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Chemistry

Organic Chemistry Laboratory Experiments

2011-07-14

Reductive Amination Reaction

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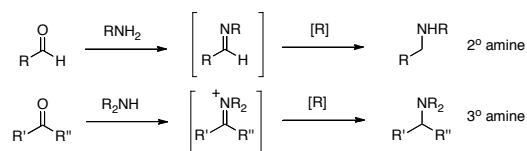
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Reductive Amination

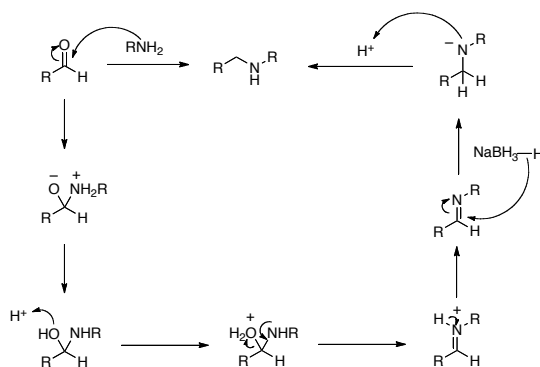
Introduction:

The reason most drugs (and by reason most drug candidates) contain nitrogen atoms is that the basic lone pairs on nitrogen can be easily protonated, making the drug cationic and thus more water soluble. By contrast, they just as often exist in neutral form, making cell membrane absorption easier since they must pass through a lipophilic barrier. Thus, many pharmaceutical companies will engineer new drugs with basic nitrogens as an important component of their design features. One way to increase the chance that these amine-containing drug candidates reach a clinical setting is to alkylate any primary and secondary amines in privileged intermediates to generate diverse scaffolds that might bind a macromolecule better and achieve an intended result.

Reductive amination is one means to achieve this goal. In this reaction, a primary (or secondary) amine is reacted with an aldehyde/ketone to generate an imine (or iminium ion) intermediate, which is then reduced to a secondary (or tertiary) amine with a reducing agent. Both steps occur by nucleophilic addition to the carbonyl and occur in the same reaction flask sequentially. This is attractive because the amount of time it takes to synthesize more complex structures is reduced considerably because multiple types of bonds are formed in one-pot. In addition, solvent and reagent waste is reduced, making these procedures more environmentally friendly, atom economical, and cost-effective.



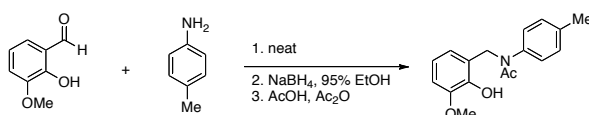
To make this process even more amenable to a wide variety of functional groups, two reagents have been prepared that are orthogonal with aldehydes, preventing their reduction into alcohols when placed in the same reaction flask from the beginning. $\text{Na}(\text{CN})\text{BH}_3$ (sodium cyanoborohydride) and $\text{NaBH}(\text{OAc})_3$ (sodium triacetoxyborohydride) are mild reducing agents that are less reactive than sodium borohydride. Thus, they can generally be added to a mixture of aldehyde and amine from the beginning and will only react with the resulting imine, not the aldehyde. Thus, these reagents are another example of chemoselective reducing agents.



In Part 1 of this laboratory, you will prepare an imine by reductive amination of *ortho*-vanillin and *para*-toluidine using NaBH₄, but a third step will also be performed, whereby the resulting secondary amine is acylated with acetic anhydride in acetic acid to generate a tertiary amide.

In addition, Part 2 of this laboratory will allow you to synthesize a variety of substituted imines and to test the effects of structure on the ¹H NMR spectrum of each compound. You will synthesize each imine individually, but the analytical work will be performed in groups. This will allow you to generate a useful structure-property relationship for this class of compounds.

Reductive Amination



Equipment:

Microscale glassware kit
NMR tubes
CDCl₃
White-capped vials
Melting tubes
MeltTemp
HPLC vials
1 mL syringes and needles

Chemicals:

ortho-Vanillin
3,4-Dimethoxybenzaldehyde
Salicylaldehyde
3-Nitrobenzaldehyde
Benzaldehyde

para-Toluidine
4-Aminophenol

Sodium borohydride
Glacial acetic acid
Acetic anhydride

95% ethanol (500 mL for reaction)
Absolute ethanol (200 mL)
Deionized water
Acetonitrile (300 mL)

Procedure: Touchette, K. M. *Journal of Chemical Education* **2006**, 83 (6), 929-930.

Part 1: Synthesis of a drug-like tertiary amide by reductive amination

Reductive Amination: In a tared beaker (100 mL or greater), combine 400 mg *para*-toluidine and 570 mg *ortho*-vanillin. Note the color of the two starting materials. Crush and mix the solids together with a spatula until a color change is observed and the solids melt together. Continue until the mixture becomes a dry powder and then transfer approximately 50 mg of this solid to a labeled test tube to save for IR analysis.

