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INFRARED AND ^{1}H NMR STUDIES OF HYDROGEN BONDING IN *N*-(2-AMINO-2-DEOXY-β-D-GLUCOPYRANOSIDE)-*N*′-CARBAMOYL-L-AMINO ACID ESTERS

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Ureido 2-deoxy-β-D-glucopyranosides with seven different amino acid ester residues were studied by means of IR and ^{1}H NMR spectroscopy. The H–D exchange rates increase in the order L-Val<L-Leu<L-Ala<Gly for both NH protons; however, the exchange rate at N-1-H (linked to the glucopyranoside) is significantly faster than that at N-3-H (at the amino acid residue). The analysis of IR spectra in the region of the NH stretching vibrations shows, in agreement with other investigations, that the signals at 3454, 3423 and 3355 cm^{-1} are due to the free and the intramolecular associated NH groups forming a five- and seven-membered ring, respectively. It was found that the C_7 associated ring is formed by hydrogen bonding between the N-1-H function and the C═O of the acetyl group at the C-3 position in the glucopyranoside. The N-3-H group is involved in a hydrogen bond with the C═O function of the ester group protecting the amino acid residue. Furthermore, it was found that the ureido sugar with L-Val exhibits a stronger C_7 hydrogen bond than the other amino acid residues. This result conforms with the lowest H–D exchange rate at the N-1 position of this compound. Steric effects resulting in the shielding of this hydrogen bond against OD attack are considered as a reason for this peculiarity. © 1997 John Wiley & Sons, Ltd.

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INTRODUCTION

Ureido sugars with nitroso groups such as streptozotocin [2-deoxy-2-(3′-methyl-3′-nitroureido)-D-glucopyranose] or chlorozotocin {2-deoxy-2-[(2′-chloroethyl)-3′-nitroso-ureido]-D-glucopyranose} are used in the clinical treatment of cancer.[1–5] However, because of the partially strong side effects of these substances, other compounds are sought which could be better tolerated by the human body, e.g. ureido sugars with amino acid residues. Since the biological activity is related to the structure, conformation and hydrogen bonding ability, information on these topics is highly desirable. In this paper we present investigations on intramolecular hydrogen bonds of the NH groups in the title compounds. Such hydrogen bonds have already been found in similar molecules by IR spectroscopy[6,7] in more apolar solvents and their existence has been derived from NMR measurements.[8] Additionally, we tried to connect ^{1}H NMR data on the kinetics of proton–deuterium (H–D) exchange with thermodynamic results of IR spectroscopy.

^{1}H NMR has been used to monitor H–D exchange in small model compounds and in biologically relevant molecules such as agiotensin, where the half-life for exchange varies from less than 1 min to 1 h.[9] In peptides, however, the rate of replacement of the NH protons by deuterons is often so rapid that it is not possible to follow the process by conventional spectroscopy.[10] In our work, preliminary results showed that the concentration of H–D exchanging partners, ureido sugar and methanol-d_4 can be adjusted so that the exchange takes 1–1·5 h and may be well monitored by NMR.[11]

Considering the possibilities of forming intramolecular hydrogen bonds, we have to take into account that all O and N atoms may act as hydrogen bond acceptors, but only two donor groups are present. Therefore, in addition to considerations about the basicities of the different acceptor sites, steric relationships should play an important role.

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EXPERIMENTAL

Ureido sugars were synthesized from methyl 3,4,6-tri-*O*-acetyl-2-deoxy-(4-nitrophenoxycarbonylamino)-*β*-D-glucopyranoside and amino acid methyl, ethyl and benzyl esters according to the described procedure.[12,13] *N*-(3,4-dichlorophenyl)ureido-*N′*-(2″-phenyl)acetic acid ethyl ester was synthesized at the Institute of Organic Chemistry of Martin Luther University[14] and used for comparison.

