

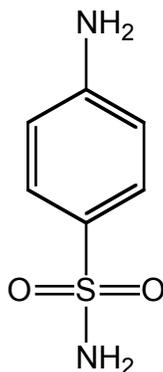
Sulfa Antibiotics - Synthesis of Sulfanilamide

INTRODUCTION

Many people think of the term chemotherapy as relating to specific modern drug treatments used to fight cancer. Actually, modern chemotherapy began in the early 1900's with the work of Paul Ehrlich, a German medical doctor and researcher, who is known as the father of immunology and chemotherapy. Ehrlich hypothesized that certain dyes could selectively "stain" harmful bacteria cells without harming host cells. He referred to such compounds as "magic bullets" and coined the term chemotherapy as a general descriptor for chemical remedies targeted to selectively kill infectious cells. This definition includes all antibiotics as well as the more recently developed chemotherapeutic agents targeted to selectively kill cancer cells. Ehrlich's pioneering work on antibiotics, led, among other remedies, to the discovery of salvarsan, an organoarsenic compound found to be highly effective for treatment of syphilis (replacing mercury which had often disastrous side effects). He was awarded the Nobel Prize for medicine in 1908.

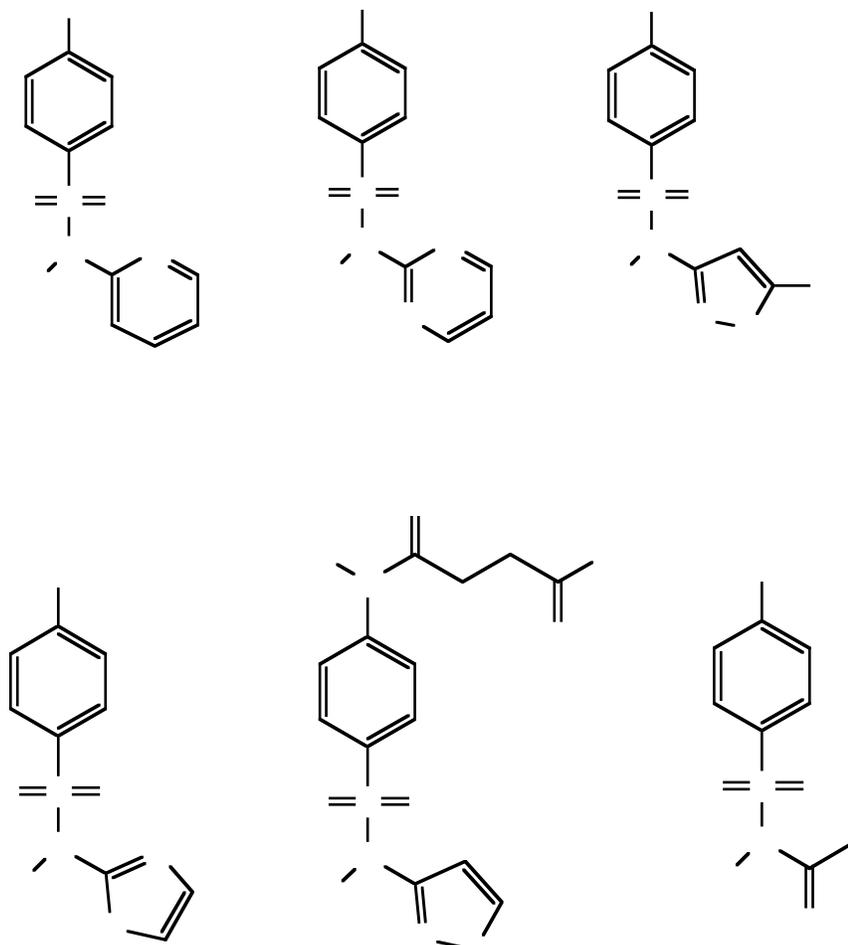
Building on Ehrlich's early work, Gerhard Domagk, a medical doctor employed by a German dye manufacturer made a breakthrough discovery by finding that a dye known as prontosil, dosed orally, was effective in curing life threatening streptococci infections in humans. He made the discovery in a desperate, but successful attempt to save his daughter who was dying of a streptococci infection. Domagk's discovery led to an extremely productive period of discovery in antibiotics. He was awarded the Nobel Prize for medicine in 1939.

