Surface Modification of G4 and G5 PAMAM Dendrimers

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Introduction

Background: Dendrimers are highly complex, branching polymers built around a core molecule. Layers of functional groups surround the core. Each successive layer is considered a generation (G2, G3, etc.)

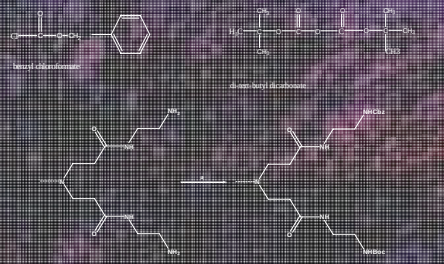
Objective: The goal of the collaborative dendrimer project is to create dendrimers that will cross the blood-brain barrier and deliver drugs. This part of the project focused on dual-functionalizing fourth- and fifth-generation poly(amino)amide (PAMAM) dendrimers, with a fluorophore.

Dual-functionalization is the process of placing two different functional groups on the dendrimers. This is accomplished by placing two different protecting groups over the terminal amine groups, removing one, attaching the desired fluorophore (dansyl group or a biotin-fluorescine molecule), then removing the second protecting group, and attaching the drug.

Fourth generation (G4) PAMAM dendrimer

1

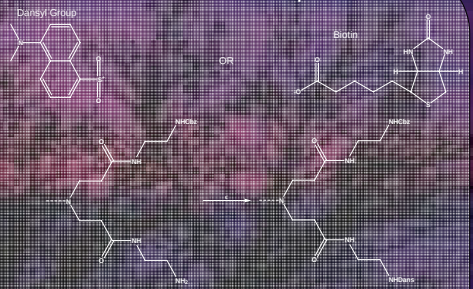
Protecting Groups



Scheme A involves attaching two protecting groups to the terminal amines, one strongly bonded, and one weakly bonded, so that they can be removed separately.

2

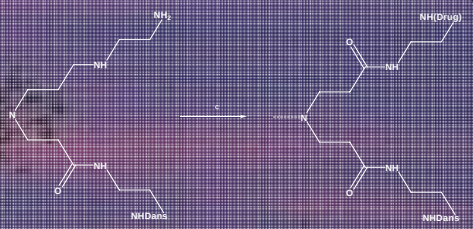
Attach the Fluorophore



Scheme B involves removing the weakly-bonded protecting group, then adding the fluorophore.

3

Drug Addition



Scheme C involves removing the more strongly-bonded protecting group, and then adding the desired drug.

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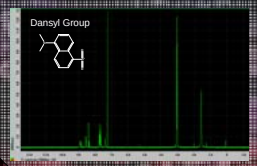
Dansylation

Most of our efforts have focused on developing a working technique for the dansylation of dendrimers. Three separate methods have been attempted, to varying degrees of success. The major problem has been purification of the product.

The purpose of the dansylation is to bind dansyl groups to the dendrimer to act as a fluorescent probe. This will allow visual tracking of the dendrimers as they cross the blood-brain barrier.



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Problems

Most of the problems encountered during this phase of the research have centered around purification of the final product. Many methods were attempted, including:

- Dialysis
- Extraction via ether immersion
- Centrifugation
- Column chromatography

Column chromatography proved to have the highest success rate.

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G4 PAMAM

without modification

This nuclear magnetic resonance (NMR) spectrum shows the number of hydrogen atoms in the dendrimer. In addition, the location on the spectrum indicates the atoms that are nearby, giving a sense of the form of the molecule.

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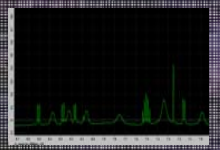
G4 PAMAM

Dansylated and unpurified

a

b



c

These NMR spectra show dansylated but unpurified G4 PAMAM dendrimers. Specrum (a) shows the full view, (b) is zoomed in on the area where the dendrimer peaks are visible, and (c) is zoomed in on the dansyl group in the aromatic region. With both clear peaks and unclear peaks, it suggests that dansyl chloride and bonded dansyl groups are present in the sample.

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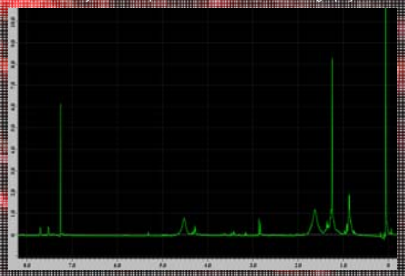
G5 PAMAM

Dansylated and purified via column chromatography



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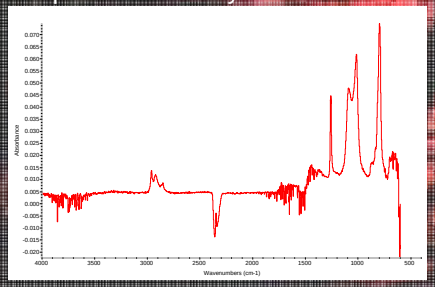
IR Spectrum of Dansylated G4 PAMAM



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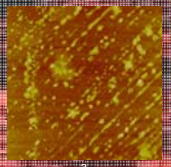
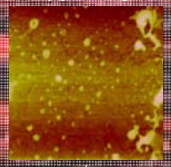
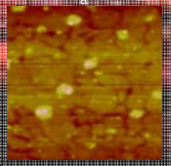
Atomic Force Microscopy Images



a



b



c

These images are 10 micrometer (a), 3.3 micrometer (b), and 1 micrometer (c) images of dansylated G5 PAMAM dendrimers.

In the images, individual dendrimers are visible, as well as clumps. Each dendrimer is approximately 5 nanometers across.

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Future Steps

To test the G4 and G5 PAMAM dendrimers' ability to cross the blood-brain barrier, continue working with the protecting groups to explore the dual-functionalization of the PAMAM dendrimers, and consider similar reactions with poly(lysine) dendrimers.

Acknowledgments

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References

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