Articles

Requirement for Hydrogen-Bonding Cooperativity in Small Polyamides: A Combined VT-NMR and VT-IR Investigation

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Received October 3, 1997

A study of intramolecular hydrogen bonding in chloroform for a small combinatorial library of nine triamides with varying connecting chain length has been completed. The starting materials for the triamides are three diacids (succinic, glutaric, and adipic acid) and three amino acids (glycine, β-alanine, and γ-aminobutyric acid). The preferences for the head-to-tail type of folding pattern are identified for the smaller triamides (**1** and **4**). The preference for the head-to-tail folding pattern can be explained by the energetic superiority of an optimal hydrogen bond geometry in which the NH---O bond angle is near linearity. The β-alanine containing triamides **2**, **5**, and **8** are resistant to intramolecular hydrogen bonding, especially to nearest neighbor hydrogen bonding. At lower temperatures, triamides **2** and **5** exhibit a small population of head-to-tail type of folding, while triamide **8** shows a significant population of bifurcated conformation. Triamide **6**, **7**, and **9** prefer bicyclic structures involving nearest neighbor hydrogen bonding. A nine-membered ring is large enough to accommodate a near linear N−H--O bond angle. Entropic effects are probably responsible for the preference of the nine-membered ring over a 12- or a 14-membered ring. The enhancement of hydrogen bonding in triamide **9** is enormous, and both NHs have a very large temperature dependence of chemical shifts (−15 ppb/K and −13.3 ppb/K for the terminal and the internal NH protons, respectively). Using appropriate temperature-dependent lower and upper limits of chemical shifts, a van't Hoff analysis gives the hydrogen bond strength for the terminal NH ($\Delta H = -3.1 \pm 0.5$ kcal/mol) and for the internal NH ($\Delta H = -2.8 \pm 0.5$ kcal/mol). The increased hydrogen bond strength is taken as evidence for hydrogen-bonding cooperativity from the two mutually enhanced individual hydrogen bonds. A near linear NH--O bond angle is required for this effect.

Introduction

It is known that the stability of α-helices has a dependence on peptide chain length. For peptides with the same amino acid compositions, longer chains lead to a greater helix content.[1] It is also known that α-helical peptides have a macrodipole.[2,3] The stability dependence on chain length of α-helices has not been rationalized satisfactorily. Recently in a theoretical study, Guo and Karplus reported that the strength of the amide−amide hydrogen bond is enhanced if both the carbonyl group and the NH have hydrogen-bonding partners.[4] It is possible that hydrogen-bonding cooperativity plays an important role in stabilizing helical conformation. Therefore, to understand the driving force of protein folding it is important to elucidate the role and the requirement of hydrogen-bonding cooperativity. However, most reported studies of α-helical conformations were carried out using macroscopic methods,[5] such as circular dichroism (CD), which although convenient to use cannot detect specific hydrogen bonding. Therefore, little is known about the requirement for hydrogen-bonding cooperativity to occur. Although the use of nonpolar solvents will not reveal hydrophobic effects, other fundamental interactions such as specific hydrogen bonds, torsional strain, electrostatic effects, and van der Waal forces can be identified.

Recently, Gellman and co-workers have reported studies of hydrogen bonding of diamides in organic solvents.[6a] It has been shown that the intramolecular amide−amide hydrogen bond is the strongest through a nine-membered ring. Thus, intramolecular amide−amide H-bonds through six- and a seven-membered rings are less favorable enthapically than H-bond through a nine-membered ring.[6a]

Another important contribution from Gellman's group is their study on derivatives of β-alanine and γ-aminobutyric acid. They found that nearest neighbor hydrogen

† Undergraduate research assistant supported by the Howard-Hughes Medical Institute.

‡ Undergraduate research assistant under the NSF-REU program.

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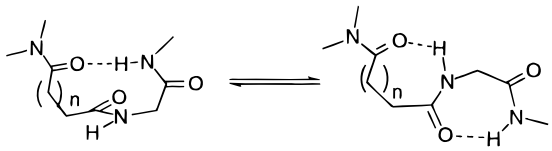
S0022-3263(97)01831-8 CCC: $15.00
Published on Web 07/29/1998

Most favorable when n = 3

bonding is favorable for the single-residue peptide of γ-aminobutyric acid and not favorable for the derivatives of β-alanine.[6b]

We have been able to study specific hydrogen bonds in a few triamides in organic solvents by using variable temperature IR and NMR techniques.[7] The formation of intramolecular amide−amide H-bonds in the triamides containing a glycine residue are more favorable through 10- and 11-membered rings than through two smaller rings.[7]



More favorable when n = 1,2

We have also reported that a triamide built from adipic acid and γ-aminobutyric acid forms a bicyclic conformation. Enhanced hydrogen bonding is observed for this triamide. This enhancement in H-bond energy has been attributed to hydrogen-bonding cooperativity. We have now completed the study of a small combinatorial library of nine triamides with varying connecting chain length. This report describes our observation for the requirement of hydrogen-bonding cooperativity in amide−amide hydrogen bonds.

Experimental Section

THF was freshly distilled from sodium benzophenone under N_2. Hexanes were freshly distilled from calcium hydride under N_2. Routine ^{1}H NMR spectra (for characterization) were obtained on a Bruker AC-200 spectrometer. Triamides were prepared by 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.

Variable-Temperature NMR Procedure. Unless specifically noted otherwise all NMR experiments were performed using $CDCl_3$ as solvent. The samples for variable-temperature (VT) ^{1}H 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 7.24 ppm at all temperatures.[8] 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.[9] 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.

Example of the Preparation of Triamides. Succinylglycyl *N*-Methylamide Methyl Ester (11). To a mixture of 30 mL of THF and 25 mL of triethylamine were added 4.19 g (30 mmol) of glycine ethyl ester hydrochloride and 3.10 g (30 mmol) of succinic anhydride. The resulting solution was stirred for 4 h and then concentrated to about 10 mL. The solution was acidified to pH = 2 with 20% hydrochloric acid and then extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure to give 4.2 g (69%) of monosuccinyl glycine ethyl ester as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.2 Hz, 3H, CH_3), 2.56 (t, J = 5.8 Hz, 2H, CH_2), 2.68 (t, J = 5.7 Hz, 2H, CH_2), 4.00 (d, J = 5.1 Hz, 2H, NCH_2), 4.18 (q, J = 7.1 Hz, 2H, OCH_2), 6.55 (s, 1H, NH), 8.87 (broad, 1H, CO_2H). This material was carried on without further purification.

A pressure-resistant glass tube was loaded with a solution of 2.03 g (10 mmol) of the ester in 10 mL of THF and was

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(8) For a similar assumption, see ref 6. Upon the request of a reviewer, we have measured the chemical shifts of $CHCl_3$ and $Si(CH_3)_4$ as a function of temperature using the instrumental frequency as a reference, i.e., recording the spectrum without lock. The chemical shifts of both $CHCl_3$ and $Si(CH_3)_4$ moved slightly upfield when the probe is cooled. After a discussion with experts from the manufacturer of the NMR instrument, the following conclusion is proposed. Since the shift difference between $CHCl_3$ and $Si(CH_3)_4$ stayed almost the same, it is most likely the shift we observed was due to the changes in the geometry of the probe and in the shim system when the temperature is lowered. In any event, the shift is small compared to the chemical shifts observed for some of the amide NH protons proposed to participate in hydrogen bonding. Therefore, the assumption that the chemical shifts of $CHCl_3$ remains constant at the experimental temperatures is valid in this context.

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Table 1. Triamides Prepared from Diacids and Amino Acids

	amino acid		
diacid	glycine	β-alanine	γ-aminobutyric acid
succinic	succinic glycine (**1**)	succinic β-alanine (**2**)	succinic γ-aminobutyric acid (**3**)
glutaric	glutaric glycine (**4**)	glutaric β-alanine (**5**)	glutaric γ-aminobutyric acid (**6**)
adipic	adipic glycine (**7**)	adipic β-alanine (**8**)	adipic γ-aminobutyric acid (**9**)

cooled to −78 °C. Anhydrous methylamine was added into the solution through a long stainless steel needle and Tygon tubing from a lecture bottle until the volume of the solution increased about 2 mL. The pressure tube was sealed and warmed to room temperature slowly. The mixture was stirred for 1 week. After being cooled to −78 °C, the seal was opened and the excess methylamine and the solvent were removed under reduced pressure to give 2.12 g (97%) of the corresponding *N*-methylamide (as its ammonium salt) as a white solid: ^{1}H NMR (DMSO) δ 2.22 (t, J = 4.0 Hz, 2H, CH_2), 2.23 (t, J = 3.9 Hz, 2H, CH_2), 2.31 (s, 3H, CH_3), 2.53 (d, J = 4.6 Hz, 3H, CH_3),3.56 (d J = 5.9 Hz, 2H, CH_2), 4.78 (broad, 3H, $MeNH_3^+$), 8.16 (q, J = 4.1 Hz, 1H, NH), 8.26 (t, J = 5.8 Hz, 1H, NH). This material was carried on without further purification.

