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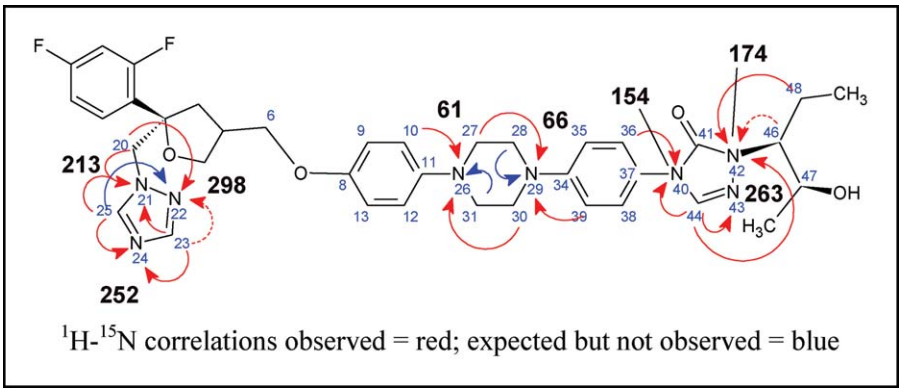

The structurally complex antifungal agent posaconazole has been well characterized by conventional ^{1}H- and ^{13}C-NMR studies. In contrast, ^{15}N resonance assignments have never been reported. We now wish to report the assignment of the eight ^{15}N resonances of posaconazole using two-dimensional long-range ^{1}H-^{15}N GHMBCAD NMR data. The ^{15}N resonance assignments were undertaken to facilitate the evaluation of the impact of ^{1}H-^{15}N heteronuclear shift correlation data in the Computer-Assisted Structure Elucidation (CASE) of complex molecular structures.

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INTRODUCTION

Nitrogen is incorporated into the structures of a significant percentage of pharmaceutical agents and can provide a valuable yet underutilized structural probe. The antifungal agent posaconazole, 1, [1] incorporates eight annular nitrogens in its structure in the form of two 1,2,4-triazolyl moieties and a piperazine. Posaconazole exhibits activity against a broad range of fungal pathogens including *Aspergillis*, *Candida*, and *Cryptococcus* [2–6]. Posaconazole and several of its oxidative degradants have been previously characterized by a combination of LC/MS and LC-NMR [7] but a systematic investigation of the molecule using ^{1}H-^{15}N heteronuclear chemical shift correlation methods has never been reported.

Although ^{15}N has a reputation of being a difficult nuclide to observe directly because of its low natural abundance (0.37%), low gyromagnetic ratio (γ_N), and for some resonances negative NOEs, the same is not true of indirect detection methods for nitrogen. There have been a number of reviews published on indirect-detection ^{15}N-NMR methods since 2000 [8–13]. For the elucidation of unknown nitrogen-containing chemical compounds, quantities of material sufficient to allow the overnight acquisition of long-range ^{1}H-^{15}N heteronuclear shift correlation 2D NMR spectra are as follows: at 600 MHz, tens of micromole quantities can be studied with conventional NMR probes; micromole quantities afford usable samples when a laboratory is equipped with a 5 mm cryoprobe [14]; and submicromole quantities can be studied when a 1.7 mm gradient triple resonance cryoprobe is available. There have also been several studies addressing the impact of the availability of long-range ^{1}H-^{15}N heteronuclear shift correlation data on the elucidation of chemical structures using Computer-Assisted Structure Elucidation (CASE) methods [15–20]. In general, the availability of long-range ^{1}H-^{15}N heteronuclear shift correlation data greatly reduces the number of structures generated by CASE programs and/or the computation time. In the case of some complex molecules, attempts to perform CASE studies without ^{1}H-^{15}N long-range heteronuclear shift correlation data have failed to produce viable structures despite computation times of up to a week [20]. Indeed, posaconazole is one such example, and the results of that investigation are the subject of the following report [21]. It is in this context that we wish to report the assignment of the ^{15}N resonances of the antifungal agent posaconazole, **1**.

