Carbohydrate Research, 180 (1988) 147–151
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

The synthesis and structure of some *N*-(L-aspart-4-oyl)-β-D-xylopyranosylamine derivatives*,†

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(Received July 15th, 1987; accepted for publication, March 5th, 1988)

The *N*-glycosylic fragment of many glycoproteins consists of 2-acetamido-2-deoxy-β-D-glucose and L-asparagine[1]. However, L-asparagine can be linked also to D-glucose[2] or 2-amino-2-deoxy-D-galactose[3] (*e.g.*, in cell-wall glycoproteins of halobacteria) and, occasionally, different *N*-glycosylic systems appear involving, for instance, L-glutamine and α-D-glucose (nephritogenic glycopeptide from the glomerular basal membrane of the rat)[4,5]. Attempts have been made to confirm these structures by synthetic approaches[5–7].

We now report the synthesis of the title compound, since D-xylose may occur in glycoproteins as well as amino acids bearing side functional groups capable of forming *N*-glycosylic linkages.

The title compound was synthesised by a pathway leading to its analogue, 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine[6]. Condensation of 2,3,4-tri-*O*-acetyl-β-D-xylopyranosylamine[8] (**1**) and 1-benzyl *N*-benzyloxycarbonyl-L-aspartate[9] (**2**) mediated by dicyclohexylcarbodi-imide gave only 35.8% of **3**, which was homogeneous. The structure of **3** was confirmed by elemental analysis and the i.r. and ^{1}H-n.m.r. spectral data (see Experimental and Table I). The f.d. and f.a.b. (positive ion) mass spectra contained peaks at *m/z* 615 for $[M + 1]^+$. The large values (~10 Hz) of $J_{1,2}$, $J_{2,3}$, $J_{3,4}$, and $J_{4,5a}$ in Table I for **3** confirm the $^4C_1$ conformation and β configuration of the sugar moiety. The ^3J coupling (9.25 Hz) of the anomeric and amide protons is indicative of an antiperiplanar orientation of the C-1–H and N-6–H bonds, and suggests at least partial hindering of rotation around the C-1–N-6 linkage. The magnetic non-equivalance of H-7,7 may be due to the neighbouring groups (chiral C-8 with the urethane and ester groupings). The high value (8.25 Hz) of $J_{8,9}$ indicates C-8–H and N-9–H to be near to antiperiplanar (see formula in Table I).

The magnetic non-equivalence of the methylene protons in one of the benzyl

*Presented at the 4th European Carbohydrate Symposium, Darmstadt, F.R.G., July 12–17, 1987.
†Glycosylamino Acids with the *N*-Glycosylic Linkage, Part I.

0008-6215/88/$ 03.50

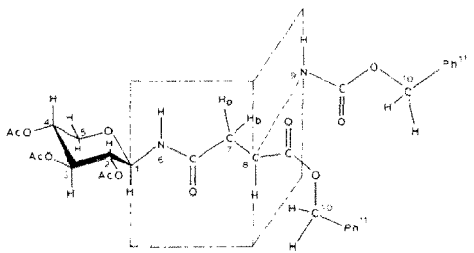
TABLE I

^{13}H-N.M.R. DATA [360 MHz, $(CD_3)_2SO$] OF **3–5**

Compound	*Chemical shifts (δ, p.p.m.)*												
	H-1[a]	*H-2*	*H-3*	*H-4*	*H-5e*	*H-5a*	*Ac*	*H-7a* β-CH_2	*H-7b* β-CH_2	*H-8* α-*CH*	*H-10a,b,c* CH_2-*Ph*	*H-6* *H-9* *(N-H)*	*H-11* *(Ar)*
3	5.227dd	4.783dd	5.265dd	4.807ddd	3.873dd	4.543dd	2.000s 1.942s 1.842s	2.670dd	2.550dd	4.493ddd	5.132d 5.084d 5.021s	8.750d 7.740d	7.400– 7.252m
4	5.144dd	4.793dd	5.201dd	4.801ddd	3.878dd	3.487dd	1.990s 1.967s 1.959s	2.744dd	2.459dd	3.472dd		9.100d 7.846– 7.169b	
5	4.588dd	3.090dd	3.155dd	3.260ddd	3.641dd	3.028dd		2.768dd	2.455dd	3.510dd		9.083d	

