

## Electronic Supplementary Information

# Hydrothermal treatment as a mean to improve solubility and enhanced diaCEST contrast efficiency

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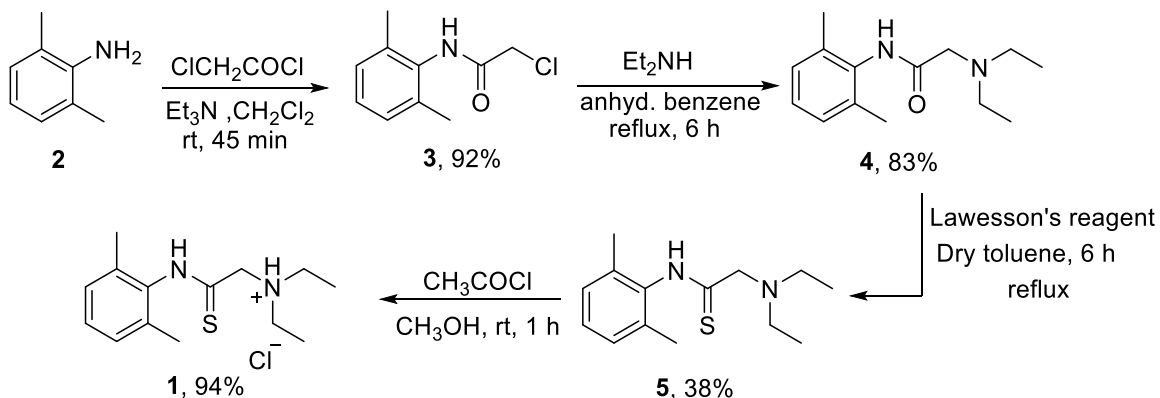
## Table of Content

- **Section S1: Synthesis and characterization of amino-thioamide**
  - **S.1A:** Procedure for preparation of 2-chloro-N-(2,6-dimethylphenyl)acetamide (**3**)
  - **S.1B:** Procedure for preparation of 2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide (**4**)
  - **S.1C:** Procedure for preparation of 2-(diethylamino)-N-(2,6-dimethylphenyl)ethanethioamide (**5**)
  - **S1.D:** Procedure for preparation of 2-((2,6-dimethylphenyl)amino)-N, N-diethyl-2-thioxoethan-1-aminium chloride (**1**)
- **Section S2 : Characterization of TCD**
- **Section S3 : Omega plot for exchange rate measurement of TCD**
- **Section S4 : 1H and 13C spectra of TCD (Batch-2)**
- **Section S5 : Z-spectra of TCD (Batch-2) at variable pH**
- **Section S6 : Re-Characterization of TCD batch-2 (repetition post 60days)**

## List of Figures

- **Section S1**
  - **Scheme S1** : Synthesis of Amino-Thioamide **1** from 2,6-Dimethylaniline **2**
  - **Fig. S1.1-S1.2** :  $^1\text{H}$  &  $^{13}\text{C}$  NMR spectrum of compound **3** in  $\text{CDCl}_3$
  - **Fig. S1.3-S1.4** :  $^1\text{H}$  &  $^{13}\text{C}$  NMR spectrum of compound **4** in  $\text{CDCl}_3$
  - **Fig. S1.5-S1.6** :  $^1\text{H}$  &  $^{13}\text{C}$  NMR spectrum of compound **5** in  $\text{DMSO-d}_6$
  - **Fig. S1.7-S1.8** :  $^1\text{H}$  &  $^{13}\text{C}$  NMR spectrum of compound **1** in  $\text{DMSO-d}_6$
  - **Fig. S1.9** : Mass spectrum of compound **1**
- **Section S2**
  - **Fig. S2.1**:  $^{13}\text{C}$  NMR spectrum of thioamide based carbon dots (TCDs) in  $\text{H}_2\text{O}$
  - **Fig. S2.2**: ESI-MS spectrum of thioamide based carbon dots (TCDs)
- **Section S3**
  - **Fig. S3.1**: Omega plot for calculation of  $k_{\text{ex}}$  for buffer solution of compound **1**, at pH 5.5.
  - **Fig. S3.2**: Omega plot for calculation of  $k_{\text{ex}}$  for buffer solution of TCDs at pH range 4.5.
  - **Fig. S3.3**: Omega plot for calculation of  $k_{\text{ex}}$  for buffer solution of TCDs at pH range 5.5.
  - **Fig. S3.4**: Omega plot for calculation of  $k_{\text{ex}}$  for buffer solution of TCDs at pH range 9.0.
  - **Fig. S3.5**: Omega plot for calculation of  $k_{\text{ex}}$  for buffer solution of TCDs at pH range 9.9.
- **Section S4**
  - **Fig. S4.1**:  $^1\text{H}$  NMR spectrum of Batch -2 thioamide based carbon dots (TCDs) in  $\text{H}_2\text{O}$ .
  - **Fig. S4.2**:  $^{13}\text{C}$  NMR spectrum of Batch -2 thioamide based carbon dots (TCDs) in  $\text{H}_2\text{O}$ .
- **Section S5**
  - **Fig. S5** : Z-spectra of TCD (Batch-2) at variable pH in 10mM PBS.
- **Section S6**
  - **Fig. S6.1**: Absorption and emission spectra of Batch -2 thioamide based carbon dots (TCDs) post 60 days.
  - **Fig. S6.2**:  $^1\text{H}$  NMR spectrum of Batch -2 thioamide based carbon dots (TCDs) in  $\text{H}_2\text{O}$ , post 60 days.
  - **Fig. S6.3**:  $^{13}\text{C}$  NMR spectrum of Batch -2 thioamide based carbon dots (TCDs) in  $\text{H}_2\text{O}$ , post 60 days.
  - **Fig. S6.4**: Z-spectrum of TCD (Batch-2) at pH 4.5 in 10mM PBS, post 60 days.

## Section S1: Synthesis and characterization of amino-thioamide



**Scheme S1:** Synthesis of Amino-Thioamide **1** from 2,6-Dimethylaniline **2**

### S1.A Procedure for preparation of 2-chloro-*N*-(2,6-dimethylphenyl)acetamide (**3**):<sup>SR1</sup>

Chloroacetyl chloride (1.4 ml, 18.1 mmol) was added to a solution of 2,6-xylydine, **2** (2 g, 16.5 mmol), and triethylamine (2.5 mL, 18.1 mmol) in dichloromethane (30 mL) slowly and was stirred at room temperature for 45 min. It is then quenched with water and extracted with DCM (3 x 15 mL). Then the organic layer was washed with hydrochloric acid (1N, 15 mL) and then with a saturated solution of NaHCO<sub>3</sub> (50 mL). It was then dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated using rota evaporator to afford chloroacetanilide, **3**. Yield: 92% (3.02 g); White solid; Melting Point: 145 - 146 °C (Lit.<sup>SR2</sup> 148 - 149 °C); R<sub>f</sub>: 0.4 in 40% ethyl acetate in hexanes; IR (KBr):  $\nu$  (cm<sup>-1</sup>) = 3438, 2375, 2331, 1637, 748, 666; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.85 (s, 1H), 7.17 - 7.09 (m, 1H), 4.25 (s, 1H), 2.24 (s, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  164.4, 135.5, 132.8, 128.5, 128.0, 42.9, 18.4; HR-MS (ESI) Calcd for C<sub>10</sub>H<sub>12</sub>ClNO [M+ H]<sup>+</sup>: 198.0680, found: 198.0692.

### S1.B Procedure for preparation of 2-(diethylamino)-*N*-(2,6-dimethylphenyl)acetamide (**4**):

Chloroacetanilide, **3** (2 g, 10.15 mmol) was refluxed with diethylamine (2.6 mL, 25.37 mmol) for 6 hours in anhydrous benzene (15 mL). The hydrochloride of the diethylamine was first filtered, then the solution was washed twice with water, and the solvent was removed with rota evaporator. Then the residue was dissolved in hydrochloric acid and was extracted using ether and then made alkaline using ammonia solution. And again, extracted using ether (4 times) and then dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed under reduced pressure. The residue was dried vacuo and purified by silica gel column chromatography to afford diethylamino acetamide, **4**.

