Glycosylamines
V.* Preparation, Structure, Anomeric Configuration and Conformation of Some *N*-Acetylglycosylamines and *N*-Acetyldiglycosylamines

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N-Acetylation of (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)amine or bis(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)amine afforded *N*-acetyl-bis(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)amine; deacetylation of the latter gave *N*-acetyl-di-β-D-glucopyranosylamine. Similarly, *N*-acetylation of bis(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl)amine led to *N*-acetyl-bis(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl)amine and its deacetylation to di-β-L-rhamnopyranosylamine. The structure, anomeric configuration and conformation of the above-mentioned compounds and also of *N*-acetyl-β-D-xylopyranosylamine, *N*-acetyl-2,3,4-tri-*O*-acetyl-β-D-xylopyranosylamine, *N*-acetyl-β-D-ribopyranosylamine, and *N*-acetyl-2,3,4-tri-*O*-acetyl-β-D-ribopyranosylamine were determined by ^{1}H, ^{13}C NMR, and mass spectrometric methods.

Recently we were engaged in the synthesis, structure, and conformation elucidations of glycosylamines and diglycosylamines of D-glucose, D-xylose [1], L-rhamnose, D-mannose, D-arabinose [2], and D-galactose [3]. As found, the preferred anomeric configuration of both glycosylamines and diglycosylamines was that having the amino group in equatorial position [1—3].

Glycosylamines and diglycosylamines are compounds of interest to enzymologists, because these amines are reported to inhibit some glycosidases [4—6]; diglycosylamines, *viz.* (α-D-glucopyranosyl)(β-D-glucopyranosyl)amine and di-β-D-glucopyranosylamine are effective inhibitors of β-glucosidase of *Trichoderma resei* [5].

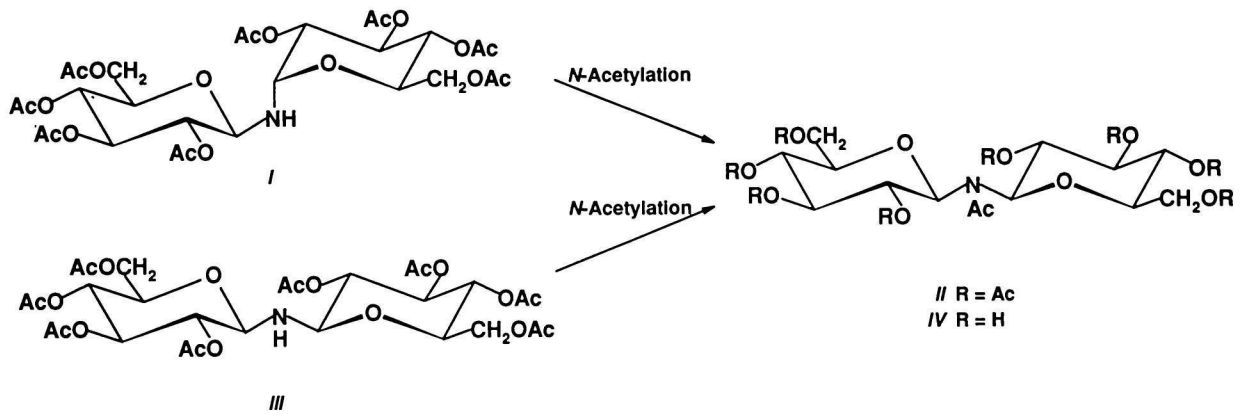
This paper was aimed to elucidate the structure, anomeric configuration and conformation of *N*-acetyl-bis(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)amine (*II*), *N*-acetyl-di-β-D-glucopyranosylamine (*IV*), *N*-acetyl-bis(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl)-amine (*VI*), *N*-acetyl-β-D-xylopyranosylamine (*VIII*), *N*-acetyl-2,3,4-tri-*O*-acetyl-β-D-xylopyranosylamine (*IX*), *N*-acetyl-β-D-ribopyranosylamine (*X*), and *N*-acetyl-2,3,4-tri-*O*-acetyl-β-D-ribopyranosylamine (*XI*).

