MAGNETIC RESONANCE IN CHEMISTRY, VOL. 35, 671–679 (1997)

Long-Range Two-Dimensional ^{1}H–^{13}N Heteronuclear Shift Correlation at Natural Abundance Using GHNMQC. A Study of the Reverse Transcriptase Inhibitor Delavirdine

Kathleen A. Farley, Gregory S. Walker† and Gary E. Martin*
Pharmacia & Upjohn, Pharmaceutical Development, Kalamazoo, Michigan 49001-0199, USA

Delavirdine is a novel BHAP [bis(heteroaryl)piperazine] RT (reverse transcriptase) inhibitor in the final stages of development for treatment of human AIDS (acquired immuno deficiency syndrome). The assignment of the six unique nitrogen resonances in the molecule and its 5-aminoindole precursor are reported at natural abundance using the two-dimensional GHNMQC (gradient hydrogen–nitrogen multiple quantum coherence) experiment. The molecule contains three protonated secondary nitrogens and three tertiary nitrogens in its structure. The protonated nitrogen of the acidic sulfonamidomethyl moiety at the 5-position of the indole substituent of the molecule is shown to be sufficiently acidic in DMSO solutions at ambient temperature to preclude the observation of a direct response for this ^{1}H–^{15}N heteronuclear pair; in contrast, direct responses for the indole and isopropyl amino nitrogens were readily observable under these conditions. Variable-temperature studies in pyridine demonstrated that it was possible to slow autoprotonation sufficiently to allow the direct response to be observed with full intensity at −35 °C. Similar, although less pronounced behavior was observed for the 5-amino group contained in the structure of the 5-aminoindole precursor of delavirdine. The broad proton multiplet for the H-2′/H-6′ methylene proton resonance of the piperazine portion of the molecule failed to afford a direct response to the piperazine N-4′ resonance in the conventional GHNMQC experiment. Modifying the pulse sequence to apply a selective pulse to the H-2′/H-6′ proton in a manner analogous to that recently reported for the selective HMBC experiment readily facilitated the observation of the otherwise absent long-range coupling to N-4′. © **1997 John Wiley & Sons, Ltd.**

Magn. Reson. Chem. **35**, 671–679 (1997) No. of Figures: 7 No. of Tables: 1 No. of References: 26

Keywords: delavirdine; ^{1}H–^{15}N shift correlation; GHNMQC

Received 10 March 1997; revised 21 April 1997; accepted 23 April 1997

INTRODUCTION

Nitrogen-15 NMR spectroscopy has had an interesting history. Early studies relied on neat liquids or solutions at or near saturation to overcome the inherent difficulties of direct ^{15}N observation. The low natural abundance of this interesting nuclide (0.37%) is further exacerbated by a low gyromagnetic ratio, γ_N (the resonant frequency is 50.68 MHz on 11.7 T magnets affording proton observation at 500.13 MHz). The intrinsic properties of the ^{15}N nuclide afford an overall sensitivity that is 0.0214 relative to ^{13}C and only 0.001 94 relative to ^{1}H.

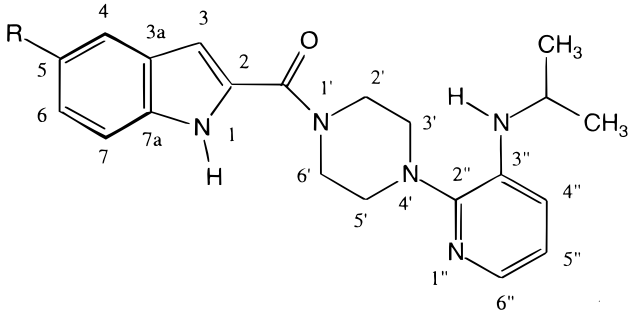
Despite problems of sensitivity, there is, surprisingly, a relative wealth of ^{15}N chemical shift data available on an extremely diverse range of nitrogen-containing moieties and heterocycles contained in several monographs[1,2] and a series of exhaustive reviews by Webb and co-workers.[3–5] To date, the bulk of chemical shift information available, aside from that of protein ^{15}N chemical shifts, has derived from direct or INEPT-facilitated observation experiments. In contrast, the work of Bax *et al.*[6,7] initially led to the development of two-dimensional inverse-detected experiments amenable to ^{15}N observation. That fertile beginning has since spawned a diverse array of far more sophisticated three- and four-dimensional NMR experiments designed specifically for the complete spectral assignment, sequencing, and tertiary structure characterization of proteins.[8]

Our previous work has taken an alternative direction, utilizing a pulse sequence that is essentially an amalgamation of the now widely utilized HMQC[9] and HMBC[10] experiments and their gradient analogs.[11] We now propose to label specifically the experiment using the acronym GHNMQC (gradient-enhanced hydrogen–nitrogen multiple quantum coherence). The experiment affords, via gradient-enhanced inverse detection, the ability to correlate ^{1}H to ^{15}N via chemical shifts either directly (via one-bond, $^{1}J_{NH}$) or long-range (via multiple bonds, $^{n}J_{NH}$, where $n = 2$, 3, etc.) in a single experiment. We suggest a separate acronym since the experiment is specifically adapted to requirements for ^{1}H–^{15}N long-range heteronuclear shift correlation and derives from

* *Correspondence to*: G. E. Martin.
E-mail: gary.e.martin@am.pnu.com.
† *Present address*: Drug Metabolism Research, Pharmacia & Upjohn, Kalamazoo, MI 49001-0199, USA.

 CCC 0749–1581/97/100671–09 $17.50

Table 1. ^{15}N Chemical shifts of nitrogen resonances of delavirdine (1) and its aminoindole precursor (4) determined using the GHNMQC experiment.



Position	1 R = $NHSO_2CH_3$ free base	1 R = $NHSO_2CH_3$ mesylate salt	4 R = $-NH_2$
1	137.7	138.9	135.5
5	117.7	117.3	53.8
1′	115.4	113.2	113.8
4′	61.3	(a)	61.3
1″	290.2	289.0	290.1
3″	78.2	76.7	78.3

[a] Not observable because of extensive homonuclear coupling of protons long-range coupled to this nitrogen resonance. Observable (see text) when a selective GHMQC experiment is performed.

components of both the GHMQC and GHMBC sequences.