Yield: 83% (1.97 g); Pale white crystals; Melting Point: 66 - 67 °C (Lit.<sup>SR2</sup> 67 - 68 °C); R<sub>f</sub>: 0.2 in 40% ethyl acetate in hexanes; IR (KBr):  $\nu$  (cm<sup>-1</sup>) = 3279, 2974, 2924, 2823, 1683, 1495, 1197, 769; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.94 (s, 1H), 7.08 (s, 1H), 3.23 (s, 1H), 2.70 (q, *J* = 4 Hz, 1H), 2.23 (s, 1H), 1.14 (t, *J* = 8 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  170.3, 135.2, 134.0, 128.3, 127.1, 57.5, 49.0, 18.6, 12.7; HR-MS (ESI) Calcd for C<sub>14</sub>H<sub>22</sub>N<sub>2</sub>O [M+ H]<sup>+</sup>: 235.1805, found: 235.1820

### **S1.C Procedure for preparation of 2-(diethylamino)-N-(2,6-dimethyl phenyl)ethanethioamide**

**(5):** Diethylamino acetamide, **4** (1g, 4.27 mmol) in dry toluene (30 mL), was treated with Lawesson's reagent (0.9g, 2.22 mmol) and refluxed at 65 °C for 6 hours, and then cooled to room temperature. The solvent was removed under reduced pressure. The residue was dried vacuo and purified by silica gel column chromatography to afford thioamide, **5**. Yield: 38% (0.41 g); Pale yellow solid; Melting Point: 105 - 106 °C; R<sub>f</sub>: 0.25 in 20% ethyl acetate in hexanes; IR (KBr):  $\nu$  (cm<sup>-1</sup>) = 3350, 1713, 1500, 1366, 1269, 771, 744; <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  11.10 (s, 1H), 7.16 – 7.09 (m, 3H), 3.56 (s, 2H), 2.62 (q, *J* = 8 Hz, 4H), 2.11 (s, 6H), 1.07 (t, *J* = 8 Hz, 6H); <sup>13</sup>C NMR (100 MHz, DMSO)  $\delta$  201.3, 137.3, 134.7, 127.7, 127.2, 65.2, 48.0, 17.7, 12.1; HR-MS (ESI) calculated for C<sub>14</sub>H<sub>22</sub>N<sub>2</sub>S [M+ H]<sup>+</sup>: 251.1576, found: 251.1599.

### **S1.D Procedure for preparation of 2-((2,6-dimethylphenyl)amino)-N, N-diethyl-2-thioxoethan-**

**1-aminium chloride (1):** Thioamide, **5** (0.15g, 0.59 mmol) was dissolved in methanol (1 ml) under nitrogen atmosphere, then kept on ice and with constant stirring acetyl chloride (0.3ml, 4.19 mmol) was added to it dropwise, and then kept at room temperature for 1h. The solvent was removed under reduced pressure. The residue was dried vacuo to afford salt, **1**. Yield: 94% (0.16 g); Pale yellow solid; Melting Point: 160 -161 °C; IR (KBr):  $\nu$  (cm<sup>-1</sup>) = 3447, 2364, 1649, 1475, 1272, 1215, 746; <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  12.91 (s, 1H), 9.58 (s, 1H), 7.16 (m, 3H), 4.6 (d, *J* = 4 Hz, 2H), 3.41 (s, 4H), 2.16 (s, 6H), 1.32 (t, *J* = 8 Hz, 6H); <sup>13</sup>C NMR (100 MHz, DMSO)  $\delta$  192.5, 136.2, 134.4, 128.1, 127.9, 58.6, 48.3, 17.7, 8.8; HR-MS (ESI) Calcd for C<sub>14</sub>H<sub>23</sub>N<sub>2</sub>SCl [M-Cl]<sup>+</sup>: 251.1576, found: 251.1597.

### **References:**

SR1. F. Cumine, S. Zhou, T. Tuttle, J. A. Murphy. *Org. Biomol. Chem.*, **2017**,*15*, 3324.

SR2. D. E. Fitzpatrick, T. Maujean, A. C. Evans, S. V. Ley, *Angew. Chem. Int. Ed.* **2018**, *57*, 15128.

