Synthesis and characterization of amphiphilic *o*-phenylene ethynylene oligomers†

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We have previously reported the synthesis of short *o*-phenylene ethynylene oligomers with polar triethylene glycol side chains which adopt a helical conformation in solution with three residues per turn. Two new oligomers have been synthesized, a hexamer and a nonamer, incorporating a repeated triad motif of polar–nonpolar–polar sidechains in order to create a hydrophobic stripe in the folded conformation which we report here for the first time. Helical folding in solution was observed and, unlike the previously-reported oligomers, these new oligomers are ordered solids at room temperature. Although these oligomers were designed to assemble into helical bundle-like structures, no evidence for a quaternary-like structure was found. The difference in polarity between alkyl and triethylene glycol side chains is likely not strong enough to induce self-association of folded helices, especially since the molecules are not water soluble where the driving force for association of the nonpolar stripe would be larger. We expect that more polar side chains, granting water solubility, represent an important target for future research.

Introduction

Foldamers have been of great interest as model systems for investigation and computational simulation of biological macromolecules in a simple and controlled context.[1–13] A great variety of foldamer systems that show secondary structure analogous to that of natural macromolecules have been investigated over the past few years. Foldamers demonstrating biomimetic tertiary and quaternary structures have been designed as well,[14–16] although there are significantly fewer examples. Extensive investigations of phenylene ethynylene foldamers have been made by various workers,[17–26] due to the simplicity and flexibility of the backbone structure.

Designed peptides have been extensively explored in order to study determinants of secondary, tertiary, and quaternary structure formation. Many peptides that fold into helical bundles in solution have been described in the literature.[27–32] The major driving force for this assembly is the burial of the hydrophobic stripe created upon helix formation. Consequently, natural as well as designed helical bundles are composed of amphiphilic helices which display a hydrophobic face in their folded conformation, inducing self-association in order to bury the hydrophobic areas.

Amphiphilic foldamers are interesting both as a means of investigating folded structure and as potential scaffolds to modulate biological activity. We have previously developed short *ortho*-phenylene ethynylene (*o*PE) oligomers which adopt helical conformations in solution[33–35] and other phenylene ethynylenes which demonstrated antimicrobial activity[36–39] similar to host defense peptides like the magainins and defensins. Here we describe new *o*PE oligomers which have both polar and nonpolar side chains to create an amphiphilic structure in the helically folded conformation. It represents our first step towards folded assemblies beyond the secondary unit. We have successfully prepared two oligomers containing six and nine *o*PE units with a repeating pattern of polar, nonpolar, polar side chains to match the three residues per turn of the helix. This places the polar and nonpolar groups on distinct faces of the folded structure. As per our earlier reports, the polar group is the ester of triethylene glycol monomethyl ether while the nonpolar group is the ester of (*S*)-2-methyl butanol.

These new oligomers were shown to adopt helical conformations in solution, representing an important milestone. Since solvophobic-like driving forces were believed to promote helix formation in our previous oligomers containing all Teg side chains, it was unclear how the replacement of one Teg chain per turn with a non-polar group would effect helix formation and stability. This solvophobic effect, proposed by Moore and Ray,[25] takes advantage of solubility differences between Teg side chains and the PE backbone. Upon changing the solvent from $CHCl_3$ to more polar solvents, like acetonitrile, the backbone folds to reduce its surface area in contact with the polar solvent. At the same time, the polar Teg side chains promote solubility of the oligomer. Therefore, the fact that these oligomers still adopt a helical conformation in acetonitrile is an important result. However, our goal remains higher order assembly and no evidence for such assemblies was observed for these two oligomers. This is attributed to their lack of water solubility where the driving force for burial of the hydrophobic stripe would be larger.

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Results and discussion

The amphiphilic oligomers **1** and **2** composed of 6 or 9 *o*PE units, respectively, as shown in Fig. 1, were synthesized in order to study their ability to fold and assemble. They are similar to our previously reported triethylene glycol monomethyl ether (Teg) substituted *o*PE oligomers, which adopt helical structures in solution; however, a new repeating 3-residue motif of polar–nonpolar–polar side chains creates a hydrophobic face in the helical conformation, as shown in Fig. 2.

