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THE SYNTHESIS OF *O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-(1→4)-*N*-ACETYLNORMURAMOYL-L-α-AMINOBUTANOYL-D-ISOGLUTAMINE*

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ABSTRACT

Benzyl 2-acetamido-3-*O*-allyl-6-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-glucopyranoside (**4**) was obtained in high yield on using the silver triflate method in the absence of base. Compound **4** was converted in six steps into benzyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-3-*O*-(carboxymethyl)-2-deoxy-α-D-glucopyranoside, which was coupled with the benzyl ester of L-α-aminobutanoyl-D-isoglutamine and the product hydrogenolyzed to afford the title compound. *O*-Benzylation of benzyl 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside with benzyl bromide and barium hydroxide in *N,N*-dimethylformamide is strongly exhanced by sonication of the reaction mixture.

INTRODUCTION

A continuing interest in the biological properties of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP) and is analogs[2] led us to attempt the synthesis of *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-*N*-acetylnormuramoyl-L-α-aminobutanoyl-D-isoglutamine† (**14**). The proposal of structure **14** was motivated by a desire to improve the biological properties of MDP by cumulation of several structural changes which increase, *per se*, the intensity and specificity of the immunoadjuvant and cancerostatic effects[3,4].

The repeating disaccharide–dipeptide unit of the bacterial cell-wall peptidoglycan has been synthesized by several methods based on the *O*-glycosylation of both cyclic[5–8] and acyclic[9] D-glucosamine derivatives. For the synthesis of compound **14**, the strategy devised by Durette and co-workers[5] appeared to be the most convenient.

*For a preliminary communication, see ref. 1.
†Normuramic acid is the trivial name used for 2-amino-3-*O*-(carboxymethyl)-2-deoxy-D-glucopyranose.

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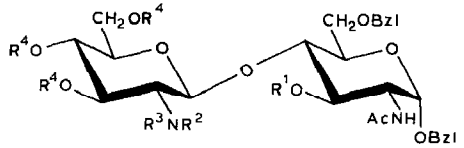
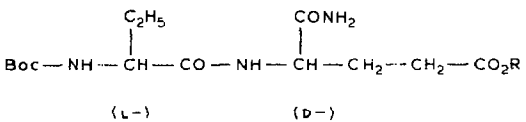


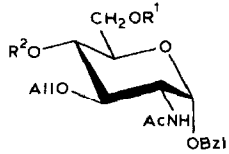
B A

4 R^1 = All, R^2, R^3 = Phth, R^4 = Ac
5 R^1 = All, R^2 = R^3 = R^4 = H
6 R^1 = All, R^2 = H, R^3 = R^4 = Ac
7 R^1 = All, R^2 = R^4 = H, R^3 = Ac
8 R^1 = All, R^2 = H, R^3 = Ac, R^4 = Bzl
9 R^1 = All, R^2 = R^4 = Bzl, R^3 = Ac
10 R^1 = R^2 = H, R^3 = Ac, R^4 = Bzl
11 R^1 = CH_2CO_2H, R^2 = H, R^3 = Ac, R^4 = Bzl
12 R^1 = $CH_2CO_2CH_3$, R^2 = H, R^3 = Ac, R^4 = Bzl
13 R^1 = CH_2CO-L-Abu-D-isoGln(OBzl), R^2 = H, R^3 = Ac, R^4 = Bzl

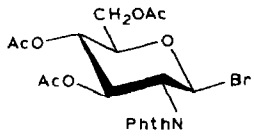


(L-) (D-)

15 R = H
16 R = Bzl



1 R^1 = Bzl, R^2 = H
2 R^1, R^2 = CHPh



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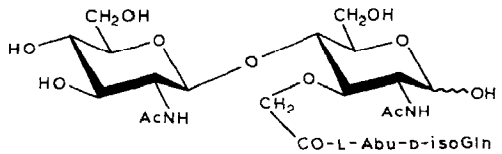

14

Ac = acetyl, All = allyl, Boc = *tert*-butoxycarbonyl,
Bzl = benzyl, Ph = phenyl, Phth = phthaloyl,
Abu = α-aminobutanoyl, isoGln(OBzl) = isoglutamine, benzyl ester