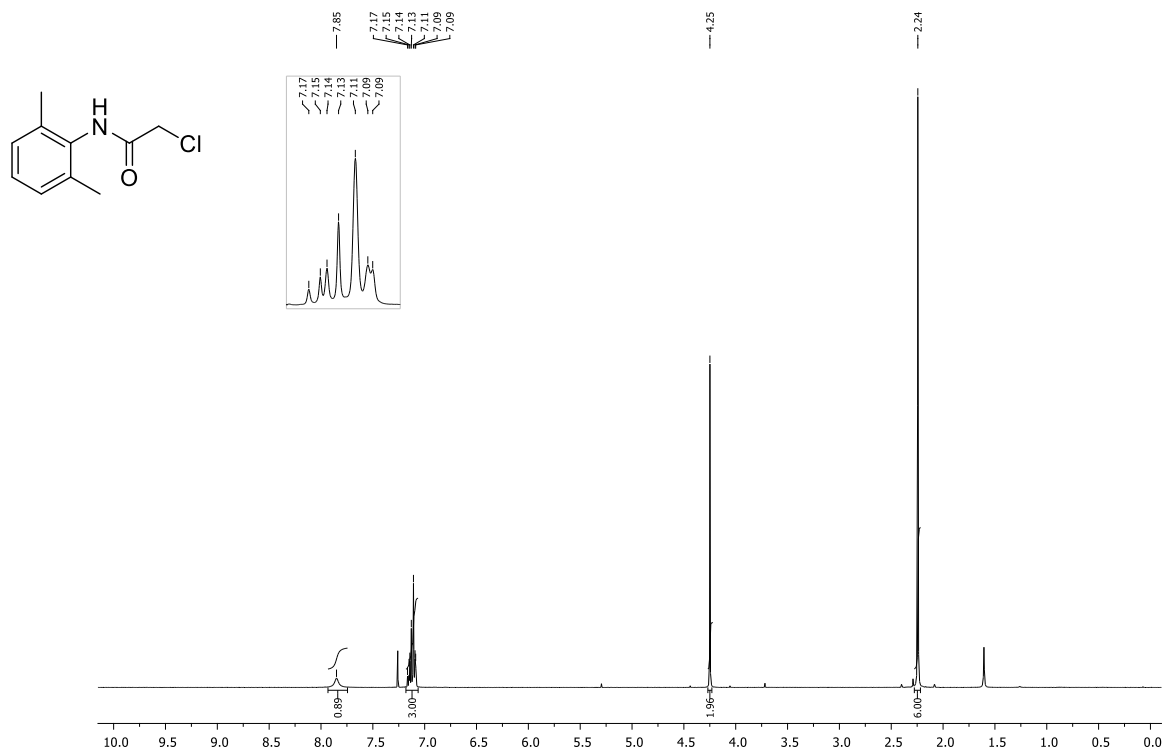


Fig. S1.1:  $^1\text{H}$  NMR spectrum of compound 3 in  $\text{CDCl}_3$

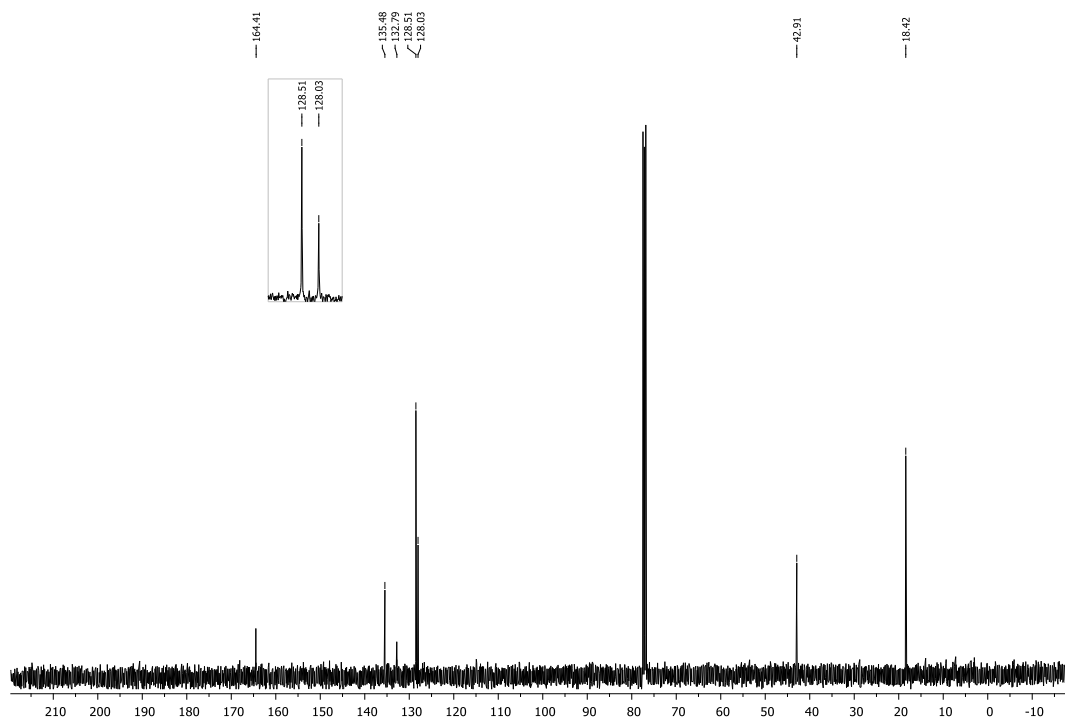
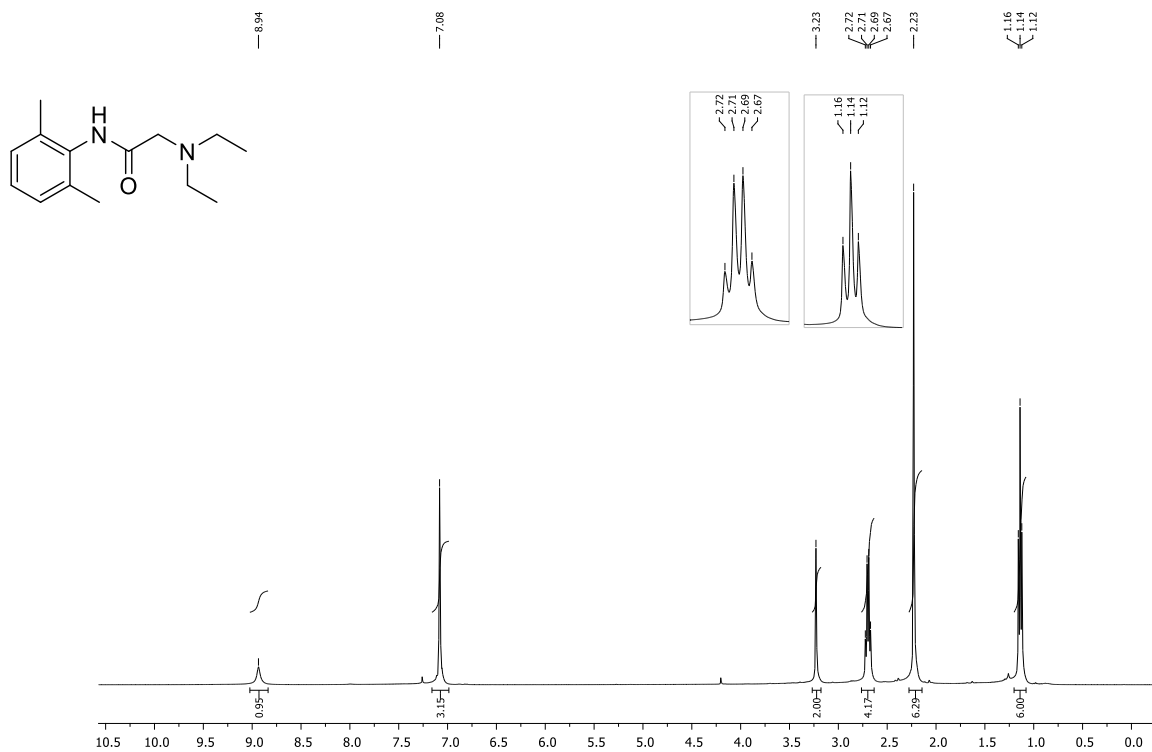
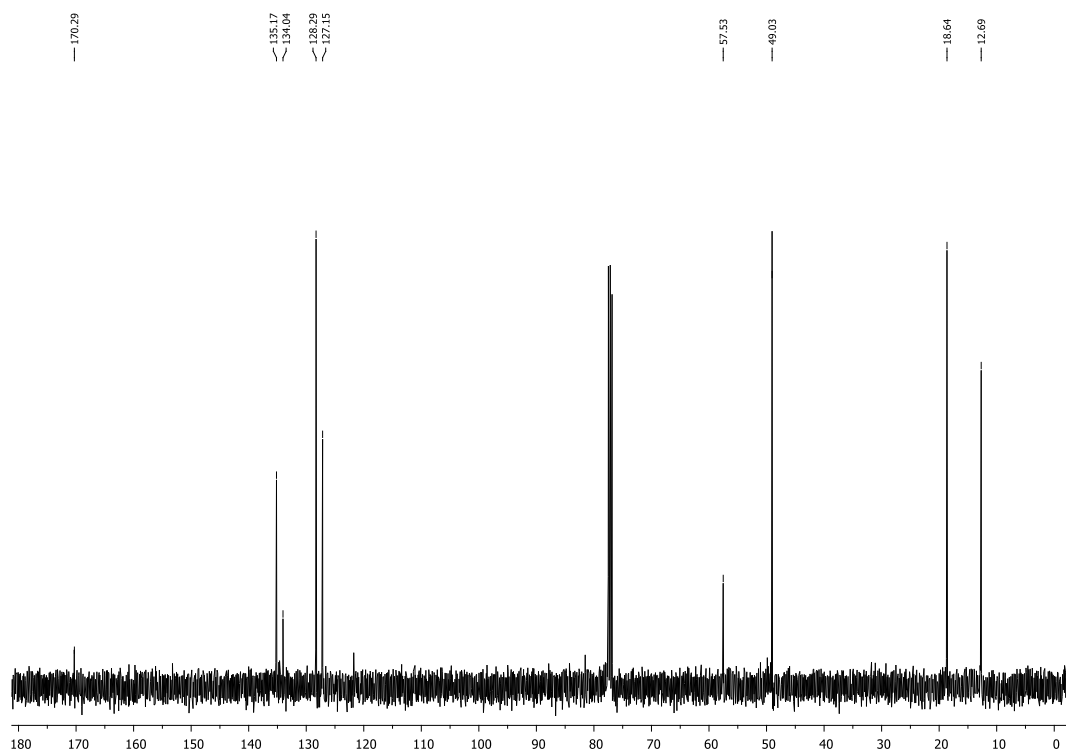


