# $^{13}C$ NMR Investigation of Tautomeric and Acid-Base Equilibria of Pyrazolone T and Three Analogs



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Pyrazolone T and three derivatives have been characterized by $^{13}C$ and, in part, $^{15}N$ nmr at several *p*H values. The $^{13}C$ chemical shifts have been assigned at, or near, the equivalence points and p$K_a$ values of these four compounds. Closely situatued quaternary carbon signals were assigned by means of a heteronuclear chemical shift correlation (FLOCK) experiment which is sensitive to, and was optimized for, 3-bond C-H couplings. The $^{13}C$ chemical shift data indicate the existence of both tautomeric and acid-base equilibria and demonstrate that the four congeners exist in surprisingly different forms at certain common *p*H values.

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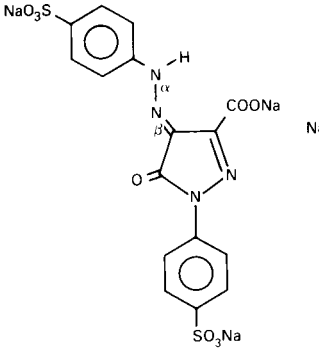
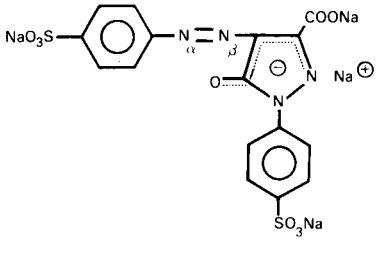
Although pyrazolone compounds have been the subject of considerable research concerning their tautomerism in a variety of organic solvent systems [1,2], their water-soluble analogs have received very little attention. This neglect is not surprising considering that much of the characterization of pyrazolone tautomers has been by ir spectroscopy, for which aqueous solutions pose analytical problems. We recently investigated the azo-hydrazone tautomerism and acid-base equilibria of FD&C Yellow No. 5 (**1**) by $^{15}N$ nmr and found it to exist predominantly in the hydrazone form below *p*H 9, as shown in the *anti*-configuration, **1a**, and almost exclusively as an anion with a predominantly azo-type structure, **1b**, above *p*H 11 [3].



**1a**



**1b**

We examined pyrazolone T (PYT, **2**), the precursor (dye intermediate) of **1**, to assist in its further characterization, *e.g.*, the identification of *syn*- and/or *anti*-hydrazone isomers below *p*H 9. However, PYT itself occurs in three *p*H-dependent tautomeric/acid-base forms, *viz.*, NH-keto, enol and trianionic species, structures **2a**, **2b** and **2c**, respectively [4-6]. Therefore, three additional pyrazolones were investigated to assist in the characterization of PYT: its 3-methyl (MePY, **3**), 3-carbethoxy (CEPY, **4**) and unsulfonated (UPY, **5**) derivatives. The $^{13}C$ nmr spectra of the four pyrazolones were recorded at certain *p*H values, and critical chemical shifts were assigned. These assignments then permitted the major forms in which the above congeners exist to be determined in acidic, neutral and basic solution.

## Results and Discussion.

As previously reported for **1** [3], the *p*H values at which the $^{13}C$ nmr spectra were recorded were selected on the basis of titration results and changes in the uv-visible spectra obtained at various *p*H values. PYT exhibits equivalence points at *p*H 4.5 and 9 (Table 1) and has a p$K_a$ value of 6.0 (carboxylate and sulfonate groups were not titrated). These data, together with those from uv-visible spectra, indicate that PYT exists as a dianion below *p*H 4.5 and as a trianion above *p*H 9. The $^{13}C$ nmr spectra showed broadening of most signals between *p*H 2.8 and 3.8, demonstrating the existence of an intermediate (on the nmr time scale) equilibrium process [7], probably NH-OH tautomeric interconversion, **2a-2b** [5]. These experimental results suggested that the $^{13}C$ nmr spectra of PYT be determined at, or near, the equivalence points and away from the p$K_a$ value of 6.0 to avoid multiple sets of resonance lines arising from differently charged species and at *p*H values on either side of the *p*H range in which $^{13}C$ signal broadening occurs. Accordingly, the following *p*H values were selected: *p*H 2.2, below which signals have started to broaden; *p*H 4.5, which is the first equivalence point and where the $^{13}C$ resonances have become sharp

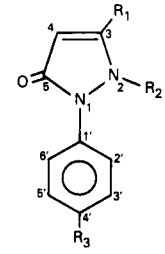
again; and *p*H 7 and 12 because of their importance in the characterization of pyrazolone dyes.

