Utilizing Long-Range ^1H - ^{15}N 2D NMR Spectroscopy for Chemical Structure Elucidation and Confirmation

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Inverse- or proton-detected heteronuclear 2D NMR methods have opened many avenues of investigation that would otherwise be closed. In particular, beginning in the mid-1990's, reports began to appear in the literature regarding preliminary investigations of long-range ^1H - ^{15}N 2D NMR at natural abundance. The subsequent development and continually-increasing access to cryogenic NMR probe technology has further facilitated the acquisition of ^1H - ^{15}N heteronuclear shift correlation data. Since 2000, the field has been reviewed several times,¹⁻⁴ and chapters have also appeared surveying the application of these methods in the field of alkaloid chemistry.^{5,6} The literature representing this area of investigation now comprises several hundred publications.

There is also a rich body of literature associated with the more difficult direct detection of ^{15}N spectra that has been the subject of several monographs⁷⁻¹⁰ and very extensive reviews by Webb and co-authors.¹¹⁻¹⁶ Direct observation of ^{15}N is fraught with difficulty as a result of the relatively low natural abundance of 0.37%, the low gyromagnetic ratio resulting in a resonance frequency of 10.13 MHz at 2.35 T, and frequently a negative NOE that requires the use of inverse gating techniques. Relative to

^1H detection for an equal number of nuclei, the relative sensitivity of ^{15}N is 0.00194; relative to ^{13}C at natural isotopic abundance, the relative sensitivity is 0.0214.⁸ These attributes conspire to make measuring times long and sample requirements rather high, with larger diameter tubes preferable. When we consider X-nucleus excitation and detection, and compare $^1\text{H}/^{13}\text{C}$ correlation experiments to $^1\text{H}/^{15}\text{N}$ the time to attain equal s/n ratios is ~100 times longer for $^1\text{H}/^{15}\text{N}$ than for $^1\text{H}/^{13}\text{C}$. Consequently, there is a relatively rich body of literature involving $^1\text{H}/^{13}\text{C}$ heteronuclear correlation with ^{13}C detection while there is virtually none for the $^1\text{H}/^{15}\text{N}$ heteronucleide pair. When we consider ^1H excitation and X-nucleus detection, the disadvantage for $^1\text{H}/^{15}\text{N}$ relative to $^1\text{H}/^{13}\text{C}$ drops to a factor of 15.6. When we compare these methods to contemporary ^1H excitation and detection, the disadvantage is erased.¹⁷ Consequently, there is a rich and growing body of applications of both direct and long-range ^1H - ^{15}N heteronuclear shift correlation in chemical structure elucidation.¹⁻⁴

Spectrometer and Probe Considerations

If an investigator has a choice of instruments on which to acquire ^1H - ^{15}N heteronuclear shift correlation data, it is usually beneficial to use higher field instruments up to a point. ^{15}N has a very wide spectral range comprising more than 900 ppm from one extreme to the other. More practically, the spectral range for a given compound is usually <500 ppm and in most cases is in the range of 300-350 ppm or less for natural products containing both aromatic and aliphatic ^{15}N resonances. Going from 400-500 MHz instruments to higher field instruments, perhaps 700-800 MHz, affords higher sensitivity but as the spectral width to be excited increases with observation frequency, so

does the inherent difficulty of uniformly exciting the entire range of potential ^{15}N shifts for a given molecule. Pulse sequences employing adiabatic ^{15}N pulses (reference) are preferred relative to conventional sequences. If one is faced with a choice of an instrument with a conventional gradient inverse probe vs. an instrument with a cryogenic probe, the latter is clearly the better choice from a sensitivity standpoint.¹⁸ Generally, not much can be done to shorten ^{15}N pulse lengths but power levels should be set to allow the shortest possible ^{15}N pulses for a given spectrometer/probe pairing. It may also be useful to determine if the instrument has attenuators in line from the transmitter to the probe and the potential impact that they may have on pulse widths if they are present.

Experiment Selection and Long-range Delay Optimization

If an investigator is interested in establishing direct ^1H - ^{15}N heteronuclear shift correlations, the choice is a relatively simple one. It is advisable to use the ^1H - ^{15}N GHSQC pulse sequence in some form. Since most protonated ^{15}N resonances are not observed across a particularly wide range, the use of a variant of the sequence that employs adiabatic ^{15}N pulses is optional. One-bond ^1H - ^{15}N coupling constants are reasonably uniform and are generally in the range of ~90-100 Hz, allowing one to set the one-bond delay to a 95 Hz average value.

