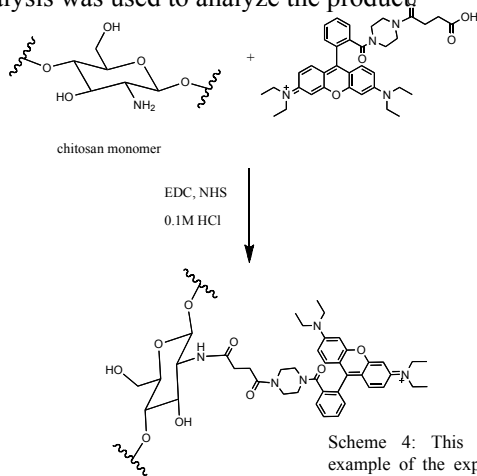


# Synthesis of fluorescent chitosan using a water soluble rhodamine B derivative

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**Background:** Chitosan is a natural product biopolymer that has been shown to possess antimicrobial properties. The mode of interaction between chitosan and the microbe has yet to be determined. In order to elucidate the interaction mechanism, our research focused on attaching a fluorescent tag to chitosan and tracking its presence in a cellular environment, using fluorescence microscopy. A suitable fluorescent tag in this case would be one that is water soluble, has a high quantum yield, and can easily be coupled to chitosan via simple peptide-bond formation. From the literature, we find that rhodamine B 4-(3-carboxypropionyl) piperazine amide suffices on each of these levels due to its straightforward synthesis (i.e. no chromatography, etc.) and succinic acid tether.

**Approach:** A three part scheme (schemes 1-3 on right) was used to synthesize the dye derivative. The first step involved the production of a rhodamine B base (scheme 1) via lactone formation from the free acid. Next, the rhodamine B piperazine amide was produced (scheme 2) by coupling piperazine to the base using trimethylaluminum. Then, rhodamine B 4-(3-carboxypropionyl)piperazine amide was synthesized (scheme 3) via acylation and ring opening of succinic anhydride. Finally, an attempt was made to couple the rhodamine B derivative to chitosan via peptide-bond formation. NMR analysis was used to analyze the product.

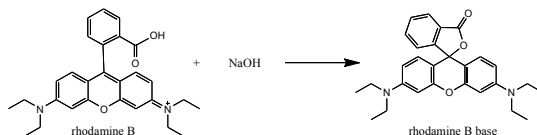


Scheme 4: This reaction shows an example of the expected product for a monomer unit of chitosan.

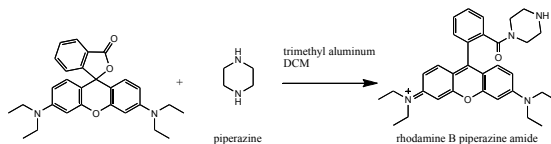
**Results:** Rhodamine B 4-(3-carboxypropionyl)-piperazine amide was synthesized as verified by NMR analysis. The coupling reaction to chitosan was performed (scheme 4), however at this time, we are unsure if we have product. NMR analysis and dialysis both produced inconclusive results.

NMR analysis did not show presence of dye. This could mean that excess dye was stuck to the sugar backbone and not covalently bound to chitosan. It could also mean that there was very low functionalization of the coupled dye, to the point that it did not show up during NMR experiments. However, somewhat low functionalization is desired because very little is necessary for the chitosan moiety to be observed under fluorescence microscopy.

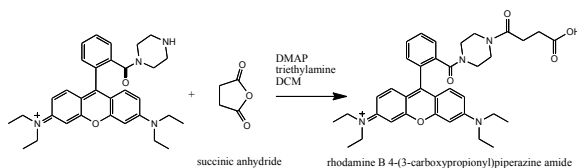
Scheme 1:



Scheme 2:



Scheme 3:



Francis, M.B.; Nguyen, T. *Organic Letters*. 2003, 5, 13, 3245-3248

Dialysis was used to dilute the concentration of chemicals that were unbound to chitosan. Ideally with each dialysis step, the product should be more purified of unbound dye than the previous dialysis iteration. This was observed by visually monitoring the degrading intensity of the dye color in the water. When the water was clear, it was expected that all excess dye had been washed out. Since the desired product material was still colored when the dialysis water was clear, it was believed that this color was due to chemically bound dye in the sample.

A control was established in order to determine if any remaining color in the sample after dialysis was indeed due to covalently bound rhodamine B derivative. The control consisted of eliminating one of the coupling agents (EDC; ethyldiisopropylcarbodiimide), which should cause none of the dye to chemically bind to chitosan. After dialysis, the control sample was significantly less colored than the experimental sample, yet still had a faint pinkish/purple color. Unfortunately, dialysis had to be rushed due to time constraints, so it is unknown if dialysis needed more time, or if dialysis is an insufficient method for washing out all unbound dye.

**Future work:** Perform more coupling reactions of the dye to chitosan, along with more controls. Try additional washing methods. Perform coupling of the dye to chitosan arginine. Obtain proton NMR and elemental analysis of the desired product.

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