Carbohydrate Research, 186 (1989) 77–85
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

CONVENIENT SYNTHESES OF *O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-(1→6 AND 4)-*N*-ACETYLMURAMOYL-L-ALANYL-D-ISOGLUTAMINE

DARKO KANTOCI, DINA KEGLEVIĆ*,

Tracer Laboratory, Department of Organic Chemistry and Biochemistry, "Ruder Bošković" Institute, 41001 Zagreb (Yugoslavia)

AND ANDREW E. DEROME

The Dyson Perrins Laboratory, University of Oxford, Oxford OX1 3QY (Great Britain)

(Received June 29th, 1988; accepted for publication, August 18th, 1988)

Catalytic hydrogenation of *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 (**1**) afforded *N*-{2-*O*-[2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,3-dideoxy-D-glucopyranos-3-yl]-(*R*)-lactoyl}-L-alanyl-D-isoglutamine *tert*-butyl ester (**2**). Carboxyl-deprotection of **1** with trifluoroacetic acid gave *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 (**3**), which, on hydrogenolysis, afforded the title (1→6)-linked disaccharide–dipeptide **4**. Coupling of benzyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside (**6**) with the benzyl ester of L-alanyl-D-isoglutamine gave *N*-{2-*O*-[benzyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-2,3-dideoxy-α-D-glucopyranosid-3-yl]-(*R*)-lactoyl}-L-alanyl-D-isoglutamine benzyl ester (**7**). *O*-Deacetylation of **7** in basic media was accompanied by α → γ transamidation reaction at the isoglutaminyl residue. Coupling of the *O*-deacetylated derivative (**8**) of **6** with the dipeptide benzyl ester yielded *N*-{2-*O*-[benzyl 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-2,3-dideoxy-α-D-glucopyranosid-3-yl]-(*R*)-lactoyl}-L-alanyl-D-isoglutamine benzyl ester (**9**) having HO-3′,4′,6′ unsubstituted. Catalytic hydrogenation of **9** afforded the title (1→4)-linked disaccharide–dipeptide **10**.

INTRODUCTION

In the preceding paper[1], it was shown that alkaline *O*-deacetylation of the benzyl α-glycoside of *O*-acetylated [β-GlcNAc-(1→6)-MurNAc]-L-alanyl-D-isoglutamine benzyl ester resulted in α → γ transamidation at the isoglutaminyl

*Author for correspondence.

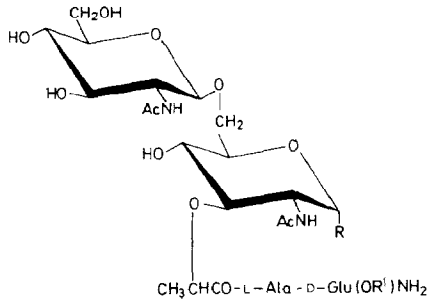
0008-6215/89/$ 03.50

residue. The base-catalysed isoglutamine ⇄ glutamine rearrangement involves a cyclic imide intermediate[2]. Deprotection of the fully acetylated benzyl α-glycoside of [β-GlcNAc-(1→6)-MurNAc]-L-alanyl-D-isoglutamine benzyl ester by application of the sequence brief catalytic hydrogenation in aqueous 90% ethanol, *O*-deacetylation with methanolic sodium methoxide, and prolonged catalytic hydrogenation has been reported[3]. The *tert*-butyl ester group at the isoglutaminyl residue of the protected disaccharide–dipeptide is not affected[1] under Zemplén conditions of *O*-deacetylation so that the *O*-acetyl groups can be removed from the sugar moiety without affecting the peptide portion. Thus, *N*-{2-*O*-[benzyl 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,3-dideoxy-α-D-glucopyranos-3-yl]-(*R*)-lactoyl}-L-alanyl-D-isoglutamine *tert*-butyl ester (**1**) was obtained in high yield, and we now describe its partial and total deprotection to give **2**–**4**.

The partially protected, unacetylated disaccharide component was used for the synthesis of the [β-GlcNAc-(1→4)-MurNAc]-L-alanyl-D-isoglutamine conjugate **10**. Compound **10** has been prepared by various routes[4–9] and we have reported[10] an efficient synthesis of benzyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (**5**) *via* the fully protected [β-GlcNPhth-(1→4)-MurNAc] methyl ester derivative. We now describe the conversion of **5** into **10**.

