taxane complexes.[13] Substituted pyridine and pyrazine ligands form stable coordination complexes with a number of redox-active d^6 metal centers, such as $[M(CN)_5]^{n-}$ and $[M(NH_3)_5]^{m+}$ with iron, cobalt, ruthenium, and osmium. Using these preassembled α-CD/ligand complexes, a great variety of symmetrical and asymmetrical α-cyclodextrin metal rotaxanes may be prepared. Kinetic and spectroscopic investigations of the ligand-substitution and electron-transfer reactions involving these α-CD metal rotaxanes are ongoing in our laboratory.

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[13] The $[(NC)_5Fe(pyz(CH_2)_n pyz{\cdot}\alpha\text{-CD})Fe(CN)_5]^{4-}$ rotaxanes are best prepared by reacting the $Fe(CN)_5OH_2^{3-}$ ions with the prethreaded $(pyz(CH_2)_n pyz{\cdot}\alpha\text{-CD})^{2+}$ complex, as the rate of dissociation of a $[Fe(CN)_5]^{3-}$ group from the $[(NC)_5Fe(pyz(CH_2)_n pyz)Fe(CN)_5]^{4-}$ dimer is an order of magnitude slower than for the bipyridinium complex.

On the Use of AM1 Calculations for the Study of Intramolecular Hydrogen Bonding Phenomena in Simple Amides

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The manner in which networks of nonbonded interactions control spatial arrangements between or among molecules (e.g., enzyme–substrate interactions, host–guest chemistry) and the juxtaposition of segments of a single molecule with respect to one another (e.g., the folding of natural and synthetic polymers) is of considerable interest to both experimentalists and theoreticians.[1] Our experimental efforts in this area have involved the folding of small oligoamides in organic solvents.[2] Because of the structural simplicity of these model systems, they provide an opportunity to evaluate the accuracy with which computational methods reproduce the balance of noncovalent forces (including solvation forces) that controls intramolecular hydrogen bond formation.

On the basis of IR and variable-temperature NMR measurements, we have proposed that triamide **1** in dilute methylene chloride solution experiences substantial temperature-dependent changes in its folding pattern.[2a] Our data indicate that the form favored at low temperature (i.e., the most enthalpically stable folding pattern) is I, in which only H_b is involved in an intramolecular hydrogen bond. Novoa and Whangbo have recently reported AM1 calculations that predict the global energy minimum to be II; III is also predicted to be energetically favorable.[3,4] In II and III, both H_a and H_b are engaged in intramolecular hydrogen bonds, which is not consistent with our data concerning the most enthalpically stable folding pattern of **1**.[2a] We point out here that comparison of the AM1 results with experimental observations and with ab initio calculations suggests that the semiempirical methodology does not accurately predict the energetics of intramolecular N—H- -O=C hydrogen bond formation in solution.

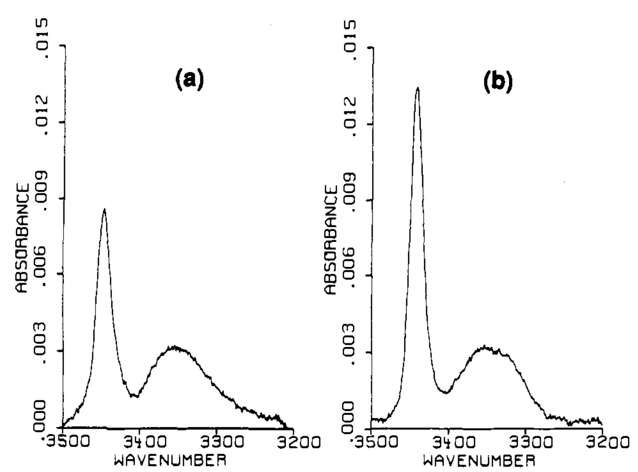
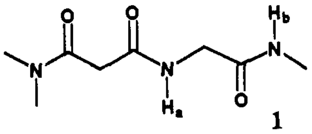
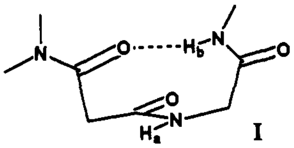