EXPERIMENTAL

The simplest form of the GHNMQC pulse sequence used in this work is shown in Fig. 1. Excitation of ^1H–^{15}N heteronuclear multiple quantum coherence is achieved in the sequence by the first two pulses (90° ^1H–D_{LR}–90° ^{15}N). The BIRD pulse[12] of the GHMQC sequence is not used in long-range correlation experiments and has thus been omitted. Likewise, the low-pass J-filter[13] normally contained in the long-range excitation phase of the GHMBC experiment has also been omitted. We find it convenient simply to allow the signals for the direct ^1H–^{15}N correlations (via $^1J_{NH}$) to be observed in the experiment, obviating the need to acquire a second experiment to establish the chemical shifts of the protonated nitrogen resonances. Wide ^{15}N chemical shift dispersion and the limited number of nitrogens contained in most molecules are such that the overlap of a direct and long-range response is very unlikely. It should also be noted that the relative intensity of the direct responses in the GHNMQC experiment can be severely modulated as a function of the optimization of the long-range coupling delay. Direct response intensity ranging from full to essentially zero intensity depending on whether the long-range delay meets $(2N + 1)/2\,^1J_{NH}$ or $N/^1J_{NH}$ conditions, respectively, can be observed. The ^1H–^{15}N heteronuclear multiple quantum coherence created by the second 90° pulse next evolves through the first half of the evolution period ($t_1/2$). Zero and double quantum terms are interchanged by the 180° ^1H pulse midway through the evolution period and then evolve during the second half of the evolution period. Multiple quantum coherence is reconverted to observable proton single quantum coherence by the final 90° ^{15}N pulse of the sequence. Data are acquired immediately following the reconversion pulse and the final gradient. Decoupling is precluded during the acquisition period as in the conventional HMBC experiment because of the variability of long-range couplings ($^nJ_{NH} \approx 2$–16 Hz for $n = 2, 3$) as in conventional HMBC experiments.[10] In the case of the ^1H–^{15}N GHNMQC experiment, coupling constant variability is more extreme than in the conventional ^1H–^{13}C HMBC experiment since direct response are specifically allowed in the experiment ($^1J_{NH} \approx 80$–100 Hz).

Gradients are applied following the 90° ^{15}N pulse, preceding and following the final 90° ^{15}N pulse in the ratio 5:5: −1. We have found sine-shaped gradients in the range 1–1.5 ms with gradient strengths in the range *ca.* 10–15 G cm^{-1} to be sufficient for purposes of

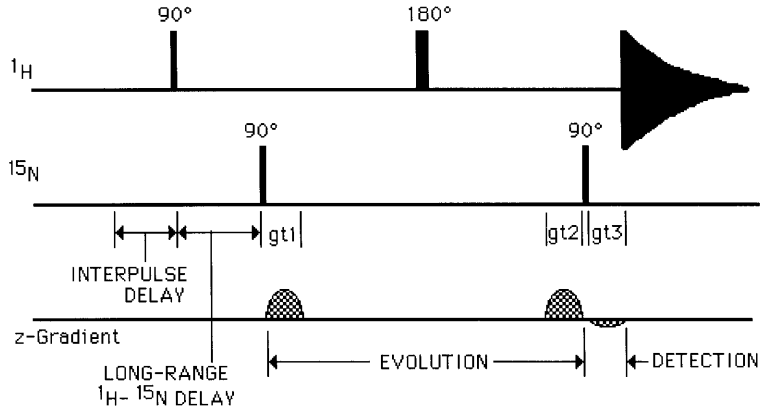


Figure 1. Gradient-enhanced hydrogen–nitrogen multiple quantum coherence two-dimensional NMR pulse sequence for which the acronym GHNMQC has been proposed. The pulse sequence is an amalgamation of the GHMQC and GHMBC pulse sequences. Both the BIRD pulse of the GHMQC sequence and the low-pass J-filter of the GHMBC sequence have been deleted in the GHNMQC experiment. Phase cycling employed was identical with that used with the GHMBC experiment. Sine-bell shaped gradients were applied in the ratio 5:5:1 to accommodate ^{15}N (13 G cm^{-1}). Both long-range ($^nJ_{NH}$, where $n = 2$, 3, etc.) and direct responses ($^1J_{NH}$, where $J = 80$–95 Hz) are observed using the pulse sequence.

^{1}H–^{15}N long-range heteronuclear correlation with the GHNMQC pulse sequence. Phase cycling used for the GHNMQC experiment was identical to that used for the conventional HMBC experiment.[10]

All experiments were performed using a Bruker AMX-500 instrument operating at a frequency of 500.13 MHz for ^{1}H observation. The spectrometer was equipped with a 5 mm Bruker inverse triple resonance probe with a single axis (*z*) gradient coil. Two-dimensional GHNMQC experiments were performed using the pulse sequence shown in Fig. 1 on samples containing *ca.* 25 mg of delavirdine free base [or the approximate molar equivalent of delavirdine mesylate or the 5-aminoindole precursor (**2**)] dissolved in 600 µl of 99.96% DMSO-d_6 (Isotec) in standard 5 mm NMR tubes (Wilmad). Pulses for ^{1}H and ^{15}N were calibrated at 6.6 and 34.0 µs, respectively. Data were typically acquired as 2048 × 128 point matrices which were zero-filled to 4096 × 256 points during data processing. The long-range delay in the GHNMQC sequence was variously optimized for 4, 6, 7 and 11 Hz (125, 83.3, 71.4 and 45.5 ms, respectively) although only data for the 4 Hz optimized spectra are shown in this paper, unless noted otherwise.

