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Spin System Assignment of Homo-*o*-Phenylene Ethynylene Oligomers

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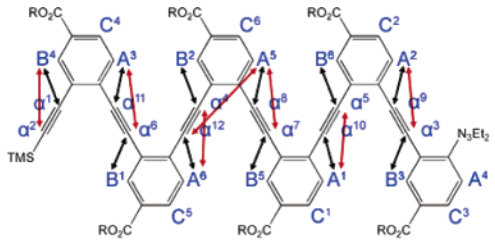

We previously reported the synthesis and solution characterization of short *o*-phenylene ethynylene (oPE) foldamers. Proton correlation techniques are not adequate for NMR assignment in these compounds as the ethynylene linkers interrupt proton connectivity. In order to facilitate structural characterization and more fully harness the power of NMR, it is necessary to know the sequence of spin systems along the molecular backbone. For example, spin system assignment is required to unambiguously assign NOE correlations for structural determination of folded forms in solution. Therefore, we developed a method to assign the aromatic spin systems in these compounds using HMBC experiments. This has been performed for tetrameric (Es_4), pentameric (Es_5), and hexameric (Es_6) oligomers and is expected to prove useful for this class of foldamers in general. The proton assignments obtained by this technique have been useful toward confirming the previous hypotheses of helical folding in oPE systems.

Introduction

Abiotic molecules designed to fold into a desired conformation in solution, known as foldamers, have been the subject of intense study in recent years.[1−4] A primary motivation for this research is to examine simple motifs that mimic the folded structures of biological macromolecules in order to decipher fundamental requirements leading to well-defined conformations in solution. Oligomeric and polymeric *m*-phenylene ethynylene (PE) foldamers have been widely synthesized and characterized by many experimental[5−19] and computational[20−24] methods. In contrast, the regioisomeric *o*-phenylene ethynylene (oPE) backbone has been studied in far less detail.[5,25−27] We recently reported a series of short oPE oligomers that showed evidence of helical folding by NMR measurements of chemical shift and NOE interactions for the first time.[27]

(1) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893−4011.
(2) Cheng, R. P. *Curr. Opin. Struct. Biol.* **2004**, *14*, 512−520.
(3) Huc, I. *Eur. J. Org. Chem.* **2004**, 17−29.
(4) Schmuck, C. *Angew. Chem., Int. Ed.* **2003**, *42*, 2448−2452.
(5) Khan, A.; Hecht, S. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 1619−1627.
(6) Gin, M. S.; Yokozawa, T.; Prince, R. B.; Moore, J. S. *J. Am. Chem. Soc.* **1999**, *121*, 2643−2644.
(7) Hecht, S.; Khan, A. *Angew. Chem., Int. Ed.* **2003**, *42*, 6021−6024.
(8) Khan, A.; Hecht, S. *Synth. Met.* **2004**, *147*, 37−42.
(9) Lahiri, S.; Thompson, J. L.; Moore, J. S. *J. Am. Chem. Soc.* **2000**, *122*, 11315−11319.
(10) Masu, H.; Sakai, M.; Kishikawa, K.; Yamamoto, M.; Yamaguchi, K.; Kohmoto, S. *J. Org. Chem.* **2005**, *70*, 1423−1431.
(11) Matsuda, K.; Stone, M. T.; Moore, J. S. *J. Am. Chem. Soc.* **2002**, *124*, 11836−11837.
(12) Prest, P. J.; Prince, R. B.; Moore, J. S. *J. Am. Chem. Soc.* **1999**, *121*, 5933−5939.
(13) Ray, C. R.; Moore, J. S. *Adv. Polym. Sci.* **2005**, *177*, 91−149.
(14) Arnt, L.; Tew, G. N. *Macromolecules* **2004**, *37*, 1283−1288.
(15) Breitenkamp, R. B.; Arnt, L.; Tew, G. N. *Polym. Adv. Technol.* **2005**, *16*, 189−194.
(16) Kim, T.; Arnt, L.; Atkins, E.; Tew, G. N. *Chem.*—Eur. J. **2006**, *12*, 2423−2427.
(17) Arnt, L.; Tew, G. N. *Langmuir* **2003**, *19*, 2404−2408.
(18) Arnt, L.; Rennie, J. R.; Linser, S.; Willumeit, R.; Tew, G. N. *J. Phys. Chem. B* **2006**, *110*, 3527−3532. .
(19) Arnt, L.; Tew, G. N. *J. Am. Chem. Soc.* **2002**, *124*, 7664−7665.
(20) Panda, M.; Chandrasekhar, J. *J. Am. Chem. Soc.* **1998**, *120*, 13517−13518.
(21) Adisa, B.; Bruce, D. A. *J. Phys. Chem. B* **2005**, *109*, 7548−7556.
(22) Adisa, B.; Bruce, D. A. *J. Phys. Chem. B* **2005**, *109*, 19952−19959.
(23) Elmer, S. P.; Pande, V. S. *J. Chem. Phys.* **2004**, *121*, 12760−12771.

