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^{1}H, ^{13}C and ^{15}N NMR Study of Nitrosoureido Sugars, Derivatives of 2-Amino-2-deoxy-β-D-glucopyranoside and Amino Acids or Peptides

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Nitrosoureido sugars with amino acid and dipeptide ester residue were characterized by ^{1}H, ^{13}C and ^{15}N NMR and their spectra were assigned using 1D and 2D NMR methods. The N-nitrosation of ureido sugars frequently yields a mixture of isomers. In the case of ureido sugars with amino acid residues preferably the N3–NO isomers were obtained, whereas for compounds with dipeptide esters the N1–NO isomers dominate. The results are in agreement with the molecular modelling (PM3) predictions, when the intramolecular hydrogen bonds, net charges at nitrogen atoms and heat of formations of particular isomers are considered.

Key words: NMR spectroscopy, antitumor agents, nitrosoureido sugars

Two series of substituted nitrosoureido sugars, the derivatives of methyl 2-deoxy-β-D-glucopyranoside and amino acid or dipeptide ester residues have been synthesized. Our aim was to search for anticancer agents, structurally similar to the clinically used nitrosoureas, such as streptozotocin or chlorozotozin [1]. One can expect that linking the ureido moiety to the amino acid or dipeptide residue will reduce strong side effects of these drugs. However, the intermediate products have two or three NH functions, whereas selective introduction of one nitroso group is desired. The selectivity of nitrosation is related to the NH protons acidity and steric requirements (accessibility of the respective nitrogen atom).

Unfortunately, despite several experiments with nitrosation conditions, only in a few cases a single nitroso derivative was obtained. Usually, conventional nitrosation yielded a mixture of isomers which are unstable and thus difficult to separate. For example, the nitrosation of 5′-[3-(2-chloroethyloureido)]-5′deoxythymidine gave a mixture of two isomers 5′-[3-(2-chloroethyl-1-nitroso)ureido]-5′deoxythymidine and 5′-[3-(2-chloroethyl-2-nitroso)ureido]-5′deoxythymidine [2]. Similarly, the nitrosation of 1-(2-bromoethyl)-3-(2-chloroethyl)urea gave a mixture of 1-(2-chloroethyl)-3-(2-bromoethyl)-1-nitrosourea and 1-(2-bromoethyl)-3-(2-chloroethyl)urea [3]. In another case, nitrosation of 1-(2-chloroethyl)-3-phenylurea afforded an iso-

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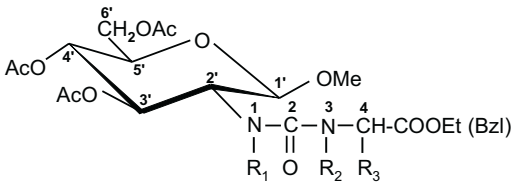
meric pair of 1-(2-chloroethyl)-1-nitroso-3-phenylurea and 3-(2-chloroethyl)-1-nitroso-3-phenylurea [4]. Nevertheless, the investigations on the reaction mechanism and improvement of selectivity require a reliable method of identification of the products [5].

The structure and conformational preferences of nitrosoureido sugars were studied by ^{1}H, ^{13}C and ^{15}N NMR spectroscopy and molecular modelling.

Scheme 1



1a R_1 = NO, R_2 = H, R_3 = H, OEt (GlyOEt)

1b R_1 = H, R_2 = NO, R_3 = H, OEt (GlyOEt)

2a R_1 = NO, R_2 = H, R_3 = H, OBzl (GlyOBzl)

2b R_1 = H, R_2 = NO, R_3 = H, OBzl (GlyOBzl)

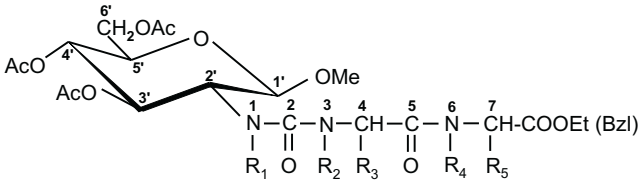
3a R_1 = NO, R_2 = H, R_3 = CH_3, OEt (AlaOEt)

3b R_1 = H, R_2 = NO, R_3 = CH_3, OEt (AlaOEt)

