Solid-State NMR and Density Functional Theory Studies of Ionization States of Thiamin

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Supporting Information

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EXPERIMENTS AND METHODS

Materials. All chemicals were purchased from Sigma-Aldrich (St.Louis, MO). All the chemicals purchased were highest purity grade available and were used without any further purification unless otherwise specified. Anhydrous solvents were either purchased from Acros Organics USA (Morris Plains, NJ) in anhydrous grade or were dried according to literature procedures.[27] ^{15}N-NH_4Cl (99.9%) was purchased from Cambridge Isotope Laboratories, Inc (Andover, MA). 3-Chloro-4-oxopentyl acetate, a gift from Hoffmann-La Roche Inc (Nutley, NJ) was purified immediately prior to use by distillation under reduced pressure (0.1 mm Hg) at 85 °C.

Synthesis of [N4' – ^{15}N] thiamin chloride hydrochloride. Compounds I, II & III (ref. Scheme S1) were synthesized with some minor modifications to the previously described methods.[29] Compound IV was synthesized according to a method described by Uray et al.[30]; and compounds V, VI & VII were synthesized with minor modifications to the method described by Contant et al.[31] and Nicewonger et a.l[32]

4-Hydroxy-5-cyano-2-methylpyrimidine (I) To a solution of sodium ethoxide (12.07 g, 177.5 mmol) in absolute ethanol (200 mL) acetamidine hydrochloride (16.75 g, 177.5 mmol) was added with stirring. The mixture was allowed to stir at 0 °C for 30 min. and the precipitate was filtered off. To the filtrate ethyl α-ethoxymethylene-α-cyanoacetate (10.0 g, 59.17 mmol) was added with stirring. After complete addition the mixture was allowed to stir 12 h at 0 °C. A crystalline precipitate formed and was filtered off. The precipitate (5 g) was treated with aqueous acetic acid (50% v/v, 10 mL) and the mixture was allowed to stir overnight. A solid (colorless needles) was formed which was filtered off and the filtrate evaporated to obtain a white solid, which could be crystallized into

colorless needles from either water or aqueous acetic acid. Yield (3 g., 30%). ^{1}H NMR (500 MHz, D_2O): δ 8.558 (s, 1H); 2.584 (s, 3H).

4-Chloro-5-cyano-2-methylpyrimidine (II) To compound I (2 g. 14.82 mmol) was added 8 mL of phosphorous oxychloride. The mixture was refluxed at 115 °C with vigorous stirring for 30 min or until the mixture turned a dark reddish brown. The stirring was stopped, the mixture was allowed to cool to RT and the excess phosphorous oxychloride was removed under reduced pressure (0.1 mm Hg). The remaining thick paste was taken into ice-cold water. The solution was neutralized with saturated potassium carbonate solution and the pH was adjusted to 10.0. Then the aqueous solution was extracted with diethyl ether (3x200 mL). The combined extracts were dried (anh. Na_2SO_4) and solvent was removed under reduced pressure. A reddish yellow oily liquid which solidifies upon cooling was obtained. Product could be recrystallized from petroleum ether. Yield (1.14 g, 50%). ^{1}H NMR (500 MHz, D_2O): δ 8.364 (s, 1H); 2.388 (s, 3H). MS (EI): m/z 153, 118, 91, 85, 51.

[4-^{15}N] 4-Amino-5-cyano-2-methylpyrimidine (III) To an ice-cold solution of sodium hydroxide (2.10 g, 52.5 mmol) in 3 mL water, ^{15}N-NH_4Cl (3 g, 55 mmol) was added in portions. After complete addition, the mixture was allowed to stir in an ice-bath with the reaction flask sealed. To this solution, a solution of compound II (0.500 g, 3.26 mmol) in ice-cold absolute ethanol (25 mL) was added drop-wise over 30 min with stirring. After complete addition, the mixture was stirred for another 60 min. The solvents were removed under reduced pressure and the residue was triturated with boiling methylene chloride (100 mL). The dry solid obtained could be recrystallized from anh. methanol.