RESULTS AND DISCUSSION

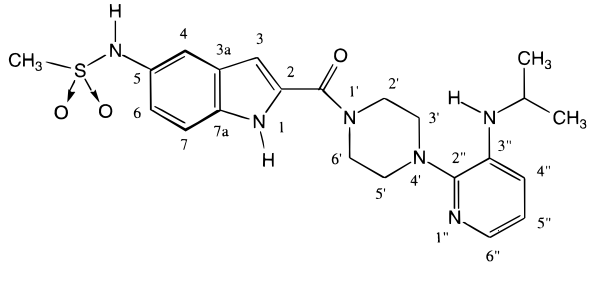
The first applications of the GHNMQC sequence in 1995 in the authors' laboratories were in the study of long-range couplings to and the chemical shifts of nitrogens contained in a variety of alkaloids, which included ajmalaine,[14] the *Strychnos* alkaloids strychnine, brucine and holstiine[15] and the bisindole semi-synthetic alkaloid vinorelbine[16] used clinically in the treatment of non-small cell carcinoma of the lung. In 1996 we reported the nitrogen resonance assignments and long-range ^{1}H–^{15}N couplings of the indoloquinoline alkaloid cryptolepine.[17] Subsequent reports have since appeared from several groups which have utilized pulse sequences similar to GHNMQC to determine the orientation of the steroidal units fused to a central pyrazine in ritterazine A,[18] the structure of a novel alkaloid, agrocybenine, from a Korean mushroom[19] and the sites of protonation of *N*-benzylideneaminopyrazoles.[20] Following these studies, we reported the ^{15}N resonance assignment and long-range coupling pathways for the alkaloid vincamine.[21]

We now direct our attention to the HIV RT (reverse transcriptase) inhibitor delavirdine (**1**), which has six unique nitrogens in its structure in a variety of different electronic environments. The proton and carbon chemical shift assignments for delavirdine were reported recently,[22] facilitating the interpretation of long-range couplings to the various nitrogens contained in the structure.

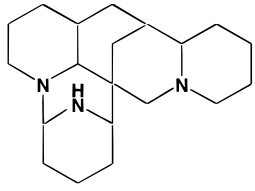
There are a variety of structural characteristics associated with ^{15}N that make it an interesting target nuclide for structure elucidation studies. Many natural products, e.g. alkaloids, incorporate aliphatic, alicyclic and/or aromatic nitrogen heterocycles of some type in their structures. Likewise, nitrogen is a fundamental structural component in >80% of all pharmaceuticals. In the elucidation of unknown structures, homonuclear and ^{1}H–^{13}C heteronuclear connectivity networks are frequently considered to 'dead end' at intervening nitrogen atoms. Typically, in such cases, an effort is made to bridge the nitrogen by resorting to some type of NOE/ROE experiment or alternatively through the use of a long-range ^{1}H–^{13}C coupling across the nitrogen in an HMBC experiment. Obviously, from a sensitivity standpoint, these approaches have far greater inherent sensitivity and should be considered before resorting to ^{1}H–^{15}N long-range heteronuclear shift correlation. However, when these more 'conventional' experiments do not work, for whatever reason, the ability to establish connectivities from protons in spatially 'disconnected' structural fragments to a common intervening ^{15}N via $^{n}J_{NH}$ ($n = 2$, 3) provides powerful structural information by allowing the homo-/heteronuclear connectivity networks to be linked to one another in an unequivocal fashion.

As an example, consider the alkaloid panamine (**2**) ($C_{20}H_{33}N_3$), which Bhacca *et al.*[23] used to demonstrate the ability of the INADEQUATE experiment to establish ^{13}C–^{13}C connectivities.

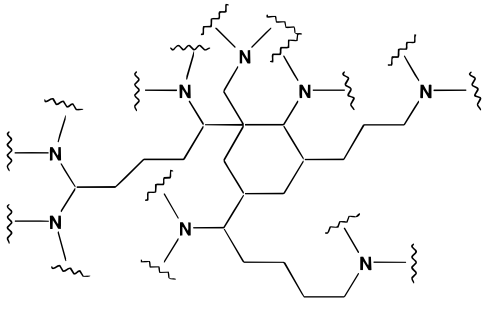
From a thorough study of the INADEQUATE data, it is possible to arrive at the molecular 'spider's web' represented by **3**,[24] in which the presence of the various nitrogen atoms are inferred on the basis of carbon chemical shifts and homonuclear ^{13}C–^{13}C connectivity pathways coming to a 'dead end' (it should be recalled, however, that 'dead ends' in the carbon–carbon connectivity network may also be explained by ^{13}C–^{13}C AB



1



2



3

spin systems when the experiment has been optimized for an AX spin system). At this point, despite the fact that the carbon skeleton of panamine (**2**) has been irrefutably 'established' from the INADEQUATE data, it still requires considerable intuition on the part of the chemist or spectroscopist, and/or a considerable amount of NOE data to link the various molecular appendages to arrive at the correct structure of panamine. In contrast, given that there are three nitrogens, which should have unique chemical shifts, linking protons contained in the various appendages to one another via long-range correlations through the intervening nitrogens should be a plausible undertaking, especially given that sufficient quantities of the alkaloid were available to perform an INADEQUATE experiment in the first place.

Another potentially interesting application of the GHNMQC experiment is in the identification of sites of *N*-oxidation or -hydroxylation when there are competing possibilities. Generally, the assignment of such structures can be envisioned on the basis of regiospecific shifts in proton and/or carbon resonances. While these approaches assuredly work in many instances, they obviously will not in every case. In such circumstances, the ability to utilize long-range ^{1}H–^{15}N correlation provides a means of unequivocally establishing the site of *N*-oxidation or -hydroxylation. It should be noted, however, that at least in the case of some diazine *N*-oxides, work by Städeli *et al.*[25] suggested that differences in ^{15}N chemical shift on *N*-oxidation alone are insufficient to allow assignments to be made and the site of *N*-oxidation to be confirmed. Whether this behavior is more generalized than the diazine heteroaromatic family remains to be demonstrated.

Finally, many nitrogen heterocycles have competing tautomeric structures. In cases where it is germane to a study to have unequivocal confirmation of the preferred tautomer, the ability to probe ^{15}N chemical shifts indirectly via long-range ^{1}H–^{15}N heteronuclear coupling pathways could provide useful structural information provided that protons are fortuitously located in the structure. In this case, the interpretational alternatives are often speculative in nature and are thus much less desirable than the hard evidence potentially afforded by ^{15}N chemical shift information.