4**a** R_1 = NO, R_2 = H, R_3 = $\overset{1''}{C}H_2\text{-}\overset{2''}{C}H(\overset{3''}{C}H_3)_2$, OEt (LeuOEt)

4**b** R_1 = H, R_2 = NO, R_3 = CH_2-$CH(CH_3)_2$, OEt (LeuOEt)

Scheme 2



5a R_1 = NO, R_2 = H, R_3 = $\overset{1''}{C}H_3$, R_4 = H, R_5 = $\overset{1'''}{C}H_3$, OEt (AlaAlaOEt)

5b R_1 = H, R_2 = NO, R_3 = CH_3, R_4 = H, R_5 = CH_3, OEt (AlaAlaOEt)

5c R_1 = H, R_2 = H, R_3 = CH_3, R_4 = NO, R_5 = CH_3, OEt (AlaAlaOEt)

6a R_1 = NO, R_2 = H, R_3 = $CH_2C_6H_5$, R_4 = H, R_5 = H, OEt (PheGlyOEt)

6b R_1 = H, R_2 = NO, R_3 = $CH_2C_6H_5$, R_4 = H, R_5 = H, OEt (PheGlyOEt)

RESULTS AND DISCUSSION

Synthesis. As mentioned above, the main task is how to perform *N*-nitrosation selectively and with a high yield. The nitrosation of the *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoylglycine ethyl ester (**1**), GlyOEt, provided a mixture (10:1) of two isomers, the dominating **1b** with N3–NO and the minor **1a** with N1–NO. The replacement of protection group OEt by OBz results in an increase of selectivity; the nitrosation of *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoylglycine benzyl ester (**2**), GlyOBz gave only isomer **2b** substituted at *N*-3. One isomer of (**3b**), also with N3–NO was obtained in the case of AlaOEt, *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoyl-L-alanine ethyl ester (**3**). The nitrosation of *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoyl-L-leucine ethyl ester (**4**), LeuOEt led to a mixture (2:1) of two nitroso products: **4b** with N3–NO and **4a** with N1–NO.

The nitrosation of ureido sugars with dipeptide ester residue provided a mixture of three isomers in the case of *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoyl-L-alanyl-L-alanine ethyl ester (**5**) AlaAlaOEt: (**5a)** with N1–NO, **5b** with N3–NO and **5c** with N6–NO. Two isomers were obtained after nitrosation of *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoyl-L-phenylalanyl-glycine ethyl ester (**6**) PheGlyOEt , **6a** with N1–NO and **6b** with N3–NO. In both cases the dominating isomer was that with N1–NO substitution. Nitrosoureas are sensitive to heating, light and air oxidation, and decompose in solution. The attempts to separate the mixture of nitrosoureido sugars **1a** and **1b** or **4a** and **4b** by column chromatography were unsuccessful, since decomposition products accompanied the outcoming isomer. From the studied compounds only **3b** was stable enough and can undergo biological tests.

Molecular modelling. An understanding of the preferred conformations of nitrosoureido sugars and a prediction of the place of nitrosation were of special interest. For this purpose: i) intramolecular hydrogen bonds, ii) charges at nitrogen atoms, and iii) relative heat of formation have been considered.

Intramolecular hydrogen bonding in ureido sugars with amino acid [6] and dipeptide esters [7] was studied in detail by infrared and ^{1}H NMR spectroscopies. Conformational analyses of ureido sugars showed that N3–H can form only one type of intramolecular hydrogen bonds (C_5) with C5=O of amino acid residue. The N1–H group does not have a chance to form five-membered cycle C_5, however it can be involved in two kinds of intramolecular interactions of C_7 (seven membered cycle) type. Therefore, from the two nitrogen atoms the N3–H seems to be more easily accessible. The conformations with intramolecular hydrogen bonds exhibit a lower energy, of lowest energy are those with two cycles: C_5 (N3–H**...**O=C5; Figure 1) and C_7 (N1–H**...**O=C5; Figure 2). Ureido sugars with a dipeptide unit can form the same hydrogen bonds as the amino acid derivatives (*e.g.* C_7 rings formed by N1–H) and another intramolecularly hydrogen bonded species involving N6–H: C_5 (N6–H···O=C-8;

Figure 1a) and C_7 (N6–H···O=C2; Figure 2b). In an extended conformation of dipeptide unit both N3–H and N6–H can form C_5 rings, which explains the difficult approach for nitrosylation agent and thus the domination of N1–NO isomers.

