# Searching for Minimum Increments of Hydrophobic Collapse: Flexible Dinaphthyl Carboxylates

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Abstract: In an effort to identify minimal units of hydrophobically induced folding, we have examined flexible molecules containing two naphthyl moieties connected by a four-atom linker that also bears a carboxyl group. Crystallographic data show that the linkers allow intramolecular edge-to-face association of the naphthyl groups without excessive strain in the backbone. For the carboxylate forms of the dinaphthyl compounds, the occurrence of intramolecular naphthyl—naphthyl proximity in aqueous solution (24 °C) was detected via upfield shifts in the aromatic region <sup>1</sup>H NMR signals, relative to mononaphthyl control compounds. The naphthyl—naphthyl proximity does not appear to be strongly "hydrophobically driven", however, because similar upfield shifts (dinaphthyl vs mononaphthyl carboxylates) were observed in 8 M aqueous urea, and for the corresponding carboxylic acids in CDCl<sub>3</sub> and C<sub>6</sub>D<sub>6</sub>. We conclude that these upfield shifts largely reflect chance encounters between the naphthyl groups resulting from random conformational motion.

## Introduction

The protection of nonpolar surfaces from aqueous solvation is widely believed to play a major role in determining the complexation behavior and conformational preferences of biopolymers<sup>1</sup> and small molecules.<sup>2,3</sup> There is still debate, however, on the extent to which hydrophobic clustering influences noncovalently controlled structural phenomena.<sup>4</sup> This uncertainty arises in part because of continued disagreement on the mechanism by which nonpolar—nonpolar interactions exert their energetically stabilizing effects, i.e., on the origin of the "hydrophobic effect". We have been trying to identify simple molecules (lowest possible molecular weight) in which hydrophobic forces influence conformation. Careful examination of folding processes in such systems should provide insight on the extent to which the hydrophobic effect determines

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structure and behavior in more complex systems. Further, the folding of small molecules in aqueous solution has become a subject of interest in its own right with the recent proposal that the biological activity of some drugs may be influenced by hydrophobically induced conformational preferences.<sup>3f</sup>

In this paper we describe the solution behavior of 1a-3a, which contain two hydrocarbon aromatic moieties connected via a flexible tether; the tether segment also contains a carboxylate to confer water solubility. These molecules were chosen because the magnetic anisotropy induced by the aromatic  $\pi$ -electrons allows for sensitive detection of intramolecular proximity via <sup>1</sup>H NMR spectroscopy. Aromatic moieties are well-represented in proteins and their natural and synthetic ligands.

We will side-step much of the current debate on the nature of hydrophobicity by employing a strictly empirical definition: the term "hydrophobic" here refers to whatever makes hydrocarbons poorly soluble in water. According to this empirical definition, aromatic hydrocarbons are hydrophobic entities, although somewhat less so than aliphatic hydrocarbons. Thus, for example, the thermodynamic signatures for the transfer of benzene and cyclohexane from the pure liquid to dilute aqueous solution are qualitatively similar to one another.<sup>4c</sup> In both cases, at 25 °C,  $\Delta G$  is positive,  $\Delta H$  is negligible,  $\Delta S$  is large and negative, and  $\Delta C_p$  is large and positive. (According to  $\Delta G$ ,  $\Delta S$ , and  $\Delta C_p$ , cyclohexane is more hydrophobic than benzene, perhaps because of the ability of the aromatic  $\pi$ -electrons to engage in weak hydrogen bonds to water.<sup>5</sup>) Further evidence for the operational hydrophobicity of hydrocarbon aromatic moieties is found in the many reported scales of amino acid hydropathy: most scales rank phenylalanine among the most hydrophobic residues.<sup>6,7</sup>

<sup>&</sup>lt;sup>®</sup> Abstract published in Advance ACS Abstracts, June 1, 1995.

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It has been suggested that aromatic amino acid side chains contribute to protein conformational stability via an intrinsic "weakly polar" attraction between the aromatic groups.<sup>8</sup> This proposal was based in part on the behavior of small aromatic hydrocarbons, which display "herringbone" or "edge-to-face" juxtapositions with their neighbors in the crystalline state.<sup>9</sup> The benzene dimer in the gas phase also appears to adopt a nonparallel arrangement.<sup>10</sup> This geometrical preference has been rationalized on the basis of polar interactions between the aromatic groups.<sup>11</sup> Two observations, however, indicate that the contribution of polar aromatic-aromatic interactions to protein stability is quite small. First, statistical surveys of aromatic-aromatic pairs in crystalline proteins reveal a nearly random orientation of the aromatic moieties relative to one another.<sup>12</sup> Second, a carefully constructed model system recently reported by Wilcox et al. shows that edge-to-face orientation of aromatic rings does not provide a significant conformation-directing force in solution.<sup>13</sup> Nevertheless, recent molecular dynamics calculations suggest that benzene molecules prefer to associate in edge-to-face rather than parallel fashion in aqueous solution.14

In a study related to the present one, we found that no intramolecular naphthyl-naphthyl stacking could be detected by <sup>1</sup>H NMR for 4 in aqueous solution (there were no upfield shifts of the arvl proton resonances of dinaphthyl 4 relative to a mononaphthyl reference compound).<sup>15</sup> In contrast, stacking could be detected by <sup>1</sup>H NMR for analogues of 4 in which one or both of the naphthyl groups was or were replaced by adenine.<sup>15</sup> The three-atom linker of **4** requires the naphthyl groups to associate intramolecularly in a parallel or near-parallel fashion. The four-atom linkers of 1a-3a, on the other hand, are long enough to allow intramolecular edge-to-face approach. These longer linkers are of particular interest because of the computational prediction that the benzene dimer prefers an edgeto-face geometry in aqueous solution.<sup>14</sup> The results provided below suggest that the four-atom tethers do allow the linked aromatic groups to find one another in solution, but that there

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**Figure 1.** Crystal structure of carboxylic acid 1b. The angle between the mean planes of the naphthyl units is  $51^{\circ}$ .



Figure 2. Crystal structure of carboxylic acid 2b. The angle between the mean planes of the naphthyl units is  $51^{\circ}$ .

is little driving force, hydrophobic or otherwise, for this aromatic-aromatic proximity.

#### Results

**Crystal Structures.** The diaryl carboxylic acids 1b-3b, their sodium salts 1a-3a, and various reference compounds discussed below were prepared by standard methods, as described in the Experimental Section. The crystal structures of 1b and 2b are shown in Figures 1 and 2. These two dinaphthyl carboxylic acids adopt nearly identical conformations in the solid state, with an intramolecular edge-to-face orientation of the naphthyl moieties. In both cases, the angle between the planes of the linked naphthyl groups (51°) is nearly identical to the interplanar angle for neighboring molecules in crystalline naphthalene itself.<sup>16</sup> The conformations observed for these flexible molecules in the solid state do not provide any direct information on conformational preferences in solution, but the presence of these folding patterns in the crystals suggests that similar folded conformations are energetically accessible in solution.

**Detection of Aromatic**—Aromatic Proximity via <sup>1</sup>H NMR. Figure 3 shows aromatic region <sup>1</sup>H NMR data for dinaphthyl carboxylate **1a** and for a 1:1 mixture of mononaphthyl carboxylates **5a** and **6a**, both samples in D<sub>2</sub>O at 24 °C. Qualitative comparison suggests that at least some of the resonances of the dinaphthyl carboxylate are shifted upfield relative to the corresponding resonances in the mixture of mononaphthyl carboxylates. (It is impossible to make assignments based upon these one-dimensional data.)

Before the <sup>1</sup>H NMR differences observed between **1a** and 5a + 6a (Figure 3) can be attributed to intramolecular naphthylnaphthyl proximity, it must be demonstrated that aggregation does not occur under these conditions. Figure 4 shows the variation of the chemical shifts of two aromatic resonances of **1a** as a function of the logarithm of concentration. Since **1a** is composed largely of aromatic subunits, one expects self-

<sup>(5)</sup> It has long been known that  $\pi$ -systems can serve as hydrogen bond acceptors toward hydroxyl groups. For historical and leading references, see: (a) Schleyer, P. v. R.; Wintner, C.; Trifan, D. S.; Backskai, R. *Tetrahedron Lett.* **1959**, *14*, 1. (b) Oki, M.; Iwamura, H. *Bull. Chem. Soc. Jpn.* **1960**, *33*, 427. (c) Bakke, J. M.; Chadwick, D. J. *Acta Chem. Scand.* **1988**, *B42*, 223. (d) Suzuki, S.; Green, P. G.; Bumgarner, R. E.; Dasgupta, S.; Goddard, W. A.; Blake, G. A. Science **1992**, *257*, 942.

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association to be signaled by upfield shifts in proton resonances. Indeed, slight upfield shifts are observed above ca. 0.5 mM, which suggests that self-association begins around this concentration. We conclude that the spectrum in Figure 3 represents monomeric **1a**, because these data were obtained at 0.2 mM, below the apparent onset of aggregation. Figure 5 shows the variation of the chemical shift of one aromatic resonance of mononaphthyl carboxylate **5a**. Aggregation is detectable only above 10 mM (we expect mononaphthyl carboxylate **6a** to behave similarly). This result suggests that aggregation is insignificant under the conditions used to obtain the spectrum of 1:1 **5a:6a** (2 mM each) in Figure 3. Therefore, we attribute the upfield shifts observed for **1a** relative to **5a** + **6a** in Figure 3 to intramolecular naphthyl-naphthyl proximity in the dinaphthyl compound.

