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Note

Synthesis and solid state ^{13}C and ^{1}H NMR analysis of new oxamide derivatives of methyl 2-amino-2-deoxy-α-D-glucopyranoside and ester of amino acids or dipeptides

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Abstract

The syntheses of new oxamide derivatives of methyl 2-amino-2-deoxy-α-D-glucopyranoside and amino acid or peptide esters are presented. The reaction of methyl 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranoside and oxalyl chloride gave *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosid-2-yl) oxamic acid chloride which on reaction with the ester of Gly, L-Ala, L-Phe, GlyGly, Gly-L-Phe and Gly-L-Ala afforded *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosid-2-yl), *N*′-oxalyl-amino acid or dipeptide esters. The structure of the oxamides was studied using ^{1}H, ^{13}C NMR in solution and solid state. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Oxamides; Synthesis; ^{13}C and ^{1}H NMR analysis, glycoconjugates; Neoglycoproteins

Compounds containing sugar and amino acid moieties such as, for example, glycoproteins are important in many biological processes. Owing to their glycosylation patterns, they are responsible for recognition and adhesion of cells as well as toxins, viruses, etc. Another important aspect of peptide glycolysation is the appearance of new factors affecting the structure and the solubility of the molecules. In naturally occurring products sugars are linked to amino acids by the N- or O-glycosidic bond, however polyfunctionality of both partners enables other connections. Amongst various possibilities we have chosen coupling by means of an oxamido bridge and synthesized a new series of non-anomeric amino acid glycoconjugates. Substituted oxamides are also useful intermediates in the synthesis of α-diketones and significant synthons in organic chemistry.[1] On the other hand, oxamides are important products due to their biological activity. Some of them have found application as pesticides,[2] plant growth regulators,[3] cephalosporin bactericides,[4] and HIV-1 protease inhibitors.[5] In the present paper we have described the preparation of glucosyloxamide derivatives of methyl 2-amino-2-deoxy-α-D-glucopyranoside and the ester of amino acids or dipeptides, a well as structural studies using NMR spectroscopy.

The treatment of methyl 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α or β-D-glucopyranosides (**1**α or **1**β) with excess oxalyl chloride in methylene chloride gave *N*-acetyl *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-α or β-D-glucopyranosid-2-yl) oxamic acid chloride. Then the addition of amine afforded *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-α- or -β-D-glucopyranosid-2-yl), *N*′-alkyl or aryloxamide.[6] In the present paper we describe the preparation of new oxamide derivatives of **1**α and L-amino acid esters or dipeptide. Acylation of **1**α with oxalyl chloride and treatment of the thus obtained oxamic acid chloride with methyl or ethyl ester of glycine, L-alanine, L-phenylalanine, glycylglycine, glycyl-L-phenylalanine and glycyl-L-alanine gave six new compounds in good yields (Scheme 1). The structures of the new oxamides were studied by means of ^{1}H, ^{13}C

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Table 1
^{13}C NMR data (δ in ppm) in $CDCl_3$ and solid state for peracetylated methyl α-D-glucopyranosyloxamides **2–7**

