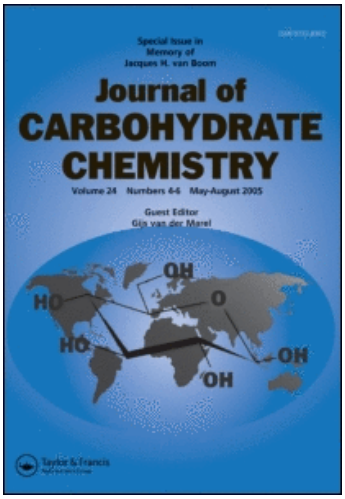

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PEPTIDOGLYCAN-RELATED DISACCHARIDE-DIPEPTIDES: DIFFERENTIATION BETWEEN GLUTAMINYL AND ISOGLUTAMINYL RESIDUES BY NMR SPECTROSCOPY

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ABSTRACT

In order to confirm that *tert*-butyl ester protection at the isoglutaminyl residue prevents the base-catalyzed isoglutamine ⇄ glutamine rearrangement in title compounds, 6-*O*-(2-acetamido-2-deoxy-ß-**D**-glucopyranosyl)-*N*-acetylmuramoyl-**L**-alanyl-**D**-glutamine (**7**) was synthesized from α-benzyl glycoside of 6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-ß-**D**-glucopyranosyl)-*N*-acetylmuramic acid (**2**) and Z-**L**-Ala-**D**-Glu(NH_2)OBu^t *via* its α-*tert*-butyl ester **5**. ^{1}H and ^{13}C NMR measurements were carried out with regioisomeric [ß-GlcNAc-(1→6)-MurNAc]-**L**-Ala-**D**-glutamine and -isoglutamine derivatives; the presented data show differences that enable distinction between the sugar-peptide regioisomers.

INTRODUCTION

Recognition of the immunoadjuvant properties of synthetically prepared *N*-acetylmuramoyl-**L**-alanyl-**D**-isoglutamine (MDP)[1,2] has stimulated synthesis of an impressive number

of compounds structurally related to bacterial cell-wall peptidoglycan fragments. Numerous synthetic methods are reported in the literature for the preparation of sugar-peptide conjugates, particularly those involving the **L**-alanyl-**D**-isoglutamine sequence in the peptide portion of the molecule.[3-5] However, although the base-catalyzed glutamine ⇄ isoglutamine rearrangement, proceeding through a cyclic glutarimide intermediate, has long been known,[6,7] less attention has been paid to the possibility of this side-reaction in the synthesis of peptidoglycan-related sugar-peptide conjugates. The glutarimide intermediate, formed by intramolecular cyclization of the glutamyl residues in alkaline media, undergoes nucleophilic attack at both carbonyl carbons of the ring to give a mixture of isomeric α- and γ-glutamyl peptides. Their separation, as well as the clear distinction between the two regioisomers poses special problems in peptide chemistry.[7,8]

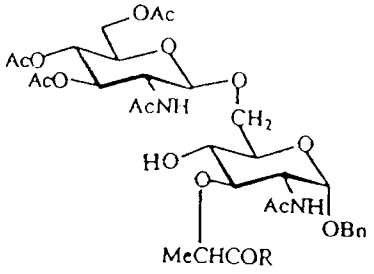
Our previous work on the synthesis of *O*-(2-acetamido-2-deoxy-β-**D**-glucopyranosyl)-(1→6)-*N*-acetylmuramoyl-**L**-alanyl-**D**-isoglutamine has shown that benzyl or methyl ester protection of the isoglutamine γ-carboxyl group is not compatible with any of the reactions requiring alkaline media (*e.g.* 0.1 *M* NaOMe/MeOH; MgO/MeOH; 0.1 *M* aq. KOH/dioxane).[9,10] A detailed study of the deacetylation of [β-GlcNAc-(1→6)-MurNAc]-**L**-alanyl-**D**-isoglutamine benzyl ester has established that deprotection of the sugar moiety is always accompanied by a α ⇄ γ transamidation of the isoglutamine residue to