The present study of the BHAP [bis(heteroaryl)-piperazine] RT inhibitor delavirdine (**1**), which has six unique nitrogens in its structure, was initially undertaken for several reasons. First, we wanted to further our understanding of the optimization of the delay used to create heteronuclear ^{1}H–^{15}N multiple quantum coherence. Second, we sought to probe the ability of the experiment to cope with the broad ^{15}N spectral width needed to encompass nitrogen species ranging from the alicyclic piperazine nitrogen to the pyridine annular nitrogen (*ca.* 50–300 ppm or 13 kHz). While a 250 ppm F_1 chemical shift range is commonly encountered in ^{1}H–^{13}C long-range correlation experiments (*ca.* 31 kHz), it must also be remembered that the excitation efficiency of the 90° heteronuclear pulse in the sequence is significantly diminished on going from ^{13}C (*ca.* 10–12 μs) to ^{15}N (*ca.* 30–34 μs). Hence it finally remained to be determined at the outset of this study if ^{15}N pulse widths in the range 30–34 μs would be sufficient to afford the excitation efficiency necessary to cope with 250–300 ppm ^{15}N spectral widths.

The GHNMQC ^{1}H–^{15}N heteronuclear correlation spectrum of delavirdine, as the free base in DMSO-d_6, is shown in Fig. 2. The long-range delay for the spectrum shown was optimized for 4 Hz (125 ms). Data were also acquired with the long-range delay optimized for 7 and 11 Hz (71.4 and 45.5 ms, respectively). At the 4 Hz optimization responses for five of the six unique nitrogen atoms in the molecule were observed, although not all were observed as expected.

The molecule contains three protonated nitrogen resonances, the isopropyl secondary amine (N-3″) attached to the 3″-position of the pyridine ring (see the numbering scheme in **1**), the indole NH (N-1) and the methylsulfonamido NH (N-5) attached to the 5-position of the indole nucleus. Direct responses were observed for N-3″ and N-1 as 85 and 99 Hz doublets, respectively, at chemical shifts of 78.2 and 137.7 ppm, respectively. (Nitrogen chemical shifts are reported to one decimal place as measured off the spectrum using a cursor. The data should not, however, be considered to be more accurate than ± 0.5 ppm because of the digital resolution limits of the experiment in F_1.) It is interesting to note that a direct response doublet was not observed for the N-5 sulfonamidomethyl NH resonance. As noted in direct contrast, long-range correlation responses were observed to all three of the protonated nitrogens.

Long-range correlations were observed from the isopropyl methyl doublet and the H-3″ proton of the pyridine ring to the N-3″ resonance. Three-bond long-range couplings were likewise observed from H-3 and H-7 to the indole N-1 resonance. Finally, and importantly, long-range coupling responses were also observed from the indole H-4 and H-6 protons to the indole N-5 resonance locating it at 117.7 ppm. A direct response NH doublet was not observed for the N-5 nitrogen resonance. This observation consequently raises the question of why N-5 should behave in an anomalous fashion. It is also worth noting that the N-5 sulfonamidomethyl NH response was also unobservable in the 7 and 11 Hz optimized experiments (not shown). As noted in the Introduction, however, the lack of a direct response could be a modulation-dependent function of the optimization of the long-range delay in the experiment for selected delays. It seems very unlikely, however, that not even a vestigial trace of a detectable response would be observed for any of the long-range optimizations utilized in this study. Finally, ^{15}N chemical shifts for the three resonances considered thus far were within the range of chemical shifts expected for these types of nitrogens.[3–5]

Before addressing the lack of an N-5 direct response, the long-range couplings to the other nitrogen resonances and the lack of couplings to N-4′ will be considered. Both the piperazine amide N-1′ and the pyridine N-1″ nitrogens were assignable on the basis of the protons to which they were long-range ^{1}H–^{15}N coupled. The N-1′ resonance was long-range coupled to the H-3′/H-5′ methylene protons of the piperazine ring and resonated at 115.4 ppm, which is reasonable for an amide nitrogen of this type. The pyridine N-1″ reso-

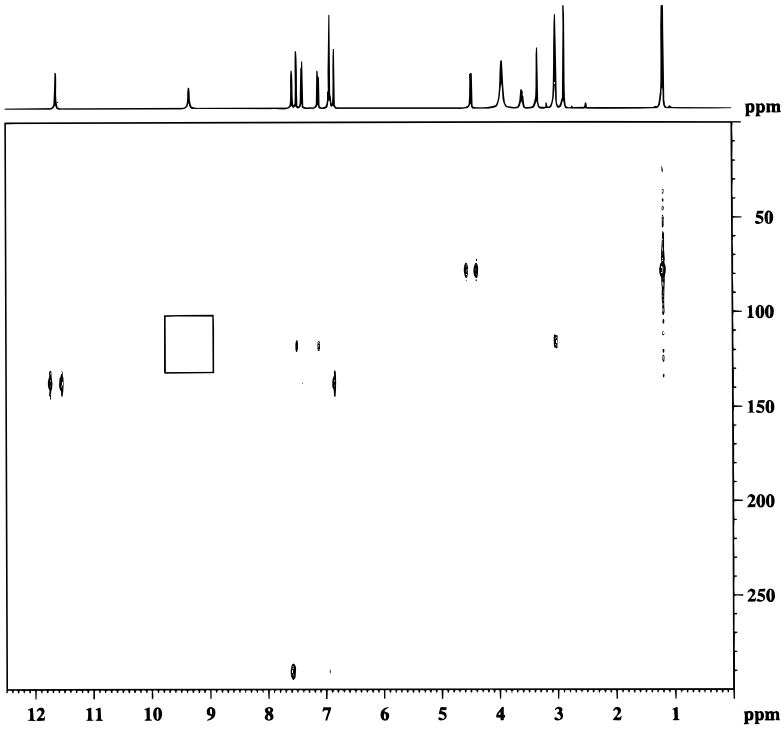


Figure 2. GHNMQC spectrum of delavirdine (**1**) as the free base optimized for a 4 Hz long-range coupling. Direct responses for the indole N-1 and isopropylamino N-3″ resonances were observed as doublets at 137.7 and 78.2 ppm, respectively. A direct response was not observed for the N-5 methylsulfonamido nitrogen (see location denoted by empty box). Long-range responses were, however, observed to N-5 from the H-4 and H-6 protons of the indole nucleus which defined the chemical shift of N-5. Long-range response were observed for the piperazine amide N-1′ and pyridine N-1″ resonances at 115.4 and 290.2 ppm, respectively. A long-range response was not observed to the piperazine N-4′ resonance, presumably owing to broadening of the H-2′/H-6′ methylene resonance of the piperazine ring.