Table 1
Titration Results

| Compound | p$k_a$ | Equivalence Points |
|---|---|---|
| PYT | 6.0 | 4.5, 9.0 |
| UPY | 6.2 | 4.5, 9.0 |
| MePY | 6.8 | 4.4, 9.5 |
| CEPY | 4.7 | 3.3, 8.3 |

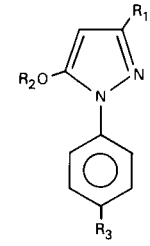
An investigation by Feeney and coworkers of a series of 1-phenyl-5-pyrazolones [8] was critical in determining the nature of the predominant tautomeric and acid-base forms in which PYT and its analogs exist at various *p*H values. They demonstrated that **6-8** occur in the enol form in dimethyl sulfoxide-$d_6$, whereas UPY and **9** are present in the NH-keto form. Their arguments are greatly aided by the fact that **8** and **9** cannot exhibit tautomerism and are thus "fixed" in the enol, **8b**, and $NCH_3$-keto, **9a**, forms, respectively. The chemical shift of C-5 is central to the issue of the forms in which pyrazolones exist. Feeney's studies have shown that the chemical shift of C-5 is expected to be *ca.* 155 ppm for pyrazolones in the enol form and *ca.* 165 ppm for those in the NH-keto form [8]. A complicating matter in the present investigation is that the chemical shift of an aromatic carbinol carbon is deshielded by *ca.* 10 ppm on conversion to the conjugate base, *e.g.*, phenol to the phenolate anion [9]. For this reason, protonated NH-keto species cannot be distinguished from their enolate conjugate bases on the basis of the value of the chemical shift of C-5. Consequently, examination of the pyrazolones was begun in acid solution, far below the p$K_a$ values, to avoid such ambiguities.

In strong acid solution, PYT (*p*H 2.2) and CEPY (*p*H 3.3) display $^{13}C$ chemical shifts which are very similar to



**2a** $R_1$ = COONa $R_2$ = H $R_3$ = $SO_3Na$
**3a** $R_1$ = $CH_3$ $R_2$ = H $R_3$ = $SO_3Na$
**5a** $R_1$ = COONa $R_2$ = H $R_3$ = H
**9a** $R_1$ = $CH_3$ $R_2$ = $CH_3$ $R_3$ = H

those reported for **6b** [8]. With the signals assigned to C-5 occurring at, or very near, 155 ppm (Table 2), both compounds are believed to exist as enol tautomers, **2b** and **4b**. As the solution *p*H of CEPY is raised through its p$K_a$ value of 4.7 to *p*H 7, the only significant change in its $^{13}C$ nmr spectrum is a downfield shift of C-5 to 163.8 ppm. This is consistent with deprotonation of an enol to produce primarily the enolate anion **4c** [9]. The $^{13}C$ nmr spectra of PYT, however, exhibit very different behavior with increasing *p*H. As the solution *p*H of PYT is increased



**2b** $R_1$ = COONa $R_2$ = H $R_3$ = $SO_3Na$
**4b** $R_1$ = $COOC_2H_5$ $R_2$ = H $R_3$ = $SO_3Na$
**6b** $R_1$ = $COOC_2H_5$ $R_2$ = H $R_3$ = H
**7b** $R_1$ = $CH_3$ $R_2$ = H $R_3$ = H
**8b** $R_1$ = $CH_3$ $R_2$ = $C_2H_5$ $R_3$ = H

