

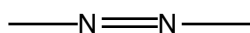
## Experiment 19: Combinatorial Synthesis of Azo Dyes

When a new pharmaceutical is needed to treat a medical condition, modern chemists sometimes utilize a research technique known as **combinatorial chemistry** to find molecules that have the necessary biological activity. In combinatorial chemistry, a large number of structurally related compounds, called *a library*, is generated using a single common reaction. The diversity of the compounds in the library can arise from varying the substituents of the molecule, the functional groups or both. For instance, a library of esters can be generated by reacting a variety of different alcohols and carboxylic acids together. Once a library has been generated, the compounds must be screened for the desired activity. An example of a class of drugs that was explored using combinatorial methods are the benzodiazepines, which are used to treat anxiety disorders.

Two approaches to combinatorial syntheses are employed. In a **parallel synthesis**, each compound is prepared individually, and the tests are performed separately on each compound. In a **split synthesis**, the library of compounds is both prepared and tested in mixtures of the compounds. In the latter case, the results of the tests are complex and usually require **deconvolution**, although a savings is realized in terms of time and materials. In either case, combinatorial chemistry allows the researcher to draw conclusions about the relationship between structure and function in molecules. The technique has been so successfully exploited that it is now utilized in other areas of chemistry besides pharmaceutical research, including catalysis and materials research.

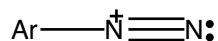
In this experiment, your lab section will generate a library of dye compounds using a parallel synthesis. Each student will carry out the same reaction, but every student in the laboratory will use a different pair of reactants. To screen the resulting products, each student will make a dye bath from his or her compound and use it to dye a variety of natural and man-made fabrics.

All of the dyes that will be synthesized contain an azo group:



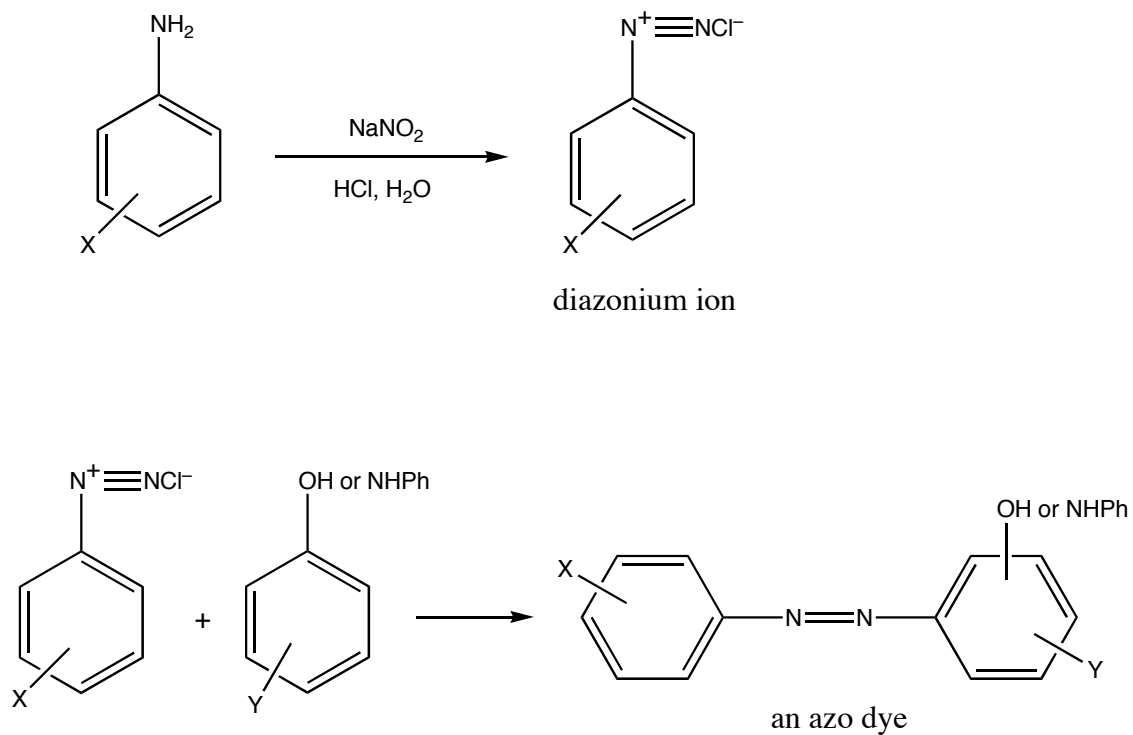
The azo group joins together aromatic rings, resulting in an extended system of conjugated multiple bonds, which is characteristic of all organic dyes.