The dinaphthyl system with a sulfur-containing linker, 2, was examined because CS-CC torsional units have weaker conformational preferences than CC-CC units,<sup>17</sup> and we suspected that torsional strain opposing internal aromatic-aromatic association might be lower in 2 than in 1. Figure 6 compares aromatic region <sup>1</sup>H NMR data for dinaphthyl carboxylate 2a with analogous data for a 1:1 mixture of mononaphthyl carboxylates 7a and 8a, in D<sub>2</sub>O at 24 °C. The upfield shifts for dinaphthyl carboxylate 2a (relative to the reference compounds) are more substantial than was observed for 1a, the analogue with the all-carbon tether (Figure 3). Figure 7 shows that there is no concentration dependence for several of the aromatic resonances of dinaphthyl carboxylate 2a between 0.01 and 0.7 mM; all other aromatic resonances were also concentration independent over this concentration range.<sup>18</sup> The limited solubility of this salt precluded evaluation of higher concentrations. We conclude from these data that 2a does not aggregate significantly up to its solubility limit in aqueous solution.<sup>19</sup>

An alternative interpretation of the concentration independence illustrated in Figure 7 is that 2a is fully aggregated



Figure 3. Aromatic region <sup>1</sup>H NMR (270 MHz) comparison between dinaphthyl carboxylate 1a (0.2 mM; lower) and a 1:1 mixture of mononaphthyl carboxylates 5a and 6a (2 mM each; upper) in  $D_2O$  at 24 °C.



Figure 4. NMR chemical shifts of H1 ( $\times$ ) and H 11 ( $\bigcirc$ ) of dinaphthyl carboxylate 1a in D<sub>2</sub>O as a function of the logarithm of concentration, between 0.01 and 2.2 mM, at 24 °C. These data suggest that aggregation occurs gradually above 1 mM. NMR samples  $\ge 1.2$  mM were turbid. A nominally 1.7 mM solution of 1a did not solubilize the hydrophobic dye orange OT.

even at 0.01 mM, but several lines of evidence argue against this possibility. First, analogue **1a** just barely begins to aggregate at 1 mM (Figure 4), and it seems unlikely that simply replacing one methylene group with a sulfur atom, to generate **2a**, would so dramatically increase self-association. Second, 0.7 mM **2a** fails to solubilize the hydrophobic dye orange OT. Solubilization of orange OT and related substances is commonly used to detect micelle formation,<sup>20</sup> and the lack of solubilization by **2a** suggests that this carboxylate is not in a micellar state at its solubility limit. Third, we know of no precedent for such avid aggregation in aqueous solution by a species of similar charge and molecular weight to **2a**.<sup>19</sup>

Concentration-dependent NMR studies of mononaphthyl carboxylates **7a** and **8a** indicate aggregation does not exert a significant effect on the data in Figure 6. Figure 8 shows the concentration-dependent variation in chemical shift of one aromatic proton resonance of mononaphthyl carboxylate **7a**.

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<sup>(18)</sup> Data may be found in the supplementary material.

<sup>(19)</sup> The failure of carboxylate **2a** to aggregate up to its aqueous solubility limit is well precedented in the behavior of ionic surfactants. Most ionic surfactants have a characteristic "Krafft temperature", below which the solubility of the monomeric surfactant is smaller than the minimum concentration for aggregation. See: Myers, D. Surfactant Science and Technology, 2nd ed.; VCH Publishers: New York, 1992.



Figure 5. NMR chemical shift of H1 of mononaphthyl carboxylate 5a in D<sub>2</sub>O as a function of the logarithm of concentration, between 1 and 250 mM, at 24 °C. These data suggest that aggregation occurs gradually above 10 mM. NMR samples  $\ge 200$  mM were turbid. The behavior of the other aryl protons was similar (see supplementary material).



Figure 6. Aromatic ring <sup>1</sup>H NMR (270 MHz) comparison between dinaphthyl carboxylate 2a (0.2 mM; lower) and a 1:1 mixture of mononaphthyl carboxylates 7a and 8a (5 mM each; upper) in D<sub>2</sub>O at 24 °C.



Figure 7. NMR chemical shifts of H1 ( $\times$ ) and H11 ( $\bigcirc$ ) of dinaphthyl carboxylate 2a in D<sub>2</sub>O as a function of the logarithm of concentration, between 0.01 and 0.7 mM, at 24 °C. These data show no sign of aggregation over this concentration range. NMR samples at 0.4 and 0.7 mM were turbid. A nominally 0.7 mM solution of 2a did not solubilize the hydrophobic dye orange OT. The behavior of the other aryl protons was similar (see supplementary material).

These data suggest that little or no aggregation occurs up to 50 mM. Analgous data for mononaphthyl carboxylate **8a** (Figure 9) indicate that aggregation begins above 10 mM. These results



Figure 8. NMR chemical shifts of H1 of mononaphthyl carboxylate 7a in  $D_2O$  as a function of the logarithm of concentration, between 0.2 and 98 mM, at 24 °C. These data suggest that aggregation occurs gradually above 10 mM. The sample of highest concentration slowly precipitated. The behavior of all six other aryl protons was similar (see supplementary material).



Figure 9. NMR chemical shifts of H11 of mononaphthyl carboxylate 8a in  $D_2O$  as a function of the logarithm of concentration, between 0.7 and 56 mM, at 24 °C. These data suggest that aggregation occurs gradually above 5 mM. The behavior of the other aryl protons was similar (see supplementary material).

indicate that there should not be significant aggregation in the solution containing 1:1 7a:8a, 5 mM each. Therefore, we attribute the upfield shifts observed for 2a relative to 7a + 8a in Figure 6 to intramolecular naphthyl-naphthyl proximity in the dinaphthyl compound.

Diphenyl carboxylate 3a, which is analogous to 2a, was also examined for intramolecular aryl-aryl proximity in aqueous solution. Figure 10 compares aromatic region <sup>1</sup>H NMR data for 3a (2 mM) and for a 1:1 mixture of monophenyl carboxylates 9a and 10a (4 mM each) in D<sub>2</sub>O at 24 °C. At least some of the aromatic resonances of the diphenyl compound appear to be shifted upfield relative to the controls. Figure 11 shows the concentration dependence of the chemical shift of one of the aromatic proton resonance for 3a, which indicates that there is no significant aggregation up to 100 mM. Variable-concentration studies were not performed for 9a and 10a, but we assume that these monophenyl carboxylates will aggregate less avidly than their naphthyl analogues, 7a and 8a. We therefore conclude that the upfield shifts observed for 3a relative to 9a + 10a arise from intramolecular phenyl-phenyl proximity in 3a.

Solvent Effects. The <sup>1</sup>H NMR data presented above indicate that the covalently linked aromatic moieties of 1a-3a spend some time near one another in aqueous solution. In order to determine whether this intramolecular proximity is hydrophobically induced, we carried out <sup>1</sup>H NMR comparisons of diaryl



Figure 10. Aromatic region <sup>1</sup>H NMR (270 MHz) comparison between the diphenyl carboxylate **3a** (2 mM; lower) and a 1:1 mixture of monophenyl carboxylates **9a** and **10a** (4 mM each; upper) in  $D_2O$  at 24 °C.



Figure 11. NMR chemical shifts of the most upfield proton resonance of diphenyl carboxylate 3a in  $D_2O$  as a function of the logarithm of concentration, between 1.0 and 100 mM, at 24 °C. These data suggest that no aggregation occurs in this concentration range (the variation of ca. 0.03 ppm is within the uncertainty of the measurement).

carboxylic acids 1b-3b with mixtures of the appropriate monoaryl carboxylic acids in  $C_6D_6$ . The hydrophobic effect is inoperative, by definition, in the absence of water, and we assume that there is no driving force for intramolecular naphthyl-naphthyl or phenyl-phenyl collapse in benzene solution. Therefore, the  $C_6D_6$  results should represent chemical shift effects arising from random internal motions of one aryl group relative to the other.

Naphthyl proton NMR data for **2b** vs (**7b** + **8b**) in C<sub>6</sub>D<sub>6</sub>, shown in Figure 12, reveal significant upfield shifts for dinaphthyl carboxylic acid **2b** relative to the 1:1 mixture of mononaphthyl carboxylic acids. None of these three carboxylic acids displays concentration dependence in aryl proton chemical shifts in this solvent.<sup>18</sup> Qualitatively similar behavior was observed for **1b** and **3b** relative to their control compounds in C<sub>6</sub>D<sub>6</sub>.<sup>18</sup> NMR comparisons were conducted for dinaphthyl system **2** under two other sets of conditions expected to "turn off" the hydrophobic effect, **2b** vs (**7b** + **8b**) in CDCl<sub>3</sub>, and **2a** vs (**7a** + **8a**) in 8 M aqueous urea. In both cases, substantial upfield shifts were observed for the dinaphthyl aromatic protons relative to the protons on the mononaphthyl reference compounds.<sup>18</sup>



Figure 12. Aromatic region <sup>1</sup>H NMR (270 MHz) comparison between dinaphthyl carboxylic acid 2b (0.5 mM; lower) and a 1:1 mixture of mononaphthyl carboxylic acids 7b and 8b (4 mM each; upper) in  $C_6D_6$  at 24 °C.

