

# Synthesis of PNA FRET Probes via an Orthogonally Protected Building Block for Post-Synthetic Labeling of PNAs at the 5-Position of U.

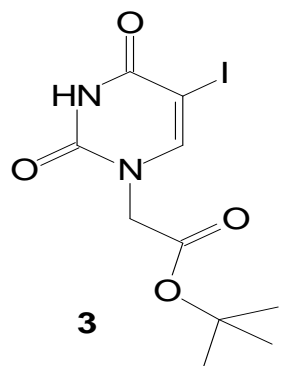
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S2

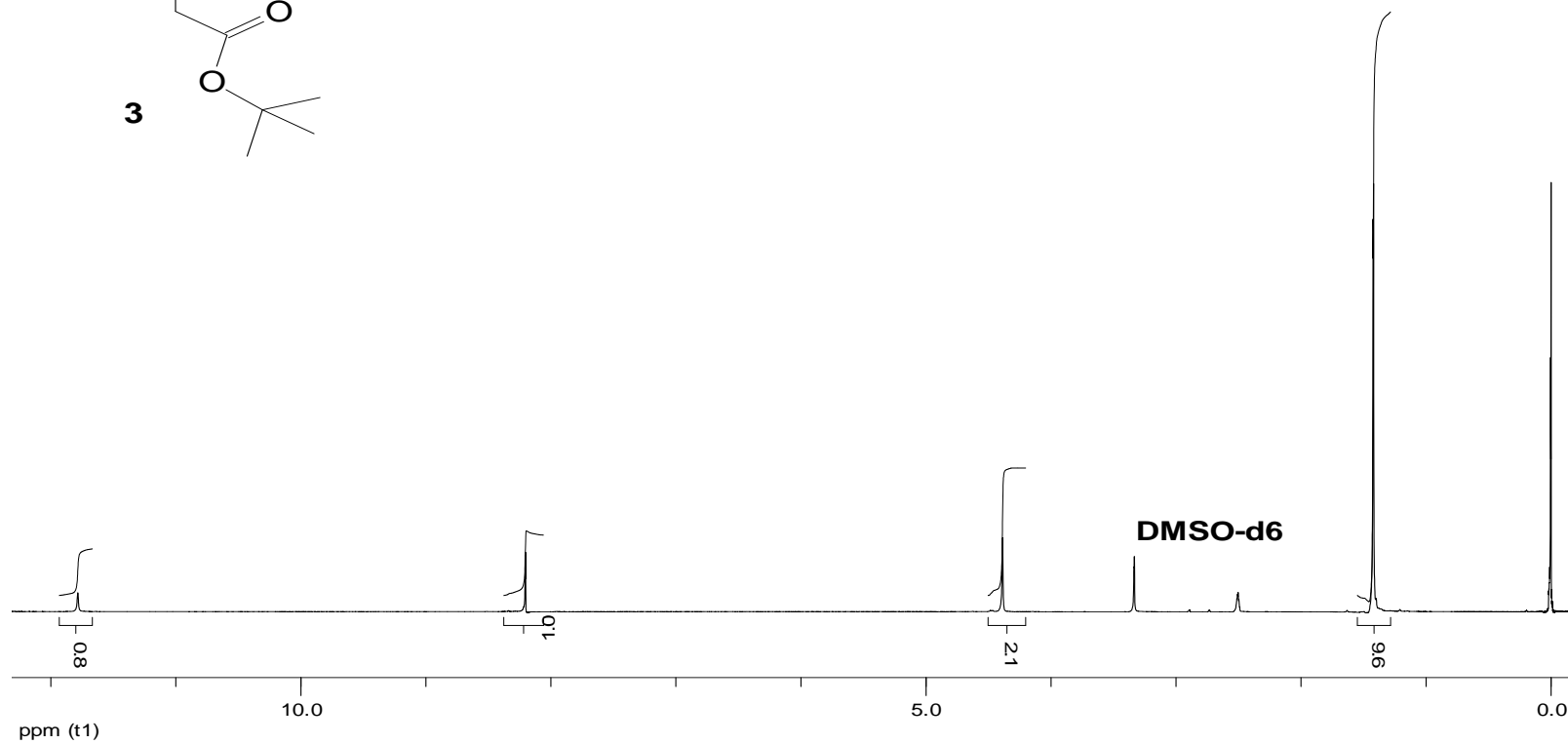
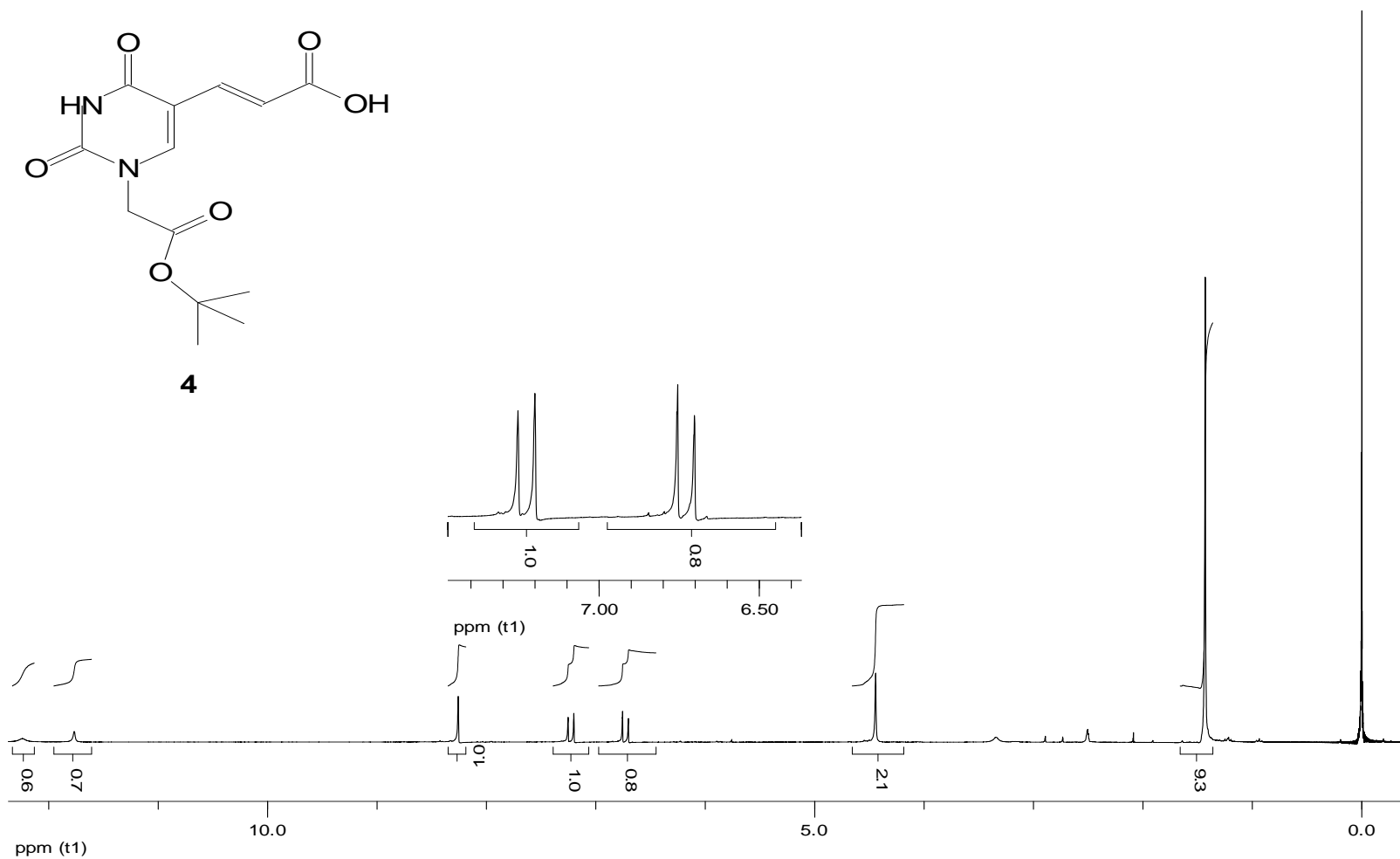
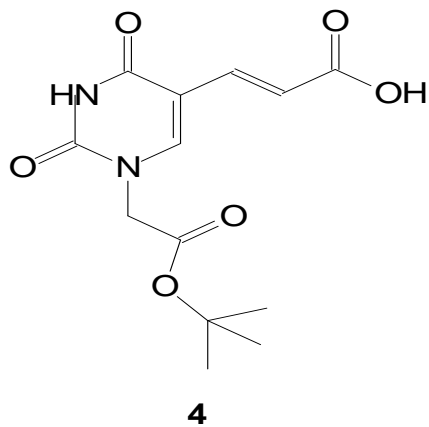


Figure S1.  $^1\text{H}$  NMR (300MHz,  $\text{DMSO-d}_6$ ) spectrum of tert-butyl 2-(5-iodo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (3).





S4

**Figure 3.3**  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-d}_6$ ) spectrum of (E)-3-(1-(2-tert-butoxy-2-oxoethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)acrylic acid (**4**).

