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Solvent-Dependent Stabilization of the E Configuration of Propargylic Secondary Amides

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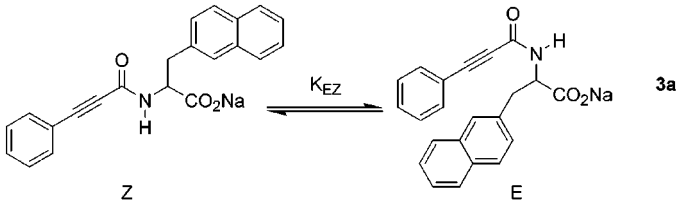
ABSTRACT



Secondary amides typically exist 98–99% in the Z rotamer to avoid steric repulsion between the substituent on the carbonyl carbon and the nitrogen. In contrast, secondary amide 3a displays 24% E rotamer at room temperature in aqueous solution. The analogous ester displays 6% E rotamer in chloroform, which suggests that the relatively high E conformer population observed for 3a in water results in part from the low steric bulk of the sp-hybridized carbons and in part from the hydrophobic effect.

The origins of stability remain mysterious for biologically important structures such as folded protein conformations and protein−ligand complexes. Identifying the contributions of specific noncovalent contacts to such large structures is difficult or impossible via experimental methods.[1] Analysis of model systems can provide an important complement to study of the authentic systems.[2] The model approach is particularly important for interactions that are still subjects of debate, such as the clustering of nonpolar ("hydrophobic") entities in water.[3]

We have recently developed a model system, exemplified by **1**, for quantitative analysis of hydrophobically induced folding.[4] In the Z rotamer, the two naphthyl groups are held apart from one another, but in the E rotamer these two groups can come into contact. Comparing the dimethyl ester (X = CH_3) and the dicarboxylate (X = Na) allowed us to examine the drive for naphthyl−naphthyl proximity as a function of solvent. A key feature of this design is the ability to determine the E:Z rotamer ratio (K_{EZ}) via NMR; interconversion is slow enough that each rotamer gives rise to a distinct set of resonances but fast enough that equilibrium is readily achieved. For **1b** and a series of related diesters with varying hydrocarbon side chains on the α-amino acid residue, $K_{EZ} = 1.6 \pm 0.1$ in chloroform.[4] In contrast, K_{EZ} values vary significantly among dicarboxylates in water;

(1) For an insightful discussion of the problems inherent in dissecting enzyme−inhibitor binding interactions, see: Morgan, B. P.; Scholtz, J. M.; Ballinger, M. D.; Zipkin, I. D.; Bartlett, P. A. *J. Am. Chem. Soc.* **1991**, *113*, 297.

(2) Examples of the use of model systems to examine noncovalent interactions: (a) Leonard, N. J. *Acc. Chem. Res.* **1979**, *12*, 423. (b) Hollingsworth, M. D.; Palmer, A. R. *J. Am. Chem. Soc.* **1993**, *115*, 5881. (c) Paliwal, S.; Geib, S.; Wilcox, C. S. *J. Am. Chem. Soc.* **1994**, *116*, 4497. (d) Newcomb, L. F.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 4993. (e) Cozzi, F.; Siegel, J. S. *Pure Appl. Chem.* **1995**, *67*, 683. (f) Newcomb, L. F.; Haque, T. S.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 6509. (g) Boyd, D. R.; Evans, T. A.; Jennings, W. B.; Malone, J. F.; O'Sullivan, W.; Smith, A. *J. Chem. Soc., Chem. Commun.* **1996**, 2269. (h) Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303. (i) Kim, E.; Paliwal, S.; Wilcox, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 11192. (j) Breinlinger, E. C.; Keenan, C. J.; Rotello, V. M. *J. Am. Chem. Soc.* **1998**, *120*, 8606. (k) Pang, Y. P.; Miller, J. L.; Kollman, P. A. *J. Am. Chem. Soc.* **1999**, *121*, 1717. (l) Mulla, H. R.; Cammers-Goodwin, A. *J. Am. Chem. Soc.* **2000**, *122*, 738.

