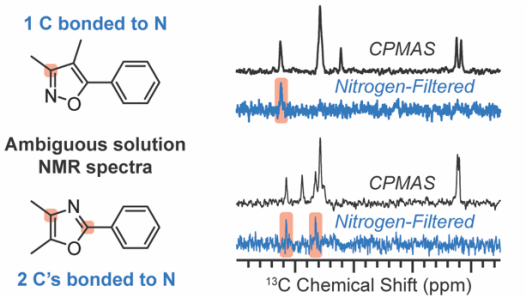
Attached Nitrogen Test by ^{13}C-^{14}N Solid-State NMR Spectroscopy for the Structure Determination of Heterocyclic Isomers

Rick W. Dorn,[1,2‡] Brendan J. Wall,[1‡] Sarah B. Ference,[1] Sean R. Norris,[1] Joseph W. Lubach,[3] Aaron J. Rossini,[1,2*] Brett VanVeller,[1*]

[1]Iowa State University, Department of Chemistry, Ames, IA, USA, 50011. [2]US Department of Energy, Ames Laboratory, Ames, IS, USA, 50011. [3]Genentech, Inc., South San Francisco, CA, USA, 94080.



ABSTRACT: Differentiation of heterocyclic isomers by solution ^{1}H, ^{13}C and ^{15}N NMR spectroscopy is often challenging due to similarities in their spectroscopic signatures. Here, $^{13}C\{^{14}N\}$ solid-state NMR spectroscopy experiments are shown to operate as an "attached nitrogen test", where heterocyclic isomers are easy to distinguish based on 1D nitrogen-filtered ^{13}C solid-state NMR. We anticipate that these NMR experiments will facilitate the assignment of heterocycle isomers during synthesis and natural product discovery.

The determination of molecular structure is a foundational pillar of synthetic chemistry and natural product discovery. Despite the suite of available techniques to probe molecular structure, we often still "see through a glass, darkly" when assigning spectroscopic data, where the vast possibilities of atomic connectivity may lead to errors in structural assignment. This ambiguity in structural assignment is particularly true in natural product discovery, where common spectroscopic techniques cannot often distinguish between isomeric products. For example, a 2011 review reported over 150 misassigned natural products between 2001–2010.[1] Multiple other reviews have also discussed the misassignment of natural products.[2-4] Several examples of natural products that were misassigned by solution NMR spectroscopy are shown in Figure 1.[5-7]

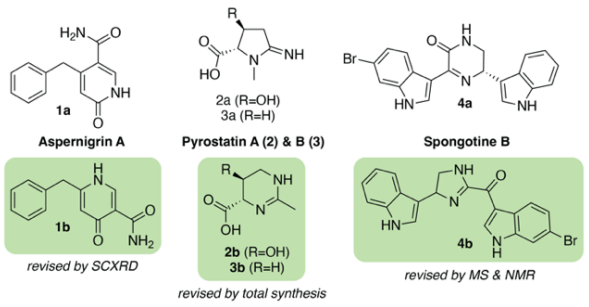


Figure 1. Selected natural products (**1**-**4**) with originally proposed structures (upper) and revised structures (lower, highlighted in green).

Total synthesis of a target molecule is a classical avenue to confirm atomic connectivity and identify errors in originally proposed structures.[2-4] A key drawback, however, is that total synthesis is a time and labor consuming endeavor. Further, even after total synthesis is completed, single crystal X-ray diffraction (SCXRD) is sometimes required to unambiguously determine molecular structure, but diffraction quality single crystals are not obtainable in all cases. Solution NMR spectroscopy is the workhorse method for probing molecular structure within organic molecules and natural products. Nearly all organic systems are suitable for NMR spectroscopy, and isotropic chemical shifts and scalar (*J*-) couplings reveal unique information on the local chemical environment of the probed nuclei. Two-dimensional (2D) homonuclear and heteronuclear correlation NMR experiments are powerful tools to determine molecular structure. However, heteronuclear correlation solution NMR experiments on organic systems are often limited to ^{1}H-^{13}C, such that ^{13}C NMR signal assignment is based solely on ^{13}C chemical shifts and ^{1}H-^{13}C scalar (*J*-) couplings. The assignment of ^{13}C NMR signals to a single isomer in systems containing nitrogen heterocycles may become ambiguous when using 2D ^{1}H-^{1}H and ^{1}H-^{13}C solution NMR spectroscopy techniques because changes in the nitrogen atom location within a heterocycle often does not alter the observed ^{1}H-^{1}H or ^{1}H-^{13}C *J*-couplings. Indeed, the misassigned natural products shown in Figure 1 differ from their corrected structures by the location and connectivity of the nitrogen atoms.

