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Note

Synthesis and characterization by ^{13}C-CP–MAS- and high resolution ^{1}H-, ^{13}C-NMR of new ureido sugars, derivatives of methyl 2-amino-2-deoxy-β-D-glucopyranose and dipeptides

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Abstract

Dipeptide ethyl and benzyl esters were used as amination agents in reaction with methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(4-nitrophenoxycarbonylamino)-β-D-glucopyranoside(**1**). Ten new ureido sugars, derivatives of GlyAla, AlaGly, AlaAla, GlyVal, ValGly, LeuGly, PheGly, GlyPhe, and AlaPhe were obtained. The new ureido sugars were studied by means of ^{1}H- and ^{13}C-NMR spectroscopy in solution, and ^{13}C-CP–MAS–NMR in the solid-state.

Keywords: Ureido sugars; Dipeptide; ^{13}C–CP–MAS solid-state NMR; ^{1}H-NMR; ^{13}C-NMR

1. Introduction

There is an increasing interest for the synthesis, biological evaluation and chemotherapeutic applications of variety of non natural derivatives of amino sugars [1]. Recently various carbohydrate-amino acid or peptide conjugates have been synthesized. As a part of our program devoted to the ureido sugars we report here the synthesis and structural analysis of sugar-peptide conjugates that combine an 2-amino-2-deoxy-D-glucosamine connected with some dipeptides by ureido bridge. The common method for the synthesis of sugar ureas is the reaction of sugar isocyanate (or isothiocyanate) with amine. Reaction of sugar isocyanate or isothiocyanate with amino acid have been reported by a number of authors [2]. The earliest work on the synthesis of D-glucosyl hydantoin involved the condensation of D-glucosyl isothiocyanate with glycin ethyl ester [3]. Reaction of the same isothiocyanate with D,L-alanine methyl ester afforded the corresponding thioureides [4]. Micheel and Schmidt [5] obtained a number of glycoproteins, derivatives with ureido sugars, for example tetra-*O*-

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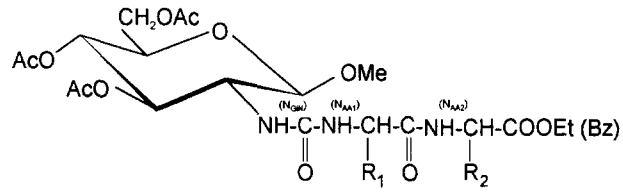
acetyl-D-glucopyranosyl cyanate was refluxed with gelatin giving a product which, on hydrolysis, yielded D-glucosylureido-gelatin. Pseudoglobulin, treated with the same acetyl D-glucosyl cyanate, yielded the analogous D-glucosylureido protein [5]. Our previous papers reported synthesis and structural analysis of the ureido sugars derivatives of 2-amino-2-deoxy-D-glucosamine and amino acid ester starting from methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(4-nitrophenoxycarbonylamino)-β-D-glucopyranoside (**1**) and some amino acid esters [6,7]. We now report on the synthesis of new ureido sugar derivatives of dipeptides and their structural analysis by means of ^{13}C-CP–MAS- and high resolution ^{1}H-, and ^{13}C-NMR.



2 R_1 = H, R_2 = CH_3, OEt

3 R_1 = CH_3, R_2 = H, OEt

4 R_1 = CH_3, R_2 = CH_3, OEt

5 R_1 = H, R_2 = $CH(CH_3)_2$, OEt

6 R_1 = $CH(CH_3)_2$, R_2 = H, OEt

7 R_1 = $CH_2CH(CH_3)_2$, R_2 = H, OEt

8 R_1 = $CH_2C_6H_5$, R_2 = H, OEt

9 R_1 = $CH_2C_6H_5$, R_2 = H, OBz

10 R_1 = H, R_2 = $CH_2C_6H_5$, OBz

11 R_1 = CH_3, R_2 = $CH_2C_6H_5$, OBz

Scheme 1.

2. Results and discussion

Synthesis.—Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(4-nitrophenoxycarbonylamino)-β-D-glucopyranoside (**1**) the key intermediate in preparation of ureido sugars was synthesized as described previously [6]. The dipeptide esters shown in Scheme 1 were prepared by condensation of *Z*-amino acid and ethyl or benzyl ester of suitable amino acids using the DCC/HOBT method. Starting from **1** and hydrochloride methyl or benzyl esters of dipeptide in the presence of triethylamine in pyridine we obtained ten new compounds in a good yields. After purification by silica gel chromatography all compounds except **7** were crystalline. The yield obtained and some physical data as well as elementary analysis for compound **2**–**11** are summarized in Table 1.

