Articles

Substituent Effect on Intramolecular Hydrogen Bonding in β-Amino Acid-Containing Polyamides

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An alkyl substitution in the β-amino acid-containing polyamides **1**, **2**, and **3** leads to an increase in the population of intramolecularly hydrogen bonded conformations. However, the most stable conformation is not always the same one when the substituent is changed from a methyl group to an isopropyl group. For the β-amino acid derivatives of succinic acid, a methyl group enhances the formation of a head-to-tail type of folding pattern through an 11-membered ring (**2b**), but an isopropyl group appears to decrease the enhancement. For β-amino acid derivatives of glutaric acid, an isopropyl group promotes the formation of a bifurcated conformation (**3c**). The thermodynamic parameters for the equilibrium between non-hydrogen-bonded states and the head-to-tail type of folding of **2a** and **2b** are obtained by a van't Hoff analysis of the variable-temperature NMR data, which gives a ΔH of −1.0 kcal/mol and a ΔS of −4.7 eu for triamide **2a** and a ΔH of −1.0 kcal/mol and a ΔS of −3.7 e.u. for triamide **2b**, each with a correlation coefficient of better than 0.99. The increased proportion of the intramolecular amide−amide hydrogen bond observed for triamide **2b** is entirely due to entropic effects according to this analysis.

Introduction

It has been shown that the formation of helical conformations by peptides of β-amino acids is dependent on the structure of individual residues.[1,2] The effect of a substituent group on intramolecular hydrogen bonding in these β-peptides might be related to the side chain effect on α-helix stability. Glycine is known to be a helix breaker, while alanine has the highest helix-forming propensity.[3−5] In addition to electrostatic and van der Waals interactions, the difference in helix-forming propensity among α-amino acids has been attributed to side chain entropic effects.[6] This is an important topic since it is directly related to theories of protein folding. However, it is not completely understood at the present time, and very little is known about the effect of substituents on hydrogen bonding. There is a lot to be learned before one will be able to predict the conformational preference of polar functional molecules. We expect it to be advantageous to study the substituent effect in the folding of model peptides to isolate the important effects. In this report, the conformational preferences of several triamides with methyl and isopropyl substituents are discussed.

There is a remarkable difference in the tendency of forming intramolecular amide−amide hydrogen bonds between a single residue peptide of β-alanine and that of a γ-amino carboxylic acid.[7] Acetyl β-alanine *N*-methylamide **1a** (R = H) does not form an intramolecular amide−amide hydrogen bond in CH_2Cl_2 either through a six-membered ring or through an eight-membered ring. Poor hydrogen bond geometry, such as a deviation from linearity of the NH- - -O bond angle,[8] and torsional strains suffered by the connecting chain in the folded conformation can account for the absence of an intramolecular hydrogen bond. We have studied β-alanine derivatives of diacids, such as **2a** (R = H) and **3a** (R = H), and found them to be resistant to forming intramolecular amide−amide hydrogen bonds as well.[9] Will the replacement of a hydrogen atom by an alkyl group make a difference in the folding behavior of these triamides? The results of this study address this question.

We have suggested that the steric bulk of the side chain substituent group (R) in certain β-amino acids could drive the dihedral angle $NC_{sp3}-C_{sp3}C(=O)$ to a gauche conformation and promote intramolecular hydrogen bonding.[9] As shown in the following Newman projections, the stretched form, where no intramolecular H-bonds are possible, is favored when the side chain R is a hydrogen

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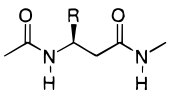
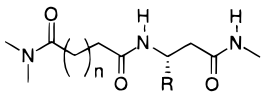
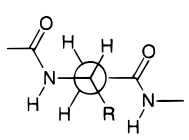


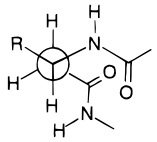


1a R = H **2a** n = 1, **3a** n = 2
1b R = CH_3 **2b** n = 1, **3b** n = 2
1c R = $(CH_3)_2CH$ **2c** n = 1, **3c** n = 2

atom. The folded form could become more favorable when



Stretched form



Folded form

the hydrogen atom is replaced with an alkyl group and allows the formation of intramolecular hydrogen bonds. We now report the effect of the substitution of an alkyl group on the intramolecular amide−amide hydrogen bonding of the homoelongated alanine (β-HAla)[10] and homoelongated valine (β-HVal) amides, **1b**,**c**, **2b**,**c**, and **3b**,**c**.

