Glycosylamines
III.* Preparation, Structure, and Conformation of Some Glycosylamines

K. LINEK, J. ALFÖLDI, and M. ĎURINDOVÁ

Institute of Chemistry, Slovak Academy of Sciences, CS-842 38 Bratislava

Received 15 October 1992

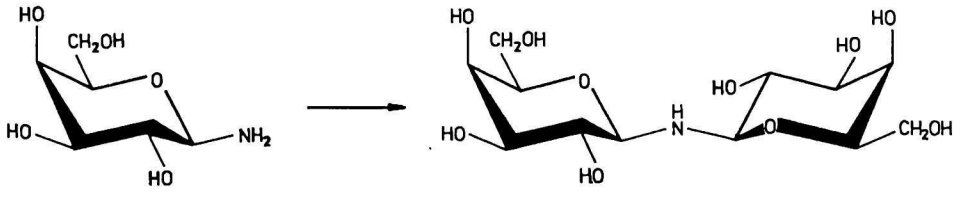
Di-β-D-galactopyranosylamine has been prepared by transglycosylation of β-D-galactopyranosylamine. By treatment of 4,6-*O*-ethylidene-α-D-glucopyranose with ammonia 4,6-*O*-ethylidene-β-D-glucopyranosylamine was prepared. The structure, anomeric configuration, and conformation of these compounds as well as of *N*-acetyl-β-D-glucopyranosylamine, *N*-acetyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine, and *N*-acetyl-β-D-mannopyranosylamine were determined by ^{1}H and ^{13}C NMR spectroscopy.

Recently, we have elucidated the problem of synthesis, structure, and conformation of glycosylamines and diglycosylamines of D-glucose, D-xylose [1], L-rhamnose, D-mannose, and D-arabinose [2]. Glycosylamines and diglycosylamines are compounds of interest for enzymologists, since they are considered as inhibitors of some glycosidases [3, 4].

Glycosylamines are prepared by treatment of concentrated alcoholic solutions of aldoses with ammonia [5]. When an unsubstituted glycosylamine is heated in a suitable solution, two molecules condense to form the corresponding diglycosylamine. This reaction is called transglycosylation of glycosylamines [6].

The anomeric configuration (α or β) of glycosylamines and diglycosylamines determines their use as inhibitors of the respective enzymes. In view of this, the present paper deals with determination by NMR spectroscopy of structure, anomeric configuration, and conformation of some glycosylamines and their derivatives. Determined were also the structures of some already described glycosylamines, the anomeric configuration and conformation of which have not yet been unambiguously assigned.

By transglycosylation of β-D-galactopyranosylamine (*I*) di-β-D-galactopyranosylamine (*II*; Scheme 1) has been prepared. The structure, anomeric configuration, and conformation of diglycosylamine *II*



I *II*

Scheme 1

Table 1. ^{13}C NMR Data of the Compounds Prepared

Compound	Chemical shift, δ								
	C-1	C-2	C-3	C-4	C-5	C-6	CH	CH_3	C=O
II	88.7	71.6	74.7	70.0	76.9	62.3			
IV	87.2	76.1	74.3	80.8	68.6	68.2	100.8	20.3	
V	80.3	72.9	77.5	70.3	78.6	61.6		23.2	176.5
VI	77.8	70.4	72.6	67.9	73.2	61.5		23.0[a]	170.4[b]
VII	78.8	71.2	74.4	67.4	78.7	61.9		23.0	175.8

a) Additional signals at δ = 20.5, 20.4, and 20.3. *b*) Additional signals at δ = 170.3, 169.5, and 169.3.

*For Part II see Ref. [2].

Table 2. ^{1}H NMR Data of the Compounds Prepared

Compound	Chemical shift, δ									
	H-1	H-2	H-3	H-4	H-5	H-6	H-6′	CH	CH_3	NH
II	4.21 d	3.46 t	3.64 dd	3.91 dd	3.68*	3.75 dd	3.62 dd			
IV	4.19 d	3.24 t	3.63 t	3.35 dd	4.18 o	3.62 m	3.46 m	4.9 q	1.38 d	
V	4.97 d	3.41 t	3.57 t	3.44 t	3.55 o	3.90 dd	3.75 dd		2.09 s	
VI	4.31 t	4.93 t	5.30 t	5.06 t	3.87 o	4.31 dd	4.09 dd		2.08 2.05 2.04 2.02 2.00 s	6.79 d
VII	5.20 d	3.95 dd	3.71 dd	3.61 t	3.46 o	3.90 dd	3.73 dd		2.07 s	

	Coupling constant J/Hz							
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$	$J_{1,NH}$
II	8.77	9.02	3.47	0.80	4.00	6.08	12.15	
IV	8.78	8.83	8.95	10.03	4.57	3.18	12.11	
V	9.03	9.10	9.19	9.25	2.22	5.08	12.32	
VI	9.47	9.56	9.66	9.90	4.53	2.05	12.52	9.32
VII	1.10	3.30	9.60	9.60	2.25	5.81	12.30	

*Overlapped.