Detailed Characterization of Naphthyl-Naphthyl Proximity. More complete comparison of diaryl folding behavior in aqueous and organic solvents required assignment of the aromatic proton resonances. The dinaphthyl compounds with the sulfur-containing linker (2) were selected for this in-depth study, because this system showed the greatest dispersion among aromatic proton resonances, the most dramatic upfield shifts relative to the mononaphthyl reference compounds, and the greatest dispersion among the proton resonances on the linking segment (this last point was crucial for assigning aromatic proton resonances to the proper naphthyl ring). Naphthyl proton NMR resonances were assigned via a combination of NOESY and TOCSY measurements, as described in the Experimental Section.

Table 1 provides a comparison of  ${}^{1}H-{}^{1}H$  chemical shift differences,  $\Delta \delta = \text{dinaphthyl } \delta - \text{mononaphthyl } \delta$ , for 2a vs (7a + 8a) in D<sub>2</sub>O and for 2b vs (7b + 8b) in C<sub>6</sub>D<sub>6</sub>. The  $\Delta\Delta\delta$ values in the rightmost column were obtained by subtracting the C<sub>6</sub>D<sub>6</sub>  $\Delta\delta$  from the D<sub>2</sub>O  $\Delta\delta$ . The substantial  $\Delta\delta$  values observed for some protons in both solvents indicate that there is significant population of conformers in which the naphthyl groups lie near their intramolecular neighbors in both water and benzene. As discussed above, we attribute the  $\Delta\delta$  values observed for **2b** vs (7b + 8b) in C<sub>6</sub>D<sub>6</sub> exclusively to stochastic internal motions that periodically bring the tethered naphthyl groups near one another, because there should be no driving force for naphthyl-naphthyl association in benzene solution. (In other words, we assume that there is no significant energetic advantage for naphthyl-naphthyl interaction over naphthylbenzene interaction.) Therefore, the fact that all of the  $\Delta\Delta\delta$ values in Table 1 are small, with only a few values significantly different from zero, suggests that there is little hydrophobic driving force for pairwise naphthyl-naphthyl association in aqueous solution at room temperature.<sup>21</sup>

Table 2 shows coupling constant data that provide further support for the conclusion that there is little "hydrophobic collapse" in dinaphthyl carboxylate **2a** in aqueous solution. This

<sup>(21)</sup> In order to estimate the limiting secondary <sup>1</sup>H chemical shift for an "edge" aryl proton in an edge-to-face aromatic pair, we have examined a series of [4.4]thiccyclophanes that promote intramolecular edge-to-face juxtaposition. These studies suggest that these secondary <sup>1</sup>H chemical shifts are >1 ppm: Schladetzky, K. D.; Haque, T. S.; Gellman, S. H. J. Org. Chem., in press.

Table 1. Upfield <sup>1</sup>H NMR Shifts of Aromatic Protons of Dinaphthyls 2a,b Relative to Mononaphthyls 7a,b and 8a,b

proton <sup>a</sup>	$\Delta \delta(\mathrm{D_2O})^b$	$\Delta \delta (C_6 D_6)^c$	$\Delta\Delta\delta^d$
1	-0.34	-0.27	-0.07
3	-0.30	-0.25	-0.05
4	-0.25	-0.15	-0.10
6	-0.09	-0.04	-0.05
7	-0.05	-0.01	-0.04
8	-0.05	-0.01	-0.04
9	-0.28	-0.14	-0.14
11	-0.15	-0.13	-0.02
13	-0.22	-0.17	-0.05
14	-0.20	-0.15	-0.05
16	-0.04	-0.03	-0.01
17	0.00	0.00	0.00
18	0.00	0.00	0.00
19	-0.10	-0.08	-0.02

<sup>a</sup> Proton on 2a or 2b. <sup>b</sup>  $\delta(2a) - \delta(7a \text{ or } 8a)$ ; 2a at 0.2 mM, 7a and 8a at 5 mM; uncertainty  $\pm 0.03$  ppm. <sup>c</sup>  $\delta(2b) - \delta(7b \text{ or } 8b)$ ; 2b at 1 mM, 7b and 8b at 5 mM; uncertainty  $\pm 0.03$  ppm. <sup>d</sup>  $[\delta(2a) - \delta(7a \text{ or } 8a)]_{D_2O} - [\delta(2b) - \delta(7b \text{ or } 8b)]_{C_6D_6}$ ; uncertainty  $\pm 0.04$  ppm.

Table 2. Vicinal Proton-Proton Coupling Constants from the Flexible Tethers of Dinaphthyls 2a,b and Mononaphthyls 7a,b

compd	solvent	$^{3}J_{\mathrm{HH}}(\mathrm{Hz})$
2a	D <sub>2</sub> O	7.7
		8.4
7a	$D_2O$	7.7
		8.1
2b	$C_6D_6$	6.8
		8.6
7b	$C_6D_6$	6.7
		8.8

table compares the two vicinal proton-proton coupling constants ( ${}^{3}J_{\rm HH}$ ) from the tethers of **2a** and of reference compound **7a** in D<sub>2</sub>O, and from the tethers of **2b** and of **7b** in C<sub>6</sub>D<sub>6</sub>. In each case, these vicinal coupling constants are consistent with random conformational averaging along the flexible carboncarbon bond under scrutiny.

Table 3 shows NMR data that provide further insight on the origin of the upfield shifts of aromatic proton resonances for dinaphthyl system 2 relative to mononaphthyl reference compounds. This table contains  $\Delta\delta$  data obtained in CDCl<sub>3</sub> for four variations on the 2 vs (7 + 8) theme, in which the branching substituent on the tether is varied from carboxylic acid [2b vs (7b + 8b) to methyl ester [2c vs (7c + 8c)] or methoxymethyl [2d vs (7d + 8d)], or eliminated [2e vs (7e + 8e)]. Comparison of the results for 2b vs (7b + 8b) in CDCl<sub>3</sub> (first column in Table 3) and  $C_6D_6$  (second column in Table 1) shows that the pattern of  $\Delta \delta$  values is indistinguishable in these two solvents, within the uncertainty of these measurements. The similarity of behavior in  $C_6D_6$  and  $CDCl_3$  suggests that there is not a significant "polar" attraction between the naphthyl groups. This result is consistent with the recent demonstration by Wilcox et al. that there is little or no intrinsic attraction between aromatic rings juxtaposed in an edge-to-face manner.13

Comparison of the results in Table 3 for 2b vs (7b + 8b)with the results for 2c vs (7c + 8c) indicates that esterification has virtually no effect on the pattern of  $\Delta\delta$  values. Conversion of the carboxylic acid group to a methoxymethyl substituent [2d vs (7b + 8b)] leads to somewhat larger changes in the  $\Delta\delta$ values, although only a few of these differences are beyond the level of uncertainty. The most significant effect on the pattern of  $\Delta\delta$  values is observed when the carboxylic acid group is replaced by a proton [2e vs (7e + 8e)]. This elimination of the branching substituent causes all eight of the  $\Delta\delta$  values that were significant in 2b to become substantially smaller; only three

Table 3.<sup>*a*</sup> Upfield <sup>1</sup>H NMR Shifts in CDCl<sub>3</sub> of Aromatic Protons of Dinaphthyls **2b**-e Relative to Mononaphthyls **7b**-e and **8b**-e

		R =				
proton <sup>b</sup>	CO <sub>2</sub> H	CO <sub>2</sub> Me	CH <sub>2</sub> OCH <sub>3</sub>	Н		
1	-0.22	-0.21	-0.15	-0.09		
3	-0.22	-0.20	-0.16	-0.08		
4	-0.14	-0.13	-0.07	-0.04		
6	-0.04	-0.02	-0.02	-0.01		
7	-0.03	-0.03	0.00	0.00		
8	-0.03	-0.03	0.00	0.00		
9	-0.17	-0.14	-0.11	-0.07		
11	-0.10	-0.07	-0.13	-0.01		
13	-0.14	-0.11	-0.12	-0.01		
14	-0.14	-0.11	-0.12	-0.01		
16	-0.03	-0.01	-0.02	-0.01		
17	+0.01	0.00	+0.01	-0.02		
18	+0.01	0.00	+0.01	-0.02		
19	-0.10	-0.07	-0.11	-0.02		

<sup>*a*</sup> Uncertainties  $\pm 0.03$  ppm. <sup>*b*</sup> Proton on **2b**-e. The reported  $\Delta \delta$  values are  $\delta(\mathbf{2}) - \delta(\mathbf{7} \text{ or } \mathbf{8})$ .

Chart 2



naphthyl protons in 2e show significant upfield shifts relative to the mononaphthyl reference compounds. These observations indicate that the pattern of  $\Delta\delta$  values observed for 2a vs (7a + 8a) in aqueous solution and for 2b vs (7b + 8b) in nonpolar solutions is due largely to the presence of a branch point in the tether. This conclusion supports our contention that the intramolecular naphthyl-naphthyl proximity in 2a in aqueous solution results predominantly from random internal motions, rather than from hydrophobic collapse.

#### Discussion

We have used aryl proton chemical shift effects to show that naphthyl and phenyl groups connected via flexible four-atom linkers spend time near one another in solution, but that this proximity is not strongly promoted by an aqueous environment relative to a nonpolar environment. Our experimental approach is based upon the fact that local magnetic anisotropy arising from aromatic  $\pi$ -electron systems can exert large effects on the chemical shifts of nearby protons. This type of secondary chemical shift has been widely used to detect intramolecular and intermolecular proximity of protons to aromatic rings.<sup>3a-c,g,22</sup>

One must be cautious in attempting to derive conformational information from secondary proton chemical shifts that arise

<sup>(22) (</sup>a) Detection of intramolecular heterocycle stacking via secondary <sup>1</sup>H NMR chemical shift effects: Constant, J. F.; Laugaa, P.; Roques, B. P.; Lhomme, J. *Biochemistry* **1988**, 27, 3997 and references therein. (b) Hostguest chemistry examples: Whitlock, B. J.; Whitlock, H. W. J. Am. Chem. Soc. **1994**, 116, 2301. Zimmerman, S. C. Top. Curr. Chem. **1993**, 165, 71.