Atom	**2**		**3**		**4**		**5**	**6**		**7**
	$CDCl_3$	CPMAS	$CDCl_3$	CPMAS	$CDCl_3$	CPMAS	$CDCl_3$	$CDCl_3$	CPMAS	$CDCl_3$
C-1	97.89	99,6	97.91	98.8	97.93	97.9	97.85	97.86.	98.7	97.79
C-2	52.62	54,6	52.23	53.2 (board)	52.51	50.5	52.26	52.52	53.8	52.21
C-3	68.24	69,9	68.24	69.6, 70.4	68.28	68.6	68.23	68.24	71.4	68.23
C-4	67.64	68,7	67.66	67.1, 56.7	67.68	67.5	67.59	67.59	68.9	67.59
C-5	70.87	72.3	70.87	72.8, 70.4	70.94	70.3	70.89	70.84	73.0	70.85
C-6	61.91	62.3	61.92	62.0	61.97	62.9	61.92	61.92	65.9	61.91
O–CH_3	55.56	56.2	55.55	55.4, 56.7	55.57	59.1	55.45	55.52	59.5	55.54
C=O	159.2, 159.1	161.9, 158.3	159.4, 158.4	161.0, 159.6	159.1, 158.6	158.8, 157.1	159.5, 159.1	159.4, 159.1	161.3, 159.8	159.4, 159.1
CH_3COO	20.74, 20.67, 20.61	21.9, 21.2, 20.5	20.73, 20.66, 20.60	20.3, 21.4, 20.8	20.72, 20.63, 20.59	20.1, 18.7	20.74, 20.70, 20.62	20.74, 20.69, 20.61	23.3, 22.4, 20.9	20.75, 20.69, 20.62
CH_3COO	170.6, 169.5, 169.0	172.1, 172.1, 171.6	170.7, 169.5	172.5, 172.0, 171.0	170.7, 169.5	169.5	169.6, 169.5, 167.6	170.7, 169.5, 166.9	171.5, 170.6, 167.5	170.1, 169.5, 166.7
COO_{ester}	170.7	169.1	171.6	170.1	170.7	168.9	170.7	170.7	170.6	171.4
CONH							170.8	171.6	172.3	172.7
$CH_{2,ester}$			61.80	62.0, 60.7			61.79			61.77
$CH_{3,ester}$	52.24	52.3	14.11	15.3, 16.0	52.64	50.5	14.12	52.53	53.8	14.11
$CH_{2,Gly}$	41.23	42.5					42.89, 41.39	42.85	45.2	42.89
$CH_{3,Ala}$			18.17	18.5, 19.3						18.43
CH_{Ala}			48.49	48.6, 49.9						48.32
CH_{Phe}					53.69	50.5		53.25	53.8	
$CH_{2,Phe}$					38.04	34.3		37.80	36.9	
C_{aromat}					135.3–127.4	136.2, 128.4, 126.5		135.3–127.4	138.1, 131.4, 128.5, 126.5	

NMR spectroscopy in solution and solid phase and NMR data are displayed in Tables 1 and 2.

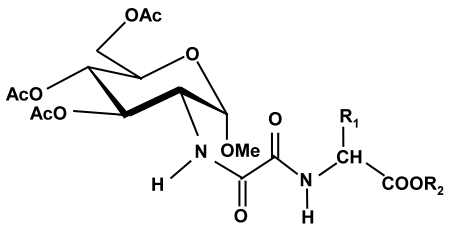
^{1}H NMR spectra of **2**–**7** in $CDCl_3$ solution recorded at 500 MHz enabled the interpretation and assignment of all multiplets of sugar protons as well as those of the dipeptide residues. Chemical shifts and coupling constants for sugar protons (Table 2) are typical for a per-*O*-acetylated glucopyranose ring with $^4C_1$ conformation and are in agreement with those reported earlier.[7,8] Small vicinal coupling constants $^3J_{H\text{-}1,H\text{-}2}$ of 3.0–4.0 Hz confirm the presence of the α-anomer. ^{1}H And ^{13}C chemical shifts and coupling constants for dipeptide ester residues might be compared with those for amino acids in linear peptides and in ureido sugars, derivatives of dipeptides[9] and are close to the values usually found. Solid compounds were obtained by crystallization from ethanol, however single crystals were not suitable for X-ray diffraction measurements, and therefore solid state NMR was considered as a source of structural information. The use of ^{1}H NMR in the solid state has been hindered due to the very strong dipolar homonuclear interactions, which result in extremely broad spectral lines. These line width could be reduced by fast spinning and/or multipulse line narrowing sequences. The ^{1}H MAS spectra of **2**,**3** and **6** and ^{13}C CP MAS spectrum of **2** are shown in Figs. 1 and 2. The ^{1}H spectra were recorded with the spinning speed as high as 32 kHz, however they did not yield high-resolution signals.

The structure of compounds **2**–**4** and **6** in the solid state is determined by the formation of intra and intermolecular hydrogen bonds of N–H···O=C type. The NH groups involved hydrogen bonds gave separate signals which could be observed at 9.1–9.4 ppm in the ^{1}H MAS spectra (Fig. 1). Distinct signals at 1.88 ppm are due to acetyl CH_3 groups of per-*O*-acetylated sugar moiety.

