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Single-Site Modifications and Their Effect on the Folding Stability of *m*-Phenylene Ethynylene Oligomers

Hirofumi Goto, Jennifer M. Heemstra, David J. Hill, 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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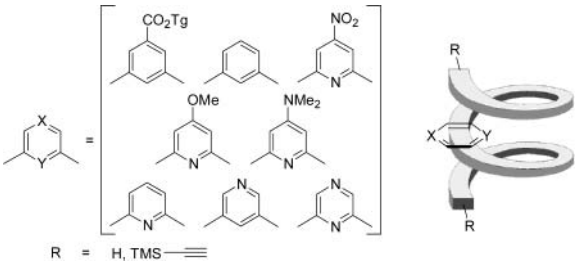
ABSTRACT



The folded structure of a *m*-phenylene ethynylene oligomer is tolerant to single-site modifications to both the backbone sequence and end groups. The helical structure is reinforced by multiple noncovalent interactions, allowing the oligomer sequence to be customized without a significant change in stability in most cases. The small changes that are observed are consistent with the expected behavior of π-stacked systems and demonstrate subtle control over folding through single-site modifications.

Enzymes rely on multiple noncovalent interactions to enforce their folded conformation and precisely orient residues in their active sites.[1] However, adopting the most active conformation may introduce unfavorable interactions, and large chain lengths are required to compensate for these interactions.[2] Foldamer research focuses on designing synthetic macromolecules that replicate the structural characteristics of their natural counterparts.[3] Analogous to enzymes, these synthetic molecules often rely on multiple noncovalent interactions to stabilize their folded conformation, although most synthetic oligomers studied to date are much smaller than enzymes. Here, we show that π-stacking interactions provide sufficient stability to maintain the folded structure of a *m*-phenylene ethynylene oligomer despite changes in backbone sequence and end group functionality.

One class of foldamers that has attracted recent interest is single-stranded oligomers that mimic peptides in their ability to undergo cooperative transitions between random and helical conformations.[4−6] The cooperative nature of the conformational transitions suggests that multiple noncovalent

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(4) Examples of single-stranded oligomers that undergo cooperative conformational transitions include: (a) Kirshenbaum, K.; Barron, A. E.; Goldsmith, R. A.; Armand, P.; Bradley, E. K.; Truong, K. T. V.; Dill, K. A.; Cohen, F. E.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4303−4308. (b) Martinez de Ilarduya, A.; Alemán, C.; García-Alvarez, M.; López-Carrasquero, F.; Muñoz-Guerra, S. *Macromolecules* **1999**, *32*, 3257−3263. (c) Cheng, J.; Deming, T. J. *Macromolecules* **2001**, *34*, 5169−5174. (d) Wu, C. W.; Sanborn, T. J.; Huang, K.; Zuckermann, R. N.; Barron, A. E. *J. Am. Chem. Soc.* **2001**, *123*, 6778−6784. (e) Fernández-Santín, J. M.; Muñoz-Guerra, S.; Rodríguez-Galán, A.; Aymamí, J.; Lloveras, J.; Subirana, J. A. *Macromolecules* **1987**, *20*, 62−68. (f) Prince, R. B.; Moore, J. S.; Brunsveld, L.; Meijer, E. W. *Chem. Eur. J.* **2001**, *7*, 4150−4154. (g) Brunsveld, L.; Prince, R. B.; Meijer, E. W.; Moore, J. S. *Org. Lett.* **2000**, *2*, 1525−1528. (h) Gin, M. S.; Moore, J. S. *Org. Lett.* **2000**, *2*, 135−138. (i) Petitjean, A.; Cuccia, L. A.; Lehn, J.-M.; Nierengarten, H.; Schmutz, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 1195−1198.

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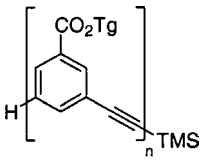
interactions are working together to stabilize the ordered structure. Phenylene ethynylene (PE) oligomers **1**, previously studied by our group, have been found to exhibit a reversible transition from random to helical conformation in response to changes in solvent quality.[7,8] The folded structure of PE oligomers is stabilized primarily by π-stacking interactions, though hydrophobic packing of the helical cavity[9] and hydrogen bonding interactions[10] can be used in certain cases to further enhance stability.



