Solvophobically Driven π-Stacking of Phenylene Ethynylene Macrocycles and Oligomers

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Abstract: Phenylene ethynylene macrocycles and oligomers with three different side-chain linking groups (ester, benzyl ether, and phenyl ether) were synthesized to investigate their tendency to undergo solvent induced π-stacked organization. ^{1}H NMR, UV, and fluorescence spectroscopies were used to probe two types of π-stacked supramolecular organizations: the intramolecular conformational ordering of the oligomers, and the intermolecular aggregation of the macrocycles. One important conclusion is that solvent can play a very dramatic role in modulating the strength of the interactions that drive the association of these π-stacked structures. The other important conclusion is that in a given solvent, the nature of the side chain linking group strongly influences the π-stacking propensities. It was found that macrocycles and oligomers with the ester side chain linking group were prone to adopt π-stacked structures in a range of solvents, whereas the corresponding macrocycles with benzyl ether and phenyl ether side chain linking groups showed only limited ability to π-stack, even in the most polar solvent examined (DMSO). In the interest of manipulating the helix-coil folding transition of phenylene ethynylene oligomers, a heterosequence consisting of monomers with ester and benzyl ether side chain linkages was synthesized. The folding transition of the heterooligomer was intermediate to that observed for the corresponding homooligomers, suggesting that the backbone sequence can be used to tune the stability of conformations that are based on π-stacked organizations.

Introduction

Macromolecular self-organization is exemplified by the ability of natural biopolymers to adopt regular conformations in solution, such as the helical, double-stranded form of DNA.[1] These ordered conformations are stabilized by noncovalent interactions such as hydrogen bonding, hydrophobic, and van der Waals forces.[2] Utilizing these same interactions to control the self-organization of nonbiological macromolecules presents an interesting challenge, especially in polar solvents where unscreened hydrogen bonding interactions are weak.[3] Unlike hydrogen bonds or metal−ligand coordination interactions for which there generally exists a good understanding of the chemical affinities and preferred bonding geometries, much remains to be learned before nonspecific supramolecular forces such as solvophobic interactions[4] and π-stacking of aromatic units can be used by design.[5,6]

It has long been known that the strength of π-stacking interactions is very sensitive to solvent.[7−10] It is also known that π-stacking tendencies are strongly influenced by the electrostatic properties of the interacting rings.[6,11] Previously in our laboratory, macrocycles consisting of phenylene ethynylene units have been shown to associate in chloroform due to π-stacking interactions (Figure 1).[12] In chloroform (the only solvent investigated prior to this study), the magnitude of the association constant depended significantly on the group that linked the phenylene ethynylene backbone to the alkyl side chain. Macrocycles with the ester linked side chains were observed to dimerize to a significant degree, whereas macrocycles with the benzyl ether and phenyl ether side chain linking groups did not exhibit observable intermolecular aggregation.

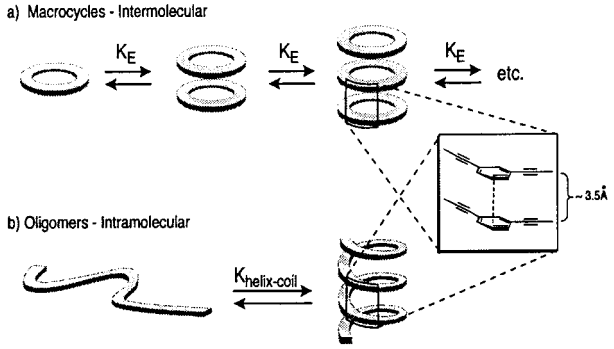

Figure 1. Schematic diagram depicting (a) the intermolecular association of macrocycles and (b) the helical conformation of oligo(phenylene ethynylenes). Solvent-induced π-stacking interactions are presumed to be the dominant force that stabilizes these supramolecular arrangements.

