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# SYNTHESIS AND REACTIONS OF *O*-ACETYLATED BENZYL α-GLYCOSIDES OF 6-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-*N*-ACETYLMURAMOYL-L-ALANYL-D-ISOGLUTAMINE ESTERS: THE BASE-CATALYSED ISOGLUTAMINE ⇄ GLUTAMINE REARRANGEMENT IN PEPTIDOGLYCAN-RELATED STRUCTURES

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ABSTRACT

Condensation of benzyl 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucopyranoside (**2**) and its 4-acetate (**4**) with L-alanyl-D-isoglutamine benzyl ester *via* the mixed anhydride method yielded *N*-{2-*O*-[benzyl 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,3-dideoxy-α-D-glucopyranosid-3-yl]-(*R*)-lactoyl}-L-alanyl-D-isoglutamine benzyl ester (**5**) and its 4-acetate (**6**), respectively. Condensation by the dicyclohexylcarbodi-imide–*N*-hydroxysuccinimide method converted **2** into benzyl 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside 1′,4-lactone (**7**). In the presence of activating agents, **7** underwent aminolysis with the dipeptide ester to give **5**. Zemplén *O*-deacetylation of **5** and **6** led to transesterification and α → γ transamidation of the isoglutaminyl residue to give *N*-{2-*O*-[benzyl 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,3-dideoxy-α-D-glucopyranosid-3-yl]-(*R*)-lactoyl}-L-alanyl-D-isoglutamine methyl ester (**8**) and -glutamine methyl ester (**9**). Treatment of **6** with MgO–methanol caused deacetylation at the GlcNAc residue to give a mixture of *N*-{2-*O*-[benzyl 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-4-*O*-acetyl-2,3-dideoxy-α-D-glucopyranosid-3-yl]-(*R*)-lactoyl}-L-alanyl-D-isoglutamine methyl ester (**11**) and -glutamine methyl ester (**12**). Benzyl or methyl ester-protection of peptidoglycan-related structures is not compatible with any of the reactions requiring alkaline media. Condensation of **2** with L-alanyl-D-isoglutamine *tert*-butyl ester gave *N*-{2-*O*-[benzyl 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,3-dideoxy-α-D-glucopyranosid-3-yl]-(*R*)-lactoyl}-L-alanyl-D-isoglutamine *tert*-butyl ester (**16**),

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deacetylation of which, under Zemplén conditions, proceeded without side-reactions to afford *N*-{2-*O*-[benzyl 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,3-dideoxy-α-D-glucopyranosid-3-yl]-(*R*)-lactoyl}-L-alanyl-D-isoglutamine *tert*-butyl ester (**17**).

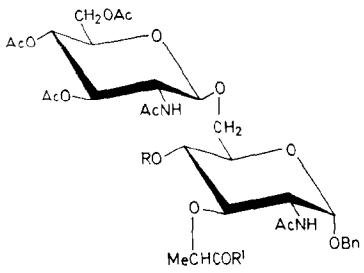
## INTRODUCTION

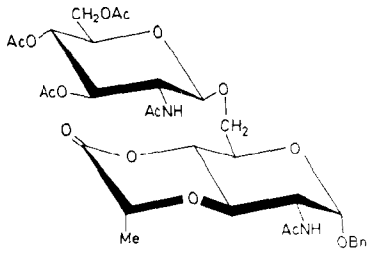
Interest in the biological properties of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine [MurNAc–L-Ala–D-Glu(OH)$NH_2$, MDP] has stimulated the preparation of an impressive number of structurally related compounds[1–3]. We have reported[4] on the synthesis of acetylated benzyl α-glycosides of [β-GlcNAc-(1→6)-MurNAc] and the related β-(1→6)-linked disaccharide lactone and also on the (imidazole + tetraalkylammonium benzoate)-catalysed aminolysis of MurNAc 1′,4-lactones with amino acid and peptide esters to give the corresponding *N*-acetylmuramoylamide derivatives[4,5]. We now report the synthesis of protected [β-GlcNAc-(1→6)-MurNAc]-dipeptides from the above disaccharide derivatives and known[6] L-Ala–D-Glu(OBn)$NH_2$. During *O*-deacetylation of the products in alkaline media, α → γ transamidation occurred at the isoglutaminyl residue and we have studied also the conditions that effect the isoglutamine ⇄ glutamine rearrangement in peptidoglycan structures. We now report also that introduction of the *tert*-butyl ester group at the carboxyl function of the isoglutaminyl residue allows *O*-deacetylation of the sugar moiety without affecting the peptide portion of the molecule.

## RESULTS AND DISCUSSION

An attempt to condense benzyl 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucopyranoside[5] (**2**), obtained by saponification of the methyl ester **1**[4], with L-alanyl-D-isoglutamine benzyl ester *via* the dicyclohexylcarbodi-imide–*N*-hydroxysuccinimide (DCC–HOSu) method failed because most of **2** underwent intramolecular cyclisation, apparently during the activation step, to give the disaccharide lactone **7**[4]. This result suggested that a coupling procedure requiring a shorter time of activation of the MurNAc carboxyl group might be more efficient. Indeed, the mixed anhydride procedure with isobutyl chloroformate as the activating agent afforded 77% of the β-(1→6)-linked disaccharide–dipeptide benzyl ester **5**, with HO-4 unsubstituted, from **2** and L-Ala–D-Glu(OBn)$NH_2$. Under these conditions, the extent of lactonisation of **2** into **7** did not exceed 10–12%. The 4,3′,4′,6′-tetra-*O*-acetyldisaccharide acid derivative **4**[5], obtained by saponification of the methyl ester **3**[4], could be condensed with the dipeptide benzyl ester by the DCC–HOSu and the mixed anhydride methods to give the disaccharide–dipeptide derivative **6** in yields of 58 and 75%, respectively. The synthesis of **6** (65%) by the former method has been reported[7].

As an alternative route to **5**, the reactivity of **7** toward L-alanyl-D-isoglutamine





7

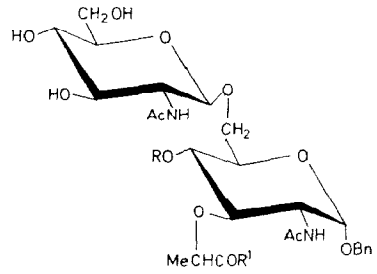
1 R = H, $R^1$ = OMe
2 R = H, $R^1$ = OH
3 R = Ac, $R^1$ = OMe
4 R = Ac, $R^1$ = OH
5 R = H, $R^1$ = L-Ala-D-Glu(OBn)$NH_2$
6 R = Ac, $R^1$ = L-Ala-D-Glu(OBn)$NH_2$

benzyl ester in the presence of imidazole and tetrahexylammonium benzoate[8] was investigated. The reaction proceeded at a rate that was considerably slower than that of the analogous MurNAc 1′,4-lactone to give, after 2 weeks, only 40% of **5**. The inertness of **7** may be ascribed to steric hindrance by the bulky GlcNAc residue at HO-6, and to the tendency of the dipeptide ester to undergo intramolecular cyclisation into the piperazine-2,5-dione derivative. Evidence for the retention of D-*gluco* configuration in the aminolysis product was obtained from the 500-MHz $^1$H-n.m.r. spectrum of its 4-acetate **6** (see Experimental).