Table 3. FAB Mass Spectral Data of the Compounds Prepared

Compound	The types of quasi-molecular ions					
	$[M + H]^+$		$[M + Glyc + H]^+$		$[2M + H]^+$	
	m/z	I_r/%	m/z	I_r/%	m/z	I_r/%
II	342	100	434	4	683	3
IV	206	100	298	33	411	4
V	222	100	314	12	443	7
VI	390	100	482	1	779	6
VII	222	100	314	26	443	7

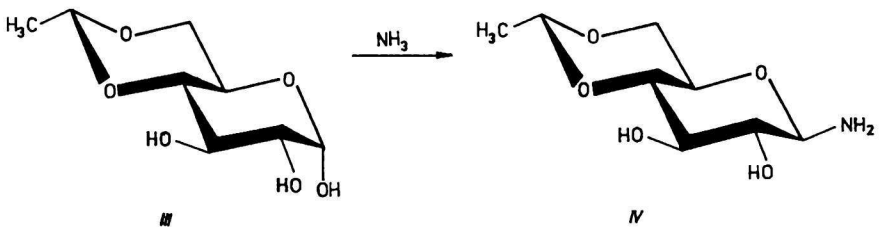
were elucidated by ^{1}H and ^{13}C NMR spectroscopy. The ^{13}C NMR spectrum of *II* (Table 1) revealed six carbon signals, pointing to a symmetrical molecule. The value of the chemical shift of C-1 (δ = 88.7) indicated a β,β-configuration of this compound [1]. The value of the anomeric coupling constant ($J_{1,2}$ = 8.77 Hz; Table 2) also confirmed this point. The value of the coupling constant $J_{1,2}$ and $J_{2,3}$ (9.02 Hz) gave evidence for a $^4\mathbf{C}_1$ conformation of *II*. The FAB mass spectrum of *II* (Table 3) was in accordance with the structure of digalactosylamine.

Treatment of the solution of 4,6-*O*-ethylidene-α-D-glucopyranose (*III*) in methanol with ammonia gave 4,6-*O*-ethylidene-β-D-glucopyranosylamine (*IV*; Scheme 2). The value of the anomeric coupling constant ($J_{1,2}$ = 8.78 Hz; Table 2) proved the β-configuration of *IV*, while the values of the coupling constants pointed to $^4\mathbf{C}_1$ conformation of the substituted glucopyranosylamine. The value of the chemical shift C-1 (δ = 87.2; Table 1) also confirmed the β-anomeric configuration of this compound.

^{1}H NMR spectroscopy proved ($J_{1,2}$ = 9.03 Hz; Table 2) the β-anomeric configuration of *N*-acetyl-β-D-glucopyranosylamine (*V*; Scheme 3). This finding is in accordance with the literature [6], where the configuration of *V* was assigned on the basis of the Hudson rule. The high values of the coupling constants $J_{1,2}$ to $J_{4,5}$ indicated a $^4\mathbf{C}_1$ conformation.

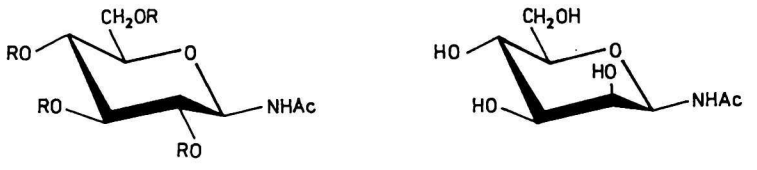
Similarly as in the case of *V*, ^{1}H NMR spectroscopy proved the β-anomeric configuration and the $^4\mathbf{C}_1$ conformation (Table 2) of *N*-acetyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (*VI*; Scheme 3).

The value of the anomeric coupling constant $J_{1,2}$ (1.10 Hz) indicated that *N*-acetyl-β-D-mannopyranosylamine (*VII*; Scheme 3) had a β-anomeric configuration. Literature [7] gives the coupling constant for β-D-mannopyranose $J_{1,2}$ = 1.1 Hz and for α-anomer $J_{1,2}$ = 1.9 Hz. The values of the coupling



III *IV*

Scheme 2



V R = H
VI R = Ac

VII

Scheme 3

constants $J_{3,4}$ (9.6 Hz) and $J_{4,5}$ (9.6 Hz) suggested a $^4\mathbf{C}_1$ conformation of *VII*. For determination of the anomeric configuration also the data of ^{13}C NMR measurements are important. The value of the chemical shift for C-5 (δ = 78.7) confirmed the β-configuration. *Voelter* and *Breitmaier* [8] give δ = 77.30 for C-5 of β-D-mannopyranose and δ = 73.55 for α-D-mannopyranose.

