

# Efficient Construction of Oligocholate Foldamers via “Click” Chemistry and Their Tolerance of Structural Heterogeneity

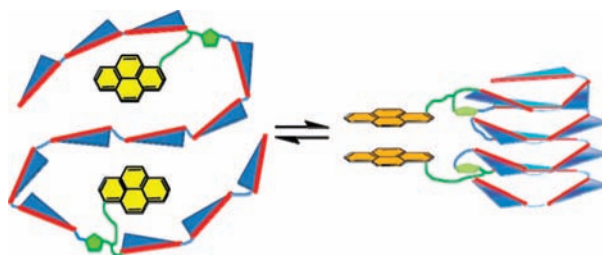
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## ABSTRACT



The 1,3-dipolar cycloaddition between an alkynyl-terminated cholate trimer and an azido-functionalized cholate hexamer readily afforded the nonamer and dodecamer derivatives, whereas amide coupling employed in previous oligocholate synthesis failed beyond the octamer. Unlike typical oligocholate foldamers with exclusively head-to-tail arrangement of the repeat units, the newly synthesized “clicked” oligocholates contained head-to-head arrangement and flexible tethers in the sequence. Despite large structural perturbations, the clicked oligocholates folded similarly as the parent foldamers, demonstrating the robustness of the solvophobically driven folding mechanism.

Much of the motivation in developing synthetic foldamers comes from the realization that biofoldamers such as proteins, nucleic acids, and polysaccharides are involved in practically all biological functions. If conformationally controllable biopolymers are entrusted by nature in structural support, binding, transport, and catalysis, their synthetic counterparts should hold promises as advanced functional materials in similar applications in chemistry, biology, and materials sciences.<sup>1,2</sup>

Although not as skillful as nature in conformational control, chemists have the advantage of working outside the

confines of genetic and biological machinery. In addition, different types of building blocks may be combined to afford heterogeneous foldamers, in which great diversity of the backbone and the side chains may be simply achieved by varying the ratio and positions of different building blocks.<sup>3,4</sup>

Our group has prepared heterogeneous foldamers containing both cholates and  $\alpha$ -amino acids—the latter mostly used as an inexpensive way to functionalize the parent foldamers.<sup>5</sup> In this paper, we report that “click” chemistry may be employed to synthesize long oligocholates inaccessible through standard amide coupling. Importantly, the same folding motif is obtained despite the large structural heterogeneity introduced during the synthesis.

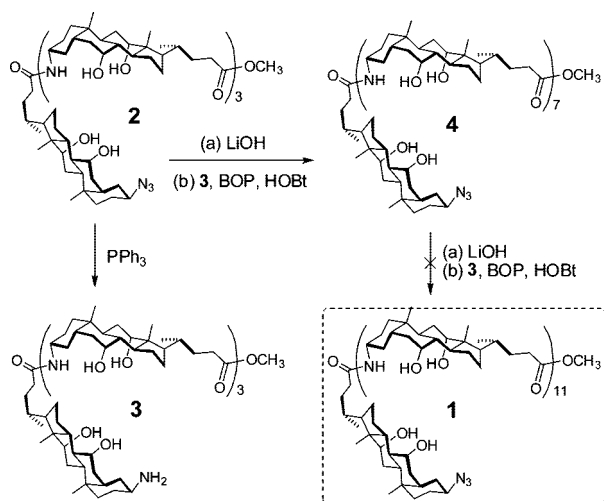
The oligocholates fold into helices in solvents such as 1–5% MeOH in hexane/ethyl acetate (EA).<sup>5</sup> The folded structure resembles a unimolecular reversed micelle (see the

(1) For some representative reviews, see: (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173–180. (b) Kirshenbaum, K.; Zuckermann, R. N.; Dill, K. 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*, 3893–4012. (d) Cubberley, M. S.; Iverson, B. L. *Curr. Opin. Chem. Biol.* **2001**, *5*, 650–653. (e) Goodman, C. M.; Choi, S.; Shandler, S.; DeGrado, W. F. *Nat. Chem. Biol.* **2007**, *3*, 252–262.

(2) Hecht, S.; Huc, I., Eds. *Foldamers: Structure, Properties, and Applications*; Wiley-VCH: Weinheim, 2007.

(3) For a recent review, see: Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399–1408.