Experimental Section

THF was freshly distilled from sodium benzophenone under N_2. Hexanes were freshly distilled from calcium hydride under N_2. Routine ^1H NMR spectra (for characterization) were obtained on a Bruker AC-200 spectrometer. Triamides were prepared following the usual coupling reactions. Commercially obtained starting materials were used without further purification. Column chromatography was carried out by using up to 5 psi air pressure with 230−400-mesh silica gel from VWR Scientific. Columns eluted with MeOH in EtOAc were slurry-packed after the slurry was stirred with 5% ethyl acetate in hexanes. All glassware was dried in an oven at 120 °C. Triamides were prepared according to the usual peptide coupling procedures. ^1H NMR data for triamides are provided in the Supporting Information.

Variable-Temperature NMR Procedure. Unless specifically noted otherwise all NMR experiments were performed using $CDCl_3$ as solvent. The samples for variable-temperature (VT) ^1H NMR experiments were dried in a desiccator (P_2O_5) under vacuum overnight. Deuterated chloroform was dried over activated 4 Å molecular sieves for 2 days. A 100 mM solution of the amide in $CDCl_3$ was prepared first, and then two 1:9 dilutions with $CDCl_3$ were performed to give a final concentration of 1 mM. Even with these precautions, the resulting samples typically contained a small peak due to H_2O.

Variable-temperature NMR measurements were performed on a Bruker AC-300 spectrometer. The VT-NMR experiments all followed a general procedure. The sample tube was placed into the NMR probe using a heavy spinner. The air line responsible for spin was disconnected, and the delivery hose from the liquid nitrogen Dewar was connected to the NMR probe. The air line responsible for lifting the NMR tube and spinner out of the NMR probe was disconnected, and the NMR probe was capped. The desired temperature was set on the variable-temperature unit (BVT 2000), and the self-tune procedure was initiated to calibrate the console. Following calibration, the temperature reading on the variable-temperature console was allowed to stabilize. The sample was equilibrated for approximately 10−15 min at the set temperature, and after the Z and Z^2 shims were adjusted, a 128-scan spectrum was obtained. Measurements were made in the temperature range of 213−323 K. In these experiments, the first measurement was made at the lowest temperature. Caution was taken to raise the temperature slowly especially when approaching 323 K to avoid the evaporation of the solvent. All chemical shifts were referenced to the signal for residual $CHCl_3$, which was assumed to be 7.240 ppm at all temperatures. The accuracy of the temperature display on the VT unit, which was measured from a thermocouple located inside the probe, was tested by measuring the chemical shifts of methanol. Calibration of the temperature dependence of the separation (in hertz) between the OH resonance and the CH_3 resonance has been reported by Becker.[11] The calibration results show a <1 K deviation in the temperature range employed.

Variable-Temperature IR Procedure. Unless specifically noted otherwise all IR experiments were performed using $CDCl_3$ as solvent. Amides were dried as described for NMR samples. $CDCl_3$ solutions were prepared by dissolving several milligrams of amide in solvent that had been dried over molecular sieves and by performing serial dilutions to 1 mM as described for NMR samples. IR measurements were performed on a Perkin-Elmer 1600 FT-IR instrument. A Specac variable-temperature cell P/N 21525 equipped with CaF_2 windows (path length = 1.0 mm) was used for variable-temperature experiments. Temperatures were maintained with a dry ice−acetone slush bath and were monitored with a thermocouple attached directly to the cell. The cell temperature was allowed to stabilize for 20 min before measurements were obtained, and the cell temperature varied less than 1 °C during data acquisition. Spectra of 32 scans were obtained with 2 cm^{-1} resolution. Solvent subtraction was carried out by using background spectra obtained at approximately the same temperatures as the sample spectra.

Results and Discussion

Variable-concentration experiments were carried out to show that no intermolecular aggregation occurred at <5 mM. A variable-temperature IR experiment was carried out for acetyl β-alanine *N*-methylamide (**1a**) and its corresponding β-methyl and β-isopropyl substituted analogues (**1b** and **1c**) in 1 mM chloroform solutions. The NH stretch region of the IR spectra for these three amides is shown in Figure 1. Consistent with a previous study in methylene chloride,[7] compound **1a** shows minimal intramolecular hydrogen bonding in chloroform at all temperatures. Diamides **1b** and **1c**, however, display low-intensity, broad IR absorptions near 3397 cm^{-1} in addition to the free NH absorptions around 3450 cm^{-1}. These broad absorptions have a lower frequency than the peaks previously reported for intramolecular amide−amide H-bonds through a near linear NH−O geometry.[7−9,12,13] The lower frequency peaks at 3393 cm^{-1} suggest weak intramolecular amide−amide hydrogen bonds through a six-membered ring, in which the NH−O bond angle is distorted from the optimal geometry. The IR spectrum of **1c** shows a more distinct broad peak at 3337 cm^{-1}, which suggests a different H-bond with a better geometry. Therefore, on the basis of the IR data amide **1a** assumes almost entirely non-hydrogen-bonded conformations, amide **1b** appears to favor an intramolecular H-bond mainly through a six-membered ring at lower temperatures, and amide **1c** appears to assume a mixture of H-bonded and nonbonded conformations and