Thus, information about direct connectivity of carbon and nitrogen atoms would be immensely valuable to discriminate between possible heterocyclic isomers. Here, we report the application of $^{13}C\{^{14}N\}$ solid-state NMR experiments that exploit ^{13}C-^{14}N dipolar couplings to identify C atoms directly bonded to N atoms.[8-15] This "attached nitrogen test" requires no isotopic labeling and the working organic

chemist will find that such spectra are easily interpretable, akin to the interpretation of NOE difference spectra. We demonstrate the utility of $^{13}C\{^{14}N\}$ solid-state NMR spectroscopy for structure determination through model case studies that address the misassignments described in Figure 1. Lastly, we demonstrate the powerful utility of N-filtered ^{13}C NMR spectra to aid in the accurate assignment of more complex molecular scaffolds relevant to natural products and pharmaceuticals.

Nitrogen has two NMR active isotopes, ^{14}N and ^{15}N, with ^{15}N being the preferred nucleus to probe in NMR spectroscopy because it is a spin $I = 1/2$ nucleus, whereas ^{14}N is spin $I = 1$ quadrupolar nucleus. Unfortunately, ^{13}C-^{15}N NMR experiments are challenging at natural isotopic abundance (0.004 % probability of having a ^{13}C-^{15}N spin pair) and are sometimes only feasible in concentrated systems and/or with sensitivity enhancement techniques, such as dynamic nuclear polarization (DNP).[16-22] ^{13}C-^{14}N NMR experiments are attractive because ^{14}N is 99.6 % abundant. However, the quadrupolar nature of ^{14}N means that one-bond ^{13}C-^{14}N *J*-couplings (^1J~ 10-15 Hz) often cannot be observed in solution NMR spectra due to the self-decoupling of ^{14}N that occurs because of the continuous alternation of the ^{14}N spin states by rapid longitudinal (T_1) relaxation.

Fortunately, ^{14}N can be readily probed in solid-state NMR experiments. Here, we use the $^{13}C\{^{14}N\}$ Resonance Echo Saturation Pulse DOuble Resonance (RESPDOR) NMR experiment to obtain 1D N-filtered ^{13}C NMR spectra (Figure 2A). We note that $^{13}C\{^{14}N\}$ solid-state NMR experiments have been used for over two decades to obtain structural information in organic and biomolecular systems.[8-15] However, the goal of our work is to demonstrate the value and simplicity of $^{13}C\{^{14}N\}$ solid-state NMR experiments to the practicing chemist to differentiate heterocyclic isomers.

We first optimized the experimental conditions for the "attached nitrogen" $^{13}C\{^{14}N\}$ RESPDOR experiments using histidine hydrochloride monohydrate (**Hist**) as a model compound. In the $^{13}C\{^{14}N\}$ RESPDOR experiment, two ^{13}C NMR spectra are recorded; one with and one without a ^{14}N phase-modulated (PM) saturation pulse.[23-24] Taking the difference of the two NMR spectra yields an N-filtered ^{13}C NMR spectrum because the ^{13}C NMR signal will have reduced intensity when pulsing on ^{14}N if it is covalently bonded to N (see SI for more discussion). Experiments on **Hist** showed that ca. 1.3 ms of ^{13}C REDOR recoupling (τ_{rec}) was optimal for maximizing the difference ^{13}C NMR signal for C atoms covalently bonded to N atoms and minimizing dephasing for C atoms not bonded to N atoms (Figure 2B and S35). For **Hist**, a total of 1 hour of spectrometer time was required to obtain the ^{14}N-filtered ^{13}C NMR spectrum that shows only ^{13}C NMR signals from C atoms exhibiting C-N covalent bonds (Figure 2C). We note that similar "attached nitrogen" $^{13}C\{^{14}N\}$ NMR spectra can be recorded without ^{13}C dipolar recoupling to cause signal dephasing by evolution of ^{13}C-^{14}N *J*-couplings and residual dipolar splittings (Figure S36).[25-26] However, the RESPDOR experiment will generally be more sensitive because the dipolar coupling is over one order of magnitude larger than the *J*-coupling and residual dipolar splitting (Figure S37, see SI for more discussion). For **Hist**, the RESPDOR experiment with dipolar recoupling was ca. two times more sensitive (SNR min$^{-1/2}$) than the analogous experiment without dipolar recoupling.