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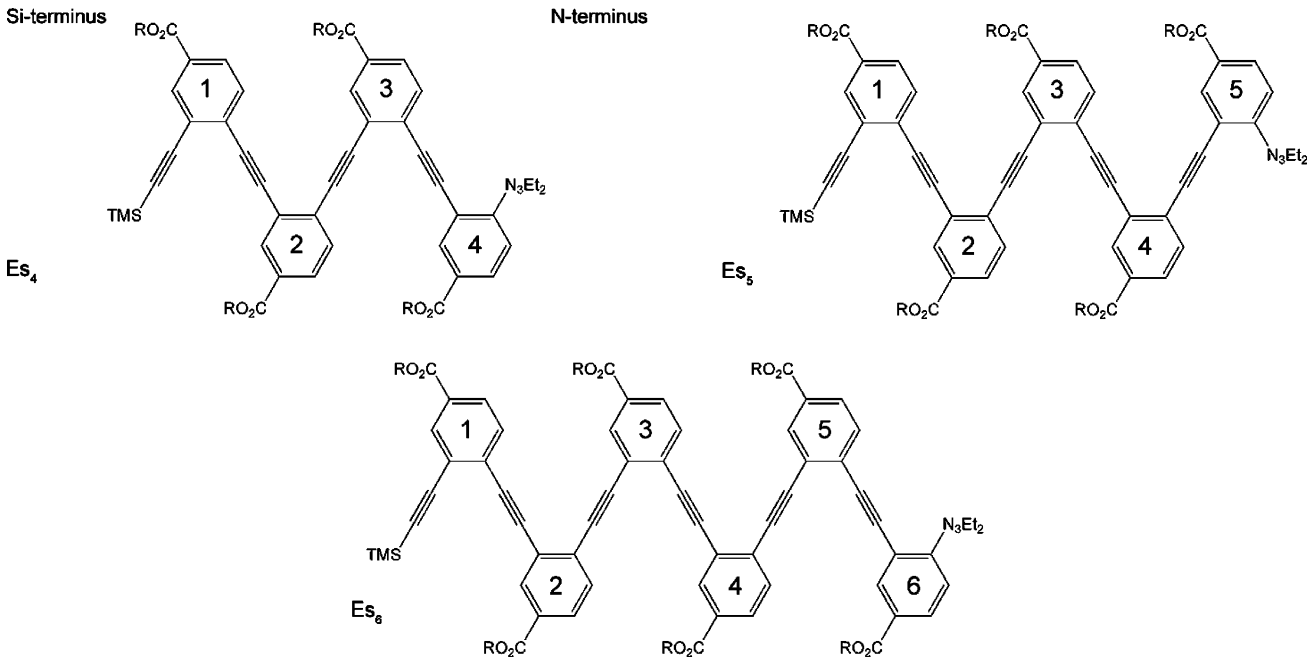

FIGURE 1. Structures of Es_4, Es_5, and Es_6 oligomers. The rings are labeled consecutively starting from the Si terminus. R = $(CH_2CH_2O)_3CH_3$.

In order to fully interpret NMR measurements of these oPE oligomers, a means of accurately assigning the proton resonances and spin systems along the aromatic backbone is necessary as these backbones consist of identical aromatic units, each with

