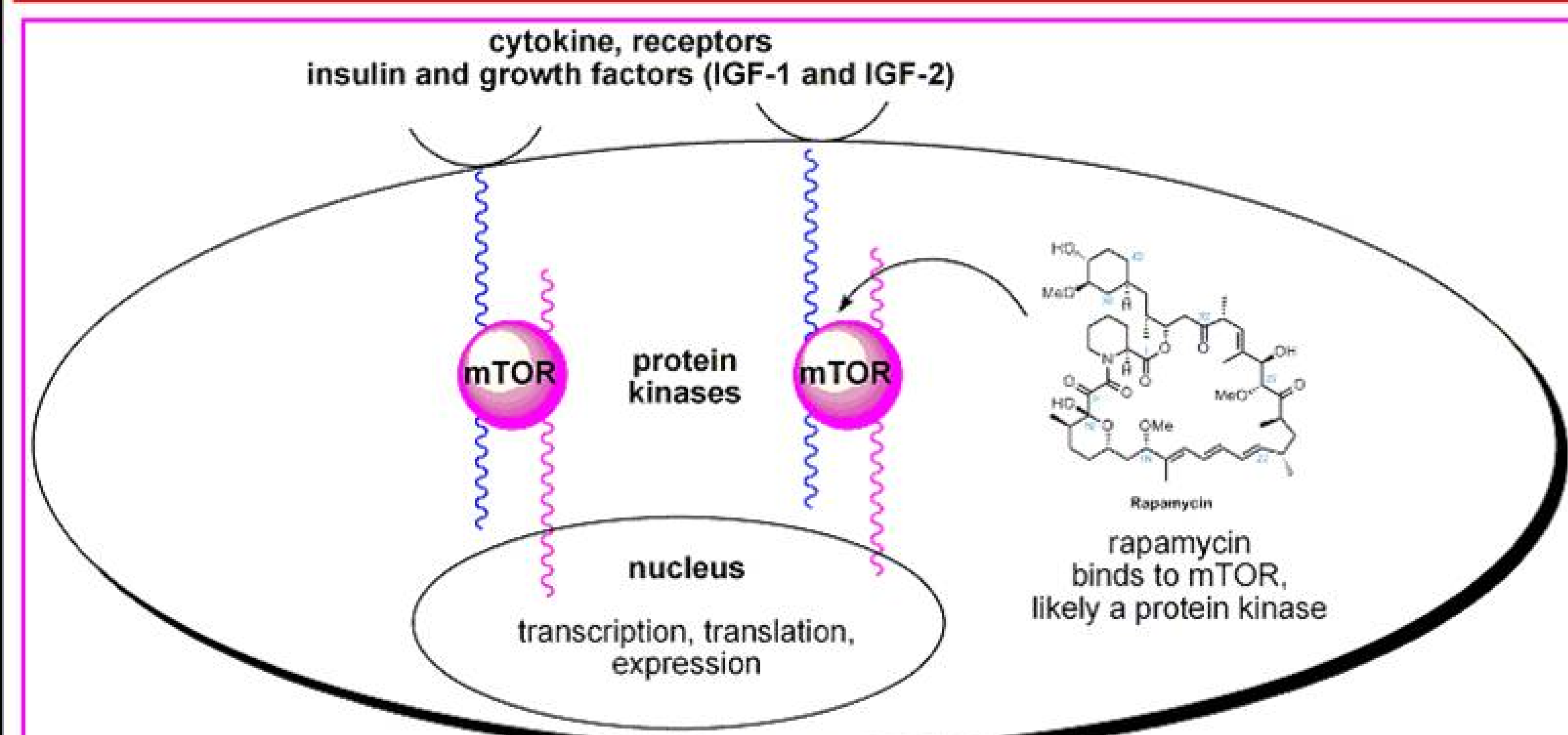


Synthesis of inhibitors of the molecular target of rapamycin (mTOR) signaling pathway