Long-range ^1H - ^{15}N heteronuclear shift correlation experiments can be performed either using the well established GHMBC pulse sequence or a variant such as IMPEACH-MBC or CIGAR-HMBC that employ accordion optimization of the long-range delays.^{19,20} While ^1H - ^{13}C long-range heteronuclear coupling constants are fairly regular, in contrast, ^1H - ^{15}N heteronuclear coupling constants are quite variable making

the use of an experiment such as GHMBC, which employs a fixed long-range evolution period, problematic. In contrast, the accordion-optimized experiments such as IMPEACH-MBC²¹ vary the long-range delay duration of a range of coupling constants. In general, we have found that optimizing the long-range delay across the range of 3-8 Hz generally provides the best results for survey conditions.^{19,20}

An example is provided by a comparison of the results obtained for the N9 amide nitrogen of strychnine (**1**) using conventional ¹H-¹⁵N GHMBC and the IMPEACH-MBC pulse sequence. The N9 amide resonance is long-range coupled to the H8, H11 α , and H13 resonances *via* two- or three-bonds. Figure 1 shows slices taken through the two spectra at the N9 chemical shift. While the data from the GHMBC spectrum shown in the bottom trace has higher overall s/n ratio of 42:1, in contrast the intensity of the correlations from the H8 and H13 resonances are considerably more intense in the IMPEACH-MBC data despite the slightly lower overall s/n ratio of the experiment.¹⁹

F1 Spectral Width Considerations

One of the inherent problems associated with setting F₁ spectral widths for long-range ¹H-¹⁵N heteronuclear shift correlation experiments is a general lack of familiarity with ¹⁵N chemical shift ranges by the majority of investigators. One approach to this problem is through the use of ¹⁵N chemical shift prediction. An example software application used in the laboratory of one of the authors (GM) is that developed by Advanced Chemistry Development (ref). At present, ACD/Labs' nitrogen NMR database (v11.0) used as the basis for development of the NNMR prediction algorithms contains > 8,500 structures and >22,000 individual ¹⁵N chemical shifts. The authors recently

evaluated the utilization of the NNMR program for the calculation of ^{15}N chemical shifts using a LOO (Leave One Out) approach in which a molecule was excluded from the database and then its ^{15}N shift(s) were calculated using the remaining data in the database.⁶ The results obtained using the NNMR database are shown in Figure 2 and on analysis gave a correlation coefficient of $R^2 = 0.97$ over the 21,400 points in the database. The figure represents the number of ^{15}N chemical shifts vs. the difference between the actual and predicted ^{15}N shift. Approximately 250 ^{15}N shifts were found in the database where the deviation between the actual and predicted ^{15}N shift using the remaining data in the database was >20 ppm.

Using the NNMR program to calculate expected ^{15}N chemical shifts of a potential molecular structure with which an investigator has no experience and then adding a buffer of ± 20 ppm to the calculated range of potential ^{15}N chemical shifts is a reasonable way to establish a starting F_1 spectral window in most cases. An example of the value of this approach is seen with the simple alkaloid harmaline, **2**. One of our colleagues ran a ^1H - ^{15}N long-range heteronuclear shift correlation spectrum of harmaline having been asked to set up the F_1 spectral window intuitively. In the first approximation, the colleague, looking at the structure and having some familiarity with ^{15}N chemical shift behavior chose to employ an F_1 spectral window of 50-200 ppm, mistakenly assuming that the N1 resonance of harmaline, **2**, would not behave in a manner terribly dissimilar to indole. Unfortunately, that is not the case and as can be seen from Figure 3A, the signal for the correlation of the methyl group to N1 is observed at approximately 150 ppm. Since the observed chemical shift is consistent with the investigator's expectation, this data could easily slip into the literature if this were part of a chemical structure

investigation. However, when the ^{15}N shift of N1 is calculated, the calculated ^{15}N shift was found to be 298 ± 9 ppm. Refining the F_1 window to 100-320 ppm, we see in Figure 3B that the N1 resonance is now observed very close to the predicted ^{15}N chemical shift for this position.

Using ^{15}N Long-range Heteronuclear Shift Correlation Experiments with Computer-Assisted Structure Elucidation Programs

There have been several comparative evaluations performed to evaluate the ability of Computer-Assisted Structure Elucidation or CASE programs to generate the correct structure with and without the availability of long-range ^1H - ^{15}N heteronuclear 2D NMR data. In 1999, Köch, Junker, and Lindel²² reported a study of the alkaloid orodin, **3**, using the COCON CASE program. Four data sets were evaluated in which the number of ^1H - ^{13}C long-range correlations fed to the program with COSY correlations was varied. In parallel, datasets that included the same numbers of ^1H - ^{13}C long-range and COSY correlations in addition to the ^{15}N long-range correlations were also evaluated. When sparse ^1H - ^{13}C data (9% of all observable correlations) and COSY data were fed to the COCON program, a remarkable 234,336 structures were generated. When the ^1H - ^{15}N long-range data were included with the same sparse ^1H - ^{13}C connectivity information, the number of structures dropped to only 10. When nearly complete ^1H - ^{13}C connectivity information (84% of all observable correlations) was fed to the program along with COSY data, the number of structures generated fell to only 60, which is a far more manageable number. When the ^1H - ^{15}N data were included, only 6 structures were generated by the program. In another study, Nuzillard and co-workers²³ reported the application of the LSD CASE program in the elucidation of the structure of acosmine

acetate, **4**, in 1999. When the data fed to the program were supplemented by ^1H - ^{15}N long-range connectivity information, the program generated only a single structure consistent with all of the spectroscopic data available.