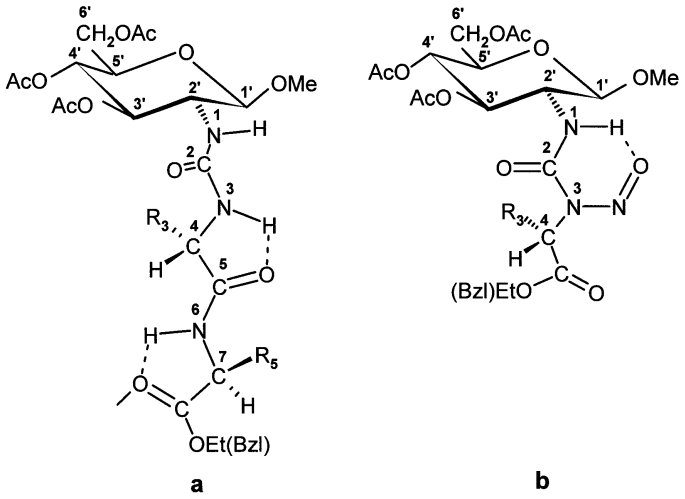


Figure 1. a. Intramolecular *N*-H...O=C hydrogen bonds (C_5 cycle) in *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoyl-L-alanyl-L-alanine ethyl ester (**5**, $R_3 = R_5 = CH_3$) or *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoyl-L-phenylalanyl-glycine ethyl ester (**6**, $R_3 = CH_2C_6H_5$, $R_5 = H$).

b. Intramolecular *N*-H...O=N hydrogen bonds in 1-*N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-3-*N*-carbamoyl-3-*N*-nitrosoglycine ethyl ester (**1b**), 1-*N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-3-*N*-carbamoyl-3-*N*-nitrosoglycine benzyl ester (**2b**), 1-*N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-3-*N*-carbamoyl-3-*N*-nitrosoalanine ethyl ester (**3b**) and 1-*N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-3-*N*-carbamoyl-3-*N*-nitrosoleucyne ethyl ester (**4b**).

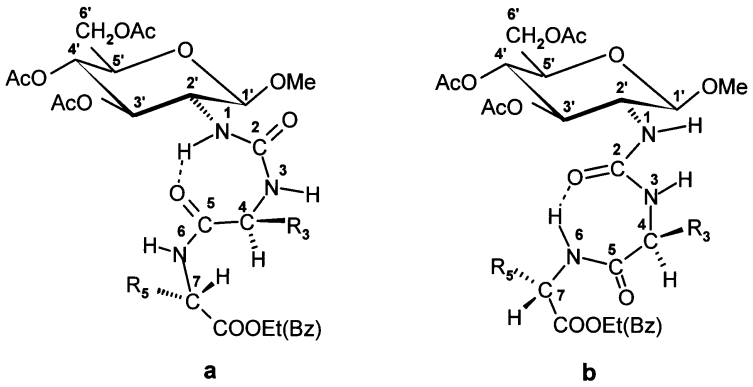


Figure 2. Two posible intramolecular *N*-H...O=C hydrogen bonds (C_7 cycle) in *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoyl-L-alanyl-L-alanine ethyl ester (**5**, $R_3 = R_5 = CH_3$) or *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoyl-L-phenylalanyl-glycine ethyl ester (**6**, $R_3 = CH_2C_6H_5$, $R_5 = H$). **a**: N1-H···O=C, (carbonyl at C5); **b**: N6-H···O=C, (carbonyl at C2).

Net charges at nitrogen atoms of nonnitrosated compounds **1–4** are in the range –0.005 to –0.018 for N1 and –0.066 to –0.080 for N3. In the nonnitrosated derivative **5** with AlaAlaOEt residue the net charges are: –0.013 at N1, –0.06 at N3 and –0.04 at N6. In all ureido sugars N-3 is more electronegative than N1. Therefore, electrophilic substitution of NO^{+} should take place preferably at N3. Neither the hydrogen bonding pattern alone nor the net charges at nitrogen atoms explain the preferences of *N*-nitrosation, especially the high yield (31%) of **4a** substituted at N1 whereas N3 should be preferred.