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from changes in molecular structure or solvent, because proton chemical shifts can be affected by multiple factors. We believe that analysis of secondary chemical shift effects for dinaphthyl compounds 2a - e is valid because our quantitative comparisons are based *not* upon absolute chemical shifts but rather upon  $\Delta\delta$ values, which are obtained from a given dinaphthyl <sup>1</sup>H chemical shift by subtracting the <sup>1</sup>H chemical shift for an analogous mononaphthyl compound. Thus, for example, comparison of the behavior of 2a in aqueous solution with the behavior of 2b in organic solvents involves a change from carboxylate to neutral carboxylic acid, and we assume that any effect of this change on absolute chemical shifts is accounted for in the  $\Delta\delta$  values. Indeed, we have monitored the behavior of 14 aryl protons for dinaphthyl system 2a,b (Table 1), and the internal consistency of the behavior of the secondary chemical shifts supports our conclusions. The lack of a substantial solvent effect on  $\Delta\delta$ values between benzene and water indicates that this dinaphthyl system is not subject to a significant hydrophobic drive for folding. The absence of a solvent effect between benzene and chloroform supports the conclusion of Wilcox et al.<sup>13</sup> that polar interactions between hydrocarbon aromatic groups exert little or no conformational influence, because such a polar effect would have been expected to manifest itself in chloroform. (Calculations by Jorgensen and Severance suggest that there might be an intrinsic polar benzene-benzene attraction in chloroform solution.<sup>11f</sup>)

It is common to use NOE or related two-dimensional measurements to detect spatial proximity between protons. ROESY<sup>23</sup> experiments were attempted for 2a in D<sub>2</sub>O, but no cross peaks could be clearly detected in the aromatic region, even between covalently adjacent protons. This difficulty stems from the very low aqueous solubility of 2a. NOESY<sup>24</sup> cross peaks could be detected for covalently adjacent protons of ester 2c, 45 mM in CDCl<sub>3</sub>, but no cross peaks were observed between protons on different naphthyl groups within this molecule (1 s mixing time). This result for 2c in CDCl<sub>3</sub>, which displays  $\Delta \delta$ values nearly identical to those for 2a in D<sub>2</sub>O, shows that secondary chemical shift effects provide more sensitive detection of intramolecular naphthyl-naphthyl proximity than do NOESY measurements. The NOESY data for 2c suggest that the secondary chemical shift effects observed for 2a-d in the various solvents arise from small populations of folded conformations.

The results reported here are important because aromatic hydrocarbon moieties (e.g., phenylalanine side chains) are commonly considered to be hydrophobic entities.<sup>1d</sup> The absence of significant hydrophobically driven folding in dinaphthyl system 2 is particularly interesting in the context of relatively large recent estimates of the thermodynamic gain associated with removal of nonpolar surfaces from contact with water.1c Comparison of the water-accessible surface area<sup>25</sup> of the crystallographically observed conformation of **2b** (568  $Å^2$ ) to the water-accessible surface area of the computationally minimized extended conformation of 2b (656 Å<sup>2</sup>) indicates that 2acould bury at least 88 Å<sup>2</sup> of nonpolar surface by folding in aqueous solution. (Comparisons involving an isolated molecule of 2b that has been minimized starting from the crystallographic conformation suggest that >100 Å<sup>2</sup> of nonpolar surface could be buried.)

Our findings are of interest also in the context of a recent hypothesis that hydrophobic collapse influences the solution conformations of low molecular weight medicinal agents.<sup>3f</sup> The lack of significant solvent-induced folding in water among the flexible diaryl carboxylates discussed here suggests that conformation-directing hydrophobic effects in drug molecules of similar size will be modest, at least when the "hydrophobic" moieties are aromatic.

Elucidation of basic chemical and biological processes and an enhanced ability to design molecules for specific functions (e.g., therapeutic agents, new catalysts) require an intimate understanding of the noncovalent interactions that control structure and function in flexible frameworks. Since many important events occur in aqueous solution, it is crucial that we learn the extent to which the hydrophobic effect can influence conformation and complexation. The results presented here provide intuitive calibration regarding the significance of hydrophobic effects involving hydrocarbon aromatic groups. These results should also be useful for quantitative calibration of computational tools that are intended to predict conformational preferences in aqueous solution. We are currently extending our experimental approach to systems containing aliphatic hydrophobic moieties.

## **Experimental Section**

General. All melting points are uncorrected. THF was freshly distilled from sodium benzophenone ketyl under N<sub>2</sub>. CH<sub>2</sub>Cl<sub>2</sub> was freshly distilled from CaH2 under N2. CH3CN was distilled from CaH2 prior to use and stored over 4-Å sieves under N<sub>2</sub>. Et<sub>3</sub>N was distilled from CaH2 prior to use and stored over KOH under N2. Reagents were used as obtained from commercial suppliers. LDA was freshly prepared by adding 1.0 equiv of n-BuLi to a solution of 1.13 equiv of diisopropylamine in THF at 0 °C. NaH, a 60% dispersion in oil, was rinsed 1-3 times with dry pentane before use. Routine <sup>1</sup>H NMR spectra were obtained on either a Bruker WP-200, WP-270, or AC-300 spectrometer and referenced to residual protonated NMR solvent, or for spectra obtained in CDCl<sub>3</sub>, to TMS. Routine <sup>13</sup>C NMR spectra were obtained on either a Bruker WP-270, AC-300, or AM-500 spectrometer and referenced to the NMR solvent. Routine FT-IR spectra were obtained on a Nicolet 740 spectrometer. High-resolution electron impact ionization mass spectrometry was performed on a Kratos MS-80. Elemental analyses were performed at Galbraith Laboratories. UV studies were performed on an HP 8452 diode array spectrophotometer. Column chromatography was carried out by using low N<sub>2</sub> pressure with either 230-400 mesh silica gel 60 from EM Science or reversed phase silica RP 18 (18-32) 60 A from ICN (this will be referred to as ODS, for octadecylsilyl).

Methyl 2-(Naphthyl)propionate. To a slurry of 15.3 g (37 mmol) of (carbomethoxymethyl)triphenylphosphonium bromide (Aldrich) in 100 mL of THF at 0 °C was added 14.1 mL (35 mmol) of n-BuLi (2.5 M in hexane) dropwise. The resulting solution was warmed to room temperature for 2 h, and then a solution of 5.0 g (32 mmol) of 2-naphthaldehyde (Aldrich) in 60 mL of THF was added via cannula. The solution was stirred at room temperature for 44 h, and then 50 mL of 1 N aqueous HCl was added. The layers were separated, and the aqueous phase was extracted with Et<sub>2</sub>O (3  $\times$  50 mL). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. After concentration of the filtrate, the crude alkene was purified by SiO<sub>2</sub> column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub> to give 6.4 g (94% yield) of a mixture of the cis and trans alkenes. The mixture of alkenes was dissolved in 150 mL of EtOAc, 0.64 g of 5% palladium on carbon was added, and the mixture was shaken under 40 psi of H<sub>2</sub> for 2.5 h and then filtered through Celite and rinsed well with EtOAc. Concentration of the filtrate gave 6.4 g (quantitative yield) of methyl 2-(naphthyl)propionate as a white solid that was recrystallized from hexane: mp 59.5 -60 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz)  $\delta$  2.70 (t, J = 7.8 Hz, 2H), 3.10 (t, J = 7.8 Hz, 2H), 3.65 (s, 3H), 7.31 (dd, J = 1.4, 8.4 Hz, 1H), 7.40-7.45 (m, 2H), 7.62 (s, 1H), 7.73-7.80 (m, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 250 MHz)  $\delta$  173.24, 137.94, 133.53, 132.09, 127.88,

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127.56, 127.45, 126.89, 125.96, 125.56, 125.06, 51.58, 35.55, 31.03; IR (KBr) 1729 cm<sup>-1</sup>; EI MS *m/e* 214.0996, calcd for  $C_{14}H_{14}O_2$  214.0994.

2-(Naphthyl)propanoic Acid. A solution of 8.1 g (38 mmol) of methyl 2-(naphthyl)propionate in 190 mL of MeOH and 190 mL of 1 N aqueous NaOH was stirred at room temperature overnight. Most of the solvent was removed by rotary evaporation, and the resulting solution was diluted with H<sub>2</sub>O and then washed with Et<sub>2</sub>O. The aqueous phase was acidified to ca. pH 1 with concentrated HCl, causing formation of a white precipitate that was isolated by filtration. After recrystallization of the solid from EtOAc, 6.7 g (88% yield) of 2-(naphthyl) propanoic acid was isolated as a white crystalline solid: mp 133.5-135 °C (lit.<sup>26</sup> mp 137-138 °C); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz)  $\delta$  2.76 (t, J = 7.7 Hz, 2H), 3.11 (t, J = 3.11 Hz, 2H), 7.33 (dd, J = 1.7, 8.5 Hz, 1H), 7.41-7.46 (m, 3H), 7.64, (s, 1H), 7.75-7.82 (m, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz) δ 179.09, 137.61, 133.58, 132.20, 128.20, 127.62, 127.52, 126.86, 126.45, 126.06, 125.46, 35.48, 30.71; IR (KBr) 1693, 1708 cm<sup>-1</sup>; EI MS m/e 200.0826, calcd for C<sub>12</sub>H<sub>12</sub>O<sub>2</sub> 281.9901.

