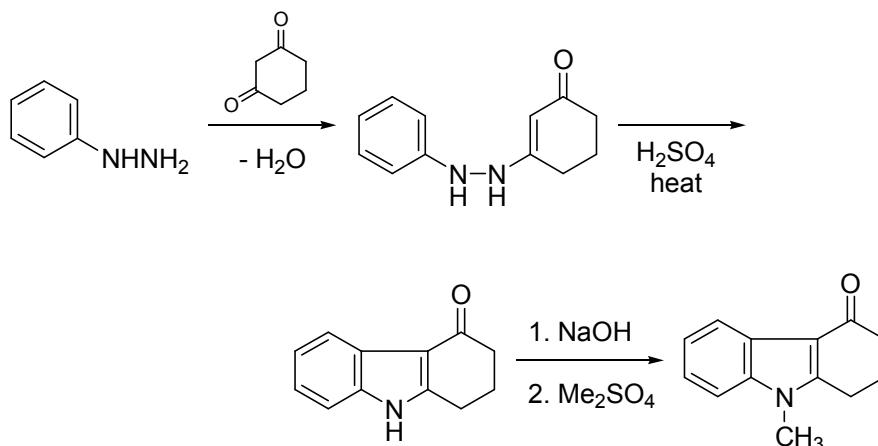


## Fischer Indole Synthesis

### Overall Reaction



### Purpose

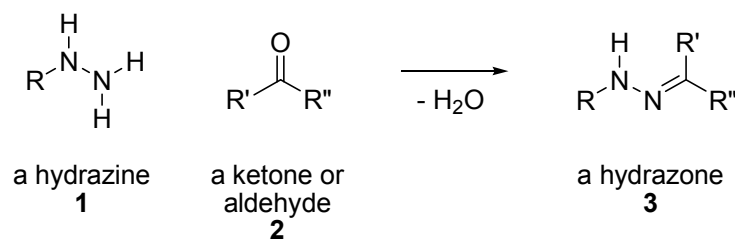
This experiment has the following goals:

- (1) demonstrate a classic heterocyclic reaction – the Fischer indole synthesis
- (2) use two-dimensional NMR to fully assign a  $^1\text{H}$  spectrum
- (3) prepare a precursor for the synthesis of a commercial drug.

### Background

#### Hydrazone Formation

Hydrazone (**3**) are formed by the condensation of a hydrazine (**1**) with an aldehyde (**2**,  $\text{R}' = \text{H}$ ) or ketone (**2**,  $\text{R}' \neq \text{H}$ ) (Scheme 1). In most cases, newly formed hydrazones are stable, solid products. In the early days of synthetic chemistry, unknown aldehydes and ketones were identified by converting them to hydrazones and related derivatives and determining the hydrazone's melting point. Tables of melting points of known hydrazones were then consulted to identify the unknown.



Scheme 1

#### Fischer Indole Synthesis

The Fischer indole synthesis was discovered in 1883 and is a classic method for assembling the indole ring system (**4**) (Figure 1). Despite its early discovery, the Fischer synthesis has withstood the test of time and remains as a favored method for synthesizing indoles. Two pharmaceuticals that may be prepared through the Fischer synthesis include indomethacin (**5**) and ondansetron (**6**), which have anti-inflammatory and anti-nausea activity, respectively.