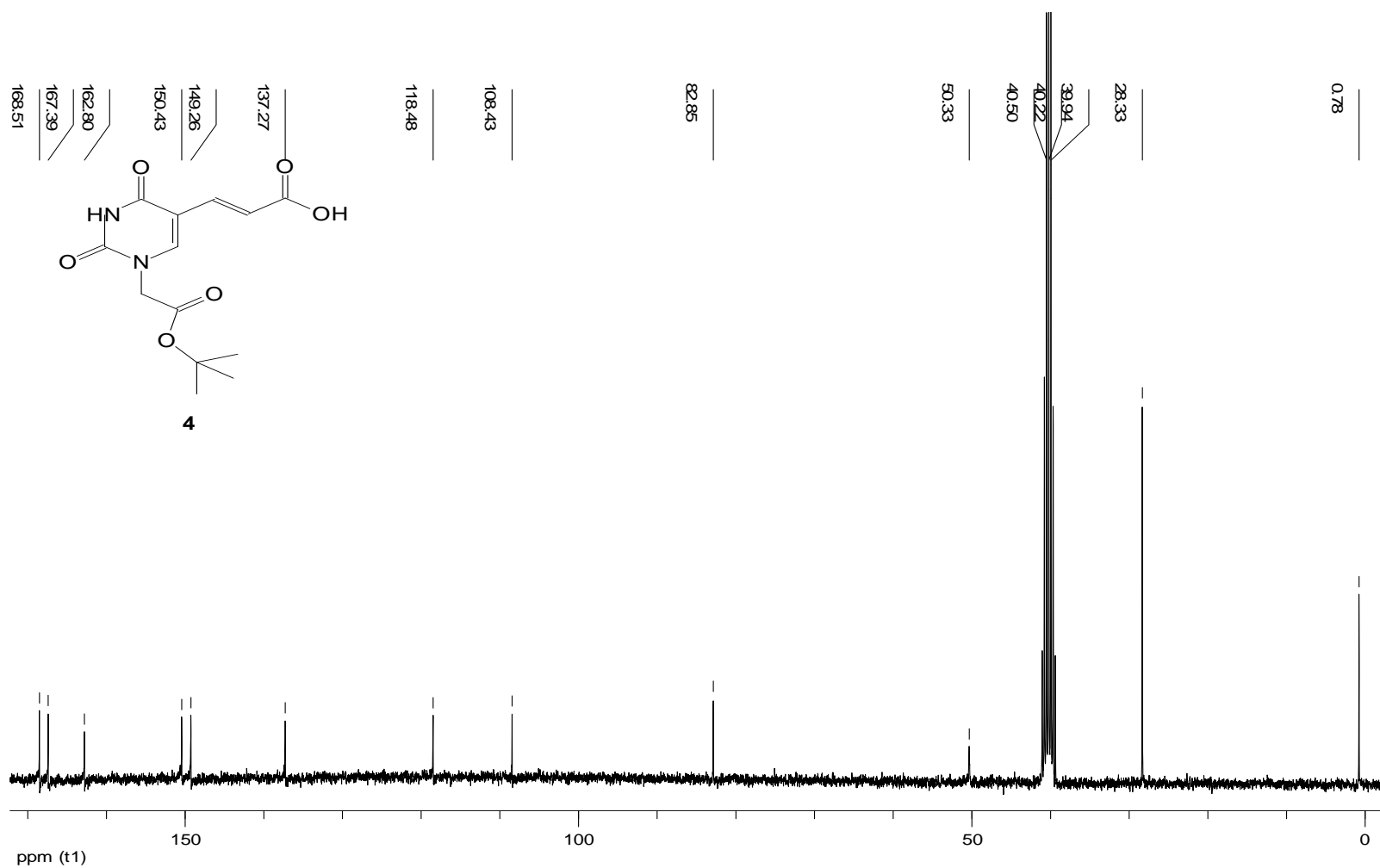
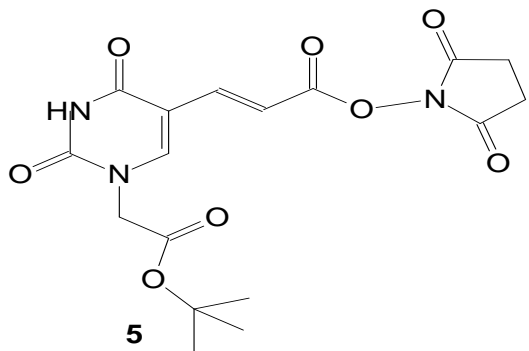
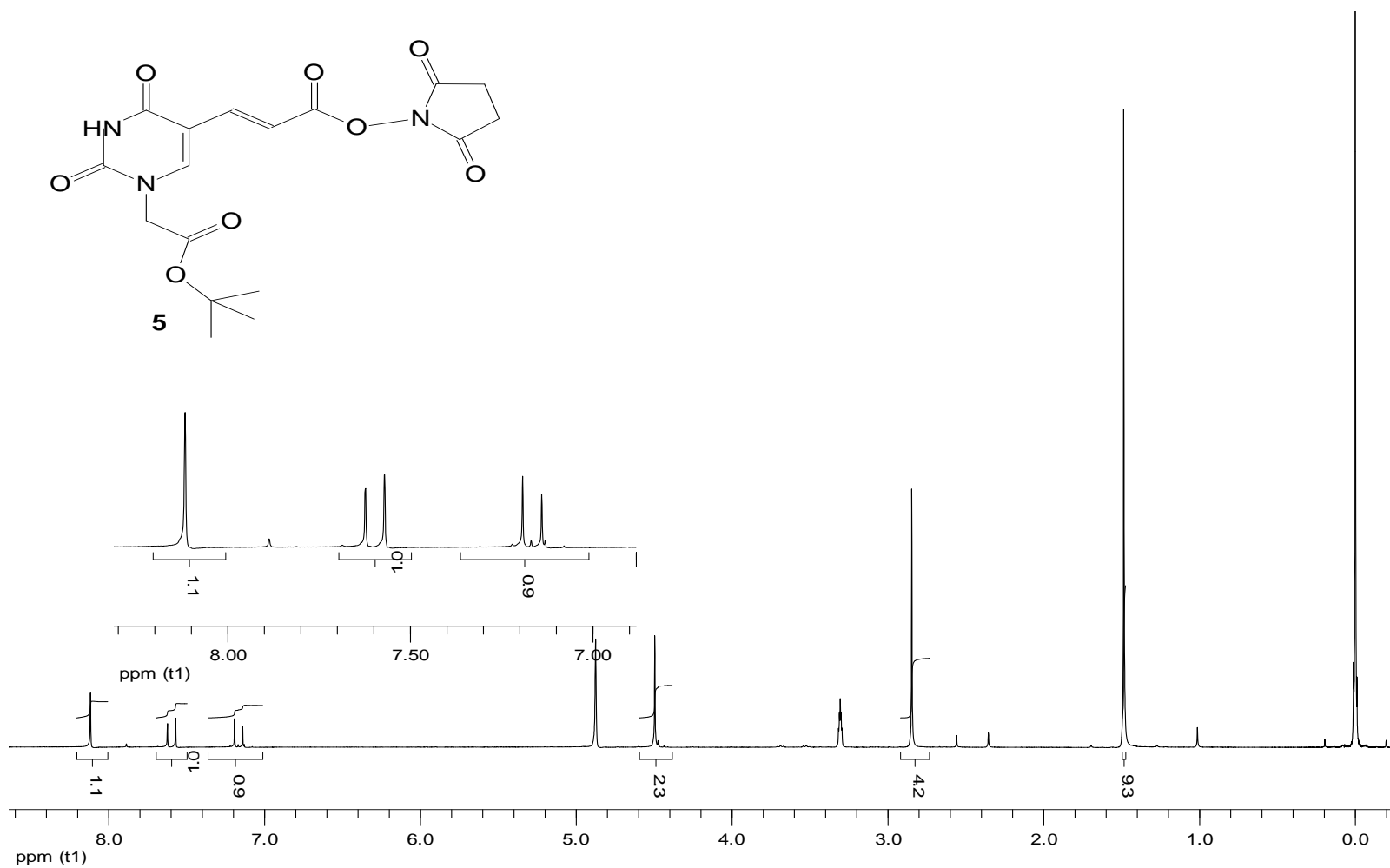


Figure S4.  $^{13}\text{C}$  NMR (300 MHz,  $\text{DMSO-d}_6$ ) spectrum of (E)-3-(1-(2-tert-butoxy-2-oxoethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)acrylic acid (4).



S6



**Figure S5.**  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-d}_6$ ) spectrum of (E)-2,5-dioxopyrrolidin-1-yl-3-(1-(2-tert-butoxy-2-oxoethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)acrylate (**5**).