Table 2
$^{13}C$ and $^{15}N$ Chemical Shifts of PYT, UPY and CEPY at Selected *p*H Values [a]

| | PYT | | | | UPY | | CEPY | |
|---|---|---|---|---|---|---|---|---|
| Position | *p*H 2.2 | *p*H 4.5 | *p*H 7 | *p*H 12 | *p*H 4.4 | *p*H 7 | *p*H 3.3 | *p*H 7 |
| 1 | -[b] | -[b] | -[b] | -175 | -[b] | -[b] | -[b] | -171 |
| 2 | -[b] | -[b] | -[b] | -115 | -[b] | -[b] | -[b] | -113 |
| 3 | 143.9 | 148.3 | 150.5 | 150.5 | 149.4 | 148.8 | 143.6 | 144.1 |
| 4 | 92 | 93 | 88 | 88 | -[c] | -[c] | -[c] | -[c] |
| 5 | 155.0 | 161.1 | 164.0 | 164.1 | 163.2 | 162.8 | 154.8 | 163.8 |
| 6 | 165.7 | 167.6 | 171.7 | 171.8 | 171.6 | 170.4 | 164.2 | 165.6 |
| 1' | 140.0 | 139.2 | 140.2 | 140.2 | 139.7 | 138.8 | 140.1 | 140.9 |
| 2'/6' | 123.9 | 124.1 [d] | 123.2 | 123.2 | 124.6 | 124.6 | 123.8 | 123.7 |
| 3'/5' | 127.5 | 127.6 [d] | 127.2 | 127.3 | 129.9 | 129.9 | 127.4 | 127.2 |
| 4' | 142.5 | 142.1 | 142.5 | 142.6 | 127.6 | 127.9 | 142.4 | 141.9 |

[a] $^{13}C$ chemical shifts referenced to dioxane at 67.4 ppm; $^{15}N$ chemical shifts referenced to the $^{15}NO_3^-$ resonance at 0 ppm. [b] Not determined at these *p*H values. [c] Not observed. [d] H-2'/6':7.56 ppm, H-3'/5':7.72 ppm; referenced to dioxane at 3.70 ppm.

from *ca.* 2.8 to 3.2, its $^{13}C$ signals broaden and generally move to lower field. With continued increasing *p*H from *ca.* 3.2 to 3.8, these signals sharpen and continue their downfield movement. This dynamic nmr behavior is indicative of tautomeric interconversion (see above) [5,7]. As the solution *p*H is increased, PYT converts from the enol, **2b**, which predominates in strong acid, to the NH-keto tautomer, **2a**, at *p*H 4.5.

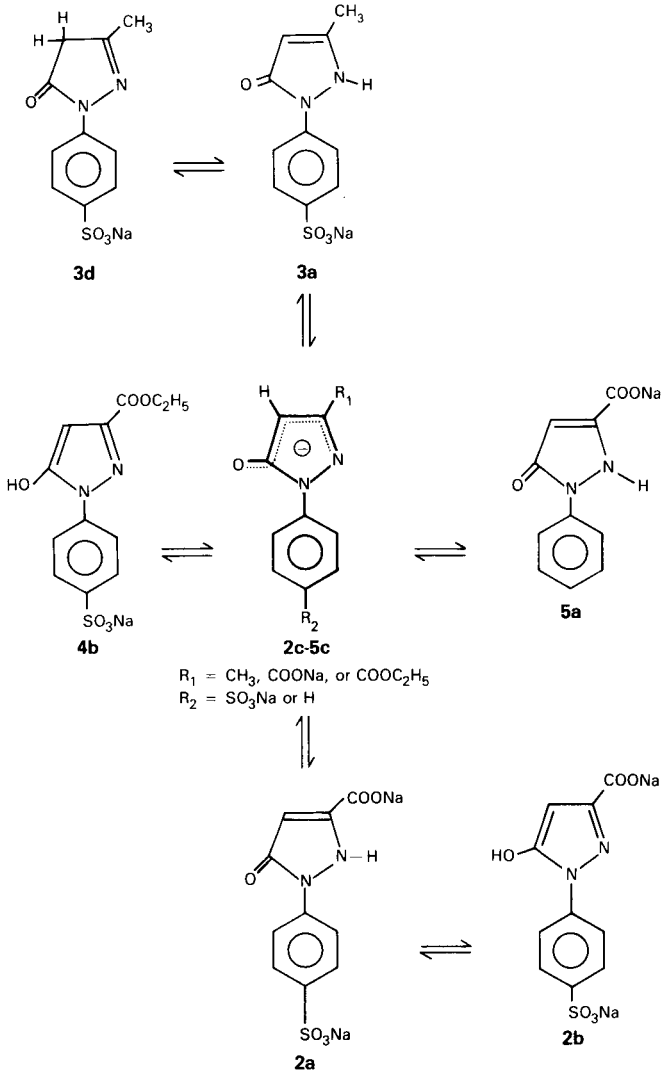
An investigation of **9**, together with present studies of MePY, provides further information concerning the identity of the *p*H 4.5 tautomer of PYT. Feeney *et al.* have demonstrated that **9** is fixed in the NH-keto form, **9a**, in dimethyl sulfoxide-$d_6$ [8]; at *p*H 4.5, MePY displays $^{13}C$ resonances which are very similar to those of **9a**. Moreover, two primary tautomers are postulated for MePY in acid solution: the NH-keto, **3a**, and CH-keto, **3d**, species [5]. Unlike **2a** and **2b**, however, they interconvert slowly enough on the nmr time scale to be observed as separate entities (in the ratio of 3:1, respectively) at *p*H 4.5. The distinctive chemical shift of the 4-methylene carbon at *ca.* 40 ppm clearly identifies **3d** as the minor component. Its non-conjugated carbonyl carbon (C-5) appears at 174.5 ppm at *p*H 4.5, whereas that of **3a** is found at 164.8 ppm. The similarity of the latter chemical shift to that of PYT at 161.1 ppm at *p*H 4.5 implies that PYT is likewise present in the NH-keto form, **2a**, at this *p*H value.

