that the γ-phosphate of ATP is positioned within the vicinity of the active site cysteine.[12]

The high $K_i$ (17.7 mM) for **1** is somewhat surprising, especially since even the simple dipeptide Arg-Arg exhibits a $K_i$ of 3.8 mM as a reversible inhibitor of the A-kinase.[13] It occurred to us that the disulfide-containing peptide might be obliged to bind in a less than optimal fashion in order to modify the active site cysteine residue.[14] This notion is consistent with our observation that the $K_i$ obtained for peptide **1** from inactivation kinetics (17.7 mM) is larger than that acquired from competitive inhibition kinetics (970 ± 26 μM).[15] The latter value is reasonable for peptides of this size.[13,16] These results suggest that more potent inhibitors may be accessible via positional isomers of Leu-Arg-Arg-Cys⇔Cys-Leu-Gly. These experiments are currently in progress.

One possible pathway for inactivation is depicted in Scheme I. Theoretical studies have predicted that the peptide bond within a Cys⇔Cys dyad should occupy the cis configuration.[17,18] We note that for peptide bonds in general, the trans arrangement is the preferred structure. In species **2**,[19] the peptide bond is now free to isomerize to the trans isomer **3**. The release of the strain inherent in a Cys⇔Cys dyad may very well explain why disulfide exchange back to the unmodified A-kinase and Leu-Arg-Arg-Cys⇔Cys-Leu-Gly is not observed.

In summary, we have described the inhibitory activity of a disulfide-containing peptide. This prototype of a potentially valuable class of affinity labels contains several attractive features. First, the preparation of the intramolecular disulfide bond is straightforward since oxidation is readily accomplished in aqueous solution. Second, the synthesis of this class of affinity labels does not require modification of a peptide by some external reagent. Therefore, even extremely long peptides, containing the natural array of nucleophilic side chains, can be converted into reagents that will covalently modify the appropriate target enzyme. Third, this class of affinity labels contains only functional groups that are present in proteins. Consequently, this suggests that it may be possible to construct *protein*-based affinity labels via site-directed mutagenesis. We note that modification is most likely specific for sulfhydryl groups. The active site cysteine-199 in the A-kinase is conserved in other protein kinases as well, including other members of the cyclic nucleotide-dependent subfamily (e.g. cGMP dependent), the calcium/phospholipid-dependent subfamily (i.e. protein kinase C), and the calcium/calmodulin-dependent subfamily (e.g. human HeLa cell serine kinase).[20] In addition, other enzymes that act upon protein substrates, such as cathepsin B (a cysteine proteinase),[21] cyclophilin (a peptidyl-prolyl-cis-trans isomerase),[22] and Factor XIIIa (a transglutaminase),[23] appear to be potential targets as well.

**Acknowledgment**. We are pleased to acknowledge the American Cancer Society (No. BC-664) for generous financial support. We also thank Professor Shahriar Mobashery for helpful advice.

**Supplementary Material Available**: A Lineweaver-Burk plot of **1** as a competitive inhibitor versus [kemptide] and a replot of the slopes of the reciprocal plot versus [**1**] (2 pages). Ordering information is given on any current masthead page.