nance was the furthest downfield at 290.0 ppm and was assigned via a long-range coupling to H-5″, which was observed via a strong response in the 4 Hz optimized spectrum, a weaker response in the 7 Hz experiment and through a very weak response in the 11 Hz data. No response was observed for the piperazine N-4′ nitrogen in any of the three long-range correlation experiments performed. Of the two missing responses, the lack of a coupling, presumably from the H-2′/H-6′ methylene protons to N-4′, was the most readily explicable and will be considered first.

Generally, long-range responses in variants of the HMBC experiment become progressively more difficult to observe as the extent of coupling of a proton or protons increases. This problem was successfully addressed by Bax *et al.*[26] in a recent paper dealing with a more conventional ^{1}H–^{13}C long-range heteronuclear correlation experiment. The experiment differed from the conventional HMBC sequence in the application of a selective 90° pulse in lieu of the first 90° ^{1}H pulse and a selective 180° ^{1}H pulse. The former is optional whereas the latter is responsible for removing the undesirable proton–proton couplings inherent to the resonance. When the proton spectrum of delavirdine as the free base is examined, the resonance for the H-2′/H-6′ methylene protons is considerably broader than that for its H-3′/H-5′ methylene counterpart (see proton trace in Fig. 4), which did give a long-range coupling response (Fig. 2) to the N-1′ piperazine amide nitrogen at 115.4 ppm. On this basis, it was logical to extrapolate that application of the selective HMBC experiment (see Fig. 3), modified for ^{1}H–^{15}N heteronuclear shift correlation, would offer a reasonable chance of observing a correlation from the H-2′/H-6′ methylene protons to N-4′. The results of this experiment are illustrated by the segment of the two-dimensional spectrum optimized for 11 Hz shown in Fig. 4, which contained the only two responses observed in the spectrum. Two responses were observed: first, the long-range correlation from the H-2′/H-6′ methylene resonance to N-4′ was now observed, as expected, at 61.3 ppm. A second one-bond doublet response was observed from the secondary isopropyl N-3″H resonance to its corresponding N-3″ nitrogen at 78.2 ppm. This response can be readily explained by the inefficiency of the selective pulse used in the experiment. Since the N-3″H proton is the closest resonance downfield in the spectrum to the H-2′/H-6′ methylene proton resonance on which the selective pulse was centered, excitation inefficiency (i.e. a lack of relative selectivity) would be expected to lead to some unavoidable but undesired excitation of the N-3′H resonance. While a more selective pulse could be used, this may not be particularly desirable.[26] The isopropyl methine resonance, which is closer still to the H-2′/H-6′ methylene resonance upfield, would also experience excitation by the selective pulse but, since it was not coupled to N-3″ in the conventional GHNMQC spectrum, it was not expected to and did not give a response in the spectrum shown in Fig. 4.

Returning to the absent direct response for N-5 in the GHNMQC spectrum shown in Fig. 2, we elected first to attempt to link the behavior to the sulfonamidomethyl moiety by acquiring data for the 5-aminoindole precursor of delavirdine (**4**).

Long-range responses were observed for **4** from the H-4 and H-6 indole protons to the N-5 amino group

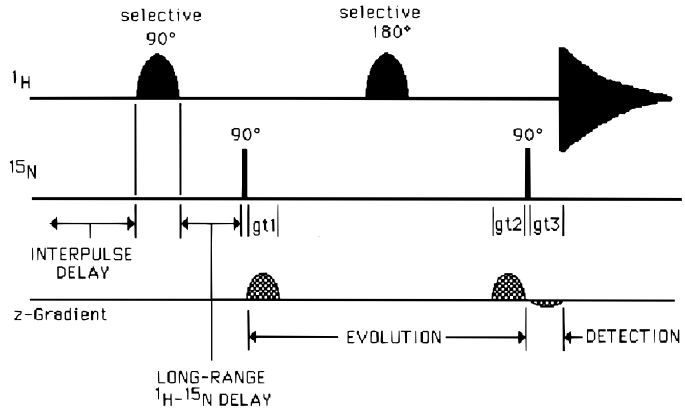
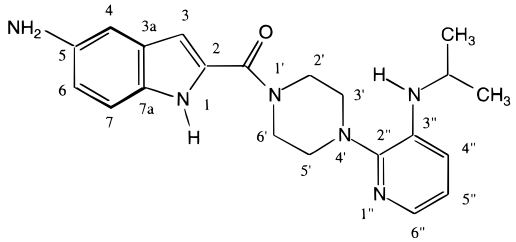


Figure 3. Selective GHNMQC pulse sequence derived from the pulse sequence shown in Fig. 1 based on the work of Bax *et al.*[26] for ^{1}H–^{15}N long-range heteronuclear shift correlation where extensive proton homonuclear coupling might be expected to preclude the observation of a long-range response.

which resonated at 53.7 ppm as shown in Fig. 5. A prominent doublet for the N-5 direct response was not observed. Instead, a strong response was observed at the chemical shift of the 5-amino group flanked by a weak doublet where the normally strong doublet would be observed in the 4 Hz spectrum. No doublet was observed in either the 7 or 11 Hz spectra. The intensity of the doublet response for the N-3″ isopropyl NH is nearby and can be readily compared with that for the N-5 amino (Fig. 5). It should also be noted that the latter does not have any response at the proton shift of the N-3″H resonance in contrast to the prominent response at the proton shift of N-5H_2. We interpreted these observations, coupled with the known acidity of the proton of the N-5 methylsulfonamido group, to suggest the possibility of exchange and/or autoprotonation of delavirdine free base in DMSO solutions.



