Elucidation of Hydrogen-Bonding Cooperativity at the Molecular Level

Benjamin W. Gung,* Zhaohai Zhu, and Brian Everingham
Department of Chemistry, Miami University, Oxford, Ohio 45056

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Recently, we have demonstrated that small changes in polyamide structure can lead to large changes in their conformation (Scheme 1).[1,2] Specifically, triamides **1** and **2** prefer the head-to-tail type of intramolecular hydrogen bonding to form a 10- and a 11-membered ring, respectively, rather than forming a 7- or an 8-membered ring. Triamide **3** prefers to form a 9-membered ring through an intramolecular hydrogen bond between the terminal NH and the internal carbonyl group rather than forming a 12-membered ring through the head-to-tail type of intramolecular hydrogen bond. Triamide **4** prefers a straight-chain conformation with no intramolecular hydrogen bonding in chloroform despite the fact that it is possible to form an 8- or a 12-membered ring. None of the previous examples displays a preference for a bicyclic conformation, another distinct possibility, in which the central amide function is hydrogen bonded to two other amide groups. Our results show the sensitivity of the polyamide conformation to the connecting chain length. We now report that triamide **5** assumes mainly the bicyclic conformation in contrast to triamides **1**−**4**. Furthermore, the hydrogen bonds have been determined to be about 1 kcal/mol stronger than other intramolecular hydrogen bonds of any diamide of similar structure.

The infrared spectra at 213 and 298 K of triamide **5** are shown in Figure 1. These IR spectra are recorded in $CDCl_3$ at 1 mmol concentration. The absorptions at ∼3450 cm^{-1} are assigned to free amide NH stretching, and the broad band at ∼3250 cm^{-1} is due to intramolecular amide−amide hydrogen-bonded NH stretching. On the basis of the IR data, there are both bonded and nonbonded NH protons at room temperature. At 213 K, the population of the hydrogen-bonded NH increases and that of the nonbonded NH decreases. When compared to the IR spectra of triamides **1**−**4**,[1,2] triamide **5** shows the largest population of intramolecular hydrogen-bonded forms.

Proton NMR spectroscopy gives information on specific NH protons. The amide NH protons of triamide **5** are distinguished from each other by a 2D COSY experiment and by their coupling patterns at lower temperatures. The terminal amide NH appears as a quartet, and the internal amide NH appears as a triplet. The chemical shift changes of the amide NH protons with temperature produce useful reduced temperature constants ($-\Delta\delta/\Delta T$) for each NH proton.[3] Displayed in Figure 2 is a graph of the amide NH protons of triamide **5** as a function of temperature. The downfield chemical shift indicates more proportions of intramolecular hydrogen bonded states in chloroform. When compared to triamides **1**−**4**, triamide **5** shows a significantly steeper slope for both the internal and the terminal NH protons. This means that both NH protons experience a greater change in the population of hydrogen-bonded forms.

A total of seven reasonable conformations are expected by taking into account the two NH groups and three C═O groups in triamide **5**. These are derived from a combination of each NH forming an H-bond to either of the two C═O groups, which would give 7-, 9-, and 14-membered rings (**B**−**E**), plus the stretched form (**A**), a possible bifurcated form (**F**), and a bicyclic form (**G**). These expected conformations are displayed in Chart 1.

From the data in Figures 1 and 2, none of the conformers **A**−**E** can be considered the most populated form because all of them require either one or two free NH protons (or solvent solvated NH protons). To serve as a control compound, we prepared diamide **6** (the left

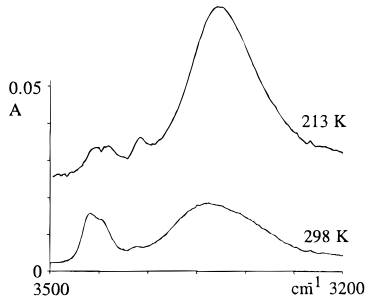


Figure 1. NH stretch region of the IR spectra at 213 and 298 K for triamide **5**. The band at 3452−3460 cm^{-1} is assigned to the free terminal NH stretch, the band at 3441−3449 cm^{-1} is assigned to the free internal NH stretch, and the broad band at 3330−3338 cm^{-1} is assigned to the intramolecularly hydrogen-bonded NH.

