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^{15}N and ^{1}H NMR study of ureido sugars, derivatives of 2-amino-2-deoxy-β-D-glucopyranosides

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ABSTRACT: The ^{15}N NMR spectra of a series of derivatives of 2-amino-2-deoxy-β-D-glucopyranose and dipeptides or secondary amines were recorded. In the dipeptide derivatives the chemical shift of nitrogen atom N-1 (linked to sugar) is essentially unchanged and the shifts of nitrogen atoms N-3 and N-6 are determined by the nature of the R_1 and R_2 substituents at C-α carbon of the amino acid unit. The highest shielding of N-3 and N-6 is observed for Gly units (R_1 or R_2 = H) and decreases in the order Gly > Val > Phe > Leu > Ala. The deshielding of the nitrogen and proton of the N-6—H group results from the intramolecular hydrogen bonding interaction with the oxygen atom of the ester group. The experiment with H–D isotopic exchange confirmed slow exchange of the N-6—H protons whereas the N-1—H and N-3—H protons, not involved in intramolecular H-bond, exchange fast.

KEYWORDS: NMR; ^{1}H NMR; ^{15}N NMR; ureido sugars

INTRODUCTION

In recent years, ureas with sugar substituents have attracted considerable attention. Ureido sugars are starting materials in the synthesis of nitrosoureido sugars, which exhibit antitumour activity. Streptozotocin[1] and chlorozotocin[2] are used in clinical treatment and nitrosoureido sugars with other substituents are being tested for this application. As part of our continuing work on the synthesis[3–5] and the determination of the structure of ureido sugars,[5–8] we report here the results of a ^{15}N NMR study of a series of derivatives of 2-amino-2-deoxy-β-D-glucopyranose and secondary amines **1**–**7** and dipeptides **13**–**22** (Scheme 1). For the sake of completeness, the results[8] for amino acid derivatives **8**–**12** are also included. The method of synthesis of ureido sugars with dipeptide residues and structure characterization by ^{1}H and ^{13}C NMR spectroscopy were reported recently.[5] Compounds with an amino acid or dipeptide contain two or three NH groups and knowledge of their properties was required prior to the next step in the synthesis, *N*-nitrosation, which should be carried out selectively.

RESULTS AND DISCUSSION

The chemical shifts and coupling constants for **2**–**4** with two alkyl groups and **13**–**22** with dipeptide residues are given in Table 1. The data for other ureido sugars are included for comparison.

Two signals were seen in the proton decoupled natural abundance ^{15}N NMR spectra of **2**–**4**, but the difference in chemical shifts was too small for reliable assignment based on alkyl group increments and the spectra were recorded using the INEPT technique. The N-1—H signal appeared as a doublet with a ^{1}J(NH) of *ca.* −87 Hz; this resonance can be assigned to the nitrogen linked to glucopyranose and the other resonance is from N-3 nitrogen. The chemical shifts of N-1 are within the range −303.5 to −306.2 ppm and nitrogen N-3 in compounds with dialkyl groups is deshielded (16–26 ppm) when compared with N-1. The nitrogen of a secondary or primary amine residue incorporated in a ureido sugar is deshielded with respect to that from the respective alkylamine. It is worth comparing ureido sugars **1** and **2** with one and two ethyl groups at N-3. The replacement of the N-3—H hydrogen by a second ethyl group results in a low-frequency shift of N-1 (of 4.7 ppm) and a high-frequency shift of N-3 (of 7.6 ppm). A small substituent effect (2 ppm) for N-1 and a high-frequency shift of N-3 (of *ca.* 20 ppm) were observed[7] for ureido sugars with aromatic substituents (**5**–**7**), in agreement with the known tendency[9] that the nitrogen resonance in arylamines is usually deshielded compared with that for alkylamines.

In the spectra of ureido sugars with one L-amino acid residue,[8] the resonance of N-1 appeared at approximately −300 ppm and the replacement of the amino acid led to no change; the resonances of N-3 are within the range −292 to −306 ppm and their chemical shifts depend on the effects of the side-chain at the C-α carbon of the amino acid unit.