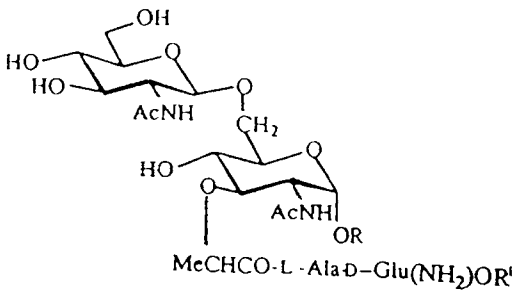
give a mixture of the corresponding disaccharide-**L**-alanyl-**D**-isoglutamine and -glutamine derivatives. However, the use of the *tert*-butyl ester[11] as a protecting group at the isoglutaminyl residue prevented the cyclic imide formation and allowed *O*-deacetylation of the sugar moiety without isoglutamine ⇄ glutamine isomerisation.[9]

In order to confirm the absence of isomerization by using *tert*-butyl ester protection and to find out whether it is possible to distinguish the glutaminyl from the isoglutaminyl residue in a disaccharide-dipeptide conjugate of the above type, we synthesized the corresponding regioisomers, *i.e.* the (1→6)-linked disaccharide-**L**-alanyl-**D**-glutamine derivatives. Availability of both members of the glutamine and isoglutamine series allowed us to compare their ^{1}H and ^{13}C NMR spectra and to find small but distinct differences between the two series.

RESULTS AND DISCUSSION

Synthesis. The routes employed for the synthesis of **3-7** were essentially those which had been successfully applied previously for the preparation of the corresponding members **III-VII**[9,10] of the isoglutamine series. The required dipeptide component was prepared from Z-**L**-Ala-OH and **D**-Glu(NH_2)OBu^t by using the DCC-hydroxybenzotriazol-Cu(II) chloride condensation method;[12] this way proved to be more efficient than the mixed anhydride method used for the synthesis of the Z-**L**-Ala-**D**-Glu(OBu^t)NH_2 regioisomer.





1 R = OMe

2 R = OH

3 R = **L**-Ala-**D**-Glu(NH_2)OBu^t

III R = **L**-Ala-**D**-Glu(OBu^t)NH_2

4 R = Bn, R' = Bu^t

5α R = H, R' = Bu^t

6 R = Bn, R' = H

7α R = R' = H

Bn = $PhCH_2$; Bu^t = $(CH_3)_3C$

Coupling of benzyl 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-ß-**D**-glucopyranosyl)-2-deoxy-3-*O*-[(*R*)-1-carboxyethyl]-α-**D**-glucopyranoside (**2**)[13] obtained by saponification of the methyl ester **1**,[14] with **L**-alanyl-**D**-glutamine α-*tert*-butyl ester, by using the mixed anhydride method, gave, after column chromatography, the disaccharide-dipeptide *tert*-butyl ester (**3**) in 68% yield. As expected, *O*-deacetylation of the sugar moiety of **3** with catalytic amounts of 0.1 *M* sodium methoxide in methanol proceeded without rearrangement of the glutaminyl residue to give the benzyl α-glycoside **4** as the exclusive product. Catalytic hydrogenation of **4**, using *tert*-butyl alcohol-acetic acid-water as solvent, provided the HO-unsubstituted

(1→6)-linked disaccharide-dipeptide α-*tert*-butyl ester **5** as an anomeric mixture (α:ß ratio ~3:1).

Cleavage of the α-*tert*-butyl ester group in **4** was achieved by a brief treatment of this compound with aqueous 90% trifluoroacetic acid (TFA); precipitation of the crude product with dry ether, followed by purification on a Sephadex G-25 column, gave the benzyl α-glycoside of ß-GlcNAc-(1→6)-MurNAc-dipeptide acid **6** (70%) as an amorphous solid. Catalytic hydrogenation of the latter yielded *N*-{2-*O*-[2-acetamido-6-*O*-(2-acetamido-2-deoxy-ß-**D**-glucopyranosyl)-2,3-dideoxy-**D**-glucopyranos-3-yl]-(*R*)-lactoyl}-**L**-alanyl-**D**-glutamine (**7**) with an α:ß ratio of ~ 3:1. In general, the prepared disaccharide-dipeptide derivatives **3-7** of the glutamine series proved to be somewhat less stable and definitely more hygroscopic than their regioisomers **III-VII**. On TLC (solvent *C*) it was shown that a member of the glutamine series moved slightly slower than its counterpart of the isoglutamine series.