In a conformational analysis with PM3, global minima were found for all nitroso isomers substituted at N1 or N3 (or N6). The conformations are stabilized by the intramolecular N–NO···H–N hydrogen bond within a six-membered, stable cycle. Heats of formation of the isomers substituted at N1–NO were taken arbitrarily as zero level (Table 1). According to the PM3, the conformer with N3–NO should dominate for **1–3**, it is most favoured in the case of **2** (indeed, it is the only nitrosation product obtained as pure compound). The high content of isomer **4a** with N1–NO (31%) is more easily understood since PM3 indicated that it is energetically preferred.

Molecular modelling of nitrosoureido sugars with dipeptide ester moiety showed that conformers with the lowest heat of formation are nitrosated at N1, the energetically less favourable isomers are those with N6–NO. The relative heats of formation of the isomers are in qualitative agreement with the yields of particular products and the predicted order is N1 > N3 > N6. The same sequence is valid when considering intramolecular hydrogen bonding whereas according to next charges the preferences should be N3 > N6 > N1. It is evident therefore that all three factors should be taken into account when the place of substitution of the NO group and the regioselectivity of nitrosation are discussed.

Table 1. The PM3 relative heats of formation and the yield of particular isomers of nitrosoureido sugars.

Compound	Relative heat of formation in kJ			Yield in %		
	a	**b**	**c**	**a**	**b**	**c**
1 (GlyOEt)	0	–2.9	—	9	91	—
2 (GlyOBzl)	0	–6.3	—	0	100	—
3 (AlaOEt)	0	–2.2	—	0	100	—
4 (LeuOEt)	0	+15.9	—	31	69	—
5 (AlaAlaOEt)	0	+6.8	+10.6	82	16.4	1.6
6 (PheGlyOEt)	0	+2.8	+12.6	33	66	0

NMR results. The ^{1}H and ^{13}C NMR data for nitrosoureido sugars with amino acid ester residue are collected in Tables 2 and 3, respectively. The analysis of ^{1}H and ^{13}C chemical shifts of *N,N′*-bis(methyl 3,4,6-tri O-acetyl-2 deoxy-β-D-glucopyranosid-2-yl)-N-nitrosourea was made [8] prior to the studies on nitrosoureas **1–6**. The effect of nitrosation was the largest for H2 (1.1 ppm) and amounted to 0.02–0.4 ppm for more distant protons. The resonances of sugar carbons, which are close to the N–NO (C-1, C-3 and C-5) appeared *ca.* 1 ppm upfield with respect to those of the nonnitrosated compound and to the resonances of sugar unit close to the NH group.