Two difficulties became apparent during preliminary deacetylation experiments with **5** and **6**, namely, the susceptibility of the isoglutaminyl residue to transamidation and the resistance of AcO-4 in **6** except under strongly basic conditions. In order to obtain more information on the relative stabilities of the sugar and peptide moieties involved, their behaviour in alkaline media was investigated.

Treatment of either **5** or **6** with catalytic amounts of 0.1M sodium methoxide in methanol (Zemplén conditions) removed all of the *O*-acetyl groups and led to transesterification and transamidation of the isoglutaminyl residue to give, in each reaction, disaccharide–dipeptides **8** and **9** having isoglutamine methyl ester and glutamine methyl ester, respectively, as the *C*-terminal amino acids. T.l.c. (solvent *B*, 5:2:1) revealed **8** ($R_F$ ~0.40) and **9** ($R_F$ ~0.35) in the ratio ~2.5:1 which were isolated after repeated column chromatography. The chemical shifts of the COOMe signal (δ 3.67 and 3.58) in the $^1$H-n.m.r. spectra of **8** in solution in $CD_3OD$ and $(CD_3)_2SO$ are at slightly higher field than those (δ 3.70 and 3.63) of the corresponding signals for **9**. The values observed agree well with those for mono- and di-methyl glutamate derivatives, measured under similar conditions.

The $\alpha \rightleftarrows \gamma$ transpeptidation of glutamyl peptides has been reported[9] and evidence provided[10,11] that the base-catalysed rearrangement involves a cyclic six-membered glutarimide intermediate. The extent of formation of the imide ring




8 R = H, $R^1$ = L-Ala-D-Glu(OMe)$NH_2$
9 R = H, $R^1$ = L-Ala-D-Glu($NH_2$)OMe
10 R = Ac, $R^1$ = L-Ala-D-Glu(OBn)$NH_2$
11 R = Ac, $R^1$ = L-Ala-D-Glu(OMe)$NH_2$
12 R = Ac, $R^1$ = L-Ala-D-Glu($NH_2$)OMe
13 R = H, $R^1$ = OMe
14 R = H, $R^1$ = OH
15 R = Ac, $R^1$ = OMe

depends on the nature of the basic catalyst and is more pronounced in glutamine than in isoglutamine derivatives[9].

When a methanolic solution of *N*-*tert*-butoxycarbonyl-L-alanyl-D-isoglutamine benzyl ester was kept with 0.1 equiv. of 0.1M NaOMe in MeOH for 4 h at room temperature, t.l.c. (solvent *A*, 9:1) showed complete conversion of the starting compound ($R_F$ ~0.6) into two products ($R_F$ ~0.5 and 0.45, ratio ~3:1) which were isolated after repeated column chromatography and characterised as Boc-L-Ala–D-Glu(OMe)$NH_2$ and Boc-L-Ala–D-Glu($NH_2$)OMe. In the $^1$H-n.m.r. spectra of these products, the resonances for the 5- and 1-methyl ester groups were clearly distinguishable and there were small differences[12,13] in values for the isoglutamine and glutamine protons.

Magnesium oxide in methanol is a mild non-selective reagent for the removal of the sugar *O*-acetyl groups[14]. When a methanolic solution of **6** was stirred with an excess of MgO (1 mg/mmol), *O*-deacetylation of the GlcNAc residue occurred, but AcO-4 on the MurNAc residue was still present even after reaction for 3 days. Fractionation of the reaction mixture afforded *N*-{2-*O*-[benzyl 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-4-*O*-acetyl-2,3-dideoxy-α-D-glucopyranosid-3-yl]-(*R*)-lactoyl}-L-alanyl-D-isoglutamine benzyl ester (**10**, 10%), the 4-acetate (**11**, 35%) of **8**, and the 4-acetate (**12**, 9%) of **9**. The $^1$H-n.m.r. spectra of each product contained one signal for OAc [δ 2.14–2.15 in $CD_3OD$ and 2.08–2.10 in $(CD_3)_2SO$]. Whereas the spectrum of **10** contained signals for two Ph groups and Ph$CH_2$, those of **11** and **12** contained signals for COOMe at δ 3.66 and 3.71, and 3.57 and 3.61, in $CD_3OD$ and $(CD_3)_2SO$, respectively. As expected, the MgO-treatment of **5** afforded **8** and **9**, but at a much lower rate and in a ratio (~4:1) different from that for the Zemplén reaction. When **10** was treated with methanolic sodium methoxide, t.l.c. (solvent *B*, 5:2:1) revealed **8** and **9** as the exclusive products.

The two deacetylation procedures were also tested on the disaccharide methyl esters **1** and **3**. Under Zemplén conditions, both **1** and **3** afforded benzyl 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (**13**; 87 and 87%, respectively). Treatment of **13** with 0.1M KOH gave the corresponding acid **14**. The MgO-catalysed methanolysis of **1** also gave, as expected, **13** (78%) as the only product. However, similar treatment of **3** for only 3.5 h gave a mixture of products which was fractionated on a silica gel column to give, besides partially deacetylated products, the 4-acetate (**15**, 62%) of **13** and **13** (10%). The presence of **13** indicates that the resistance of the AcO-4 group in the disaccharide is considerably lower than in its peptide conjugate **6**.

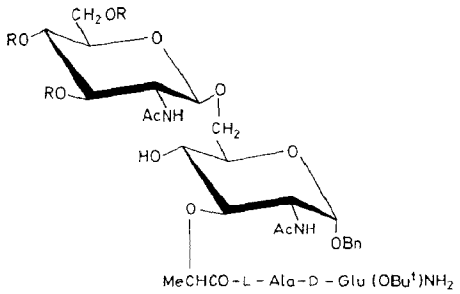
The structures of **13–15** were established from analytical and spectral data. Only the $^1$H-n.m.r. spectrum of **15** contained a signal for OAc at δ 2.176 and 2.082 in $CD_3OD$ and $(CD_3)_2SO$, respectively. The comparison of the $^{13}$C-n.m.r. data (see Experimental) for **13** and **15** showed that, in the latter, the resonance assigned to C-4 was shifted (1.3 p.p.m.) downfield and the resonances for C-3 and C-5 were shifted upfield (~1.7 and 2.1 p.p.m., respectively), thus indicating O-4 of the MurNAc residue to be the site of acetylation.