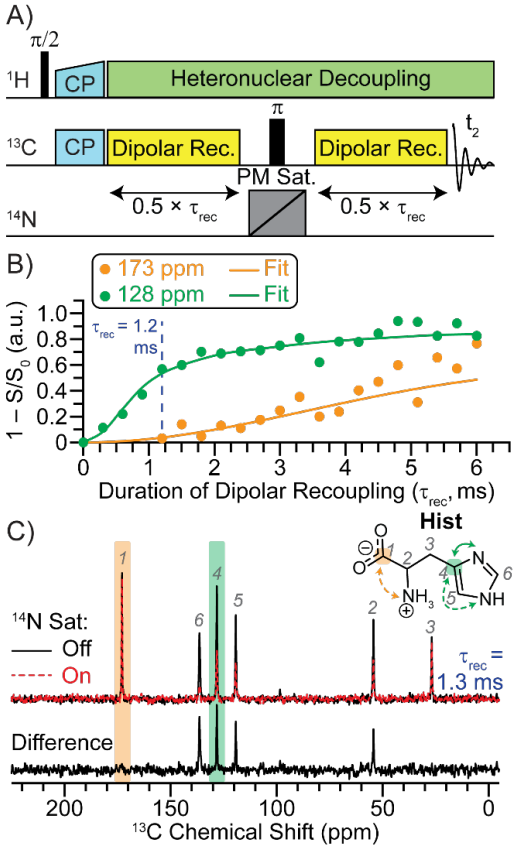


Figure 2. (A) $^{13}C\{^{14}N\}$ PM-RESPDOR pulse sequence. (B) $^{13}C\{^{14}N\}$ RESPDOR curves for the ^{13}C NMR signals of **Hist** at (orange) 173 ppm and (green) 128 ppm. The experimental data points are shown as circles and numerical simulations are shown as solid lines. (C) $^{13}C\{^{14}N\}$ RESPDOR spectra of **Hist** recorded (red, dashed) with or (black, solid) without a ^{14}N PM saturation pulse. The difference spectrum is shown below.

The core atoms of heterocycles typically do not display characteristic ^1H or ^{13}C chemical shifts that would allow for their straightforward identification through 1D $^1H/^{13}C$ NMR spectroscopy without prior knowledge of chemical shifts (Figure S38). Even with modern 2D ^1H homonuclear and/or ^1H-^{13}C heteronuclear correlation solution NMR experiments, spectral interpretation is still often ambiguous due to similarities in the observed correlations for different isomers, making it challenging to differentiate isomers on unknown, highly substituted heterocyclic systems (see SI for solution NMR spectra).

For example, the structure of Aspernigrin A (**1**) was initially assigned via $^1H\{^{13}C\}$ HMBC but was later corrected by SCXRD (Figure 1).[5, 27-28] The ambiguity in assigning the 2- or 4-pyridone heterocyclic cores could have been easily addressed with an "attached nitrogen" $^{13}C\{^{14}N\}$ RESPDOR NMR experiment. To test this hypothesis, we recorded $^{13}C\{^{14}N\}$ RESPDOR NMR spectra of acridinone (**5**) and phenanthridinone (**6**) as model compounds for Aspernigrin A (Figure 3A-B).