N-Acetyl-bis(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)amine (*II*) was prepared by *Brigl* and *Keppler* [7]; its anomeric configuration presumed as being β,β determined by *Tóth et al.* [8] by ^{1}H and ^{13}C NMR spectral methods.

We prepared compound *II* by $ZnCl_2$-catalyzed *N*-acetylation of (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-amine (*I*) or bis(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)amine (*III*) with acetic anhydride (Scheme 1). *N*-Acetylation of compound *I* was associated with the change of anomeric configurations from α,β to β,β. The reaction was carried out under milder acetylation conditions than described in paper [7]. This change in reaction conditions resulted in a higher yield of compound *II*. The ^{13}C NMR spectrum of compound *II* in $CDCl_3$ showed at 25 °C signals at δ = 85.3 and δ = 80.2 attributable to anomeric carbon (Table 1); in DMSO-d_6 at 60 °C they appeared as a broad singlet at δ = 82.7, this being due to a partially restricted rotation of the *N*-acetyl group. The broadened and unresolved anomeric proton signal in the ^{1}H NMR spectrum did not allow to estimate the configuration at C-1 and therefore the undecoupled ^{13}C NMR spectrum was recorded. The $J_{C\text{-}1,H\text{-}1}$ value of 158.9 Hz showed unequivocally the β configuration of this symmetric molecule in a $^4C_1$ conformation [9].

N-Acetyl-di-β-D-glucopyranosylamine (*IV*) was obtained by *Brigl* and *Keppler* [7] in a sirupy form but no evidence of its structure was given. We prepared this *N*-acetyl derivative *IV* by deacetylation of compound *II* in a crystalline form. Compound *IV*, similarly as *II*, revealed two signals of the anomeric carbon at δ = 88.9 and δ = 84.2 in D_2O at 25 °C; at 60 °C both signals merged into a broad singlet at δ = 86.5. The

For Part IV see Ref. [5].

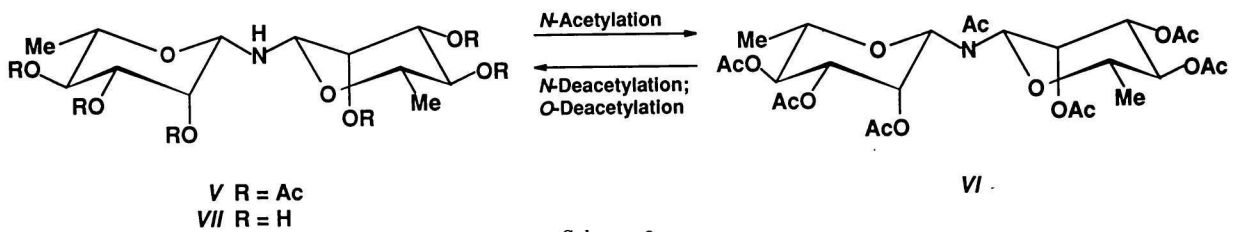


Scheme 1

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

Compound	Chemical shift, δ								
	C-1	C-2	C-3	C-4	C-5	C-6	C=O	CH_3 (NAc)	CH_3 (OAc)
II	85.3[a]	70.5	74.4	67.7	74.9	62.1	171.4	22.6	20.6
	80.2						170.4		20.5
							170.2		
							169.1		
IV	88.9[b]	72.0	77.8	70.5	79.8	62.0	177.6	23.9	
	84.2								
VI	80.5	69.9	71.1	69.7	73.7	17.6	173.0	25.7	20.8
							169.7		20.6
									20.4
VIII	81.0	72.7	77.6	70.1	67.8				
IX	78.5	70.7	72.4	68.9	64.3		170.6	23.0	20.4
							170.5		20.3
							169.6		
							169.5		
X	77.3	69.8	71.4	67.4	64.7		176.6	23.2	
XI	75.2	66.2	68.1	68.1	62.3		171.1	23.0	20.58
							170.2		20.51
							169.9		
							169.5		

a) These signals appear as a broad singlet at $\delta = 82.7$ in DMSO-d_6 at 60 °C.
b) These signals appear as a broad singlet at $\delta = 86.5$ in D_2O at 60 °C.