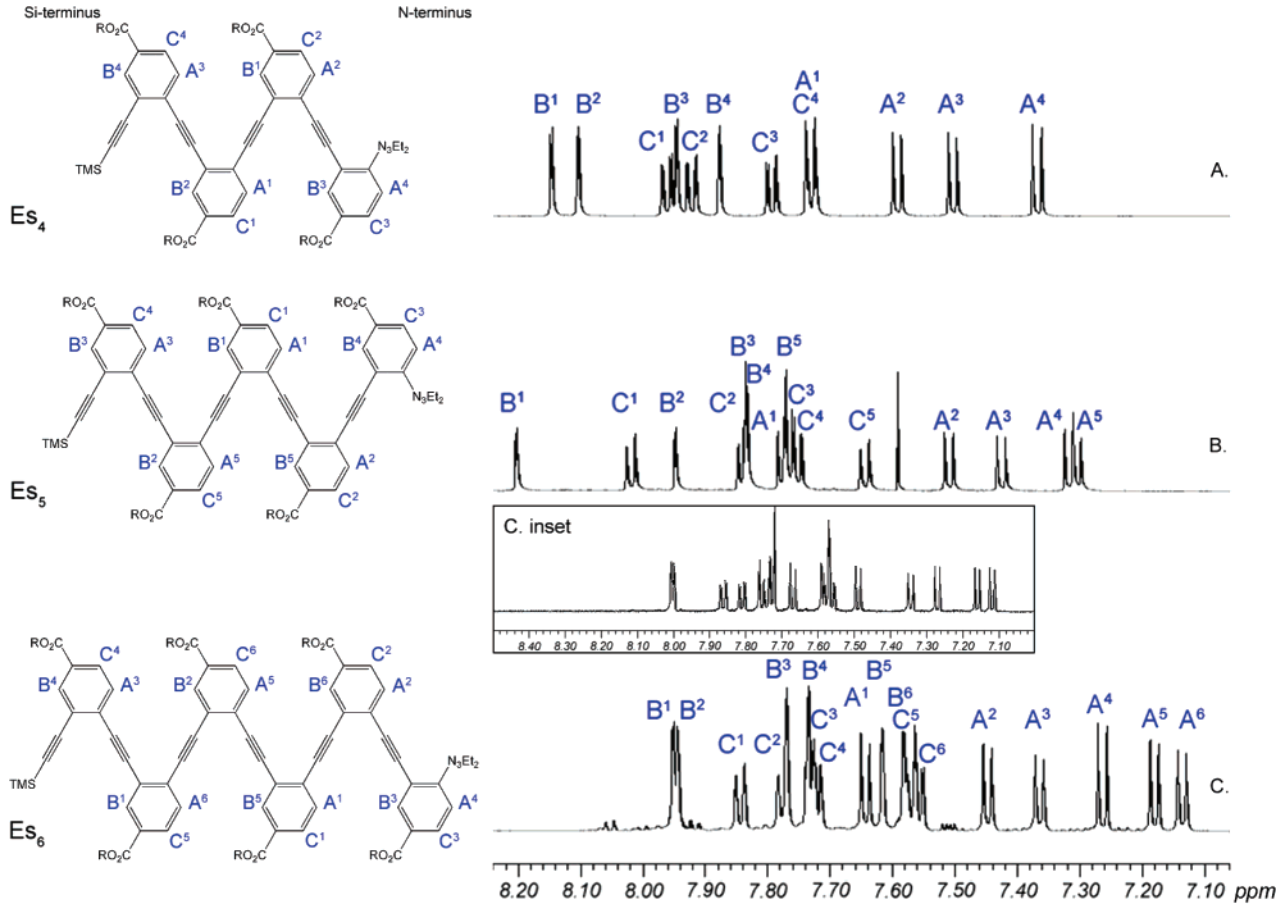

FIGURE 2. (Left) Structures Es_4, Es_5, and Es_6, with final ^1H assignments. (Right) Aromatic ^1H regions of Es_4, Es_5, and Es_6, labeled in order of chemical shift. (Inset) Spectrum of Es_6 at 1.25 mM, lacking the small peaks visible in the more concentrated spectrum which are attributed to aggregation. When the sample is diluted, the peaks shift downfield, further supporting aggregation. Letter labels indicate splitting pattern of each proton—A denotes a wide (≈8.4 Hz) doublet, B denotes a narrow (≈2.5 Hz) doublet, and C denotes a doublet of doublets ($J_1 \approx 2.5$ Hz, $J_2 \approx 8.4$ Hz). Splitting patterns unambiguously identify ring position of each proton.

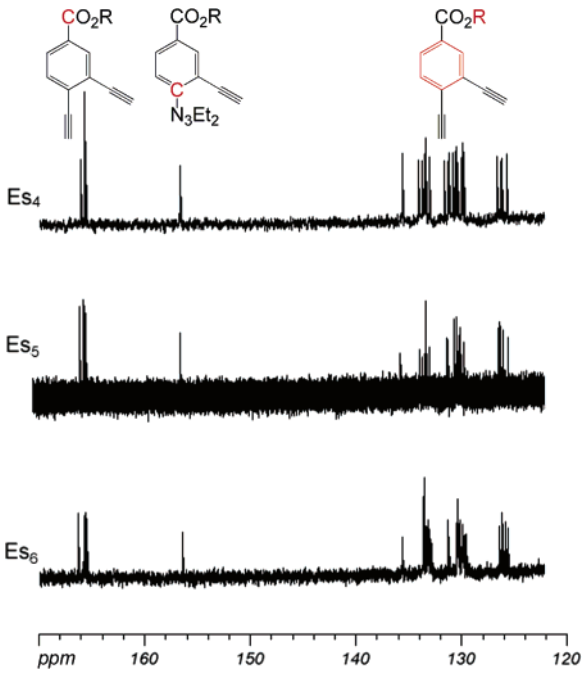

FIGURE 3. Ester, side chain, and aromatic ^{13}C signals for Es_4, Es_5, and Es_6 oligomers. Red carbon atoms indicate the type of peak(s) in each region.