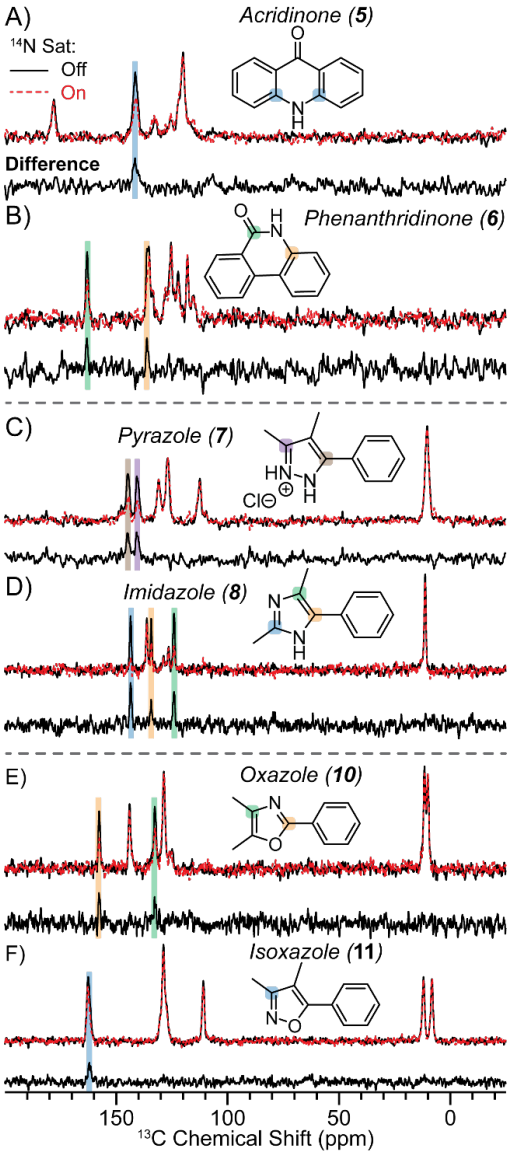


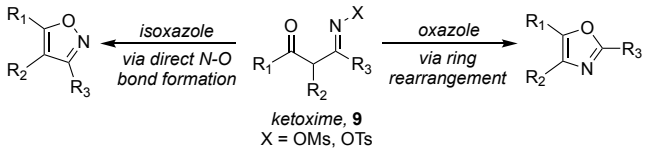
Figure 3. Comparison of $^{13}C\{^{14}N\}$ RESPDOR NMR spectra of (A) acridinone (**5**), (B) phenanthridinone (**6**), (C) pyrazole (**7**), (D) imidazole (**8**), (E) oxazole (**10**) and (F) isoxazole (**11**). $^{13}C\{^{14}N\}$ RESPDOR spectra were recorded with 1.28 ms of total dipolar recoupling and (red, dashed) with or (black, solid) without a ^{14}N PM saturation pulse. The difference spectrum is shown below. NMR signals correspond to the highlighted C atoms on the structures.

The ^{14}N-filtered ^{13}C NMR spectra allow for clear differentiation of the 2- versus 4-pyridone core. Isomer **6** displays two ^{13}C NMR signals exhibiting a C-N covalent bond (Figure 3B). Importantly, one of the ^{13}C NMR signals attached to N in **6** clearly shows a diagnostic chemical shift associated with a carbonyl carbon (> 150 ppm), while that of **5** does not. **5** displays only one ^{13}C NMR signal exhibiting a C-N covalent bond due to the C_2 symmetry of the compound (Figure 3A). In the more substituted Aspernigin A (**1a** and **1b**), the corrected structure will show two carbonyl ^{13}C NMR signals in the ^{14}N-filtered ^{13}C NMR spectrum as opposed to one in the misassigned structure (Figure 1).

We next examined the differentiation of azole-type heterocycles using the "attached nitrogen" $^{13}C\{^{14}N\}$ RESPDOR experiment. Model heterocycles pyrazole (**7**) and imidazole (**8**) form the core of numerous drug scaffolds (Figure 3C-D). Particularly for highly substituted rings, ^{1}H-^{13}C correlations can be ambiguous, and without prior knowledge of ^{1}H and/or ^{13}C chemical shifts, the assignment of the $^{1}H/^{13}C$ NMR spectra to a single isomer can lead to error (Figure S38). Alternatively, comparison of the ^{14}N-filtered ^{13}C NMR spectra enable the easy assignment of the two heterocyclic isomers, where **7** displays two ^{13}C NMR signals exhibiting C-N covalent bonds, while imidazole **8** displays three ^{13}C NMR signals exhibiting C-N covalent bonds (Figure 3C-D).

Differentiation of isoxazole and oxazole heterocyclic isomers provide a compelling illustration of the utility of the "attached nitrogen" $^{13}C\{^{14}N\}$ RESPDOR NMR experiment. Even when assisted by 2D solution NMR experiments, conclusive assignment of the oxazole can be elusive without comparison to the isoxazole isomer (and vice versa), a form of structure determination by total synthesis (Figure S3-5 and S8-10). Alarmingly, however, such an approach is more complicated when one considers that both the oxazole and isoxazole can be prepared from the same starting ketoxime (**9**, Scheme 1).[29]

Scheme 1. Preparation of Oxazole and Isoxazole Heterocyclic Isomers from Ketoxime.



The ^{14}N-filtered ^{13}C NMR spectra of oxazole (**10**) and isoxazole (**11**) enable the straightforward differentiation of the heterocyclic isomers (Figure 3E-F). **10** displays two ^{13}C NMR signals with C-N bonds, while **11** only exhibits one ^{13}C NMR signal with a C-N bond.