Treatment of **5** and **6** in aqueous alkaline media (1,4-dioxane–0.1M KOH, $Et_3N$–1,4-dioxane–water, $NH_3$–MeOH–water) gave complex mixtures; monitoring by t.l.c. (solvents *B* and *C*) and $^1$H-n.m.r. spectroscopy of the mixtures indicated that saponification of the peptide ester group, accompanied by $\alpha \rightarrow \gamma$ transamidation at the isoglutaminyl residue, proceeded much faster than *O*-deacetylation of the sugar moiety. When the disaccharide–dipeptide methyl ester **8** (which carries no *O*-acetyl groups on the sugar moiety) was treated in 1,4-dioxane with one equivalent of 0.1M KOH, t.l.c. (solvent *C*) showed the immediate formation of two products ($R_F$ ~0.2 and 0.24) of which the former, associated with the rearranged product, preponderated after ~1 h. This result is consistent with the finding[9] that opening of the glutarimide intermediate in aqueous alkaline media gives mainly glutamine derivatives [the lower electron density of the carbonyl group attached to the –CH(NHR)(R′) carbon atom favours the attack of the hydroxyl ions at that site] and the process is accompanied by partial racemisation[9]. Under the above conditions, AcO-4 in **6** resisted cleavage, even after addition of a five-fold excess of the base and prolongation of the time of reaction to 1 week.

The foregoing results indicate that benzyl- or methyl-ester-protection of peptidoglycan-related derivatives is not compatible with any of the reactions requiring basic media. Zemljakov *et al.*[7] reported selective removal of the benzyl ester group in **6** by brief catalytic hydrogenolysis in aqueous 90% ethanol, followed by Zemplén *O*-deacetylation and prolonged catalytic hydrogenolysis, but, in our hands, this procedure was not straightforward.

In order to prevent intramolecular transpeptidations of peptides with esterified aspartyl and glutamyl residues under basic conditions, Schwyzer[15] recommended the use of the *tert*-butyl ester as a protecting group instead of methyl

or benzyl groups. Therefore, *N*-benzyloxycarbonyl-L-alanyl-D-isoglutamine *tert*-butyl ester was synthesised and, in parallel experiments with Boc-L-Ala–D-Glu(OBn)$NH_2$, its stability toward transesterification and transamidation reactions under Zemplén conditions was confirmed. The disaccharide **2** was then coupled with L-Ala–D-Glu(OBu$^t$)$NH_2$ *via* the anhydride method to give 87% of *N*-{2-*O*-[benzyl 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,3-dideoxy-α-D-glucopyranosid-3-yl]-(*R*)-lactoyl}-L-alanyl-D-isoglutamine *tert*-butyl ester (**16**). Treatment of **16** under Zemplén conditions afforded an almost quantitative yield of the benzyl α-glycoside of the HO-unsubstituted disaccharide–dipeptide *tert*-butyl ester **17**, indicating that no transesterification and transamidation reactions occurred during the *O*-deacetylation step.




16 R = Ac
17 R = H

EXPERIMENTAL

*General.* — Melting points were determined in capillaries and are uncorrected. Solvents were removed under reduced pressure at <45°. Column chromatography was performed on Silica Gel (Merck 0.040–0.063 mm) and t.l.c. on Silica Gel 60 and 60 $F_{254}$ (Merck) with detection by charring with sulphuric acid and the chlorine–iodine reagent for peptides. The solvents used were *A*, chloroform–methanol; *B*, ethyl acetate–ethanol–water; *C*, chloroform–methanol–water in proportions given in the text. Optical rotations were determined for 1% solutions if not stated otherwise. N.m.r. spectra (internal $Me_4Si$) were recorded with a Jeol FX 90 F.t. spectrometer operating at 100 ($^1H$) and 22.5 MHz ($^{13}C$), if not stated otherwise. The 500-MHz $^1H$-n.m.r. spectra were recorded with a Bruker AM-500 spectrometer operating in the F.t. mode and equipped with a Bruker Aspect 3000 computer.

*Amino acids and peptides.* — (*a*) N-*Benzyloxycarbonyl*-D-*isoglutamine* tert-*butyl ester.* Obtained in 70% yield from Z-D-Glu(OH)$NH_2$[16] (1 g) and *tert*-butyl acetate (100 mL) containing aqueous 70% (w/v) perchloric acid (0.5 mL) as

described[12] for the L isomer but for a longer (~30 h) reaction period, this compound had m.p. 140–142°, $[\alpha]_D$ +4.3° (*c* 2, methanol). N.m.r. data, $^1H$ ($CD_3OD$): δ 4.15 (m, α-CH), 2.42, 2.40, 2.33, 2.28 (m, 2 H, γ-$CH_2$), 1.93 (m, 2 H, β-$CH_2$), 1.427 (s, 9 H, $^t$Bu); $^1H$ [$(CD_3)_2SO$]: δ 3.95 (m, α-CH), 2.32, 2.30, 2.23, 2.15 (m, 2 H, γ-$CH_2$), 1.81 (m, 2 H, β-$CH_2$), 1.383 (s, 9 H, $^t$Bu); $^{13}C$ ($CD_3OD$): δ 177.14, 174.21, 158.52 (3 CO), 82.11 (*C*$Me_3$), 68.06 (O*C*$H_2$Ph), 55.18 (α-*C*H), 32.45 (γ-*C*$H_2$), 28.95 (β-*C*$H_2$), 28.61 [C(*C*$H_3$)$_3$]. Lit.[17] for the L-isomer, m.p. 132–133°, $[\alpha]_D$ −4.5°.

*Anal.* Calc. for $C_{17}H_{24}N_2O_5$: C, 60.70; H, 7.19; N, 8.33. Found: C, 60.64; H, 7.23; N, 8.40.

(*b*) N-*Benzyloxycarbonyl*-L-*alanyl*-D-*isoglutamine* tert-*butyl ester*. Application of the mixed anhydride method with Z-L-AlaOH (440 mg, 2 mmol) and H-D-Glu(OBu$^t$)$NH_2$ [obtained by hydrogenolysis of Z-D-Glu(OBu$^t$)$NH_2$ (673 mg, 2 mmol) in 90% acetic acid[18]] as described for **5**, column chromatography [solvent *A* (4:1)], and crystallisation of the product (652 mg, 80%) from ethyl acetate–light petroleum gave the title compound, m.p. 164–166°, $[\alpha]_D$ −3° (*c* 1.5, methanol). N.m.r. data ($CD_3OD$): $^1H$, δ 7.33 (Ph), 5.08 (O*C*$H_2$Ph), 4.37 (m, α-CH Glu), 4.11 (q, α-CH Ala), 2.41, 2.37, 2.31, 2.26 (m, 2 H, γ-$CH_2$ Glu), 1.9 (m, 2 H, β-$CH_2$ Glu), 1.438 (s, 9 H, $^t$Bu), 1.33 (d, *J* 7 Hz, Me Ala); $^{13}C$, δ 176.50, 176.08, 174.04, 158.60 (4 CO), 82.06 (*C*$Me_3$), 68.0 (O*C*$H_2$Ph), 53.78 (α-*C*H Glu), 52.54 (α-*C*H Ala), 32.90 (γ-*C*$H_2$ Glu), 28.61 [C(*C*$H_3$)$_3$], 28.44 (β-*C*$H_2$ Glu), 18.23 (Me Ala).