To a solution of 2.12 g (9.7 mmol) of the ammonium carboxylate in 10 mL of methanol was added 2.58 g (13.4 mmol) of *p*-toluenesulfonic acid monohydrate. The resulting solution was cooled to 0 °C and then added to ethereal diazomethane. The completion of the reaction was monitored by TLC (20% MeOH/EtOAc). The mixture was concentrated and purified by column chromatography and eluted with 20% MeOH in EtOAc to give 1.14 g (58%) of the corresponding diamide ester (**11**) as a white solid (mp 115−125 °C): ^{1}H NMR ($CDCl_3$) δ 2.48 (t, J = 6.7 Hz, 2H, CH_2), 2.71 (t, J = 6.6 Hz, 2H, CH_2), 2.79 (d, J = 4.8 Hz, 3H, NCH_3), 3.67 (s, 3H, OCH_3), 3.91 (d, J = 5.7 Hz, 2H, NCH_2), 6.55 (broad at rt, triplet at 213 K, 1H, NH), 6.62 (broad at rt, quartet at 213 K, 1H, NH).

Triamide 2. A pressure-resistant glass tube was loaded with 0.90 g (4.2 mmol) of the diamide ester (**11**) and cooled to −78 °C. Anhydrous dimethylamine was added through a long stainless steel needle and Tygon tubing from a lecture bottle until the volume of the solution increased about 5 mL. The tube was sealed and warmed to room temperature slowly. The mixture was stirred for 1 week. After the mixture was cooled to −78 °C, the seal was opened and the excess dimethylamine and solvent were removed under reduced pressure to give 1.00 g (100%) of the desired triamide **2** as a white solid (mp 100−115 °C): ^{1}H NMR ($CDCl_3$) δ 2.36 (t, J = 6.7 Hz, 2H, CH_2),2.43 (t, J = 5.9 Hz, 2H, CH_2), 2.63 (t, J = 6.1 Hz, 2H, CH_2), 2.76 (d, J = 4.7 Hz, 3H, CH_3), 2.90 (s, 3H, CH_3), 2.99 (s, 3H, CH_3), 3.47 (dt, J = 6.4 Hz, 5.3 Hz, 2H, NCH_2), 6.50 (broad at rt, triplet at 213 K, 1H, NH), 6.62 (broad at rt, quartet at 213 K, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 26.2, 28.7, 31.2, 35.4, 36.0, 36.1, 37.0, 171.9, 172.3, 172.9; IR (1 mM in $CDCl_3$) 3457 (NH), 3351 (broad, NH), 1662 (C═O), 1639 (C═O) cm^{-1}.

Other triamides are prepared using similar procedures from the appropriate starting materials indicated in Table 1. The following are characterizations of the remaining triamides.

Triamide 1: ^{1}H NMR ($CDCl_3$) δ 2.42 (t, J = 5.5 Hz, 2H, CH_2), 2.74 (t, J = 5.5 Hz, 2H, CH_2), 2.75 (d, J = 4.7 Hz, 3H, CH_3), 2.91 (s, 3H, CH_3), 3.00 (s, 3H, CH_3), 3.92 (d, J = 6.4 Hz, 2H, NCH_2), 6.44 (broad, 1H, NH), 7.36 (broad, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 26.1, 29.1, 31.3, 35.3, 37.0, 43.2, 170.0, 172.2, 173.2; IR (1 mM in $CDCl_3$) 3452 (NH), 3350 (broad, NH), 1668 (C═O), 1628 (C═O) cm^{-1}.

Triamide 3: ^{1}H NMR ($CDCl_3$) δ 2.36 (t, J = 6.7 Hz, 2H, CH_2),2.43 (t, J = 5.9 Hz, 2H, CH_2), 2.63 (t, J = 6.1 Hz, 2H, CH_2), 2.76 (d, J = 4.7 Hz, 3H, CH_3), 2.90 (s, 3H, CH_3), 2.99 (s, 3H, CH_3), 3.47 (dt, J = 6.4 Hz, 5.3 Hz, 2H, NCH_2), 6.50 (broad, 1H, NH), 6.62 (broad, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 26.2, 28.7, 31.2, 35.4, 36.0, 36.1, 37.0, 171.9, 172.3, 172.9; IR (1 mM in $CDCl_3$) 3457 (NH), 3351 (broad, NH), 1662 (C═O), 1639 (C═O) cm^{-1}.

Triamide 4: ^{1}H NMR ($CDCl_3$) δ 1.95 (quintet, J = 6.8 Hz, 2H, CH_2), 2.31 (t, J = 6.9 Hz, 2H, CH_2), 2.39 (t, J = 6.8 Hz, 2H, CH_2), 2.76 (d, J = 4.8 Hz, 3H, CH_3), 2.90 (s, 3H, CH_3), 3.00 (s, 3H, CH_3), 3.89 (d, J = 5.8 Hz, 2H, NCH_2), 6.82 (broad, 1H, NH), 7.00 (broad, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 20.7, 26.1, 32.3, 35.2, 35.4, 37.4, 43.2, 169.9, 172.7, 173.3; IR (1 mM in $CDCl_3$) 3450 (NH), 3412 (broad, NH), 3324 (broad, NH), 1667 (C═O), 1630 (C═O) cm^{-1}.

Triamide 5: ^{1}H NMR ($CDCl_3$) δ 1.90 (quintet, J = 7.0 Hz, 2H, CH_2), 2.22 (t, J = 6.9 Hz, 2H, CH_2), 2.35 (t, J = 6.8 Hz, 2H, CH_2), 2.38 (t, J = 5.7 Hz, 2H, CH_2), 2.76 (d, J = 4.7 Hz, 3H, NCH_3), 2.90 (s, 3H, CH_3), 2.98 (s, 3H, CH_3), 3.47 (q, J = 5.5 Hz, 2H, NCH_2), 6.38 (broad, 1H, NH), 6.68 (broad, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 21.0, 26.2, 32.3, 35.3, 35.6, 35.7, 37.2, 43.2, 172.2, 172.5, 173.0; IR (1 mM in $CDCl_3$) 3458 (NH), 3317 (broad, NH), 1665 (C═O), 1632 (C═O) cm^{-1}.

Triamide 6: ^{1}H NMR ($CDCl_3$) δ 1.79 (quintet, J = 6.1 Hz, 2H, CH_2), 1.92 (quintet, J = 7.0 Hz, 2H, CH_2), 2.20 (t, J = 7.1 Hz, 2H, CH_2), 2.25 (t, J = 7.4 Hz, 2H, CH_2), 2.38 (t, J = 6.8 Hz, 2H, CH_2), 2.76 (d, J = 4.8 Hz, 3H, NCH_3), 2.91 (s, 3H, CH_3), 2.99 (s, 3H, CH_3), 3.25 (q, J = 6.2 Hz, 2H, NCH_2), 6.69 (broad, 2H, NH); ^{13}C NMR ($CDCl_3$) δ 21.2, 25.7, 26.2, 32.2, 33.5, 35.4, 35.6, 37.3, 38.6, 172.7, 173.5, 173.6; IR (1 mM in $CDCl_3$) 3460 (NH), 3320 (broad, NH), 1662 (C═O), 1632 (C═O) cm^{-1}.

Triamide 7: ^{1}H NMR ($CDCl_3$) δ 1.64 (quintet, J = 3.4 Hz, 4H, $2CH_2$), 2.28 (t, J = 6.5 Hz, 2H, CH_2), 2.31 (t, J = 6.2 Hz, 2H, CH_2), 2.77 (d, J = 4.8 Hz, 3H, NCH_3), 2.90 (s, 3H, CH_3), 2.97 (s, 3H, CH_3), 3.89 (d, J = 5.5 Hz, 2H, NCH_2), 6.75 (broad, 1H, NH), 7.12 (broad, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 24.2, 24.9, 25.1, 26.1, 32.7, 35.4, 35.9, 37.2, 43.4, 169.9, 172.8, 173.6; IR (1 mM in $CDCl_3$) 3452 (NH), 1670 (C═O), 1632 (C═O) cm^{-1}.