RESULTS AND DISCUSSION

Deprotection of the anomeric position in **1** was effected by catalytic hydrogenolysis (10% Pd–C) in *tert*-butyl alcohol–acetic acid–water (the use of *tert*-butyl alcohol in place of ethanol prevents[11] transesterification). In this solvent system, *O*-debenzylation proceeded much faster than in aqueous acetic acid or aqueous acetic acid–ethanol to give *N*-{2-*O*-[2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,3-dideoxy-αβ-D-glucopyranos-3-yl]-(*R*)-lactoyl}-L-alanyl-D-isoglutamine *tert*-butyl ester (**2**, 89%), the structure of which was confirmed by the ^{1}H- and ^{13}C-n.m.r. data (Tables I and II). The αβ-ratio was 3:1.



1	R = OBn	R^1 = Bu^t
2	R = OH,H	R^1 = Bu^t
3	R = OBn	R^1 = H
4	R = OH,H	R^1 = H

TABLE I

^{1}H-N.M.R. DATA FOR THE SUGAR MOIETY PROTONS IN **2**[a]

Unit	*Chemical shifts in δ and (J) in Hz*									
	H-1 ($J_{1,2}$)	*H-2* ($J_{2,3}$)	*H-3* ($J_{3,4}$)	*H-4* ($J_{4,5}$)	*H-5* ($J_{5,6a}$)	*H-6a* ($J_{5,6b}$)	*H-6b* ($J_{6a,6b}$)	*NAc*	*Me* (J)	*CH* (J)
MurNAc α	5.13d (3.5)	3.84dd (10.5)	3.61dd (9)	3.44dd (10)	3.93dd (2)	4.08dd (5.5)	3.72dd (11.5)	1.950s	1.40d (7)	4.39q (7)
MurNAc β	4.52d (8.5)	3.68dd (10.5)	3.41dd (9)	3.38dd (10)	3.45m (2)	4.14dd (5.5)	3.88dd (11.5)	1.964s	1.40d (7)	4.33q (7)
GlcNAc β	4.49d[b] (8.5) 4.50d[c] (8.5)	3.65dd (10.5)	3.47dd (9.5)	3.35dd (10.5)	3.28ddd (2)	3.88dd (5.5)	3.69dd (12)	2.004		

[a]Measured at 500 MHz for a solution in CD_3OD (internal Me_4Si); data for the peptide moiety are given in the Experimental. [b]Linked to α-MurNAc. [c]Linked to β-MurNAc.

TABLE II

^{13}C-N.M.R. CHEMICAL SHIFTS (δ) DATA[a] FOR COMPOUNDS **2**, **4**, AND **10**

Atom	**2**		**4**		**10**	
	α	β	α	β	α	β
C-1	92.43	97.20	92.27	97.03	91.07	96.58
C-2	55.48	58.13	55.46	57.92	55.76	57.25
C-3	80.26	83.27	80.03	83.18	77.09[c]	79.42
C-4	72.01	71.69	71.97	71.41	76.64[c]	
C-5	72.30	77.02	72.23	76.83	72.52[d]	
C-6	70.32	70.43	70.19	70.19	61.29	
C-1′	103.19	103.35	103.17		101.64	
C-2′	57.53		57.31		57.85	
C-3′	76.10		75.99		75.34	
C-4′	72.18		71.96		72.52[d]	
C-5′	78.01		77.88		77.88	
C-6′	62.83		62.68		63.04	
CH_3-NAc	22.88, 23.15		22.97 (2×)		22.69, 22.91	
CO-NAc	173.41, 173.82		173.31, 173.95		173.93, 174.27	
Lactyl						
α-*C*H	78.08	78.42	77.98	78.36	78.05	
CH_3	19.74	19.64	19.82	19.71	19.53	19.72
Alanyl						
α-*C*H	50.08	50.73	50.76	50.67	50.22	
CH_3	17.68	17.77	17.85	18.00	17.55	
Isoglutamyl						
α-*C*H	53.71	53.56	54.25	54.09	53.72	
β-CH_2	28.08	28.09	28.47	28.67	27.82	
γ-CH_2	32.72	32.72	32.73	32.73	31.09	
Me_3 Bu^t	28.36	28.36				
Cq Bu^t	81.89	81.89				

[a]Relative to the central CD_3OD peak at 49.00 p.p.m. in methanol-d_4; compound **2** recorded at 125 MHz, **4** at 70 MHz, and **10** at 22.5 MHz; carbonyl carbon resonances of the peptide portion are not shown. [b]For a solution of **10** measured in D_2O referenced to CD_3OD. [c]The signals may be interchanged. [d]Duplicated.