*Anal.* Calc. for $C_{20}H_{29}N_3O_6$: C, 58.95; H, 7.17; N, 10.31. Found: C, 58.74; H, 7.40; N, 10.39.

N-{2-O-[*Benzyl 2-acetamido-6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,3-dideoxy-α-D-glucopyranosid-3-yl*]-(R)-*lactoyl*}-L-*alanyl*-D-*isoglutamine benzyl ester* (**5**). — (*a*) To a solution of dry **2** [prepared from 145 mg (0.2 mmol) of **1**] in dry tetrahydrofuran (8 mL) at −16° were added, with stirring, *N*-methylmorpholine (25 μL, 0.22 mmol) and isobutyl chloroformate (30 μL, 0.22 mmol). After 15 min, a precooled solution of L-Ala–D-Glu(OBn)$NH_2$ [liberated with $Et_3N$ from the trifluoroacetate, prepared from 102 mg (0.25 mmol) of Boc-L-Ala–D-Glu(OBn)$NH_2$[6]] in *N,N*-dimethylformamide (3 mL) was added, the mixture was stirred for 1 h at −15° and for 5 h at room temperature, then concentrated, and traces of *N,N*-dimethylformamide were removed by co-distillation with water. Column chromatography [solvent *A* (9:1)] of the residue gave, first, a mixture ($R_F$ ~0.7, 20 mg) of **7** and **1** (formed on the silica gel by methanolysis of **7**), and then **5** ($R_F$ ~0.3, 155 mg, 77%). Crystallisation from methanol–di-isopropyl ether afforded **5**, m.p. 236–238°, $[\alpha]_D$ +45.5° (methanol). $^1H$-N.m.r. data (MeOD + $CDCl_3$): δ 7.3 (10 H, 2 Ph), 5.13 (d, $J_{1,2}$ 3 Hz, H-1), 5.12 (s, $CO_2C\mathit{H}_2$Ph), 2.043, 2.005, 1.975, 1.896, 1.880 (15 H, 2 NAc + 3 OAc), 1.36 (d, 6 H, Me Lact + Me Ala).

*Anal.* Calc. for $C_{47}H_{63}N_5O_{19}$: C, 56.34; H, 6.34; N, 6.99. Found: C, 56.17; H, 6.30; N, 7.17.

(*b*) To a stirred suspension of **7** (139 mg, 0.2 mmol) and imidazole (41 mg, 0.6 mmol) in dry 1,4-dioxane (10 mL) were added, at room temperature, a solution

of tetrahexylammonium benzoate[8] (131 mg, 0.27 mmol) in 1,4-dioxane (6 mL) and L-Ala–D-Glu(OBn)$NH_2$ [prepared from Boc-L-Ala–D-Glu(OBn)$NH_2$ (123 mg, 0.3 mmol) *via* the trifluoroacetate which was neutralised with *N*-methylmorpholine immediately before addition]. The additions were repeated after 2 days, the mixture was then kept at room temperature, with occasional stirring, for 12 days [monitoring by t.l.c., solvent *A* (9:1)] and then concentrated. Column chromatography [twice with solvent *A* (9:1)] of the residue gave **5** (88 mg, 44%), m.p. 235–238° (from MeOH–di-isopropyl ether), $[\alpha]_D$ +52.5° (*N,N*-dimethylformamide).

Conventional treatment of **5** (50 mg) with $Ac_2O$–pyridine (1:1, 5 mL) gave, after column chromatography [solvent *A* (9:1)], **6** identical to the product described below. $^1$H-N.m.r. (500 MHz) data (pyridine-$d_5$): δ 5.70 (dd, $J_{3',4'}$ 9, $J_{3',2'}$ 10 Hz, H-3'), 5.34 (dd, $J_{4',5'}$ 10 Hz, H-4'), 5.13 (d, $J_{1,2}$ 3.2 Hz, H-1), 5.06 (s, $CO_2CH_2Ph$), 5.04 (dd, $J_{4,5}$ 10.5, $J_{4,3}$ 9.5 Hz, H-4), 4.99 (d, $J_{1',2'}$ 8.5 Hz, H-1'), 4.99 (m, α-CH Glu), 4.88, 4.45 (2 d, 2 H, $J_{gem}$ 12 Hz, $OCH_2Ph$), 4.70 (q, *J* 7 Hz, α-CH Ala), 4.65 (ddd, $J_{2,3}$ 10.5 Hz, H-2), 4.49 (ddd, $J_{2',3'}$ 10 Hz, H-2'), 4.43 (dd, $J_{6'a,6'b}$ 12, $J_{6'a,5'}$ 5 Hz, H-6'a), 4.36 (q, *J* 7 Hz, α-CH Lact), 4.18 (dd, $J_{6'b,5'}$ 2.5 Hz, H-6'b), 4.14 (dd, $J_{6a,6b}$ 11, $J_{6a,5}$ 7 Hz, H-6a), 4.13 (dq, $J_{5,6b}$ 2 Hz, H-5), 3.99 (dd, $J_{2,3}$ 10.5 Hz, H-3), 3.86 (dq, H-5'), 3.67 (dd, $J_{6b,5}$ 2 Hz, H-6b), 2.68–2.62 (m, γ-$CH_2$ Glu), 2.32–2.22 (m, β-$CH_2$ Glu), 1.935, 1.932, 1.922, 1.897, 1.876, 1.866 (6 s, 2 NAc + 4 OAc), 1.58 (d, *J* 7 Hz, Me Ala), 1.28 (d, *J* 7 Hz, Me Lact).

N-{*2*-O-[*Benzyl 2-acetamido-6*-O-*(2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy*-β-D-*glucopyranosyl)-4*-O-*acetyl-2,3-dideoxy*-α-D-*glucopyranosid-3-yl*]-(R)-*lactoyl*}-L-*alanyl*-D-*isoglutamine benzyl ester* (**6**). — Conversion of **4** [prepared from 385 mg (0.5 mmol) of **3**] into the mixed anhydride and treatment with L-Ala–D-Glu(OBn)$NH_2$ was performed as described above for **5**. Most of the solvent was evaporated, the concentrate was diluted with water (100 mL), and the precipitate (411 mg, 78%) was collected, washed (water), dried, and crystallised from methanol–chloroform–di-isopropyl ether to give **6**, m.p. 284–286°, $[\alpha]_D$ +60° (*N,N*-dimethylformamide). $^{13}$C-N.m.r. data (pyridine): δ 101.8 (C-1'), 97.3 (C-1), 79.0, 78.8 (C-3, α-C Lact), 73.6 (C-4), 72.2 (C-5'), 71.1, 70.0, 69.6 (C-5,6,3',4'), 69.2 ($OCH_2Ph$), 66.18 ($CO_2CH_2Ph$), 62.4 (C-6'), 54.8, 54.1 (C-2,2'), 52.9 (α-C Glu), 50.4 (α-C Ala), 31.1 (γ-C Glu), 28.0 (β-C Glu), 19.4 (Me Lact), 17.6 (Me Ala). Lit.[7] m.p. 268–271°, $[\alpha]_D$ +61° (acetic acid); no analytical data given.