Scheme 2

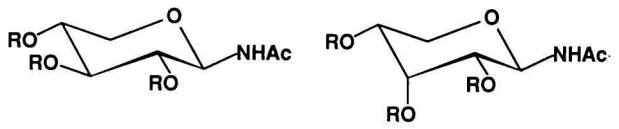
anomeric configuration and conformation of *N*-acetyl derivative *IV* was found to be identical with that of compound *II* [1, 3].

N-Acetyl-bis(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl)amine (*VI*) was synthesized by *N*-acetylation of bis(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl)amine under the same reaction conditions as described with compound *II* (Scheme 2). The ^{13}C NMR spectrum of compound *VI* ($CDCl_3$, 25 °C) disclosed only one a little broadened signal of the anomeric carbon at $\delta = 80.5$

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

Compound	Chemical shift, δ									
	H-1	H-2	H-3	H-4	H-5	H-5′	CH_3 (C-6)	NH	CH_3 (NAc)	CH_3 (OAc)
VI	5.52 d	5.43 dd	5.14 dd	5.03 dd	3.67 o		1.32 d			2.18[a] s
VIII	4.89 d	3.51 t	3.39 t	3.64 o	3.85 dd	3.98 dd			2.08 s	
IX	5.22 t	4.92 t	5.31 t	5.01 o	4.08 dd	3.46 dd		6.88 d		2.06[b] s
X	5.09 d	3.62 dd	4.21 t	3.87 o	3.65—3.75 m				2.07 s	
XI	5.47 t	4.89 dd	5.68 t	5.01 se	3.86 d			6.98 d	2.20	2.03[c]
	Coupling constants J/Hz									
	$\{J_{1,2}\}$	$\{J_{2,3}\}$	$\{J_{3,4}\}$	$\{J_{4,5}\}$	$\{J_{4,5'}\}$	$\{J_{5,5'}\}$	$\{J_{5,6}\}$	$\{J_{1,NH}\}$		
VI	1.15	3.44	10.10	9.41			6.20			
VIII	8.99	9.00	9.20	5.26	10.14	11.30				
IX	9.37	9.45	9.52	5.67	10.68	11.50		9.24		
X	9.34	2.91	2.75	5.60	10.40					
XI	9.55	2.82	2.85	8.54	8.54			9.35		

a) Additional signals at $\delta = 2.08$ and 1.96. *b*) Additional signals at $\delta = 2.04$, 2.02, and 1.99. *c*) Additional signal at $\delta = 2.02$.



VIII R = H
IX R = Ac

X R = H
XI R = Ac

Scheme 3

(Table 1), thus indicating a greater rotational freedom around the *N*-acetyl group when compared with that of compounds *II* and *IV*. Compound *VI* was ascribed the β-L-configuration and $^1C_4$ conformation [2, 10] on the basis of coupling constant values in its ^{1}H NMR spectrum (Table 2).

Deacetylation of *N*-acetyl derivative *VI* did not afford the anticipated *N*-acetyl-di-β-L-rhamnopyranosylamine but di-β-L-rhamnopyranosylamine (*VII*).

This compound was identical with di-β-L-rhamnopyranosylamine obtained by transglycosylation of β-L-rhamnopyranosylamine [2]. Finding that deacetylation of derivative *II* resulted in elimination of *O*-acetyl group only whilst deacetylation of compound *VI* was associated also with the loss of *N*-acetyl group can be rationalized by different nonbinding interactions of this group with equatorial and axial *O*-acetyl groups at C-2 of compounds *II* and *VI*, respectively.

N-Acetyl-β-D-xylopyranosylamine (*VIII*, Scheme 3) was prepared and its anomeric configuration was determined by *Isbell* and *Frush* [11] on the basis of optical rotation value according to Hudson's rule. The coupling constant value $J_{1,2} = 8.99$ Hz evidenced β-configuration of the *N*-acetyl group at the anomeric carbon. The high values of coupling constants $J_{1,2}$ to $J_{3,4}$ indicated the $^4C_1$ conformation of the pyranose ring [1].

N-Acetyl-2,3,4-tri-*O*-acetyl-β-D-xylopyranosylamine (*IX*, Scheme 3) has a β-anomeric configuration and $^4C_1$ conformation like compound *VIII* as followed from $J_{1,2} = 9.37$ Hz (Table 2) and $J_{2,3}$, $J_{3,4}$ coupling constant values [1].