In 1936, a year after Domagk's discovery of the anti-streptococcus activity of the dye prontosil, Ernest Fourneau of the Pasteur Institute in Paris discovered that prontosil breaks down in the human body to produce sulfanilamide which is the active agent that kills streptococcus bacteria. Fourneau's discovery triggered a flurry of research on structural derivatives of sulfanilamide which resulted in development of a family of highly successful antibiotics that have saved millions of lives.



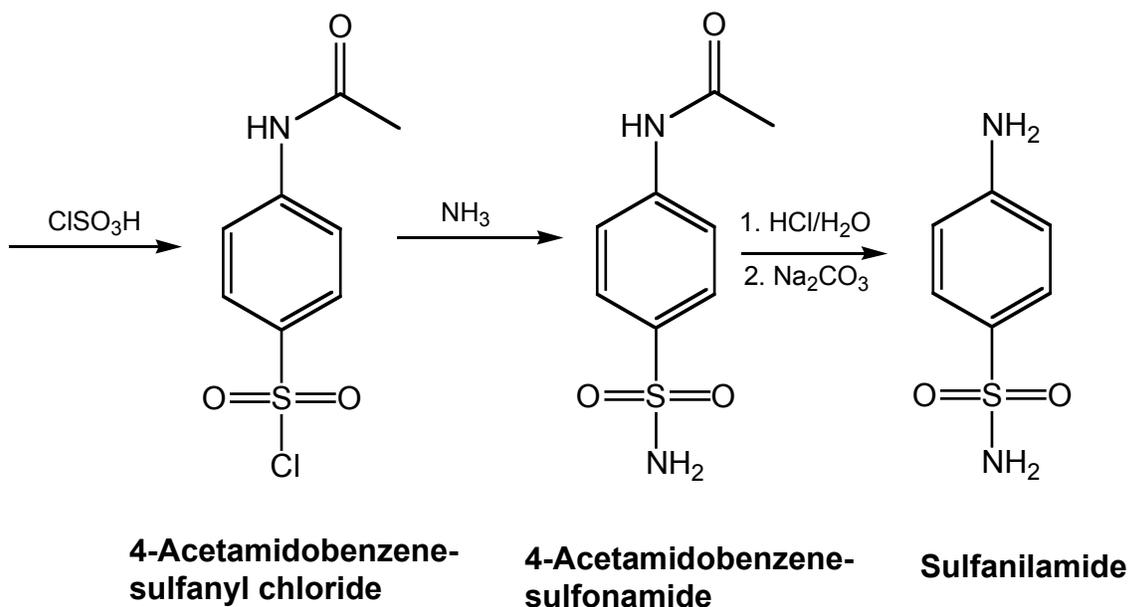
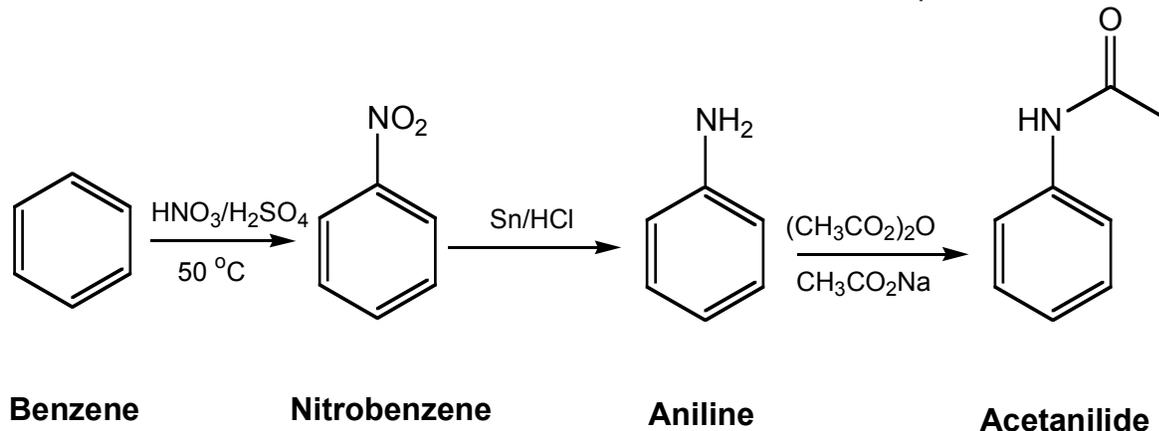
Sulfanilamide