S7

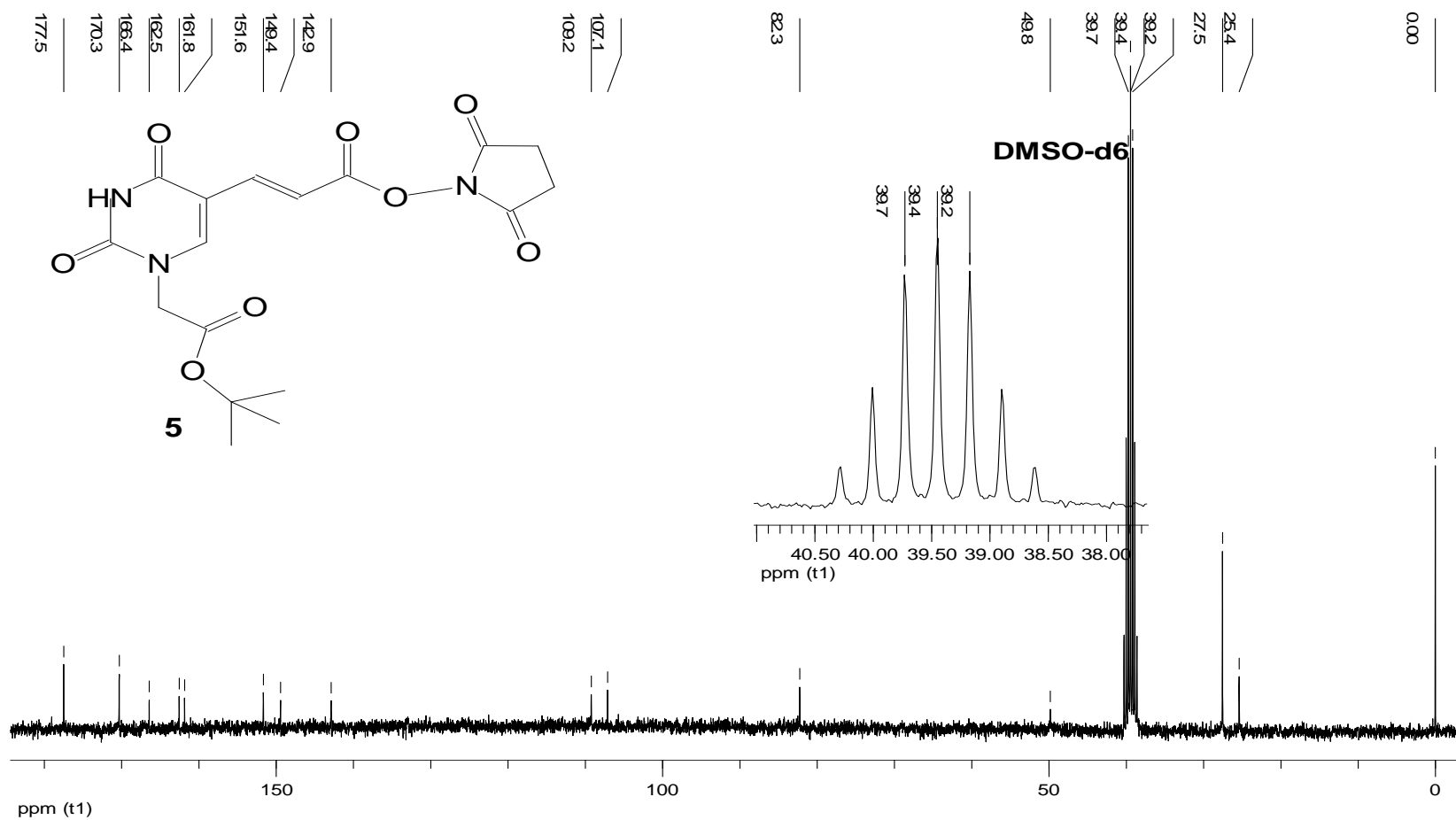
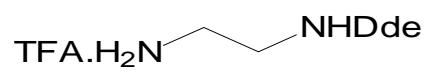
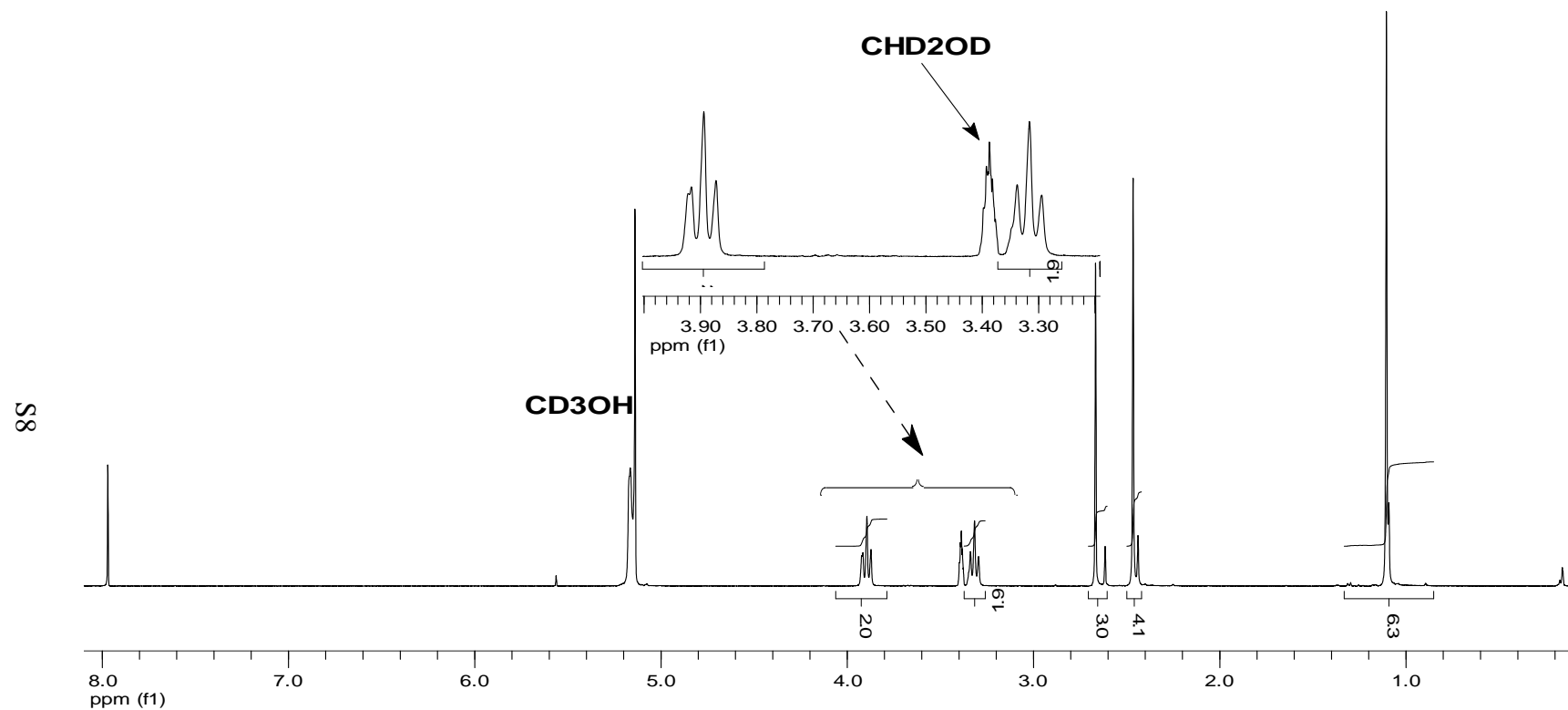


Figure S6. <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>) spectrum of (E)-2,5-dioxopyrrolidin-1-yl-3-(1-(2-tert-butoxy-2-oxoethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)acrylate (5).

