An undeniably important part of undergraduate chemistry courses is the associated laboratory period, which aids in developing a deeper understanding of classroom theory using lab experiments. It is with this hands-on experience that students become exposed to fundamental concepts such as solubility, polarity, extraction/isolation techniques, and characterization using spectroscopic methods (\(^1\)H and \(^{13}\)C magnetic resonance spectroscopy, infrared spectroscopy, etc.), among other analytical techniques. Specifically, nuclear magnetic resonance (NMR) spectroscopy is a topic introduced in early organic chemistry courses; however, students are commonly exposed to software-generated or idealized spectra, and many undergraduate students still do not have direct access to an NMR spectrometer due in part to their significant upfront and recurring costs.\(^1\) However, with the advent of benchtop NMR, students are able to work directly with an NMR instrument, gathering and interpreting their own spectra within a typical 3-hour laboratory block. Time and time again, NMR has proven itself to be one of the most important analytical tools in chemistry due to its extensive structural elucidation capabilities, and benchtop NMR provides unparalleled access to this technique.

One of the most studied reactions in organic chemistry is the Fischer esterification, which is usually taught in first-year organic chemistry courses.\(^2\) It is a reversible condensation reaction, involving the reaction of a carboxylic acid with an alcohol under acidic conditions to produce an ester and water, outlined in Scheme 1.

![Scheme 1. General reaction scheme for the Fischer esterification reaction.](image)

In this sample experiment, a simple Fischer esterification reaction is performed, based on work published by Schweiker et al. in The Journal of Chemical Education.\(^3\) The \(^1\)H and \(^{13}\)C(\(^1\)H) NMR spectra are acquired using a 60 MHz benchtop spectrometer, illustrating the benefits of incorporating this technology into undergraduate teaching labs. In addition, this experiment emphasizes the pedagogic goals of liquid-liquid extractions, solubility, polarity and offer a closer look into benchtop NMR analysis.
PROCEDURE

**Materials**

4-Amino-3-nitrobenzoic acid (97%), sulfuric acid (95-98%), anhydrous methanol (99.8%), sodium bicarbonate (≥99.5%), anhydrous ethyl acetate (99.8%), pentane (98%) and DMSO-d6 (99.9%) were purchased from Sigma Millipore and used without further purification.

**Instrumentation**

All NMR data was obtained using a Nanalysis 60PRO instrument. The 1H experiments were performed using the following parameters: spectral width, 20 ppm; spectral center, 5 ppm; number of points, 4096; number of scans, 4 for 4-amino-3-nitrobenzoic acid (1), 16 for methyl 4-amino-3-nitrobenzoate (2); dummy scans, 0; interscan delay, 5 seconds for 4-amino-3-nitrobenzoic acid (1), 7 seconds for methyl 4-amino-3-nitrobenzoate (2); pulse angle, 90°; receiver gain, auto. The 13C{1H} experiments were performed using the following parameters: spectral width, 220 ppm; spectral center, 100 ppm; number of points, 4096; number of scans, for 4-amino-3-nitrobenzoic acid (1), 16384 for methyl 4-amino-3-nitrobenzoate (2); dummy scans, 0; interscan delay, 0 seconds; pulse angle, 67.70° for 4-amino-3-nitrobenzoic acid (1), 30° for methyl 4-amino-3-nitrobenzoate (2); receiver gain, auto. All spectra were manually corrected for phase and baseline distortions using the MestReNova software (v14.1.1).

**Synthesis**

The synthesis of methyl 4-amino-3-nitrobenzoate (2) from 4-amino-3-nitrobenzoic acid (1) was adapted from a literature procedure described by Schweiker et al. and is outlined in **Scheme 2**.3

**Scheme 2.** Preparation of methyl 4-amino-3-nitrobenzoate (2) from the reaction of 4-amino-3-nitrobenzoic acid (1) with methanol under reflux conditions and using concentrated sulfuric acid as a catalyst.

