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Spacer-Dependent Folding and Aggregation of Oligocholates in SDS Micelles

Yan Zhao*

Department of Chemistry, Iowa State University, Ames, Iowa 50011-3111

zhaoy@iastate.edu

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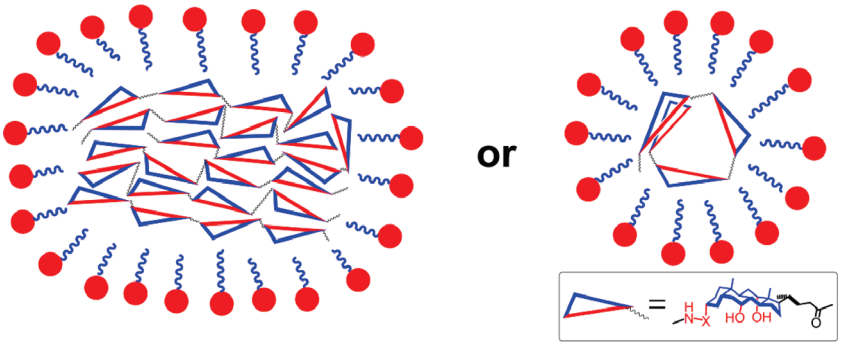

Insertion of flexible, 4-aminobutyroyl spacers in between the cholate repeat units had been found previously to enhance the folding of cholate oligomers in homogeneous solution (Zhao, Y. *J. Org. Chem.* **2009**, *74*, 834−843). The opposite effect was observed when the oligomers were solubilized in aqueous solutions of sodium dodecyl sulfate (SDS). The spacers enabled formation of tight aggregates of the oligocholates in SDS solutions when the surfactant was below its critical micelle concentration (CMC). Above the CMC, SDS micelles formed and dissociated the oligocholate aggregates. The parent oligocholates (without spacers in between the repeat units) also aggregated when they were too short to fold (e.g., dimer). The longer tetramer and hexamer preferred to fold, as their rigid, awkwardly shaped backbones prevented tight packing needed in the formation of stable aggregates. Folding was favored both below and above the CMC of SDS and was enhanced by an increase in the chain length.

Introduction

Foldamers, the conformational mimics of proteins and nucleic acids, have attracted the attention of scientists in many disciplines.[1] Recent research efforts include continued search for novel building blocks and folding motifs. Rigid bis-amino acids,[2] ferrocene-containing amino acids,[3] *N*-arylglycine,[4] spirobi(indane),[5] and indole[6] are but a few examples of new building blocks that appeared in recent literature. Utilization of two or more types of building blocks in the construction has also become a powerful way to tune the structure and function of foldamers.[7] Inserting a second set of monomers into the original, homogeneous foldamer sequence was shown to modify the conformational property,[8] introduce desired molecule- or metal-binding feature,[9] and overcome synthetic limitations in the original building blocks.[10] In addition, as chemists acquire fundamental learning in the structural design and

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conformational control of synthetic foldamers, they become increasingly interested in the practical applications of these molecules. Tunable anion receptors,[11] antimicrobial materials,[12] bioactive ligands,[13] organogellators,[14] vesicles,[14a] and biomimetic catalysts[15] were created from foldamers. Folding has also been used to modulate interactions of organic semiconductors[16] and control macrocyclization.[17]

The environment of a molecule has enormous impact on its conformation. Nonetheless, foldamer research so far has focused almost exclusively on the conformational control of molecules in homogeneous solution and in the solid state; folding in other environments such as surfactant micelles or lipid bilayers is largely unexplored. Although foldamers have been synthesized to interact with lipid bilayers,[12] their conformation has rarely been studied in detail.[18] However, as chemists explore new applications of synthetic foldamers, understanding how foldamers behave in different environments is crucial. In nature, for example, both water-soluble and membrane-associated proteins play important biological functions.

Micelles have been frequently used by biochemists to study how membrane-associated proteins/peptides fold in a membrane-like environment.[19] Membrane proteins play critical roles in numerous biological activities including ion conduction, photosynthesis, signal transduction, vision, fertilization, and immune response but are notoriously difficult to study. Understanding how synthetic foldamers behave in a membrane-like environment will not only expand the scope of potential applications of synthetic foldamers but also help us gain insight into how membrane proteins fold in similar environments.

In this paper, we continue our investigation of amphiphilic oligocholate foldamers in surfactant micelles. The most interesting discovery is that oligocholates with flexible spacers in between the cholates behave completely differently from the parent, more rigid foldamers without any spacers. The folding or aggregation of a particular oligocholate in SDS solutions additionally depends on the surfactant concentration and the chain length of the oligomer. The interplay between these parameters highlights the different rules that govern the folding of the same molecules in different environments.

Results and Discussion

4-Aminobutyroyl-Spaced Oligocholates in SDS Micelles. Oligocholates are amphiphilic molecules with distinct distribution of hydrophilic and hydrophobic groups. In solution, their folding is controlled by preferential solvation of the hydrophilic and hydrophobic surfaces.[20] Without additional stabilizing forces such as an internal

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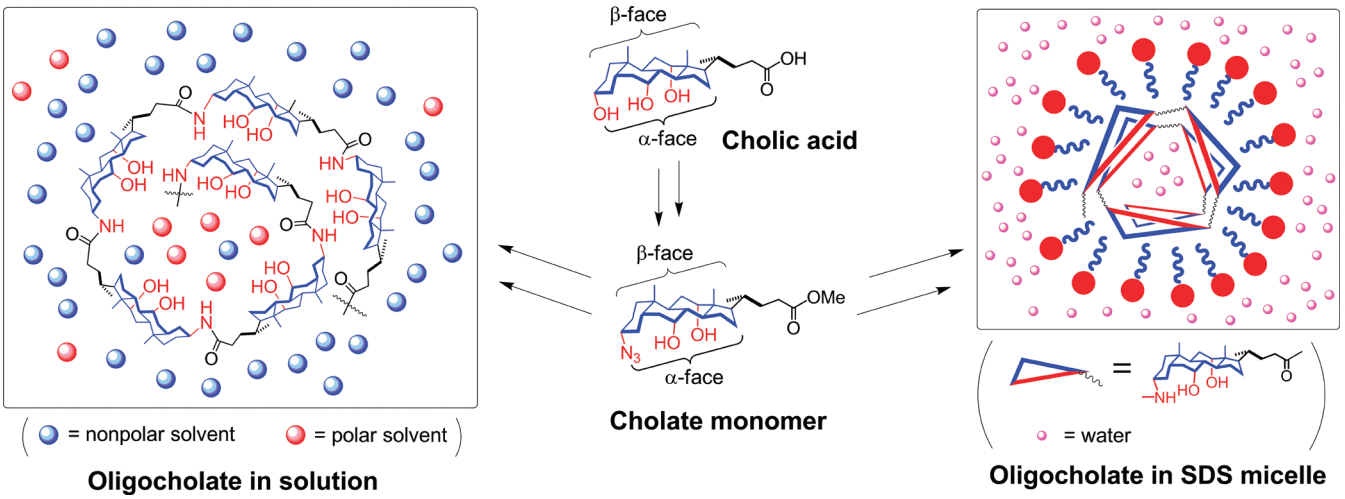
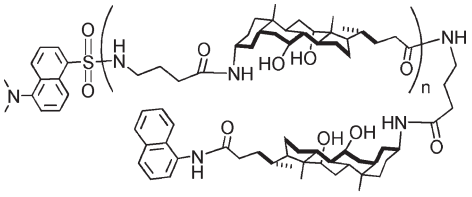
SCHEME 1. Cholic Acid and Oligocholate



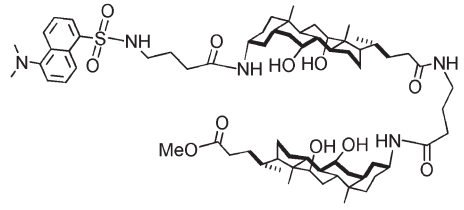
salt bridge[20d] or metal−ligand complexation,[20b,e] oligocholates typically fold in nonpolar solvents (e.g., ethyl acetate, ethyl acetate/hexane, or CCl_4) containing a small amount of a polar solvent (DMSO, methanol, or other small alcohol). During folding, the minor, polar solvent microphase-separates from the bulk into the nanometer-sized hydrophilic cavity, providing efficient solvation to the polar groups in a largely nonpolar environment (Scheme 1, left).