2-(2-Mesylethyl)naphthalene. To a solution of 1.0 g (5.8 mmol) of 2-naphthylethanol (Aldrich) and 1.2 mL (8.7 mmol) of Et<sub>3</sub>N in 30 mL of CH<sub>2</sub>Cl<sub>2</sub> at 0 °C was added 0.67 mL (8.7 mmol) of methanesulfonyl chloride. The solution was stirred for 2 h, and then 1 N aqueous HCl was added to the cloudy yellow mixture. The layers were separated, and the aqueous phase was extracted with  $CH_2Cl_2$  (3 × 15 mL). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. After concentration of the filtrate, the residue was purified by SiO<sub>2</sub> column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub> to give 1.3 g (90% yield) of the desired mesylate as a white solid that was recrystallized from EtOAc and hexane to give large clear plates: mp 75-76 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz) δ 2.83 (s, 3H), 3.22 (t, J = 6.9 Hz, 2H), 4.54 (t, J = 6.9 Hz, 2H), 7.35 (dd, J = 1.8, 8.5 Hz, 1H), 7.45-7.50 (m, 2H), 7.69 (s, 1H), 7.77-7.84 (m, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz) & 133.65, 133.26, 132.18, 128.16, 127.46, 127.44, 127.34, 126.85, 126.07, 125.62, 70.05, 36.96, 35.45; IR (KBr) 1123, 1467 cm<sup>-1</sup>; EI MS *m/e* 250.0667, calcd for  $C_{13}H_{14}O_3S$  250.0664.

**2-(2-Iodoethyl)naphthalene.** A mixture of 1.7 g (6.6 mmol) of 2-(2mesylethyl)naphthalene and 6.0 g (40 mmol) of NaI in 35 mL of CH<sub>3</sub>CN was stirred for 36 h, and then 20 mL of H<sub>2</sub>O was added. The layers were separated, and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. After concentration of the filtrate, the residue was purified by SiO<sub>2</sub> column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub> to give 1.6 g (88% yield) of the desired iodide as an off-white solid that was recrystallized from hexane to give small off-white crystals: mp 77.5–79 °C (lit.<sup>27</sup> mp 81.5–83 °C); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz)  $\delta$  3.29–3.49 (m, 4H), 7.31 (dd, J = 1.7, 8.4 Hz, 1H), 7.44– 7.51 (m, 2H), 7.64 (s, 1H), 7.78–7.83 (m, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  134.51, 133.18, 132.60, 128.28, 128.21, 127.73, 127.70, 127.23, 126.21, 125.94, 43.59; IR (KBr) no major bands; EI MS *m/e* 155.0844, calcd for C<sub>12</sub>H<sub>11</sub> (M<sup>+</sup> – I) 155.0861.

Compound 1b. To a solution of 2.0 g (10 mmol) of 2-(naphthyl)propanoic acid in 30 mL of THF at 0 °C was added 22.7 mL of 1 M LDA (22.7 mmol) at 0 °C followed by 1.6 mL (13.1 mmol) of DMPU. The resulting dark solution was warmed to room temperature over 0.5 h, and then a solution of 5.7 g (20 mmol) of 2-(2-iodoethyl)naphthalene in 20 mL of THF was added. The reaction mixture, which immediately became a clear yellow solution, was stirred 2 h, and then 30 mL of 10% aqueous HCl was added. The layers were separated, and the aqueous phase was extracted with EtOAc (3  $\times$  30 mL); the aqueous phase was saturated with NaCl before the last extraction. The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. After concentration of the filtrate, the residue was purified twice by SiO<sub>2</sub> column chromatography, eluting the first column with EtOAc and the second column with 1:1 EtOAc followed by EtOAc. Acid 1b was isolated as a pale yellow solid, which was recrystallized from EtOAc and hexane to give 1.8 g (50% yield) of white crystals: mp 122-123 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 1.88–2.00 (m, 1H), 2.06–2.18 (m, 1H), 2.72-3.00 (m, 4H), 3.20 (dd, J = 7.3, 13.5 Hz, 1H), 7.23-7.29 (m, 2H), 7.37–7.46 (m, 4H), 7.55 (s, 1H), 7.59 (s, 1H), 7.71–7.80 (m, 6H);  $^{13}$ C NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  181.80, 138.60, 136.16, 133.51, 133.42, 132.19, 132.00, 128.08, 127.94, 127.57, 127.54, 127.51, 127.41, 127.35, 127.20, 127.14, 126.51, 125.98, 125.88, 125.44, 125.20, 46.53, 38.21, 33.59, 33.01; IR (KBr) 1689, 1705 cm<sup>-1</sup>; EI MS *m/e* 354.1606, calcd for C<sub>25</sub>H<sub>22</sub>O<sub>2</sub> 354.1620. Anal. Calcd for C<sub>25</sub>H<sub>22</sub>O<sub>2</sub>: C, 84.71; H, 6.26. Found: C, 84.66; H, 6.33.

2-Naphthylmethyl Disulfide. To a solution of 15 g (68 mmol) of 2-(bromomethyl)naphthalene (Aldrich) in 180 mL of CH<sub>3</sub>CN was added 5.2 g (68 mmol) of thiourea. The mixture was stirred for 2 h, during which time a substantial amount of white solid formed, and then the solvent was removed by rotary evaporation. The white solid was slurried with 200 mL of H<sub>2</sub>O and 100 mL of 1 M aqueous NaOH, refluxed for 3 h, and then stirred at room temperature overnight. The solution was heated until the solid became an oil. After cooling, 200 mL of CHCl3 were added. Iodine was added until a dark color persisted, and then  $Na_2S_2O_3$  was added until the dark color was gone. The layers were separated, and the aqueous phase was extracted with CHCl<sub>3</sub> (3  $\times$  100 mL). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. After concentration of the filtrate, 11.6 g (98% yield) of 2-naphthylmethyl disulfide was isolated as a pale yellow solid that was recrystallized from CHCl<sub>3</sub> and hexane: mp 127-128 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 200 MHz) δ 3.71 (s, 4H), 7.33 (dd, J = 1.8, 8.5 Hz, 2H), 7.43-7.54 (m, 6H), 7.74-7.84 (m, 6H);<sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz) δ 134.51, 133.18, 132.60, 128.28, 128.21, 127.73, 127.70, 127.23, 126.21, 125.94, 43.59; EI MS m/e 346.0850, calcd for C<sub>22</sub>H<sub>18</sub>S<sub>2</sub> 346.0850.

Compound 2c. A solid which contained acid 2b was prepared from 2-(naphthyl)propanoic acid and 2-naphthylmethyl disulfide via a procedure analogous to that used for 1b. The crude product was adsorbed onto SiO<sub>2</sub> and cleaned by SiO<sub>2</sub> column chromatography eluting with 2:1 EtOAc:hexane, EtOAc, 5% MeOH in EtOAc, and 35% MeOH in EtOAc. The middle fractions were collected, adsorbed onto SiO<sub>2</sub>, and repurified by SiO<sub>2</sub> chromatography eluting with 2:1 EtOAc: hexane, 5% MeOH in EtOAc, 10% MeOH in EtOAc, and 50% MeOH in EtOAc. The middle fractions were concentrated to give 4.9 g of a crude solid containing acid 2b. This material was dissolved in 50 mL of 1:1 MeOH:dioxane. Concentrated HCl (7 mL) was added, and the solution was stirred overnight. The reaction was quenched with water, some of the solvent was removed by rotary evaporation, and the remaining aqueous layer was extracted with ether (3  $\times$  30 mL). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. After concentration of the filtrate the crude product was purified on five successive SiO2 columns; the first was eluted with CH<sub>2</sub>Cl<sub>2</sub> and the next four were eluted with toluene to give 1.15 g (21% yield from 2-naphthylpropanoic acid) of ester 2c as a clear oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  3.02 (dd, J = 7.1, 14.1 Hz, 1H), 3.31 (dd, J = 8.5, 14.1 Hz, 1H), 3.52 (dd, J = 7.1, 8.5 Hz, 1H), 3.62 (s, 3H), 3.95 (ABq, J = 13.6, 2H), 7.12 (dd, J = 1.8, 8.4 Hz, 1H), 7.36 (dd, J= 1.8, 8.5 Hz, 1H), 7.40-7.42 (m, 2H), 7.43 (s, 1H), 7.44-7.46 (m, 2H), 7.62 (d, J = 7.7, 1H), 7.62–7.63 (m, 1H), 7.65 (s, 1H), 7.68 (d, J = 8.5, 1H), 7.71–7.72 (m, 1H), 7.74–7.75 (m, 1H), 7.77–7.79 (m, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  171.57, 135.18, 134.53, 133.34, 133.14, 132.57, 132.27, 128.35, 127.95, 127.62, 127.52, 127.12, 127.00, 126.16, 125.93, 125.85, 125.52, 52.22, 47.18, 37.69, 36.62; IR (film) 1733 cm<sup>-1</sup>; EI MS m/e 386.1334, calcd for C<sub>25</sub>H<sub>22</sub>O<sub>2</sub>S 386.1341.