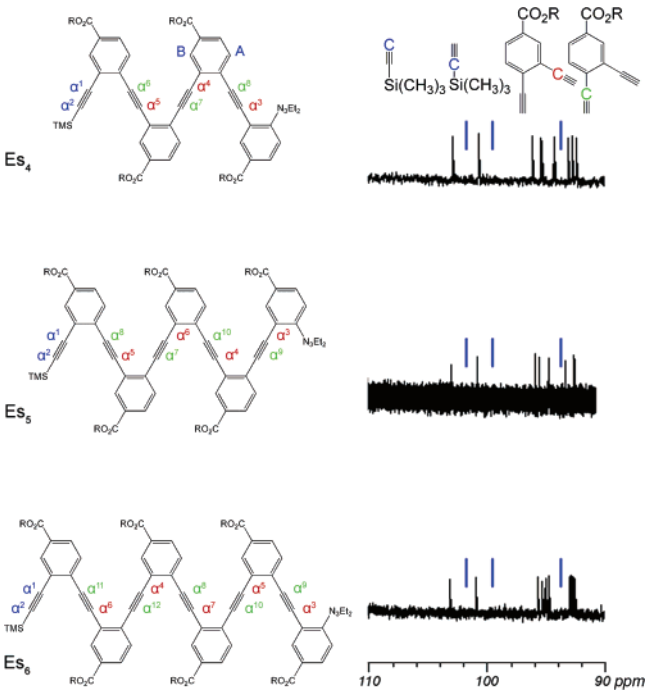

FIGURE 4. Assignment of all ^{13}C acetylene signals in Es_4, Es_5, and Es_6 oligomers, labeled in order of chemical shift. Each acetylenic carbon is referred to either as β to proton B or β to proton A as shown on the structure of Es_4. The internal acetylene carbons, for example, α^3 to α^8 in Es_4, separate into two regions with those β to proton B between 97 and 94 ppm and those β to proton A between 94 and 92 ppm.

protons of similar chemical shift. This decoding of the primary sequence along the backbone is required to more fully harness the power of NMR. For example, through-space NOE correlations become extremely powerful when they can be assigned to specific protons on the molecular backbone. This was demonstrated earlier on tetrameric oPE foldamers.[27] However, as the oligomers become larger, distinguishing the aromatic protons in the molecular backbone becomes more challenging. Classical methods of assigning the primary sequence of peptides and proteins via local NOE interactions between amide and alpha protons[28] have been adapted to peptidomimetic and related foldamers.[29,30] However, these methods do not suffice for foldamers with backbones containing a series of tertiary carbon atoms. In the case of oPE oligomers, the aromatic spin systems are spaced too far apart to be linked by sequential NOE interactions. Heteronuclear multiple bond correlation (HMBC) spectroscopy may be used to correlate ^1H resonances to ^{13}C resonances that are separated by multiple bonds and was expected to allow assignment of oPE foldamer backbones.

Recent work demonstrated the use of HMBC to assign backbone protons in quinoline and pyridine-derived oligomers.[31] These foldamers are also composed of aromatic backbones with many tertiary carbon atoms, and although connected by amide linkages, standard NOE connectivity methods could not be used. HMBC methods, using the amide linkage to bridge spin systems, allowed precise assignment of the aromatic rings along the backbone. In the oPE oligomers described here, it was unclear if HMBC methods would be able to bridge the longer couplings across the carbon−carbon triple bonds. A few examples are documented in the literature where HMBC couplings spanning five or even six bonds,[32] including across carbon−carbon triple bonds,[33,34] have been used for structural assignment. For oPE, HMBC couplings across a minimum of four bonds would be required to establish spin system connectivity leading to determination of the primary sequence. Therefore, it seemed reasonable that HMBC methods would prove useful.

The ester-substituted oPE oligomers under investigation in this report are shown in Figure 1, and the rings are labeled consecutively starting from the Si terminus. The tetramer, denoted as Es_4, has previously shown evidence of a helical structure in solution by chemical shift measurements, which indicated ring stacking, and ROESY (rotating-frame Overhauser effect spectroscopy) interactions between the terminal N_3Et_2 and $Si(CH_3)_3$ groups.[27] Reasonable assignments of primary sequence could be made for Es_4 based on ^1H chemical shifts of model compounds and monomeric, dimeric, and trimeric precursors. However, this method of assignment becomes difficult or impossible with increasing oligomer length. This report confirms our assignments for Es_4 and provides the full assignment of the aromatic protons for Es_5 and Es_6. Assignments of the

(24) Lee, O. S.; Saven, J. G. *J. Phys. Chem. B* **2004**, *108*, 11988−11994.
(25) Blatchly, R. A.; Tew, G. N. *J. Org. Chem.* **2003**, *68*, 8780−8785.
(26) Jones, T. V.; Blatchly, R. A.; Tew, G. N. *Org. Lett.* **2003**, *5*, 3297−3299.
(27) Jones, T. V.; Slutsky, M. M.; Laos, R.; de Greef, T. F. A.; Tew, G. N. *J. Am. Chem. Soc.* **2005**, *127*, 17235−17240.
(28) Wuthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.
(29) Seebach, D.; Hook, D. F.; Glattli, A. *Biopolymers* **2006**, *84*, 23−37.
(30) Seebach, D.; Mathad, R. I.; Kimmerlin, T.; Mahajan, Y. R.; Bindschadler, P.; Rueping, M.; Jaun, B.; Hilty, C.; Etezady-Esfarjani, T. *Helv. Chim. Acta* **2005**, *88*, 1969−1982.
(31) Dolain, C.; Grelard, A.; Laguerre, M.; Jiang, H.; Maurizot, V.; Huc, I. *Chem.*—Eur. J. **2005**, *11*, 6135−6144.
(32) Araya-Maturana, R.; Delgado-Castro, T.; Cardona, W.; Weiss-Lopez, B. E. *Curr. Org. Chem.* **2001**, *5*, 253−263.
(33) Cavin, A.; Potterat, O.; Wolfender, J. L.; Hostettmann, K.; Dyatmyko, W. *J. Nat. Prod.* **1998**, *61*, 1497−1501.
(34) Zgoda, J. R.; Freyer, A. J.; Killmer, L. B.; Porter, J. R. *J. Nat. Prod.* **2001**, *64*, 1348−1349.