Finally, to illustrate the ability of "attached nitrogen" $^{13}C\{^{14}N\}$ RESPDOR experiments to aid in the determination of more complex molecules relevant to natural products and pharmaceuticals, we performed experiments on a multi-component API, where the free-base molecule forms a co-crystal with phosphoric acid (**12**, Figure 4A).[30] The $^{13}C\{^{14}N\}$ RESPDOR experiments were performed with either conventional NMR at room temperature or with dynamic nuclear polarization (DNP) at *ca.* 100 K. In a DNP experiment, the NMR signal intensity is enhanced by 1-2 orders of magnitude by transferring the polarization of electron spins from a polarizing agent (e.g., TEKPol) to the nuclear spins. $^{1}H\rightarrow^{13}C$ CPMAS DNP enhancements were $\geq$ 10, meaning that a $^{1}H\rightarrow^{13}C$ CPMAS NMR spectrum with the same signal-to-noise ratio could be acquired c*a.* 100 times faster with DNP than conventional room temperature NMR spectroscopy (Figure S39).

1D ^{14}N-filtered ^{13}C NMR spectra of **12** were obtained in *ca.* 40 min with DNP and a 3.2 mm rotor or *ca.* 17 hours with conventional room temperature solid-state NMR spectroscopy and 2.5 mm rotor (Figure 4B and S40, respectively). The 1D ^{14}N-filtered ^{13}C NMR spectrum reveals all ^{13}C NMR signals exhibiting C-N covalent bonds. We note that *C*14 has reduced intensity in the DNP spectrum due to ^{13}C signal overlap with the DNP solvent (see SI for more discussion).

The large sensitivity gains provided by DNP also enabled the acquisition of $^{13}C\{^{14}N\}$ RESPDOR curves that provide detail as to the rate of signal build-up and the extent of signal dephasing (Figure 4C and S42). In turn, the shape of these curves are dependent on the type and number of nitrogen atoms within a ca. 4 Å radius (Table S2). Therefore, fitting of the experimental $^{13}C\{^{14}N\}$ RESPDOR curves

with numerical simulations facilitates the assignment of all ^{13}C NMR signals spatially proximate to nitrogen atoms (Figure 4C and S42). This capability permits complete ^{13}C signal assignment by comparing the $^{13}C\{^{14}N\}$ RESPDOR curves with ^{1}H-^{13}C heteronuclear correlation NMR spectra and plane-wave DFT GIPAW[31] calculated ^{13}C chemical shifts (Figure S43-45).

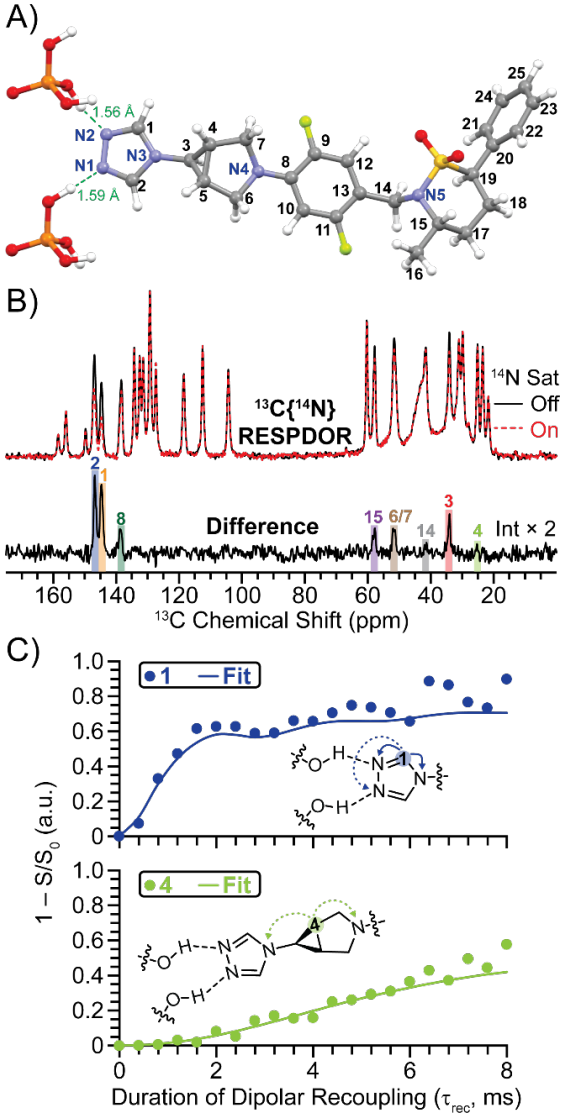


Figure 4. (A) Crystal structure of co-crystal **12**. H, C, N, O, F, P and S atoms are white, grey, blue, red, green, orange and yellow, respectively. (B) DNP-enhanced $^{13}C\{^{14}N\}$ RESPDOR spectra recorded with 1.2 ms of dipolar recoupling and (red) with or (black) without a ^{14}N saturation pulse. The difference spectrum is shown below. (C) DNP-enhanced $^{13}C\{^{14}N\}$ RESPDOR curves of co-crystal **12** (see Figure S42 for all RESPDOR curves). The circles correspond to the experimental data points and the solid lines correspond to numerical simulations.