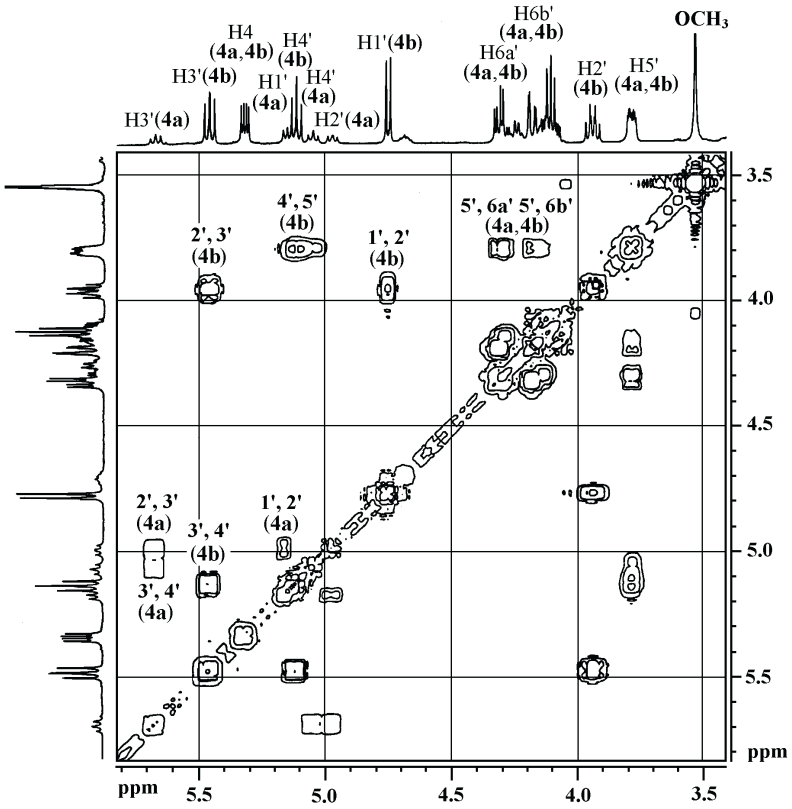


Figure 3. $^{1}H - {}^{1}H$ COSY NMR spectrum of the mixture of 1-*N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-1-*N*-nitroso-3-*N*-carbamoyl-L-leucine ethyl ester (**4a**) and 1-*N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-3-*N*-carbamoyl-3-*N*-nitroso-L-leucine ethyl ester (**4b**).

The assignment of NMR signals for nitroso compounds **1–6** was performed using the 2D $^{1}H–{}^{1}H$ COSY, heteronuclear $^{1}H–{}^{13}C$ and $^{1}H–{}^{15}N$ HMQC and $^{1}H–{}^{13}C$ HMBC techniques. The assignment of all signals was not an easy task, especially in the case when the reaction product contained a mixture of two, or even three isomers. The $^{1}H–{}^{1}H$ connectivities were found by the analyses of the COSY spectra. A fragment of the COSY spectrum of **4a, 4b** is presented in Figure 3. The ^{1}H detected HMQC spectra correlated all the proton resonances with the corresponding nitrogens (Figure 4).

In the ^{1}H spectrum of **1** a doublet of N1–H appeared at 4.69 ppm and a triplet of N3-H at 5.45 ppm. The nitrosation of N3 resulted in a disappearance of the N3–H signal and a high frequency shift of N1–H to 7.45 ppm in the spectrum of **1b**. Similarly, in the ^{1}H spectrum of **1a** the signal of N3–H appeared at 7.34 ppm. The significant high frequency shift (of 2.76 and 1.89 ppm) is due to the intramolecular hydrogen bonds N1–H···ON–N3 and N3–H···ON–N1 in **1b** and **1a**, respectively. The positions of the NH signals at 7.0–7.5 ppm (Table 2) in nitroso compounds indicate that such hydrogen bonds (Figure 1b) exist in all isomers.

The largest changes in ^{13}C chemical shifts for the N3–NO isomers occur for ureido C2=O, low frequency shift of *ca.* 5 ppm (157–158 ppm in nonnitrosated ureas [9] in comparison with 152 ppm in **1a–4a**) and for C5=O of ester group (2.5–5 ppm). Significant effects can also be noticed for carbons of amino acid residues (low frequency shift of 4.9 ppm for C1" and 5.4 ppm for C2"). The nitrosation of N1, which is close to sugar unit, produces largest chemical shift changes for sugar carbons and therefore this isomer is easy to recognise. The increase in shielding of C2 (3.5 ppm), C1 (3.1 ppm) and C3 (1 ppm) is observed.

The evidence of *N*-nitrosation was provided by the analysis of ^{15}N NMR spectra and the **2b** ^{1}H–^{15}N correlations. The ^{15}N chemical shifts for a series of methyl 2-deoxy-β-D-glucopyranosyl ureas with amino acid or dipeptide ester residues were reported [10]. The chemical shift of N1–H was almost constant (–299 to –301 ppm), δ N3–H (–280 to –307 ppm) was more dependent on the kind of substituent. The resonance of N6–H in the spectra of dipeptide derivatives appeared ca. 20 ppm downfield, at –261 to –279 ppm. The ^{15}N spectra of nonnitrosated compounds contained two (or three) resonances in the narrow range, typical of NH. After nitrosation the chemical shifts were remarkably different; most characteristic being the resonance of NO at 177–180 ppm and of its neighbour, which appeared at –106 to –117 ppm (Figure 4). The ^{1}H–^{15}N correlation for **2b** showed that the *N*-1 peak at –296 ppm is bonded to N1–H (^{1}J = 93 Hz), H3, H1 and H2; cross peak between N3 at –117 ppm and H4 of Gly residue as well as between NO at 177 ppm and H1 and H4 confirm the localization of the NO group. The ^{15}N shifts of nitrosoureas are in agreement with those found for nitrosamines [11] δ NO between 200 and 155 ppm, chemical shift of *N* linked to NO in the range – 195 to – 95 ppm. It is worth noticing that the ^{15}N chemical shifts of the anticancer drug, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), are: δ NH = – 276.9 ppm, δ *N* = –111.6 ppm and δ NO = 180.2 ppm [5].