*Anal.* Calc. for $C_{49}H_{65}N_5O_{20}$: C, 56.37; H, 6.27; N, 6.71. Found: C, 56.20; H, 6.39; N, 6.77.

N-{*2*-O-[*Benzyl 2-acetamido-6*-O-*(2-acetamido-2-deoxy*-β-D-*glucopyranosyl)-2,3-dideoxy*-α-D-*glucopyranosid-3-yl*]-(R)-*lactoyl*}-L-*alanyl*-D-*isoglutamine methyl ester* (**8**) *and -glutamine methyl ester* (**9**). — (*a*) To a cooled (ice–water) solution of **5** (100 mg, 0.1 mmol) in dry methanol (7 mL) was added methanolic 0.1M NaOMe (1 mL) in two portions during 1 h. The solution was kept (~3 h) at room temperature, until t.l.c. [solvent *B* (5:2:1)] indicated (peptide reagent) the presence only of products with $R_F$ ~0.45 and 0.4. The solution was neutralised with Amberlite IR-120 ($H^+$) resin, filtered, and concentrated. Column chromatography [solvent *B*

(5:2:1)] of the residue gave, first, **8** (35 mg, 44%), then a mixture (15 mg, 19%) of **8** and **9**, and then **9** (15 mg, 19%).

Compound **8** had m.p. 224–226° (from 9:1 methanol–water–ether), $[\alpha]_D$ +55° (9:1 methanol–water). $^1$H-N.m.r. data ($CD_3OD$): δ 7.36 (Ph), 4.87 (d, $J_{1,2}$ 3 Hz, H-1), 3.672 (s, $CO_2Me$-5), 2.49, 2.41, 2.35 (t, γ-$CH_2$ Glu), 1.989, 1.915 (2 NAc), 1.39, 1.37 (2 d, 6 H, *J* 7.08 and 6.8 Hz, 2 *Me*CH); [$(CD_3)_2SO$]: δ 7.36 (Ph), 4.75 (d, $J_{1,2}$ 2.9 Hz, H-1), 2.31, 2.21 (m, γ-$CH_2$ Glu), 3.577 (s, $CO_2Me$-5), 1.798 (s, 6 H, 2 NAc), 1.26, 1.23 (2 d, *J* 7 Hz, 2 *Me*CH).

*Anal.* Calc. for $C_{35}H_{53}N_5O_{16}$: C, 52.55; H, 6.68; N, 8.76. Found: C, 52.26; H, 6.50; N, 8.62.

Compound **9** had m.p. 199–202° (from 98:2 methanol–water–ether), $[\alpha]_D$ +60° (9:1 methanol–water). $^1$H-N.m.r. data ($CD_3OD$): δ 7.36 (Ph), 4.88 (d, $J_{1,2}$ 3.4 Hz, H-1), 3.695 (s, $CO_2Me$-1), 2.31, 2.21, 2.17 (m, γ-$CH_2$ Glu), 1.961, 1.915 (2 NAc), 1.38, 1.37 (2 d, 6 H, *J* 7 and 6.8 Hz, 2 *Me*CH); [$(CD_3)_2SO$]: δ 7.35 (Ph), 4.74 (d, $J_{1,2}$ 3.2 Hz, H-1), 3.606 (s, $CO_2Me$-1), 2.11, 2.06, 1.98 (m, γ-$CH_2$ Glu), 1.794 (s, 6 H, 2 NAc), 1.27, 1.24 (2 d, 6 H, *J* 7 Hz, 2 *Me*CH).

*Anal.* Found: C, 52.39; H, 6.79; N, 8.87.

(*b*) Treatment of **6** (105 mg, 1 mmol) as in (*a*) gave, after column chromatography, **8** (38.5 mg, 48%) and **9** (13.6 mg, 17%).

*O-Deacetylations with MgO.* — (*a*) *N-{2-O-[Benzyl 2-acetamido-6-O-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-4-O-acetyl-2,3-dideoxy-α-*D*-glucopyranosid-3-yl]-(*R*)-lactoyl}-*L*-alanyl-*D*-isoglutamine benzyl ester* (**10**), *-isoglutamine methyl ester* (**11**), *and -glutamine methyl ester* (**12**). To a stirred solution of **6** (209 mg, 0.2 mmol) in dry methanol–chloroform (3:1, 28 mL) was added MgO (200 mg), and the suspension was stirred for 48 h at room temperature [monitoring by t.l.c. (peptide reagent), solvent *B* (5:2:1)]. The mixture was then filtered and concentrated. Column chromatography (twice with solvent *B*) of the residue gave **10** (22 mg, 12%), **11** (60 mg, 36%), and **12** (17 mg, 10%).

Compound **10** had m.p. 228–230° (from MeOH–$Et_2O$), $[\alpha]_D$ +57° (*c* 0.5, 9:1 methanol–water). $^1$H-N.m.r. data ($CD_3OD$): δ 7.33 (10 H, 2 Ph), 5.11 (s, $CO_2CH_2Ph$), 4.89 (d, $J_{1,2}$ 3 Hz, H-1), 2.51, 2.44, 2.38 (t, γ-$CH_2$ Glu), 2.119 (s, 3 H, AcO-4), 1.972, 1.871 (2 NAc), 1.35, 1.25 (2 d, 6 H, *J* 7.08 and 6.8 Hz, 2 *Me*CH); [$(CD_3)_2SO$]: δ 7.33 (10 H, 2 Ph), 5.07 (s, $CO_2CH_2Ph$), 4.76 (s, $J_{1,2}$ 3 Hz, H-1), 2.076 (s, 3 H, AcO-4), 1.798, 1.772 (2 NAc), 1.21, 1.14 (2 d, 6 H, *J* 7.03 and 6.8 Hz, 2 *Me*CH).

*Anal.* Calc. for $C_{43}H_{59}N_5O_{17}$: C, 56.26; H, 6.48; N, 7.63. Found: C, 56.16; H, 6.61; N, 7.59.

Compound **11**, m.p. 240–242° (from MeOH–$Et_2O$), was still contaminated by a small amount of **12**. $^1$H-N.m.r. data ($CD_3OD$ + $D_2O$): δ 7.37 (5 H, Ph), 3.701 (s, ~0.3 H, $CO_2Me$-1), 3.665 (s, ~2.7 H, $CO_2Me$-5), 2.51, 2.42, 2.34 (t, γ-$CH_2$ Glu), 2.142 (AcO-4), 1.999, 1.890 (2 NAc), 1.39, 1.27 (2 d, 6 H, *J* 7.08 and 6.8 Hz, 2 *Me*CH); [$(CD_3)_2SO$]: δ 7.37 (5 H, Ph), 4.75 (d, $J_{1,2}$ 3 Hz, H-1), 3.614 (s, ~0.3 H, $CO_2Me$-1), 3.573 (s, ~2.7 H, $CO_2Me$-5), 2.099 (AcO-4), 1.788 (s, 6 H, 2 NAc), 1.24 (s, 6 H, *J* 7 Hz, 2 *Me*CH).