In conclusion, the determination of molecular structure is a foundational pillar of organic synthesis and natural product discovery. However, typical ^{1}H-^{1}H and ^{1}H-^{13}C scalar (J-) correlation solution NMR experiments reveal the same homonuclear/heteronuclear correlations for many heterocyclic isomers, meaning that spectroscopic assignment to a single isomer without prior knowledge of ^{1}H and/or ^{13}C chemical shifts is often ambiguous. Here, $^{13}C\{^{14}N\}$ RESPDOR solid-state NMR spectroscopy experiments are shown to enable the easy acquisition of 1D ^{14}N-filtered ^{13}C solid-state NMR spectra, which effectively operates as an "attached nitrogen test".

The practical utility of $^{13}C\{^{14}N\}$ RESPDOR solid-state NMR spectroscopy experiments to differentiate heterocyclic isomers was demonstrated for three different model systems. In all three examples, the heterocyclic isomers could be easily distinguished from the ^{14}N-filtered ^{13}C NMR spectra, where 1D and 2D ^{1}H and ^{13}C correlation solution NMR spectroscopy were ambiguous, particularly if one did not have prior knowledge of the molecular structure. We also demonstrated how ^{14}N-filtered ^{13}C NMR spectra can aid in the structural characterization of more complex molecular scaffolds relevant to natural products and pharmaceuticals.

We anticipate that $^{13}C\{^{14}N\}$ RESPDOR solid-state NMR spectroscopy experiments will provide practicing chemists with a simple method to obtain 1D ^{14}N-filtered ^{13}C solid-state NMR spectra that can greatly aid in ^{13}C NMR signal assignment and differentiation of heterocyclic isomers. 1D $^{13}C\{^{14}N\}$ NMR experiments on **12** were performed at room temperature with 2.5 mm rotors, ca. 20 mg of material and 17 hours of spectrometer time. Therefore, even in the absence of sensitivity enhancement by DNP, $^{13}C\{^{14}N\}$ NMR experiments can be feasibly applied to molecules with comparable size and complexity as natural products. Therefore, "attached nitrogen tests" could be especially useful in natural product discovery because they will reduce structural ambiguities and misassignments.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. Methods (synthesis, NMR and DFT), solid-state NMR experimental parameters, solution NMR spectra, additional solid-state NMR spectra and SIMPSON numerical simulation input files (ZIP)

AUTHOR INFORMATION

Corresponding Author

*email: arossini@iastate.edu, 515-294-8952
*email: bvv@iastate.edu, 515-294-7613

Author Contributions

‡R.W.D. and B.J.W. contributed equally.

ACKNOWLEDGMENT

Solid-state NMR spectroscopy experiments (R.W.D., S.F. and A.J.R.) were supported by the National Science Foundation under Grant No. 1709972. A.J.R. acknowledges additional support from the Alfred P. Sloan Foundation through a Sloan research fellowship. We thank Genentech, Inc. and its Innovation Fund, for providing additional financial support for this work. Heterocyclic isomer syntheses and solution NMR spectroscopy experiments (B.J.W., S.N. and B.V.) were supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R35 GM142883. We are grateful to Dr. Paroma Chakravarty and Dr. Lauren Sirois (Genentech) for providing cocrystal **12**.

REFERENCES

1. Suyama, T. L.; Gerwick, W. H.; McPhail, K. L., Survey of marine natural product structure revisions: A synergy of spectroscopy and chemical synthesis. *Bioorg. Med. Chem.* **2011,** *19* (22), 6675-6701.
2. Nicolaou, K. C.; Snyder, S. A., Chasing Molecules That Were Never There: Misassigned Natural Products and the Role of Chemical

Synthesis in Modern Structure Elucidation. *Angew. Chem. Int. Ed.* **2005,** *44* (7), 1012-1044.

3. Maier, M. E., Structural revisions of natural products by total synthesis. *Nat. Prod. Rep.* **2009,** *26* (9), 1105-1124.