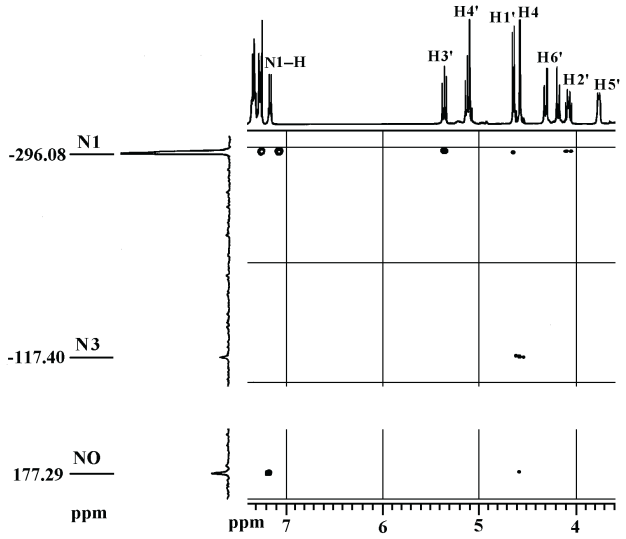


Figure 4. 2D gs – HMQC $^{1}H/^{15}N$ NMR spectrum of 1-*N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-3-*N*-carbamoyl-3-*N*-nitrosoglycine benzyl ester (**2b**).

Table 2 ^{1}H NMR data (δ in ppm, *J* in Hz) for nitrosoureido sugars.

Atom	**1a** N1–NO	**1b** N3–NO	**2b** N3–NO	**3b** N3–NO	**4a** N1–NO	**4b** N3–NO	**5a** N1–NO	Ref. [8] N1–NO
H1’		4.66d	4.64d	4.72d	5.13	4.73	5.19d	5.16
$J_{1',2'}$		8.3	8.5	8.3	7.9	8.3	7.7	8.1
H2’	4.94dd		4.07ddd	3.95ddd	4.95dd	3.91ddd	4.97dd	4.93
$J_{2',NH}$	8.2	9.1	9.1	9.0	—	8.7	—	
$J_{2',3'}$	10.4	10.5	10.8	10.5	10.1	10.1	10.6	10.7
H3’	5.63dd	5.38dd	5.37dd	5.41dd	5.64dd	5.43dd	5.57dd	5.67
$J_{3',4'}$		9.4	9.6	9.4	9.0	9.4	9.3	9.7
H4’		5.11dd	5.14dd	5.10dd	5.02dd	5.09dd	5.08dd	5.12
$J_{4',5'}$		9.5	10.2	9.5	9.0	9.5	4.4	9.7
H5’		3.77ddd	3.76ddd	3.75ddd	3.77ddd	3.75ddd	3.77dd	3.78ddd
$J_{5',6a'}$		4.9	4.7	4.7	4.6	4.8	5.0	4.4
$J_{5',6b'}$		2.6	2.3	2.5	2.3	2.3	2.5	2.3
H6a’		4.29dd	4.32dd	4.29dd	4.23dd	4.28dd	4.25dd	4.32dd
H6b’		4.18dd	4.18dd	4.18dd	4.16dd	4.15dd	4.10	4.18dd
$J_{6a',6b'}$		12.3	12.5	12.3	12.2	12.0	12.5	12.2
OMe	3.35	3.49	3.51	3.51	3.40	3.53	3.37	3.43
N1–H	—	7.45d	7.11d	7.21d	—	7.14d	—	—
N3–H	7.34	—	—	—	7.16d	—	7.21	7.0s
H4		4.48, 4.52	4.56, 4.60	5.28		5.29	4.62dd	—
H1”		—	—	1.40		1.76, 1.89[a]	1,51	—
$J_{4,4a}$ or $J_{4,1"}$		18.0	17.0	7.0		9.8	7.0	
N6–H	—	—	—	—	—		7.05d	—
$J_{6,7}$							7.2	
H7	—	—	—	—	—		4.51dd	—
$J_{7,1'''}$							7.4	
H1’’’	—	—	—	—	—	—	1,12dd	—

[a]H2” = 1.25; H3” = 0.81d, 0.83d.