Fig. S1.2:  $^{13}\text{C}$  NMR spectrum of compound 3 in  $\text{CDCl}_3$



**Fig. S1.3:** <sup>1</sup>H NMR spectrum of compound 4 in CDCl<sub>3</sub>



**Fig. S1.4:** <sup>13</sup>C NMR spectrum of compound 4 in CDCl<sub>3</sub>

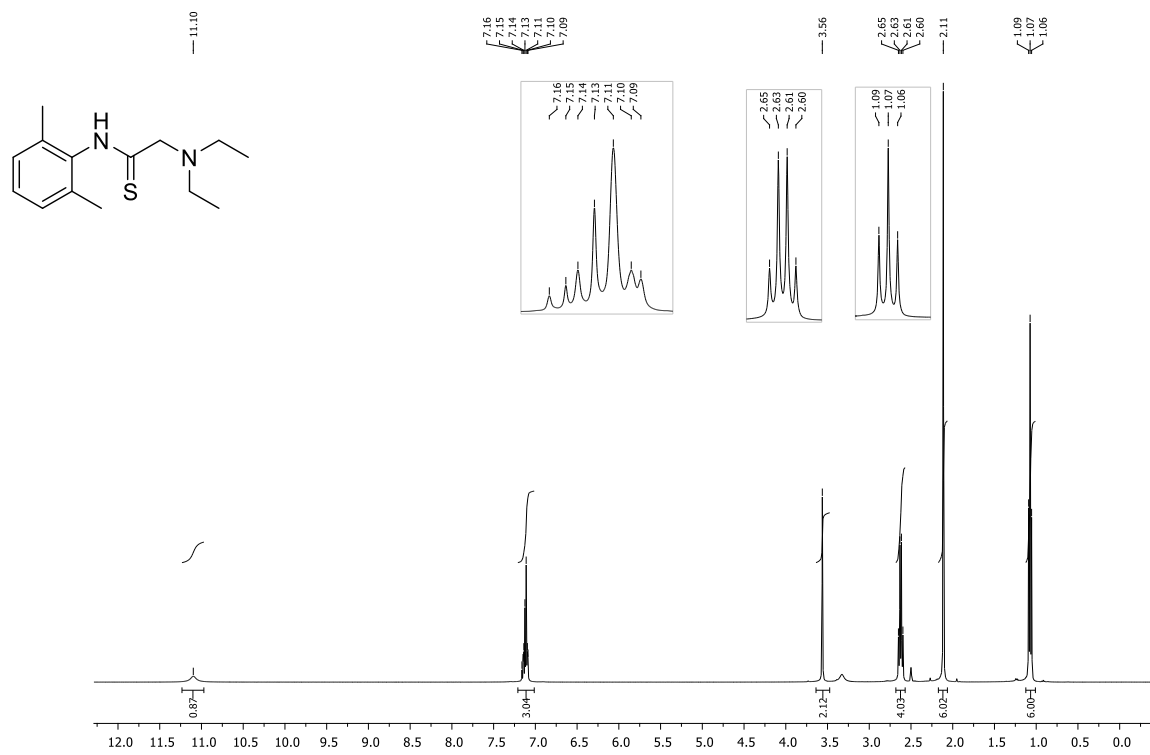


Fig. S1.5: <sup>1</sup>H NMR spectrum of compound 5 in DMSO-d<sub>6</sub>

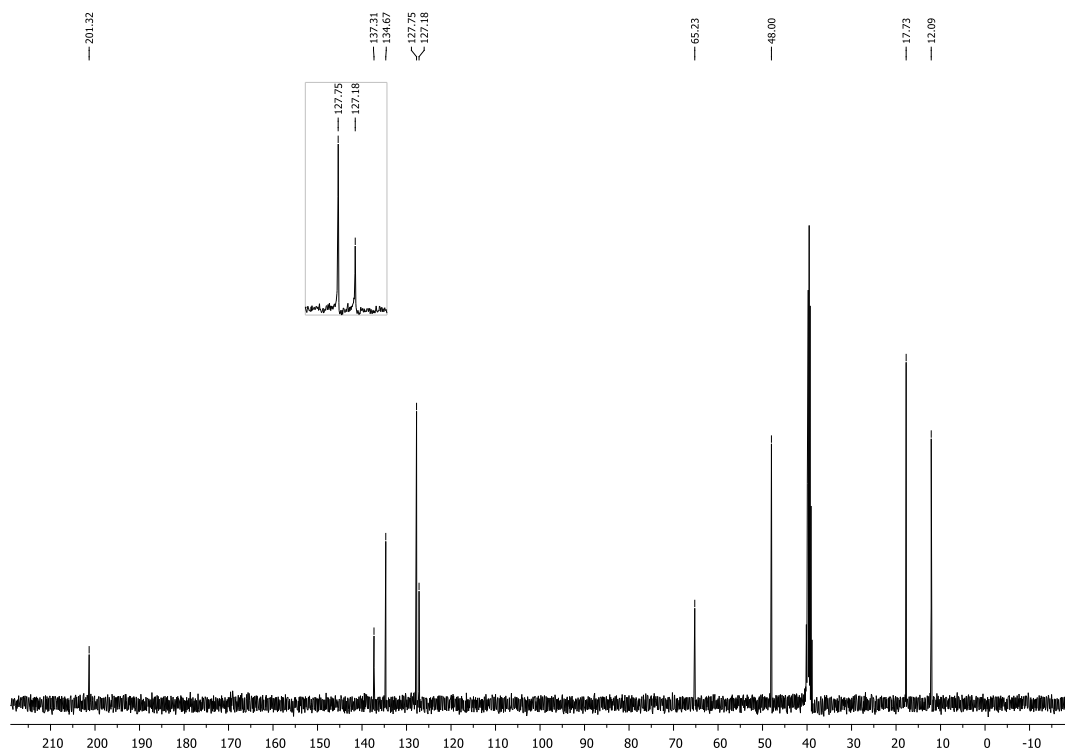


Fig. S1.6: <sup>13</sup>C NMR spectrum of compound 5 in DMSO-d<sub>6</sub>

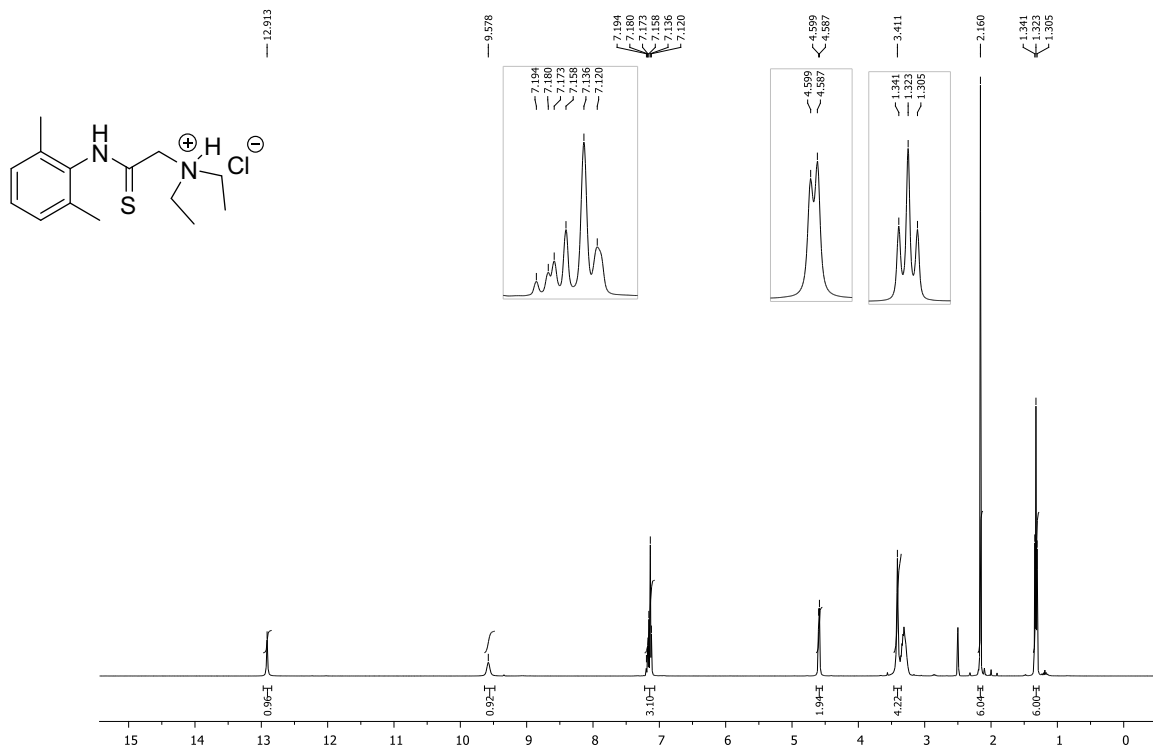


Fig. S1.7: <sup>1</sup>H NMR spectrum of compound 1 in DMSO-d<sub>6</sub>

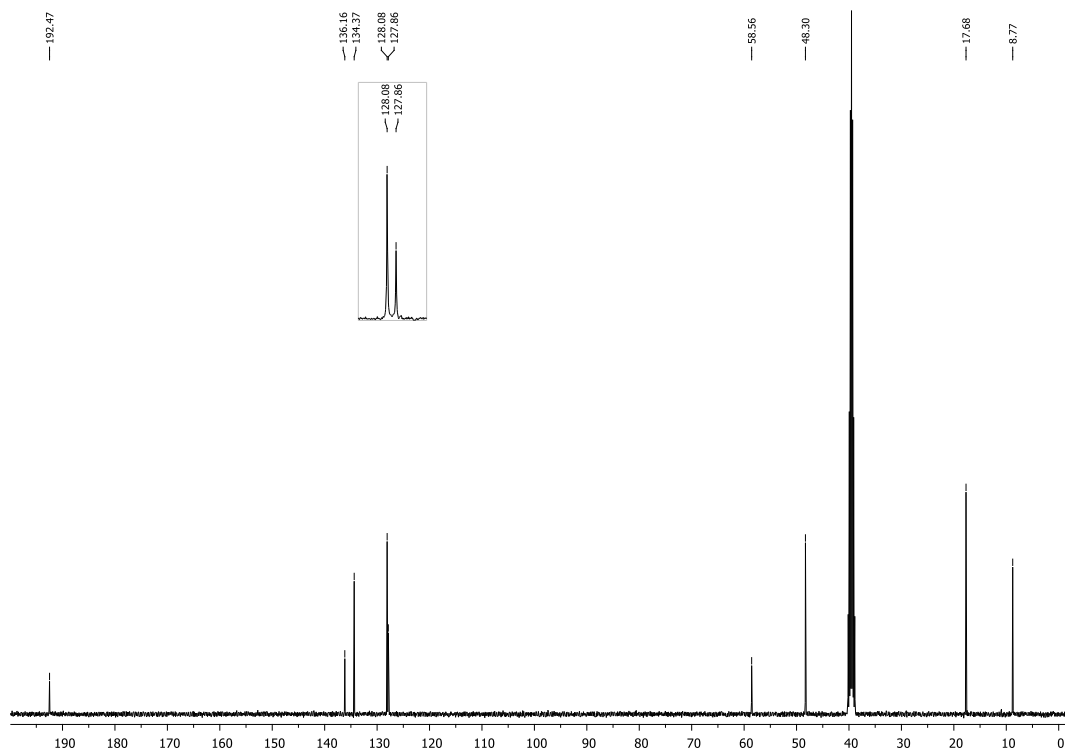
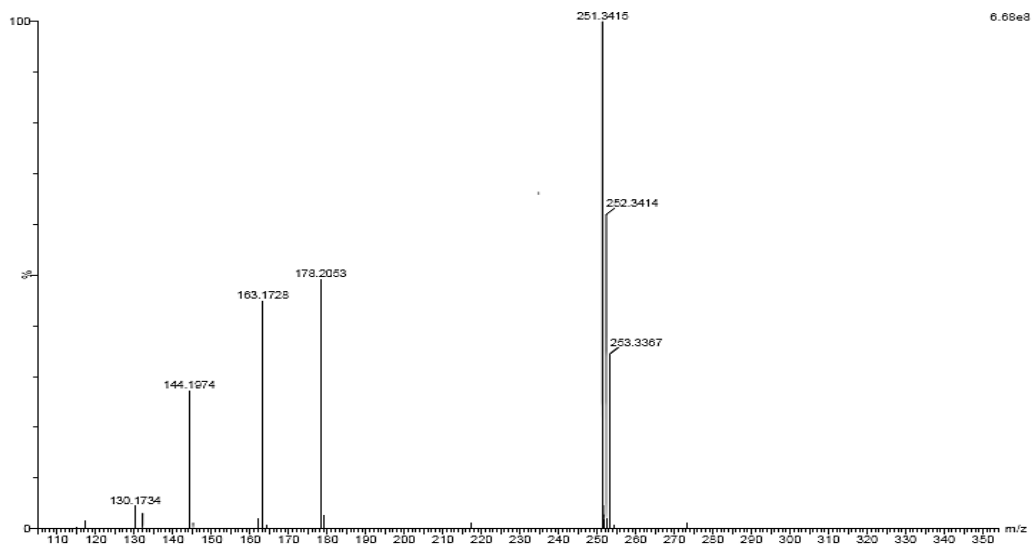