**Compound 2b.** To a solution of 0.24 g (0.61 mmol) of ester 2c in 3 mL of MeOH was added 3 mL of 1 N aqueous NaOH. The cloudy white mixture was stirred at room temperature overnight and then heated to reflux for 30 min. The resulting clear solution was cooled to room temperature, and concentrated HCl was added dropwise until the solution was ca. pH 1, causing formation of a white precipitate. This precipitate was isolated by filtration and dried under vacuum in the presence of  $P_{2}O_5$  for 5 h to give 0.20 g (89% yield) of acid 2b as a white solid that was recrystallized from EtOAc and hexane: mp 160–161 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  3.01 (dd, J = 7.5, 14.0 Hz, 1H), 3.32 (dd, J = 7.9, 14.0 Hz, 1H), 3.50 (t, J = 7.7, 1H), 3.99 (ABq, J = 13.6 Hz, 2H), 7.12 (dd, J = 1.6, 8.4 Hz, 1H), 7.34 (dd, J = 1.7, 8.5 Hz, 1H), 7.41–7.49 (m, 5H), 7.61–7.80 (m, 7H); <sup>13</sup>C NMR (DMSO- $d_6$ , 500 MHz)  $\delta$  172.91, 135.95, 135.26, 132.85, 132.75, 132.01, 131.75, 128.04, 127.56, 127.50, 127.46, 127.41, 127.30, 127.26,

<sup>(26)</sup> Nakabayashi, T. Nippon Kagaku Zasshi **1960**, 81, 121. (27) Pataki, J.; Harvey, R. G. J. Org. Chem. **1982**, 47, 20.

127.21, 127.14, 126.21, 125.96, 125.83, 125.46, 47.53, 37.47, 35.36; IR (KBr) 1680, 1703 cm<sup>-1</sup>; EI MS *m/e* 372.1189, calcd for  $C_{24}H_{20}O_2S$  372.1184.

**Compound 3b** was prepared from hydrocinnamic acid (Aldrich) and benzyl disulfide (Aldrich) via a procedure analogous to that used for **1b**. The crude product was purified by SiO<sub>2</sub> column chromatography eluting with 1:2 EtOAc:hexane and then 2:1 EtOAc:hexane containing a drop of AcOH to give 1.14 g (63% yield) of acid **3b** as a yellow oil that solidified upon standing. Recrystallization from hexane gave an off-white solid: mp 62.5–63.5 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  2.87 (dd, J = 6.9, 14.2 Hz, 1H), 3.16 (dd, J = 8.6, 14.1 Hz, 1H), 3.39 (dd, J = 6.9, 8.6 Hz, 1H), 3.84 (ABq, J = 13.4 Hz, 2H), 7.05–7.10 (m, 2H), 7.18–7.32 (m, 8H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  178.83, 137.50, 136.86, 129.05, 128.88, 128.40, 128.32, 127.16, 126.64, 47.05, 36.91, 36.23; IR (KBr) 1702, 1709 cm<sup>-1</sup>; EI MS *m/e* 272.0864, calcd for C<sub>16</sub>H<sub>16</sub>O<sub>2</sub>S 272.0871.

**Compound 5b** was prepared from 2-(naphthyl)propanoic acid and ethyl iodide via a procedure analogous to that used for **1b**. The crude product was purified by SiO<sub>2</sub> column chromatography eluting with 1:1 EtOAc:hexane and then EtOAc to give 3.91 g (85% yield) of acid **5b** as a yellow oil that solidified upon standing. The product was repurified using bulb-to-bulb distillation: bp ca. 168–172 °C (0.20 mmHg); mp 49–50 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.96 (t, J = 7.4 Hz, 3H), 1.55–1.75 (m, 2H), 2.67–2.76 (m, 1H), 2.90 (dd, J = 7.0, 13.7 Hz, 1 H), 3.14 (dd, J = 7.7, 13.7 Hz, 1H), 7.31 (dd, J = 1.8, 8.5 Hz, 1H), 7.42–7.45 (m, 2H), 7.63 (s, 1H), 7.74–7.81 (m, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 270 MHz)  $\delta$  182.24, 136.56, 133.44, 132.15, 127.95, 127.23, 125.92, 125.34, 48.70, 37.70, 24.70, 11.54; IR (KBr) 1702 cm<sup>-1</sup>; EI MS *m/e* 228.1149, calcd for C<sub>15</sub>H<sub>16</sub>O<sub>2</sub> 228.1150. Anal. Calcd for C<sub>15</sub>H<sub>16</sub>O<sub>2</sub>: C, 78.92; H, 7.06. Found: C, 78.96; H, 7.20.

**Compound 6b** was prepared from butyric acid and 2-(2-iodoethyl)naphthalene via a procedure analogous to that used for **1b**. The crude product was purified by SiO<sub>2</sub> column chromatography eluting with 5% acetone in CHCl<sub>3</sub> to give 0.18 g (13% yield) of the desired acid **6b** as a pale yellow oil. The oil was repurified by preparative scale TLC eluting with 1:2 acetone:CHCl<sub>3</sub> and then crystallized from hexane to give off-white crystals: mp 58–59.5 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.96 (t, J = 7.5 Hz, 3H), 1.59–1.76 (m, 2H), 1.85–1.91 (m, 1H), 2.04–2.12 (m, 1H), 2.37–2.43 (m, 1H), 2.77–2.86 (m, 2H), 7.33 (dd, J = 1.7, 8.5 Hz, 1H), 7.37–7.46 (m, 2H), 7.63 (s, 1H), 7.75–7.80 (m, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  182.81, 139.02, 133.56, 132.01, 127.93, 127.56, 127.41, 127.19, 126.47, 125.89, 125.17, 46.46, 33.71, 33.20, 25.19, 11.62; IR (KBr) 1703 cm<sup>-1</sup>; EI MS *m/e* 242.1327, calcd for C<sub>16</sub>H<sub>18</sub>O<sub>2</sub> 242.1307.

Compound 7c. A mixture of the acid 7b and 2-naphthylpropanoic acid was prepared from 2-(naphthyl)propanoic acid and ethyl disulfide via a procedure analogous to that used for 1b. After several attempts at purification of 7b on SiO<sub>2</sub> and ODS columns, the isolated material was still a mixture of these two acids. Ester 7c was prepared from crude 7b via a procedure analogous to that used for 2c. The crude product was purified by SiO<sub>2</sub> column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub>. After purification on two more SiO<sub>2</sub> columns, each eluted with toluene, 0.36 g (13% yield based on 2-naphthylpropanoic acid) of the desired ester 7c was isolated as a clear oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  1.21 (t, J = 7.4 Hz, 3H), 2.63 (dq, J = 1.0, 7.4 Hz, 2H), 3.11 (dd, J = 6.3, 13.8 Hz, 1H), 3.36 (dd, J = 9.4, 13.8 Hz, 1H), 3.61 (s, 3H), 3.63 (dd, J = 6.3, 9.4 Hz, 1H), 7.30 (dd, J = 1.6, 8.3 Hz, 1H), 7.37-7.44 (m, 2H), 7.63 (s, 1H), 7.73-7.77 (m, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz) δ 172.16, 135.25, 133.10, 132.00, 127.73, 127.26, 127.25, 127.20, 126.82, 125.67, 125.23, 51.65, 47.39, 37.62, 25.26, 14.05; IR (film) 1733 cm<sup>-1</sup>; EI MS *m/e* 274.1017, calcd for C<sub>16</sub>H<sub>18</sub>O<sub>2</sub>S 274.1027.

**Compound 7b** was prepared from ester 7c via a procedure analogous to that used for 2b. The crude product was purified by preparative scale TLC eluting with EtOAc to give acid 7b as a clear oil that solidified upon standing: mp 70.5–72.5 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 250 MHz)  $\delta$  1.19 (t, J = 7.5 Hz, 3H), 2.64 (dq, J = 1.1, 7.5 Hz, 2H), 3.09 (dd, J = 6.6, 14.0 Hz, 1H), 3.34 (dd, J = 8.7, 14.0 Hz, 1H), 3.61 (dd, J = 6.7, 8.6 Hz, 1H), 7.31 (dd, J = 1.7, 8.5 Hz, 1H), 7.38–7.46 (m, 2H), 7.65 (s, 1H), 7.71–7.79 (m, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  178.31, 135.15, 133.36, 132.34, 128.14, 127.63, 127.60, 127.08, 126.04, 125.61, 47.59, 37.45, 25.96, 14.21; IR (film) 1706 cm<sup>-1</sup>; EI MS *m/e* 260.0865, calcd for C<sub>15</sub>H<sub>16</sub>O<sub>2</sub>S 260.0871.

**Compound 8b** was prepared from butyric acid and 2-naphthylmethyl disulfide via a procedure analogous to that used for **1b**. The crude product was adsorbed onto SiO<sub>2</sub> and purified by SiO<sub>2</sub> column chromatography eluting with 1:1 EtOAc:hexane and then 4:1 EtOAc: hexane to afford 0.8 g (7% yield) of acid **8b** as a pale yellow solid that was recrystallized from CHCl<sub>3</sub> and hexane: mp 84.5–85 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.94 (t, J = 7.4 Hz, 3H), 1.61–1.70 (m, 1H), 1.82–1.92 (m, 1H), 3.07 (dd, J = 7.2, 7.9 Hz, 1H), 4.02 (ABq, J = 13.4 Hz, 2H), 7.44–7.51 (m, 2H), 7.52 (dd, J = 1.7, 8.4 Hz, 1H), 7.77 (s, 1H), 7.80–7.84 (m, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 270 MHz)  $\delta$  179.25, 134.62, 133.16, 132.59, 128.42, 127.78, 127.73, 127.65, 127.07, 126.19, 125.88, 46.99, 36.31, 24.05, 11.72; IR (film) 1703 cm<sup>-1</sup>; EI MS *m/e* 260.0886, calcd for C<sub>15</sub>H<sub>16</sub>O<sub>2</sub>S 260.0871.

