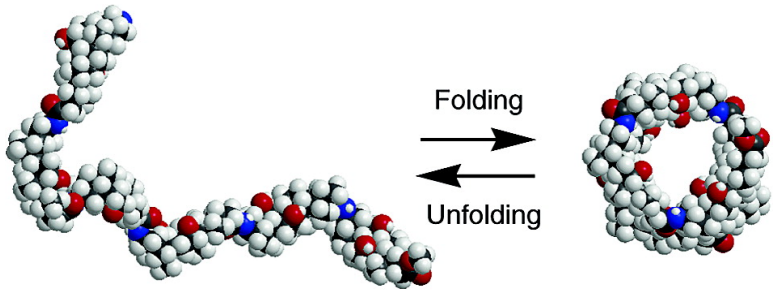
Article

Oligomeric Cholates: Amphiphilic Foldamers with Nanometer-Sized Hydrophilic Cavities

Yan Zhao, and Zhenqi Zhong

J. Am. Chem. Soc., **2005**, 127 (50), 17894-17901• DOI: 10.1021/ja056151p • Publication Date (Web): 24 November 2005



J|A|C|S
ARTICLES
Published on Web 11/24/2005

Oligomeric Cholates: Amphiphilic Foldamers with Nanometer-Sized Hydrophilic Cavities

Yan Zhao* and Zhenqi Zhong

Contribution from the Department of Chemistry, Iowa State University, Ames, Iowa 50011-3111

Received September 15, 2005; E-mail: zhaoy@iastate.edu

Abstract: The hydroxyl at the C-3 of cholic acid was converted to an amino group, and the resulting amino-functionalized cholic acid was used as a monomer to prepare amide-linked oligomeric cholates. These cholate oligomers fold into helical structures with nanometer-sized hydrophilic internal cavities in solvent mixtures consisting of mostly nonpolar solvents such as carbon tetrachloride or ethyl acetate/hexane and 2−5% of a polar solvent such as methanol or DMSO. The conformations of the foldamers were studied by UV, fluorescence, fluorescence quenching, and fluorescence resonance energy transfer. The nature of the polar/nonpolar solvents and their miscibility strongly influenced the folding reaction. Folding was cooperative, as evidenced by the sigmoidal curves in solvent denaturation experiments. The folded conformers became more stable with an increase in the chain length. The folding/unfolding equilibrium was highly sensitive toward the amount of polar solvent. One percent variation in the solvent composition could change the folding free energies by 0.5−1.4 kcal/mol.

Introduction

Conformational control in biomolecules such as polypeptides is at the heart of biology. The binding and catalytic functions of many protein receptors and enzymes are regulated through their controlled conformational changes.[1] Biological systems rely on these conformationally responsive molecules to sense and react to constantly fluctuating environmental conditions. As chemists, however, we have difficulty controlling the conformations of even small−medium-sized molecules. With an emerging recognition for the importance of conformational control in synthetic molecules, chemists have directed significant efforts in recent years to foldamers, or synthetic molecules with biomolecule-like, compact, ordered conformations.[2] It is anticipated that advancement in foldamer chemistry may not only shed new light on the folding and function of biomolecules but also allow the design of biomolecule-like, stimuli-responsive materials based on abiotic backbones.

We have been interested in using cholic acid (**1**) to construct environmentally responsive amphiphiles.[3] Cholic acid has a hydrophilic (α) face and a hydrophobic (β) face pointing to opposite directions.[4,5] It has a curvature toward the hydrophilic face as a result of the cis-fused A,B rings of the steroid backbone. We hypothesized that combination of these two features would make cholic acid a suitable monomer for foldamer synthesis. The contrafacial hydrophilic and hydrophobic faces allow the utilization of solvophobic interactions in conformational control; the curvature of the backbone creates an intrinsic preference for oligomeric cholates to adopt folded conformations. Another distinguishing feature of cholic acid is its size. The distance between the carboxyl tail and the hydroxyl group at C-3 is about 1.4 nm. Therefore, oligomers with just a few repeating units would reach several nanometers in dimension. A large monomer unit will not only improve the synthetic efficiency dramatically, but also provide a strong solvophobic driving force in the conformational control, as the strength of

(1) (a) Koshland, D. E., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1958**, *44*, 98−104. (b) Koshland, D. E., Jr. *Nature Med.* **1998**, *4*, 1112−1114 and references therein. (c) Hervé, G., Ed. *Allosteric Enzymes*; CRC Press: Boca Raton, Florida, 1989. (d) Perutz, M. F. *Mechanisms of Cooperativity and Allosteric Regulation in Proteins*; Cambridge University Press: Cambridge, 1990. (e) Hervé, G., Ed. *Allosteric Enzymes*; CRC Press: Boca Raton, Florida, 1989. (f) Kvamme, E., Pihl, A., Eds. *Regulation of Enzyme Activity and Allosteric Interactions*; Academic Press: New York, 1968.

(2) For several representative reviews, see: (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180. (b) Kirschenbaum, K.; Zuckerman, R. N.; Dill, D. A. *Curr. Opin. Struct. Biol.* **1999**, *9*, 530−535. (c) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893−4011. (d) Cubberley, M. S.; Iverson, B. L. *Curr. Opin. Chem. Biol.* **2001**, *5*, 650−653. (e) Sanford, A. R.; Gong, B. *Curr. Org. Chem.* **2003**, *7*, 1649−1659.

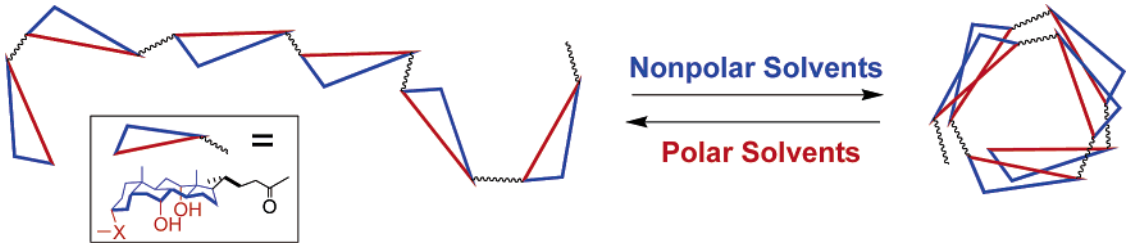
(3) (a) Ryu, E.-H.; Zhao, Y. *Org. Lett.* **2004**, *6*, 3187−3189. (b) Zhong, Z.; Yan, J.; Zhao, Y. *Langmuir* **2005**, *21*, 6235−6239. (c) Zhao, Y.; Ryu, E.-H. *J. Org. Chem.* **2005**, *70*, 7585−7591.