In a previous work, surfactant micelles were found to fold oligocholates by a completely different mechanism.[21] The SDS micelle has a strong preference for spherical shape to minimize the charge density on the micellar surface.[22] The hydrophobic core of an SDS micelle is about 3 nm in diameter, as determined by the chain length of the dodecyl chain. A small, spherical micelle has difficulty accommodating the unfolded oligocholate, which can extend to several nanometers in length. The folded form, on the other hand, is < 2 nm in diameter and can be easily included within a small, spherical micelle. A cholate hexamer was found to remain completely folded in 1−70 mM SDS aqueous solution.[21] Mechanistically, folding of an oligocholate in an SDS micelle (Scheme 1, right) is analogous to forcing a snake into a small cage, in which the snake has no choice but to coil up.

The current investigation initially focused on 4-aminobutyrol-spaced oligocholates **1**−**5**. In homogeneous solution, insertion of the flexible spacers was found to facilitate the folding. Whereas the parent oligocholates (without any spacers) require at least 5 repeat units to fold, the 4-aminobutyroyl-spaced oligocholates fold well with 3 or 4 cholate groups, even in more challenging solvents.[23] The enhanced foldability was attributed to a less strained folded helix. The explanation was supported by our earlier work on cholate−calixarene-based molecular baskets, which adopt similar reversed micelle-like conformation in nonpolar environments.[24]



1, n = 3; **2**, n = 2; **3**, n = 1



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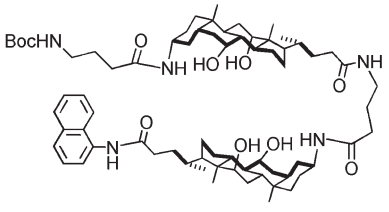

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The main question asked at the outset of this investigation was whether the flexible spacers could enhance the folding of **1**−**5** in micelles just as they did in solution. The deeper questions, of course, were (a) whether similar rules govern the folding of oligocholates in micelles and in homogeneous solution, and (b) if the rules are different, in what aspects and for what reasons are they different.

Fluorescence resonance energy transfer (FRET) is a powerful tool to study the conformation of molecules.[25] Similar to NOE-based techniques, FRET depends on distances (between a donor

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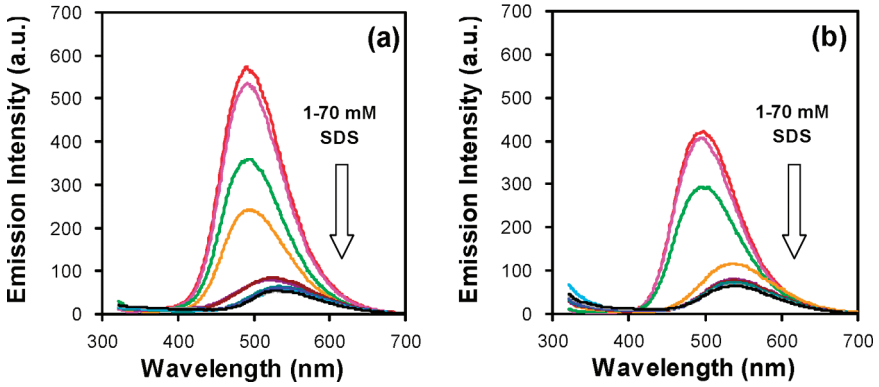
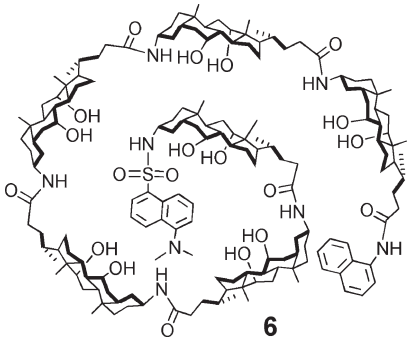


FIGURE 1. Emission spectra of (a) **1** and (b) **2** in 1, 2, 4, 6, 8, 10, 14, 20, 30, 50, and 70 mM SDS solutions. [oligomer] = 2.0×10^{-6} M. λ_{ex} = 287 nm.

and an acceptor fluorophore). The energy-transfer efficiency (E) is related to the D−A distance (r) by equation $E = R_o^6/(R_o^6 + r^6)$, in which R_o is the Förster distance for a specific D−A pair and corresponds to the distance at which the energy-transfer efficiency is 50%. Because R_o typically ranges from 1 to 10 nm and such a distance is comparable to the size of most biofoldamers, FRET is widely used to study the conformation of proteins and DNAs. FRET is especially suitable for the oligocholates because of their nanometer-sized dimension and highly dynamic conformation.[20,21,23] With the R_o value equal to 2.2 nm,[26] the naphthyl−dansyl pair can easily detect distances between 1.5 nm (E = 0.9) and 3.2 nm (E = 0.1). FRET can be measured either by an increase in the acceptor emission or the decrease of the donor emission. Our previous work indicates that the naphthyl emission is weak under most conditions and FRET is better detected by the enhancement of the dansyl emission.[20,21,23]

Naphthyl has the maximum absorption (λ_{max}) at 300 nm and emits around 360 nm.[27] Because the largest difference in absorption between naphthyl and dansyl occurs at 287 nm, we first recorded the emission spectra of tetramer **1** and trimer **2** at this wavelength. When the concentration of SDS is increased from 1 to 70 mM, dansyl emission near 500 nm becomes weaker and meantime shifts to the red (Figure 1). Notably, the red shift occurs abruptly around 6−8 mM of SDS. These behaviors differ greatly from those of the parent, more rigid hexamer **6**, whose emission stayed nearly constant in 1−70 mM SDS.[21]



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Thus, the flexible spacers greatly altered the behavior of the oligocholates in SDS solutions. The weakening of the dansyl emission may result from two possible processes: (a) the oligocholates fold at low SDS but unfold at higher SDS, or (b) the oligocholates aggregate at low SDS and the aggregates dissociate at higher SDS. During a folding−unfolding transition, dansyl no longer benefits from intramolecular FRET, which transfers the excited energy of the donor to the dansyl acceptor and enhances its emission. Weakening of dansyl emission is frequently observed in homogeneous solution when an oligocholate is unfolded by the addition of a polar solvent.[20,23] Alternatively, dansyl may become less fluorescent during an aggregation−dissociation process for the following reasons. First, dansyl may become more exposed to water. The aggregate of an oligocholate can bury the dansyl in the interior and shield it from water. As the aggregate dissociates, the environment around the dansyl may become more polar; it is known that dansyl fluoresces less strongly in more polar environments.[28] Second, an individual molecule moves faster than its aggregate and, as a result, collides with more molecules (oxygen, solvent) per unit time. A higher collision frequency in the lifetime of an excited molecule increases its probability of being quenched and is expected to lower its fluorescence quantum yield. Third, if intermolecular FRET occurs within the aggregate, dissociation of the aggregate can lower the acceptor's emission by stopping the energy transfer.