Fig. S1.8: <sup>13</sup>C NMR spectrum of compound 1 in DMSO-d<sub>6</sub>





**Fig. S1.9:** Mass spectrum of 2-((2,6-dimethylphenyl)amino)-*N,N*-diethyl-2-thioxoethan-1-aminium chloride (**1**)

## Section S2 : Characterization of TCD

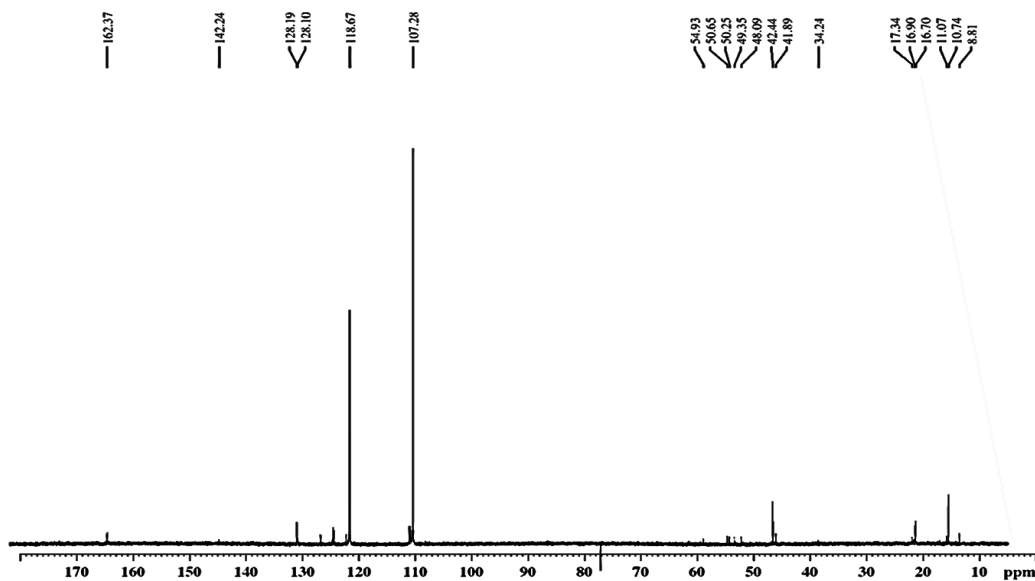


Fig. S2.1:  $^{13}\text{C}$  NMR spectrum of hydrothermal thioamide based carbon dots (TCD) in  $\text{H}_2\text{O}$ .

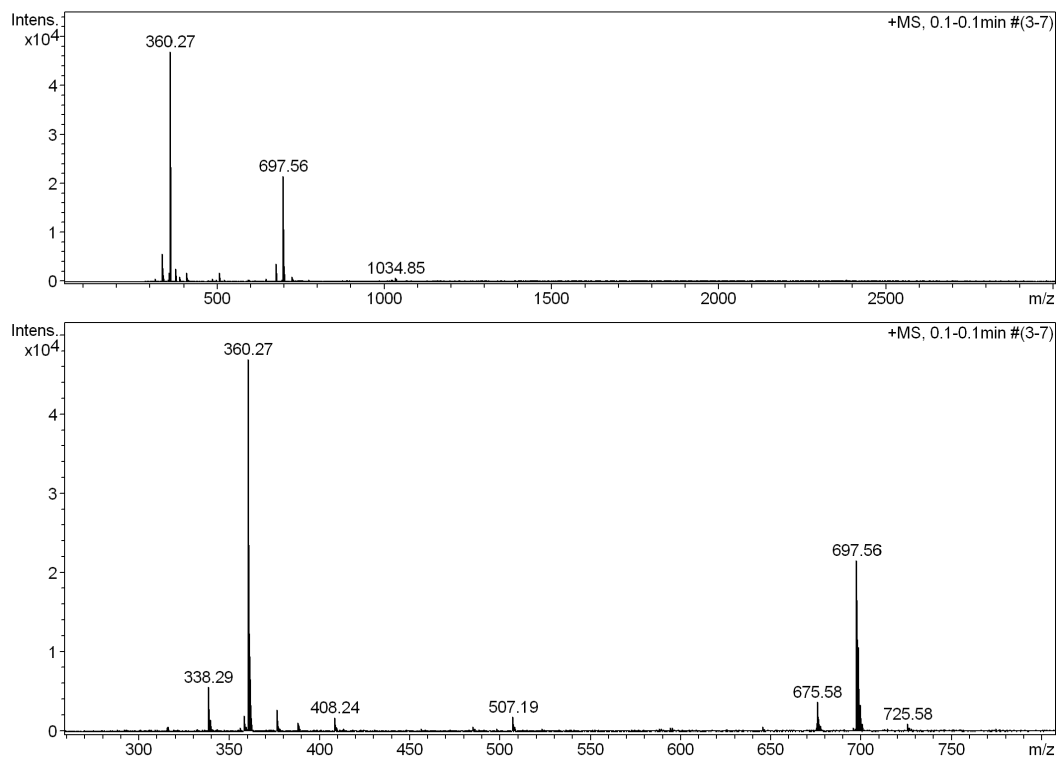
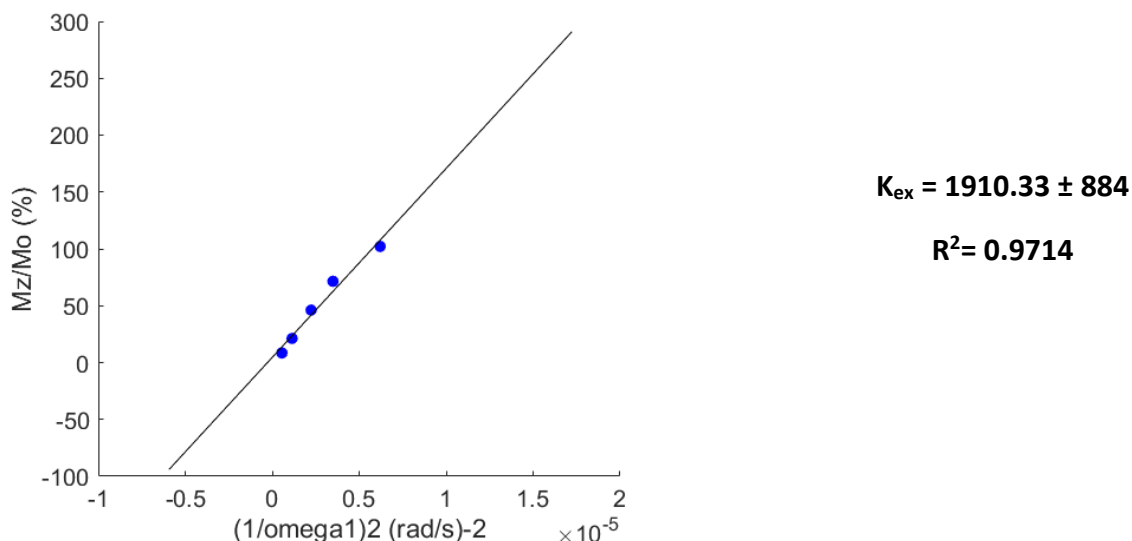
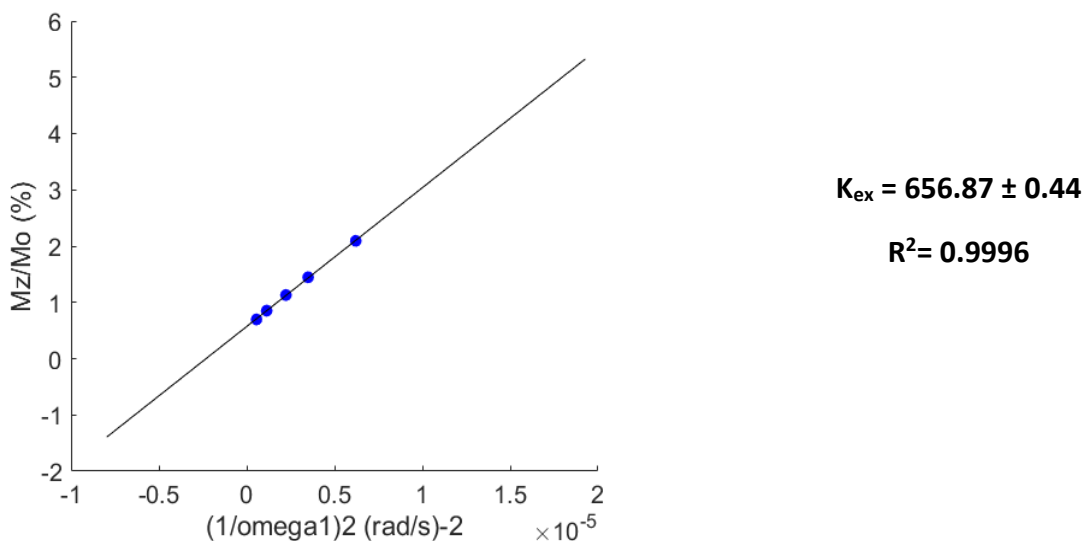