NMR Spectroscopy. - The ^{13}C chemical shifts of compounds **3-7** were assigned by comparison with the data for related mono- and disaccharides, dipeptides and the corresponding regioisomers **III-VII**; assignments for compounds **4-7** are given in Table 1. Chemical shifts for sugar carbons are in accordance with the position of glycosidic linkage (upfield shift for C-6, downfield shift for C-5), the presence of reducing *N*-acetylmuramyl residue in compounds **5** and **7** (two sets of resonances for α and ß anomers), and the α-benzyl glycosylated forms of **4** and **6** (downfield shift for

TABLE 1. ^{13}C NMR Spectral Data[a] for Compounds **4-7**

Compound	**4**	**5**		**6**	**7**	
		α	β		α	β
C-1	97.4	92.27	97.15	97.32	92.4	97.1
C-2	54.9	55.52	58.08	54.84	55.5	58.0
C-3	80.4	79.91	82.90	80.44	80.0	82.8
C-4	71.9	71.93	71.61	71.78	71.6	71.4
C-5	72.9	72.19	76.84	72.91	72.2	76.8
C-6	70.2[b]	70.12		70.23	70.2	
C-1'	103.3	103.24	103.26	103.3	103.4	
C-2'	57.4	57.34		57.4	57.3	
C-3'	76.1	76.05		76.1	75.9	
C-4'	72.1	72.00		72.01	71.9	
C-5'	78.0	78.02		78.03	78.0	
C-6'	62.8	62.78		62.8	62.6	
CH_3-NAc	23.3,22.9	23.1,22.84		23.3,22.8	23.3,22.9	
OCH_2Ph	70.2[b]	___		70.17	___	
Lactyl						
α-*CH*	78.3	78.05	78.24	78.2	78.04	78.24
CH$_3$	19.7	19.63		19.6	19.7	
Alanyl						
α-*CH*	50.4	50.40		50.4	50.5	
CH$_3$	18.5	18.34		18.6	18.2	
Glutaminyl						
α-*CH*	54.1	54.11	54.03	54.1	54.4	
ß-CH_2	28.0	27.83		28.2	28.3	
γ-CH_2	32.3	32.32		32.5	32.6	
$(CH_3)_3C$	28.3	28.12		___	___	

[a]Relative to the central CD_3OD peak at 49.00 ppm in methanol-d_4; compd. **4** recorded at 22.5 MHz, compds. **5-7** at 75 MHz; carbonyl resonances and quarternary C-atom of Bu^t ester are not shown.

[b] Duplicated

C-1). Chemical shifts of the signals of the peptide carbon atoms also support the proposed structures.

On comparing the above data with those for the corresponding members of the isoglutamine series (*e.g.* **5** *vs.* **V**), no differences in the chemical shifts of carbohydrate carbons could be observed. In the peptide portion, a small downfield shift ($\Delta\delta$ 0.4-0.5 ppm) of the α-*C*H glutaminyl carbon relative to α-*C*H isoglutaminyl in a given pair of regioisomers has been observed; this difference appears to be too small to be taken into account for structural assignments. However, a characteristic and consistent chemical shift difference ($\Delta\delta$ 1.1-1.2 ppm) between the two series is exhibited by the quarternary carbon (C_q) of the *tert*-butyl ester groups: the C_q resonances corresponding to α-Bu^t esters are shifted downfield relative to those corresponding to the γ-Bu^t esters (Table 2). A difference in C_q resonances characteristic of α- *vs.* γ-monoamides of *N*-substituted L-glutamic acid monoamide *tert*-butyl esters has been reported.[8] The effect is now seen to be very useful for distinction between the two sugar-peptide regioisomers.

The complexity of ^{1}H NMR spectra of **3-7** required the use of homonuclear and heteronuclear spectroscopy for reliable results. As expected, the chemical shifts and coupling constants exhibited by sugar protons in the spectra of **3-7** showed no noticeable differences from the data found[9,10] for their regioisomers. On the contrary, the signals associated with the α-C*H* resonances of the glutaminyl and isoglutaminyl residues were clearly dis-

TABLE 2. ^{13}C NMR Chemical Shifts (δ)[a] for the Quarternary Carbon of Glutamine and Isoglutamine *tert*-Butyl Esters Series

D-Glutamine series		D-Isoglutamine series[b]	
Compound	α-CO_2CMe_3	Compound	-CO_2CMe_3
3	83.01	**III**	81.83
4	83.03	**IV**	81.87
5	83.06	**V**	81.89
Z-D-Glu(NH_2)OBu^t	82.86	Z-D-Glu(OBu^t)NH_2	81.81
Z-L-Ala-D-Glu(NH_2)OBu^t	83.04	Z-L-Ala-D-Glu(OBu^t)NH_2	81.79

[a]Relative to the central CD_3OD peak at 49.00 ppm in methanol-d_4; compds. **4** and **IV** recorded at 22.5 MHz, all others at 75 MHz.