(4) For other examples of facial amphiphiles, see: (a) Stein, T. M.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 3943−3950. (b) Cheng, Y.; Ho, D. M.; Gottlieb, C. R.; Kahne, D.; Bruck M. A. *J. Am. Chem. Soc.* **1992**, *114*, 7319−7320. (c) Venkatesan, P.; Cheng, Y.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 6955−6956. (d) McQuade, D. T.; Barrett, D. G.; Desper, J. M.; Hayashi, R. K.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 4862−4869. (e) Broderick, S.; Davis, A. P.; Williams, R. P. *Tetrahedron Lett.* **1998**, *39*, 6083−6086. (f) Isaacs, L.; Witt, D.; Fettinger, J. C. *Chem. Commun.* **1999**, 2549−2550. (g) Taotafa, U.; McMullin, D. B.; Lee, S. C.; Hansen, L. D.; Savage, P. B. *Org. Lett.* **2000**, *2*, 4117−4120. (h) Arnt, L.; Tew, G. N. *J. Am. Chem. Soc.* **2002**, *124*, 7664−7665. (i) Zhong, Z.; Yan, J.; Zhao, Y. *Langmuir* **2005**, *21*, 6235−6239.

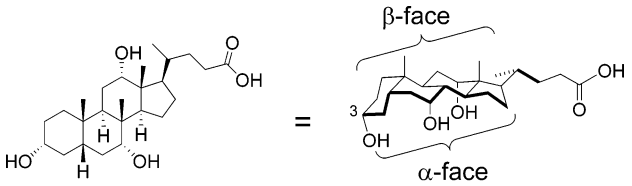
(5) For some examples of supramolecular systems constructed from cholic acid, see: (a) Davis, A. P.; Bonar-Law, R. P.; Sanders, J. K. M. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davis, J. E. D., MacNicol, D. D., Vögtle, F., Eds.; Elsevier: Oxford, 1996; Vol. 4, Chapter 7, and references therein. (b) Li, Y.; Dias, J. R. *Chem. Rev.* **1997**, *97*, 283−304 and references therein. (c) Maitra, U. *Curr. Sci.* **1996**, *71*, 617−624. (d) Smith, B. D.; Lambert, T. N. *Chem. Commun.* **2003**, 2261−2268, and references therein. (e) Davis, A. P.; Joos, J.-B. *Coord. Chem. Rev.* **2003**, *240*, 143−156 and references therein. (f) Burrows, C. J.; Sauter, R. A. *J. Inclusion. Phenom.* **1987**, *5*, 117−121. (g) Janout, V.; Lanier, M.; Regen, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 1573−1574. (h) Ariga, K.; Terasaka, Y.; Sakai, D.; Tsuji, H.; Kikuchi, J.-I. *J. Am. Chem. Soc.* **2000**, *122*, 7835−7836. (i) Werner, F.; Schneider, H.-J. *J. Inclusion Phenom. Macro. Chem.* **2001**, *41*, 37−40. (j) Yoshino, N.; Satake, A.; Kobuke, Y. *Angew. Chem., Int. Ed.* **2001**, *40*, 457−459.

10.1021/ja056151p CCC: $30.25

Scheme 1



solvophobic interaction is directly proportional to the buried solvophobic area.[6]



1, Cholic acid

In this paper, we report the synthesis of cholic acid based foldamers and the characterization of their conformational behavior by several spectroscopic methods.[7] An unusual feature of these cholate foldamers is that cavities 1 nm in diameter and 1.5 nm in length are formed with as few as six repeating units. Many foldamers in the literature adopt helical structures, but very few have internal cavities with nanometer-sized internal cavities.[8]

Results and Discussion

Design of Cholate Foldamers. We previously synthesized amphiphilic baskets by attaching four cholate groups to a calixarene scaffold.[3a,c] The molecules adopt micellelike conformations in polar solvents, with the hydroxyl groups facing outward, and reversed-micelle-like conformations in nonpolar solvents, with the hydroxyl groups inward. Similar solvophobically driven conformational changes may also occur if several cholates are linked in a head-to-tail fashion (Scheme 1). If the solvent mixture has similar preference for the α and the *β* faces, the oligomer should adopt extended, random conformations to maximize its entropy. In a mostly nonpolar solvent mixture, which preferentially solvates the hydrophobic *β* faces, the molecule is expected to bury the α faces. Several possible scenarios exist including aggregation/precipitation. If sufficiently low concentration is used and the possibility of precipitation excluded, the molecule may adopt collapsed but disordered conformations or, as shown in Scheme 1, fold into a helical structure with a hydrophobic exterior and a hydrophilic interior. Choice between the disordered or the helical conformer is not obvious at the outset of the project and probably depends on several factors including the intrinsic curvature and the rigidity of the monomer units.

Whereas we have little control over the intrinsic folding propensities of the cholate backbone, we have complete control in the solvent composition, which should also have profound influence on the conformations. A folded cholate oligomer resembles a unimolecular reversed micelle with a hydrophobic exterior and a hydrophilic interior. As in the molecular baskets, the most suitable solvents for such conformers consist of mostly a nonpolar solvent such as carbon tetrachloride and a small amount of a polar solvent such as methanol or dimethyl sulfoxide (DMSO).[3a,c]

In this study, we also used ternary solvent mixtures (DMSO/ethyl acetate/hexane) based on the postulation that folding is more likely to occur in partially miscible solvent mixtures than completely miscible ones. This is because the folded conformer would enrich the polar solvents in its hydrophilic interior and such demixing should be easier with low solvent miscibility. DMSO is completely miscible with ethyl acetate but immiscible with hexane. By varying the ratio between ethyl acetate and hexane, we can easily tune the solvent miscibility. For example, DMSO is miscible at all ratios with ethyl acetate/hexane (1/1) but is miscible only up to 5 vol % in ethyl acetate/hexane (1/2) in our hands.

Typical Synthesis of Cholate Oligomers. Among the three hydroxyl groups, the most reactive one is the hydroxyl at the C-3 position. Following a procedure reported by Davis and colleagues,[9] we prepared the *β* mesylate **3** from methyl cholate **2** using triphenylphosphine and diisopropyl azodicarboxylate (DIAD). Sodium azide attacks the mesylate by an S_N2 reaction to afford azide ester **4**, which is reduced by triphenylphosphine in aqueous THF to give **5** (Scheme 2). Standard amide coupling between **5** and carboxylic acid **6** using benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) yields the dimeric azide ester **7**. Repetition of the procedures gives the tetrameric azide ester **9**. Other oligomeric cholates could be synthesized in similar fashions, e.g., hydrolysis of **9** and amide coupling between the resulting acid and dimer amine **8** gives the hexamer.