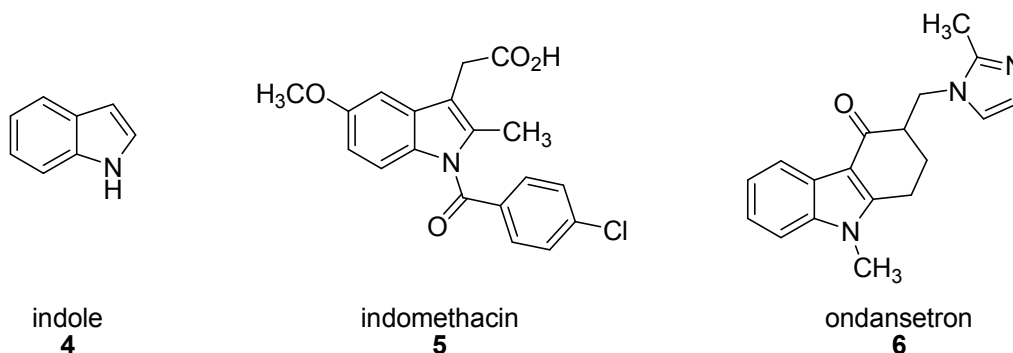
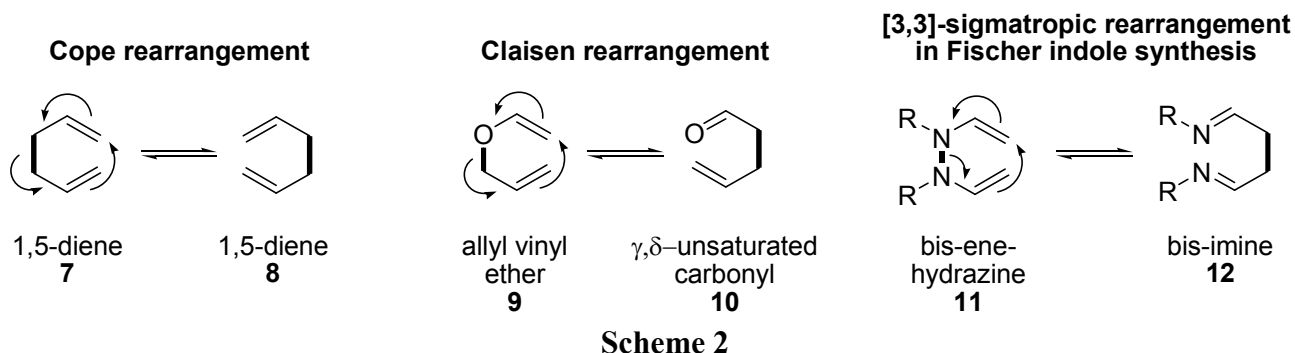
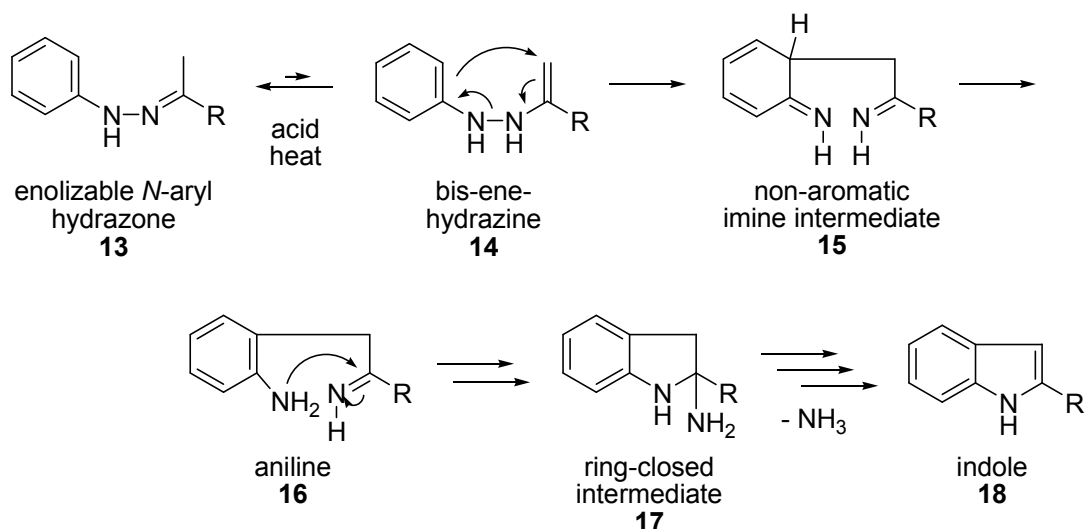


Figure 1

The mechanism of the Fischer indole synthesis involves a [3,3]-sigmatropic rearrangement. As the name implies, sigmatropic rearrangements involve the movement (tropism) of a sigma bond. The numbers in the brackets indicate the number of atoms each end of the sigma bond has moved. The simplest [3,3]-sigmatropic rearrangement is the Cope rearrangement (Scheme 2). In this reaction, a 1,5-diene (**7**) rearranges to form a new 1,5-diene (**8**). In the related Claisen rearrangement, an allyl vinyl ether (**9**) forms a  $\gamma,\delta$ -unsaturated carbonyl (**10**). During the Fischer reaction, a bis-enehydrazine (**11**) rearranges to form a bis-imine (**12**), which undergoes further reaction. All three of these reactions are [3,3]-sigmatropic rearrangements.



