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Identification of the Major Tautomer for an Etheno Adduct of 2,6-Diaminopurine by Determination of the Sign of $^{n}J_{H,C}$

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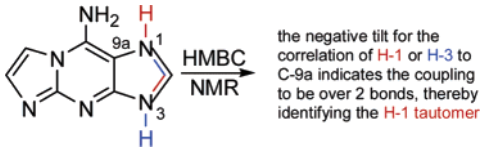
ABSTRACT



The major of two solution-state tautomers observed for an etheno product of 2,6-diaminopurine was identified as the tautomer H-1 on the basis of the recognition of the two-bond coupling between the NH proton and C-9a and the three-bond coupling between the NH proton and C-3a. The couplings were distinguished as being over two- or three bonds by determination of the sign of the coupling using two-dimensional heteronuclear NMR, negative in the former case and positive in the latter case.

The mere qualitative detection of long-range couplings between ^{1}H and X nuclei, notably ^{13}C but now also pervasively ^{15}N, has been essential for structure elucidation and NMR signal assignment itself. In particular, HMBC NMR experiments[1] have been at the forefront of the assault on structures known or unknown and are the unparalleled method of choice for detecting long-range couplings. This is despite laboring under the burden of their most notable limitation now that pulsed field gradient technology has vanquished the problem of interfering ^{12}C-proton magnetization, namely, the distinction between two- and three-bond couplings, and for which there is still not a general solution. However, this problem is generally true for all manner of experiments that perform the same function. In any event, when confronted by the necessity of making such a distinction, this limitation may not always represent a serious obstacle. For example, cases involving only proton-bearing carbons and where this distinction is often necessary due to overlapping proton spins, one can resort to hybrid sequences, e.g., HSQC-TOCSY or the recently developed HMBC-RELAY,[2] which can effect a distinction between correlations arising from $^{2}J_{H,C}$ or $^{n}J_{H,C}$. Interestingly, quantification of $^{n}J_{H,C}$ was formerly, due to the manner in which analyses were conducted (coupled carbon and selective ^{1}H irradiation), performed much more frequently but has quietly, and understandably, fallen out of favor due to the emergence and eventual domination of HMBC, an experiment which, in its basic form, is not well suited for such purposes. The persistent demands for such valuable structural information though has led to the development of a number of sequences (including HMBC-based ones) designed explicitly to redress this deficiency.[3] The next logical step after quantification of the magnitude of $^{3}J_{H,C}$ (or $^{2}J_{H,C}$) is obviously determination of the sign of the coupling, and indeed an elegant solution to this problem has been reported,[4] though with the limitation that the proton involved must also possess homonuclear coupling. Herein we demonstrate a simple approach to this problem and use the derived sign of the coupling to render a distinction between $^{2}J_{H,C}$ and $^{3}J_{H,C}$ thereby effecting identification of the major tautomer. This is based on the

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(1) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.
(2) Sprang, T.; Bigler, P. *Magn. Reson. Chem.* **2004**, *42*, 55−60.
(3) For a review and comparative testing of pulse sequences designed for the quantification of $^{n}J_{H,C}$, see: Marquez, B. L.; Gerwick, W. H.; Williamson, R. T. *Magn. Reson. Chem.* **2001**, *39*, 499−530.
(4) Otting, G. *J. Magn. Reson.* **1997**, *124*, 503−505.

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premise that $^2J_{H,C}$ is quite usually negative in sign, though sometimes it is positive, but for such a condition it is very often small in magnitude (<5 Hz); for $^3J_{H,C}$, it is almost always positive, but when it is negative it is almost certainly small in magnitude (<3 Hz).[5] Thus, a large (greater than several Hz) negative coupling can be construed as being indicative of $^2J_{H,C}$ and a large positive coupling indicative of $^3J_{H,C}$.

For the system under study, as part of our broad examination[6] of the etheno adducts of purine-based systems, we had isolated, out of a number of compounds arising from the reaction of chloroacetaldehyde with 2,6-diaminopurine, one compound that we have unambiguously determined to have the linear structure indicated in Figure 1. The compound was

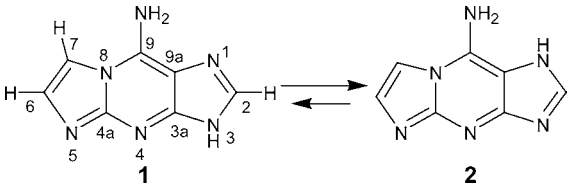

Figure 1. Equilibrium of the two tautomers observed for the etheno adduct under study. While the major tautomer is clearly only either the H-1 or the H-3 tautomer, the minor tautomer is only presumed to be the alternative. Conceivably, the equilibrium could also be between the H-1 (or H-3) and H-4 tautomers.