(10) A homoelongated amino acid is defined as β-HXaa, where Xaa is the usual three letter symbol for a natural amino acid. For example, a homoelongated phenylalanine is named as β-HPhe.[1]

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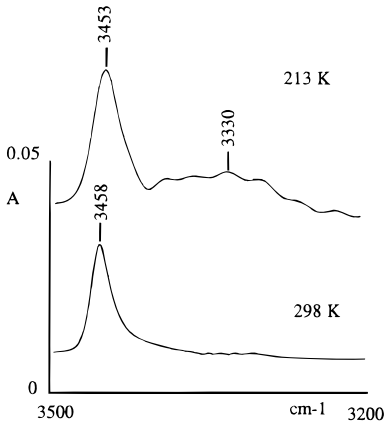
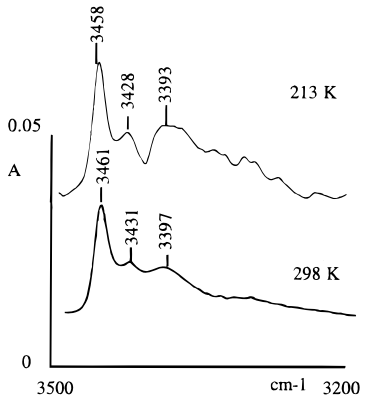
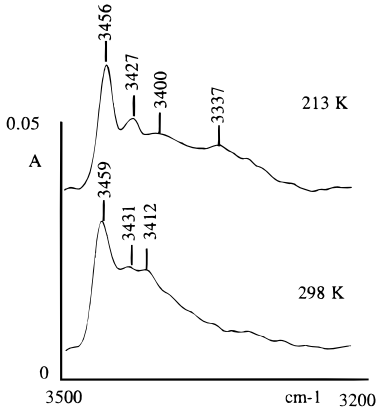


(a) acetyl β-alanine N-methylamide **1a**



(b) Acetyl β-HAla[10] N-methylamide **1b**



(c) Acetyl β-HVal[10] N-methylamide **1c**

Figure 1. NH stretch region of IR spectra for 1 mM chloroform solution of **1a** (top, maximum at 3453 and 3330 cm^{-1}; bottom, maximum at 3458 cm^{-1}), **1b** (top, maximum at 3458 and 3393 cm^{-1}; bottom, maximum at 3461, 3431, and 3397 cm^{-1}), and **1c** (top, maximum at 3456, 3427, 3400, and 3337 cm^{-1}; bottom, maximum at 3459, 3431, and 3412 cm^{-1}) at 298 K.

the H-bonded conformations contain both a six- and an eight-membered ring.

The amide proton NMR chemical shifts as a function of temperature for **1a** (triangles), **1b** (circles), and **1c** (squares) are displayed in Figure 2. The trend for the C-terminal NH proton chemical shifts of these three amides is a consistent downfield shift from **1a** to **1c** at all temperatures. However, the chemical shifts of the N-terminal NH protons do not follow this order. The

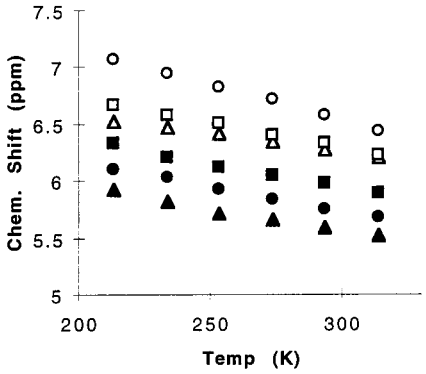
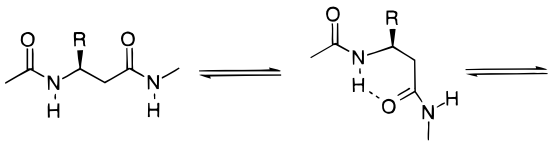


Figure 2. Amide proton NMR chemical shifts as a function of temperature for *N*-acetyl *β*-alanine *N*-methyl amide (**1a**, triangles), *N*-acetyl *β*-HAla[10] *N*-methyl amide (**1b**, circles), and *N*-acetyl *β*-HVal[10] *N*-methyl amide (**1c**, squares). Temperature dependency: ($-\Delta\delta NH/\Delta T$, ppb/K): N-terminal NH, (△) **1a** (3.2), (○) **1b** (6.2), (□) **1c** (4.5); C-terminal NH, (▲) **1a** (4.1), (●) **1b** (4.3), (■) **1c** (4.4).