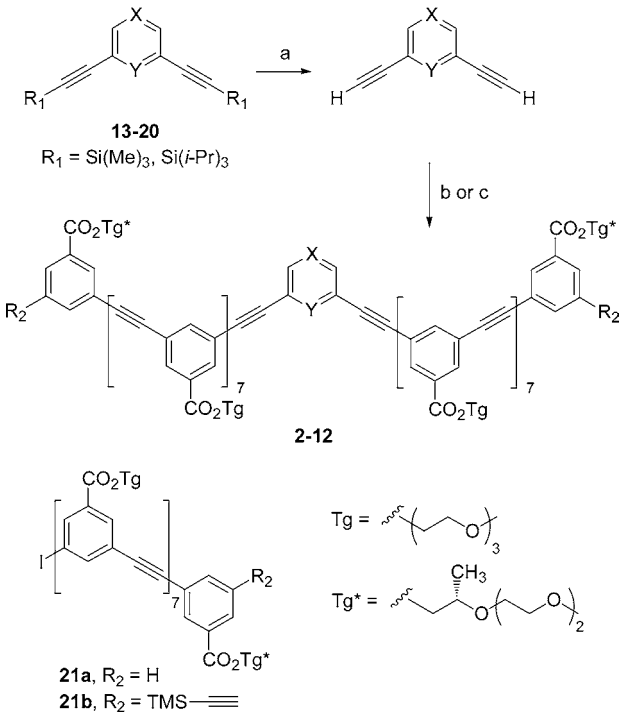
1, n≥10

Folding of PE oligomers into a helical conformation generates an interior cavity capable of binding small, hydrophobic guest molecules.[11] More recently, we have demonstrated that the surface of the binding cavity can be functionalized by incorporation of modified monomers into the backbone sequence, expanding the potential use of the oligomers in molecular recognition and catalysis.[12] We hypothesize that multiple π-stacking interactions cooperatively stabilizing the helical conformation of the oligomer will allow single-site modifications to be made without significant disruption of the folded structure. To test the robustness of the helical structure, oligomers **2**−**12** were synthesized, with **2** and **3** composed entirely of unmodified monomers to serve as control oligomers and **4**−**12** incorporating one modified monomer each. The resulting changes in folding stability were monitored to determine the impact of each of the modifications.

Synthesis of oligomers **2**−**12** is outlined in Scheme 1. Monomers **13**−**20** were subjected to TBAF for removal of the silyl protecting groups. Then, Pd-catalyzed cross-coupling with 2 equiv of iodide-terminated PE octamer (**21**)[10] gave the desired oligomers with yields as listed in Table 1. Oligomers **2** and **4**−**10** were synthesized having hydrogen capping groups on the terminal phenyl rings, whereas **3**, **11**, and **12** were capped with trimethylsilyl acetylene groups. This modification allows the effect of the capping group on folding stability to be evaluated directly.

The solvophobically induced folding of oligomers **2**−**12** can be monitored using either circular dichroism (CD) or UV spectroscopy. In acetonitrile, the PE backbone is poorly solvated, causing the oligomer to collapse into a helical

Scheme 1 [a]



[a] Reagents and conditions: (a) TBAF, AcOH, THF; (b) **21**, $Pd_2(dba)_3$, CuI, PPh_3, Et_3N, CH_3CN, 50 °C; (c) **21**, $Pd_2(dba)_3$, CuI, PPh_3, piperidine, CH_3CN, 60 °C → rt.

conformation, whereas in chloroform the PE backbone is better solvated and the oligomer unfolds into a random conformation. Each oligomer is equipped with chiral triethyleneglycol side chains on the terminal phenyl rings, and upon folding the chiral information in the side chains is transferred to the PE backbone, biasing the handedness of the helical conformation.[13] This twist-sense bias produces a Cotton effect in the CD spectrum, as shown in Figure 1 (top) for oligomer **4** in acetonitrile. In chloroform, no CD signal

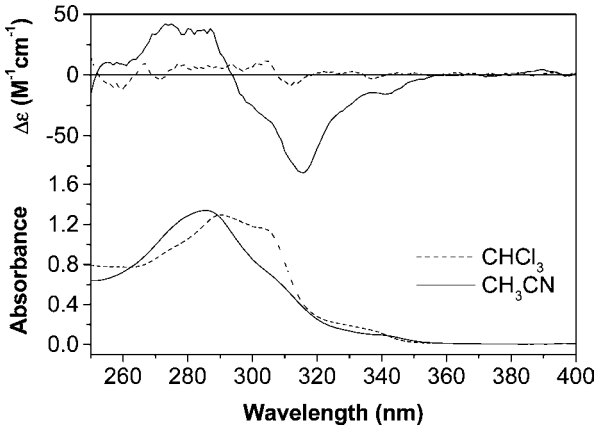


Figure 1. CD spectra (top) and UV−vis spectra (bottom) of **4** in chloroform and acetonitrile.