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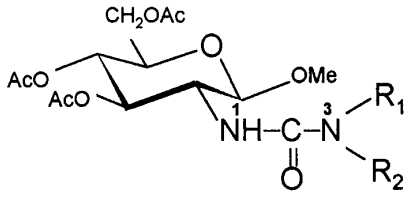
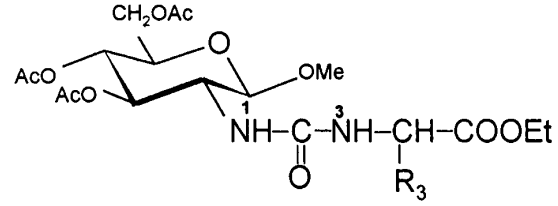
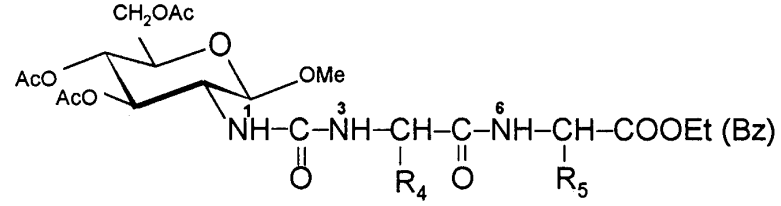


1. $R_1 = CH_2CH_3$, $R_2 = H$
2. $R_2 = CH_2CH_3$, $R_2 = CH_2CH_3$
3. $R_1 = C_6H_{13}$, $R_2 = C_6H_{13}$
4. $R_1 = C_6H_{11}$, $R_2 = C_6H_{11}$
5. $R_1 = C_6H_5$, $R_2 = H$
6. $R_1 = p\text{-}CH_3C_6H_4$, $R_2 = H$
7. $R_1 = p\text{-}CH_3OC_6H_4$, $R_2 = H$



8. $R_3 = H$
9. $R_3 = CH_3$
10. $R_3 = CH(CH_3)_2$
11. $R_3 = CH_2CH(CH_3)_2$



13. $R_4 = H$, $R_5 = CH_3$ (Et)
14. $R_4 = CH_3$, $R_5 = H$ (Et)
15. $R_4 = CH_3$, $R_5 = CH_3$ (Et)
16. $R_4 = H$, $R_5 = CH(CH_3)_2$ (Et)
17. $R_4 = CH(CH_3)_2$, $R_5 = H$ (Et)
18. $R_4 = CH_2CH(CH_3)_2$, $R_5 = H$ (Et)
19. $R_4 = CH_2C_6H_5$, $R_5 = H$ (Et)
20. $R_4 = CH_2C_6H_5$, $R_5 = H$ (Bz)
21. $R_4 = H$, $R_5 = CH_2C_6H_5$ (Bz)
22. $R_4 = H$, $R_5 = CH_3$ (Bz)
23. $R_4 = CH_2CH(CH_3)_2$, $R_5 = CH_2CH(CH_3)_2$ (Et)

Scheme 1. Structures of the compounds studied.

High-resolution and solid-state ^{15}N NMR techniques are being increasingly applied to studies of peptides;[10] chemical shifts were found to depend on the conformation, nature of the amino acid, amino acid sequence and the kind of hydrogen bonding. It seemed interesting to measure ^{15}N NMR spectra for ureido sugars **13–22** because these compounds are soluble in $CDCl_3$, and the chemical shifts of dipeptide residues can be obtained in a weakly interacting solvent whereas most available data are for DMSO or water.

^{15}N NMR spectra of dipeptide derivatives were recorded with the INEPT technique and three doublets appeared in the typical spectra in the narrow range from −290 to −310 ppm. This difference in chemical shifts of a few ppm is too small for unambiguous assignment. Single-frequency $^{15}N\{^1H\}$ decoupling experiments were first performed to differentiate the N-CH_2 nitrogen in Gly-containing compounds from the remaining nitrogens with CH neighbours. The values of ^2J(N-C,H) are 0.7–1.5 Hz[11] and such small splittings into triplets or doublets were not clearly seen in the resonances. The intensity of the N-1 signal was usually slightly smaller, probably because of the longer relaxation time of this nitrogen, bound to the bulky and relatively rigid sugar moiety. The assignment of the ^{15}N shifts of the three NH groups was confirmed by the correlation with the shifts of their attached proton in a 2D heteronuclear experiment (gs-HSQC). The most deshielded ^{15}N resonance assigned to N-6 is correlated, as expected, with the most deshielded proton N-6—H (Fig.1). The chemical shift of N-1, linked to glucose, is *ca.* −300 ppm and is not responsive (as in ureido sugars with one amino acid) to the changes of the substituent at N-3. The chemical shifts of the N-3 nitrogens in ureido sugars **13–22** are more variable. The highest shielding is observed for nitrogen in Gly (R = H) and

