

Trapping of Integral Membrane Proteins with Amphipathic Polymers

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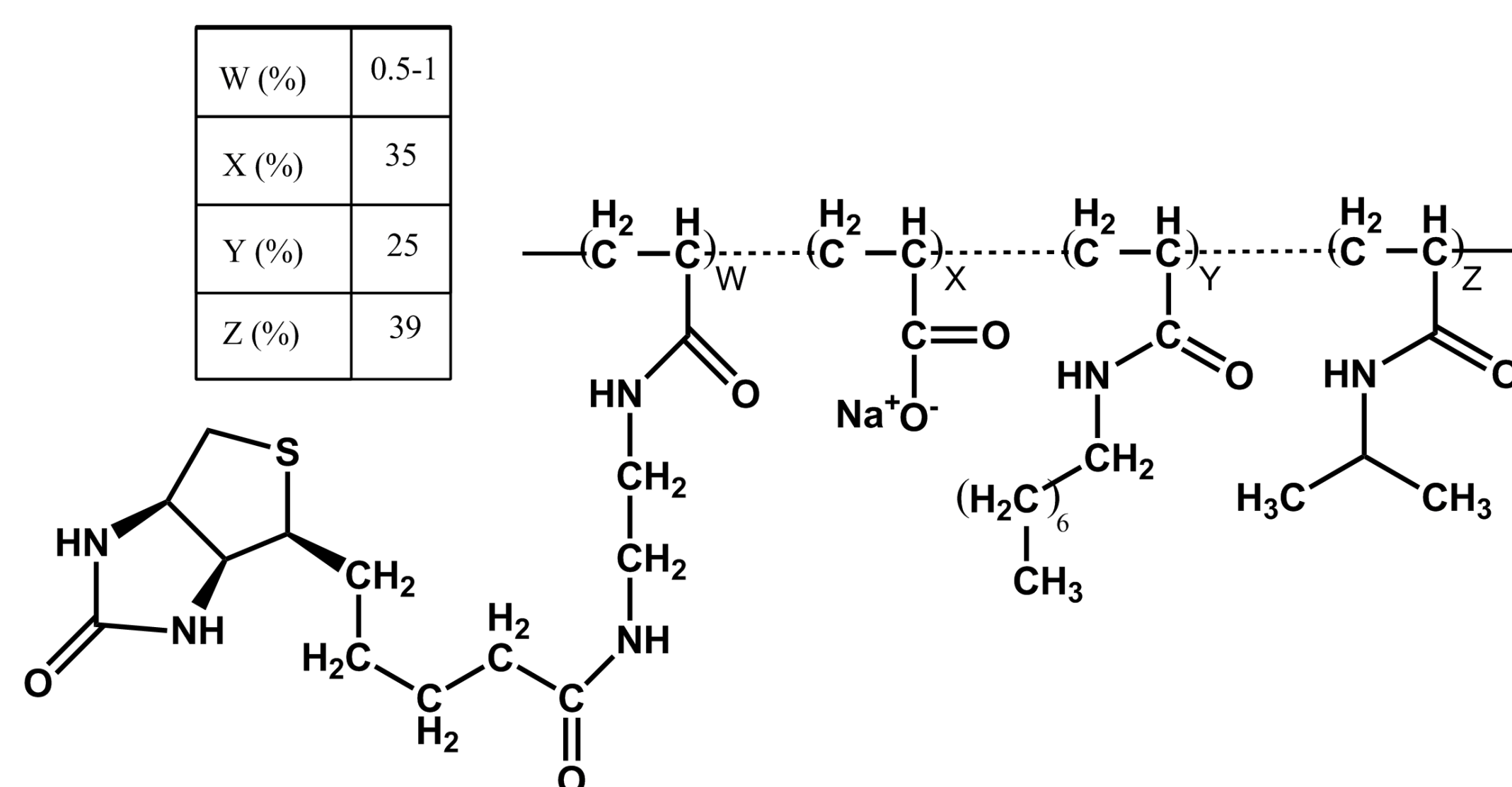
Introduction

