Intrastrand Foldamer Crosslinking by Reductive Amination

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ABSTRACT: A series of *m*-phenylene ethynylene (mPE) foldamers were crosslinked in their helical conformation using a reductive amination-based strategy. This was accomplished by placing aldehyde moieties in the backbone of the oligomer at specific residues, which allowed a diamine crosslinker to covalently link the helical loops together. Three different foldamers with crosslinks placed at different locations in the backbone were synthesized and characterized by mass spectrometry, ^{1}H NMR, and gel permeation chromatography. The effect of the crosslinking on the stability of the folded state was evaluated through solvent denaturation studies. These studies show a reduction in the oligomer's ability to unfold of up to 30% relative to an unmodified mPE oligomer of the same length in solvents that promote unfolding. © 2010 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 48: 927–935, 2010

KEYWORDS: crosslinking; oligomers; self-organization; supramolecular structures

INTRODUCTION

In biological molecules, stabilization of the secondary structure is critical for their ability to fold and function correctly.[1–6] This stabilization can come from the arrangement of nonpolar functional groups to minimize unfavorable solvent contacts and incorporation of salt bridges, hydrogen bonds, or covalent crosslinks that are most commonly found as cys-cys disulfide linkages.[7] Synthetic covalent crosslinks have also been used to stabilize the helical structure of peptides and to improve their lifetime *in vivo*.[8–10] Similarly, the flexibility and structural robustness of supramolecular host molecules can affect their molecular recognition properties and their reactive behavior.[11] Developing effective methods for controlling the reactive and structural properties of these synthetic systems has been a goal of supramolecular chemists for several decades.[12–16]

m-Phenylene ethynylene (mPE) foldamers are a class of amphiphilic oligomers that are capable of folding into a solvophobically driven helical conformation. This group and others have studied these molecules for their molecular recognition and reactive properties.[17–26] Our group has investigated one particular reactive property, known as reactive sieving,[17] in which functionalized mPE oligomers are capable of differentiating between reactive substrates based on subtle differences in the guest's structure when it is bounded in the cavity of the foldamer. Although the exact nature of this selectivity is not completely understood, we hypothesized that the flexible nature of the mPE foldamer backbone could affect its reactivity with bound guests. To more rigorously test the contribution of conformational flexibility to the observed behavior, it was necessary to develop foldamers with restricted conformational freedom.

One other approach for covalently crosslinking mPE foldamers using UV light to promote a [2 + 2] cycloaddition has been reported in the literature. However, this method results in significant decomposition of the foldamer and poor crosslinking efficiency.[27] The method was applied to polydisperse oligomers and is unlikely to be suitable for synthesizing discrete oligomers needed in reactivity or molecular recognition studies. Previously, our group used reductive amination chemistry to form two-dimensional molecular ladders with mPE oligomers.[28] Because mPE foldamers can be made using an iterative solid-phase methodology, they are amenable to rapid, modular synthesis, and precise incorporation of crosslinking sites by sequence design.[29] Using these methods, we synthesized several crosslinkable mPE foldamers and tested a reductive amination-based crosslinking strategy for its ability to restrict the unfolding of the helical conformation.

OLIGOMER DESIGN AND SYNTHESIS

Several strategies were considered with respect to the placement of the crosslink junction (illustrated in Fig. 1). A single crosslink can be placed into the backbone to lock only one loop of the helix (left) or to span several turns (right).

Additional Supporting Information may be found in the online version of this article. Correspondence to: J. S. Moore (E-mail: jsmoore@illinois.edu)
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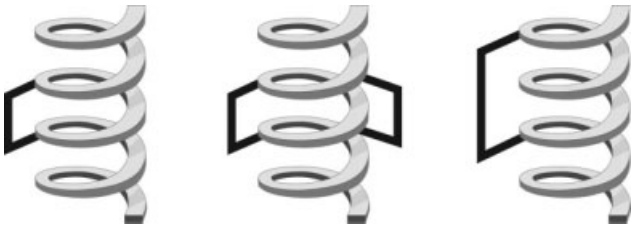


FIGURE 1 Cartoon illustrations of potential mPE foldamer crosslinking designs.

Multiple crosslinks could also be used to make the structure even more rigid (middle). To test each of these possibilities (Fig. 2), three sequences were synthesized and subjected to a reductive amination crosslinking.

Monomers containing an aldehyde moiety in place of the typical T_g-ester group were used to introduce the crosslink junction. The oligomer sequence determines the relative location of crosslinkable monomers, and these sequences are constructed efficiently using our established solid-phase methods along with a fragment coupling approach The syntheses of the aldehyde monomers and oligomers **1–3** are shown in Schemes 1 and 2, respectively. The aldehyde monomer synthesis was adapted from monomer syntheses that have been previously reported by this group.[28] Monomers **7** and **10** are used in the solid-phase synthesis.