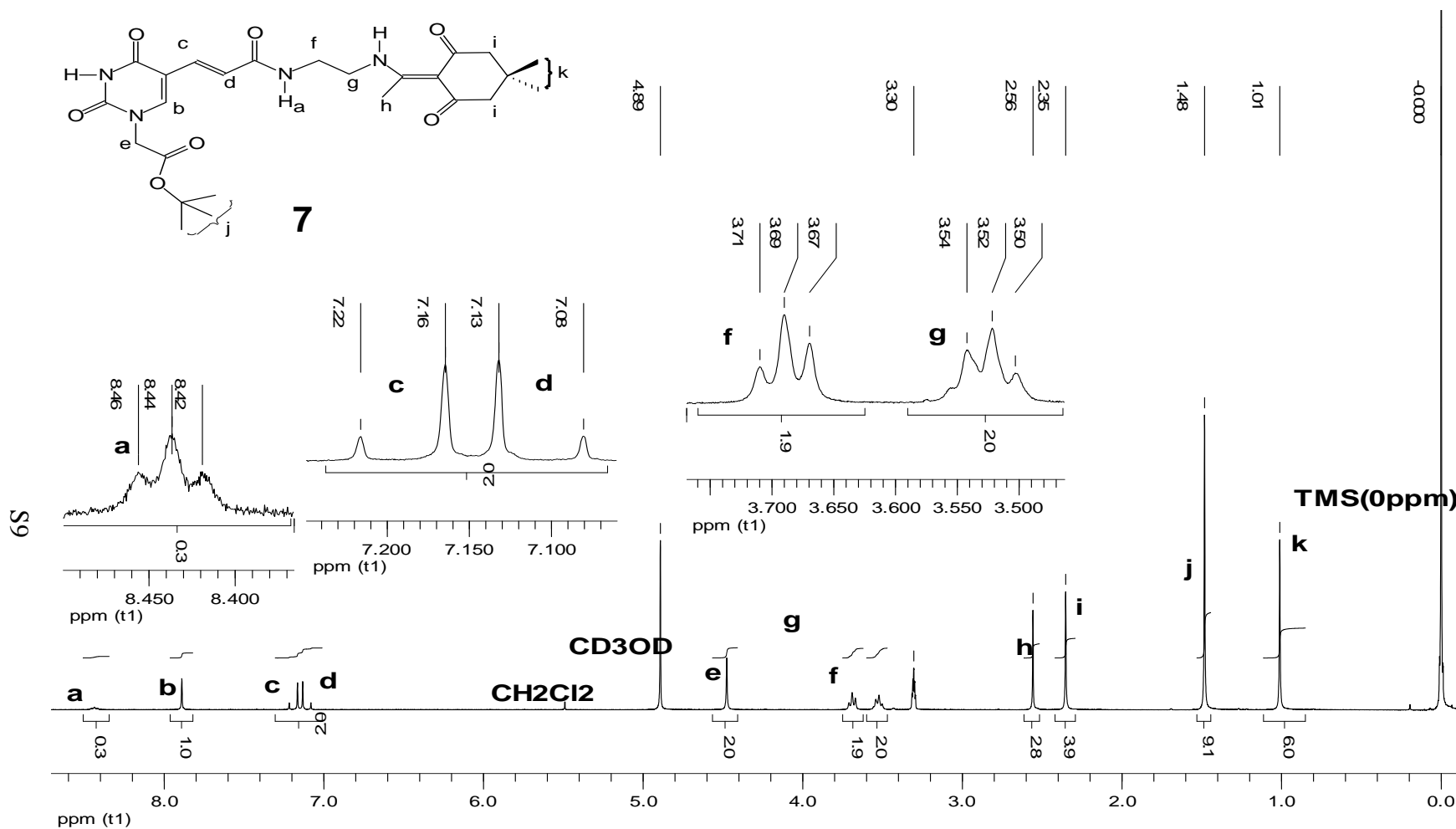


**6**

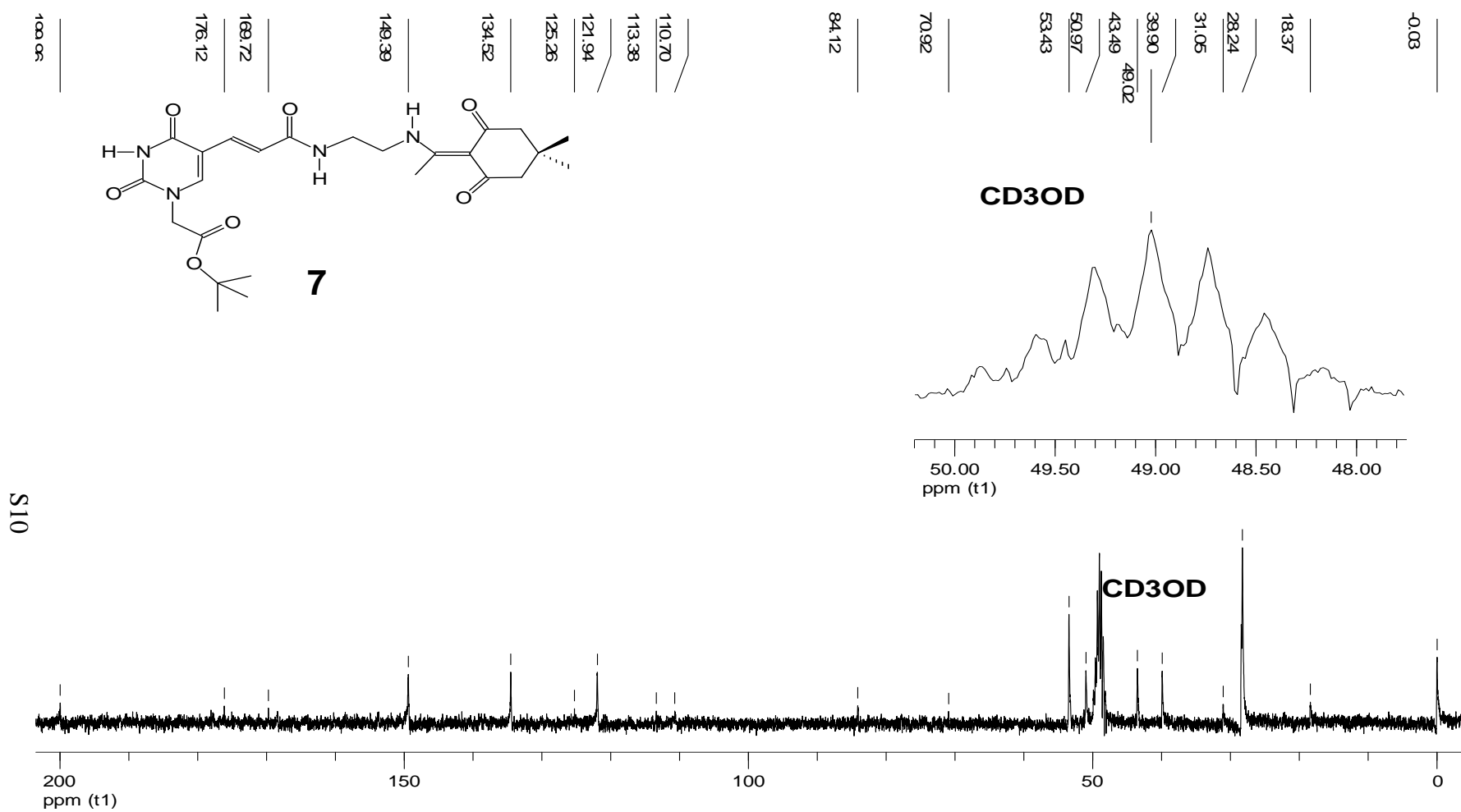


**Figure S7.** <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) spectrum of Synthesis of 2-(1-(2-aminoethylamino)ethylidene)-5,5-dimethyl-1,3-cyclohexanedione (**6**).





**Figure S8. <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) spectrum of (E)-tert-butyl 2-(5-(3-(2-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino)ethylamino)-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (7).**



**Figure S9.**  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ) spectrum of (E)-tert-butyl 2-(5-(3-(2-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino)ethylamino)-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**7**).

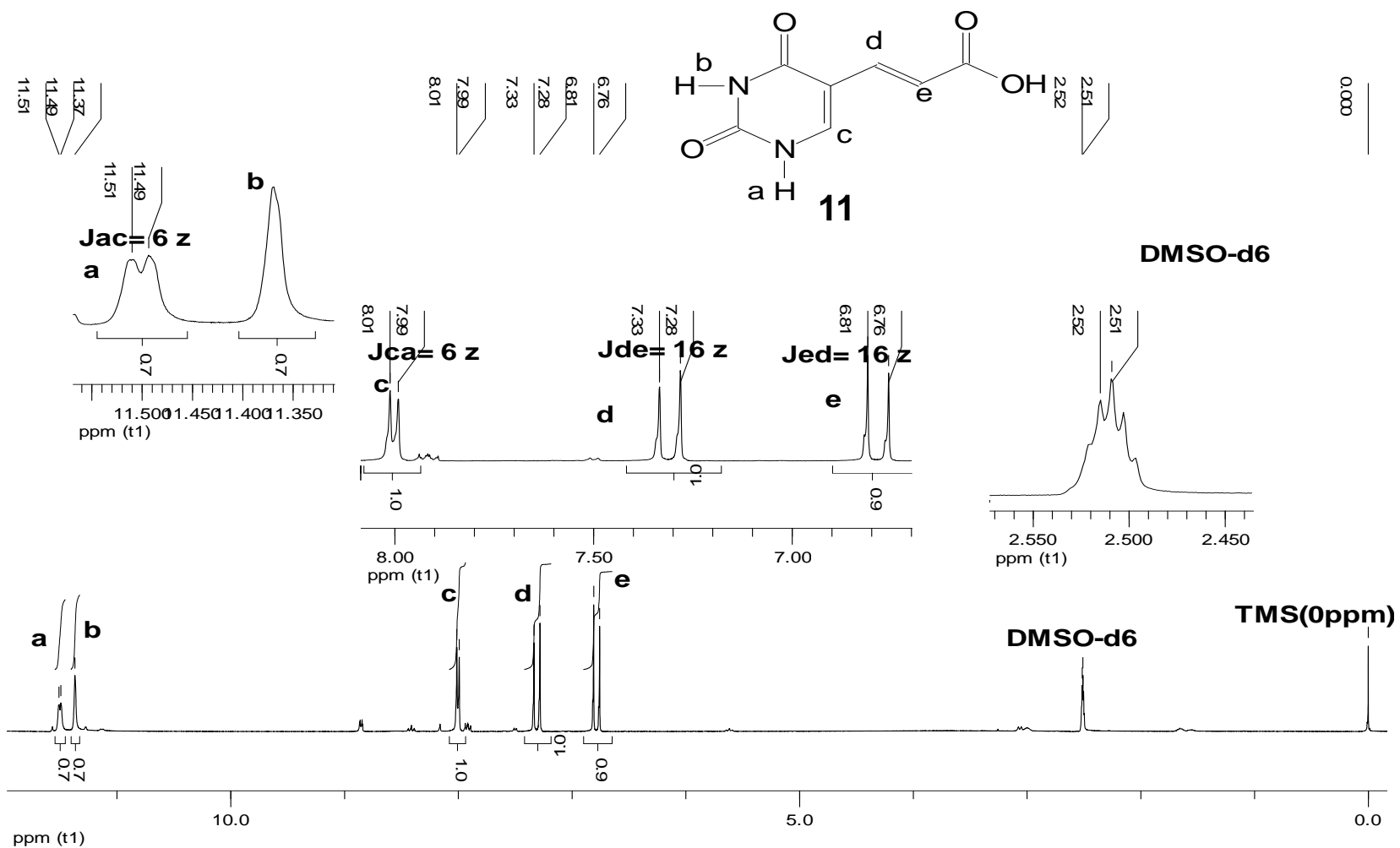


Figure S10. <sup>1</sup>H NMR(300 MHz, DMSO-d<sub>6</sub>) spectrum of (E)-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)acrylic acid (11).