Structural characterization by NMR.—The presence of Ac groups enabled the assignments of all multiplets of sugar CH protons in the ^{1}H-NMR 500 MHz spectra and the Me group at the anomeric carbon prevented epimerization in solution. The ^{1}H-NMR chemical shifts and coupling constants are given in Table 2. Large vicinal coupling constant $^{3}J_{H\text{-}1,H\text{-}2}$ of 8.0–8.5 Hz confirm the presence of β anomer in all compounds. The replacement of one dipeptide residue by another has some influence on shielding of H-2, proximal to the place of substitution to Glc*p*; for example, in the sequence of amino acid Ala–Gly, Gly–Ala and Ala–Ala chemical shifts of H-2 are 3.50, 3.65 and 3.71, respectively. No significant influence on other proton chemical shifts and

Table 1
Analytical data for methyl β-D-glucopyranosyl ureas **2**–**11**

Compound	Yield/%	mp/°C	$[\alpha]_D^{20}$/degrees (c 1, chloroform)	Formula	Analytical data					
					Calc.			Found		
					C	H	N	C	H	N
2	45	181–184	+10.9	$C_{21}H_{33}O_{12}N_3$	48.55	6.40	8.09	48.26	6.69	8.46
3	53	210–212	−5.4	$C_{21}H_{33}O_{12}N_3$	48.55	6.40	8.09	48.27	6.60	8.40
4	62	224–226	−8.2	$C_{22}H_{35}O_{12}N_3$	49.52	6.61	7.88	49.23	6.79	8.11
5	95	207–210	−2.2	$C_{23}H_{37}O_{12}N_3$	50.44	6.81	7.67	50.44	6.93	7.42
6	74	232–234	−8.4	$C_{23}H_{37}O_{12}N_3$	50.44	6.81	7.67	50.38	6.93	7.63
7	83	sirup	−5.0	$C_{24}H_{39}O_{12}N_3$	51.33	7.00	7.48	51.37	7.15	7.27
8	88	207–210	−1.4	$C_{27}H_{37}O_{12}N_3$	54.45	6.26	7.05	54.44	6.26	6.96
9	45	222–224	+8.1	$C_{32}H_{39}O_{12}N_3$	58.44	5.98	6.39	58.23	5.90	6.45
10	92	217–219	+12.3	$C_{32}H_{39}O_{12}N_3$	58.44	5.98	6.39	58.33	5.88	6.25
11	54	211–213	+4.2	$C_{33}H_{41}O_{12}N_3$	59.01	6.15	6.26	58.91	6.19	6.24

Table 2
^{1}H-NMR data (δ in ppm, J in Hz) in $CDCl_3$ for peracetylated methyl β-D-glucopyranosyl ureas **2–11**