Infrared spectra of different ureido sugars were recorded as solutions in $CHCl_3$ (*ca* 10^{-3} mol/l^{-1}) using an IFS 66 spectrometer (Bruker). The resolution of the overview spectra was 2 cm^{-1}. For more detailed analysis, smaller regions of the NH stretching vibration (3500–3300 cm^{-1}) were monitored using a resolution of 0·25 cm^{-1}.

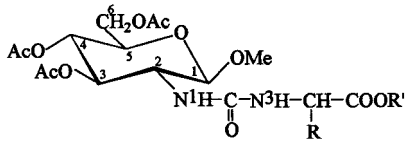
The band profiles of the donor groups of the molecules were fitted with the help of a peak-fit program 'for non-linear curve fitting' (Jandel) using Voigt functions.

For the unambiguous assignment of the observed NH bands, a small amount of the L-Val and the L-Phe derivatives were deuterated. The sample was dissolved in a few drops of deuterated methanol (CH_3OD) and subsequently the solvent was evaporated. This procedure was repeated several times. Thus partial exchange of the NH through ND took place. The product was dissolved in $CDCl_3$ and studied spectroscopically as described above.

The temperature effect on the position of the NH signals was studied from IR measurements performed in the temperature range 303–243 K using commercial cooling equipment.

^{1}H NMR spectra were recorded on a Bruker AMX-500 spectrometer equipped with a variable temperature unit. A constant temperature of 303 K was maintained during H–D exchange measurements. Ureido sugar (0.08 mmol) dissolved in 0.9 ml of acetone-d_6 and methanol-d_4 (2·5 mmol) was added with a microsyringe. The recording of the ^{1}H NMR spectra started 1 min later and the exchange was monitored at 10 min time intervals. The integrated areas of the NH signals were compared with the neighbouring signals of the immobile sugar hydrogen atoms H-3 and H-4.

Semi-empirical AM1 calculations were carried out using a program implemented in the HyperChem program package.[15]



Scheme 1. General structure of the ureido sugars investigated. For clarification the glucopyranoside protons are not shown. Ac = acetyl

Table 1. The *N*-(3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside)-*N′*-carbamoyl-L-amino acid esters investigated, with their protecting groups (Scheme 1)

Amino acid	Residue R	Residue R′
Gly	H	CH_2CH_3
L-Ala	CH_3	CH_2CH_3
L-Val	$CH(CH_3)_2$	CH_2CH_3
L-Leu	$CH_2CH(CH_3)_2$	CH_2CH_3
L-Ile	$CH(CH_3)(CH_2CH_3)$	CH_2CH_3
L-Phe	CH_2Ph	CH_3
D-Val	$CH(CH_3)_2$	CH_2CH_3

RESULTS AND DISCUSSION

The ureido sugars studies were derivatives of methyl-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside with the amino esters shown in Scheme 1 and Table 1.

NMR measurements. The compounds were previously characterized by ^{1}H, ^{15}N and ^{13}C NMR spectroscopy in order to confirm their structure.[8] Chemical shifts of the N-1-H (linked to the sugar residue) are in the region 4·5–5·0 ppm and those of N-3-H (linked to the amino acid) 5·3–5·8 ppm. The N-3-H always appears at lower field. Taking into account only the net charges of the adjacent nitrogen atoms, this result is surprising. As can be seen in Table 2, AM1 calcualtions give higher net charges for the N-1-proton. Hence its NMR signal should appear at the lower field, in contrast to the experimental results. To explain this behavior, we have to discuss the appearance and the influence of hydrogen bonds.

The position of the NH signals in the ^{1}H NMR spectra indicates that the NH functions are involved in hydrogen bonding, but the results gave no insight into the kind of hydrogen bonding, intra- or intermolecular, or into the dynamics of the proton exchange in these systems. Moreover, the NMR lines are narrow and no exchange broadening was observed.

In general, the H–D exchange experiments were expected to deliver more precise information about the kind of hydrogen bonding interaction. The change of the NH intensities during the H–D exchange reaction is shown in Fig. 1 with the example of the ureido sugar with an L-Val residue.

The rate constants obtained from the time dependence of the intensities of the signals are summarized in Table 3 for four representative compounds.