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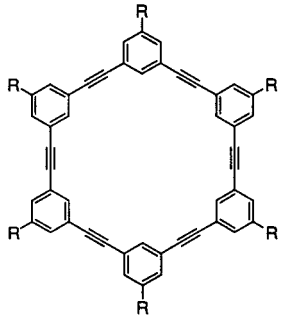
Phenylene ethynylene oligomers with an ester group linking the aromatic backbone to a polar tri(ethylene glycol) side chain have been demonstrated to collapse into a π-stacked helical conformation in polar solvents due to solvophobic interactions (Figure 1).[13,14] If the trends observed for the macrocycles carry over to phenylene ethynylene oligomers, then a strategy to manipulate the conformational transitions of the oligomers is apparent. To explore this issue, oligomeric sequences with widely varying solvophobic and π-stacking propensities are required. To this end, we selected three different groups (ester, phenyl ether, benzyl ether) to link a polar tri(ethylene glycol) side chain to the backbone. Macrocycles and oligomers were synthesized from the corresponding monomers, and the stability of their π-stacked arrangement was probed. We show that the stability of these solvent-induced π-stacked arrangements is dramatically altered by subtle changes in monomer structure. These findings open the way to modulating the folding transitions of the oligomers by altering monomer composition, analogous to the way that α-helix stability depends on the peptide's amino acid sequence[15] or the melting transition of DNA strands depends on the stacking preferences of the nucleotide bases.[16]

Results and Discussion

Previous studies on phenylene ethynylene macrocycles were conducted on macrocycles possessing simple *n*-alkyl side chains.[12] Due to poor solubility, their association behavior could only be tested in a limited range of solvents. To address this issue, a more polar side chain was sought. Tri(ethylene glycol) side chains function as powerful solubilizing appendages making it possible to study macrocycle association in a wide range of solvents and thus explore π-stacking tendencies under stronger solvophobic conditions. Following this line of reasoning, macrocycles **1**−**3** were synthesized using procedures previously developed in our laboratory.[17]



1 R = $COO(CH_2CH_2O)_3CH_3$

2 R = $CH_2O(CH_2CH_2O)_3CH_3$

3 R = $O(CH_2CH_2O)_3COCH_3$

All three macrocycles exhibited marked solubility in a wide selection of solvents, ranging in polarity from toluene to methanol. They were not soluble, however, in hydrocarbon solvents such as hexanes, nor were they significantly soluble in pure water. The aggregation behavior of **1**−**3** was qualitatively assessed by measuring the ^{1}H NMR chemical shifts in various solvents at a concentration of 0.6 mM. It is well established that upfield chemical shifts are a signature of intermolecular association involving π-stacking.[18] As can be seen from Figure 2, macrocycles **2** and **3** did not exhibit significant changes in chemical shifts over a range of solvents tested.[19] This implies that either **2** and **3** are not prone to stacking in these solvents or that the difference in chemical shift between the monomer and aggregate is negligible. In contrast to **2** and **3**, the chemical shift of macrocycle **1** exhibited significant upfield shifting as the solvent polarity increased (Figure 2). The chemical shifts of **1** were plotted against several bulk solvent parameters;[20] solvent dielectric gave the best correlation for a single bulk solvent parameter.

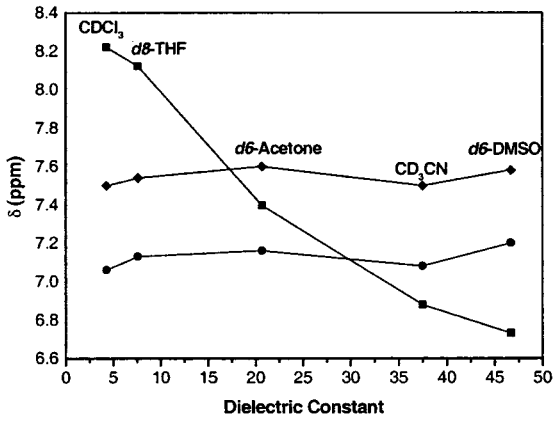
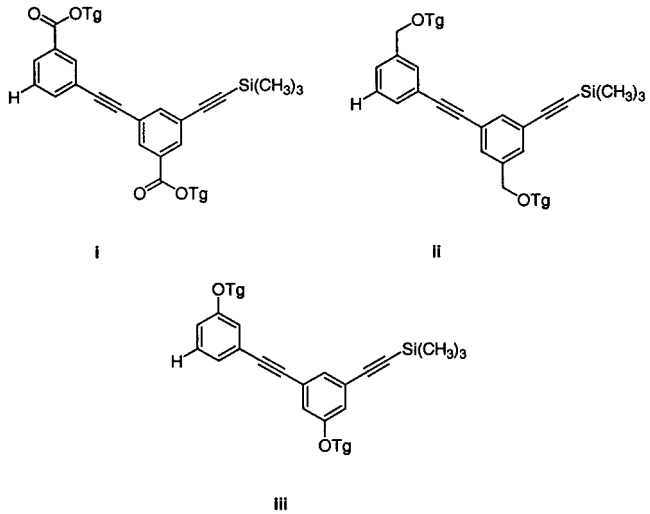


Figure 2. Plot of ^{1}H NMR chemical shifts of the aromatic protons at 0.6 mM and room temperature for macrocycles **1**−**3** as a function of solvent dielectric constant **1** (■), **2** (◆), and **3** (●).