The best way to extract the contribution of FRET is through the excitation spectrum, in which the emission of the dansyl acceptor is monitored while the excitation wavelength is varied. FRET is signified by the appearance of the *donor's absorption* peaks in the acceptor's emission spectrum. Figure 2a and b are the as-recorded excitation spectra of donor−acceptor-labeled tetramer **1** and acceptor-labeled dimer **4** in different SDS solutions when the dansyl emission is monitored at 500 nm. Dimer **4** is essentially "half" of tetramer **1**, used as a control compound to measure the effect of SDS on dansyl in the absence of the donor. Emissive intensity decreases in both cases as the concentration of SDS is increased. The weakening of dansyl emission in **4** by higher SDS does not have anything to do with FRET, since the donor is absent, but the weakening in **1** clearly does. In Figure 2a, the peak near 300 nm is from the donor's absorption and gradually disappears as more SDS is added.

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(27) For the UV spectra of these compounds, see: Zhao, Y. *J. Org. Chem.* **2009**, *74*, 834–843.

(28) Li, Y.-H.; Chan, L.-M.; Tyer, L; Moody, R. T.; Himel, C. M.; Hercules, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 3118–3126.

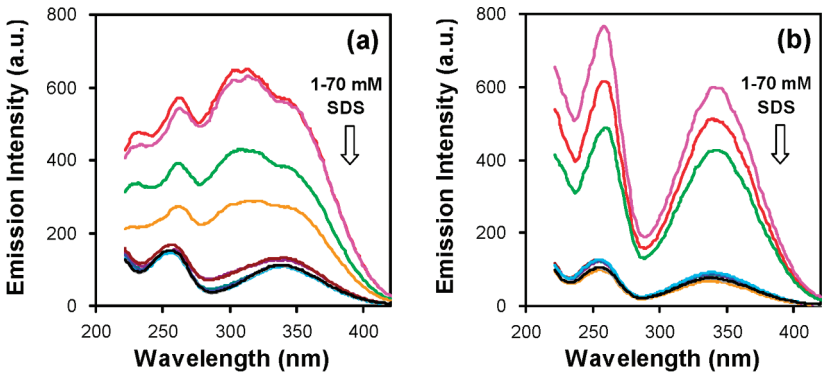


FIGURE 2. Excitation spectra of (a) **1** and (b) **4** in 1, 2, 4, 6, 8, 10, 14, 20, 30, 50, and 70 mM SDS solutions. [oligomer] = 2.0×10^{-6} M. The acceptor emission at 500 nm was monitored.

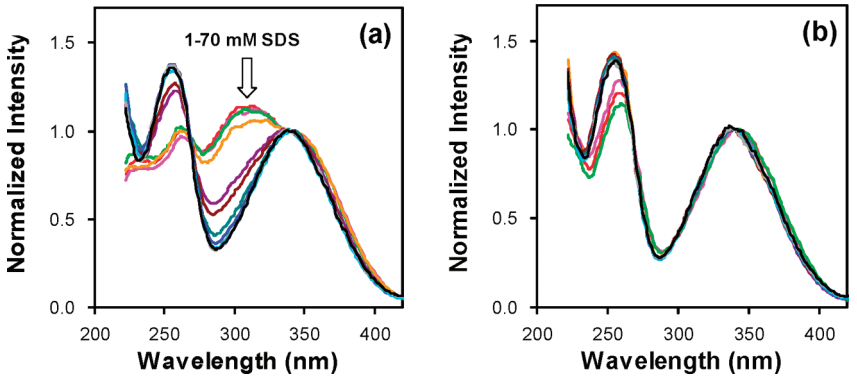


FIGURE 3. Normalized excitation spectra of (a) **1** and (b) **4** in 1, 2, 4, 6, 8, 10, 14, 20, 30, 50, and 70 mM SDS solutions. The acceptor emission at 500 nm was monitored. The intensity at 340 nm (the λ_{max} of dansyl in absorption spectrum) was set to 1. [oligomer] = 2.0×10^{-6} M.

In both compounds, the largest change occurs near the CMC (8 mM) of SDS.[29] When the excitation spectra are normalized so that the intensity at 340 nm (the λ_{max} of dansyl) equals 1, the effect of SDS on dansyl itself is removed (Figure 3b) and the contribution from FRET becomes more clear. The peak near 300 nm from the donor is observable in 1−6 mM SDS for **1** but disappears with >8 mM SDS (Figure 3a).

Thus, FRET does occur in **1** below the CMC of SDS. Nevertheless, energy transfer from the naphthyl to dansyl only indicates the proximity of the two groups; a more important question is whether the FRET is caused by folding, as in the parent cholate hexamer (**6**),[21] or aggregation. Because λ_{max} for the donor and acceptor is 300 and 340 nm,[27] respectively, the F_{300}/F_{340} ratio in the excitation spectrum is a good indicator for the energy-transfer efficiency.[30] A higher F_{300}/F_{340} ratio corresponds to a larger contribution of naphthyl to the dansyl's emission and translates to a shorter D−A distance. Figure 4a and b compare the F_{300}/F_{340} ratio of various oligocholates in different SDS solutions. Tetramer **1** and trimer **2** both start with high F_{300}/F_{340} that decreases quickly above 6−8 mM SDS to a low plateau (Figure 4a). The average D−A distance thus increases with higher SDS. Energy transfer disappears almost completely with >30 mM SDS, and the F_{300}/F_{340} is very similar to that of the dimer acceptor **4**.

The F_{300}/F_{340} curves for **1** and **2** in SDS solutions are surprisingly similar to what was obtained when methanol was added to unfold these oligocholates in ethyl acetate/hexane.[23] Although it is tempting to imagine that a similar unfolding may have been caused by the increasing SDS, the change in F_{300}/F_{340} is consistent with an aggregation−dissociation transition as well. As long as naphthyl and dansyl are sufficiently close within the aggregate, intermolecular FRET also could give a high F_{300}/F_{340} value. Oligocholate are dominated by hydrophobic groups. Their aggregation is favored by hydrophobic interactions in water. Below the CMC, SDS molecules prefer to stay at the air−water interface and have very little solubilizing power for hydrophobic molecules. Above the CMC, SDS begins to form micelles, which have much higher solubilizing power for hydrophobic molecules in comparison with the individual surfactant.[31] Under such a condition, an oligocholate may satisfy its "hydrophobic needs" by either interacting with other oligocholates (via aggregation) or entering an SDS micelle. If the latter process becomes sufficiently competitive, micellization of SDS can cause dissociation of the (oligocholate) aggregates. As long as the individual

(29) Rosen, M. J. *Surfactants and Interfacial Phenomena*, 2nd ed.; Wiley: New York, 1989; Chapter 3.