Fig. S2.2: Mass spectrum of thioamide based carbon dots (TCDs). No trace of signal corresponding to the presence of precursor **1** was observed.

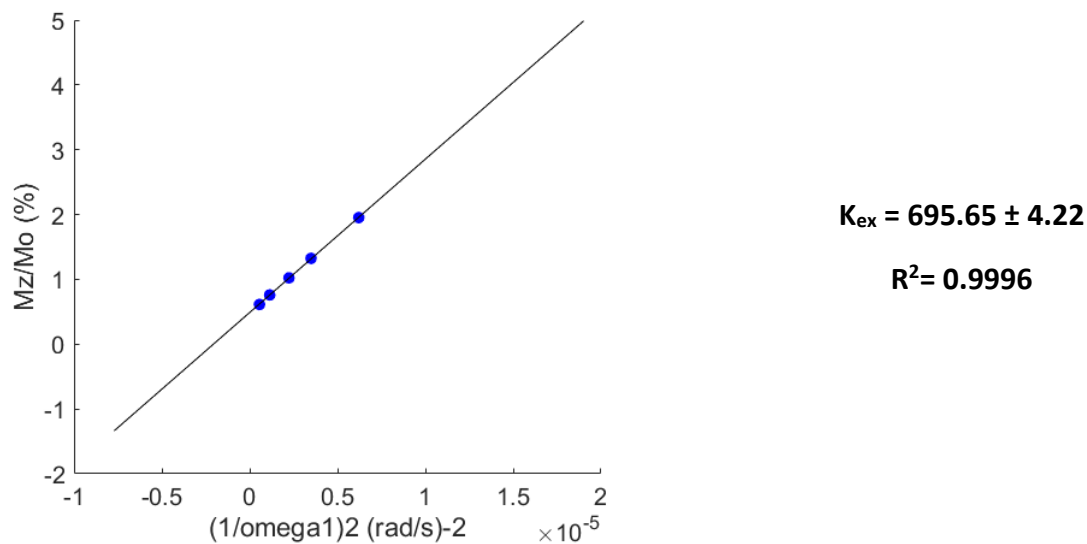
### Section S3: Omega plot for exchange rate measurement of TCD



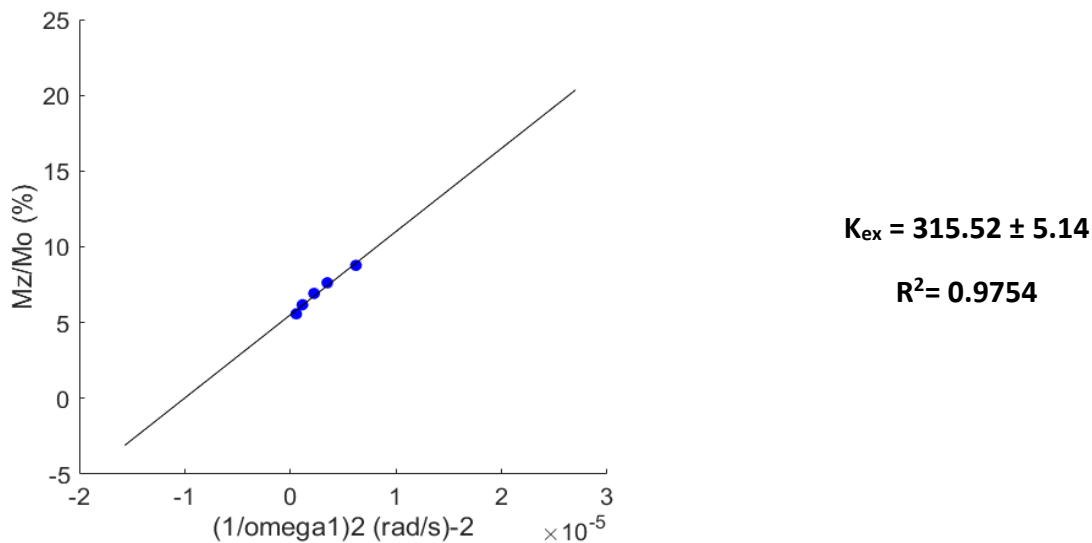
**Fig. S3.1:** Omega plot for exchange rate measurement. The expected linear relationship of  $M_z/(M_0-M_z)$  as a function of  $1/\omega_1^2$  (rad/sec)<sup>-2</sup>  $\times 10^{-5}$  was obtained when recorded at 9.4 T of 8.5 mM compound in 0.01M PBS at pH 5.5. RF saturation pulse was applied for 6 s ensuring complete saturation.



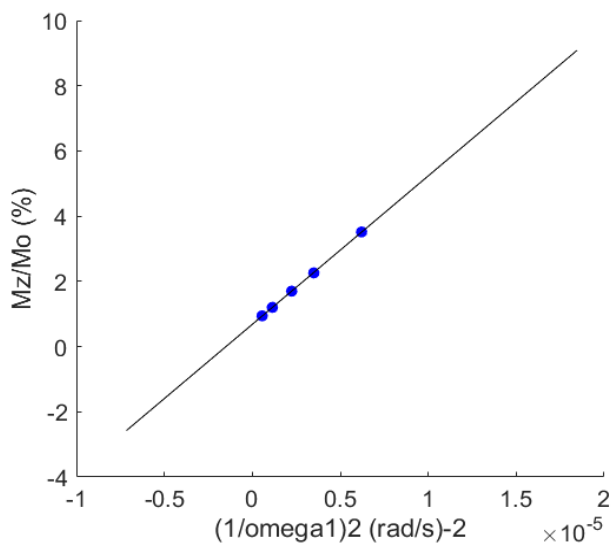
**Fig. S3.2:** Omega plot for exchange rate measurement. The expected linear relationship of  $M_z/(M_0-M_z)$  as a function of  $1/\omega_1^2$  (rad/sec)<sup>-2</sup>  $\times 10^{-5}$  was obtained when recorded at 9.4 T of ~8.5 mM TCD solution in 0.01M PBS at pH 4.5. RF saturation pulse was applied for 6 s ensuring complete saturation.



**Fig. S3.3:** Omega plot for exchange rate measurement. The expected linear relationship of  $M_z/(M_0-M_z)$  as a function of  $1/\omega_1^2$  (rad/sec)<sup>-2</sup>  $\times 10^{-5}$  was obtained when recorded at 9.4 T of ~ 8.5 mM TCD solution in 0.01M PBS at pH 5.5. RF saturation pulse was applied for 6 s ensuring complete saturation.



**Fig. S3.4:** Omega plot for exchange rate measurement. The expected linear relationship of  $M_z/(M_0-M_z)$  as a function of  $1/\omega_1^2$  (rad/sec)<sup>-2</sup>  $\times 10^{-5}$  was obtained when recorded at 9.4 T of ~ 8.5 mM TCD solution in 0.01M PBS at pH 9.0. RF saturation pulse was applied for 6 s ensuring complete saturation.



$$K_{ex} = 816.92 \pm 27.44$$

$$R^2 = 0.9996$$

**Fig. S3.5:** Omega plot for exchange rate measurement. The expected linear relationship of  $M_z/(M_0-M_z)$  as a function of  $1/\omega_1^2$  (rad/sec)<sup>-2</sup> x 10<sup>-5</sup> was obtained when recorded at 9.4 T of ~ 8.5 mM TCD solution in 0.01M PBS at pH 9.9. RF saturation pulse was applied for 6 s ensuring complete saturation.