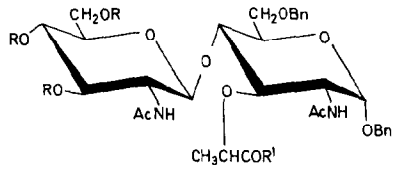
Glycosylation of the MurNAc residue in **2** at HO-6 caused a downfield shift for the signals of H-6a,6b, as compared to those of 2-acetamido-2-deoxy-D-glucopyranose[12]. The large (9.5–10.5 Hz) $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ values observed for each anomer indicated that the $^4C_1$(D) conformation of the disaccharide moiety was unaffected by the peptide substitution.

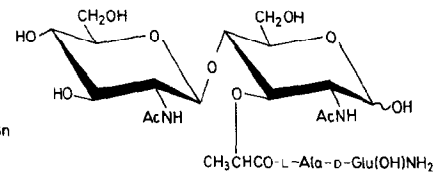
The acidic conditions required for the cleavage of the *tert*-butoxy group may also hydrolyse glycosidic bonds[13,14]. In the glycopeptide series, involving di-*N*-acetylchitobiose bound *N*-glycosylically to the peptide chain, removal of the *N*-*tert*-

butoxycarbonyl (Boc) group in the HO-unsubstituted molecule was accompanied by partial cleavage of the *O*-glycosylic bond[15]. However, brief treatment of **1** with aqueous 90% trifluoroacetic acid at room temperature, followed by immediate precipitation of the product with dry ether, ensured selective hydrolysis of the Bu^t ester group to give 80% of the benzyl α-glycoside (**3**) of the [β-GlcNAc-(1→6)-MurNAc]-dipeptide. The removal of trifluoroacetic acid by distillation, or the use of formic acid, resulted in degradation.

Catalytic hydrogenation of **3** afforded 80% of *N*-{2-*O*-[2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,3-dideoxy-αβ-D-glucopyranos-3-yl]-(*R*)-lactoyl}-L-alanyl-D-isoglutamine (**4**). The anomers were separable by t.l.c. [chloroform–methanol–water–acetic acid (30:30:5:1)] and the αβ-ratio was ~3:1. The structure of **4** was confirmed by analytical and ^{1}H- and ^{13}C-n.m.r. data (Table II).

The protected [β-GlcNAc-(1→4)-MurNAc]-L-alanyl-D-isoglutamine benzyl ester **7** was synthesised (in yields of 79 and 78%, respectively, after column chromatography) by two routes (Woodward reagent K and mixed anhydride method, respectively) using benzyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside (**6**) (obtained[10] by saponification of the methyl ester group of **5**) and L-alanyl-D-isoglutamine benzyl ester.





5 R = Ac, R^1 = OMe

6 R = Ac, R^1 = OH

7 R = Ac, R^1 = α

8 R = H, R^1 = OH

9 R = H, R^1 = α α = L-Ala-D-Glu(OBn)NH$_2$

10

As expected[1], *O*-deacetylation of **7** in alkaline media was accompanied by α → γ transamidation of the isoglutaminyl residue and t.l.c. of the reaction mixtures revealed the isoglutamine and glutamine derivatives. In order to avoid the use of alkaline media, the disaccharide component, having the GlcNAc residue unsubstituted, was synthesised. Treatment of a 1,4-dioxane solution of **5** with 0.1M KOH for 2 days resulted in *O*-deacetylation of the GlcNAc residue and saponification of the MurNAc methyl ester group to give benzyl 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-[(*R*)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside (**8**, 90%) as a highly hygroscopic solid.

Coupling of **8** with L-alanyl-D-isoglutamine benzyl ester, performed with the Woodward reagent K in acetonitrile–*N*,*N*-dimethylformamide, gave, after column

chromatography, 85% of the partially protected disaccharide–dipeptide benzyl ester **9**. Catalytic hydrogenation of **9** in *tert*-butyl alcohol–acetic acid–water removed the benzyl groups to afford, after purification on Biogel P-2, 81% of *N*-{2-*O*-[2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,3-dideoxy-αβ-D-glucopyranos-3-yl]-(*R*)-lactoyl}-L-alanyl-D-isoglutamine (**10**), which was characterised by analytical and n.m.r. spectral data (Table II).

