Adventures with Fluorescent Compounds

Fatou Bintou Kebbeh Njie and Anna K. Y. Giebink

(Faculty mentor: David E. Lewis)

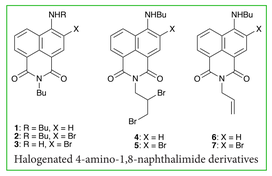
Department of Chemistry, University of Wisconsin-Eau Claire, Eau Claire WI 54702-4004.

Introduction: *N*-allyl-4-amino-1,8-naphthalimides

4-Amino-1,8-naphthalimide derivatives1 are highly fluorescent compounds whose relatively simple synthesis and high quantum yields have led to their widespread use, for example, as fluorescent sensors for metal ions and pH, and as elements in the design of molecular logic gates. The emission properties of these systems are strongly influenced by the solvent and by the nature and degree of substitution on the nitrogen5 since the ability of the compounds to form twisted charge transfer states may be a major determinant of the excited state properties. The emission properties of the naphthalimide nucleus is also sensitive to environment even when conformationally locked, as shown by the fluorescence emission of Tröger's bases based on this fluorophore.

The bromination of saturated 4-alkylamino-*N*-alkyl-1,8-naphthalimides (e.g **1**) proceeds well in halogenated solvents, but in typical experiments several hours are required for the complete discharge of the color of the halogen. The first product formed with bromine is the 3-bromo compound (**2**); using excess bromine and long reaction times leads to the 4-amino compound (**3**) rather than a second ring bromination.

In the process of developing site-selective probes for use in fluorescence microscopy of live cells, we needed a dibromide of general structure 4, which we expected should be easily obtained by addition of bromine to an *N*-allyl-4-alkylamino-1,8-naphthalimide (**6**). This addition reaction did not, however, proceed as expected.

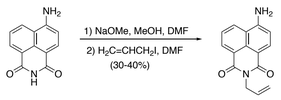


1: R = Bu, X = H
2: R = Bu, X = Br
3: R = H, X = Br
4: X = H
5: X = Br
6: X = H
7: X = Br

Halogenated 4-amino-1,8-naphthalimide derivatives

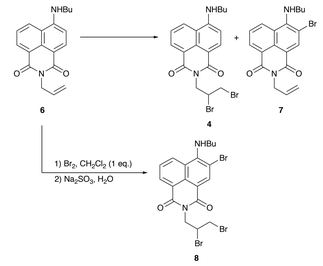
Results

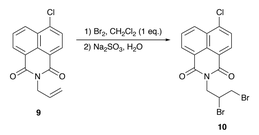
The required *N*-allyl-4-amino-1,8-naphthalimide was prepared by our previous methods based on the modified Gabriel approach, using the conjugate base of the parent 4-amino-1,8-naphthalimide with allyl bromide. The yields of pure product were not particularly high, but were in line with the results of other chemists.

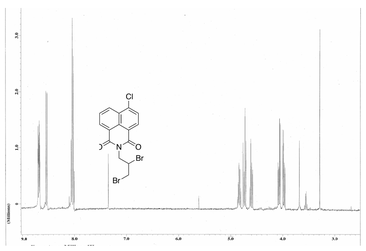


What is surprising about this observation is that the ***bromination of the aromatic ring has occurred in preference to the addition of halogen to the double bond of the allyl group***. To put this into perspective, the addition of one equivalent of bromine to *N*-allyl-4-chloro-1,8-naphthalimide (**9**) under the same conditions was complete within minutes, and the expected dibromide (**10**) was obtained as the only product. This shows that the substituent at the 4- position of the dye exerts a substantial influence over the course of the reaction.