The resonances of sugar protons contribute to the resonance at ca 3.5 ppm. Aromatic protons of the phenylalanine substituent of compound **6** appear at 6.75 ppm (Fig. 1a). ^{13}C CP MAS spectra of **2**,**4** and **6** reveal no polymorphism, however the solid-state spectrum of **3** contains more signals than the liquid state

Table 2
^{1}H NMR data (δ in ppm, J in Hz) in $CDCl_3$ for peracetylated methyl α-D-glucopyranosyloxamides **2**–**7**

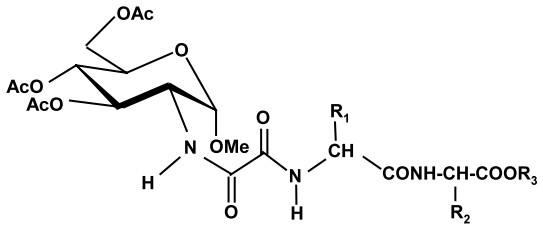
Atom	**2**	**3**	**4**	**5**	**6**	**7**
H-1	4.76d	4.75d	4.74d	4.75d	4.76d	4.76d
$J_{1,2}$	3.5	4.0	4.0	3,5	3.5	3.0
H-2	4.31–4.26 m	4.35–4.14m	4.35–4.14m	4.32–4.21m	4.32–4.26m	4.30–4.24m
$J_{2,NH}$	9.5	9.0	9.5			
$J_{2,3}$	11.0	9.5	10.0			
H-3	5.31dd	5.32dd	5.29dd	5.32dd	5.12dd	5.12dd
$J_{3,4}$	10.0	10.0	10.5	10.5	10.0	10.0
H-4	5.12dd	5.12dd	5.10dd	5.12dd	5.32dd	5.32dd
$J_{4,5}$	9.5	9.5	9.5	9.5	9.5	9.5
H-5	3.98ddd	4.00ddd	3.97ddd	3.98ddd	4.03–3.90m	3.99m
$J_{5,6b}$			2.0		2.5	
H-6a	4.31–4.26 m	4.35–4.14m	4.35–4.14m	4.32–4.21m	4.32–4.26m	4.30–4.24m
$J_{6a,6b}$			12.0			
H-6b	4.16–4.09m	4.35–4.14m	4.12dd	4.16–4.09m	4.12dd	4.04m
CH_3O	3.42s	3.42s	3.42s	3.42s	3.42s	3.42s
$CH_{2,ester}$		4.35–4.14m		4.16–4.09m		4.04m
$CH_{3,ester}$	3.79s	1.29t	3.72s	1.29t	3.73s	1.29t
CH_{Ala}		4.55m				4.13–4.10m
$CH_{3,Ala}$		1.48d				1.43d
CH_{Phe}			4.82m		4.88	
$CH_{2,Phe}$			3.14m		3.11d	
N–H_{GlN}	7.56d	7.55d	7.48d	7.60d	7.55d	7.58d
N–H_{AA}	7.80t	7.83d	7.72d	8.04t	7.95t	7.99t
$CH_{2,Gly}$	4.16–4.09m			4.32–4.21m 4.16–4.09m	4.03–3.90m	4.24–4.19m
Ar			7.31–7.11m		7.30–7.06m	
CH_3COO	2.11, 2.03, 1.98, 3s	2.11, 2.03, 1.98, 3s	2.10, 2.02, 1.95, 3s	2.11, 2.03, 1.98, 3s	2.11, 2.03, 1.97, 3s	2.03, 2.02, 2.01, 3s
CONH				6.57t	6.37d	6.56d



2 R_1= H, R_2= Me

3 R_1= CH_3, R_2= Et

4 R_1= CH_2Ph, R_2= Me



5 R_1= H, R_2= H, R_3 = Et

6 R_1= H, R_2 =CH_2Ph, R_3 = Me

7 R_1= H, R_2= CH_3, R_3 = Et

Scheme 1.

spectrum. Thus, in the solid **3** at least two different molecules exist in the crystals.

The ^{13}C resonances could be assigned by comparison with solution data (Table 1). The signals of carbons linked to nitrogen atoms N–C=O, N–C-l, $N–CH_{2gly}$ are broader due to the residual $^{13}C–^{14}N$ coupling (Fig. 2) and confirm the assignment of N–C=O, N–C-l, $N–CH_{2gly}$ (the $^{13}C–^{14}N$ dipolar and ^{14}N quadrupolar interaction cannot be eliminated simultanously by magic angle spinning). The ^{13}C MAS chemical shifts of sugar carbons are close to those for solution (within $\pm$ 1 ppm). The downfield shift of C-1 of (1–2 ppm) and OMe (1–3 ppm) can be related to the formation of $NH\cdots OCH_3$ hydrogen bond, similarly as observed in ureido sugars with dipeptide chains.[9] The ^{13}C shifts of C=O provide sensitive probes for hydrogen bonding, the downfield shift with respect to solution data usually indicates the formation of NH···O=C hydrogen bond in the solids.[10] The differences $\delta_{solid} - \delta_{solution}$ are small (0.5–1.2 ppm) for one of the C=O (located near sugar part) and slightly larger (1.5–2.7 ppm) for its neighbour. It is probable that these two C=O groups are involved in the formation of intramolecular hydrogen bonds, both in solution and in the solid state.