As can be seen, the H–D exchange rates at the N-3 position are smaller than those at N-1. It should be mentioned that the rate constant for N-1-H varies less than threefold, but that at the N-3-H position varies more than fivefold. Considering only shielding effects, this result should not be expected. Therefore, influences of hydrogen bonding and steric hindrance should be considered.

Table 2. Net charges at the N and H atoms as found by AM1 calculations and the ^{1}H chemical shifts of the NH functions

	Charges on		Charges on		^{1}H chemical shift[a] at	
Substance	N-1	H at N-1-H	N-3	H at N-3-H	N-1-H	N-3-H
L-Ala	−0·3717	0·2430	−0·3594	0·2270	4·47	5·29
L-Val	−0·3834	0·2416	−0·3908	0·2303	4·53	5·70

[a] Ref. 8.

Molecular modeling[1–3] has shown that an intramolecular hydrogen bond is possible between N-1-H and the carboxyl oxygen of the acetyl group at C-3. This would lead to the formation of a seven-membered ring. IR spectroscopy is suitable for proving the results of the theoretical investigations.

Infrared measurements. Our IR measurements were based on the following considerations. We know from previous work that the NH functions in peptides may form intramolecular hydrogen bonds, which act as linking parts in associated rings of different sizes. Based on steric considerations, we may expect the formation of a seven-membered ring of the N-1-H function and the CO ester bond at the C-3 position of the sugar and a five-membered ring between the N-3-H group and the CO ester function of the amino acid in the ureido sugars investigated. Five-membered hydrogen bond-aiding arrangements which result in an extended conformation of the peptide unit may be expected for short-chain peptides and their derivatives. A possible arrangement of C_7 and C_5 associates as described above with the example of the L-Val-substituted ureido sugar is shown in Fig. 2.

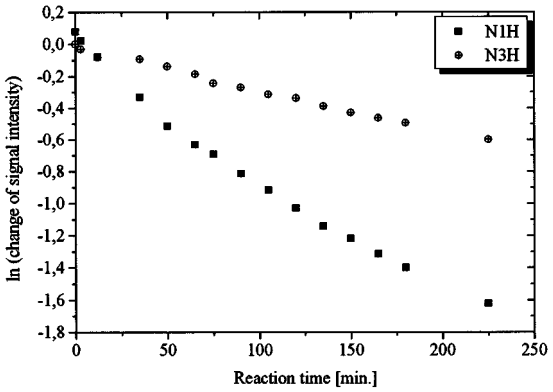


Figure 1. Intensity changes of the NH protons in the ureido sugar with L-Val residue during the exchange reaction (monitored by ^{1}H NMR)

The formation of a C_7 arrangement demands the back-folding of the amino acid residue and, therefore, brings the bulky residues R close to the acetyl-substituted glucopyranoside ring. Hence, the nature of this residue may strongly affect the formation of the C_7 associate by repulsion between the residue and other substituents. In Figure 3 we have tried to simulate an arrangement which realizes a hydrogen bond between the acetyl group at the C-6 position of the glucopyranoside. By varying the torsion angles it is not possible to bring the acceptor and donor atoms closer than 4 Å. Hence, an intramolecular hydrogen bond to this acceptor function can be excluded. Additionally, the lone pairs of the oxygen atom of the acetyl group and the ethyl oxygen in the ring come close to each other, precluding this steric arrangement by the repulsion between two groups.

Figure 4 shows the NH region of the Gly, L-Val and L-Phe derivatives dissolved in $CHCl_3$. In general, the band profile of the Phe compound represents the shape of the analogous bands of all the ureido sugar derivatives investigated.