[b]Syntheses reported in ref. 9 and 10.

tinguishable.[15] In the spectrum of **5**, the α-C*H* proton of glutaminyl residue is observed at δ 4.26 whereas that of the isoglutaminyl residue is shifted downfield to δ 4.32 in **V**: the change from α- to γ-carboxamide group also affects the α-C*H* resonances of **L**-alanyl and **D**-lactyl residues, which shift in the opposite direction from glutaminyl α-CH proton (Fig. 1). As a consequence, the order of the α-CH protons in **5** is different from that in **V**. Assignments based upon the chemical shift differences between ß-methylene protons of glutamine and isoglutamine were hampered by the overlap of these resonances with *N*-acetyl methyl signals. Selected ^1H NMR data for the reducing disaccharide-dipeptides **5** and **7**, their regioisomers **V** and **VII**, and the starting peptide components are given in Table 3.

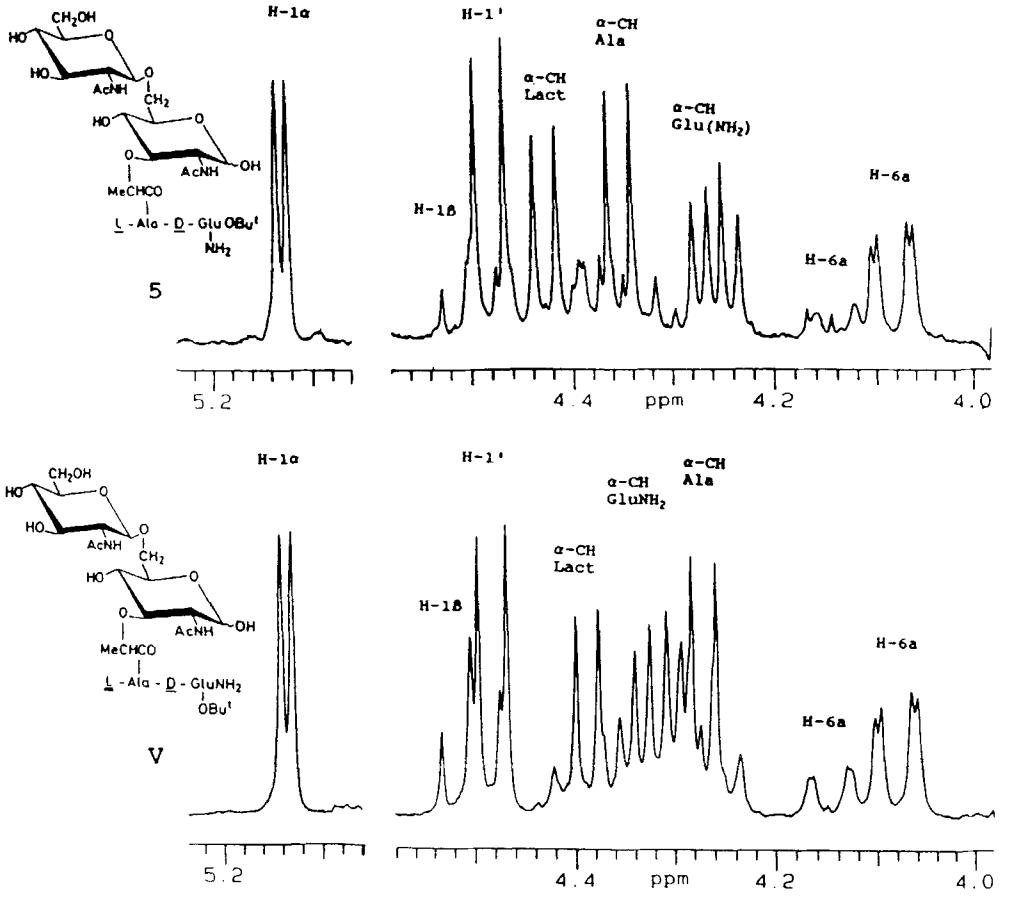


FIG. 1 ^{1}H NMR spectra of the anomeric and α-CH protons of **5** and **V** i CD_3OD at 300 MHz.