In another example, Grube and Köck²⁴ reported the utilization of long-range ^1H - ^{15}N heteronuclear shift correlation data in conjunction with the COCON CASE program to elucidate the structure of oxocyclostyridol, **5**, an intramolecular cyclized orodin derivative from a marine sponge. The full complement of 2D NMR data acquired included both long-range ^1H - ^{15}N heteronuclear shift correlation data and 1,1-ADEQUATE data. When the authors performed the COCON calculation with the full spectroscopic data set, only four structures, **5** – **8**, were generated by the program. When the ^1H - ^{15}N data were excluded from the calculation, the number of structures generated rose to 50,000. When both the ^1H - ^{15}N and 1,1-ADEQUATE data were excluded, the number of structures generated rose to more than 150,000 clearly underscoring the importance of these data. The ^{15}N chemical shifts and observed long-range correlations are shown on the correct structure, **5**, of the output set.

Even incomplete long-range ^1H - ^{15}N heteronuclear chemical shift data can have a very beneficial impact on the performance of CASE programs. The complex spiro non-cyclic alkaloid cryptospirolepine (**9**) underwent very complex degradation on long-term storage of an NMR sample in d_6 -DMSO.²⁵ One of the major degradation products has been identified as cryptoquindoline (**10**). Using the CASE program Structure Elucidator v7.0, the program took 8 h and generated ~3300 structures, of which 355 remained after filtering and removal of duplicates. The correct structure was in the second position of the output table with a ^{13}C cumulative chemical shift deviation of $\text{d}^{13}\text{C} = 3.9$ ppm.

Another application of partial ^1H - ^{15}N chemical shift correlation data was seen in the application of the Structure Elucidator CASE program to the dimeric indoloquinoline alkaloid cryptomisine, **11**. Without any ^1H - ^{15}N chemical shift correlation data, the program ran for 210 h generating >75 million structures, of which >22,000 remained after filtration and removal of duplicates. In contrast, when only simple ^1H - ^{15}N HMQC direct correlation data were added to the input data, only 5 structures were generated in ~1 m, the correct structure among them.²⁶

In the opinion of the authors, the results just discussed clearly underscore the value of using ^1H - ^{15}N heteronuclear chemical shift correlation data in the investigation of unknown, nitrogen-containing structures. The impact of these data on multiple CASE program applications in terms of the reduction of the numbers of structural possibilities generated as output very dramatically illustrates the benefit of having these data available.

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Differentiating Structures on the Basis of ^{15}N Chemical Shift or Long-Range Coupling Information

Another very powerful application of long-range ^1H - ^{15}N heteronuclear chemical shift data is in the differentiation of potentially isomeric structures or in determining the site(s) of reaction when several are possible. There are many more examples than can be reviewed in the space of the contribution. The interested reader is thus referred to the reviews cited at the beginning of this contribution for additional information.

The two largest groups of applications of long-range ^1H - ^{15}N heteronuclear 2D in structure elucidation/confirmation have been in the areas of diazine chemistry and in the characterization of N-oxide compounds. These segments of the literature will be discussed as groups, followed by selected applications that, in the opinion of the authors, highlight the utilization of long-range ^1H - ^{15}N heteronuclear chemical shift correlation techniques.

Applications in Diazine Chemistry

One area of investigation that has seen the frequent application of long-range ^1H - ^{15}N heteronuclear shift correlation data has been in the determination of substitution patterns in diazine compounds that are of particular importance in the flavor and fragrance industry. Pyrazines are the product of the Maillard reaction during the browning of sugars and proteins; these reactions occur during roasting, cooking, and baking of food products. Some of the early work done in this area utilized both ^{15}N INEPT as well as long-range ^1H - ^{15}N 2D heteronuclear shift correlation experiments and is captured in the chapter by Zviely, Kern, Gozlan, and Frim.²⁷ Examples of the identification of a minor impurity component contained in 2-chloro-3-methylpyrazine (**12**) using both techniques is described in another report by the same group of authors.²⁸ Fundamentally, the problem is one of differentiating 2-chloro-5-methylpyrazine (**13**) from 2-chloro-6-methylpyrazine (**14**). If the correct structure is that represented by **13**, the N1 resonance should be coupled only to the H6 resonance while N4 would be expected to be long-range coupled to both the H3 and 5-methyl resonances. For **14**, N1 would long-range couple only to the 6-methyl group while N4 would be coupled to both H3 and H5, assuming that all $^2J_{\text{NH}}$ and $^3J_{\text{NH}}$ correlations are observed. Both the ^{15}N INEPT and long-range ^1H - ^{15}N GHMBC connectivity information supported 2-chloro-6-methylpyrazine (**14**) as the correct structure of the minor component of the sample.

