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A Water-Soluble *m*-Phenylene Ethynylene Foldamer

Matthew T. Stone and Jeffrey S. Moore*

Department of Chemistry and Materials Science & Engineering, 600 South Mathews Avenue, The University of Illinois at Urbana−Champaign, Urbana, Illinois 61801

moore@scs.uiuc.edu

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ABSTRACT

$K_{1:1} = 1.4 \pm 0.090 \times 10^6\ M^{-1}$, 90% water in acetonitrile

A water-soluble *m*-phenylene ethynylene (*m*PE) foldamer was realized by appending hexaethylene glycol side chains to the backbone repeat unit. UV spectra of the oligomer in aqueous solutions were consistent with a helical conformation. The association constant of the oligomer with (–)-α-pinene increased dramatically with increasing water composition, peaking at 90% water by volume in acetonitrile. The rate of the host/guest system's approach to equilibrium was found to decrease considerably with increasing water content.

Previous studies have established that *m*-phenylene ethynylene (*m*PE) oligomers are capable of binding small molecules such as terpenes, presumably by adopting a helical conformation in solution, which generates a hydrophobic binding pocket within the helix interior.[1−3] The association constant was found to vary linearly with water composition, indicating that hydrophobic interactions are a primary driving force for this binding event.[1] However, the poor water solubility of *m*PE dodecamer **1** precluded determination of the association constant at higher compositions of water where this driving force would be greatest. This letter describes the synthesis of water-soluble *m*PE dodecamer **2**, its spectroscopic properties, and binding characteristics with (−)-α-pinene (Figure 1).

A common approach to increasing water solubility of a molecule is the addition of charged functional groups.[4] However, introduction of multiple charges on an oligomer backbone may destabilize the helical conformation due to electrostatic repulsion. Previously, Meijer et al. demonstrated the ability of neutral pentaethylene glycol side chains to solubilize a hydrophobic core in water.[5] Following this lead, *m*PE dodecamer **2** was developed by replacing the triethylene glycol side chains of *m*PE dodecamer **1** with longer hexaethylene glycol side chains. A hexaethylene glycol side chain was chosen because of its synthetic accessibility in two steps from inexpensive, commercially available materials analogous to a previously reported synthesis of the compound.[6]

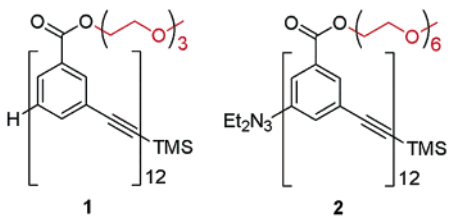

Figure 1. Previously studied *m*PE dodecamer **1** and water-soluble *m*PE dodecamer **2** with longer hexaethylene glycol side chains.

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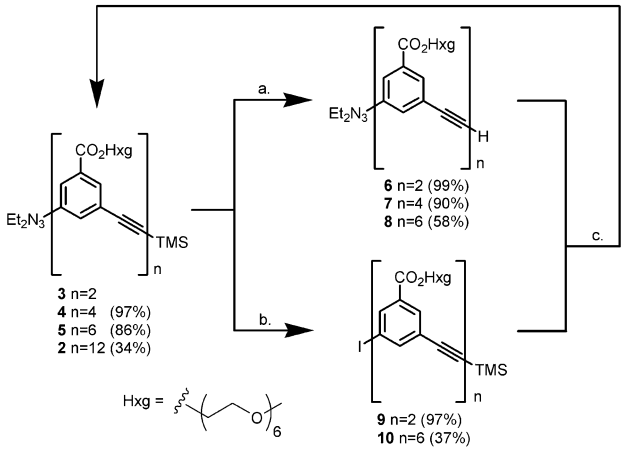
The synthesis of dodecamer **2** was based on an established strategy for the construction of *m*PE oligomers.[7] Orthogonally protected dimer **3** was synthesized and then extended using the divergent/convergent strategy outlined in Scheme 1. One

Scheme 1



Reagents and Conditions: a) TBAF, THF, AcOH b) CH_3I, 110°C c) $Pd_2(dba)_3$, CuI, PPh_3, Et_3N, 50°C.