Hexamers **11a–c** were made using solid-phase synthesis and then coupled to difunctional monomer unit **12** through Sonogashira coupling.[30] Deprotection of the aldehydes was achieved using *p*-toluenesulfonic acid in acetone to give oligomers **1–3**. The coupled products were purified by preparative-scale GPC.

CROSSLINKING OPTIMIZATION AND CHARACTERIZATION

A deprotected oligomer (**1–3**) was dissolved in solution at a concentration of ~5 μM along with 0.1 mg of scandium(III)-triflate, which acts as a catalyst for the formation of the imine. The dilute conditions were used to prevent aggregation of the foldamer, thus reducing the opportunities for interstrand coupling. A diamine was then added to the mixture and allowed to stir for 2.5 h before the concomitant imines were reduced using sodium cyanoborohydride in large excess (100 equiv). The reduction was allowed to proceed overnight at room temperature. The solvents were then removed under vacuum, and the residue was dissolved in chloroform and washed with water to remove any aqueous soluble material from the sample. The residue obtained from evaporating the chloroform fraction was analyzed by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) using the 2-(4′-hydroxyphenylazo)benzoic acid matrix.

Previous studies indicate that the pitch of an mPE oligomer is ~3.6 Å,[31] and computational models suggested that the optimal crosslinker to span one turn would be an ethane or propane diamine. Therefore, a series of diamines that met these criteria were tested under reductive amination conditions (sodium cyanoborohydride). In addition to finding the correct diamine crosslinker, a set of optimal reaction conditions for the reductive amination were also needed. A series of small-scale reactions were performed, and the crude products were analyzed by MALDI-MS. Although mass

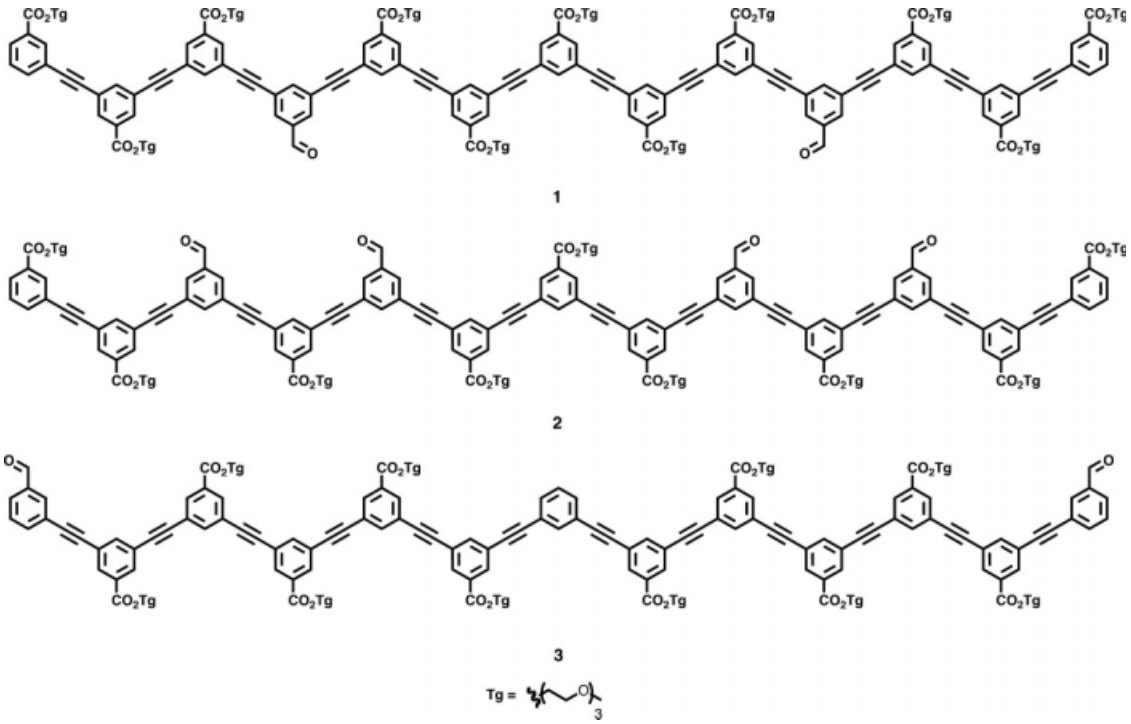
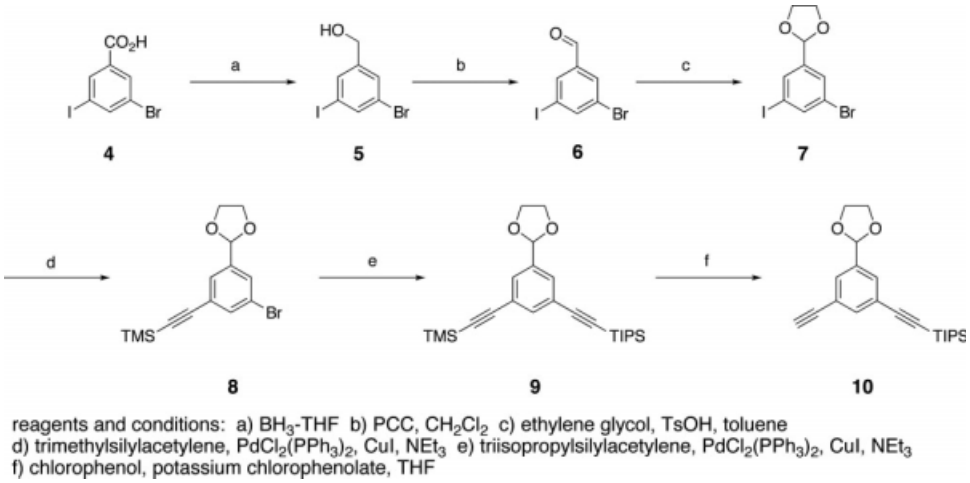