Oligomers **1** and **2** were synthesized using a partially convergent strategy of deprotection and Sonogashira couplings. Acetylene groups were trimethylsilyl (TMS) protected, while triazene groups were used to provide masked aryl iodide functionality, as in previous PE synthetic work. Protected (*S*)-2-methylbutyl ester monomers **9** and **10** were prepared as shown in Scheme 1 from previously reported intermediate **6**,[34] using chemistry similar to that used to prepare previously reported Teg-ester monomers **3** and **4**. Aryl iodide-terminated *o*PE dimers **12** and **14** were synthesized as shown in Scheme 2, similarly to the preparation of previously reported Teg-ester dimer iodide **5**.[35] In our hands, the methyl iodide reaction to convert the triazene to an aryl iodide provides the lowest yield. In fact, as the oligomer length increased, this reaction became more problematic and influenced the synthetic route. With dimers **12** and **14** in hand, along with **5**, construction of the hexamer and nonamer was performed. Schemes 3 and 4 outline the synthesis of hexamer **1** and nonamer **2**, respectively. The sequential coupling steps used dimeric units instead of trimeric units due to the very low yield obtained during the unmasking of triazene-terminated trimers. Scheme 3 combined

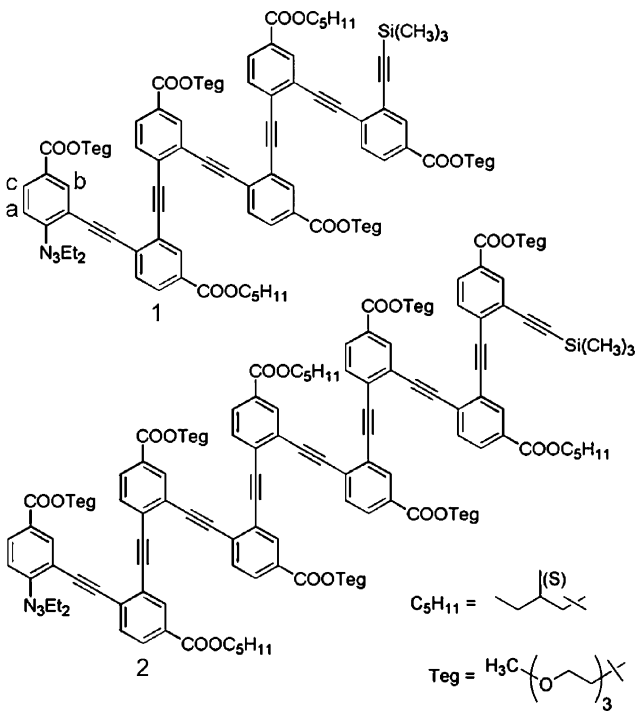


Fig. 1 Triethylene glycol monomethyl ether and alkyl ester-substituted *o*PE oligomers, hexamer (**1**) and nonamer (**2**). Each ring has three protons that are labeled by their NMR splitting pattern: a (8.4 Hz, d); b (2.1 Hz, d); c (8.4 Hz and 2.1 Hz, dd).

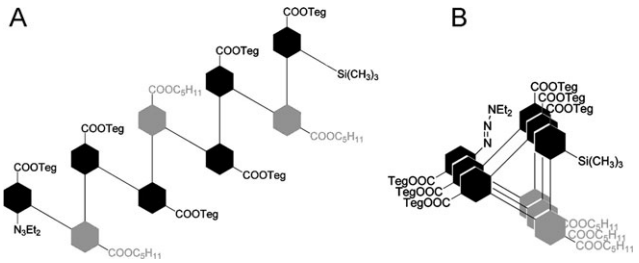
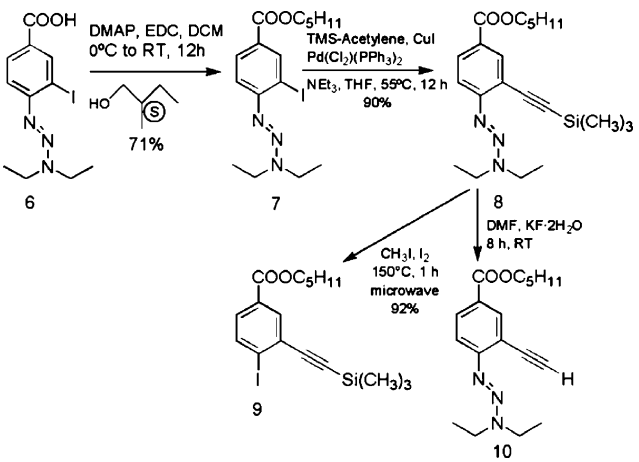


