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

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Received January 20, 1989
Accepted March 7, 1989

Silver triflate-promoted condensation of 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (*VIII*) with benzyl 2-acetamido-6-O-benzoyl-2-deoxy-3-O-(methoxycarbonyl)-methyl-α-D-glucopyranoside (*IV*) afforded benzyl 2-acetamido-4-O-(3,4,6-tri-O-acetyl-2-deoxy-2--phthalimido-β-D-glucopyranosyl)-6-O-benzoyl-2-deoxy-3-O-(methoxycarbonyl)methyl-α-D-glucopyranoside (*IX*) which, after deprotection, was converted into the acid *XI*. Condensation of acid *XI* with L-α-aminobutanoyl-D-isoglutamine benzyl ester and subsequent hydrogenolysis of the product *XIII* furnished compound *XIV*. Benzyl 2-acetamido-6-O-benzoyl-2-deoxy-3-O--(methoxycarbonyl)methyl-α-D-glucopyranoside (*IV*) was prepared by partial benzoylation of benzyl 2-acetamido-2-deoxy-3-O-(methoxycarbonyl)methyl-α-D-glucopyranoside (*III*) with benzoyl cyanide.

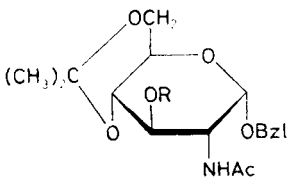
Some time ago we described[2] the preparation of O-(2-acetamido-2-deoxy-β-D--glucopyranosyl)-(1→4)-N-acetylnormuramoyl-L-α-aminobutanoyl-D-isoglutamine (*XIV*) using the synthetic method suggested by Durette and coworkers[3] for the preparation of repeating disaccharide-dipeptide unit of the bacterial wall peptidoglycan (β-D-GlcNAc-(1→4)-MurNAc-L-Ala-D-isoGln). The compound *XIV* exhibits a markedly higher immunoadjuvant activity than MDP (MurNAc-L-Ala-D-isoGln) with simultaneous suppression of undesired side-effects such as pyrogenicity and thrombocytolysis[4-8]. The immunomodulatory activity of compound *XIV* was determined by the delayed hypersensitivity assay with ovalbumin as antigen, by the comitogennic test with phytohemaglutinin-stimulated thymocytes and by the experimental allergic encephalitis induction test. In the construction of the sugar unit, the mentioned synthesis of compound *XIV* requires the introduction of a glycoloyl ether residue into the position 3 of a suitably protected derivative of chitobiose (β-D-GlcNAc-(1→4)-D-GlcNAc). Syntheses of the repeated disaccharide unit of

* For a preliminary communication see ref.[1]

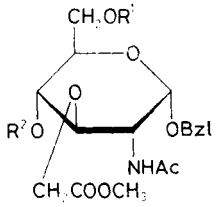
** Normuramic acid is the trivial name for 2-amino-3-O-carboxymethyl-2-deoxy-D-glucopyranose. The symbols and abbreviations obey the published recommendations (Biochemical Nomenclature and Related Documents. International Union of Biochemistry, London 1978).

peptidoglycan, starting from suitably protected muramic acid derivative as glycosyl acceptor, have been described only recently. The oxazoline-promoted β-(1→4) coupling of GlcNAc and MurNAc residues requires an ester bond between the COOH group of the MurNAc moiety and the C(6)—OH group of the GlcNAc residue and affords the disaccharide in a yield of only 20% (ref.[9]). An effective glycosidation of the little reactive C(4)—OH group of N-acetylmuramic acid has been described by Kantoci and collaborators[10]: silver triflate-catalyzed reaction of 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride with benzyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-[(*R*)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside afforded the disaccharide in 66% yield. 1,6-Di-O-benzyl derivative of methyl N-acetylmuramate was prepared by reductive opening of the acetal ring in benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-[(*R*)-1-(methoxycarbonyl)ethyl]-α-D--glucopyranoside with sodium cyanoborohydride[11].

To simplify the preparation of compound *XIV*, we elaborated a procedure which starts from the well accessible benzyl 2-acetamido-6-O-benzoyl-2-deoxy-3-O--(methoxycarbonyl)methyl-α-D-glucopyranoside (*IV*) as the glycosyl acceptor.



