latter at 292 K ($t_{1/2}$ ca. 12 min, $\Delta G^{\ddagger} \approx 21.1$ kcal/mol),[14a] whereas quadricyclane derivatives, like quadricyclane itself ($t_{1/2} > 14$ h at 413 K, $\Delta G^{\ddagger} > 33.6$ kcal/mol),[16] are not thermally labile at room temperature. Since the activation barriers for the neutral molecules are much higher than those for the radical cations, the neutral transition states may reside further along the reaction coordinate. In this case, relief of strain could assume a dominant role in the neutral molecule reactions,[18] whereas altogether different structural factors might be expected to come into play for early transition states in the radical cation reactions.[19]

In summary, the present work has shown for the first time that the radical cation of a fused dicyclopropane ($\mathbf{1}^{\bullet+}$) can be formed photochemically by cycloaddition from its diene radical cation counterpart ($\mathbf{2}^{\bullet+}$). The observation of $\mathbf{1}^{\bullet+}$ is also significant as a novel example of a radical cation identified with a highly strained and *previously unrealized* molecule.[10]

Acknowledgment. We thank Professor Thomas Bally for his comments on the preprinted version of this paper. This work was supported by the Division of Chemical Sciences, Office of Basic Energy Sciences, U. S. Department of Energy (Grant No. DE-FG05-88ER13852).

(18) Computational results[10] suggest that the heat of reaction for the cleavage of neutral **1** is more exothermic than that of quadricyclane by ca. 10 kcal/mol. Accordingly, the greater reactivity shown by the neutral derivatives of **1** relative to those of its lower homologue is in keeping with a late transition state accompanied by relief of strain. Cf. Wiberg, K. B. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 312. Wiberg, K. B.; Waddell, S. T. *J. Am. Chem. Soc.* **1990**, *112*, 2194.

(19) An early transition state for the cleavage of the radical cations may be associated with a surface crossing from a $^2B_1$ to a $^2B_2$ state.[3]

A Dibenzofuran-Based Amino Acid Designed To Nucleate Antiparallel β-Sheet Structure: Evidence for Intramolecular Hydrogen-Bond Formation

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β-Sheets are found in almost all proteins, yet this secondary structure is poorly understood when compared to the α-helix.[1] We have recently reported the synthesis of amino acid **1** as part of our effort to develop small antiparallel β-sheets which are amenable to biophysical examination.[2a] The dibenzofuran-based amino acid was designed to form a specific intramolecular hydrogen bond of the type that will nucleate β-sheet formation within a polypeptide sequence.[3] We now report ^{1}H NMR and FT-IR evidence for an intramolecular hydrogen bond of this nature in diamide derivatives of **1**.[2b]

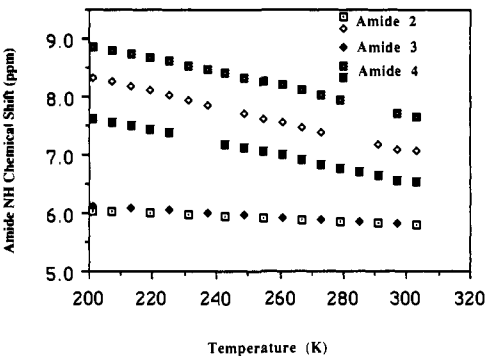
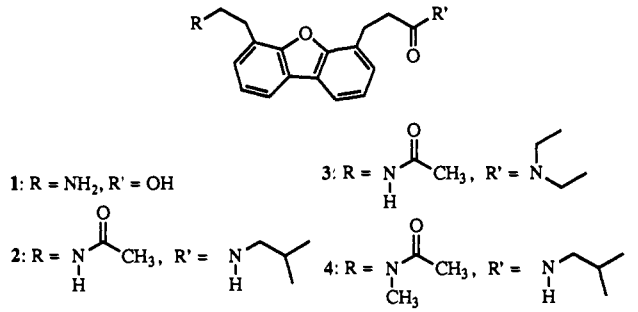


Figure 1. Temperature dependence of the amide proton NMR chemical shift for diamides **2**, **3**, and **4** in 1.5 mM CD_2Cl_2 solutions. Alternate data points for diamides **2** and **3** (around δ 6 ppm) were omitted for clarity. Data points around δ 7.2 ppm and δ 7.8 ppm are missing due to overlap with the aromatic region of dibenzofuran. The data were obtained on a Varian XL-400 spectrometer.