(12) Jimenez, J. S.; Kupfer, A.; Gani, V.; Shaltiel, S. *Biochemistry* **1982**, *21*, 1623.
(13) Feramisco, J. R.; Krebs, E. G. *J. Biol. Chem.* **1978**, *253*, 8968.
(14) Plapp, B. V. *Methods Enzymol.* **1982**, *87*, 469.
(15) Covalent modification of the enzyme is precluded under the assay conditions employed to assess the $K_i$ of **1** via competitive inhibition kinetics (i.e. in the presence of MgATP): Assays contained 100 mM MOPS, 150 μM [γ-$^{32}$P]ATP (200 cpm/pmol), 12.5 mM $MgCl_2$, 150 mM KCl, 0.125 mg/mL BSA, kemptide (10-40 μM), and peptide **1** (0-1200 μM) in a total volume of 80 μL at pH 7.1 and 30 °C. Kinase reactions were initiated by addition of the A-kinase to a final concentration of 5 nM. Reactions were terminated as described in ref 8.
(16) It should be possible to enhance the affinity of this peptide for the enzyme-active site by addition of appropriate amino acids on the N-terminus. See: Glass, D. B.; Cheng, G.-H.; Mende-Mueller, L.; Reed, J.; Walsh, D. A. *J. Biol. Chem.* **1989**, *264*, 8802.
(17) Chandrasekaran, R.; Balasubramanian, R. *Biochem. Biophys. Res. Commun.* **1968**, *188*, 1.
(18) We have recently determined that compound **1** exists as both the cis and trans isomers in solution (based on NOESY NMR experiments, Sukumaran, D. K.; Prorok, M.; Lawrence, D. S., manuscript submitted for publication). The trans isomer constitutes a sizable percentage (35%) of the total cis/trans population. Consequently, it appears likely that this species also contributes to the inactivation of the A-kinase.
(19) Compound **2** is the result of only one of two possible modes of nucleophilic attack. Experiments are in progress to determine the relative ratios of the two potential products.
(20) (a) Hanks, S. K. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 388. (b) Hanks, S. K.; Quinn, A. M.; Hunter, T. *Science* **1988**, *241*, 42.
(21) Rich, D. H. In *Proteinase Inhibitors*; Barrett, A. J., Salvesen, Eds.; Elsevier: New York, 1986; p 153.
(22) Although the active site cysteine in cyclophilin does not appear to be essential for catalysis, modification of this residue does result in the elimination of enzymatic activity. See: (a) Fisher, G.; Wittman-Liebold, B.; Lang, K.; Kiefhaber, T.; Schmid, F. X. *Nature* **1989**, *337*, 476. (b) Harrison, R. K.; Stein, R. L. *Biochemistry* **1990**, *29*, 1684. (c) Liu, J.; Albers, M. W.; Chen, C.-M.; Schreiber, S. L.; Walsh, C. T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 2304.
(23) Folk, J. E. *Adv. Enzymol.* **1983**, *54*, 1.

## Synthesis and X-ray Crystal Structure of a Highly Strained Anti-Bredt Olefin/Anti-Bredt Lactam. *exo*-2-Carbomethoxy-1-aza-8-oxobicyclo[3.3.1]non-4-ene

K. J. Shea,* Timothy G. Lease, and Joseph W. Ziller

*Department of Chemistry*
*University of California, Irvine*
*Irvine, California 92717*

*Received July 30, 1990*

Bridgehead double bonds experience topologic constraints that can result in nonplanar olefin geometries.[1] Such distortions can also profoundly influence chemical reactivity. These anti-Bredt olefins may be isolated only when the trans double bond is contained in a ring of eight or more atoms. We report the synthesis and X-ray crystal structure of *exo*-2-carbomethoxy-1-aza-8-oxobicyclo[3.3.1]non-4-ene (**1**), a molecule that contains both a bridgehead olefin and a bridgehead amide in an eight-membered ring. *This is the first X-ray structure of a bridgehead olefin containing a trans-cyclooctene ring,[2] and it represents one of the most highly strained isolable anti-Bredt molecules prepared to date.*

Anti-Bredt olefin **1** was prepared by a type 2 intramolecular Diels-Alder cycloaddition.[3] Condensation of diene amide **2** with methyl glyoxalate followed by acetylation of the resulting methylol according to the procedure of Weinreb[4] afforded diene acetate **3**, the Diels-Alder precursor (Scheme I). In situ thermolytic elimination of acetic acid generated the *N*-acylimine intermediate **4**, which cyclized to give **1**. The cycloadduct was produced in optimum yield (35%) by heating dilute solutions of **3** (0.01 M,

(1) Bridgehead olefin reviews: (a) Warner, P. M. *Chem. Rev.* **1989**, *89*, 1067-1093. (b) Wentrup, C. *Reactive Molecules*; John Wiley and Sons: New York, 1984; pp 265-308. (c) Szeimies, G. In *Reactive Intermediates*; Abramovitch, R. A., Ed; Plenum: New York, 1983; Vol. 3, pp 299-366. (d) Shea, K. J. *Tetrahedron* **1980**, *36*, 1683-1715.
(2) For several recent examples of X-ray crystal structures of transition-metal complexes of strained olefins see: (a) Bly, R. S.; Hossain, M. M.; Lebioda, L. *J. Am. Chem. Soc.* **1985**, *107*, 5549-5550. (b) Kumar, A.; Lichtenhan, J. D.; Critchlow, S. C.; Eichinger, B. E.; Borden, W. T. *J. Am. Chem. Soc.* **1990**, *112*, 5633-5634. (c) For the X-ray crystal structure of a 1-azabicyclo[3.3.1]nonan-2-one derivative see: Buchanan, G. L.; Kitson, D. H.; Mallinson, P. R.; Sim, G. A.; White, D. N. J.; Cox, P. J. *J. Chem. Soc., Perkin Trans. II* **1983**, 1709-1712.
(3) (a) Shea, K. J.; Burke, L. D. *Tetrahedron Lett.* **1987**, *28*, 735-738. (b) Shea, K. J.; Burke, L. D. *J. Org. Chem.* **1985**, *50*, 725-727. (c) Shea, K. J.; Wise, S.; Burke, L. D.; Davis, P. D.; Gilman, J. W.; Greley, A. C. *J. Am. Chem. Soc.* **1982**, *104*, 5708-5715. (d) Shea, K. J.; Wise, S. *J. Am. Chem. Soc.* **1978**, *100*, 6519-6521.
(4) Weinreb, S. M. *Acc. Chem. Res.* **1985**, *18*, 16-21 and references therein.