I, R = H
II, R = CH_2COOCH_3

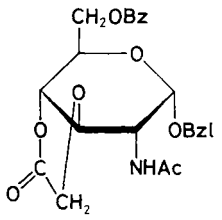


III, $R^1 = R^2 = H$
IV, R^1 = Bz ; R^2 = H
V, $R^1 = R^2$ = Bz
VI, R^1 = Bz ; R^2 = Ac

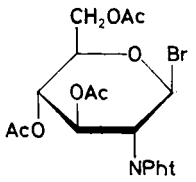
The starting 6-O-benzoyl derivative *IV* was prepared by partial benzoylation[12] of methyl N-acetyl-1-α-O-benzylnormuramate (*III*) with benzoyl cyanide in dichloromethane at 0—5°C. The subsequent benzoylation in the position 4, leading to the 4,6-di-O-benzoate *V*, took place at elevated temperature and with use of at least two equivalents of benzoyl cyanide. On heating to 185°C, the monobenzoate *IV* was converted into the 6-membered 1,4-lactone *VII* which can be methanolyzed in the presence of triethylamine to give the starting compound *IV*. Lactonization of compound *IV* proves that the benzoyl group is present in position 6. For characterization, the compound *IV* was converted into its 4-O-acetyl derivative *VI* by reaction with acetic anhydride in pyridine. The structure of compounds *IV*—*VII* has been proven by ^{1}H NMR spectra.

Methyl N-acetyl-1-α-O-benzylnormuramate (*III*) was prepared from sodium salt of benzyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranoside (*I*) (gene-

rated by sodium hydride) by O-alkylation with sodium chloroacetate in dioxane[13], subsequent esterification of the formed acid with diazomethane and hydrolytic removal of the isopropylidene group from the ester *II* by treatment with Dowex 50 (H^+-form) in methanol. The isopropylidene derivative *I* (ref.[14]) was prepared by reaction of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[15] with acetone and ethyl orthoformate in the presence of trifluoromethanesulfonic acid.



VII

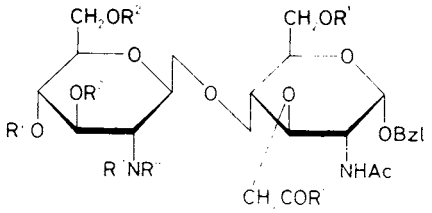


VIII

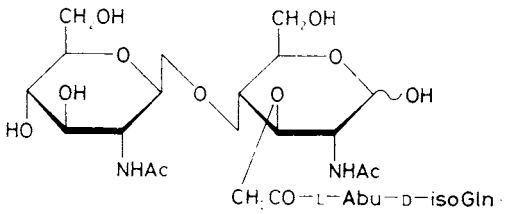
The disaccharide *IX* was prepared by our modified[2] triflate procedure in the absence of base which inhibits glycosidation of the not very reactive hydroxyl groups[16–18]. By reaction of compounds *IV* and *VIII* in the presence of silver triflate (in molar ratio 1 : 2 : 2) in dichloromethane at −45°C we obtained benzyl 2-acetamido-4-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-6-O-benzoyl-2-deoxy-3-O-(methoxycarbonyl)methyl-α-D-glucopyranoside (*IX*) in 62% yield. The lower yield of the glycosidation than that stated in ref.[2] is caused by lower reactivity of the hydroxyl in the glycosyl acceptor *IV* due to the neighbouring electronegative benzoyl group[19].

The disaccharide *IX* was O-deacetylated with methanolic sodium methoxide, the ester group was saponified with aqueous sodium hydroxide and the phthaloyl group was removed by heating with butylamine in methanol. The deblocked compound *X* was selectively N-acetylated with acetic anhydride in water to give compound *XI*. The attempted N-acetylation of *X* with acetic anhydride in methanol was accompanied by partial esterification of the carboxyl. The disaccharide acid *XI*, which crystallized as the monohydrate, was characterized by conversion into the well crystallizable tetra-O-acetate methyl ester *XII* by reaction with diazomethane and subsequent acetylation.

We coupled the acid *XI* with L-α-aminobutanoyl-D-isoglutamine benzyl ester in the presence of N,N′-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole and obtained the disaccharide-dipeptide *XIII* in 33% yield. Instead of the sirupy trifluoroacetate[2], the dipeptide benzyl ester was used as its crystalline *p*-toluenesulfonate *XV*. The use of the *p*-toluenesulfonate *XV* increased the yield of the coupling reaction which is unfavourably influenced[20] by the presence of two primary hydroxyl groups in the molecule of acid *XI*. Hydrogenolysis of the benzyl groups in compound *XIII* on a palladium catalyst in 50% acetic acid afforded the desired compound *XIV*.