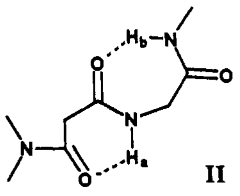
Figure 1. N–H stretch region FT-IR spectral data for 1 mM diamide **2** in CH_2Cl_2, after subtraction of the spectrum of pure CH_2Cl_2 at the same temperature: (a) 23 °C (absorption maxima at 3447 and ca. 3355 cm^{-1}); (b) −69 °C (absorption maxima at 3442 and ca. 3350 cm^{-1}). Samples prepared and data obtained on a Nicolet 740 spectrometer as described in ref 2c. Control experiments indicate that little or no aggregation of **2** occurs under these conditions.



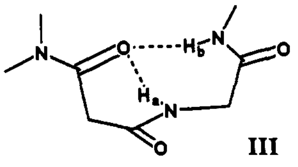
1



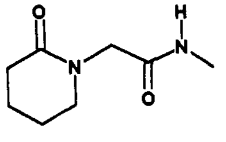
I



II



III



2

Our folding hypothesis for **1** was derived in part from the behavior of diamide **2**, which can form a seven-membered-ring hydrogen bond that is similar to the seven-membered-ring hydrogen bond available to H_b of **1**.[2a,5] Since only one intramolecular N—H- -O=C pairing is available to **2**, deducing this

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[1] For leading references see: (a) Rebek, J. *Acc. Chem. Res.* **1990**, *23*, 399. (b) *Cyclophanes*; Diederich, F., Ed.; The Royal Society of Chemistry: Cambridge, 1991. (c) Jorgensen, W. L. *Acc. Chem. Res.* **1989**, *22*, 184. (d) Kollman, P. A.; Merz, K. M. *Acc. Chem. Res.* **1990**, *23*, 246. (e) Dill, K. A. *Biochemistry* **1990**, *31*, 7133. (f) *Prediction of Protein Structure and the Principles of Protein Conformation*; Fasman, G. D., Ed.; Plenum Press: New York, 1989.

[2] (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. (c) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164. (d) Liang, G.-B.; Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1991**, *113*, 3994.

[3] Novoa, J. J.; Whangbo, M.-H. *J. Am. Chem. Soc.* **1991**, *113*, 9017.

[4] Similar computational predictions for **1** and **2** have been made on the basis of AMBER/MacroModel calculations, using a continuum solvation model for CH_2Cl_2: Smith, D. A.; Vijayakumar, S. *Tetrahedron Lett.* **1991**, *32*, 3613, 3617. For a critical evaluation of these computational results, see: Gellman, S. H.; Dado, G. P. *Tetrahedron Lett.* **1991**, *32*, 7737.

[5] The seven-membered-ring hydrogen bond available to **2** is closely related to the "C_7" folding pattern available to peptides, which has been the subject of considerable experimental and theoretical investigation. For leading references, see: (a) Böhm, H.-J.; Brode, S. *J. Am. Chem. Soc.* **1991**, *113*, 7129. (b) Perczel, A.; Angyan, J.; Kajtar, M.; Viviani, W.; Rivail, J.-L.; Marcoccia, J.-F.; Csizmadia, I. G. *J. Am. Chem. Soc.* **1991**, *113*, 6256. (c) Head-Gordon, T.; Head-Gordon, M.; Frisch, M. J.; Brooks, C. L.; Pople, J. A. *J. Am. Chem. Soc.* **1991**, *113*, 5989. (d) Roterman, I. K.; Lambert, M. H.; Gibson, K. D.; Scheraga, H. A. *J. Biomol. Struct. Dynam.* **1989**, *7*, 421. (e) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1. (f) Maxfield, F. R.; Leach, S. J.; Stimson, E. R.; Powers, S. P.; Scheraga, H. A. *Biopolymers* **1979**, *18*, 2507.

