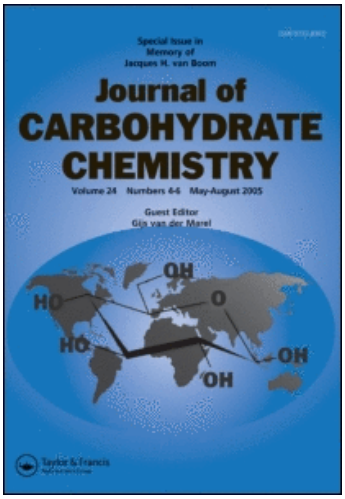

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INTRAMOLECULAR HYDROGEN BONDING IN *N*-(2-AMINO-2-DEOXY-β-D-GLUCOPYRANOSIDE)-*N'*-CARBAMOYL-L-DIPEPTIDYLESTERS: AN INFRARED AND ^{1}H NMR STUDY

M. Plass,[a*] M. Weychert,[b] I. Wawer,[b] B. Piekarska-Bartoszewicz[c] and A. Temeriusz[c]

[a] Institute of Physical Chemistry, Martin-Luther-University, Kurt-Mothes-Str. 2, 06120 Halle (Saale), Germany;
[b] Department of Physical Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, 02097 Warsaw, Poland
[c] Department of Chemistry, Warsaw University, 02093 Warsaw, Pasteura 1, Poland

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ABSTRACT

Ten *N*-(2-amino-2-deoxy-β-D-glucopyranoside)-*N'*-carbamoyl-L-dipeptidylesters with different amino acid sequences in the dipeptide unit were studied by means of IR and ^{1}H NMR spectroscopy. In the IR spectra three bands at 3453, 3420 and 3390 cm^{-1} were observed which could be assigned to the free NH, the intramolecularly hydrogen bonded NH species forming five-membered, C_5, and seven-membered, C_7, rings, respectively. Comparing the NH band positions which correspond to the C_7 rings of the Gly-Xaa and the Xaa-Gly dipeptidylesters, the signals of the Xaa-Gly sequence were shifted by 10 cm^{-1} to lower wave numbers indicating stronger hydrogen bonds. The temperature effect dν/dT was an order of magnitude larger for the C_7 associates than for C_5 showing the highest enthalpy of the C_7 hydrogen bond. The ^{1}H NMR spectra give three separate signals for the NH groups. The temperature coefficient $\Delta\delta/\Delta T$ was the

largest for N-1-H indicating the formation of less stable hydrogen bonds (C_7). The solvent induced changes of the chemical shift of the NH signals was lowest for the N-3-H signal. Obviously the deshielding properties on this function do not vary in dependence of the solvent polarity. The hydrogen/deuterium exchange rate was lowest for the N-6-H proton indicating the lower accessibility of this proton. Combining the results of both spectroscopic methods it can be concluded that the N-1-H forms only C_7 rings whereas N-6-H can participate in C_5 and C_7 intramolecular hydrogen bonds. The strength of the formed C_7 associates depends on the amino acid sequence in the dipeptide residue.

INTRODUCTION

Combining glycopyranoside moieties with peptide residues is a widely used approach in drug development since glycosylation increases the solubility and oral availability[1,2] of the compounds compared to their non-glycosylated analogues. Furthermore, the biological activity of the glycosylated product covers a wider spectrum[3] and from the point of conformational heterogeneity a broader set of variants is available than for peptides alone. [4,5]

Regarding the potential of the glycoside to influence the conformation, two opposite mechanisms have been described.[6] One approach can be defined as a simple reduction of the variety of conformers of otherwise flexible peptide chains by bending of the peptide backbone away from the glycoside.[7] As a consequence turns are introduced in the peptide residue. The nature of the glycoside can influence the character of the turn. [8,9] Furthermore, specific interactions, e.g. hydrogen bonds between the sugar and the peptide function, can result in "glyco-turns".[10] However, there are also examples in the literature where no changes in the peptide backbone are observed due to glycosylation compared to the parent molecule.[11]

The title compounds are structurally different from the glycopeptides since the dipeptidyl residue is linked via a ureido function on the glucopyranoside (Fig. 1). Due to the reinforcement of a planar arrangement of the urethane function, the possibility of sugar-peptide interactions will differ from those of linear glycopeptides.