IX, R^1 = Bz ; R^2 = Ac , $R^3 + R^4$ = Pht , R^5 = OCH_3
X, $R^1 = R^2 = R^3 = R^4 = H$, R^5 = OH
XI, $R^1 = R^3 = R^4 = H$; R^2 = Ac ; R^5 = OH
XII, $R^1 = R^2 = R^4$ = Ac ; R^3 = H ; R^5 = OCH_3
XIII, $R^1 = R^2 = R^3 = H$; R^4 = Ac ; R^5 = L–Abu–D–isoGln(OBzl)



XIV

Also the partially blocked disaccharide-dipeptide *XIII* exhibited a higher immunoadjuvant activity than MDP, was apyrogenic and did not induce thrombocytolysis[4–8].

Structure of the key disaccharide *IX* has been proven by 1D and 2D homocorrelated ^{1}H NMR spectra (Table I). In the 1D spectrum we unequivocally assigned the NH-doublet (δ 7·64, J(2, NH) = 9·4 Hz). This allowed assignment of all protons of the disaccharide skeleton by the 2D homocorrelated spectra. Doublet of the anomeric proton H-1′ (δ 5·49, J(1′, 2′) = 8·4 Hz) proves the β-(1→4) disaccharide bond, doublet of the second anomeric proton H-1 (δ 5·25, J(1, 2) = 3·0 Hz) shows the α-configuration of the benzyl group on C-1. The higher chemical shift of the H-3′ proton than that of the H-1′ proton results from the extensive shielding effect of the phthalimido group bonded to the neighbouring carbon atom C-2′. Signals of the individual carbon atoms of the sugar skeleton were assigned using the ^{13}C–^{1}H heterocorrelated spectra.

EXPERIMENTAL

The melting points were determined on a Kofler block and are not corrected. Optical rotations were measured on a Perkin–Elmer 141 polarimeter. The NMR spectra were obtained with a Varian 200 XL instrument in the FT mode (^{1}H spectra at 200 MHz, ^{13}C spectra at 50·3 MHz)

in deuteriochloroform, using tetramethylsilane and deuteriochloroform (δ 77·0) as internal standard for the ^1H and ^{13}C spectrum, respectively. Chemical shifts are given in ppm (δ-scale) and coupling constants (J) in Hz. The IR spectra were recorded on a UR 20 (Carl Zeiss, Jena) spectrometer, wavenumbers are given in cm^{-1}. Thin-layer chromatography was performed on Silufol UV_{254} sheets, column chromatography on silica gel Silpearl (both Kavalier, Votice, Czechoslovakia). High performance liquid chromatography was carried out on 250 × 4 mm and 250 × 17 mm column packed with Separon SGX C18 (5 μm and 10 μm, respectively, Laboratorní přístroje, Prague, Czechoslovakia). Amino acid analyses were obtained with an amino acid analyzer Durrum; the samples were hydrolyzed with 4M-HCl at 110°C for 8 h. Analytical samples were dried at 25°C/6·5 Pa for 8 h.

Dichloromethane was distilled from phosphorus pentoxide and stored over molecular sieves Merck 4A. Silver trifluoromethanesulfonate was recrystallized from toluene.

Benzyl 2-Acetamido-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranoside (*I*)

Trifluoromethanesulfonic acid (500 μl) was added at room temperature to a stirred suspension of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[15] (20 g, 64·2 mmol) in a mixture of ethyl orthoformate (66 ml) and dry acetone (500 ml). After stirring for 2 h, the solution was neutralized with triethylamine (2 ml) and taken down. The residue was dissolved in chloroform (400 ml) and the solution was washed with water (2 × 150 ml), dried over sodium sulfate and stripped of the solvent. Crystallization of the residue from chloroform–diethyl ether–light petroleum afforded 14·7 g (65·2%) of compound *I*, m.p. 138°C. $[\alpha]_D^{25}$ +107° (*c* 0·4, chloroform). Reported[14] m.p. 138°C and $[\alpha]_D^{25}$ +117° (*c* 1·0, chloroform). For $C_{18}H_{25}NO_6$ (351·4) calculated: 61·52% C, 7·17% H, 3·99% N; found: 61·57% C, 7·23% H, 4·00% N.