Yield (0.352 g, 80%). ^{1}H NMR (500 MHz, d_6-DMSO): δ 8.499 (s, 1H); 7.756 (br. d, J = 89 Hz, 2H); 2.382 (s, 3H).

[4-^{15}N] 4-Amino-5-aminomethyl-2-methylpyrimidine (IV) To a solution of Raney-Nickel (250 mg.) in 20 mL dimethyl formamide (saturated with ammonia) was added compound III (0.400 g, 2.97 mmol). The yellowish-orange mixture was hydrogenated in a bomb reactor at 60 °C under 3 bar hydrogen gas pressure with vigorous stirring overnight. After completion, the catalyst was filtered out and the solvent from the filtrate was removed under reduced pressure (0.1 mm Hg). The yellowish precipitate obtained was sublimed at 115 °C under reduced pressure (0.1 mm Hg) to obtain a white powder. Yield (0.372 g., 90%). ^{1}H NMR (500 MHz, d_6-DMSO): δ 7.875 (s, 1H); 6.672 (d, J = 88.5 Hz, 2H); 3.523 (s, 2H); 2.277 (s, 3H); 1.891 (br. s, 2H).

3-Mercapto-4-oxopentyl acetate (V) To a suspension of NaSH (0.941 g, 16.8 mmol) in anh. methanol (7 mL) maintained at 0 °C, a solution of 3-chloro-4-oxopentyl acetate (3.0 g, 16.81 mmol) in anhydrous methanol (7 mL) was added drop-wise under N_2 protection. The temperature was maintained at 0-2 °C and the mixture was stirred vigorously during addition. After the addition was complete the reaction mixture was allowed to warm up to RT slowly and while stirring for 2.5 h. A white precipitate formed and the mixture was filtered under N_2 and the filtrate was evaporated. The oily residue was dissolved in anh CH_2Cl_2 and filtered again under N_2 to remove any insoluble material. The filtrate was evaporated under reduced pressure to yield a slightly yellow oil. Upon distillation under reduced pressure (0.1 mm Hg) at 90 °C a clear liquid was obtained. Yield (1.78 g, 60%).

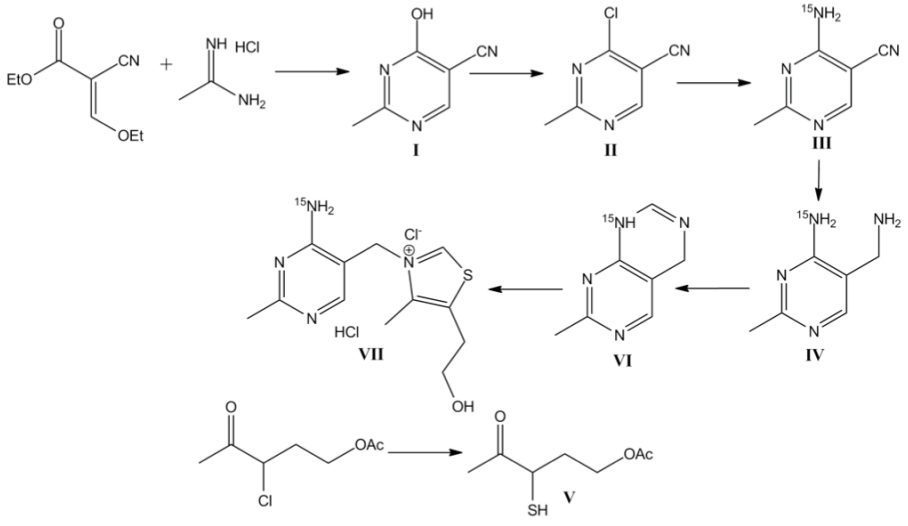
^{1}H NMR (500 MHz, $CDCl_3$): δ 4.201 (t, J = 6 Hz, 2H); 3.411 (dt, J = 7.5 Hz, 11 Hz, 1H); 2.332 (s, 3H); 2.270 (m, J = 6Hz, 7.5 Hz, 15 Hz, 1H); 2.033 (s, 3H); 1.937 (m, J = 6 Hz, 7.5 Hz, 15 Hz, 1H); 1.726 (d, J = 11 Hz, 1H). MS (EI): m/z 134, 116, 73, 43.