UV and Fluorescence of NBD-Labeled Oligomeric Cholates. Since a folded cholate oligomer should enrich polar solvent molecules within its internal cavity, we reasoned that a solvent-sensitive chromophore attached to the oligomer should detect such a change in local solvent composition. We prepared cholate monomer, trimer, pentamer, and heptamer (**10**−**13**) labeled with the *N*-(7-nitrobenz-2-oxa-1,3-diazo-4-yl) or NBD group, whose UV and fluorescence are both sensitive to solvent polarity.[10] Because short oligomers cannot fold, any folding-derived "unusual" solvent effects should only occur in the longer oligomers (**12**, **13**). Shorter oligomers (**10**, **11**), therefore, serve

(6) (a) Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd ed.; Wiley: New York, 1980. (b) Ben-Naim, A. *Hydrophobic Interactions*; Plenum Press: New York, 1980. (c) Blokzijl, W.; Engberts, J. B. F. N. Hydrophobic Effects: Opinions and Facts. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1545−1579.

(7) We mainly use UV and fluorescence spectroscopy to characterize the conformational transitions of our foldamers. ^{1}H NMR spectroscopy is not useful as the cholate units are completely aliphatic and do not show noticeable changes in chemical shifts upon folding. In addition, severe overlapping of signals exists in the ^{1}H NMR spectra. Circular dichroism spectroscopy of the unlabeled oligomeric cholates is not possible due to interference of the amide absorptions by the solvents (e.g., CCl_4, ethyl acetate, and DMSO) needed to promote folding.

(8) To the best of our knowledge, well-characterized foldamers with large (nanometer-sized) internal cavities have only been achieved recently: Gong, B., et al. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11583−11588.

(9) Davis, A. P.; Dresen, S.; Lawless, L. J. *Tetrahedron Lett.* **1997**, *38*, 4305−4308.

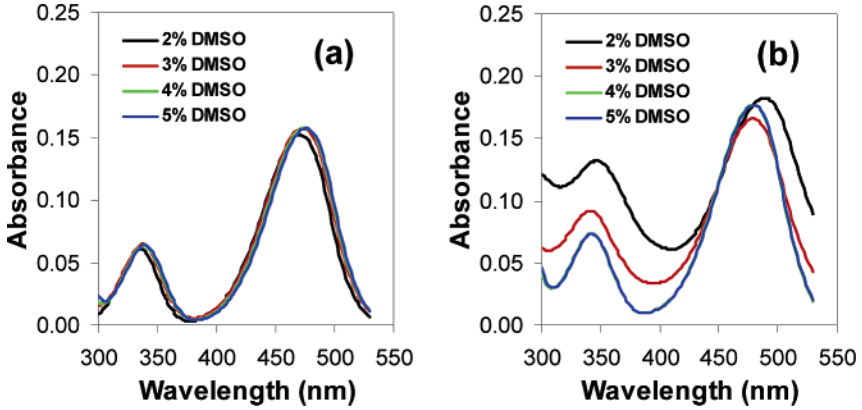
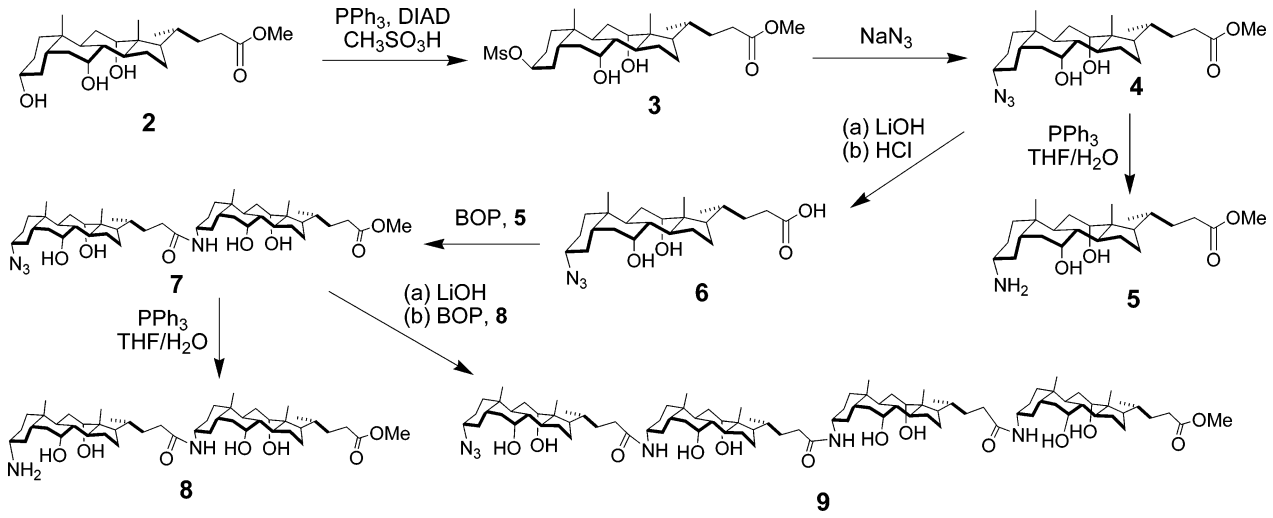
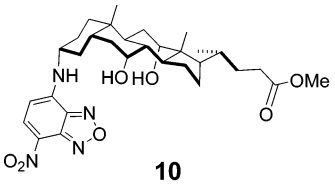


Figure 1. UV spectra of (a) monomer **10** and (b) heptamer **13** in DMSO/CCl_4 mixtures. [**10**] = [**13**] = 10 μM.

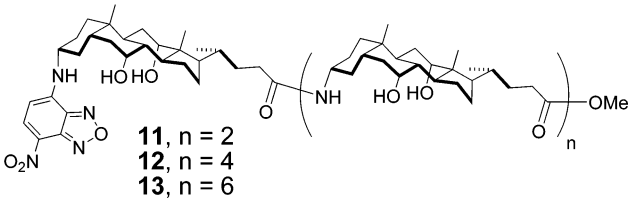
Scheme 2. Typical Synthesis of Cholate Oligomers



as control compounds. As in the molecular baskets, an increase in the polar solvent is expected to destabilize the reversed-micelle-like conformers.[3a] Hence, the unusual solvent effects should diminish with higher percentage of the polar solvent. In the end, all the oligomers should behave similarly if sufficient amount of the polar solvent is added to unfold the longer oligomers. These predictions are indeed confirmed in the NBD-labeled oligomers. When the volume percentage of DMSO is increased from 2 to 5% in CCl_4, the UV spectrum of the monomer (Figure 1a) stays nearly the same, but that of the heptamer displays large changes (Figure 1b). Moreover, the difference between the two is most significant in low percentage of DMSO and quickly disappears with higher (4−5%) DMSO.