A more quantitative analysis was performed by determining the association constants of the macrocycles in different solvents. ^{1}H NMR chemical shifts were measured as a function of concentration to determine the association constants of the macrocycles (Table 1, Figure 3).[21] These data were analyzed using nonlinear least-squares regression and the equal *K* or isodesmic (K_E) model of indefinite self-association.[22] For macrocycle **1**, the room-temperature K_E values were extremely



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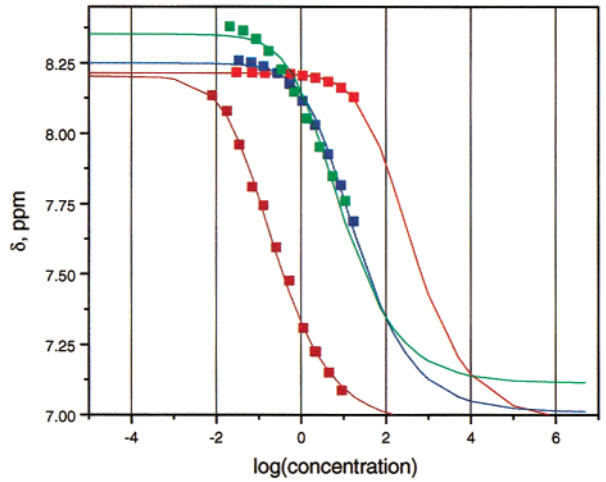


Figure 3. Plot of chemical shift vs log(concentration) (mg/mL) of **1** in different solvents. The curves represent the best fit of these data to the equal *K* model of indefinate association (brown, acetone; blue, THF; green, benzene; red, chloroform).

Table 1. Association Constants of Macrocycles **1** and **3** in Different Solvents

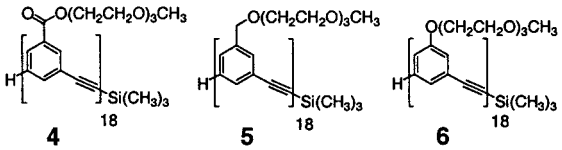
macrocycle	solvent	K_e [a] (M^{-1})
1	$CHCl_3$	50
1	THF	350
1	benzene	1200
1	acetone	15000
3	acetone	140

[a] K_E values were determined by nonlinear least-squares fitting of chemical shift data to the equal *K* (K_E) model of indefinite self-association.[22]

solvent dependent, ranging from 50 M^{-1} in $CDCl_3$ to 15 000 M^{-1} in acetone-d_6. In general, K_E increased with solvent polarity. A surprising result, however, was that the association constant of **1** in benzene was intermediate to that measured in THF and acetone. The reason for this behavior is presently unknown.

In contrast to the strong association of **1**, macrocycle **3** exhibited a K_E value of only 140 M^{-1} in acetone-d_6. We conclude from this that the data for **3** in Figure 2 reflect its weak self-association. The most likely interpretation of these results is that the stacking propensity of the hexakis(phenylene ethynylene) macrocycles in a given solvent is very sensitive to the nature of the side-chain linking group and the macrocycle with the ester side chain linkage displays the greatest tendency to aggregate. Certain linking groups (e.g., phenyl ether, benzyl ether) severely disfavor π-stacking, even under strongly solvophobic conditions. Whether these linking groups disfavor π-stacking because of electrostatic reasons (e.g., electron-rich rings) or because of steric effects (e.g., conformational differences at the ring/side-chain juncture) cannot be determined at this time.