### Scheme 1. Synthesis of Oligocholates by Amide Coupling



TOC graphic), in which some polar solvent molecules are microphase-separated from the bulk into the hydrophilic cavity to solvate the inwardly facing polar groups. The folded helix is extremely sensitive to solvent composition. One percent variation in the solvent composition could change the folding free energy of the parent oligocholates by over 1 kcal/mol.<sup>5a</sup> We reasoned that one way of stabilizing the folded helix is to increase the chain length<sup>6</sup> and became interested in long oligocholates such as dodecamer **1**.<sup>7</sup>

Construction of oligocholates typically follows a segment-doubling strategy (Scheme 1). One batch of **2** was reduced with  $\text{PPh}_3$  to give tetramer amine **3**. Another batch was hydrolyzed with  $\text{LiOH}$ . The resulting tetramer acid and amine were coupled using benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate ( $\text{BOP}$ ) to afford octamer **4**. Hydrolysis of the methyl ester in the oligocholates typically requires 5–10 equiv of  $\text{LiOH}$  in  $\text{MeOH}/\text{H}_2\text{O}$  at

room temperature for <24 h. To our surprise, the same condition was completely ineffective toward **4**, even though the hydrolysis proceeded smoothly in a cholate hexamer.<sup>5a</sup> Harsher reaction conditions did not solve the problem. A large excess of  $\text{LiOH}$  (>100 equiv) in refluxing  $\text{THF}/\text{MeOH}/\text{H}_2\text{O}$ , for example, afforded little hydrolysis in 24 h but caused extensive decomposition in **4** over several days. As an alternative route to **1**, we also attempted to reduce the azido group of **4** (and then couple the amino octamer with the tetramer acid). Again, neither the Staudinger reaction with >20 equiv  $\text{PPh}_3$  in refluxing  $\text{THF}/\text{H}_2\text{O}$  nor hydrogenation with >500 psi  $\text{H}_2$  over  $\text{Pd}/\text{C}$  was able to reduce the normally reactive azido group.

Regardless of the exact reason for the unreactivity of **4**,<sup>8</sup> a more efficient synthesis is needed to access longer oligocholates. Amide coupling clearly is not possible. Considering the difficulty encountered in the ester hydrolysis in **4**, even if the acid can be obtained, its coupling with a bulky amine such as **3** would be problematic. With an azido group already at one end of the oligocholates (see **2** and **4**), it is natural to consider the  $\text{Cu}(\text{I})$ -catalyzed “click” reaction between an azide and a terminal alkyne.<sup>9,10</sup> With an extraordinary thermodynamic driving force of >20 kcal/mol, this reaction has been used widely in efficient syntheses of sterically crowded compounds such as dendrimers and bulky polymers.<sup>11</sup>

The synthesis of nonamer **5a** and dodecamer **5b** is shown in Scheme 2. Hexamer **6a**, terminated with an azido group and a pyrene, was synthesized from the hexamer methyl ester (**6**,  $\text{R}' = \text{OMe}$ ) by standard transformations.<sup>5a</sup> Very similar chemistry yielded hexamer **6b** carrying two azido groups at

(8) The lack of reactivity in the terminal groups (azide or methyl ester) of **4** is peculiar. The compound is not expected to fold in the solvents used for the reactions. Our current hypothesis is that the curvature in the repeat unit makes an oligocholate “curl back” as the chain lengthens. Such a structure is disordered, different from the folded helix formed in special solvent mixtures. Derived from the monomer curvature, this “curling back” probably has little dependence on the solvents, should increase rapidly with an increase of the chain length, and may have buried the terminal groups of **4** to shield them from the chemical reagents. Unfortunately, the solubility of the oligocholates in the folding solvents (e.g., 2:1 hexane/EA with a few percent  $\text{MeOH}$ ) is insufficient for us to test whether the reactivity is enhanced in the folding conditions. Further study is underway to investigate this effect.

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(10) For recent examples of foldamers prepared by “click” chemistry, see: (a) Meudtner, R. M.; Ostermeier, M.; Goddard, R.; Limberg, C.; Hecht, S. *Chem.—Eur. J.* **2007**, *13*, 9834–9840. (b) Holub, J. M.; Jang, H.; Kirshenbaum, K. *Org. Lett.* **2007**, *9*, 3275–3278. (c) Juwarker, H.; Lenhardt, J. M.; Pham, D. M.; Craig, S. L. *Angew. Chem., Int. Ed.* **2008**, *40*, 3740–3743. (d) Meudtner, R. M.; Hecht, S. *Angew. Chem., Int. Ed.* **2008**, *47*, 4926–4930. (e) Li, Y.; Flood, A. H. *J. Am. Chem. Soc.* **2008**, *130*, 12111–12122.