The ^{1}H- and ^{13}C-NMR resonance assignments of posaconazole, **1**, were obtained straightforwardly using a

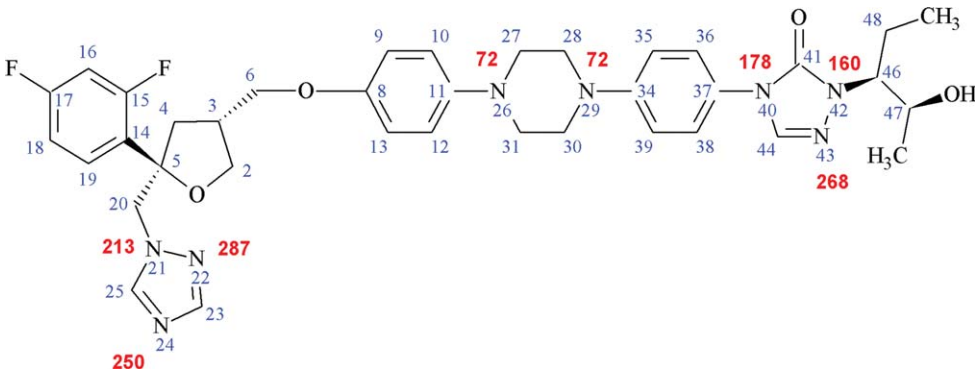

Figure 1. 15N Chemical shifts of posaconazole, 1, calculated using the neural net method with the NNMR program, v12.01, developed by Advanced Chemistry Development (ACD) Laboratories. 15N shifts shown are in ppm downfield from liquid NH3 with a shift of 0.0 ppm (−380.2 ppm relative to nitromethane).

combination of homo- and ^{1}H-^{13}C heteronuclear 2D NMR spectra. These data were acquired using a Varian 600 MHz three channel NMR spectrometer equipped with a 5 mm gradient inverse triple resonance ColdProbe^{TM} using a sample containing 2 mg of posaconazole dissolved in ~200 μL of d_6-DMSO (Cambridge Isotope Laboratories) in a Wilmad 3 mm NMR tube. These assignments provided the basis for the subsequent assignment of the eight ^{15}N resonances of the molecule. Examination of the substructural components containing nitrogen leads to the expectation that the resonances of the 1,2,4-triazole should be in the range of ~200–300 ppm (downfield of liq. NH_3 with a shift of 0.0 ppm). For the 1,2,4-triazol-3-one, the resonances would be expected in the range of ~150–300 ppm. Finally, in the case of the two piperazine nitrogens, the resonances would be expected in the range of ~50–80 ppm. Consistent with these expectations, ^{15}N chemical shift calculations done using the NNMR program, v12.01, developed by Advanced Chemistry Development (ACD) Laboratories gave the calculated ^{15}N shifts shown in Figure 1. Using the expectations of the authors, combined with the calculated ^{15}N chemical shifts for posaconazole, the 6 Hz optimized ^{1}H-^{15}N GHMBCAD spectrum used to assign the ^{15}N shifts was acquired with the F_1 spectral window set to cover the range from 50 to 350 ppm.

Long-range ^{1}H-^{15}N heteronuclear correlations are generally observed *via* either $^{2}J_{NH}$ or $^{3}J_{NH}$ couplings. On this basis, the expected long-range couplings to the various nitrogen resonances of posaconazole are shown in Figure 2. Generally, all of the expected correlations will not be observed, but it is useful to begin the examination of long-range ^{1}H-^{15}N data relative to the expected correlations to the nitrogens in a given structure when that structure is known. It should be noted that ^{15}N chemical shifts are much less accurately predicted than those of ^{13}C resonances. Although the database used by the NNMR program for ^{15}N chemical shift prediction contains ~25,000 shifts, in contrast, the database used for ^{13}C chemical shift prediction by the C-NMR program contains > 1.5 million chemical shifts.