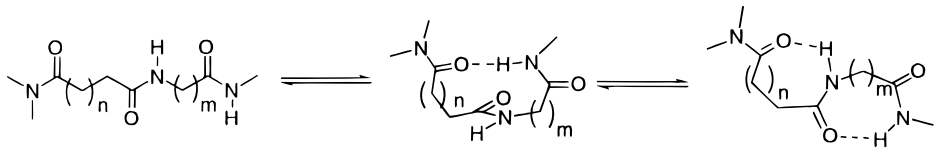
Triamide 8: ^{1}H NMR ($CDCl_3$) δ 1.59 (m, 4H, $2CH_2$), 2.17 (t, J = 6.3 Hz, 2H, CH_2), 2.28 (t, J = 5.2 Hz, 2H, CH_2), 2.38 (t, J = 6.2 Hz, 2H, CH_2), 2.74 (d, J = 4.8 Hz, 3H, NCH_3), 2.89 (s, 3H, CH_3), 2.96 (s, 3H, CH_3), 3.48 (q, J = 6.0 Hz, 2H, NCH_2), 6.62 (broad, 1H, NH), 6.84 (broad, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 24.3, 24.9, 25.2, 26.2, 32.8, 33.1, 35.4, 35.6, 35.8, 36.2, 37.2, 172.2, 172.7, 173.1; IR (1 mM in $CDCl_3$) 3457 (NH), 3307 (broad, NH), 1662 (C═O), 1633 (C═O) cm^{-1}.

Triamide 9: ^{1}H NMR ($CDCl_3$) δ 1.61 (m, 4H, $2CH_2$), 1.82 (quintet, J = 6.3 Hz, 2H, CH_2), 2.18 (m, 2H, CH_2), 2.25 (m, 2H, CH_2), 2.33 (m, 2H, CH_2), 2.77 (d, J = 4.7 Hz, 3H, $HNCH_3$), 2.93 (s, 3H, CH_3), 2.99 (s, 3H, CH_3), 3.30 (q, J = 6.1 Hz, 2H, NCH_2), 6.80 (broad, 1H, NH), 6.94 (broad, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 24.2, 25.1, 25.9, 26.1, 32.7, 33.6, 35.4, 36.2, 37.2, 38.5, 172.9, 173.4, 173.6; IR (1 mM in $CDCl_3$) 3460 (NH), 3338 (broad, NH), 1658 (C═O), 1630 (C═O) cm^{-1}.

Results

To study the cooperative effect in amide−amide hydrogen bonding, one must determine the strength of the amide−amide hydrogen bond in the presence and in the absence of this effect. The systems that we employ mesure the difference between the strength of an intramolecular H-bond in a diamide and the strengths of the same type of H-bonds in a series of triamides where the central amide function has opportunity to hydrogen bond to two other amide functional groups. As shown in Figure 1, such a mutual enhancement of hydrogen bonding can be achieved in the bicyclic conformation (**C**) of the triamides.

A parallel synthesis scheme was employed in our study to prepare nine triamides with similar structures but varying connecting chain lengths. The starting materials are three diacids (succinic, glutaric, and adipic acid) and three amino acids (glycine, β-alanine, and γ-aminobutyric

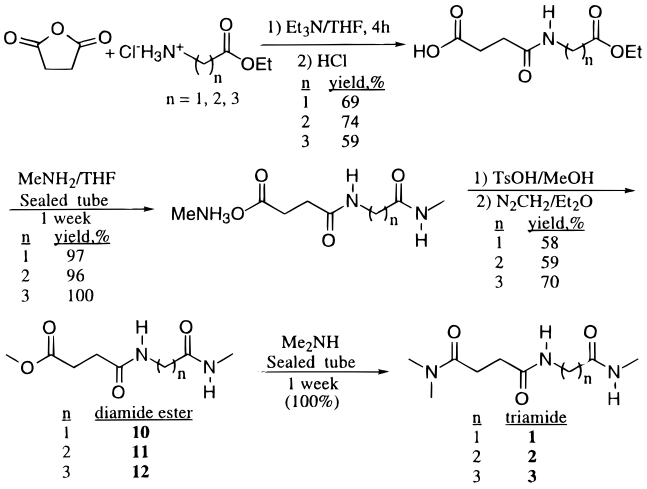


A **B** **C**

Triamide **1:** n = 1; m = 1 **4:** n = 2; m = 1 **7:** n = 3; m = 1
2: n = 1; m = 2 **5:** n = 2; m = 2 **8:** n = 3; m = 2
3: n = 1; m = 3 **6:** n = 2; m = 3 **9:** n = 3; m = 3

Figure 1. Equilibria among extended chain (**A**), head-to-tail folding (**B**), and bicyclic (**C**) conformations for triamides **1**−**9**.

Scheme 1



acid) as shown in Table 1. A typical sequence of reactions used to prepare these triamides is depicted in Scheme 1.

Each of the commercially available hydrochloric acid salts of amino acid derivatives (ethyl esters of glycine, β-alanine, and 4-aminobutyric acid) was allowed to react with succinic anhydride in the presence of triethylamine, yielding the corresponding amide ester. The intermediate was sealed in a pressure-resistant sealed tube with anhydrous methylamine to give corresponding diamide carboxylates. The carboxylates were acidified with 1 equiv of toluenesulfonic acid followed by treatment with diazomethane to give diamide esters **10**, **11**, and **12**. These diamide esters were converted to desired triamides **1**, **2**, and **3** by treatment with dimethylamine in a sealed tube. A similar methodology was applied for the preparation of other triamides.

Infrared Spectroscopy. As reported before, all IR and NMR spectra are recorded in $CDCl_3$ at 1 mmol concentration. Variable concentration IR experiments were carried out for each triamide to ascertain that no intermolecular aggregation is present. In all cases, hydrogen-bonded NH absorptions remain constant when the concentration is in the range of 1−5 mmol.

Infrared spectroscopy can provide straightforward information on the association of the amide NH group with acceptor groups. In chloroform, which we used for our experiments, association of the amide NH proton with the solvent is weak. The IR absorption for this solvent-exposed NH occurs at ∼3450 cm^{-1} and is referred to as a free NH.[6] If the amide NH is hydrogen bonded to an amide C═O group, the IR absorption occurs at ∼3320 cm^{-1}.[6] The free NH peak is sharp and in certain cases the resolution is high enough to differentiate two types of free NH stretch. In combination with ^1H NMR

Table 2. Amide NH Stretching Frequencies (cm^{-1}) in $CDCl_3$ (1 mM) for Triamides 1−9[a]

	298 K		213 K	
tri-amide	free NH $\nu_{NH(int)}$ $\nu_{NH(ter)}$	H-bonded NH(broad)	free NH $\nu_{NH(int)}$ $\nu_{NH(ter)}$	H-bonded NH (broad)
1	3453 (s)	3350 (s)	3449 (s)	3336 (s)
2	3457 (s, overlap)	3351 (w)	3451 (s, overlap)	3337 (m)
3	3444 (s), 3460 (s)	3338 (m)	3440 (s), 3454 (m)	3324 (s)
4	3451 (s, overlap)	3325 (w)	3446 (m)	3304 (s)
5	3458 (s, overlap)	3319 (w)	3452 (s, overlap)	3304 (w)
6	3446 (m), 3460 (s)	3320 (m)	3442 (m), 3453 (m)	3307 (s)
7	3452 (s, overlap)	broad (m)	3447 (s, overlap)	3270 (m)
8	3457 (s, overlap)	3307 (w)	3447 (s), 3451 (s)	3309 (m)
9	3441 (m), 3452 (m)	3330 (s)	3449 (w), 3460 (w)	3338 (s)

[a] s = strong, m = medium, w = weak.

Table 3. Amide Carbonyl Stretching Frequencies (cm^{-1}) in $CDCl_3$ (1 mM) for Triamides 1−9

triamide	$\nu_{(C=O)NHR}$	$\nu_{(C=O)NMe2}$ (298 K)	$\nu_{(C=O)NHR}$	$\nu_{(C=O)NMe2}$ (213 K)
1	1668	1628	1660	1624
2	1662	1639	1653	1635
3	1662	1637	1650	1631
4	1667	1632	1659	1619
5	1665	1632	1657	1629
6	1662	1632	1653	1635
7	1670	1632	1659	1625
8	1662	1633	1653	1623
9	1658	1630	1650	1616

methods, the stretching at ∼3460 cm^{-1} is assigned to the terminal amide NH and the stretching at ∼3440 cm^{-1} to the internal amide NH (see Table 2).

Furthermore, two types amide carbonyl groups can be identified in the carbonyl stretching region by their difference in frequencies (∼1630 cm^{-1} for tertiary amide C═O group and ∼1665 cm^{-1} for secondary amide carbonyl group, see Table 3). However, in most cases IR cannot distinguish individual NH or CO stretching. We have recently reported that triamide **9** assumes the bicyclic conformation **C** with two consecutive intramolecular hydrogen bonds each through a nine-membered ring and that a cooperative effect is observed to enhance the amide−amide hydrogen bond strength.[7c] The conclusion was based on the VT-IR and VT-NMR data and the amide carbonyl stretching frequencies.[7c] We have modified the listing of the carbonyl stretching frequencies in Table 1 of ref 7c from the ambiguous terms $\nu_{internal}$ and $\nu_{terminal}$ to the more precise terms $\nu_{(C=O)NHR}$ and $\nu_{(C=O)NMe2}$ (see Table 3). The absorptions around 1650−1670 cm^{-1} are the results of two overlapping peaks due to the internal and the terminal $\nu_{(C=O)NHR\ groups}$.