[1-^{15}N] 7-Methyl-1,4-dihydropyrimido [4,5-d]pyrimidine (VI) In a round bottom flask equipped with a vigreux column and a Hickman still head under N_2, compound IV (0.420 g, 3.04 mmol) and freshly distilled toluene sulfonicacid (10 mg) were placed. Anh triethyl orthoformate (1.2 mL, 7.3 mmol) was added. The mixture was heated at 110 °C with stirring, and the ethanol produced was distilled off until a thick white paste was observed. This was left to stand at 110 °C for an additional 45 min. Then 2 mL of anh toluene was added and the mixture was refluxed at 90 °C for 2.5 h. The solvent was evaporated and the residue was dried for one half h under reduced pressure (0.1 mm Hg). A sample was removed for NMR analysis. Yield (>90%). ^{1}H NMR (500 MHz, d_6-DMSO): δ 8.019 (s, 1H); 7.209 (s, 1H); 4.501 (s, 2H); 2.384 (s, 3H).

[N4' – ^{15}N] Thiamin chloride hydrochloride (VII) To compound VI in the same pot, anh formic acid (15 mL) was added. Compound V (0.558 g., 3.17 mmol) was added drop-wise and temperature was maintained below 35 °C. The mixture was stirred at RT under N_2 for 45 min. A freshly prepared solution of HCl in absolute ethanol (3 mL) was added slowly to the mixture. The mixture was stirred at RT for 45 min. The volatiles were removed under reduced pressure and the solid green residue was suspended in 5 mL absolute ethanol. 2 mL aqueous HCl (25 %) was added and the reaction mixture was slowly warmed until a clear solution was obtained. The solution was cooled to room temperature and then kept at 4 °C overnight for crystallization. The white crystals formed were collected by filtration and the filtrate was evaporated under reduced pressure. The

residue was dissolved in 2 mL distilled water and was washed with chloroform (3x4mL). The aqueous phase was evaporated under reduced pressure and the resultant dark green solid was recrystallized as above. The crystals were collected by filtration and dried in vacuo. Yield (0.830 g, 90%). ^{1}H NMR (500 MHz, d_6-DMSO): δ 9.930 (s, 1H); 9.106 (br. d, J = 91.5 Hz, 2H); 8.345 (s, 1H); 5.572 (s, 2H); 3.660 (t, J = 5.5 Hz, 2H); 3.071 (t, J = 5.5 Hz, 2H); 2.565 (s, 3H); 2.524 (s, 3H).

[N4'– ^{15}N] Thiamin To a suspension of [N4'– ^{15}N] thiamin chloride hydrochloride (0.020 g., 0.06 mmol) in 5 mL absolute ethanol under N_2 protection, triethyl amine (0.022 g., 0.20 mmol) was added drop-wise. The clear solution obtained was stirred for 10 min. To this solution anh diethyl ether was added and immediate precipitation was observed. The addition of diethyl ether was continued until no further precipitation was apparent. The precipitate was collected by filtration and washed with anh diethyl ether and dried in vacuo.



Scheme S1. Synthetic route to [N4' -^{15}N] thiamin chloride hydrochloride.