FIGURE 2 Oligomer sequences containing aldehyde functionality that can be used to test each crosslinking strategy.

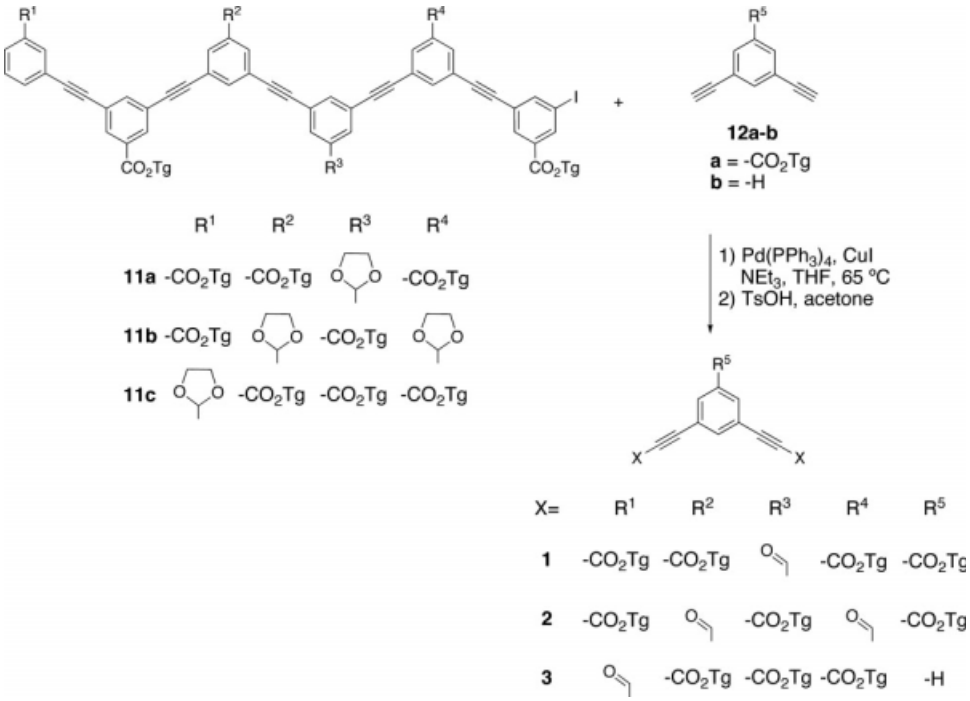


SCHEME 1 Synthesis of protected aldehyde monomers.

spectrometry is not a quantitative method for determining the absolute composition of a mixture, it was used in this case to determine whether the given reaction conditions were able to produce the crosslinked product.

Initially, 1,2-diaminoethane and 1,3-diamino-2,2-dimethylpropane were tested as crosslinkers for **1**, and the results are shown below in Figure 3.[32] A number of partially aminated but uncrosslinked products were observed using 1,2-diaminoethane, indicating that a two carbon crosslinker may be too short. Using 1,3-diamino-2,2-dimethylpropane, some uncrosslinked material was observed along with starting material, and the partially aminated product was not observed. This indicated that when the reaction conditions were not optimal, a three carbon spacer was the correct length to bridge a single turn of the helix. Comparable aromatic spacers (e.g., 1,2-diaminobenzene and 1,3-diaminobenzene) did not give any crosslinked product. Longer diamines (e.g., 1,4-butanediamine) were not tested for the single-loop crosslinking strategy because of the large deformations observed in the computational models of the folded structure.

Once we determined that 1,3-diamino-2,2-dimethylpropane was the ideal length crosslinker for a single helical turn, the reaction conditions were optimized. Because some uncrosslinked material was still visible by MS, we added chloroform (as a cosolvent that promotes the unfolded conformation of the mPE oligomers) in an attempt to relax the conformation



SCHEME 2 Synthesis of crosslinkable oligomers **1**–**3**.