The tertiary amide carbonyl group ($\nu_{(C=O)NMe2)}$ in each triamide does give rise to a distinct peak in the IR spectrum. Therefore these tertiary amide carbonyl stretching frequencies are useful in conformational iden-

Table 4. Amide Proton NMR Chemical Shift Temperature Dependence in $CDCl_3$ (1 mM)

	$\Delta\delta/\Delta T$ (ppb/K)		
compd	NH_t[a]	NH_i	ref
1	−7.7	−0.8	this work
2	−8.7	−1.2	this work
3	−11.8	−1.5	this work
4	−13.0	−2.0	this work
5	−5.5	−3.8	this work
6	−11.9	−6.9	this work
7	−5.9	−10.7	this work
8	−9.2	−6.2	this work
9	−15.0	−13.3	this work
13	−5.3		this work
14	−5.2		this work
16	−11.9[b]		this work
17	−11.3[c]		6b
18	−9.8[c]		6b
19	−11.9		this work

[a] NH_t and NH_i stand for terminal and internal NH, respectively. [b] Value is for the s-trans isomer. For the s-cis isomer: $\Delta\delta NH/\Delta T = -3.5$ ppb/K. [c] Determined in CH_2Cl_2.

tifications. For example, triamides **1** and **4** assume the head-to-tail type of folding pattern to a large extent at low temperatures and their tertiary amide carbonyl stretching frequencies are 1624 and 1619 cm^{-1}, respectively, at 213 K. Triamide **2** assumes the head-to-tail folding also but only to a small extent based on its VT-NMR data and its tertiary amide carbonyl stretching frequency is 1635 cm^{-1} at 213 K. Triamide **3** has a nine-membered ring intramolecular H-bond but does not involve the tertiary amide carbonyl group. Its tertiary amide carbonyl stretching frequency is 1631 cm^{-1} at 213 K. Thus, a fully amide−amide hydrogen-bonded tertiary amide carbonyl group should be expected to absorb infrared light at ∼1620 cm^{-1}.

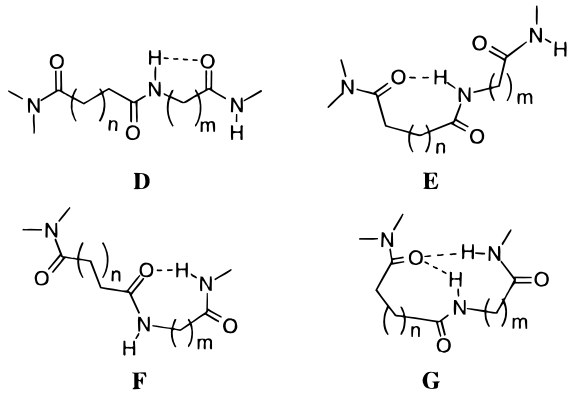
^{1}H NMR Spectroscopy. Although NMR has a slow time scale and gives a weighted average of chemical shifts of the free and the H-bonded NH for each amide proton, its resolution is high enough to identify specific NH protons. The two amide NH protons of each triamide (**1**−**9**) are distinguished from each other by their coupling patterns at lower temperatures and are confirmed by 2D COSY experiments. The terminal amide NH appears as a quartet, and the internal amide NH appears as a triplet at below −30 °C. At room temperature, the amide NH proton appears as a broad peak. The chemical shift change of the amide NH proton with temperature produces useful temperature coefficient ($-\Delta\delta/\Delta T$, Table 4) for each NH proton,[10a,b] from which qualitative and, in certain cases, quantitative information on the population of the hydrogen-bonded NH can be obtained. It is useful to compare the temperature dependence of NH chemical shift of a given amide NH proton to that of the *N*-methylacetamide (NMA), which shows only a free NH absorption in its IR spectrum at 1 mmol concentration in chloroform and gives a $\Delta\delta/\Delta T$ of −3.3 ppb/K in chloroform.

Discussion

Assignment of Preferred Conformations. In addition to the three conformations depicted in Figure 1, three other conformations are possible for these triamides

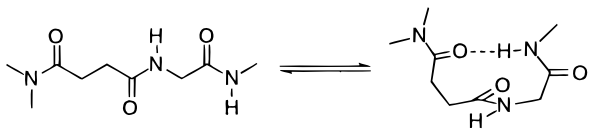
(10) (a) Stevens, E. S.; Sugawara, N.; Bonara, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7048. (b) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, 512.

Chart 1



if one accounts for all combinations of hydrogen bonding possibile among two NH groups and three C═O groups in each triamide. One NH may form an H-bond to its nearest C═O groups while the other NH is free, such as those shown in Chart 1 (conformations **D**, **E**, and **F**). These six structures (**A**−**F**) plus a possible bifurcated form (**G**), give a possibility of seven distinct conformations.

Triamide 1. After a study of the VT-NMR and VT-IR data of the nine triamides and a few control compounds, it is relatively straightforward to single out the triamides with major populations of an extended chain (**A**) and/or with a head-to-tail conformation (**B**). Triamide **1** assumes almost entirely the head-to-tail conformer (**B**) as indicated by both its IR and NMR data (see Tables 2−4). It is the only triamide in this group whose relative intensities of NH peaks show little change with temperature. Its ^{1}H NMR spectrum shows that the internal NH appears upfield (∼6 ppm) even at low temperature and the terminal NH appears downfield (∼8 ppm) even at high temperature. It is therefore deduced that the sharp NH peak in the IR spectrum is due to the internal NH while the broad peak is due to the terminal NH. These data are consistent with the following folding pattern.



1 Preferred conformation (**B**)

Triamides 2 and 4. Triamides **2** and **4** display an equilibrium between the extended chain (**A**) and the head-to-tail conformer (**B**). An example of the VT-NMR data for such a folding pattern (triamide **2**) is shown in Figure 2b, in which the chemical shift of the terminal NH proton changes with temperature while that of the internal NH does not. The temperature coefficient for triamide **4** is greater than that for triamide **2** (see Table 4). The greater intensity of the broad NH absorption at low temperature (see Table 2) is in agreement with the NMR data. The stretching frequency of the carbonyl group (C═ONMe$_2$) of **4** is also lowered more than that of **2** at low temperature. All of these data indicates that triamide **4** is more tightly folded than triamide **2** at lower temperatures. Since both lead to an 11-membered ring when an intramolecular H-bond is formed, the less favorable formation of an intramolecular H-bond in triamide **2** should be due to torsional strains created by

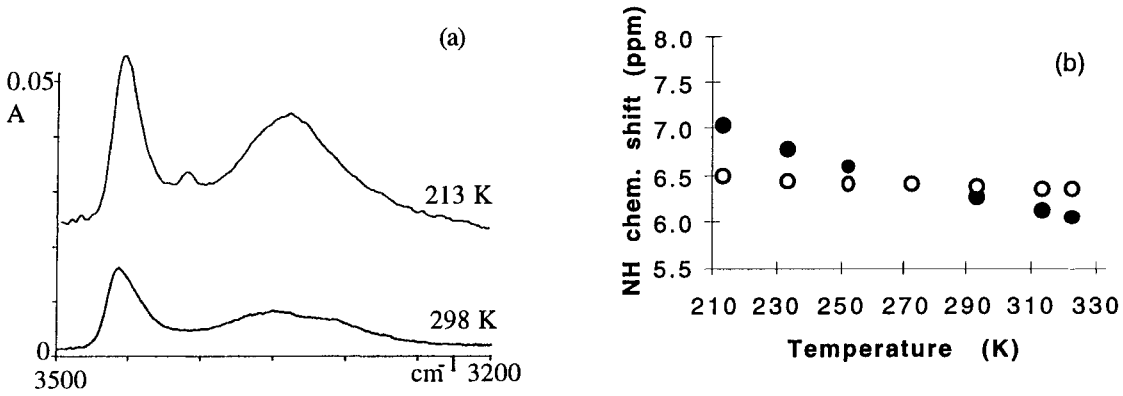
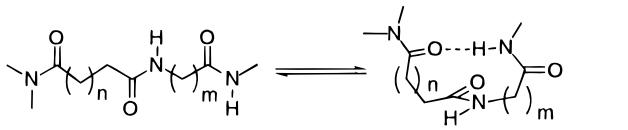


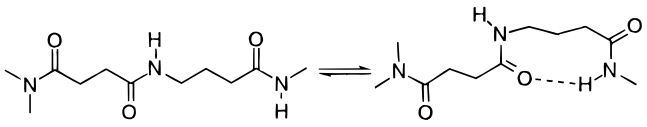
Figure 2. (a) NH stretch region of the IR spectra at 213 and 298 K for *N*,*N*-dimethylaminosuccinyl β-alanyl *N*-methylamide, **2**. The sharp band at 3451−3457 cm^{-1} is assigned to the free NH stretch and the broad band at 3337−3351 cm^{-1} to the intramolecularly hydrogen-bonded NH. (b) Amide proton NMR chemical shifts as a function of temperature for triamide **2**. Internal NH (○, $\Delta\delta NH/\Delta T = -1.2$ ppb/K). Terminal NH (●, $\Delta\delta NH/\Delta T = -8.7$ ppb/K).