(30) A ratiometric treatment is also advantageous because it is not as susceptible to artifacts (e.g., quenching) and small inaccuracies in concentration.

(31) Rosen, M. J. *Surfactants and Interfacial Phenomena*, 2nd ed.; Wiley: New York, 1989; Chapter 4.

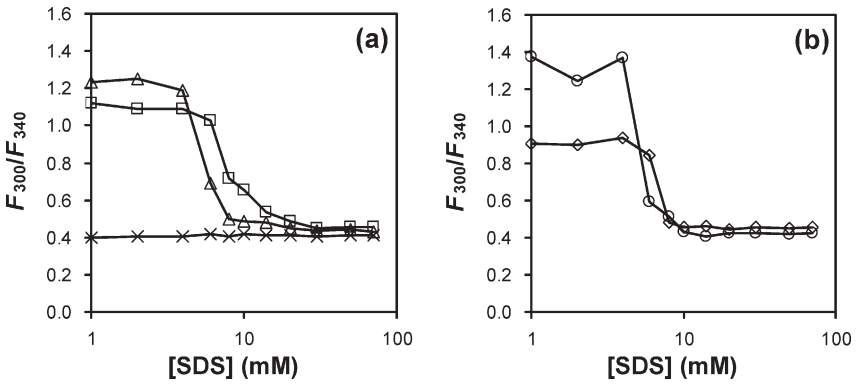


FIGURE 4. F_{300}/F_{340} of (a) **1** (□), **2** (△), **4** (×), and (b) **3** (◇) and 1:1 mixture of **4** and **5** (○) as a function of SDS concentration. [oligomer] = 2.0×10^{-6} M. F_{300} and F_{340} represent the emissive intensity of dansyl at 500 nm in the excitation spectrum when λ_{ex} is 300 and 340 nm, respectively. The data points are connected to guide the eye.

oligocholate is *unfolded* in the SDS micelle, F_{300}/F_{340} would stay low above the CMC, as seen in Figure 4a.

Figure 4b allows us to distinguish the two processes (i.e., folding−unfolding and aggregation−dissociation) more clearly. Herein, F_{300}/F_{340} is shown to follow a similar high−low transition for donor−acceptor labeled dimer **3** below and above the CMC of SDS. In solution, dimer **3** is too short to fold, even under most favorable conditions.[23] If folding is impossible for **3**, the observed FRET is most likely caused by the alternative process, aggregation. More definitive evidence for aggregation comes from a control experiment using a 1:1 mixture of dansyl-labeled dimer **4** and naphthyl-labeled dimer **5**. Because the donor and the acceptor are on separate molecules, an intramolecular process such as folding cannot contribute to FRET. Yet, Figure 4b clearly shows a similar high−low transition in F_{300}/F_{340} for the **4**/**5** mixture. In fact, the initial F_{300}/F_{340} ratio is even higher for **4**/**5** than for **3**.[32] Regardless of the exact reason for the difference in F_{300}/F_{340} between **4**/**5** and **3**, the fact that strong FRET is observed in the binary mixture indicates that aggregation occurs in the **4**/**5** mixture (most likely in other oligocholates as well) below the CMC of SDS.

The large drop in F_{300}/F_{340} at >8 mM indicates that the oligocholate aggregates are dissociated by the SDS micelles. In addition, the dissociated oligocholate must be unfolded within the SDS micelle (or high F_{300}/F_{340} would have been obtained). During this transition, the maximum emission wavelengths (λ_{em}) of these compounds all show distinct changes, jumping from ca. 495 nm to ca. 540 nm near the CMC of SDS (Figure 5). The λ_{em} for dansyl is an indicator for its environmental polarity and shifts to red in more polar environments.[28] The observed change in λ_{em} suggests that the oligocholate aggregate has a less polar interior than the SDS micelle. In the literature, cholate-derived micelles are indeed reported to have "drier" interior than SDS micelles.[33,34] This conclusion is also consistent with Figures 1 and 2. Since dansyl fluoresces less strongly in more polar environments,[28] its emission should decrease as it moves from the oligocholate aggregate to a more polar environment (i.e., SDS micelle). Finally, according to both Figures 4a and 5, a higher concentration of SDS is needed to dissociate the tetramer aggregate than those of the shorter oligomers. In other words, the aggregates of the tetramer are more stable than those of the shorter oligomers toward SDS dissolution, a reasonable result considering the area of the hydrophobic surface area involved in the dissociation.

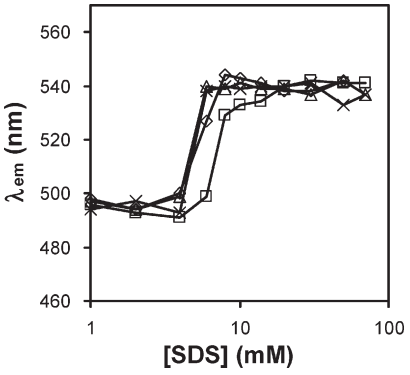


FIGURE 5. Maximum emission wavelength (λ_{em}) of dansyl in **1** (□), **2** (△), **3** (◇), and **4** (×) as a function of SDS concentration. [oligomer] = 2.0×10^{-6} M. λ_{ex} = 350 nm. The data points are connected to guide the eye.

Parent Oligocholates in SDS Micelles. Insertion of 4-aminobutyroyl spacers has a large impact on the conformation of oligocholates. In homogeneous solution, the spacers enhance the foldability.[23] In SDS aqueous solution, the opposite effect is apparently operating. Instead of folding, the flexible oligocholates (**1**−**5**) form intermolecular aggregates below the CMC of SDS and are solubilized by SDS micelles as individual, unfolded oligomers above the CMC. The behavior contrasts that of the parent, more rigid cholate hexamer (**6**), which stays folded and unaggregated both below and above the CMC of SDS.[21] What makes the two types of oligocholates behave so differently in SDS solutions? Although the 4-aminobutyroyl spacers seem to be the obvious culprit, we have to rule out other factors, such

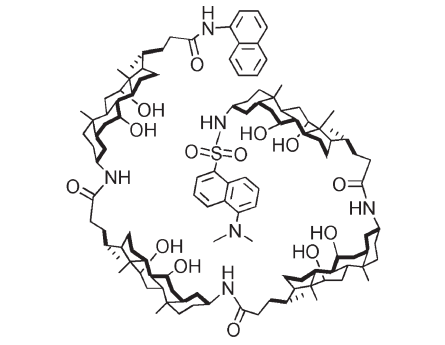
(32) The difference is presumably a result of different concentrations of oligocholates. Because all oligomers were used at 2.0 μM in this experiment, the concentrations of naphthyl and dansyl were the same but the concentration of *oligocholate* was twice in the **4**/**5** mixture than in **3**.

(33) (a) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039–2044. (b) Zana, R.; Guveli, D. *J. Phys. Chem.* **1985**, *89*, 1687–1690.