Relative Orientations of C6' and N4' CSA Tensors

The orientation of C6' CSA tensor in Th•HCl is such that the δ_{33} component lies perpendicular to the 4'-aminopyrimidine ring while both δ_{11} and δ_{22} lie in the plane of the ring. The δ_{11} component is inclined at an angle of 18-25° with the C6'-H bond vector. Interestingly, the orientation remains largely unchanged upon deprotonation with only the direction of δ_{11} moving closer to the C6'-H bond, by about 10°. The absence of the two chloride anions does not affect the orientation of the C6' CSA tensor, and each component retains the same position as with chloride anions (figure not shown).

The δ_{22} component of the N4' CSA tensor is perpendicular to the 4'-aminopyrimidine ring. The δ_{11} component is collinear with the C4'-N4' bond vector. Interestingly and similar to the C6' results, this orientation remains unchanged upon deprotonation. However, the absence of the chloride ions significantly affects the N4' CSA orientation. In the protonated form, the δ_{22} component moves away from the normal to the 4'-aminopyrimidine plane by about 50° with only δ_{11} lying in the 4'-aminopyrimidine plane. In thiamin, all three components change their orientation, with both δ_{11} and δ_{33} moving away from the plane by 13° and 10°, respectively, while δ_{22} moves away from the normal by 17°.

In summary, we observed no major differences in the orientation of the C6' and N4' CSA tensors in the two ionization states in the presence of chloride anions. The absence of chloride ions while having no impact on the ^{13}C tensors, affects the N4' CSA tensor resulting in different orientations of the principal components in the protonated and unprotonated forms.

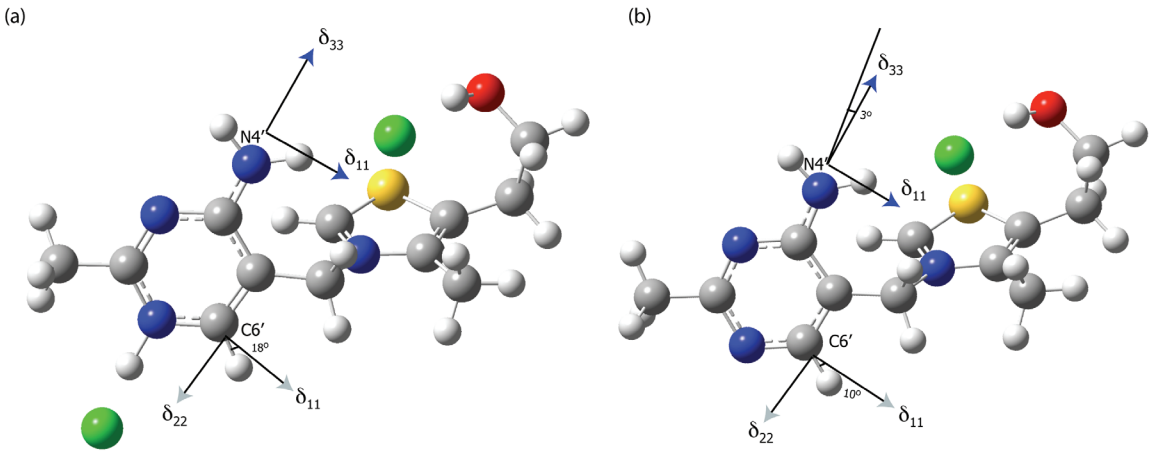
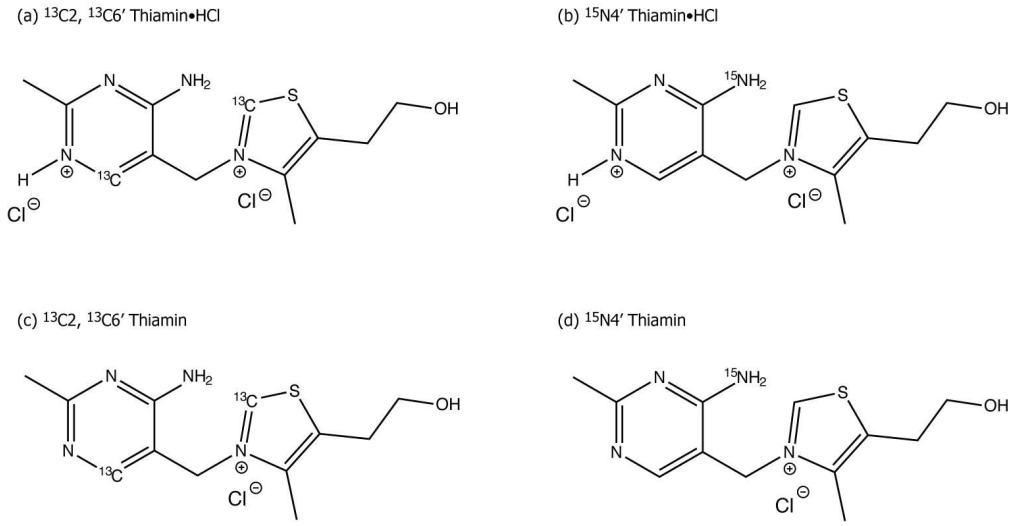