N-Acetyl-β-D-ribopyranosylamine (*X*, Scheme 3) was ascribed the β-anomeric configuration ($J_{1,2} = 9.34$ Hz, Table 2) and $^4C_1$ conformation [10].

Similarly as compound *X* also 2,3,4-tri-*O*-acetyl-β-D-ribopyranosylamine (*XI*, Scheme 3) possessed, on the basis of ^{1}H NMR data, β-anomeric configuration and $^4C_1$ conformation (Table 2).

Electron impact (EI), fast atom bombardment (FAB), and chemical ionization (pyridine; CI (pyr)) mass spectra of compound *VI* are illustrated in Fig. 1. Assignment of the FAB and CI spectra above $m/z =$ 331 (*II*) or 273 (*VII*) is shown in Table 3.

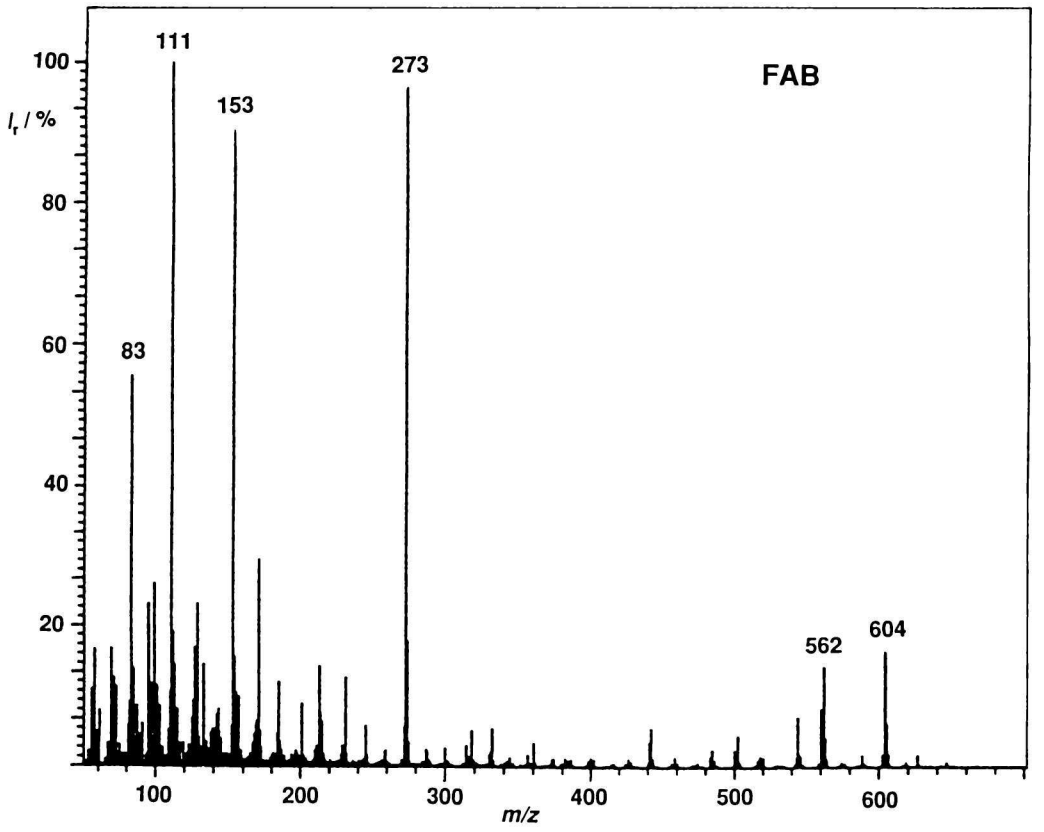
The molecular ions absented in the EI mass spectra and the most prominent recognizable ions were formed by the loss of 102 mass units $[M - AcOH - CH_2CO]^+$.