Since the putative helical conformation of phenylene ethynylene oligomers involves π-stacked structures, the intramolecular folding transition is expected to track closely with intermolecular aggregation tendencies of the macrocycles (Figure 1). To test this idea, octadecamers **5** and **6** were synthesized. UV spectroscopy has previously been shown to be useful for monitoring the conformational transition of this backbone in dilute solution where unimolecular species predominate.[14,23] In particular for **4**, the absorbance ratio of the 303 to 288 nm $\pi \rightarrow \pi^*$ bands decreases abruptly upon helix formation.[14,23] This behavior is believed to be due to differences in oscillator strengths for the cisoid and transoid conformations of diphenylacetylene. A decrease in the ratio of these bands was observed in the UV spectra of **5**; however, compared to **4** solvents of greater polarity were required in order to induce the conformational transition (inset Figure 4a,b). The UV spectra of **6** were substantially



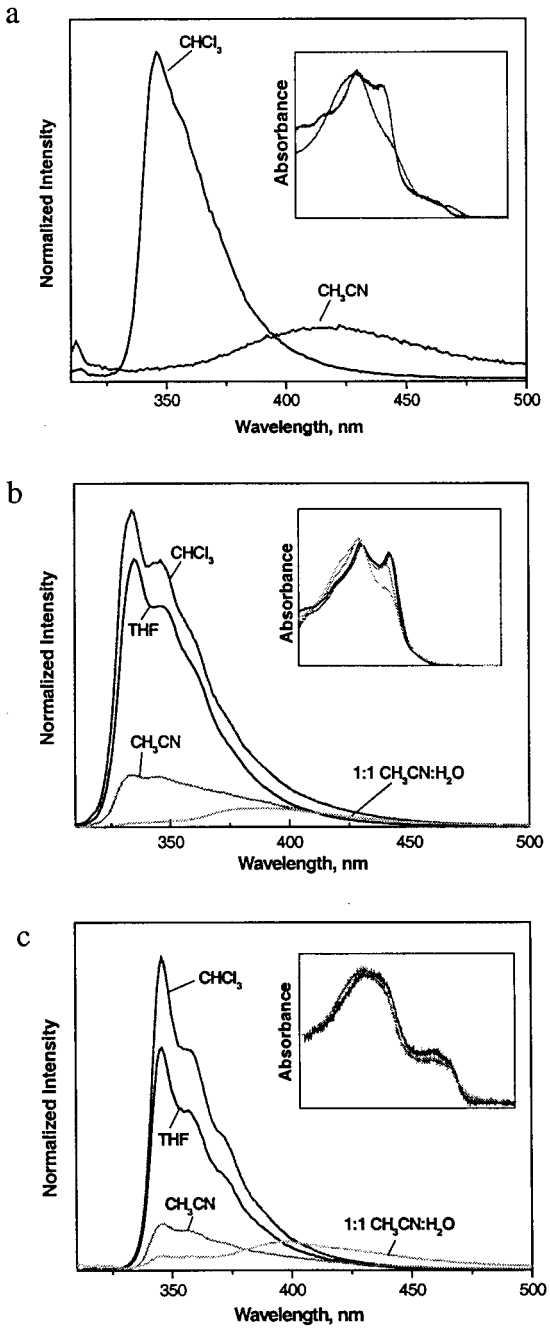


Figure 4. Normalized fluorescence spectra (excitation wavelength = 290 nm) at room temperature of (a) **4**, (b) **5**, and (c) **6** in the indicated solvents. Spectra were normalized to a constant optical density of 0.1 at 290 nm. The inset shows the corresponding absorption spectra. The aqueous acetonitrile solvent mixtures are 1:1 by volume.

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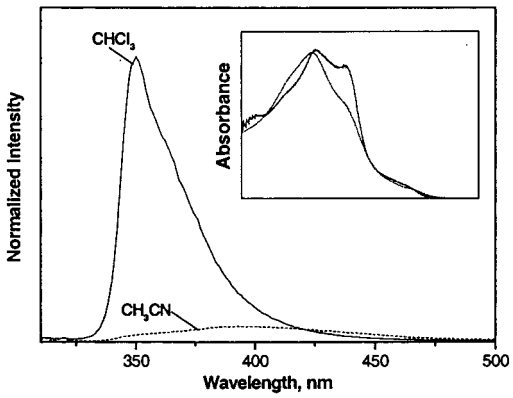


Figure 5. Normalized fluorescence spectra (excitation wavelength = 290 nm) at room temperature of **7** in $CHCl_3$ and CH_3CN. Spectra were normalized to a constant optical density of 0.1 at 290 nm. The inset shows the corresponding absorption spectra.

different in that only one broad band was observed, and therefore, these data did not provide any information about the backbone conformation (inset Figure 4c).