## Section S4: $^1\text{H}$ and $^{13}\text{C}$ spectra of TCD (Batch-2)

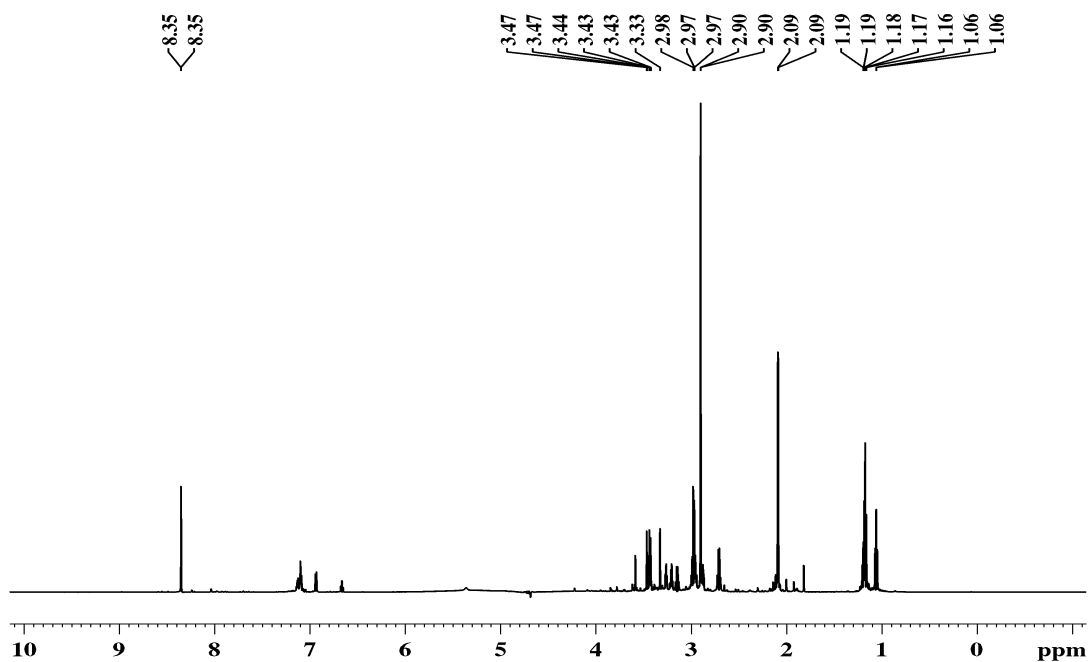


Fig. S4.1:  $^1\text{H}$  NMR spectrum of second batch of thioamide based carbon dots (TCD) in  $\text{H}_2\text{O}$ .

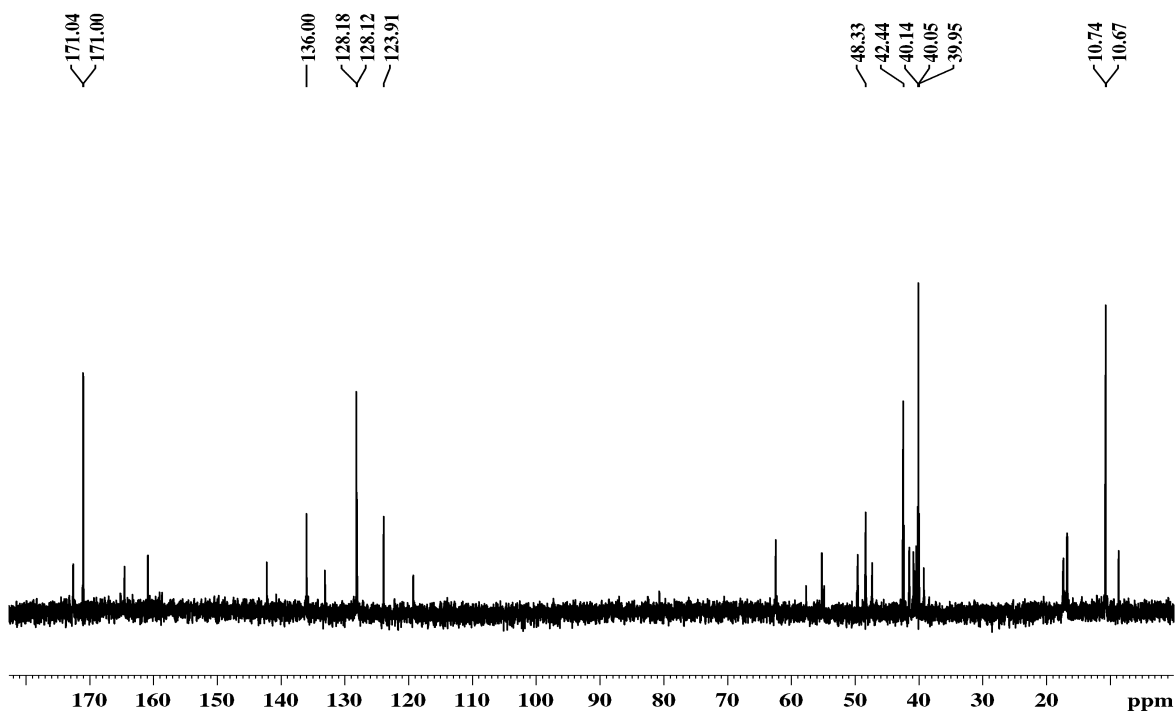
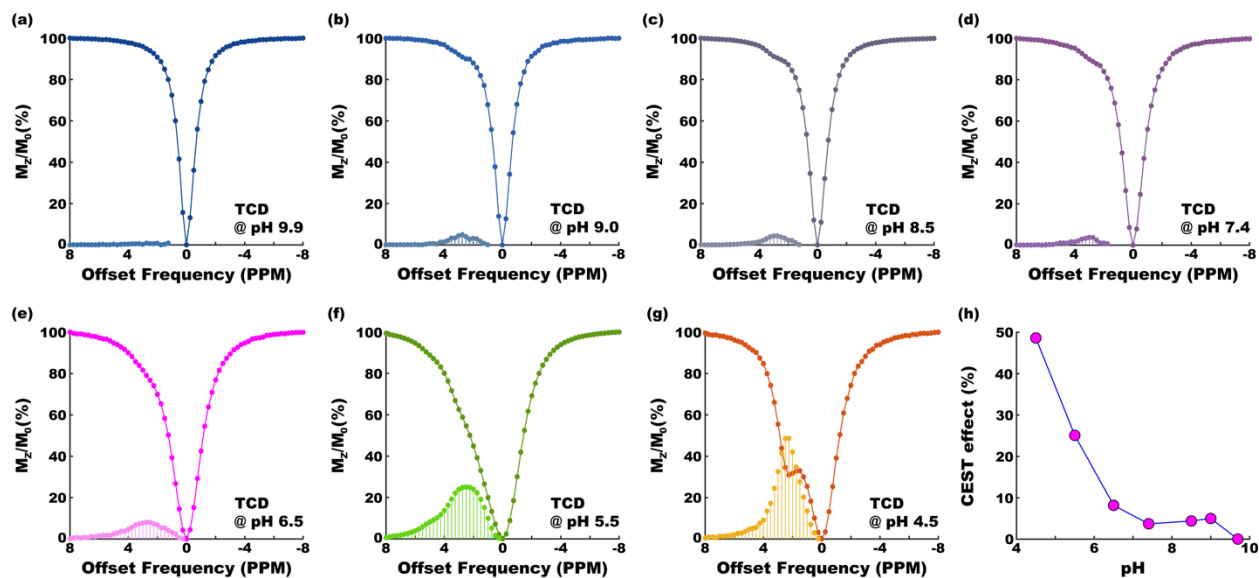


Fig. S4.2:  $^{13}\text{C}$  NMR spectrum of second batch of thioamide based carbon dots (TCD) in  $\text{H}_2\text{O}$ .

## Section S5: Z-spectra of TCD (Batch-2) at variable pH



**Fig. S5:** CEST Z-spectra of 8.5 mM solution of the carbon quantum dots at different pH in PBS. Variable pHs at which Z-spectra were recorded are 9.9(a), 9.0(b), 8.5(c), 7.4(d), 6.5(e), 5.5(f) and 4.5(g). All the experiments were carried out at 37 °C. The experiments were performed using 3 s saturation at 5  $\mu$ T RF field strength with 0.25 PPM resolution. The corresponding direct saturation subtracted CEST peaks are plotted on the x-axis. The CEST efficiencies as a function of pH are plotted in (h).