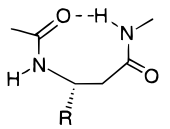
Table 1. Amide Proton NMR Chemical Shift Temperature Dependence in $CDCl_3$ (1 mM)

	$\Delta\delta/\Delta T$ (ppb/K)				
compd	$NH_{N-terminal}$	$NH_{C-terminal}$	compd	$NH_{internal}$	$NH_{terminal}$
1a	−3.2	−4.1	**2a**	−1.3	−8.7
1b	−6.2	−4.3	**2b**	−0.7	−9.0
1c	−4.5	−4.4	**2c**	−2.1	−7.8
			3a	−3.8	−5.5
			3b	−6.3	−7.1
			3c	−8.5	−10.1

N-terminal NH of the methyl-substituted amide **1b** has the most downfield chemical shift and the greatest temperature dependence of chemical shift (see Table 1). Consistent with the IR data, the lower field chemical shifts and the greater temperature dependence of the N-terminal NH (open circles in Figure 2) suggest that the most favored folding pattern of **1b** is from the N-terminal NH to the C-terminal carbonyl group through a six-membered ring, eq 1. However, the homoelongated valine derivative **1c** (squares) appears to also form a small amount of intramolecular H-bonds through an eight-membered ring involving the C-terminal NH. This assignment is based on the relatively downfield chemical shifts of the C-terminal NH and the relatively upfield chemical shifts of the N-terminal NH.



predominant when R = H (**1a**) favored when R = Me (**1b**)



(1)

present when R increases in size (**1c**)

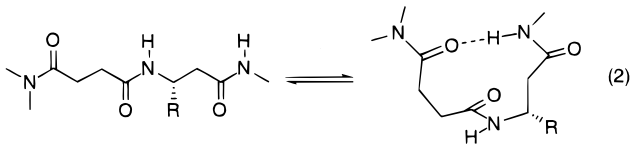
The fact that a methyl substitution seems to enhance the stability of a single conformation while an isopropyl group appears to promote a mixture of conformations is

also observed for triamide **2**. This is interesting because alanine is the amino acid that has the highest helix-forming propensity and the side chain of alanine is a methyl group.[3−5]

The NH stretch regions of the IR spectra for triamides **2a, 2b,** and **2c** in 1 mM chloroform solutions at two temperatures (213 and 298 K) are displayed in Figure 3 to show the changes in amide NH stretching. Triamide **2a** shows little hydrogen-bonded NH stretching at 298 K, while triamides **2b** and **2c** show a significant broad absorption in the region of 3340 cm^{-1}. The two free NH stretches are resolved in the IR spectrum of triamides **2b** and **2c**. The higher frequency peak (3462 cm^{-1}) is due to the terminal NH. This assignment is based on our previous study and is supported by the VT-NMR data of **2b** and **2c**. Thus, at 213 K the higher frequency free NH peak has decreased in intensity, suggesting that the terminal NH of triamides **2b** and **2c** becomes more involved in amide−amide hydrogen bonding at lower temperatures.

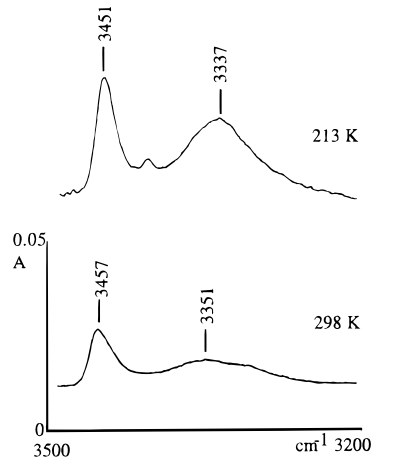
The VT-NMR data in Figure 4 show that the internal NH's of both **2a** and **2b** (open triangles and open circles) have little involvement in amide−amide hydrogen bonding, while the terminal NH's of all three triamides (**2a**, **2b**, and **2c**) are increasingly involved in hydrogen bonding as the temperature is decreased. The terminal NH can form an intramolecular hydrogen bond either to the center carbonyl group through an eight-membered ring or to the tertiary amide carbonyl group through an 11-membered ring.

On the basis of the results reported by Gellman[7] and the results from diamide **1b**, it is clear that for such a β-amino acid-containing structure an intramolecular hydrogen bond through an eight-membered ring is not favorable. Since the internal amide NH is not H-bonded, the terminal NH's of both **2a** and **2b** must be hydrogen bonded to the tertiary carbonyl group through an 11-membered ring. There are two advantages to this conformation. First, a tertiary amide carbonyl group is a better H-bond acceptor. Second, an 11-membered ring allows a better hydrogen bonding geometry.

