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New Journal of Chemistry

Electronic Supplementary Information

The role of hydrogen bonding in tuning CEST contrast efficiency: A comparative study of intra and inter molecular hydrogen bonding

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Figure S1.1: ¹H-NMR spectrum of *N N*'-(1,2-phenylene)diacetamide (1) in DMSO-D₆.



Figure S1.2: ¹³C NMR spectrum of *N*,*N*'-(1,2-phenylene)diacetamide (1) in DMSO-D₆.



Figure S1.3: Expanded ESI Mass spectra of N,N'-(1,2-Phenylene)diacetamide.

N,N'-(1,2-Phenylene)diacetamide (1): ¹H NMR (700 MHz, DMSO-d₆) δ 9.37 (s, 1H), 7.61 – 7.58 (m, 1H), 7.18 – 7.16 (m, 1H), 2.13 (s, 3H); ¹³C NMR (175 MHz, DMSO-d₆) δ 169.1, 130.9, 125.2, 125.0, 24.1. HR-MS (ESI): calculated for C₁₀H₁₂N₂O₂ [M+H]⁺: 193.0972, found, 193.1014.



Figure S1.4: ¹H-NMR spectrum of *N*,*N*'-(1,3-phenylene)diacetamide (2) in DMSO-D₆.



Figure S1.5: ¹³C NMR spectrum of *N*,*N*'-(1,3-phenylene)diacetamide (2) in DMSO-D₆.



Figure S1.6: Expanded ESI Mass spectra of N,N'-(1,3-Phenylene)diacetamide.

N,N'-(1,3-Phenylene)diacetamide (2): ¹H NMR (700 MHz, DMSO-d₆) δ 9.92 (s, 1H), 7.88 (s, 1H), 7.26 (d, J = 8.0 Hz, 2H), 7.17 (t, J = 8.0 Hz, 1H), 2.03 (s, 6H); ¹³C NMR (175 MHz, DMSO-d₆) δ 168.7, 140.0, 129.2, 114.3, 110.2, 24.4. HR-MS (ESI): calculated for C₁₀H₁₂N₂ O₂ [M+H]⁺: 193.0972, found, 193.1007.



Figure S1.7: ¹H NMR spectrum of *N*,*N*'-(1,4-phenylene)diacetamide (3) in DMSO-D₆.



Figure S1.8: ¹³C NMR spectrum of *N*,*N*'-(1,4-phenylene)diacetamide (3) in DMSO-D₆.

N,N'-(1,4-Phenylene)diacetamide (3): ¹H NMR (700 MHz, DMSO-d₆) δ 9.79 (s, 1H), 7.47 (s, 2H), 2.01 (s, 3H); ¹³C NMR (175 MHz, DMSO-d₆) δ 169.3, 135.0, 119.8, 24.2.



Figure S2.0: Logarithm of normalized water peak (at 4.7 ppm) intensity as a function of echo delay for determining the transverse relaxation of buffer solution.



Figure S2.1: Logarithm of normalized water peak (at 4.7 ppm) intensity as a function of echo delay for determining the transverse relaxation of 15mM solution of **1**, in buffer.



Figure S2.2: Logarithm of normalized water peak (at 4.7 ppm) intensity as a function of echo delay for determining the transverse relaxation of half diluted saturated solution of **1**, in buffer.



Figure S2.3: Logarithm of normalized water peak (at 4.7 ppm) intensity as a function of echo delay for determining the transverse relaxation of saturated solution of **1**, in buffer.



Figure S2.4: Logarithm of normalized water peak (at 4.7 ppm) intensity as a function of echo delay for determining the transverse relaxation of 15mM solution of **2**, in buffer.



Figure S2.5: Logarithm of normalized water peak (at 4.7 ppm) intensity as a function of echo delay for determining the transverse relaxation of half diluted saturated solution of **2**, in buffer.



Figure S2.6: Logarithm of normalized water peak (at 4.7 ppm) intensity as a function of echo delay for determining the transverse relaxation of saturated solution of **2**, in buffer.



Figure S3: (a) Dependence of CEST percentage on saturation field strength ranging from $1.5 \,\mu\text{T}$ to $5.5 \,\mu\text{T}$ for *N*,*N*'-(1,2-phenylene)diacetamide (1) at 298K and pH 7.4 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of $1/\omega_{1^2}$ (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 7.4. RF saturation pulse was applied for 6 s ensuring complete saturation