10



11, n = 2
12, n = 4
13, n = 6

Our folding model predicts a higher local concentration of DMSO for a folded oligomer. Thus, our initial thought was that the unusual UV absorptions of the heptamer (in 2% DMSO/CCl_4) should be modeled simply by the monomer in solvent mixtures with higher DMSO. However, the UV spectrum of the monomer is almost identical in 10/90, 50/50, and 90/10 mixtures of DMSO and CCl_4 (Figure 1S in the Supporting Information). Apparently, it is not simply an increase in solvent polarity that caused the unusual UV of the heptamer. The result is not a total surprise because, even if DMSO is enriched inside the folded heptamer, they are not evenly distributed around the NBD group. It is quite possible that such an unsymmetrical solvation shell cannot be modeled by the NBD group of the monomer in homogeneous DMSO/CCl_4 mixtures. Even though the exact cause of the unusual UV of the heptamer is unclear at this point, this local environment is clearly destroyed with the addition of just a few percent of DMSO. Such behavior is consistent with the unfolding of the cholate backbone, which will destroy the internal cavity and local enrichment of DMSO.

We then studied the effect of chain length on the UV absorption. In Figure 2a, the UV absorbance at 334 nm (which is from the $\pi-\pi^*$ transition and most sensitive toward DMSO percentage) is plotted against DMSO percentage for the various oligomers. A distinctive chain-length dependence is observed, as none of the shorter oligomers (**10**−**12**) display solvent-sensitive absorptions. Most significantly, the plot for the

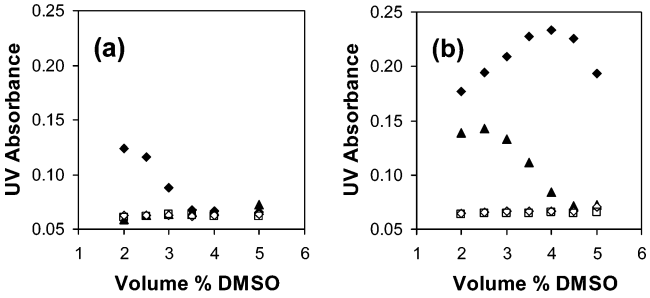


Figure 2. UV absorbance at 334 nm for monomer **10** (□), trimer **11** (◇), pentamer **12** (▲), and heptamer **13** (◆) as a function of DMSO in (a) CCl_4 and (b) ethyl acetate/hexane = 1/2. [Oligomer] = 10 μM.

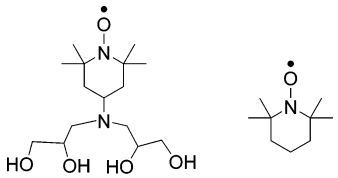
heptamer (◆) has a sigmoidal shape, a hallmark of cooperative phenomena typically observed in protein denaturation.[11] The data supports a helix-coil transition, which is cooperative and predicts higher stability with an increase in the chain length.[12] At this point, we tentatively assign the higher-absorbing state of the heptamer as the folded state and the monomerlike, lower-absorbing state as the unfolded state. As will be shown in later sections, the helix-coil model is completely consistent with all of our studies.

Another prediction from the solvophobically driven folding is that demixing and folding should be easier in the partially miscible DMSO/(ethyl acetate/hexane = 1/2) mixtures than the completely miscible DMSO/CCl_4. Indeed, as shown in Figure 2b, the pentamer (data shown in ▲), which seems to be unable to fold in DMSO/CCl_4 mixtures, displays the heptamerlike sigmoidal transitions in the ternary solvents. Not surprisingly, the monomer and the trimer show little changes in their UV with variation of DMSO, as they are too short to fold. The heptamer, on the other hand, remains in the higher-absorbing state throughout the experiments. However, instead of a sigmoidal curve, the UV absorbance of the heptamer in the ternary solvents shows an initial increase and a subsequent decrease (Figure 2b). This behavior is actually reasonable with the proposed folding process. According to Figure 2b, the pentamer is mostly folded with up to 3% DMSO. Because the folded heptamer is more stable and has a longer internal cavity, it should enrich DMSO more efficiently than the pentamer. Provided higher absorption of the NBD group is caused by local enrichment of DMSO, the heptamer should have higher absorption as well. During the initial increase in DMSO, the heptamer is mostly folded. Therefore, an increase in DMSO in the bulk solvent should increase its local concentration even more (and enhance the UV absorption of NBD). Further increase in DMSO, however, would unfold the heptamer and thus should decrease the local concentration of DMSO (and reduce the UV absorption of NBD). In ethyl acetate/hexane (1/2), we cannot go beyond 5% DMSO due to limited miscibility. Otherwise, we expect that all four oligomers will have the same UV eventually, as they did in the DMSO/CCl_4 mixtures.

Similar solvent effects were also observed in the fluorescence spectra. For example, the emission of the monomer is nearly unchanged with 2−5% DMSO in CCl_4 (Figure 3a), but that of the heptamer starts out with an intensity 1/6 of that of the monomer in 2% DMSO and gradually gains strength, approaching that of the monomer in 5% DMSO. In addition, a chain-length dependence similar to that found in UV was also observed in the fluorescence (Figure 4), except that the higher-absorbing (folded) state in the UV corresponds to the lower-fluorescing state in the fluorescence.

The UV and fluorescence data are consistent with solvent-induced folding/unfolding of the cholate oligomers. Importantly, the unusual behavior of the longer oligomers does not depend on their concentrations. The sample concentration was 10 μM for the UV and 2 μM for the fluorescence. When even lower concentration (0.2 μM) is used, similar DMSO-dependent fluorescence was observed for the heptamer and not for the monomer (Figure 2S in the Supporting Information). Therefore, the "unusual" solvent effects in the longer oligomers are unlikely to result from concentration-dependent aggregation or disordered conformational changes, which are unlikely to be cooperative. Instead, they probably come from cooperative, helix-coil transitions. The following experiments provide additional evidence for this hypothesis.

Fluorescence Quenching of NBD-Labeled Oligomeric Cholates. If folding indeed creates internal hydrophilic cavities, the emission of the NBD group should be efficiently quenched by a hydrophilic quencher (**14**) and the quenching efficiency should increase with chain lengths, as the folded state is more stable for the longer oligomers. Quenching by the parent quencher, TEMPO (**15**), however, should be independent of chain lengths because its hydrophobicity forbids it to enter the cavity generated by folding.