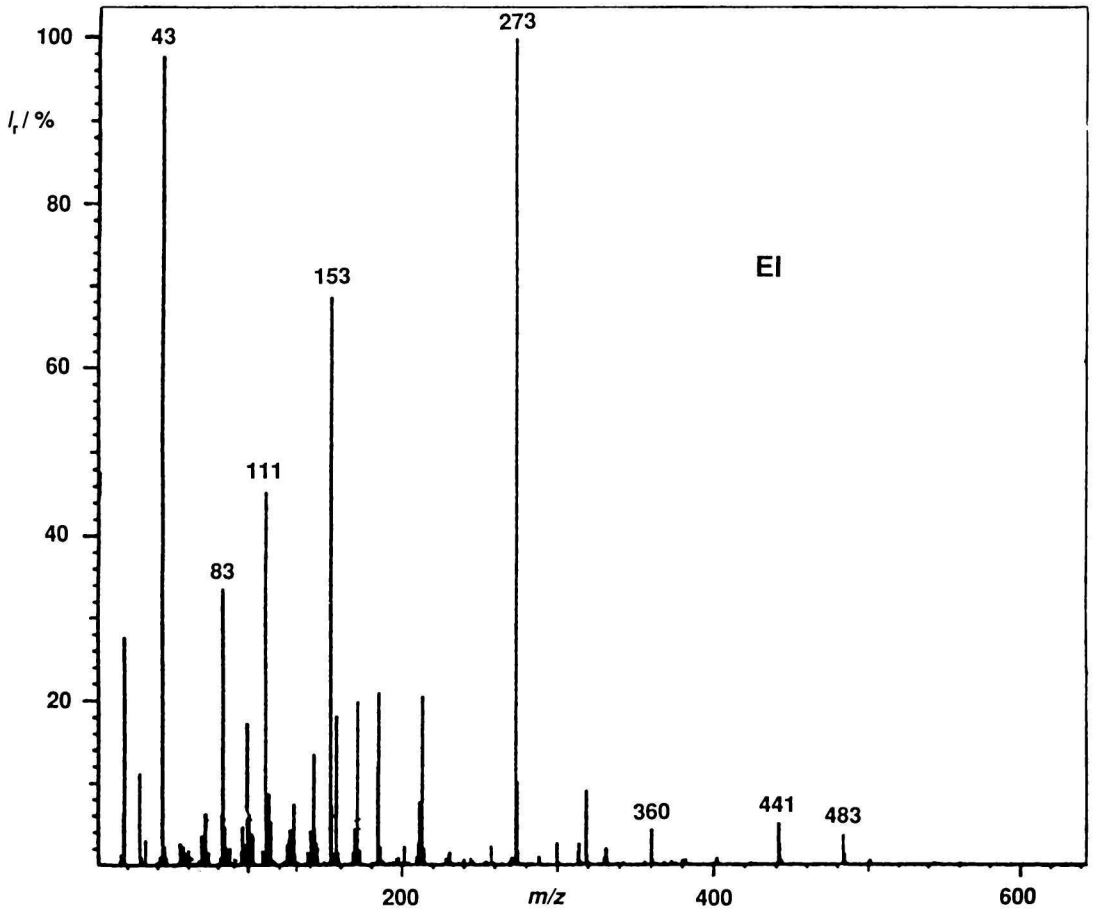
The molecular mass in the FAB mass spectra was proved by the presence of an $[M + H]^+$ ion. All ions observed in the spectra of compounds *II* and *VI* were discussed earlier [3].

The molecular mass was determined also under CI conditions with protonated pyridine as a reagent gas [12]; observed were ions $[A_1 + Pyr]^+$ originating from ion-molecule reactions between A_1 type of the sample ions and reagent gas molecules as well [13, 14].