The ^{13}C-resonances of the isomeric β-(1→6)- and β-(1→4)-linked disaccharide–dipeptides **2**, **4**, and **10** were assigned (Table II) by comparison with data for related mono- and di-saccharides and dipeptides. The reducing *N*-acetylmuramoyl residue exhibited two sets of resonances for the α and β anomers; in the spectrum of **2** (recorded at 125 MHz), two resonances for each of the six MurNAc carbon atoms could be assigned. The chemical shifts of the signals associated with the 2-acetamido-2-deoxy-β-D-glucopyranosyl residue agree well with those for the parent disaccharides[1,10] and the reported values[16] (adjusted to the methanolic central peak). The sites of glycosidation are clearly reflected in the downfield chemical shifts for the C-6 resonances in the spectra of **2** and **4** and for the C-4 resonance in the spectrum of **10**, as well as in the upfield chemical shifts for C-5 in the spectra of each of the compounds and for C-3 in the spectrum of **10**. The resonances observed for the peptide moiety agree well with the values observed for the parent dipeptides.

EXPERIMENTAL

The general conditions were as reported in the preceding paper[1]. The ^{1}H-(100 MHz) and ^{13}C-n.m.r. (22.5 MHz) spectra were recorded with a Jeol FX 90 F.t. spectrometer for the solutions (internal Me_4Si) indicated, if not stated otherwise. The ^{1}H- (500 MHz) and ^{13}C-n.m.r. (125 MHz) spectra were recorded with a Bruker AM-500 spectrometer operating in the F.t. mode and equipped with a Bruker Aspect 3000 computer. Solvent systems for chromatography were *A*, 9:1 chloroform–methanol; *B*, ethyl acetate–ethanol–water (proportions are given in the text); *C*, 12:12:1 chloroform–methanol–water; *D* 30:30:5:1 chloroform–methanol–water–acetic acid; *E*, 1:1:1:1 1-butanol–ethyl acetate–acetic acid–water.

N-{*2*-O-[*2-Acetamido-6*-O-*(2-acetamido-2-deoxy*-β-D-*glucopyranosyl)-2,3-dideoxy*-D-*glucopyranos-3-yl*]-(R)-*lactoyl*-}-L-*alanyl*-D-*isoglutamine* tert-*butyl ester* (**2**). — A mixture of **1**[1] (180 mg) and 10% Pd–C (180 mg) in *tert*-butyl alcohol–acetic acid–water (3:3:2, 8 mL) was shaken under H_2 at 1 atms. and room temperature until t.l.c. [solvent *B* (5:3:1)] indicated (~20 h) complete conversion of **1** (R_F ~0.8) into αβ-**2** (R_F ~0.50 and 0.4, α,β-ratio 3:1). The suspension was centrifuged, the catalyst was washed with 1:1 EtOH–H_2O (5 mL), and the combined supernatant solutions were concentrated. The dry (P_2O_5) residue was dissolved in the minimum amount of hot methanol, dry ether was added to the cooled solution, and the precipitate was collected by centrifugation and washed (3 ×) with ether to give **2** as a powder (140 mg, 89%), m.p. 214–216°, $[\alpha]_D$ +20° (*c* 1, methanol). ^{1}H-N.m.r. data

(500 MHz, CD_3OD): δ 2.32 (m, γ-CH_2 Glu), 2.18 and 1.87 (2 m, 2 H, β-CH_2 Glu), 4.32 (m, α-CH Glu), 4.28 (q, *J* 7 Hz, α-CH Ala), 1.38 (d, 3 H, *J* 7 Hz, Me Ala), 1.449 (s, 9 H, Me_3C), other signals are given in Table I and the ^{13}C signals in Table II.

Anal. Calc. for $C_{31}H_{53}N_5O_{16}$: C, 49.52; H, 7.11; N, 9.32. Found: C, 49.78; H, 7.30; N, 9.17.