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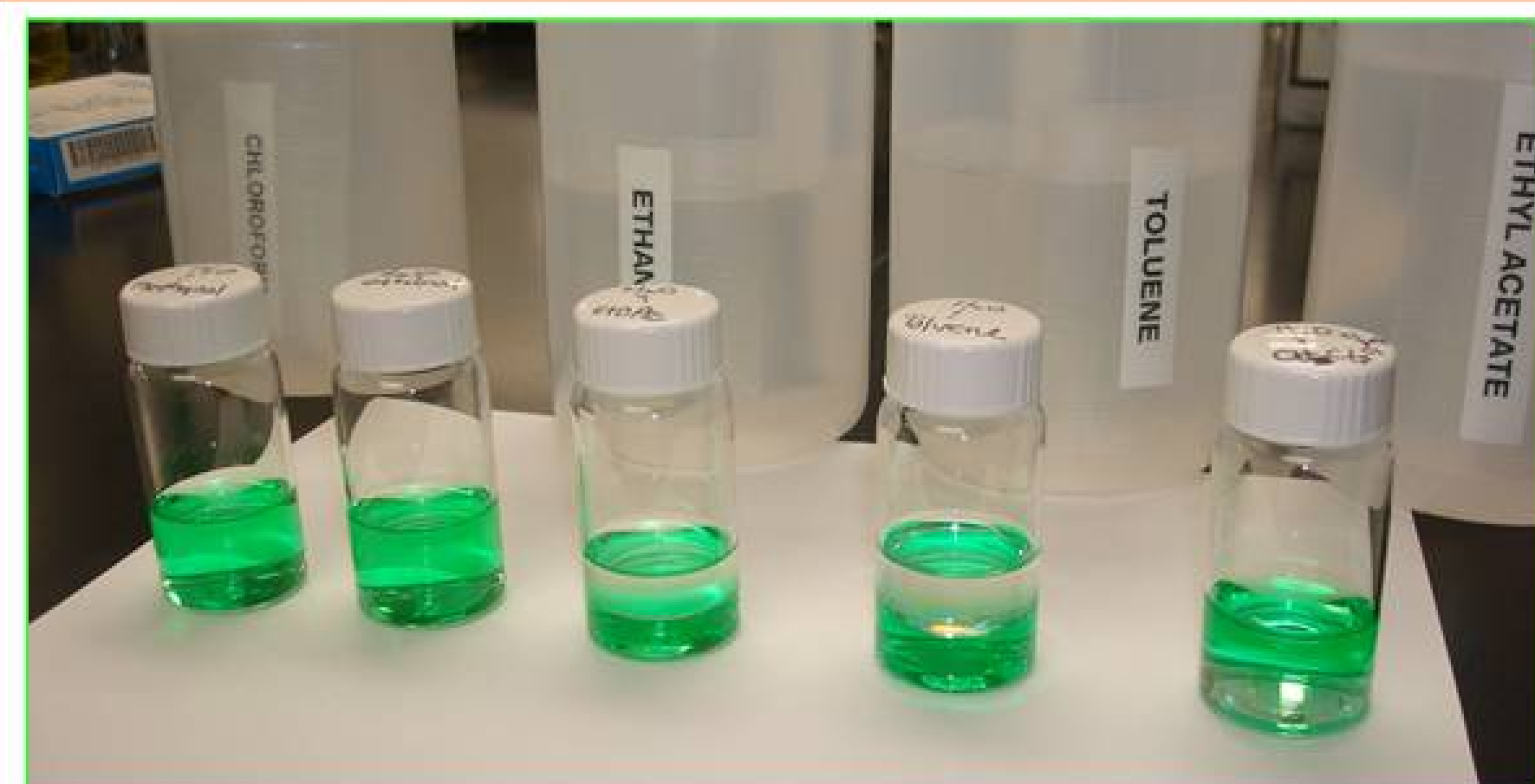
Abstract: The molecular target of rapamycin (mTOR) cell signaling pathway is believed to be important in the process of cancer cell growth and proliferation.^{1,2} Chemical compounds that would inhibit the mTOR pathway might be expected to suppress tumor growth and thus would potentially be new drug candidates for the treatment of cancer in humans. Our project involved the synthesis and purification of an organic chemical compound believed to be one in a series of mTOR inhibitors. This project taught organic chemistry laboratory techniques including **solvent selection, thin layer chromatography (TLC), organic chemistry reactions, reaction mechanisms, column chromatography, proton NMR (¹H NMR), and carbon NMR (¹³C NMR)**. Ultimately, compounds prepared in this project will be tested for activity in a cellular assay of mTOR pathway activity.³



In the cell, mTOR is a serine/threonine protein kinase that regulates cellular signaling that leads to cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription. Rapamycin inhibits these processes by binding to mTOR and interfering with cellular signaling.