Sulfapyridine was shown to be effective against pneumonia in 1938. Sulfacetamide found highly successful use in fighting urinary tract infections starting in 1941. Succinoylsulfathiazole has been used against gastrointestinal tract infections since 1942. Sulfathiazole was used very effectively during World War II to fight infection in soldiers with battle wounds. Sulfanilamide itself, a potent antibiotic, never gained widespread use due to its greater human toxicity versus its various derivatives.



The total synthesis of sulfanilamide from benzene can be carried out in six steps using reactions that are very familiar to intermediate level organic chemists. In addition to providing a great example of the synthetic utility of these reactions, the synthesis demonstrates the use of protecting group chemistry, and taking advantage of steric and electronic directing effects (in electrophilic aromatic substitution) and differing rates of

hydrolysis to optimize the yield of the desired product. The synthesis to be performed here will start from nitrobenzene to make sulfanilamide in five steps:



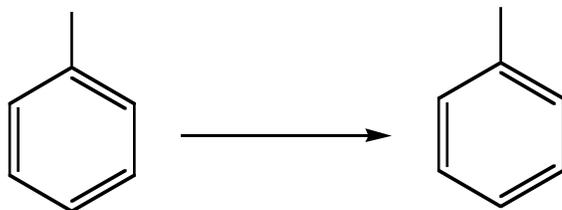
If we have sufficient time this semester, you will have an opportunity to measure the antibiotic activity of sulfanilamide against selected strains of pathogenic bacteria. You will perform standard zone of inhibition assays using filter paper discs impregnated with sulfanilamide and placed on lawns of growing bacteria. You will be provided with information on the biological assay conditions later in the semester.

SYNTHETIC APPROACH

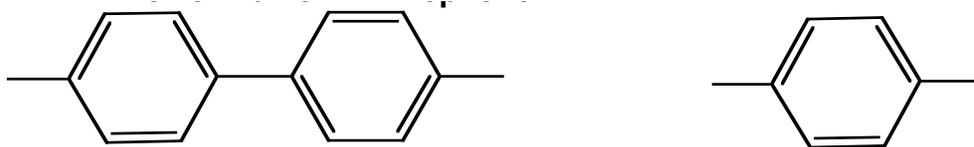
Preparation of Aniline via Reduction of Nitrobenzene

A wide range of nitrobenzene derivatives can be easily made via regioselective nitration of substituted benzenes. Reduction of the nitrobenzene derivatives using tin/hydrochloric acid provides a convenient and high yielding route to a wide variety of

aromatic amines. The simplest case is the synthesis of aniline from nitrobenzene. In this reaction, tin metal serves as the reducing agent and is oxidized to stannic chloride, SnCl_4 .

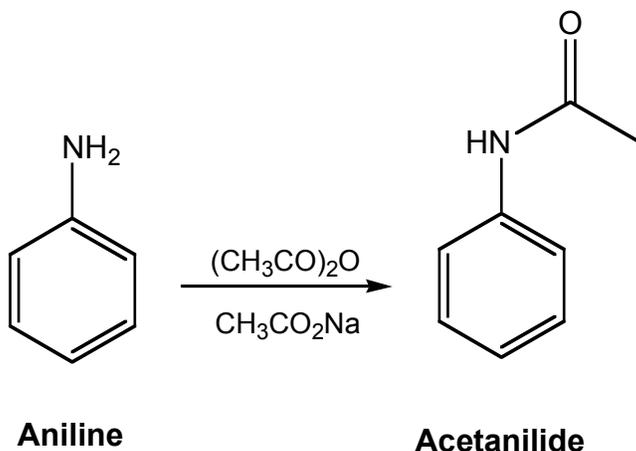


The crude aniline product is contaminated with unreacted nitrobenzene as well as benzidine and 4-aminophenol formed as byproducts in the reduction. It is purified using a combination of steam distillation and extraction.