1. Experimental

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 dichloromethane–methanol (4:1). Dipeptide esters were synthesized by conventional procedures.[11]

^{13}C And ^{1}H spectra for $CDCl_3$ solutions were recorded on a Varian UNITY-500 spectrometer. ^{1}H And ^{13}C spectra of solids were recorded on a Bruker DSX-400 spectrometer at 400.13 and 100.16 MHz respectively. In order to measure ^{13}C CP MAS spectra

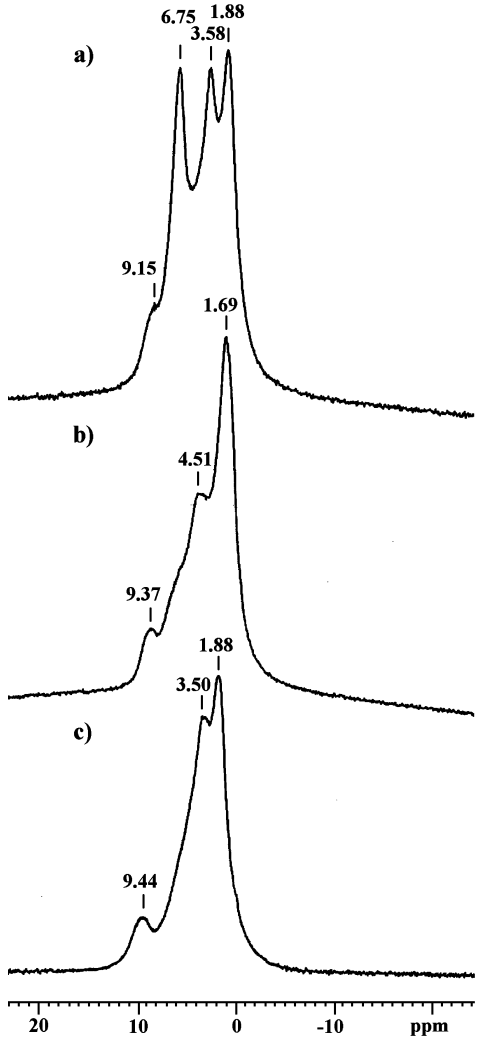


Fig. 1. ^{1}H MAS spectra of (a) *N′*-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosid-2-yl), *N*-oxalylglycyl-L-phenylalanine methyl ester (**6**), (b) *N′*-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosid-2-yl), *N*-oxalyl-L-alanine ethyl ester (**3**), (c) *N′*-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosid-2-yl), *N*-oxalylglycine methyl ester (**2**).

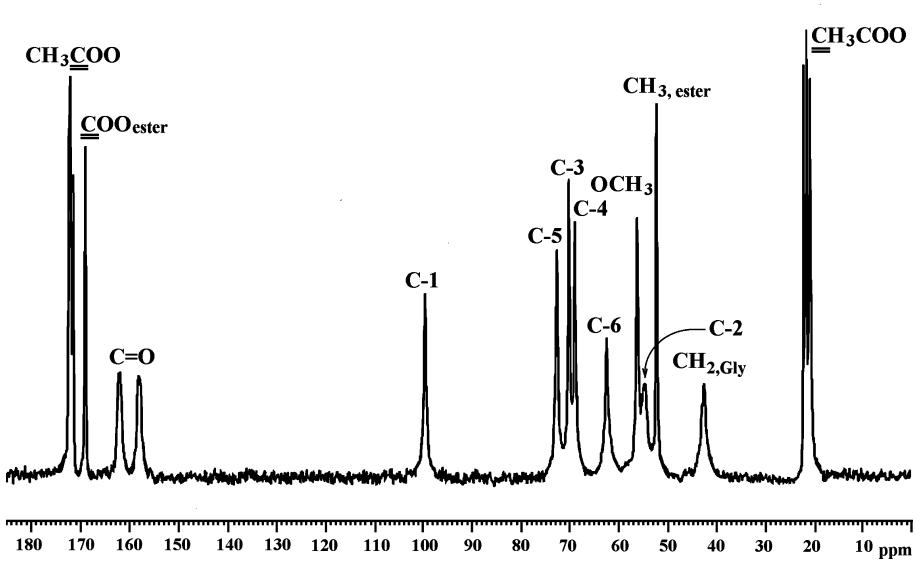


Fig. 2. ^{13}C CP MAS spectrum of *N*′-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosid-2-yl), *N*-oxalylglycine methyl ester (**2**).