(11) For examples of using “click” chemistry on dendrimers or polymers, see: (a) Helms, B.; Mynar, J. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2004**, *126*, 15020–15021. (b) Parrish, B.; Breitenkamp, R. B.; Emrick, T. *J. Am. Chem. Soc.* **2005**, *127*, 7404–7410. (c) Diaz, D. D.; Rajagopal, K.; Strable, E.; Schneider, J.; Finn, M. G. *J. Am. Chem. Soc.* **2006**, *128*, 6056–6057. (d) White, M. A.; Johnson, J. A.; Koberstein, J. T.; Turro, N. J. *J. Am. Chem. Soc.* **2006**, *128*, 11356–11357. (e) Whittaker, M. R.; Urbani, C. N.; Monteiro, M. J. *J. Am. Chem. Soc.* **2006**, *128*, 11360–11361.

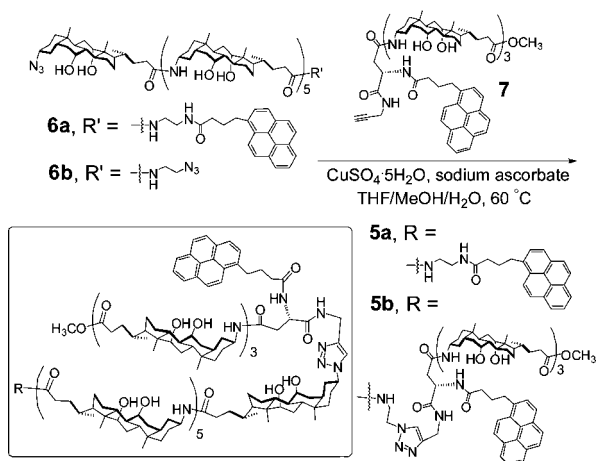
(4) For some other examples of heterogeneous foldamers, see: (a) Hagihara, M.; Anthony, N. J.; Stout, T. J.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 6568–6570. (b) Krauthäuser, S.; Christianson, L. A.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1997**, *119*, 11719–11720. (c) Huck, B. R.; Fisk, J. D.; Gellman, S. H. *Org. Lett.* **2000**, *2*, 2607–2610. (d) Gong, B. *Chem.—Eur. J.* **2001**, *7*, 4336–4342. (e) Gopi, H. N.; Roy, R. S.; Raghothama, S. R.; Karle, I. L.; Balam, P. *Helv. Chim. Acta* **2002**, *85*, 3313–3330. (f) Huc, I. *Eur. J. Org. Chem.* **2004**, 17–29. (g) Shamala, N.; Balam, P. *J. Am. Chem. Soc.* **2005**, *127*, 16668–16674. (h) Ananda, K.; Vasudev, P. G.; Sengupta, A.; Raja, K. M. P.; Baldauf, C.; Günther, R.; Hofmann, H. J. *J. Org. Chem.* **2006**, *71*, 1200–1208. (i) Sharma, G. V. M.; Jadhav, V. B.; Ramakrishna, K. V. S.; Jayaprakash, P.; Narsimulu, K.; Subash, V.; Kunwar, A. C. *J. Am. Chem. Soc.* **2006**, *128*, 14657–14668. (j) Rodriguez, J. M.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2007**, *46*, 8614–8617. (k) Cubberley, M. S.; Iverson, B. L. *Curr. Opin. Chem. Biol.* **2001**, *5*, 650–653. (l) Zhang, W.; Moore, J. S. *J. Am. Chem. Soc.* **2005**, *127*, 11863–11870. (m) Elliott, E. L.; Ray, C. R.; Kraft, S.; Atkins, J. R.; Moore, J. S. *J. Org. Chem.* **2006**, *71*, 5282–5290.

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(6) A longer oligocholate has more polar groups, which can concentrate the polar solvent more effectively into the hydrophilic interior of the folded helix. These polar solvent molecules in turn can solvate the introverted hydroxyl groups of the cholates more effectively in a nonpolar environment.

(7) The longest oligocholate synthesized previously was a heptamer (ref 5a).

## Scheme 2. Synthesis of Oligocholates by “Click” Chemistry

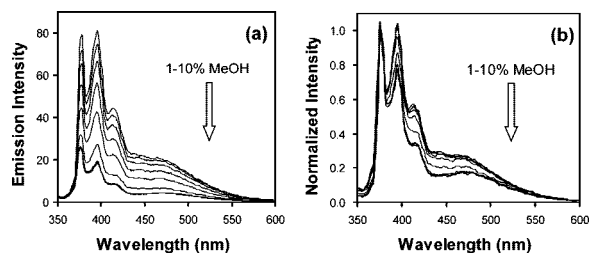


the chain ends. Compound **7** has three parts linked through an aspartic acid—a propargyl group for the 1,3-dipolar cycloaddition with azide, a pyrenyl to characterize the folding (vide infra), and a cholate trimer. To our delight, the “click” reaction indeed worked extremely well. Hexamer **6** and trimer **7** were stirred with  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and sodium ascorbate in THF/MeOH/ $\text{H}_2\text{O}$  at  $60^\circ\text{C}$ .<sup>12</sup> The reaction proceeded smoothly and was complete within 2 days. Preparative TLC afforded the final products in 50–70% isolated yield.

