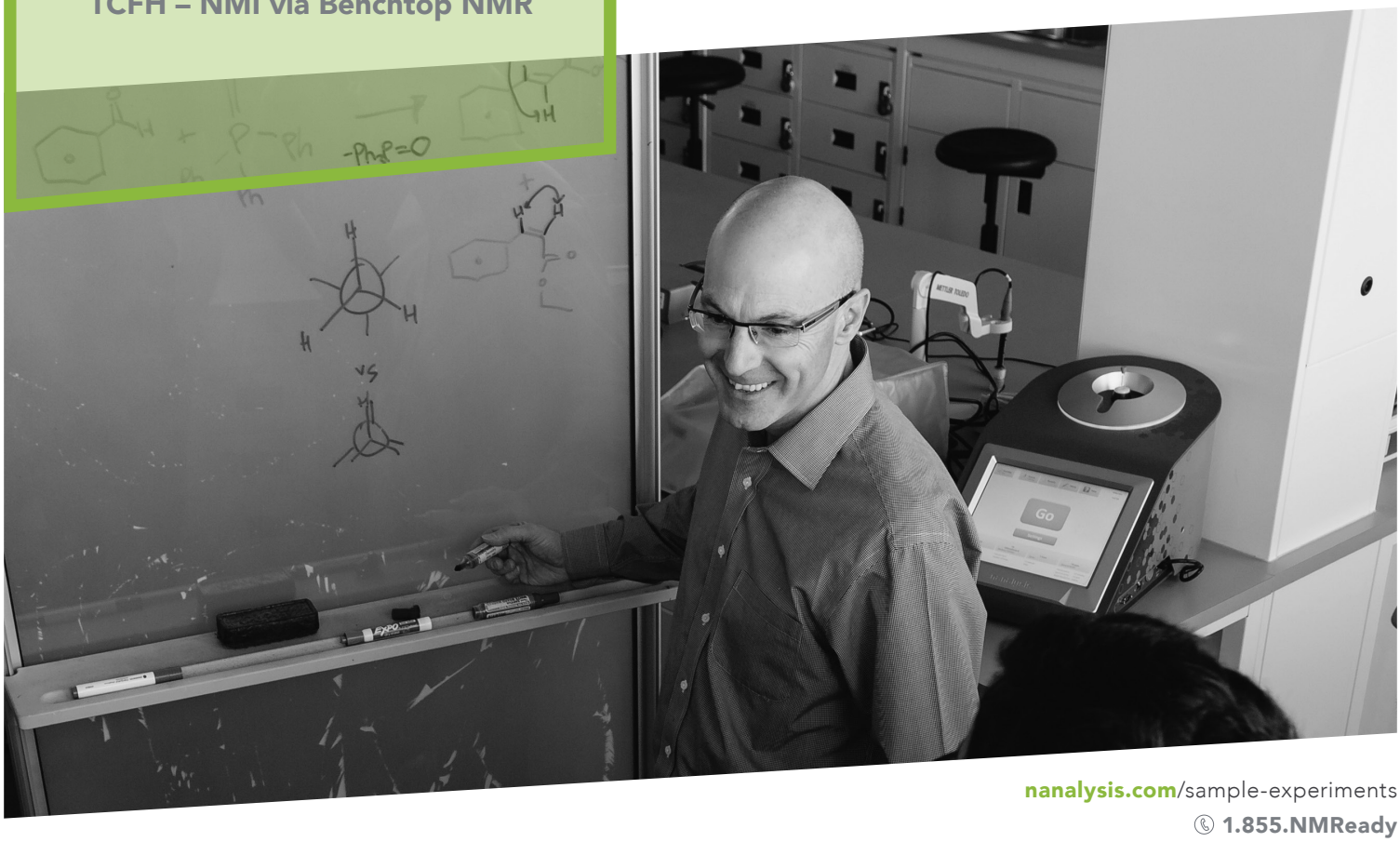
ORGANIC

UNDERGRADUATE EXPERIMENT

Greener Approach to the Synthesis of Amides Using TCFH – NMI via Benchtop NMR



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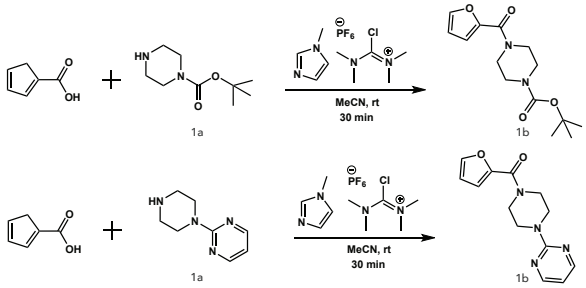
INTRODUCTION

