

Helical Structures

Helical structures represent one of the most common structural motifs in biology. They are used to design and hold functional groups at defined locations in 3-dimensional space. In addition, the helix represents a 1-dimensional building block for higher order structures. Our research objectives are to create helical structures from the non-natural phenylene ethynylene backbone and learn to assemble these helical structures into larger bundles reminiscent of naturally occurring protein structures. For example, a four-helix bundle from the core of a natural protein is shown below.

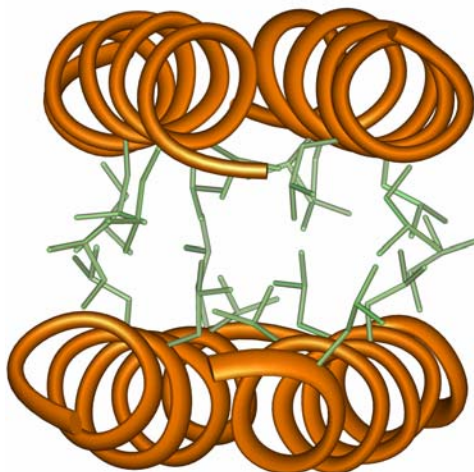


Figure 1. A four helix bundle core of the natural Lac repressor protein.

Helical bundles, in which two or more helices associate, are ubiquitous in nature and commonly found as dimers, trimers, and tetramers. They perform an impressive number of functions from structural to catalytic to DNA binding. In most cases, the helix is amphiphilic and traditionally described by the heptad repeat, *abcdefg*. The *a* and *d* positions contain NP amino acids leading to a hydrophobic strip along the face of the helical cylinder. It is the shielding of this hydrophobic strip from aqueous solution that drives inter-helical association and burying of hydrophobic residues is generally accepted to contribute $50 \text{ cal}/\text{\AA}^2$ to folding stability. This corresponds to approximately 5 kcal/mol at room temperature for a single Leucine (Leu) residue. As the association number increases toward a four helix bundle, the hydrophobic surface area also increases. In fact, the four helix bundle often has hydrophobic residues located at the *e* and/or *g* position as shown in Figure 2. The helix used to form bundles are typically shorter than those in dimeric bundles because of the increased hydrophobic surface area found in the larger bundle.

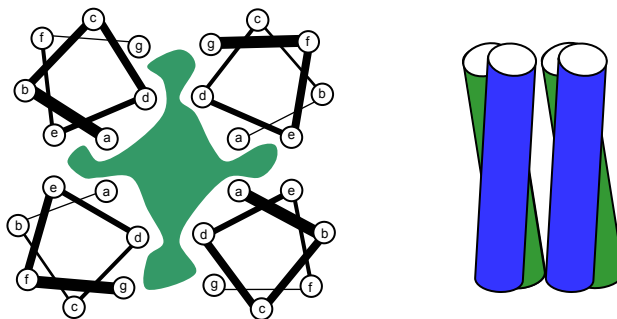


Figure 2. Four helices self assemble to form the bundle. The hydrophobic surface area is illustrated schematically.

Because of our large platform of expertise in phenylene ethynylene chemistry and the elegant nature of these molecules to adopt a variety of conformations, we have focused our efforts on designing *ortho*-PE derivatives. The selection of this backbone was inspired for many reasons including:

1. the *ortho* substitution leads to the shortest possible helical repeat based on aromatic substitution thereby minimizing the synthetic effort and enabling long oligomers to be synthesized,

2. the helical structure of a *meta* 18-mer, one of the longest prepared, more closely resembles a puck as opposed to a tall cylinder due to the large helical repeat of the *meta* series. The aspect ratio of a 12-mer is 0.25 vs. 1.33 for the *meta* and *ortho*, respectively, as shown in Figure 3. This is critical for helical bundle formation since it requires tall cylinder-like objects. The specific requirement of a high aspect cylinder is two fold. One is that a cylinder provides a large hydrophobic surface area patterned with NP groups that supply the enthalpy contribution for self-assembly of the helical bundle (Leu is worth 5 kcal/mol).

3. the PE backbone is strongly hydrophobic, we must be concerned with end-to-end stacking of helical structures due to the presence of aromatic faces at each ends of the helix and, in fact, Moore as observed this interaction. By selecting *ortho* isomers, the helical repeat is reduced from six (*meta*) to three (*ortho*) and results in a smaller hydrophobic end so less enthalpy is gained upon stacking.

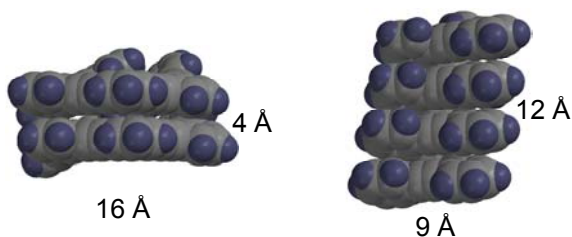


Figure 3. Comparison of a 12-mer sequence for *meta* vs. *ortho* PE. It is clear that the *ortho* series makes a tighter and taller helix.