(3) For leading references on the hydrophobic effect, see: (a) Schellman, J. A. *Biophys. J.* **1997**, *73*, 2960. (b) Makhatadze, G. I.; Privalov, P. L. *Adv. Protein Chem.* **1995**, *47*, 307. (c) Spolar, R. S.; Record, M. T. *Science* **1994**, *263*, 777. (d) Wiley: R. A.; Rich, D. H. *Med. Res. Rev.* **1993**, *13*, 327. (e) Blokzijl, W.; Engberts, J. B. F. N. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1545. (f) Muller, N. *Trends Biochem. Sci.* **1992**, *17*, 459. (g) Pace, C. N. *J. Mol. Biol.* **1992**, *226*, 29. (h) Dill, K. A. *Biochemistry* **1990**, *29*, 7133.

(4) Gardner, R. R.; Christianson, L. A.; Gellman, S. H. *J. Am. Chem. Soc.* **1997**, *119*, 5041.

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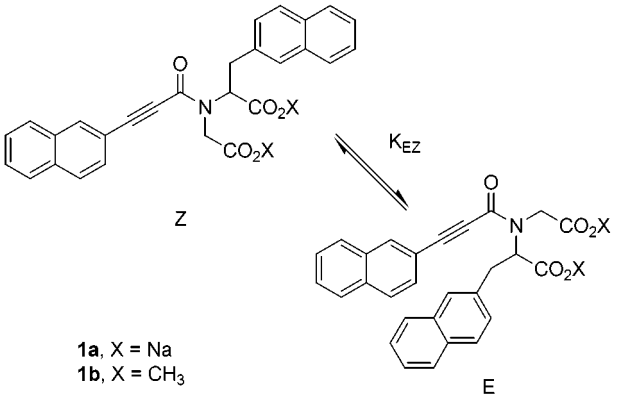
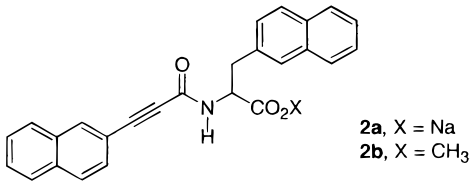
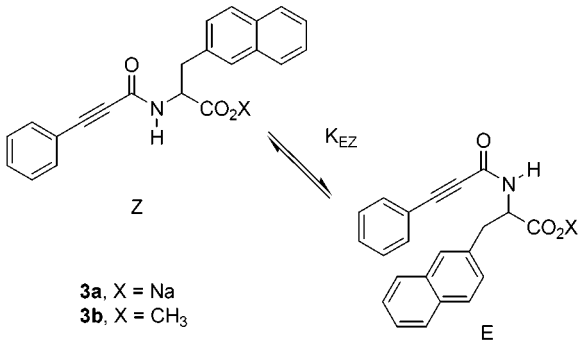
larger hydrocarbon side chains on the α-amino acid residue lead to larger K_{EZ} (for **1a**, $K_{EZ} = 2.9$).



The design of **1** and related molecules incorporates a tertiary amide group because the E and Z rotamers were expected to be (and are) populated to similar extents when there is no drive for association of the hydrocarbon appendages. Secondary amide groups, in contrast, usually display a strong intrinsic preference for the Z rotamer because of steric repulsions between the substituent on the amide carbon and the substituent on the amide nitrogen in the E rotamer.[5] Here we report that secondary amides related to **1a** display enhanced population of the unusual E conformation in aqueous solution.



Compound **2a** was initially examined as a model for the Z rotamer of **1a**, because we assumed that the secondary amide group would ensure complete population of the Z form. Carboxylate **2a** was insoluble in water, but NMR characterization of ester **2b** in $CDCl_3$ suggested a small but significant population of the E rotamer (in addition to a major set of resonances consistent with the expected structure, there was also a minor set of resonances). Analogous carboxylate **3a** proved to be soluble in water, although variable concentration NMR analysis indicated that **3a** self-associates above



5 mM at 297 K (detected via upfield shifts in proton resonances at higher concentrations, Figure 1). At 1 mM,

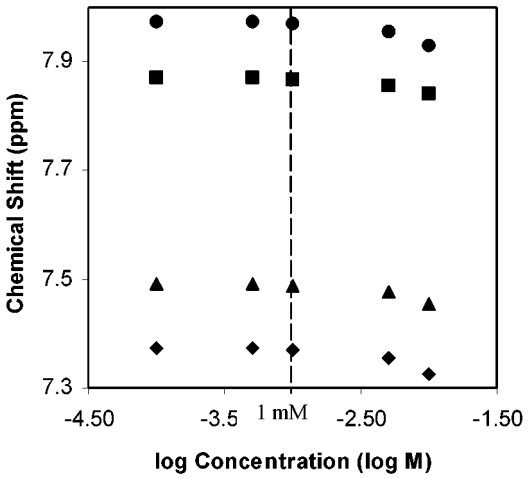


Figure 1. Concentration dependence of selected aromatic resonances of **3a** in D_2O. At 1mM **3a** does not self-associate significantly.