**Compound 9b** was prepared from hydrocinnamic acid (Aldrich) and ethyl disulfide via a procedure analogous to that used for **1b**. The crude product was purified by SiO<sub>2</sub> column chromatography eluting with 1:4 acetone:CHCl<sub>3</sub> to give 0.34 g (24% yield) of acid **9b** as a yellow oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  1.23 (t, J = 7.4 Hz, 3H), 2.67 (dq, J = 1.9, 7.4 Hz, 2H), 2.95 (dd, J = 6.6, 13.9 Hz, 1H), 3.20 (dd, J = 8.9, 13.9 Hz, 1H), 3.52 (dd, J = 6.6, 8.9 Hz, 1H), 7.21–7.31 (m, 5H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  178.39, 137.62, 128.86, 128.40, 126.76, 47.62, 37.26, 25.85, 14.15; IR (film) 1705 cm<sup>-1</sup>; EI MS *m/e* 210.0720, calcd for C<sub>11</sub>H<sub>14</sub>O<sub>2</sub>S 210.0714.

**Compound 10b** was prepared from butyric acid and benzyl disulfide (Aldrich) via a procedure analogous to that used for **1b**. The crude product was purified by SiO<sub>2</sub> column chromatography eluting with 1:1 acetone:CHCl<sub>3</sub> to give a crude mixture that contained the desired product. This mixture was purified twice more by SiO<sub>2</sub> column chromatography eluting successively with EtOAc and then CHCl<sub>3</sub> followed by 3% MeOH in CHCl<sub>3</sub> to give 0.22 g (15% yield) of acid **10b** as a yellow oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  0.92 (t, J = 7.4 Hz, 3H), 1.59–1.69 (m, 1H), 1.79–1.89 (m, 1H), 3.10 (t, J = 7.4 Hz, 1H), 3.80 (ABq, J = 13.3 Hz, 2H), 7.19–7.33 (m, 5H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  178.92, 137.29, 129.07, 128.47, 127.22, 47.25, 36.05, 24.16, 11.74; IR (film) 1704 cm<sup>-1</sup>; EI MS *m/e* 210.0712, calcd for C<sub>11</sub>H<sub>14</sub>O<sub>2</sub>S 210.0715.

**Compound 8c** was prepared from acid **8b** via a procedure analogous to that used for **2c**. The crude product was purified by preparative scale TLC eluting with CH<sub>2</sub>Cl<sub>2</sub> to give ester **8c** as a clear oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.90 (t, J = 7.3 Hz, 3H), 1.61–1.71 (m, 1H), 1.81–1.91 (m, 1H), 3.11 (dd, J = 6.8, 8.2 Hz, 1H), 3.69 (s, 3H), 3.95 (ABq, J = 13.4 Hz, 2H), 7.45–7.50 (m, 3H), 7.74 (s, 1H), 7.79–7.83 (m, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  173.07, 134.93, 133.19, 132.55, 128.33, 127.67, 127.63, 127.58, 127.08, 126.16, 125.81, 52.11, 47.66, 36.20, 24.58, 11.85; IR (film) 1734 cm<sup>-1</sup>; EI MS *m/e* 274.1035, calcd for C<sub>16</sub>H<sub>18</sub>O<sub>2</sub>S 274.1027.

Compound 2d. A solution of 0.32 g (0.82 mmol) of ester 2c in 3 mL of THF was added dropwise to a slurry of 48 mg (1.3 mmol) of LiAlH<sub>4</sub> in 2 mL of THF. The resulting slurry was stirred at room temperature for 1 h and then at reflux for 1 h. After this solution had cooled to room temperature, a freshly prepared solution of saturated aqueous Na<sub>2</sub>SO<sub>4</sub> was added dropwise until no additional solid formed. The solids were removed by filtration and rinsed well with THF. Concentration of the filtrate gave 0.25 g of the slightly impure alcohol as a clear oil. This oil was dissolved in 3 mL of THF and added dropwise to a slurry of 34 mg (0.84 mmol) of NaH in 2 mL of THF at 0 °C, followed by 0.24 mL (3.8 mmol) of CH<sub>3</sub>I. The resulting solution was stirred at room temperature for 6 h, and then 2 mL of 1% aqueous citric acid was added. The layers were separated, and the aqueous phase was extracted with EtOAc ( $3 \times 5$  mL). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. After concentration of the filtrate, the residue was purified by preparative scale TLC eluting with CH<sub>2</sub>Cl<sub>2</sub>. The desired product 2d was isolated as 0.18 g (57% yield) of a yellow oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$ 2.86-2.99 (m, 2H), 3.13 (dd, J = 5.8, 12.7 Hz, 1H), 3.28 (s, 3H), 3.37-3.40 (m, 2H), 3.79 (ABq, J = 13.4 Hz, 2H), 7.19 (dd, J = 1.7, 8.5, 1H), 7.34 (dd, J = 1.8, 8.4 Hz, 1H), 7.37-7.44 (m, 4H), 7.49 (s, 1H), 7.54 (s, 1H), 7.63-7.67 (m, 4H), 7.72-7.76 (m, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 270 MHz) δ 136.48, 135.62, 133.40, 133.14, 132.47, 132.15, 128.29, 127.80, 127.78, 127.68, 127.59, 127.30, 127.08, 126.10, 125.87, 125.71, 125.33, 76.54, 58.85, 45.56, 38.42, 36.30; EI MS m/e 372.1541, calcd for C<sub>25</sub>H<sub>24</sub>OS 372.1548.

**Compound 7d** was prepared from ester 7c via a procedure analogous to that used for 2d. The crude product was purified by preparative scale TLC eluting with CH<sub>2</sub>Cl<sub>2</sub> to afford 0.90 g (53% yield) of the desired product 7d as a yellow oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  1.18 (t, J = 7.4 Hz, 3H), 2.52 (q, J = 7.4, 2H), 2.97 (dd, J = 6.4, 12.6 Hz, 1H), 3.06–3.35 (m, 2H), 3.35 (s, 3H), 3.38–3.49 (m, 2H), 7.36 (dd, J = 1.6, 8.4 Hz, 1H), 7.38–7.47 (m, 2H), 7.66 (s, 1H), 7.75–7.81 (m, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  136.58, 133.36, 132.11, 127.74, 127.67, 127.52, 127.47, 125.81, 125.26, 74.69, 58.74, 46.26, 38.50, 25.37, 14.79; EI MS *m/e* 260.1236, calcd for C<sub>16</sub>H<sub>20</sub>OS 260.1235.

Compound **8d** was prepared from acid **8b** via a procedure analogous to that used for **2d**. The crude product was purified by SiO<sub>2</sub> column chromatography eluting with 1:5 EtOAc:hexane to afford 0.20 g (51% yield) of the desired product **8b** as a yellow oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.82 (t, J = 7.0 Hz, 3H), 1.33–1.65 (m, 4H), 2.67–2.76 (m, 1H), 3.31 (s, 3H), 3.41–3.94 (m, 2H), 3.94 (ABq, J = 13.4, 2H), 7.43–7.47 (m, 2H), 7.51 (dd, J = 2.0, 8.6, 1H), 7.72 (s, 1H), 7.77–7.83 (m, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  136.04, 133.18, 132,43, 128.22, 127.59, 127.55, 127.19, 127.15, 126.03, 125.62, 76.27, 58.80, 44.13, 36.00, 33.86, 19.89, 13.91; EI MS *m/e* 274.1375, calcd for C<sub>17</sub>H<sub>22</sub>OS 274.1391.

Compound 2e. A mixture of 0.47 g (2.7 mmol) of freshly prepared 2-naphthylmethanethiol,<sup>28</sup> 0.38 g (2.7 mmol) of K<sub>2</sub>CO<sub>3</sub>, and 0.56 g (2.2 mmol) of 2-(2-mesylethyl)naphthalene in 12 mL of CH<sub>3</sub>CN was stirred at room temperature for 12 h, during which time a fluffy white solid formed. The mixture containing the starting mesylate was then heated at reflux for 4 h and stirred at room temperature for 20 h. After rotary evaporation of the solvent, the residue was partitioned between CHCl<sub>3</sub> and H<sub>2</sub>O, and the solution was acidified with 8 mL of 10% aqueous HCl. The layers were separated and the aqueous phase was extracted with CHCl<sub>3</sub> (2  $\times$  8 mL). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. After concentration of the filtrate, the crude product was purified by SiO<sub>2</sub> column chromatography eluting with 3% EtOAc in hexane to give a mixture of the desired product and 2-naphthylmethyl disulfide. The mixture was dissolved in 1 mL of THF, and a few drops of sec-BuLi were added. The resulting solution was loaded onto preparative scale TLC plates, which were eluted with 1:2 CH2Cl2:hexane. The desired product 2e was isolated as a white solid (19 mg, 3% yield) that was recrystallized from CHCl<sub>3</sub>: mp 102-103.5 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 2.71-2.76 (m, 2H), 2.96-3.02 (m, 2H), 3.87 (s, 2H), 7.24 (dd, J = 1.7, 8.4, 1H), 7.40-7.50 (m, 5H), 7.53 (s, 1H), 7.66 (s, 1H), 7.70-7.83 (m, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 500 MHz) δ 137.90, 135.68, 133.48, 133.21, 132.52, 132.13, 128.41, 127.98, 127.67, 127.58, 127.47, 127.27, 127.08, 127.04, 126.75, 126.17, 125.95, 125.74, 125.33, 36.80, 36.20, 32.59; EI MS m/e 328.1284, calcd for C<sub>23</sub>H<sub>20</sub>S 328.1286.