The full mechanism of the Fischer indole synthesis starts with an enolizable *N*-aryl hydrazone (**13**), i.e. a hydrazone with an  $\alpha$ -hydrogen and an *N*-aryl group. Hydrazone **13** exists in equilibrium with its less favorable bis-enehydrazine tautomer (**14**). With heating and under acidic conditions, the tautomer undergoes a [3,3]-sigmatropic rearrangement to form a non-aromatic, unstable intermediate (**15**). Tautomerization restores aromaticity. Intramolecular addition of the aniline nitrogen onto an imine forms a new ring. Loss of ammonia aromatizes the second ring and completes the indole synthesis.



Scheme 3

## Multi-dimensional NMR Spectroscopy

Most NMR spectra, like a standard  $^1\text{H}$  spectrum, are considered one-dimensional because they have one axis relating the chemical shift of the observed nucleus. Therefore, two-dimensional NMR has two axes with units of ppm. The most common example of 2D NMR is the  $^1\text{H}$ - $^1\text{H}$  COSY (correlation spectroscopy) spectrum. A COSY spectrum has the same  $^1\text{H}$  spectrum projected on both axes. A sample COSY spectrum for *O*-cinnamyl salicylaldehyde (**19**), a reasonably complex molecule, is shown below in Figure 2.