Solvent properties and thin layer chromatography (TLC)

- Studied relative **density** of volatile organic solvents
- Studied relative **polarity** and **water miscibility** of solvents
- Studied **hydrophilic** and **hydrophobic** properties of solvents
- Choose TLC mobile phase based on these properties



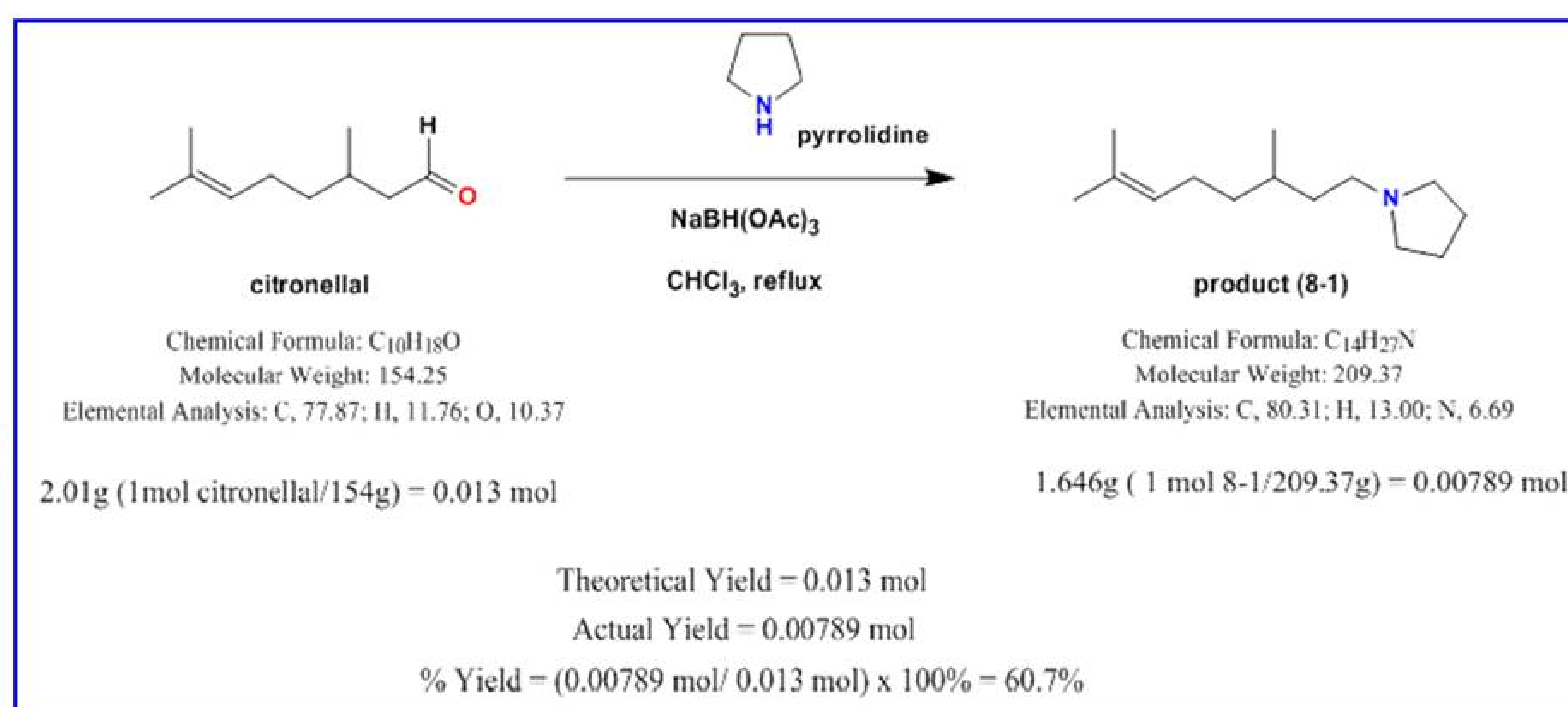
Methanol – highly polar, water miscible
Ethanol – highly polar, water miscible
Ethyl Acetate – non-polar, not water miscible, less dense than water
Toluene – non-polar, not water miscible, less dense than water
Chloroform – non-polar, not water miscible, more dense than water

- TLC plate has a **highly polar silica gel stationary phase**
- TLC plate is **developed in a chamber** with a solvent mobile phase
- TLC plates were developed using **5% NH₃:MeOH/CHCl₃ mobile phase**
- TLC helps determine **reaction progression and compound purity**

Carlos and Katia prepare **TLC chambers** that contain the organic solvent **mobile phase**. TLC plates spotted with our compound mixture are developed in the chambers and then visualized by **UV light and PMA stain**.