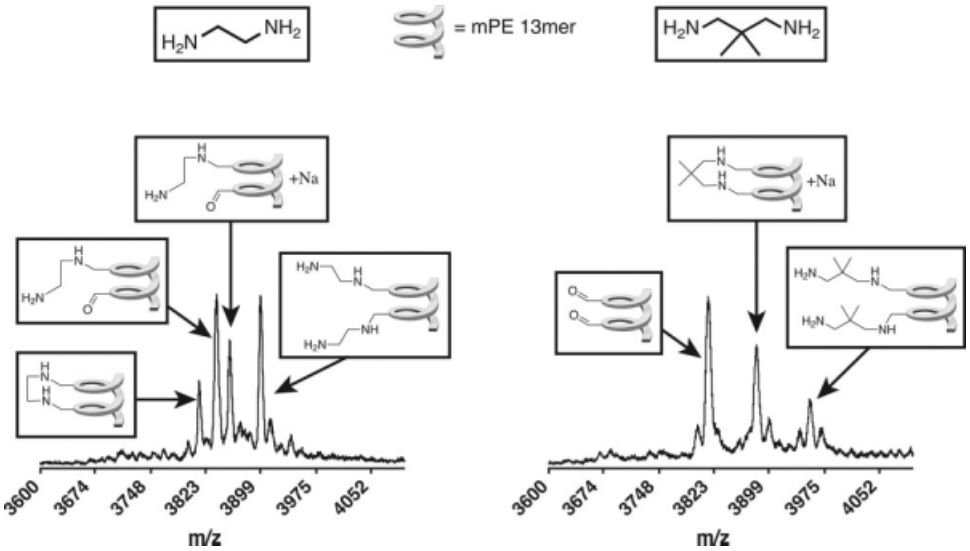


FIGURE 3 Spectra of **1** following reductive amination conditions with ethane and propane diamines.

and impart flexibility to the folded structure. The crosslinking reaction was carried out on oligomer **1** using 1,3-diamino-2,2-dimethylpropane using solvent mixtures of 50:50 and 90:10 acetonitrile/chloroform. Reactions that were performed in 100% chloroform did not give any of the crosslinked products. The resulting mass spectral data are shown below in Figure 4. The 90:10 mixture showed very little starting material and no observable amounts of partially aminated products.

Using the reaction conditions that were optimized for **1**, oligomer **2** was crosslinked at two positions. Because the aldehyde moieties on **1** and **2** are in the same relative distance, further reaction optimization was not performed. The crosslinked product was observed by mass spectrometry (Fig. 5). However, some unidentified higher molecular weight peaks were also present. The first higher molecular weight peak has a *m/z* ratio that roughly corresponds to partially crosslinked structure shown in Figure 5. The other two peaks are impurities that could not be definitively assigned.

As crosslinking two loops of the helix in oligomer **3** would require a longer diamine, 1,4-diaminobutane and 1,5-diaminopentane were tested using the optimized reaction conditions (Fig. 6). Although both diamines gave crosslinked product, the five carbon spacers appear to be better suited for crosslinking across two loops of the folded conformation.

Shown in Figure 7 are the final optimized crosslinking conditions used, along with the crosslinked oligomers (**13–15**) used to test if the crosslinked architecture offers resistance toward denaturation. Larger amounts of crosslinked foldamers were prepared using the optimized conditions and purified by preparative gel permeation chromatography (GPC). Further characterization of the crosslinked oligomers **13–15** was completed using UV–vis spectrophotometry and GPC. GPC was used to qualitatively determine the presence of crosslinked material, and UV–vis was used to quantitatively determine the ability of the crosslinker to reduce the unfolding capability of the oligomer.

Solvent denaturation titrations have been used previously to characterize the stability of the folded state of mPE oligomers.[21,33] Hence, these experiments were used to determine the extent of unfolding of crosslinked oligomers compared with uncrosslinked ones. By varying the polarity of the solvent using different ratios of acetonitrile (folding solvent) to chloroform (denaturing solvent), the helix transition of the

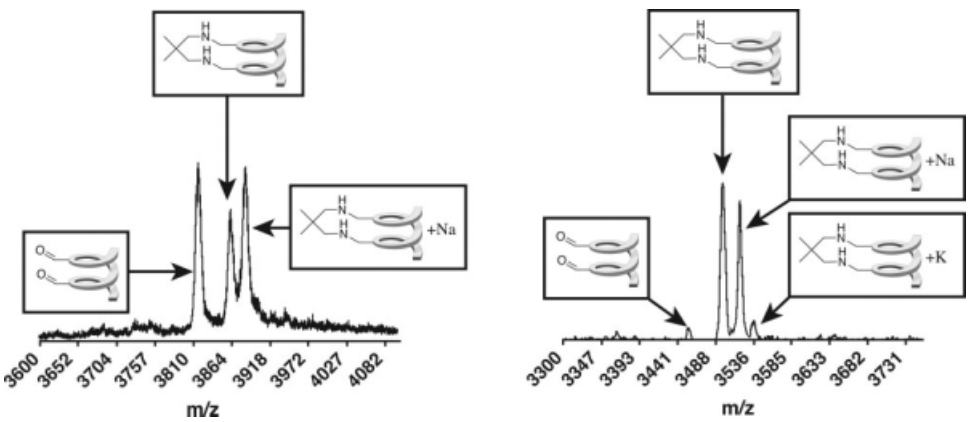


FIGURE 4 MALDI MS spectra of the crosslinked product for reactions conducted in 50:50 acetonitrile/chloroform (left) and 90:10 acetonitrile/chloroform (right).