Beyond academic interest, our work also has a high practical value. Initiated by the task to find anticancer drugs structurally similar to streptozotocin and chlorozotocin, [12-17] the 2-deoxy-2-(3-nitrosoureido)-D-glucopyranoses with amino acid and di-peptidyl

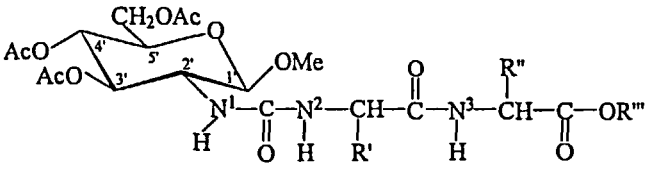

Fig. 1 Sketch of the title compounds

side chains were synthesised. It is hoped that by linking the ureido side chain to natural substances such as peptides or amino acids, the strong side effects of the drugs can be reduced with preservation of drug effectiveness. In the synthesis of the 3-nitrosoureido glucopyranoside derivatives, the acetylated ureido sugar peptide derivatives shown in Fig. 1 are formed as intermediate products. In order to prepare 3-nitrosoureido sugar derivatives, the selective introduction of a nitroso function on N-3 is demanded. The selectivity of the nitrosylation will be directed by the proton acidity of the NH function and by the accessibility of the reactive centre, e.g., its steric properties. For those reasons it would be worthwhile to know if intramolecular hydrogen bonds occur and influence the properties of the NH function.

To study the intramolecular hydrogen bonds in a more detailed fashion a number of acetylated products with different side chains as listed in Table 1 were synthesised and characterised.

This paper is strongly related to an article we have published about the intramolecular hydrogen bonding behaviour in the analogue amino acid derivatives.[18] Hence, this work is a continuation of our spectroscopic studies (FTIR, ^{1}H NMR and ^{13}C CP/MAS) which were applied to gain information about the conformational and hydrogen bonding properties of these compounds.[19,20]

RESULTS and DISCUSSION

Infrared measurements. The NH region of the ureido sugars with Ala-Gly and Gly-Ala substitution in the side chain is shown in Fig. 2 at 298K. In general, all substances exhibit similar band profiles, which indicates the overlapping of different NH

Table 1. Residues of the dipeptide derivatives.

Dipeptide sequence	R'	R''	R'''
Gly-Ala	H	CH_3	CH_2CH_3
Gly-Val	H	$CH(CH_3)_2$	CH_2CH_3
Gly-Val	H	$CH(CH_3)_2$	CH_2Ph
Gly-Phe	H	$CH_2C_6H_5$	CH_2CH_3
Ala-Gly	CH_3	H	CH_2CH_3
Val-Gly	$CH(CH_3)_2$	H	CH_2CH_3
Leu-Gly	$CH_2CH(CH_3)_2$	H	CH_2CH_3
Phe-Gly	$CH_2C_6H_5$	H	CH_2CH_3
Ala-Ala	CH_3	CH_3	CH_2CH_3
Ala-Phe	CH_3	$CH_2C_6H_5$	CH_2Ph

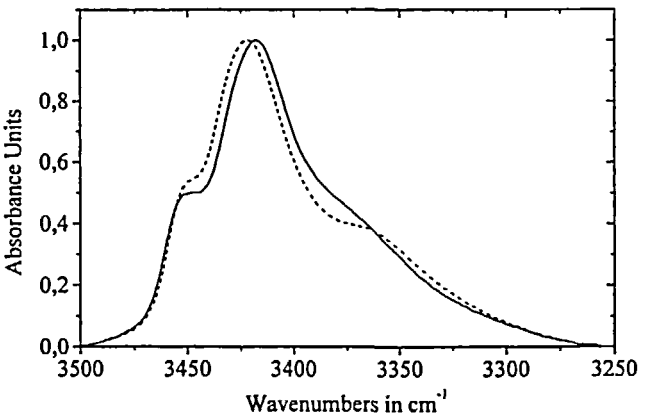

Fig. 2. NH stretching bands of the Ala-Gly (dashed) and Gly-Ala (solid) derivatives in $CHCl_3$ solution of at 298 K.