Fluorescence spectroscopy has also been used to assess the solution conformation of the oligomers in different solvents at low concentration (unassociated). Previously, it was shown that the 350 nm emission of **4** in its folded form was quenched and red-shifted (i.e., excimer-like emission) relative to the random coil conformation.[14] The fluorescence spectra of **5** and **6** in chloroform indicate disordered conformations as expected (Figure 4). In acetonitrile, partial quenching at 350 nm was observed, but there was no substantial excimer-like emission at longer wavelengths. Therefore, fluorescence spectra were recorded for **5** and **6** in a 1:1 mixture of acetonitrile and water. The spectra of the octadecamers in this more polar solvent were both quenched and red-shifted suggesting the possibility of helix formation. However, it is also possible that in this highly polar aqueous solvent composition nonspecific aggregation occurs as well.

From the UV and fluorescence data it is evident that the nature of the side chain linking group plays an important role in determining the stability of the ordered helical conformation of the phenylene ethynylene oligomers. This behavior correlates well with that observed for macrocycle association, supporting the notion that solvophobically induced π-stacking interactions are the dominant force stabilizing the folded conformation.

In the interest of manipulating the folding transition of these oligomers, a heterooligomer (**7**, Chart 1) consisting of monomers with ester and benzyl ether side chain linkages was synthesized. In acetonitrile, the UV spectrum of **7** exhibited a significant decrease in the absorbance of the 305 nm band relative to the 289 nm band. In chloroform these bands were of equal intensity. These results suggest that **7** collapses into a helical conformation in acetonitrile (Figure 5, inset). The fluorescence spectrum of **7** was consistent with the UV results in that an excimer-like emission in acetonitrile was observed, implying helix formation.

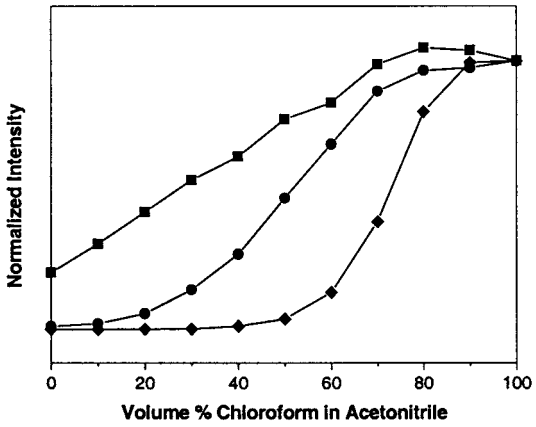


Figure 6. Fluorescence titration data for oligomers **4** (◆), **5** (■), and **7** (●). Fluorescence intensities at 350 nm are plotted against the volume percent chloroform in acetonitrile. Intensities were normalized to a constant optical density of 0.1 at 290 nm (the excitation wavelength).

Table 2. Solvent Denaturation of **4** and **7**[a]

oligomer	$[CHCl_3]_{1/2}$ (vol %)	m (cal·mol^{-1})	ΔG (CH_3CN) (kcal·mol^{-1})
4	72	99	−7.1
7	57	42.1	−2.5

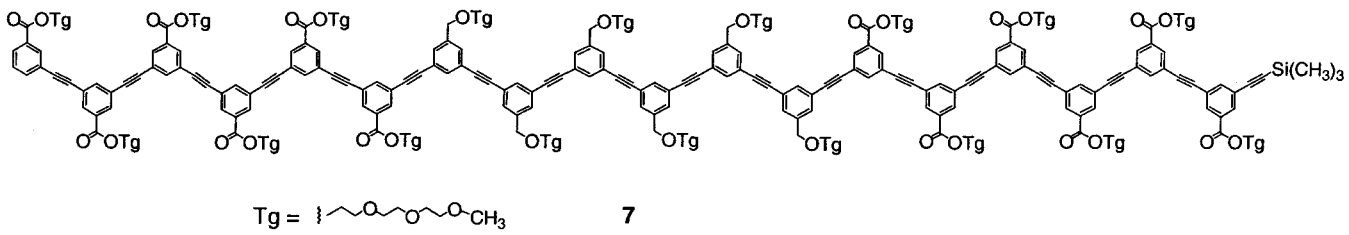
[a] $[CHCl_3]_{1/2}$ is the solvent composition required to reach the midpoint of transition. m describes how rapidly the free energy of transition changes with solvent composition.[14]