powdered samples were placed in 4 mm cylindrical ZrO_2 rotor and spun at 10 kHz; 200 scans with a contact time of 2 sec and spectral width of 40 kHz were accumulated. Chemical shifts were calibrated indirectly through the glycine CO signal observed at 176.3 ppm relative to TMS. ^1H Spectra of solids were measured using MAS technique, the samples were spun in 2.5 mm rotor at 32 kHz.

1.1. Typical procedures for the synthesis of oxamido sugar derivatives

To a solution of **1**α[12] in dichloromethane was added a solution of three-fold excess of oxalyl chloride in dichloromethane. The reaction mixture was stirred at 0 °C for 10 min and at room temperature during 30 min. Tlc then indicated the absence of **1**. Next the reaction mixture was evaporated *in vacuo* dissolved again in dichloromethane and amino acid ester or dipepdide ester was added. The mixture was stirred at room temperature for 2 h. The resulting mixture was successively washed with hydrochloric acid (1M), water, and the saturated solution of sodium hydrogen carbonate, and then dried over magnesium sulfate. The solvent was evaporated *in vacuo* and gave the desired product as a viscous oil which was purified by chromatography with dichloromethane–methanol (4:1) as eluent. The following compounds were prepared in this manner.

1.2. *N*′-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosid-2-yl), *N*-oxalyl-glycine methyl ester (2)

Yield 88%, $[\alpha]_D^{22}$ + 78.8° (*c*, $CHCl_3$); mp. 143–144 °C; LSIMS (+) NBA *m*/*z* 463 $[M+H]^+$, Calc. for $C_{18}H_{26}O_{12}N_2$ 462.4 $[M]^+$.

1.3. *N*′-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosid-2-yl), *N*-oxalyl-L-alanine ethyl ester (3)

Yield 46%, $[\alpha]_D^{22}$ + 64.9° (*c*, $CHCl_3$); mp. 138–140 °C; LSIMS (+) NBA *m*/*z* 491 $[M+H]^+$, Calc. for $C_{20}H_{30}O_{12}N_2$ 490.4 $[M]^+$.

1.4. *N*′-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosid-2-yl), *N*-oxalyl-L-phenylalanine methyl ester (4)

Yield 60%, $[\alpha]_D^{22}$ +99.7° (*c*, $CHCl_3$); mp. 110–111 °C; LSIMS (+) NBA *m*/*z* 553 $[M+H]^+$, Calc. for $C_{25}H_{32}O_{12}N_2$ 552.5 $[M]^+$.

1.5. *N*′-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosid-2-yl), *N*-oxalyl-glycylglycine ethyl ester (5)

Yield 62%, $[\alpha]_D^{22}$ +65.9° (*c*, $CHCl_3$); white foam, LSIMS (+) NBA *m*/*z* 556 $[M+Na]^+$, Calc. for $C_{21}H_{31}O_{13}N_3$ 533.5 $[M]^+$.

1.6. *N*′-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosid-2-yl), *N*-oxalyl-glycyl-L-phenylalanine methyl ester (6)

Yield 58%, $[\alpha]_D^{22}$ +66.7° (*c*, $CHCl_3$); mp. 144–146 °C, white solid; LSIMS (+) NBA *m*/*z* 610 $[M+H]^+$, Calc. for $C_{27}H_{35}O_{13}N_3$ 609.6 $[M]^+$.

1.7. *N*′-(methyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosid-2-yl), *N*-oxalyl-glycyl-L-alanine ethyl ester (7)

Yield 20%, $[\alpha]_D^{22}$ + 74.6° (*c*, $CHCl_3$); white foam, LSIMS (+) NBA *m*/*z* 548 $[M+H]^+$, Calc. for $C_{22}H_{33}O_{13}N_3$ 547.5 $[M]^+$.

Acknowledgements

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