The molecular mass of compounds *II*, *IV*—*VII* was proved by the presence of intensive quasi-molecular ions $[M + H]^+$, $[M + Glyc + H]^+$, and $[2M + H]^+$ in the FAB mass spectra. The respective *m/z* values for the individual compounds and their relative intensities (%) are presented in Table 3.

In the FAB spectrum of *IV* a distinct signal was observed at *m/z* = 394 which corresponded to the $[2M - NH_3 + H]^+$ ion and indicated the presence of a dimer. The FAB mass spectrum of *IV* is illustrated in Fig. 1.

As expected, *VI* underwent a deeper fragmentation due to the presence of five acetyl groups than the other compounds studied: *m/z* = 348 $[M + H - CH_2CO]^+$, *m/z* = 288 $[M + H - CH_2CO - AcOH]^+$, *m/z* = 228 $[M + H - CH_2CO - 2AcOH]^+$, *m/z* = 168 $[M + H - CH_2CO - 3AcOH]^+$, *m/z* = 330 $[M + H - AcOH]^+$, *m/z* = 270 $[M + H - 2AcOH]^+$, and *m/z* = 210 $[M + H - 3AcOH]^+$. In the spectrum there were observed also an oxonium ion of the A_1 type [9] at *m/z* = 331 and the ions with *m/z* = 271, 211, 289, 229, 169, 109, 139, corresponding to the series A.

EXPERIMENTAL

Melting points were determined on a Kofler micro hot-stage. The solutions were evaporated under diminished pressure at 30—40 °C. NMR spectra were recorded on an AM-300 FT (Bruker) spectrometer. Compound *VI* was measured in $CDCl_3$ containing Me_4Si. The other compounds were measured in D_2O solutions using methanol as the internal standard (δ = 50.15). The spectra were recorded at the following instrumental parameters: for ^1H (^{13}C) spectra frequency 300.13 MHz (75.46 MHz), spectral width 3.5 kHz (17 kHz), data points 16 K (32 K). The signals of the protons were assigned using 2D-COSY 45 homocorrelated spectra, and those of the carbon atoms by 2D-X-H correlated spectra. Mass

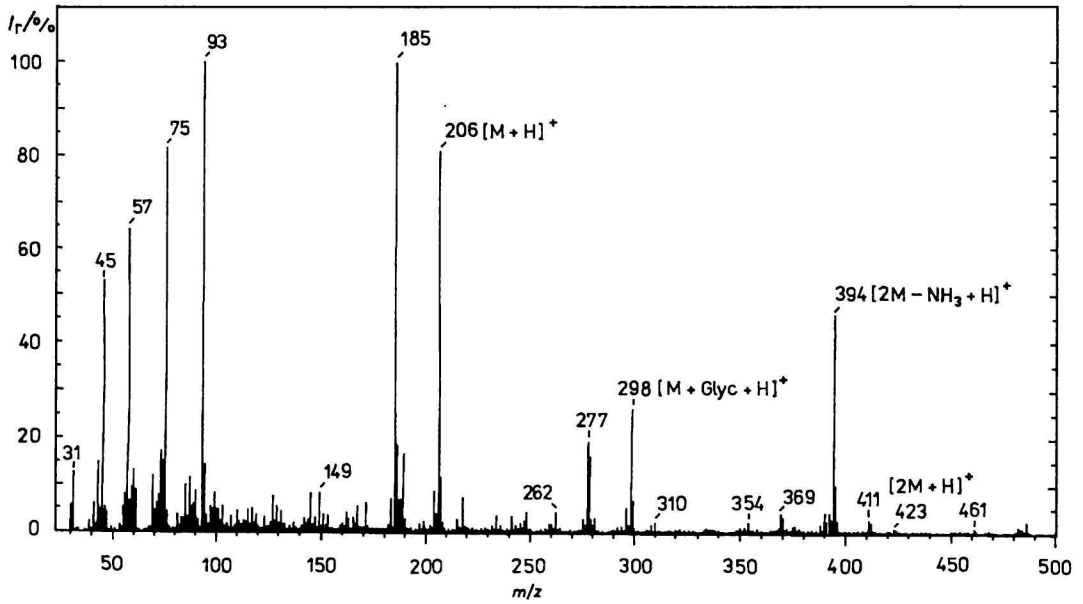


Fig. 1. FAB mass spectrum of 4,6-*O*-ethylidene-β-D-glucopyranosylamine (*IV*).