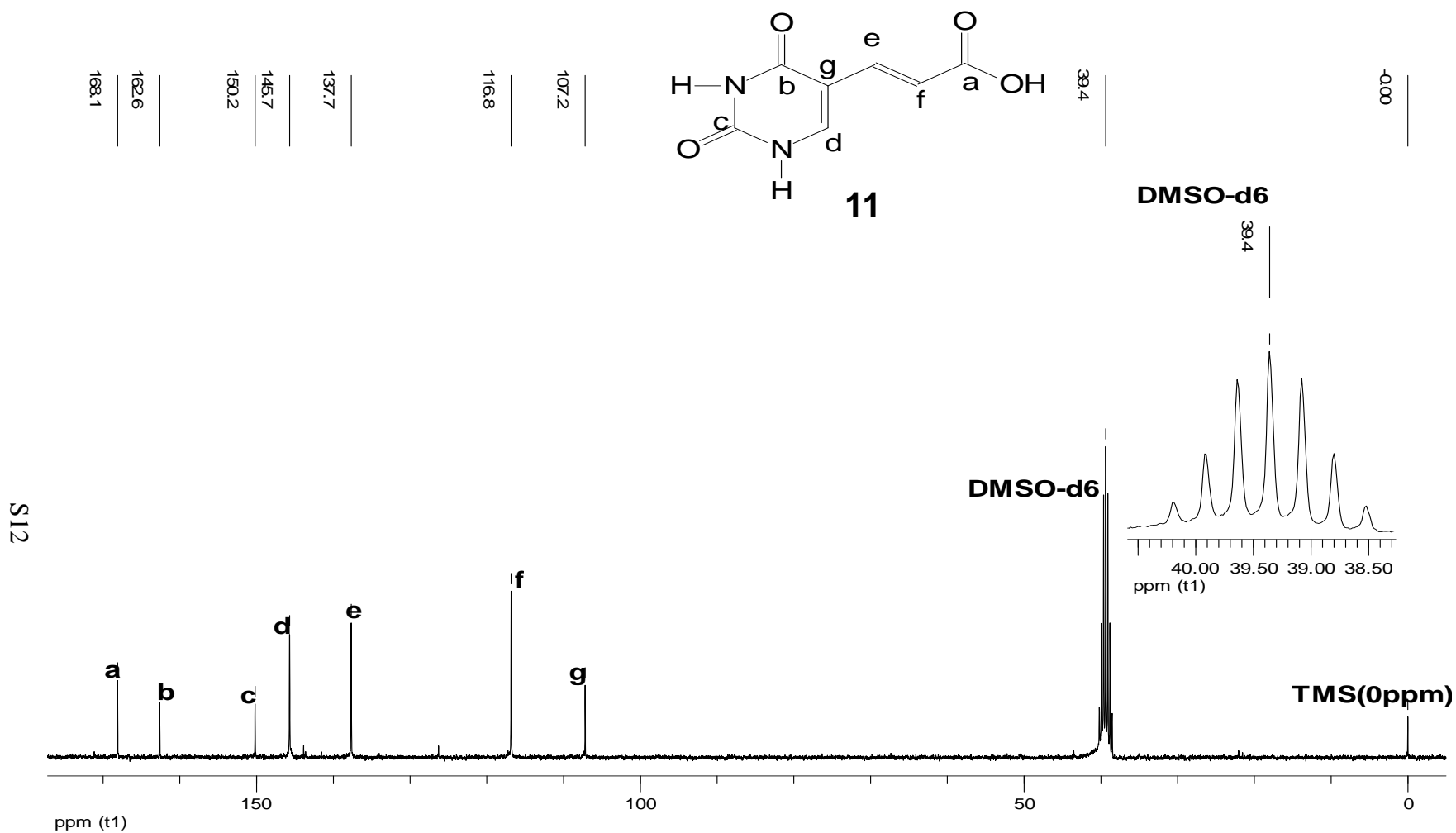
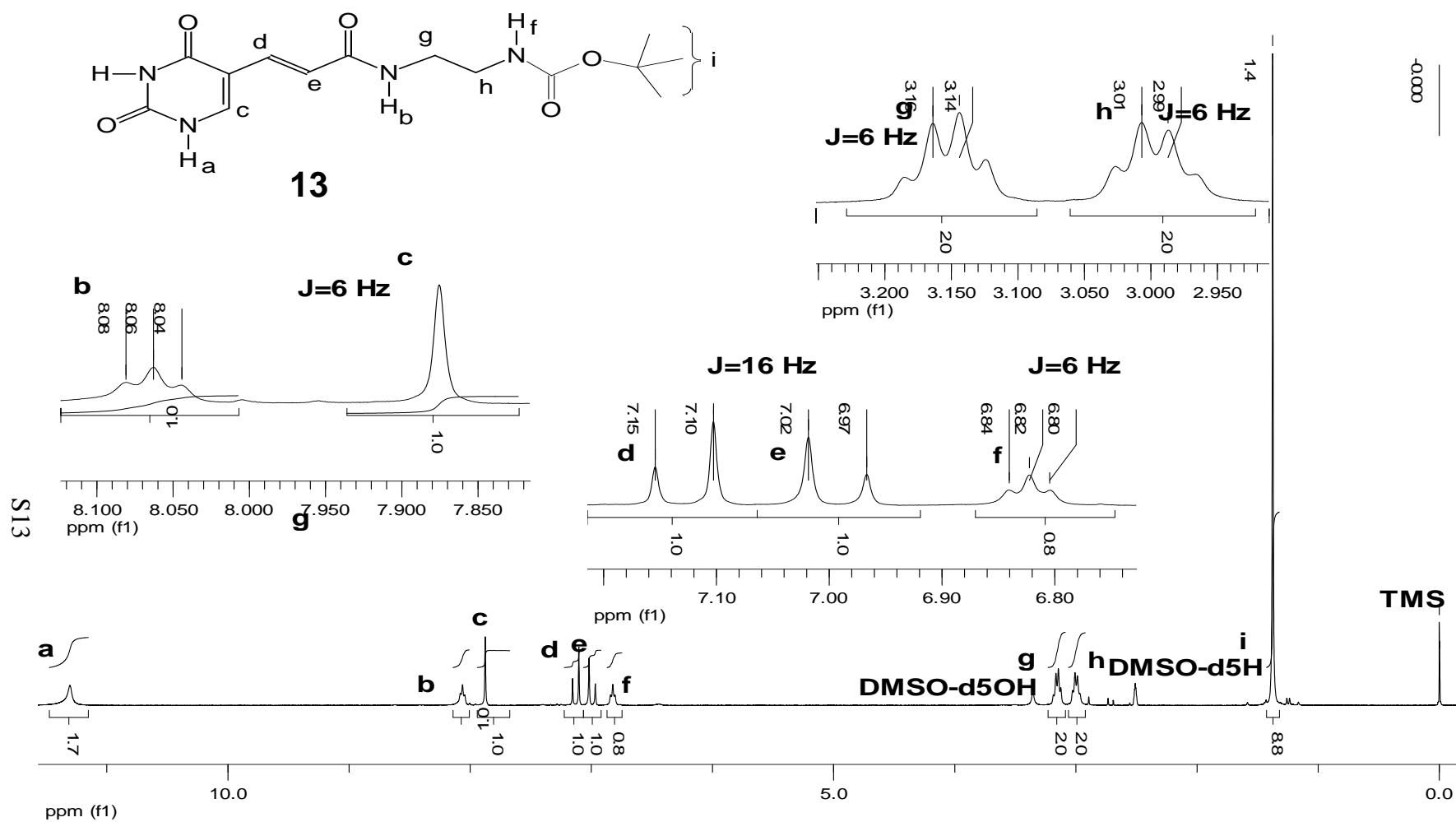
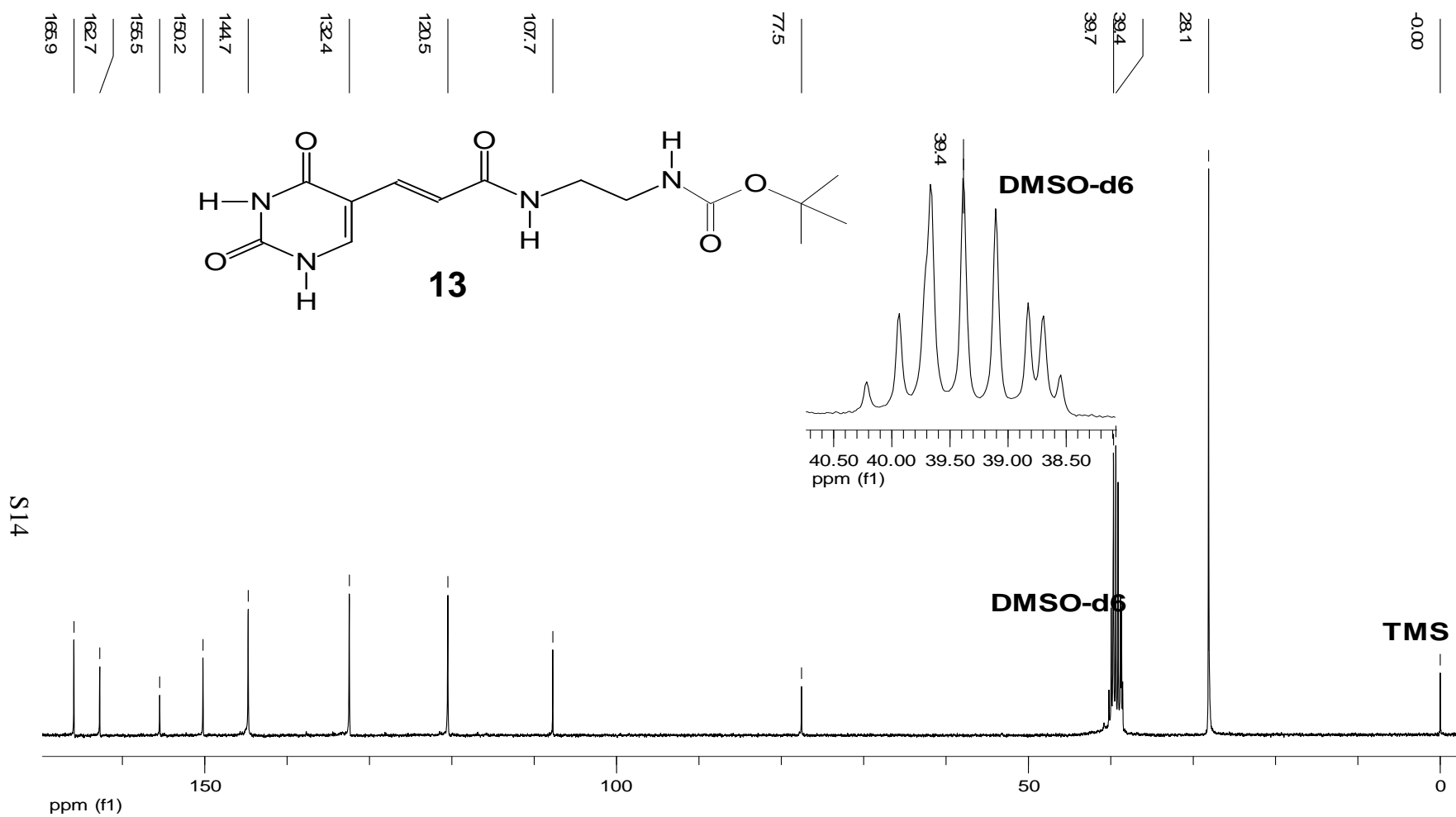


Figure S11. <sup>13</sup>C NMR(75 MHz, DMSO-d<sub>6</sub>) spectrum of (E)-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)acrylic acid (11).