For the assignment of the NH signals observed in the ureido sugar derivatives, we recorded the spectra of an unequally substituted urea, the *N*-(3,4-dichlorphenyl)ureido-N′-(2″-phenyl)acetic acid ethyl ester. As shown in the Figure 5, our model, compound only exhibits a symmetrical signal at 3432 cm^{-1}. Thus, in the ureido sugars both NH

Table 3. Rate constants for the H–D exchange at the NH groups of the ureido sugars

Substance	Rate constant k_1 (min^{-1}) at N-1 (N-1 linked to the sugar residue)	Rate constant k_3 (min^{-1}) at N-3 (N-3 linked to the amino acid residue)
Gly	$2{\cdot}1 \times 10^{-2}$	15×10^{-3}
L-Ala	$2{\cdot}5 \times 10^{-2}$	$5{\cdot}1 \times 10^{-3}$
L-Leu	$1{\cdot}68 \times 10^{-2}$	$3{\cdot}31 \times 10^{-3}$
L-Val	$0{\cdot}86 \times 10^{-2}$	$2{\cdot}9 \times 10^{-3}$

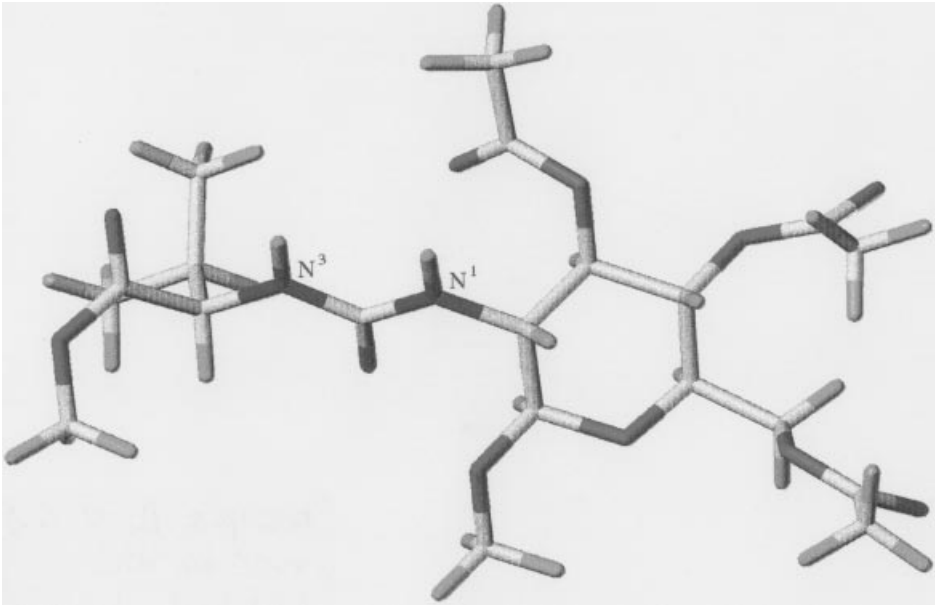


Figure 2. One possible conformation of *N*-(3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside)-*N′*-carbamoyl-L-valyl ethyl ester showing the C_7 and C_5 associated rings