Establishing greener methods and increasing visualization of complicated concepts within chemistry are essential in developing successful undergraduate experiments. Regarding safety, green chemistry aims to reduce the amounts of hazardous chemicals used in an experiment.[1] Pairing this with benchtop NMR in a laboratory space provides a safe, efficient, and interactive environment in which to conduct experiments that provide appreciable information that can be used in higher levels of academia or industry.[2] Traditionally, NMR samples that students submit are analyzed by specialists and subsequent handouts are given. This process with high-field spectrometers, whilst effective in generating data for students, lacks the element of hands-on experience. Providing an interactive display where students can manually manipulate parameters (*e.g.*, phasing, baseline correction, integration and peak picking) directly on an instrument provides students with knowledge towards increasing their chemical repertoire. Furthermore, this allows for for an easier transition into industrial and higher academic settings.

In some institutions, the spectra that students receive are unphased or the baseline is distorted. Moreover, advanced concepts in NMR (*e.g.*, labile protons,[3] amide bonds,[4] and relaxation delays[5]) are sometimes omitted. Benchtop NMR spectrometers can be valuable instruments to perform experiments and aid in structural elucidation.

In this sample experiment, rapid amide bond formation using the combination of *N,N,N′,N′*-tetramethylchloroformamidium hexafluorophosphate (TCFH) and *N*-methylimidazole (NMI) are conducted as outlined in **Scheme 1**. This experiment has been adapted from the work published by Baldwin and Conrad-Marut in *The Journal of Chemical Education*.[6] The role of this experiment is to provide students with an example of a large-scale industrial process that is reproducible and minimizes the use of an excess of solvents. Additionally, it provides an opportunity to learn about the structural elucidation of the products, which are important medicinal precursors in other reactions.[6] In **Scheme 1**, 2-furoic acid reacts with a mono-substituted piperazine with an excess of TCFH – NMI to form an amide under ambient temperatures.



***Scheme 1**. Reaction schemes for the amide bond formation of N-boc-piperazine (**1a**) and 1-(2-pyrimidyl) piperazine (**2a**) with 2-furoic acid and TCFH – NMI to form the respective amide products (**1b**) and (**2b**).*

The ^{1}H, $^{13}C\{^{1}H\}$, COSY, and HSQC NMR spectra were acquired using a 100 MHz benchtop NMR instrument to highlight the range of different experiments instructors can implement in a laboratory setting. Each experiment provides information that aids in the structural elucidation of the amide products.

Procedure

Materials

2-furoic acid (98%) and acetonitrile (99.8%) were purchased from Sigma Aldrich. DMSO-d_6 (99.8%) was purchased from Deutero GmbH. Distilled water was purchased from a local grocery store. Furthermore, *N*-boc piperazine (98%); 1-(2-pyrimidyl) piperazine (98%); TCFH (98%); and NMI (98%) were purchased from BLD Pharmatech. All reagents were used without further purification.

Hazards

As most of reagents used in the experiment can cause skin, respiratory and eye irritation, it is recommended that lab coats, gloves, and protective goggles are worn during this laboratory session. Thus, it is recommended to use a fume hood for handling the reagents. Furthermore, Safety Data Sheets (SDS) of all reagents should be reviewed prior this experiment.

Instrumentation

All NMR data was obtained using a Nanalysis 100 MHz instrument. The ^{1}H NMR experiments were performed using the following parameters: spectral width: 20 ppm, spectral center: varied; number of points: 8192; number of scans: varied; dummy scans: 0; interscan delay: varied; pulse angle: 90°; and receiver gain: auto. To note, 1 scan and a spectral center of 5 ppm should be sufficient for most experiments. For accurate integration, a longer interscan delay should be used.[7] The $^{13}C\{^{1}H\}$ NMR experiments were performed with the following parameters: spectral width: 220 ppm, spectral center: 100 ppm; number of points: 4096; number of scans: 4096; dummy scans: 0; interscan delay: 1 second; pulse angle: 45°; and receiver gain: auto. The parameters of the 2D experiments framed in reference to the parameters in the ^{1}H and $^{13}C\{^{1}H\}$. All the spectra presented were manually corrected for phase and baseline distortions using MestReNova software (v14.2.3).