Azo compounds are synthesized by first reacting a primary aromatic amine with nitrous acid,  $\text{HNO}_2$ , to produce an aryldiazonium ion:


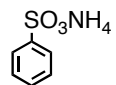


This ion can couple with a nucleophilic aromatic compound, such as an aryl amine or a phenol, to produce the azo compound. On page 4, the top row of Table 1 shows the primary amines that will be used for one reactant, and the first column in the table shows the aryl phenol and secondary amine compounds that will be used for the other starting material. The general sequence of reactions is shown at the top of page 2.

Dye molecules bind to the fiber molecules in cloth in one of the following ways: via electrostatic attraction, by van der Waals forces, through hydrogen bonding or even by covalent bonds. In general, the greater the number of polar groups that are present in the fiber molecule, the easier the fabric is to dye. A variety of methods are available for



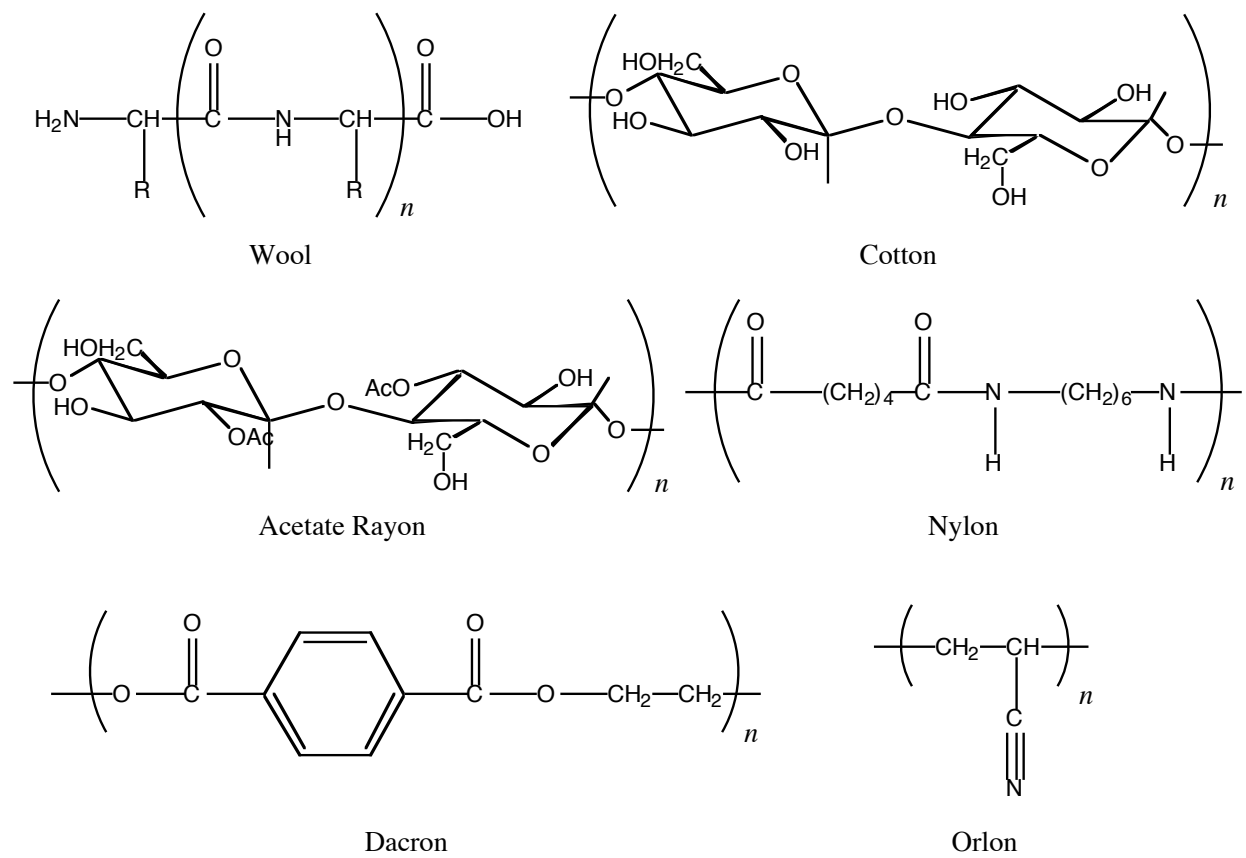
X = SO<sub>3</sub> or NO<sub>2</sub>

Y = CO<sub>2</sub>H or  or 

**Figure 1. Generalized synthesis of an azo dye**

dyeing cloth, depending on the nature of the dye itself. *Direct dyes* attach themselves to the fibers directly; that is, in the absence of any facilitating molecules.

In today's experiment, you will use your azo dye product as a direct dye to color a number of different fabrics whose structures are shown on page 3.



**Figure2. Structures of fabric molecules**

**Table 1. Illustration of the combinatorial synthesis of azo dyes with structures**


**Table 2. Illustration of the combinatorial synthesis of azo dyes with chemical names**

	2-aminobenzene-sulfonic acid	3-aminobenzene-sulfonic acid	4-aminobenzene-sulfonic acid	4-nitroaniline
1-naphthol				
2-naphthol				
salicylic acid				
8-anilino-1-naphthalene-sulfonic acid, ammonium salt				

## Procedure for making an azo dye using an aminobenzenesulfonic acid

### *Diazotization of Aminobenzenesulfonic acid*

In an 18 x 150 mm test tube, dissolve 0.49 g of 2-, 3- or 4-aminobenzenesulfonic acid in 5 mL of 2.5% Na<sub>2</sub>CO<sub>3</sub> solution. Place the test tube in a beaker of boiling water on a hot plate and stir to completely dissolve the solid. Cool the solution under the tap, and then add 0.2 g of sodium nitrite, NaNO<sub>2</sub>. Stir until it is dissolved. Add this solution dropwise to a 25 mL flask that contains about 3 g of ice and 0.53 mL of concentrated HCl. **Use care when handling concentrated acids. Wear gloves!