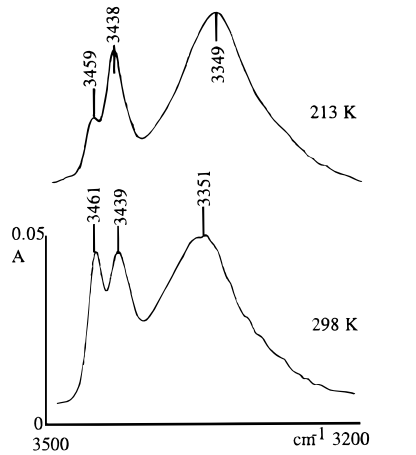


(2)

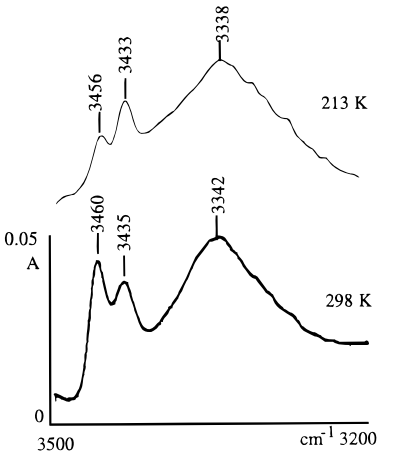
The IR spectrum of triamide **2c** also shows more hydrogen-bonded NH stretching at lower temperatures. Both the IR and the NMR data show an appreciable difference in the folding pattern between **2b** and **2c**. The internal free NH absorption of **2c** (3435 cm^{-1}) is smaller than that of **2b** at all temperatures, while the terminal free NH (3455 cm^{-1}) of **2b** is smaller than that of **2c** at 213 K. This suggests that triamide **2b** favors more a single stable conformation at lower temperatures through its terminal NH proton, while triamide **2c** experiences more of an equilibrium among several conformations. The internal NH of **2c** is, to a certain degree, involved in amide−amide hydrogen bonding on the basis of both the IR and the NMR data. The reduced temperature dependence of the terminal NH chemical shift of **2c** (compared to **2a** and **2b**, see Table 1) is added evidence that



(a) succinyl β-alanyl N-methylamide, **2a**



(b) succinyl β-HAla[10] N-methylamide **2b**



(c) succinyl β-HVal[10] N-methylamide **2c**

Figure 3. NH stretch region of the IR spectra for a 1 mM chloroform solution of **2a** (top, maximum at 3451 and 3337 cm^{-1}; bottom, maximum at 3457 and 3351 cm^{-1}), **2b** (top, maximum at 3459, 3438, and 3349 cm^{-1}; bottom, maximum at 3461, 3439, and 3351 cm^{-1}), and **2c** (maximum at 3460, 3435, and 3342 cm^{-1}) at 298 K.

intramolecular hydrogen bonds involving the internal NH are competing with the head-to-tail type of H-bond which involves the terminal NH only. It is reasonable to suggest that an isopropyl group destabilizes the head-to-tail type of folding and allows alternative intramolecular H-bonds, perhaps through a six- and a seven-membered ring, to occur (eq 3). Gellman has shown that an intramolecular

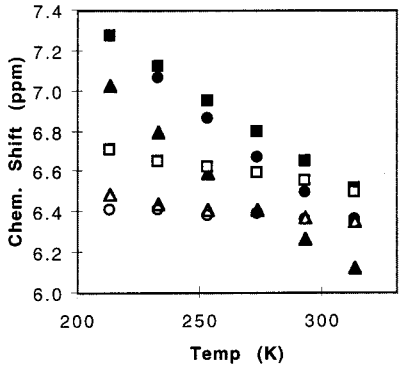
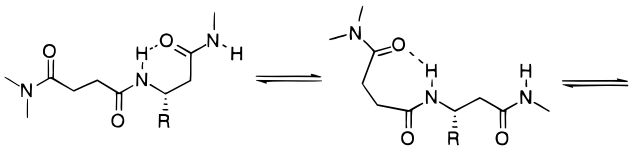
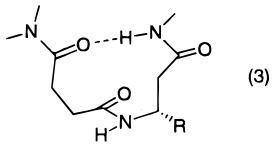


Figure 4. Amide proton NMR chemical shifts as a function of temperature for (a) *N*,*N*-dimethylamino succinyl *β*-alanyl *N*-methylamide (**2a**, triangles), *N*,*N*-dimethylamino succinyl *β*-HAla *N*-methylamide (**2b**, circles), and *N*,*N*-dimethylamino succinyl *β*-HVal *N*-methylamide (**2c**, squares). Temperature dependency ($-\Delta\delta NH/\Delta T$, ppb/K): Terminal NH, (▲) **2a** (8.7), (●) **2b** (9), (■) **2c** (7.8); internal NH, (△) **2a** (1.3), (○) **2b** (0.7), (□) **2c** (2.1).