To properly study the *in vivo* action of proteins, cellular conditions must be replicated. Integral membrane proteins (MPs) exist suspended in a lipid bilayer which stabilizes the hydrophobic domain. To study these proteins there have been few options to immobilize functional proteins. The proposed method would need to be simple and versatile as to accommodate many different proteins without major adaptations. The use of short (~70 unit) Acrylic Acid Polymers (PAA) substituted with hydrophobic side chains has been studied and appears to be successful in stabilizing and sequestering MPs. If these polymers can be biotinylated it is then possible to immobilize these protein-amphipol complexes onto a surface. This stabilization and immobilization is not possible with any current technique over a range of temperatures, including physiological temperature. In this project the goal is to synthesize a biotinylated amphipol and trap and immobilize a 5-HT_{3A} receptor.

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Research Plan

In the past a specific amphipol (A8-35) has been the most extensively studied, and has been shown to successfully trap and stabilize MPs. To immobilize complexes formed with A8-35 the amphipol must be biotinylated. Below is the desired final product.



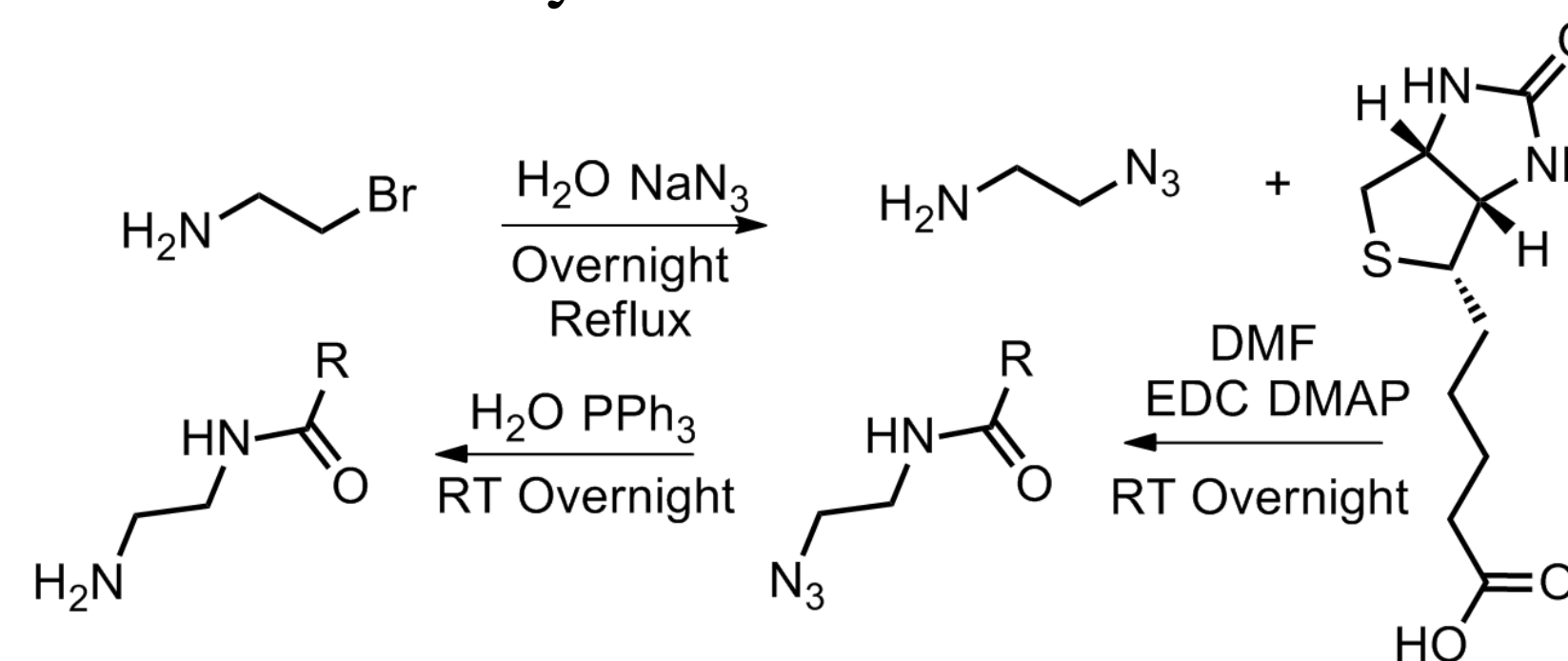
Acknowledgements

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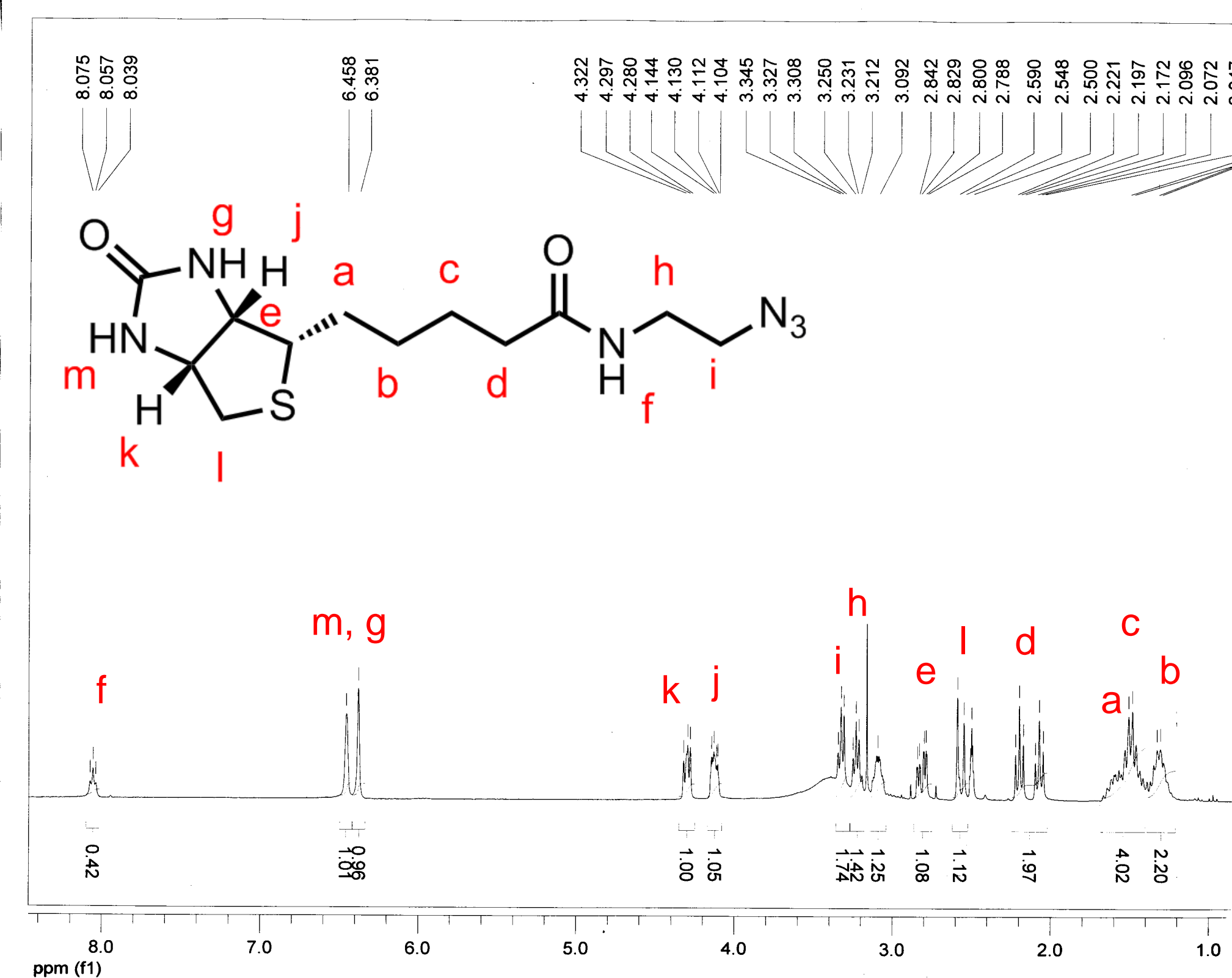
Synthesis of N-(2-Aminoethyl)-biotinamide

To create a biotinylated amphipol a modified biotin molecule must be synthesized.