** Place the flask in ice, and a white precipitate of diazonium salt should appear. If solid does not appear after 20 minutes, stir the solution with a glass rod, scratching the sides and bottom of the beaker. The product is not collected but is used in the preparation of the dye while in suspension. It is more stable than most diazonium salts and will keep for a few hours.

### *Coupling Reaction*

Weigh out 0.0026 mol of your assigned coupling agent (1- or 2-naphthol, salicylic acid or 8-anilino-1-naphthalenesulfonic acid, ammonium salt) and place it in a 50 mL flask. Add 2 mL of 2.5 M NaOH solution. Place the flask in an ice bath. Add the suspension of diazonium salt (solid *and* liquid) to this solution in small portions using a Pasteur pipet. Stir after each addition. You should notice a change in color. Remove the reaction mixture from the ice bath, and allow it to sit for 10 minutes with occasional stirring. Heat the mixture on a hot plate to boiling, at which point the solid material should dissolve. Add 1 g of NaCl and continue to heat to dissolve it. Remove the flask from the hot plate and let it cool to room temperature, then place it on ice. Collect the product by vacuum filtration on a Buchner funnel. Wash the solid with two 2 mL portions of saturated NaCl solution.

If no solid material is collected, ask your TA for a piece of Grade 2 filter paper. Re-filter the filtrate using this fine grade paper.

## Procedure for making an azo dye using 4-nitroaniline

### *Diazotization of 4-Nitroaniline*

In an 18 x 150 mm test tube, place 1.5 mL of water and then add 1.5 mL of concentrated HCl. **Use care when handling concentrated acids. Wear gloves!** Set this test tube in an ice bath. In another 18 x 150 mm test tube, place 0.7 g of 4-nitroaniline, 0.38 g of sodium nitrite, NaNO<sub>2</sub>, and 1.5 mL of water. Stir the mixture thoroughly with a glass rod, then quickly add this suspension (solid *and* liquid) to the ice-cold HCl solution using a Pasteur pipet. Keep the test tube in the ice bath, and stir the reaction mixture with a glass rod while making this addition. After the addition is completed, continue to stir occasionally for 10 minutes. Filter the solution through a filter pipet, and collect the filtrate, which contains the diazonium salt, in a 13 x 100 mm test tube.

