Preparation and ^{13}C NMR spectra of some disaccharide glycosylmethylamines

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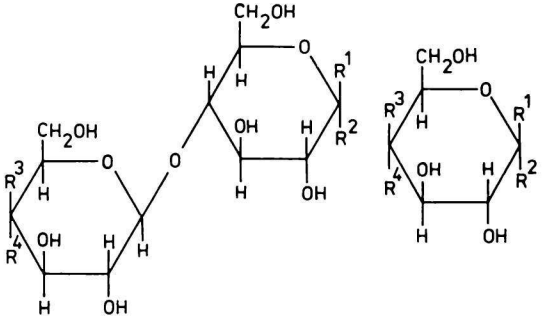
Reduction of β-lactosylnitromethane, β-cellobiosylnitromethane, and β-maltosylnitromethane with ferrous hydroxide *in situ* afforded β-lactosylmethylamine, β-cellobiosylmethylamine, and β-maltosylmethylamine. In the prepared derivatives the primary amino group, in comparison to the primary hydroxyl group, causes a shift of the ^{13}C NMR signal of the α-carbon towards higher values of magnetic field. The primary nitro group in the nitro-precursors exhibits an opposite effect. The INEPT pulse technique was used to assign the signals of the nitromethyl carbon atoms.

Восстановлением β-лактозилнитрометана, β-целлобиозилнитрометана и β-мальтозилнитрометана гидроокисью железа(II) *in situ* были получены β-лактозилметиламин, β-целлобиозилметиламин и β-мальтозилметиламин. В полученных соединениях первичная аминогруппа, по сравнению с первичной гидроксильной группой, вызывает сдвиг ^{13}C ЯМР сигнала α-углерода в сторону более сильного магнитного поля. Первичная нитрогруппа в исходных нитросоединениях обладает противоположным эффектом. Для соотнесения сигналов нитрометильных атомов углерода был применен импульсный метод INEPT.

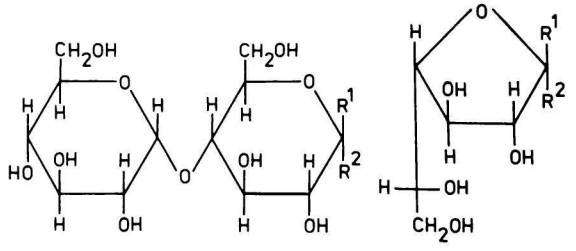
Glycosylmethylamines, as saccharide derivatives suitable *e.g.* for protein glycosylation, are known only for monosaccharides [1—4]. In a previous paper [5] we have described an efficient way of the preparation of glycosylmethylamines by reduction of the corresponding nitro-precursors with ferrous hydroxide *in situ*. Application of this method to some disaccharides and a study of ^{13}C NMR effects of the nitromethyl and aminomethyl groups in disaccharide and monosaccharide derivatives form the subjects of the present communication.

A treatment of β-lactosylnitromethane in aqueous solution with ferrous sulfate and ammonia at 100 °C for 15 min gave β-lactosylmethylamine (*I*) which was isolated from the reaction mixture in 70 % yield. The same reaction with β-cellobiosylnitromethane or β-maltosylnitromethane afforded β-cellobiosylmethylamine (*II*) and β-maltosylmethylamine (*III*) in 73 % and 63 % yields, respectively.

Chromatographically homogeneous amino derivatives *I*—*III*, as well as β-D-glucopyranosylmethylamine (*IV*), β-D-galactopyranosylmethylamine (*V*), and nitro derivatives β-lactosylnitromethane (*VI*), β-cellobiosylnitromethane (*VII*), β-maltosylnitromethane (*VIII*), β-D-glucopyranosylnitromethane (*IX*), β-D-galactopyranosylnitromethane (*X*), α-D-galactopyranosylnitromethane (*XI*), β-D-galactofuranosylnitromethane (*XII*), and α-D-galactofuranosylnitromethane (*XIII*) were subjected to ^{13}C NMR spectral measurements.



I $R_1 = CH_2NH_2$, $R_2 = R_4 = H$, $R_3 = OH$
II $R_1 = CH_2NH_2$, $R_2 = R_3 = H$, $R_4 = OH$
VI $R_1 = CH_2NO_2$, $R_2 = R_4 = H$, $R_3 = OH$
VII $R_1 = CH_2NO_2$, $R_2 = R_3 = H$, $R_4 = OH$
IV $R_1 = CH_2NH_2$, $R_2 = R_3 = H$, $R_4 = OH$
V $R_1 = CH_2NH_2$, $R_2 = R_4 = H$, $R_3 = OH$
IX $R_1 = CH_2NO_2$, $R_2 = R_3 = H$, $R_4 = OH$
X $R_1 = CH_2NO_2$, $R_2 = R_4 = H$, $R_3 = OH$
XI $R_1 = R_4 = H$, $R_2 = CH_2NO_2$, $R_3 = OH$