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In related work, Quéguiner, *et al.*,²⁹ have used long-range ^1H - ^{15}N 2D methods to assign the product resulting from the metalation of pyrazine thiocarboxamides followed by reaction with electrophiles. Beginning with pyrazine-2-*N*-*t*-butylthiocarboxamide, **15**, the proton and nitrogen resonances were first assigned by 2D methods. Following reaction of the metalated pyrazine with an electrophile, the products were unequivocally determined to be the 2,5-disubstituted pyrazines such as **16** on the basis of long-range ^1H - ^{15}N GHMBC spectra.

Later the same year, Quéguiner and co-workers³⁰ utilized long-range ^1H - ^{15}N 2D NMR methods in the determination of the site of metalation in the benzene portion of some benzodiazines. The site of reaction with electrophiles was established based on the analysis of the 3 Hz long-range optimized ^1H - ^{15}N GHMBC spectra. The authors began by unequivocally assigning the N1 and N4 shifts of 2-methoxy- and 2-phenylquinoxaline (**17**). With these assignments in hand it was possible to unequivocally establish the identities of the H5 and H8 resonances of 2-methoxy-3-phenylquinoxaline, **18**. These data were finally employed to establish the site of electrophilic substitution of **19** based on the long-range correlations of the H7 and H8 protons to N1.

A somewhat similar and interesting study was reported by Gawinecki and co-workers³¹ in which they employed long-range ^1H - ^{15}N 2D methods to characterize pyrazoloquinoxaline products (**20**) formed from the condensation of pyrazoline-4,5-diones (**21**) with aromatic 1,2-diamines (**22**). A further comprehensive study of an extensive series of di- and tri-substituted pyrazines was reported in 2000 by Sommer and co-workers.³²

Pyrazine-containing natural products of various types have also been a structure characterization challenge. One of the earliest such examples that employed long-range ^1H - ^{15}N 2D NMR techniques was in the structure characterization of ritterazine-A, **23**, a

highly cytotoxic pyrazine-containing natural product isolated from a Japanese tunicate. The H1 β and H1' β protons are long-range coupled to the N2 and N3 nitrogen resonances at 332.0 and 332.8 ppm, respectively, allowing the unambiguous determination of the fusion of the two steroidal constituents to the central pyrazine ring.³³

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Chill, Akinin, and Kashman³⁴ utilized long-range ^1H - ^{15}N heteronuclear chemical shift data in the determination of the structure of barrenazine-A, a pyrazine derived natural product isolated from an unidentified tunicate collected at Barren Island, Madagascar. The problem focused on differentiation between two isomeric structures shown by **24** and **25**. Long-range correlations were observed and used to differentiate the structures are shown by the correlation arrows for **24**, the identified structure.

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Structural Characterization of N-oxides

N-oxides of drug molecules and other chemical structures of interest are frequently encountered either as the result of oxidative metabolism or chemical degradation. Quite often, when there were alternative sites of N-oxidation possible, arguments have been advanced to establish the site of N-oxidation based on perturbations of the ^1H shifts of protons in the vicinity of the site of N-oxidation. While this approach is workable when both nitrogens are in environments where flanking proton resonances can be perturbed, there are certainly many cases in which the development of such arguments is not possible because of the absence of protons to serve as reporters of the site of N-oxidation. An example of establishing the site of N-oxidation based on ^1H and ^{13}C chemical shift and ^1H - ^{13}C coupling constant perturbations is found in a study of the N-oxidation of 3-aryl-1,2,4,5-tetrazines by Adam, van Barneveld and Golsh.³⁵

Rather than using perturbations of neighboring ^1H or even ^{13}C shifts, a more direct and sensitive measure of the site of N-oxidation is found in the ^{15}N shift of the nitrogen resonances themselves. Witanowski and Webb⁷ reported that ^{14}N resonances are deshielded by 70-100 ppm following N-oxidation. An early study by Städeli, *et al.*,³⁶ of a series of pyrimidines and their respective N-oxides suggested that perturbations following N-oxidation might have only limited utility in determining the site of N-oxidation. One of the authors, reasoning that the effects of N-oxidation might be dependent on the hybridization state of the nitrogen in question, reported the utilization of long-range ^1H - ^{15}N connectivities to determine the sites of N-oxidation of an antibiotic, **26**, and a potential antipsychotic, **27**.³⁷ The ^{15}N shifts of the parent molecule, **26**, and the N1''-oxide, **27**, are shown in Table 1 accompanied by the incremental change of the ^{15}N chemical shifts following N-oxidation.