RESULTS AND DISCUSSION

As starting material for the synthesis of **14**, we employed benzyl 2-acetamido-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**1**), which was prepared by reduction of the readily accessible benzyl 2-acetamido-3-*O*-allyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[10] (**2**) with sodium cyanoborohydride[11]. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide[12] (**3**), used as a glycosyl donor, was prepared by an improved procedure. For the glycosylation of compound **1**, a modification of the silver triflate method according to Garegg and Norberg[13] was used, as it had earlier been successfully applied for the glycosylation of hexopyranosyl acceptors having a poorly reactive OH-4 group[14,15]. On reaction of compounds **1** and **3** in the molar ratio of 1:2, using silver triflate in dichloromethane at −45° in the absence of base, the desired benzyl 2-acetamido-3-*O*-allyl-6-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-glucopyranoside (**4**) was obtained in 94% yield (referred to compound **1**).

The alkali-labile groups of compound **4** were removed by means of butylamine, and the benzyl 2-acetamido-3-*O*-allyl-4-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**5**) formed was converted into the highly crystalline derivative **6** with acetic anhydride in pyridine. When *O*-deacetylated with sodium methoxide in methanol, it gave benzyl 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**7**).

O-Benzylation[16] of compound **7** with benzyl bromide and barium hydroxide in *N,N*-dimethylformamide took place very slowly, and after 20 h, the reaction mixture contained partially benzylated derivatives, and the desired benzyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**8**) was isolated in only 30% yield. Using ultrasonication, we succeeded in achieving complete conversion of compound **7** into **8** after 10 h, and compound **8** was obtained, after chromatography, in 72% yield. The rate of *O*-benzylation of **7** was increased by using sodium hydride as the base. In this case, however, there was formed, to a considerable extent, a product that had a higher chromatographic mobility, and to which we ascribe the tentative structure **9** on the basis of its mass spectrum. Benzylation of an acetamido group when using sodium hydride had been observed by Japanese authors[17].

Deallylation of **8** was achieved by catalytic isomerization of the allyl group with tris(triphenylphosphine)rhodium(I) chloride and subsequent acid hydrolysis[18]. Hydroxy derivative **10** was alkylated[19] with sodium chloroacetate in 1,4-dioxane in the presence of sodium hydride, and the benzyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-3-*O*-(carboxymethyl)-2-deoxy-α-D-glucopyranoside (**11**) formed was converted with diazomethane into the crystalline methyl ester **12** for characterization. The coupling of **11** with the trifluoracetate salt of the benzyl ester of L-α-aminobutanoyl-D-isoglutamine was

TABLE I

^{1}H-N.M.R. DATA FOR DISACCHARIDE **4**, DETERMINED FROM 2D HOMOCORRELATED SPECTRUM IN $CDCl_3$, USING TETRAMETHYLSILANE AS INTERNAL STANDARD

Sugar ring	*Chemical shifts*[a] *(in p.p.m.)*							
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*	*NH*
A	4.84	4.19	3.54	4.06	3.56	3.99	4.27	5.51
B	5.52	4.26	5.73	5.14	3.55	3.41[b]	3.41[b]	—
	Coupling constants[a] *(in Hz)*							
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$	$J_{2,NH}$
A	3.6	10.5	8.0	10.0	—[c]	3.9	−12.3	9.5
B	8.5	10.7	9.1	10.2	—[c]	—[c]	—[c]	—

[a]Chemical shifts and coupling constants of the protecting groups determined from 1D ^{1}H-n.m.r. spectrum: 1.83, 2.01, 2.05, and 1.93 (4 s, 12 H, 4 Ac), 4.36 and 4.58 (2 d, J_{gem} = −11.9 Hz, 4 H, 2 $PhCH_2$–), 5.04–5.37 (m, 2 H, $=CH_2$), 5.65–6.03 (m, 1 H, –CH=), 7.17–7.39 (m, 10 H, 2 Ph), 7.67–7.89 (m, 4 H, phthalimido group). [b]Strongly coupled system, only approximate values are given. [c]The parameter values were not determined.

conducted in 1,4-dioxane in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole[20], and the *N*-{2-*O*-[benzyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-2,3-dideoxy-α-D-glucopyranosid-3-yl]glycoloyl}-L-α-aminobutanoyl-D-isoglutamine benzyl ester (**13**) obtained was hydrogenolyzed, to give the final, deprotected product **14**. The starting Boc-L-α-aminobutanoyl-D-isoglutamine benzyl ester (**16**) was prepared by using a procedure[21] described for analogous dipeptides derived from D-isoglutamine benzyl ester.