III $R_1 = CH_2NH_2$, $R_2 = H$
VIII $R_1 = CH_2NO_2$, $R_2 = H$
XII $R_1 = CH_2NO_2$, $R_2 = H$
XIII $R_1 = H$, $R_2 = CH_2NO_2$

^{13}C NMR chemical shifts of the carbon atoms of the CH_2NH_2 group in compounds *I*—*V* (Table 1) occur between 42.5 and 43.1 ppm, thus pointing to a strong shielding effect of the amino group of the equatorial aminomethyl group. A comparison to the chemical shifts of the equatorial hydroxymethyl groups of these compounds shows that, due to the negative α-effect of the equatorial aminomethyl group, the signals are shifted to higher values of magnetic field by 18.9—19.8 ppm. A similar effect of the amino group has been reported with some other amino derivatives of saccharides [6]. Other signals were assigned on the basis of ^{13}C NMR spectral data of unsubstituted saccharides [7]. The selective decoupling could not be used because the 100 MHz ^1H NMR spectra of all studied compounds were of a high order.

The signals of the nitromethyl groups of derivatives *VI*—*XIII* occur in the region of the resonance of the majority of carbon atoms of the pyranoid or furanoid rings (Table 1). In such cases the OFF resonance technique could not be applied for the

Table 1

^{13}C NMR chemical shifts of compunds *I*—*XIII* and α-effects of the primary NH_2 and NO_2 groups

Compound	Chemical shift δ_r/ppm													α-Effect** δ_r/ppm
	CH_2R	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	
I	43.0	81.0	73.8	79.5*	80.1*	77.2	62.3	104.2	72.3	72.3	69.9	76.7	61.7	−19.3
II	42.9	80.3	72.4	77.2	79.5	76.8	61.8	103.8	74.4	77.2	70.7	76.8	61.8	−18.9
III	42.9	80.6	73.0	79.0*	79.2*	78.6	62.3	101.0	72.5	73.9*	70.6	74.1*	61.7	−19.4
IV	42.5	80.6	72.6	78.4	71.0	78.4	62.3	—	—	—	—	—	—	−19.8
V	43.1	81.8	70.4	75.3	70.1	79.7	62.9	—	—	—	—	—	—	−19.8
VI	77.7	79.6	73.8	79.2	79.2	77.0	62.3	104.1	71.5	72.2	69.8	76.6	61.9	+15.4
VII	77.7	79.5	71.5	77.2	79.5	76.7	61.1	103.7	74.4	77.5	70.7	76.7	61.9	+16.6
VIII	77.8	79.4	71.6	77.8*	78.7	77.0*	61.7	101.0	70.5	74.1	70.5	74.1	61.7	+16.1
IX	77.3	80.9	71.8	78.4	70.7	77.2	61.8	—	—	—	—	—	—	+15.5
X	77.8	80.1	70.2	75.0	69.0	77.8	62.4	—	—	—	—	—	—	+15.4
XI	74.3	74.7	68.1	70.9*	70.0*	73.5	62.2	—	—	—	—	—	—	+12.1
XII	73.5	79.1*	80.1*	78.1	83.4	72.1	63.8	—	—	—	—	—	—	+ 9.7
XIII	76.3	86.0	78.3	77.9	79.0	72.3	63.8	—	—	—	—	—	—	+12.3

R = NH_2 for *I*—*V*, R = NO_2 for *VI*—*XIII*.

* Chemical shifts can be reversely assigned.

** Calculated as a difference $\Delta\delta_r = \delta_r(CH_2R) - \delta_r(C\text{-}6)$.

assignments of the signal of the nitromethyl groups since the signals of the CH_2 and CH groups lie very closely or even overlap. Therefore, the INEPT pulse technique [8] was used for their differentiation. A noise decoupled spectrum and an INEPT spectrum of compound *X* is shown in Fig. 1.

As it follows from Table 1, the amino and nitro groups exhibit reverse effects on the shift of the ^{13}C NMR signals. Due to the deshielding effect of the nitro group the signals of the equatorial nitromethyl groups of compounds *VI*—*X* are shifted to lower values of the magnetic field and occur between 77.3 and 77.8 ppm. The value of the observed positive α-effect is 15.4—16.6 ppm. In the compound with the axial nitromethyl group (compound *XI*) the value of the α-effect is lower by 3 to 4 ppm. Differences in the chemical shifts and α-effects were also observed with nitromethyl groups differently bound in the furanoid structures of isomers *XII* and *XIII*.

From the above analysis of the ^{13}C NMR spectral data of amino derivatives *I*—*V* and nitro derivatives *VI*—*XIII* it follows that ^{13}C NMR spectra can be used for determination of the stereochemistry of both nitromethyl and aminomethyl groups linked to a saccharide pyranoid ring. Since ^{13}C NMR spectrometry affords also data for amino derivatives, it appears to be a more general method for elucidation of stereochemistry of the nitromethyl group in glycosylnitromethanes than the method based on the measurement of circular dichroism spectra [9].