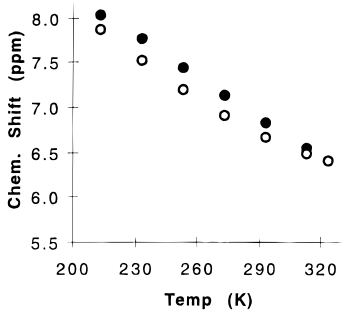
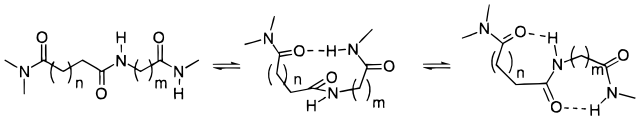


Figure 2. Amide proton NMR chemical shifts as a function of temperature for triamide **5**: internal NH (○, $\Delta\delta NH/\Delta T = -13.3$ ppb/K); terminal NH (●, $\Delta\delta NH/\Delta T = -15.0$ ppb/K).

Scheme 1

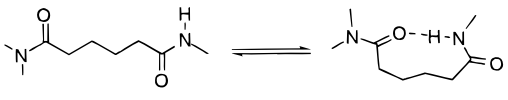


1: n = 1, m = 1 **3**: n = 2, m = 2 **5**: n = 3, m = 3
2: n = 2, m = 1 **4**: n = 1, m = 3

Table 1. Amide Carbonyl Stretching Frequencies (cm^{-1}) in $CDCl_3$ (1 mM) for Triamides 1−5

triamide	$\nu_{internal}$, $\nu_{terminal}$ (298 K)	$\nu_{internal}$, $\nu_{terminal}$ (213 K)
1	1668, 1628	1660, 1624
2	1662, 1639	1653, 1635
3	1662, 1637	1650, 1631
4	1665, 1632	1657, 1629
5	1658, 1630	1650, 1616

From the data in Figures 1 and 2, none of the conformers **A**−**E** can be considered the most populated form because all of them require either one or two free NH protons (or solvent solvated NH protons). To serve as a control compound, we prepared diamide **6** (the left



6a **6b**

(1) Gung, B. W.; Zhu, Z. *J. Org. Chem.* **1996**, *61*, 6482.
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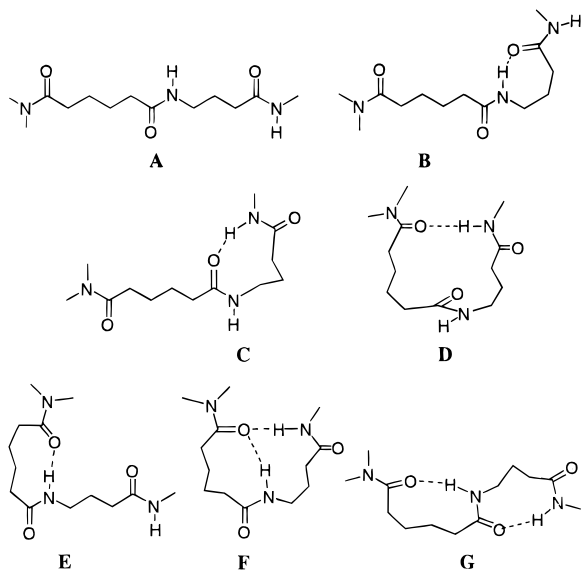
Table 2. Amide Proton NMR Chemical Shift Temperature Dependence and Thermodynamic Parameters

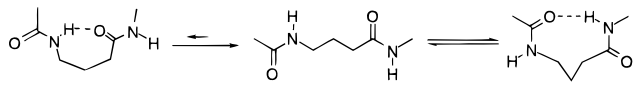
triamide	$NH_{terminal}$ (ppb/K)	ΔH (kcal/mol)	ΔS (eu)	$NH_{internal}$ (ppb/K)	ΔH (kcal/mol)	ΔS (eu)
5	−15	−3.1 ± 0.5	−9.9 ± 2	−13.3	−2.3 ± 0.5	−7.5 ± 2
6	−11.9	−2.0 ± 0.5	−8.7 ± 2			
8	−11.9	−2.1 ± 0.5	−6.1 ± 2			

Chart 1



half of triamide **5**) and determined its amide NH reduced temperature constant in chloroform. Similar to Gellman's results in methylene chloride,[4a] diamide **6** displays a significant temperature dependence of the amide NH chemical shift. The reduced temperature constant is −11.9 ppb/K in chloroform, slightly larger than the value obtained in methylene chloride (−10 ppb/K). Although these are considered large temperature effects, they are still smaller than that of the amide NH protons of triamide **5**.

