ORGANIC

UNDERGRADUATE EXPERIMENT

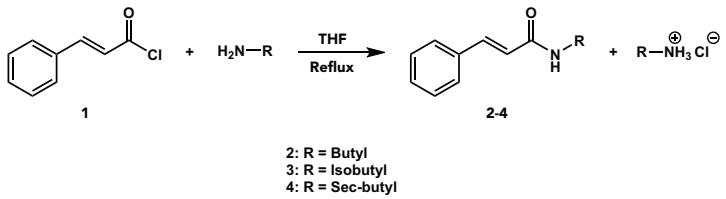
2D Techniques in the Structural Elucidation of Cinnamamides

INTRODUCTION

In most undergraduate chemistry courses, students are required to relate in-class theory to real-life scenarios brought up within the laboratory component. Students use those connections to obtain hands-on experience with fundamental concepts of chemistry that include, but are not limited to, polarity, solubility, extraction/isolation, and characterization techniques (*e.g.*, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, ultraviolet-visible (UV-vis) spectroscopy, mass spectroscopy, *etc.*). Unfortunately, arguably one of the most important characterization tools, NMR spectroscopy, continues to be difficult to access for many students. A contributing factor is that most post-secondary institutions only employ high-field NMR spectrometers, which require extensive training to operate, as well as significant upfront and recurring costs to maintain the instrument. As a result, access to the instrument is only granted to specialists and/or graduate students who have already built up the knowledge and understanding of the technique. With these accessibility issues, undergraduate students are instead given printouts of spectral reports in their quest for structural elucidation and validation, instead of obtaining much-needed hands-on experience with the instrument.

With the advent of benchtop NMR, students can gather and interpret their own spectra immediately after synthesis. Not only can students perform basic 1D ^{1}H and ^{13}C{^{1}H} experiments, but they can also explore 2D structural elucidation approaches (*i.e.*, homonuclear correlation spectroscopy (COSY, ^{1}H-^{1}H), heteronuclear single quantum correlation – multiplicity enhanced (HSQC-ME, ^{1}H-^{13}C), *etc.*), giving them the ability to fully elucidate the structures of their compounds. This provides students with hands-on experience to NMR instrumentation during their undergraduate studies and can prove to be advantageous in future endeavours with graduate studies or within industry.

In this sample experiment, a series of simple nucleophilic acyl substitutions are performed and a general reaction scheme is outlined in **Scheme 1**. The transformation presented is based on work published by Carroll and Shuldburg in *The Journal of Chemical Education*.[1] The goal of this sample experiment is to show that students can perform basic structural elucidation experiments by themselves to understand connectivity in their products or to determine the identity of an unknown product, thereby garnering NMR experience early in their careers. In **Scheme 1**, cinnamoyl chloride (**1**) is reacted with a butyl-branched amine group in excess, yielding a cinnamamide.



Scheme 1. *Reaction scheme for the nucleophilic acyl substitution of cinnamoyl chloride (**1**) with butylamine (**2**), isobutylamine (**3**), or sec-butylamine (**4**) in tetrahydrofuran (THF) to yield the respective cinnamamide.*

The ^{1}H, $^{13}C\{^{1}H\}$, distortionless enhancement by polarization transfer (DEPT), COSY, and HSQC-ME NMR spectra were acquired using a 100 MHz benchtop spectrometer to illustrate the benefits of incorporating benchtop NMR into an undergraduate laboratory. As these structures are highly similar, the series of experiments conducted here will allow for full characterization of the different amide products.

Procedure

Materials

Cinnamoyl chloride (97%, predominantly *trans*) was purchased from BLD Pharmatech; chloroform-*d* (99.8%) was purchased from Deutero GmbH; and butylamine (99.5%), isobutylamine (99%), *sec*-butylamine (99%), tetrahydrofuran (≥99.9%, anhydrous), dichloromethane (≥99.8%, anhydrous), sodium hydroxide (≥98%), hydrochloric acid (≥37%), and magnesium sulfate (≥99.5%) were purchased from MilliporeSigma. All chemicals were used without further purification.