the connecting chain during folding. It is noteworthy that triamide **2** contains a β-alanine unit which has been reported to resist the formation of nearest neighbor intramolecular hydrogen bond.[6b]



2 n = 1, m = 2; **4** n = 2, m = 1 Preferred at lower temperatures (**B**)

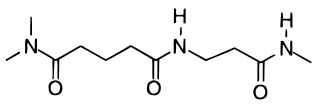
Triamide 3. The IR and NMR data in Tables 2−4 indicate that triamide **3** favors conformation **F**, where an intramolecular amide−amide H-bond between the terminal NH and the internal carbonyl group through a



3 Preferred conformation (**F**)

nine-membered ring (**F**) is preferentially formed, rather than the head-to-tail (**B**) type of intramolecular hydrogen bond through a 12-membered ring. Evidence for this conformation includes (1) large terminal NH chemical shift−temperature dependence, (2) strong absorption of the free internal NH in the IR spectra, and (3) constant stretching frequency for the tertiary amide carbonyl group ($C{=}ONMe_2$).

Triamide 5. The IR and NMR spectra of triamide **5** show little H-bonded NH, indicating a conformer with no intramolecular hydrogen bonding. Again, triamide **5** contains a β-alanine unit and it resists the formation of intramolecular amide−amide hydrogen bond even in nonpolar solvents, such as chloroform.



5 Preferred conformation (**A**)

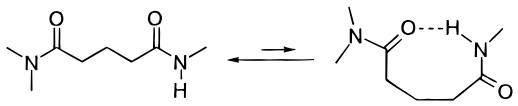
Thus, for triamides **1**−**3** (Figure 1, $n = 1$, $m = 1-3$) and **4**−**5** ($n = 2$, $m = 1-2$), it was relatively simple to identify the most stable conformation by examining the VT-IR and VT-NMR data and comparing them to the available literature results. However, it becomes more complicated to identify the most populous conformer for triamides **6**−**9** where the connecting chains become longer ($n = 2$, $m = 3$ and $n = 3$, $m = 1-3$). While for triamides **1**−**5**, only the terminal NH proton has a significant chemical shift dependence on temperature, triamides **6**−**9** show a significant temperature effect for both the terminal and the internal NH protons. This would exclude the head-to-tail type of folding pattern and point to the bicyclic conformation **C** and the bifurcated conformation **G**. These two conformations would exhibit a downfield chemical shift and a large temperature dependence of chemical shift for both the terminal and the internal NH protons.

Triamide 6. By comparison to the IR spectra of triamide **3**, which shows two distinct free NH stretchings, it is possible to assign each of the two free NH absorptions to a specific amide NH stretching for triamide **6**. Thus, the higher frequency (~3460 cm^{-1}) absorption is due to the terminal NH and the lower frequency (~3446 cm^{-1}) is due to the internal NH. The broad band at ~3320 cm^{-1} is due to intramolecular amide−amide hydrogen-bonded NH stretching. On the basis of the IR data, there are both H-bonded and nonbonded NH protons at room temperature, but the internal NH appears to have a smaller population of free NH than the terminal NH at 298 K. At 213 K, the population of the hydrogen-bonded NH increases and that of the nonbonded NH decreases, especially for the terminal NH.

The VT-NMR data in Table 4 show that both the terminal NH ($\Delta\delta NH/\Delta T = -11.9$ ppb/K) and the internal NH ($\Delta\delta NH/\Delta T = -6.9$ ppb/K) of triamide **6** are involved in hydrogen bonding. The terminal NH can form an intramolecular amide−amide H-bond through a nine-membered ring to the center carbonyl group. The significant reduced temperature coefficient observed for the internal NH should exclude the head-to-tail folding pattern **B** and should be due to the changes in the population of hydrogen-bonded internal NH. Therefore only two conformations are consistent with the VT-NMR data for triamide **6**: the bicyclic form **C** and the bifurcated form **G**.

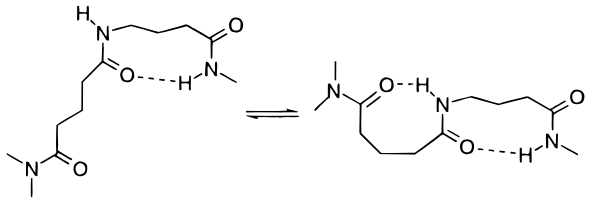
To serve as a control experiment, the VT-NMR data in chloroform for diamide **13** are collected (see Table 4). Diamide **13** is the same as the left portion of triamide **6** and gives an NH temperature dependence of chemical shift of −5.3 ppb/K. Conformation **13b** is a model for

form **E** ($n = 2$) of triamide **6** and it is also part of the bicyclic form **C** of triamide **6**. On the basis of the VT-NMR data, the eight-membered ring **13b** only occurs to a small extent.



13a **13b**

The internal NH of triamide **6** has a temperature coefficient of −6.9 ppb/K. This increase in temperature dependence comparing to **13**, which has a temperature coefficient of −5.3 ppb/K, can be explained by the improved H-bond donor ability of the center NH group. Thus, as the temperature decreases, the favorable nine-membered ring conformation **F** becomes more heavily populated, which leads to a more polarized internal amide function and a better H-bond donor, which in turn leads to a more populated bicyclic conformer **C**.



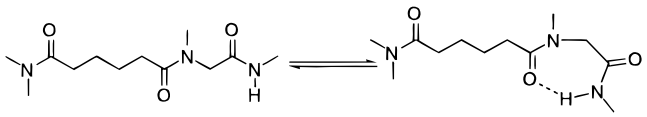
6 **F** Preferred conformation **C**

The bifurcated form **G**, which contains an eight- and a 13-membered ring, is also consistent with the VT-NMR data if the temperature coefficient from the intramolecular H-bond through a 13-membered ring is similar to that through a nine-membered ring. Entropic effects should limit the populations of the head-to-tail form **B** and the bifurcated form **G**. Molecular modeling using MacroModel with Amber* forcefield gives a strain energy difference of 0.5 kcal/mol between the bifurcated form and the bicyclic form.[11] The results from the modeling show that both conformation **C** and conformation **G** contain little torsional strain on the connecting chain and both can accommodate near linear N−H- - -O bond angles. However, at the present time the force field does not take into account of hydrogen-bonding cooperativity. Therefore we have to look into other experimental evidences.

The IR carbonyl stretching supports the bicyclic conformation **C**. The tertiary amide carbonyl group of triamide **6** shows an absorption at 1635 cm^{-1}, which indicates the absence of a strongly H-bonded tertiary C═O group. It is more consistent with a tertiary amide carbonyl group that is hydrogen bonded through an eight-membered ring (poor H-bond geometry) than through a 13-membered ring (better H-bond geometry). Thus, the bifurcated conformation **G** is inconsistent with the experimental facts: the VT-NMR data show a strongly bonded terminal NH proton at 213 K and the low-temperature IR shows a weakly bonded tertiary amide C═O group. On the other hand, these facts are more consistent with the bicyclic conformation **C**. The bicyclic conformation **C** is also consistent with the rule of forming the nearest neighbor intramolecular amide−amide H-bond.[6,10] Thus, we conclude that triamide **6** assumes a bicyclic conformation at lower temperatures and that a cooperative effect is observed for the intramolecular amide−amide hydrogen bond, which leads to a more populated eight-membered ring.

Triamide 7. An increase in intensity in the 3300 cm^{-1} region of the IR spectra for triamide **7** is evident when temperature is lowered. Unlike most other triamides in this series, the internal NH proton of triamide **7** shows a greater temperature dependence of chemical shift ($\Delta\delta NH/\Delta T = -10.7$ ppb/K) than that of the terminal NH proton ($\Delta\delta NH/\Delta T = -5.9$ ppb/K). This result is not surprising since the internal NH can form an intramolecular hydrogen bond through a nine-membered ring in **7** while the internal NH in triamides **1**−**6** cannot.