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diamide's behavior on the basis of spectroscopic data should be more straightforward than for **1**. Previously reported variable-temperature ^{1}H NMR measurements ($\Delta\delta(NH)/\Delta T$) indicated that the intramolecularly hydrogen-bonded and non-hydrogen-bonded[6] states of **2** are of very similar enthalpy in CD_2Cl_2.[2a] In contrast, when an attempt was made to account for solvation by including three CH_2Cl_2 molecules in a "supermolecule" calculation, AM1 predicted the minimum energy intramolecularly hydrogen-bonded conformation of **2** to be 1.9 kcal/mol more enthalpically favorable than the minimum energy non-hydrogen-bonded conformation.[3]

In order to provide a more quantitative comparison with the calculations, we have now carried out an IR-based van't Hoff analysis of the intramolecular hydrogen bonding equilibrium occurring in a 1 mM CH_2Cl_2 solution of **2** over the temperature range −69 to 23 °C. Figure 1 shows the N–H stretch region of the IR spectra obtained at high and low temperatures. Both hydrogen-bonded (3340–50 cm^{-1}) and non hydrogen-bonded[6] (3443–8 cm^{-1}) bands are observed at each temperature. No hydrogen-bonded N–H stretch band can be detected at any temperature for a 1 mM sample of *N*-methylcyclohexylacetamide (**3**) in CH_2Cl_2; therefore, we used this compound to estimate the extinction coefficient of the non-hydrogen-bonded N–H stretch band of **2** as a function of temperature. van't Hoff analysis (intramolecularly hydrogen-bonded vs non-hydrogen-bonded states; each "state" comprises a set of conformations) indicated that the internally hydrogen-bonded state of **2** is 0.25 ± 0.06 kcal/mol less enthalpically favorable and 0.67 ± 0.48 eu more entropically favorable than the non-hydrogen-bonded state.[7]

Since CH_2Cl_2 is relatively nonpolar, it is interesting that *the internally hydrogen-bonded and non-hydrogen-bonded states of **2** have very similar enthalpies, with the state containing the N—H- -O=C interaction slightly less enthalpically favorable.* An ideal amide–amide hydrogen bond should be enthalpically superior to any interaction between the amide group and the solvent. The enthalpic similarity of the internally hydrogen-bonded and non-hydrogen-bonded states of **2** may result from at least two factors: (1) the geometry of the seven-membered-ring hydrogen bond is not optimal for the amide–amide interaction (e.g., a nonlinear N–H- -O angle is unavoidable); (2) closure of the hydrogen-bonded ring may involve the development of torsional strain and/or other enthalpically unfavorable interactions. The entropic similarity between the internally hydrogen-bonded and non-hydrogen-bonded states may arise from the fact that these two states enjoy similar degrees of conformational mobility[2c] and/or from desolvation associated with intramolecular hydrogen bond formation.[8] (The breadth and asymmetry of the hydrogen-bonded N–H stretch band in Figure 1 is consistent with the existence of multiple hydrogen-bonded ring conformations.)

Why does AM1 overestimate the enthalpic favorability of the intramolecularly hydrogen-bonded state of **2**? One potential source of error is indicated by the comparison between ab initio and AM1 results for the hydrogen-bonded formamide dimer reported by Novoa and Whangbo.[3] When interaction energy was examined as a function of the N–H- -O angle, the ab initio calculations predicted that the hydrogen bond energy becomes increasingly unfavorable as the angle decreases below 150°.[9] For an N–H- -O angle of 120° (the smallest angle examined), the ab initio interaction energy was 1.5 kcal/mol less favorable than in the 150–180° region. In contrast, AM1 predicted only small variations in interaction energy over the N–H- -O angle range 120–180°, with a shallow minimum around 130°. (The ab initio results are in line with experimental observations concerning the geometric preferences of N—H- -O=C hydrogen bonds.[10])

