is 36 K above room temperature, with a worst case lower limit of 12 K and an upper limit of 63 K. This estimate is not needed to support the observation of cooling but is useful as an indication that we are observing the later stages of cooling within our 8-ps laser pulse. The 532-nm photon causes a 460 K temperature jump.[12] Within 8 ps, most of the cooling is over. However, the molecule is still vibrationally hot and relaxes with a 2-5-ps $1/e$ time constant.[1]

Anatomy of a Stable Intramolecularly Hydrogen Bonded Folding Pattern†

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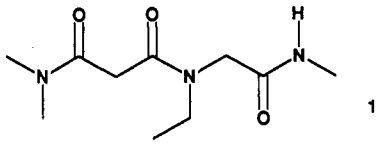
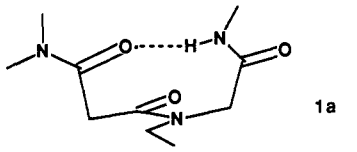
We recently reported that triamide **1** exists predominantly in the intramolecularly hydrogen bonded folding pattern **1a** in nonpolar and moderately polar solvents (CH_2Cl_2 and CH_3CN) at room temperature.[1] The stability of form **1a** is remarkable in light of the molecule's inherent conformational flexibility; formation of the internal hydrogen bond restricts rotations about one C–N and three C–C single bonds. We now describe a series of experiments that elucidates the origin of this folding pattern's stability. These studies are part of our continuing effort to understand, from the physical organic chemist's perspective, the interplay of noncovalent forces that controls protein folding patterns.[1-3]



1



1a

Diamide **2** has available to it a nine-membered-ring hydrogen bond similar to the one that occurs in **1**, but as we have previously shown, **2** experiences little internal hydrogen bonding in CH_2Cl_2 or CH_3CN at room temperature.[2] Diamide **2** is more flexible than **1**, because the central, rotationally restricted C–N bond in **1** is replaced with an sp^3–sp^3 C–C bond in **2**. We prepared diamide **3**[4] in order to determine whether simply abolishing rotation about the central bond of the covalent skeleton would stabilize the internally hydrogen bonded state. FT-IR analysis of **3** (10 mM in CH_3CN) reveals that the trans double bond does not substantially stabilize the folded state. Under these conditions, the N–H stretch regions for **2** and **3** are quite similar, showing only one distinct band, at about 3400 cm^{-1}, indicating hydrogen bonding to the solvent.[2b] In contrast, triamide **1** in CH_3CN shows a dominant band at 3313 cm^{-1}, indicating an intramolecular N—H···O=C interaction (Figure 1a–c).

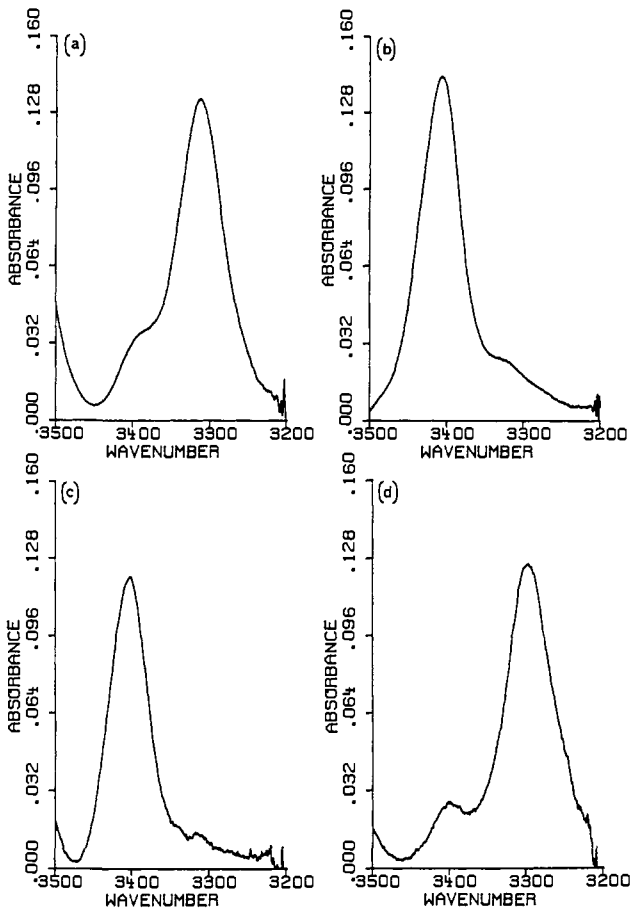
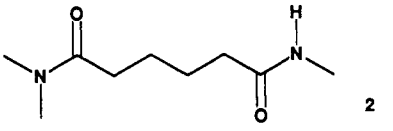
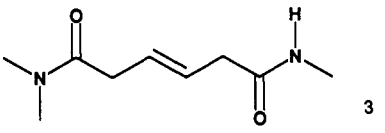