portion of dimer **3** was reacted with methyl iodide at 110 °C to convert the diethyltriazene group to an aryl iodide providing **9** in good yield,[8] while a second portion of **3** was treated with tetrabutylammonium fluoride to convert the trimethylsilylethynylene group to an ethynylene, giving **6**. Dimers **3** and **9** were then joined through palladium-catalyzed Sonogashira coupling[9] resulting in tetramer **4**. By iteration of this process, the oligomer was extended to 12 repeat units, providing dodecamer **2**. Characterization by mass spectrometry (FAB or MALDI), ^{1}H NMR spectroscopy, and high-performance liquid chromatography confirmed the structure and purity of compounds **2**−**10**. In addition, gel permeation chromatography of dodecamer **2** was found to agree with the molecular weight determined by mass spectrometry. The measured polydespersity of 1.02 (M_w/M_n) is consistent with a discrete molecular species. Oligomers **2**−**5** were found to be soluble in water at room temperature. Solutions of the compounds in water displayed a clouding behavior upon heating, similar to polyethene glycol.[10]

The UV spectra of dodecamer **2** in acetonitrile and chloroform were congruent with previously studied *m*PE dodecamer **1**, indicating a folded helical structure in acetonitrile and a denatured, random conformation in chloroform.[3,4] The UV spectra of dodecamer **2** collected with increasing water composition showed a shift to shorter

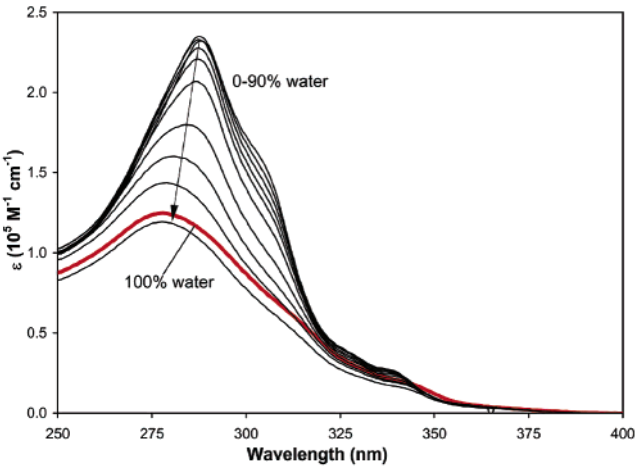


Figure 2. UV spectra of dodecamer **2** in varying percentages of water in acetonitrile (100% water in red).

wavelengths and a depression of the overall optical density (Figure 2). This effect, referred to as hypochromicity, is observed in the UV spectra of DNA during thermal denaturation and commonly attributed to the close packing of aromatic chromophores in solution.[11] For *m*PE oligomers, close packing of the backbone chromophores could be caused by tightening of the helical conformation and/or aggregation of helical dodecamer **2**. Prior investigations of *m*PE oligomers revealed this hypochromic effect in solutions of water and acetonitrile, but the limited solubility of the oligomers prevented tracking this effect into pure water.[12] The UV spectrum of dodecamer **2** in 100% water deviated from the overall trend, with a slightly greater optical density than in 90% water and noticeable differences in the spectral band shape. This deviation is possibly an effect of the absence of acetonitrile molecules that may preferentially solvate around and within the folded helix. The complete absence of acetonitrile might cause dodecamer **2** to alter its conformation in order to minimize unfavorable interactions with water.

Once it had been established that water-soluble dodecamer **2** had solvent-dependent UV characteristics similar to those of the previously studied dodecamer **1**, the binding characteristics of the oligomer were explored as a function of water composition. To determine the association constant of dodecamer **2** with a chiral guest, the induced circular dichroism (CD) was measured.[13] The CD signal is the result of a preferential association of the chiral guest with one handedness of the helix, which gives rise to a shift from a dynamic racemate to diastereomeric complexes that favor one helical twist sense. (−)-α-Pinene was chosen as the guest so that results could be compared with previous studies.[1]

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To survey the magnitude of the guest-induced CD signal over the full range of solvent compositions, solutions were prepared containing 4 μM dodecamer **2** and 100 equiv of (−)-α-pinene in varying percentages of water in acetonitrile (Figure 3a). Rather unexpectedly, no induced CD signal was