TABLE I

Proton NMR parameters of disaccharide *IX* determined by 2D homocorrelated spectrum in $CDCl_3$ (tetramethylsilane as internal standard)

Sugar ring	Chemical shifts[a] (ppm)							
	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	NH
A	5·25	3·85	3·76	3·96	3·69	3·84	4·34	7·64
B	5·49	4·29	5·75	5·18	3·82	4·04	4·49	—

	Coupling constants[a] (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	3·0	[b]	8·1	10·0	4·6	1·6	−12·3	9·4
B	8·4	10·5	9·1	10·2	2·3	4·5	−12·1	—

[a] Chemical shifts of the protecting groups, determined from the 1D ^1H NMR spectrum: 1·83, 2·01 and 2·09 3 s, 9 H (3 × OAc); 2·02 s, 3 H (NAc); 3·79 s, 3 H (OCH_3); 7·15–7·97 m, 14 H (2× phenyl, phthalimido group); [b] not determined.

Benzyl 2-Acetamido-2-deoxy-4,6-O-isopropylidene-3-O-
-(methoxycarbonyl)methyl-α-D-glucopyranoside (*II*)

Sodium hydride (9 g, 375 mmol) was added at room temperature to a stirred solution of compound *I* (24 g, 70 mmol) in dioxane (270 ml). After stirring at 90°C for 2 h, the mixture was cooled to room temperature, mixed with chloroacetic acid (9·45 g, 100 mmol), stirred at 65°C for 6 h and cooled to room temperature. Solid carbon dioxide was added, the excess hydride was decomposed with water and the solvent was evaporated. The residue was dissolved in water (450 ml) and the stirred and ice-cooled solution was neutralized with a solution of potassium dihydrogen phosphate, which had been adjusted to pH 3 with sulfuric acid (60 g of KH_2PO_4 and 40 g of H_2SO_4 in 200 ml of water). The separated product was extracted with ethyl acetate (3 × 200 ml), the extract was dried over sodium sulfate and concentrated to about 100 ml. To the stirred and ice-cooled residue was added diazomethane in diethyl ether to persisting yellow colouration. After standing for 30 min at room temperature, the excess diazomethane was decomposed with acetic acid and the mixture was evaporated. Crystallization of the residue from ethyl acetate–ether–light petroleum afforded 21·2 g (72%) of compound *II*, m.p. 114°C; $[\alpha]_D^{25}$ +153°, (*c* 0·2, chloroform). For $C_{21}H_{29}NO_8$ (423·5) calculated: 59·56% C, 6·90% H, 3·31% N; found: 59·33% C, 6·74% H, 3·26% N.

Benzyl 2-Acetamido-2-deoxy-3-O-(methoxycarbonyl)methyl-α-D-glucopyranoside (*III*)

Dowex 50 (H^+-form, 13 g, pre-dried in vacuo over potassium hydroxide) was added to a solution of compound *II* (14·9 g, 35·2 mmol) in methanol (200 ml). The mixture was refluxed for 2 h under stirring. The Dowex was filtered off, washed with hot methanol (2 × 100 ml) and the filtrate was evaporated in vacuo. The residue was co-evaporated with water (2 × 50 ml), dissolved in water (100 ml) and the solution was filtered with charcoal and concentrated to crystallization. After standing at +5°C for 12 h, the product was collected on filter and dried in vacuo over potassium hydroxide to give 9·8 g (73·6%) of compound *III*, m.p. 143—145°C; $[\alpha]_D^{25}$ +124° (*c* 0·2, water). For $C_{18}H_{25}NO_8$ (383·4) calculated: 56·39% C, 6·57% H, 3·65% N; found: 56·20% C, 6·45% H, 3·56% N.

Benzyl 2-Acetamido-6-O-benzoyl-2-deoxy-3-O-(methoxycarbonyl)methyl-α-D-
-glucopyranoside (*IV*)