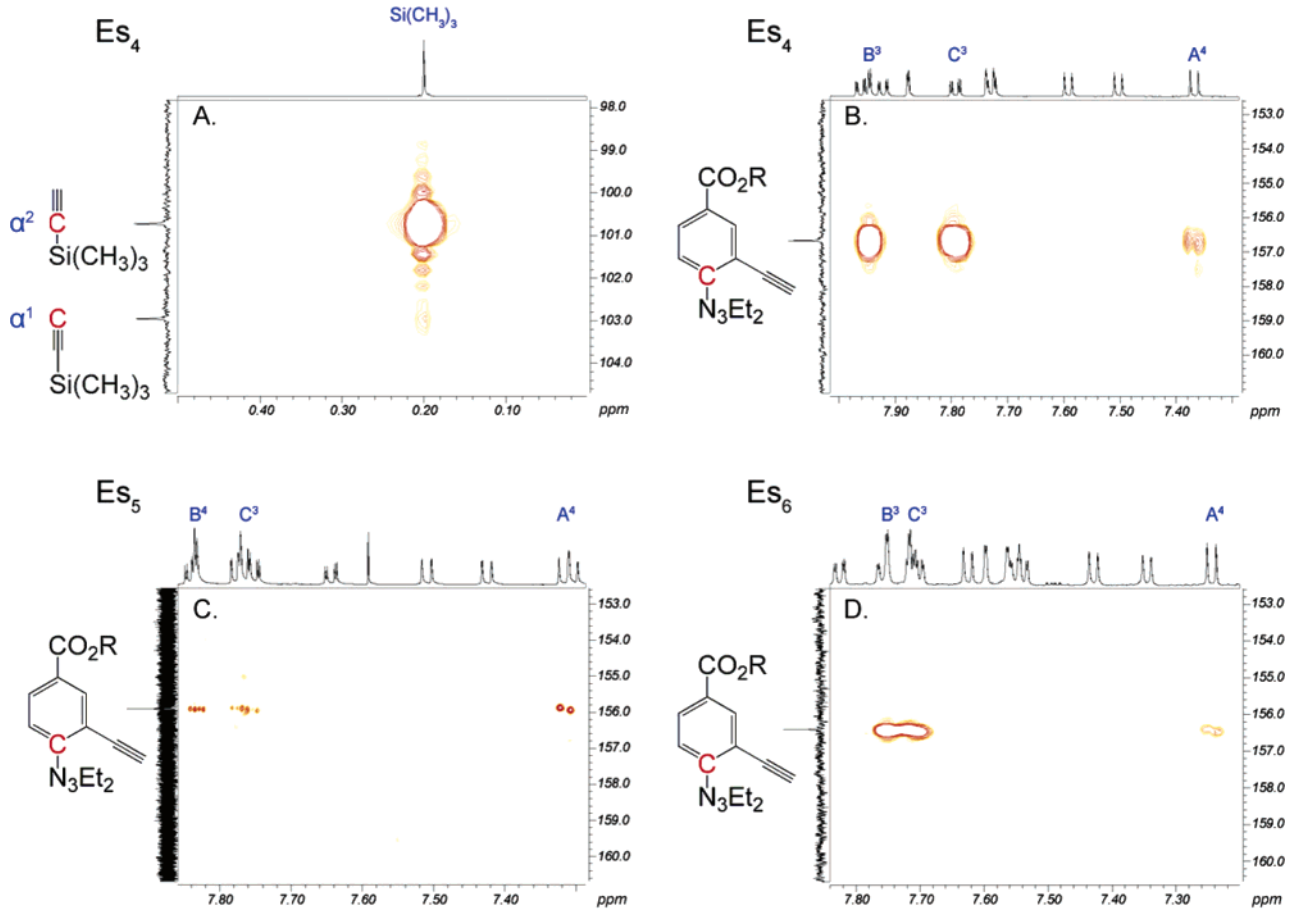

FIGURE 5. Assignment of TMS and N_3Et_2 termini for Es_4, Es_5, and Es_6. (A) HMBC interaction between the $Si(CH_3)_3$ protons and neighboring acetylene carbons in Es_4—similar peaks are observed in Es_5 and Es_6 as well. (B, C, and D) HMBC correlations between the aromatic C−N_3Et_2 carbon and nearby ring protons in Es_4, Es_5, and Es_6, respectively.

aromatic ^1H protons in Es_4, Es_5, and Es_6 oligomers were performed by a combination of 1D, COSY, and HMBC measurements. Splitting patterns of each 1D signal were used to identify ring position, COSY measurements were taken to identify spin systems, and in order to assign the primary sequence of spin systems, HMBC measurements were taken. Shorter-range HMBC couplings were used to assign the ^{13}C signals for acetylenic carbons adjacent to each ring, and afterward, longer range HMBC couplings across the carbon−carbon triple bonds were used to place the rings in order.