Synthesis

Disubstituted piperazine

In a vial (≥20 mL recommended), 2-furoic acid (300 mg, 2.68 mmol) was added with the mono-substituted piperazine (**1a**: 498 mg, 1 equiv.) or (**2a**: 439 mg, 1 equiv.). A stir bar was added to the vial. 3 mL of acetonitrile was added to dissolve the contents of the vial. 0.45 mL of NMI (2.1 equiv.) was added to the mixture. The resulting mixture was stirred and TCFH (825 mg, 1.1 equiv.) was added to the vial. The vial was capped, and the speed of the stirring were increased. After 30 minutes of stirring the mixture, 9 mL of distilled water was added to the vial. The vial was vigorously shaken for a minute, then placed in an ice-bath for 10 minutes. Using gravity filtration, the resulting white crystals were washed with distilled water, air-dried, then isolated. It should be noted that for a faster synthesis, vacuum filtration is recommended. No further purification was conducted.

Results and Discussion

The experiments were conducted under an hour, and the acylation reactions resulted in average yields of 70% among both amide products. From the products, a saturated sample (~500 mM) is sufficient for all NMR experiments.

During the experiments, ^{1}H NMR spectra of the starting reagents can be collected from 250 mM concentrations in DMSO-d_6. With exception to 2-furoic acid, ~25 mg of starting reagents in 0.6 mL of DMSO-d_6 is also sufficient for a ^{1}H NMR analysis (**Figures 1** and **2**).

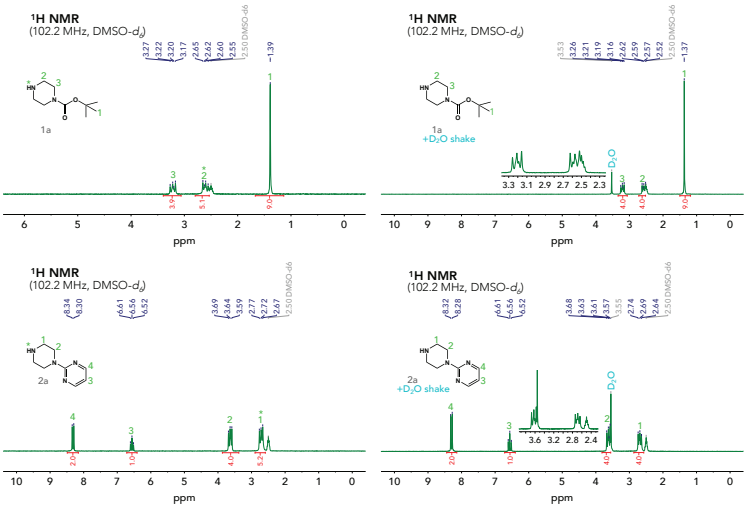


Figure 1. *^{1}H (102.2 MHz) NMR spectra of monosubstituted piperazines (**1a**) and (**2a**) in DMSO-d_6. The asterisk in the ^{1}H spectrum represents the labile proton on the secondary amine. A drop of D_2O was added into both NMR samples to diminish the signal from exchangeable protons.*[8]

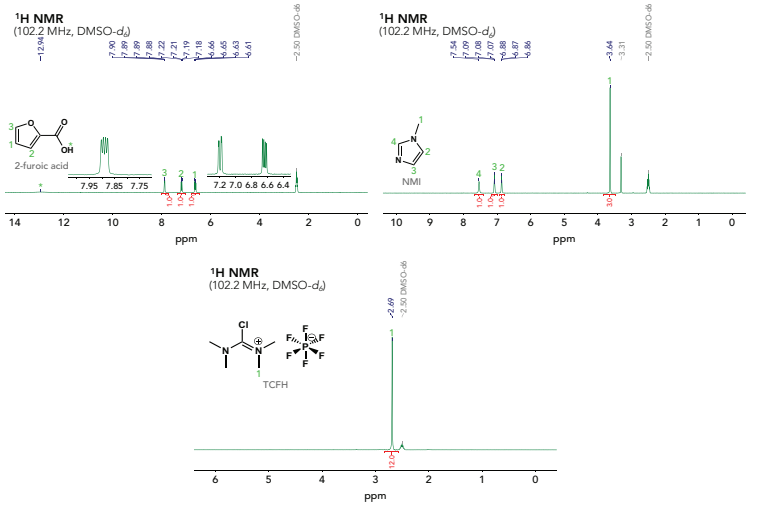


Figure 2. *^{1}H (102.2 MHz) NMR spectra of 2-furoic acid, NMI, and TCFH in DMSO-d_6.*

The ^{1}H NMR spectra of both **1b** and **2b** (**Figures 3** and **4**) contain a furoyl group (integrating to three protons) as well as the piperazine methylene groups (integrating to eight protons). The furoyl protons can be differentiated by their aromaticity. Due to their environment the signals of the furoyl protons have a higher chemical shift relative to the other protons.