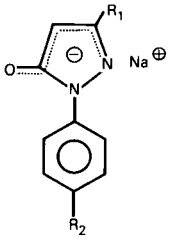
As the solution *p*H of MePY is increased from 4.5 to 7, the equilibrium for the protonated species shifts farther in favor of **3a**, whereas the $^{13}C$ chemical shift values remain essentially constant. However, the $^{13}C$ resonances observed for PYT at *p*H 4.5 and 7 are sensitive to this change in *p*H. These differences may arise because the chemical shifts of PYT were obtained for the dianion **2a** at its first equivalence point (*p*H 4.5) and also at one *p*H unit above the $pK_a$ of 6.0, where PYT exists largely as the trianion **2c**.

When the solution *p*H is increased to the second equivalence points (*p*H 8-9), PYT, MePY, CEPY and UPY apparently occur in a common polyanionic form, **2c-5c** (Scheme). This contention is supported by $^{15}N$ nmr data which were obtained for PYT and CEPY at *p*H 10 and 7, respectively, and which are essentially identical. The N-1 chemical shifts for **2c** and **4c** are -175 and -171 ppm, respectively, whereas those of N-2 are -115 and -113 ppm, respectively. These resonances are similar to those reported by El Khadem and Coxon for the pyrazolone nitrogens of certain dehydro-L-ascorbic bisphenylhydrazones: -184.2 (N-1) and -85.2 ppm (N-2) [10].

The $^{13}C$ signals of the pyrazolones were assigned in the manner described below. The following range of $^{13}C$ chemical shifts has been observed for C-5: *ca.* 155 ppm for enol species (*e.g.*, **2b**, **4b** at low *p*H) and *ca.* 165 ppm for NH-keto entities (*e.g.*, **2a**, **3a** at *p*H 4.5) and mixtures of these NH-keto species and their conjugate bases (*e.g.*, **2c**, **3c**) at *p*H 7. The other downfield signals appearing at 164-172 ppm from *p*H 2.2 to 12 were consequently ascribed to the remaining oxygen-bearing carbon (C-6). In addition, very weak resonances occurring as quintets at 88-93 ppm were assigned to C-4. This carbon is hightly acidic [6,11], and its protons undergo rapid exchange in deuterium oxide.

**SCHEME**



**2c** $R_1$ = COONa $R_2$ = $SO_3Na$
**3c** $R_1$ = $CH_3$ $R_2$ = $SO_3Na$
**4c** $R_1$ = $COOC_2H_5$ $R_2$ = $SO_3Na$
**5c** $R_1$ = COONa $R_2$ = H