The ability of amides composed of **1** to undergo intramolecular hydrogen bonding in nonpolar solvents at room temperature is required, but is not sufficient, to predict their efficacy as nucleators in aqueous solutions. Other factors such as optimized geometry and hydrophobic interactions also play an important role in aqueous solvents. This paper presents evidence that satisfies the hydrogen-bonding criteria that a sheet nucleator must meet.[3,4] In order to examine the potential of amino acid **1** to nucleate antiparallel β-sheet formation, diamide **2** was synthesized. The degree of intramolecular amide–amide hydrogen bonding was determined by analyzing the temperature dependence of the amide proton NMR chemical shift and the amide N–H IR stretch region (3200–3500 cm^{-1}).[5]

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(2) (a) Díaz, H.; Kelly, J. W. *Tetrahedron Lett.* **1991**, *32*, 5725. (b) Diamides **2** and **3** were prepared from the *t*-Boc pentafluorophenol active ester of **1** and the corresponding amine followed by TFA deprotection and acetylation. Diamide **4** was prepared by methylation of the *t*-Boc derivative of **1** as described in ref 2c. All compounds were purified by crystallization or RP-HPLC and characterized by NMR and high-resolution mass spectroscopy. Full experimental details will be published elsewhere. (c) Cheung, S. T.; Benoiton, N. L. *Can. J. Chem.* **1977**, *55*, 906.

(3) According to studies reported recently, the incorporation of a rigid template (with the appropriate dimensions and geometry) into a polypeptide strand should decrease the chain entropy penalty associated with the nucleation of secondary structures. For details, see: (a) Kemp, D. S.; Curran, T. P.; Davis, W. M.; Boyd, J. G.; Mundel, C. *J. Org. Chem.* **1991**, *56*, 6672. (b) Kemp, D. S.; Curran, T. P.; Boyd, J. G.; Allen, T. J. *J. Org. Chem.* **1991**, *56*, 6683. (c) Kemp, D. S.; Boyd, J. G.; Mundel, C. *Nature* **1991**, *352*, 451. (d) Williams, D. H.; Cox, J. P. L.; Doig, A. J.; Gardner, M.; Gerhard, U.; Kaye, P. T.; Lal, A. R.; Nicholls, I. A.; Salter, C. J.; Mitchell, R. C. *J. Am. Chem. Soc.* **1991**, *113*, 7020.

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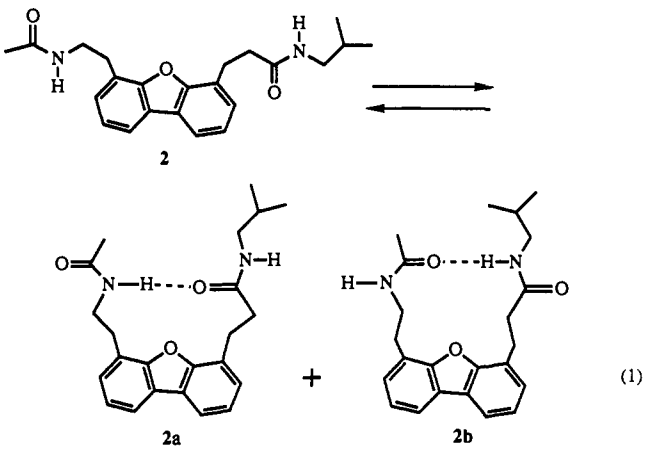
Inspection of CPK models suggests that both amide groups in compound **2** are available (as donors or acceptors) for intramolecular hydrogen bonding (eq 1). The ^{1}H NMR spectrum of **2**



(1)

(1.5 mM in CD_2Cl_2, room temperature) shows resonances for the amide protons at δ 7.05 ppm and δ 5.79 ppm. Furthermore, the signal at lower field shows a large temperature dependence ($\Delta\delta/\Delta T$ = −0.013 ppm/K) whereas the signal at higher field is not significantly affected by temperature ($\Delta\delta/\Delta T$ = −0.003 ppm/K) (Figure 1). It has been previously demonstrated that the amide proton chemical shift (δ 5.49 ppm) of a mixture of *N*-methylacetamide and *N*,*N*-dimethylacetamide in non-hydrogen-bonding solvents (1 mM CD_2Cl_2) shows a small temperature dependence ($\Delta\delta/\Delta T$ = −0.003 ppm/K).[5] The observed chemical shift and the small $\Delta\delta/\Delta T$ have been attributed to the absence of amide–amide hydrogen-bonding interactions under these conditions.