Atom	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
H-1	4.57d	4.61	4.55d	4.56d	4.53d	4.57d	4.53d	4.53d	4.52d	4.74d
$J_{1,2}$	8.0	8.0	8.0	8.5	8.5	8.5	8.5	8.5	8.5	8.5
H-2	3.65ddd	3.50ddd	3.71ddd	3.65ddd	3.65ddd	3.59ddd	3.50ddd	3.58ddd	3.72ddd	3.73ddd
$J_{2,NH}$	7.5	7.0	8.5	8.0	7.0	8.0	8.0	7.5	7.5	7.0
$J_{2,3}$	10.0	11.0	11.0	10.5	10.0	10.0	10.0	10.5	10.0	10.0
H-3	5.27dd	5.29dd	5.29dd	5.24dd	5.21dd	5.25dd	5.25dd	5.25dd	5.26dd	5.19dd
$J_{3,4}$	10.0	9.5	9.5	9.0	9.0	9.5	9.5	9.5	9.5	9.5
H-4	5.07dd	5.07dd	5.07dd	5.07dd	5.08dd	5.07dd	5.06dd	5.05dd	5.07dd	5.09dd
$J_{4,5}$	9.5	9.5	9.5	10.0	10.0	10.0	10.0	10.5	10.0	9.5
H-5	3.73ddd	3.71ddd	3.75ddd	3.72ddd	3.72ddd	3.72ddd	3.68ddd	3.67ddd	3.73ddd	3.71ddd
$J_{5,6a}$	5.0	4.5	5.0	5.0	5.0	5.5	5.0	5.0	5.0	5.0
$J_{5,6b}$	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.0	2.5
H-6a	4.28dd	4.28dd	4.28dd	4.29dd	4.39dd	4.29dd	4.23dd	4.27dd	4.28dd	4.14dd
$J_{6a,6b}$	12.5	12.5	12.5	12.5	12.0	12.0	12.5	12.5	12.5	12.0
H-6b	4.14dd	4.13dd	4.13dd	4.14dd	4.14dd	4.14dd	4.12dd	4.17dd	4.14dd	4.28dd
CH_3O	3.52s	3.53s	3.51s	3.52s	3.53s	3.51s	3.41s	3.40s	3.45s	3.48s
$N\text{–}H_{GlN}$	5.44d	5.15d	5.57d	5.36d	5.18d	5.24d	5.01d	4.95d	5.61d	5.39d
$N\text{–}H_{AA1}$ [a]	5.93t	5.56d	6.07d	5.93t	5.70d	5.61d	5.48d	5.41d	6.08t	5.85d
$N\text{–}H_{AA2}$ [a]	7.10d	7.11d	7.28d	6.96d	7.06t	7.15t	6.86t	6.89t	7.04d	7.04d
OCH_{2ester}	4.19dd	4.20dd	4.20dd	4.19dd	4.21m	4.20′	4.18dd			
CH_{3ester}	1.28t	1.28t	1.27t	1.28t	1.28t	1.28	1.27t			
OCH_2Ph								5.15dd	5.12d	5.14d
CH_3	1.42d	1.37d	1.43d	0.96d	0.98d	0.94d				1.27d
			1.34d	0.93d	0.92d	0.93d				1.27d
CH_2						1.68m				
CH				2.18m	2.15m	1.49m				
CH_2Phe							3.11dd	3.12dd	3.09dd	3.09d
							3.04dd	3.03dd	3.04dd	
Ar							7.29–7.19	7.36–7.18	7.34–7.04	7.33–7.18
CH_3COO	2.09	2.09	2.08	2.08	2.08	2.08	2.08	2.07	2.07	2.08
	2.08	2.03	2.03	2.04	2.02	2.02	2.01	2.01	2.05	2.00
	2.02	2.02	2.02	2.01	2.01	2.01	1.98	1.95	1.97	1.98
$N\text{–}CH_{2Gly}$	3.94dd	4.06dd		3.92dd	4.06dd	4.02dd	4.02dd	4.08dd	3.85dd	
	3.82dd	3.97dd		3.87dd	3.93dd	3.96dd	3.92dd	3.94dd	3.74dd	
$N\text{–}CH_{AA}$ [a]	4.49m	4.39m	4.46m	4.56m	4.21m	4.38m	4.59dd	4.59dd	4.80dd	$4.39m_{Ala}$
										$4.78dd_{Phe}$

[a] AA = amino acid; AA1 = amino acid 1; AA2 = amino acid 2; see Scheme 1.

coupling constants within the sugar part was observed. ^{1}H chemical shifts of N_{GIN}–H are in the range 4.95–5.61, of N_{AA1}–H 5.48–6.08 and those of N_{AA2}–H 6.86–7.28. The signals of NH protons from the ureido fragment appear in separate spectral ranges approximately 1 ppm upfield from that of the amide type N_{AA2}–H. The ^{13}C chemical shifts are given in Table 3. The influence of peptide substituents can be noticed on C-2 with chemical shifts from 55.2 to 56.8 ppm.

^{13}C-CP–MAS–NMR.—High resolution ^{1}H- and ^{13}C-NMR are the standard tools for obtaining structural information, however for solution spectra conformational information is often lost because of fast internal rotation averaging chemical shifts. In the solid state rigid conformations are usually observed and, additionally, it seemed interesting to gain some insight into structure and intermolecular interactions without the influence of solvent. Solid compounds were obtained by crystallization from ethanol, however the attempts to grow single crystals suitable for X-ray diffraction have until now failed and solid state NMR remain the only source of structural information.

In the absence of X-ray diffraction data, the assignments of resonances are not straightforward when the solid state spectrum contains more signals (after elimination of rotational side bands) than the liquid state spectrum. The resonances spectra could be directly assigned by comparison with solution data. The signals of carbons linked to nitrogen atoms are broader and/or split into asymmetric doublets due to the residual ^{13}C–^{14}N coupling [8]. These residual splittings or broadening allow the assignment of C-2–N_{GIN}, $N_{GIN}CON_{AA1}$ and N_{AA}–C_{α} carbons (Fig. 1).