Since AM1 predictions for very simple systems are at odds with both experimental and ab initio results, it seems unlikely that AM1 calculations can provide useful insight on the behavior of triamide **1**. Indeed, the predicted minimum energy form of $\mathbf{1}\cdot 4(CH_2Cl_2)$ contains six- and seven-membered hydrogen-bonded rings, with N–H- -O angles of 120° and 141°.[3,11]

Predicting the structural consequences stemming from the operation of complex networks of noncovalent interactions remains an important avenue of research.[1] The comparisons discussed here suggest that caution must be exercised in applying computational methods to such systems.

Acknowledgment. We thank Professor M.-H. Whangbo for sending manuscripts before publication and Professor William Saunders for helpful comments. This work was supported by the National Science Foundation (CHE-9014488). G.P.D. is the recipient of a National Research Service Award (T32 GM08923) from the National Institute of General Medical Sciences. S.H.G. is grateful to the Searle Scholars Program, the NSF PYI Program (CHE-9157510), the Eastman Kodak Company, and the Upjohn Company for support. The FT-IR spectrometer was purchased with funds provided by the Office of Naval Research (N00014-90-1902).

Supplementary Material Available: Representative van't Hoff plot for intramolecular hydrogen bonding of **2** in CH_2Cl_2 (1 page). Ordering information is given on any current masthead page.

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[6] We use the term "non-hydrogen-bonded" to signify the absence of an N—H- -O=C interaction. Such protons are presumably engaged in weak interactions with the solvent.

[7] A representative van't Hoff plot is included as supplementary material. Only the non-hydrogen-bonded N–H stretch band was used for quantification, because appropriate reference compounds were not available for determining the extinction coefficient of the hydrogen-bonded N–H stretch band. We have previously shown that this type of IR-based van't Hoff analysis of intramolecular hydrogen bonding in simple diamides provides results quantitatively similar to those of ^{1}H NMR-based van't Hoff analysis; see ref 2c.

[8] For a dramatic example of solvation effects on noncovalent association phenomena in CD_2Cl_2, see: Bryant, J. A.; Knobler, C. B.; Cram, D. J. *J. Am. Chem. Soc.* **1990**, *112*, 1254.

[9] See Figure 2a of ref 3.

[10] (a) Baker, E. N.; Hubbard, R. E. *Prog. Biophys. Mol. Biol.* **1984**, *44*, 97 and references therein. (b) Taylor, R.; Kennard, O.; Versichel, W. *Acta Crystallogr.* **1984**, *B40*, 280.

[11] Geometrically problematic hydrogen-bonding predictions by AM1 have been reported previously: (a) Rzepa, H. S.; Yi, M. *J. Chem. Soc., Perkin Trans. 2* **1991**, 531 and references therein. (b) Schröder, S.; Daggett, V.; Kollman, P. *J. Am. Chem. Soc.* **1991**, *113*, 8922.

Diffusion-Ordered Two-Dimensional Nuclear Magnetic Resonance Spectroscopy

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A noninvasive analytical method is needed that can identify molecular components of mixtures *and* simultaneously characterize the sizes of aggregates and other structures present. Consider, for example, a broth containing vesicles, micelles, and protein fragments. Light-scattering experiments cannot resolve such mixtures, and chromatographic methods may disrupt fragile structures such as micelles and vesicles. In any case hydrodynamic methods typically do not directly identify the molecular components. Even highly selective NMR methods are at a disadvantage with mixtures of species having complicated spectra. Here we report an alternative analysis method, a 2D-NMR experiment that displays an NMR chemical shift spectrum in one direction and a "spectrum" of diffusion coefficients or particle sizes in the other, i.e., for each radius the NMR spectrum of associated molecular species is displayed. This experiment complements existing analytical methods and provides a global view of particle sizes in the sample. It is effective at detecting impurities and aggregates in samples and, furthermore, is relatively easy to implement on modern NMR spectrometer systems.

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