(6) Nelson, J. C.; Saven, J. G.; Moore, J. S.; Wolynes, P. G. *Science* **1997**, *277*, 1793−1796.
(7) Hill, D. J.; Moore, J. S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5053−5057.
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(9) Prince, R. B. Ph.D. Thesis, University of Illinois at Urbana-Champaign, 2000.
(10) Cary, J. M.; Moore, J. S. *Org. Lett.* **2002**, *4*, 4663−4666.
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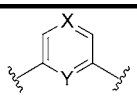
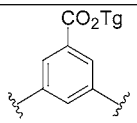
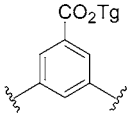
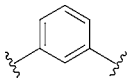
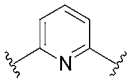
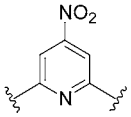
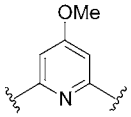
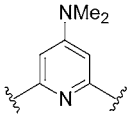
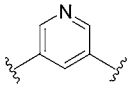
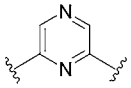
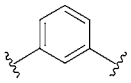
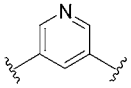
(12) Heemstra, J. M.; Moore, J. S. *Org. Lett.* In press.

Table 1. Yields and Comparison of Folding Stability for Oligomers **2**−**12**

oligomer		R_2	monomer, reaction conditions[a]	yield (%)	ΔG (CH_3CN) (kcal·mol^{-1})	m (cal·mol^{-1})	$[CHCl_3]_{1/2}$ (vol %)
2		H—	**13**,[b] c	69	-5.6 ± 0.4	85 ± 5	66 ± 1
3		TMS—≡—	**13**,[b] b	97	-5.0 ± 0.3	82 ± 4	61 ± 1
4		H—	**14**,[c] c	98	-5.0 ± 0.1	78 ± 1	63 ± 1
5		H—	**15**,[d] c	98	-5.0 ± 0.3	77 ± 5	66 ± 2
6		H—	**16**,[d] b	37	-6.0 ± 0.7	83 ± 9	73 ± 1
7		H—	**17**,[d] b	80	-5.2 ± 0.4	76 ± 6	68 ± 1
8		H—	**18**,[d] b	57	-4.7 ± 0.3	70 ± 4	67 ± 1
9		H—	**19**, c	68	-5.9 ± 0.3	90 ± 6	63 ± 1
10		H—	**20**, c	11	-5.1 ± 0.2	76 ± 3	67 ± 1
11		TMS—≡—	**14**,[c] b	50	-4.3 ± 0.1	70 ± 1	61 ± 1
12		TMS—≡—	**19**, b	63	-5.2 ± 0.2	83 ± 3	63 ± 1

[a] See Scheme 1. [b] Zhao, D.; Moore, J. S. *J. Org. Chem.* **2002**, *67*, 3548−3554. [c] Neenan, T. X.; Whitesides, G. M. *J. Org. Chem.* **1988**, *53*, 2489−2496. [d] Heemstra, J. M.; Moore, J. S. *Org. Lett.* In press.

is observed, consistent with a random conformation. In the UV spectra of the PE oligomers, the ratio of absorbance bands at 303 and 289 nm is indicative of the conformation of the oligomer backbone, with low values of $A_{303/289}$ signifying a high degree of folding.[5,6] Accordingly, the spectra in Figure 1 (bottom) suggest that **4** adopts a helical conformation in acetonitrile and a random conformation in chloroform, consistent with the CD results.