The chemical shifts of C-6 resonances are sensitive probes of conformations because of rotation around the C-5–C-6 bond. Compared to the averaged value in the liquid state, the signal of C-6 is shifted downfield (3.2 ppm in **3**). ^{13}C shifts were found to correlate with the torsional angle O-6–C-6–C-5–C-5 and the sequence of chemical shifts for conformations of D-glucopyranose in cyclomalto–polyoses [9] was tg > gt > gg. Assuming the same direction of changes for D-Glc*p* in ureido derivative we can suggest the tg conformation in **3** characterized by the C-6 chemical shift value 65.4 ppm.

The structure of peptide derivatives in solid state is determined mainly by the formation of intra- and intermolecular hydrogen bonds. Dipeptides prefer two conformations: (i) An extended conformation with proton donor (NH) and acceptor (C=O) groups at-

Table 3
^{13}C-NMR data (δ in ppm,) in $CDCl_3$ for peracetylated methyl β-D-glucopyranosyl ureas **2–11**, and ^{13}C-CP–MAS–NMR (δ in ppm,) for compound **3** [b]

Atom [a]	**2**	**3**	**3** [b]	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
C-1	102.58	102.53	104.2	102.66	102.45	102.84	102.62	102.62	102.51	102.67	102.83
C-2	55.63	56.30	53.4	55.45	55.37	55.05	56.83	55.21	55.37	55.53	55.48
C-3	72.70	72.70	73.3	72.72	73.00	73.36	73.09	73.00	72.82	72.82	73.09
C-4	68.75	68.75	71.7	68.99	69.13	69.41	69.24	69.35	69.19	69.08	69.92
C-5	71.61	71.72	72.7	71.64	71.63	71.46	71.57	71.57	71.57	71.63	71.63
C-6	62.17	62.21	64.4	62.38	62.42	62.74	61.27	62.69	62.58	62.36	62.36
OCH_3	57.17	57.12	59.1	57.00	56.94	57.22	57.05	57.05	57.05	57.00	57.00
N_{GIN}–CO–N_{AA1}	158.11	157.49	158.0	157.64	158.31	158.84	157.88	157.71	157.61	158.21	157.61
CH_3COO	20.77	20.73	20.5	20.66	20.75	20.64	20.64	20.70	20.70	20.75	20.70
C*OOR*	171.05	171.16	172.4	172.58	171.05	170.61	170.61	170.56	170.72	171.05	171.05
	170.77	170.70	170.2	170.67	170.93	170.34	170.45	169.70	170.61	170.72	170.90
	170.23	169.96	169.7	169.44	170.72	169.85	169.80	169.53	170.50	170.50	170.83
	169.49	169.42	168.2		169.49	169.60	169.53		169.58	169.48	169.48
C–C*O*–N_{AA}	172.84	173.47	174.7	173.27	171.70	173.75	174.40	173.21	173.00	171.26	173.16
$CH_{2,ester}$	61.49	61.40	60.4	61.35	61.17	61.28	61.28	61.22	67.00	67.00	67.20
$CH_{3,ester}$	14.12	14.14	13.4	14.08	14.19	14.10	14.10	14.14			
CH–N_{AA1}	43.91	49.60	47.2	49.37	43.94	58.68	52.01	54.67	54.67	43.89	49.52
CH–N_{AA2}	48.30	41.36	40.5	48.45	57.70	41.83	42.37	41.50	41.45	53.69	53.85
CH_{AA}					30.83	32.40	41.72				
$CH_{2,AA}$								39.28	38.85	37.36	37.71
CH_3	17.86	18.38	19.9	19.00	18.96	19.07	22.97				19.02
				17.60	17.93	18.31	22.48				

[a] AA = amino acid; AA1 = amino acid 1; AA2 = amino acid 2; see Scheme 1.