3a displayed two complete sets of proton NMR signals in D_2O. To determine whether the doubling of resonances arose from the presence of two rotamers that interconvert slowly on the NMR time scale, we monitored the proportion of the minor resonances as a function of temperature. Immediately after dissolution of **3a** in D_2O at 277 K, 19% of the minor species was present. After warming to 297 K, the equilibrated minor species population was 24%, and further warming to 323 K resulted in an equilibrated minor species population of 29%. The minor species population returned to 24% upon cooling to 297 K. These reversible changes show that the two species are in equilibrium, as expected for amide rotamers. We assign the major species as the Z rotamer and the minor species as the E rotamer, for reasons described below. The temperature-dependent trend is consistent with stabilization of the E rotamer by the hydrophobic effect (i.e., burial of hydrophobic surface area from intramolecular phenyl−naphthyl interactions), because the hydrophobic effect typically becomes more pronounced as temperature rises.[6] We observed no sign of coalescence over this temperature range. (It should be noted that all of the amides discussed here have a greater conformational diversity than is represented in the two-state equilibria drawn. We focus on the E and Z rotameric states because they can be distinguished by NMR, while conformations differing by rotation about other bonds are not distinguishable by NMR at room temperature.)

The rotamer assignment for **3a** stems from comparison of the spacing between the chemical shifts of the methylene protons adjacent to the naphthyl group of **3a** (Figure 2a) and

(5) (a) Stewart, W. E.; Siddall, T. H. *Chem. Rev.* **1970**, *70*, 517. (b) Radzicka, A.; Pedersen, S.; Wolfenden, R. *Biochemistry* **1988**, *27*, 4538.
(6) See ref 3a and citations therein.

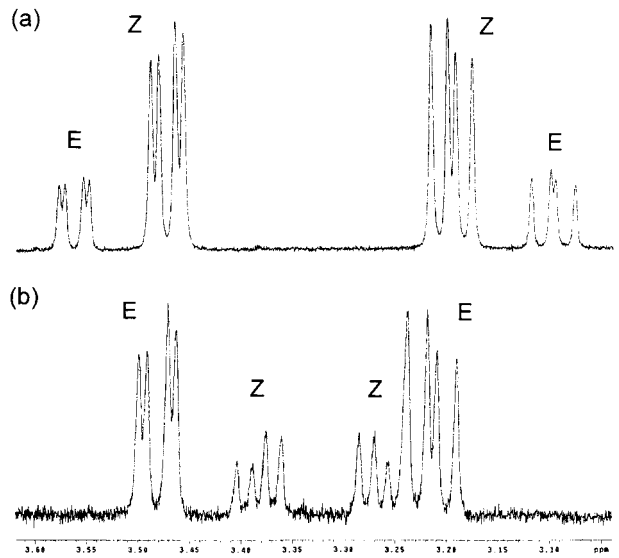
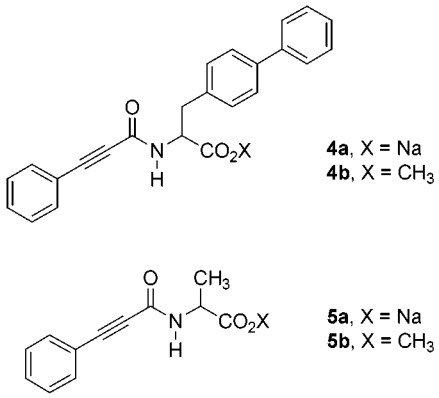


Figure 2. NMR spectra of the methylene protons adjacent to the naphthyl group of **3a** (a) and **1a** (b). The resonances are assigned to the Z or E rotamer as discussed in the text.

the spacing between the analogous methylene chemical shifts for **1a** (Figure 2b). For **1a**, the rotamers were assigned on the basis of observation of naphthyl−naphthyl NOEs for only one rotamer (assigned as E).[4] The NMR spectrum of **1a** showed that the methylene protons of the Z rotamer had relatively similar chemical shifts, while the methylene proton resonances of the E rotamer were more widely spaced. A similar methylene chemical shift pattern was observed for the E and Z rotamers of six analogues of **1a**, suggesting that this trend is general.[4] For **3a**, the major rotamer methylene resonances are more closely spaced than are the minor rotamer methylene resonances, which leads to our assignment of the major rotamer as Z.