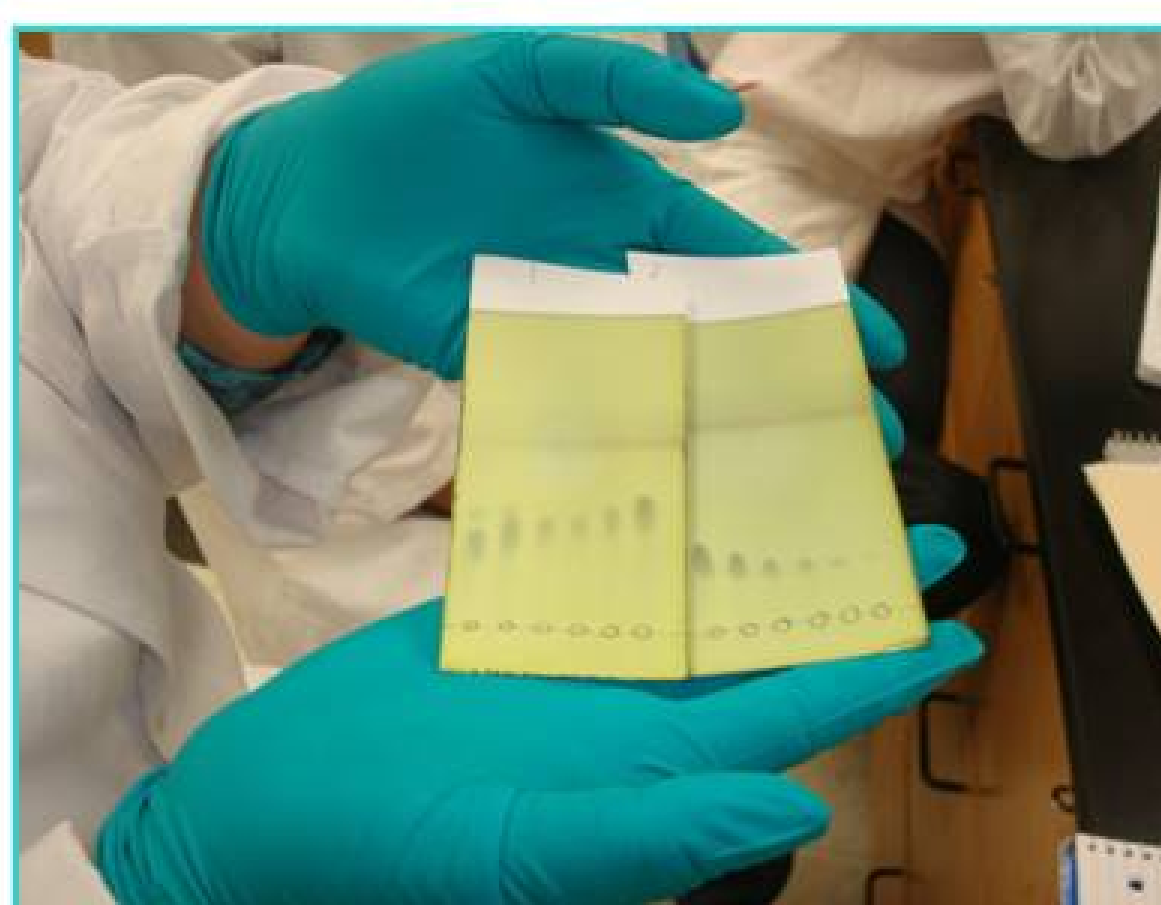


'REDUCTIVE AMINATION' USING SODIUM TRIACETOXY BOROHYDRIDE⁴



Claudina performs **silica gel column chromatography**. A solvent mobile phase selected by **TLC analysis** effects **resolution** of our product mixture and we obtain a **purified product compound**.

Katia performs **rotary evaporation**. A vacuum pump is used to create reduced pressure and effect a **vacuum distillation** to remove solvent and concentrate our purified product.