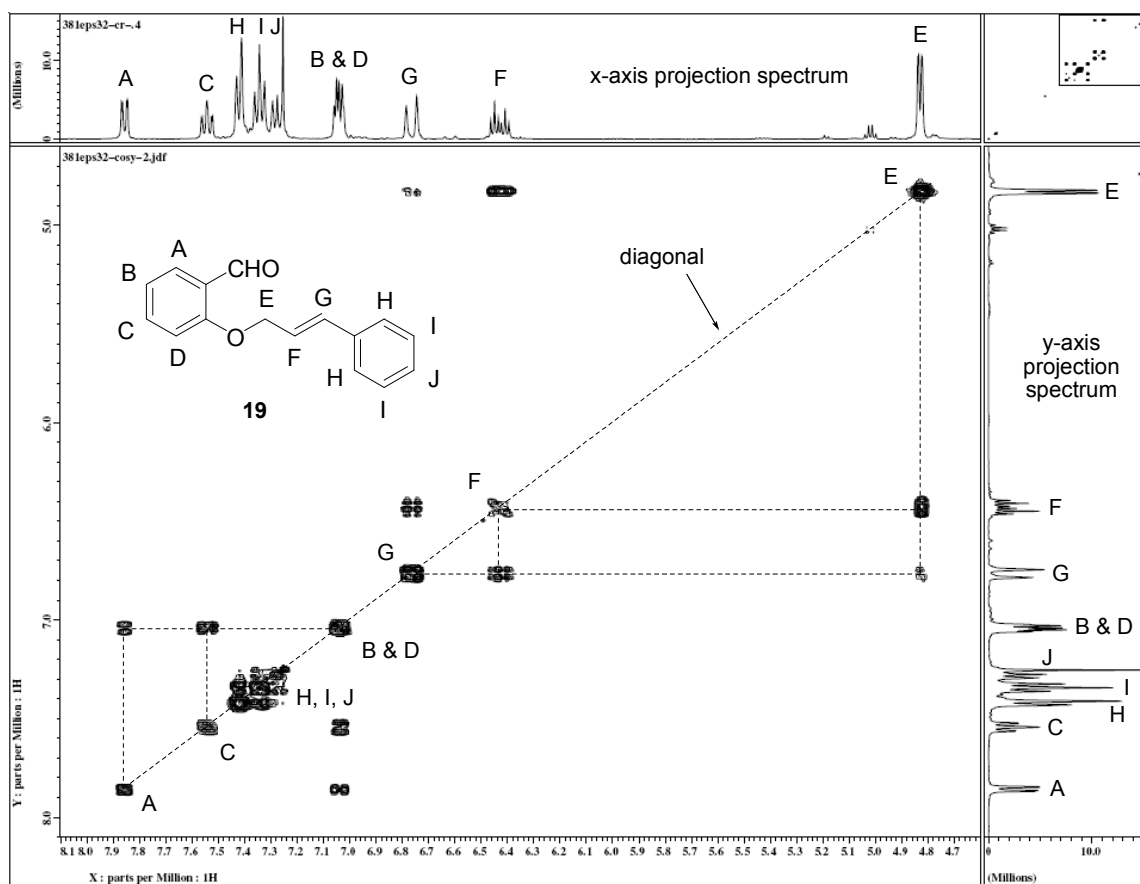


Figure 2

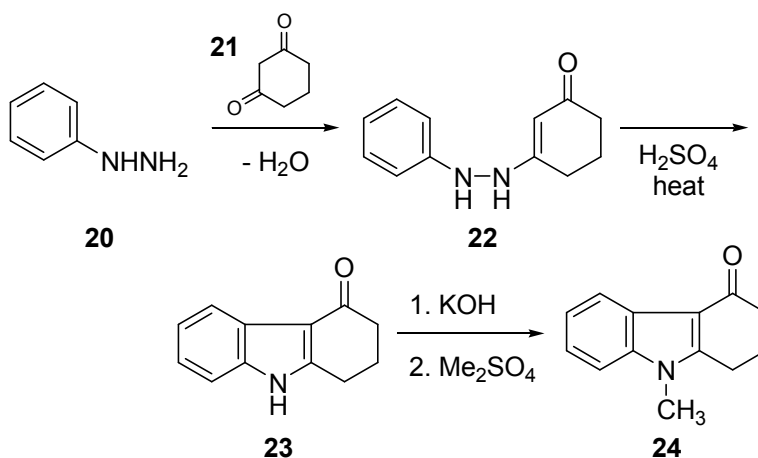
Figure 2 demonstrates all the common features of a COSY spectrum. The standard 1D  $^1\text{H}$  spectrum of the molecule is shown along both axes for reference. These are called *projection spectra*. Each peak in the projection spectra will have a corresponding signal along the diagonal of the COSY spectrum. The central, rectangular region of the spectrum contains the COSY data, which are shown in a top-down view in a contour plot. More intense signals are larger and darker. In addition to the expected peaks on the diagonal, COSY spectra have any number of *off-diagonal* signals. The off-diagonal signals represent *J*-coupling between hydrogens in the molecule and are symmetrically oriented about the spectrum diagonal.

Interpreting COSY spectra requires making connections between the signals on and off the diagonal. For example, in Figure 2,  $\text{H}_\text{G}$  and  $\text{H}_\text{F}$  are vicinal hydrogens on the alkene of **19**. They couple to one another and provide a corresponding off-diagonal peak in the COSY spectrum. The off-diagonal peak is connected to both the diagonal  $\text{H}_\text{F}$  and  $\text{H}_\text{G}$  signals with dotted lines. In turn,  $\text{H}_\text{F}$  and  $\text{H}_\text{E}$  *J*-couple and show an off-diagonal peak. Hydrogens F, G, and H collectively represent a *spin system*, a group of hydrogens that are interconnected by *J*-coupling. Similarly, hydrogens A, B, C, and D are a spin system. Hydrogens H, I, and J are also a spin system, but they are so closely clustered that their off-diagonal peaks are difficult to distinguish.

In most cases, *J*-coupling only occurs between vicinal hydrogens. *J*-Coupling is also sometimes called *three-bond coupling* because the interacting hydrogens are separated by three bonds, i.e. H-C-C-H. *J*-Coupling can occur through four and even five bonds. Such long range couplings are more common with hydrogens on rings and double bonds. In Figure 2,  $\text{H}_\text{E}$  and  $\text{H}_\text{G}$  are separated by four bonds and yet show an off-diagonal peak. While the peak is present, it is also very weak. Long range couplings normally give weak signals in a COSY spectrum.

A second form of 2D spectroscopy is NOESY (nuclear Overhauser effect spectroscopy). A NOESY spectrum is much like a COSY spectrum. Both the x- and y-axes show a  $^1\text{H}$  projection spectrum with all peaks appearing on the diagonal. The key difference is that a NOESY spectrum measures the nuclear Overhauser effect, a phenomenon in which magnetization of one proton transfers to other nearby protons. Magnetization transfer only occurs over very short distances. Therefore, observation of an NOE between two hydrogens indicates that the hydrogens are very close to each other. NOE signals are very weak and can be hard to observe. NOESY spectra tend to have far fewer off-diagonal peaks than COSY spectra.