### *Coupling Reaction*

Weigh out 0.0051 mol of your assigned coupling agent (1- or 2-naphthol, salicylic acid or 8-anilino-1-naphthalenesulfonic acid, ammonium salt) and place it in a 50 mL flask. Add 10 mL of 2.5 M NaOH solution. Place the flask in an ice bath. Add the

solution of diazonium salt to the solution of the coupling agent dropwise with stirring. Allow the reaction mixture to sit for 10 minutes on ice. If a color change occurs, make a note of it. Add 1.5 mL of concentrated HCl slowly until the pH is between 3 and 4, as indicated by Alkacid paper. Add 1 g of NaCl and heat the mixture to the boiling point on a hot plate. Remove the flask from the hot plate and let it cool to room temperature, then place it on ice. Collect the product by vacuum filtration on a Buchner funnel. Wash the solid with two 2 mL portions of water.

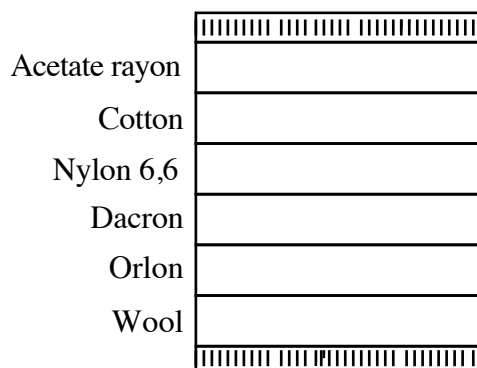
If no solid material is collected, ask your TA for a piece of Grade 2 filter paper. Re-filter the filtrate using this fine grade paper.

### Procedure for dyeing the fabrics

You will be given a rectangular piece of testing cloth which contains a number of different strips of fabric. The figure below explains which strip corresponds to which fabric type. The bottom strip (wool) is light beige in color and the upper strip (acetate rayon) is white. There should be a black line near the acetate rayon strip.

Prepare the dye bath by mixing 50 mg of your dye with 20 mL of water in a 150 mL beaker. [Note: If you did not obtain solid material, place the pieces of filter paper that were used to collect the product in the beaker with the water.] Heat the mixture on a hot plate to the boiling point. Once the mixture is boiling, stir it with a glass rod.

Immerse a piece of the test fabric in the bath, and use the glass rod to keep it submerged in the boiling mixture for *exactly* 10 minutes. Add water from a wash bottle as needed to maintain the volume of liquid in the bath. Using tweezers, remove the fabric from the dye bath, allow it to cool for 5 minutes, then wash it thoroughly under *cold* running water. Pat the cloth dry with a paper towel.



Dispose of the dye bath and any solid azo dye compounds into the **Laboratory Byproducts** jar.

### Comparing the Dyes

Attach your dyed piece of cloth to the small card provided by stapling it on both ends. Write your name and the reactants on the back, and give it to your lab instructor. He/she will arrange these fabrics in a 4 x 4 grid according to the tables shown on page 4. Your TA will take a digital picture of the fabrics that will be e-mailed to each student in the lab section. Please be sure there is enough room in your mailbox to receive the .jpg file.

Name \_\_\_\_\_ Date \_\_\_\_\_

T. A. \_\_\_\_\_ Lab period \_\_\_\_\_

**Results and Calculations** (to be handed in at the next lab period)

What conclusions can you draw about the relationship between chemical structure and dye color from the results of the combinatorial experiment?

Which dye(s) produced the most consistent color from one fabric to the next?

Which dye(s) produced the least consistent color from one fabric to the next?

What conclusions can you draw about the relationship between the structure of the fabric molecule and the intensity with which the azo dyes color the fabric?

Which dye(s) adhered to the greatest number of fabrics?

Which dye(s) adhered to the smallest number of fabrics?

Write a balanced equation for the preparation of the diazonium ion that *you* made. Draw a possible structure for your azo dye.