Table 1. ^{15}N chemical shifts of **26** and the N1''-oxide, **27**, in d_6 -DMSO and the difference in the observed ^{15}N shift following N-oxidation ($\Delta \delta^{15}\text{N}$).

Position	26 $\delta^{15}\text{N}$ (ppm)	27 $\delta^{15}\text{N}$ (ppm)	$\Delta \delta^{15}\text{N}$ (ppm)
N3	98.1	101.7	+3.6
N7	109.3	109.2	-0.1
N1''	52.3	120.8	+68.5
N4''	111.0	105.8	-5.2

Similar perturbations in the ^{15}N chemical shift following oxidation were observed in the case of the potential antipsychotic, **28**, and its N11-oxide, **29**. The ^{15}N shifts of the parent molecule, **28**, and the N11-oxide, **29**, are shown in Table 2 accompanied by the incremental change of the ^{15}N chemical shifts following N-oxidation. Based on the observed behavior for the two piperidine containing molecules shown in these examples, the ^{15}N chemical shift perturbation following N-oxidation is a reliable means of establishing the site of N-oxidation in a molecule when more than one aliphatic nitrogen may be the site of N-oxidation.

28 (parent - no N11-oxide) **29** (N11-oxide)

Table 2. ^{15}N chemical shifts of **28** and the N11-oxide, **29**, in $\text{d}_6\text{-DMSO}$ and the difference in the observed ^{15}N shift following N-oxidation ($\Delta \delta^{15}\text{N}$).

Position	28 $\delta^{15}\text{N}$ (ppm)	29 $\delta^{15}\text{N}$ (ppm)	$\Delta \delta^{15}\text{N}$ (ppm)
N11	44.9	113.0	+68.1
N14	72.1	75.0	-7.1
N20	96.5	96.2	-0.3

Later in 1999, Martin, *et al.*,³⁸ reported a study of the effects of N-oxidation on the ^{15}N shifts of a partially degraded sample of the indoloquinoline alkaloid cryptolepinone **30** and what was identified in the study as the N5-oxide, **31**.

observed in both the parent and N5-oxide

observed only for the parent

30 (parent - no N5-oxide) **31** (N5-oxide)

Table 3. ^{15}N chemical shifts of **30** and the N5-oxide, **31**, in d_6 -DMSO and the difference in the observed ^{15}N shift following N-oxidation ($\Delta \delta^{15}\text{N}$).

Position	30 $\delta^{15}\text{N}$ (ppm)	31 $\delta^{15}\text{N}$ (ppm)	$\Delta \delta^{15}\text{N}$ (ppm)
N5	111.4	135.3	+23.9
N10	103.4	188.1	+84.7

The *Strychnos* alkaloids strychnine (**32**) and brucine (**33**) have been shown to exhibit very similar behavior on N-oxidation of the aliphatic N19 except that oxidation at N19 produced even larger downfield shifts of the N19 resonance. The chemical shift behavior of the ^{15}N resonances of strychnine, **32**, brucine, **33**, and their respective N19-oxides is shown in Table 4.

Table 4. ^{15}N chemical shifts of strychnine, **32**, brucine, **33**, and their respective N19-oxides, in d_6 -DMSO and the difference in the observed ^{15}N shift following N-oxidation ($\Delta \delta^{15}\text{N}$).

32 R = -H, N19 not oxidized

33 R = -OCH₃, N19 not oxidized

Position	32 $\delta^{15}\text{N}$ (ppm)	32 N-oxide $\delta^{15}\text{N}$ (ppm)	$\Delta \delta^{15}\text{N}$ (ppm)	33 $\delta^{15}\text{N}$ (ppm)	33 N-oxide $\delta^{15}\text{N}$ (ppm)	$\Delta \delta^{15}\text{N}$ (ppm)
N9	148.0	146.5	-1.5	147.9	145.3	-2.6
N19	35.0	136.3	+101.3	35.9	135.5	+99.6

Laihia, *et al.*,³⁹ reported a study of the effects of N-oxidation on a series of 2,4-disubstituted pyridines and their respective N-oxides using long-range ^1H - ^{15}N 2D NMR methods. The results of this study showed much smaller perturbations of the ^{15}N shift of the pyridine nitrogen following oxidation that ranged from -1.2 to +27.4 ppm. The results of this study were consistent with those reported by Städeli, *et al.*³⁶ da Silva and co-workers⁴⁰ also reported the results of a study of a series of picolinic acids and their corresponding N1-oxides. This group of authors, however, focused their attention on the ^{15}N shift perturbations of the 3-amino substituent on the parent vs. the N1-oxide, which were substantial and attributed to the hydrogen bonding of the 2-carboxyl group to the N1-oxide, the N3-NH group hydrogen bonded, in turn, to the carbonyl of the 2-carboxylic acid substituent. Laihia and co-workers⁴¹ reported again on the effects of N-oxidation on a series of nitramino and N-alkylamio-4-nitro derivatives of pyridine and pyridine-1-oxide.