Figure S4: (a) Dependence of CEST percentage on saturation field strength ranging from $1.5 \,\mu\text{T}$ to $5.5 \,\mu\text{T}$ for *N*,*N*'-(1,2-phenylene)diacetamide (1) at 304K and pH 7.4 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of $1/\omega_1^2$ (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 7.4. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S5: (a) Dependence of CEST percentage on saturation field strength ranging from $1.5 \,\mu\text{T}$ to $5.5 \,\mu\text{T}$ for *N*,*N*'-(1,2-phenylene)diacetamide (1) at 310K and pH 7.4 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of $1/\omega_1^2$ (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 7.4. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S6: (a) Dependence of CEST percentage on saturation field strength ranging from $1.5 \,\mu\text{T}$ to $5.5 \,\mu\text{T}$ *N*,*N'*-(1,2-phenylene)diacetamide (1) at 316K (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of $1/\omega_1^2$ (rad/sec)⁻² x 10^{-5} was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 7.4. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S7: (a) Dependence of CEST percentage on saturation field strength ranging from $1.5 \,\mu\text{T}$ to $5.5 \,\mu\text{T}$ for *N*,*N*'-(1,2-phenylene)diacetamide (1) at 322K and pH 7.4 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of $1/\omega_1^2$ (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 7.4. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S8: (a) Dependence of CEST percentage on saturation field strength ranging from $1.5 \,\mu\text{T}$ to $5.5 \,\mu\text{T}$ for *N*,*N*'-(1,3-phenylene)diacetamide (**2**) at 298K and pH 7.4 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of $1/\omega_1^2$ (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 7.4. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S9: (a) Dependence of CEST percentage on saturation field strength ranging from $1.5 \,\mu\text{T}$ to $5.5 \,\mu\text{T}$ for *N*,*N*'-(1,3-phenylene)diacetamide (**2**) at 304K and pH 7.4 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of $1/\omega_1^2$ (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 7.4. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S10: (a) Dependence of CEST percentage on saturation field strength ranging from 1.5 μ T to 5.5 μ T for *N*,*N*'-(1,3-phenylene)diacetamide (**2**) at 310K and pH 7.4 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of $1/\omega_1^2$ (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 7.4. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S11: (a) Dependence of CEST percentage on saturation field strength ranging from 1.5 μ T to 5.5 μ T for *N*,*N*'-(1,3-phenylene)diacetamide (**2**) at 316K (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of $1/\omega_1^2$ (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 7.4. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S12: (a) Dependence of CEST percentage on saturation field strength ranging from 1.5 μ T to 5.5 μ T for *N*,*N'*-(1,3-phenylene)diacetamide (**2**) at 322K and pH 7.4 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of $1/\omega_1^2$ (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 7.4. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S13: (a) Dependence of CEST percentage on saturation field strength ranging from 1.5 μ T to 5.5 μ T for *N*,*N*'-(1,2-phenylene)diacetamide (1) at 310K and pH 6.5 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of 1/ ω ₁² (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 6.5. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S14: (a) Dependence of CEST percentage on saturation field strength ranging from 1.5 μ T to 5.5 μ T for *N*,*N'*-(1,2-phenylene)diacetamide (1) at 310K and pH 7.0 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of 1/ ω ₁² (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 7.0. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S15: (a) Dependence of CEST percentage on saturation field strength ranging from 1.5 μ T to 5.5 μ T for *N*,*N*'-(1,2-phenylene)diacetamide (**1**) at 310K and pH 7.4 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of 1/ ω 1² (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 7.4. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S16: (a) Dependence of CEST percentage on saturation field strength ranging from 1.5 μ T to 5.5 μ T for *N*,*N*'-(1,2-phenylene)diacetamide (1) at 310K and pH 8.1 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of 1/ ω ¹² (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 8.1. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S17: (a) Dependence of CEST percentage on saturation field strength ranging from 1.5 μ T to 5.5 μ T for *N*,*N*'-(1,3-phenylene)diacetamide (**2**) at 310K and pH 6.5 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of $1/\omega_1^2$ (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 6.5. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S18: (a) Dependence of CEST percentage on saturation field strength ranging from 1.5 μ T to 5.5 μ T for *N*,*N*'-(1,3-phenylene)diacetamide (**2**) at 310K and pH 7.0 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of 1/ ω_1^2 (rad/sec)⁻² x 10⁻⁵ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 7.0. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S19: (a) Dependence of CEST percentage on saturation field strength ranging from 1.5 μ T to 5.5 μ T for *N*,*N'*-(1,3-phenylene)diacetamide (**2**) at 310K and pH 7.4 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of 1/ ω 1² (rad/sec)⁻² x 10⁻⁷ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 7.4. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S20: (a) Dependence of CEST percentage on saturation field strength ranging from 1.5 μ T to 5.5 μ T *N,N'*-(1,3-phenylene)diacetamide (**2**) at 310K and pH 8.1 (b) Omega plot for exchange rate measurement. The expected linear relationship of M_z/(M₀-M_z) as a function of $1/\omega_1^2$ (rad/sec)⁻² x 10⁻⁷ was obtained when recorded at 9.4 T of 15 mM compound in 0.01M PBS buffer at pH 8.1. RF saturation pulse was applied for 6 s ensuring complete saturation.



Figure S21: Dependence of CEST effect of *N*,*N*'-(1,3-phenylene)diacetamide (**2**) on pH. Overlaid Z-spectra with pH ranging from 6.5 to 8.1, RF saturation of 5 μ T was applied for 3s to obtain the z-spectra.



Figure S22: Dependence of k_{ex} for 1, as a function of pH of buffer solution.



Figure S23.1: Normalized water peak (at 4.7 ppm) intensity as a function of relaxation delay for determining the longitudinal relaxation time constant of water in normal PBS buffer solution.



Figure S23.2: Normalized water peak (at 4.7 ppm) intensity as a function of relaxation delay for determining the longitudinal relaxation time constant of water in 15mM solution of **2** in PBS buffer.