Application of the Randomly Optimized RDSQC Experiment for the Elucidation of an Unknown Compound

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The application of the randomly optimized RDSQC (Randomly optimized Direct correlation Single Quantum Coherence) experiment for the detection of direct correlations facilitated the characterization of an unknown compound. The expected structure consisted of purely aliphatic moieties. The actual, identified compound contained the desired structure plus an adenosine functionality with two protons whose direct proton-carbon couplings were over 200 Hz. Application of a 130 Hz optimized direct heteronuclear GHSQC experiment afforded no correlations for the adenine responses. The RDSQC experiment allowed for the simultaneous optimization of multiple couplings in a range of 130 to 220 Hz producing a direct correlation spectrum with all the expected responses.

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Introduction.

In the pharmaceutical industry, the need often arises for structural identification of an unknown compound. Such a necessity may originate from an impurity, degradant, incorrectly labeled samples, or even a negative identification from a screening hit. Subsequently, the spectroscopic data must then follow through well thought-out optimization as improper or insufficient data can lead to an incorrect identification.

Following the interpretation of the one-dimensional proton NMR data, elucidation of an unknown structure typically proceeds with the identification of all homonuclear spin systems, using a COSY or TOCSY data set. These spin systems can then be correlated to their directly attached carbons *via* a direct heteronuclear shift correlation experiment such as an HSQC or HMQC. The lack of a response in a ^{1}H-^{13}C direct correlation data set generally suggests that the proton in question is attached to a heteroatom other than carbon (usually nitrogen or oxygen). The defined spin systems (now containing the direct correlations) can next be correlated to each other *via* heteronuclear long-range ^{1}H-^{13}C HMBC data. Other, more "exotic" experiments such as NOESY / ROESY, HSQC-TOCSY, ^{1}H-^{15}N GHMBC, *etc*., may be acquired as necessary to complement the aforementioned assignments.

Direct heteronuclear shift correlation experiments employed to identify the carbons directly attached to the protons are dependent on delays optimized as a function of the one-bond heteronuclear coupling ($^{1}J_{CH}$). For the statically optimized [1] experiment (such as the HSQC [2] or HMQC [3]) this becomes, at best, an educated guess when working with an unknown. The typical range of aliphatic $^{1}J_{CH}$ couplings is 120 to 140 Hz, and simple aromatic $^{1}J_{CH}$ couplings are from 155 to 175 Hz. This would lead to optimizations of 130 and 165 Hz, respectively, for the purely aliphatic and purely aromatic systems. A value of 140 or 145 Hz is generally used for systems with both functionalities. Unfortunately, some proton-carbon pairs (especially in heteronuclear aromatic systems) exhibit couplings much larger than the 140 Hz average; the proton in the 2-position of pyridine exhibits a $^{1}J_{CH}$ of 180 Hz, and also the proton in the 2-position of furan is coupled by ~210 Hz. A direct correlation experiment optimized for 140 Hz could potentially not observe all proton-carbon pairs in such a molecule with a wide range of $^{1}J_{CH}$ couplings. A second experiment would have to be re-optimized for a more appropriate value to observe the missing responses with convincing intensity.

A different approach involves sampling of a range of optimizations in a single experiment [4]. The randomly optimized RDSQC [5] (Randomly optimized Direct correlation Single Quantum Coherence) experiment can be optimized for couplings in a user-defined range, thereby providing all potential one-bond correlations in a single experiment. Values within the optimization range are sampled in a random order to remove artifacts spread into the F_1 domain. Employing such optimization techniques allows for the simultaneous observation of all direct ^{1}H-^{13}C correlations in a single experiment.

Results and Discussion.

A recent antibacterial screening assay produced a positive hit but with a negative identity. Structural identification of the compound was then necessary before subsequent studies could be completed. A sample thought to be **1** was determined to have a mass of 740 Da, 330 Da above the expected value. Tandem mass spectroscopy identified many fragment ions consistent with the proposed structure.