### The Experiment



Scheme 4

Our experiment will follow the three steps outlined in Scheme 4 above. The reaction begins with the formation of a hydrazone (**22**) from 1,3-cyclohexanedione (**21**) and phenylhydrazine (**20**).

**(CAUTION – Phenylhydrazine is a strong irritant and a suspected carcinogen.)** Like many hydrazones, compound **22** forms quickly and rapidly precipitates from the reaction as it forms. Hydrazone **22** has several possible tautomeric structures including **22**, **25**, and **26** (Scheme 5). Structure **25** is most similar to a typical hydrazone, but the carbonyl favors tautomerization to the fully conjugated forms of **22** and **26**. The  $^1\text{H}$  NMR spectrum is consistent with both **22** and **26** but not **25**.

Heating hydrazone **22** in aqueous  $\text{H}_2\text{SO}_4$  affects a Fischer indole synthesis to afford indole **23**. The reaction proceeds quickly and is easily monitored by TLC. The product is precipitated from the reaction mixture by adding dilute base. The  $^1\text{H}$  spectrum of **23** has a very downfield peak at approximately 12 ppm. This peak corresponds to the N-H, which is pulled downfield by the strongly deshielding conjugated carbonyl. The identity of this signal at 12 ppm may be confirmed by adding a drop of  $\text{D}_2\text{O}$  to the NMR sample and then taking another NMR spectrum. In the presence of  $\text{D}_2\text{O}$ , protons on nitrogen and oxygen will undergo exchange and become deuterated. Because deuterium ( $^2\text{H}$ ) does not give a signal in a  $^1\text{H}$  NMR spectrum, exchangeable protons seem to disappear when treated with  $\text{D}_2\text{O}$ . This process is often called the *D<sub>2</sub>O and shake* experiment.

Indole **23** may be readily methylated by deprotonating the N-H with KOH followed by treatment with dimethyl sulfate. **(CAUTION – Dimethyl sulfate is a strong irritant and a suspected carcinogen.)** The reaction may be monitored by TLC. Additional portions of KOH and dimethyl sulfate may be needed to force the reaction to completion. Dilution of the reaction with water precipitates the product, which may be isolated by filtration. The  $^1\text{H}$  NMR spectrum of **24** is very similar to that of **23** with the exception of a methyl singlet near 4 ppm.

The new methyl group provides a nice handhold to allow full characterization of the  $^1\text{H}$  NMR spectrum of **24**. Compound **24** contains two different spin systems, the four individual protons on the aromatic ring and three pairs of protons on the cyclohexanone ring. The methyl hydrogens are sufficiently close in space to hydrogens in the two spin systems to show an NOE. Careful study of the NOESY spectrum of **24**, especially the off-diagonal peaks that correlate to the *N*-methyl group, allows complete assignment of the  $^1\text{H}$  spectrum.

## Experiment

### Hydrazone Formation

Procedure modified from: Martin, M. J.; Trudell, M. L.; Arauzo, H. D.; Allen, M. S.; LaLoggia, A. J.; Deng, L.; Schultz, C. A.; Tan, Y. C.; Bi, Y.; Narayanan, K.; Dorn, L. J.; Koehler, K. F.; Skolnick, P.; Cook, J. M. Molecular yardsticks. Rigid probes to define the spatial dimensions of the benzodiazepine receptor binding site. *J. Med. Chem.* **1992**, *35*, 4105-4117.

Monday/Wednesday (week 0): Add 1,3-cyclohexanedione (40 mmol) by powder funnel to a 250 mL Erlenmeyer flask containing water (70 mL) and a 1" stir bar. The contents of the flask should be stirring efficiently. Use water (50 mL) to rinse any solids through the powder funnel. In a 20 mL scintillation vial weigh out phenylhydrazine (30 mmol) with a Pasteur pipet. Add the hydrazine in a slow, dropwise fashion to the cyclohexanedione solution, which should still be stirring rapidly. Rinse the vial with water (5 mL) to complete the transfer. Let the reaction stir.