Table 3 ^{13}C NMR chemical shifts (δ in ppm, $CDCl_3$) for nitrosoureido sugars.

Atom	**1b** N3–NO	**2b** N3–NO	**3b** N3–NO	**4a** N1–NO	**4b** N3–NO	**5a** N1–NO	**5b** N3–NO	**6a** N1–NO	**6b** N3–NO	ref. [8]
C1’	101.7	101.8	101.7	99.7	101.5	100.0	101.1	99.8	100.1	100.0
C2’	55.69	55.61	55.93	52.46	55.92	53.03		52.57	55.25	52.80
C3’	71.90	71.90	71.97	70.88	71.91	71.46	73.04	71.98	71.86	71.22
C4’	68.90	68.53	68.76	69.36	68.64	68.97	68.63	69.35	68.64	68.92
C5’	72.33	71.96	72.24		72.22	71.88	71.99	70.71	71.44	71.83
C6’	62.25	62.00	62.15		61.59	62.05	62.24	62.36	61.99	62.08
OMe	57.11	57.16	57.28		57.15	57.23	57.36	57.27	57.42	59.5
C2=O	152.7	152.4	152.5		152.6	153.2	153.0	153.2	153.5	158.0
C4	40.53	40.41	13.85		51.20	50.11	52.62	54.32	53.07	—
C5=O	171.0	164.4	171.52		170.68	172.7	172.9	171.8	174.2	—
C1”	—	—	—	24.61	25.20	18.35	18.79	38.51	37.55	—
C2”	—	—	—	42.86	36.70	—	—	—	—	—
C3”	—	—	—		22.70, 21.69	—	—	—	—	—
C7	—	—	—	—	—	48.71	49.69	40.16	41.58	—
C1’’’	—	—	—	—	—	17.70	18.02	—	—	—

Table 4 ^{15}N NMR data (δ in ppm, ^{1}J in Hz, in $CDCl_3$) for nitrosoureido sugars.

A-tom	**1a** N1–NO	**1b** N3–NO	**2b** N3–NO	**3b** N3–NO	**4a** N1–NO	**4b** N3–NO	**5a** N1–NO	**5b** N3–NO	**5c** N6–NO	**6a** N1–NO	**6b** N3–NO	Ref. [8] N1–NO
N1		–295.5 (92.9)	–296.1 (93.0)	–291.1 (92.8)	–116.9	–296.7 (92.9)	–116.6	–296.9 (93.0)	–294.7	–116.6	–288.5	–116.4
N3	–298.8 (93.3)	–116.9	–117.4	–116.6	–288.4 (92.9)	–108.6	–284.8	–105.9	–286.7	–291.7		–297.1 (93.2)
N6	—	—	—	—	—	—	–264.2	–268.0		–282.7	–278.5	—
NO		177.9	177.3	177.0	181.8	176.4	181.4	178.8				180.0

EXPERIMENTAL

The ureido sugars were synthesized from methyl 3,4,6-tri-O-acetyl-2-deoxy-2-(4-nitrophenoxy-carbonylamino)-β-D-glucopyranoside and amino acid or respective dipeptide (protected by the OEt or OBz group) according to the described procedures [12]. The following urea were prepared in this manner: *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy- β-D-glucopyranoside-2-yl)-*N*-carbamoylglycine ethyl ester (**1**), *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoylglycine benzyl ester (**2**), *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoyl-L-alanine ethyl ester (**3**), *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoyl-L-leucine ethyl ester (**4**), *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoyl-L-alanyl-L-alanine ethyl ester (**5**), *N*-(methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside-2-yl)-*N*-carbamoyl-L-phenylalanyl-glycine ethyl ester (**6**). Then, the compounds were treated with the N_2O_3 solution (in chloroform or acetic anhydride) to afford the nitroso derivatives.