Table 1. ^{15}N chemical shifts (in $CDCl_3$, δ in ppm, reference CH_3NO_2) and ^{1}J(N,H) coupling constants (Hz) (in parentheses) for peracetylated methyl β-D-glucopyranosyl ureas with various substituents

Compound	N-1	N-3	N-6	Ref.
1	−301.2 (88.7)	−294.1 (88.7)		6
2	−305.9 (87.7)	−286.5		
3	−306.2 (87.3)	−279.8		
4	−303.5 (87.3)	−286.9		
5	−297.9 (89.5)	−277.4 (89.4)		7
6	−298.3 (89.3)	−278.0 (89.2)		7
7	−299.2 (89.8)	−280.9 (89.0)		7
8	−300.2 (89.4)	−307.6 (90.1)		8
9	−300.2 (89.3)	−292.2 (90.0)		8
10	−299.2 (88.2)	−298.9 (89.1)		8
11	−300.1 (89.2)	−294.1 (89.8)		8
12	−300.1 (89.1)	−296.9 (89.9)		8
13	−300.7 (88.7)	−306.4 (89.5)	−263.7 (93.9)	
14	−300.9 (91.5)	−289.8 (90.6)	−279.3 (91.8)	
15	−300.2 (88.3)	−290.7 (89.1)	−261.9 (92.9)	
16	−300.8 (89.2)	−306.2 (90.6)	−269.6 (93.3)	
17	−300.5 (87.8)	−296.1 (89.5)	−273.3 (92.0)	
18	−300.8 (89.7)	−291.2 (91.0)	−276.4 (94.0)	
19	−300.8 (88.8)	−294.4 (89.5)	−276.0 (93.8)	
20	−300.8 (90.1)	−294.2 (89.5)	−277.1 (93.7)	
21	−300.6 (89.8)	−306.4 (90.7)	−267.3 (90.3)	
22	−301.0 (88.0)	−290.0 (90.5)	−278.9 (93.9)	

decreases in the order Gly > Val > Phe > Leu > Ala. This order can be explained in terms of the β-effect of the alkyl group R_4 or R_5 linked to the C-α carbon of the amino acid unit and is in agreement with the sequence observed in *N*-acyl- and *N*-carbobenzyloxyamino acids[11] and in ureido sugars **8**–**12** with L-amino acid residues.[8]

The plot of the nitrogen chemical shifts for ureido sugars with dipeptides *versus* the above amino acid sequence (Fig. 2) characterizes the behaviour of three types of NH groups: (i) the chemical shift of N-1, linked to sugar, is essentially unchanged; (ii) the chemical shift of N-3 is determined by the effect of R_4, in similar way as in other peptide compounds since the relationship is linear; (iii) the chemical shift of N-6 is dependent on the nature of R_5, but the proximity of the ester group results in deshielding (approximately 20 ppm).

The plots shown in Fig. 2 give the possibility of predicting nitrogen chemical shifts for the ureido sugars with other amino acid sequences such as those synthesized by us (**13**–**22**, Scheme 1) for example, for ureido sugar **23** with Leu–Leu the values −300, −291 and −264 ppm can be expected for N-1, N-3 and N-6, respectively.

Since the δ ^{15}N measurements were carried out in dilute $CDCl_3$ solutions, the contribution of intermolecular hydrogen bonding can be neglected; however,

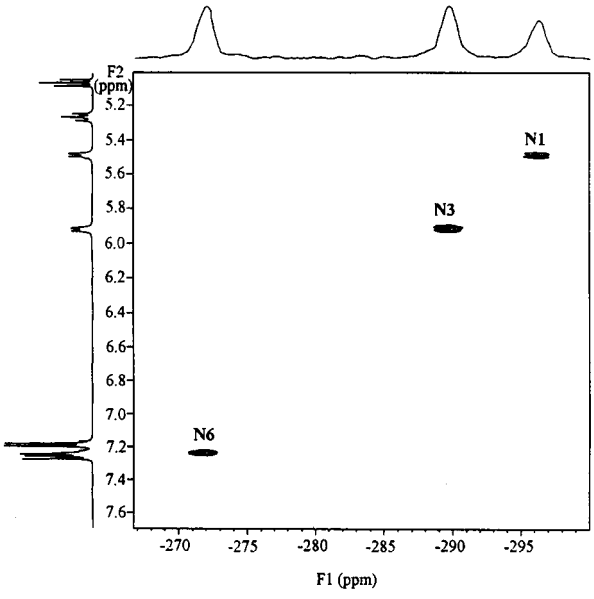


Figure 1. 2D gs-HSQC ^{1}H/^{15}N NMR spectrum of **19**.