For the structure determination of the key disaccharide **4**, the 1 D and 2 D homocorrelated ^{1}H-n.m.r. spectrum was employed (see Table I). In the 1 D spectrum, the doublet of the NH-group proton (δ 5.51, J 9.5 Hz) can be identified unambiguously. This fact permitted us to assign, by means of the 2 D homocorrelated spectrum, the signals of all protons on the disaccharide skeleton. The doublet of the anomeric H-1′ (δ 5.52, $J_{1',2'}$ 8.5 Hz) indicated the presence of a β-(1→4)-disaccharide bond. The doublet of the second anomeric proton (H-1; δ 4.84, $J_{1,2}$ 3.5 Hz) gave evidence for the α configuration of the benzyl glycoside moiety. The fact that the chemical shift of H-3′ is higher than that of H-1′ is a consequence of the long-range shielding-effect of the phthalimido group bound to C-2′. The H-6′a and H-6′b atoms constitute a strongly coupled system, whereas H-6a and H-6b are chemically non-equivalent in consequence of the anisotropy of the shielding effect of the benzyl group.

The immunoadjuvant activity of compound **14** was estimated in guinea pigs by the delayed-type hypersensitivity assay using ovalbumin as an antigen. When applied with Freund's incomplete adjuvant, compound **14** produced an immunoadjuvant effect 3.4 times that of MDP. The undesirable pyrogenic effect of **14** is, unlike that of MDP, very low[22].

General methods. — Melting points were determined on a Kofler block; the melting points are not corrected. Optical rotations were measured with a Perkin–Elmer 141 spectrometer, and c.d. spectra with a Roussel–Jouan Dichrographe CD 185. Preparative column chromatography was performed with silica gel (30–60 μm) or C18 silica gel (10–15 μm). High performance liquid chromatography was conducted in a Spectra-Physics SP-8700 instrument with an SP-8400 detector, using a column (25 × 0.4 cm) of C18 silica gel and methanol–water as the eluant. ^{1}H-N.m.r. spectra were recorded with a Varian XL-200 (200 MHz) instrument, with tetramethylsilane as the internal standard. Amino-acid analyses were obtained with a Durrum amino-acid analyzer following hydrolysis for 10 h at 110° with 4M HCl.

Dichloromethane was distilled from phosphorus pentaoxide, and kept over molecular sieves 4A (Merck). Silver trifluoromethanesulfonate was recrystallized from toluene, and stored in the dark over phopshorus pentaoxide.

*Benzyl 2-acetamido-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (**1**). — To a stirred suspension of **2** (14.91 g; 33.9 mmol) and sodium cyanoborohydride (13.3 g, 229 mmol) in dry tetrahydrofuran (500 mL), was added dropwise an ethereal 2.5M solution of hydrogen chloride (about 120 mL) at room temperature until the mixture was acidic, as shown by moistened pH-paper. The mixture was stirred for 5 h at room temperature, poured onto ice–water (~1 kg), and extracted with chloroform (4 × 200 mL). The extracts were combined, washed with saturated sodium hydrogencarbonate solution, dried (Na_2SO_4), and evaporated *in vacuo*. A solution of the residue in 80% acetic acid (100 mL) was heated for 1 h at 100°, cooled, and evaporated, co-distilled with 1:1 methanol–benzene, and the residue chromatographed on a column of silica gel with 1:9 (v/v) acetone–chloroform. Crystallization from 2-propanol–light petroleum gave 11.4 g (76%) of compound **1**; m.p. 151–152° (from ethyl acetate), $[\alpha]_D^{25}$ +102.8° (*c* 0.21 chloroform); lit.[10] m.p. 149–150°, $[\alpha]_D$ +103° (*c* 1, chloroform).

*3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl bromide* (**3**). — A gentle stream of hydrogen bromide was introduced during 1 h at 0° into a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose[23] (10.0 g) in dichloromethane (50 mL), the mixture was kept for 2 h at 15° and evaporated *in vacuo*, and a solution of the resulting syrup in dry diethyl ether (50 mL) kept for 12 h at −10°. Compound **3** crystallized; it was filtered off on a fritted filter, and dried over potassium hydroxide; yield 9.31 g (89%); m.p. 135–136° (dichloromethane–ether), $[\alpha]_D^{25}$ +50.28° (*c* 0.25, chloroform); lit.[12] m.p. 122–123°, $[\alpha]_D^{24}$ +57.3° (*c* 1, chloroform).