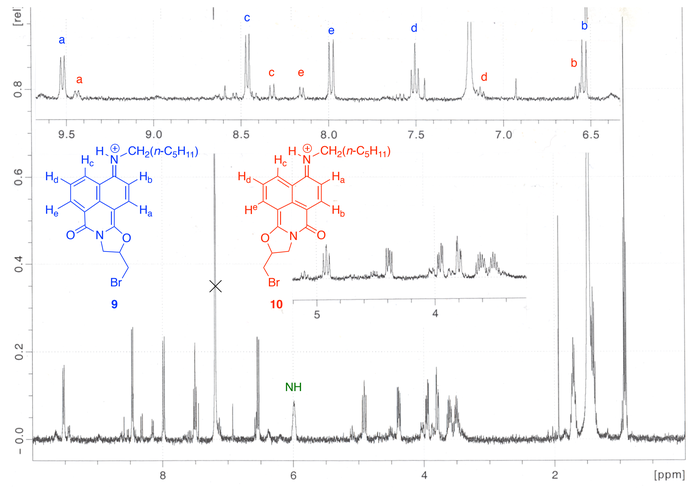
On the addition of one equivalent of bromine to *N*-allyl-4-butylamino-1,8-naphthalimide (**6**) in dichloromethane, the red color of the halogen persisted for several hours. Reductive work-up of the reaction mixture with sodium sulfite gave two products: a minor product identified as the expected dibromide (**4**), and the major product, identified as ring-brominated compound **7**. Similar results were obtained using one equivalent of bromine in acetic acid. When two equivalents of bromine in dichloromethane were used, the tribromide **5** was obtained as the product of the reaction.





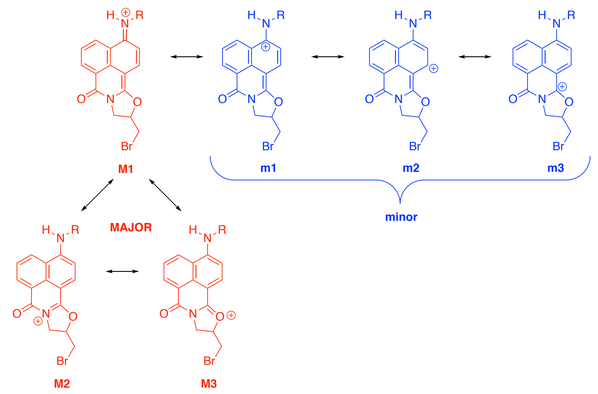


Mechanistic Considerations

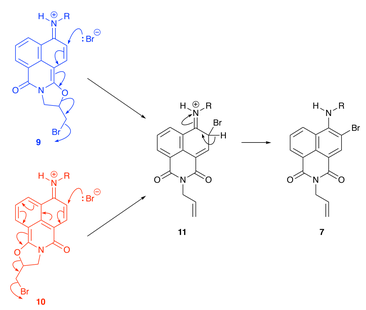


The results of the ^{1}H NMR monitoring show that within minutes of mixing, all the starting naphthalimide has been converted to a 4:1 mixture of two products. The ^{1}H NMR spectrum reveals that neither compound retains the olefinic double bond of the allyl group in the starting material, so the alkene must have reacted with the halogen. In addition, there are now two resonances close to δ=9.5 ppm, at least 1 ppm downfield from any resonance in the starting naphthalimide. On the other hand, both ions retain the resonance near δ=6.5 characteristic of the 4-amino-1,8-naphthalimide skeleton. Based on these observations, we propose that the most reasonable structures for these products are the cations **9** and **10**, respectively

The simplest rationalization consistent with these observations is that the naphthalimide ring system participates in the reaction as a neighboring group. Taking cation **9** (the major regioisomer) as the model, we see that the cation is strongly delocalized. There are three major "onium ion" contributors to the structure of the cation (**M1**, **M2** and **M3**), and three minor "carbocation" contributors (**m1**, **m2** and **m3**). In **M2** and **M3**, the naphthalene ring of the original 4-amino-1,8-naphthalimide is intact, so this can be used to rationalize the retention of the resonance at 6.5 ppm. In contributor m2, on the other hand, there is a positive charge at C2, which should shift the resonance of proton a well downfield from its position in the starting material.



Approximately 60% of the starting material is converted to the observed products **5** (major) and **6** (minor) within the first few minutes. The formation of **5** as the major product this early in the reaction means that the reaction products cannot be formed directly from the bromonium ion, so another intermediate must be involved in the reaction. For the reasons above, we believe that this intermediate is the mixture of cations **9** and **10**. The slower conversion of these ions to the final product may be a result of solubility: the cation mixture frequently precipitates from the solution, so the rate of the final conversion may be determined by the the rate at which the cation returns to solution.