Instrumentation

All NMR data was obtained using a Nanalysis 100 MHz instrument. The ^{1}H experiments were performed using the following acquisition parameters: spectral width, 20 ppm; spectral center, 5 ppm; number of points, 8096; number of scans, 1; dummy scans, 0; interscan delay, 1 second; pulse angle, 90°; receiver gain, auto. The $^{13}C\{^{1}H\}$ experiments were performed using the following acquisition parameters: spectra width: 220 ppm; spectral center, 100 ppm; number of points, 8096; number of scans, vary; interscan delay, 0 seconds; pulse angle, 45°, 90°, 135°; receiver gain, auto. The COSY and HSQC-ME experiments were performed using adaptations of their respective ^{1}H and $^{13}C\{^{1}H\}$ experiment parameters. All spectra were manually corrected for phase and baseline distortions using the MestReNova software (v14.2.3).

Synthesis

Cinnamamide

The amine (1.50 mL, ~2.5 equiv.) was mixed with tetrahydrofuran (2 mL) in a 25 mL round-bottomed flask equipped with a magnetic stirring bar. In a separate vial, cinnamoyl chloride (~1.00 g, ~1.0 equiv.) was dissolved in tetrahydrofuran (2 mL) and added dropwise into the round-bottomed flask containing the amine mixture over a period of 5 minutes. Upon addition, a light, clear yellow colour was immediately observed. The solution was heated to reflux for 30 minutes.

The reaction mixture was removed from heat, and dichloromethane (20 mL) was added to the reaction mixture then allowed to cool to room temperature. The mixture was then washed with 5% sodium hydroxide (3 x 10 mL), 5% hydrochloric acid (3 x 10 mL), and finally with water (10 mL). The organic layer was collected, dried with magnesium sulfate, and gravity filtered. The solution was concentrated *in vacuo*, yielding a pale-yellow to yellow solid for each product. No further purification was necessary.

Results and Discussion

The ^{1}H, $^{13}C\{^{1}H\}$ (1D and DEPT stacked), COSY, and HSQC-ME NMR spectra of **2**, **3**, and **4** were collected and are shown in **Figures 1**, **2**, and **3**, respectively. As expected, within each spectrum, the aromatic and alkene double bond regions ($\delta(^{1}H)$ = 6.54 ppm to 7.80 ppm, $\delta(^{13}C\{^{1}H\})$ = 120 ppm to 141 ppm) appear to have almost identical chemical shifts. The protons of the alkene double bond, labelled as 5 and 6, appear as doublets centered approximately at 7.67 ppm and 6.54 ppm (overlapping with the broad nitrogen proton in amide 3), respectively. As expected, the coupling constant of these olefinic protons ($^{3}J_{H\text{-}H}$= ~15.7 Hz) is characteristic of a *trans* position.[2] In the $^{13}C\{^{1}H\}$ NMR spectrum, the carbons related to the aromatic and alkene regions appear from 121.45 ppm to 140.42 ppm. Additionally, the quaternary carbons related to the phenyl ring and carbonyl of the amide appear at approximately 135 ppm and 166 ppm, respectively. As the COSY experiment details which protons are spin-coupled to each other, within the same general region, we observe correlations between H_1-H_2, H_2-H_3, and H_5-H_6. Finally, the HSQC-ME experiment details directly bonded proton-carbon correlations, where in the aromatic and alkene double bond region, we observe correlations between H_1-C_1, H_2-C_2, H_3-C_3, H_5-C_5, and H_6-C_6. As these structures are constitutional isomers and have highly similar atom connectivity, the main differences are detailed in the butyl chain of each molecule and can be easily differentiated through NMR.

Specifically, the DEPT experiments combined with the 1D $^{13}C\{^{1}H\}$ experiment can aid in differentiating primary, secondary, tertiary, and quaternary carbons. Within the DEPT-90 experiment, only tertiary carbons are observed, whereas in DEPT-135, primary and tertiary carbons are shown with a positive phase, while secondary carbons are shown with a negative phase.[3]