Preparation of Acetanilide

Aniline can be easily converted to acetanilide by acetylation reaction using acetic anhydride in the presence of sodium acetate.

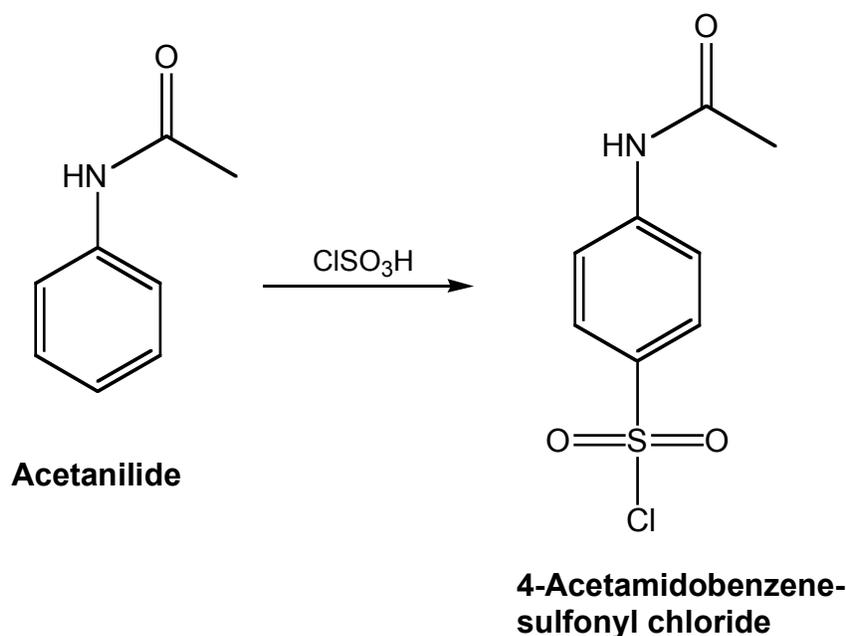


The purpose of this step is to protect the amine functional group from entering into unwanted reactions during the subsequent chlorosulfonation and amination steps.

Without protection, the free amine, under the strong acid conditions of chlorosulfonation, would protonate or react with strong Lewis acids present (such as SO_3), resulting in deactivation of the ring toward chlorosulfonation and loss of regioselectivity. Using amidation as a protecting group strategy allows the free amine to be regenerated under mild conditions in the final step without hydrolysis of the more hydrolytically stable sulfonamide group.

Preparation of 4-Acetamidobenzenesulfonyl Chloride

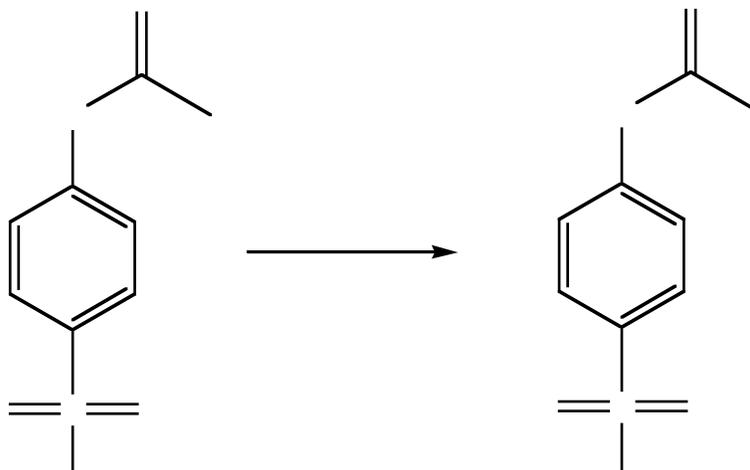
The chlorosulfonyl group can be introduced para to the acetamide group in one step using the electrophilic aromatic substitution reaction known as chlorosulfonation:



This reaction is more complicated than it looks at first inspection. The electrophile that initially adds to the ring is probably SO_3 , forming the sulfonic acid. The initially formed substitution product is the sulfonic acid. Substitution is essentially all para due to combined electronic and steric effects. The sulfonic acid is then converted to 4-acetamidobenzenesulfonyl chloride by reaction with excess chlorosulfonic acid, generating sulfuric acid as the co-product.