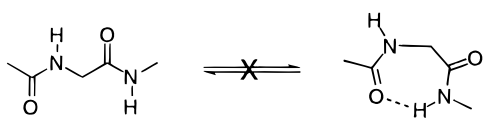
The terminal NH of triamide **7** shows a small but significant temperature dependence of chemical shift ($\Delta\delta NH/\Delta T = -5.9$ ppb/K). This dependence of both NH chemical shifts on temperature is again only consistent with either the bicyclic conformation **C** or the bifurcated form **G** as the most enthalpically stable conformer. Since the bifurcated form **G** contains a nine- and a 12-membered ring, we have prepared triamide **14** to evaluate the relative stability of conformation **B** for triamide **7**. The internal NH proton of triamide **7** is replaced with



14

a methyl group so that triamide **14** has the opportunity to form an intramolecular hydrogen bond through a 12-membered ring without the interference of an internal NH group. The temperature dependence of the NH chemical shift is more consistent with a C_7 form (see Table 4) for triamide **14**. If an intramolecular amide−amide H-bond was formed through a 12-membered ring, a much greater temperature dependence of chemical shift should have been observed due to a larger entropic effect. Therefore triamide **14** exists in a C_7 conformation in chloroform rather than forming an intramolecular hydrogen bond through a 12-membered ring.

Although the C_7 form was not observed in previous studies for diamide **15**,[12] the formation of the C_7 form is in accord with other previous results,[13,14] which show that if a tertiary amide carbonyl group is the H-bond acceptor, the formation of the C_7 conformation becomes more favorable. Thus, a subtle change in the capacity of the



15

H-bond acceptor makes a difference in the conformational preference of polyamides. Although the center amide function in triamide **7** is secondary, rather than tertiary, the formation of the nine-membered ring of the adipic

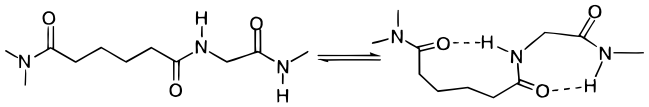
(11) McDonald, D. Q.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 11550−11553.

(12) Maxfield, F. R.; Leach, S. J.; Stimson, E. R.; Powers, S. P.; Scheraga, H. A. *Biopolymers* **1979**, *18*, 2507.

(13) Mizushima, S.; Shimanouchi, T.; Tsuboi, M.; Arakawa, T. *J. Am. Chem. Soc.* **1957**, *79*, 5357.

(14) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *114*, 3138.

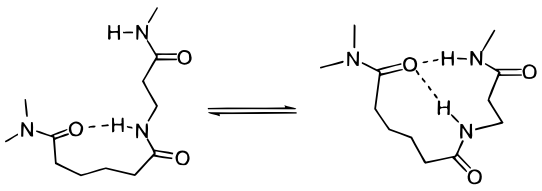
acid moiety appears to improve the H-bond acceptor ability of the center amide. It is a situation that is similar to triamide **6**. Thus, on the basis of the VT-IR and VT-NMR data and on the results of triamide **14**, bicyclic conformation **C** is more likely to be the most enthalpically favored conformer for triamide **7**. The observed temperature dependence of the terminal NH is evidence for the polarization of the center amide function.



7 Preferred conformation (**C**)

Triamide 8. At room temperature there is a small amount of hydrogen-bonded NH absorption on the IR spectrum of triamide **8**, which becomes more intense at 213 K. An interesting point about triamide **8** comes from the VT-NMR data, which show that at higher temperatures the terminal NH is mostly free. However, it moves rapidly downfield at lower temperatures and has a larger temperature dependence of chemical shift ($\Delta\delta$NH/ΔT = −9.2 ppb/K) than the internal NH ($\Delta\delta$NH/ΔT = −6.2 ppb/K).

This is interesting because on the basis of previous studies the internal NH should be forming an intramolecular amide−amide H-bond through a nine-membered ring to the tertiary amide C═O group and should have a temperature dependence of chemical shift similar to that of triamide **7** ($\Delta\delta$NH/ΔT = −10.7 ppb/K). The small magnitude of temperature coefficient of the internal NH is most likely due to the presence of a mutually exclusive competing conformation. The VT-NMR data of triamide **8** is best explained by assuming an equilibrium between conformations **E** and **G**. At higher temperatures, the nine-membered ring **E** is more favorable, while at lower temperatures, the bifurcated conformation **G** becomes more stable. The stretching frequency of the tertiary amide carbonyl is 1625 cm^{-1}, which is consistent with a strong association with H-bond donors.

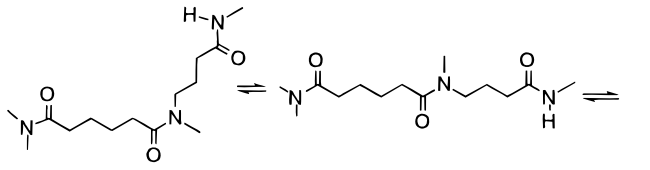


8 **E** **G**

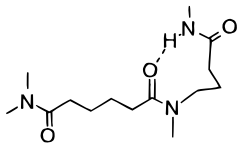
It is noteworthy that triamide **8** contains a β-alanine unit and it behaves differently from other triamides in this series. The fact that a bicyclic conformation **C** is absent is consistent with the rule of unfavorable formation of nearest neighbor by a β-alanine structure unit.[6b,15] This is probably the first observation of a triamide forming a bifurcated structure in organic solvent.

Triamide 9. The VT-IR and VT-NMR data for triamide **9** have been reported in a recent communication.[7c] Different from any of the other triamides, **9** shows a near absence of the free NH absorption in the IR spectrum at 213 K. Triamide **9** also shows the greatest temperature dependence of chemical shifts for both the terminal and the internal NH protons. Variable concentration IR shows a constant ratio of free vs H-bonded NH absorptions in the range of 1−10 mmol concentrations, which indicates that the amide−amide hydrogen bonds are intramolecular. The IR spectrum shows that at 213 K the tertiary amide carbonyl group of triamide **9** has a stretching at 1616 cm^{-1}. This is the lowest frequency of this series, indicating a strong hydrogen-bonded C═O group.

A few details from our study that were not reported in the communication should provide more evidence on the conformational preference of triamide **9** in chloroform. Like the analysis of triamide **7**, we prepared triamide **16** in order to explore the possibility of a bifurcated form.



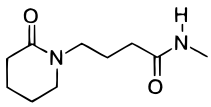
16 ***s-cis*** **16** ***s-trans***



16

Triamide **16** is identical to **9** except that the internal amide proton is replaced with a methyl group to eliminate conformations **C**, **D**, and **E**. Along with the normal s-trans (around the center C−N bond) isomer, a minor s-cis isomer was also detected for triamide **16**. The fact that s-cis isomer becomes stable enough to be observed for tertiary amides has been widely reported for proline derivatives in the literature.[16] The temperature dependence of chemical shifts of the NH protons of both the s-trans and the s-cis isomers can be found in Table 4.

The amide proton of the s-trans isomer can interact with either the internal carbonyl to form a nine-membered ring or the *tertiary* terminal amide carbonyl to form a 14-membered ring. The temperature dependence of the chemical shift of the major isomer is similar to that of diamide **17** (−11.3 ppb/K in CH_2Cl_2).[6]



17

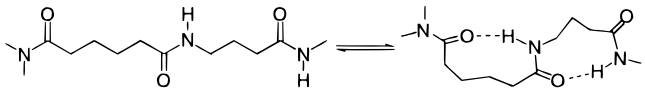
It is noteworthy that both triamide **16** and diamide **17** have a tertiary amide carbonyl to serve as the hydrogen-bond acceptor, which is a stronger acceptor than a secondary amide carbonyl group. The similarity between **16** and **17** suggests that a nine-membered ring, rather than a 14-membered ring, is assumed by triamide **16**. The NH in the s-cis isomer of **16** suffers from torsional strain when forming an intramolecular hydrogen bond through a nine-membered ring. However, it does not appear to suffer from any strain when forming a 14-membered ring. In fact, the first 11 most stable conformers of triamide **16** are intramolecularly hydrogen bonded 14-membered rings according MacroModel with Amber*

(15) Gung, B. W.; Zhu, Z. *J. Org. Chem.* **1997**, *62*, 6100.

(16) For an example, see: Liang, G. B.; Rito, C. J.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 4440−4442.

force field. On the basis of its NH chemical shifts, the s-cis isomer assumes mostly non-hydrogen-bonded conformations. Therefore, the results of both the s-trans and the s-cis isomers of **16** indicate that a 14-membered intramolecular hydrogen bond suffers from considerable entropic disadvantage.

Other evidence against the 14-membered ring comes from comparison to the results of the other triamides in this series, such as **3**, **6**, and **7**. All of these triamides can form either a 12- or a 13-membered ring through an intramolecular hydrogen bond. However, the most favorable conformation for all of them contains an intramolecular amide−amide hydrogen bond through a nine-membered ring. Therefore one can conclude that a triamide containing a γ-aminobutyric acid unit prefers to form an intramolecular hydrogen bond through a nine-membered ring, rather than through a greater loop. This once again follows the rule of the formation of the nearest neighbor hydrogen bond for a peptide containing a γ-aminobutyric acid unit.[6] Such is the case for triamides **3**, **6**, and **9**. All the above evidence suggests that conformation **C**, the bicyclic form, is the most enthalpically favored structure for triamide **9**.