The 6 Hz optimized ^{1}H-^{15}N GHMBCAD spectrum of posaconazole in d_6-DMSO at a temperature of 25°C recorded at 600 MHz using a Bruker 1.7 mm TCI cryoprobe^{TM}. The data were acquired using a 330 μg sample

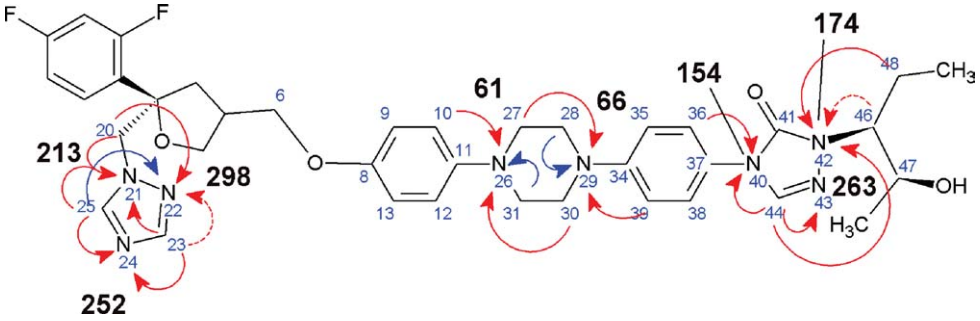

Figure 2. Possible 2JNH and 3JNH long-range 1H-15N heteronuclear long-range couplings for posaconazole, 1. 4JNH Couplings are less frequently observed and generally are only observed via correlations from sharp methyl singlets or aromatic methine protons with minimal homonuclear coupling. Long-range correlations designated by red arrows were observed in the spectrum shown in Figure 3; dashed red arrows indicate weak correlations observed in Figure 3; expected correlations that were not observed are denoted by blue arrows. Assigned 15N chemical shifts are shown in black and compare favorably with the calculated shifts shown in Figure 1.