On the basis of the large difference of the amide proton chemical shifts and the temperature dependences (Figure 1), it can be inferred that intramolecular hydrogen bonding to one amide group is preferred over hydrogen bonding to the other amide group in **2**. Analysis of the amide N–H IR stretch region of **2** (1.5 mM in CH_2Cl_2, room temperature) reveals two bands with maxima at 3447 cm^{-1} (nonbonded N–H) and 3336 cm^{-1} (hydrogen-bonded N–H) (Figure 2a). Since we found it difficult to establish whether structure **2a** or **2b** (or a combination thereof) is the favored hydrogen-bonded conformation, compounds **3** and **4** were prepared.

In diamide **3** the acetamide functionality is the only potential hydrogen-bond donor while the diethylamide serves as the only conceivable hydrogen-bond acceptor. The inverse situation regarding possible hydrogen-bond contributions exists in diamide **4**. The tertiary acetamide group can act only as the hydrogen-bond acceptor while the isobutylamide is the only possible hydrogen-bond donor.

The ^{1}H NMR analysis of **3** displays a signal for the acetamide N–H at δ 5.80 ppm and a small temperature dependence ($\Delta\delta/\Delta T$ = −0.003 ppm/K) (Figure 1). As expected, however, the proton NMR spectrum of compound **4** revealed two amide signals in a nearly 1:1 ratio (δ 6.83 ppm, $\Delta\delta/\Delta T$ = −0.011 ppm/K; δ 8.02 ppm, $\Delta\delta/\Delta T$ = −0.012 ppm/K). This observation is consistent with *E*/*Z* isomerization around the tertiary amide bond in **4**. According to these data, both isomers appear to be intramolecularly hydrogen-bonded in CH_2Cl_2 solutions.

Examination of the amide N–H IR stretch region of **3** shows a single band with a maximum at 3446 cm^{-1} (nonbonded N–H) (Figure 2b). Interestingly, compound **4** appears to be nearly completely hydrogen-bonded, revealing a weak band at 3446 cm^{-1} (nonbonded N–H) and an intense band at 3324 cm^{-1} (hydrogen-bonded N–H) (Figure 2c). These data suggest that the isobutylamide functionality in **2** is the preferred hydrogen-bond donor and the acetamide is the hydrogen-bond acceptor (structure **2b**).

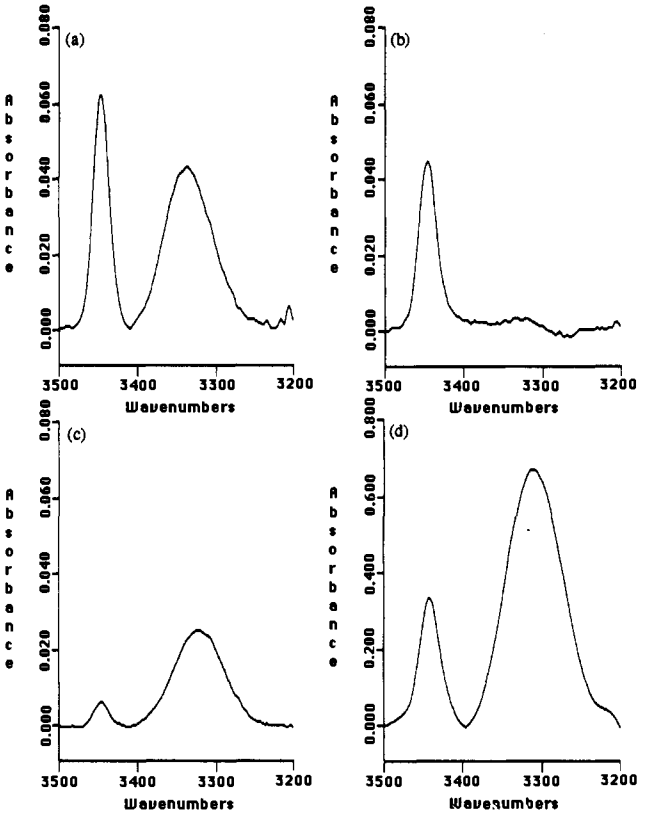
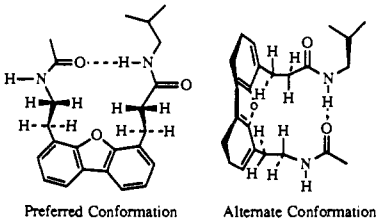