(34) SDS micelles do contain an appreciable amount of water; see: (a) Menger, F. M. *Acc. Chem. Res.* **1979**, *12*, 111–17. (b) Martens, F. M.; Verhoeven, J. W. *J. Phys. Chem.* **1981**, *85*, 1773–1777. (c) Turro, N. J.; Okubo, T. *J. Am. Chem. Soc.* **1981**, *103*, 7224–7228. (d) Fadnavis, N.; Engberts, B. F. N. *J. Org. Chem.* **1982**, *47*, 152–154. (e) Szajdzinska-Pietek, E.; Maldonado, R.; Kevan, L.; Jones, R. R. M. *J. Am. Chem. Soc.* **1984**, *106*, 4675–4678.

as chain length, as the cause for the difference. After all, the main reason for the folding of **6** was attributed to its reduced size upon folding (which allows it to be "comfortably" encapsulated in a small spherical micelle).[21]

To answer the above question, we synthesized donor−acceptor-labeled tetramer **7** without the 4-aminobutyroyl spacers. Its emission in SDS solutions is different still from that of the flexible counterpart (**1**). Figure 6 plots the emissive intensity of dansyl at 500 nm against [SDS] for the two tetramers. Although some fluctuation in intensity does occur, the nearly flat curve for rigid tetramer **7** is similar to that of rigid hexamer **6**[21] and is in sharp contrast to that of flexible tetramer **1**. Thus, chain length is NOT responsible for the difference between the rigid and flexible oligocholates.



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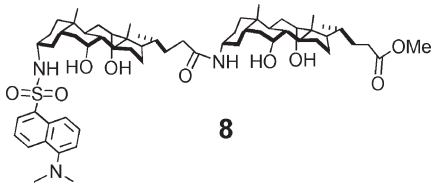

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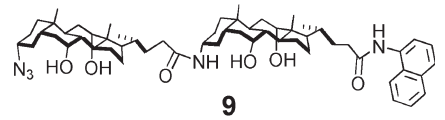

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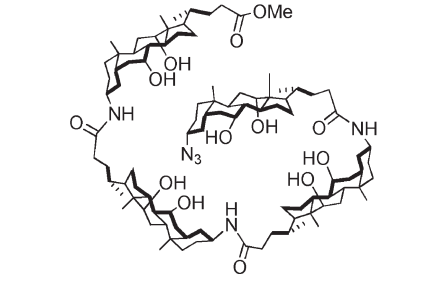

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In addition to different emissive intensity, the rigid and flexible tetramers differ in their energy-transfer profiles (Figure 7). The data for flexible tetramer **1** was taken out of Figure 4a, showing a quick drop in F_{300}/F_{340} above 8 mM SDS. Rigid tetramer **7** gives a different curve. Relatively high F_{300}/F_{340}, resulted from FRET, persists above the CMC and disappears only at 50−70 mM SDS. Meanwhile, both the flexible and the rigid dimer-acceptor

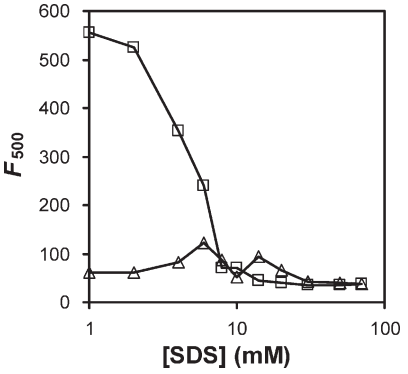


FIGURE 6. Emissive intensity of dansyl at 500 nm as a function of SDS concentration for **7** (△) and **1** (□). [oligomer] = 2.0×10^{-6} M. λ_{ex} = 287 nm. The data points are connected to guide the eye.

(**4** and **8**) give low F_{300}/F_{340} in all SDS solutions in the absence of FRET.

The data so far demonstrate that the 4-aminobutyroyl spacers strongly affect both the emissive intensity of dansyl and the energy transfer in the oligocholates. If the flexible oligocholates (**1**−**5**) aggregate below the CMC and become individually solubilized by SDS micelles above the CMC, what does the rigid tetramer **7** do in different SDS solutions? The SDS-independent emission in Figure 6 seems to suggest that **7** may be in more or less the same microenvironment in all SDS solutions. Similar SDS-independent emission was observed in rigid hexamer **6**, which stays folded in all SDS solutions.[21] Is it possible that **7** might be folded within the SDS micelle as well, at least in < 50 mM SDS?

Because folding of the oligocholates is strongly dependent upon chain length in solution,[20a,23] we plotted F_{300}/F_{340} as a function of SDS concentration for various rigid oligocholates in Figure 8. The acceptor-labeled dimer **8**, again, has a low and flat curve in the absence of FRET. For the 1:1 dimer mixture **8**/**9**, the high F_{300}/F_{340} (resulted from *intermolecular* FRET) below the CMC of SDS suggests that aggregation takes place similarly as the flexible **4**/**5** dimer mixture. Apparently, once folding becomes impossible (due to too short a chain length), aggregation is the only effective way to satisfy the hydrophobic needs of the oligocholates. Energy transfer disappears above the CMC, indicating that the aggregates are dissociated by SDS micelles, just as those of the flexible oligocholates.

Hexamer **6** is distinctively different from all others in Figure 8, maintaining a high F_{300}/F_{340} value, indicating a close D−A distance and efficient FRET in all SDS solutions. Tetramer **7** overall has much lower F_{300}/F_{340} than the hexamer. Note that a smaller F_{300}/F_{340} alone is *not* a sign for poor folding; small F_{300}/F_{340} could be caused also by a large D−A distance in the folded state. The cholate backbone prefers trimeric periodicity according to Sanders' work.[35] Because of the periodicity, cholate heptamer has a longer end-to-end distance in the folded state than the hexamer, even though the former folds better.[20a] On the other hand, since hexamer **6** is able to maintain FRET better than tetramer **7** upon increasing SDS (Figure 8), it is likely

(35) Brady, P. A.; Bonar-Law, Ri. P.; Rowan, S. J.; Suckling, C. J.; Sanders, J. K. M. *Chem. Commun.* **1996**, 319–320.

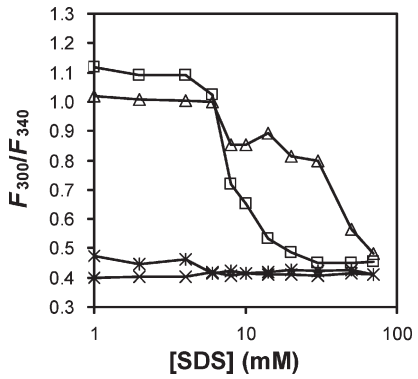


FIGURE 7. F_{300}/F_{340} in **7** (△), **1** (□), **8** (∗), and **4** (×) as a function of SDS concentration. F_{300} and F_{340} represent the emissive intensity of dansyl at 500 nm in the excitation spectrum with λ_{ex} = 300 and 340 nm, respectively. [oligomer] = 2.0×10^{-6} M. The data points are connected to guide the eye.

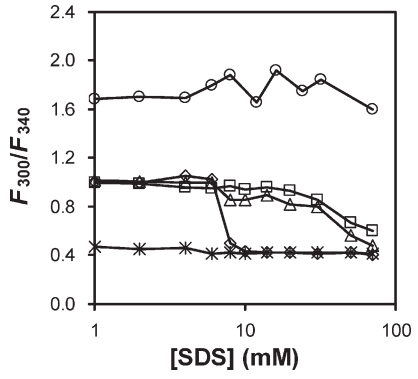


FIGURE 8. F_{300}/F_{340} of **6** (○), **7** (△), **8** (∗), 1:1 mixture of **8** and **9** (◊), and 1:3 mixture of **7** and **10** (□) as a function of SDS concentration. F_{300} and F_{340} represent the emissive intensity of dansyl at 500 nm in the excitation spectrum with λ_{ex} = 300 and 340 nm, respectively. [oligomer] = 2.0×10^{-6} M. The data points are connected to guide the eye.

that a longer chain length is advantageous to folding (vide infra).