14

15

Figure 5a shows the quenching of **10**−**13** by **14** in 5% DMSO/CCl_4. We used 5% DMSO because the quencher had to be used in millimolar concentrations but was fairly insoluble with lower DMSO. As expected, positive deviations from linear Stern−Volmer quenching profiles are observed, and quenching efficiency clearly increases with the chain length. The basic conclusions drawn from Figure 5a—that the longer the chain length, the more stable the folded state (and the better it can bind a hydrophilic guest)—are the same from our UV and fluorescence studies. Subtle differences do exist, particularly for the pentamer. For example, UV (Figure 2a) seems to indicate that the pentamer is completely unfolded in DMSO/CCl_4. Fluorescence (Figure 4a) appears to suggest that pentamer is partly folded at least in 2% DMSO. Figure 5a, on the other hand, clearly shows enhanced quenching of the pentamer even in 5% DMSO/CCl_4, seemingly suggesting that the pentamer is folded even in 5% DMSO/CCl_4. Such "discrepancies" between different experimental methods are quite normal because these methods probably detect different folding-related events (e.g., DMSO enrichment or binding of a hydrophilic guest) and are expected to have different sensitivities for the conformational

(10) Fery-Forgues, S.; Fayet, J.-P.; Lopez, A. *J. Photochem. Photobiol. A* **1993**, *70*, 229−2463.
(11) Chan, H. S.; Bromberg, S.; Dill, K. A. *Philos. Trans. R. Soc. London, Ser. B* **1995**, *348*, 61−70.
(12) (a) Schellman, J. A. *J. Phys. Chem.* **1958**, *62*, 1485−1494. (b) Zimm, B. H.; Bragg, J. K. *J. Chem. Phys.* **1959**, *31*, 526−535. (c) Lifson, S.; Roig, A. *J. Chem. Phys.* **1961**, *34*, 1963−1974. (d) Poland, D. C.; Scheraga, H. A. *Theory of the Helix-Coil Transition*; Academic Press: New York, 1970.

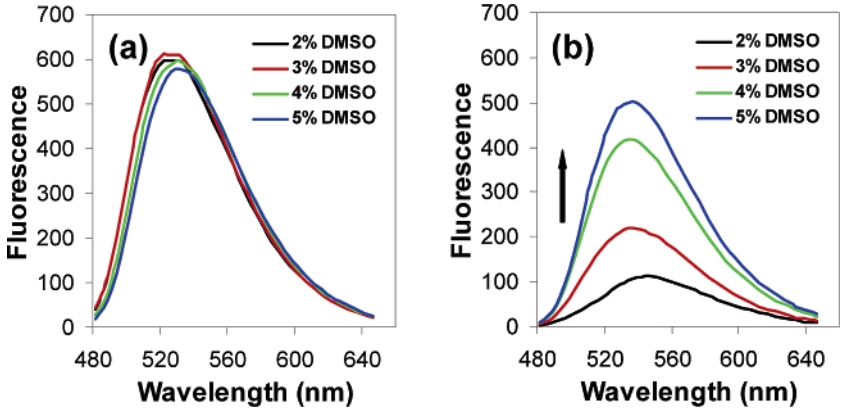


Figure 3. Fluorescence spectra of (a) monomer **10** and (b) heptamer **13** in DMSO/CCl_4 mixtures. [**10**] = [**13**] = 10 μM.

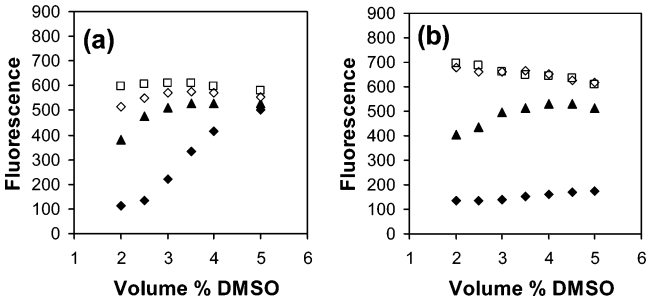


Figure 4. Maximum fluorescence intensity of monomer **10** (□), trimer **11** (◇), pentamer **12** (▲), and heptamer **13** (◆) as a function of DMSO in (a) CCl_4 and (b) ethyl acetate/hexane = 1/2. [Oligomer] = 10 μM.

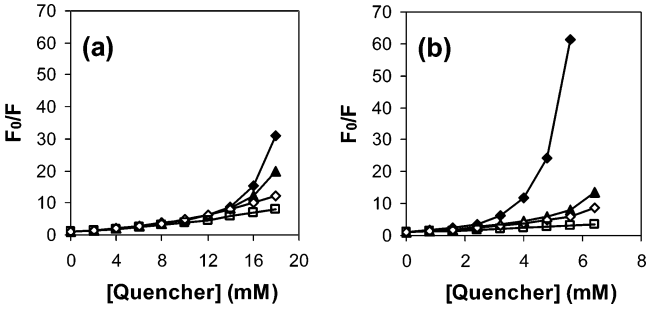


Figure 5. Quenching of monomer **10** (□), trimer **11** (◇), pentamer **12** (▲), and heptamer **13** (◆) by hydrophilic quencher **14** in (a) 5% DMSO/CCl_4 and (b) 2% MeOH/CCl_4. [Oligomer] = 12.5 μM.

transitions. In addition, these events may not be synchronous and have their own unique solvent-dependences. Therefore, the observed solvent effects are "composite" effects from multiple factors and should not be simply assigned to the folding/unfolding equilibrium.

Little enhancement in quenching of **10**−**13** by **14** is observed in 5% methanol/CCl_4. In 2% methanol, however, similar positive deviations appear again (Figure 5b). There are three important differences between the two solvent mixtures: (a) Quenching efficiency is much higher for the same cholate oligomer in 2% methanol/CCl_4 than in 5% DMSO/CCl_4. For example, the fluorescence of heptamer **13** (◆) is reduced by 60-fold with 5.6 mM of **14** in 2% methanol (Figure 5b) but only by <3-fold with the same concentration of the quencher in 5% DMSO. Therefore, binding between the heptamer and the quencher is much stronger in the former solvent. (b) When the most "foldable" heptamer (◆) and the least "foldable" monomer (□) are compared, difference in quenching is only obvious with >12 mM of quencher in 5% DMSO but is clearly observable at 3 mM in 2% methanol. Apparently, the quencher has difficulty entering the binding site formed by the heptamer in 5% DMSO. This difference again suggests stronger binding in 2% methanol than in 5% DMSO. (c) Difference between the more stable heptamer (◆) and the less stable pentamer (▲) is much larger in 2% methanol than in 5% DMSO. This result, together with the lack of quenching enhancement in 5% methanol, suggests that DMSO/CCl_4 mixtures are more amenable to folding than methanol/CCl_4 mixtures.

Interestingly, very similar solvent effects were observed in our cholate-based calixarene basket. The basket binds phenyl β-D-glucopyranoside in the reversed-micelle-like conformation. Binding is fairly strong (K_a = 290 M^{-1}) in 10% methanol/CCl_4 but is too weak to be detected in 10% DMSO/CCl_4, even though the latter mixture stabilizes the guest-binding conformer more than the former.[3c] This unusual solvent effect is actually quite normal, if one realizes that preferential solvation of the hydrophilic faces of cholates by DMSO or methanol is responsible for both the formation of the reversed-micelle-like conformer and its binding property. Whereas stronger preferential solvation stabilizes this conformer initially, it also makes it an inferior host during subsequent binding, because the strongly solvating DMSO molecules cannot be displaced easily by the guest. In other words, for a conformationally mobile, solvophobically based supramolecular host, the same interaction that helps the formation of the ordered conformer actually works against it during binding.