present in DMSO-d_6 solution as an equilibrium of two tautomers, possibly **1** and **2** (see Supporting Information for the ^{1}H NMR spectrum). One of these two tautomers, the major tautomer, was readily realized to be a tautomer where an N-bound H was positioned at either N-3 or N-1 (i.e., **1** or **2**, respectively). This determination was based on a number of experiments (^{1}H{^{13}C}-HSQC; ^{1}H{^{13}C}-HMBC, $^nJ_{H,C}$ = 8 and 2 Hz; ^{1}H{^{15}N}-HMQC; ^{1}H{^{15}N}-HMBC, $^nJ_{H,N}$ = 8 and 2 Hz, etc.), but what were not forthcoming were the identities of each tautomer as, other than NOEs between the etheno bridge protons (H-6 and H-7), $\eta_{H,H}$s were not observed at all (and these absences were also apparent for other similar structures also isolated as part of this work). Neither could a correlation from the NH proton of either tautomer to C-9 or N-4 be observed, which would otherwise potentially render the identification of the tautomers. It was of interest to us to determine the identity of at least the major tautomer, and since the NH signal for the major tautomer showed correlations to both C-3a and C-9a, it was necessary only to distinguish between a two-bond and a three-bond coupling to effect the structural distinction. For a ^{1}H{^{13}C}-HMBC experiment run with the long-range coupling optimized for 8 Hz, one of the relevant correlations for the major tautomer, that to C-9a (identifiable by its characteristic shielding), was clearly much larger than the other, that to C-3a, on the basis of the relative intensities of the correlations. This suggested to us that if we could determine the sign of the larger coupling, then this would lead to the identification of that tautomer, as a negative sign would implicate the coupling to be over two bonds and hence consistent with structure **2**, while a positive sign would implicate the coupling to be over three bonds and thereby infer structure **1**. The structure of the minor tautomer would then remain as either the alternative H-1, H-3, or even H-4 tautomer.[7] Presumably though, one of the former pairs is more likely, given that adenine is widely considered [8−10] to exist in solution predominantly as the H-7/H-9 tautomeric pair (equivalent to H-1/H-3 in this system), though contribution of the latter tautomer cannot be discounted, given that the presence of the H-3 tautomer for adenine (equivalent to H-4 in this system) in solution is strongly inferred.[8,10] It is also worth noting that any sign determination of the smaller couplings would be ineffectual with regard to tautomer identification, and consequently, contradiction cannot arise.

Accomplishment of this task was simply managed by standard magnitude-mode HMBC pulse sequence[11] acquired with high resolution in both dimensions (but with the low-pas *J* filter removed, i.e., the first 90° carbon pulse). During t1, due to the multiple-quantum state of the system, only the passive couplings (e.g., homonuclear ^{1}H−^{1}H coupling) evolve, whereas during t2, both the active (i.e., $J_{H,C}$) and passive couplings evolve. If the couplings have the same sign, then a positive tilt is imparted to the correlation; conversely, if they have opposite signs, a negative tilt is imparted.

(5) Kalinowski, H. O.; Berger, S.; Braun, S. *Carbon-13 NMR Spectroscopy*; Wiley: Chichester, 1988.

(6) (a) Mäki, J.; Klika, K. D.; Sjöholm, R.; Kronberg, L. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1216−1219. (b) Mäki, J.; Tähtinen, P.; Kronberg, L.; Klika, K. D. *J. Phys. Org. Chem.* DOI: 10.1002/poc.840. (c) Virta, P.; Holmström, T.; Roslund, M. U.; Mattjus, P.; Kronberg, L.; Sjöholm, R. *Org. Biomol. Chem.* **2004**, 821−827. (d) Virta, P.; Holmström, T.; Munter, T.; Nyholm, T.; Kronberg, L.; Sjöholm, R. *Nucleosides Nucleotides Nucl.* **2003**, *22*, 85−97.

(7) For the minor tautomer, only a correlation for the NH proton to C-3a was observed, a correlation consistent with either the H-3 or the H-4 tautomer. Needless to say, due to the rate of exchange (still sufficiently fast at 18 °C in DMSO-d_6), scalar coupling was not evident between the NHs and H-2 in either tautomer. Indeed, the intermediate rate of exchange precludes the observation of even the coupling between H-6 and H-7, though this coupling has been observed for other samples of this compound where the rate of exchange is fast and the two tautomers are not observed as separate sets of signals, other isomeric structures within this study, and similar adducts in a previous study.[6c] Typically the magnitude of this vicinal coupling, when observable, is ca. 1.5 Hz, appropriate for vicinal protons within an imidazole ring.

(8) Laxer, A.; Major, D. T.; Gottlieb, H. E.; Fischer, B. *J. Org. Chem.* **2001**, *66*, 5463−5481.