4

To probe the behavioural similarity and consistency of the N-5 methylsulfornamido and N-5 amino groups, in conjunction with the known acidity of the former, we

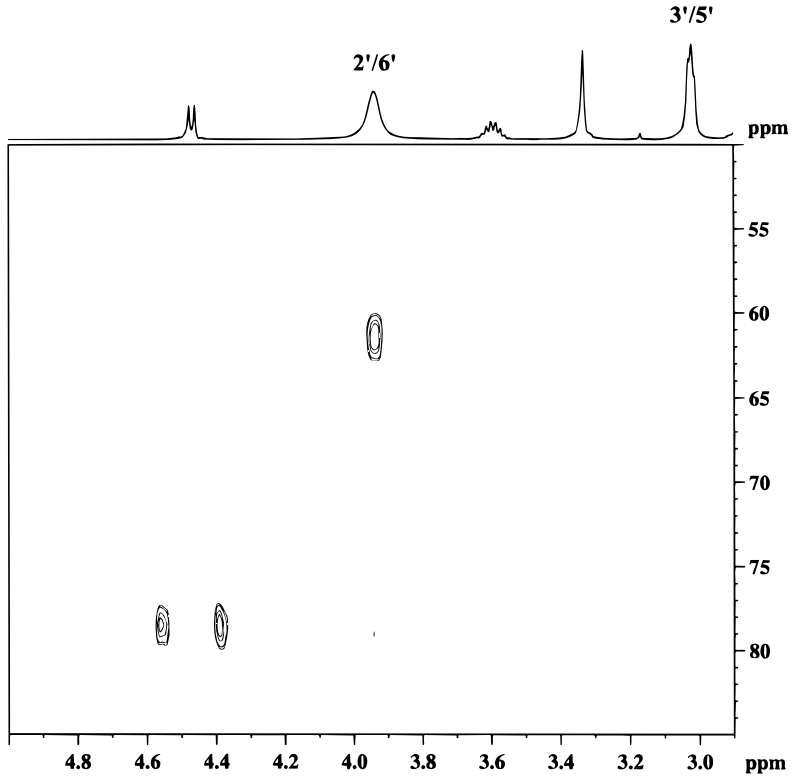


Figure 4. Selective GHNMQC spectrum of delavirdine (**1**) free base in DMSO-d_6 recorded with the selective ^{1}H 90°/180° pulses applied (see text and Ref. 26) to the H-2′/H-6′ methylene resonance, affording the correlation observed at 61.3 ppm. The 'selective' pulses utilized were not rigorously optimized in regard to proximate, non-coupled protons allowing some excitation of the N-3″H doublet, thereby giving rise to the long-range correlation response to the N-3″ nitrogen resonating at 78.2 ppm.

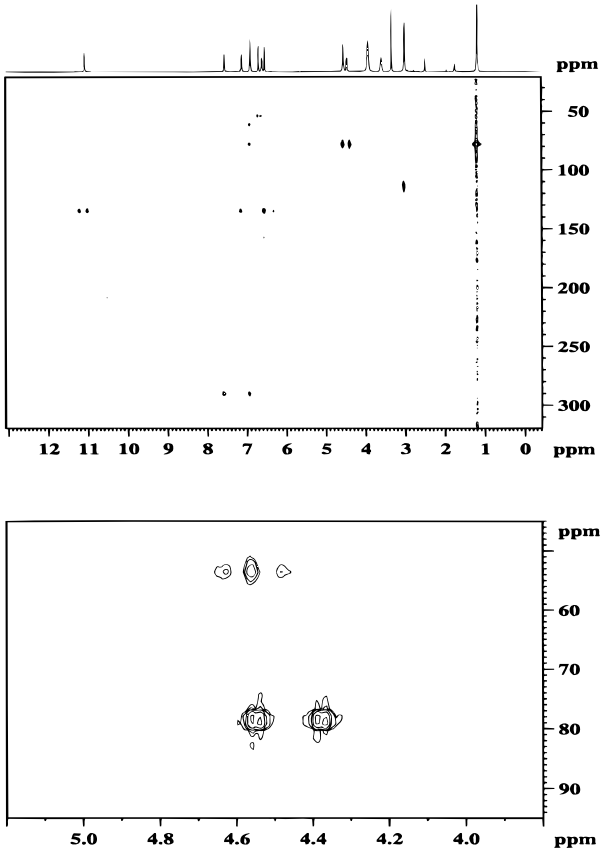


Figure 5. GHNMQC spectrum of the 5-aminoindole precursor (**4**) of delavirdine (**1**) optimized for 4 Hz. The full spectrum is shown at the top; an expansion of the aliphatic nitrogen region of the spectrum from 45 to 95 ppm (5 Hz optimized) is shown at the bottom. The N-3″H isopropylamino nitrogen gave the strong doublet observed at 78 ppm in the bottom panel; the N-5 amino-indole nitrogen gave only the weak doublet flanking the stronger on-resonance signal at 57.3 ppm in the bottom panel. The weak doublet was unobservable in both the 7 and 11 Hz optimized spectra.