*Anal.* Calc. for $C_{37}H_{55}N_5O_{17}$: C, 52.79; H, 6.58; N, 8.32. Found: C, 52.74; H, 6.63; N, 8.59.

Compound **12** had m.p. 202–204°, $[\alpha]_D$ +51° (*c* 0.5, methanol). $^1$H-N.m.r. data ($CD_3OD$): $\delta$ 7.36 (5 H, Ph), 3.697 (s, $CO_2$Me-1), 2.21 (m, $\gamma$-$CH_2$ Glu), 2.133 (AcO-4), 1.980, 1.874 (2 NAc), 1.38, 1.27 (2 d, 6 H, *J* 7.08 and 6.8 Hz, 2 *Me*CH); [$(CD_3)_2SO$]: $\delta$ 7.37 (5 H, Ph), 4.74 (d, $J_{1,2}$ 3.0 Hz, H-1), 3.613 (s, $CO_2$Me-1), 2.101 (AcO-4), 1.783 (s, 6 H, 2 NAc), 1.24 (d, 6 H, *J* 7 Hz, 2 *Me*CH).

*Anal.* Found: C, 52.59; H, 6.68; N, 8.41.

(*b*) Treatment of **5** (100 mg, 1 mmol) as in (*a*) gave **8** (32 mg, 40%) and **9** (9.5 mg, 12%).

*Studies with the peptide component.* — (*a*) *With NaOMe–MeOH.* To a stirred solution of Boc-L-Ala-D-Glu(OBn)$NH_2$ (305 mg, 0.75 mmol) in dry MeOH (10 mL) was added methanolic 0.1M NaOMe (0.75 mL). After 4 h at room temperature, t.l.c. [solvent *A* (9:1)] revealed no starting compound ($R_F$ ~0.8), but two products [$R_F$ ~0.50 (major) and ~0.45 (minor)]. The solution was neutralised with Amberlite IR-120 ($H^+$) resin, filtered, and concentrated. Column chromatography [twice with solvent *A* (9:1)] of the residue gave the 5-$CO_2$Me derivative (100 mg, 40%), then a mixture (75 mg, 30%) of the 5- and 1-$CO_2$Me isomers, and then the 1-$CO_2$Me derivative (42 mg, 17%).

Boc-L-Ala-D-Glu(OMe)$NH_2$ had m.p. 115–117° (from EtOAc–light petroleum), $[\alpha]_D$ −12° (methanol). $^1$H-N.m.r. data ($CD_3OD$): $\delta$ 4.40 (m, $\alpha$-CH Glu), 4.02 (q, *J* 7 Hz, $\alpha$-CH Ala), 3.656 (s, $CO_2$Me-5), 2.51, 2.42, 2.35 (t, $\gamma$-$CH_2$ Glu), 2.0 (m, $\beta$-$CH_2$ Glu), 1.433 (s, 9 H, $^t$Bu), 1.30 (d, *J* 7 Hz, *Me*CH); [$(CD_3)_2SO$]: $\delta$ 7.54 and 7.23 (2 d, 2 H, *J* 8.5 and 12 Hz, 2 NH), 7.10 (d, 2 H, *J* 10.5 Hz, $NH_2$), 4.05 (m, $\alpha$-CH Glu), 3.88 (q, *J* 7 Hz, $\alpha$-CH Ala), 3.570 (s, $CO_2$Me-5), 2.39, 2.29, 2.20 (t, $\gamma$-$CH_2$ Glu), 1.85 (m, $\beta$-$CH_2$ Glu), 1.369 (s, $^t$Bu), 1.15 (d, *J* 7 Hz, *Me*CH).

*Anal.* Calc. for $C_{14}H_{25}N_3O_6$: C, 50.74; H, 7.61; N, 12.68. Found: C, 50.65; H, 7.19; N, 12.66.

Boc-L-Ala-D-Glu($NH_2$)OMe had m.p. 110–111° (from EtOAc–light petroleum), $[\alpha]_D$ −22° (methanol). $^1$H-N.m.r. data ($CD_3OD$): $\delta$ 4.38 (m, $\alpha$-CH Glu), 4.06 (q, *J* 7 Hz, $\alpha$-CH Ala), 3.713 (s, $CO_2$Me-1), 2.23 (m, $\gamma$-$CH_2$ Glu), 2.0 (m, $\beta$-$CH_2$ Glu), 1.433 $^t$Bu), 1.30 (d, *J* 7 Hz, *Me*CH); [$(CD_3)_2SO$]: $\delta$ 8.17 (d, *J* 11 Hz, NH), 7.24 (NH), 6.79 (2 H, $NH_2$), 4.12 (m, $\alpha$-CH Glu), 3.95 (q, *J* 7 Hz, $\alpha$-CH Ala), 3.619 ($CO_2$Me-1), 2.15 (m, $\gamma$-$CH_2$ Glu), 1.89 (m, $\beta$-$CH_2$ Glu), 1.369 ($^t$Bu), 1.17 (d, *J* 7 Hz, *Me*CH).

*Anal.* Found: C, 50.79; H, 7.70; N, 12.55.

(*b*) *With MgO.* Treatment of Boc-L-Ala-D-Glu(OBn)$NH_2$ (204 mg) with MgO (500 mg) in dry MeOH (10 mL) for 24 h and column chromatography [solvent *A* (9:1)] of the products gave Boc-L-Ala-D-Glu(OBn)$NH_2$ (61 mg, 30%), Boc-L-Ala-D-Glu(OMe)$NH_2$ (66 mg, 40%), and Boc-L-Ala-D-Glu($NH_2$)OMe (20 mg, 12%).

(*c*) *With 0.1M KOH.* To a solution of Boc-L-Ala-D-Glu(OBn)$NH_2$ (204 mg, 0.5 mmol) in 1,4-dioxane (6 mL) was added 2.5 mL of 0.1M KOH. T.l.c. [solvent

*B* (5:2:1)] showed the immediate formation of two products ($R_F$ ~0.55 and 0.5). The faster co-chromatographed with Boc-L-Ala–D-Glu(OH)$NH_2$[19]. After 10 min, the ratio of the products was 1:3.

(*d*) *With NaOMe–MeOH.* A methanolic solution of Z-L-Ala–D-Glu(OBu$^t$)$NH_2$ (81.5 mg, 0.2 mmol) was treated with methanolic 0.1M NaOMe (2 mL) for 3 h at room temperature. Work-up, as described in (*a*), gave the starting compound only. Similar treatment of Boc-L-Ala–D-Glu(OBn)$NH_2$ gave the transesterification and transamidation products as in (*a*).