(9) Wilson, R. W.; Callis, P. R. *Photochem. Photobiol.* **1980**, *31*, 323−327.

(10) Mishra, S. K.; Shukla, M. K.; Mishra, P. C. *Spectrochim. Acta A* **2000**, *56*, 1355−1384.

(11) (a) Müller, L. *J. Am. Chem. Soc.* **1979**, *101*, 4481−4484. (b) Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Magn. Reson.* **1983**, *55*, 301−315.

(12) **Experimental Procedures.** NMR experiments were performed at 14.1 T using an NMR spectrometer equipped with a *z*-axis field gradient 5 mm inverse broadband probe operating at 600.13, 150.92, and 60.81 MHz for ^{1}H, ^{13}C, and ^{15}N, respectively. Spectral widths for the two-dimensional experiments were optimized from the one-dimensional spectra and acquired with an appropriate level of resolution: for PHOESY, this was the absolute minimum necessary to resolve separate signals in either dimension; for HMBC, the resolution was required to be less than the magnitude of the coupling constant in both dimensions. All two-dimensional spectra were acquired in magnitude-mode, and processing consisted of forward linear prediction, Q-sine functions, and appropriate exponential broadening applied in both dimensions. The length of the mixing time was set to 1 s for the PHOESY experiments.

The sequence was first tested[12] on a sample of acetaldehyde, which conveniently has two long-range $J_{H,C}$ couplings,[5] one of which is positive ($^2J_{H1,C2} = 26.2$ Hz) and the other which is negative ($^2J_{H2,C1} = -6.6$ Hz) with respect to the homonuclear vicinal coupling, which is assumed to be positive. The experimental observations using HMBC optimized on a $^nJ_{H,C}$ value of 8 Hz were in accordance with the reported values (see Figure 2), i.e., the correlation between

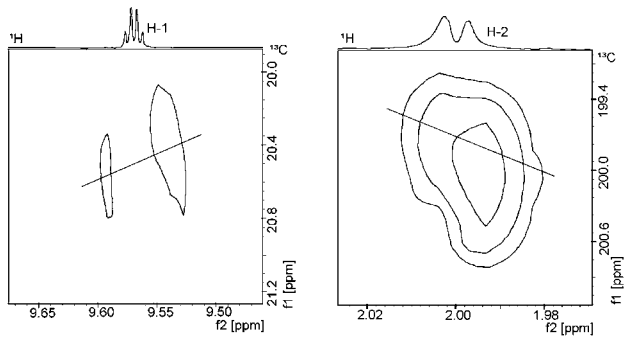

Figure 2. Correlations between H-1 and C-2 (left) and H-2 and C-1 (right), confirming the reported signs of these couplings, positive for the former and negative for the latter.

H-1 and C-2 displayed a positive tilt, while the correlation between H-2 and C-1 displayed a negative tilt.

For the etheno adduct at hand, an unresolved coupling exists between H-2 and the NH proton when it is located on either N-1 or N-3, thus rendering the method amenable to this system. Depicted in Figure 3a for the sample of the etheno adduct (5 mg, 3:1 ratio of tautomers) is the correlation between the NH proton of the major tautomer and C-9a obtained using this methodology (the HMBC experiment was again optimized on a $^nJ_{H,C}$ value of 8 Hz). The negative sign of the coupling (relative to the $J_{H2,NH}$ coupling, which is assumed to be positive) responsible for this $J_{H,C}$ correlation confers a negative tilt to the correlation. Thus, the coupling is considered to extend over only two bonds, and the structure of the major tautomer should thus be **2**. It should be noted that the less than clear result, though unmistakable, is only a consequence of the broad width of the NH signal (ca. 9 Hz) that all but masks the tilt. For comparison, the correlation of the NH proton to C-2 is also shown (Figure 3b), and it too has a negative tilt arising from a negative value of the coupling constant, as one would expect for a two-bond coupling. The correlation of the NH proton to C-3a is also displayed in Figure 3c, and though its tilt is positive as one might expect, this is structurally uninformative, as the small magnitude implies that there is some possibility of the coupling happening to take on a negative value.

To confirm these results, the sample was also subjected to PFG-enhanced inverse-detected HOESY[13,14] (hereafter referred to as PHOESY), a so-far under-utilized methodology for structure elucidation quite probably due to its very low sensitivity for systems involving a rare spin despite the use of proton detection. Correlations for the NH proton of the major tautomer were observed in this experiment[12] using the version presented in ref 14 to both C-2 and, importantly, C-9a, thereby reaffirming the results of the sign determination.