To rule out the possibility of aggregation in the tetramer, we need to demonstrate that the FRET observed in **7** comes from an intramolecular rather than an intermolecular process. For this purpose, we measured the F_{300}/F_{340} ratio for a 1:3 mixture of donor−acceptor-labeled tetramer **7** and unlabeled tetramer **10** in different SDS solutions. If aggregation is responsible for the FRET in **7**, its mixing with an unlabeled tetramer should dilute the donor/acceptor in the aggregate and lower the (intermolecular) energy-transfer efficiency. Such mixing should not affect aggregation, as the total oligocholate concentration is the same (2.0 μM). Figure 8 shows nearly identical F_{300}/F_{340} curves for **7** and the **7**/**10** mixture. Intermolecular aggregation thus did not occur under these conditions, making folding the only logical cause for the FRET observed in the tetramer.

The maximum emission wavelength of dansyl reveals additional detail in the behavior of these rigid oligocholates (Figure 9). The λ_{em} (480−500 nm) for hexamer **6** is nearly independent of [SDS], consistent with its folding in all SDS solutions. The dimers, whether the dimer acceptor **8** or the **8**/**9** dimer mixture, behave the same, with λ_{em} jumping abruptly

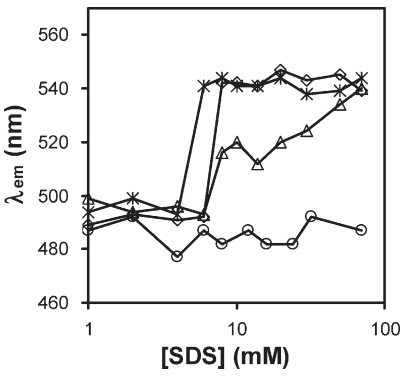


FIGURE 9. Maximum emission wavelength (λ_{em}) of **6** (○), **7** (△), **8** (∗), and 1:1 mixture of **8** and **9** (◊) as a function of SDS concentration. [oligomer] = 2.0×10^{-6} M. The data points are connected to guide the eye.

from 500 to 540 nm around the CMC of SDS. As discussed earlier, the red shift near the CMC results from the change of the dansyl from a less polar environment (i.e., inside oligocholate aggregates) to a more polar environment (i.e., inside SDS micelle). The λ_{em} of tetramer **7** stays at ca. 500 nm below the CMC of SDS, jumps to 520 nm above the CMC, and slowly reaches 540 nm at 70 mM SDS. Because **7** is folded at low SDS and unfolded in 50−70 mM SDS (vide supra), the change in its λ_{em} upon increasing SDS probably results from a transition from the folded to unfolded conformation. The λ_{em} of dansyl is known to correlate with its environmental polarity.[28] Figure 9 suggests that the dansyl on the hexamer is located in a more hydrophobic environment than that of the tetramer above the CMC of SDS. Provided the micelles that contain the hexamer and the tetramer do not differ significantly, the most probable cause for the different environmental polarity for the two oligocholates is the location of the dansyl within the micelle. Hexamer **6** is completely folded in all SDS solutions. With a hydrophobic exterior, the folded hexamer is expected to be located in the hydrophobic core of the SDS micelle. Both the folded and unfolded conformers of **7** probably exist above the CMC. Because the unfolded oligocholate has some of its hydrophilic groups exposed, even after it is solubilized in an SDS micelle, it should prefer the surface of the micelle where its hydrophilic groups can be better solvated by water.

A major reason for the folding of the rigid hexamer was postulated to be its small size upon folding, which allows it to be better accommodated within a small spherical micelle than the unfolded conformer.[21] This is probably also the reason why the hexamer folds better than the tetramer, as the size difference in the folded and unfolded tetramer is not as significant. Why do the flexible oligocholates prefer aggregation instead of folding? The reason does *not* lie in the intrinsic foldability, since the flexible oligocholates (**1** and **2**) fold much better than the rigid ones in solution.[23] The excitation spectra give some clues to this question. As shown by Figure 10, the contribution from the donor (at 300 nm) is much more pronounced in the flexible **4**/**5** mixture (Figure 10b) than in the rigid **8**/**9** mixture (Figure 10a). Note that none of the dimers (**4**, **5**, **8**, or **9**) can fold. Since the donor and acceptor are located on different molecules, FRET can come only from aggregation. The different (intermolecular) energy-transfer efficiencies in the two mixtures thus suggest

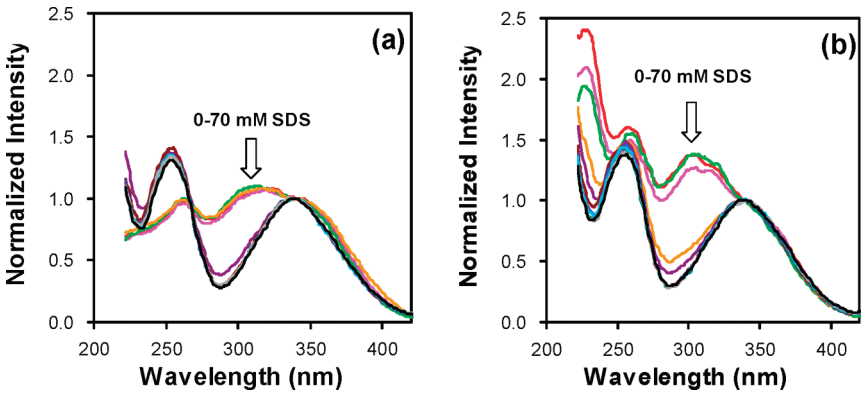


FIGURE 10. Normalized excitation spectra of (a) 1:1 mixture of **8** and **9** and (b) 1:1 mixture of **4** and **5** in 1, 2, 4, 6, 8, 10, 14, 20, 30, 50, and 70 mM SDS solutions. [oligomer] = 2.0×10^{-6} M. The acceptor emission at 500 nm was monitored.

that the aggregates of the flexible dimers are more tightly packed than those of the rigid ones. Bile acid derivatives, including cholates, are known to pack loosely in the solid state and tend to include guest molecules. The feature is widely exploited in the formation of inclusion complexes between bile acids and guest molecules.[36] If tight packing is difficult for a monomeric cholate derivative, it should be even more challenging for the rigid oligocholates joined together by short covalent bonds. Insertion of flexible spacers is expected to alleviate the problem, as the cholate groups have more freedom to move around and adjust themselves in the aggregate.

Conclusions

Different rules indeed govern the folding of the oligocholates in homogeneous solution and in micelles. The important lesson learned is about the different competitions involved in the different environments. In solution, the main competition lies in the solvation of the facially amphiphilic cholates by the polar and nonpolar solvents. Preferential solvation of the hydrophilic groups by the minor, polar solvent drives the folding and the flexible spacers promote the folding by reducing the strain in the folded helix.