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* (**3**). — Trifluoroacetic acid (90%, 0.8 mL) at +5° was added to **1** (125 mg, 0.15 mmol) under nitrogen, and the mixture was shaken until all of **1** had dissolved (~5 min). The clear solution was kept for 20 min at room temperature, cold dry ether was added, and the precipitate was collected by centrifugation, washed (4 ×) with dry ether, and dried overnight. A solution of the solid residue (118 mg) in the minimum amount of methanol was diluted with dry ether and the precipitate was treated as described for **2**, to give **3** as an amorphous powder (94 mg, 80%), m.p. 198–200°, $[\alpha]_D$ +64° (*c* 1, 4:1 methanol–water). ^1H-N.m.r. data (CD_3OD): δ 7.36, 7.34 (Ph), 4.85 (d, $J_{1,2}$ 3.2 Hz, H-1), 2.45, 2.38, 2.31 (2 H, γ-CH_2 Glu), 1.956, 1.909 (2 NAc), 1.38 and 1.36 (2 d, 6 H, *J* 7 Hz, 2 *Me*CH). Lit.[3] m.p. 196–198°, $[\alpha]_D$ +55° (water); no analytical data given.

Anal. Calc. for $C_{34}H_{51}N_5O_{16}$: C, 51.97; H, 6.54; N, 8.91. Found: C, 51.83; H, 6.70; N, 8.82.

N-{2-O-[*2-Acetamido-6-O-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-2,3-dideoxy-*D*-glucopyranos-3-yl*]-(R)-*lactoyl*}-L-*alanyl*-D-*isoglutamine* (**4**). — Hydrogenation of **3** (102 mg) in *tert*-butyl alcohol–acetic acid–water (3:3:2) over 10% Pd–C (110 mg) was performed as described for **2**. The product was precipitated from solution in the minimum amount of methanol with dry ether to afford **4** as a hygroscopic powder, m.p. 174–178°, $[\alpha]_D$ +18° (*c* 1, methanol), 0° (*c* 1, water), that turned gradually into a syrup if exposed to the atmosphere. T.l.c. (solvent *D*, peptide reagent) R_F ~0.32 (strong, α anomer) and ~0.29 (weak, β anomer), R_F ~0.3 (solvent *E*). Lit[3]: amorphous, $[\alpha]_D$ +5° (H_2O). For ^{13}C-n.m.r. data, see Table II.

Anal. Calc. for $C_{27}H_{45}N_5O_{16}\cdot 2.5\ H_2O$: C, 43.78; H, 6.80; N, 9.46; H_2O, 6.08. Found: C, 44.00; H, 7.08; N, 9.41; H_2O, 6.25.

N-{2-O-[*Benzyl 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-glucopyranosyl)-6-O-benzyl-2,3-dideoxy-α-*D*-glucopyranosid-3-yl*]-(R)-*lactoyl*}-L-*alanyl*-D-*isoglutamine benzyl ester* (**7**). — (*a*) To a solution of **6**[10] (77 mg) and Et_3N (20 μL) in acetonitrile–*N*,*N*-dimethylformamide (2:1, 2 mL) were added at 0°, with stirring, Woodward reagent K (28 mg, 0.1 mmol) and, after dissolution, L-Ala–D-Glu(OBn)NH_2 [liberated from 32 mg of the HCl salt with Et_3N (20 μL) in the same solvent (1 mL)]. The mixture was stirred overnight at room temperature and then concentrated, and water (20 mL) was added to the residue. Column chromatography (solvent *A*) of the precipitate gave **7** (82.5 mg, 79%), m.p. 226–228° (from EtOAc–Me_2CO–di-isopropyl ether), $[\alpha]_D$ +32° (*c* 0.7, chloroform).

N.m.r. data ($CDCl_3$): ^1H, δ 7.5–7.3 (15 H, 3 Ph), 5.11 (s, CO_2CH_2Ph), 2.04, 2.00, 1.93 (15 H, 2 NAc + 3 OAc), 1.40 and 1.36 (2 d, 6 H, *J* 7 Hz, 2 *Me*CH); ^{13}C, δ 99.2 (C-1′), 96.5 (C-1), 61.6 (C-6′), 54.3, 53.7 (C-2,2′), 52.5 (α-C Glu), 49.7 (α-C Ala), 30.7 (γ-C Glu), 26.4 (β-C Glu), 18.8 (CH_3 Lact), 17.0 (CH_3 Ala).

Anal. Calc. for $C_{54}H_{69}N_5O_{19}$: C, 59.39; H, 6.37; N, 6.41. Found: C, 59.63; H, 6.57; N, 6.58.