**Compound 7e** was prepared from 2-(2-iodoethyl)naphthalene and ethanethiol via a procedure analogous to that used for **2e**. The crude product was purified by SiO<sub>2</sub> column chromatography eluting with 5% EtOAc in hexane to afford 0.24 g (32% yield) of the desired product **7e** as a yellow oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  1.27 (t, J = 7.4 Hz, 3H), 2.57 (q, J = 7.4 Hz, 2H), 2.83–2.88 (m, 2H), 3.01–3.07 (m, 2H), 7.33 (dd, J = 1.7, 8.4 Hz, 1H), 7.41–7.45 (m, 2H), 7.64 (s, 1H), 7.76–7.81 (m, 2H), 7.77 (d, J = 8.6 Hz, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  138.13, 133.52, 132.15, 128.02, 127.61, 127.47, 127.03, 126.65, 125.97, 125.33, 36.44, 33.07, 26.10, 14.76; EI MS *m/e* 216.0964, calcd for C<sub>14</sub>H<sub>16</sub>S 216.0973.

**Compound 8e** was synthesized from freshly prepared 2-naphthylmethanethiol<sup>28</sup> and iodobutane via a procedure analogous to that used for **2e**. The crude product was purified twice by SiO<sub>2</sub> column chromatography eluting first with 2% EtOAc in hexane and then with hexane to give 0.57 mg (59% yield) of the desired product **8e** as a clear oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  0.86 (t, J = 7.3 Hz, 3H), 1.31–1.39 (m, 2H), 1.50–1.57 (m, 2H), 2.40 (t, J = 7.4 Hz, 2H), 3.84 (s, 2H), 7.43–7.50 (m, 3H), 7.68 (s, 1H), 7.76–7.82 (m, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  135.94, 133.17, 132.42, 128.24, 127.58, 127.52, 127.07, 126.03, 36.43, 31.21, 30.84, 21.91, 13.63; EI MS *m/e* 230.1124, calcd for C<sub>15</sub>H<sub>18</sub>S 230.1129.

Preparation of Na Salts. To prevent contamination with divalent cations, all glassware was soaked for a minimum of 12 h in a

Nochromix (Gooax Laboratories) acid bath and then rinsed three times with distilled  $H_2O$  and then three times with Millipore  $H_2O$ . Acids were dried at room temperature in the presence of  $P_2O_5$  under vacuum and then weighed in air on a pre-tared balance. The acids were slurried in Millipore  $H_2O$ , and 1.02-1.20 equiv of a standardized solution of semiconductor grade NaOH in Millipore  $H_2O$  was added. The dinaphthyl salt solutions were stirred for 24 h. If undissolved material was present, it was removed by gravity filtration through filter paper.  $H_2O$  was removed by lyophilization.

**Preparation of D<sub>2</sub>O NMR Samples.** All glassware was acid washed (see above). NMR tubes were soaked in either H<sub>3</sub>PO<sub>4</sub> or HNO<sub>3</sub> for a minimum of 12 h and then rinsed multiple times with Millipore H<sub>2</sub>O and dried under a stream of N<sub>2</sub>. The salts were dried at room temperature or 100 °C in the presence of P<sub>2</sub>O<sub>5</sub> under vacuum. All manipulations were performed in a N<sub>2</sub> glovebag. Salts were dissolved in D<sub>2</sub>O, and the concentration of the solution was determined by UV spectroscopy ( $\epsilon$ (276) = 4600 for 2-naphthyl,  $\epsilon$ (258) = 200 for substituted phenyl), and then the stock solution was diluted to the desired concentration. For variable-concentration <sup>1</sup>H NMR studies, all dilutions were made from the stock solution.

**Preparation of NMR Samples in Organic Solvents.** The appropriate compound was dissolved in either CDCl<sub>3</sub>, for studies in CDCl<sub>3</sub>, or CHCl<sub>3</sub>, for studies in other solvents, and the concentration of the solution was determined by UV spectroscopy ( $\epsilon$ (276) = 4600 for 2-naphthyl,  $\epsilon$ (258) = 200 for substituted phenyl). For studies performed in CDCl<sub>3</sub>, the stock solution was diluted to the desired concentration. For studies performed in other solvents, a known amount of the stock solution was placed in a small round-bottom flask, the solvent was removed by evaporation, and the compound was placed under vacuum for 12 h. A known amount of the appropriate solvent was then added to the compound to make a solution of the desired concentration.

**NMR Studies.** The solubility of Na salts in D<sub>2</sub>O was found to vary depending on the lot of D<sub>2</sub>O, presumably because the amount of divalent counterions varies by lot. D<sub>2</sub>O from Cambridge Isotope Laboratories (either 99.9% D in glass bottles, or 99.96% D that had been glass distilled and stored in plastic bottles) in which salt **2a** was soluble to at least 0.10 mM was used for these studies. Since an excess of NaOH was used in the preparation of the Na salts, solutions of the salts were basic; for example, a 200 mM solution of salt **5a** in Millipore-filtered H<sub>2</sub>O was pH 11.9. Sequanal grade urea (from Pierce) was deuterated in an acid-washed flask (see above) by three lyophilizations from D<sub>2</sub>O. The urea-d<sub>4</sub> was dried under vacuum in the presence of P<sub>2</sub>O<sub>5</sub> for 48 h and then stored under N<sub>2</sub> in a desiccator containing CaSO<sub>4</sub> (Drierite).

Spectra obtained in D<sub>2</sub>O or 8 M urea in D<sub>2</sub>O were referenced to an external standard of a dilute solution of TSP (sodium 3-(trimethylsilyl)propionate-2,2,3,3-d<sub>4</sub>) in D<sub>2</sub>O. The z and  $z^2$  shims for the reference could be adjusted so that the TSP signal was a singlet without changing the absolute frequency of the TSP signal. Since the TSP signal is temperature sensitive (it changes 29.2 Hz over 5 °C), the temperature was controlled for all samples and the reference. Room temperature spectra were obtained at 24 °C. <sup>1</sup>H NMR studies of dilute D<sub>2</sub>O solutions and all 2-D NMR studies were performed on a Varian Unity 500 spectrometer. All other <sup>1</sup>H NMR studies were performed on either a Bruker WP-270 or AM-500 spectrometer. All spectra for a concentration study of a specific compound were acquired on the same day. Due to line widths, instrument drift, and uncertainty associated with assigning the chemical shifts of peaks that are multiplets, the uncertainty in NMR assignments is  $\pm 0.02$  ppm. All assignments were made from at least two independently referenced spectra.



NOE signals observed in 2c

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## Searching for Minimum Increments of Hydrophobic Collapse

Two-Dimensional <sup>1</sup>H NMR Studies. All signals in <sup>1</sup>H NMR spectra of 2a-c were assigned using both NOESY and TOCSY experiments. The spectra of 2a-c are similar in all solvents examined, and the spectra of acid 2b and ester 2c in CDCl<sub>3</sub> are nearly identical. Since ester 2c is substantially more soluble in CDCl<sub>3</sub>, without apparent aggregation, than acid 2b, a NOESY<sup>24</sup> experiment was performed on a 45 mM sample of ester 2c in CDCl<sub>3</sub> using a sweep width of 5000 Hz and a mixing time of 1.0 s. The protons between which NOE enhancements were observed are shown above. The two naphthyl rings were differentiated by the NOE enhancements between the methylene protons on the linking chain and the aromatic protons H-1 and H-3, or H-11 and H-13. Further assignments were made by a TOCSY<sup>29</sup> experiment on the aromatic region using a sweep width of 1275 Hz. The TOCSY spectrum showed four coupled spin systems: (H-1, H-3, H-4); (H-11, H-13, H-14); (H-6, H-7, H-8, H-9); (H-16, H-17, H-18, H-19). The TOCSY spectrum allowed for assignment of most of the naphthyl proton resonances, but H-6 and H-9 could not be differentiated, nor could H-16 and H-19. The resonances of H-6 and H-9, and of H-16 and H-19, were assigned on the basis of NOE enhancements observed in the NOESY spectrum. All chemical shift assignments in D<sub>2</sub>O at 24 °C, D<sub>2</sub>O at 88 °C benzene-d<sub>6</sub>, and CDCl<sub>3</sub> were made from TOCSY spectra of the appropriate mononaphthyl or dinaphthyl compound.

**Dye Uptake Studies.** An aqueous solution containing a carboxylate of known concentration and suspended orange OT [(1-*o*-tolylazo)-2-naphthol], was rocked for a minimum of 24 h. Solid dye was removed

(29) Braunschweiler, L.; Ernst, R. R. J. Magn. Reson. 1983, 53, 521.

by filtration through cotton, and the filtrate was examined visually for the presence of color. If any color was present, the solution was checked by UV spectroscopy for absorbance at 500 nm.

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Supplementary Material Available: <sup>1</sup>H NMR comparisons involving dinaphthyl and diphenyl and appropriate monoaryl reference compounds, data from aggregation control studies, and crystallographic data for 1b and 2b (32 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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