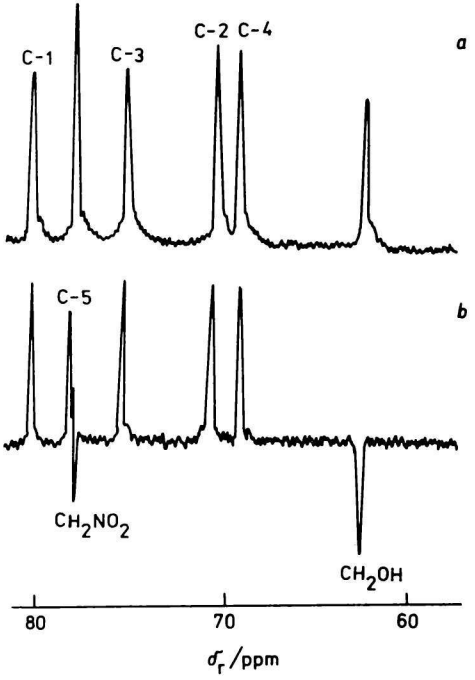


Fig. 1. ^{13}C NMR spectra ($\nu = 25.14$ MHz) of β-D-galactopyranosylnitromethane (*X*). *a*) Noise decoupled; *b*) INEPT.

Experimental

Specific rotations were measured on a Perkin—Elmer 141 polarimeter and the melting points were determined on a Kofler stage. Elemental analyses were done with an automatic Perkin—Elmer 240 analyzer. ^{13}C NMR spectra were recorded with a FT-NMR spectrometer Jeol FX-100 in deuterium oxide at compound concentrations of 70 mg cm^{-3} and room temperature using methanol as internal standard (its chemical shift related to TMS is 50.15 ppm). Other parameters of measurements: pulse width 4 μs, sweep width 2500 Hz, flip angle 30°, repetition time 1.5 s, and average number of accumulations 2000. The pulse sequence INEPT ($J_{C,H} = 130$ Hz) was used to resolve the signals of the nitromethyl and hydroxymethyl groups. All glycosylnitromethanes and monosaccharide glycosylmethylamines were prepared according to the literature [5, 10].

β-Lactosylmethylamine (I)

A solution of β-lactosylnitromethane (2 g) in water (10 cm^3) was added into a stirred boiling solution of ferrous sulfate heptahydrate (10.1 g) in water (24 cm^3). While still agitated intensively, the solution was alkalized (pH > 8) with concentrated aqueous solution of ammonia added by portions (à 3 cm^3) and then boiled for another 10 min. The alkalinity of the solution was maintained by addition of ammonia. The precipitate was filtered off and washed with a 2 % aqueous solution of ammonia (50 cm^3). The filtrate was cooled and mixed with an anion exchanger in the hydroxide form (*ca.* 50 g) and the liquid phase was then concentrated *in vacuo* (*ca.* 5 kPa) to about a half volume. The anex was removed by filtration and washed with water (3 × 50 cm^3). The filtrate was evaporated to give pure β-lactosylmethylamine (yield = 1.3 g (70 %)) which crystallized from methanol in the atmosphere of carbon dioxide on the vessel walls as β-lactosylmethylammonium hydrogen carbonate, m.p. 139—140 °C, [α] (D; 20 °C; water; $\varrho = 20$ g dm^{-3}) = + 19°

For $C_{13}H_{25}NO_{10} \cdot H_2CO_3$ w_i(calc.): 40.28 % C, 6.52 % H, 3.36 % N; w_i(found): 40.36 % C, 6.72 % H, 3.36 % N.

β-Cellobiosylmethylamine (II)

The procedure for the preparation of *I* carried out with β-cellobiosylnitromethane (1 g) up to the stage of the concentration of the final filtrate (5 kPa, 40 °C, 5 h) afforded amorphous glassy hygroscopic β-cellobiosylmethylamine monohydrate (yield = 0.7 g (73 %)), [α] (D; 20 °C; water; $\varrho = 20$ g dm^{-3}) = + 2°.

For $C_{13}H_{25}NO_{10} \cdot H_2O$ w_i(calc.): 41.82 % C, 7.29 % H, 3.75 % N; w_i(found): 42.17 % C, 7.39 % H, 3.53 % N.

β-Maltosylmethylamine (III)

The procedure for the preparation of *II* carried out with β-maltosylnitromethane (2 g) gave β-maltosylmethylamine monohydrate (yield = 1.2 g (63 %)), an amorphous glassy

hygroscopic compound, $[\alpha]$ (D; 20 °C; water; $\varrho = 20$ g dm^{-3}) = +93°.
For $C_{13}H_{25}NO_{10} \cdot H_2O$ w_i(found): 42.04 % C, 7.43 % H, 3.50 % N.

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