Gellman and co-workers also reported the variable-temperature NMR data for diamide **7**,[4b] which resembles the right half of triamide **5**. Diamide **7** can form either



a seven- or a nine-membered ring. Gellman has shown that the C-terminal amide proton of **7** shows a larger temperature dependence (−9.8 ppb/K) than the N-terminal proton of **7** (−2.5 ppb/K), which indicates that formation of the nine-membered ring (**7c** or **C** in Chart 1) is enthalpically more favorable than formation of the seven-membered ring (**7a** or **B** in Chart 1). Such is the case with triamide **5** except that triamide **5** displays a much greater reduced temperature constant (−15 ppb/K) than diamide **7** for the corresponding amide NH proton.

After comparison of the IR and NMR data of **5** with that of **6** and **7**, conformers **A**−**E** can be ruled out as the most enthalpically favored form. To distinguish between the bifurcated form **F** and the bicyclic form **G**, we then compared the IR frequencies of the center amide carbonyls for triamides **1**−**5**. The stretch frequency of the center amide carbonyl of triamide **5** is 1658 cm^{-1} at rt, which is the lowest among the five triamides (Table 1). The stretching frequency moves to 1650 cm^{-1} at 213 K, which ties the lowest one with that of triamide **3**. This information suggests that the internal carbonyl group is hydrogen bonded, rather than being free in conformation **F**.

Once we have identified the most enthalpically favored conformer, a van't Hoff analysis of the ^1H NMR variable-temperature data should produce the thermodynamic parameters for the equilibrium between the states in which the NH protons are free and conformation **G** for triamide **5**. Interpretation and limitation of obtaining thermodynamic parameters from variable-temperature NMR data have been discussed in the literature.[1−6] An important step in this analysis is to find *temperature-dependent* upper and lower limits of chemical shifts.[7] The chemical shifts of a 1:1 mixture of *N*-methylacetamide and *N*,*N*-dimethylacetamide in $CDCl_3$ at 1 mM concentration are deemed proper to serve as the limiting value for non-hydrogen-bonded states.[4] For the limiting chemical shifts of intramolecularly hydrogen-bonded states, compound **8** from ref 8 is used to produce a ΔH of −3.1 ± 0.5 kcal/mol and a ΔS of −9.9 ± 2 eu for the terminal NH and a ΔH of −2.3 ± 0.5 kcal/mol and a ΔS of −7.5 ± 2 eu for the internal NH (Table 2). Compound **8** from ref 8 has been shown to be completely intramolecularly hydrogen bonded at all temperatures, and its structure is similar to triamide **1**.[8] Analysis of the VT-NMR data of diamide **6** using the same *temperature-dependent* upper and lower limits of chemical shifts gives a 2 ± 0.5 kcal/mol ΔH and a ΔS of −8.7 ± 2 eu for the equilibrium in chloroform. The correlation coefficients in the van't Hoff plots are better than 0.97. The errors are estimated considering the uncertainties in the choice of upper and lower limiting chemical shifts. It is noteworthy that none of the other triamides (**1**−**4**) forms a bicyclic conformation, which leads us to conclude that hydrogen-bonding cooperative effects only operate when an individual H-bond is allowed to assume a favorable N−H−O geometry. In an α-helical peptide, each backbone hydrogen bond is in a 13-membered ring, which allows a near-linear N−H- -O bond angle. When other conditions, such as steric and electrostatic, are nonprohibiting, the strength of the individual hydrogen bond should increase as the helical chain length increases. Each amide function becomes increasingly more polarized as the sequential hydrogen-bonding network increases its length, which leads to the macrodipole typical of α-helices.

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Supporting Information Available: Experimental procedures, ^1H, ^{13}C, and two-dimensional NMR (COSY), and IR spectra for **5** (7 pages).

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