To compare the folding properties of **2**−**12**, UV absorption spectra of each of the oligomers were obtained in a series of solvent mixtures ranging from pure acetonitrile to pure chloroform. Assuming that all of the oligomers undergo a complete transition between their folded and unfolded states over the observed range of solvent mixtures, the UV absorbance data can be fitted to give the fraction of oligomer in the unfolded state for each solvent mixture.[5]

Figure 2 displays the absorption data obtained for **6**−**8** as a representative example of the relationship between solvent

(13) Prince, R. B.; Brunsveld, L.; Meijer, E. W.; Moore, J. S. *Angew. Chem., Int. Ed.* **2000**, *39*, 234−236.

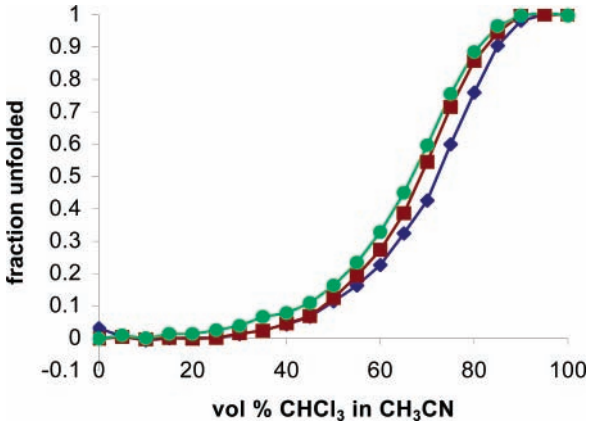


Figure 2. UV titration curve of **6** (blue ◆), **7** (red ■), and **8** (green ●).

composition and fraction of oligomer in the unfolded state. Assuming that the free energy difference between the folded and unfolded states depends linearly on solvent composition,[14] the free energy of folding in pure acetonitrile, $\Delta G(CH_3CN)$, can be determined from eq 1 where $[CHCl_3]$ represents the chloroform composition expressed as vol % and m indicates how rapidly the stabilization energy of the helix (ΔG) changes in response to changes in solvent composition.[5]

$$\Delta G = \Delta G(CH_3CN) - m[CHCl_3] \qquad (1)$$

The composition of chloroform required to reach the midpoint of denaturation ($[CHCl_3]_{1/2}$) is given by $\Delta G(CH_3CN)/m$. A comparison of the free energy of folding and the midpoint of denaturation for **2**−**12** is presented in Table 1. The data reveal that incorporating a modified monomer into the backbone sequence or changing the capping groups on the terminal phenyl rings has little effect on the folding stability of the oligomer. However, comparison of the values of $\Delta G(CH_3CN)$ for the oligomers reveals two general trends in folding stability. First, changing the end group from hydrogen to TMS acetylene (**3**, **11**, **12**) has a slight destabilizing effect on the folded conformation. Second, for oligomers having a 2,6-diethynylpyridine ring, the folding stability may be influenced by the electronics of the substituents on the ring, with electron-withdrawing substituents (**6**) stabilizing the helix and electron-donating substituents (**7** and **8**) destabilizing the helix. This observation agrees with well-documented $\pi-\pi$ stacking arguments;[15] however, the experimental errors associated with the $\Delta G(CH_3CN)$ measurements preclude establishment of a definite relationship between pyridine ring electronics and folding stability.

This work demonstrates that a wide variety of modified monomers can be incorporated into the backbone of PE oligomers, expanding the available monomer alphabet and allowing functionalization of the interior binding cavity. Future studies will focus on utilizing these monomers to design oligomers with potential to act as synthetic enzyme mimics by directing substrate recognition and reaction catalysis.

The helical structure of a PE oligomer is only minimally impacted by single-site modifications to the backbone sequence and changes in end group functionality. This robustness of the helical structure may result from multiple π-stacking interactions working cooperatively to stabilize the folded structure of the oligomer. The ability of multiple noncovalent interactions to enforce the folded structure despite potentially unfavorable modifications provides a possible rationale for the typically large chain lengths of functional biomolecules. Furthermore, these results support the hypothesis that careful engineering of noncovalent interactions can provide synthetic foldamers that adopt stable secondary structures at significantly shorter chain lengths than those characteristic of biomolecules.

Acknowledgment. This research was funded by the U.S. Department of Energy, Division of Material Science (Grant DEFG02-91-ER45439). H.G. thanks JSR Corporation for financial support. J.M.H. thanks the University of Illinois for a doctoral fellowship.

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

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