4. Yoo, H.-D.; Nam, S.-J.; Chin, Y.-W.; Kim, M.-S., Misassigned natural products and their revised structures. *Arch. Pharmacal Res.* **2016,** *39* (2), 143-153.

5. Ye, Y. H.; Zhu, H. L.; Song, Y. C.; Liu, J. Y.; Tan, R. X., Structural Revision of Aspernigrin A, Reisolated from Cladosporium herbarum IFB-E002. *J. Nat. Prod.* **2005,** *68* (7), 1106-1108.

6. Castellanos, L.; Duque, C.; Zea, S.; Espada, A.; Rodríguez, J.; Jiménez, C., Isolation and Synthesis of (−)-(5S)-2-Imino-1-methylpyrrolidine-5- carboxylic Acid from Cliona tenuis: Structure Revision of Pyrostatins. *Org. Lett.* **2006,** *8* (21), 4967-4970.

7. Bao, B.; Sun, Q.; Yao, X.; Hong, J.; Lee, C.-O.; Cho, H. Y.; Jung, J. H., Bisindole Alkaloids of the Topsentin and Hamacanthin Classes from a Marine Sponge Spongosorites sp. *J. Nat. Prod.* **2007,** *70* (1), 2-8.

8. Grey, C. P.; Veeman, W. S., The detection of weak heteronuclear coupling between spin 1 and spin 1/2 nuclei in MAS NMR; $^{14}N/^{13}C/^{1}H$ triple resonance experiments. *Chem. Phys. Lett.* **1992,** *192* (4), 379-385.

9. Grey, C. P.; Veeman, W. S.; Vega, A. J., Rotational echo $^{14}N/^{13}C/^{1}H$ triple resonance solid-state nuclear magnetic resonance: A probe of $^{13}C–^{14}N$ internuclear distances. *J. Chem. Phys.* **1993,** *98* (10), 7711-7724.

10. Grey, C. P.; Eijkelenboom, A. P. A. M.; Veeman, W. S., ^{14}N Population transfers in two-dimensional ^{13}C-^{14}N-^{1}H triple-resonance magic-angle spinning nuclear magnetic resonance spectroscopy. *Solid State Nucl. Magn. Reson.* **1995,** *4* (2), 113-120.

11. Gullion, T., Measurement of Dipolar Interactions Between Spin-1/2 and Quadrupolar Nuclei by Rotational-Echo, Adiabatic-Passage, Double-Resonance NMR. *Chem. Phys. Lett.* **1995,** *246* (3), 325-330.

12. Hughes, E.; Gullion, T.; Goldbourt, A.; Vega, S.; Vega, A. J., Internuclear Distance Determination of S=1, I=1/2 Spin Pairs Using REAPDOR NMR. *J. Magn. Reson.* **2002,** *156* (2), 230-241.

13. Ba, Y.; Kao, H.-M.; Grey, C. P.; Chopin, L.; Gullion, T., Optimizing the $^{13}C–^{14}N$ REAPDOR NMR Experiment: A Theoretical and Experimental Study. *J. Magn. Reson.* **1998,** *133* (1), 104-114.

14. Gan, Z., Measuring multiple carbon–nitrogen distances in natural abundant solids using R-RESPDOR NMR. *Chem. Commun.* **2006,** (45), 4712-4714.

15. Pope, G. M.; Hung, I.; Gan, Z.; Mobarak, H.; Widmalm, G.; Harper, J. K., Exploiting $^{13}C/^{14}N$ solid-state NMR distance measurements to assign dihedral angles and locate neighboring molecules. *Chem. Commun.* **2018,** *54* (49), 6376-6379.

16. Jackalin, L.; Kharkov, B. B.; Komolkin, A. V.; Dvinskikh, S. V., Experimental strategies for $^{13}C–^{15}N$ dipolar NMR spectroscopy in liquid crystals at the natural isotopic abundance. *Phys. Chem. Chem. Phys.* **2018,** *20* (34), 22187-22196.

17. Cifelli, M.; Domenici, V.; Chizhik, V. I.; Dvinskikh, S. V., $^{15}N–^{13}C$ Dipole Couplings in Smectic Mesophase of a Thermotropic Ionic Liquid. *Appl. Magn. Reson.* **2018,** *49* (6), 553-562.

18. Cheatham, S.; Kline, M.; Kupče, E., Exploiting natural abundance $^{13}C–^{15}N$ coupling as a method for identification of nitrogen heterocycles: practical use of the HCNMBC sequence. *Magn. Reson. Chem.* **2015,** *53* (5), 363-368.