4-Amino-3-nitrobenzoic acid (307 mg, 1.69 mmol) was dissolved in anhydrous methanol (40 mL). Concentrated sulfuric acid was added dropwise (6 drops) to the reaction mixture and was heated to reflux for 24 hours. It should be noted that the experimental procedure presented by Schweiker et al. indicates that the reaction can be done in 1 hour (suitable for an undergraduate laboratory), but for maximum yield, 16 hours is optimal.3 The reaction was quenched with a saturated solution of sodium bicarbonate (40 mL) and transferred to a separatory funnel. Ethyl acetate (40 mL) was used to extract 2 and was further washed with the sodium bicarbonate solution. This was repeated until the sodium bicarbonate layer (bottom layer) remained clear (shown in wash 3 of **Figure 1**).

**RESULTS AND DISCUSSION**

Using 1H and 13C{1H} NMR spectroscopy, both the starting material 1 and product 2 were elucidated, and their spectra were compared. **Figures 3** and 4 depict the 1H (top) and 13C (bottom) spectra for 1 and 2, respectively.
Figure 3. ¹H (top, 60 MHz) and ¹³C{¹H}(bottom, 15.1 MHz) NMR spectra of 4-amino-3-nitrobenzoic acid (1) in DMSO-d₆.

Upon comparison of the ¹H spectra of the starting material and product, we observe the loss of a broad peak at 12.74 ppm from Figure 3 (top) and the appearance of a singlet at 3.81 ppm in Figure 4 (top). The loss of the broad peak corresponds to the OH group in the carboxylic acid functionality, whereas the singlet peak represents the methoxy methyl group. Aside from these two groups, the spectra for the starting material and product look very similar, which is expected, as they are structurally almost identical. With the help of the other peaks in the ¹H spectra, the structures can be easily elucidated. The doublet centered at 7.04 ppm in Figure 3 (7.05 in Figure 4) relates to the meta proton of the aryl ring, as it is the most shielded due to its proximity to the electron donating amine group, in addition to the observed splitting caused by coupling to its neighboring proton environment. The broad peak at 7.90 ppm in Figure 3 (7.94 in Figure 4) corresponds to the amine group in the molecule. The proton signal centered at 7.84 ppm refers to the ortho proton furthest from the nitro group, which should theoretically be a doublet of doublets due to coupling with its neighboring proton as well as long-range coupling with the other ortho proton, which is hidden due to overlapping with the amine signal. Finally, the most deshielded doublet at 8.54 ppm in both ¹H spectra refer to the other ortho proton, which is situated between two strong electron withdrawing groups (nitro and ester groups) and is split through long-range coupling.

For the ¹³C spectra, we once again see a defining characteristic evident in the product, but not present in the starting material. In the spectrum of the product 2, we see the methoxy carbon resonance appear at 51.79 ppm, which is not present in the starting material 1. Aside from the methoxy carbon peak in the ¹³C spectrum of 2, there are 7 total peaks: 6 between 116 ppm and 149 ppm, relating to the aromatic peaks and 1 at 166.08 ppm for 1 (164.80 ppm for 2), corresponding to the carbonyl resonance.

Figure 4. ¹H (top, 60 MHz) and ¹³C{¹H}(bottom, 15.1 MHz) NMR spectra of methyl 4-amino-3-nitrobenzoate (2) in DMSO-d₆.

CONCLUSION

Using benchtop NMR spectroscopy, students are able to directly work with an NMR spectrometer in their labs and obtain ¹H and ¹³C data within minutes after collecting their product. In this sample experiment, a Fischer esterification reaction was completed to yield the product, methyl 4-amino-3-nitrobenzoate (2). The ¹H and ¹³C spectra of this product were obtained, analyzed and compared with the starting material, 4-amino-3-nitrobenzoic acid. Upon analysis, it was confirmed that the final product was successfully synthesized due to the appearance of the methoxy protons and carbons in the ¹H and ¹³C spectra of methyl 4-amino-3-nitrobenzoate (2), respectively, that did not appear in that of the 4-amino-3-nitrobenzoic acid (1) starting material. As NMR spectroscopy is one of the most powerful structural elucidation tools available to chemists, this experiment demonstrates the potential of benchtop NMR instruments as tools for hands-on learning in an undergraduate laboratory.

References