Assignment of the signals due to C-3 and the sulfophenyl carbons is incidental to questions concerning the primary tautomeric and acid-base form(s) in which the above pyrazolones exist at various *p*H values. In addition, the designation of resonances to specific quaternary or methine carbons is not entirely definitive. This ambiguity derives from the similarity of the chemical shifts of carbons 3, 1′ and 4′, carbons 2′/6′ and 3′/5′, and protons 2′/6′ and 3′/5′ of PYT and CEPY (Table 2) and MePY (Table 3). A heteronuclear chemical shift correlation experiment showed that the downfield protons of PYT (7.72 ppm at *p*H 4.5) are directly coupled to the downfield carbons at 127.6 ppm, but neither the 2′/6′ nor the 3′/5′ signals, for either protons or carbons, can be unequivocally assigned. The unsulfonated analog of PYT (UPY) was used to differentiate signals arising from the sulfophenyl methine carbons at *p*H 4.5, where both pyrazolones exist predominantly as NH-keto tautomers, **2a** and **5a**. The *ortho*-substituent constant for the sulfonate group was previously determined to be -2.3 ppm [12]. Addition of this quantity to the chemical shifts of both methine carbons of UPY (124.6 and 129.9 ppm at *p*H 4.5) yielded a calculated *ortho*-chemical shift of either 122.3 or 127.6 ppm for C-3′/5′ of PYT. The observed methine-carbon shifts for PYT are 124.1 and 127.6 ppm at 4.5 (Table 2). The latter agrees very well with the more deshielded, calculated chemical shift (127.6 ppm), and C-3′/5′ was ascribed this value at *p*H 4.5.

Table 3

$^{13}$C Chemical Shifts of MePY [a]

| | *p*H 4.5 | | *p*H 7 | | *p*H 12 |
|---|---|---|---|---|---|
| Position | **3a** | **3d** | **3a/3c** | **3c/3d** | **3c** |
| 3 | 152.1 | 162.8 | 152.2 | 162.8 | 153.6 |
| 4 | 94 | 40 | 94 | 40 | 88 |
| 5 | 164.8 | 174.5 | 164.7 | 174.5 | 164.9 |
| 6 | 12.3 | 16.6 | 12.5 | 16.6 | 14.4 |
| 1′ | 138.1 | 139.6 | 138.6 | 139.6 | 138.9 |
| 2′/6′ | 122.4 | 120.8 | 122.1 | 121.4 | 121.8 |
| 3′/5′ | 127.6 | 127.3 | 127.5 | 127.3 | 127.3 |
| 4′ | 141.4 | 140.5 | 141.1 | 141.0 | 142.8 |

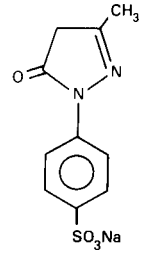
[a] Referenced to dioxane at 67.4 ppm.

With the downfield carbon signal thus assigned to C-3′/5′, the downfield proton resonance then belongs to H-3′/5′ (see above). An indirectly bonded heteronuclear chemical shift correlation experiment (FLOCK) [13] was next used to correlate the respective aromatic protons with quaternary carbons to which they are coupled through three bonds. This experiment is also sensitive to 2-bond C-H couplings. However, these tend to be very small in most aromatic systems [14,15], and none were observed for PYT. This experiment demonstrated that H-3′/5′ are coupled to the carbon at 139.2 ppm (C-1′) and H-2′/6′, to C-4′ at 142.1 ppm. The remaining signal at 148.3 ppm was then assigned to C-3 (Table 2, *p*H 4.5). The chemical shifts of the three PYT analogs at the acidic, neutral and basic *p*H conditions employed, in addition to those of PYT at *p*H 2.2 and 12, are generally similar to the shift values of PYT at *p*H 4.5 (discussed above) and have been assigned accordingly (Tables 2 and 3).

Conclusion.

Although the four pyrazolones in this study have similar structures, they exhibit markedly different behavior as their solution *p*H is raised from *ca.* 2 to 7 (Scheme). PYT exists primarily as a dianionic enol, **2b**, at *p*H values below 2.8. Between *p*H 2.8 and 3.8 it interconverts with the NH-keto form, **2a**, at an intermediate rate on the nmr time scale. At the first equivalence point of *p*H 4.5, it is predominantly in the dianionic NH-keto form, **2a**. As the *p*H is further increased, removal of the N-2 proton occurs as the p$K_a$ is reached at *p*H 6. Conversion to the trianion **2c** continues with increasing *p*H until the second equivalence point is reached at *p*H 9.