The tautomeric ratio thus appears to be in stark contrast to the case of adenine where it is the H-9 tautomer that dominates in DMSO solution,[8-10] and the other NH signal present in the ^{1}H NMR spectrum of adenine is assigned to the H-7 tautomer. In adenine, the NH proton of the major tautomer is deshielded in comparison to its counterpart in the minor tautomer. In the case of the two tautomers in this study, H-1 of the major tautomer is shielded in comparison

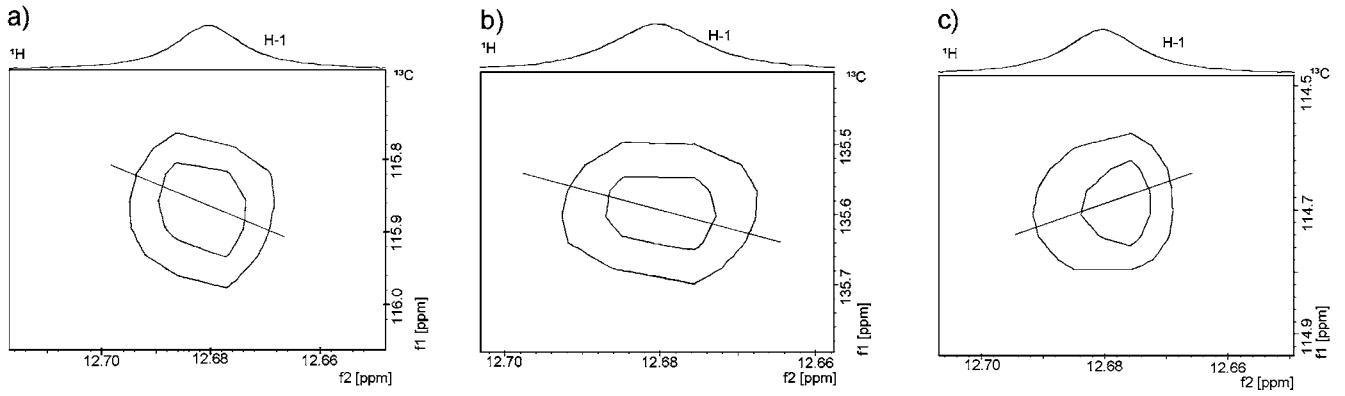

Figure 3. Correlations between the NH proton of the major tautomer and C-9a (a), C-2 (b), and C-3a (c). The negative tilts for a and b arise from the negative values of the coupling constants that exist between the NH proton and both C-9a and C-2 (as expected for two-bond couplings). Conversely, the positive tilt for the correlation between the NH proton and C-3a arises from the positive value of this coupling, as is typical for three-bond couplings, though structurally this is not informative given the small magnitude of the coupling. The delay for the evolution of $J_{H,C}$ was optimized for 8 Hz.

to the NH signal from the minor tautomer, and it was this observation that initially led to the suspicion of a tautomeric shift.

It is worth emphasizing the one caveat regarding HMBC, the need for high resolution in both dimensions to reliably observe the tilt. This should be of the order of the expected magnitude of $J_{H,C}$ (in principle, less than) in both dimensions. This high demand for resolution can lead to extended acquisition times due both to the sequential nature of f1 acquisition and the need to regain detrimental loss of S/N by a substantial increase in the number of scans. The former aspect, though, can be time-managed by the judicious contraction of f1 even to the point of multiple folding of the signals, as invariably sign determination will likely only be of interest for a few, or even a sole, coupling(s) present in the molecule. Both aspects can also be effectively combated by the application of an appropriate amount of forward linear prediction in both dimensions. Thus, as an alternative it more than effectively competes with PHOESY and represents a viable methodology for distinguishing between $^2J_{H,C}$ and $^3J_{H,C}$, which is important as this can aid or even be instrumental in structural determinations, as was the case here.

Acknowledgment. Prof. Todd Alam is kindly thanked for supplying the code for an even more improved version of the PHOESY pulse sequence,[14] which yields better results for ^{1}H{^{7}Li} studies[15] but is seemingly indifferent for ^{1}H-{^{13}C} studies, at least for the one examination performed on the test sample (10% ethylbenzene in $CDCl_3$) in this work. This pulse sequence and its forerunner[14] are available in code form (Bruker Avance series) in Supporting Information. Financial support from the Finnish Graduate School of Bioorganic and Medicinal Chemistry (P. V.) is gratefully acknowledged.

Supporting Information Available: Proton spectrum of the tautomers **1** and **2** in DMSO-d_6 at 18 °C; ^{1}H, ^{13}C, and ^{15}N chemical shifts for both tautomers; a pictorial representation of the HMBC pulse sequence; and the program codes (Bruker Avance series) for the pulse sequences described herein. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Bauer, W. *Magn. Reson. Chem.* **1996**, *34*, 532−537.
(14) Alam, T. M.; Pedrotty, D. M. Boyle, T. J. *Magn. Reson. Chem.* **2002**, *40*, 361−365.
(15) Alam, T. M. Personal communication.