Figure S1. Structures of Th•HCl (a) and Th (b) used for DFT calculations and depicting orientations of C6' and N4' CSA tensors. For both atoms, the third principal component (δ_{33} for C6', δ_{22} for N4') is perpendicular to the 4'-aminopyrimidine plane.

Complete Gaussian citation:

[1] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, V.G. Zakrzewski, J.A.J. Montgomery, R.E. Stratmann, J.C. Burant, S. Dapprich, J.M. Millam, A.D. Daniels, K.N. Kudin, M.C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G.A. Petersson, P.Y. Ayala, Q. Cui, K. Morokuma, P. Salvador, J.J. Dannenberg, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J. Cioslowski, J.V. Ortiz, A.G. Baboul, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, J.L. Andres, C. Gonzalez, M. Head-Gordon, E.S. Replogle and J.A. Pople. Revision A.1***x*** ed., Gaussian, Inc., Pittsburgh PA 2001.



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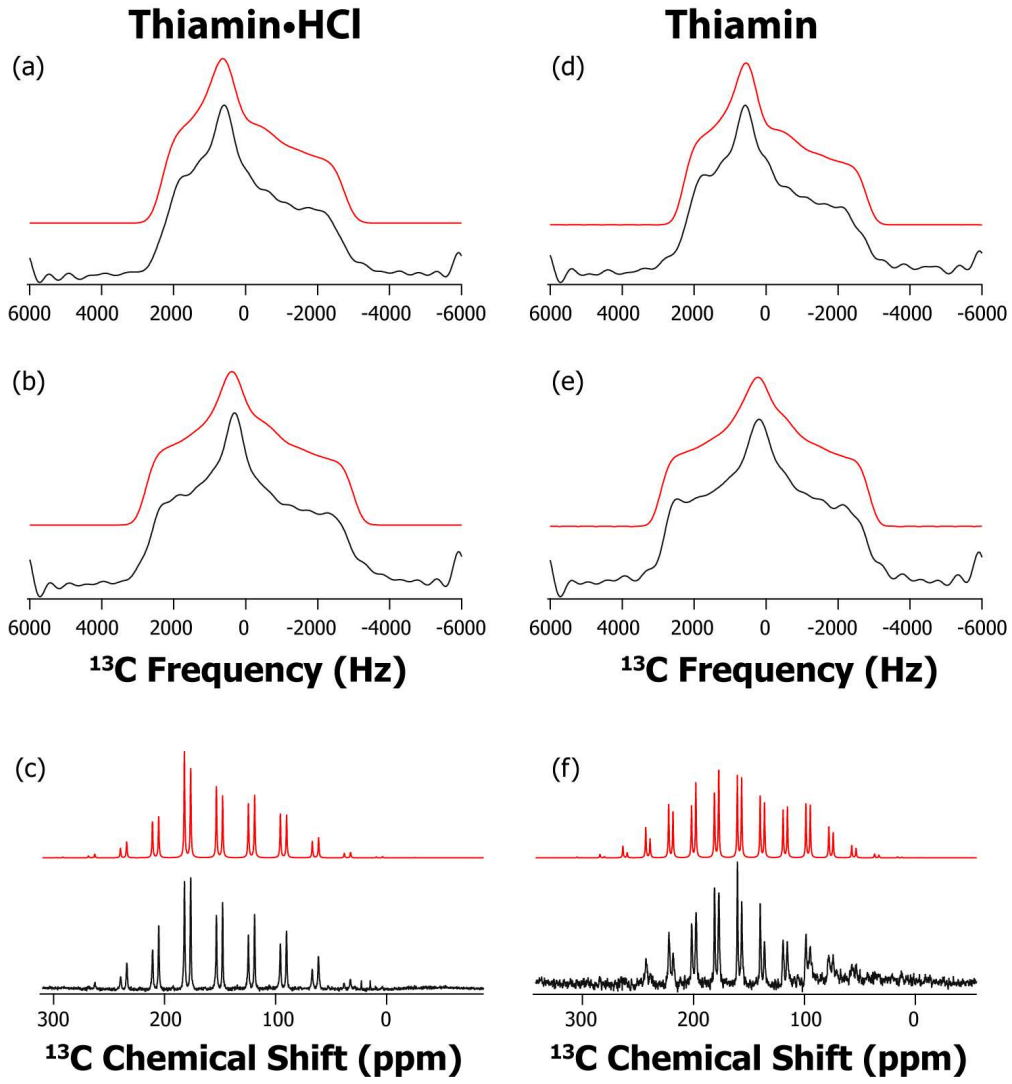