0002-7863/90/1512-8627$02.50/0 

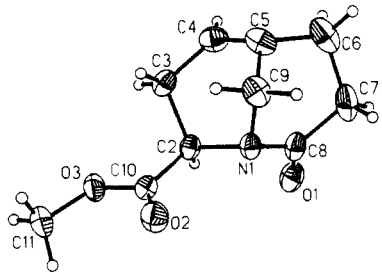
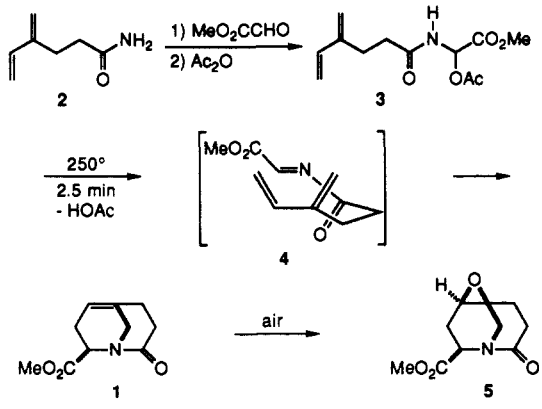



**Figure 1.** ORTEP plot of **1** at the 50% probability level.

**Scheme I**




xylenes) to 250 °C for 2.5 min. The crude pyrolysis mixture could be chromatographed under $N_2$ allowing isolation of the colorless, crystalline cycloadduct.

The anti-Bredt olefin is a highly reactive compound and efforts to manipulate it in air resulted in formation of epoxide **5**. Handling the cycloadduct in a nitrogen drybox permitted recrystallization by the vapor diffusion method: slow diffusion of hexane into a benzene solution of **1** at −30 °C resulted in formation of monoclinic crystals suitable for X-ray crystallography.[5]

An ORTEP plot of bridgehead alkene **1** is shown in Figure 1. The cycloadduct contains the bridgehead olefin and bridgehead amide in a trans,trans-1,5 relationship in an eight-membered ring. The structural data, therefore, permit an evaluation of the response of each functional group to the strain of occupying the bridgehead position of the bicyclo[3.3.1]nonane framework.


The X-ray data reveal the bridgehead olefin and bridgehead amide are significantly distorted from planarity. The extent of the distortions can be quantified by the torsion angle $\tau$ between the p-orbitals of the olefin and amide $\pi$ systems and by the pyramidalization angles $\chi$ of the constituent atoms of the two functional groups.[6] At the C–C double bond the torsion angle $\tau_{C4-C5}$ is 10.8 (5)°. The pyramidalization angle of the bridgehead carbon $\chi_{C5}$ is 39.0 (2)° whereas $\chi_{C4}$ is 17.9 (11)°. The torsion and pyramidalizations of the double bond are readily discerned from Figure 2 (parts a and b). The C=C bond distance, 1.333 (2) Å, is within error of the value for cyclohexene, 1.335 (3) Å,[7] and is much smaller than the distance of 1.543 Å for a single bond at the same (boat cyclohexane) position in a bicyclo[3.3.1]nonane derivative.[8] Since it is indistinguishable from that of a normal olefin, the bond distance is not a sensitive function of the distortions.