Anal. Calc. for $C_{20}H_{20}BrNO_9$ (498.3): C, 48.42; H, 4.07; Br, 16.30; N, 2.57. Found: C, 48.21; H, 4.04; Br, 16.04; N, 2.81.

Benzyl 2-acetamido-3-O-allyl-6-O-benzyl-2-deoxy-4-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-glucopyranoside (**4**). — A mixture of **1** (6.0 g, 13.59 mmol) and silver trifluoromethanesulfonate (8.16 g, 31.8 mmol) was dried, under intensive magnetic stirring in an apparatus provided with a septum, for 4 h at room temperature and 1.32 Pa. The apparatus was purged twice with argon and dichloromethane (45 mL) was added through the septum; then a solution of bromide **3** (13.5 g, 27.09 mmol) in dichloromethane (45 mL) was added to the stirred solution during 2 h at −45°. The mixture was stirred for another 50 min at −45° and 30 min at −20°, pyridine (2 mL) was added and the reaction vessel was withdrawn from the cooling bath; when the mixture reached ambient temperature, it was diluted with chloroform (750 mL), and the suspension was filtered. The filtrate was washed successively at +5° with 0.5M HCl (3 × 150 mL) and saturated sodium hydrogencarbonate solution (2 × 150 mL), dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel with 2:1 (v/v) ethyl acetate–toluene; yield of **4**, 11.0 g (94.2%, referred to **1**), in the form of a solid foam; it was chromatographically homogeneous in h.p.l.c. using 3:1 (v/v) methanol–water; $[\alpha]_D^{25}$ +49.0° (*c* 0.21, chloroform); lit.[5] $[\alpha]_D^{25}$ +50° (*c* 1.2, chloroform); for ^{1}H-n.m.r. spectrum, see Table I.

Anal. Calc. for $C_{45}H_{50}N_2O_{15}$ (858.9): C, 62.93; H, 5.87; N, 3.26. Found: C, 62.72; H, 5.96; N, 3.09.

Benzyl 2-acetamido-3-O-allyl-4-O-(2-amino-2-deoxy-β-D-glucopyranosyl)-6-O-benzyl-2-deoxy-α-D-glucopyranoside (**5**). — A solution of **4** (3.6 g, 4.2 mmol) in 4:1 (v/v) methanol–butylamine (70 mL) was heated in a sealed ampoule for 12 h at 85°. The mixture was cooled and evaporated, and the solid residue was triturated with ether (3 × 100 mL), dissolved in methanol (50 mL), adjusted to pH 4 by addition of formic acid, and placed on a column of Dowex 50 (H^+) ion-exchange resin (500 mL). After washing with 9:1 methanol–water (500 mL), compound **5** was eluted with 1:7 (v/v) 25% ammonia–methanol. Evaporation of the eluate (500 mL) yielded 1.97 g (78%) of **5** as an amorphous powder. Crystallization of the crude product from methanol–diethyl ether afforded the hemihydrate of **5**; m.p. 200°, $[\alpha]_D^{25}$ +82.1° (*c* 0.2, methanol).

Anal. Calc. for $C_{31}H_{42}N_2O_{10}\cdot 0.5\ H_2O$ (611.7): C, 60.87; H, 7.09; N, 4.58. Found: C, 60.70; H, 6.87; N, 4.59.

Benzyl 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-allyl-6-O-benzyl-2-deoxy-α-D-glucopyranoside (**6**). — A solution of compound **5** (1.95 g, 3.15 mmol) in 2:1 (v/v) pyridine–acetic anhydride (30 mL) was kept for 20 h at room temperature. Ethanol (10 mL) was added, and after 20 min, the mixture was evaporated, and traces of solvent co-evaporated with toluene (3 × 20 mL). The residue crystallized from ethanol; yield 2.04 g (82%) of **6**; m.p. 247° (ethanol), $[\alpha]_D^{25}$ +40.6° (*c* 0.2, chloroform).

Anal. Calc. for $C_{39}H_{50}N_2O_{14}$ (770.8); C, 60.77; H, 6.54; N, 3.63. Found: C, 60.66; H, 6.46; N, 3.68.