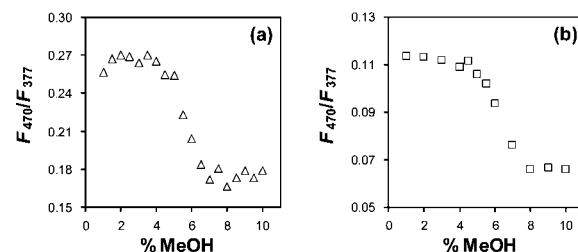
Although these long heterogeneous oligocholates (**5a** and **5b**) can be readily synthesized, there are serious concerns for their foldability. It is unclear how the triazole groups would affect the conformation. The aspartate spacer also brings considerable flexibility to the structure. In comparison to the parent oligocholates, the aspartate–triazole linker(s) introduces five additional rotatable bonds into **5a** and more than 10 into **5b**. Instead of the exclusively head-to-tail arrangement of the cholates in the parent foldamers, head-to-head arrangement is present in **5a** and **5b**.

The pyrenyl groups in oligocholates **5a** and **5b** were included to evaluate the folding of these compounds. The parent oligocholates have three units per turn according to our previous study.<sup>5a</sup> The trimeric periodicity is consistent with Sanders’ work in cyclic oligocholate esters.<sup>13</sup> According to the CPK model, the end-to-end distance of the fully folded hexamer is 1–1.5 nm. Separated by six cholates in between, the two pyrenyl groups of **5a** and **5b** should be reasonably close in space to form an excimer intramolecularly, provided the long aspartate–triazole tethers and the head-to-head arrangement of cholates do not alter the folding motif significantly.

To evaluate the folding of these heterogeneous oligocholates, we studied the emission of **5a** in 2:1 hexane/ethyl acetate (EA) with 1–10% MeOH. This solvent system represents one of the most “folding-friendly” for the (parent)



**Figure 1.** (a) Fluorescence and (b) normalized fluorescence spectra of nonamer **5a** in different mixtures of methanol and hexane/EA (2/1).  $[\mathbf{5a}] = 0.1 \times 10^{-6}$  M.  $\lambda_{\text{ex}} = 336$  nm.



**Figure 2.** Excimer/monomer ratio in (a) **5a** and (b) **5b** as a function of % MeOH in 2:1 hexane/EA.

oligocholates.<sup>5,14</sup> As methanol is added, the pyrene emission becomes weaker (Figure 1a). In low methanol solutions, a broad band is visible near 470 nm that corresponds to the excimer of pyrene. At a low concentration of  $0.1 \mu\text{M}$ , **5a** is unlikely to aggregate, as the parent oligocholates show no signs of aggregation in similar solvents even at ca.  $10 \mu\text{M}$ .<sup>5a</sup> Thus, the excimer in **5a** should come from an intramolecular process such as folding. Once the spectra are normalized to the intensity at 377 nm (i.e., monomer emission), addition of methanol is clearly seen to lower the excimer/monomer ratio (Figure 1b), indicating a transition to the unfolded conformation. The other vibronic bands of pyrene (at 400 and 420 nm) also showed changes during the methanol titration. This is a reasonable result because, as mentioned earlier, the local composition of solvents is expected to be different for the folded and the unfolded conformer and the vibronic bands of pyrene are known to be sensitive to solvent polarity.<sup>15</sup>

Since the excimer/monomer ratio ( $F_{470}/F_{377}$ ) is the best indicator for the folding/unfolding transition, it is useful to compare its change during the methanol titration for **5a** (Figure 2a) and **5b** (Figure 2b). These solvent titration curves have a distinctive sigmoidal shape, characteristic of cooperative conformational changes.<sup>16</sup> Note that similar sigmoidal

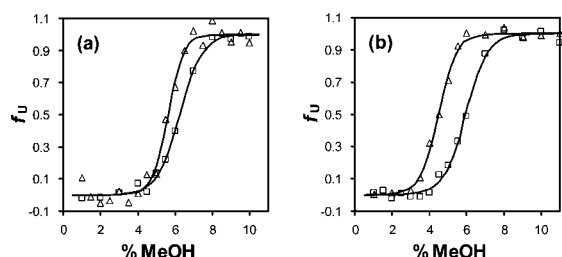
(12) This condition previously afforded 1,4-triazoles exclusively in our synthesis of water-soluble calixarenes; see: Ryu, E.-H.; Zhao, Y. *Org. Lett.* **2005**, *7*, 1035–1037.