Friday (week 0): Filter the resulting solid with a 500 mL side-arm flask with a 5.5 cm Büchner funnel. Rinse the solid with water (2×25 mL). Spread the solid to dry on a tared watch glass. At some later point, obtain a <sup>1</sup>H NMR spectrum (solvent should be *d*<sub>6</sub>-DMSO) and perform a satisfactory TLC plate (10% methanol/90% EtOAc) on the product. Also, determine your recovered mass and percent yield.

### Fischer Indole Synthesis

Procedure modified from: Rodriguez, J.-G.; Temprano, F.; Esteban-Calderon, C.; Martinez-Ripoli, M.

Synthesis of 4-(*N,N*-dimethylaminoethyl)-1,2,3,4-tetrahydrocarbazole: molecular structure and reactivity of the 1,2-dihydrocarbazol-4(3*H*)-one and derivatives. *J. Chem. Soc., Perkin Trans. 1* **1989**, 2117-2122.

Monday/Wednesday (week 1): Determine the mass and yield of the dry product. Heat 20% aqueous H<sub>2</sub>SO<sub>4</sub> (125 mL) to between 80 and 100° in a 250 mL Erlenmeyer flask with a sand bath. Add the hydrazone (~3.0 g) to the hot solution and allow the reaction to stir for 10 min. Monitor the reaction with a three-lane TLC plate (developing solvent: 10% methanol/90% EtOAc). [Mix a couple drops of

the reaction with EtOAc (1-2 mL) in a test tube. Sample the top layer (EtOAc) for the TLC plate.] If the reaction is not complete, continue to sample the reaction and check by TLC until all the starting material has been consumed. Once it is complete, cool the reaction on ice, and then carefully pour the liquid into a 500 mL Erlenmeyer flask. Try to decant the liquid and leave as much of the solids in the original reaction flask. Dissolve NaOH (25 g) into water (250 mL). Add a stir bar to the reaction solution, stir the mixture, and slowly add the NaOH solution. Cool the mixture on ice. Filter the product with a 500 mL side-arm flask and a 5.5 cm Büchner funnel. Rinse the solid with a solution of NaHCO<sub>3</sub> (3 g) in water (50 mL) followed by water (2×25 mL). Spread the solid to dry on a tared watch glass. At some later point, obtain a <sup>1</sup>H NMR spectrum (solvent should be *d*<sub>6</sub>-DMSO), perform a satisfactory TLC plate (EtOAc) on the product, and record the product's melting range. After the <sup>1</sup>H spectrum has been obtained, add two drops of D<sub>2</sub>O to the NMR tube and run another <sup>1</sup>H spectrum. Determine your recovered mass and percent yield.

### ***N*-Methylation**

Dissolve KOH (6.6 mmol) in a mixture of methanol (6 mL) and water (1 mL) in a 20 mL scintillation vial. In a separate vial, dissolve the indole from the previous step (5.4 mmol) in DMSO (6 mL). Transfer the KOH solution by Pasteur pipet into the vial containing the indole solution. Rinse the KOH solution with methanol (1 mL). Allow the mixture to stir for 10 min and then add Me<sub>2</sub>SO<sub>4</sub> (8.1 mmol) by pipetter. After 30 min, place a drop of the reaction in test tube. Add EtOAc (1 mL) and 5% HCl (1 mL) and swirl the mixture. Take a TLC (developing solvent: EtOAc) of the top layer in the test tube. If the reaction is not complete, add additional KOH (2.1 mmol) and Me<sub>2</sub>SO<sub>4</sub> (2.1 mmol) and re-sample the reaction after another 30 min. Repeat until the TLC shows no remaining starting indole. Once complete, pour the reaction into a solution of KOH (2.5 g) and water (50 mL). Rinse the reaction vial with DMSO (2 mL). Filter the precipitated product with a 125 mL side-arm flask and a Büchner funnel. Rinse the solid with water (2×10 mL). Spread the solid to dry on a tared watch glass. At some later point, obtain <sup>1</sup>H NMR and COSY spectra (solvent should be *d*<sub>6</sub>-DMSO), perform a satisfactory

TLC plate (EtOAc) on the product, and record the product's melting range. A NOESY spectrum will be provided. Determine your recovered mass and percent yield.

**Lab Report**

In the Results/Discussion Section, go into some detail interpreting the COSY and NOESY spectra for full characterization of the  $^1\text{H}$  NMR. Also, report the overall percent yield of all three steps.