To further explore the effect of the polar solvent on quenching, we mixed the heptamer or the monomer with the hydrophilic quencher **14** in 2% methanol/CCl_4 and then added aliquots of methanol to this mixture. The two oligomers differ enormously in their response, as shown in Figure 6. Quenching efficiency for the monomer stays nearly the same with 2−10% of methanol, but that for the heptamer shows a huge decrease within a very narrow range (2−3%) of methanol percentage. In solvophobically based hosts with rigid structures, binding energies typically correlate linearly to the solvent solvophobicity parameters (which are linearly related to volume percentages in binary solvent mixtures).[13] Therefore, the abrupt solvent response cannot be explained by changes in solvent solvophobicity alone. Instead, unfolding of the heptamer is the most likely

(13) (a) Abraham, M. H. *J. Am. Chem. Soc.* **1982**, *104*, 2085−2094. (b) Abraham, M. H.; Grellier, P. L.; McGill, R. A. *J. Chem. Soc., Perkin Trans. 2* **1988**, 339−345. (c) Schneider, H.-J.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442−6448.

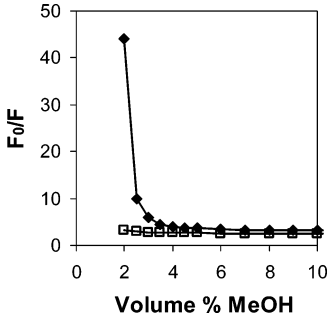


Figure 6. Quenching of monomer **10** (□) and heptamer **13** (◆) by hydrophilic quencher **14** as a function of the percentage of methanol in CCl_4. **[10]** = **[13]** = 12.5 μM, **[14]** = 5.6 mM.

reason. The conclusion is consistent with the earlier UV and fluorescence data.

As expected, when the hydrophobic **15** is used as the quencher, quenching becomes much less efficient, and the difference between the heptamer and the monomer completely disappear in either DMSO/CCl_4 or MeOH/CCl_4 mixtures (see Figure 3S in the Supporting Information). This is in line with our folding model because the hydrophilic cavity created by folding cannot bind hydrophobic guests.

Finally, to probe the effect of solvent miscibility on folding, we studied quenching in 5% DMSO in three different nonpolar solvents: ethyl acetate/hexane (1/2), CCl_4, and ethyl acetate. As discussed above, folding should be most favorable in ethyl acetate/hexane = 1/2 due to easy demixing of DMSO. Even though both ethyl acetate and CCl_4 are miscible with DMSO at all ratios, DMSO certainly is more "like" the polar ethyl acetate than the nonpolar CCl_4. Therefore, demixing of DMSO and folding should be easier in the latter. Our data clearly supports such a notion, with much more efficient quenching in ethyl acetate/hexane = 1/2 than in CCl_4 (see Figure 4S in the Supporting Information). The heptamer is probably completely unfolded in 5% DMSO/ethyl acetate, as no quenching enhancement is observed at all in this mixture.

Characterization of Conformations by Fluorescence Resonance Energy Transfer (FRET). The data so far suggest that the cholate foldamers (with ≥5 repeating units) form internal hydrophilic cavities upon folding. In agreement with helix-coil transitions, the folding seems to be cooperative. Examination of the molecular models suggests that three repeating units make one turn in the cholate foldamers. According to the models, the end-to-end distance is ca. 1 nm for the hexamer and ca. 2 nm for both the pentamer and the heptamer in the fully folded states. These distances can be easily distinguished by FRET,[14] whose transfer efficiency (E) is related to the donor−acceptor (D−A) distance (r) by equation $E = R_0^6/(R_0^6 + r^6)$, in which R_0 is the Förster distance for a specific D−A pair. Because typical R_0 (2−6 nm) is comparable to the diameter of many proteins, FRET has been widely used in the conformational study of biomolecules.[14] Note that distance measurement has been used to study foldamer conformations as well. For example, Moore and co-workers characterized the helical pitch of their *m*-phenylene ethynylene foldamers using distance-dependent spin−spin interactions.[14]

(14) In general, FRET is better used for measuring relative instead of absolute distances, see: (a) Stryer, L. *Annu. Rev. Biochem.* **1978**, *47*, 819−846. (b) Selvin, P. R. *Methods Enzymol.* **1995**, *246*, 300−334. (c) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer: New York, 1999; Chapter 13.

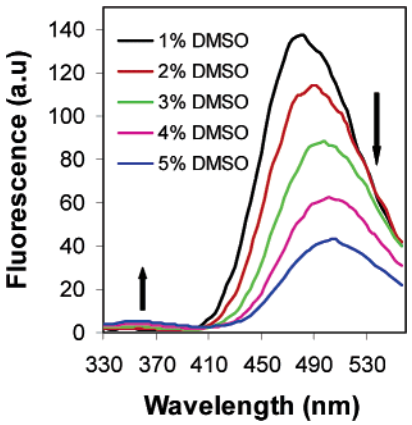
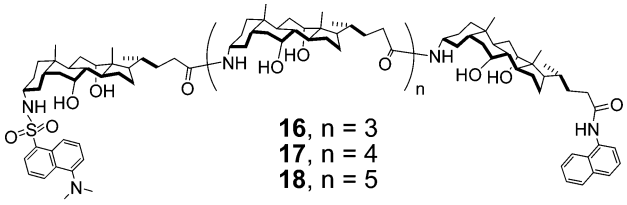
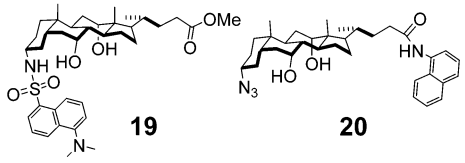


Figure 7. Fluorescence spectra of HXDA **17** in ethyl acetate/hexane (1/2) with different percentages of DMSO.

We choose the naphthalene−Dansyl D−A pair[16] and synthesized pentamer-DA **16** (PDA), hexamer-DA **17** (HXDA), heptamer-DA **18** (HPDA), monomer-A **19** (MA), and monomer-D **20** (MD).[17] Because CCl_4 interferes with the fluorescence of naphthalene, we mostly employ the ternary DMSO/ethyl acetate/hexane mixtures instead. As shown in Figure 7, FRET is clearly observable in HXDA in 1% DMSO, with the donor emission at 350 nm close to zero and the acceptor emission at 480 nm extremely strong. FRET becomes less efficient with higher DMSO, as shown by an increase of the donor emission and a decrease of the acceptor emission. Reduction in FRET suggests an increase in the D−A distance, consistent with a transition from a more compact folded helix to an unfolded coil.





The donor emission is very weak in our systems. Therefore, we use the acceptor emission as a relative indicator for the transfer efficiency—a stronger acceptor emission corresponds to a more efficient FRET. Parts a and b of Figure 8 show the maximum emission intensity of the acceptor vs DMSO percentage in two mixtures (1:2 and 1:1) of ethyl acetate/hexane.