Benzyl 2-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-3-O-

allyl-6-O-benzyl-2-deoxy-α-D-glucopyranoside (**7**). — A suspension of **6** (1.96 g, 2.54 mmol) in 0.01M sodium methoxide in methanol (100 mL) was stirred at room temperature until the solid dissolved (~1 h), the solution kept for 12 h at 5°, the base neutralized with Dowex 50 (H^+) ion-exchange resin, and the resin filtered off, and washed with methanol. The filtrate and washings were combined and evaporated to give 1.52 g of **7** (92%) as a syrup which was chromatographically homogeneous (h.p.l.c. with 7:3 (v/v) methanol–water); $[\alpha]_D^{25}$ +73.1° (*c* 0.20, methanol); lit.[5] m.p. 268–270° (dec.), $[\alpha]_D^{25}$ +73° (*c* 1.2, methanol).

Anal. Calc. for $C_{33}H_{44}N_2O_{11}$ (644.7): C, 61.48; H, 6.88; N, 4.35. Found: C, 61.06; H, 6.96; N, 4.39.

Benzyl 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-3-O-allyl-6-O-benzyl-2-deoxy-α-D-glucopyranoside (**8**). — *Procedure A.* A solution of **7** (1.17 g; 1.82 mmol) in *N,N*-dimethylformamide (12 mL) and benzyl bromide (2.16 g; 12.6 mmol) were added successively under argon through a septum to powdered barium hydroxide [prepared by equilibration of barium oxide (6.13 g, 40 mmol) and barium hydroxide octahydrate (1.6 g, 5 mmol)] and the mixture was sonicated in a Branson Ultrasonic Cleaner (Model B-12E, 50 W) for 10 h at room temperature. The mixture was diluted with chloroform (200 mL), successively washed with 10% acetic acid (2 × 30 mL) and water, dried (Na_2SO_4), and evaporated. The residue was purified on a column of silica gel with 2:1 (v/v) chloroform–ethyl acetate; yield, 1.18 g (71%) of **8** (solid); m.p. 205–209° (dec.), $[\alpha]_D^{25}$ +53.3° (*c* 0.20, chloroform); lit.[5] m.p. 214–218° (dec.), $[\alpha]_D^{25}$ +58° (*c* 1.1, chloroform); *m/z*: 914 (M), 823 (M − C_7H_7), 807 (M − C_7H_7O), 474 ($C_{29}H_{32}NO_5$), and 424 ($C_{25}H_{30}NO_5$).

Anal. Calc. for $C_{54}H_{62}N_2O_{11}$ (915.1): C, 70.87; H, 6.83; N, 3.06. Found: C, 70.35; H, 6.79; N, 2.93.

Procedure B. *N,N*-Dimethylformamide (1 mL) and benzyl bromide (0.12 mL, 1 mmol) under argon were added through a septum closure to a mixture of **7** (100 mg, 155 μmol) and sodium hydride (24 mg, 1 mmol), and the mixture was stirred with a magnetic stirrer for 20 h at room temperature. After work-up as described in procedure *A*, and preparative h.p.l.c. on silica gel with 2:1 (v/v) chloroform–ethyl acetate, a solid residue (49 mg, 29%) with the proposed structure **9** was obtained; *m/z* 1004 (M), 564 ($C_{36}H_{38}NO_5$), 563 ($C_{36}H_{37}NO_5$), 473 (564 − C_7H_7), 472 (563 − C_7H_7), 424 ($C_{25}H_{30}NO_5$), and 423 ($C_{25}H_{29}NO_5$). From a fraction having a lower chromatographic mobility was isolated **8** (58 mg); the mass spectrum and chromatographic mobility were identical with those of **8** prepared by procedure *A*.

Benzyl 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-6-O-benzyl-2-deoxy-α-D-glucopyranoside (**10**). — Compound **8** (1.2 g, 1.32 mmol) and tris(triphenylphosphine)rhodium(I) chloride (0.14 g, 0.15 mmol) were refluxed for 3 h in 7:3:2 (v/v) ethanol–toluene–water (40 mL). After addition of formic acid (1 mL), the mixture was refluxed for 1 h, cooled, and evaporated, and the residue chromatographed on silica gel with 50:1 (v/v) chloroform–

methanol; yield, 1.05 g (91%) of **10**; m.p. 228–229° (methanol). $[\alpha]_D^{25}$ +73.2° (*c* 0.78, chloroform); lit.[5] m.p. 220.5–221.5°, $[\alpha]_D^{25}$ +74° (*c* 1.5, chloroform).