Figure 1. N–H stretch region FT-IR spectra for 10 mM amide solutions in CH_3CN (room temperature), after subtraction of the spectrum of pure CH_3CN. Data obtained on a Nicolet 740 spectrometer: (a) triamide **1** (maximum at 3313 cm^{-1}); (b) diamide **2** (maximum at 3406 cm^{-1}); (c) diamide **3** (maximum at 3403 cm^{-1}); (d) diamide **7** (maximum at 3298 cm^{-1}).

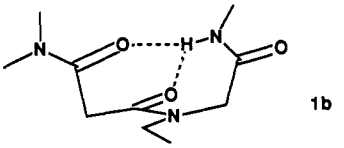


2



3

The behavior of **3** caused us to wonder whether the central amide group of **1** stabilizes the folded conformation by participating in a bifurcated hydrogen bond (**1b**). This uncertainty could



1b

by resolved by comparing the amide I regions of the IR spectra of **1** and methylated derivative **4**,[4] since hydrogen-bond acceptance by a carbonyl shifts the amide I band (largely C=O stretch) to lower wavenumber. These experiments required samples of **1** and **4** selectively labeled with ^{13}C, in order to eliminate band overlap. The positions of the amide I bands (cm^{-1}) are shown in the following structures (an asterisk indicates ^{13}C labeling; all samples 1 mM in CH_2Cl_2). Replacing the lone amide proton of **1** with

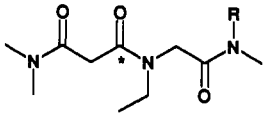
† Dedicated to Professor Ronald Breslow on the occasion of his 60th birthday.

(1) (a) Gellman, S. H.; Adams, B. R.; Dado, G. P. *J. Am. Chem. Soc.* **1990**, *112*, 460. (b) Dado, G. P.; Desper, J. M.; Gellman, S. H. *J. Am. Chem. Soc.* **1990**, *112*, 8630.

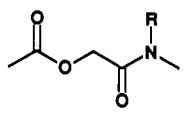
(2) (a) Gellman, S. H.; Adams, B. R. *Tetrahedron Lett.* **1989**, *30*, 3381. (b) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164.

(3) For a recent review on the role of noncovalent interactions in determining protein folding patterns, see: Dill, K. A. *Biochemistry* **1990**, *31*, 7133.

(4) The covalent structures assigned to all new compounds are consistent with NMR, IR, and MS analysis. Synthetic routes and full characterization will be reported elsewhere.



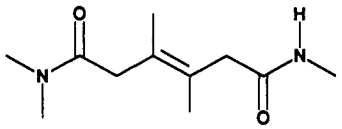
1, R = H	1641	1614	cm^{-1}
4, R = CH_3	1650	1606	cm^{-1}
Δ	+9	-8	



5, R = H	1757	cm^{-1}
6, R = CH_3	1751	cm^{-1}
Δ	-6	

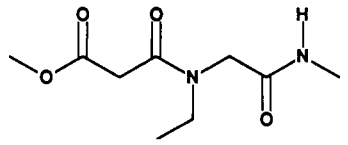
a methyl group in **4** results in the amide I band of the nine-membered-ring acceptor moving 9 cm^{-1} to higher wavenumber, which is consistent with the loss of a hydrogen bond accepting role for this carbonyl. The labeled amide group, on the other hand, moves 8 cm^{-1} to *lower* wavenumber in **4**. The direction of this shift is not consistent with a hydrogen bond accepting role for the labeled carbonyl in **1**. The -8-cm^{-1} shift is apparently a general result of the covalently remote methylation, as indicated by the ester C=O stretch data for **5** and **6**. (The lone NH stretch of 1 mM **5** in CH_2Cl_2 occurs at 3454 cm^{-1}, demonstrating that there is no intramolecular hydrogen bonding in this amide–ester under these conditions.) These IR data indicate that the internal hydrogen bond of **1** is not bifurcated in solution.[5]