19. Märker, K.; Pingret, M.; Mouesca, J.-M.; Gasparutto, D.; Hediger, S.; De Paëpe, G., A New Tool for NMR Crystallography: Complete $^{13}C/^{15}N$ Assignment of Organic Molecules at Natural Isotopic Abundance Using DNP-Enhanced Solid-State NMR. *J. Am. Chem. Soc.* **2015,** *137* (43), 13796-13799.

20. Cheatham, S.; Gierth, P.; Bermel, W.; Kupče, Ē., HCNMBC – A pulse sequence for H–(C)–N Multiple Bond Correlations at natural isotopic abundance. *J. Magn. Reson.* **2014,** *247*, 38-41.

21. Smith, A. N.; Märker, K.; Hediger, S.; De Paëpe, G., Natural Isotopic Abundance ^{13}C and ^{15}N Multidimensional Solid-State NMR Enabled by Dynamic Nuclear Polarization. *J. Phys. Chem. Lett.* **2019,** *10* (16), 4652-4662.

22. Smith, A. N.; Märker, K.; Piretra, T.; Boatz, J. C.; Matlahov, I.; Kodali, R.; Hediger, S.; van der Wel, P. C. A.; De Paëpe, G., Structural Fingerprinting of Protein Aggregates by Dynamic Nuclear Polarization-Enhanced Solid-State NMR at Natural Isotopic Abundance. *J. Am. Chem. Soc.* **2018,** *140* (44), 14576-14580.

23. Nimerovsky, E.; Gupta, R.; Yehl, J.; Li, M.; Polenova, T.; Goldbourt, A., Phase-modulated LA-REDOR: A robust, accurate and efficient solid-state NMR technique for distance measurements between a spin-1/2 and a quadrupole spin. *J. Magn. Reson.* **2014,** *244*, 107-113.

24. Duong, N. T.; Rossi, F.; Makrinich, M.; Goldbourt, A.; Chierotti, M. R.; Gobetto, R.; Nishiyama, Y., Accurate ^{1}H-^{14}N Distance Measurements by Phase-Modulated RESPDOR at Ultra-Fast MAS. *J. Magn. Reson.* **2019,** *308*, 106559.

25. Gan, Z., Measuring Amide Nitrogen Quadrupolar Coupling by High-Resolution $^{14}N/^{13}C$ NMR Correlation under Magic-Angle Spinning. *J. Am. Chem. Soc.* **2006,** *128* (18), 6040-6041.

26. Cavadini, S.; Lupulescu, A.; Antonijevic, S.; Bodenhausen, G., Nitrogen-14 NMR Spectroscopy Using Residual Dipolar Splittings in Solids. *J. Am. Chem. Soc.* **2006,** *128* (24), 7706-7707.

27. Hiort, J.; Maksimenka, K.; Reichert, M.; Perović-Ottstadt, S.; Lin, W. H.; Wray, V.; Steube, K.; Schaumann, K.; Weber, H.; Proksch, P.; Ebel, R.; Müller, W. E. G.; Bringmann, G., New Natural Products from the Sponge-Derived Fungus Aspergillus niger. *J. Nat. Prod.* **2004,** *67* (9), 1532-1543.

28. Hiort, J.; Maksimenka, K.; Reichert, M.; Perović-Ottstadt, S.; Lin, W. H.; Wray, V.; Steube, K.; Schaumann, K.; Weber, H.; Proksch, P.; Ebel, R.; Müller, W. E. G.; Bringmann, G., New Natural Products from the Sponge-Derived Fungus Aspergillus niger. *J. Nat. Prod.* **2005,** *68* (12), 1821-1821.

29. Ning, Y.; Otani, Y.; Ohwada, T., Contrasting C- and O-Atom Reactivities of Neutral Ketone and Enolate Forms of 3-Sulfonyloxyimino-2-methyl-1-phenyl-1-butanones. *J. Org. Chem.* **2018,** *83* (1), 203-219.

30. Zhao, L.; Hanrahan, M. P.; Chakravarty, P.; DiPasquale, A. G.; Sirois, L. E.; Nagapudi, K.; Lubach, J. W.; Rossini, A. J., Characterization of Pharmaceutical Cocrystals and Salts by Dynamic Nuclear Polarization-Enhanced Solid-State NMR Spectroscopy. *Cryst. Growth Des.* **2018,** *18* (4), 2588-2601.

31. Pickard, C. J.; Mauri, F., All-Electron Magnetic Response with Pseudopotentials: NMR Chemical Shifts. *Phys. Rev. B* **2001,** *63* (24), 245101.