A stirred suspension of compound *III* (7·62 g, 20 mmol) in dichloromethane (100 ml) was cooled with ice and mixed with triethylamine (2·02 g, 20 mmol). A solution of benzoyl cyanide (2·94 g, 22 mmol) in dichloromethane (20 ml) was gradually added during 45 min. After standing for 12 h at 5°C, the mixture was evaporated and the residue chromatographed on a column of silica gel (400 g) in ethyl acetate–toluene (3 : 1). The product *IV* was obtained as a sirup which crystallized on addition of diethyl ether; yield 6·51 g (67%), m.p. 124—125°C. An analytical sample was recrystallized from dipropyl ether–light petroleum, m.p. 133—134°C; $[\alpha]_D^{25}$ +72° (*c* 0·3, chloroform). IR spectrum (chloroform): 3 440 (NH); 3 365 (OH bonded); 1 741 ($COOCH_3$); 1 721 shoulder (C_6H_5CO); 1 681 (amide I); 1 603 (aromate ring); 1 526 (amide II); 1 379 (CH_3); 1 278 (COOR). ^{1}H NMR spectrum: 2·00 s, 3 H (NAc); 3·75 s, 3 H (OCH_3); 3·90 m, 1 H (H-5, $J(5, 6) = 2·2$; $J(5, 6') = 4·0$; $J(4, 5) = 9·3$); 4·07 ddd, 1 H (H-2, $J(1, 2) = 3·5$; $J(2, 3) = 10·4$; $J(2, NH) = 7·5$); 4·43 dd, 1 H (H-6, $J(5, 6) = 2·2$; $J(6, 6') = -12·2$); 4·73 dd, 1 H, (H-6', $J(5, 6') = 4·0$; $J(6, 6') = -12·2$); 5·19 d, 1 H (H-1, $J(1, 2) = 3·5$); 6·70 d, 1 H (NH, $J(2, NH) = 7·5$); 7·26—8·10 m, 10 H (2× phenyl). For $C_{25}H_{29}NO_9$ (487·5) calculated: 61·59% C, 6·00% H, 2·87% N; found: 61·88% C, 6·06% H, 2·64% N.

Benzyl 2-Acetamido-4,6-di-O-benzoyl-2-deoxy-3-O-
-(methoxycarbonyl)methyl-α-D-glucopyranoside (*V*)

Triethylamine (100 μl, 0·72 mmol) and benzoyl cyanide (576 mg, 4·4 mmol) were added at room temperature to a stirred suspension of compound *III* (763 mg, 2 mmol) in dry dichloromethane (10 ml). After stirring for 2 h at room temperature, the mixture became homogeneous and after 12 h contained about 50% of monobenzoate *IV* (according to TLC in ethyl acetate–toluene (3 : 1). Another portion of benzoyl cyanide (576 mg, 4·4 mmol) was added, the mixture was refluxed for 4 h and processed as described in the preceding experiment. Yield 660 mg (56%) of compound *V*, m.p. 140–141°C. An analytical sample was recrystallized from ethanol, m.p. 141°C; $[\alpha]_D^{25}$ +104° (*c* 0·3, chloroform). ^{1}H NMR spectrum: 2·04 s, 3 H (NAc); 3·69 s, 3 H (OCH_3); 4·03–4·29 m, 5 H (H-2, H-3, H-5, CH_2COOCH_3); 4·32 dd, 1 H (H-6, *J*(5, 6) = 4·8; *J*(6, 6′) = −12·0); 4·47 dd, 1 H (H-6′, *J*(5, 6′) = 3·0; *J*(6, 6′) = −12·0); 4·56 and 4·72 2 × d, 2 H ($C_6H_5CH_2$, *J*(gem) = −11·8); 5·41 d, 1 H (H-1, *J*(1, 2) = 2·9); 5·49 dd, 1 H (H-4, *J*(3, 4) = = 8·9; *J*(4, 5) = 10·1); 7·30–8·05 m, 15 H (3× phenyl). For $C_{32}H_{33}NO_{10}$ (591·6) calculated: 64·97% C, 5·62% H, 2·37% N; found: 65·07% C, 5·43% H, 2·30% N.

Benzyl 2-Acetamido-4-O-acetyl-6-O-benzoyl-2-deoxy-3-
-O-(methoxycarbonyl)methyl-α-D-glucopyranoside (*VI*)

4-Dimethylaminopyridine (2 mg) was added to a stirred solution of compound *IV* (100 mg, 0·2 mmol) in acetic anhydride (2 ml). After standing at room temperature for 12 h, the excess acetic anhydride was destroyed with methanol (2 ml) and the mixture was evaporated. Crystallization of the residue from ethanol afforded 92 mg (87%) of compound *VI*, m.p. 147–148°C; $[\alpha]_D^{25}$ +111° (*c* 0·3, chloroform). ^{1}H NMR spectrum: 2·02 s, 3 H (NAc); 2·11 s, 3 H (OAc); 3·85 s, 3 H (OCH_3); 3·90 dd, 1 H (H-3, *J*(2, 3) = 10·9; *J*(3, 4) = 8·8); 4·02 ddd, 1 H (H-5 *J*(4, 5) = 10·2; *J*(5, 6) = 4·8; *J*(5, 6′) = 2·8); 4·06 ddd, 1 H (H-2, *J*(1, 2) = 3·4; *J*(2, 3) = 10·9; *J*(2, NH) = 5·8); 4·27 dd, 1 H (H-6, *J*(5, 6) = 4·8; *J*(6, 6′) = −12·2); 4·40 dd, 1 H (H-6′, *J*(5, 6′) = 2·8; *J*(6, 6′) = −12·2); 4·53 and 4·77, 2 × d, 2 H ($C_6H_5CH_2$, *J*(gem) = −11·8); 5·20 dd, 1 H (H-4, *J*(3, 4) = 8·8; *J*(4, 5) = 10·2); 5·32 d, 1 H (H-1, *J*(1, 2) = 3·4); 7·24–8·09 m, 10 H (2× phenyl). For $C_{27}H_{31}NO_{10}$ (529·5) calculated: 61·24% C, 5·90% H, 2·65% N; found: 61·42% C, 5·92% H, 2·59% N.