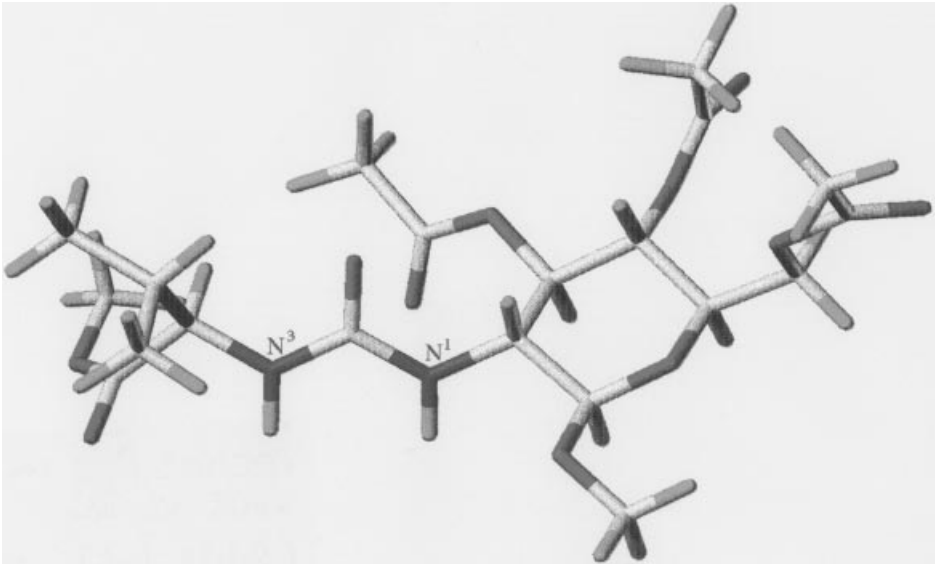


Figure 3. Open conformation of *N*-(3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside)-*N′*-carbamoyl-Lvalyl ethyl ester. The hydrogen bond between N-1-H and the acetyl function at the C-6 atom of the glycopyranoside cannot be formed

groups should not cause different NH bands. Additionally, *N*-(3,4-dichlorphenyl)-ureido-*N′*-(2″-phenyl)acetic acid ethyl ester does not show any tendency to form intra- or intermolecular hydrogen bonds if used at the same concentration as the sugar derivatives. Hence the NH groups are not sufficiently acidic for the formation of strong hydrogen bonds. Therefore, the asymmetric band profile is rather due to the overlapping of different NH species.

The best fitting results can be obtained by using three absorption bands contributing to the observed band shape. Table 4 summarizes the positions and the intensity ratios of the fitting results.

As the comparison with literature data on protected amino acid and dipeptide derivatives has shown, the signals at 3454, 3423 and 3355 cm^{-1} should be assigned to the free and the intramolecularly C_5 and C_7 associated NH functions, respectively.[6,16] Intermolecular association can be excluded from concentration-dependent measurements of the carefully dried samples. Also, the partial deuteration of the L-Val and L-Phe compounds leads to a similar band profile in the ND region (Figure 6).

Table 5 summarizes the temperature dependence of the NH signals for selected compounds, and demonstrates that the temperature effect is in general very small, indicating that we have indeed only free and intramolecularly associated NH species. As expected, the shift of the NH (C_5) and NH (C_7) signals is larger than that for NH (free). Hence, the increasing flexibility of the backbone with increasing temperature takes effect here. In some cases (L-Ala, L-Val and Gly), another less intense band occurs at lower wavenumbers (*ca* 3308 cm^{-1}) at low temperatures, which should be due to the formation of intermolecular hydrogen bonds.

The NIR spectrum of the L-Phe compound dissolved in chloroform presents one asymmetrical signal, in which the overlapping signals, which were analyzed after peak fitting, are separated by 52 cm^{-1}. In the MIR region the difference between the free NH and the C_5 NH band position was determined as 31 cm^{-1}. Considering the anharmonic oscillator model for vibrations, this value is reasonable. A C_7 signal cannot be observed in the NIR region. This behavior is not unexpected because the absorption coefficient of a C_7 NH harmonic is low and comparable to those of intermolecular NH associates bands.

It is well known that protons in hydrogen bonds are activated for reaction. The extent of activation increases with increasing hydrogen bond enthalpy and, using the Badger–Bauer rule,[17] with increasing shift of the associated NH band in the IR spectra. Therefore, we have to expect higher H–D exchange rates at the N-1 atom, which is