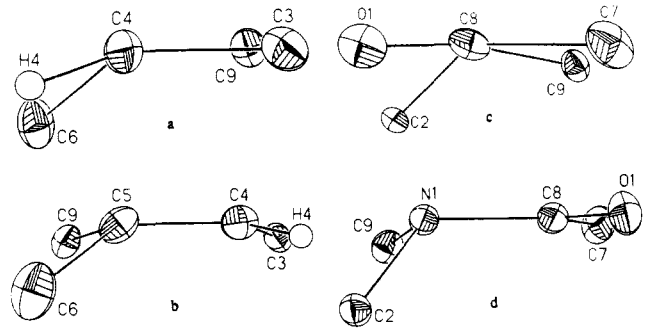



**Figure 2.** ORTEP plots (at 20% probability for clarity) of the olefin and amide bonds (and their respective substituents) in **1** with all other atoms omitted: (a) olefin viewed along the axis of the C=C bond with $C_4$ in front and (b) the side view; (c) amide viewed along the axis of the C–N bond with $C_8$ in front and (d) the side view.

A soft bending potential at nitrogen and a weak torsion potential about the C–N bond result in a more extensive distortion of the bridgehead amide.[9] Pyramidalization at the bridgehead nitrogen is essentially complete: $\chi_{N1}$ = 54.9 (1)°. The carbonyl carbon, however, undergoes almost no pyramidalization: $\chi_{C8}$ = 1.4 (2)°. The torsion angle $\tau_{N1-C8}$ is 16.7 (1)° (Figure 2, parts c and d). The distortion of the amide linkage results in a lengthening of the amide C–N bond to 1.399 (2) Å, compared to 1.333 (2) Å for 4-*tert*-butylvalerolactam;[10] the C–N bond distance in piperidine is 1.472 (11) Å.[11] Thus the amide C–N bond distance in **1** is halfway between a C–N single bond and a normal C–N amide bond.

The distortions in **1** result in a significant modification of the chemistry of both the olefin and amide linkages. A report of this behavior and structural studies of related compounds will be given in the full account of this work.

**Acknowledgment.** We thank the National Science Foundation for financial support of this work. Funds for the purchase of the Siemens R3m/V diffractometer system were made available from the National Science Foundation under Grant CHE-85-14495. We also thank Professor William Evans for his assistance with the recrystallization procedure and Professor Robert Doedens for

(5) X-ray diffraction data for $C_{10}H_{13}NO_3$: The crystal belongs to the monoclinic system with unit cell parameters at 183 K: $a$ = 8.4717 (16) Å, $b$ = 6.5737 (10) Å, $c$ = 17.496 (3) Å, $\beta$ = 94.195 (15)°, and $V$ = 971.7 (3) Å$^3$. The space group is $P2_1/n$ with $Z$ = 4 formula units/unit cell and $D$(calc) = 1.33 Mg/m$^3$. Intensity data (2023 total) were collected on a Siemens R3m/V diffractometer system with monochromatized Mo K$\alpha$ radiation ($\lambda$ = 0.710730 Å) by a $\theta$–$2\theta$ scan technique.[5a] All 1647 reflections with $|F_o| > 0$ were considered observed. The structure was solved by direct methods and refined by full-matrix least-squares techniques.[5b,c] Hydrogen atoms were located from a difference-Fourier map and included with isotropic temperature parameters. At convergence, $R_F$ = 4.1%, $R_{wF}$ = 4.9%, and GOF = 1.71 for 180 variables. A final difference-Fourier map was featureless. Pyramidalization and torsion angles were calculated with use of the OR FFE program.[5d] (a) Churchill, M. R.; Lashewycz, R. A.; Rotella, F. *J. Inorg. Chem.* **1977**, *16*, 265–271. (b) UCLA Crystallographic Computing Package, University of California, Los Angeles, 1981, C. Strouse, personal communication. (c) SHELXTL PLUS Program set; Siemens Analytical X-Ray Instruments, Inc.; Madison, WI, 1989. (d) Busing, W. R.; Martin, K. O.; Levy, H. A. *OR FFE: A Fortran Crystallographic Function and Error Program*; Oak Ridge National Laboratory, U.S. Atomic Energy Commission: Oak Ridge, TN, 1964; ORNL-TM-306.

(6) Winkler, F. K.; Dunitz, J. D. *J. Mol. Biol.* **1971**, *59*, 169–182. For a distorted double bond A the pyramidalization angle $\chi_B$ is found by defining


A

the plane ZBA and measuring its acute angle to the plane YBA. Note that for a planar sp$^2$–sp$^2$ double bond $\chi \approx 0°$; for a fully pyramidalized (sp$^3$) atom $\chi \approx 60°$. The p-orbital torsion angle $\tau_{A-B}$ is the average of the four-atom torsion angles $\Phi_{WABY}$ and $\Phi_{XABZ}$. When there is no $\pi$-orbital misalignment $\tau = 0°$.