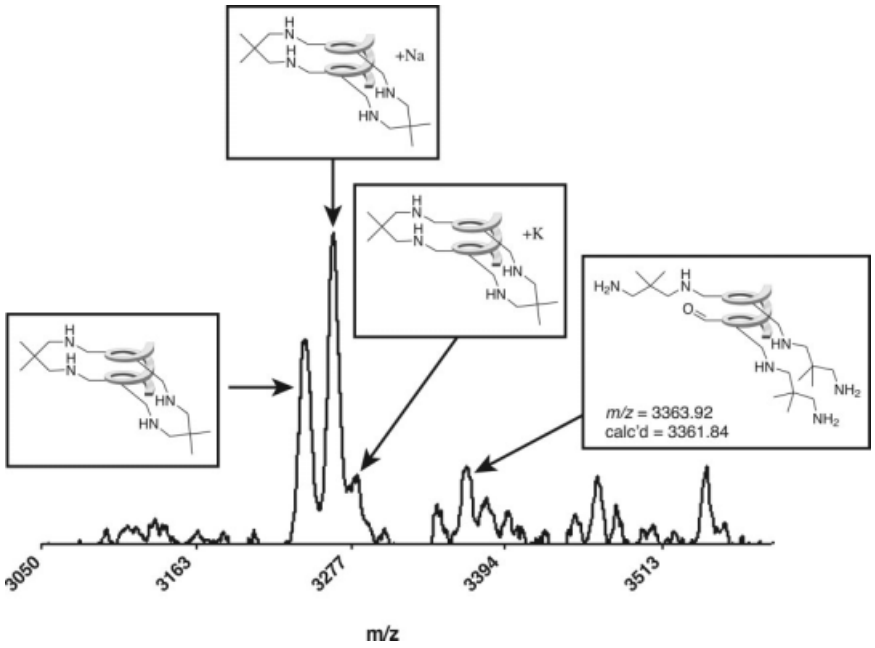


FIGURE 5 MALDI-MS chromatogram of doubly crosslinked products of oligomer **2** using 1,3-diamino-2,2-dimethylpropane.

oligomer can be monitored. The ratio of absorbance at 305–289 nm represents the *cisoid* versus *transoid* conformational equilibrium (Fig. 8). A larger absorbance ratio corresponds to the unfolded (or *transoid*) conformation.

Shown in Figure 9 are the folding titrations for uncrosslinked oligomer **1** and crosslinked oligomers **13**–**15**. A small shoulder begins to appear in the UV spectrum as the concentration of chloroform increases. This is representative of the shift in conformation from predominantly *cisoid* to *transoid.*

GPC analysis of both the crosslinked and uncrosslinked material was performed. Each chromatogram shows the uncrosslinked starting material with its corresponding crosslinked analog (Fig. 10). Crosslinking of the foldamer should affect the radius of gyration of the molecule, resulting in a shift in the retention time, which can be seen in the chromatograms in Figure 10. This shift is discussed in further detail in the discussion section.

DISCUSSION

The most useful method for quantifying the crosslinker's ability to maintain the folded conformation is solvent denaturation. Formation of a shoulder in the spectrum with increasing concentration of chloroform is indicative of oligomer unfolding. Upon visual analysis of the raw UV spectra for the denaturation studies, crosslinked oligomers **13**–**15** do appear to unfold somewhat, but the shoulder

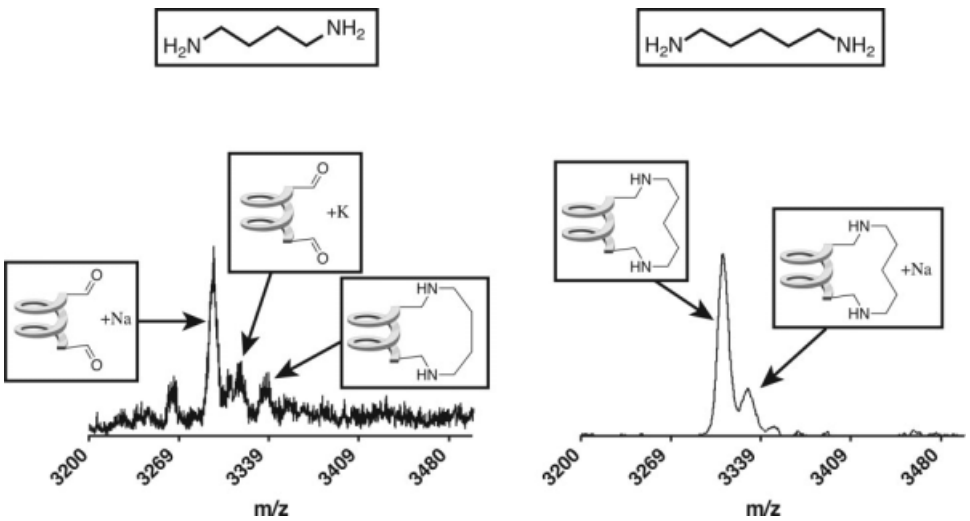


FIGURE 6 MALDI-MS spectrum of oligomer **3** crosslinked with 1,4-diaminobutane (left) and 1,5-diaminopentane (right).