## Section S6: Characterization of TCD batch-2 (repetition post 60days)

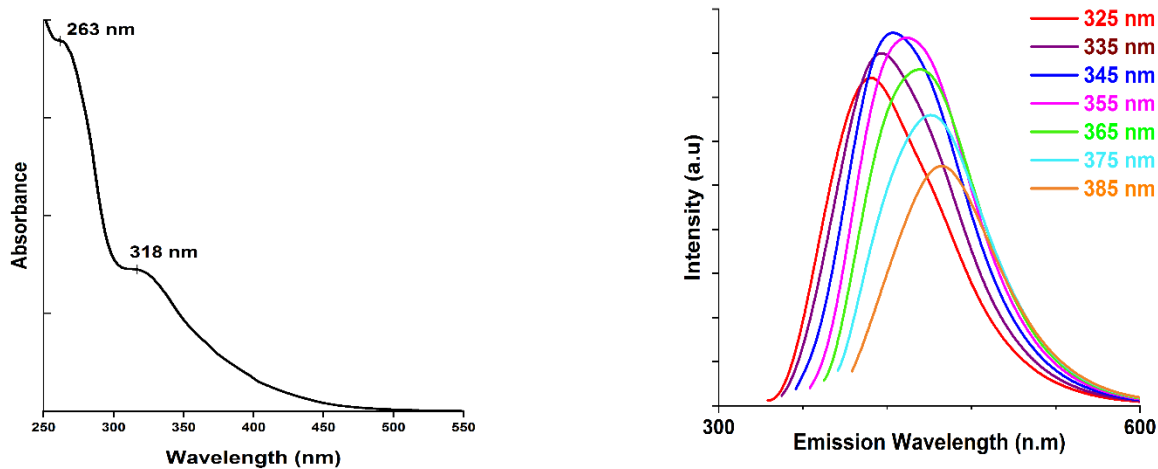


Fig. S6.1: Absorption and emission spectra of thioamide based carbon dots (TCD) .

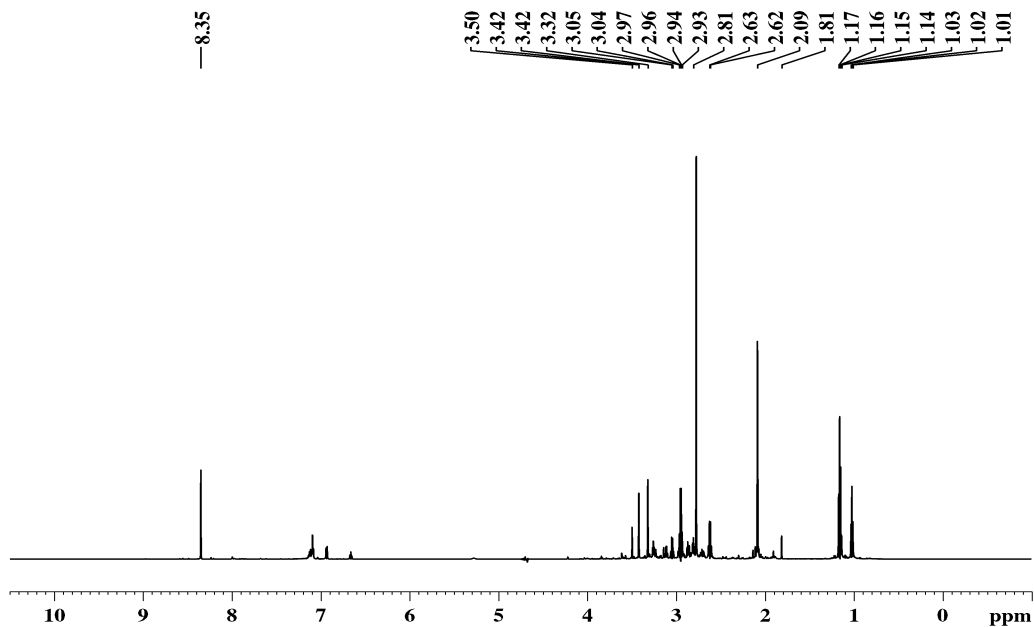
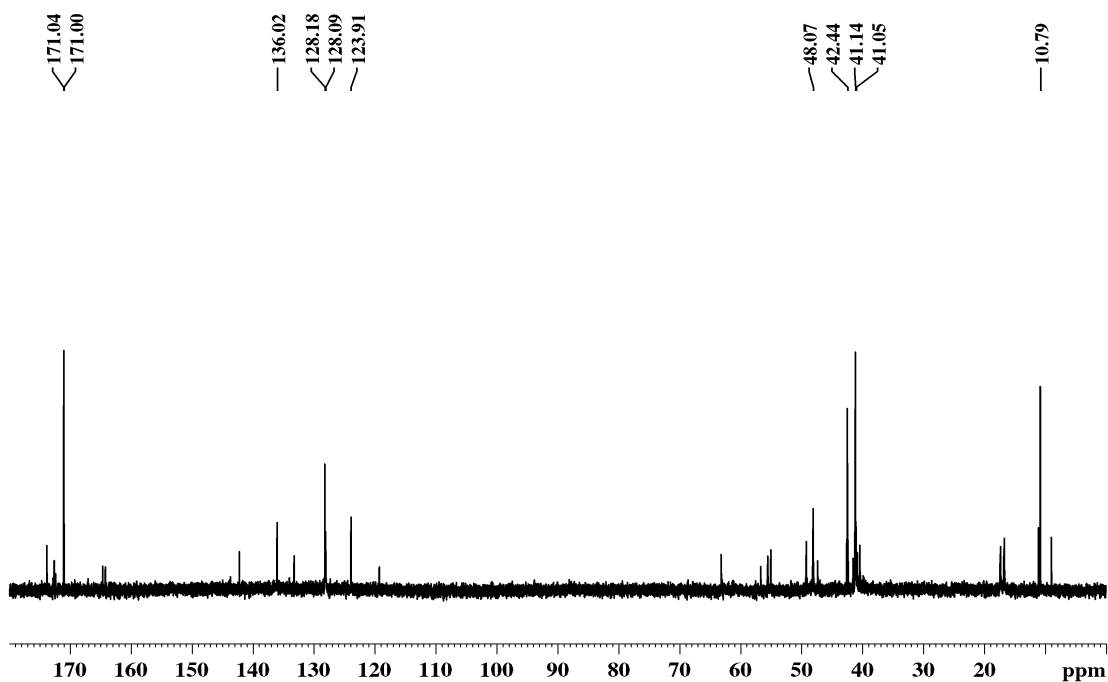
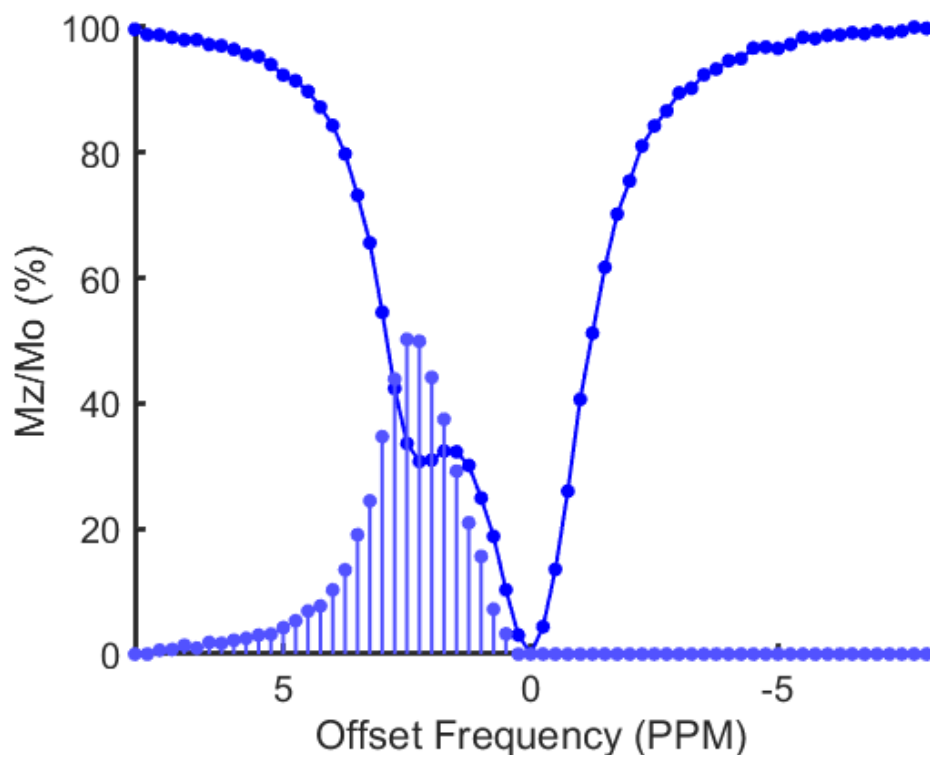


Fig. S6.2: <sup>1</sup>H NMR spectrum of second batch of thioamide based carbon dots (TCD) in H<sub>2</sub>O.





**Fig. S6.3:**  $^{13}\text{C}$  NMR spectrum of second batch of thioamide based carbon dots (TCD) in  $\text{H}_2\text{O}$ .



**Fig. S6.4:** Z-spectra of 8.5 mM solution of thioamide based carbon dots TCD at pH 4.5 in PBS.