Benzyl 2-Acetamido-6-O-benzoyl-2-deoxy-3-O-carboxymethyl-α-D-glucopyranoside
1,4′-Lactone (*VII*)

Compound *IV* (1·0 g, 2 mmol) was stirred under nitrogen at 185°C for 4 h. During the heating the originally molten substance solidified. The cold mixture was dissolved in chloroform and the solution was filtered and taken down. Crystallization of the residue from chloroform–diethyl ether afforded 790 mg (87%) of compound *VII*, m.p. 212–213°C; $[\alpha]_D^{25}$ +94° (*c* 0·3, chloroform). IR spectrum (chloroform): 3 442 (NH); 1 764 (CO lactone); 1 724 (C_6H_5CO); 1 682 (amide I); 1 603, 1 585 (aromate ring); 1 520 (amide II); 1 277 (COOR). ^{1}H NMR spectrum: 2·00 s, 3 H (NAc); 3·79 dd, 1 H (H-3, *J*(2, 3) = 10·5; *J*(3, 4) = 9·1); 4·12 ddd, 1 H (H-5, *J*(4, 5) = 10·2; *J*(5, 6) = 4·4; *J*(5, 6′) = 2·2); 4·42 ddd, 1 H (H-2, *J*(1, 2) = 3·6; *J*(2, 3) = 10·5; *J*(2, NH) = = 9·4); 4·49 dd, 1 H (H-4, *J*(3, 4) = 9·1; *J*(4, 5) = 10·2); 4·49 dd, 1 H (H-6, *J*(5, 6) = 4·4; *J*(6, 6′) = −12·3); 4·66 dd, 1 H (H-6′, *J*(5, 6′) = 2·2; *J*(6, 6′) = −12·3); 7·27–8·08 m, 10 H (2× phenyl). For $C_{24}H_{25}NO_8$ (455·5) calculated: 63·29% C, 5·53% H, 3·08% N; found: 63·00% C, 5·56% H, 3·07% N.

A solution of lactone *VII* (46 mg, 0·1 mmol) in methanol (2 ml) was mixed with triethylamine (200 μl). After standing for 3 h at room temperature, the solvent was evaporated and the residue chromatographed on a column of silica gel (15 g) in chloroform–ethyl acetate (20 : 1) to give 40 mg (82%) of compound *IV*, which after crystallization from dipropyl ether–light petroleum melted at 133—134°C without depression with an authentic sample of *IV*.

Benzyl 2-Acetamido-4-O-(tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-6-O--benzoyl-2-deoxy-3-O-(methoxycarbonyl)methyl-α-D-glucopyranoside (*IX*)

A mixture of compound *IV* (4·87 g, 10 mmol) and silver trifluoromethanesulfonate (6·0 g, 23·3 mmol) was dried under vigorous stirring at 20°C/1·32 Pa for 4 h in an apparatus equipped with a septum. The apparatus was flushed with argon (2×) and dry dichloromethane (30 ml) was added through the septum. After dissolution, the mixture was cooled to −45°C and a solution of bromide *VIII* (ref.[2]; 9·54 g, 20 mmol) in dry dichloromethane (30 ml) was gradually added through the septum under stirring during 1 h. The mixture was stirred at −45°C for 1 h and at −20°C for another hour. Pyridine (10 ml) was added at −20°C and, after warming to room temperature, the mixture was diluted with chloroform (700 ml) and filtered. The filtrate was washed with 0·5M-HCl (3 × 200 ml) and saturated solution of sodium hydrogencarbonate (200 ml), dried over sodium sulfate and the solvent was evaporated. Chromatography on a column of silica gel (400 g) in ethyl acetate–toluene (2 : 1) afforded 5·65 g (62%) of chromatographically homogeneous fraction. Crystallization from ethanol gave 5·09 g (56%) of the compound *IX*, m.p. 155—156°C; $[\alpha]_D^{25}$ +54° (*c* 0·2, chloroform). For ^{1}H NMR spectrum see Table I; ^{13}C NMR spectrum: 53·87 (C-2); 55·03 (C-2′); 61·35 (C-6′); 62·41 (C-6); 68·46 (C-4′); 68·68 (C-5); 70·40 (C-3′); 71·44 (C-5′); 77·17 (C-3); 78·29 (C-4); 96·11 (C-1); 97·75 (C-1′). For $C_{45}H_{48}N_2O_{18}$ (904·9) calculated: 59·72% C, 5·34% H, 3·09% N; found: 59·81% C, 5·27% H, 3·08% N.