(7) Determined by electron diffraction: Chiang, J. F.; Bauer, S. H. *J. Am. Chem. Soc.* **1969**, *91*, 1898–1901.

(8) Determined by X-ray crystallography: Tamura, C.; Sim, G. A. *J. Chem. Soc. B* **1968**, 1241.

(9) For a review of bridgehead lactams see: Greenburg, A. In *Structure and Reactivity*; Liebman, J. F., Greenburg, A., Eds.; VCH: New York, 1988; Chapter 4.

(10) Determined by X-ray crystallography: van der Helm, D.; Ekstrand, J. D. *Acta Crystallog.* **1979**, *B35*, 3101–3103.

(11) Determined by electron diffraction: Gundersen, G.; Rankin, D. W. H. *Acta Chem. Scand., Ser. A* **1983**, *A37*, 865–874.

his assistance with the torsion angle calculations.

**Supplementary Material Available**: Description of the X-ray diffraction experiment and tables of experimental data, atomic coordinates, thermal parameters, interatomic distances, and bond angles (5 pages); listing of structure factor amplitudes (6 pages). Ordering information is given on any current masthead page.

## The 2-Pyridone Dimer, a Model Cis Peptide. Gas-Phase Structure from High-Resolution Laser Spectroscopy

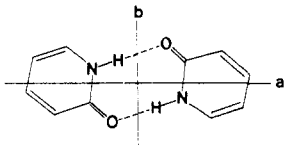
Andrew Held and David W. Pratt*

*Department of Chemistry, University of Pittsburgh Pittsburgh, Pennsylvania 15260*

*Received July 27, 1990*
*Revised Manuscript Received September 24, 1990*

One of the criteria for the formation of a satisfactory polypeptide configuration is that each carbonyl and imino group be involved in the formation of a hydrogen bond with the N—H···O distance approximately 2.8 Å and with the oxygen atom nearly on the N-H axis.[1] This requirement is met by many proteins, including those containing the rare cis amide linkage.[2] Linkages of this type also are exploited widely in molecular recognition.[3] In this report, we show that at least one precursor to such structures also exists in the gas phase, that it is planar or nearly so, and that its ground-state geometry is very similar to that determined by X-ray crystallography.

The model cis peptide is the 2-pyridone dimer I, formed in the




I

gas phase by expansion of a supersonic jet seeded with 2-hydroxypyridine (2-HP). Figure 1A shows a portion of the $S_1 \leftarrow S_0$ fluorescence excitation spectrum (FES) of the resulting gas-phase mixture, probed ~15 mm downstream of the nozzle with a tunable pulsed dye laser operating in the ultraviolet. There are three prominent bands in this spectrum at a resolution of ~1 $cm^{-1}$. The two lower frequency bands, at 29831 and 29930 $cm^{-1}$, are the electronic origins of two conformers of the 2-pyridone monomer (2-PY, the lactam form of 2-HP), differing in the degree of nonplanarity at the nitrogen atom.[4,5] The higher frequency band, displaced 945 $cm^{-1}$ to the blue of the first monomer origin, belongs to the 2-PY dimer, $(2\text{-PY})_2$.

Proof that this high-frequency band is that of $(2\text{-PY})_2$ was provided by examining it at higher resolution, sufficiently high to expose the underlying rotational structure of the band so that the inertial parameters of the molecule (in both its $S_0$ and $S_1$ states) could be measured. A molecular beam CW laser spectrometer[6] was employed for this purpose. Figure 1, spectra B and C, shows the rotationally resolved FES of the high-frequency band. The