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(14) Folding of an oligocholate requires the phase separation of the polar solvent (MeOH) into the hydrophilic cavity in the folded helix. The phase separation of methanol is easier with hexane in the solvent because hexane is immiscible with methanol but EA is.

(15) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039–2044.

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**Figure 3.** Fraction of the unfolded conformer ( $f_U$ ) for nonamer **5a** ( $\Delta$ ) and dodecamer **5b** ( $\square$ ) as a function of % MeOH in (a) 2:1 hexane/EA and (b) 1:1 hexane/EA. The theoretical curves are nonlinear least-squares fitting to a two-state transition model (see the Supporting Information for details).

titration curves were observed in the parent oligocholates.<sup>5a</sup> The difference is that the parent oligocholates (pentamer through heptamer) did not show a plateau on the left side of the titration curve.<sup>17</sup>

A two-state model has been used to describe the conformational changes of solvophobic foldamers<sup>18</sup> including the oligocholates.<sup>5a</sup> Two-state analysis allows one to calculate the percentages of the unfolded ( $f_U$ ) or folded conformer at any given solvent composition. Figure 3a shows the two-state fitting of the titration data. Both the nonamer ( $\Delta$ ) and the dodecamer ( $\square$ ) remain fully folded in <4% MeOH. In contrast, the parent oligocholates (with up to seven cholates) were already in the transition region even with 1% MeOH.<sup>5c</sup> Thus, both **5a** and **5b** fold better than the parent homogeneous oligocholates. One reason must be their longer chain lengths.<sup>19</sup> Among the two clicked oligocholates, the longer **5b** ( $\square$ ) folds better than the shorter **5a** ( $\Delta$ ). According to the data fitting, it takes 5.6 and 6.2% MeOH to unfold 50% of **5a** and **5b**, respectively.

Both the cooperativity and the chain length-dependency in the solvent titration suggest that these clicked oligocholates and the parent oligocholates follow the same folding mech-

anism. To further confirm the folding mechanism, we performed MeOH titration and data fitting in 1:1 hexane/EA (Figure 3b). Because the folding of oligocholates require the phase separation of methanol from the nonpolar mixture, lowering hexane in the solvent mixture makes the folding more difficult.<sup>5a,14</sup> When the two solvent systems are compared (Figures 3a and 3b), even though both foldamers become less stable in 1:1 hexane/EA, the shorter nonamer ( $\Delta$ ) is clearly affected more. For example, 50% unfolding requires 4.5 and 6.0% MeOH for **5a** and **5b**, respectively (compare with the 5.6 and 6.2% MeOH for these compounds in 2:1 hexane/EA).

Click chemistry once again demonstrated its power in highly crowded constructs. A dodecameric oligocholate, inaccessible through the amide coupling, was readily prepared by the 1,3-dipolar cycloaddition. The cooperativity, chain length effect, and solvent dependency in the clicked oligocholates are completely in line with the solvent-driven folding observed in the parent foldamers. The folded hexamer has two helical turns with three cholates per turn.<sup>5a</sup> Driven by the same solvent effect, the folded nonamer and dodecamer most likely are helical as well, with one and two helical turns added to the two turns already formed by the hexamer in the middle. The tolerance of structural heterogeneity by the oligocholates is remarkable.<sup>20</sup> Neither long tethers nor head-to-head arrangement of repeat units posed any problems to the folding. Given the outstanding tolerance of functional groups, the high yields, and the modularity of the click reaction, this synthetic strategy will greatly simplify the synthesis of functionalized oligocholates.

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**Supporting Information Available:** Experimental details, two-state analysis, fluorescence spectra, and NMR spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(17) The plateaus on the left and the right side of the titration curve generally correspond to the fully folded and the fully unfolded state for a two-state transition.

(18) Prince, R. B.; Saven, J. G.; Wolynes, P. G.; Moore, J. S. *J. Am. Chem. Soc.* **1999**, *121*, 3114–3121.

(19) Another possible reason is the flexibility introduced by the aspartate-triazole linker(s).

(20) This is probably because solvophobic forces do not require precise alignment of functional groups to be effective; see: Zhao, Y.; Moore, J. S. In *Foldamers*; Hecht, S., Huc, I., Eds.; Wiley-VCH: Weinheim, 2007; pp 75–108.