*N-butylcinnamamide (**2**)*

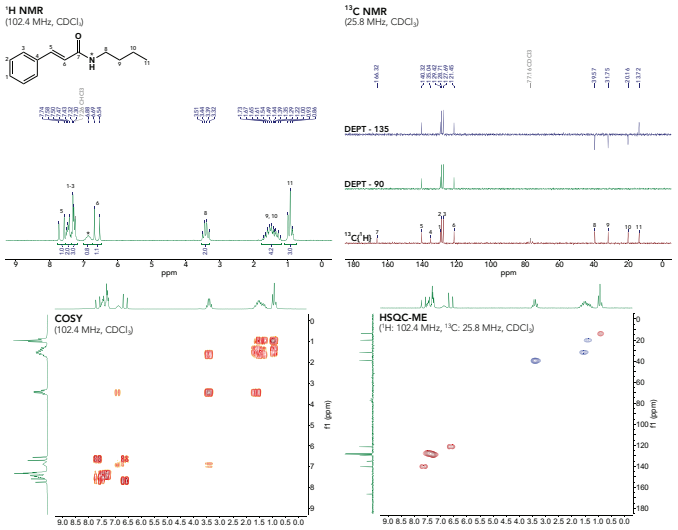


Figure 1. *^{1}H (102.4 MHz), $^{13}C\{^{1}H\}$ (25.8 MHz), COSY (102.4 MHz) and HSQC-ME (^{1}H: 102.4 MHz, ^{13}C: 25.8 MHz) NMR spectra of n-butylcinnamamide (**2**) in $CDCl_3$. The asterisk in the ^{1}H spectrum represents the proton bound to the nitrogen in the amide.*

Product **2** consists of a linear butyl group, which contains 4 different types of protons and carbons (**Figure 1**). The methylene group adjacent to the nitrogen atom appears as a quartet centered at 3.42 ppm in the ^{1}H spectrum and at 39.57 ppm in the $^{13}C\{^{1}H\}$ spectrum. The next two -methylene moieties overlap in the ^{1}H spectrum where they are centered at 1.48 ppm, as they are in similar chemical environments, which is further shown in its integration to 4 protons. These two functionalities, however, are observed as two separate peaks in the ^{13}C spectrum at 31.75 ppm and 20.16 ppm. Lastly, the methyl couples to the two adjacent protons and appears as a triplet centered at 0.93 ppm in the ^{1}H spectrum and 13.72 ppm in the ^{13}C spectrum. The COSY of **2** shows distinctive correlations for this isomer between H_8-H_9, H_9-H_{10}, and H_{10}-H_{11}.

*Isobutylcinnamamide (**3**)*

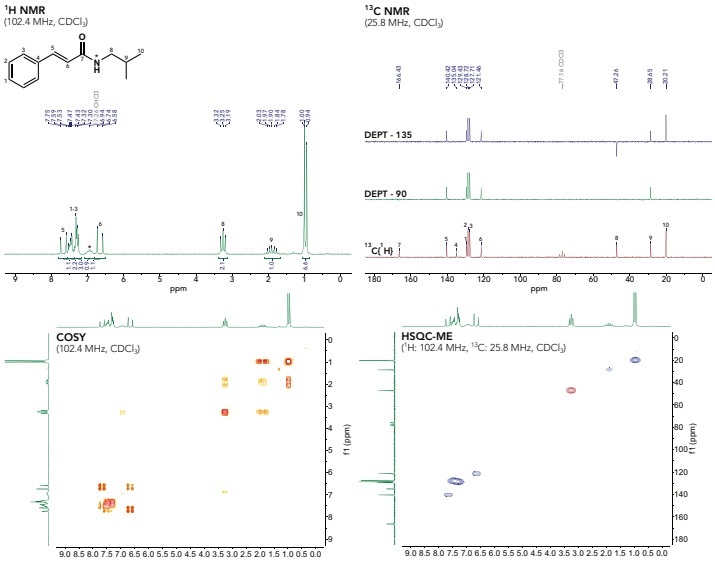


Figure 2. *^{1}H (102.4 MHz), ^{13}C (25.8 MHz), COSY (102.4 MHz) and HSQC-ME (^{1}H: 102.4 MHz, ^{13}C: 25.8 MHz) NMR spectra of isobutylcinnamamide (**3**) in $CDCl_3$. The asterisk in the ^{1}H spectrum represents the proton bound to the nitrogen in the amide.*

Product **3** contains an *iso*-butyl group with three different types of protons and carbons (**Figure 2**). The methylene group adjacent to the nitrogen atom appears as a triplet at 3.25 ppm in the ^{1}H spectrum and at 47.26 ppm in the $^{13}C\{^{1}H\}$ spectrum. The methine fragment directly bonded to the -CH_2- and two -CH_3 groups appear as a multiplet from 1.70 ppm to 2.03 ppm in the ^{1}H spectrum and at 28.65 ppm in the $^{13}C\{^{1}H\}$ spectrum. Lastly, the two -CH_3 groups are chemically equivalent and appear centered at 0.97 ppm in the ^{1}H spectrum and 20.21 ppm in the $^{13}C\{^{1}H\}$ spectrum. As expected, the COSY of **3** shows two distinct correlations between H_8-H_9 and H_9-H_{10}.