(*b*) To a solution of **6** (80 mg) in dry tetrahydrofuran (6 mL) at −18° were added, with stirring, *N*-methylmorpholine (11 μL) and isobutyl chloroformate (13 μL) and, after 10 min, a precooled solution of L-Ala–D-Glu(OBn)NH_2 [liberated from 36 mg of the HCl salt with Et_3N in *N,N*-dimethylformamide (1 mL)]. Stirring was continued for 1 h at −15° and, after attaining room temperature, for an additional 3 h. The solvent was evaporated, and water (20 mL) and saturated aqueous $NaHCO_3$ (0.5 mL) were added to the residue. Column chromatography (solvent *A*) of the precipitate gave **7** (85 mg, 78%).

Benzyl 2-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-O-benzyl-[(R)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside (**8**). — To a solution of **5** (82 mg) in 1,4-dioxane (4 mL) was added 0.5M KOH (2 mL), and the solution was kept, under occasional stirring, for 2 days at room temperature [t.l.c. monitoring, solvent *B* (17:10:1)], then neutralised with Amberlite IR-120 (H^+) resin, filtered, and concentrated. Column chromatography (17:10:1 EtOAc–EtOH–AcOH) of the residue gave **8** (61 mg, 90%) as an amorphous and highly hygroscopic solid, $[\alpha]_D$ +29° (*c* 1, methanol). N.m.r. data (CD_3OD): ^1H, δ 7.36, 7.29 (10 H, 2 Ph), 5.14 (d, $J_{1,2}$ 2 Hz, H-1), 1.95 (s, 6 H, 2 NAc), 1.48 (d, *J* 6.8 Hz, Me Lact); ^{13}C, δ 180.9 (CO_2H), 173.3, 171.9 (2 CO NAc), 101.9 (C-1′), 97.18 (C-1), 69.2 (C-6), 61.7 (C-6′), 58.0 (C-2), 55.6 (C-2′), 23.1, 22.7 (2 NAc), 19.7 (CH_3 Lact).

Anal. Calc. for $C_{33}H_{44}N_2O_{13}\cdot 2\,H_2O$: C, 55.61; H, 6.79; H_2O, 5.06. Found: C, 55.94; H, 6.88; H_2O, 4.80.

N-{2-O-[*Benzyl 2-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-O-benzyl-2,3-dideoxy-α-D-glucopyranosid-3-yl*]-(R)-*lactoyl*}-L-*alanyl*-D-*isoglutamine benzyl ester* (**9**). — A solution of dry **8** (74 mg) and Et_3N (20 μL) in acetonitrile–*N,N*-dimethylformamide (2:1, 4 mL) was treated with the Woodward reagent K (30 mg) and L-Ala–D-Glu(OBn)NH_2 [liberated from its HCl salt (40 mg)] as described for **7**. After concentration of the mixture, the residue was triturated with dry ether (50 mL). Column chromatography (20:15:6:2:4 $CHCl_3$–^{i}PrOH–MeOH–AcOH–H_2O) of the crude product gave **9** (90 mg, 85%), m.p. 152–154°, $[\alpha]_D$ +13° (*c* 1, chloroform). N.m.r. data: ^1H (CD_3OD), δ 7.3–7.2 (15 H, 3 Ph), 5.1 (s, CO_2CH_2Ph), 4.94 (d, $J_{1,2}$ 2.9 Hz, H-1), 2.4–2.3 (m, γ-CH_2 Glu), 1.96, 1.89 (2 NAc), 1.39 and 1.38 (2 d, *J* 7 Hz, 2 *Me*CH); ^{13}C (CD_3OD + $CDCl_3$), δ 101.2 (C-1′), 97.4 (C-1), 69.3 (C-6), 63.0 (C-6′), 55.9, 54.6 (C-2,2′), 49.5 (α-C Ala), 30.6 (γ-C Glu), 27.9 (β-C Glu), 19.4 (CH_3 Lact), 17.9 (CH_3 Ala).

Anal. Calc. for $C_{48}H_{63}N_5O_{16}$: C, 59.68; H, 6.57; N, 7.25. Found: C, 59.40; H, 6.83; N, 7.10.