In a study of pyrrolizidine alkaloids from *Onosma leptantha*, Chinou, *et al.*,⁴² utilized long-range ^1H - ^{15}N 2D NMR measurements in establishing the structures of leptanthine N-oxide, **34**, and the N-oxide of 3'-O-acetylechihumiline, **35**, and the known alkaloid echihumiline N-oxide, **36**. The ^1H - ^{15}N GHMBC spectra of the N4-oxide species gave correlations to a ^{15}N resonance in the vicinity of 152 ppm that was correlated to the H2 vinyl, H6 and H7 aliphatic protons, which shifted upfield to 71.1 ppm in the parent alkaloid leptanthine, which does not have an N4-oxide. The chemical shift perturbation following N-oxidation in this case is +80.1 ppm.

In 2006, Laihia and co-workers⁴³ reported a further study of a series of methyl and alkylamino derivatives of 4-halopyridine N-oxides that may be of utility in the elucidation of pyridine N-oxide containing structures. Later in 2006 a curious application of long-range 1H - ^{15}N 2D NMR was reported in the characterization of [60]Fullerene-pyrrolidine N-oxides, **37**, that exhibit a +85 ppm downfield ^{15}N shift relative to the parent.⁴⁴

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Later in 2006, Boyd, Hay, and Boyd⁴⁵ reported a study of tirapazamine and related 1,2,4-benzotriazine N-oxides that employed ^1H - ^{15}N 2D NMR methods in establishing the structures of these molecules, noting the potential ambiguities in ^{15}N chemical shift differences pointed out much earlier by Städeli, *et al.*³⁶ Despite the challenges, these workers were able to record long-range ^1H - ^{15}N spectra that confirmed the structure of 3-amino-1,2,4-benzotriazine 4-oxide, **38**. Using the long-range correlations shown by **38**, it was also possible to assign the structures of the parent 1,2,4-benzotriazole, **39**, the 1-oxide, **40**, the 2-oxides, **41**, and the 1,4-dioxide, **42**. The ^{15}N chemical shift assignments for these molecules are collected in Table 5. It is also interesting to note that in some cases (see Table 5) the oxidize ^{15}N resonance was shifted upfield by nearly -150 ppm.

In 2007, González and co-workers⁴⁶ reported a study of the structures of a series of indazole 1-oxides using a variety of methods, including long-range ^1H - ^{15}N 2D NMR measurements in conjunction with other spectroscopic techniques including X-ray crystallography. Long-range ^1H - ^{15}N correlations allowed the unambiguous differentiation and assignment of the H4 and H7 aromatic protons as in the previous work of Quéguiner, *et al.*³⁰ discussed above. Again

some interesting comparisons were made in terms of the effects of N-oxidation on the N1 and N2 shifts of the indazoles studied that are summarized in Table 6.

The most recent study we are aware of was the 2007 report of Potmischii, *et al.*⁴⁷ that dealt with the effects of N-oxidation and N-quaternarization on the ^{15}N chemical shifts of N-methylpiperidine-derived mono-, bi-, and tri-cycloaliphatic tertiary amines, which are common in many types of natural products. The ^{15}N chemical shift data contained in the report of Potmischii, *et al.* will be useful for example in the characterization of molecules such as leptanthine N-oxide (**34**) discussed above and the synthetically prepared fumiquinaoline alkaloids reported by Wang and Ganesan⁴⁸ who utilized long-range ^1H - ^{15}N 2D methods to characterize an N-oxide, **43**, that formed at one of the two nitrogens in the alkaloid structure.

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Table 5. ^{15}N chemical shifts of 1,2,4-benzotriazoles and various N-oxide analogues, in $\text{d}_6\text{-DMSO}$ and the difference in the observed ^{15}N shift following N-oxidation ($\Delta \delta^{15}\text{N}$). All ^{15}N shifts are reported in ppm downfield of liq. ammonia.