**Figure S12.**  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-d}_6$ ) spectrum of (E)-tert-butyl-2-(3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)acrylamido)ethylcarbamate (**13**).



**Figure S13.**  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO-d}_6$ ) spectrum of (*E*)-tert-butyl 2-(3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)acrylamido)ethylcarbamate (**13**).

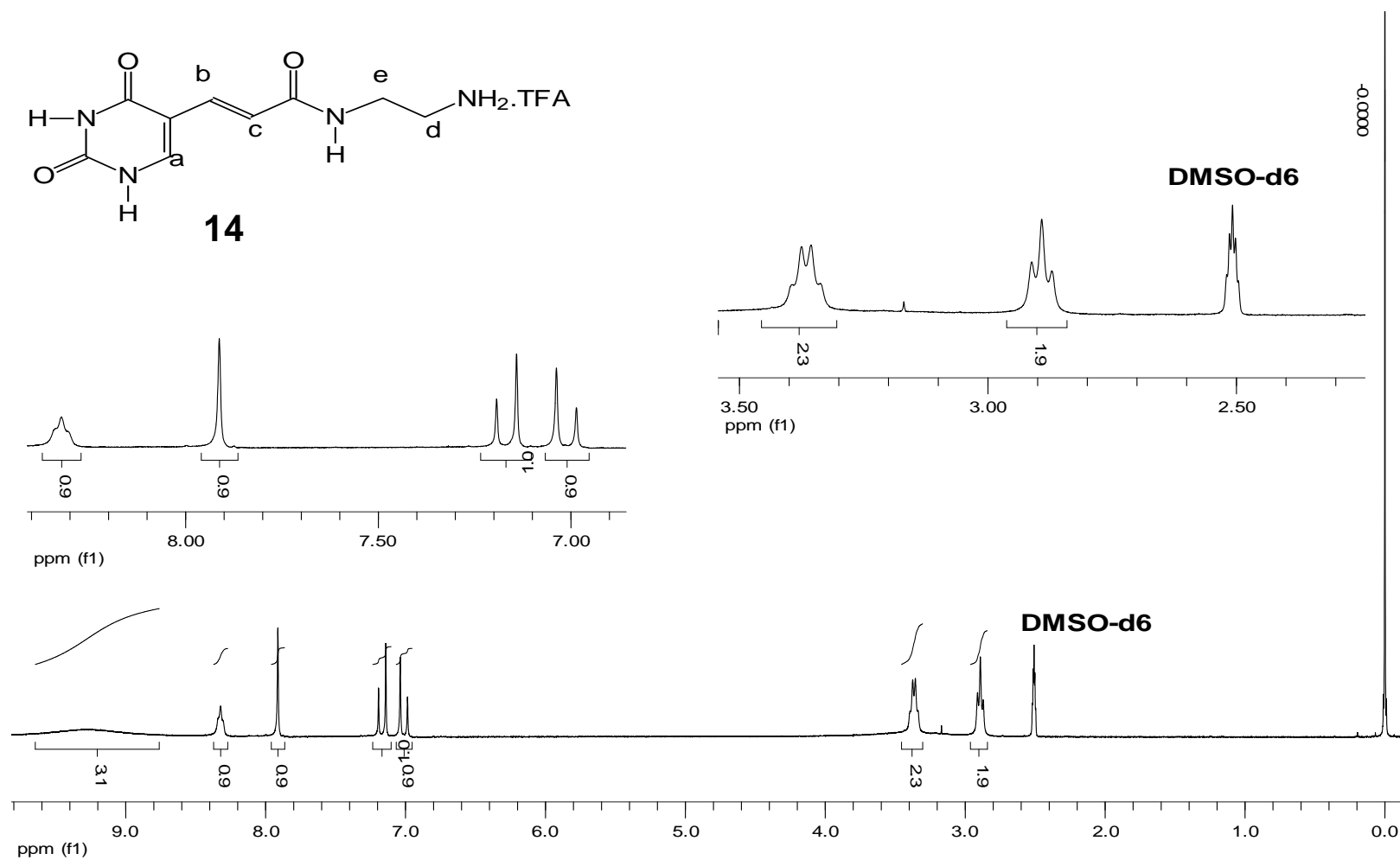


Figure S14. <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) spectrum of (E)-N-(2-aminoethyl)-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)acrylamide (**14**).

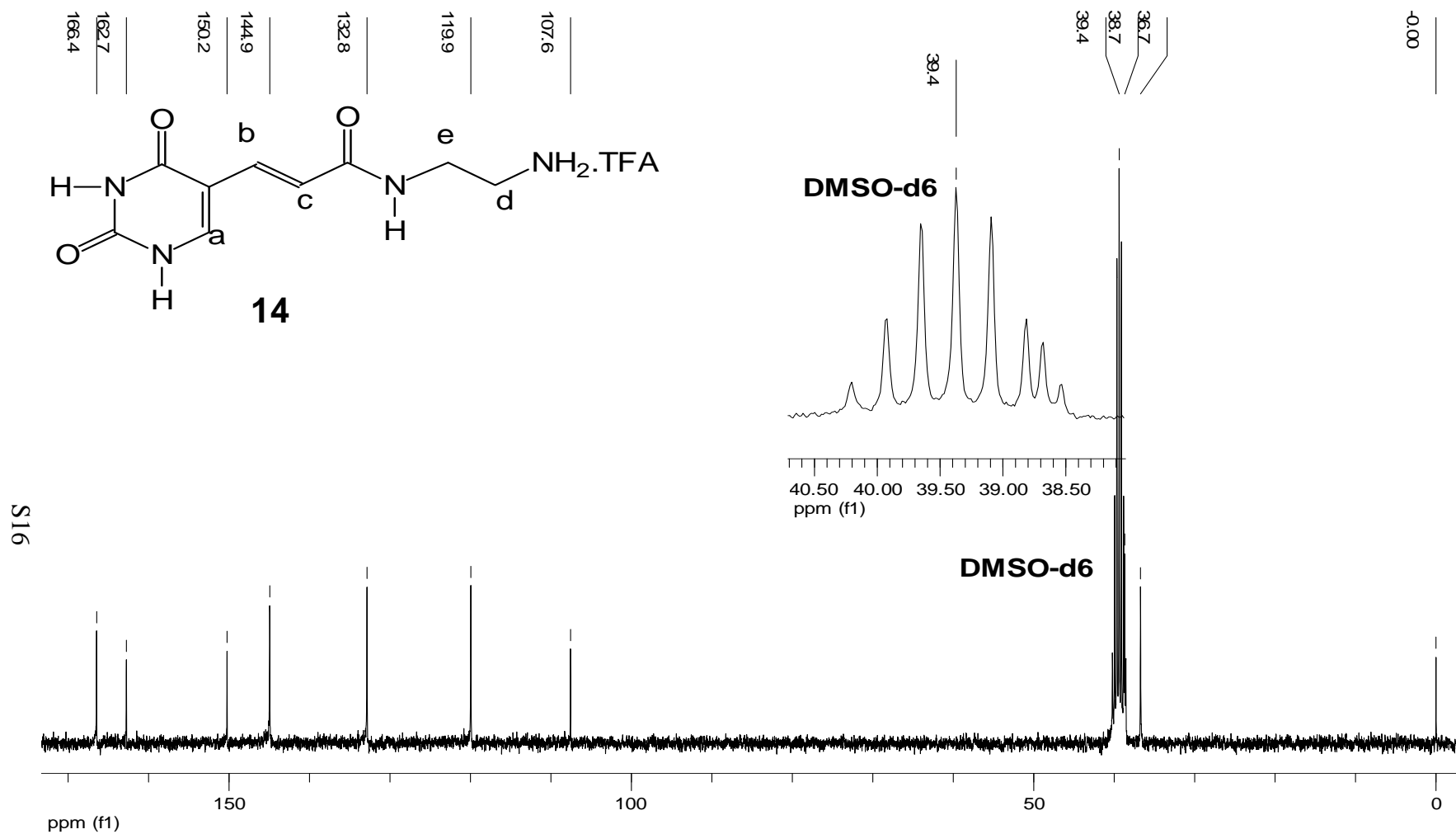
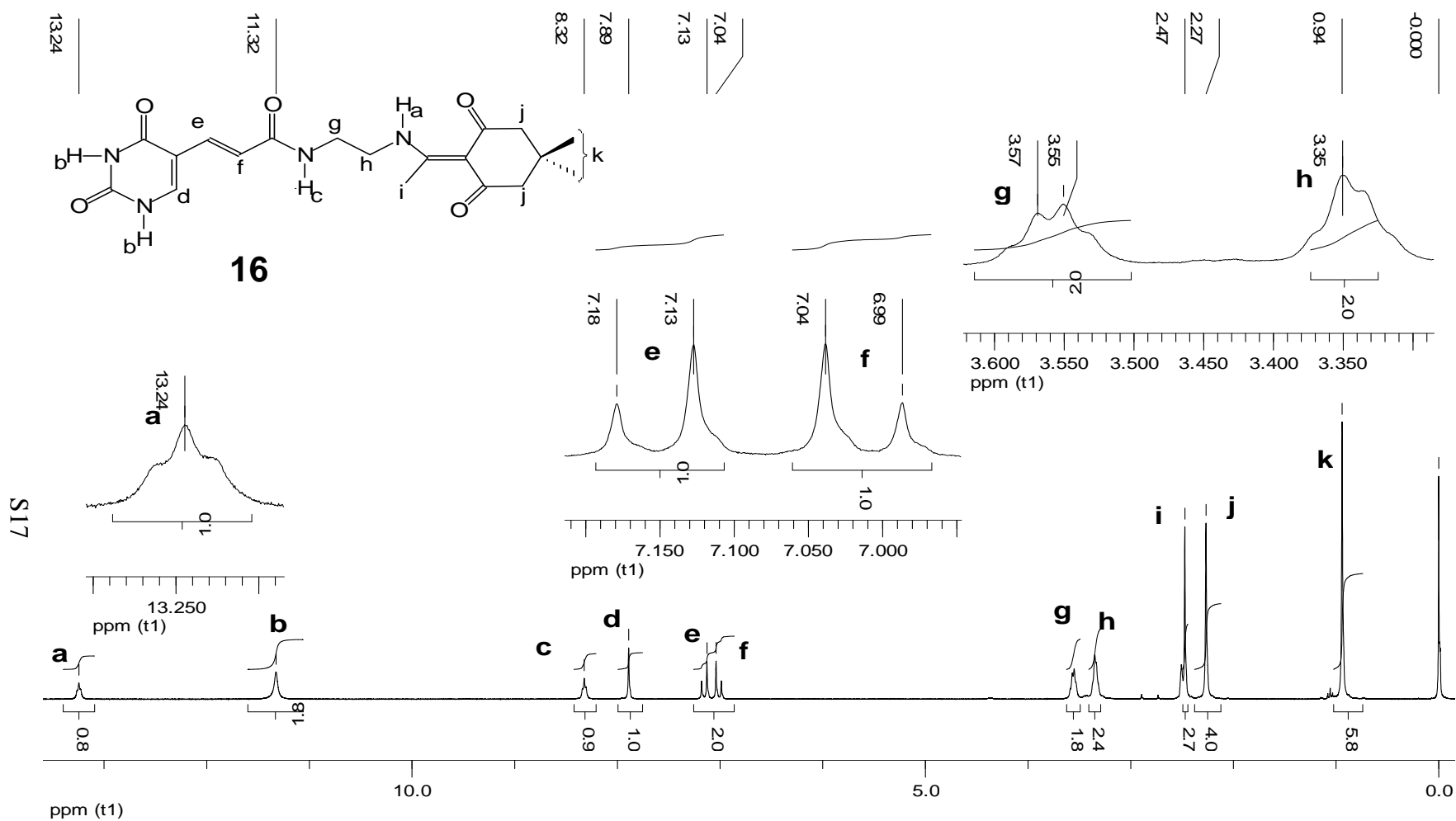


