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Title: Synthesis and Temperature-dependent Phase Behavior of a Dendritic Dipeptide

Category: Chemistry

Research Plan

Rationale

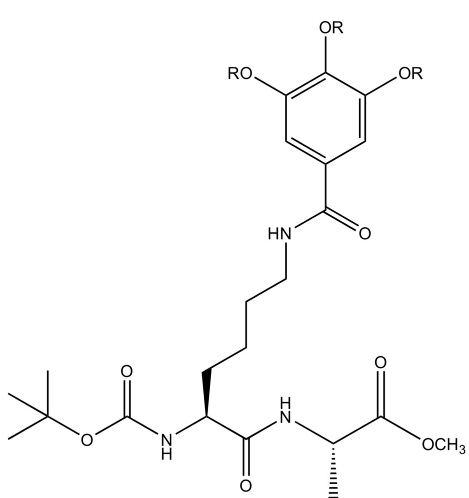
Proteins are naturally occurring polymers that are found in almost every system of the world. They act as catalysts, convert solar to chemical energy, and transport molecules in and out of the cell in membranes. Polymer membranes are synthetic membranes made of repeating organic molecules utilized in water purification, solar cells, and drug delivery: in many of which embedded proteins play a crucial role. Due to its versatility, transmembrane proteins are often synthesized to better help understand the structure and function of other complex proteins (Percec et al., 2007).

In order for synthetic porous structures to successfully mimic the functions of natural transmembrane proteins, the architecture of the synthetic polymer must be carefully controlled on all levels. Monomers must successfully self-assemble in solution and in the solid state through intramolecular interactions, such as H-bonding, ionic interactions, and π-π interactions (Rosen et al., 2011). Currently, existing synthetic porous structures that self-assemble stably in both the membrane environment and the solid state have not been synthesized (Percec et al., 2004). Dendron-peptide hybrids are monomers with distinct folding properties that ultimately control the hierarchy of assembly (Barkley et al., 2017), which means changes to an individual dendritic peptide can affect the stability of the structure. Adjustments in the length of the dendron, the protective groups on the dipeptide, and the stereochemistry of the dendron can lead to altercations in the pore size, the type of intramolecular interactions, and the shape of the overall structure. (Percec et al., 2007).

In solution, the self-assembling dendrons in the dendritic peptide promote the formation of a liquid crystalline mesophase, an intermediate state of matter between liquid and solid (Barkley et al., 2017). Dendritic peptides can form different types of liquid crystals, including columnar and cubic, but the columnar structure in the shape of a hexagon is the most common and optimal for transporting ions in and out of the membrane (Percec et al., 2005).

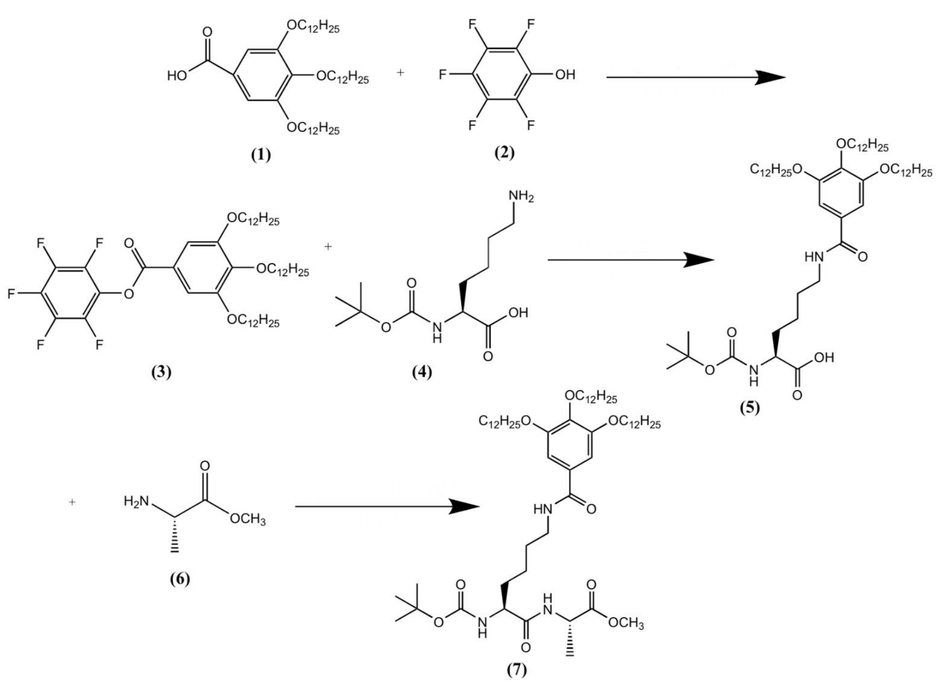
Barley et al. (2017) organized a hexagonally columnar mesophase by covalently attaching the amphiphilic dendrons to the dipeptides in an ɑ-helical protein; however, when done on a β-sheeted peptide-dendron using the same amino acids, no liquid crystalline structure was formed (Barley et al., 2018). This was attributed to a greater amount of hydrogen bonding, which prevented a liquid crystalline structure from forming.