spectra were recorded in the FAB-ionization mode (Xe, accelerating potential 3 kV, resolution 1500, glycerol (Glyc) matrix) on a JMS-AX 505 W (Jeol) apparatus with double focusing. β-D-Galactopyranosylamine (*I*) was prepared according to [10], 4,6-*O*-ethylidene-α-D-glucopyranose (*III*) according to [11], *N*-acetyl-β-D-glucopyranosylamine (*V*) and *N*-acetyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (*VI*) according to [6, 12], and *N*-acetyl-β-D-mannopyranosylamine (*VII*) according to [6].

Di-β-D-galactopyranosylamine (*II*)

I (5.38 g; 0.03 mol) in anhydrous methanol (300 cm^3) was refluxed with the exclusion of external moisture for 4 h. The solution was concentrated to 100 cm^3 and acetone was added until the first turbidity. The compound *II*, which crystallized at 0 °C within 24 h, was filtered off and dried over P_2O_5 (4.5 g; 43.9 %). Recrystallization from the mixture of methanol—acetone gave a compound of m.p. = 120—122 °C, [α] (D, 20 °C, ρ = 20 g dm^{-3}, water, 2 min) = + 45° [13]. For $C_{12}H_{23}O_{10}N$ (M_r = 341.31) w_i(calc.): 42.23 % C, 6.79 % H, 4.10 % N; w_i(found): 42.13 % C, 6.91 % H, 4.01 % N.

4,6-*O*-Ethylidene-β-D-glucopyranosylamine (*IV*)

III (5 g; 0.024 mol) was suspended in anhydrous methanol (50 cm^3). Then NH_4Cl (0.5 g) was added and gaseous ammonia was introduced at 0 °C until complete dissolution of *III*. From the solution kept at 0 °C the compound *IV* crystallized within maximum seven days. It was filtered off and dried over P_2O_5 (4.1 g; 82.4 %). After recrystallization from methanol it had m.p. = 154 °C, [α] (D, 20 °C, ρ = 20 g dm^{-3}, water, 2 min) = − 30°. For $C_8H_{15}O_5N$ (M_r = 205.21) w_i(calc.): 46.82 % C, 7.37 % H, 6.83 % N; w_i(found): 46.70 % C, 7.49 % H, 6.69 % N.

Acknowledgements. The authors thank the BIJVOET CENTER FOR BIOMOLECULAR RESEARCH, Faculty of Chemistry, Utrecht University (Dr. W. Heerma) for allowing FAB mass spectral measurements. Technical assistance was provided by H. Borská. Our thanks are due also to K. Paule for microanalyses and G. Košický for optical rotation measurements. K. Linek thanks the Slovak Academy of Sciences in Bratislava for the Grant No. 2/999396/92.

REFERENCES

1. Linek, K., Alföldi, J., and Defaye, J., *Carbohydr. Res. 164,* 195 (1987).
2. Linek, K., Alföldi, J., and Defaye, J., *Carbohydr. Res. 247,* 329 (1993).
3. Lalégerie, P., Legler, G., and Yon, J. M., *Biochimie 64,* 977 (1982).
4. Linek, K., Alföldi, J., Farkaš, V., Hrmová, M., Kolarová, N., and Defaye, J., *Abstracts of Papers, 3rd European Symposium on Carbohydrates, Grenoble, 1985,* p. 281.
5. Paulsen, H. and Pflughaupt, K. W., in *The Carbohydrates,* Vol. *1B.* (Pigman, W. and Horton, D., Editors.) P. 881. Academic Press, New York, 1980.
6. Isbell, H. S. and Frush, H. L., *J. Org. Chem. 23,* 1309 (1958).
7. Altona, C. and Haasnoot, C. A. G., *Org. Magn. Reson. 13,* 417 (1980).
8. Voelter, W. and Breitmaier, E., *Org. Magn. Reson. 5,* 311 (1973).
9. Kováčik, V., Petráková, E., Mihálov, V., Tvaroška, I., and Heerma, W., *Biomed. Mass Spectrom. 12,* 49 (1985).
10. Frush, H. L. and Isbell, H. S., *J. Res. Natl. Bur. Stand. (U.S.) 47* 239 (1951).
11. Helferich, B. and Appel, H., *Ber. 64,* 1841 (1931).
12. Brigl, P. and Keppler, H., *Z. Physiol. Chem. 180,* 38 (1929).
13. Linek, K. and Alföldi, J., *Czech. Appl.* 2196/92 (1992).

Translated by A. Kardošová