Nitrosation of ureido sugars with amino acid or dipeptide ester residue. To a solution of urea (0.5 mmol) in pyridine (5 mL) was added a solution of N_2O_3 (2.5 mmol) in $CHCl_3$ (5 mL). The reaction mixture was stirred at –5°C for 15 min and at room temperature during 1 h. Tlc then indicated the absence of substrate. Next 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 the product was analyzed by NMR spectroscopy. The ^{1}H, ^{13}C and ^{15}N NMR spectra were recorded on a Bruker DRX-500 spectrometer for $CDCl_3$ solutions, in 5 mm tubes. The two-dimensional COSY, HMQC, HMBC correlations were recorded using standard pulse sequences from Bruker library. The 2D nitrogen-proton correlation experiments were performed using a phase-sensitive gradient-selected HMQC inverse technique, optimised for $^{1}J_{NH}$ of 90 Hz. The remaining parameters were as follows: acquisition time 0.25 s, relaxation delay 1 s, observe pulse width 7.4 μs, spectral width (^{1}H) 4 kHz, number of points 2048. The experiments were 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 nitrogen direction. Chemical shifts were referenced against internal TMS (^{1}H, ^{13}C) and external neat CH_3NO_2 (^{15}N). The semi-empirical calculations of geometries and energies were performed on IBM PC using the PM3 method as implemented in HyperChem 5.02 program [13]. Polak-Ribiere conjugate gradient with RHF spin pairing, 0.01 convergence limit in *vacuo*, and RMS gradient of 0.1 kcal/[A mol] were used during modelling process. According to Steward [14], heats of formation and hydrogen bonding are reproduced by the PM3 level of theory with chemically useful accuracy for molecules with bonds involving C, H, N and O atoms.

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REFERENCES

1. Gnewuch C.T. and Sosnowsky G., *Chem. Rev.,* **97**, 829 (1997).
2. Lin T.S., Fischer P.H., Shiau G.T. and Prusoff W.H., *J. Am. Chem. Soc.,* **21**, 130 (1978).
3. Johnston T.P., McCaleb G.S., Opliger P.S. and Montgomery J.A., *J. Med. Chem.,* **9**, 892 (1966).
4. Johnston T.P., McCaleb G.S. and Montgomery J.A., *J. Med. Chem.,* **18**, 104 (1975).
5. Lown J.W. and Chauhan S.M.S., *J. Org. Chem.*, **48**, 507 (1983).
6. Plass M., Wawer I., Piekarska-Bartoszewicz B. and Temeriusz A., *J. Phys. Organ. Chem.,* **10,** 747 (1997).
7. Plass M., Weychert M., Wawer I., Piekarska-Bartoszewicz B. and Temeriusz A., *J. Carbohydr. Chem.,* **19**, 1059 (2000).
8. Temeriusz A., Piekarska-Bartoszewicz B., Weychert M. and. Wawer I., *Polish J. Chem.,* **73,** 1011 (1999).
9. Wawer I., Piekarska-Bartoszewicz B. and Temeriusz A., *Carbohydr. Res.*, **279**, 83 (1995); Temeriusz A., Piekarska-Bartoszewicz B. and Wawer I., C*arbohydr. Res*., **304,** 334 (1997).
10. Weychert M., Klimkiewicz J., Wawer I., Piekarska-Bartoszewicz B. and Temeriusz A., *Magn. Reson. Chem.***, 36,** 727 (1998).
11. Witanowski M., Stefaniak L. and Webb G.A., *Ann. Rep. NMR Spectroscop.*, **25,** 382 (1992).
12. Piekarska-Bartoszewicz B. and Temeriusz A., *J. Carbohydr. Chem*., **12,** 913 (1993); Temeriusz A., Piekarska-Bartoszewicz B. and Wawer I., C*arbohydr. Res*., **304,** 334 (1997).
13. HyperChem 5.02 package, Hypercube Inc., Canada 1997.
14. Steward J.J.P., *J. Comput. Chem.,* **10**, 221 (1989).