species. After application of a deconvolution and peak fitting procedure, three NH signals can be identified. Their band maxima are given in Table 2. For comparison, the NH band position of two amino acid derivatives are also presented. As can be seen, the band positions of the dipeptide ureido glucopyranosides are similar to those of the amino acid derivatives allowing small deviations of the band positions due to the mass effect. For protected linear oligopeptides, intramolecular hydrogen bonds forming a seven-membered, C_7, and five-membered, C_5, rings have been observed.[21-24] These small hydrogen bonded rings are energetically favoured by linear oligopeptides with a chain length of two and three amino acid units, since they are too short to form more stable β-turns or other hydrogen bonds typical for proteins. C_7 and C_5 associate rings in tripeptide derivatives exist in apolar solvents such as carbon tetrachloride and *n*-hexane, whereas in chloroform the interactions with the solvent molecules prevent the formation of C_7 intramolecular associates for peptides with apolar side groups. Obviously the energy stabilisation of these hydrogen bonds is small.

Without doubts, the same types of intramolecular hydrogen bonds occur in the dipeptide derivatives as are described in reference 18. Hence the bands at about 3422 and 3375 cm^{-1} were assigned to intramolecularly associated NH species forming a five-membered ring (C_5) and a seven-membered ring (C_7), respectively. Furthermore, on the high frequency side of the C_5 NH band, the free NH signal could be observed at 3453 cm^{-1}.

Although the type of the hydrogen bonds are the same as for the amino acid derivatives, the variety of potential hydrogen bond forming functions is increased by the enlargement of the peptide residue. Thus, in the extended peptide conformation the N-3-H and N-6-H can form C_5 rings which are represented by the intense band at 3420 cm^{-1}. Furthermore, the interaction of the N-1-H and the acetyl function at C-3 of the glucopyranoside, the N-1-H and peptide carbonyl group and the N-6-H with the ureido CO result in C_7 hydrogen bond arrangements. Additionally, an interaction of the methoxy function at C-1 and the N-3-H group is theoretically possible but, to our knowledge, this association was only observed in the solid phase of ureido sugars with D-valine substitution.[20] For steric reasons other intramolecular hydrogen bonds to other acetyl functions are rather unlikely and, therefore, were not taken into account.

Table 2. Positions of the NH signals after peak fitting [in cm^{-1}].

Dipeptide residue	ν_{NHfree}	ν_{NHC5}	ν_{NHC7}
Gly-Ala	3453	3420	3384
Gly-Val[b]	3451	3421	3390
Gly-Val[a, b]	3452	3419	3391
Gly-Phe[a]	3452	3418	3387
Ala-Gly	3453	3423	3374
Val-Gly[b]	3453	3424	3380
Leu-Gly	3453	3424	3382
Phe-Gly	3452	3421	3371
Ala-Ala	3454	3421	3376
Ala-Phe[a]	3455	3420	3379
Gly[18]	3454	3424	3372
Val[18]	3455	3420	3355

a. R'''=Bzl

b. Here the identification of the NH signals was ambiguous; also two different C_7 signals would fit the band profile. For the sake of equivalent treatment, the C_7 band was represented by only one Voigt function.

Considering Table 2 in terms of the Badger-Bauer rule,[27] it turns out that the differences between ν_{NHC5} and ν_{NHfree} are more or less the same for all compounds under investigation. Therefore, ΔH should also be the same or similar for all substances. In contrast, there are two groups in $\Delta\nu_{(NHC7\text{-}NHfree)}$. If R''=H, $\Delta\nu$ is ca. 10 to 16 cm^{-1} higher than for the compounds with Gly-Xaa sequence. This may be interpreted as due to the steric ineffectiveness of the Gly residue, which allows the NH group to approach to the CO groups without hindrance. On the other hand, a bulky residue in the R' position evidently diminishes the mobility in the whole molecule and, thus, influences the entropy term in the thermodynamic consideration of the association. This explains the $\Delta\nu$ value for Ala-Ala and Ala-Phe. In Fig. 3 one conformation is proposed in order to illustrate the steric aspects when considering intramolecular hydrogen bonds. However, other conformers with only one C_7 associate ring or combinations of C_5 and C_7 rings will be found in the solutions as well as discussed in reference 18.