The novel aspect ratio of the *ortho* PEs is critical because the energetic driving force for helix self-assembly is association of the hydrophobic domains away from the polar (or water) environment. Because this is an intermolecular process, it is dependent on the binding constant, or association energy, which is related to the size of the hydrophobic domains, and controllable with concentration. In contrast, helix formation is intramolecular and therefore concentration independent. As a result, it should be able to control the hierarchy of organization from an unfolded, linear sequence to helix and helix to bundle self-assembly with concentration. Further, the *ortho* derivatives favor helix formation compared to *meta* structures based on conformational entropy considerations. If we make a simple assumption that the PE backbone can adopt only a transoid or cisoid conformation, then the *meta* derivative must confine 5 bond torsions to form a helix compared to only 2 for *ortho* derivatives. To support this hypothesis, we have performed NOESY experiments where we are able to collect NOE data at 24 °C compared to *meta* structures which required -10 °C, even with the presence of multiple hydrogen bonds to stiffen the conformation. Additionally, NOE studies show folding at only 4 rings, less than half the length needed for *meta* derivatives. Figure 4 shows the helical pitch of an *ortho*-PE in more detail.



Figure 4. Crystal structures including a side and end-on view of *o*-PE.

The chemical synthesis of these oligomers is shown below. Excellent work by graduate students and post-docs has resulted in great progress with this difficult problem.

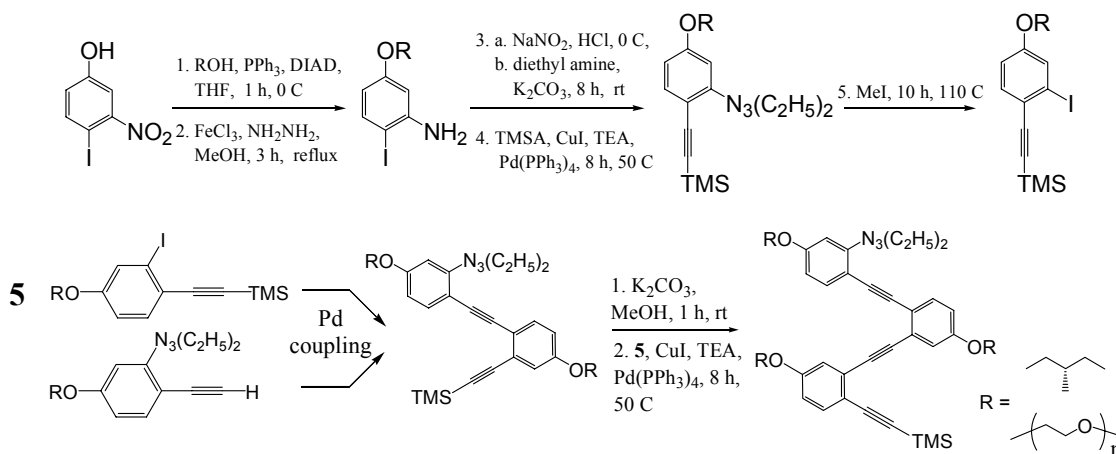


Figure 5a. Synthesis of *o*-PEs monomers and oligomers. Reaction of monomer **5** with the acetylene monomer, obtained from TMS deprotection, yields dimer. This dimer is deprotected and coupled with **5** producing trimer.

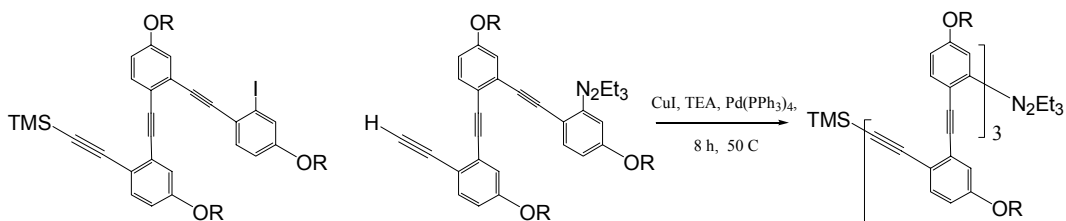
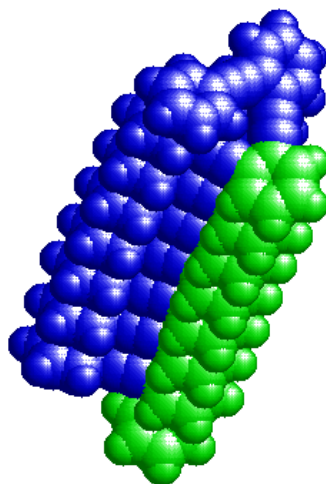


Figure 5b. Trimer in 7a allows convergent-divergent synthesis of longer oligomers as illustrated here for a hexamer.

Successful chemical synthesis of monomers containing both polar and nonpolar groups enable amphiphilic helical sequences to be generated as shown below.



The successful design of these structures will have important fundamental and practical implications. Learning how molecules fold and adopt discrete structures will reveal basic scientific principles. At the same time, new materials properties will be realized including liquid crystals, catalysts, amphiphiles, etc.