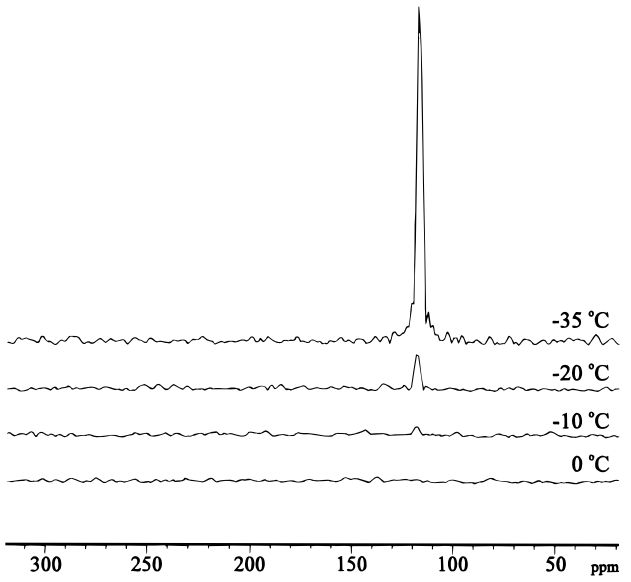


Figure 6. F_1 traces from a series of variable-temperature GHNMQC spectra of delavirdine (**1**) in pyridine-d_5. The N-5H sulfonamidomethyl direct response was unobservable in the 28 °C ambient probe spectrum that was shown as a contour plot in Fig. 2. When spectra were acquired at 0 and −10 °C, no response and a weak direct response were observed, respectively, at the proton chemical shift of the sulfonamindomethyl proton. The direct response intensified on going to −20 °C and was at full intensity at −35 °C.

prepared a solution of delavirdine free base in pyridine-d_5 to allow variable temperature studies to be performed. A series of GHNMQC ^{1}H–^{15}N long-range heteronuclear correlation spectra optimized for 6 Hz were acquired under identical conditions except for the temperature, which was varied from ambient probe temperature (27 °C) to 0, −10, −20 and −35 °C. From this series of experiments, slices were extracted from the F_1 frequency domain at the ^{15}N chemical shift of the N-5 methylsulfonamido resonance. As with the DMSO data, the pyridine spectrum at ambient temperature did not exhibit any sign of a direct response for N-5. There was a weak direct response at the proton chemical shift of N-5H observed in the 0 °C spectrum, which grew progressively in intensity on going first to −10 °C and then to −20 °C. The resonance took on what was assumed to be full response intensity in the −35 °C spectrum. These data are shown in Fig. 6 and clearly substantiate the temperature dependence of the N-5H direct response.

In an effort to determine whether the phenomenological behavior of the N-5H resonance was simply zwitterionic autoprotonation, a GHNMQC ^{1}H–^{15}N long-range correlation spectrum of delavirdine as the mesylate salt in DMSO-d_6 was acquired under conditions identical (4 Hz optimization and equivalent digitization) with those for the spectrum shown in Fig. 2 in which no direct response was observed for the sulfonamidomethyl N-5H resonance. When processed, the sulfonamidomethyl N-5H resonance was observed as an 84 Hz doublet at 118.0 ppm in conjunction with long-range responses from the H-4 and H-6 indole protons. Interestingly, calculations based on the now available 84 Hz one-bond coupling rule out modulation as an explanation for the lack of a direct response. Indeed, the calculations instead mandate that the response should have high intensity based on the 84 Hz coupling. These data are shown in Fig. 7. By converting the molecule to its mesylate salt, the basic site, which the acidic N-5H proton could logically be expected to autoprotonate zwitterionically in the case of the free base, was blocked, thereby precluding autoprotonation and effectively forcing the acidic N-5H proton to reside largely, if not exclusively, on the sulfonamidomethyl nitrogen. It was also observed from the proton reference spectrum plotted above the contour plot in Fig. 6, that the isopropyl amino N-3″ resonance has shifted downfield relative to the free base in addition to some broadening; both results are consistent with protonation at this site. As a consequence, the N-3″ direct response was observed as a 90 Hz doublet at 76.2 ppm in conjunction with long-range coupling observed to the isopropyl methyl groups as expected. Overall, N-3″ experienced an upfield shift of 2.0 ppm relative to the free base, a change which is hardly sufficient to be diagnostically useful in determining sites of *N*-protonation.

Finally, an alternative explanation of the behavior of the N-5H direct response observed, although we feel it is less plausible than the explanation just presented,

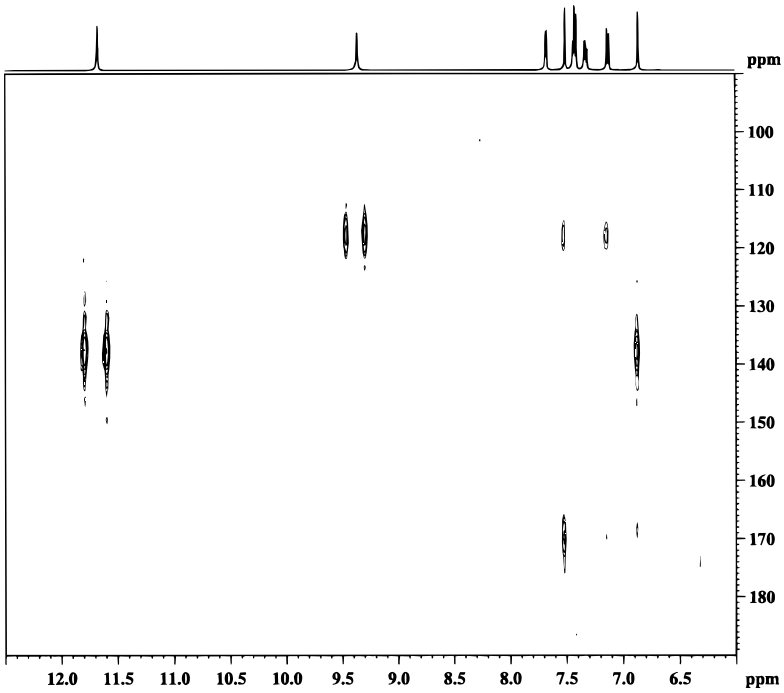


Figure 7. Expansion from 90 to 190 ppm of the 4 Hz optimized spectrum of delavirdine as the mesylate salt in DMSO-d_6. A pronounced 84 Hz direct response doublet is observed at 118.0 ppm in addition to the long-range responses from the H-4 and H-6 indole resonances which were also observed in the spectrum of the free base.

requires invoking a change in the size of the one-bond ^1H–^{15}N coupling as a function of the observation temperature. Changing the size of the coupling by 2–4 Hz at room temperature could give a null response (see discussion of modulation of direct response intensities above) relative to the full intensity response observed at $-35\,^\circ C$. The behavior of the N-5H one-bond coupling as a function of temperature, however, was not probed based on the observation of the direct response at room temperature in the spectrum of delavirdine mesylate shown in Fig. 7.