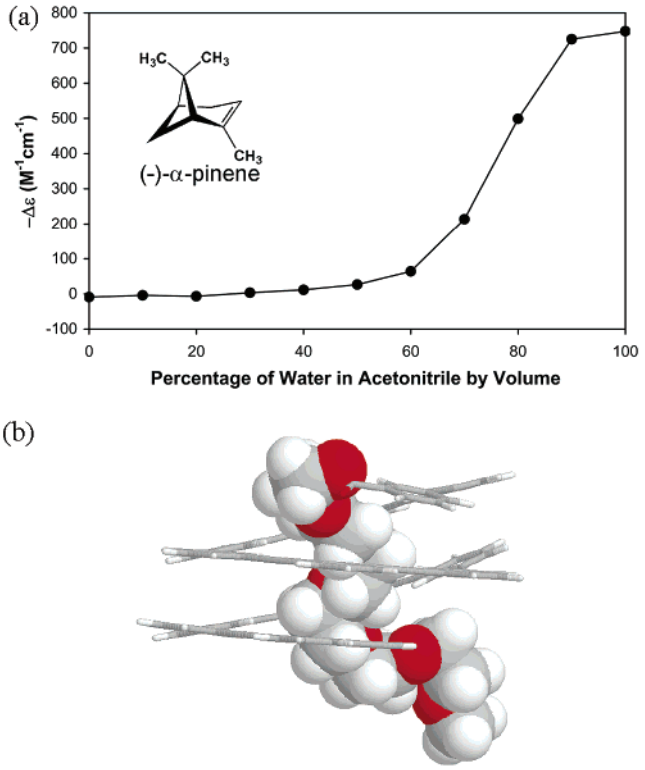

Figure 3. (a) Molar ellipticity at 315 nm for 4 μM dodecamer **2** and 100 equiv of (−)-α-pinene in varying compositions of water in acetonitrile. (b) Computer model of dodecamer **2** as determined by a Monte Carlo search for minimum energy conformations. Side chain is displayed as a space-filling model within the cavity.

observed when the solvent composition contained less than 50% water; above this composition, the signal increased sharply and leveled off at 90% water. This result deviated significantly from previous studies where an induced CD signal was evident at 10% water.[1] From this observation, it can be concluded that the hexaethylene glycol side chains of dodecamer **2** have more than just an ancillary role with regard to guest binding.

One possible explanation for the difference in behavior for *m*PE dodecamers **1** and **2** is that at lower compositions of water, the longer side chains fill the helical cavity and compete with (−)-α-pinene binding. Presumably at higher percentages of water, the binding of (−)-α-pinene in the helical cavity is favored and the side chains are displaced into solution. To further explore this hypothesis, a Monte Carlo search of minimum energy conformations was performed on the structure of dodecamer **2** (Figure 3b). To simplify the calculation, all side chains except the one attached to the last repeat unit were removed and reorganization of the helix was excluded from the model. The minimum energy conformation shows that the side chain threaded through the center of the helix. Although this model is overly simplified, it does demonstrate the capacity of the side chains to fill the cavity.

At solvent compositions having 60% water or more, the CD signal was strong enough to reliably determine the association constant with (−)-α-pinene. The association constant was measured by preparing several solutions with a constant concentration of dodecamer **2** while the concentration of (−)-α-pinene was incrementally increased (Figure 4). The resulting CD spectra displayed an isodichroic point

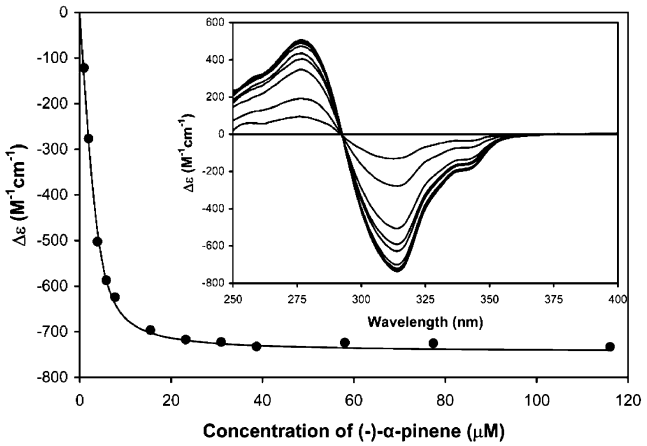

Figure 4. Plot of the $\Delta\epsilon$ at 315 nm against the concentration of (−)-α-pinene with 4 μM dodecamer **2** in 90% water in acetonitrile. The line represents the nonlinear least-squares fit. The inset shows the corresponding CD spectra. Spectra shown are averages of two solutions of identical composition.

at 293 nm, indicative of a single equilibrium between the bound and unbound dodecamer **2**. The CD signal at 315 nm was then plotted against the concentration of (−)-α-pinene, and the association constant was determined using a nonlinear least-squares fitting routine.[14] The stoichiometry of the complex was determined to be 1:1 for the solvent compositions examined on the basis of the linearity of Benesi−Hildebrand plots and from the quality of the nonlinear least-squares fitting to the 1:1 binding isotherm.[15,16] Although the oligomer's state of aggregation likely changes at higher compositions of water, the stoichiometry of the complex does not appear to be affected.