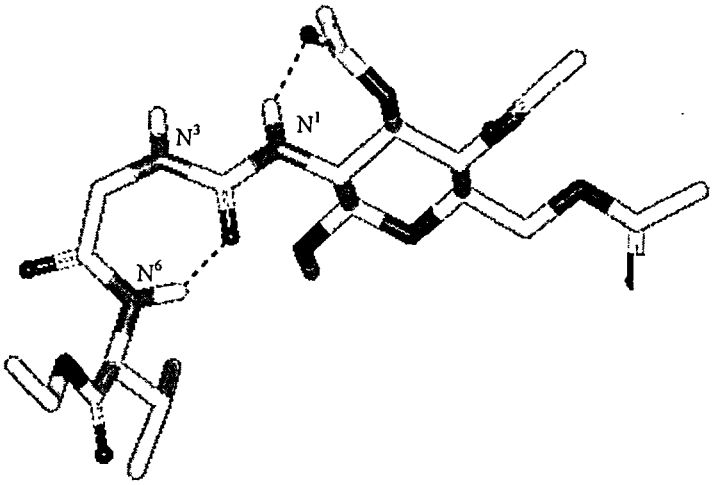

Fig. 3. One possible conformation of the Gly-Val ureido sugar derivative. [28] With exception of the NH the protons are not shown. Intramolecular C_7 hydrogen bonds are indicated. (Colour code: black: oxygen, white: carbon, grey: nitrogen atoms).

In order to judge the concentration of the associated species, the extinction coefficient is needed, but these values are not available from these spectra. From previous investigations on oligopeptide derivatives, it was estimated that the NH extinction coefficient of a C_5 associated group is 1.5 to 3 times larger than ε_{NHfree}.[29] For NH signals involved in a C_7 associate ring, ε_{NHC7} will be even larger and can be approximated as up to 10 times of ε_{NHfree}. Based on these simplifications the relative concentration of non associated, C_5 and C_7 associated NH species are given in Table 3 using a factor of 2 and 7 for the correction of the NH extinction coefficients of C_5 and C_7 signals, respectively.

The data in columns 2 and 3 show that the percentage of free and C_5 associated NH species are of the same order for Xaa-Gly and Gly-Xaa sequences. Thus, the peptide chain is mainly in the extended conformation which is even more favoured in the cases of the Ala-Ala and the Ala-Phe derivatives. Obviously, the molecules are forced to arrange in the extended form due to repulsion of the bulky residues on the C-α, although the C_7 associates would be enthalpically more stable as indicated in the paragraph above.

The temperature effect on the positions of the NH signals gives an indication about the enthalpy of the associate formation. As expected the shift of the NH position

Table 3. Relative concentration of free, C_5 and C_7 NH functions at 293 K [in %].

Dipeptide residue	NH_{free}	NH_{C5}	NH_{C7}
Gly-Ala	43.6	48.5	7.6
Gly-Val	40.5	51.3	8.2
Gly-Val[a]	31.9	59.9	8.2
Gly-Phe	29.8	63.1	7.0
Ala-Gly	37.3	56.5	6.2
Val-Gly	42.3	52.1	5.6
Leu-Gly	40.6	53.0	6.3
Phe-Gly	39.5	54.9	5.5
Ala-Ala	18.6	71.5	9.9
Ala-Phe[a]	15.9	75.6	8.4
Gly	35.4	58.4	6.1
L-Val	22.7	64.7	12.7
L-Phe	25.0	68.5	6.5

a. R'''=Bzl

with temperature is generally very small for the C_5 and the free NH signals but by an order of magnitude larger for the C_7 associates.

For the amino acid derivatives we have found a strong dependence $\Delta\nu/\Delta T$ on the kind of amino acid residue. In the case of the dipeptide derivatives the stability of the intramolecular hydrogen bonds seems to follow different rules. Here the amino acid sequence determines $\Delta\nu/\Delta T$. Thus, compounds with an Xaa-Gly sequence (Xaa = Ala, Val, Phe) have a stronger temperature effect than the corresponding Gly-Xaa derivatives. Obviously, the bulkiness of the amino acid Xaa lowers the enthalpy effect of stable C_7 associates. Gly-Xaa sequences are less affected; the acetyl functions at the glycoside in the backfolded conformer might be repelled by the bulky residue of the second amino acid as sketched in Fig. 3. Thus, the C_7 type of intramolecular hydrogen bonds are not supported in Gly-Xaa sequences and the dipeptidyl function favours the extended conformation. When comparing the data of the ureido sugar with the amino acid combination of Gly and Val, it seems surprising that $\Delta\nu/\Delta T$ for the free NH is larger than for the C_5 signal. However, we have to recall that the signal defined as free NH is indeed

Table 4. Temperature coefficients $\Delta\nu/\Delta T$ of the NH signals [in 10^{-3} cm^{-1}/K].