EXPERIMENTAL

Melting points were determined on a Kofler micro hot-stage. Solutions were evaporated under diminished pressure at 30—40 °C. Compounds *IX*, *X*, and *XI* were prepared according to [11] and [15], respectively. The NMR spectra were run with an AM-300 FT (Bruker) spectrometer. Compounds *IX* and *XI* were


FAB
I_r / %
100
80
60
40
20
111
273
153
83
562
604
100
200
300
400
500
600
m/z


EI
I_r / %
100
80
60
40
20
43
273
153
111
83
360
441
483
200
m/z
400
600

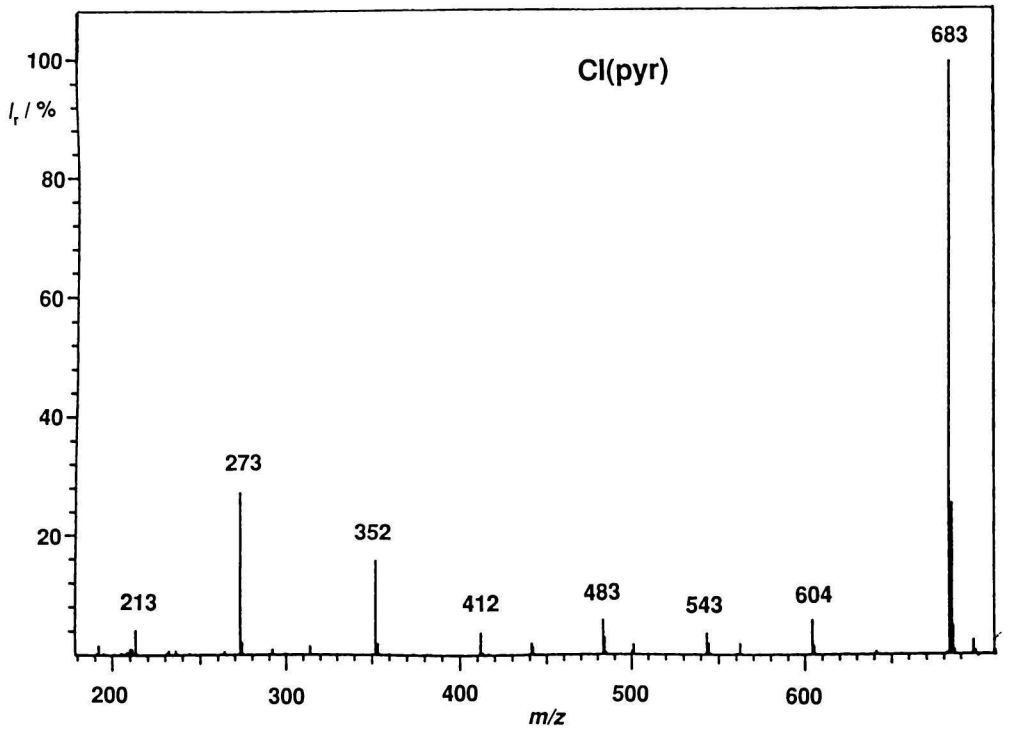


Fig. 1. Mass Spectra of *N*-acetyl-bis(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl)amine (*VI*).

Table 3. FAB and CI (pyr) Mass Spectral Data of Compounds *II* and *VI*

II			*VI*			Type of ions
m/z	FAB	CI (pyr)	m/z	FAB	CI (pyr)	
	I_r/%			I_r/%		
799	–	100	683	–	100	$[M + PyrH]^+$
720	8	7	604	16	5	$[M + H]^+$
678	7	2	562	12	2	$[M + H - CH_2CO]^+$
677	1	0	561	6	0	$[M - CH_2CO]^+$
660	4	1	544	5	2	$[M + H - 2AcOH]^+$
659	0	4	543	2	3	$[M - AcOH]^+$
618	3	3	502	3	2	$[M + H - CH_2CO - AcOH]^+$
617	1	1	501	1	1	$[M - CH_2CO - AcOH]^+$
600	1	3	484	2	3	$[M + H - 2AcOH]^+$
599	0	3	483	1	5	$[M - 2AcOH]^+$
558	3	1	442	4	1	$[M + H - CH_2CO - 2AcOH]^+$
557	2	1	441	3	2	$[M - CH_2CO - 2AcOH]^+$
410	–	11	352	–	16	$[A_1 + Pyr]^+$
331	35	23	273	98	27	$[A_1]^+$

measured in $CDCl_3$ (25 °C) containing Me_4Si, compounds *VIII* and *X* in D_2O (25 °C) with methanol as an internal standard (δ = 50.15). The spectra were recorded as the same instrumental parameters as reported in Ref. [3]. EI and CI (pyr) mass spectra were measured with a Finnigan MAT SSQ 710 instrument (70 eV, 200 μA, source temp. 150 °C, reagent gas pressure 160 Pa). The FAB mass spectra were recorded with a JMS-AX 505 W (Jeol) apparatus with double focusing (Xe, accelerating potential 3 kV, 1-thioglycerol matrix).