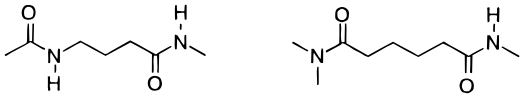
9 Preferred conformation (**C**)

Origins of Conformational Preferences. The preference for the head-to-tail type of folding is preferred by the smaller triamides. The highest preference for this conformation is exhibited by triamide **1** followed by triamide **4**. The preference for the head-to-tail folding pattern can be explained by the energetic superiority of an optimal hydrogen bond geometry in which the NH- - -O bond angle is near linearity. The alternative folding patterns would involve 7/7 and 8/7 bicyclic structures. These results are consistent with previous studies of diamides with varying connecting chain length.[6]

However, either when a γ-aminobutyric acid or when an adipic acid is part of the structure, nearest H-bond formation occurs to form a nine-membered ring. Triamides **6**, **7**, and **9** prefer bicyclic structures involving nearest neighbor hydrogen bonding. A nine-membered ring is large enough to accommodate a nearly linear N−H- - -O bond angle. Entropic effects are probably responsible for the preference of the nine-membered ring over a 12- or a 14-membered ring.

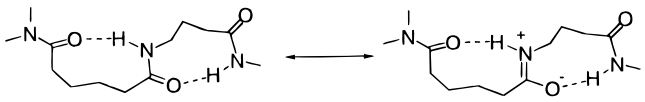
The β-alanine-containing triamides **2**, **5**, and **8** are resistant to intramolecular hydrogen bonding, especially to nearest neighbor hydrogen bonding. At lower temperatures, triamides **2** and **5** exhibit a small population of head-to-tail type of folding, while triamide **8** shows a significant population of bifurcated conformation. The connecting chain of β-alanine has two sp^3 carbons. The conformation of the Csp^3-Csp^3 bond includes one anti and two gauche rotamers. The anti rotamer is favored when there is no substituent on these two carbons. The anti rotamer does not permit an intramolecular amide−amide hydrogen bond. The gauche rotamers allow intramolecular hydrogen bonding but the resulting NH- -O bond angle is far from linearity if nearest neighbor hydrogen bonding occurs. However, if the α or β carbons of the β-alanine unit have substituent groups, the gauche rotamer can become more favorable and intramolecular hydrogen bonds may form between distal hydrogen bond partners. Such cases are documented in the literature as β-peptides readily form helical conformation.[19]

Therefore, although triamides **1**−**5** and **8** could form intramolecular hydrogen bonds through a bicyclic conformation, the enthalpical preferred form for these amides is the head-to-tail folding. In other words, hydrogen-bonding cooperativity does not occur in these triamides. For triamides **6** and **7**, an enhancement of hydrogen bonding for the respective smaller rings is observed. Namely, the internal NH of **6** has a temperature dependence of −6.9 ppb/K (comparing to −5.3 ppb/K for diamide **13**) and the internal NH of **7** has a temperature dependence of −5.9 ppb/K. This is a considerable increase of intramolecular hydrogen bonding since normally a C_7 conformation does not exist (comparing to −3.3 ppb/K for *N*-methylacetamide). The hydrogen bonds in the respective nine-membered rings of triamides **6** and **7** are not enhanced compared to the temperature dependence of corresponding diamides (**18** has a C-terminal NH temperature dependence of chemical shift of −9.8 ppb/K in CH_2Cl_2 and **19** has a value of −11.9 ppb/K in chloroform, see Table 4).



18 **19**

However, the enhancement of hydrogen bonding in triamide **9** is enormous and both NHs have a very large temperature dependence of chemical shifts (−15 and −13.3 ppb/K for the terminal and the internal NH protons, respectively, see Table 4). These enormous temperature constants reflect a significant increase in hydrogen-bonding energy. We attribute this increase in hydrogen bonding energy to hydrogen-bonding cooperativity. The bicyclic conformation of **9** contains two nine-membered rings. Each of these two nine-membered rings is capable of forming a strong intramolecular hydrogen bond with a nearly linear NH- -O bond angle. The resulting two intramolecular amide−amide hydrogen bonds are mutually enhanced, or are synergistic in terms of enthalpy. These observations can be rationalized using the resonance theory of amide structure. When a strong amide−amide hydrogen bond is formed in the case of optimal NH- -O bond geometry, such as in the nine-membered rings, the contribution from the ionized structure is increased. The center amide function becomes both a better H-bond donor and a better H-bond acceptor



(17) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 4228−4245.

(18) Winningham, M. J.; Sogah, D. Y. *J. Am. Chem. Soc.* **1994**, *116*, 11173−11174.

(19) (a) Seebach, D.; Overhand, M.; Kuhnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913−943. (b) See also: Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. *J. Am. Chem. Soc.* **1996**, *118*, 13071−72.

to its respective partners. However, when the NH- -O bond geometry is not close to linearity, such as in thecase of smaller rings, the contribution from the ionized form is small and the influence on its neighboring H-bonds is small. Therefore, a nearly linear NH- -O bond angle is the requirement for hydrogen-bonding cooperative effects to occur.

Quantification of the VT-NMR Data. Although qualitatively we have shown that hydrogen-bonding cooperativity occurs in triamide **9**, it is desirable to have this effect evaluated quantitatively. The quantitative results are useful for re-parametrization of force fields for computations. Currently, hydrogen-bonding energy is treated as additive by most force fields, which is one of the reasons why molecular modeling at the present time cannot predict the preferred conformations of peptide mimetics.

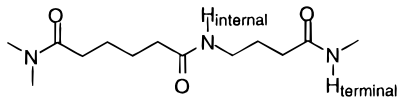
As previously shown by Gellman,[6] in certain cases a van't Hoff analysis of the ^{1}H NMR variable temperature data can produce the thermodynamic parameters for the equilibrium between the states in which the NH protons are free and the intramolecular hydrogen bonded state. In contrast to variable temperature experiments conducted in water, a large temperature dependence of NH proton chemical shift does not mean that the NH is solvent exposed. It usually indicates that an initially shielded NH proton is being transferred to a less shielded state or vice versa.[10a] In other words, a large temperature coefficient indicates the change of population in hydrogen-bonded conformations. This equilibrium in conformational populations is related to the strength of the hydrogen bond. The equilibrium constant K_{eq} is related to the observed chemical shifts by the following equations:

$$K_{eq} = (\delta_{obs} - \delta_n)/(\delta_b - \delta_{obs}) \quad (1)$$

$$\ln K_{eq} = (-\Delta H°/R)(1/T) + \Delta S°/R \quad (2)$$

where δ_{obs} is 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.

Caution and limitation of obtaining thermodynamic parameters from variable temperature NMR data have been discussed in the literature.[17,18] The most important step in this analysis is to find *temperature-dependent* upper (δ_b) and lower (δ_n) limits of chemical shifts. The chemical shifts of *N*-methylacetamide (NMA) in CD_2Cl_2 at 1 mM concentration have been used previously as the limiting value for non-hydrogen-bonded states for a triamide.[6] We have also chosen to use NMA as our standard except that we run the experiments in $CDCl_3$.



9

For the limiting chemical shifts of the intramolecularly hydrogen bonded state, compound **20** was used as the standard for both the terminal and the internal NH protons in our recent communication since it shows no free NH stretching and has a structure similar to that of triamide **9**.[7] We are satisfied to use NMA and compound **20** to serve as the lower and upper limiting chemical

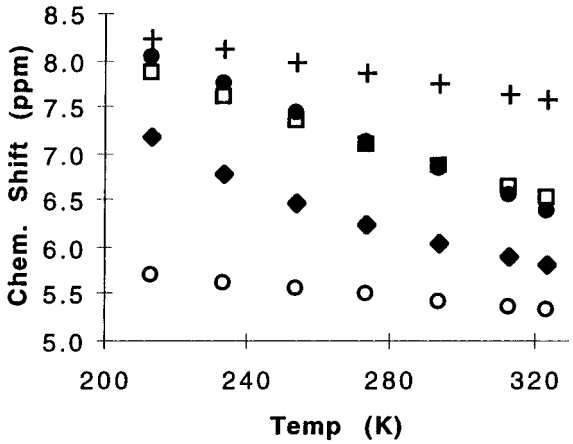
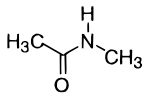
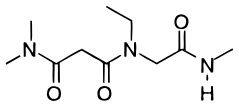


Figure 3. Amide proton NMR chemical shifts as a function of temperature for the terminal NH proton of triamide **9** (●), the NH proton of triamide **16** (○), the NH proton for triamide **20** (+), the NH proton of diamide **19** (♦), and the NH proton of *N*-methylacetamide (○).

shifts for the terminal NH, respectively. However, we



NMA



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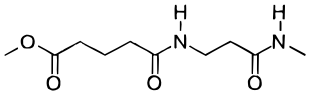
are not satisfied with these standards for the internal NH. To improve the quality of the quantification, we now choose to use the internal NH of diamide ester **21** to serve as the nonbonded state since its VT-IR spectrum shows no hydrogen bonding at room temperature and its chemical environment is more similar to the internal NH of triamide **9** than that of NMA.