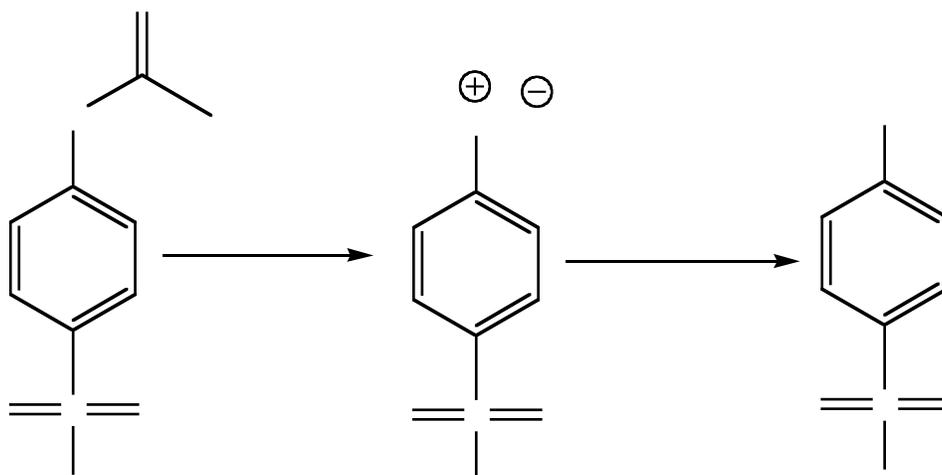
Preparation of 4-Acetamidobenzenesulfonamide

4-acetamidobenzenesulfonyl chloride is converted to the corresponding sulfonamide by reaction with aqueous ammonia. The reaction equation is shown at the top of the following page. This step is carried out immediately after isolating the 4-acetamidobenzenesulfonyl chloride to prevent losses due to hydrolysis of the sulfonyl chloride.



Preparation of Sulfanilamide

The acetamide group of 4-acetamidobenzenesulfonamide can be hydrolyzed under acidic conditions without affecting the sulfonamide group, which hydrolyzes much more slowly. As a final step, the acidic hydrolysis solution is neutralized with sodium carbonate to isolate sulfanilamide:



EXPERIMENTAL

Synthesis of Aniline

Tin powder (13.1 g) and nitrobenzene (5.2 mL) are added to a 250 mL round-bottom flask equipped with a football-shaped magnetic stir bar and a thermometer extending down into the flask to above the stir bar. The slurry is rapidly stirred while concentrated hydrochloric acid (28 mL) is added to the flask (all at once). The temperature is monitored and the flask is cooled in an ice bath as necessary to maintain the temperature below 60 °C. After the exothermic stage of the reaction is complete (about 15 min) the thermometer is removed, the flask is fitted with a water-cooled reflux condenser, and the solution is heated at reflux for 20 minutes with constant stirring.

The solution is cooled in an ice bath and 6 M NaOH solution (50 mL) is slowly added. The pH of the solution is checked (using pH paper) to confirm that it is basic. If necessary, more NaOH solution is added to achieve a basic solution. The flask is fitted for simple distillation and steam distilled until the condensate no longer appears oily. About 35-40 mL of distillate should be collected.