Research Question/Hypothesis



in order to further explore the desired characteristics of peptide-dendron hybrids, a dendritic dipeptide with lysine, alanine, and nonpolar protective groups (t-BOC and OMe) will be synthesized.

Procedure



- Esterification of Benzoic Acid
    - Add 1.0 g of benzoic acid (1) and 0.3 g of PFP (2) to a round bottom flask (RBF) with a magnetic stirrer
    - Add 4 mL of DCM and 0.22 g DPTS to the RBF
    - Place RBF into an ice bath at 0℃, and add DCC drop-by-drop
    - Remove RBF from ice bath and let stir for 2 days
    - Run a TLC in 100% DCM and an H NMR in deuterated chloroform (chloroform-D) to determine reaction completion

- Attachment of $_L$-Lysine
  - Add 0.4 g of pentafluorophenol ester and 0.122 g of $_L$-lysine to a 25 mL RBF with a magnetic stirrer
  - Pipette 0.120 mL DIEA and 12 mL THF into the RBF
  - Attach the RBF to a reflux condenser and submerge it in an oil bath that will be heated to 55℃
  - Let stir for 4 days
  - Run a TLC in 100% DCM and an H NMR in chloroform-D to determine reaction completion
- Coupling of Alanine
  - Add 0.3 g of the dendritic lysine and 0.035 g of $_L$-alanine in a 10 mL RBF with a magnetic stirrer
  - Add 5 mL DCM and 0.075 g DCC into RBF
  - Weigh 0.05 g DPTS in a separate tube and use 2 mL DCM to dissolve
  - Place RBF in an ice bath and add the DPTS solution drop-by-drop into the RBF
  - Remove RBF from ice bath and let stir for 2 days
  - Run a TLC in 100% DCM and an H NMR in chloroform-D to determine reaction completion
- Polarized Optical Microscopy (POM) Experiments
  - Prepare a sample of the dendritic dipeptide onto a slide
  - Place slide in a heating chamber and under the microscope
  - Set the heating rate at 5 °C/min, starting at 25 °C to 120 °C

    - Observe and take pictures of the sample as it changes phases
    - Once the chamber reaches 120 °C, start the cooling cycle at 5 °C/min back down to 25 °C
    - Observe and take pictures of the sample as it changes phases
- Differential Scanning Calorimetry (DSC) experiments
    - Send sample of the dendritic dipeptide to another lab for experimentation

Risk and Safety

Most of the reagents used in the reactions have low health and safety ratings, but any chemical with a health hazard rating of 2 or higher will be handled by a supervisor. Because this project requires the synthesis of multiple unprecedented molecules, there are no existing MSDS sheets for these said molecules. However, proper equipment, including safety goggles, gloves, and lab coats, will be worn when handling any sample. During experimentation, all chemicals will be handled and all reactions done under a fume hood. Any leftover chemicals after experimentation will be organized into organic and inorganic liquids and poured into a 20L waste bottle that is emptied once every week.

The following reagents will be used: dichloromethane (DCM), N,N'-Dicyclohexylcarbodiimide (DCC), 4-(Dimethylamino)pyridinium 4-toluenesulfonate (DPTS), and tetrahydrofuran (THF). DCM has a health rating of 2 meaning it is hazardous; a fire rating of 1, meaning it is a flammable above 200F; and a reactivity rating of 0, meaning it is a stable compound. DCC has a health rating of 3, meaning it is extremely hazardous; a fire rating of 1, meaning it is a flammable above 200F; and a reactivity rating of 0, meaning it is a stable compound. DCC will only be handled in small amounts and in the fume hood. THF has a health rating of 2, meaning it is hazardous; a fire rating of 3, meaning it is a flammable below 100F; and a reactivity rating of 1, meaning it is unstable when heated. THF will only be handled in the fume hood. DPTS may cause skin and eye irritation.

Bibliography

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Addendum

- During the coupling of alanine, I used 0.064 g of EDC-HCl and 0.041 g of DMAP in 1 mL DCM instead of 0.075 g DCC and 0.05 g DPTS in 9 mL DCM .
    - Risks:
        - N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC-HCl) has a health rating of 2, meaning it is hazardous; a fire rating of 1, meaning it is a flammable above 200F; and a reactivity rating of 0, meaning it is a stable compound.
        - 4-Dimethylaminopyridine (DMAP) is toxic if swallowed, inhaled, or touched, so DMAP will only be handled under the fume hood by a graduate student.