Dipeptide residue	NH ($_{free}$)	NH (C_5)	NH (C_7)
Ala-Gly	29.3	57.1	299
Gly-Ala	11.6	25.7	156
Val-Gly	31.4	15.8	153
Gly-Val	26.9	13.3	148
Phe-Gly	19.0	32.9	170
Gly-Phe	[a]	38.0	86
Gly	21.7	41.7	312
L-Ala	[a]	1.0	107
L-Val	31.4	60.4	478

a. Intensity of the band is very low at 243 K.

a result of three different NH functions. What is observed here might be also a result of the different temperature behaviour of the associates because the acidity of the three NH functions will be slightly different, although the NH signal cannot be resolved sufficiently by the IR technique.

The detailed analysis of the IR spectra is limited since we cannot observe the individual NH functions but only characterise the type of hydrogen bond. Here the advantage of the NMR spectroscopy can be used where the time scale does not allow the observation of associated and non-associated species but only the separation of the particular NH groups in the molecule.

^{1}H NMR measurements. Hydrogen bonding affects the shielding properties of the proton donor group in the molecule and, thus, the chemical shift δ of the NH groups in a ^{1}H NMR spectrum can be used as a diagnostic tool for hydrogen bonding. In the case of the substances under investigation the NH functions, N-1-H and N-3-H of the ureido group and the N-6-H of the peptide function seem worth monitoring in regard to the dependence on temperature, solvent polarity and H/D exchange kinetic.

Table 5 shows the chemical shifts of the NH protons and their temperature coefficients determined in solutions of $CDCl_3$. The assignments of the signals are based on reference 25.

Table 5. ^{1}H NMR chemical shifts of the NH groups at room temperature and their temperature coefficients [δ in ppm, Δδ/ΔT in ppb/K].

Amino acid sequence	N-1-H		N-3-H		N-6-H	
	δ	Δδ/ΔT	δ	Δδ/ΔT	δ	Δδ/ΔT
Gly-Ala	5.44	-8.31	5.93	-6.89	7.10	-6.67
Gly-Val	5.36	-7.80	5.93	-6.13	6.96	-6.41
Gly-Phe	5.61	-5.42	6.08	-4.15	7.04	-3.19
Ala-Gly	5.15	-6.51	5.56	-5.02	7.11	-5.04
Phe-Gly	4.95	-7.40	5.41	-6.65	6.89	-6.79
Val-Gly	5.18		5.70		7.06	
Gly[18]	4.69		5.45			
Ala[18]	4.47		5.29			
Phe[18]	4.55		5.26			

Comparing the chemical shifts of the amino acid and the dipeptide derivatives, the N-1-H and N-3-H signals are shifted to higher fields indicating that the electronic properties of the whole molecule have been changed by enlarging the ureido sugar derivatives. On a relative scale in comparison to the glycine derivative the signals of both NH functions, N-1-H and N-3-H, are shifted by ca. 12 %.

Similarly as for the amino acid derivatives, the N-1-H signals were observed at higher field than the N-3-H signals. Obviously, the kind of amino acid residue and the sequence of the amino acids have an effect on the deshielding properties of these functions. Note that the chemical shifts of the N-1-H and N-3-H protons vary also in the case of the Gly-Xaa sequence and, thus, not only the nearest environment of the substances must be considered when explaining the behaviour of the NH functions. As was expected the N-6-H proton of the peptide bond was found at lower field than the two other NH signals.

The temperature dependence of the amide proton NMR shift has been used as a criterion of hydrogen bonding in peptides[30] and other compounds.[31] In our case the temperature coefficients (Δδ/ΔT) are rather small for hydrogen bonded species in $CDCl_3$. Indeed, they are an order of magnitude smaller than those of intermolecularly bonded alcohol or amine associates. However, the -Δδ/ΔT values usually found for intramolecular

Table 6. ^{1}H NMR chemical shift of the NH functions [in ppm].