In conclusion, results from our measurements show that NMR spectroscopy can be used for differentiation between glutaminyl and isoglutaminyl residues in the isomeric sugar-peptide conjugates.

EXPERIMENTAL

General procedures. - Melting points are determined in capillaries and are uncorrected. Optical rotations were re-

TABLE 3. Selected ^1H NMR Spectral Data[a] for Compounds of the Glutamine and Isoglutamine Series

Compound	Anomeric protons				Aliphatic chain α-CH protons			
	H-1		H-1'*					
	α-form	ß-form	α-	ß-	Lactyl	Alanyl	Glu(NH_2)OR	Glu(OR)NH_2
5	5.13d	4.51d	4.48d	4.5 d	4.43q	4.35q	4.26dd	___
R=OBu^t	(3.2)	(8.5)	(8.5)	(8.5)	(7)	(7)	(4.8,8.9)	
V[b]	5.13d	4.52d	4.48d	4.49d	4.39q	4.28q	___	4.32dd
R=OBu^t	(3.4)	(8.5)	(8.5)	(8.5)	(7)	(7)		(4.6,9.4)
7	5.13d	4.55d	4.48d	n.d.[c]	4.44q	4.38q	4.28dd	___
R=OH	(2.9)	(8.5)	(8.5)		(7)	(7)	(5, 9)	
VII	5.14	n.d.[c]	4.49d	n.d.[c]	4.39q	4.30q	___	4.38dd
R=OH	(3.0)		(8.4)		(7)	(7)		(5, 9)
Peptides								
Z-L-Ala-D-Glu(NH_2)OBu^t						4.15q	4.20dd	___
						(7)	(5, 9)	
Z-L-Ala-D-Glu(OBu^t)NH_2						4.11q	___	4.37dd
						(7)		(5, 9)
D-Glu(NH_2)OBu^t						___	4.06dd	___
							(4.9, 9.1)	
D-Glu(OBu^t)NH_2						___	___	4.16dd
								(5.2,8.8)

[a]Measured at 300 MHz in CD_3OD (internal Me_4Si); chemical shifts are in δ units, coupling constants (J) in Hz; *linked to α- and ß-MurNAc

[b]Data taken from ref. 10.

[c]Not determined

corded with an Optical Activity LTD automatic AA-10 polarimeter for 1% solutions, if not stated otherwise. Column chromatography was performed on silica gel (Merck), gel filtration on Sephadex G-25 (Pharmacia) and TLC on silica gel 60 and $60F_{254}$ (Merck) by charring with sulphuric acid and the chlorine-iodine reagent for peptides. The solvents used were: *A*, $CHCl_3$—MeOH; *B*, EtOAc—EtOH—H_2O; *C*, $CHCl_3$—MeOH—AcOH—H_2O (24:24:1:3); proportions for *A* and *B* are given in the text.

NMR spectra were recorded for solutions in methanol-d_4 (internal Me_4Si) with a Varian VXR 300 spectrometer operating at 300 (^1H) and 75 MHz (^{13}C) or a Jeol FX 90 spectrometer operating at 100 (^1H) and 22.5 MHz (^{13}C), respectively. The ^1H chemical assignments were based on 2D experiments. Chemical shifts are expressed in ppm (δ) relative to Me_4Si (δ=0.00 ^1H spectra) or MeOD (central peak at δ=49.00, ^{13}C spectra) with the notations indicating the multiplicity of the signal. Evaporations were carried at <45 °C under diminished pressure.