N-{2-O-[*2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,3-di-*

deoxy-D-glucopyranos-3-yl]-(R)-*lactoyl*}-L-*alanyl*-D-*isoglutamine* (**10**). — Catalytic hydrogenation of **9** (90 mg) was performed in *tert*-BuOH–AcOH–H_2O (3:3:2, 6 mL) with 10% Pd–C (70 mg), as described for **2**, to give a residue which was passed through a column (2.5 × 90 cm) of Biogel P-2 with aqueous 1% acetic acid. The appropriate fractions were concentrated, the residue was dissolved in a small amount of dry methanol, and dry ether was added to precipitate **10** as a hygroscopic solid (52 mg, 81%), m.p. 170–174°, $[\alpha]_D$ +12° (*c* 1.8, methanol), +1° (*c* 1.4, H_2O), R_F ~0.31 (strong, α anomer) and 0.29 (weak, β anomer) [t.l.c. (peptide reagent), solvent *D*]; R_F ~0.45 (solvent *E*); lit.[4,16] m.p. 170–178°, $[\alpha]_D$ +0.6° (water); $[\alpha]_D$ −2° (water)[17]. ^{1}H-N.m.r. data (D_2O): δ 5.23 (d, $J_{1,2}$ 2 Hz, H-1), 2.4–2.3 (m, γ-CH_2 Glu), 2.04 (s, 3 H, NAc GlcNAc), 1.96, 1.94 (3 H, ~2:1, NAc MurNAc), 1.43 (d, *J* 7.08 Hz, Me Lact), 1.38 (d, *J* 6.6 Hz, Me Ala). The ^{13}C-n.m.r. data are given in Table II. For analysis, **10** was dried at 60° and 0.1 Torr for 48 h.

Anal. Calc. for $C_{27}H_{45}N_5O_{16}$: C, 46.62; H, 6.52; N, 10.08. Found: C, 46.79; H, 6.75; N, 9.99.

ACKNOWLEDGMENTS

We thank Dr. Jurka Kidrič ("Boris Kidrič" Institute, Ljubljana, Slovenia) for providing the 70-MHz ^{13}C-n.m.r. spectrum, Mrs. B. Metelko and Mr. Z. Marinić for recording the other n.m.r. spectra, and Mrs. D. Orlić for technical assistance.

REFERENCES

1 D. Keglević and A. E. Derome, *Carbohydr. Res.*, 186 (1989) 63–75.
2 M. Bodanszky and J. Martinez, in E. Gross and J. Meienhofer (Eds.), *The Peptides*, Vol. 5B, Academic Press, New York, 1983, pp. 111–216.
3 A. E. Zemljakov, V. J. Czirova, and A. J. Khorlin, *Bioorg. Chem.*, 12 (1986) 1637–1640.
4 S. Kusumoto, K. Yamamoto, and T. Shiba, *Tetrahedron Lett.*, (1978) 4407–4410.
5 P. L. Durette, E. P. Meitzner, and T. Y. Shen, *Tetrahedron Lett.*, (1979) 4013–4016.
6 P. L. Durette, E. P. Meitzner, and T. Y. Shen, *Carbohydr. Res.*, 77 (1979) c1–c4.
7 M. Kiso, Y. Kaneda, R. Shimizu, and A. Hasegawa, *Carbohydr. Res.*, 83 (1980) c8–c11.
8 L. I. Rostovtseva, T. M. Andronova, V. P. Malkova, I. B. Sorokina, and V. T. Ivanov, *Bioorg. Chem.*, 7 (1981) 1843–1858.
9 J. Farkaš, M. Ledvina, J. Brokeš, J. Ježek, J. Zajíček, and M. Zaoral, *Carbohydr. Res.*, 163 (1987) 63–72.
10 D. Kantoci, D. Keglević, and A. E. Derome, *Carbohydr. Res.*, 162 (1987) 227–235.
11 P. C. Crofts, J. H. H. Markes, and H. N. Rydon, *J. Chem. Soc.*, (1959) 3610–3616.
12 S. J. Perkins, L. N. Johnson, D. C. Phillips, and R. A. Dwek, *Carbohydr. Res.*, 59 (1977) 19–34.
13 W. A. R. van Heeswijk, H. G. J. Visser, and J. F. G. Vliegenthart, *Carbohydr. Res.*, 58 (1977) 494–497.
14 G. O. Aspinall and M. C. Knebl, *Carbohydr. Res.*, 157 (1986) 261–268.
15 H. Kunz and H. Waldmann, *Angew. Chem. Int. Ed. Engl.*, 24 (1985) 883–885.
16 S. Kusumoto, K. Yamamoto, M. Imoto, M. Imoto, M. Inage, M. Tsujimoto, S. Kotani, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 59 (1986) 1411–1417.
17 M. Kiso, Y. Kaneda, R. Shimizu, and A. Hasegawa, *Carbohydr. Res.*, 104 (1982) 253–269.