Figure S15. <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>) spectrum of (E)-N-(2-aminoethyl)-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)acrylamide (13).





**Figure S16.**  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-d}_6$ ) spectrum of (E)-N-(2-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino)-ethyl)-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)acrylamide (**16**).

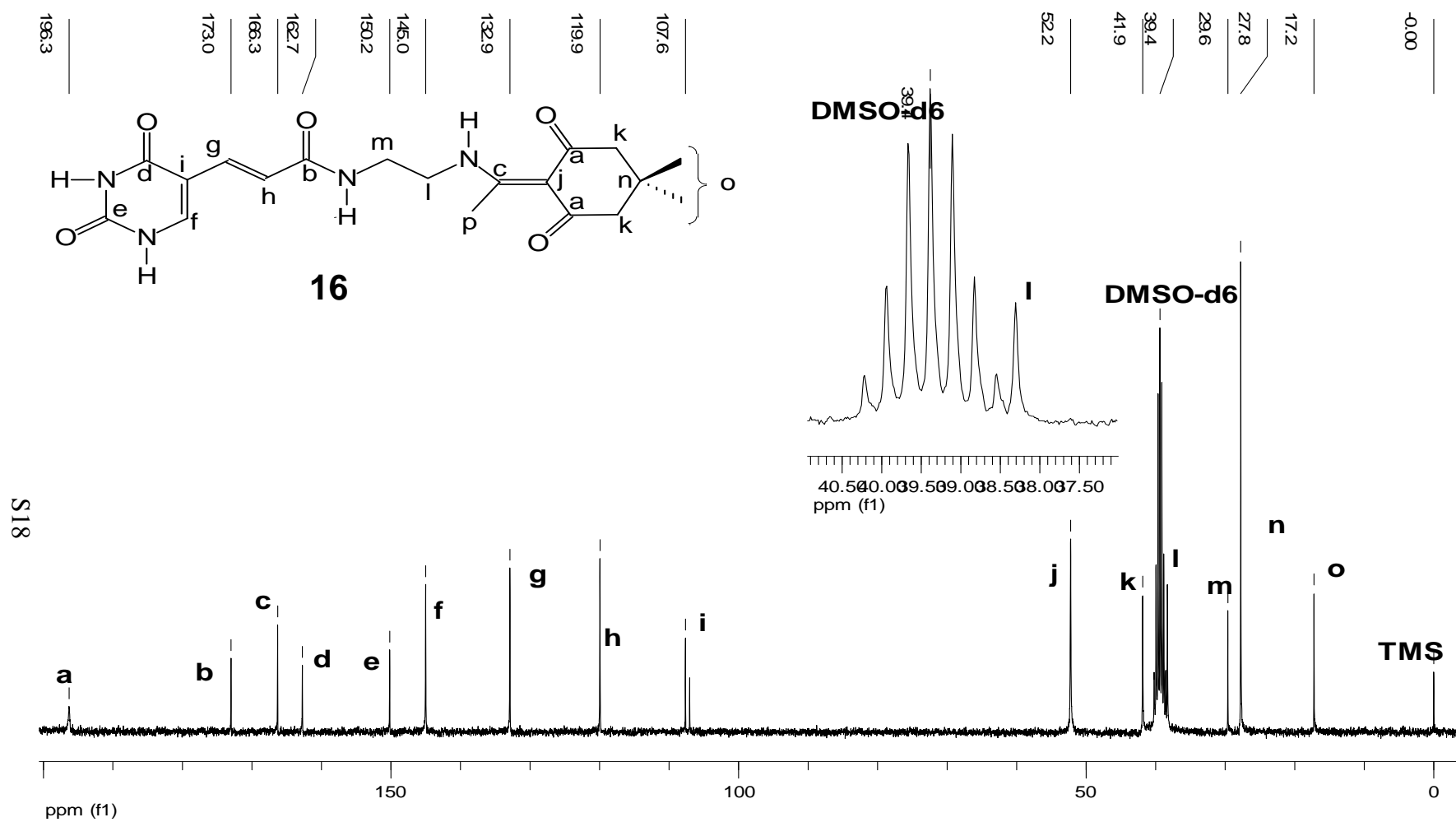


Figure S17.  $^{13}\text{C}$  NMR (75 MHz, DMSO- $\text{d}_6$ ) spectrum of (E)-N-(2-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino)-ethyl)-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)acrylamide (16).