MePY exhibits analogous behavior except that the tautomeric interconversion is of the CH-NH variety (rather than the NH-OH type of PYT), which is sufficiently slow on the nmr time scale that both tautomers are observed [5,7]. At the first eqivalence point at *p*H 4.5, the NH-keto, **3a**, and CH-keto, **3d**, forms are present in a 3:1 ratio, and both are monoanions. As the *p*H is raised, the equilibrium shifts in favor of **3a**. Removal of the N-2 proton of **3a** and the C-4 protons of **3d** occurs until only trace signals of **3d** and its conjugate remain at the p$K_a$ of 6.8. Conversion to the dianion **3c** continues with increasing *p*H until the second equivalence point is reached at *p*H 9.5.




**3d**

CEPY exists only as a monoanionic enol, **4b**, below its first equivalence point at *p*H 3.3. Above this *p*H value, removal of the C-5 hydroxyl proton occurs until the p$K_a$ is reached at *p*H 4.7. Conversion to the common dianionic form **4c** is essentially complete at *p*H 7 with hydrolysis of the ester group occurring in basic solution.

## EXPERIMENTAL

4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-1*H*-pyrazole-3-carboxylic acid, sodium salt (pyrazolone T, PYT, Hilton-Davis), 3-methyl-

pyrazolone T (MePY, Pfaltz and Bauer), 3-carbethoxypyrazolone T (CEPY, Stange) and the unsulfonated analog of PYT (UPY, Pfaltz and Bauer) were used without further purification. Test portions (300 mg) of these compounds were titrated with 0.1 *N* hydrochloric acid by using a Radiometer RT 5622 automatic titrator. Starting solution *p*H values ranged from 11 to 12; final values were 2-3. The $^{13}C$ nmr spectra of compounds, in deuterium oxide at various *p*H values were recorded at 20 MHz, on a Varian Associates FT-80A spectrometer. Proton-decoupled spectra, described by 4096 data points (real part), were obtained with broad-band irradiation at 80 MHz. Single-frequency off-resonance decoupled spectra were obtained with proton-decoupling frequencies set at *ca.* -5 ppm and at full decoupling power levels. Pulse widths of 10 microseconds were employed, which correspond to tip angles of 45° with 10-mm sample tubes. Spectral widths of 4 kHz were used, corresponding to acquisition times of *ca.* 1 second. Dioxane was the internal standard. Chemical shifts are reported relative to TMS.

The $^{15}N$ nmr spectra of **2c** and **4c** in deuterium oxide were recorded at 40.6 MHz on a Varian Associates XL-400 spectrometer. Proton-decoupled spectra, described by 30,000 data points (real part), were obtained with gated (nOe-suppressed) broad-band irradiation at 400 MHz. Pulse widths of 7 microseconds were employed, which correspond to tip angles of 30° with 10-mm sample tubes. Spectral widths of 16 kHz were used, corresponding to acquisition times of *ca.* 1.88 seconds. Pulse delay times of 4 seconds were also employed for nOe suppression. Aqueous, saturated ammonium [$^{15}N$] nitrate solution was the external standard. Chemical shifts are reported relative to the $^{15}NO_3$-resonance.

Directly bonded, heteronuclear chemical shift correlation nmr spectra were obtained at 100.6 MHz (XL-400) with spectral widths of 603.5 and 160 Hz in the carbon and proton dimensions, respectively, and with 256 data points in the $^{13}C$ dimension. Sixty-four incremented $^{13}C$ spectra of 32 scans each were acquired using 11.5-microsecond (90°) $^{13}C$ and 17-microsecond (90°) $^{1}H$ pulse widths and a 1-second repetition rate. Free-induction decays in both dimensions were processed as a 128 x 512 matrix with appropriate zero filling and modified pseudo-echo weighting. A value of $^{1}J(CH) = 165$ Hz was employed for calculating the delays $\Delta_1$ and $\Delta_2$.

FLOCK nmr spectra were recorded at 100.6 MHz (XL-400) with spectral widths of 4773 and 1520 Hz in the carbon and proton dimensions, respectively, and 1024 data points in the $^{13}C$ dimension; 192 incremental $^{13}C$ spectra of 256 scans each were acquired using a 1-second repetition rate. Free-induction decays in both dimensions were processed as a 512 x 2048 matrix with appropriate zero filling and modified pseudo-echo weighting. A value of $^{n}J(CH) = 7.5$ Hz was used for calculating the delays $\Delta_1$ and $\Delta_2$, and $^{1}J(CH) = 165$ Hz was used for $\tau$ in the bilinear rotation decoupling (BIRD) [16] pulses.

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