*Benzyl 2-acetamido-6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-3-O-[(R)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside* (**13**). — To a stirred solution of **3** (77 mg, 0.1 mmol) in dry methanol (5 mL) was added methanolic 0.1M NaOMe (1 mL) at room temperature. After 2 h, t.l.c. [solvent *A* (4:1)] indicated that a single slower-moving product had been formed. The solution was neutralised with Amberlite IR-120 ($H^+$) resin, filtered, and concentrated, and the residue was crystallised from acetone–methanol–di-isopropyl ether to give **13** (52 mg, 87%), m.p. 228–230° (softening at 224°), $[\alpha]_D$ +84° (methanol). N.m.r. data ($CD_3OD$): $^1$H, δ 7.33 (Ph), 5.03 (d, $J_{1,2}$ 2.9 Hz, H-1), 3.732 (s, $CO_2Me$), 1.959, 1.948 (2 NAc), 1.35 (d, *J* 6.8 Hz, *Me*CH); $^{13}$C, δ 177.3, 174.5, 174.0 (CO), 103.8 (C-1′), 97.2 (C-1), 80.1 (C-3), 77.9, 77.3 (C-5′, α-C Lact), 76.0 (C-3′), 72.9 (C-5), 72.3 (C-4′), 72.0 (C-4), 70.7 (C-6), 70.0 ($OCH_2Ph$), 62.7 (C-6′), 57.3 (C-2′), 55.6 (C-2), 53.3 ($CO_2CH_3$), 23.4, 23.1 ($CH_3$ NAc), 19.5 ($CH_3$ Lact).

*Anal.* Calc. for $C_{27}H_{40}N_2O_{13}$: C, 53.99; H, 6.71; N, 4.66. Found: C, 53.71; H, 6.71; N, 4.50.

(*b*) Treatment of **1** (145 mg) with methanolic 0.1M NaOMe (2 mL) as in (*a*) gave **13** (105 mg, 87%).

(*c*) Treatment of a methanolic solution of **1** (73 mg) with MgO (100 mg), as described for **15**, afforded **13** (47 mg, 78%).

*Benzyl 2-acetamido-6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-3-O-[(R)-1-carboxyethyl]-α-D-glucopyranoside* (**14**). — To a solution of **13** (78 mg) in 1,4-dioxane (5 mL) was added 0.1M KOH (1.4 mL), and the solution was stirred at room temperature until t.l.c. [solvent *B* (5:2:1)] showed that the starting material had disappeared (~1 h). Work-up, as described for **13**, gave a product which was dried over $P_2O_5$ and then crystallised from acetone–methanol–di-isopropyl ether to give **14** (56 mg, 73%), m.p. 230–232° (softening at 214°), $[\alpha]_D$ +87° (*c* 0.5, methanol). $^1$H-N.m.r. data ($CD_3OD$): δ 7.31 (Ph), 5.10 (d, $J_{1,2}$ 2.9 Hz, H-1), 1.975, 1.961 (2 NAc), 1.36 (d, *J* 7 Hz, *Me*CH).

*Anal.* Calc. for $C_{26}H_{38}N_2O_{13}$: C, 53.23; H, 6.56; N, 4.78. Found: C, 53.29; H, 6.82; N, 4.70.

*Benzyl 2-acetamido-6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-4-O-acetyl-2-deoxy-3-O-[(R)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside* (**15**). — A suspension of **3** (100 mg) and MgO (130 mg) in dry methanol (6 mL) was stirred for 3.5 h at room temperature. T.l.c. [solvent *A* (4:1)] then showed the presence of a major product migrating faster than **13**. The suspension was filtered, the insoluble

material was washed with methanol, and the combined filtrate and washings were concentrated. Column chromatography [solvent *A* (4:1)] of the residue gave a mixture (~20 mg) of partially deacetylated **3, 15** (52 mg, 62%), and **13** (8 mg, 10%). Crystallisation of homogeneous fractions from acetone–methanol–di-isopropyl ether afforded **15**, m.p. 248–250° (softening at 220°) as the monohydrate, $[\alpha]_D$ +67° (*c* 1.1, methanol). N.m.r. data ($CD_3OD$): $^1H$, $\delta$ 7.39 (Ph), 5.05 (d, $J_{1,2}$ 2.8 Hz, H-1), 3.760 (s, $CO_2Me$), 2.176 (s, 3 H, AcO-4), 2.016, 1.989 (6 H, 2 NAc), 1.33 (d, *J* 6.8 Hz, *Me*CH); [$(CD_3)_2SO$]: $\delta$ 4.77 (d, $J_{1,2}$ 3.3 Hz, H-1), 3.609 (s, $CO_2Me$), 2.082 (s, AcO-4), 1.809, 1.788 (6 H, 2 NAc), 1.18 (d, *J* 7 Hz, *Me*CH); $^{13}C$, $\delta$ 176.0, 174.1, 173.6, 171.9 (4 CO), 103.2 (C-1′), 97.44 (C-1), 78.4, 78.2 (C-3, $\alpha$-C Lact), 77.3 (C-5′), 76.5 (C-3′), 73.3 (C-4), 72.3 (C-4′), 70.8 (C-5), 70.7 (C-6), 69.4 ($OCH_2Ph$), 62.9 (C-6′), 57.5 (C-2′), 55.3 (C-2), 53.1 ($CO_2CH_3$), 23.5, 23.0 (2 NAc), 21.3 (OAc), 19.6 ($CH_3$ Lact).

*Anal.* Calc. for $C_{29}H_{42}N_2O_{14}\cdot H_2O$: C, 52.72; H, 6.71; N, 4.32; $H_2O$, 2.73. Found: C, 52.56; H, 6.81; N, 4.32; $H_2O$, 2.71.

N-{*2*-O-[*Benzyl 2-acetamido-6*-O-*(2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl)-2,3-dideoxy-α*-D-*glucopyranosid-3-yl*]-(R)-*lactoyl*}-L-*alanyl*-D-*isoglutamine* tert-*butyl ester* (**16**). — A solution of **2** (214 mg, 0.3 mmol) in dry tetrahydrofuran (15 mL) was treated at −17° with *N*-methylmorpholine (40 μL) and isobutyl chloroformate (45 μL) as described for **5**. After 15 min, a precooled solution of L-Ala–D-Glu($OBu^t$)$NH_2$ [prepared by catalytic hydrogenation of Z-L-Ala–D-Glu($OBu^t$)$NH_2$ (144 mg, 0.4 mmol) in 90% AcOH[18], isolation of the AcOH salt, and treatment with $Et_3N$ in *N,N*-dimethylformamide (3 mL)] was added and the mixture was treated as described for **5**. Column chromatography [solvent *A* (9:1)] of the residue afforded **16** (253 mg, 87%), m.p. 258–260° (from MeOH–di-isopropyl ether), $[\alpha]_D$ +56° (*c* 1, methanol). $^1H$-N.m.r. data ($CD_3OD$ + $CDCl_3$): $\delta$ 7.34 (Ph), 5.25 (t, *J* 10 Hz, H-3), 5.13 (d, $J_{1,2}$ 3 Hz, H-1), 2.06, 2.02, 1.99, 1.91, 1.89 (5 s, 2 NAc + 3 OAc), 1.44 (s, 9 H, $^t$Bu), 1.39 and 1.36 (2 d, 6 H, *J* 7 Hz, 2 *Me*CH).