To the remaining imine, add 7.5 mL 95% EtOH (the imine will only partially dissolve). Carefully add 150 mg NaBH₄ in portions so that the bubbling is constant, but not too vigorous. Occasionally swirl the mixture until no more bubbling occurs and the solution is homogeneous (it may be necessary to break up some of the small pieces of imine and/or NaBH₄ with a spatula). Note any change in color.

To the clear solution, add 1.1 mL acetic acid and 1.1 mL acetic anhydride, in that order. Gently warm the resulting solution in a hot water bath with swirling for 5-10 minutes. Remove the product mixture from the heat and cool to room temperature.

Work-up: Add ~20 mL of deionized water to the solution. A white solid should precipitate. Filter and collect the solid by vacuum filtration. Allow the solid to dry until the end of the lab period and obtain a yield.

Analysis: Obtain all of the standard analytical data for your tertiary amide product (¹H NMR, IR, and LC/MS). For LC/MS, transfer one small crystal (~1 mg) into a test tube and dilute with acetonitrile (5 mL). Transfer 1 mL of this solution into an HPLC vial and run LC/MS. In addition, take a melting point of your product. Finally, take an IR spectrum of the imine intermediate you saved from the first step in Part 1 and note its color in your notebook.

Part 2: NMR analysis of differentially substituted imines

Imine Formation: In a vial, combine 200 mg 4-aminophenol and one equivalent of a substituted aldehyde according to your assigned letter (see Pre-Lab lecture). Dissolve the mixture in 2 mL 1:1 EtOH:H₂O and heat on a water bath for 5 minutes.

Work-up: Allow the sample to cool to room temperature and crystallize (you may need to add 1-2 mL H₂O to fully crystallize your product). Isolate the solid by vacuum filtration and allow to dry briefly before performing analysis. Determine the yield of your imine before proceeding to analysis.

Analysis: Work with the classmates (in your lab section only) who were given the same starting aldehyde for Part 2 for the rest of the laboratory period, splitting up the work equally. First, determine the color of your sample and compare this to the imine from Part 1. Next, obtain an IR spectrum of your sample. Then, obtain a ¹H NMR spectrum of your sample and note the chemical shifts of the protons on the iminophenol ring.

Before you leave lab, get together with the rest of your classmates and share the relevant ^1H NMR analytical data to develop a structure-property relationship for substituted imines (ie. the chemical shifts of the protons in the iminophenol ring). Be sure to write down the structure that corresponds to this data. You will be held responsible for all of this data, even though you may not have prepared the compound yourself.

Required Analytical Data for Lab Report:

1. Yield, ^1H NMR, IR, LC/MS, and melting point for the tertiary amide (this should be your own data)
2. Yield, ^1H NMR, and IR data for your group imine (share this data with members of your assigned group)
3. ^1H NMR chemical shifts of the iminophenol ring for those imines prepared by the other members of the class.

Pre-lab Questions:

- 1) Draw a full mechanism for the reaction you will perform in Part 1 to generate the tertiary amide.
- 2) Why is it necessary to add the NaBH_4 after formation of the imine? Why could we not just add the aldehyde, amine, and NaBH_4 all at once?
- 3) Based on what you know about IR stretching frequencies, would you expect the C=N stretch or the C=O stretch to have a higher wavenumber?
- 4) How does the melting point of an impure substance compare to the melting point of a pure substance?
- 5) Describe the lowest energy transition for the imine formed during Part 1 of this experiment that would lead to the absorption of light in the visible spectrum.
- 6) Capsaicin, whose structure is shown below, is found in many hot chili peppers and is responsible for the activation of sensory neurons for pain. It is an active ingredient in topical creams to treat arthritis, back pain, ligament sprains, and psoriasis, among others. Look up the structure of capsaicin and devise a multistep synthesis of it using chemistry analogous to that which you will explore in this experiment.

Post-Lab Questions: There will be no specific post-lab questions for this experiment. Instead, answer the following questions as part of a more detailed discussion section in your lab report. This section should be written as if you were presenting data in a research paper, so be sure to indicate your understanding of your data.

- 1) Compare the melting point of your isolated tertiary amide to that reported in the literature.
- 2) How does the IR spectra compare between the intermediate imine and the product in Part 1? Why does the amide C=O come at so low frequency ($\sim 1630\text{ cm}^{-1}$)? Why does the C=N come at even lower frequency ($\sim 1250\text{ cm}^{-1}$)?
- 3) Using the data from the entire class for aminophenol-containing imines, comment on how the chemical shifts of the protons on the iminophenol ring are influenced by the electronic nature of the starting aldehyde.
- 4) Judging from the color of both your intermediate imine and product from Part 1, what trend would you expect from the UV-Vis spectrum (hint: look at p. 170 in your textbook and then answer this questions – when you reduce the imine to the amine, what happens to the conjugation and why would this effect the UV-Vis spectrum?)? What electronic transitions would be responsible for these effects?