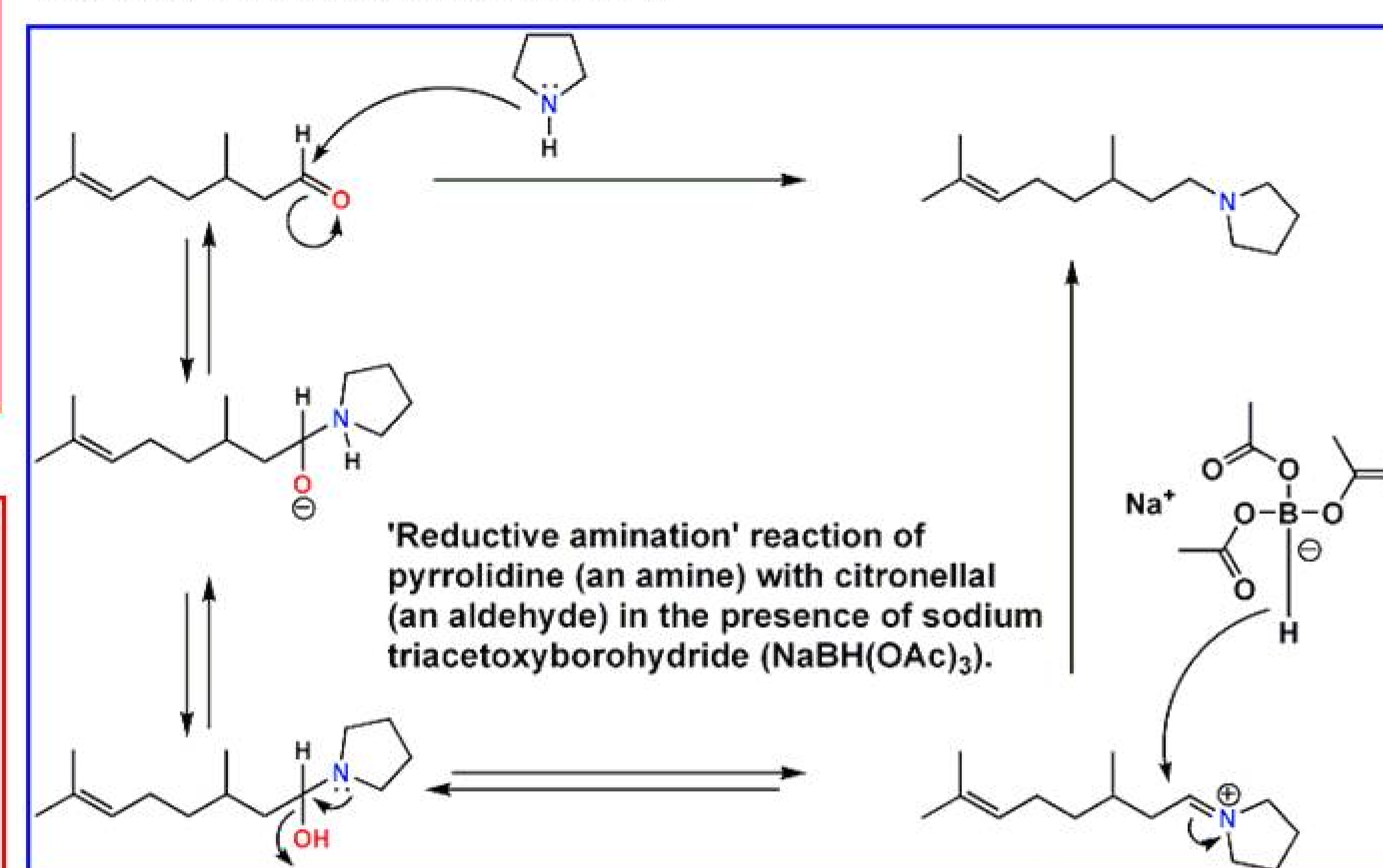


Claudina inserts a sample into the **500 MHz NMR** to obtain **¹H NMR (proton) and ¹³C NMR (carbon) spectra** to help identify the chemical structure of our product compound.