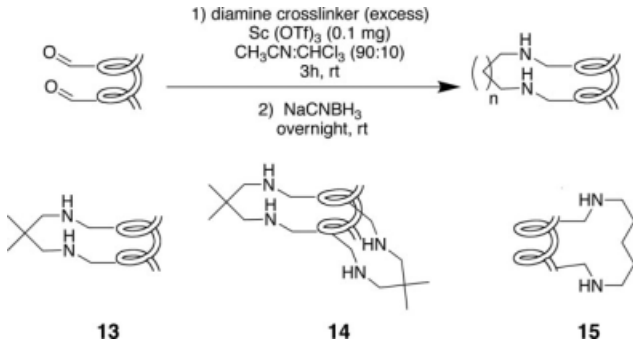


FIGURE 7 Optimized reaction conditions for crosslinking and the crosslinked products (**13**–**15**) of oligomers **1**–**3** that were used in further analysis.

formation is not as sharp compared with uncrosslinked oligomer **1**. To further quantify this interaction, the relative fraction of oligomer that is unfolded can be calculated using eq 1 based on the A_{305}/A_{289} ratio from the UV spectrum. This analysis assumes that the samples consist of pure, crosslinked oligomers. A_F is the A_{305}/A_{289} ratio for oligomer **1** in 100% acetonitrile, A_U is the ratio in 100% chloroform, and A is the A_{305}/A_{289} ratio of the oligomer being examined at the given concentration of chloroform in acetonitrile. The results of this study for oligomer **1** and **13**–**15** are plotted in Figure 11.

$$f_u = \frac{A_F - A}{A_F - A_U}. \quad (1)$$

In comparison with the uncrosslinked oligomer **1**, the singly crosslinked foldamers **13** and **15** appear to restrict the oligomers ability to unfold by ~30%. Surprisingly, crosslinking the oligomer at its ends (**15**) rather than in the middle did not provide a significant advantage in terms of reducing the unfolding capability of the mPE foldamer. It is possible that both of these oligomers have similar, partially unfolded structural conformations in chloroform that cannot be distinguished by UV–vis.

However, doubly crosslinked oligomer **14** has a much different denaturation profile than the other two foldamers. It does not undergo the expected sigmoidal unfolding process that is typically seen and appears to be more unfolded than the other oligomers in 100% acetonitrile. It is possible that the extra crosslink could be destabilizing the folded conformation. However, because this sample could not be completely purified because of unknown high-molecular-weight oligomers, we are unable to draw conclusions about this.

GPC can provide a unique method of characterizing the extent of crosslinking. As mentioned previously, the GPC traces were collected using THF as a mobile phase, which does not promote the folded conformation in mPE oligomers. Because crosslinked foldamers would not have the same radius of gyration, when unfolded as their uncrosslinked analogs they would appear to be smaller in molecular weight and therefore have a longer retention time. This behavior is in fact observed with **13** and **15**. Oligomer **13** appears to have a large amount of uncrosslinked material that overlaps with starting material **1**, followed by a shoulder of what appears to be material of lower molecular weight, which is likely the fully crosslinked foldamer **13**. The large shoulder overlapping with **1** indicates that the extent of crosslinking in this case appears to be relatively poor. This is confirmed by the presence of some residual benzyl aldehyde proton peak in the ^{1}H NMR for all of the crosslinked oligomers (see Experimental Section in Supporting Information). Oligomer **3** appears to have undergone a more efficient crosslinking reaction to give **15**, as the smaller molecular weight peak appears to be larger than the preceding shoulder that corresponds to uncrosslinked material. The GPC analysis of **14** shows a broad, relatively featureless peak adding further evidence that a combination of products, varying in their degree of crosslinking, were formed by the reductive amination reaction. Unfortunately, we were unable to separate the uncrosslinked material from the fully crosslinked using preparative GPC. As these peaks could not be resolved chromatographically, the degree of crosslinking could not be quantified. Although this reaction was performed under high dilution conditions, larger molecular weight products still formed as evidenced by the short, broad peaks with shorter retention times than the main peaks, which are likely interstrand coupling products. Unfortunately, these products could not be definitively identified, as they were not observed by MALDI-MS. These higher molecular weight peaks likely contribute to the lower isolated yields of the purified oligomers, which ranged from ~20–35% (See Supporting Information for specific crosslinking reaction yields).