Benzyl 2-Acetamido-4-O-(2-amino-2-deoxy-β-D-glucopyranosyl)--2-deoxy-3-O-carboxymethyl-α-D-glucopyranoside (*X*)

A solution of compound *IX* (3·62 g, 4 mmol) in methanolic 0·01M-CH_3ONa (100 ml) was set aside at 5°C for 12 h, neutralized with acetic acid and the solvent was evaporated. The residue was dissolved in 0·5M-NaOH (80 ml) and heated to 60°C for 3·5 h. After cooling, the mixture was neutralized by addition of Dowex 50 (H^+-form) and the suspension was poured on a column of the same ion-exchanger (150 ml). The material was eluted with water (600 ml), the eluate was taken down and the residue was dried at room temperature and 1·32 Pa for 2 h. The residue was dissolved in a mixture of methanol and butylamine (4 : 1; 60 ml) and the solution was heated in a sealed ampoule to 85°C for 15 h. After cooling, the mixture was evaporated and the residue was extracted with ether (3 × 80 ml). The insoluble residue was dissolved in water (50 ml), the solution was adjusted to pH 4 with formic acid and poured on a column of Dowex 50 (NH_4^+--form; 170 ml). The column was washed with water (600 ml) and the product was eluted with 5% aqueous ammonia (700 ml). Evaporation and crystallization of the residue from water–methanol afforded 1·9 g (87%) of compound *X*. An analytical sample was recrystallized from water; m.p. 180°C (decomp.); $[\alpha]_D^{25}$ +94° (*c* 0·2, water). For $C_{23}H_{34}N_2O_{12}.H_2O$ (548·5) calculated: 50·35% C, 6·61% H, 5·11% N; found: 50·16% C, 6·27% H, 5·40% N.

Benzyl 2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)--2-deoxy-3-O-carboxymethyl-α-D-glucopyranoside (*XI*)

Acetic anhydride (2 ml) was gradually added during 10 min to a stirred suspension of compound *X* (1·1 g, 2 mmol) in water (15 ml) at room temperature. After 30 min, another portion of acetic

anhydride (2 ml) was added and after stirring for 2 h the mixture was evaporated. The residue was dissolved in water and the solution was applied onto a column of Dowex 50 (H^+-form; 30 ml). The column was washed with water (200 ml), the eluate was evaporated, and the sirupy residue was mixed with methanol (20 ml) and set aside at 5°C overnight. The separated crystalline product was collected on filter and dried over potassium hydroxide in vacuo. Yield 730 mg (62%) of compound *XI*. An analytical sample was obtained by crystallization from 90% methanol; m.p. 183°C; $[\alpha]_D^{25}$ +71° (*c* 0·2, water). For $C_{25}H_{36}N_2O_{13}.H_2O$ (590·6) calculated: 50·84% C, 6·49% H, 4·74% N; found: 50·77% C, 6·20% H, 4·63% N.

Benzyl 2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-
-6-O-acetyl-2-deoxy-3-O-(methoxycarbonyl)methyl-α-D-glucopyranoside (*XII*)

Ethereal diazomethane was added at 0°C to a stirred solution of compound *XI* (118 mg, 0·2 mmol) in methanol (12 ml) until the colour remained permanently yellow. After standing for 30 min at 0°C, the excess diazomethane was decomposed with acetic acid. The mixture was evaporated, the residue was dissolved in a mixture of pyridine and acetic anhydride (2 : 1, 6 ml) and the solution was allowed to stand at room temperature for 12 h. The unreacted acetic anhydride was decomposed with methanol, the mixture was evaporated and the residue was co-evaporated with toluene (3 × 20 ml). Crystallization from methanol–water afforded 76 mg (50%) of compound *XII*, m.p. 272°C; $[\alpha]_D^{25}$ +62° (*c* 0·8, chloroform). For $C_{34}H_{46}N_2O_{17}$ (754·7) calculated: 54·11% C, 6·14% H, 3·71% N; found: 54·18% C, 6·12% H, 3·51% N.