Figure 2. N–H stretch FT-IR region for diamides **2**, **3**, and **4** in CH_2Cl_2 solutions at room temperature: (a) 1.5 mM diamide **2**; (b) 1.5 mM diamide **3**; (c) 1.5 mM diamide **4**; (d) 1200 mM diamide **3** (this demonstrates that, at 1.5 mM concentrations (room temperature), no intermolecular hydrogen bonding should be expected in the series of diamides studied). All spectra were collected on a Galaxy 6021 spectrometer. Base-line corrections were applied in each case.

A computational study suggests that an ideal intramolecular hydrogen bond can form in conformation **2b** which is free of significant torsional and nonbonded strain in CH_2Cl_2, whereas the hydrogen bond shown in conformer **2a** cannot form without imposing a significant amount of strain in the rest of the molecule.[6] The intramolecular hydrogen bonding capability of **1** fulfills the first requirement of a potential β-sheet nucleator. The ability of peptides containing **1** to cooperatively fold in aqueous solvents remains to be demonstrated.

(6) Molecular dynamics runs of 25 ps were carried out at 700 K without constraints using the CVFF force field in Discover (Biosym Technologies). Conformations were sampled at 1-ps intervals. Annealing of each structure at 300 K for 2 ps was performed to remove strain. Each structure was then minimized first using steepest descents and later with conjugate gradients using cross terms and Morse bond potentials until the derivative reached 0.0001 kcal mol^{-1} Å^{-1}. Constraints were used in some cases to test the energy of the hydrogen-bonded isomeric forms, but were always released in the end before final minimization. The dielectric constant used in the minimizations was that of CH_2Cl_2. The preferred intramolecularly hydrogen bonded conformation shown below facilitates an ideal hydrogen bond without imposing strain in the molecule and resulted from the majority of conformers sampled during dynamics. One other low-energy conformation was observed; however, the hydrogen bonding within this conformation is not ideal.



Preferred Conformation Alternate Conformation

Acknowledgment. We thank the referees for their thoughtful and thorough review, Professor Donald Darensbourg for providing access to FT-IR facilities, and Earl Atnip for his assistance with the IR instrumentation. We gratefully acknowledge the financial support of the Robert A. Welch Foundation, the Searle Scholars Program/The Chicago Community Trust (J.W.K.), and the Texas Higher Education Coordinating Board's Advanced Research Program.

Liposomal Stabilization of Camptothecin's Lactone Ring

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Camptothecin displays unprecedented antitumor activities against human colon cancer.[1] To date its full therapeutic utility has been limited by poor water-solubility and the aqueous instability of the lactone ring moiety. Ring opening is rapid, resulting in a complete loss of biological activity.[2] In this communication, we demonstrate that liposome-bound camptothecin is stable, thus suggesting that liposomes may serve as useful drug delivery systems for solubilizing camptothecin and conserving both its lactone ring and antitumor activity.

In order to study the equilibrium associations of camptothecin with lipid bilayers, we exploited the drug's intense intrinsic fluorescence. Fluorescence is associated with the extended conjugation of the quinoline ring system. Upon association with small unilamellar vesicles (SUVs) composed of L-α-dimyristoyl phosphatidylglycerol (DMPG), the λ_{max} value of camptothecin's emission spectrum shifts to lower wavelength, or blue shifts, some 16 nm (Figure 1).

While these spectral changes provide qualitative evidence that camptothecin binds membranes, fluorescence anisotropy titration offers the most sensitive means for quantitatively assessing the extent of drug binding. For example, Figure 2 shows a 19-fold enhancement in camptothecin's steady-state anisotropy (a) value upon drug association with DMPG vesicles. Similar results are shown for drug binding to electroneutral L-α-dimyristoyl phosphatidylcholine (DMPC). Analysis of the data using double-reciprocal plots[3] gave identical overall association constants of 100 M^{-1}.