*Disubstituted piperazine (**1b**)*

The ^{1}H, $^{13}C\{^{1}H\}$, and COSY NMR spectra of **1b** are shown in **Figure 4**. In the ^{1}H NMR spectrum, we can observe the expected *tert*-butyl group at δ = 1.4 ppm, and the methylene and aromatic resonances at 3 – 4 ppm and 6.5 – 8 ppm, respectively. This is further supported by confirming the integrations. From the $^{13}C\{^{1}H\}$ NMR spectrum, we can hypothesize the chemical shift of the quaternary carbons from their relative intensities to other peaks, then use the HSQC to support that assessment. The quaternary carbon of the *tert*-butyl group is observed at δ = 79.1 ppm. The COSY experiment highlight spin-coupling between protons within the same vicinity or environment as one another. From the COSY, we can observe H_4-H_5, and H_4-H_6 correlations of the furoyl group. The HSQC experiment details correlations between proton and carbon directly bonded. We can observe H_1-C_1, H_2-C_2, H_3-C_3, H_5-C_6, H_4-C_5, and H_6-C_7.

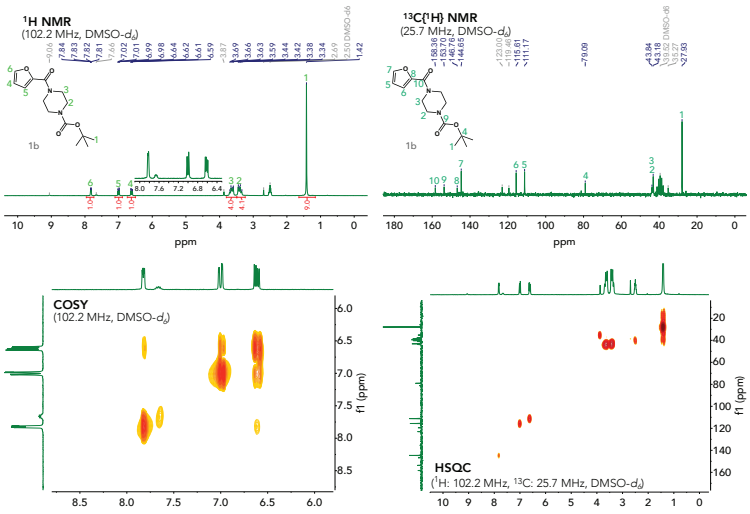


Figure 3. *^{1}H (102.2 MHz); $^{13}C\{^{1}H\}$ (25.7 MHz); ^{1}H, ^{1}H COSY (102.2 MHz); and (^{1}H: 102.2 MHz), (^{13}C: 25.7 MHz) HSQC NMR spectra of **1b** in DMSO-d_6.*

*Disubstituted piperazine (**2b**)*

The ^{1}H, $^{13}C\{^{1}H\}$, and COSY NMR spectra of **2b** were collected are shown in **Figure 4**. In the ^{1}H NMR spectrum, we can observe methylenes at δ = 3.8 ppm. Interestingly, the product contains two aromatic groups: furoyl and pyrimidyl, that are in the same region (6.5 – 9 ppm). From the integrations, H_6 can be identified because it integrates to two and the signal is a doublet. Two signals overlap in between δ = 6.5 and 6.8 ppm. These signals can be differentiated based on their coupling patterns. from their coupling patterns. H_2 is a doublet of doublets and H_3 is a triplet. The coupling constant between of the triplet H_3 and doublet of H_6 are both (~4.8 Hz). In the $^{13}C\{^{1}H\}$ NMR spectrum, three quaternary carbons are observed. Further, the characteristic methylenes are observed at δ = 43.3 ppm. In the COSY spectrum, we can observe the furoylic correlations (H_2-H_4 and H_2-H_5). The HSQC spectrum, identifying proton-carbon single bond correlations shows H_1-C_1, H_2-C_2, H_3-C_3, H_4-C_4, H_5-C_5 and H_6-C_7.