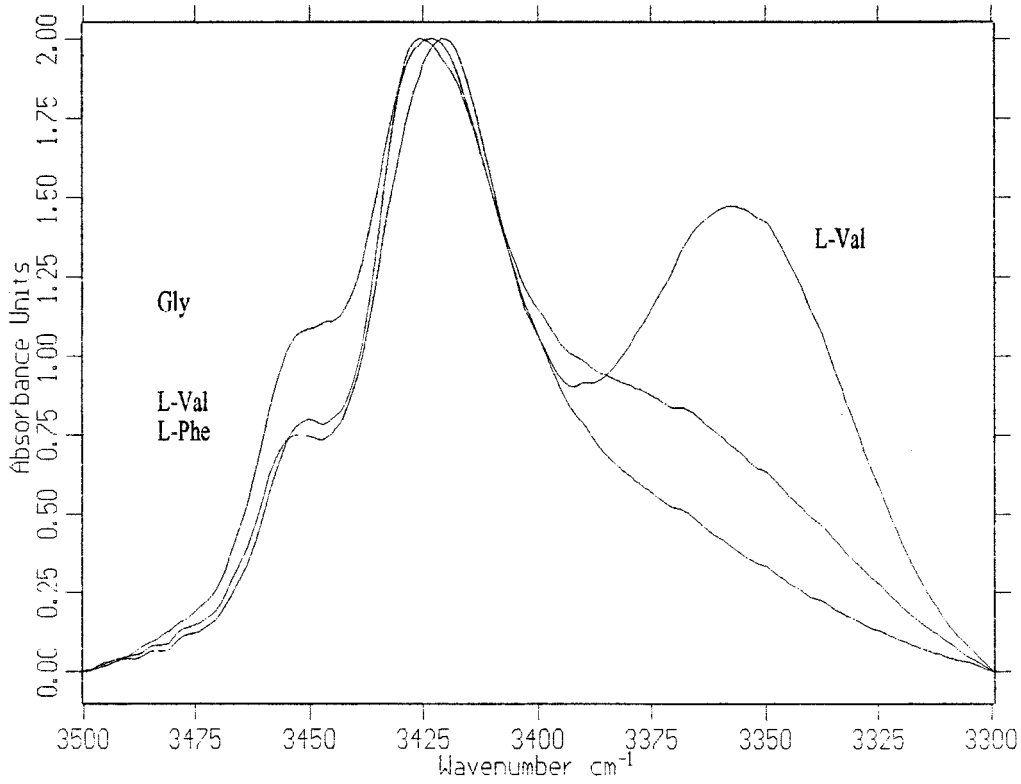


Figure 4. IR spectrum of the IR region of ureido sugars with Gly, L-Val and L-Phe residues