Results and Discussion

The aromatic ^1H signals from 1D ^1H NMR were labeled first according to their splitting pattern on the ring as A, B, or C. This assignment is consistent with our previous nomenclature and retained here for clarity. Next, the individual signals in the spectra were numbered according to their chemical shift order as shown in Figure 2. This follows conventional assignment procedures and is not associated with ring numbers along the backbone, as can be seen with Es_4 in which the Si-terminal ring contains A^3, B^4, and C^4. On examining Figure 2c some smaller peaks are observed in the aromatic region of the Es_6 sample which are due to aggregation at high concentration and not an impurity, as the spectrum of a more dilute Es_6 sample is shown in the inset, and HPLC analysis showed good (>95%) purity. As the time required for COSY and HMBC acquisition at a given resolution and S/N is inversely proportional to the square of the concentration and these additional peaks were not large enough to interfere with structural assignment, this concentrated sample was used for data collection. Assignment of A, B, and C to individual spin systems or aromatic rings was achieved using COSY. In the case of Es_6, COSY data was confirmed by the observation of HMBC correlations between ester carbons and aromatic ring protons. This enabled each set of A, B, and C protons, connected to an individual ring, to be grouped together, although it did not provide any sequence order along the backbone.

^{13}C signals, shown in Figures 3 and 4, could be identified as ester, aromatic, or acetylenic by chemical shift, and each type is highlighted by color in the chemical structure. Signals from the two end groups had unique chemical shifts that aided in structure determination. The aromatic carbon connected to the N_3Et_2 group at the N terminus has a chemical shift at roughly 156 ppm which falls between that of the ester carbons and the other aromatic carbons, as shown in Figure 3, while the Si-$(CH_3)_3$ carbons at the Si terminus of the oligomer provide a strong signal close to 0 ppm.

The acetylenic carbons between 105 and 90 ppm were labeled according to chemical shift order, from α^1−α^8, α^1−α^{10}, or α^1−α^{12} in Es_4, Es_5, and Es_6 respectively, as shown in Figure 4. Within the acetylenic carbon region, there appears to be three groups of signals, the two unique carbons located on the terminal acetylene (shown in blue) and then two regions with several

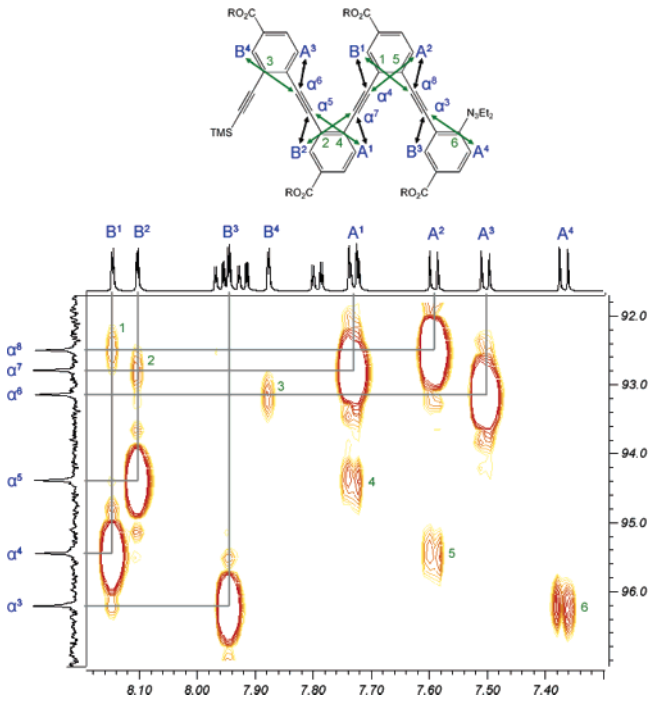

FIGURE 6. Short (crossing two carbon−carbon bonds) HMBC interactions between A and B protons of Es_4 and acetylene carbons adjacent to each ring are shown in black arrows on the structure and connected by lines on the spectrum. Weaker cross-peaks are labeled with numbered green arrows on the structure and correspond to the number on the spectrum. These indicate medium-range (crossing three carbon−carbon bonds) HMBC interactions between A and B protons of each ring and acetylene carbons adjacent to each ring.