N-{2-O-[Benzyl 2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,3-
-dideoxy-α-D-glucopyranosid-3-yl]-glycoloyl}-L-α-aminobutanoyl-D-
-isoglutamine Benzyl Ester (*XIII*)

A mixture of compound *XI* (768 mg, 1·3 mmol), 1-hydroxybenzotriazole monohydrate (176 mg, 1·3 mmol) and L-α-aminobutanoyl-D-isoglutamine benzyl ester *p*-toluenesulfonate (*XV*; 642 mg, 1·3 mmol) was dried for 3 h at room temperature and 1·32 Pa in a flask equipped with a septum and magnetic stirrer. The flask was flushed with argon (2×), N,N-dimethylformamide (4 ml) was added through the septum and the solution was cooled to −30°C. Triethylamine (181 μl, 1·3 mmol), followed by a solution of N,N′-dicyclohexylcarbodiimide (722 mg, 3·5 mmol) in N,N-dimethylformamide (5 ml), was added through the septum. The stirred mixture was warmed to room temperature during 2 h and set aside at this temperature for 2 days. The unreacted N,N′-dicyclohexylcarbodiimide was decomposed with 10% acetic acid (20 ml), the separated N,N′-dicyclohexylurea was filtered off and washed with 10% acetic acid. The filtrate was evaporated, the residue was mixed with acetone (50 ml) and left to stand overnight at +5°C. The product was filtered, dried in vacuo over potassium hydroxide and chromatographed on silica gel C18 in methanol–water (3 : 2). Evaporation of the product fraction gave 387 mg (33%) of powdery compound *XIII*; $[\alpha]_D^{25}$ +50° (*c* 0·4, 50% methanol). Amino acid analysis: glutamic acid 1·00, α-aminobutanoic acid 0·95, normuramic acid 1·07, glucosamine 1·04. For $C_{41}H_{57}.N_5O_{16}.H_2O$ (893·9) calculated: 55·08% C, 6·65% H, 7·84% N; found: 55·39% C, 6·53% H, 7·78% N.

O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-N-
-acetylnormuramoyl-L-α-aminobutanoyl-D-isoglutamine (*XIV*)

Compound *XIII* (268 mg, 0·3 mmol) was hydrogenolyzed in acetic acid (40 ml) over 5% palladium catalyst on charcoal (600 mg) at room temperature for 12 h. (The catalyst was prepared by reduc-

tion of an aqueous solution of $PdCl_2$ with hydrogen in the presence of charcoal and washed with water to neutral reaction.) The catalyst was filtered, washed with 50% acetic acid (50 ml) and then with water (50 ml). The filtrate was evaporated in vacuo at 40°C and the residue was chromatographed on silica gel C18 in methanol–water (3 : 97). The homogeneous fraction was concentrated and the residue was lyophilized from water to give 151 mg (71%) of compound *XIV*. Its mobility (silica gel C18, in methanol–0·1% trifluoroacetic acid (5 : 95)) and CD spectrum were identical with those of an authentic sample[2] of compound *XIV*. For $C_{27}H_{45}N_5O_{16}.H_2O$ (713·7) calculated: 45·44% C, 6·64% H, 9·81% N; found: 45·25% C, 6·40% H, 9·62% N.

L-α-Aminobutanoyl-D-isoglutamine Benzyl Ester *p*-Toluenesulfonate (*XV*)

A solution of N-(tert-butoxycarbonyl)-L-α-aminobutanoyl-D-isoglutamine benzyl ester[2] (843 mg, 2 mmol) in a mixture of dichloromethane–trifluoroacetic acid (5 : 2; 14 ml) was allowed to stand at room temperature for 45 min and then evaporated. The sirupy residue was mixed with dichloromethane (2 ml) and *p*-toluenesulfonic acid monohydrate (380 mg, 2 mmol) was added. The formed precipitate was triturated with ether, filtered, washed with ether and dried over potassium hydroxide in vacuo, affording 960 mg (97%) of compound *XV*; $[\alpha]_D^{25}$ +20° (*c* 0·4, water). For $C_{23}H_{31}N_3O_7S$ (493·6) calculated: 55·97% C, 6·33% H, 8·51% N, 6·50% S; found: 55·67% C, 6·23% H, 8·39% N, 6·61% S.

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Translated by M. Tichý.