Several important conclusions can be drawn from Figure 8. First, the acceptor emission of MA **19** is the same in the absence (□) or the presence (×) of MD **20** (see the Supporting Information for the actual fluorescent spectra). Thus, no intermolecular transfer occurs under the experimental conditions

(15) Matsuda, K.; Stone, M. T.; Moore, J. S. *J. Am. Chem. Soc.* **2002**, *124*, 11836−11837.
(16) This D−A pair was used as a spectroscopic ruler in the characterization of oligomeric l-prolines, see: Stryer, L.; Haugland, R. P. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 719−726.
(17) See the Supporting Information for the details of synthesis.

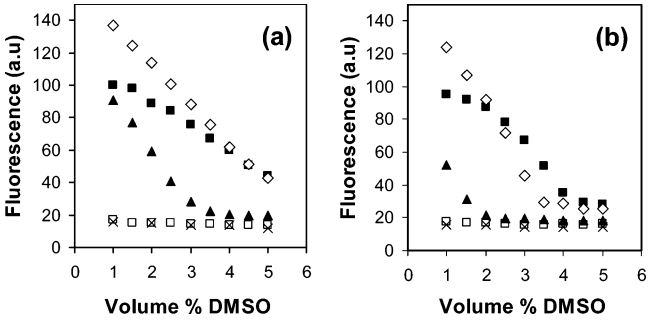


Figure 8. Maximum emission intensity of the acceptor in PDA **16** (▲), HXDA **17** (◇), HPDA **18** (■), MA **19** (□), and a 1:1 mixture (**19** + **20**) of MA and MD (×) as a function of the volume percentage of DMSO in (a) ethyl acetate/hexane (1/2) and (b) ethyl acetate/hexane (1/1).

in either solvent system. Also, the two curves for MA and (MA + MD) are nearly flat, indicating that the effect of DMSO on the acceptor emission is small. Second, FRET becomes less efficient in all three oligomers (**16**−**18**) with higher DMSO. On the basis of the slopes of the curves, stability of the folded state follows the order of heptamer > hexamer > pentamer. Third, stability of all three foldamers decreases in the more miscible DMSO/(ethyl acetate/hexane = 1/1) mixtures. For example, the pentamer loses its folded conformer with about 4% DMSO in ethyl acetate/hexane = 1/2 but does so with as little as 2% DMSO in ethyl acetate/hexane = 1/1. This behavior is fully consistent with our miscibility hypothesis. Fourth, the hexamer has the most efficient FRET in both mixtures of ethyl acetate/hexane with 1% DMSO but, because of its lower stability compared to the heptamer, ends up with lower FRET than the heptamer. It is not surprising for the hexamer to have a shorter D−A distance than the heptamer—any collapsed conformations probably will give such a result. It is, however, quite unusual for the hexamer to have a shorter D−A distance than the pentamer. The result once again confirms the helix-folding model, which predicts the closest end-to-end distance in the hexamer if three repeating units make one turn and the hexamer has two full turns. Such a periodicity is probably a result of the curvature of monomer created by the *cis*-fused A−B rings of the cholate backbone. Similar preference for trimeric structures was discovered by Sanders and co-workers, who reported trimeric cyclic cholate esters were more stable than other cyclic oligomers under thermodynamic control.[18]

The curves in Figure 8 once again have sigmoidal shapes, suggesting cooperative transitions in these "solvent denaturation experiments". The experimental data fit quite well to a two-state model (eq 1).[19,20] As shown in parts a and b of Figure 9, the stability of the foldamer follows the order of heptamer (■) > hexamer (◇) > pentamer (▲). In addition, the free energies in the transition regions of the solvent denaturation curves are linearly related to the concentration of DMSO (parts a and b of Figure 10). Such behavior is frequently seen in proteins which display two-state transitions in their folding and unfolding.[11,19]

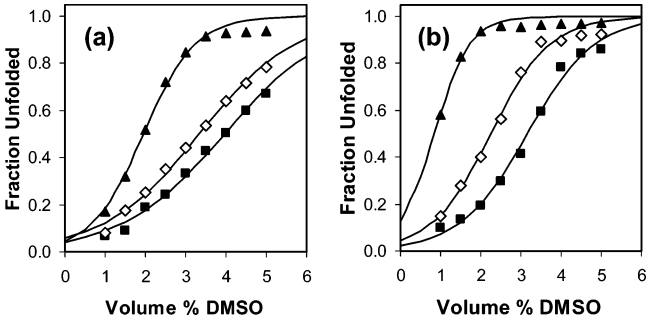


Figure 9. Fraction of the unfolded conformer in PDA **16** (▲), HXDA **17** (◇), and HPDA **18** (■) as a function of the volume percentage of DMSO in (a) ethyl acetate/hexane (1/2) and (a) ethyl acetate/hexane (1/1). The theoretical curves are nonlinear least-squares fitting to a two-state transition model (see the Supporting Information for details).

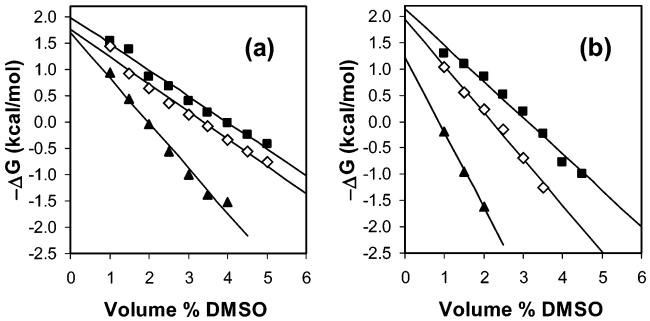


Figure 10. Unfolding free energies for PDA **16** (▲), HXDA **17** (◇), and HPDA **18** (■) as a function of the volume percentage of DMSO in (a) ethyl acetate/hexane (1/2) and (a) ethyl acetate/hexane (1/1).

It is quite remarkable that the cholate oligomers can display cooperative folding with as few as five repeating units.

$$\text{folded (helix)} \overset{K_{eq}}{\rightleftharpoons} \text{unfolded (coil)} \qquad (1)$$

From the curves in Figures 9 and 10, we can extract the thermodynamic parameters (Table 1) for the folding/unfolding equilibrium in all three cholate foldamers. In both solvents, the foldamers become more resistant to DMSO denaturation with longer chainlength, as *m* decreases with increasing chain length (entries 1−3 and 4−6). The ΔG_0 values generally reflect the same trend of stability (i.e., heptamer > hexamer > pentamer). Also, *m* is smaller for the same foldamer in the less miscible DMSO/(ethyl acetate/hexane = 1/2) than the more miscible DMSO/(ethyl acetate/hexane = 1/1)—compare entries 1 vs 4, 2 vs 5, and 3 vs 6. Therefore, the folded conformation does become more stable as demixing of the polar solvent becomes easier, as our initial postulation has predicted.