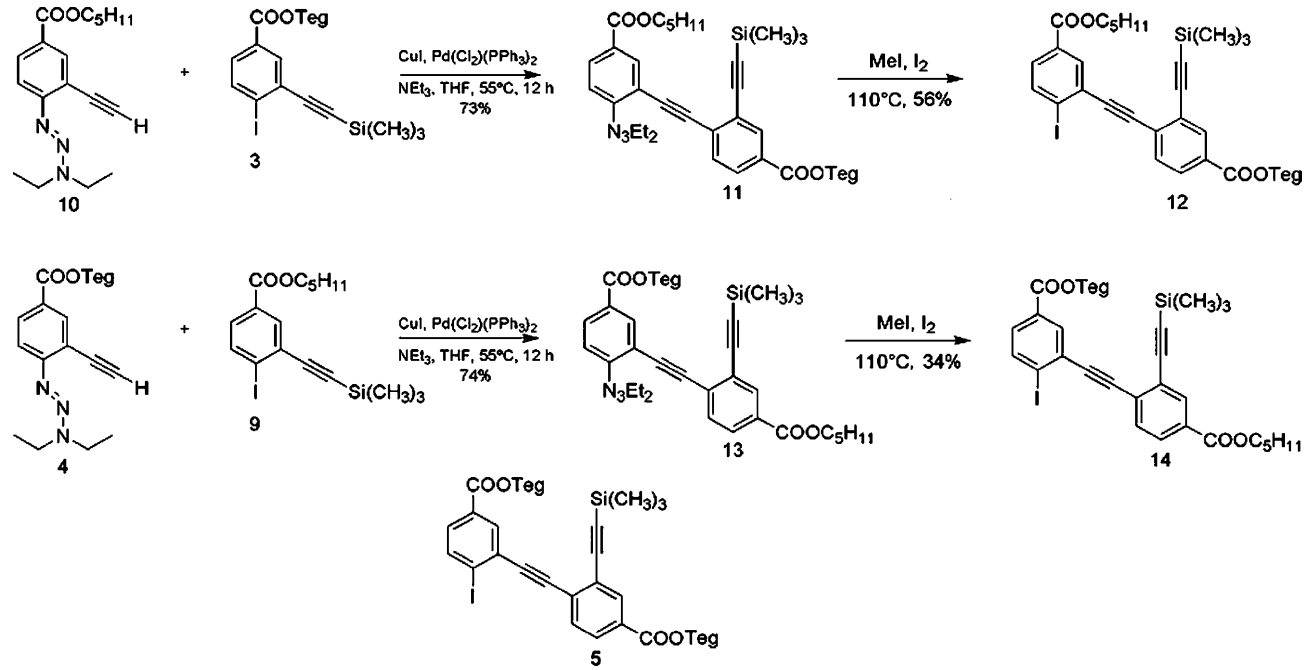
Fig. 2 Nonamer (**2**) in two potential conformations, fully extended (A) and fully helical (B).

4 and **12** to generate the amphiphilic trimer, **15**, which was converted to the free acetylene **16** and reacted with **14** to generate pentamer **17**. Another cycle of acetylene deprotection to form **18** followed by Sonogashira coupling, this time with **3**, yielded the hexamer **1** in 21% overall yield for the five steps shown in Scheme 3. Sonogashira coupling of intermediate **18** with **5** followed by another acetylene deprotection step and a final Sonogashira coupling with **12** gave nonamer **2** in only 7% yield over 7 steps, as shown in Scheme 4.