N-Acetyl-bis(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)amine (*II*)

Method A. A freshly fused $ZnCl_2$ (1 g) was added to compound *I* (4 g; 5.9 mmol) in acetic anhydride (100 cm^3). This solution was heated to 50 °C within

15 min and then allowed to stand at 20 °C for 1 h, poured onto crushed ice (800 g), and extracted with chloroform (3 × 100 cm^3). The solvent was evaporated and ethanol (200 cm^3) was added to the residue.

Compound *II*, crystallizing during concentration of the solution, was filtered off and dried over P_2O_5. Yield 3.6 g (84.7 %). Recrystallization of the crude product from ethanol—water produced compound of m.p. = 191—192 °C, [α](D, 20 °C, ρ = 20 g dm^{-3}, chloroform) = −8.2°. Ref. [7] reports m.p. = 192 °C, [α](D, chloroform) = −9.2°.

Method B. Per-*O*-acetyl derivative *III* (4 g; 5.9 mmol) was *N*-acetylated and worked out by the same procedure as given with compound *I*. Yield of *N*-acetyl derivative *II* 3.4 g (80.0 %, m.p. = 192 °C, [α](D, 20 °C, ρ = 20 g dm^{-3}, chloroform) = −8.4°. The ^1H and ^{13}C NMR spectra accorded with those of compound *II* prepared by method *A*.

N-Acetyl-di-β-D-glucopyranosylamine (*IV*)

Compound *II* (1.3 g; 1.8 mmol) was treated with methanol—ammonia (40 cm^3) [16] at 0 °C for 48 h, the solvent was evaporated and the residue was dissolved in ethanol (20 cm^3). Acetone was added to the first turbidity and the solution was left to stand at 0 °C. The separated crystals of compound *IV* were filtered off (0.6 g, 86.6 %) and recrystallized from ethanol. M.p. = 154—155 °C, [α](D, 20 °C, ρ = 20 g dm^{-3}, H_2O) = + 20.1°. For $C_{14}H_{25}O_{11}N$ (M_r = 383.35) w_i(calc.): 43.86 % C, 6.57 % H, 3.65 % N; w_i(found): 43.77 % C, 6.52 % H, 3.71 % N.

N-Acetyl-bis(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyl)amine (*VI*)

A freshly fused $ZnCl_2$ (1 g) was added to compound *V* (2 g; 3.6 mmol) in acetic anhydride (80 cm^3); the solution was then heated to 60 °C and kept standing at 20 °C for 2 h. The mixture was worked out as specified with compound *II*. Product *VI* was separated during concentration of the ethanolic solution; yield 1.5 g (69.8 %). The compound crystallizing from ethanol—water had m.p. = 105 °C, [α](D, 20 °C, ρ = 20 g dm^{-3}, chloroform) = −19.5°. For $C_{26}H_{37}O_{15}N$ (M_r = 603.58) w_i(calc.): 51.74 % C, 6.18 % H, 2.32 % N; w_i(found): 51.65 % C, 6.27 % H, 2.42 % N.

Di-β-L-rhamnopyranosylamine (*VII*)

Compound *VI* (1.0 g; 1.7 mmol) was treated with methanol—ammonia (30 cm^3) at 0 °C for 48 h [16]. The solvent was evaporated and ethanol (10 cm^3) was added to the residue. Acetone was dropped into the solution till the first turbidity. Crystals separated during standing at 0 °C were filtered off. Yield of crude compound *VII* was 0.38 g (74.2 %); m.p. of product recrystallized from ethanol—acetone was 117—118 °C, [α](D, 20 °C, ρ = 20 g dm^{-3}, H_2O, 2 min) = + 51.0°. Ref. [2] reports m.p. = 118 °C, [α](D, 25 °C, ρ = 20 g dm^{-3}, H_2O, 2 min) = + 52.0°.

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