: C, 62.69; H, 5.72; N, 2.79.

*Benzyl 2-acetamido-4-*O*-(6-acetamido-6-deoxy-β-*D*-glucopyranosyl)-2-deoxy-3-*O*-[*D*-1-(methoxycarbonyl)ethyl]-α-*D*-glucopyranoside* (**37**). — Compound **36** (190

mg) was treated with methanolic sodium methoxide for 30 min at room temperature, and the base was neutralized with Amberlite IR-120 (H^+) ion-exchange resin. The resin was filtered off, and the filtrate was evaporated to a syrup which was chromatographed on a column of silica gel with 20:1 chloroform–methanol, to afford amorphous **37** (99 mg), $[\alpha]_D$ +106.3° (*c* 1, methanol); ν_{max}^{KBr} 3600–3100 (OH and NH), 1720 (C=O), and 1630 and 1520 cm^{-1} (amide); n.m.r. data (in CD_3OD): δ 1.38 (d, 3 H, MeCH), 1.96 (s, 6 H, 2 MeCO), 3.73 (s, 3 H, CO_2Me), and 7.3 (s, 5 H, Ph).

Anal. Calc. for $C_{27}H_{40}N_2O_{13}$: C, 53.99; H, 6.71; N, 4.67. Found: C, 54.15; H, 6.76; N, 4.60.

Benzyl 2-acetamido-2-deoxy-4-O-β-D-glucopyranosyl-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine benzyl ester)-α-D-glucopyranoside (**40**). — To a solution of **32** (679 mg) in methanol (40 mL) was added 0.1M potassium hydroxide (10 mL), and the solution was stirred for 10 min at room temperature, and then treated with Amberlite IR-120 (H^+) ion-exchange resin to remove the base. The resin was filtered off, and the filtrate was evaporated, to afford amorphous **38** in quantitative yield, $[\alpha]_D$ +24.5° (*c* 0.51, methanol); this was used, without purification, for the next reaction. To a solution of **38** (270 mg) in dry *N,N*-dimethylformamide (5 mL) were added HOSu (70 mg) and DCC (110 mg), and the mixture was stirred for 1 h at room temperature. L-Alanyl-D-isoglutamine benzyl ester trifluoroacetate (282 mg) and triethylamine (62 mg) were added to the mixture, and it was stirred overnight at room temperature. 1,4-Dioxane was added, the 1,3-dicyclohexylurea formed was filtered off, and the filtrate was evaporated. The residue was chromatographed on a column of silica gel with (*a*) chloroform, (*b*) 50:1, and (*c*) 10:1 chloroform–methanol. Eluant (*c*) gave amorphous **40** (300 mg), $[\alpha]_D$ +76.3° (*c* 0.28, methanol); ν_{max}^{KBr} 3600–3100 (OH and NH), 1700 (C=O), 1650 and 1530 (amide), and 690 cm^{-1} (Ph).

Anal. Calc. for $C_{39}H_{54}N_4O_{16}$: C, 56.10; H, 6.52; N, 6.71. Found: C, 55.92; H, 6.34; N, 6.57.

Benzyl 2-acetamido-4-O-(6-acetamido-6-deoxy-β-D-glucopyranosyl)-2-deoxy-3-O-(D-2-propanoyl-L-alanyl-D-isoglutamine benzyl ester)-α-D-glucopyranoside (**41**). — To a solution of **37** (95 mg) in 9:1 1,4-dioxane–water was added 0.1M potassium hydroxide (2 mL), and the mixture was processed as just described, to give amorphous **39**, $[\alpha]_D$ +88.7° (*c* 0.9, methanol). Compound **39** (76.5 mg) in dry *N,N*-dimethylformamide (8 mL) was activated with HOSu (21 mg) and DCC (60 mg), and coupled with L-alanyl-D-isoglutamine benzyl ester trifluoroacetate (100 mg) in the presence of triethylamine (24 mg) as described for **40**, to afford compound **41** (93 mg), $[\alpha]_D$ +43.0° (*c* 0.607, methanol); ν_{max}^{KBr} 3600–3100 (OH and NH), 1700 (C=O), 1650 and 1540 (amide), and 690 cm^{-1} (Ph); n.m.r. data (in CD_3OD): δ 1.36 (2 d, 6 H, *J* 7 Hz, 2 MeCH), 1.93 and 1.97 (2 s, 6 H, 2 MeCO), 1.9–2.6 (m, 4 H, $-CH_2CH_2-$), and 7.3 (near s, 10 H, 2 Ph).

Anal. Calc. for $C_{41}H_{57}N_5O_{16}$: C, 56.22; H, 6.56; N, 8.00. Found: C, 56.53; H, 6.48; N, 7.71.

2-Acetamido-2-deoxy-4-O-β-D-glucopyranosyl-3-O-(D-2-propanoyl-L-alanyl-D-

isoglutamine)-D-*glucopyranose* (**42**). — To a solution of **40** (450 mg) in 10:10:1 methanol–water–acetic acid (30 mL) was added 10% Pd–C catalyst (300 mg), and hydrogen was bubbled through for 3 h at 20–25°. The catalyst was filtered off, and successively washed with methanol and water. The filtrate and washings were combined, and evaporated at 40°, to give amorphous **42** (quantitative), $[\alpha]_D$ +32° (*c* 0.3, methanol); n.m.r. data (in D_2O): complete disappearance of the phenyl protons (at δ 7.3).

Anal. Calc. for $C_{25}H_{42}N_4O_{16}$: C, 45.87; H, 6.47; N, 8.56. Found: C, 45.46; H, 6.68; N, 8.22.

*2-Acetamido-4-*O-(*6-acetamido-6-deoxy-β-*D-*glucopyranosyl*)-*2-deoxy-3-*O-(D-*2-propanoyl-*L-*alanyl-*D-*isoglutamine*)-D-*glucopyranose* (**43**). — Hydrogenation of **41** (57 mg) in 10:10:1 methanol–water–acetic acid (10 mL) in the presence of 10% Pd–C catalyst (50 mg) was achieved as described in the previous section, to afford **43** (quantitative) as an amorphous mass, $[\alpha]_D$ +55.2° (*c* 0.46, methanol); n.m.r. data (in D_2O): loss of the phenyl protons.

Anal. Calc. for $C_{27}H_{45}N_5O_{16}$: C, 46.61; H, 6.52; N, 10.07. Found: C, 46.13; H, 6.29; N, 9.81.

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