Peptide components. a) ***N*-Benzyloxycarbonyl-D-glutamine *tert*-butyl ester** was prepared (60%) from Z-D—Glu-(NH_2)OH (1 g) and *tert*-butyl acetate (100 mL) as described[16] for the L-isomer; m.p. 93-94 °C, $[\alpha]_D$ +21°(EtOH). Lit. for the L-isomer: 93-94 °C,[8] $[\alpha]_D$ -20.6°(EtOH).[16] ^1H NMR δ 7.3 (Ph), 5.08 (CH_2Ph), 2.32-2.30-2.28 (m, 2 H, γ-CH_2 Gln), 2.03, 1.98 (2 m, 2 H, ß -CH_2 Gln), 1.44 (s, 9 H, Me_3 of Bu^t). ^{13}C NMR δ 177.6 (γ-$CONH_2$), 172.9 (α-CO), 158.6 (CO of Z), 67.6 ($PhCH_2O$), 55.9 (α-C), 32.6 (γ-C),

28.4 (ß-C), 28.2 (Me_3 of Bu^t). Other data are given in Tables 2 and 3.

Anal. Calcd for $C_{17}H_{24}N_2O_5$: C, 60.70; H, 7.19; N, 8.33. Found: C, 60.56; H, 7.23; N, 8.36.

(*b*) ***N*-Benzyloxycarbonyl-L-alanyl-D-glutamine *tert*-butyl ester** Z-D-Glu(NH_2)OBu^t (673 mg, 2 mmol) was hydrogenated (10% Pd/C in 90% AcOH) and after the removal of the catalyst and solvent, the residue was dried over P_2O_5 *in vacuo*. To the resulting acetate in dry tetrahydrofuran (THF, 25 mL) were added, under stirring, one equivalent each of *N*-ethylmorpholine (0.22 mL), 1-hydroxybenzotriazole (342 mg) and Z-L-Ala-OH (446 mg). The solution was cooled to 5 °C, $CuCl_2$ (269 mg) and dicyclohexylcarbodiimide (DCC) (450 mg) were added, and the reaction was run at 5 °C for 2 h and then at room temp. overnight. Dicyclohexylurea was filtered off, the filtrate was concentrated, and the residue was chromatographed on a silica gel column with solvent *A* (9:1) to give a homogenous product (570 mg, 70%) that was recrystallized from EtOAc—light petroleum (473 mg, 58%, m.p. 158–159 °C, $[\alpha]_D$ +2° (c 1.5, EtOH). ^{1}H NMR δ 7.3 (Ph), 5.09 (CH_2Ph), 2.32-2.31-2.25 (m,2 H, γ-CH_2 Gln), 2.10, 1.90 (2 m, 2 H, ß-CH_2 Gln), 1.44 (s, 9 H, Me_3 of Bu^t) 1.35 (d, 3 H, *J*=7 Hz, *Me*-Ala). ^{13}C NMR δ 175.9 (γ-CO Gln + CO Ala), 172.4 (α-CO Gln), 158.4 (CO of Z), 67.8 (PhCH_2O), 54.0 (α-C Gln), 52.3 (α-C Ala), 32.5 (γ-C Gln), 28.3 (ß-C Gln + Me_3 of Bu^t), 18.4 (Me Ala) Other data are given in Tables 2 and 3.

Anal. Calcd for $C_{20}H_{29}N_3O_6$: C, 58.95; H, 7.17; N, 10.31. Found: C, 58.97; H, 7.40; N, 10.35%.

***N*-{2-*O*-[Benzyl 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-ß-D-glucopyranosyl)-2,3-dideoxy-α-D-glucopyranosid-3-yl]-(*R*)-lactoyl}-L-alanyl-D-glutamine *tert*-butyl ester (3)**. To a solution of 2,[13] prepared by saponification of 1[14] (242 mg, 0.33 mmol), in dry THF (6 mL) were added with stirring at -16 °C, *N*-methylmorpholine (38 μL) and isobutyl chloroformate (45 μL). After 15 min of vigorous stirring the reaction mixture was combined with a precooled solution of L-Ala-D-Glu(NH_2)OBu^t [obtained by catalytic hydrogenation of Z-Ala-D-Glu(NH_2)OBu^t (138 mg, 0.34 mmol) in 90% AcOH and liberation of the AcOH salt with Et_3N in DMF (2 mL)], and the reaction mixture was allowed to reach room temp. (~ 5 h). The solution was concentrated, traces of DMF were removed by co-distillation with water, and the residue was chromatographed on a silica gel column with solvent *A* (9:1) to give **3** (220 mg, 68%), m.p. 203-205 °C (softening at 200 °C, $[\alpha]_D$ +48° (MeOH). ^{1}H NMR δ 7.35 (Ph), 5.25 (dd, $J_{3',4}$=9.5 $J_{3',2'}$=10 Hz, H-3'), 4.99 ($J_{4',5'}$=10 Hz, H-4'), 4.87(d, $J_{1,2}$=3.2 Hz, H-1), 4.73 (d, $J_{1',2'}$=8.5 Hz, H-1'), 2.34-2.26-2.19 (m, 2 H, γ-CH_2 Gln), 2.050, 2.007, 1.974, 1.909, 1.880 (5 s, 15 H, 2 NAc + 3 OAc), 1.451 (s, 9 H, *Me*$_3$ of Bu^t), 1.38 and 1.37 (2 d, 6 H; *J*=7 Hz, 2 *Me*CH). ^{13}C NMR δ 102.9 (C-1'), 97.3 (C-1), 80.4 (C-3), 78.3 (α-C Lact), 54.9 (C-2), 54.1 (α-C Gln), 50.3 (α-C Ala), 32.3 (γ-C Gln), 28.2 (*Me*$_3$ of Bu^t), 27.9 (ß-C Gln), 23.0, 22.8