CONCLUSIONS

The present study has further demonstrated the viability, at natural abundance, of the GHNMQC ^1H–^{15}N heteronuclear shift correlation experiment with a complex nitrogenous pharmaceutical agent. While $^2J_{NH}$ (two-bond) coupling pathways have tended to be predominant in much of the work that we have previously reported dealing with aliphatic nitrogens contained in alkaloidal structures, $^3J_{NH}$ (three-bond) couplings were far more prevalent in the present study. Some of the responses were shown to be at least somewhat sensitive to the optimization of the GHNMQC long-range delay over the range 4–11 Hz. The behavior of the highly acidic sulfonamidomethyl N-5H proton was investigated in an effort to explain the enigmatic absence of the direct response doublet for this nitrogen resonance in the DMSO spectrum of the free base at ambient temperature. From a variable-temperature study performed in pyridine, it was shown that an 'exchange' process leads, presumably, to at least the partial autoprotonation (on the time-scale of the GHNMQC NMR experiment) of the N-3″ isopropyl amino nitrogen resulting in the formation of a zwitterionic species which can account for the absence of the N-5H direct response doublet. It has further been demonstrated that it is possible to slow 'exchange' almost, if not completely, between -20 and $-30\,^\circ C$ in pyridine-d_5 solutions of delavirdine and to block the process by converting delavirdine to its mesylate salt, which allowed the N-5H direct response doublet to be readily observed in DMSO at ambient temperature. Finally, it is also worth noting that even the substantially less acidic 5-aminoindole precursor (**2**) of delavirdine is prone to engage in zwitterionic autoprotonation in an analogous fashion to that prevalent with the 5-sulfonamidomethyl substituent.

REFERENCES

1. G. C. Levy and R. L. Lichter, *Nitrogen NMR Spectroscopy*. Wiley, New York (1979).
2. G. J. Martin, M. L. Martin and J.-P. Gouesnard, *^{15}N-NMR Spectroscopy, NMR Basic Principles and Progress*, Vol. 18, edited by P. Diehl, E. Fouck and R. Kosfeld. Springer, New York (1981).
3. M. Witanowski, L. Stefaniak and G. A. Webb, *Annu. Rep. NMR Spectrosc.* **11B**, 000 (1981).
4. M. Witanowski, L. Stefaniak and G. A. Webb, *Annu. Rep. NMR Spectrosc.* **18**, 000 (1986).
5. M. Witanowski, L. Stefaniak and G. A. Webb, *Annu. Rep. NMR Spectrosc.* **25**, 000 (1993).

6. A. Bax, R. H. Griffey and B. L. Hawkins, *J. Magn. Reson.* **55**, 301 (1983).
7. A. Bax, R. H. Griffey and B. L. Hawkins, *J. Am. Chem. Soc.* **105**, 7188 (1983).
8. C. M. Griesinger, H. Schwalbe, J. Schleucher and M. Sattler, in *Two-Dimensional NMR Spectroscopy—Applications for Chemists and Biochemists*, edited by W. R. Croasmun and R. M. K. Carlson, 2nd edn, pp. 457–580, and references cited therein. VCH, New York (1994).
9. A. Bax and S. Subramanian, *J. Magn. Reson.* **67**, 565 (1986).
10. A. Bax and M. F. Summers, *J. Am. Chem. Soc.* **101**, 2093 (1986).
11. R. E. Hurd and B. K. John, *J. Magn. Reson.* **91**, 648 (1991).
12. J. R. Garbow, D. P. Weitekamp and A. Pines, *Chem. Phys. Lett.* **93**, 504 (1982).
13. H. Kogler, O. W. Sørensen and R. R. Ernst, *J. Magn. Reson.* **55**, 157 (1983).
14. G. E. Martin and R. C. Crouch, *J. Heterocycl. Chem.* **32**, 1665 (1995).
15. G. E. Martin, R. C. Crouch and C. W. Andrews, *J. Heterocycl. Chem.* **32**, 1759 (1995).
16. G. E. Martin and R. C. Crouch, *J. Heterocycl. Chem.* **32**, 1839 (1995).
17. G. E. Martin, R. C. Crouch, M. H. M. Sharaf and P. L. Schiff, Jr, *J. Nat. Prod.* **59** 2 (1996).
18. S. Fukuzawa, S. Matsunaga and N. Fusetani, *Tetrahedron Lett.* **37**, 1447 (1996).
19. H. Koshino, I.-K. Lee, J.-P. Kim, W.-G. Kim, J. Uzawa and I.-D. Yoo, *Tetrahedron Lett.* **37**, 4549 (1996).
20. E. Kolehmainen, A. Puchala, R. Suontamo, D. Rasala and R. Lysek, *J. Chem. Soc., Perkin Trans. 2* **00**, 2383 (1996).
21. G. E. Martin, *J. Heterocycl. Chem.* in press.
22. K. A. Farley, G. S. Walker, R. H. Robins, J. E. Kupstas-Guido, W. K. Duholke, D. L. Romero, R. A. Morge, P. A. Meulman, R. S. Chao, J. L. Havens, J. G. Gage, M. A. Lystger, P. E. Fagerness and G. E. Martin, *J. Heterocycl. Chem.* **33**, 493 (1996).
23. N. S. Bhacca, M. F. Balandrin, A. D. Kinghorn, T. A. Frenkiel, R. Freeman and G. A. Morris, *J. Am. Chem. Soc.* **105**, 2538 (1983).
24. G. E. Martin and A. S. Zektzer, *Two-Dimensional NMR Methods for Establishing Molecular Connectivity—A Chemist's Guide to Experiment Selection, Performance and Interpretation*, pp. 405–407 and 464–471. VCH, New York (1989).
25. W. Städeli, W. von Philipsborn, A. Wick and I. Kompis, *Helv. Chim. Acta* **63**, 504 (1980).
26. A. Bax, G. S. Walker and K. A. Farley, *J. Magn. Reson. A* **119**, 134 (1996).