H-bond through a seven-membered ring is possible if the H-bond acceptor is a tertiary amide carbonyl group.[12]



Favored at higher temperatures



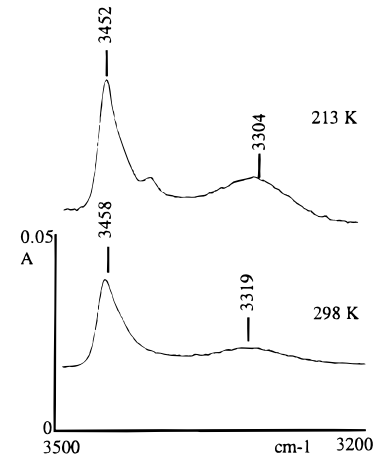
(3)

favored at lower temperatures

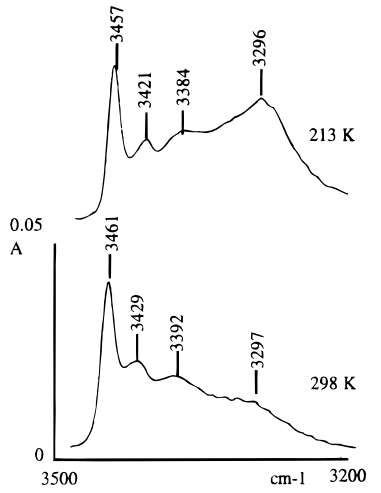
R = an isopropyl group

It is noteworthy that, similar to amide **1b**, the replacement of a hydrogen atom by a methyl group enhances the stability of a single conformation (the head-to-tail form) for triamide **2**, while the replacement of a methyl group by an isopropyl group promotes an equilibrium mixture. *β*-Branched amino acids, such as valine, are known to destabilize α-helical conformations due to entropic effects.[6] Namely, the free rotation of the *β*-branched substituents is hindered in a helix. Similar effects could also be operating here. The free rotation of the isopropyl group could be restricted in the head-to-tail type of folding pattern.

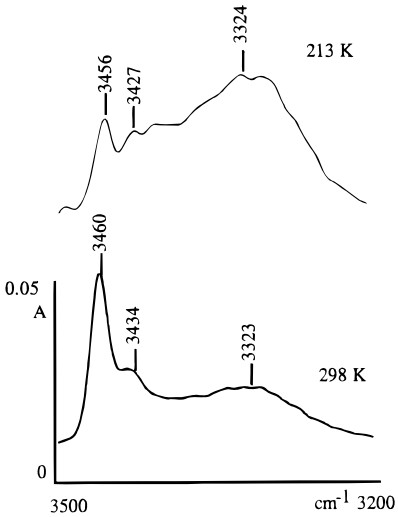
The IR data for the glutaryl *β*-amino acid *N*-methylamides **3a**,**b**,**c** are displayed in Figure 5. Little amide−amide hydrogen-bonded NH is observed for **3a** at room temperature, and a small broad peak is observed at 3304 cm^{-1} at 213 K. Unlike triamides **2b** and **2c**, the IR spectra of **3b** and **3c** lack a distinct strong absorption at ~3300 cm^{-1}. A total of four peaks are observed for **3b** in the NH stretching region. The higher frequencies (3457, 3461 cm^{-1}) are due to the free NH absorptions. The two lower frequency broad peaks are assigned to the H-bonded NH's. At room temperature, the absorption at 3392 cm^{-1}, which is most consistent with an H-bond through a six-membered ring, is more prominent than



(a) Glutaryl β-alanine N-methylamide **3a**



(b) Glutaryl β-HAla[10] N-methylamide **3b**



(c) Glutaryl β-HVal[10] N-methylamide **3c**

Figure 5. NH stretch region of the IR spectra for 1 mM chloroform solution of **3a** (top, maximum at 3452 and 3304 cm^{-1}; bottom, at 3458 and 3319 cm^{-1}), **3b** (top, maximum at 3457, 3421, 3384, and 3296 cm^{-1}; bottom, maximum at 3461, 3429, 3392, and 3297 cm^{-1}), and **3c** (maximum at 3460, 3434, and 3323 cm^{-1}) at 298 K.

