A Final Report on the Scholarly Activity Improvement Fund
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by

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Title: The Study of DNA Cleavage Activity of Diazonium Salts
1. **The results of the project**

My research plan is to investigate structure-activity relationships in the cleavage of DNA by arenediazonium salts. A series of arenediazonium salts with various kinds of substituents, different in electronic nature and hydrophilic nature, on the phenyl ring were selected. Syntheses of these compounds, since they are not available commercially, are the major task of this research. Last year a chemistry-major student worked with me on the synthesis of these compounds. So far we have synthesized two arenediazonium salts. They are 4-fenoldiazonium tetrafluoroborate and 4-benzoicaciddiazonium tetrafluoroborate. Their structures are listed below. The $^1$H NMR spectra of these two compounds are shown in Figure 1 to Figure 2.

![Chemical structures](image)

4-benzoicaciddiazonium tetrafluoroborate

4-fenoldiazonium tetrafluoroborate
Figure 1. $^1$H NMR spectrum of 4-benzoicacid Diazonium tetrafluoroborate in D$_2$O.
Figure 2. $^1$H NMR spectrum of 4-fenoldiazonium tetrafluoroborate in D$_2$O.
Arenediazonium salts are chosen in this proposed research, because they have been shown to be water soluble and thermally stable, and earlier studies have shown that benzenediazonium salt was able to cleave DNA in the presence of cuprous salts. In this proposal, we intend to synthesize a series of arenediazonium salts with various kinds of substituents, different in electronic nature and hydrophilic nature, on the phenyl ring and study their activities in the cleavage of DNA. The selection of the substituents will range from electron-donating groups, such as –CH₂CH₃, –OCH₃, –OH, –N(CH₃)₂, to electron-withdrawing groups, such as –Cl, –CO₂H, –COCH₃, –CF₃. Among these substituents, –OH and –CO₂H are hydrophilic, and –CH₂CH₃ and –CF₃ are hydrophobic. So far we have synthesized two arenediazonium salts with a hydrophilic group attached to the phenyl ring. The ¹H-NMR, IR and melting point of the two compounds were obtained. The results showed that the two compounds we got are actually 4-benzoicaciddiazonium tetrafluoroborate and 4-fenoldiazonium tetrafluoroborate.

With the two arenediazonium salts successfully synthesized and their identification verified, the electrophoresis experiments were started. 1% agarose electrophoresis gels were made, and a Tris-EDTA running buffer as well as a 50% glycerol loading buffer were made to be used for running with the electrophoresis gel to attempt cleaving DNA with the synthesized arenediazonium salts. The DNA samples were prepared by diluting with the Tris-EDTA buffer and taking a portion of this into Eppendorf tubes to give a final concentration of 500 ng/mL. The DNA samples were incubated with or without exposure to the synthesized salt and all were incubated for 1 hour. The loading buffer was then added and the samples added to the gel. The gel was
run under standard electrophoresis parameters for 40 minutes. No obvious cleavage of DNA was observed. The conditions of the electrophoresis experiments need to be further investigated. More electrophoresis experiments with various concentrations of arenediazonium salts will be performed in the future. The results of the successful electrophoresis study of these compounds will shed light on the structure-activity relationships of arenediazonium salts for DNA cleavage. This project will answer questions not yet answered by other published results. For example, what would be the effect on DNA cleaving activity of arenediazonium salts by introducing a substituent on the phenyl ring? And what kind of substituent would make it a more effective DNA cleaving reagent, a hydrophilic or a hydrophobic group, an electron-withdrawing or an electron-donating group? Most importantly, more efficient DNA cleaving reagents may be obtained through this proposed research.

2. Benefit to Individual, Department, College or University

The proposed project needs all kinds of organic techniques as well as techniques of electrophoresis experiments. Students involved get hands-on experience in not only organic synthesis and purification techniques but also electrophoresis experiments. My student, Elaine Hildebrandt, has learned the basic organic synthetic techniques and also has learned the techniques of electrophoresis experiments. This project provides students with good research backgrounds and better employment opportunities, which will attract more students to enroll in our university as chemistry majors. This project also gave me a good start on my new research project.
3. Presenting the Results

The results of this project were presented at the annual UW-Platteville Research/Poster Day and were also presented at the Posters in the Rotunda in 2007. This project will be continued by other chemistry-major students.