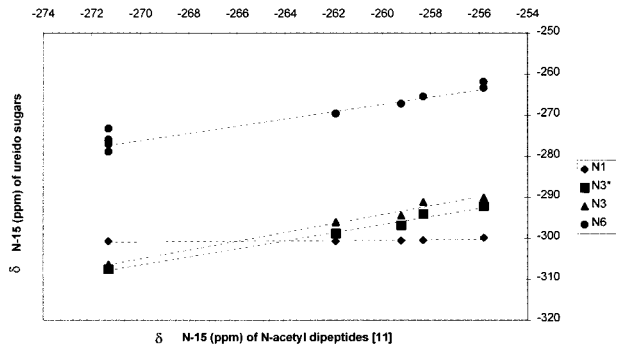


Figure 2. Plot of ^{15}N chemical shifts for ureido sugars with dipeptides *versus* the amino acid sequence (from Ref. 11).

intramolecular hydrogen bonds are still present. IR and ^{1}H NMR spectroscopy have frequently been applied to characterize the inter- and intramolecular interactions of amino acid derivatives in solution. A Fourier transform IR study of ureido sugars with amino acid residues[12] indicated that the NH group is involved in an intramolecular hydrogen bond, a pentagon (C_5 type), which is formed by association with the oxygen atom of the ester group.

In ureido sugars with dipeptides, the ^{1}H NMR chemical shifts of N-1—H are in the range 4.95–5.61 ppm and those of N-3—H are 5.45–6.08 ppm, whereas those of N-6—H are in the narrower range of 6.86–7.28 ppm; usually N-6—H is at least 1 ppm more deshielded than N-3—H. The relationship between δ ^{1}H and δ ^{15}N for the three NH groups is illustrated in Fig. 3. The deshielding of N-6—H protons can be related to the intramolecular hydrogen bonding interaction. In order to confirm this supposition, an experiment with H–D isotopic exchange was carried out.

The NH protons are usually rapidly exchanged with deuterium; however, in molecules where amide protons are involved in intramolecular hydrogen bonds the rate of the exchange reactions with the solvent will be considerably slower. The H–D exchange effect for labile protons of biomolecules was used as an indicator of the exposure of these protons to the solvent and yielded information on the peptide conformations.[13] Part of the ^{1}H NMR spectrum of **19** is illustrated in Fig. 4; after the addition of CD_3OD (50 µl) the signals of N-1—H and N-3—H lost 95% of their intensity whereas only a slight decrease of the intensity of N-6–H was observed.

The ^1J(N,H) coupling constants, given in Table 1, are of interest because they could be related to the torsional angles in conformations of peptides in solution. According to a general dependence, ^1J(N,H) decreases from *ca.* 95 Hz for a planar *trans* peptide group to *ca.* 67 Hz for an angle of 90° and increases again to *ca.* 90 Hz for a planar *cis* arrangement, found in cyclic peptides. In the linear dipeptides, ^1J(N,H) is between 92.1 and 94.5 Hz.[13] The extended conformation with an all-*trans* orientations is far more probable than *cis* orientations of NH and C=O groups for the dipeptide residue of ureido sugars, hence the values of ^1J(N,H) can be related

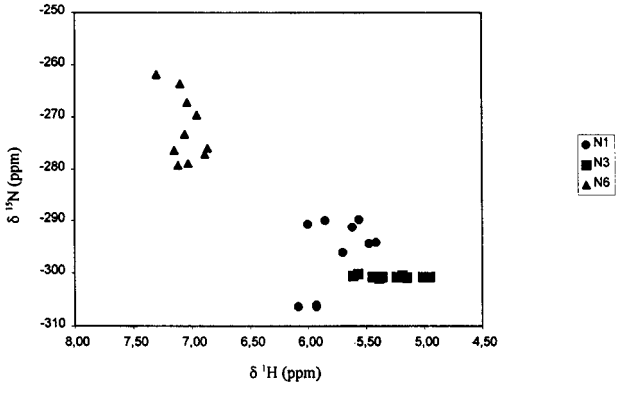


Figure 3. Relationship between δ ^{1}H and δ ^{15}N for the three NH groups in **13–22**.