Once the oligocholates are dissolved in SDS aqueous solution, completely different competitions come into play. The main issues are 2-fold: (a) the oligomers want to hide their hydrophobic faces from water, and (b) the SDS micelles have a strong preference for small, spherical shape. The flexible oligocholates (**1**−**5**) are able to form tight, stable aggregates and satisfy their hydrophobic needs below the CMC of SDS. Although SDS has a long hydrophobic tail, its ability to solubilize nonpolar molecules remains low until its concentration exceeds the CMC.[31] This is probably the reason why the oligocholate aggregates disintegrate above the CMC of SDS.

The rigid oligocholates (**8** and **9**) that are too short to fold have no choice but to aggregate below the CMC of SDS. Above the CMC, the aggregates dissociate just as those of the flexible oligocholates. The longer hexamer **6** and tetramer **7** can fold into a helix with introverted (i.e., shielded) hydrophilic groups and satisfy their hydrophobic needs by entering the SDS micelle. Folding of the oligocholates, both below and above the CMC of SDS, is favored mainly because the competing processes are unfavorable. Intermolecular aggregation of the oligocholates is disfavored by the poor packing of the cholate groups in water, and unfolding by the difficulty of the unfolded conformer to be encapsulated within the highly preferred, small, spherical micelle of SDS. For the same reason, a large size difference between the folded and the unfolded conformer favors the folded form. This is probably the reason why hexamer **6** folds better than tetramer **7** and also provides some explanation for the unfolding of the flexible oligocholates (**1** and **2**) in SDS micelles. Although there is no intrinsic reason for the flexible oligocholates to fold poorly—quite the opposite is true in solution—an unfolded, flexible oligocholate can easily adjust its shape to fit within a small, spherical SDS micelle. Once the reason to disfavor the unfolded conformer is eliminated, it easily becomes more stable than the folded due to higher conformational entropy.

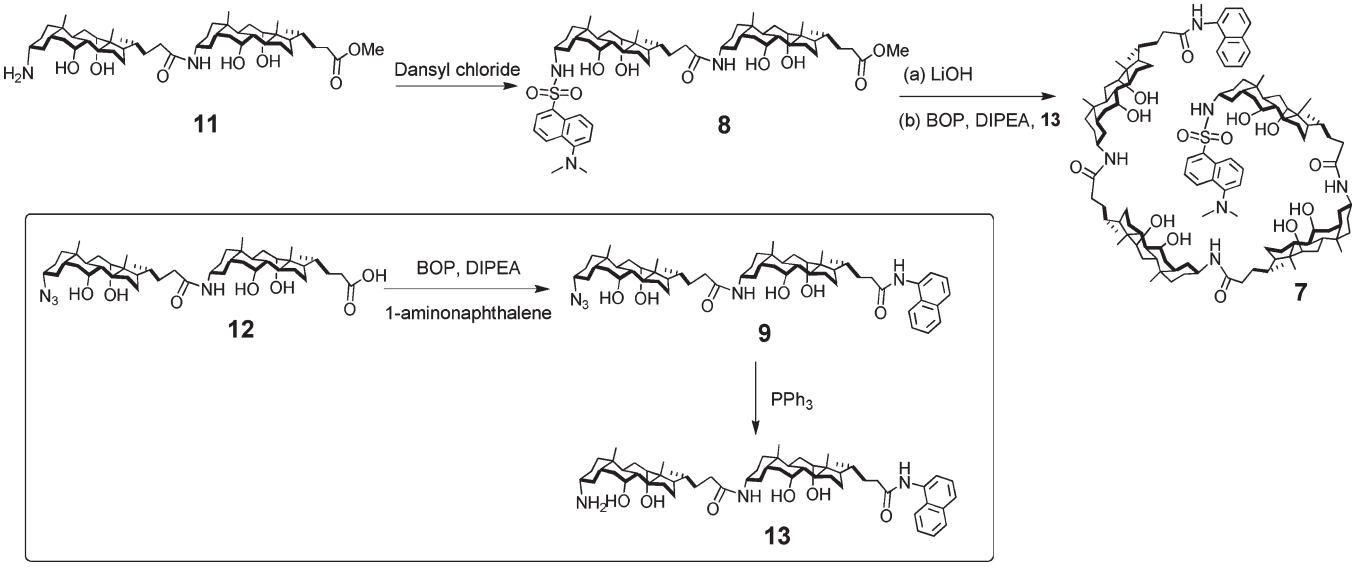
Experimental Section

The syntheses of compounds **1**−**5**[23] and **6**[20a] were reported previously.

Compound 8. Compound **11**[20a] (0.192 g, 0.237 mmol) and Et_3N (0.060 g, 0.59 mmol) were dissolved in anhydrous DMF (4 mL). Dansyl chloride (0.062 g, 0.23 mmol) was added in one portion. The mixture was stirred at room temperature overnight. The mixture was poured into acidic water (4 mL of 2 N HCl in 30 mL of water). The solid was collected by suction filtration and purified by column chromatography over silica gel with CH_2Cl_2/MeOH = 15/1 to 7/1 as the eluents to give a light yellow glass (0.170 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD = 3:1, δ): 8.44 (d, J = 8.8 Hz, 1H), 8.23 (d, J = 8.8 Hz, 1H), 8.16 (d, J = 7.2 Hz, 1H), 7.49−7.43 (m, 2H), 7.12 (d, J = 7.6 Hz, 1H), 7.05 (d, J = 8.0 Hz, 1H), 3.89 (br, 1H), 3.82 (br, 1H), 3.75 (br, 1H), 3.68 (br, 1H), 3.60 (s, 3H), 3.49 (br, 1H), 3.28 (br, 1H), 2.81 (br, 7H), 2.22−0.66 (series of m, 58H), 0.62 (s, 3H), 0.56 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD, δ): 175.1, 174.5, 151.7, 137.0, 129.8, 129.7, 129.6, 128.5, 127.6, 123.0, 119.6, 115.0, 72.5, 67.6, 67.5, 54.2, 50.8, 49.7, 46.6, 46.2, 46.1, 44.6, 42.5, 42.3, 41.7, 41.6, 39.6, 39.5, 37.3, 36.1, 35.8, 35.6, 35.5, 34.6, 34.5, 34.35, 34.31, 32.9, 32.1, 30.9, 30. 6, 28.3, 28.2, 27.4, 27.2, 26.6, 26.4, 22.94, 22.88, 22.1, 21.9, 16.5, 16.4, 11.8, 11.7. MALDI-TOFMS (m/z): $[M+H-H_2]^+$ calcd for

(36) For two reviews, see: (a) Miyata, M.; Sada, K. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davis, J. E. D., MacNicol, D. D., Vögtle, F. Eds.; Elsevier: Oxford, 1996; Vol. 6, Chapter 6. (b) Miyata, M.; Sada, K.; Yoswathananont, N. In *Encyclopedia of Supramolecular Chemistry*; Atwood, J. L., Steed, J. W. Eds.; Marcel Dekker: New York; p 441.

SCHEME 2. Synthesis of Oligocholates 7−9



$C_{61}H_{92}N_3O_9S$, 1043.5; found, 1043.1. $[M+Na-H_2]^+$ calcd for $C_{61}H_{91}N_3NaO_9S$, 1065.4; found, 1066.1.