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Position	$\delta^{15}\text{N}$ (ppm)	$\delta^{15}\text{N}$ (ppm)	$\Delta \delta^{15}\text{N}$ (ppm)	$\delta^{15}\text{N}$ (ppm)	$\Delta \delta^{15}\text{N}$ (ppm)	$\delta^{15}\text{N}$ (ppm)	$\Delta \delta^{15}\text{N}$ (ppm)	$\delta^{15}\text{N}$ (ppm)	$\Delta \delta^{15}\text{N}$ (ppm)
1	455.5	332.7	-122.8	335.4	-120.1	400.5	-55.0	307.1	-148.4
2	383.7	293.6	-90.1	297.4	-86.3	350.4	-33.3	283.9	-99.8
4	209.9	104.8	-05.1	216.0	+6.1	236.9	+27.0	220.6	+10.7
3-NH ₂	79.0	79.2	+0.2	73.4	-5.6	69.6	-9.4	71.2	-7.8

Table 6. ^{15}N chemical shifts of some indazoles and their respective N1-oxide analogues, in d_6 -DMSO and the difference in the observed ^{15}N shift following N-oxidation ($\Delta \delta^{15}\text{N}$). All ^{15}N shifts are reported in ppm downfield of liq. ammonia.

R = - $p\text{C}_6\text{H}_4\text{I}$

43

R = - $p\text{C}_6\text{H}_4\text{I}$

44

R = - $p\text{C}_6\text{H}_4\text{NO}_2$

45

R = - $p\text{C}_6\text{H}_4\text{NO}_2$

46

R =

47

Position	$\delta^{15}\text{N}$ (ppm)	$\Delta \delta^{15}\text{N}$ (ppm)	$\delta^{15}\text{N}$ (ppm)	$\delta^{15}\text{N}$ (ppm)	$\Delta \delta^{15}\text{N}$ (ppm)	$\delta^{15}\text{N}$ (ppm)	$\delta^{15}\text{N}$ (ppm)
1	274.5	-29	301.5	265.5	-34	299.5	263.5
2	293.6	-9	297.4	212.5	-12	224.5	211.5
-NO ₂	---	---	---	367.5	+3	364.5	---

Natural Product Structure Assembly Based on Long-Range ^1H - ^{15}N Heteronuclear Correlations

An early but elegant utilization of long-range ^1H - ^{15}N heteronuclear correlations in natural product structure assembly is seen in the work of Ōmura and co-workers⁴⁹ in their report on the elucidation of the structure of amphistin, **44**. Structural fragments assembled from HMQC and COSY spectra, **45** – **47**, were linked to the intervening nitrogen atoms of the structure using long-range ^1H - ^{15}N GHMBC correlations.

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Differentiation Between Isomeric Structures on the Basis of Long-Range ^1H - ^{15}N Heteronuclear Chemical Shift Correlation Data

A very interesting report by McDonnell and co-workers⁵⁰ illustrated the differentiation of pyrazole regioisomers, **48**, and **49**, through the use of long-range ^1H - ^{15}N heteronuclear correlations.

Another very interesting application of long-range ^1H - ^{15}N 2D NMR for the revision of the structure of tetrapetalone-A was reported in 2003 by Hirota and co-workers.⁵¹ The structure of tetrapetalone was originally reported to be **50** using a suite of NMR experiments that consisted of COSY, HMQC, ^1H - ^{13}C HMBC and ^{13}C - ^{13}C INADEQUATE by same group of authors.⁵² The structure had to be revised to that shown by **51** when long-range ^1H - ^{15}N 2D NMR data became available to account for the observed long-range ^1H - ^{15}N couplings. It should also be noted that had structure **50** been correct, the ^1H - ^{15}N GHMBC spectrum would have shown the NH resonance as an approximately 95 Hz doublet unless the low-pass J-filter had been specifically set to suppress these correlations. In our experience, there is generally so little spectral

crowding in a long-range ^1H - ^{15}N 2D NMR spectrum that it is far more beneficial to allow these correlations to leak into the GHMBC spectrum.

Another application of long-range ^1H - ^{15}N 2D NMR for the differentiation of structures is seen in the characterization of the antineoplastic agent cribostatin 6 by Petit and co-workers.⁵³ During the course of the elucidation of the structure, it was not possible to distinguish between alternative structures **52** and **53** from the homonuclear and ^1H - ^{13}C heteronuclear shift correlation data available. The acquisition of a 5 Hz optimized GHMBC spectrum did, however, resolve the structural ambiguity, allowing the structure to be correctly assigned as **52**, that assignment verified by an X-ray crystal structure. Weak correlations from the 1-position are denoted by dashed arrows.