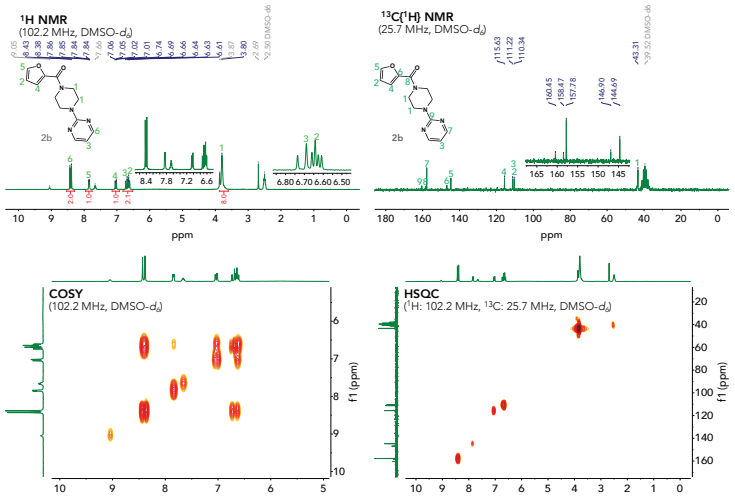


Figure 4. *^{1}H (102.2 MHz); $^{13}C\{^{1}H\}$ (25.7 MHz); ^{1}H, ^{1}H COSY (102.2 MHz); and (^{1}H: 102.2 MHz), (^{13}C: 25.7 MHz) HSQC NMR spectra of **2b** in DMSO-d_6.*

Conclusion

This experiment focuses on the facile synthesis of amide products at room temperature and facilitating constant interaction with a benchtop NMR instrument. Building on the elements of green chemistry, this experiment does not require an inert atmosphere and limits exposure to harmful reagents.[6] With the advent of benchtop NMR, students can explore different parameters and acquire their own data for experiments. Paired with the visual display and hands-on experience, NMR can provide a range of experiments that aid in the structural elucidation of compounds, especially medicinally relevant compounds. This laboratory experiment highlights a range of 1D (^{1}H, and $^{13}C\{^{1}H\}$) and 2D (COSY, and HSQC) experiments on two disubstituted piperazines. Other 2D experiments such as NOESY and TOCSY can be implemented to illustrate more advanced concepts in NMR.

References

[1] Ganesh, K.N.; Zhang, D.; Miller, S.J.; Rossen, K., Chirik, P.J.; Kozlowski, M.C., Zimmerman, J.B.; Brooks B.W.; Savage, P.E.; Allen, D.T.; Voutchkova-Kostal, A.M. *Org. Process Res. Dev.* **2021**, *25*, 1455–1459

[2] Castaing-Cordier, T.; Bouillaud, D.; Farjon, J.: Giraudeau, P. *Annual Reports on NMR spectroscopy.* **2021**, *103*, 191-258.

[3] Labile protons and J-Coupling. https://www.nanalysis.com/nmready-blog/2023/2/7/labile-protons-and-j-coupling Accessed May 4, 2023)

[4] Using NMR to observe the restricted rotation in amide bonds. https://www.nanalysis.com/nmready-blog/2023/3/20/using-nmr-to-observe-the-restricted-rotation-in-amide-bonds (Accessed May 4, 2023)

[5] NMR acquisition parameters and qNMR. https://www.nanalysis.com/nmready-blog/2022/9/27/nmr-acquisition-parameters-and-qnmr (Accessed May 19, 2023)

[6] Baldwin, O.W.M.; Conrad-Marut, L.H.; Beutner, G.L.; Vosburg, D. A. *J. Chem. Educ.* **2022**, *99*, 3747–3751

[7] Beyond Structural Elucidation – Introduction to qNMR Part III – Relaxation Delays https://www.nanalysis.com/nmready-blog/2019/5/30/beyond-structural-elucidation-introduction-to-qnmr-part-iii-relaxatiojhyn-delays (Accessed May 4, 2023)

[8] To D_2O or not to D_2O? https://www.nanalysis.com/nmready-blog/2017/11/30/to-d2o-or-not-to-d2o (Accessed May 4, 2023)

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