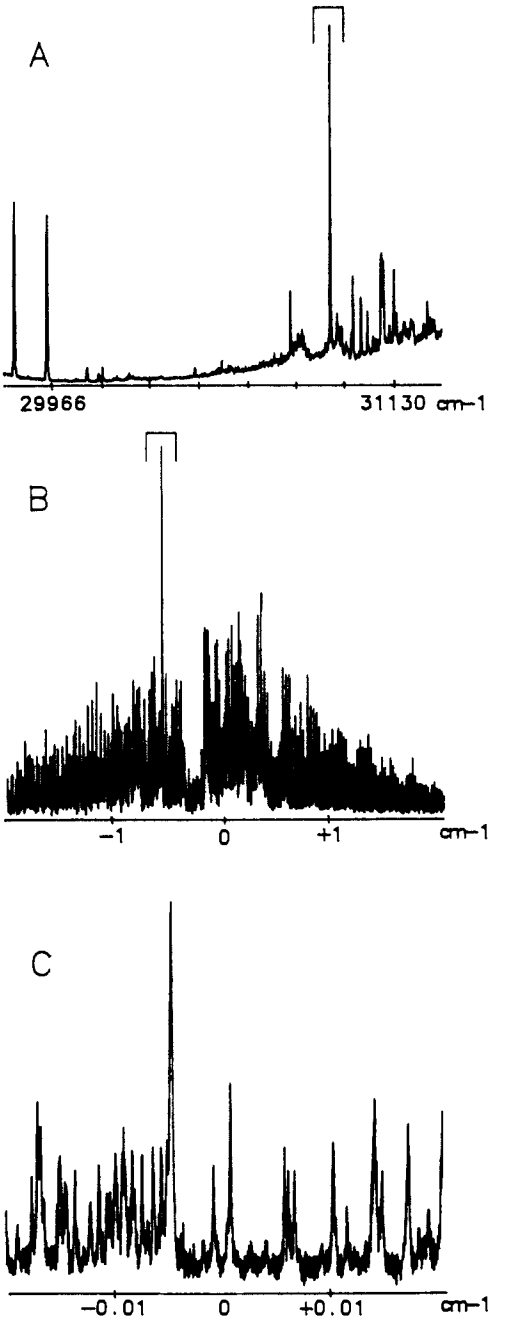



**Figure 1.** (A) A portion of the vibrationally resolved fluorescence excitation spectrum (FES) of 2-hydroxypyridine (2-HP) in a supersonic jet. The heated sample of 2-HP (at ~120 °C) was seeded in 5 atm of He, expanded through a 2 mm diameter pulsed nozzle, and probed 15 mm downstream by a frequency-doubled pulsed dye laser. The two strong lower frequency bands are the $S_1 \leftarrow S_0$ origins of the 2-HP tautomer 2-pyridone (2-PY); the strong high-frequency band is a $S_1 \leftarrow S_0$ vibronic transition of the model cis peptide, 2-pyridone dimer $[(2\text{-PY})_2]$. (B) The rotationally resolved FES of the $(2\text{-PY})_2$ $S_1 \leftarrow S_0$ vibronic band, recorded in the collision-free environment of a molecular beam. In this case, 2-HP was heated to ~200 °C, seeded in 300 Torr of Ar, expanded through a 240-μm quartz nozzle, skimmed twice, and probed 100 cm downstream by a tunable CW laser operating in the UV. The resulting FES, detected with a photon counter, was calibrated with use of a mode-matched confocal interferometer and the $I_2$ absorption spectrum. All data were processed and analyzed with a Concurrent computer system and specially designed software. (C) A portion of spectrum B illustrating the resolved rotational lines of $(2\text{-PY})_2$. In spectra A and B, brackets denote the piece of the spectrum that is expanded in spectra B and C, respectively.

entire spectrum of this band (Figure 1B) spans ~90 GHz (3 $cm^{-1}$) and exhibits more than 1500 resolved lines. The lifetime-limited line width (fwhm) of single lines (Figure 1C) is 18 MHz (~0.0006 $cm^{-1}$). Notably, these lines exhibit spacings that are significantly

(1) Pauling, L.; Corey, R. B. *Proc. Natl. Acad. Sci. U.S.A.* **1951**, *37*, 729.
(2) Bairaktari, E.; Mierke, D. F.; Mammi, S.; Peggion, E. *J. Am. Chem. Soc.* **1990**, *112*, 5383 and references therein.
(3) See, for example: Jeong, K.-S.; Tjivikua, T.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 3215.
(4) Nimlos, M. R.; Kelley, D. F.; Bernstein, E. R. *J. Phys. Chem.* **1989**, *93*, 643.
(5) Held, A.; Plusquellic, D. F.; Tomer, J. L.; Pratt, D. W. Work in progress.
(6) Majewski, W. A.; Plusquellic, D. F.; Pratt, D. W. *J. Chem. Phys.* **1989**, *90*, 1362.

0002-7863/90/1512-8629$02.50/0 