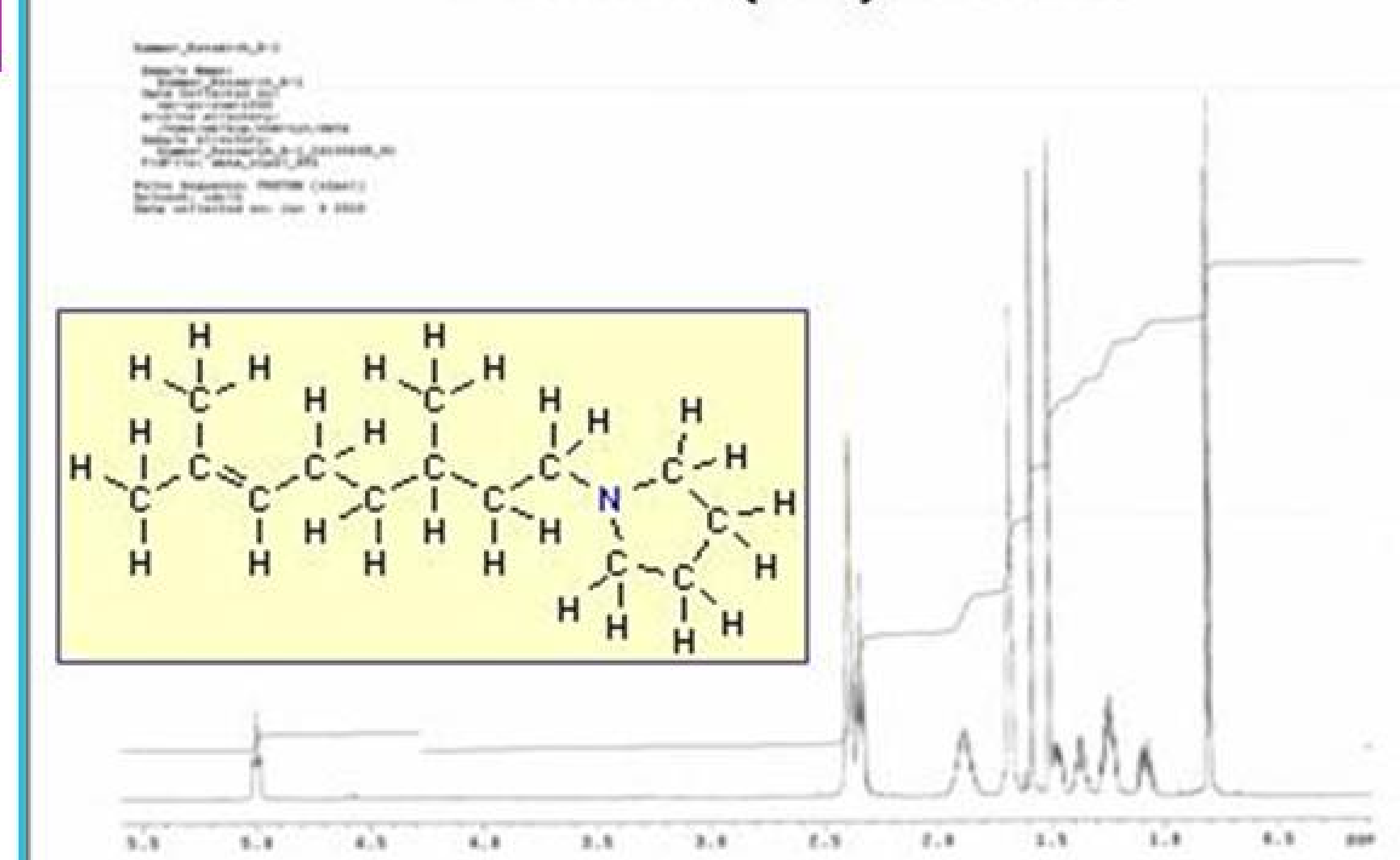
Nuclear Magnetic Resonance Spectroscopy (NMR)

Chemical Shift: Electronegative atoms cause **de-shielding** so resonances occur **downfield**. Electropositive atoms cause shielding so resonances occur **upfield**. The **(n + 1) rule:** Splitting depends on the number of neighboring protons 'n' and follows the (n + 1) rule. **Proton NMR (¹H)** focuses on hydrogen atoms. **Carbon NMR (¹³C)** focuses on carbon 13 atoms. Our spectra support the proposed structure.