The proton spectrum is shown on top of Figure 1. Resonances consistent with the structure of **1** are present. There are, however, a number of additional unidentified resonances. The COSY experiment identified the homonuclear spin systems; the overlapped regions made

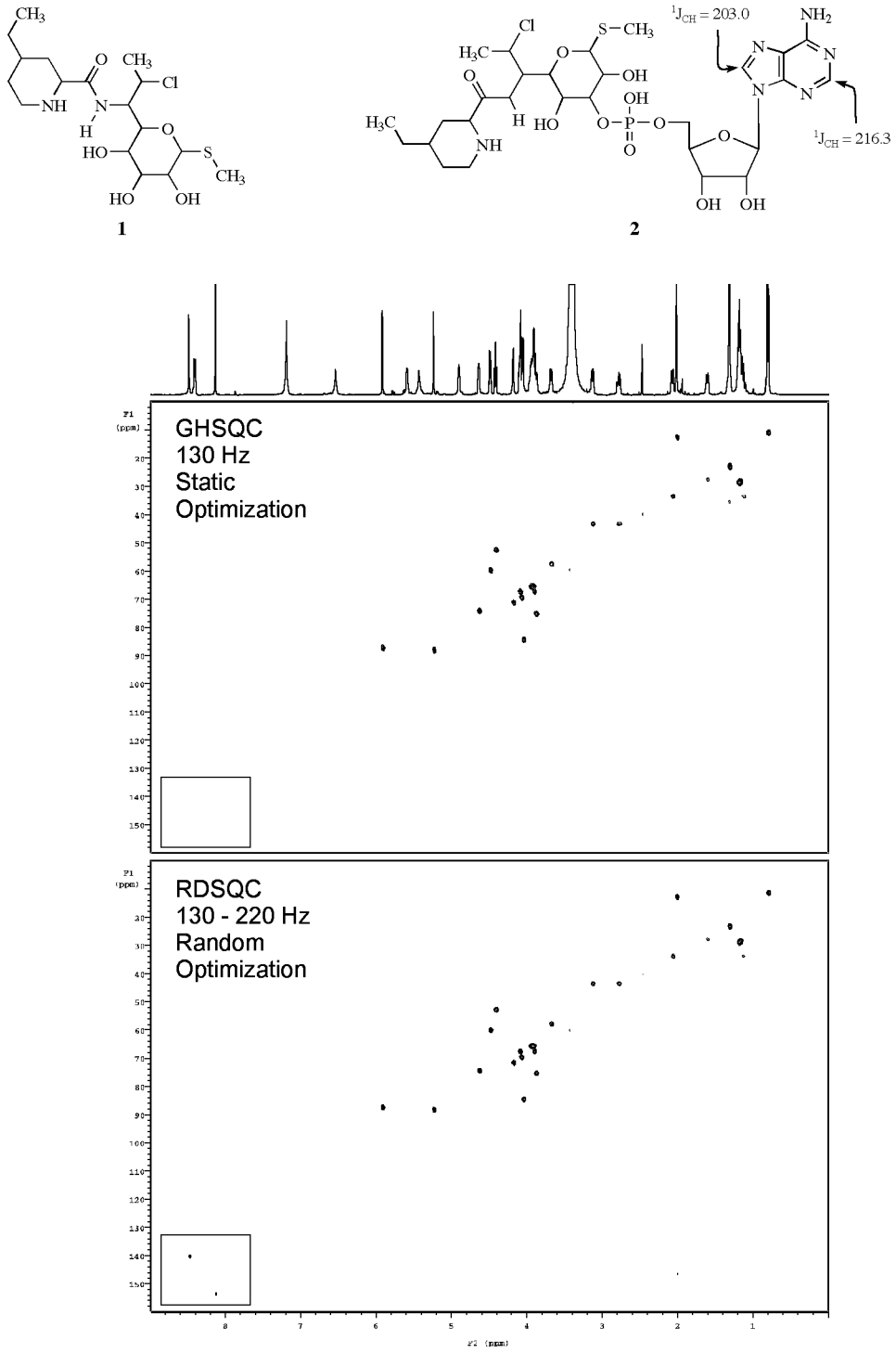

Figure 1. Comparison of the contour plots for the statically optimized GHSQC [top] and randomly optimized RDSQC [bottom] for **2**. The single optimization of 130 Hz for the GHSQC experiment did not allow for the observation of the adenine responses (boxed), which have $^1J_{CH}$ couplings of 203 and 216 Hz. The random optimization of a range of couplings from 130 to 220 Hz afforded both correlations. The top spectrum could potentially lead to misassignment of a proton attached to a heteroatom such as nitrogen or oxygen, while the bottom leaves no ambiguity.

data interpretation difficult. These systems were then correlated to their directly bound carbons. Proper experiment selection at this point is an important consideration. For an aliphatic molecule such as **1**, a 130 Hz statically optimized GHSQC might have been used to acquire the direct correlation data. Potential aromatic resonances observed in the

proton spectrum would be observed with a $^1J_{CH}$ static optimization of 130 Hz.