Compound 9. Compound **12**[20a] (0.296 g, 0.360 mmol), BOP (0.209 g, 0.473 mmol), HOBt (0.096 g, 0.711 mmol), and DIPEA (0.254 g, 1.96 mmol) were dissolved in anhydrous DMF (4 mL). After 0.5 h at room temperature, 1-aminonaphthale (0.106 g, 0.740 mmol) was added. The reaction was allowed to continue at 65 °C for 4 d. The mixture was poured into acidic water (4 mL of 2 N HCl in 30 mL of water). The solid was collected by suction filtration and purified by column chromatography over silica gel with ethyl acetate/hexane/MeOH = 2/2/0.1 and CH_2Cl_2/MeOH = 15/1 as the eluents to give an off-white powder (0.201 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 3:1, δ): 7.87 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.60 (d, J = 7.2 Hz, 1H), 7.48−7.36 (m, 3H), 3.94 (br, 1H), 3.87 (br, 1H), 3.75 (br, 2H), 3.51 (br, 1H), 3.15 (br, 1H), 2.58−0.76 (series of m, 60H), 0.67 (s, 3H), 0.60 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD = 3:1, δ): 174.2, 174.1, 134.3, 132.8, 128.5, 128.3, 126.2, 126.0, 125.6, 122.2, 121.8, 73.2, 73.1, 68.3, 68.1, 61.7, 60.7, 47.0, 46.7, 46.6, 46.2, 42.0, 41.9, 41.8, 39.5, 39.3, 36.4, 36.0, 35.7, 35.5, 35.1, 34.8, 33.8, 32.6, 32.0, 31.7, 31.6, 28.2, 28.1, 27.7, 27.4, 26.8, 26.7, 26.6, 23.4, 22.70, 22.67, 21.1, 17.43, 17.40, 14.2, 12.6, 12.4. MALDI-TOFMS (m/z): $[M + Na - H_2]^+$ calcd for $C_{58}H_{83}N_5NaO_6$, 969.3; found, 969.4.

Compound 13. Compound **9** (0.234 g, 0.280 mmol) and PPh_3 (0.242 g, 0.92 mmol) were dissolved in MeOH (5 mL). The mixture was heated to reflux for 3 h. The solvent was removed in vacuo. The residue was purified by column chromatography over silica gel with CH_2Cl_2/MeOH = 7/1 and CH_2Cl_2/MeOH/Et_3N = 2/1/0.2 as the eluents to give a white powder (0.192 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 3:1, δ): 7.87 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 7.2 Hz, 1H), 7.69−7.58 (m, 2H), 7.49−7.36 (m, 3H), 3.93 (br, 1H), 3.88 (br, 1H), 3.75 (br, 2H), 3.48 (br, 1H), 2.73 (br, 1H), 2.52 (m, 1H), 2.41 (m, 1H), 2.20−0.74 (series of m, 58H), 0.66 (s, 3H), 0.60 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$ = 3:1, δ): 179.3, 174.2, 174.0, 134.3, 133.5, 132.8, 128.5, 128.4, 126.2, 126.0, 125.6, 122.3, 121.9, 73.2, 72.9, 69.3, 68.0, 51.1, 50.0, 49.4, 46.9, 46.8, 46.5, 41.9, 41.7, 41.6, 39.4, 36.0, 35.7, 35.6, 35.3, 35.1, 34.8, 34.7, 34.4, 33.7, 33.5, 32.0, 31.7, 28.2, 27.73, 27.71, 27.66, 27.4, 26.6, 26.5, 26.3, 24.4, 23.3, 22.8, 22.7, 22.5, 17.44, 17.36, 14.2, 12.6, 12.5. MALDI-TOFMS (m/z): $[M + H - H_2]^+$ calcd for $C_{58}H_{86}N_3O_6$, 921.3; found, 921.4. $[M + Na - H_2]^+$ calcd for $C_{58}H_{85}N_3NaO_6$, 943.3; found, 943.4.

Compound 7. Compound **8** (0.123 g, 0.118 mmol) was dissolved in a mixture of MeOH (3 mL) and THF (1.5 mL). LiOH (2.0 M, 0.5 mL) was added. After 24 h, the solvents were removed in vacuo and dilute HCl aqueous solution was added to precipitate the carboxylic acid derivative. The yellow solid was collected by suction filtration and air-dried to give the corresponding acid (0.109 g, 90%), which was used in the next step without further purification. The acid derivative of **8** (0.069 g, 0.067 mmol), **13** (0.062 g, 0.067 mmol), BOP (0.073 g, 0.165 mmol), HOBt (0.010 g, 0.074 mmol), and DIPEA (0.066 mg, 0.51 mmol) were dissolved in anhydrous DMF (1.5 mL). The mixture was stirred at 65 °C for 3 d and was poured into acidic water (6 mL of 2 N HCl in 30 mL of water). The solid was collected by suction filtration and purified by column chromatography over silica gel with CH_2Cl_2/MeOH = 16/1 to 6/1 as the eluents to give a light yellow powder (0.049 g, 38%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 3:1, δ): 8.45 (d, J = 8.4 Hz, 1H), 8.23 (d, J = 8.8 Hz, 1H), 8.16 (d, J = 6.8 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.67 (d, J = 8.0 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.52−7.35 (m, 4H), 7.19−7.02(m, 2H), 3.94 (br, 1H), 3.89 (br, 2H), 3.82 (br, 1H), 3.79−3.65 (m, 4H), 3.47 (br, 3H), 2.82 (br, 7H), 2.54 (br, 1H), 2.42 (br, 1H), 2.19−0.70 (series of m, 118H), 0.67 (s, 3H), 0.61 (s, 3H), 0.60 (s, 3H), 0.56 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD = 3:1, δ): 170.51, 170.48, 170.2, 132.4, 130.3, 128. 8, 126.0, 125.9, 125.7, 125.0, 124.52, 124.45, 124.0, 122.3, 122.2, 122.0, 121.6, 119.3, 118.6, 118.0, 115.4, 111.2, 95.7, 69.2, 69.1, 69.0, 64.2, 64.1, 50.1, 45.7, 43.1, 42.9, 42.8, 42.5, 41.43 38.2, 37.9, 37.8, 37.7, 35.4, 33.5, 32.4, 32.0, 31.6, 30.8, 30.6, 30.5, 29.7, 29.54, 29.46, 29.35, 28.1, 28.0, 27.9, 24.4, 24.2, 24.0, 23.6, 23.4, 22.6, 22.4, 19.3, 19.2, 18.7, 18.5, 13.3, 13.2, 8.54, 8.46, 8.4. MALDI-TOFMS (m/z): $[M+Na-H_2]^+$ calcd for $C_{118}H_{174}N_6NaO_{14}S$, 1955.7; found, 1956.3.

Fluorescence. A typical procedure is as follows. Stock solutions of the oligocholates (2.0×10^{-4} M in MeOH) and SDS (70.0 mM in Millipore water) were prepared. Aliquots (20.0 μL) of a stock solution of an oligocholate were added to separate glass vials. MeOH was allowed to evaporate overnight in air. Different amounts of the SDS stock solution and Millipore water were added to the vials, so that concentration of the oligocholate was 2.0 μM and the concentrations of the SDS were 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 14.0, 20.0, 30.0, 50.0, and 70.0 mM,

respectively. The samples were allowed to sit at room temperature for several hours for dissolution of the oligocholates. The fluorescence and the excitation spectra were recorded. The excitation wavelength was 287 nm for the fluorescence spectra. For the excitation spectrum, the dansyl emission at 500 nm was monitored as the excitation wavelength was scanned from 220 to 460 nm.

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Supporting Information Available: UV and NMR spectra of compounds **7**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.