Unsymmetrical Indirect Covariance ^{13}C - ^{15}N Heteronuclear Correlation Plots

Covariance processing of 2D NMR spectra has been a rapidly growing field of investigation over the past several years, expanding from the initial report of Brüschweiler and Zhang in 2004⁵⁴ to more than 30 papers at present. A communication that described indirect covariance processing of HSQC-TOCSY spectra to derive ^{13}C - ^{13}C correlation plots in late 2004⁵⁵ caught the attention of the authors and co-workers, prompting them to initiate their own investigations into this area of research first focusing on artifacts observed due to proton resonance overlap in the indirect covariance processed ^{13}C - ^{13}C correlation plots that are derived from HSQC-TOCSY spectra.⁵⁶ That first study was followed by a communication that described a technique that the authors termed unsymmetrical indirect covariance processing, which allowed two discretely acquired 2D NMR spectra to be processed together. In that first foray into this area of investigation, an HSQC and HMBC spectra were co-processed to give an experimental result equivalent to what would be obtained from a 1,1-ADEQUATE experiment.⁵⁷ Germane to the subject of this contribution, Martin, *et al.*⁵⁸ subsequently focused their attention on the coprocessing of ^1H - ^{13}C GHSQC and ^1H - ^{15}N GHMBC spectra of strychnine to derive what would otherwise be the experimentally inaccessible ^{13}C - ^{15}N heteronuclear shift correlation data shown in Figure 4. Work by Kupče and Freeman⁵⁹ has addressed the derivation of ^{13}C - ^{15}N heteronuclear shift correlation data by an alternative projection reconstruction method using cyanocobalamin, vitamin B-12, as a model compound for their study.

The experimental result of subjecting a multiplicity-edited ^1H - ^{13}C GHSQC spectrum (panel B) and a ^1H - ^{15}N GHMBC spectrum (panel A) to unsymmetrical indirect

covariance processing is shown in Figure 4, the resulting ^{13}C - ^{15}N heteronuclear shift correlation plot shown in panel C. It is important to stress that no new information is generated during unsymmetrical indirect covariance processing of NMR spectra. Rather, the covariance processing provides an alternative method of visualizing data contained in the spectra that are coprocessed, hopefully providing a data presentation from which it is easier to extract atom-to-atom connectivity information. It remains to be seen how much of an impact unsymmetrical indirect covariance processing methods will have on the utilization and interpretation of ^{15}N chemical shift information for structure elucidation purposes.

Conclusions

The field of long-range ^1H - ^{15}N 2D NMR at natural abundance now extends to several hundred published reports since the first reports appeared in the mid-1990's. This contribution has touched on the utilization of these techniques in several prevalent areas of investigation and the reader wishing to delve more deeply into this field is advised to consult the multiple reviews and chapters that have thus far appeared for a more comprehensive treatment of this area of investigation.

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Figure 1. Comparison of the responses for the N9 resonance of strychnine from an 8 Hz optimized GHMBC experiment (bottom trace) and a 3-16 Hz optimized IMPEACH-MBC experiment (top trace).¹⁹ (need permission from Wiley)

Figure 2. Plot of the results achieved using a LOO (Leave One Out) analysis of the ^{15}N chemical shift database employed by ACD/Labs' NNMR program (v11.0). The correlation coefficient for the analysis was $R^2 = 0.97$ with ~250 shifts out of the 21,400 shifts contained in the database deviating by >20 ppm between experimental and predicted ^{15}N shifts.

Figure 3.

Figure 3. Intuitive *vs.* calculated ^{15}N F_1 spectral windows. The F_1 spectral window from 50-200 ppm was selected by an investigator with some knowledge of ^{15}N chemical shifts based on the premise that the N1 nitrogen resonance of harmaline (**2**), would be similar to that of indole, in the range of ~130 ppm. The experimental result is shown in panel A; the N1 resonance was observed near 150 ppm *via* a long-range correlation from the vinyl methyl group. The observed ^{15}N chemical shift is however folded and since it was reasonable relative to the spectroscopist's intuitive thinking, that incorrect ^{15}N chemical shift could be propagated in the literature. However, when the ^{15}N shifts of harmaline (**2**) were calculated, the calculated ^{15}N shifts were found to be 131.6 for the indole N6 and 298.1 for the N1 resonance. Opening the F_1 spectral window to 100-320 ppm gave the experimental result shown in Figure 3B. The N1 resonance is "unfolded" and now appears quite near the predicted ^{15}N chemical shift value.

Figure 4.

Figure 4. Calculation of a ^{13}C - ^{15}N heteronuclear shift correlation plot for strychnine. The discretely acquired ^1H - ^{15}N GHMBC and multiplicity-edited ^1H - ^{13}C GHSQC spectra used in the covariance calculation are shown in panels A and B, respectively. The GHSQC spectrum shown in panel B has been transposed to orient it identical to the transposition imposed on this data matrix during the covariance processing to better illustrate the distribution of correlation responses in the ^{13}C - ^{15}N plot shown in panel C. The correlations observed in panel C are shown on the inset structure of strychnine. Red correlation arrows denote correlations from a methylene carbon to ^{15}N ; black arrows denote correlations from methine carbons to ^{15}N . It must be recalled that no new information is created during covariance processing. All of the correlation information contained in panel C can be extracted manually from the data contained in panels A and B.