*Anal.* Calc. for $C_{44}H_{65}N_5O_{19}$: C, 54.59; H, 6.77; N, 7.23. Found: C, 54.34; H, 6.50; H, 7.20.

N-{*2*-O-[*Benzyl 2-acetamido-6*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-2,3-dideoxy-α*-D-*glucopyranosid-3-yl*]-(R)-*lactoyl*}-L-*alanyl*-D-*isoglutamine* tert-*butyl ester* (**17**). — To a stirred solution of **16** (194 mg, 0.2 mmol) in dry methanol (8 mL) (ice–water bath) was added methanolic 0.1M NaOMe (1 mL) during 10 min; after ~2.5 h, t.l.c. [solvents *B* (5:2:1) and *A* (3:1)] revealed a single, slower-moving product. The solution was worked-up as described for **8** and **9**, to give a product (165 mg, 98%), column chromatography [solvent *A* (3:1)] of which gave **17**, m.p. 228–232° (dec.), $[\alpha]_D$ +49° (*c* 1, 9:1 methanol–water). N.m.r. data ($CD_3OD$ + $CDCl_3$): $^1H$, $\delta$ 7.34 (Ph), 4.89 ($J_{1,2}$ 3.4 Hz, H-1), 1.99, 1.92 (2 NAc), 1.45 (9 H, $^t$Bu), 1.40 and 1.38 (2 d, *J* 7 Hz, 2 *Me*CH); $^{13}C$, ($CD_3OD$ + $D_2O$ 9:1), $\delta$ 103.3 (C-1′), 97.5 (C-1), 82.7 (*C*$Me_3$), 80.9 (C-3), 78.7 ($\alpha$-C Lact), 77.8 (C-5′), 76.0 (C-3′), 72.9 (C-5), 72.0, 71.3 (C-4,4′), 70.5 (C-6), 70.1 ($OCH_2Ph$), 62.7 (C-6′),

57.3 (C-2′), 54.8 (C-2), 53.7 (α-C Glu), 50.5 (α-C Ala), 32.8 (γ-C Glu), 28.6 [C($CH_3$)$_3$], 28.2 (β-C Glu), 23.5, 23.1 (NAc), 19.9 ($CH_3$ Lact), 18.1 ($CH_3$ Ala).

*Anal.* Calc. for $C_{38}H_{59}N_5O_{16}$: C, 54.31; H, 7.06; N, 8.23. Found: C, 54.05; H, 7.29; N, 8.43.

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REFERENCES

1 P. LEFRANCIER AND E. LEDERER, *Pure Appl. Chem.*, 59 (1987) 449–454.
2 V. T. IVANOV, T. M. ANDRONOVA, M. V. BEZRUKOV, V. A. RAR, E. A. MAKAROV, S. A. KOZMIN, M. A. ASTAPOVA, T. I. BARKOVA, AND V. A. NESMEYANOV, *Pure Appl. Chem.*, 59 (1987) 317–324.
3 S. KOTANI, M. TSUJIMOTO, T. OGAWA, K. NEROME, A. OOYA, T. TAKAHASHI, Y. GOTO, T. SHIBA, S. KUSUMOTO, AND T. SHIMAMOTO, in M. ZAORAL (Ed.), *Synthetic Immunomodulators and Vaccines, Proc. Int. Symp.*, Třeboň, Czechoslovakia, October 14–18, 1985, Institute of Organic Chemistry and Biochemistry of Czechoslovak Academy of Sciences, Prague, 1986, pp. 40–64; J. FARKAŠ, M. LEDVINA, J. BROKEŠ, J. JEŽEK, J. ZAJIČEK, AND M. ZAORAL, *ibid.*, pp. 123–128.
4 D. KEGLEVIĆ AND M. PONGRAČIĆ, *Carbohydr. Res.*, 135 (1984) 85–99.
5 D. KEGLEVIĆ, M. PONGRAČIĆ, AND D. KANTOCI, *Croat. Chem. Acta*, 58 (1985) 569–581.
6 P. LEFRANCIER AND E. BRICAS, *Bull. Soc. Chim. Biol.*, 49 (1967) 1257–1271; S. KUSUMOTO, Y. TAZUMI, K. IKENAKA, AND T. SHIBA, *Bull. Chem. Soc. Jpn.*, 49 (1976) 533–539; L. R. PHILLIPS, O. NISHIMURA, AND B. A. FRASER, *Carbohydr. Res.*, 132 (1984) 275–286.
7 A. E. ZEMLJAKOV, V. J. CZIROVA, AND A. J. KHORLIN, *Bioorg. Chem.*, 12 (1986) 1637–1640.
8 F. M. MENGER AND A. C. VITALE, *J. Am. Chem. Soc.*, 95 (1973) 4931–4934.
9 M. BODANSZKY AND J. MARTINEZ, in E. GROSS AND J. MEIENHOFER (Eds.), *The Peptides*, Vol 5B, Academic Press, New York, 1983, pp. 111–216.
10 A. R. BATTERSBY AND J. C. ROBINSON, *J. Chem. Soc.*, (1955) 259–269.
11 E. SONDHEIMER AND R. W. HOLLEY, *J. Am. Chem. Soc.*, 79 (1957) 3767–3770.
12 D. J. ANTONJUK, D. K. BOADIE, H. T. A. CHEUNG, AND T. G. TRAN, *J. Chem. Soc., Perkin Trans. 1*, (1984) 1989–2003.
13 I. KRISTENSEN AND P. O. LARSEN, *Acta Chem. Scand.*, 27 (1973) 3123–3125.
14 I. J. HERZIG AND A. NUDELMAN, *Carbohydr. Res.*, 153 (1986) 162–167.
15 R. SCHWYZER AND H. KAPPELER, *Helv. Chim. Acta*, 44 (1961) 1991–2002; R. SCHWYZER AND H. DIETRICH, *ibid.*, 2003–2006.
16 R. STRAKA AND M. ZAORAL, *Collect. Czech. Chem. Commun.*, 42 (1977) 560–563.
17 E. KLIEGER AND H. GIBIAN, *Liebig's Ann. Chem.*, 655 (1962) 195–210.
18 J. S. MORLEY, *J. Chem. Soc., C*, (1967) 2410–2421.
19 M. ZAORAL, J. JEŽEK, V. KRCHNAK, AND R. STRAKA, *Collect. Czech. Chem. Commun.*, 45 (1980) 1424–1446.