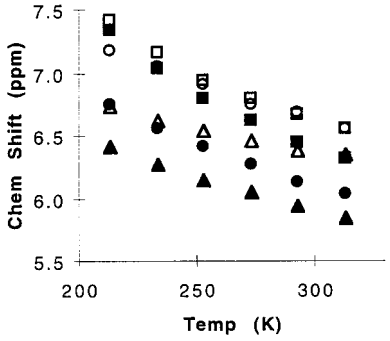


Figure 6. Amide proton NMR chemical shifts as a function of temperature for (a) *N*,*N*-dimethylamino glutaryl β-alanyl *N*-methylamide, (**3a**, triangles), *N*,*N*-dimethylamino glutaryl β-HAla[10] *N*-methylamide (**3b**, circles), and *N*,*N*-dimethylamino glutaryl β-HVal[10] *N*-methylamide (**3c**, squares). Reduced temperature constants (−Δδ NH/Δ*T*, ppb/K): Terminal NH, (▲) **3a** (5.5), (●) **3b** (7.1), (■) **3c** (10.1); Internal NH, (△) **3a** (3.8 ppb/K), (○) **3b** (6.3 ppb/K), (□) **3c** (8.5).

the one at 3297 cm^{-1}, which is most consistent with an H-bond of optimal NH−O bond angle.

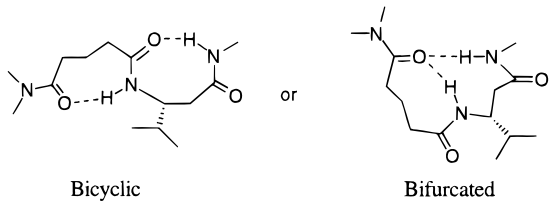
At 213 K, the peak at 3297 cm^{-1} becomes stronger in intensity. The lower frequency absorption should be due to a stronger intramolecular amide−amide H-bond. Therefore it is reasonable to assign a mixture of conformations to **3b** with more H-bonded conformations at lower temperatures. Triamide **3c** appears to have a similar distribution of conformations except **3c** has more H-bonded conformations than **3b**.

The VT-NMR data for triamides **3a**,**b**,**c** are displayed in Figure 6. Unlike triamides **2a**,**b**, the internal NH of **3a**,**b** (compare the open triangles and open circles in Figure 6 with those in Figure 4) shows consistently lower field chemical shifts than the terminal NH protons. These observations are consistent with the IR data implicating the involvement of the internal NH in hydrogen bonding. The internal NH of **3** can form an intramolecular H-bond through a six-membered ring to the secondary amide carbonyl group or through an eight-membered ring to the tertiary amide carbonyl group.

The trend in Figure 6 for both the C-terminal and the N-terminal NH proton chemical shifts of the three triamides (**3a**,**b**,**c**) is a gradual downfield shift from **3a** to **3c** at all temperatures. There is also a gradual increase in temperature dependence of chemical shifts from **3a** to **3c**. Triamide **3c** shows more H-bonded NH absorption in its IR spectrum (Figure 5c) than either **3a** or **3b** at lower temperatures.

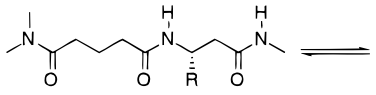
To sum it up, the formation of an intramolecular hydrogen bond for triamide **3a** is minimal because it is energetically unfavorable to form an intramolecular H-bond through a six-membered ring and it is entropically unfavorable to form an intramolecular H-bond through an eight- or a 12-membered ring. However, with an alkyl substitution there is a mixture of hydrogen-bonded and nonbonded conformations for **3b** and **3c**. The VT-NMR data in Figure 6, in combination with the IR data, provide valuable information concerning the preferred conformation of **3b** and **3c**. To a different degree, both the internal NH and the terminal NH protons of **3b** and **3c** are involved in hydrogen bonding.

The terminal NH protons of all three triamides have a greater temperature constant than their corresponding internal NH protons. The chemical shifts of the internal NH and the terminal NH of **3c** nearly merge at 213 K. The fact that the chemical shift of the internal NH changes with temperature rules out the head-to-tail type of hydrogen bond. Therefore, the VT-NMR data of **3a**,**b**,**c** should be explained by assuming either a bicyclic or a bifurcated conformation, which are more favorable at lower temperatures. The fact that the terminal NH protons of the three triamides have a greater temperature constants (see Table 1) suggests that the bifurcated conformation is favored, in which the terminal NH enjoys a more favorable H-bond geometry.