Solvent denaturation studies previously have been used to quantify the conformational transitions of these oligomers.[14] Earlier results on octadecamer **4** showed that this oligomer undergoes a cooperative conformational transition as the solvent composition changes from chloroform to acetonitrile.[14] To determine the folding transition and thus the free energy of folding for heterooligomer **7**, a similar solvent denaturation experiment was performed (Figure 6, Table 2). For pure acetonitrile, solvent denaturation studies indicate that the helical conformation of **7** is ca. 2.5 kcal/mol more stable than the unfolded conformation. When compared to the 7.1 kcal/mol value measured for **4**,[14] it is apparent that the incorporation of the six benzyl ether units has significantly destabilized the heterooligomer's helical conformation. This example illustrates how it is possible to tune the helix-coil transition of these oligomers.

Conclusions

Solvophobicity can be an effective way to drive supramolecular organizations involving π-stacked structures. However, certain aromatic ring systems are much more prone to solvent-induced stacking than others. This was demonstrated by studying the intermolecular association of phenylene ethynylene macrocycles in different solvents as a function of side chain linking group. For a given type of linking group, there is at least qualitative correlation between the intermolecular association

Chart 1



of macrocycles, and the stability of the helical conformation of the corresponding oligomers. The folding transitions of these phenylene ethynylene oligomers can be tuned by combining different monomers to make heterosequences which exhibit folding behavior intermediate to that observed for their corresponding homosequences.

Experimental Section

Synthesis and Characterization. Compounds **2**, **3**, and **5**−**7** were synthesized according to previously reported procedures using a divergent/convergent growth strategy (see the Supporting Information). The syntheses of **1** and **4** have already been reported.[13,14] All compounds were purified by silica gel column chromatography. Characterization of intermediates leading to the monomers and oligomers included methods of ^{1}H NMR, ^{13}C NMR, mass spectrometry (EI), and elemental analysis. Oligomers **5**−**7** and macrocycles **2** and **3** were characterized by ^{1}H NMR and MALDI-TOF mass spectrometry and were assessed for purity by high-performance liquid chromatography (HPLC). HPLC was performed using a Rainin binary gradient system equipped with two SD-200 pumps, a Si 80−125-C5 analytical column (4.6 × 250 mm), and a UV detector operating at 290 nm. All compounds were greater than 99% pure based on peak areas.

^{1}H NMR Experiments To Determine Association Constants of Macrocycles. In a given solvent, NMR samples were prepared of each macrocycle over a range of concentrations. The concentration range was determined by the solubility of the macrocycles, detection limit on the 600 MHz NMR, and sample availability. The association constants were calculated based on an equal *K* model described by Martin (eq 1), where P is the observed chemical shift, $P_{monomer}$ is the chemical shift of monomer, K_E = association constant/(molecular weight of macrocycle), c_t is the concentration (mg/mL) of the sample, and Δ is the difference in chemical shift between monomer and dimer.[22] The chemical shifts vs concentration data were analyzed by nonlinear least-squares fitting using Mathematica 3.0 (Wolfram Research).

$$P = P_{monomer} - \Delta(1 + (1 - (4K_E c_t + 1)^{1/2})/(2K_E c_t)) \quad (1)$$

Fluorescence and Absorption Measurements. Fluorescence spectra were recorded on a Photon Technology International (PTI) QM-1 fluorimeter using a 1-cm quartz cuvette in the right angle geometry at room temperature (23 °C). The absorption of the solutions for the fluorescence measurements was approximately 0.1 at 290 nm (excitation wavelength). The UV absorption spectra were recorded on a Shimadazu (model UV-160A) spectrophotometer using 1-cm quartz cells. The solvent denaturation experiment on compound **7** and calculations of free energy were conducted based on previously reported methods.[14]

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Supporting Information Available: Synthesis and characterization data of macrocycles **2** and **3** and oligomers **5**−**7**, calculations of free energy of folding of **7**, and calculations for association constants of macrocycles **1** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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