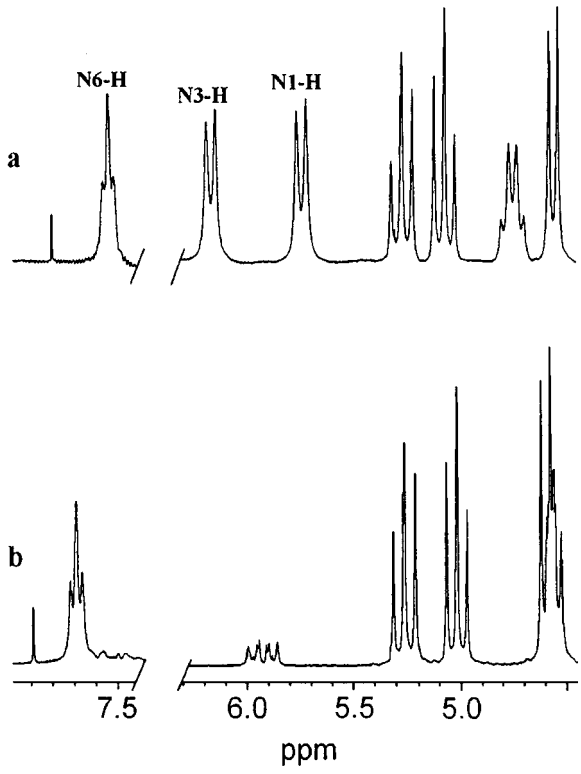


Figure 4. Part of ^{1}H NMR spectrum of **19**, (a) before and (b) after addition of CD_3OD.

to the more pyramidal or trigonal geometry of the nitrogen. The value of ^1J(N,H) is linearly dependent on the percentage of *s* character of the nitrogen orbitals,[9,11] the higher the value of ^1J(N,H), the higher is the bond order of the nitrogen. Values of 87–90 Hz were measured for ^1J(N,H) for nitrogens N-1 and N-3 of the ureido bridge (Table 1), in agreement with those usually observed for ureas. The shielding of >300 ppm and the smaller ^1J(N,H) of 87–88 Hz for N-1 suggest a more pyramidal geometry of nitrogen linked to glucose. The value of ^1J(N,H) between 91.5 and 94 Hz and the lowest shielding indicates that the geometry for N-6 is more trigonal.

EXPERIMENTAL

The ureido sugars were synthesized from methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(4-nitrophenoxycarbonylamino)-β-D-glucopyranoside and a secondary amine or respective dipeptide (protected by an OMe, OEt or OBz group) according to published procedures.[3–5]

^{15}N NMR spectra were recorded on a Bruker AM-500 spectrometer at 50.7 MHz, using the standard INEPT Bruker sequence with following parameters: ^{15}N pulse width 30 µs (90°), relaxation delay 1.5 s, acquisition time 2.95 s, spectral width 5 kHz and digital resolution 0.17 Hz per point. The pulse sequence was optimized for the average ^1J(N,H) of *ca.* 90 Hz (t = 1/4 ^1J(N,H)). Spectra were recorded using a 10 mm probe and the concentration was 0.5 M in $CDCl_3$. ^{15}N chemi-

cal shifts were referenced against neat external CH_3NO_2 ($\delta_{liq.ammonia} = 379.5 - \delta_{nitromethane}$).

^{1}H NMR spectra were recorded on a Bruker AMX-500 spectrometer for 0.05 M solutions in $CDCl_3$; hydrogen to deuterium exchange rates were measured upon the addition of CD_3OD.

The two-dimensional nitrogen–proton correlation experiment was performed on a Bruker DRX-500 spectrometer, using the phase-sensitive gradient-selected HSQC inverse technique. Spectra were measured in 5 mm tubes. The experiment was optimized for ^{1}J(N,H) of 90 Hz. The remaining parameters were as follows: acquisition time 0.25 s, relaxation delay 1 s, observed pulse width 7.4 μs, spectral width (^{1}H) 4 kHz and number of points 2048. The experiment was performed with four scans of 128 echo and four scans of 128 anti-echo accumulations; 2D experimental data were zero-filled to 512 points along the nitrogen direction. Chemical shifts were referenced against internal TMS (^{1}H) and external neat CH_3NO_2 (^{15}N).

Acknowledgements

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