Dipeptide sequence	$CDCl_3$ [25]			DMSO-d6		
	N-1-H	N-3-H	N-6-H	N-1-H	N-3-H	N-6-H
Ala-Gly	5.15	5.56	7.11	6.15	6.13	8.3
Phe-Gly	5.01	5.48	6.89	6.17	6.14	8.42
Val-Gly	5.18	5.70	7.06	6.17	6.06	8.37
Leu-Gly	5.24	5.61	7.15	6.09	6.05	8.36
Gly-Ala	5.44	5.93	7.10	6.21	6.12	8.24
Gly-Phe[a]	5.61	6.08	7.04	6.15	6.07	8.39
Gly-Val	5.36	5.93	6.96	6.22	6.17	7.98

associated hydrogen bonds were less than 1 ppb/K. Thus, the data presented in Table 5 show that there exists an equilibrium between intramolecularly associated species C_7 and non-associated NH. C_5 associates do not contribute to the $\Delta\delta/\Delta T$ coefficient since in this arrangement the NH function is still exposed to the solvent. The temperature coefficients are slightly higher for N-1-H, which can be involved only in hydrogen bonds of C_7 type. Obviously, the associates show a stronger enthalpy effect than the other groups. The smaller coefficients ($\Delta\delta/\Delta T$) are found for the N-3-H and N-6-H, which are involved in C_5 rings.

At low concentration of ca. 10^{-3} mol/L homomolecular association can be neglected. Thus, the chemical shift data given in Table 6 are determined by the compound-solvent interactions at 298 K.

Although we are aware of the fact that the change of the solvent polarity causes a complex correspondence of the molecules in solution (change of the associate behaviour, conformation, etc.) some conclusions can be drawn from the chemical shift of the NH protons listed in Table 6. First, the polar solvent results in a low field shift of the NH signals due to the solvent-substance interaction. This effect was observed for all substances. Second, the amount of the solvent induced shift might indicate that intramolecular hydrogen bonds exist. Mostly hydrogen bonding causes a low field shift of the NH signals. Two possibilities must be considered here. If an NH function is involved

in intramolecular hydrogen bonds which is stable in both solvents, the change of the chemical shift of the NH functions should be comparably small. If, however, no intramolecular hydrogen bond exists in the tested solvents the solvent induced shift of δ should be also small. If the hydrogen bonds break apart in the more polar solvent DMSO-d6 the signal of the corresponding NH function should show a large low field shift due to the formation of intermolecular hydrogen bonds to the solvent.

For the amino acid derivatives the solvent induced chemical shift of the NH group was found to be ca. 30 % and ca. 20% for the N-1-H and N-3-H group, respectively. The variation of the side chain residue did not influence the deshielding properties significantly. In a previous paper we have shown that these derivatives form seven membered intramolecular hydrogen bonds, C_7, to the acetyl function on the C-3 of the glucopyranoside ring.[18] Thus, the large solvent effect for the N-1-H function indicate changes of the conformation and intramolecular association due to the solvent interaction.

For the dipeptide derivatives the NH functions have to be discussed individually. Whereas the solvent effect on the chemical shift of the N-1-H and N-6-H was calculated as 13 to 20 % and, therefore, similar to the effects observed on the N-1-H of the amino acid residues. The N-3-H varies its resonance frequency by less than 5 % for the ureido sugars with Gly-Xaa sequence, and ca. 8 to 12 % for the Xaa-Gly sequence. Especially in the case of the benzyl ester of the Gly-Phe ureido sugar the solvent induced shift of δ_{N3H} was less than 1%. Hence the N-3-H group is not influenced by the solvent attack. Since this function would be only able to form eight-membered hydrogen bonds to the C-terminal ester function which are energetically favoured, it is concluded that N-3-H is exposed to the solvent molecules in both solvents, $CDCl_3$ and DMSO-6. The question arises why the solvent effect is stronger in the Gly-Xaa sequences than in the Xaa-Gly substances. It may be understood as a secondary effect: in $CDCl_3$ the formation of intramolecular hydrogen bonds of the N-1-H and the N-6-H proton donor function requires a conformational response of the residues at the C-α of the first amino acid. Thus, the N-3-H function might be more shielded in Xaa-Gly sequences. In the polar DMSO-d6 those intramolecular interactions do not exist anymore and, thus, also affect the shielding of the N-3-H function.

In biopolymers greater exchange rates were usually interpreted as greater solvent exposure of particular NH, slow exchange rates take place for protons involved in

intramolecular hydrogen bonds.[32] Thus, H/D exchange experiments might give further hint to locate the interacting proton donor and acceptor function in the molecule.