(2 x Me NAc), 20.7, 20.6, 20.55 (3 x Me OAc) 19.7 (Me Lact), 18.4 (Me Ala).

Anal. Calcd for $C_{44}H_{65}N_5O_{19}$: C, 54.59; H, 6.77; N, 7.23. Found: C, 54.40; H, 6.84; N, 6.98.

N{2-*O*-[**Benzyl 2-acetamido-6-*O*-(2-acetamido-2-deoxy-ß-D-glucopyranosyl)-2,3-dideoxy-α-D-glucopyranosid-3-yl]-(*R*)-lactoyl}-L-alanyl-D-glutamine *tert*-butyl ester (4). To a stirred solution of **3** (225 mg, 0.23 mmol) in dry methanol (7 mL, ice-water bath) was added 0.1 *M* NaOMe (1.5 mL); after ∼1.5 h, TLC monitoring (solvent *B*, 5:2:1) showed a single slower-moving product. The solution was neutralised with Amberlite IR 120 (H^+) resin, filtered, concentrated, and the residue was dried (180 mg, 90%) over P_2O_5 *in vacuo*. The analytical sample, chromatographed on a silica gel column with solvent *B*, contained one molecule of water; m.p. 196-200 °C (softening at 129 °C), $[\alpha]_D$ +55° (MeOH). ^1H NMR δ 4.88 (d, $J_{1,2}$=3.2 Hz, H-1), 2.32-2.26-2.19 (m, 2 H, γ-CH_2 Gln), 1.956, 1.907 (2 x Me NAc), 1.422 (s, 9 H, Me_3 of Bu^t), 1.39, 1.37 (2 d, 6 H, Me Ala + Lact). ^{13}C NMR δ 177.6, 176.0, 174.9, 173.8, 173.3, 172.1 (6 CO: 2 x Gln, Ala, Lact + 2 x NAc); other data are given in Table 1.

Anal. Calcd for $C_{38}H_{59}N_5O_{16}$ x H_2O: C, 53.07; H, 7.15; N, 8.15; H_2O, 2.09. Found: C, 52.95; H, 7.07; N, 8.08; H_2O 2.2.

N-{2-*O*-[**2-Acetamido-6-*O*-(2-acetamido-2-deoxy-ß-D-glucopyranosyl)-2,3-dideoxy-D-glucopyranos-3-yl]-(*R*)-lactoyl}-L-alanyl-D-glutamine *tert*-butyl ester (5). A mixture of **4** (195 mg, 0.23 mmol) and 10% Pd/C (190 mg) in *tert*-butyl alcohol-acetic acid-water (3:3:2)[17] was shaken with H_2 [∼24 h, moni-