A reasonable mechanism for the formation of the product with the intact allyl group is shown at left. In this case, the species that attacks the cation is the bromide anion, which leads to the iminium ion **11**. Since it is generated in the same step as the addition, overall, bromide anion is a catalyst for the formation of **11**. The tautomerization of the iminium ion then completes the formation of **7**.

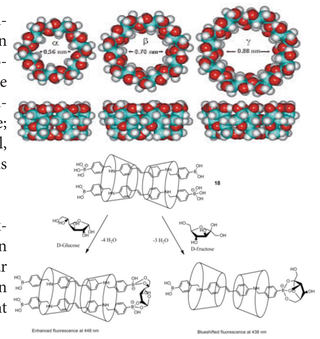
A New Direction

Encapsulated fluorescent naphthalimides, I

Cyclodextrins

Cyclodextrins are produced from starch by a series of enzymatic reaction and are used in pharmaceutical, food, drug delivery, agriculture and genetic engineering. It comes in three forms: alpha, beta, and gamma cyclodextrins.

Cyclodextrins interact with a range of other molecules to form supramolecular assemblies. The fluorescent cyclodextrin in aqueous solution alone usually adopts a self-inclusion conformation in which the chromophore is located in the interior of the cyclodextrin cavity. We can vary the properties of the inclusion compounds by varying the number and location of positively-charged and negatively-charged groups on either side; if these are equal, for example, the molecule will be electrically neutral, which we expect will mean that its mobility during gel electrophoresis will be determined by its complexation partner.



To date, most workers have specifically focused on the beta cyclocloddextrin, which has a diameter of 0.70Å The larger diameter allows insertion of particularly larger molecules. These molecules contribute to molecular recognition in terms of size shape and hydrophobicity. This cyclodextrin has already been used successfully by other workers to form a fluorescent rotaxane.

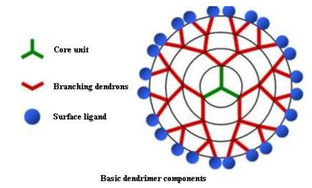
Encapsulated fluorescent naphthalimides, II

Dendrimer-based fluorescent dyes: probes of biomlecules

An alternative potential method for forming fluorescent molecules that do not, for example, intercalate into the DNA double helix. Naphthalimide dyes are well known for their propensity to intercalate, which is responsible for the antineoplastic action of amonifide, for example. The photophysics of intercalating naphthalimide dyes with DNA has been extensively studied, but the same studies where the dye is complexed to, but not intercalated into the double helix aere not available.

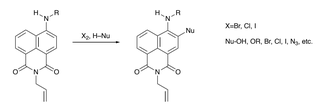
We believe that an approach to obtaining these types of molecules is to incorporate dendrimers with charged groups into the fluorescent probe. By modulating the positive and negative charges on the probe (e.g., by changing pH), we expect to see the effects of major- and minor-groove binding on the fluorescence properties of the dye.

Although this has been accomplished by other research groups, we have been less successful. The problem with this synthetic pathways is that it requires a lot of polymer chemistry and since there is not a lot of experience with polymer chemistry within the group this has turned out to be challenging.

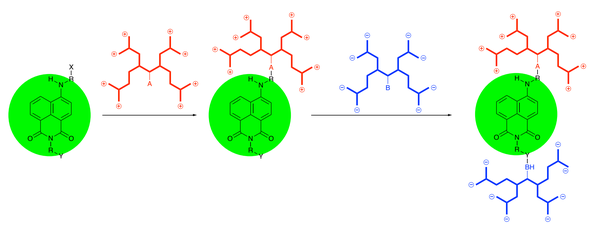


Future Directions

- The results of our experiments with bromine and the *N*-allyl-4-alkylamino-1,8-naphthalimides suggests a potential method for using nucleophiles to substitute the parent ring system at the 3- position. Typical nucleophilic groups include –OH, –OR (from alcohols), $–N_3$, –Br, –Cl, –I, and so on.



- Our problems with the synthesis of dendrimer-based fluorescent dyes may be alleviated by using pre-formed dendrimers with groups allowing derivatization by suitable fluorescent naphthalimide precursors. Again, by choosing dendrimers with different charge types, it may be possible to modulate the photophysics of the fluorophore.



Acknowledgments

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^{1}H NMR spectra were recorded by Ms. Kelsey L. Dunkle Smrstick.