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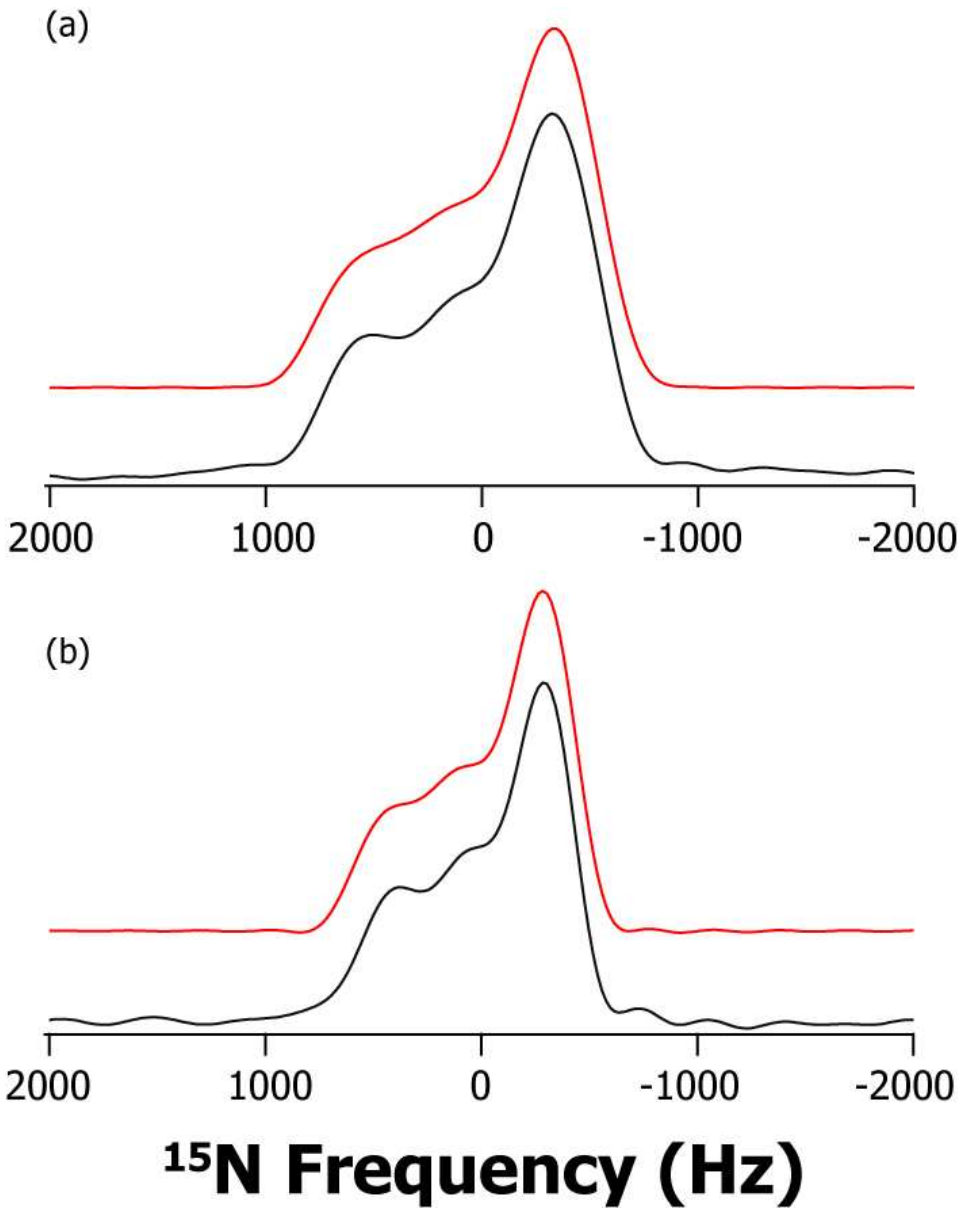