Folding of these two new oligomers, **1** and **2**, was studied using NMR spectroscopy. Similar to peptides, a main driving force for folding is burial of the hydrophobic backbone. In our case, the aromatic backbone of these foldamers is strongly hydrophobic and only weakly solvated by acetonitrile, which tends to promote a helically folded conformation as the *o*PE attempts to minimize the surface area in contact with the solvent. In contrast, a solvent such as chloroform, which strongly solvates aromatic backbones, tends to promote a more unfolded, or randomly coiled, conformation. Due to the precise chemical synthesis, NMR spectroscopy provides an excellent handle for studying conformational changes in these *o*PE foldamers. The three protons on each ring are expected to shift upfield in the event of ring stacking, due to ring current effects. Our previous studies on shorter *o*PE foldamers confirmed that this upfield-shift was due to folding by using simultaneous 2D NOE measurements.[34] Therefore, since these oligomers would represent 2 and 3 full turns upon folding into a fully helical conformation, the entire aromatic region of each spectra was expected to shift upfield.

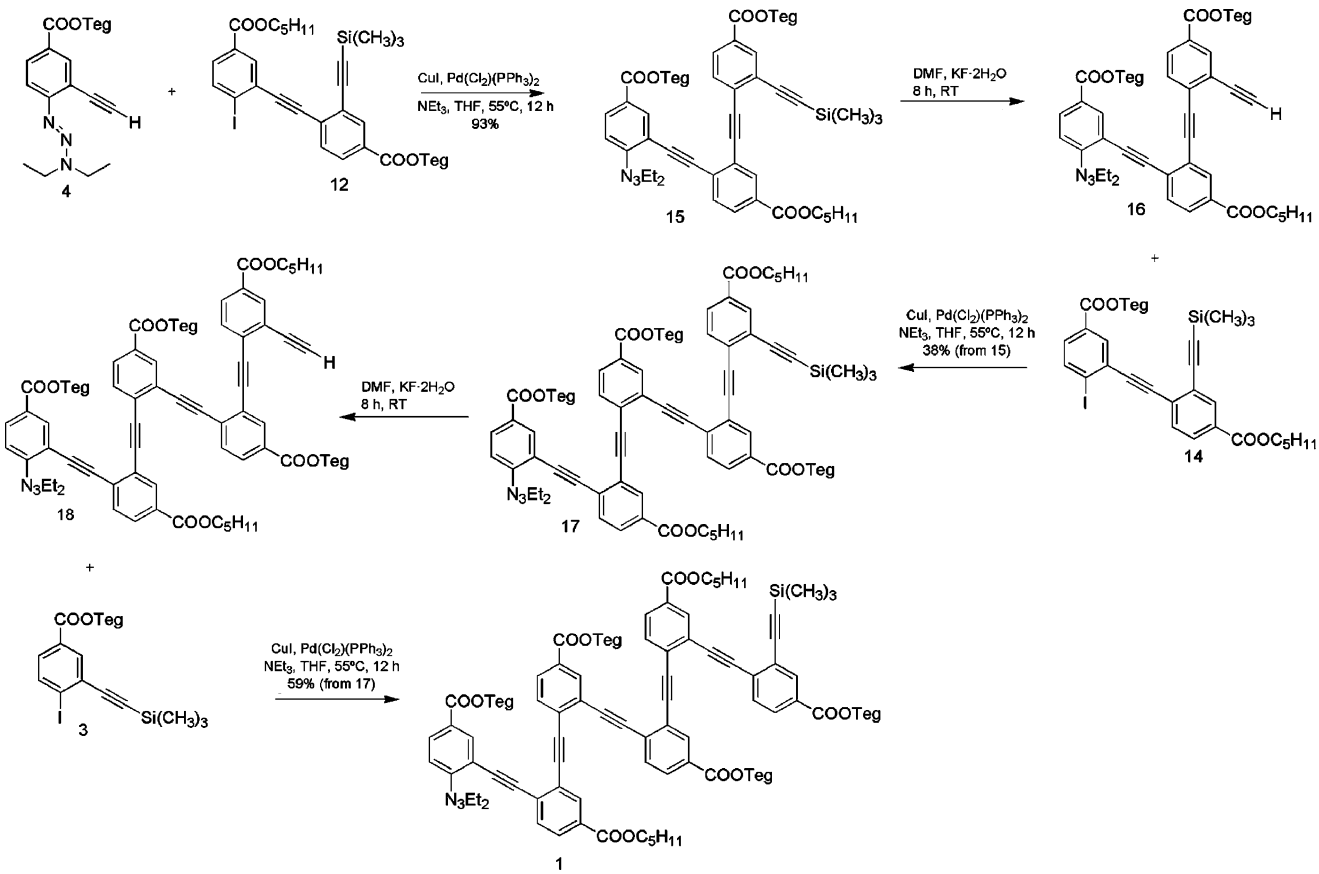


Scheme 1 Synthesis of (*S*)-2-methylbutyl ester nonpolar monomers **9** and **10**.

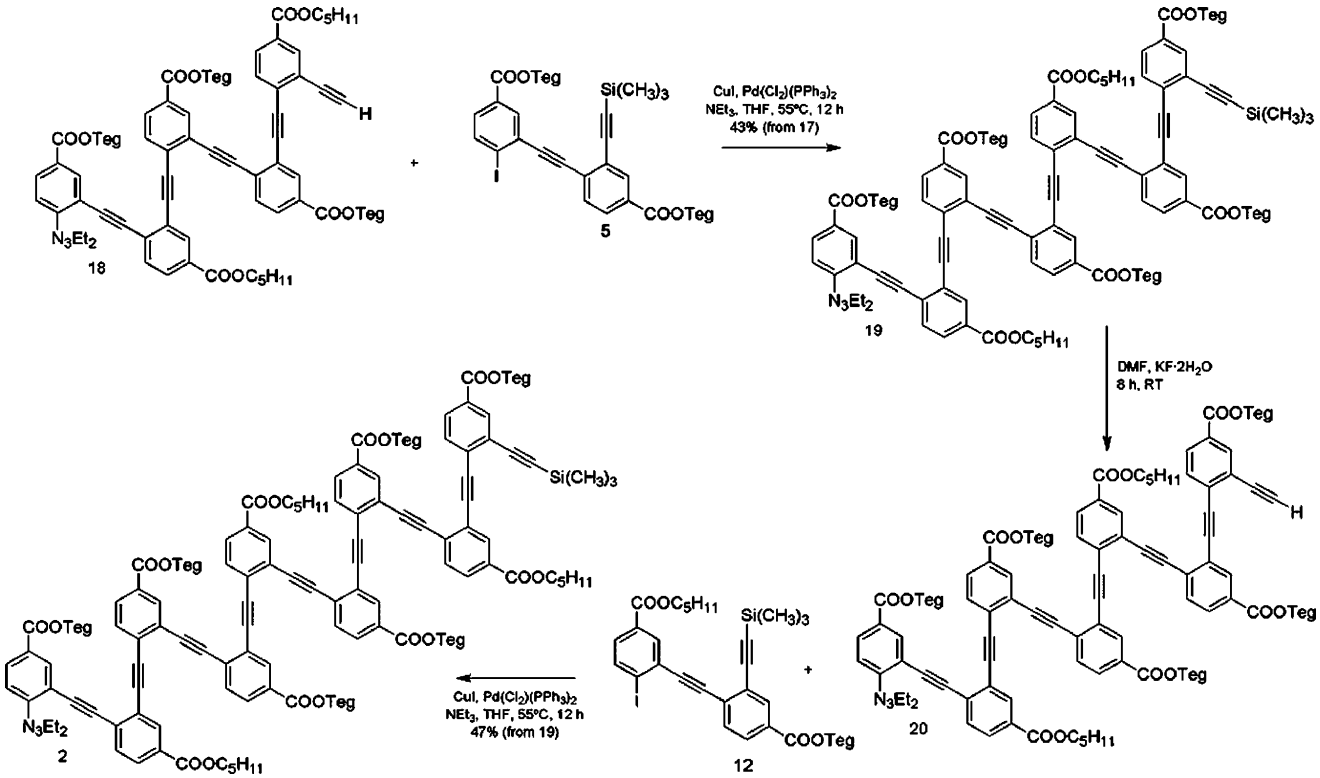


Scheme 2 Synthesis of aryl iodide-terminated dimers **12** and **14**. Previously reported dimer **5** shown as well.