The product of step one was extracted from the aqueous into diethyl ether after basification with NaOH. The solvent was then removed by rotary evaporation to give a yellow oil with a 50% yield. The appearance and ¹H/¹³C NMR spectra agreed with literature.

The product of step two was purified in two ways. First the DMF was removed by rotary evaporation. The resulting solid, was washed with DCM and filtered out. The solid was then washed with DCM and MeOH to remove any EDC or DMAP. To the filtrate, a flash silica column was performed (DCM→10%MeOH/DCM). The combined isolated products give a white powder in 75% yield. ¹H-NMR in DMSO yielded the following spectra.



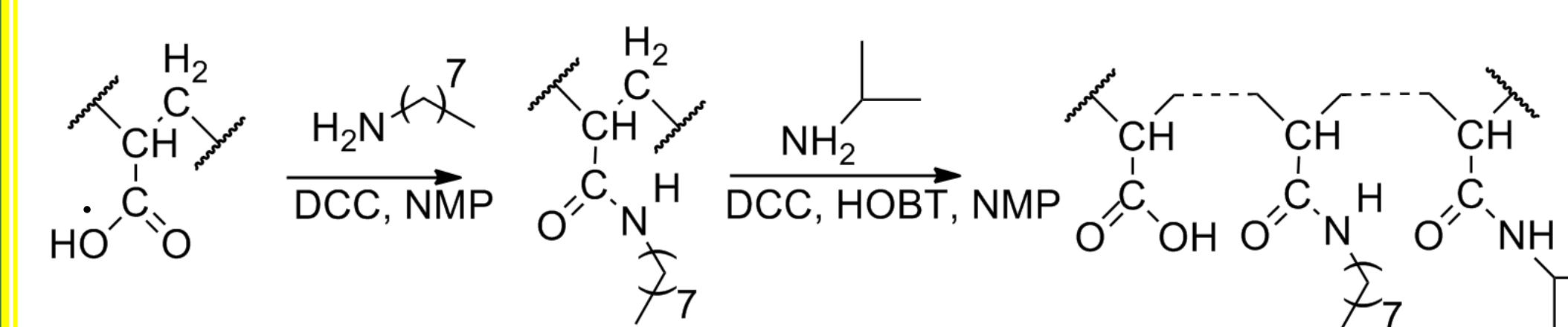
N-(2-Azo-Ethylamine)Biotinamide ¹H NMR Spectra at 300 MHz

The reduction of the azide, however has not been attempted, due to solubility issues and an inability to use Pd catalyzed reactions. Currently a reduction using triphenylphosphine has been proposed as the best option despite potentially low yields.

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Synthesis of A8-35

First the PAA which comes as a 50% by weight in water solution must be lyophilized to create a dry powder. The synthesis of biotinylated A8-35 (BA8-35) is then done in 2 additions.



The planned purification was as follows, as it is highly cited in literature. By adding an excess of water and sodium methanoate it is possible to filter out DCU crystals and then precipitate out the polymer. Through three steps of acidification with 10% HCl and dissolution in NaOH solution the product is purified. The supposedly soluble polymer however appeared to complex with the highly hydrophobic DCU which created a colloid as opposed to a truly base soluble compound. This was confirmed by ¹H-NMR analysis.

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Future Work

The reduction of the azide containing biotinamide is planned along with the optimization of the purification of A8-35. Once A8-35 is synthesized successfully a biotinylated version will be synthesized. If serotonin receptors can be complexed, then single molecule spectroscopy is planned. This SMS which look at ligand (dis)association constants.