Anal. Calc. for $C_{51}H_{58}N_2O_{11}$ (875.0): C, 70.00; H, 6.68; N, 3.20. Found: C, 70.23; H, 6.48; N, 2.93.

Benzyl 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-6-O-benzyl-3-(carboxymethyl)-2-deoxy-α-D-glucopyranoside (**11**). — A mixture of **10** (700 mg, 0.8 mmol) and sodium hydride (96 mg, 4 mmol) in 1,4-dioxane (7.0 mL) was heated for 1 h at 96° under magnetic stirring, and cooled to 65°, monochloroacetic acid (100 mg, 1.06 mmol) was added, and the mixture was stirred for 3.5 h at this temperature. After being kept for 12 h at room temperature, water (1 mL) was added, and the solution was evaporated. The residue was dissolved in 5% acetic acid (60 mL), the solution extracted with chloroform (3 × 80 mL), and the extracts were combined, dried (Na_2SO_4), and evaporated, and the residue chromatographed on silica gel C18 with 9:1 (v/v) methanol–1% formic acid; yield, 0.556 g (74.5%) of **11** (syrup).

Anal. Calc. for $C_{53}H_{60}N_2O_{13}$ (933.0): C, 68.22; H, 6.48; N, 3.00. Found: C, 68.03; H, 6.51; N, 2.68.

Benzyl 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-6-O-benzyl-2-deoxy-3-O-[(methoxycarbonyl)methyl]-α-D-glucopyranoside (**12**). — A 0.4M solution of diazomethane in ether was added at room temperature, and under stirring, to a solution of **11** (150 mg, 0.15 mmol) in 1:1 (v/v) chloroform–methanol (10 mL) until the yellow color persisted. The mixture was decolorized by addition of a few drops of acetic acid, and evaporated, and the residue was chromatographed on silica gel C18 with 3:2 methanol–water; yield, 143 mg (94%) of crude **12**. Crystallization from methanol gave pure **12** (95 mg, 62.7%); m.p. 223° (methanol); $[\alpha]_D^{25}$ +67.3° (*c* 0.21, chloroform); *m/z* 474 ($C_{29}H_{32}NO_5$) and 456 ($C_{25}H_{30}NO_7$).

Anal. Calc. for $C_{54}H_{62}N_2O_{13}$ (974.1): C, 68.48; H, 6.60; N, 2.96. Found: C, 68.47; H, 6.54; N, 2.95.

N-{2-O-[*Benzyl 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-6-O-benzyl-2,3-dideoxy-α-D-glucopyranosid-3-yl*]-*glycoloyl*}-L-*α-aminobutanoyl-D-isoglutamine benzyl ester* (**13**). — A solution of the benzyl ester of (*tert*-butoxycarbonyl)-L-α-aminobutanoyl-D-isoglutamine (210 mg, 0.5 mmol) in 17:3 (v/v) dichloromethane–trifluoroacetic acid (10 mL) was kept for 45 min at room temperature. After evaporation, the syrupy residue was washed with ether (2 × 30 mL), and the insoluble part was dried for 1 h at room temperature/132 Pa. The syrup obtained was dissolved in 1,4-dioxane (8 mL) and the resulting solution of the trifluoroacetate of the benzyl ester of L-α-aminobutanoyl-D-isoglutamine was used immediately for coupling with the acid **11**.

1-Hydroxybenzotriazole (54 mg, 0.4 mmol) and the solution of the trifluoroacetate salt were added to a solution of **11** (0.33 g, 0.36 mmol) in dichloromethane (3 mL), and then M triethylamine in dichloromethane (0.7 mL) and M dicyclohexylcarbodiimide in dichloromethane (0.36 mL) were added under

stirring and cooling at 0°. The mixture was stirred for 2 h at 0°, kept for 10 h at room temperature, filtered, and the filtrate evaporated; the residue was chromatographed on silica gel with 25:1 (v/v) chloroform–methanol; yield, 0.405 g (91%) of **13** (syrup).

Anal. Calc. for $C_{69}H_{81}N_5O_{16}$ (1236.4): C, 67.02; H, 6.60; N, 5.56. Found: C, 66.67; H, 5.58; N, 5.62.