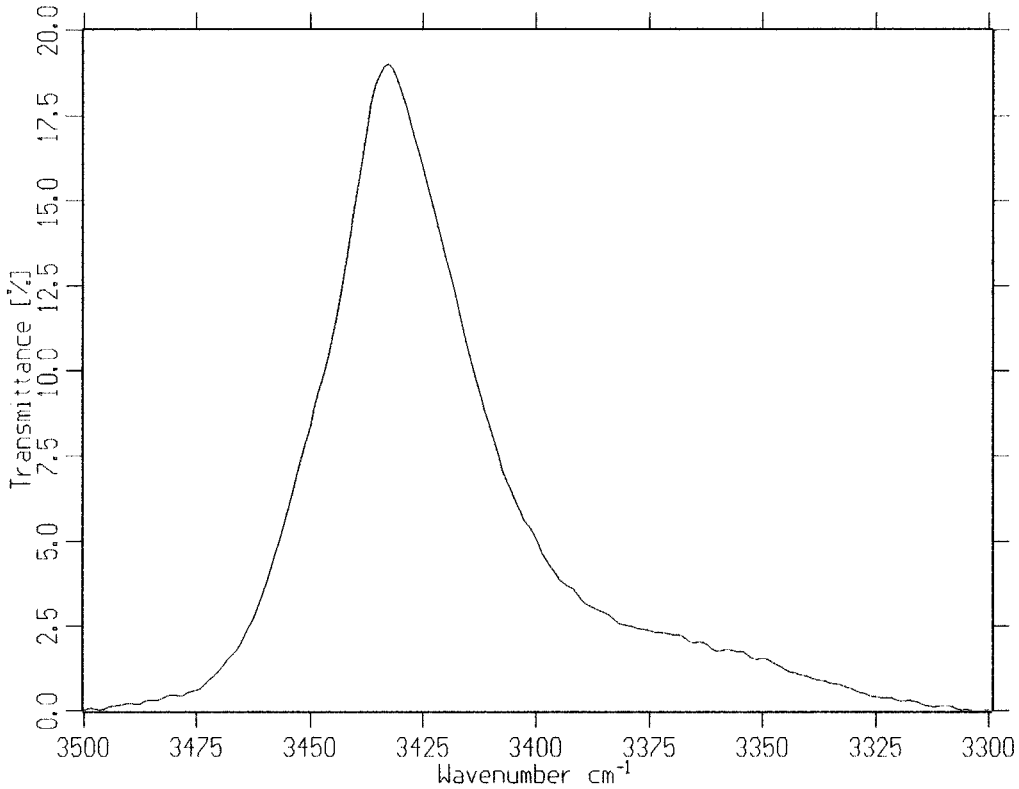


Figure 5. IR spectrum of the NH region of *N*(3,4-dichlorphenyl)-ureido-*N′*-(2″-phenyl)acetic acid ethyl ester dissolved in chloroform.

involved in a C_7 hydrogen bond, than at the N-3 atom in the C_5 associate; the latter also exhibits a lower bathochromic shift of the associated NH band in the IR spectra. This means, considering the hydrogen bond formation, that the principal differences in the exchange rate can be understood. To a certain extent steric hindrance due to the different bulkiness of the residue R may be an explanation for the differences in the exchange rate comparing the same nitrogen atom in different compounds. However, steric hindrance by the residue R does not influence the two NHs in the same manner owing to their positions. Therefore, in the discussion of the results, steric influences and hydrogen bonding have to be considered as overlapping effects. The remaining problem, however, is the interpretation of the behavior of the L-Val compound.

As can be seen from the NH intensity tratios in Table 4 and Figure 2, the non-congruent behavior of the L-Val compound persists also in the IR spectra. From the shift of the NH (C_7) signal with respect to the position of the NH (free) band and from the intensity ratios it can be deduced

Table 4. Positions (in cm^{-1}) and ratios of the separated NH bands at 20°C obtained from peak fitting

Substance	NH (free)	NH (C_5)	NH (C_7)[a]	NH (free):NH (C_5)	NH (C_7)
Gly	3454	3424	3372	3·30	1·21
L-Ala	3454	3423	3391	7·58	1·80
L-Val	3455	3420	3355	5·70	3·91
L-Leu	3454	3423	3393	8·06	2·00
L-Ile	3454	3423	3386	7·48	1·20
L-Phe	3453	3423	3395	5·47	1·82
D-Val	3454	3421	3373	1·63	0·86

[a] This band is independent on concentration.