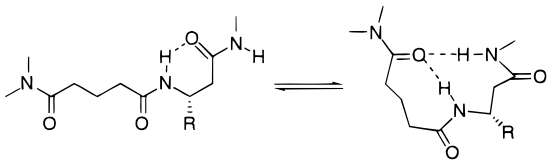


Bicyclic Bifurcated

The bicyclic conformation requires the formation of two eight-membered rings. As discussed previously, the eight-membered ring involving the H-bond from the terminal NH to the internal amide carbonyl is not favorable. Furthermore, the temperature dependency of the chemical shifts on the terminal NH proton of **3c** is −10.1 ppb/K, which suggests a large enthalpic effect. This is more consistent with a 12-membered ring. Therefore, the bifurcated conformation appears to be the preferred form for **3b** and **3c**, in which both the terminal and the internal amide NH's are, to a different degree, hydrogen-bonded to the tertiary amide carbonyl group. The substituent effect on the conformation of glutaryl β-amino acid-containing triamide **3** can be depicted by the following equilibrium.



Favored when R = H



present when R = Me and at high temperature

favored when R = *i*-Pr and at low temperature

Quantification Using VT-NMR Data. As previously shown by Gellman,[12] in certain cases a van't Hoff analysis of the ^1H NMR variable-temperature data can produce the thermodynamic parameters for the equilibrium between the states in which the NH protons are free and intramolecularly hydrogen bonded. The equilibrium constant K_{eq} is related to the observed chemical shifts by the following equations:

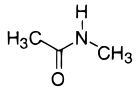
$$K_{eq} = (\delta_{obs} - \delta_n)/(\delta_b - \delta_{obs}) \quad \text{(a)}$$

$$\ln K_{eq} = (-\Delta H°/R)(1/T) + \Delta S°/R \quad \text{(b)}$$

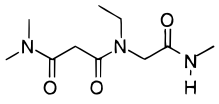
where the δ_{obs} are the observed chemical shifts of the amide proton involved in hydrogen bonding, δ_n is the limiting chemical shift for the non-hydrogen-bonded

state, and δ_b is the chemical shift for the fully hydrogen bonded state. For triamide **2a** and **2b**, a two-state equilibrium can be assumed (see eq 2) since the internal NH does not participate in hydrogen bonding.

The chemical shifts of *N*-methylacetamide (NMA) in CD_2Cl_2 at 1 mM concentration have been used previously as the limiting values for non-hydrogen-bonded states for a triamide.[9] We have also chosen to use NMA as our standard except that we run the experiments in $CDCl_3$.



NMA



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For the limiting chemical shifts of the intramolecularly hydrogen bonded state, compound **4** has been used as a standard since it shows no free NH stretching and has a structure similar to triamide **2**.[13] Thus, the chemical shifts of NMA and triamide **4** are obtained at various temperatures and used in eq a as respective lower and upper limits of chemical shifts. The equilibrium constants for eq 2 are obtained using the VT-NMR data in Figure 4 for triamides **2a** and **2b.** The thermodynamic parameters for the process depicted in eq 2 are obtained using eq b. van't Hoff plots give a ΔH of -1.0 ± 0.2 kcal/mol and a ΔS of -4.7 ± 1 eu for triamide **2a** and a ΔH of -1.0 ± 0.2 kcal/mol and a ΔS of -3.7 ± 1 eu for triamide **2b**, each with a correlation coefficient of better than 0.99. The uncertainties are estimated considering the choice of the imperfect upper and lower limiting chemical shifts. These temperature-dependent upper and lower limits of chemical shifts are the best available values.

It is interesting to note that the increased proportion of the intramolecular amide−amide hydrogen bond observed for triamide **2b** appears to be entirely due to entropic effects. This is reasonable since both **2a** and **2b** form intramolecular hydrogen bonds through an 11-membered ring. Therefore both should have an identical H-bond geometry and the H-bond should be similar in strength. However, upon methyl substitution, the conformational space is reduced for triamide **2b** in the stretched form, while the conformational freedom in the folded form does not change from **2a** to **2b**.

Conclusions

An alkyl substitution in the β-amino acid-containing amides **1**, **2**, and **3** leads to an increase in the population of intramolecularly hydrogen bonded conformations. However, there is no simple pattern to predict which conformation will become more populated. For triamide **2**, a methyl group greatly enhances the formation of a head-to-tail type of folding pattern through an 11-membered ring (compare **2b** with **2a**), but an isopropyl group appears to decrease the enhancement. For triamide **3**, the formation of a bifurcated conformation appears to be proportional to the steric size of the alkyl substitution. Therefore, an alkyl substitution on the connecting chain of a hydrogen bond donor and a hydrogen bond acceptor should promote intramolecular hydrogen bonding by reducing the conformational space for the stretched conformation. Whether a particular conformation is enhanced by the substituent appears to depend on the size of the substituent. A larger substituent is not necessarily a better promoter for the formation of a single stable conformation. Will the substituent effect observed here for the small polyamides be applicable to larger peptides? Study along this line is in progress in our laboratories.

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