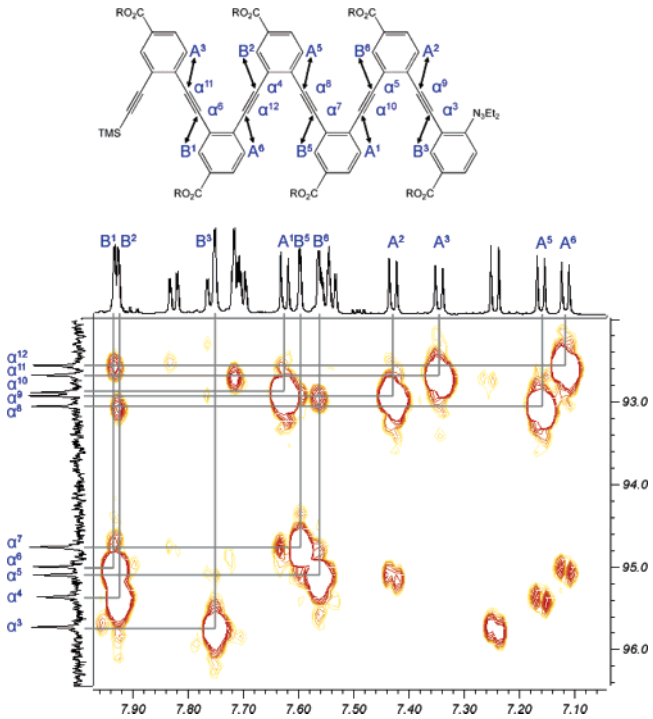

FIGURE 7. Short (crossing two carbon−carbon bonds) HMBC interactions between A and B protons of Es_6 and acetylene carbons adjacent to each ring, shown in black arrows on the structure and connected by lines on the spectrum. Weaker, unlabeled cross-peaks indicate medium-range (crossing three carbon−carbon bonds) HMBC interactions between A and B protons of each ring and the adjacent acetylene carbons.

carbon signals between 97 and 94 ppm (shown in red) and 94−92 ppm (shown in green).

On the basis of the number of signals in each of these two groups, we speculated that they might be the acetylenic carbons β to the B proton on the adjacent ring (red) and the acetylenic carbons β to the A proton on the adjacent ring (green), as shown for Es_4 in Figure 4. This was confirmed after complete assignment of the structures, resulting in the observation that the chemical shifts of acetylenic carbons β to protons at position B on the ring are well separated from those β to protons at position A.

With the 1D ^1H and ^{13}C spectra completed, as well as COSY spectra, HMBC experiments were performed to assign the primary sequence. Starting with the unique acetylenic carbon signals at the Si terminus of Es_4, the $Si(CH_3)_3$−acetylene assignment was confirmed as shown in Figure 5a. These terminal acetylene carbons were used to assign the protons in ring 1. A strong HMBC signal between α^1 and B^4 along with weaker signals between α^2 and A^3 as well as between α^1 and A^3 confirmed the assignment. At the other end of the Es_4 molecule the ring 4 protons, B^3, C^3, and A^4, were assigned based on HMBC couplings to the ring carbon bonded to the N_3Et_2 group, as shown in Figure 5b. Similar procedures were used for assignment of Es_5 and Es_6. During assignment of rings 5 and 6 in Es_5 and Es_6, respectively, some ambiguity in HMBC signals was observed due to ^1H overlap, as shown in Figure 5c and 5d; however, this was easily resolved using the COSY data to select the set of A, B, and C protons from a single spin system. For example, in Es_5, a clear HMBC signal between the aromatic carbon bonded to N_3Et_2 and A^4 is seen. Two other couplings from this aromatic carbon are also observed—one that could indicate C^2, B^3, or B^4 and another that could indicate B^5, C^3, or C^4. COSY data assigns A^4, B^4, and C^3 to a single spin system which combined with the HMBC signals allows definite assignment of these protons to ring 5.

Short-range HMBC couplings, defined here as spanning two carbon−carbon bonds, from A and B protons on each ring were used to assign the acetylene carbons β to each of these protons as shown in Figures 6 and 7 and Supporting Information Figure S14. Medium-range couplings, defined here as spanning three carbon−carbon bonds but not crossing a triple bond, were used to cross-check these assignments. For example, six weaker signals are also shown in Figure 6. These HMBC signals correspond to couplings between A and B protons and the other acetylene carbon attached to the ring. The HMBC spectrum for Es_6 shown in Figure 7 shows very similar features with 10 short- and 10 medium-range couplings. Figure S14 shows the HMBC spectrum for Es_5, which was collected with a different pulse sequence due to an upgrade of the NMR facility. This pulse program was optimized for longer range couplings and did not always show the short and medium range with significantly different intensities as observed for Es_4 and Es_6 in Figures 6 and 7. Nonetheless, unambiguous assignment can still be made using the knowledge gained from assigning Es_4 and Es_6, followed by cross checking with long-range HMBC couplings across the carbon−carbon triple bond. Another cross check is that these assignments are consistent with the separation of the acetylene carbons into two groups. With each set of acetylene carbons assigned to the spin systems with which they are connected, it only remains to make the connections between ring systems across the triple bond.