One-dimensional ^{1}H NMR spectra were collected from 1.25 mM solutions of **1** and **2** in $CDCl_3$ and CD_3CN, as shown in Fig. 3, and a clear upfield shifting of the aromatic ring protons is observed upon changing the solvent from $CDCl_3$ to CD_3CN. The shift of all rings is consistent with a helical conformation in CD_3CN. Using two-dimensional correlation



Scheme 3 Synthesis of hexamer. Sequential Sonogashira coupling of **12** and **14** followed by TMS deprotection produces intermediate **18**, which is used in a final coupling reaction to yield **1**. This intermediate, **18**, is also used to synthesize **2**.



Scheme 4 Synthesis of nonamer **2** from **18** by sequential Sonagashira coupling of **5** and **12**.

spectroscopy (COSY) methods, it is relatively straightforward to connect each spin system, A, B and C, on the aromatic rings. In contrast, it is more difficult to determine the exact primary sequence by NMR; although we previously showed that heteronuclear multiple bond correlation (HMBC) could be used for absolute primary sequence assignment of *o*PE oligomers.[35] For the two oligomers reported here, we have not performed HMBC experiments, but COSY experiments were performed and Fig. 4 shows the average chemical shift of each spin system for **1** and **2** in $CDCl_3$ and CD_3CN. Globally, the upfield shift in CD_3CN is clearly observed; however since the exact primary sequence has not been determined it is difficult to track each ring from $CDCl_3$ to CD_3CN. In other words, COSY easily allows us to determine the a, b, and c protons of each ring (spin system) and thus the average chemical shift of each ring, but quantitative information on the primary sequence prevents definite assignment of the rings in the primary sequence. Therefore, we cannot say that every ring proton shifts upfield but obviously the overall trend is for substantial upfield shifts and it therefore seems reasonable to assume all rings are involved in the folded conformation. This is further supported by the fact that no ring appears to shift downfield which is the intrinsic nature of going from $CDCl_3$ to CD_3CN for the PE unit, as shown previously with model dimer and trimer oligomers which cannot fold.[34]

This helical folding of **1** and **2** enables the hydrophobic alkyl side chains to occupy one face of the folded molecule and gives the molecule an overall amphiphilic character which is commonly used in peptide design to drive self-association into a

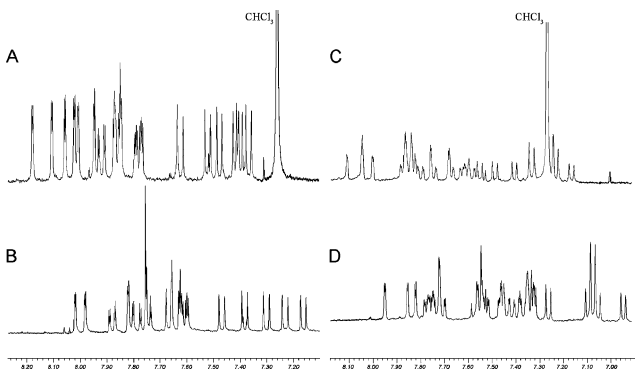


Fig. 3 ^{1}H NMR spectra showing all aromatic protons for hexamer **1** in $CDCl_3$ (A) and CD_3CN (B), and of nonamer **2** in $CDCl_3$ (C) and CD_3CN (D).

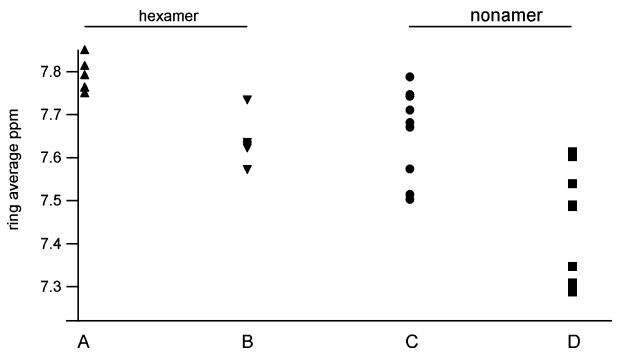


Fig. 4 Average chemical shifts for the a, b, and c protons of each aromatic ring in oligomers **1** and **2**. The average chemical shifts of the 6 rings of **1** in $CDCl_3$ (A) and in CD_3CN (B), and the average chemical shifts of the 9 rings of **2** in $CDCl_3$ (C) and CD_3CN (D). Due to overlap, not all average chemical shifts are distinctly visible in all cases.