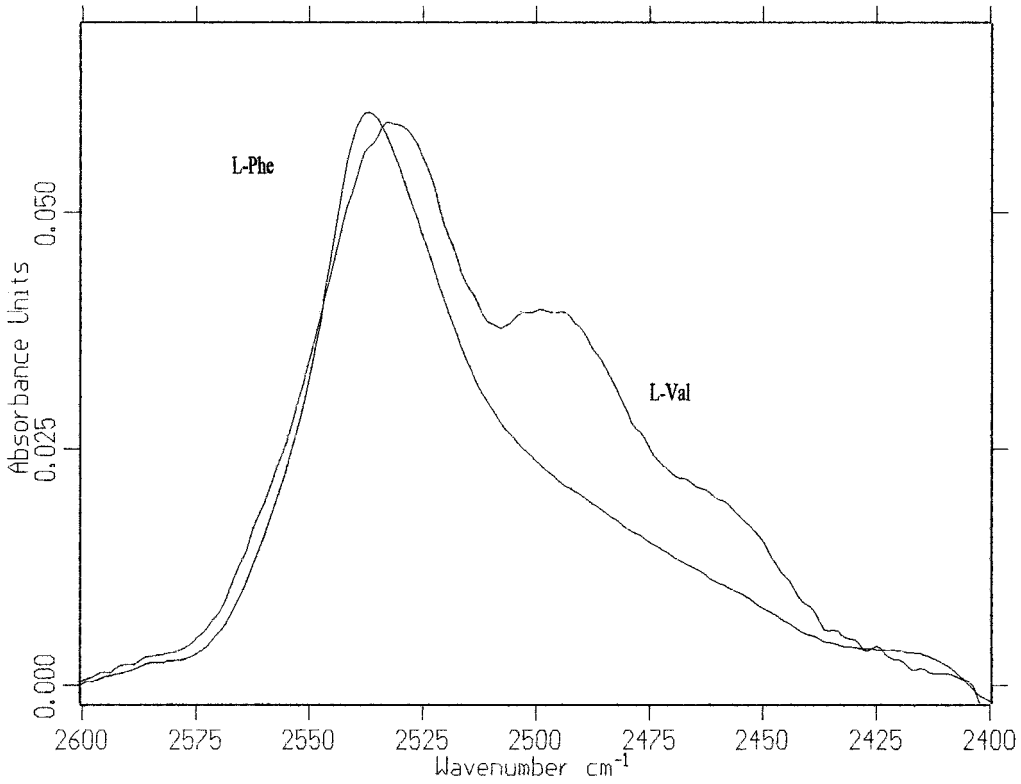


Figure 6. IR spectrum of the ND region of partially deuterated ureido sugars with L-Val and L-Phe residues

that the L-Val compound forms the stronger C_7 associated rings to a greater extent than the other ureido sugar derivatives. Keeping in mind that the intensity given in the fifth column of Table 4 is a sum function of intensities of two different NH absorptions, whereas the values in the sixth and seventh columns are ratios of the band intensity of only one NH function, the data for the L-Val compound will be lower than given in the table; because of the large amount of N-1-H groups involved in a C_7 hydrogen bond, only a low concentration of this function adds to the intensity of the free NH signal. Therefore, compounds with L-Val and Gly excluded, the NH (C_5):NH (free) ratios are almost equal for all L-substances. Hence, the equilibrium between five-membered associated rings and non-associated molecules is nearly independent of the amino acid residue in those cases.

However, the NH (C_5):NH (free) ratio calculated for Gly indicates a larger amount of free NH groups and, therefore, a lower tendency for the intramolecular formation of C_5 rings. Additionally, Table 3 shows the largest H–D exchange rate at the N-3 of the Gly compound compared with the equivalent position in the other compounds. This may be taken as evidence of the importance of unhindered accessibility for the increase in the reaction rate compared with NH activation by hydrogen bonding. We have to admit that in our interpretation all other L-compounds (except L-Val) possess more or less the same hindrance to perform this exchange. In the case of L-Val we might have a steric situation which may be described by a key–lock relationship. We assume that a conformation which strongly prefers the C_7 ring is stabilized. The compound bearing the D-Val residue cannot be used for the comparison without limitations, because in this diastereoisomer the intramolecular interactions might be seriously altered.

The search for the reasons for these differences might be

Table 5. $\Delta\nu/\Delta T$ of the different NH bands (in cm^{-1}/K^{-1}) calculated from the positions of the NH bands at 30 and −30°C (after peak-fitting)

Substance	$\Delta\nu/\Delta T$ of NH (free)	$\Delta\nu/\Delta T$ of NH (C_5)	$\Delta\nu/\Delta T$ of NH (C_7)
Gly	$21{\cdot}7\times10^{-3}$	$41{\cdot}7\times10^{-3}$	$31{\cdot}2\times10^{-2}$
LAla	–[a]	$1{\cdot}0\times10^{-3}$	$10{\cdot}7\times10^{-3}$
L-Val	$31{\cdot}4\times10^{-3}$	$60{\cdot}4\times10^{-3}$	$47{\cdot}8\times10^{-3}$
D-Val	$7{\cdot}2\times10^{-3}$	$13{\cdot}0\times10^{-3}$	$7{\cdot}9\times10^{-3}$

[a]Intensity of the NH (free) is very low at −30°C.

focused on steric problems; further NMR and IR studies on these compounds are in progress.

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