Invalidation of the bifurcation hypothesis suggested that the trans double bond of diamide **3** is not an adequate model for the purely structural role played by the central amide in stabilizing folding pattern **1a**. The central rotationally restricted C—N bond of **1** has four non-hydrogen substituents, while the analogous C=C bond of **3** has only two non-hydrogen substituents. We prepared diamide **7**,[4] containing a tetrasubstituted C=C bond, in order to determine whether additional sterically demanding substituents around the central rotationally restricted linkage could affect the stability of the internally hydrogen bonded state.[6] The IR N–H stretch signature of **7** in CH_3CN (Figure 1d) reveals this molecule to be predominantly internally hydrogen bonded, in contrast to diamide **3**, but similar to triamide **1**.[7]



7

One might now ask whether the intramolecular hydrogen bond itself imparts any stability to folding pattern **1a**.[8] We addressed this question by examining **8**,[4] in which the terminal dimethyl amide moiety, a strong hydrogen-bond acceptor, is replaced by a methyl ester, a much weaker acceptor.[9] The N–H stretch region for **8** (1 mM in CH_2Cl_2) shows two bands, at 3392 and 3445 cm^{-1}. The former arises from N–H hydrogen bonded to an ester carbonyl; the latter arises from a non-hydrogen-bonded N–H. The 3392-cm^{-1} band is the larger of the two, indicating that **8** experiences substantial nine-membered-ring hydrogen bonding under these conditions. However, the non-hydrogen-bonded N–H stretch band of **8** is significantly larger than the analogous band for **1** under identical conditions, which demonstrates that the stability of folding pattern **1a** does indeed depend in part on the strength of the intramolecular hydrogen bond.



8

We have shown that at least three distinct factors contribute to the stability of folding pattern **1a**. The strong intramolecular hydrogen bond, the central nonrotating bond in the segment linking the hydrogen-bond donor and acceptor, and additional, more subtle torsional restrictions about sp^3–sp^2 bonds in the linking segment all appear to play important roles in stabilizing the conformation containing a nine-membered hydrogen-bonded ring. We are unaware of previous studies in which the network of internal noncovalent forces stabilizing the folding pattern of a natural or unnatural peptide has been elucidated at this level of detail. Parallel experiments with larger peptides are in progress.

Acknowledgment. We are grateful for partial support from the National Science Foundation (CHE 9104488) and the Searle Scholars Program. S.H.G. thanks the American Cancer Society for a Junior Faculty Research Award, and G.-B.L. thanks BP America for a fellowship.

(5) A referee has suggested that the geometry of the hydrogen bond involving the central amide carbonyl in hypothetical conformation **1b** could be so distorted (proton far out of the plane of the acceptor carbonyl) that the usual amide I shift to lower wavenumber would not be observed. We do not known of any published data that bear upon this suggestion.

(6) For a recent review covering the literature on torsional barriers associated with variously substituted sp^2–sp^3 bonds, see: Berg, U.; Sandström, J. *Adv. Phys. Org. Chem.* **1989**, *25*, 1.

(7) Crystallographic analysis of diamide **7** reveals that the nine-membered-ring hydrogen bond is retained in the solid state (Liang, G.-B.; Desper, J. M., unpublished results).

(8) Computational results led Synder to conclude that two observed intramolecular hydrogen bonds in the cyclic depsipeptide roseotoxin B are not a primary source of conformational rigidity; rather, these hydrogen bonds appear to be largely a consequence of other rigidifying factors. Snyder, J. P. *J. Am. Chem. Soc.* **1984**, *106*, 2393.

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Reactions and Rate Constants between Hydrated Electrons and the Monomer and Dimer of 2-Methyl-2-nitrosopropane Determined by the Pulse Radiolysis Method

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The spin trapping method[1] is now widely applied in chemistry, biology, and medicine regarding free radicals. In particular, free radicals induced in biologically significant molecules by ionizing radiation have been identified by this method to elucidate the primary processes in radiation biology.[2-9] In spin trapping studies, 2-methyl-2-nitrosopropane (MNP) has been frequently employed as the spin trap because this is suitable for identification of the radical structures. Ionizing radiation produces hydroxyl radicals ($OH^•$), hydrated electrons (e_{aq}^-), and atomic hydrogen ($H^•$) in an aqueous system. These radicals react not only with biological molecules but also with the spin trap. This means that MNP traps free radicals from biological molecules to form spin adducts and free radicals from MNP itself to induce byproducts. Sargent and

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‡Department of Atomic Science and Nuclear Engineering, Faculty of Engineering.

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