helical bundle or other higher order structures. The chiral alkyl side chains were incorporated in order to induce a chiral bias upon helical folding which might be detectable by circular dichroism (CD) spectroscopy with the *o*PE backbone acting as a chromophore.[20] Unfortunately, significant CD signals were not observed under solvent and temperature conditions similar to those used for the NMR experiments. Even with helical *o*PEs containing chiral side chains at every position, we have never observed a significant CD signal. Another way in which higher order association might reasonably be detected would be by examination of the ^{1}H NMR signals of the alkyl chains. If they were tightly packed together, such as in the middle of a hydrophobically-collapsed structure, their signals would be expected to be weakened by an increase in relaxation time. However, although shorter signal acquisition times were tried, no weakening of these signals was observed. Therefore, by CD and NMR spectroscopy, there is no evidence for assembly of these helical foldamers into higher order structures.

Dynamic light scattering (DLS) was used in an attempt to detect aggregation due to amphiphilicity upon change of solvent. Changing solvent from $CHCl_3$ to the more polar CH_3CN would be expected to induce aggregation in order to bury the hydrophobic side chains. As the NMR measurements showed evidence of helical folding in CH_3CN, it would be reasonable to expect aggregation due to self-association of the resulting amphiphilic helix, which would appear as increased particle size. Regrettably, no evidence for increased particle size with increasing solvent polarity was found by DLS.

Although **1** and **2** displayed similar solution behavior to our previously reported homo-Teg *o*PE oligomers, their solid-phase properties were strikingly different, possibly due to their amphiphilic nature. The previously reported homo-Teg oligomers (up to hexamer) were all viscous liquids at room temperature. However, **1** and **2** are solids at room temperature and, when solvent cast onto glass slides, showed birefringence by polarized optical microscopy (POM) with a microcrystalline appearance, as shown in Fig. 5. In contrast, the homo-Teg oligomers showed no birefringence when examined by POM. Utilizing a heating stage, the birefringence of **1** and **2** disappeared between 55–60 °C and 120–130 °C, respectively. After cooling a melted slide of **1** under vacuum, larger birefringence patterns with long-range order appeared, although this was not observed with **2** which regained the microcrystalline appearance after this treatment.

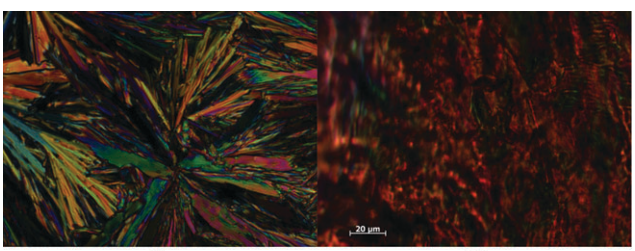


Fig. 5 Optical photograph of **1** under crossed polarizers, showing longer-range order (A). Optical photograph of **2** under crossed polarizers, showing microcrystalline birefringence.

Conclusions

The synthesis of two new amphiphilic *o*PE oligomers was reported for the first time. Despite the amphiphilic character, the oligomers still undergo folding into a helical conformation when the solvent is changed from $CDCl_3$ to CD_3CN. Although helical folding of these amphiphilic oligomers is an important milestone, they did not show any evidence by CD, NMR, or DLS that they further assembled into higher ordered structures like a helical bundle. This is most likely due to the fact that they are not water-soluble which limits the hydrophobic driving force. We are building amphiphilic oligomers with more polar side chains than Teg and expect that if they are water soluble, helical bundle-like assembly will occur. It was observed that these oligomers formed ordered solids unlike the homo-Teg derivatives, suggesting there is a fundamental difference between these new amphiphilic oligomers and their earlier analogs. It is too early to know if the molecules in the solid are helical in nature and if they are ordered into bundles. X-Ray studies should help address these questions, but they are beyond the scope of this report.

Acknowledgements

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