Concentrated HCl (5 mL) is added to the distillate, and the pH is checked to confirm that it is acidic. More acid is added if necessary. The aqueous solution is transferred to a separatory funnel and extracted sequentially with two 15 mL portions of diethyl ether. This removes any unreacted nitrobenzene; note, the protonated aniline remains in the aqueous layer! The aqueous layer is transferred to an Erlenmeyer flask containing a magnetic stir bar, and cooled in an ice-water bath. The aqueous solution is made basic by slow addition of 12 M NaOH solution until the solution tests basic to pH paper. Next, this basic solution is saturated with NaCl by addition of solid NaCl with stirring. The solution is then transferred to a clean separatory funnel and extracted with two 15 mL portions of diethyl ether; note, aniline should now be in the organic layer. These combined organic extracts are then dried over anhydrous sodium sulfate. The dried solution is transferred to a dry, weighed round-bottom flask, and the diethyl ether is removed on a rotary evaporator. The % yield is calculated and IR and NMR (CDCl_3) spectra of the aniline product are obtained.

Synthesis of Acetanilide

Hydrochloric acid (0.4 M, 100 mL) is added to a 250 mL Erlenmeyer flask equipped with a magnetic stir bar. Aniline (3.6 mL) is added to the flask with stirring and the mixture is warmed to 50 °C. A separate solution of sodium acetate is prepared by dissolving 6.0 g of the sodium acetate trihydrate in 20 mL of water. In a separate clean, dry beaker or flask, 4.4 mL of acetic anhydride is measured out.

The acetic anhydride is added in one portion with rapid stirring to the warm solution of aniline hydrochloride salt, and immediately after, the sodium acetate solution is added in one portion. The mixture is stirred and cooled in an ice bath until the crystalline product

completely precipitates. The acetanilide is collected by vacuum filtration, washed with a small amount of ice-cold water and air dried on the filter funnel. The dry product is weighed, % yield calculated, and IR and NMR (DMSO- d_6) spectra are obtained.

Synthesis of 4-Acetamidobenzenesulfonyl Chloride

Dry acetanilide (2.7 g) is placed in a clean, dry, 50 mL round-bottom flask equipped with a stir bar and fitted with a Claisen adapter. The Claisen adapter is in turn fitted with a dropping funnel on the straight arm and a vacuum distillation adapter on the side arm connected to a water aspirator as a vapor sweep to remove HCl evolved during the reaction (see figure on page 10). The dropping funnel is removed, set into a beaker as a secondary container and taken to a fume hood where chlorosulfonic acid (8.0 mL) is added. **(Caution! Wear gloves!)** The dropping funnel is stoppered and carefully checked (while in hood) to ensure that there is no leaking, after which it is stoppered and carefully carried back to the bench (in the beaker) where it is fitted back onto the Claisen adaptor.

The reaction flask is cooled in a cold water bath (not an ice bath) maintained between 10 and 15 °C. The stopcock of the dropping funnel is opened fully to add the chlorosulfonic acid rapidly all at once. The solution is rapidly stirred keeping it in the cooling bath with the water temperature below 20 °C. After the acetanilide is mostly dissolved and the initial exothermic reaction has subsided, the cooling bath is removed and the solution allowed to warm to room temperature with continuous stirring. Then the flask is heated in a hot water bath at 70-80 °C for 20 min to complete the reaction.

The reaction mixture is cooled in an ice bath to room temperature or below. The reaction flask is transferred to a fume hood where it is carefully poured into a 500 mL beaker containing 150 grams of crushed ice. **(Caution: wear gloves and pour the reaction solution over the ice slowly and carefully to avoid splattering.)** The ice slurry is stirred using a stir rod to prevent formation of large lumps as the product precipitates. Any large lumps that form should be carefully broken up using the stir rod. The precipitate is collected by vacuum filtration and washed with 15 mL portions of cold water until the filtrate tests neutral to pH paper. The product is air dried on the filter funnel by pulling air through it for at least 10 minutes. A small sample of the product is saved to obtain IR and NMR spectra. The remainder of the product is used immediately for synthesis of 4-acetamidobenzene-sulfonamide.

Synthesis of 4-Acetamidobenzenesulfonamide.