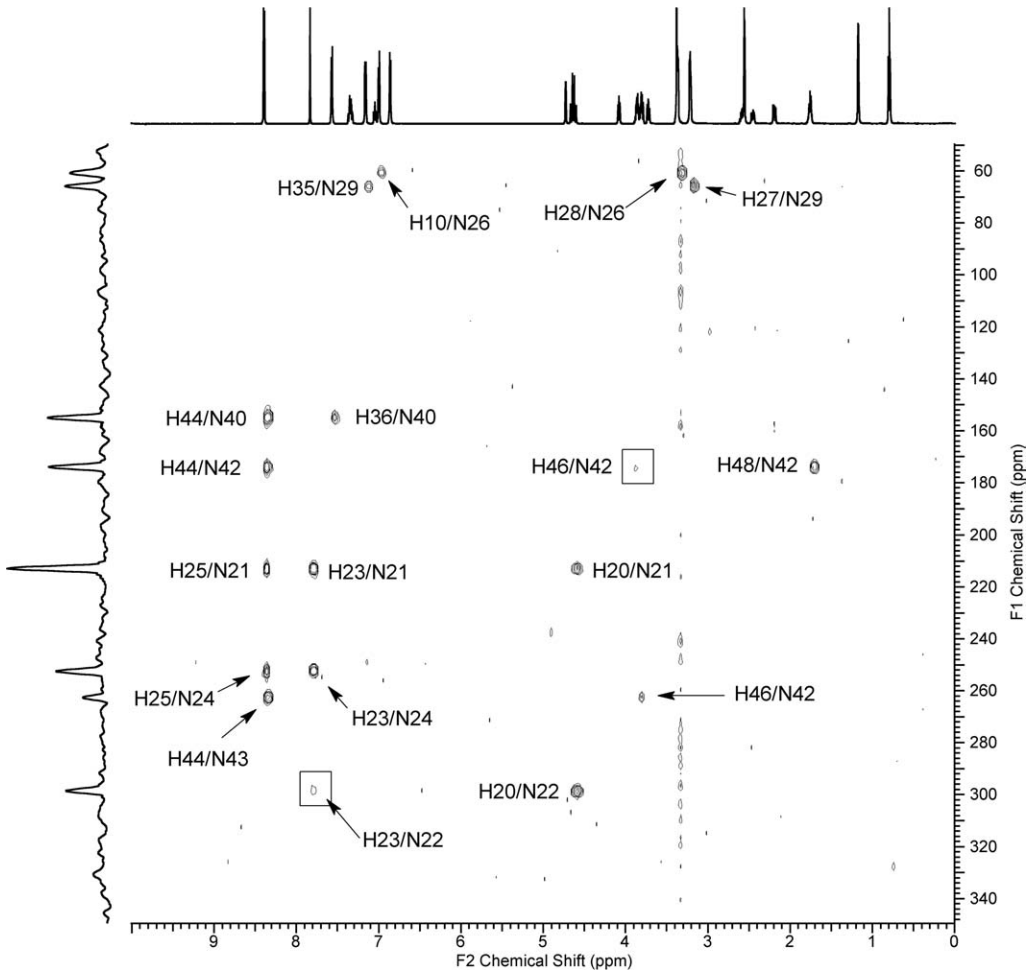

Figure 3. Contour plot of the 6 Hz optimized GHMBCAD spectrum of posaconazole recorded in d_6-DMSO at an observation frequency of 600 MHz with the sample temperature regulated at 25°C. The data were recorded using a 330 μg sample of posaconazole in 30 μL of d_6-DMSO prepared by serial dilution. The data were acquired as 4096 × 128 points in 3 h 15 min using a Bruker 600 MHz NMR equipped with a 1.7 mm triple resonance inverse-detection TCI CryoProbe™ and were processed to 2K × 1K points. The 600 MHz 1H reference spectrum is plotted along the F_2 axis; the projection of the 15N spectrum through F_1 is plotted vertically along that axis. Resonance assignments are specified as 1H/15N; weak correlations are boxed.

of posaconazole in 30 μL of d_6-DMSO (Cambridge Isotope Laboratories) prepared by serial dilution. The data were acquired in 3 h 15 min as 4096 × 128 points and were processed to 2K × 1K.

The assignment of the ^{15}N resonances of the 1,2,4-triazole ring was straightforward, using the anisochronous 20-methylene proton resonances at 4.60 and 4.56 ppm to assign the N21 and N22 resonances, which were assigned at 213 and 298 ppm, respectively. The H23 proton resonance at 7.78 ppm also exhibited correlations to the N21 and N22 resonances as well as to the N24 resonance at 252 ppm. Finally, the H25 proton resonating at 8.34 ppm exhibited two-bond long-range correlations to the N21 and N24 resonances but did not exhibit the expected three-bond correlation to the N22 resonance.

As shown in Figure 3, two aliphatic nitrogen resonances corresponding to the piperazine N26 and N29 resonances were observed at 61 and 65 ppm in reasonable agreement with the predicted chemical shift of these resonances at ~72 ppm (Fig. 1). The N26 resonance was assigned as the nitrogen resonating at 61 ppm based on the identification of the H10/H12 resonance *via* the following series of ^{1}H-^{13}C and ^{1}H-^{15}N GHMBC correlations. The H6 methylene was coupled long-range to the oxygen-bearing C8 quaternary aromatic resonance, to which the H10/H12 aromatic protons were also coupled. The H10/H12 resonances were, in turn, long-range coupled to the nitrogen resonating at 61 ppm, allowing the assignment of that resonance as N26. The H35/H39 resonances associated with the other phenyl ring were coupled long-range to the other aliphatic nitrogen resonating at 66 ppm, which was assigned as N29.

The three remaining nitrogen resonances from the 1,2,4-triazolyl-3-one, N40, N42, and N43, resonated at

154, 174, and 263 ppm, respectively. Correlations from the H36/H38 aromatic resonances and H44 allowed the assignment of N40 to the resonance at 155 ppm. Correlations from the H44, H46, and H48 resonances allowed the assignment of N42 as the resonance at 174 ppm, and finally, the correlation from the H44 resonance allowed the assignment of N43 as the remaining resonance observed at 263 ppm. Although the ^{15}N chemical shift of N43 is much more accurately predicted by the neural network calculations (*vs*. 306 ppm *via* HOSE code), the chemical shifts of N40 and N42 are more accurately predicted by simple HOSE code calculations as 168 and 178 ppm, respectively, essentially the reverse of the 178 and 160 ppm predictions obtained with the neural network method.

The assignments of the ^{15}N resonances of posaconazole, **1**, have been reported. The impact of the availability of ^{1}H-^{15}N heteronuclear connectivity data on the CASE of posaconazole and degradants thereof is being evaluated; the results of that investigation will form the basis of a following report [21].

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