Having determined the concentrations of DMPC and DMPG required to assure a bound drug fraction in excess of 97%, we then used an HPLC assay[4] to evaluate the stability of free and liposome-bound drug (Figure 2, inset). We observed rapid hydrolysis of free camptothecin in PBS at 37 °C ($t_{1/2}$ = 16.6 min). In

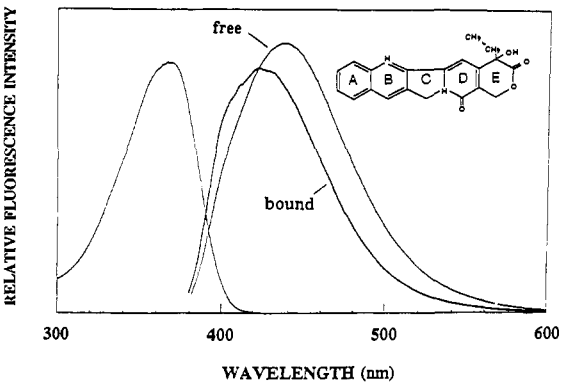

Figure 1. Fluorescence excitation and emission spectra of camptothecin (1 μM, PBS buffer, 37 °C). Also shown is the emission spectra for DMPG-bound drug (0.29 M lipid).

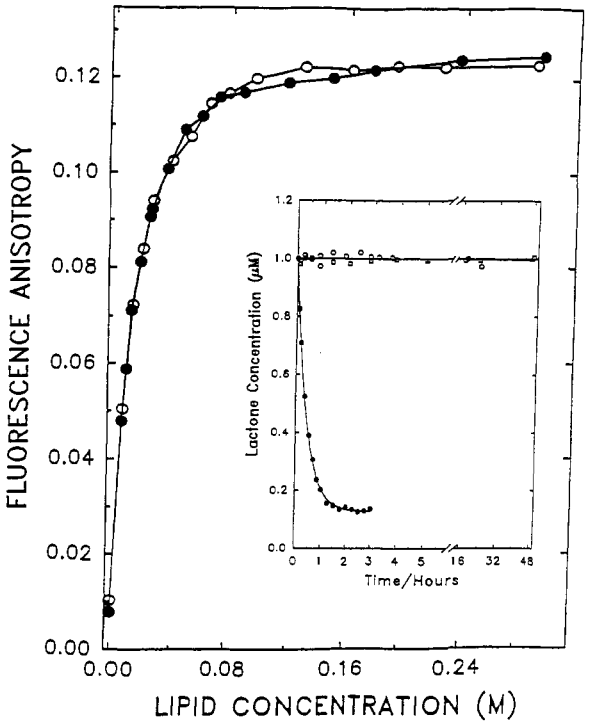

Figure 2. Equilibrium binding of camptothecin to SUVs composed of DMPG (○) and DMPC (●). The inset shows the change in lactone concentration as a function of time for free drug (●), DMPC-bound drug (○), and DMPG-bound drug (□). Drug and lipid concentrations of 1 μM and 0.29 M, respectively, were used (PBS buffer, 37 °C).

marked contrast, however, camptothecin was found to be stable in both DMPC and DMPG bilayers, with no evidence of ring opening even at time points as long as 48 h. Stabilization was also achieved in liposomal formulations containing drug concentrations of 2 mM, where the lipid:drug ratio was reduced to 150.

The fact that liposome-associated camptothecin is stable suggests that the drug's lactone ring penetrates into the bilayer. Two types of spectroscopic data are available which support this notion. The first type of evidence comes from Figure 1, where blue shifting of the drug's emission spectrum is observed upon association with membrane. Such a spectral shift is indicative of a change in the dielectric constant of the medium surrounding the fluorophore, as when a compound leaves an aqueous environment and intercalates in between the lipid acyl chains.[5]

Additional evidence that camptothecin's fluorochrome penetrates into the lipid bilayer comes from iodide quenching data.[6]

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(2) Wani, M. C.; Nicholas, A. W.; Manikumar, G.; Wall, M. E. *J. Med. Chem.* **1987**, *30*, 1774-1779.

(3) Burke, T. G.; Tritton, T. R. *Biochemistry* **1985**, *24*, 1768-1776.

(4) The kinetics of lactone ring opening was monitored by HPLC with fluorescence detection. Separation of parent drug from carboxylate form was achieved on an Ultrasphere C-18 column using an isocratic mobile phase consisting of 32% acetonitrile, 67% 0.1 M acetate buffer (pH 5.5), and 1% 0.1 M sodium dodecyl sulfate. The detergent sodium dodecyl sulfate was added to samples prior to injection.

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