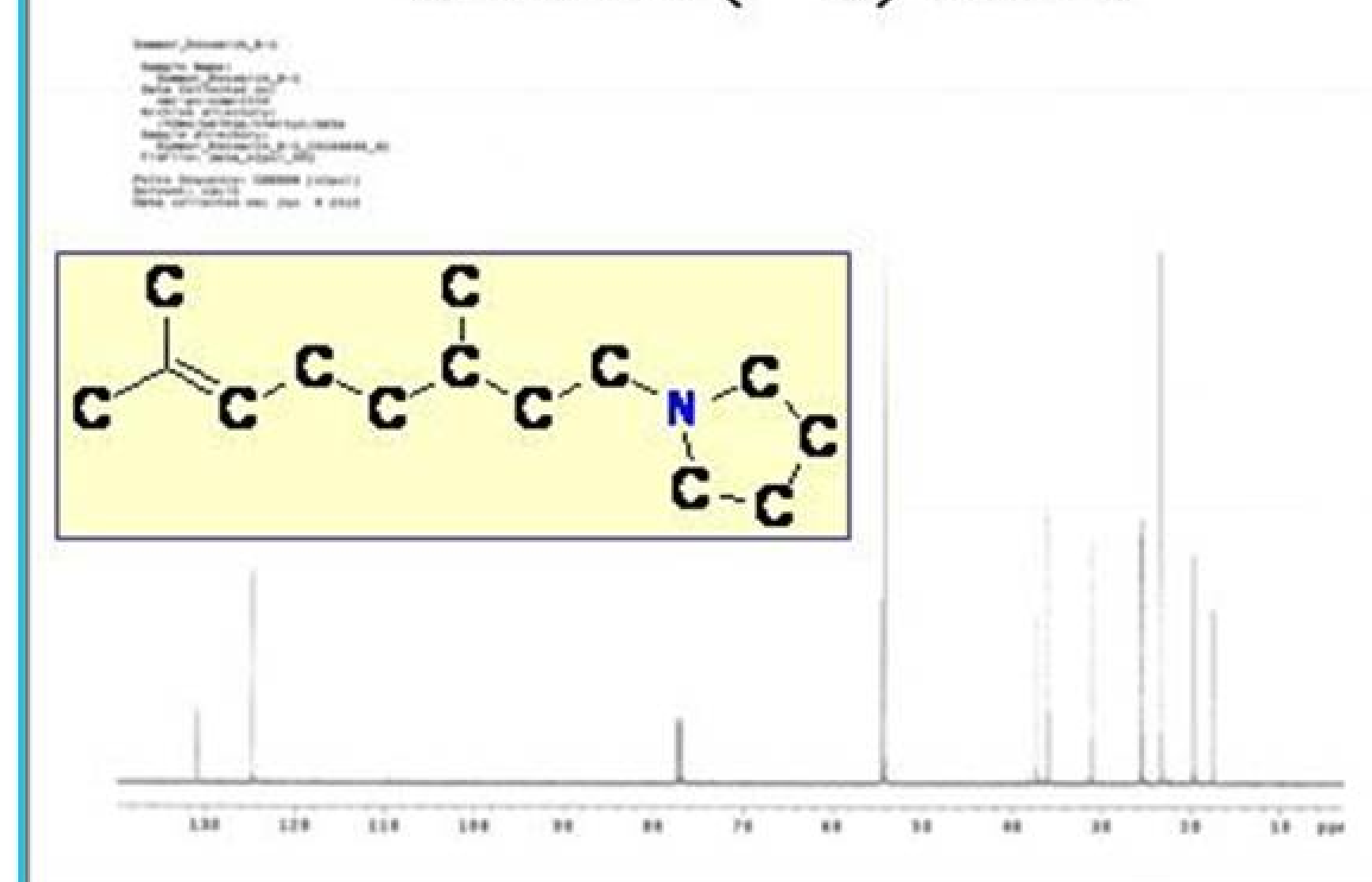
reaction mechanism:



Proton (¹H) NMR



Carbon (¹³C) NMR



Carlos, Claudina, and Katia dissolve purified samples in **deuteriochloroform (CDCl₃)** in NMR tubes. These are inserted into the **500 MHz superconducting magnet** to obtain **¹H and ¹³C NMR spectra** to characterize our product compound.

References:

- 1) Harrison, C. **Anticancer drugs: Actively targeting mTOR**. *Nature Reviews Drug Discovery* 2010 9, 193.
- 2) Janes, M. R. *et al.* **Effective and selective targeting of leukemia cells using a TORC1/2 kinase inhibitor**. *Nature Medicine* 2010 16, 205–213.
- 3) Studies performed in the laboratory of Prof. Nitika Parmar.
- 4) Abdel-Magid, A.F., Carson K.G, Harris, B.D., Maryanoff, C.A.; Shah, R.D. **Reductive Amination of Aldehydes and Ketones with Sodium Triacetoxyborohydride. Studies on Direct and Indirect Reductive Amination Procedures**. *J. Org. Chem.* 1996, 61, 3849-3862.

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