Compound	*Coupling constants (Hz)*										
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5e}$	$J_{4,5a}$	$J_{5e,5a}$	$J_{1,6}$	$J_{7a,7b}$	$J_{7a,8}$	$J_{7b,8}$	$J_{8,9}$	$J_{10a,10b}$
3	9.25	9.25	9.25	5.5	11.0	−11.0	9.25	−15.5	6.0	7.75	8.25	12.3
4	9.25	9.25	9.5	5.5	10.0	−11.5	~8	−16.2	3.75	8.25		
5	8.75	8.75	5.75	5.25	10.5	−11.0	8.5	−16.5	4.0	8.75		

[a]Numbering of atoms:

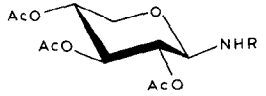


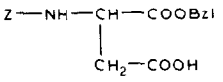
groups in **3** is striking. The PhCH_2 signals appeared at δ 5.021 (s) and at δ 5.132 and 5.084 (AB system). The assignment of these signals to the urethane and ester moieties is tentative and is based on literature evidence[10,11].

Hydrogenation of **3** over Pd/C in dilute acetic acid removed the protecting groups from the amino acid moiety to give **4** (88%), which crystallised as an ethanolate. The mass spectra of **4** contained peaks for $[M + 1]^+$ at *m/z* 391 (f.d. and positive ion f.a.b.). The β configuration and the $^4C_1$ conformation of the sugar ring were indicated by the n.m.r. data (Table I).

O-Deacetylation of **4** with 0.6M LiOH was rapid and 68% of crystalline **5** was isolated subsequently as a hydrate. The mass spectra of **5** contained peaks for $[M + 1]^+$ at *m/z* 265 (f.d. and positive ion f.a.b.) and for $[M - 1]^-$ at *m/z* 263 (negative ion f.a.b.).

The structure assigned to **5** was verified by the ^{1}H-n.m.r. spectra (standard and COSY 45, and after deuterium exchange). The large ^3J values of the sugar ring protons indicated the β configuration and $^4C_1$ conformation. Well-shaped signals for the protons in the sugar and the amino acid moieties are displayed, and the absence of doubling indicated that anomerisation at C-1, inversion of configuration of C_α, or isomerisation of the amino acid residue had not occurred.



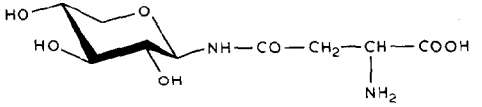


2

1 R = H

3 R = $COCH_2CH(NHZ)COOBzl$

4 R = $COCH_2CH(NH_2)COOH$



5

EXPERIMENTAL

Reactions were monitored by t.l.c. on Kieselgel 60 (Merck, 5553) or 60 F_{254} (Merck, 5719), using *A*, benzene–acetone (3:1); *B*, ether–hexane (10:1); *C*, benzene–acetic anhydride–acetic acid (20:3:1); *D*, carbon tetrachloride–acetone (1:1); and *E*, 1-butanol–acetic acid–water (12:3:5); and detection with iodine vapour, by heating to 200°, with u.v. light, or with ninhydrin. Optical rotations were measured on a Hilger–Watt polarimeter or on a JASCO J-20 spectropolarimeter. I.r. spectra were recorded (Nujol) with a Specord 71 spectrophotometer. ^{1}H-N.m.r. spectra (standard and COSY 45 spectra) were recorded with a Bruker (360 MHz) instru-

ment for solutions in $(CD_3)_2SO$ (internal Me_4Si). Mass spectra were obtained with an MAT 711 (f.d.), 8222, or CH-7A (f.a.b.) spectrometer; glycerine was used as the matrix.

Melting points are uncorrected.

1-Benzyl N-*benzyloxycarbonyl*-L-*aspartate* (**2**). — *N*-Benzyloxycarbonyl-L-aspartic acid anhydride[12] (5.43 g) was treated with benzyl alcohol (3.3 mL) at 90–105° by the method of Bergmann *et al.*[13]. The crude product (6.5 g, 83.4%; m.p. 72–82°) was suspended in ether (17.3 mL), and a solution of dicyclohexylamine (3.6 mL) in ether (5.2 mL) was added dropwise during 15 min with vigorous stirring. The mixture was then stirred for 80 min at room temperature. The ether was distilled off and the residual material was recrystallised twice from ethyl acetate to give the dicyclohexylammonium salt of 1-benzyl Z-aspartate (4.42 g, 45.6%), m.p. 107–110.5°. A suspension of the salt (4.31 g) in ice-cooled M sulphuric acid (15 mL) was stirred for 1 h, ether (25 mL) was added, the aqueous layer was washed 3 times with ether, and the combined ethereal solutions were washed 4 times with water to pH 5, then dried ($MgSO_4$), and concentrated. The residue was crystallised from ether to give **2** (1.2 g, 45.1%), m.p. 76–86°, $[\alpha]_D$ −10° (*c* 1.2, acetic acid), identical with that reported[9].