An interesting feature of our system is the role played by DMSO. At low concentrations, it is needed for both solubility and preferential solvation of the hydrophilic faces of cholates—the latter provides the fundamental driving force to folding. At higher concentrations, however, it destabilizes the folded conformer, with one percent change in DMSO shifting the folding free energy by 0.5−1.4 kcal/mol. One percentage change in solvent composition is unlikely to significantly change bulk solvent properties such as dielectric constants. Quite possibly, DMSO is enriched around the cholate foldamers by their polar hydroxyl and amide groups. Two types of interactions between DMSO and the cholate oligomers may exist. One type, serving to contract the cholate chains, is from the DMSO molecules

(18) Brady, P. A.; Bonar-Law, Ri. P.; Rowan, S. J.; Suckling, C. J.; Sanders, J. K. M. *Chem. Commun.* **1996**, 319−320.
(19) (a) Pace, C. N. *Methods in Enzymology*; Hirs, C. H. W., Timasheff, S. N., Eds.; Academic Press: New York, 1986; Vol. 131, pp 266−280. (b) Pace, C. N.; Shirley, B. A.; Thomson, J. A. *Protein Structure: A Practical Approach*; Creighton, T. E., Ed.; IRL Press: New York, 1989; pp 311−330.
(20) The two-state model seems to be reasonable for foldamers with relatively rigid repeating units, see: Prince, R. B.; Saven, J. G.; Wolynes, P. G.; Moore, J. S. *J. Am. Chem. Soc.* **1999**, *121*, 3114−3121 and references therein.

Table 1. Values of ΔG_0 and m Determined from Solvent Denaturation Curves[a]

entry	chain length	solvent composition	ΔG_0 (kcal/mol)	m (kcal/mol)
1	$n = 5$	DMSO in ethyl acetate/hexane (1/2)	1.8 ± 0.1 (1.7)	0.93 ± 0.06 (0.86)
2	$n = 6$	DMSO in ethyl acetate/hexane (1/2)	1.7 ± 0.1 (1.8)	0.50 ± 0.02 (0.52)
3	$n = 7$	DMSO in ethyl acetate/hexane (1/2)	1.9 ± 0.1 (2.0)	0.47 ± 0.02 (0.50)
4	$n = 5$	DMSO in ethyl acetate/hexane (1/1)	1.1 ± 0.2 (1.2)	1.35 ± 0.20 (1.42)
5	$n = 6$	DMSO in ethyl acetate/hexane (1/1)	1.8 ± 0.1 (1.9)	0.81 ± 0.06 (0.89)
6	$n = 7$	DMSO in ethyl acetate/hexane (1/1)	2.2 ± 0.1 (2.1)	0.71 ± 0.04 (0.69)

[a] Data with errors are determined from Figure 9 by nonlinear least-squares fitting to a two-state transition model. Data in parentheses are determined from Figure 10 by linear fitting of the unfolding free energies as a function of denaturant concentration. The data slightly underestimate the thermodynamic stability of the folded states. See the Supporting Information for details of data analysis.

within the hydrophilic cavities. These DMSO molecules selectively solvate the α faces of the cholates and essentially act as solvophobic "glue" to pull the otherwise extended chains together to form the helix. The other type is from those DMSO molecules outside the cavities, presumably serving to solvate the amide bonds and relax the cholate chains. It is probably the balance between the two that determines the folding/unfolding equilibrium. The ultrahigh sensitivity of the foldamers toward DMSO is unusual but is not unexpected considering similar behavior of our amphiphilic basket.[3a] The difference is that the latter is much better preorganized and, in consequence, requires higher percentage of DMSO to induce the conformational change.

Many other polar solvents can unfold the cholate helixes (see Figure 5S in the Supporting Information). In general, the unfolding ability of the solvent seems to follow its polarity and/or hydrogen-bonding ability. For example, the unfolding ability of the solvent follows the order of methanol > ethanol > 2-propanol > *tert*-butyl alcohol. For aprotic solvents, neither dioxane nor tetrahydrofuran has strong influence on the folding/unfolding equilibria (with dioxane being a slightly stronger denaturant among the two). *N*,*N*-Dimethylformamide, on the other hand, has very strong unfolding abilities, probably due to its strong hydrogen-bonding ability to solvate the amide linkages of the cholate foldamers.

Conclusions

The cholate oligomers can be synthesized easily from the amino-derived cholic acid using standard amide coupling reactions. The UV and the fluorescence studies of the NBD-labeled oligomeric cholates suggest cooperative conformational transitions in the longer oligomers ($n \geq 5$) from a higher-absorbing, lower-fluorescing state to a lower-absorbing, higher-fluorescing state, as the polar solvent (DMSO) is increased in a mostly nonpolar solvent mixture. The fluorescence-quenching experiments demonstrate that the higher-absorbing, lower-fluorescing conformer has internal hydrophilic cavities capable of binding a hydrophilic quencher. Binding affinity increases with the chain length. In addition, the binding site is quickly destroyed with even a few percent increase of the polar solvent. These data are consistent with folding of the cholate oligomers into helix structures with hydrophilic internal cavities in low DMSO and unfolding of the helix in high DMSO. Intermolecular aggregation is probably not important under the experimental conditions, as the changes in fluorescence and UV are independent of concentration over 50-fold dilution (from 10 to 0.2 μM).

To gain more definitive evidence for the folding process, we labeled the two ends of the cholate oligomers with a fluorescent donor and an acceptor and used FRET to measure the end-to-end distance in the cholate oligomers. The results strongly support a helix model with three monomer units making one turn, as the hexamer has closer end-to-end distance than either the pentamer or the heptamer. The thermodynamic parameters for the folding reactions obtained from the FRET data are consistent with cooperative helix-coil transition. In general, the folded conformers become more stable with longer chainlengh and are not as susceptible to the denaturant.

With three repeating units making one turn, the cholate foldamers can grow rapidly along the helical axis. This effect is further magnified by the large size of the repeating unit and its sideway alignment along the helical axis. As a result, every three repeating units contribute ca. 0.7 nm to the helical axis. On the basis of Corey−Pauling−Koltun models, foldamers with six repeating units can form a hydrophilic cavity about 1 nm in diameter and nearly 1.5 nm in length. It is remarkable that nanosized structures can be obtained in these cholate foldamers available in just a few steps from the monomer. The dimension of the internal cavity, the easy synthesis, and the readily tunable folding/unfolding of the cholate foldamers should make them very useful as novel supramolecular hosts and responsive materials.

Acknowledgment. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, and to Iowa State University for support of this research.

Supporting Information Available: The entire Experimental Section including the general Experimental Section, data analysis, synthesis and characterization of all the cholate oligomers, UV and fluorescence data, and ref 8 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA056151P