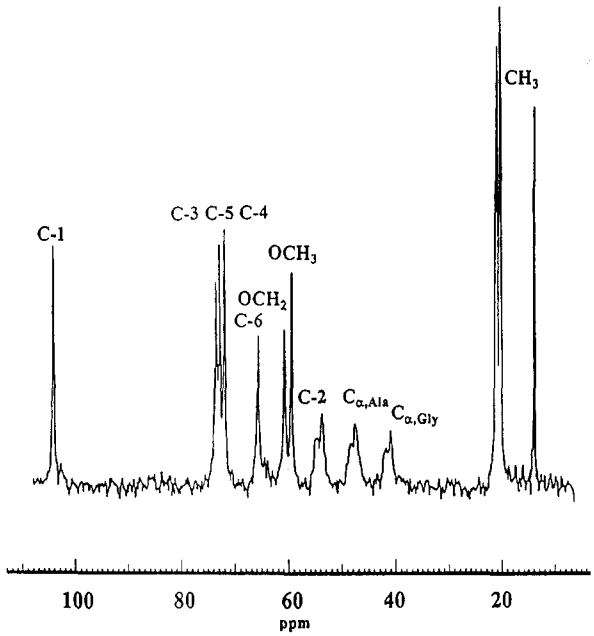


Fig. 1. ^{13}C-CP–MAS–NMR spectrum of sugar carbon region of *N*-(methyl-3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside)-*N'*-carbonyl-L-alanylglycine ethyl ester (**3**).

tached to the same C_α carbon atom forming five-membered ring (C_5) with weak interaction and (ii) a folded seven-member ring conformation (C_7) with a stronger intramolecular hydrogen bond. The C_5 structure was found in solution by means of FT–IR in ureido sugars with the L-amino acid residue [10].

The downfield shift of C-1 (1.7 ppm) followed by downfield shift of OMe (2.0 ppm) can be related to the formation of intermolecular NH $\cdots$ OCH_3 hydrogen bond involving anomeric oxygen atom. Such type of interaction was first found in the crystal of *N*-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside)-*N'*-carbamoyl-D-valine ethyl ester [11], one can assume that chemical shift of OMe carbon of 59.1 ppm found in compounds Ala–Gly (**3**) is indicative of this arrangement.

The analysis of chemical shifts of the three CO signals revealed that the differences between solution and solid state are negligible for ureido-type N–CO–N and for ester COOR whereas NCO carbonyl exhibit downfield shift of 5.3 ppm. It is quite probable, therefore that these CO groups are mainly involved in the formation of intermolecular hydrogen bonding in the solid.

3. Experimental

Melting points are uncorrected. Optical rotations were measured on a Perkin–Elmer Model 241 polarimeter. TLC was performed on Silica Gel 60 F_{254} (Merck), using chloroform–acetone (4:1) and detection by UV light or by charring with sulfuric acid. Column chromatography was conducted on Silica Gel 60 (Merck 230–400 mesh) in chloroform–acetone (4:1). Dipeptide esters were synthesized by conventional procedures [12]. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AMX-500 for 0.05 M solutions in $CDCl_3$. ^{13}C-NMR spectra of solids were recorded on a Bruker MSL-300 spectrometer at 75.5 MHz using magic angle spinning and cross-polarization technique. Powdered samples were placed in 7 mm cylindrical ZrO_2 rotor and spun at 3–4 kHz; 500–2100 scans with a contact time of 5 ms, a repetition time of 10 s and spectral width of 20 kHz were accumulated. Chemical shifts were calibrated indirectly through the glycine CO signal observed at 176.3 ppm relative to TMS.

Synthesis of ureido derivatives.—To a solution of **1** (2 mmol) in pyridine (30 mL) was added the hydrochloride of an ethyl ester or benzyl ester of dipeptide and triethylamine (160 μL, 2 mmol). The mixture was stirred at room temperature for 48 h, TLC then indicated the absence of **1**. After removal of the solvent under reduced pressure, the residue was dissolved in dichloromethane (40 mL) and was washed with ammonia (1 M), and water, then dried, and concentrated. Column chromatography of the residue and recrystallization from ethanol gave a ureido sugar. The following compounds were prepared in this manner. *N*-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside)-*N'*-carbonyl-glycyl-L-alanine ethyl ester (**2**), *N*-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside)-*N'*-carbonyl-L-alanyl-glycine ethyl ester (**3**), *N*-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside)-*N'*-carbonyl-L-alanyl-L-alanine ethyl ester (**4**), *N*-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside)-*N'*-carbonyl-glycyl-L-valine ethyl ester (**5**), *N*-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside)-*N'*-carbonyl-L-valyl-glycine ethyl ester (**6**), *N*-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside)-*N'*-carbonyl-L-leucyl-glycine ethyl ester (**7**), *N*-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside)-*N'*-carbonyl-L-phenyl-alanyl-glycine ethyl ester (**8**), *N*-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside)-*N'*-carbonyl-L-phenyl-

alanyl-glycine benzyl ester (**9**), *N*-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside)-*N'*-carbonyl-glycyl-L-phenyl-alanine benzyl ester (**10**), *N*-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside)-*N'*-carbonyl-L-alanyl-L-phenyl-alanine benzyl ester (**11**).

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