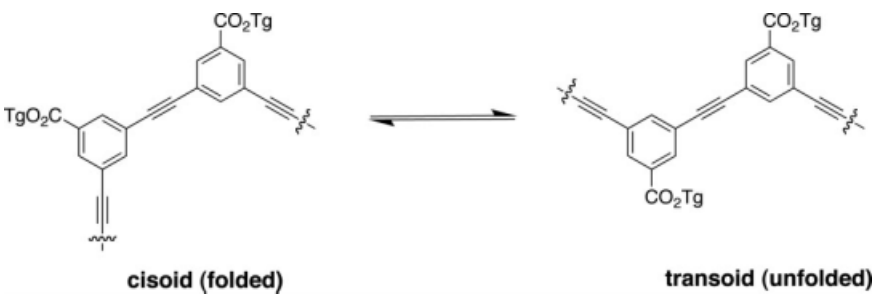


FIGURE 8 *Transoid* versus *cisoid* structures corresponding to the unfolded and folded conformations of the mPE oligomer backbone.

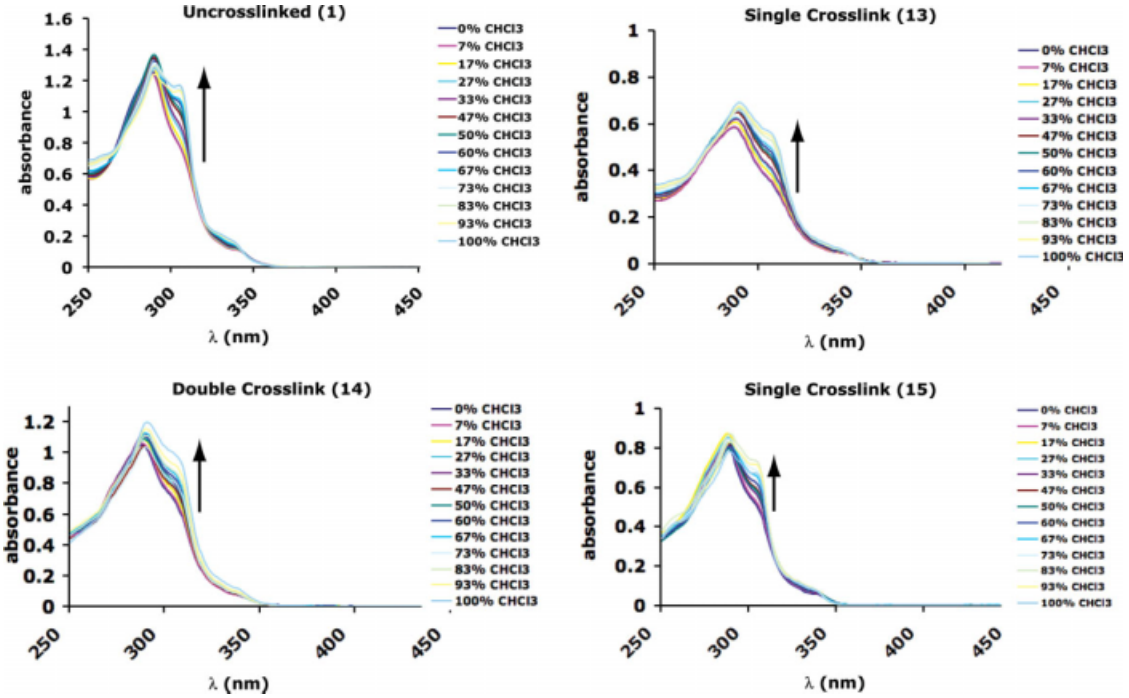


FIGURE 9 UV–vis solvent denaturation titrations for uncrosslinked oligomer **1** and crosslinked oligomers **13**–**15**.

These impurities could be removed to some degree, although not completely, using preparative GPC.

Based on these data, crosslinking an oligomer with two loops (oligomer **15**) reduces the unfolding to the same degree as crosslinking a single loop (**13**). This behavior is also observed for the crosslinking of helical peptides. Verdine and coworkers found that the yield of the crosslinking reaction was highest when the peptide was restricted across the i and $i + 7$ positions.[9] This orientation is analogous to the placement of aldehyde groups in the backbone of oligomer **15**, which are at the i and $i + 13$ positions.