toring by TLC in solvent *B* (5:3:1)]. The catalyst was centrifuged off, washed with 1:1 EtOH—H_2O, the combined solutions were concentrated, and the residue, dissolved in a minimum amount of 0.1 *M* AcOH was passed through a Sephadex G-25 column with the same solvent. The relevant fractions were collected, and a solution of the residue in the minimum amount of hot absolute ethanol was diluted with dry ether; the precipitate was collected by centrifugation, washed with dry ether (3 x) and dried over P_2O_5. Yield: 138 mg (80%) of a white powder, m.p. 194-196 °C (softening at 192 °C), $[\alpha]_D$ +19° (MeOH), ^{1}H NMR δ 4.13, 4.16, (2 d, 0.3 H, $J_{5,6a}$=2, $J_{6a,6b}$= 11 Hz, H-6a MurNac-ß), 4.10, 4.05 (2 d, 0.7 H, $J_{5,6a}$ 1.9, $J_{6a,6b}$=10.9 Hz, H-6a MurNAc-α), 2.001 (s, 1 H, GlcNAc), 1.969, 1.949 (2 s, 0.3 + 0.7 H, MurNAc—ß,α), 1.465 (s, 9 H, Me_3 of Bu^t), 1.401, 1.389 (2 d, 6 H, *J*=7 Hz, Me Lact + Ala). Other data are given in Tables 1-3.

Anal. Calcd for $C_{31}H_{53}N_5O_{16}$: C, 49.52; H, 7.11; N, 9.32. Found: C, 49.48; H, 7.20; N, 9.12.

N-{2-*O*-[**Benzyl 2-acetamido-6-*O*-(2-acetamido-2-deoxy-ß-D-glucopyranosyl)-2,3-dideoxy-α-D-glucopyranosid-3-yl**](*R*)-**lactoyl**}-**L-alanyl-D-glutamine** (**6**). A centrifuge tube, containing **4** (127 mg, 0.15 mmol) was flushed with dry nitrogen; precooled trifluoroacetic acid (90%, 0.3 mL) was added, and the mixture was shaken until all of **4** had dissolved (~5 min). The clear solution was kept at 4 °C for 30 min, whereupon cold dry ether (~20 mL) was added and the precipitate was collected by centrifugation, washed (4 x) with dry ether and passed through a Sephadex G-25 column, as described for

5; dissolution in a minimum amount of absolute ethanol and precipitation with dry ether gave **6** (92 mg, 78%) as an amorphous powder, m.p. 194-196 °C (decomp.), $[\alpha]_D$ +58° (MeOH). ^{1}H NMR δ 7.35(m, 5 H, Ph), 4.89 (d, $J_{1,2}$=3.2 Hz, H-1), 2.19 (m, 2 H, γ-CH_2 Gln), 1.959, 1.910 (2 s, 2 x NAc), 1.39 (d, 6 H, J=7 Hz, Me Lact + Ala); ^{13}C NMR data are given in Table 1.

Anal. Calcd for $C_{34}H_{51}N_5O_{16}$: C, 51.97; H, 6.54; N, 8.91. Found: C, 51.82; H, 6.69; N, 8.79.

N-{2-*O*-[**2-Acetamido-6-*O*-(2-acetamido-2-deoxy-ß-D-glucopyranosyl)-2,3-dideoxy-D-glucopyranos-3-yl**](*R*)-**lactoyl**}-**L-alanyl-D-glutamine** (**7**). Catalytic hydrogenation of **6** (92 mg, 0.12 mmol) over 10% Pd/C (90 mg) and gel filtration of the crude product over a Sephadex G-25 column were performed as described for **5**. The product was precipitated from a solution in the minimum amount of dry methanol with dry ether to afford **7** as an amorphous hygroscopic powder with no definite m.p.; if exposed to the atmosphere, it turned gradually into a syrup. TLC (solvent *C*, peptide reagent): R_F ~0.30 (strong, α anomer), and ~ 0.28 (weak, ß-anomer), $[\alpha]_D$ +17°(MeOH). For analysis the compound was dried at 50 °C and 0.1 Torr for 48 h. ^{1}H NMR δ 4.14 (dd, ~0.3 H, $J_{6a,6b}$=11 Hz, H-6a MurNAc-ß), 4.09, 4.04 (dd,~0.7 H, $J_{5,6a}$=1.5 Hz $J_{6a,6b}$= 11 Hz, MurNAc-α), 2.30-2.25-2.20 (m, 2 H, γ-CH_2 Gln), 2.025, 1.960 (2 s,2 x NAc), 1.40, 1.38 (2 d, J=7 Hz, Me Lact + Ala); other data are given in Tables 1 and 3.

Anal. Calcd for $C_{27}H_{45}N_5O_{16}$: C, 46.61; H, 6.52; N, 10.07. Found: C, 46.40; H, 6.72; N, 9.87.

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