Ester **3b** in $CDCl_3$ at 297 K displayed a major and minor set of ^1H resonances that were attributed to Z and E rotamers; the minor rotamer accounted for 6% of the population. The 4-fold increase in E rotamer population for **3a** in D_2O relative to **3b** in $CDCl_3$ supports the conclusion that the E rotamer of **3a** is favored in aqueous solution by a hydrophobic effect. There is presumably little or no drive for intramolecular association of the aromatic groups in **3b** in $CDCl_3$, since hydrocarbons are well solvated in this medium. Very similar behavior was observed for biphenyl compounds **4** at 297 K; carboxylate **4a** in D_2O displayed 26% of the minor rotamer (presumably E), while ester **4b** in $CDCl_3$ displayed 5% of the minor rotamer.[7]

Compounds **5** were examined to obtain further insight on the origin of solvent-dependent differences in rotamer populations for **3a** vs **3b** and **4a** vs **4b**. Compounds **5** bear a proton in place of the naphthyl unit of **3** and the biphenyl unit of **4**. Thus, little or no hydrophobic drive for folding is possible for **5a**. Indeed, the rotamer populations are similar for carboxylate **5a** in D_2O and ester **5b** in $CDCl_3$, 8% and



5%, respectively. The similarity among esters **3b**, **4b,** and **5b** shows that the rotamer populations in $CDCl_3$ reflect local interactions among atoms immediately surrounding the amide C−N bond and that interactions between hydrocarbon appendages are not energetically important in $CDCl_3$. The small difference in rotamer proportions for **5a** in D_2O vs **5b** in $CDCl_3$ suggests that the larger differences observed for **3a** vs **3b** and **4a** vs **4b** result at least in part from hydrophobically promoted clustering between the hydrocarbon moieties at either end of these molecules in aqueous solution. However, we cannot rule out the possibility that differences in carboxylate solvation between the E and Z amide rotamers of **3a** and **4a** contribute to the observed ratios in a way that is not accounted for by control compound **5a**.[8]

K_{EZ} values were used to estimate the free energy difference between E and Z rotamers (ΔG_{EZ}) of the carboxylates in D_2O at 297 K. ΔG_{EZ} values were similar for **3a** and **4a**, ca. 0.6 kcal/mol preference for the "extended" Z rotamer relative to the more compact E rotamer. For **5a**, the Z rotamer displayed greater relative stability, ca. 1.4 kcal/mol. Thus, partial clustering of the two aromatic groups in **3a** or **4a**, perhaps reinforced by differences in carboxylate solvation, stabilizes the unusual E rotamer relative to the Z rotamer by ca. 0.8 kcal/mol in aqueous solution.

Z rotamers of secondary amides are generally favored by roughly 2 kcal/mol relative to the E rotamers.[5] The strong Z preference is manifested in the very low E rotamer frequency of secondary amides in protein backbones.[9] Among simple secondary amides, the E rotamer population grows if steric repulsions are reduced in this conformation. For example, *N*-methylacetamide (NMA) displays 1.5% E rotamer in water,[5b] while *N*-methylformamide (NMF) displays 8% E rotamer;[5b] this difference presumably arises because the methyl/methyl replusion in (*E*)-NMA is greater than the hydrogen/methyl repulsion in (*E*)-NMF. The observation of 5−6% E rotamer for esters **3b**, **4b**, and **5b** in chloroform, relative to 2.8% (*E*)-NMA in chloroform,[5b] indicates that the

(7) We examined compounds **4** to ensure that similar behavior would be seen when the naphthyl group of **3** was replaced with another hydrophobic group.

(8) Reference 5b shows that the E/Z rotamer ratios of simple secondary amides are not strongly affected by solvation. See also: Morgan, K. M.; Kopp, D. A. *J. Chem. Soc., Perkin Trans. 2* **1998**, 2759.

(9) Weiss, M. S.; Jabs, A.; Hilgenfeld, R. *Nat. Struct. Biol.* **1998**, *5*, 676.

alkynyl carbon/methyl repulsion is diminished relative to repulsions between two sp^3 carbons. This diminution makes it possible to detect solvent-dependent stabilization in the E rotamers of **3b** and **4b**.

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