In contrast to the previous study with dodecamer **1**, the overall trend of the association constant as a function of water composition was found to be nonlinear, increasing sharply by approximately 2 orders of magnitude when the water

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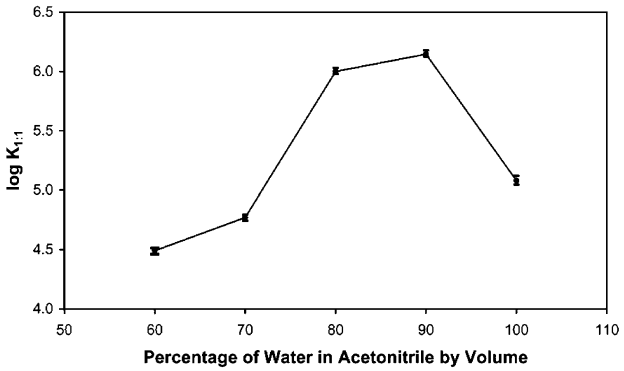

Figure 5. Plot of association constant for dodecamer **2** at 4 μM with (−)-α-pinene against the percentage of water in acetonitrile. Note that the association constant for 100% water was collected at 2 μM due to the relative insolubility of (−)-α-pinene in water.

content of the solution was changed from 70 to 80% (Figure 5).[1] The association constant peaked at 90% water in acetonitrile with a value of $1.4 \pm 0.090 \times 10^6$ M^{-1}, which is significantly higher than the maximum value predicted by extrapolation to 100% water in the previous study (6×10^4 M^{-1}).[1] In pure water, the association constant was an order of magnitude lower than the maximum value, suggesting that some impedance to binding exists. It is possible that the helical conformation in 100% water is tighter and has a smaller interior cavity, which disfavors binding with (−)-α-pinene. This hypothesis is consistent with the differences observed in the UV spectrum collected in 100% water, which may also be attributed to a tighter helical conformation. The wide range of association constants (10^4−10^6 M^{-1}) demonstrates the tremendous sensitivity of binding affinities based on nonspecific, noncovalent interactions to solvent composition. The association constants measured for dodecamer **2** with (−)-α-pinene are similar in magnitude to those exhibited by enzymes binding their substrates, suggesting that *m*PE oligomers might have potential as scaffolds for engineering artificial enzymes.[17,18]

In addition to the strength of the association constant, the approach of the host/guest system to equilibrium was found to be significantly different for water-soluble dodecamer **2** in comparison with previous guest binding studies. In prior investigations at lower percentages of water, the induced CD signal reached equilibrium nearly instantaneously upon mixing with a chiral guest; however, at higher compositions of water, the CD signal of **2** was much slower to reach a constant value.[19,20] The relationship between solvent composition and the kinetics of binding was investigated by tracking the CD signal at 315 nm vs time at various compositions of water containing 4 μM dodecamer **2** and 20 equiv of (−)-α-pinene. To determine the half-life of the process, the CD signal at 315 nm was normalized by its final value and fit to a psuedo-first-order kinetics model.

In the case of 60 and 70% water in acetonitrile, the CD signal reached its maximum before the first measurement could be taken or within a minute of mixing. The half-lives of the process in 80, 90, and 100% water were on the order of seconds, minutes, and hours, respectively. It is evident that the rate of the process was highly dependent on the composition of the solution. The role of the longer side chains is difficult to determine because *m*PE oligomers with shorter triethylene glycol side chains are not soluble at these solvent compositions. However, the strong dependence on water composition would seem to support a higher energy, unfolded intermediate that slows the evolution of the preferred helical handedness of binding.

In conclusion, water-soluble *m*PE dodecamer **2** was achieved by replacing triethylene glycol side chains of dodecamer **1** used in previous studies with longer hexaethylene glycol side chains. The UV spectra of dodecamer **2** showed a large hypochromic effect that increased with increasing water composition. This hypochromic effect was attributed to close stacking of the backbone chromophores possibly through the tightening of the helical conformation and/or aggregation of the folded dodecamer **2**. Varying the water composition of the solvent allowed access to a large range of association constants (10^4−10^6 M^{-1}). The maximum association constant of dodecamer **2** with (−)-α-pinene was $1.4 \pm 0.090 \times 10^6$ M^{-1} at a solvent composition of 90% water in acetonitrile, which is the largest association constant measured for a *m*PE foldamer with a guest molecule. Finally, the evolution of the induced CD signal upon mixing was also found to be highly dependent on solvent composition, and the rate at which equilibrium was reached decreased dramatically as the solvent composition approached pure water. Thus, increasing the solubility range of *m*PE oligomers provides an opportunity to express considerable control over the host−guest binding event through the modulation of solvent composition.

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Supporting Information Available: Detailed descriptions of all experimental procedures and results. This material is available free of charge via the Internet at http://pubs.acs.org.

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