2,3,4-Tri-O-*acetyl*-N-[*1-benzyl*-N-*(benzyloxycarbonyl)*-L-*aspart-4-oyl*]-β-D-*xylopyranosylamine* (**3**). — To a solution of **2** (0.2538 g, 0.95 mmol) and 2,3,4-tri-*O*-acetyl-β-D-xylopyranosylamine[8] (**1**; 0.1998 g, 0.73 mmol) in dichloromethane (5 mL) was added dicyclohexylcarbodi-imide (0.1589 g, 0.77 mmol). The mixture was stored for 23 h at room temperature; no **1** was then detected (t.l.c.). Glacial acetic acid (0.1 mL) was added, the dicyclohexylurea was collected and washed with dichloromethane, the combined filtrate and washings were concentrated, and a solution of the residue in ethyl acetate was washed successively with M HCl, water, saturated aqueous sodium hydrogencarbonate, and water, then dried ($MgSO_4$), and concentrated. The residue was crystallised from ethanol (1.5 mL) to give a product (0.1596 g, 35.8%) having m.p. 150–174°. Recrystallisation from ethanol gave needles of **3**, m.p. 180–183°, $[\alpha]_D$ +25° (*c* 0.3, chloroform); ν_{max} 3220 (urethane NH), 1740 (ester C=O), 1700 (urethane C=O), 1670 (Amide I), and 1550 cm^{-1} (Amide II).

Anal. Calc. for $C_{30}H_{34}N_2O_{12}$: C, 58.61; H, 5.57; N, 4.59. Found: C, 58.36; H, 5.60; N, 5.35.

2,3,4-Tri-O-*acetyl*-N-*(*L-*aspart-4-oyl)*-β-D-*xylopyranosylamine* (**4**). — To a solution of **3** (0.3075 g, 0.5 mmol) in acetic acid (36 mL) and water (4 mL) was added 5% Pd/C (~0.1 g), and a stream of hydrogen was passed through the suspension for 2 h at room temperature, when **3** had reacted completely (t.l.c.). The catalyst was removed, the filtrate was concentrated at 40°(bath)/13 mmHg, and the residue was crystallised from ethanol to give **4** (0.1721 g, 88.2%), m.p. 224–225° (dec.); ν_{max} 3260 (amide), 3140 (NH_3^+), 1745 (ester C=O), 1610 (COOH C=O), and 1690 cm^{-1} (Amide I).

Anal. Calc. for $C_{15}H_{22}N_2O_{10}\cdot C_2H_5OH$: C, 46.78; H, 6.47; N, 6.42. Found: C, 47.27; H, 6.15; N, 6.99.

N-*(L-Aspart-4-oyl)-β-D-xylopyranosylamine* (**5**). — A solution of **4** (0.10 g, 0.26 mmol) in 0.6M LiOH (2.6 mL) was stored at room temperature. T.l.c. showed that reaction was complete after a few min. After 4 h, the mixture was eluted from a column of Amberlite IRC-50 (H^+) resin (2 g) slowly with water. The eluate was concentrated at 35°/20 mmHg, and the residue was crystallised from ethanol–water to give **5** (0.0469 g, 68.3%), m.p. 215–217°; ν_{max} 3500–2500 (HO, NH), 1605 and 1570 (COOH C=O), 1670 (Amide I), and 1635 cm^{-1} (NH_3^+).

Anal. Calc. for $C_9H_{16}N_2O_7 \cdot H_2O$: C, 38.30; H, 6.43; N, 9.92. Found: C, 38.22; H, 6.38; N, 9.57.

ACKNOWLEDGMENTS

This work was carried out under contract C.P.B.P.-01.13.2.13 PAN. We thank Professor D. Leibfritz and his co-workers (Dr. P. Schulze, Dr. H. M. Schiebel, Dipl.-Chem. J. Stelten, and Miss I. Erxleben) from the University of Bremen for the recording and preliminary interpretation of the ^{1}H-n.m.r. and f.a.b.-mass spectra.

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