It is difficult to find a suitable model compound for the intramolecular bonded state of the internal NH proton of triamide **9**. We decided to use diamide ester **22** to serve as the upper limit for the chemical shift of the completely hydrogen-bonded NH. The internal NH of **22** forms a six-membered ring to the tertiary amide carbonyl group of the malonic unit at all temperatures studied. Thus for the internal NH of triamide **9** we use the internal NH of **21** as the non-hydrogen-bonded standard and the NH of **22** as the hydrogen-bonded standard.



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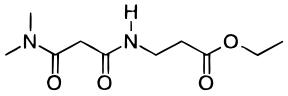

22

The upper (+) and lower (○) limits of chemical shifts along with that of triamide **16**, diamide **19**, and the terminal NH of triamide **9** are shown in Figure 3. The internal NH proton chemical shifts of triamide **9** and the chosen standards are shown in Figure 4. An example of a typical van't Hoff plot is shown in Figure 5, and the resulting thermodynamic parameters are displayed in Table 5. The correlation coefficients in the van't Hoff plots are better than 0.99. The errors are estimated considering the uncertainties in the choice of upper and lower limiting chemical shifts. These temperature-dependent upper and lower limits of chemical shifts are the best available values and are not perfect for every compound.

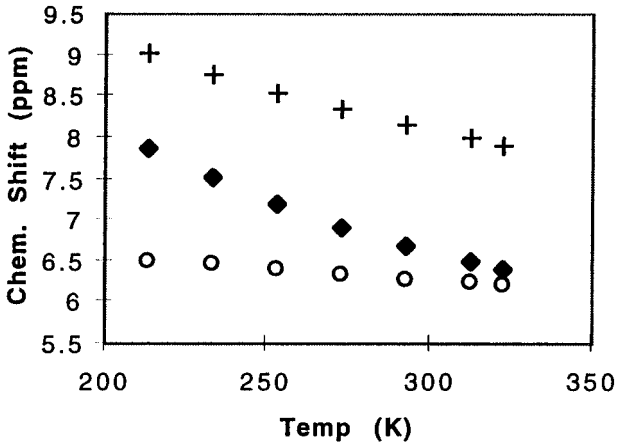

Figure 4. Amide proton NMR chemical shifts of as a function of temperature for the internal NH proton of triamide **9** (♦), the NH proton of diamide ester **19** (+), and the internal NH proton of diamide ester **18** (○).

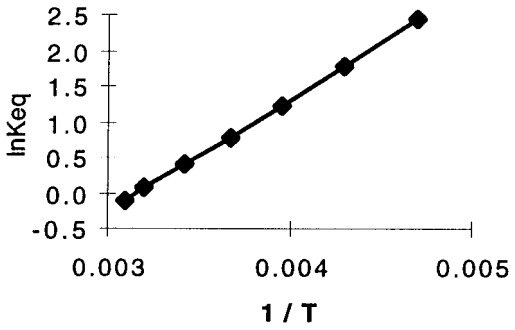

Figure 5. van't Hoff plot from NH proton NMR data obtained in 1 mM samples in $CDCl_3$, 213−323 K (data shown in Figure 3). K_{eq} determined according to eq 1 in the text (correlation coefficient = 0.999).

Table 5. Amide Proton NMR Chemical Shift Temperature Dependence and Thermodynamic Parameters

triamide	$\Delta\delta/\Delta T$ (NH_i) (ppb/K)	ΔH (kcal/mol)	ΔS (eu)	$\Delta\delta/\Delta T$ (NH_i) (ppb/K)	ΔH (kcal/mol)	ΔS (eu)
9	−15	−3.1 ± 0.5	−9.9 ± 2	−13.3	−2.8 ± 0.5	−12.2 ± 2
16	−11.9	−2.0 ± 0.5	−5.7 ± 2			
19	−11.9	−2.0 ± 0.5	−8.7 ± 2			

The NH proton of triamide **16** has the same temperature dependence of chemical shift as that of diamide **19**. However, they have different absolute chemical shifts at all temperatures. Triamide **16** has more downfield chemical shifts than that of diamide **19**. This is a difference in entropic factors as it becomes clear after obtaining the thermodynamic parameters through the process of van't Hoff analysis. The formation of an intramolecular hydrogen bond gives rise to a more negative entropy term for diamide **19** than for triamide **16**. The calculated results are reasonable when one examines the structures of these two compounds. The formation of a hydrogen bond through a nine-membered ring in diamide **19** would restrict the rotation of five single bonds, while the same process in triamide **16** only constrains the free rotation of three single bonds. The N−C bond next to the center amide group in **16** is partially constrained even in the extended form due to the presence of the *N*-methyl group. Thus, the constant downfield chemical shift exhibited by a di- or triamide in nonpolar solvents is an indication of the formation of an intramolecular hydrogen bond that is not strongly opposed by entropic factors.

Both the terminal and the internal NH protons of triamide **9** have a greater temperature dependency of chemical shifts than the model compounds **16** and **19**. Using the respective lower and upper limits of chemical shifts, the terminal NH gives a ΔH of −3.1 kcal/mol and the internal NH gives a ΔH of −2.8 kcal/mol. However, the internal NH gives a more negative entropy term. This again is reasonable if one counts the number of single bonds becoming constrained in the intramolecular hydrogen bonded form **C**. The formation of a hydrogen bond through a nine-membered ring by the terminal NH will restrict the free rotation of four single bonds while that of the internal NH will restrict the free rotation of five single bonds.

To compensate for uncertainty in choosing the upper limit of the temperature dependency of chemical shifts, we have estimated the error conservatively in the resulting thermodynamic parameters. However, the experimental data show that the difference between triamide **9** and the model compounds **16** and **19** is real. We believe that any systematic errors should cancel. Therefore, the about 1 kcal/mol increase in amide−amide hydrogen bond energy observed for triamide **9** in chloroform when compared to **16** and **19** is due to the cooperative effect occurring during the formation of two consecutive hydrogen bonds in triamide **9**.

Conclusions

In conclusion, the enhanced intramolecular hydrogen bonds in **9** are supported by a variety of evidence. The IR spectra of **9** show more population of H-bonded NH than that of triamides **1**−**8** at both room temperature and 213 K. The chemical shifts of the amide NH protons of **9** are more downfield shifted than that of triamides **1**−**8** and diamide **16** at all temperatures. The temperature dependence of chemical shifts of **9** is greater than that of triamides **1**−**8** and diamide **16** and triamide **13**. Finally, a van't Hoff analysis of the variable temperature ^{1}H NMR data gives greater hydrogen bond energies for both hydrogen bonds in **9**. We believe that the increased hydrogen bond strength is evidence for hydrogen-bonding cooperativity from the two mutually enhanced individual hydrogen bonds. This enhancement of hydrogen bonding is a result of an improved H-bond donor and acceptor property of the center amide function. The contribution from the ionized form of the amide function is the origin of the improved H-bond donor and acceptor property. A near linear NH- -O bond angle is required for this effect.

Hydrogen-bonding cooperative effects are only observable when at least one of the participating H-bonds assumes a near linear N−H- -O geometry. The backbone intramolecular amide−amide hydrogen bonds in an α-helical peptide is from the CO group of residue *i* to the NH group of residue *i* + 3 in a 13-membered ring, which allows an optimal N−H- -O bond angle. The strength of the individual hydrogen bond should increase as the helical chain length increases. Each peptide bond becomes increasingly more polarized as the sequential hydrogen bonding network increases its length, which leads to the macrodipole typical of α-helices. Currently, hydrogen-bonding cooperativity has not been recognized in the analysis of helix stability. As suggested by

Karplus,[4] force field parameters should include a correction term to take into account this cooperative effect.

In summary, it is interesting to note that each of these nine triamides displays its own unique intramolecular hydrogen-bonding pattern and that not all of the model peptides prefer the intramolecular hydrogen-bonded conformation. Conformational preference of these triamides cannot be easily predicted on the basis of current knowledge. Therefore more systematic studies in this area should be carried out to uncover the fundamental factors that control molecular conformations of polar flexible organic compounds. Appropriate model systems that allow the elucidation of hydrogen-bonding cooperativity in water should be designed to study the difference of conformational preference in organic and in aqueous solutions. Work along this line is in progress in our laboratories.

Acknowledgment. This research is supported in part by a grant from the National Institutes of Health (GM49745) and by the Committee on Faculty Research of Miami University. We thank the National Science Foundation for a grant (CHE-9012532) for the purchase of the 300 MHz Bruker FT-NMR.

JO971831Z