The crude 4-acetamidobenzenesulfonyl chloride is placed in a 125 mL Erlenmeyer flask and 15 mL of concentrated (28%) aqueous ammonia (“ammonium hydroxide”) is added. **(Caution: a vigorous reaction may occur if the crude starting material was not previously washed enough to remove strong acid impurities.)** The resulting thick suspension is stirred with a stirring rod to break up any lumps, and the mixture is heated

at 70-80 °C for 30 minutes. The mixture is then cooled in an ice bath, and the product is collected by vacuum filtration. The product is washed with ice cold water and air dried. The product is weighed and % yield is determined. An IR and ^1H NMR spectrum is obtained.

Synthesis of Sulfanilamide.

4-acetamidobenzenesulfonamide from the previous step is weighed and placed in a 50 mL round-bottom flask equipped with a magnetic stir bar. Dilute hydrochloric acid (6 M) is added to the flask in an amount equal to twice the weight of the 4-acetamidobenzene-sulfonamide. The flask is fitted with a cold water condenser and heated at reflux with constant stirring for 45 minutes, after which it is allowed to cool to room temperature. If any solid appears upon cooling, the mixture is reheated at reflux for another 15 minutes. After cooling, the reaction mixture is neutralized by slow addition of a saturated Na_2CO_3 solution with stirring until it tests slightly alkaline to pH paper.

A precipitate may have begun to form during neutralization. Cool the beaker in an ice bath to complete the precipitation of product. It may be necessary to gently scratch the inside bottom of the beaker to induce crystallization. The product is collected by vacuum filtration, washed with a small amount of ice cold water and air dried. A small sample of the crude product is saved, and the remainder is purified by recrystallization by dissolving in a minimum amount of boiling hot water, allowing the solution to slowly cool to room temperature, then further cooling in an ice bath to crystallize as much product from solution as possible. The recrystallized product is collected by vacuum filtration. The product is weighed and % yield is calculated. The overall yield for the five-step synthesis of sulfanilamide from nitrobenzene is calculated and reported. IR and NMR spectra are obtained on the final product.

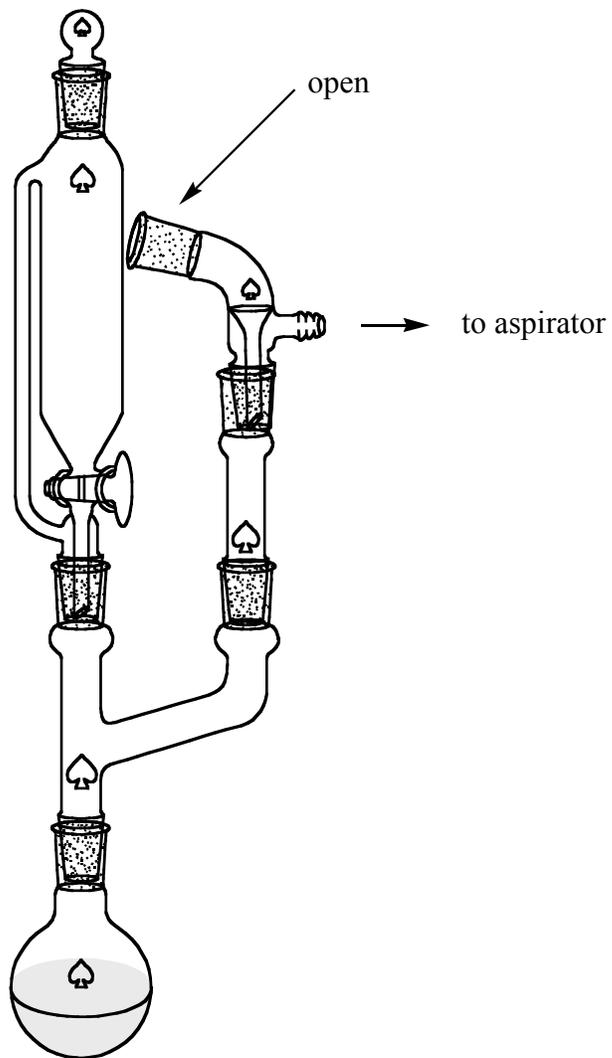


Figure: Reaction vessel with dropping funnel and gas trap.