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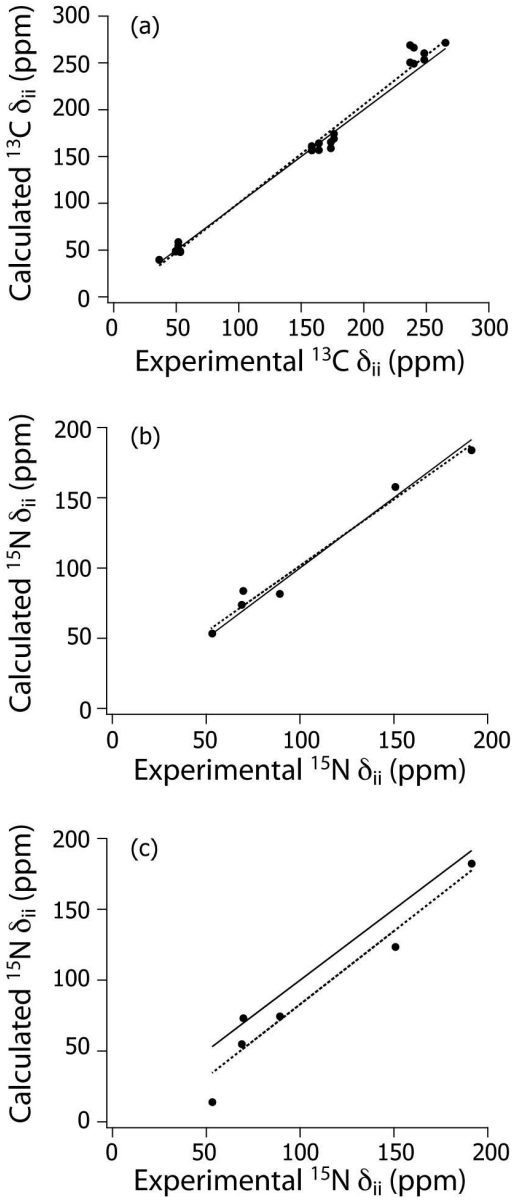

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