The amide proton exchange to deuterium is relatively slow and could be followed in ^{1}H NMR spectra. In our previous H/D experiments with glucopyranoside-*N'*-carbamoyl-L-leucine ethyl ester,[19] slower exchange of N-3-H was observed and explained by the existence of intramolecular hydrogen bonding.

The H/D exchange measurements were carried out on three compounds with dipeptide units: Val-Gly, Phe-Gly and Leu-Gly. In all experiments the H/D exchange was the slowest at N-6-H position. After the first 3 min. the signals of N-1-H and N-3-H retain only ca. 30-40% of their initial intensity whereas the signal of N-6-H 80-93%. Surely the acidity of the NH functions plays a dominating role in the exchange kinetic. As we know from the investigations on amino acid derivatives an intramolecular hydrogen bond enhances the exchange rate. The H/D equilibrium between three positions is reached after 25-30 min. A differentiation of the H/D effects in dependence on the sequence or amino acid residue would be vague because of the small deviations and, thus, we defer details of these effects until completion of further investigation.

CONCLUSIONS

FTIR investigations have shown that the ureido sugars with dipeptide side groups form intramolecular hydrogen bonds. Those intramolecular interactions could be identified as seven-membered (C_7) and five-membered (C_5) hydrogen bond rings. In comparison to the analogue sugar derivatives with amino acid side chains the extent of C_7 rings is lower. Furthermore, the amino acid sequence seems to play a major role for the C_7 association. Hence, in Xaa-Gly (Xaa = Ala, Phe, Val) the corresponding NH C_7 signal was observed at lower wave numbers than in the corresponding Gly-Xaa sequences indicating a larger enthalpy value for these associates. Contrary, the concentration of C_7 was slightly lower in these sequences representing a smaller equilibrium constant. The diverse tendency of the equilibrium constant and the association enthalpy is obviously caused by an entropy effect, e.g., steric hindrance. Additionally, the low $\Delta\nu/\Delta T$ of the C_7 band in Gly-Xaa sequences supports the entropy concept. The ^{1}H NMR experiments

show that the N-3-H is always exposed to the solvent and, therefore, not involved in intramolecular hydrogen bonds except for C_5. The shielding properties of N-3-H do not change in the Gly-Xaa sequences but in Xaa-Gly compounds. This peculiarity might be understood as a secondary effect of the intramolecular hydrogen behaviour of N-1-H with the acetyl function at C-3, and N-6-H with the ureido function which is favoured in derivatives with Xaa-Gly sequence.

EXPERIMENTAL

Materials. The substances were synthesised from methyl 3,4,6-tri-*O*-acetyl-2-deoxy-(4-nitrophenoxy-carbonylamino)-β-D-glucopyranoside and dipeptide ethyl or benzyl esters according to the described procedure.[25, 26] The ureido sugars studied were derivatives of methyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside with different amino acid sequences in the dipeptide unit. The dipeptidyl residues containing only L-amino acids are specified in Table 1.

FT IR spectroscopy. Infrared spectra of different ureido sugars were recorded as $CHCl_3$ solutions in concentrations of ca. 10^{-3} mol/L. The measurement parameters of the IFS 66 (Bruker) spectrometer were chosen as follows: The resolution in the spectra was 2 cm^{-1}. Fifty scans were accumulated in order to receive a reasonable signal/noise ratio. A NaCl cell with a thickness of 3 mm was used for all investigations. The region between 3550 and 3250 cm^{-1} was fitted using a set of three Voigt functions with the help of a peakfit program "for non-linear curve fitting" (Jandel) Vs. 5. The fitting was accepted with a root square coefficient better than 0.999.

The influence of temperature on hydrogen bonding was tested on selected substances using a thermostated sample holder which allowed measurements between 273 and 323 K. The accuracy of the temperature was checked by a separate thermocouple and did not exceed the chosen temperature by more than ± 0.5 K. As a cooling agents a methanol/ dry ice mixture was used.

NMR spectroscopy. ^1H NMR spectra were recorded on a Varian Unity Plus 500 MHz spectrometer equipped with a variable temperature probe. The concentration of the

ureido glucoside derivatives dissolved in $CDCl_3$ and in DMSO-d6 was chosen according to the IR experiment. The temperature was varied in the range between 298 and 333K in 5 K steps. The H/D exchange measurements were performed in $CDCl_3$ and followed the procedure described in reference 18. During the experiment the temperature was kept constant at 298 K.

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