O-*(2-Acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→4)*-N-*acetylnormuramoyl-*L-*α-aminobutanoyl-*D-*isoglutamine* (**14**). — Compound **13** (2.30 g, 1.86 mmol) was hydrogenolyzed in acetic acid (200 mL) in the presence of 5% palladium–charcoal catalyst (5.0 g) for 12 h at room temperature. (The catalyst had been prepared by reduction with hydrogen of an aqueous solution of palladium(II) chloride in the presence of charcoal, and washed until neutral). At the end of the hydrogenolysis, the apparatus was evacuated, and purged with nitrogen. The catalyst was filtered off, successively washed with 50% acetic acid (200 mL) and water (200 mL), and the filtrate and washings were combined, evaporated *in vacuo* at 40°, and the residue was chromatographed on silica gel C18 with 3:97 methanol–water. After lyophilization of the chromatographically homogeneous fraction, 0.921 g (69.4%) of **14** was obtained; c.d. spectrum (water; deg.cm^2.dmol^{-1}): θ_{194} +29710, θ_{204} 0, and θ_{213} −3510; amino acid analysis: glutamic acid, 1.00; 2-aminobutanoic acid, 1.03; normuramic acid, 0.98; glucosamine, 1.02.

Anal. Calc. for $C_{27}H_{45}N_5O_{16}\cdot H_2O$ (713.7): C, 45.44; H, 6.64; N, 9.81. Found: C, 45.12; H, 6.27; N, 9.54.

N-*(*tert*-Butoxycarbonyl)-*L-*α-aminobutanoyl-*D-*isoglutamine* (**15**). — *p*-Nitrophenol (6.2 g, 44.5 mmol) and a cooled solution of dicyclohexylcarbodiimide (9.5 g, 44.5 mmol) in ethyl acetate (60 mL) were added to a stirred solution of *N-(tert*-butoxycarbonyl)-L-α-aminobutanoic acid (9.0 g, 44.5 mmol) in ethyl acetate (60 mL), and the mixture was kept for 1 h at 0°, and the separated 1,3-dicyclohexylurea filtered off, and washed with ethyl acetate. The filtrate and washings were combined, and evaporated, and the residue was dissolved in 1,4-dioxane (45 mL). D-Isoglutamine hydrobromide (10.1 g, 44.5 mmol) in water (30 mL) and 2M NaOH (44.5 mL) were added to the mixture, under stirring, and cooling with ice. After stirring for 20 h at room temperature, water (100 mL) was added, the *p*-nitrophenol was extracted with ether (3 × 60 mL), and the pH of the aqueous phase was brought to 3 with hydrochloric acid. The aqueous phase was extracted with ethyl acetate (6 × 300 mL). The extracts were combined, washed with water (3 × 40 mL), dried (Na_2SO_4), and concentrated. Addition of light petroleum to the concentrate precipitated **15**; yield, 8.4 g (57%); m.p. 178–182°. Crystallization from ethyl acetate–ethanol–light petroleum gave 6.8 g (46%) of pure **15**; m.p. 182–183°, $[\alpha]_D^{25}$ −6.1° (*c* 0.3, methanol); amino acid analysis: glutamic acid, 1.00; 2-aminobutanoic acid, 0.955.

Anal. Calc. for $C_{14}H_{25}N_3O_6$ (331.4): C, 50.75; H, 7.60; N, 12.68. Found: C, 50.72; H, 7.59; N, 12.46.

N-*(tert-Butoxycarbonyl)*-L-*α-aminobutanoyl*-D-*isoglutamine, benzyl ester* (**16**). — Benzyl bromide (2.76 mL, 23 mmol) was added to a solution of **15** (6.9 g, 20.8 mmol) and dicyclohexylamine (4.2 mL, 20.8 mmol) in *N,N*-dimethylformamide (53 mL), and the mixture was stirred for 2 h at 70°. After cooling, the dicyclohexylamine hydrobromide formed was filtered off, and washed with *N,N*-dimethylformamide (2 × 15 mL), and the filtrate and washings were combined, and evaporated. The syrupy residue was dissolved in ethyl acetate (250 mL), and the solution was dried (Na_2SO_4), and evaporated. Trituration of the residual syrup with light petroleum gave 6.95 g (79%) of **16**; m.p. 102–106°. After crystallization from ethyl acetate–light petroleum, compound **16** had m.p. 104–105°. $[\alpha]_D^{25}$ −5.5° (*c* 0.5, methanol).

Anal. Calc. for $C_{21}H_{31}N_3O_6$ (421.5): C, 59.84; H, 7.41; N, 9.97. Found: C, 60.04; H, 7.44; N, 10.08.

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