Identification of an unknown requires careful optimization. Instead of a statically optimized GHSQC, a randomly optimized RDSQC sampling a one-bond coupling constant range from 130 to 220 Hz was employed. These data are shown in the bottom panel of Figure 1. Note that the proton responses at 8 and 8.5 ppm exhibit responses at 140 and 150 ppm, respectively. Hence, these protons are correlated to sp^2 carbons.

A 130 Hz GHSQC experiment (Figure 1, top) was acquired for comparison to the randomly optimized RDSQC data. This optimization was chosen because the 'suspected' structure was purely aliphatic in its functionality. The lack of responses for the 8.0 and 8.5 ppm protons (boxed region) could have been misleading and would have required additional experiments to be acquired to determine that these proton resonances are indeed attached to carbons. Significant savings in time can be gained through the ability of the RDSQC experiment to obviate the need of a reoptimization of the direct, heteronuclear shift correlation experiment. The observation of the downfield responses in the RDSQC spectrum was succeeded by an RDSQC satellite spectrum to determine the $^1J_{CH}$ coupling constants of the proton-carbon pairs at 203 and 216 Hz.

It is worth noting that there are two responses (Figure 1, ^{1}H / ^{13}C pairs at 1.5 / 36 ppm and 2.5 / 39 ppm) in the GHSQC data that are not observed in the RDSQC. Since the statically optimized GHSQC experiment was optimized for 130 Hz and will thus achieve a better signal intensity for that optimization than the RDSQC experiment when optimized for a range of 130 to 220 Hz. The RDSQC data, however, will have a better signal intensity average for the entire range of couplings sampled as shown by the boxed region in Figure 1.

Conclusion.

The application of the RDSQC experiment in the determination of the unknown **2** allowed for the efficient acquisition of the necessary direct correlation data. Under certain circumstances, such as low concentrations, short lifetimes, limited magnet time, *etc.*, the acquisition of a second statically optimized direct correlation data set is impossible. The RDSQC allows for all correlations to be observed in a single experiment.

EXPERIMENTAL

The sample of **2** was prepared as approximately 1 mg dissolved in 150 µL dimethylsulphoxide-d_6 (CIL, 99.996 %). All NMR experiments were acquired on a Varian INOVA 600 MHz NMR spectrometer, operating at a proton frequency of 599.75 MHz, and equipped with a Nalorac Z•Spec™ MIDTG-600-3 inverse geometry NMR probe. The 90° pulse lengths were as follows; 5.5 µs at 51 dB (63 dB max) for ^{1}H, and 13.8 µs at 59 dB (63 dB max) for ^{13}C.

The optimization values were calculated as follows. The step-size between points was determined by [1 / (2 * (($^1J_{CHmin}$ - $^1J_{CHmax}$) / ni))], where $^1J_{CHmin}$ is the smallest coupling in the desired range, $^1J_{CHmax}$ is the largest coupling in the desired range, and ni is the number of experimental increments. This value is then successively subtracted from the starting point of [1 / (2 * ($^1J_{CHmin}$)]. The RDSQC then randomized this series of optimizations with the C program command "srand." The srand random generator provides a reproducible (otherwise known as pseudo-random) set of variable numbers based on the array of values supplied to the function.

REFERENCES AND NOTES

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[1] Static-optimization refers to the experimental optimization for a single coupling, as opposed to random optimization of a range of couplings.

[2] L. Müller, *J. Am. Chem. Soc*., **101**, 4481 (1979); A. Bax and S. Subramanian, *J. Magn. Reson*., **67**, 565 (1986); R. E. Hurd and B. K. John, *J. Magn. Reson*., **91**, 648 (1991); A. L. Davis, J. Keeler, E. D. Laue and D. Moskau, *J. Magn. Reson.*, **98**, 207 (1992).

[3] G. Bodenhausen and D. J. Ruben, *Chem. Phys. Lett.*, **69**, 185 (1980); A. Bax and Pochapsky, *J. Magn. Reson.*, **99**, 638 (1992).

[4] C. E. Hadden and D. T. Angwin, *Magn. Reson. Chem.*, **39**, 1, (2001).

[5] C. E. Hadden, J. B. Moon, and D. T. Angwin, *J. Heterocylcic Chem.* **38**, 843 (2001).