CONCLUSIONS

In conclusion, we have demonstrated a reductive amination strategy for crosslinking the backbone of mPE oligomers. Based on the model studies presented here, it appears that

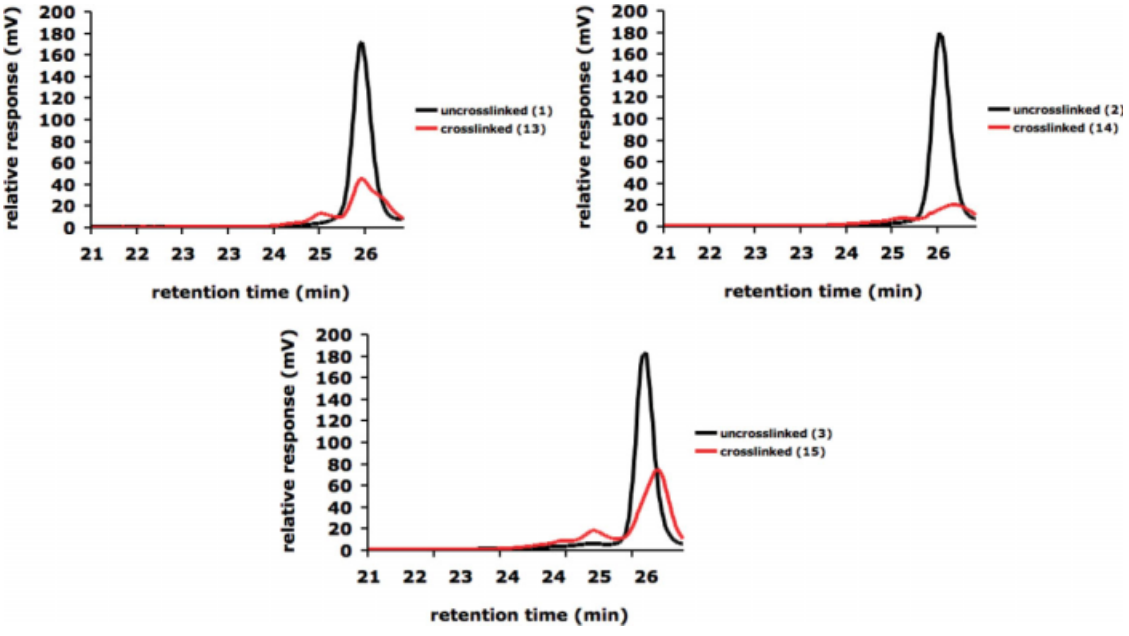


FIGURE 10 GPC comparison of mPE foldamers **1**–**3** and their crosslinked analogs **13**–**15**.

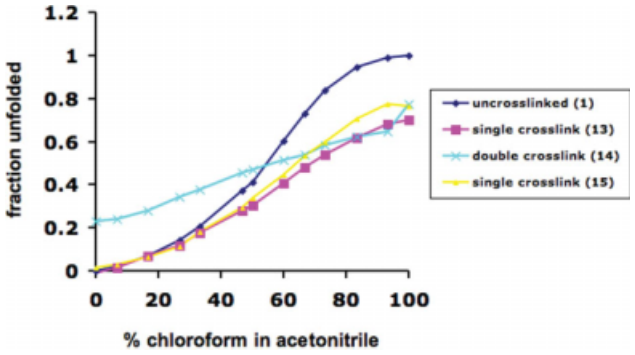


FIGURE 11 Solvent denaturation of uncrosslinked oligomer **1** and crosslinked oligomers **13**–**15**. The fraction of oligomer that is unfolded is relative to uncrosslinked oligomer **1**. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the efficiency of the crosslinking reaction depends not only on the length of the diamine tether but also on the placement of the crosslink points (i.e., benzyl aldehyde moieties) in the oligomer sequence. Placing a single crosslink in the backbone across one turn of the helix provides some helix stability, but results in a low degree of crosslinking. Adding an additional staple using this strategy is generally ineffective both in terms of conversion and rigidifying the folded structure. The most effective crosslinking strategy, in terms of both conversion to product and restricting the unfolded conformation, is to staple the foldamer across two turns of the helix. This behavior has been observed previously in peptide crosslinking studies,[8,9] and it is interesting that it appears to be conserved here using an entirely nonbiological system.

Future applications of this methodology can include the synthesis of foldamers that are incapable of unfolding entirely where the folded conformation would remain persistent in solvents that are known to promote the random coil conformation (i.e., chloroform and methylene chloride). This would also provide an opportunity to lock in stereochemistry to an mPE foldamer biased with an excess of either its *M* or *P* twist sense, creating a supramolecular host with a helically chiral binding site. Chiral bias can be induced through the use of either a chiral guest molecule or incorporation of chiral elements into the foldamer structure itself.[20,23,34,35] Additionally, foldamers with reduced backbone flexibility could be used to systematically explore the effects of host flexibility on their reactivity with guest molecules.

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30 Since the T_g-ester sidechain that is attached to the monomer in the adjacent loop would obstruct the ability of the diamine crosslinker to reach to the aldehyde handle, it was deleted from the sequence by using monomer **12b**.

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32 During the initial studies, oligomer **1** contained trimethylsilyl acetylene end groups as an artifact of the solid-phase synthesis (see Ref. 29), resulting in the different *m/z* ratios observed in Figure 3 in comparison with the crosslinked oligomer used in the more detailed studies later on. These were later deleted from the sequence for convenience. These end groups do not have a measurable effect on the folding capability of the oligomer and their absence or presence does not affect the crosslinking reaction.

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