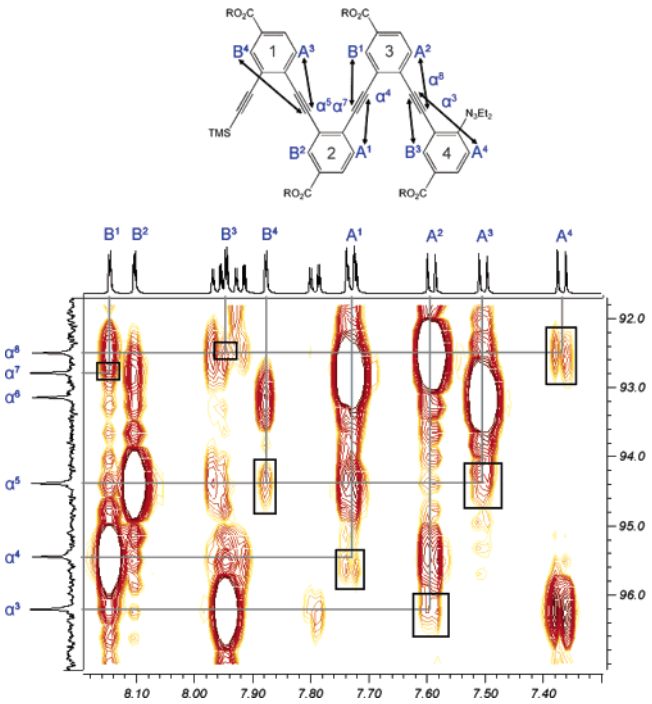

FIGURE 8. Long-range HMBC interactions across carbon−carbon triple bonds between aromatic protons of Es_4 and acetylene carbons are shown in black arrows on the structure and connected by lines on the spectrum.

Using long-range interactions across the triple bond, it was possible to connect each spin system with its neighbors such that unambiguous assignment of the entire primary sequence was obtained. Figure 8 and Supporting Information Figures S15 and S16 contain the long-range HMBC spectra used for assignment of Es_4, Es_5, and Es_6 respectively. For example, in Figure 8 the seven clear long-range interactions that were used to assign Es_4 are shown. As rings 1 and 4 of Es_4 have already been assigned from the end groups, the remaining task is to assign rings 2 and 3. HMBC couplings between α^5 and B^4 as well as between α^5 and A^3 allow the assignment that ring 2 is connected to ring 1. HMBC couplings between α^3 and A^2 as well as between α^8 and B^3 and α^8 and A^4 allow the assignment that ring 3 is connected to ring 4. Connections between ring 2 and ring 3 are indicated by HMBC couplings between α^7 and B^1 as well as between α^4 and A^1. These were used to confirm the assignments of the aromatic protons of Es_4. Following this procedure, working from the terminal rings inward, full assignment of the aromatic protons of Es_5 and Es_6 was made.

The assignment of Es_4 is extremely easy to make, as three or four HMBC couplings across each triple bond are present, and clear differentiation of signal strengths based on distance is shown. The signal-to-noise ratio was not as high in the case of Es_6, but weak signals were readily distinguished from noise by the characteristic shape from their ^1H splitting pattern. More detail is provided in the Supporting Information. HMBC interactions in Es_5 showed a high signal-to-noise ratio but due to the use of a long-range pulse program did not display a clear dependence on distance. Furthermore, some extremely long-range interactions were visible in the spectra of Es_5, which made assignment more difficult. However, there are only six possible primary sequences after the relatively easy assignment of rings 1 and 5 by end-group interactions, and only one potential assignment of rings 2−4 adequately explained all observations. In all oligomers, at least one HMBC coupling across every triple bond was present, ensuring that no assignment was dependent upon any single HMBC coupling.

Conclusions

HMBC, COSY, 1D ^1H, and 1D ^{13}C NMR methods provided the necessary information to assign the primary sequence of three oPE oligomers. These results confirm the use of HMBC to assign the sequence of homo-oligomers that contain an abundance of tertiary carbon centers. In addition to establishing the full assignment of the aromatic protons, HMBC revealed that these oligomers have directionality along the backbone that remains resolved even in the hexamer, likely due to the electron-withdrawing effect of the ester group. This is clearly seen in the acetylene carbons in which those β to A protons (the N-terminus side of the ring) are located upfield compared to the acetylene carbons β to B protons (the Si-terminus side of the ring).

This total assignment of the aromatic protons has proven invaluable for interpretation of chemical shift and NOE data collected during studies to determine the solution conformation of these foldamers. In particular, investigation of solvent- and temperature-dependent folding of Es_5 and Es_6, analogous to our previously published work with Es_4,[27] has been greatly assisted. A particular point of interest is ring 3 of Es_5. In a polar solvent such as the CD_3CN used in these measurements a helical conformation with three rings per turn may be expected. In this conformation ring 3 would be the one ring remaining unstacked with any other and therefore not subject to the resulting upfield shifting as observed for the other rings. It is only by this HMBC assignment that the protons on ring 3 can be assigned with accuracy. Thus, the fact that only protons on ring 3 remain unshifted upon folding supports the helical conformation. It is expected that similar techniques will allow assignment of aromatic protons in other PE oligomers with various side chains and end groups.

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Supporting Information Available: Experimental Section; synthetic procedures for all oligomers reported here; tables of all observed HMBC and COSY interactions used for assignment; HPLC and NMR measurements used for confirmation of Es_6 purity; HMBC data for Es_6 in ester-carbon aromatic-hydrogen region, used to confirm COSY assignments for Es_6; COSY data for all oligomers; HMBC data for Es_4−6, α1-α2-carbon aromatic-proton region. HMBC for Es_4−6 acetylene-carbon aromatic-proton region; details and discussion for assignment of long-range HMBC peaks. This material is available free of charge via the Internet at http://pubs.acs.org.

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