*Sec-butylcinnamamide (**4**)*

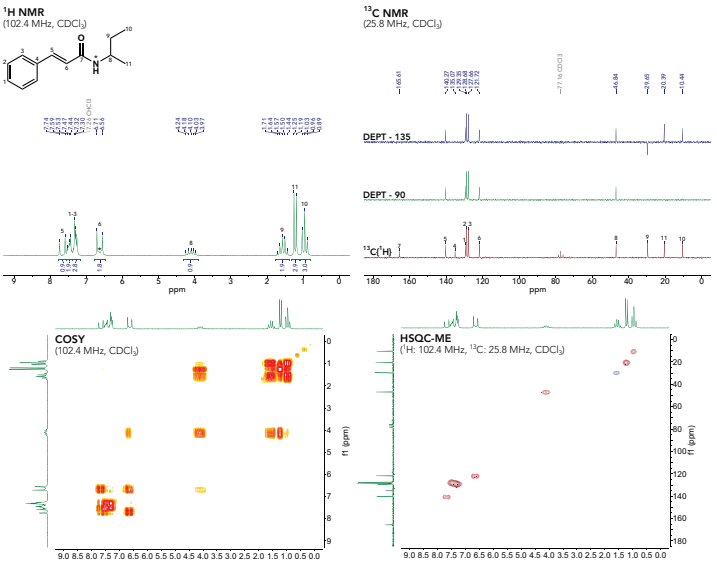


Figure 3. *^{1}H (102.4 MHz), ^{13}C (25.8 MHz), COSY (102.4 MHz) and HSQC-ME (^{1}H: 102.4 MHz, ^{13}C: 25.8 MHz) NMR spectra of sec-butylcinnamamide (**4**) in $CDCl_3$. The asterisk in the ^{1}H spectrum represents the proton bound to the nitrogen in the amide.*

Product **4** contains a *sec*-butyl chain, with four different types of protons and carbons (**Figure 3**). The methine group adjacent to the nitrogen atom appears as a multiplet from 3.97 ppm to 4.24 ppm in the ^{1}H spectrum and at 46.84 ppm in the $^{13}C\{^{1}H\}$ spectrum. The methylene and methyl groups adjacent to that methine proton appear as a multiplet and a doublet centered at 1.57 ppm 1.22 ppm, respectively, in the ^{1}H spectrum. In the $^{13}C\{^{1}H\}$ spectrum, these appear at 29.65 ppm and 10.44 ppm. Finally, the last methyl group appears as a triplet centered at 0.96 ppm in the ^{1}H spectrum and 20.39 ppm in the $^{13}C\{^{1}H\}$ spectrum. The COSY of **4** shows strong correlations between H_8-H_9, H_8-H_{11}, H_9-H_{10}, and likely a 4-bond long-range correlation between H_8-H_{10} due to these protons residing in close spatial proximity to each other.

Conclusion

With the incorporation of benchtop NMR spectroscopy into undergraduate chemistry courses, students can obtain hands-on experience with NMR and therefore build a foundation of NMR knowledge before they pursue graduate studies or a career in industry. With the power of NMR, students can setup their own basic structural elucidation routes within their laboratory period, gain knowledge in data interpretation, and finally, discern structurally related compounds with their new NMR abilities. In this sample experiment, a series of 1D and 2D experiments were conducted (^{1}H, $^{13}C\{^{1}H\}$, DEPT-90, DEPT-135, COSY, and HSQC-ME) on three constitutional isomers of butylcinnamamides. Using the range of experiments, students can determine direct connectivity and coupling of nuclides, which will aid in the determination of which butylcinnamamide they have synthesized.

References

[1] Shuldburg, S.; Carroll, J. *J. Chem. Educ.* **2017**, *94*, 1974-1977.

[2] Silverstein, R.M.; Webster, F.X.; Kiemle, D.J. "Spectrometer Identification of Organic Compounds" 7th Ed. John Wiley & Sons Inc.: USA

[3] DEPT: A tool for ^{13}C peak assignments. https://www.nanalysis.com/nmready-blog/2020/6/22/dept-a-tool-for-13c-peak-assignments (Accessed March 21, 2023)

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