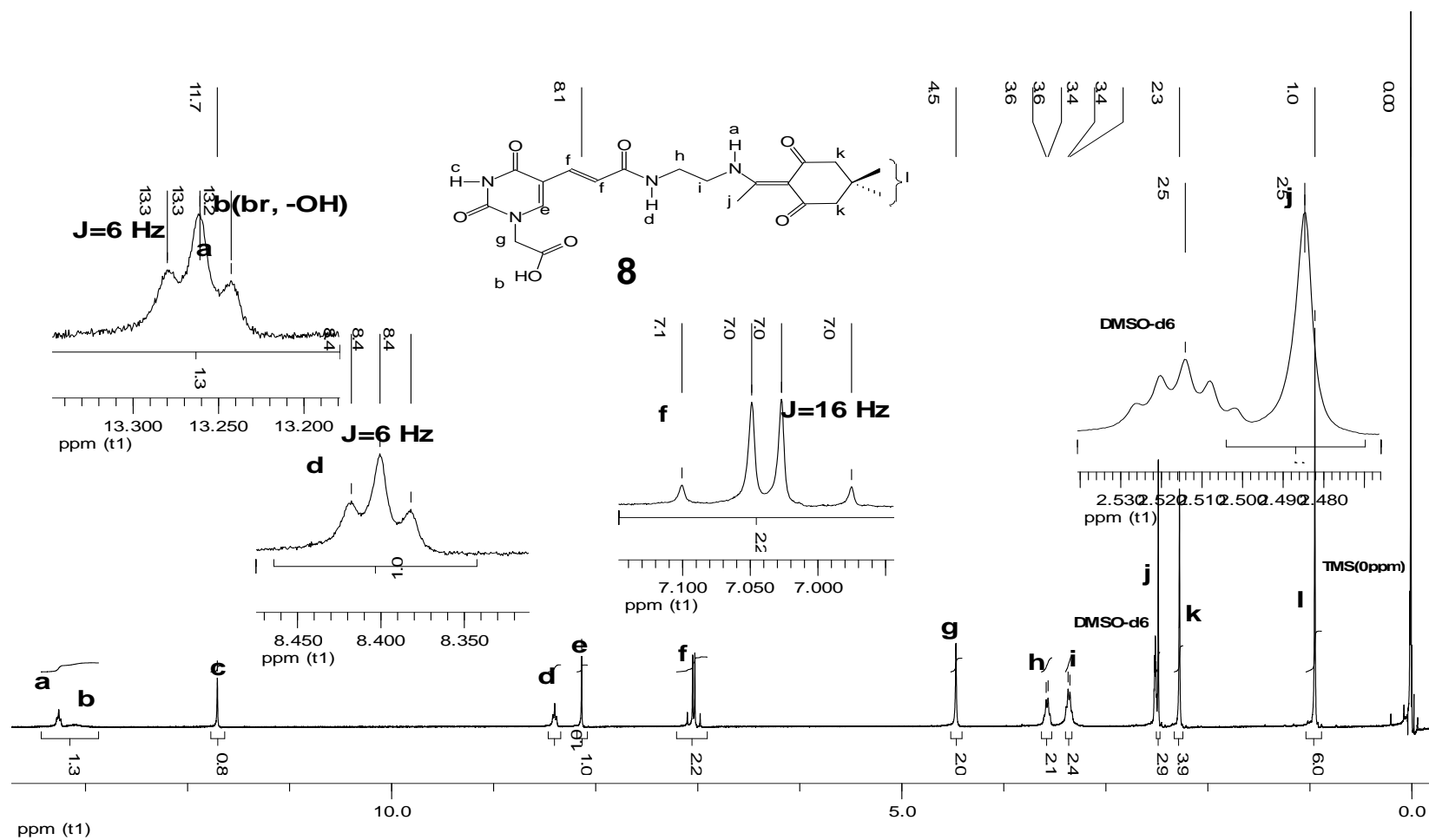


Figure S18.  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-d}_6$ ) spectrum of (E)-2-(5-(3-(2-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino)ethylamino)-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) acetic acid (**8**)

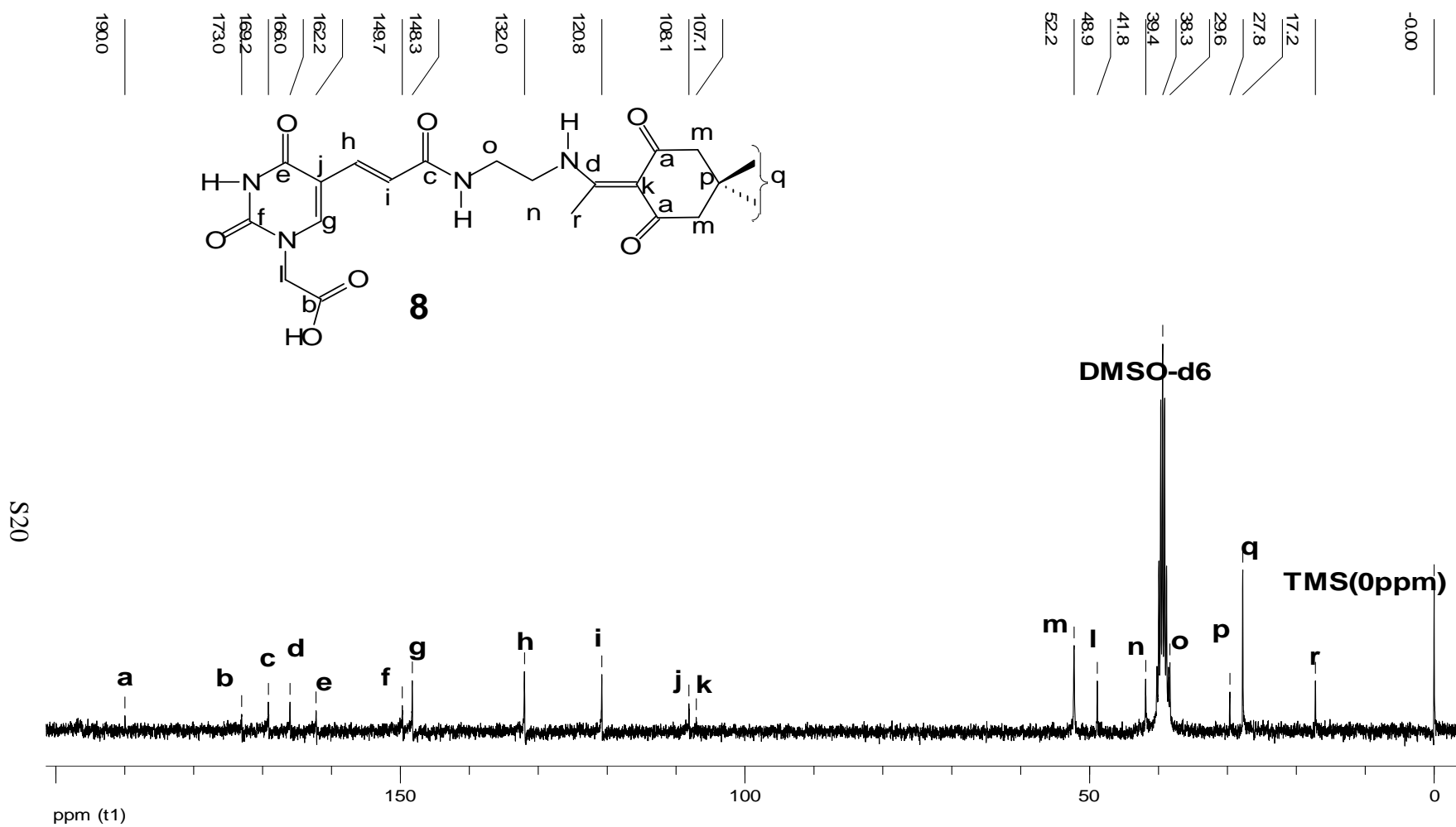
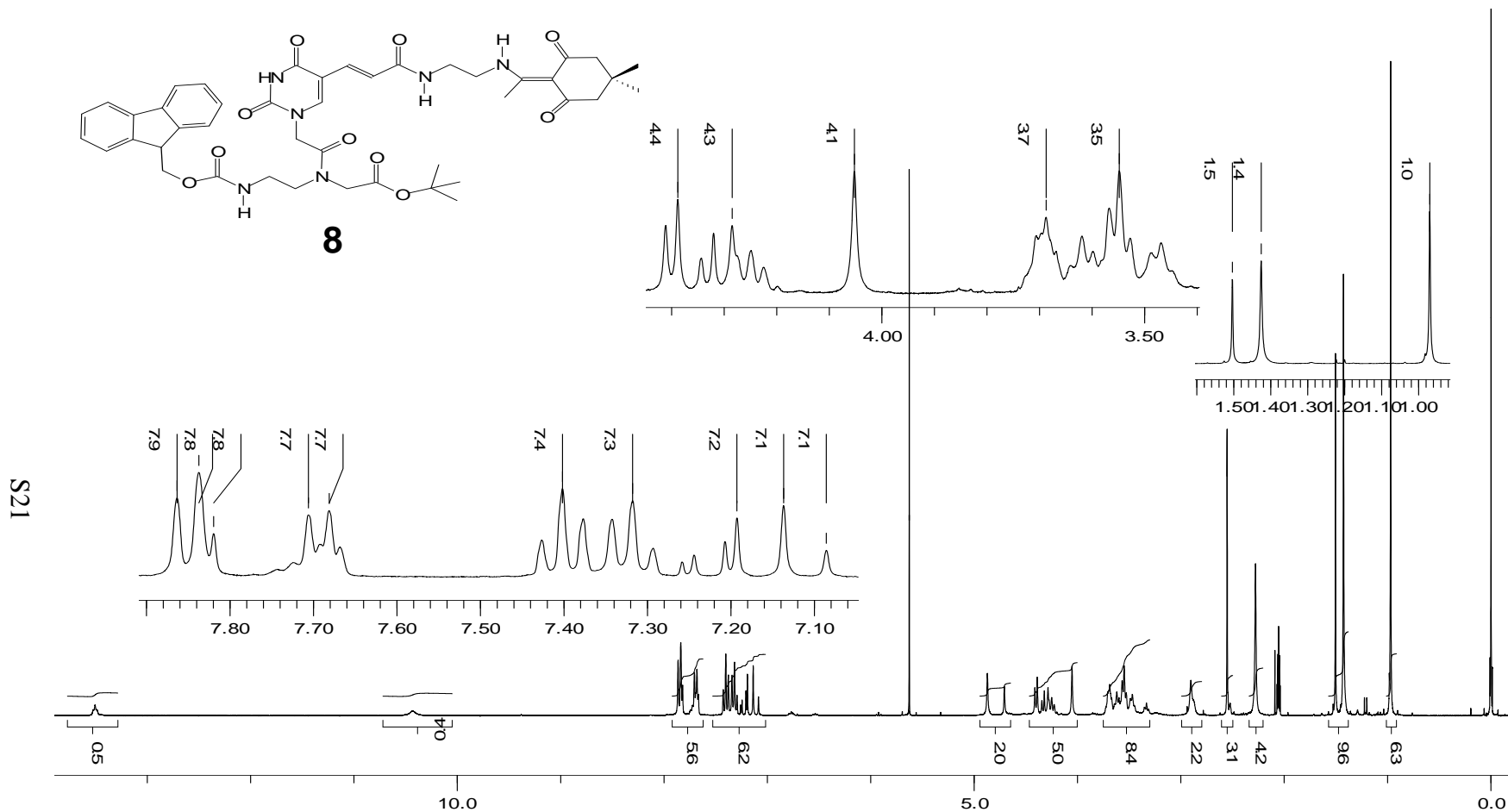


Figure S19.  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO-d}_6$ ) spectrum of (E)-2-(5-(3-(2-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino)ethylamino)-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) acetic acid (**8**).



**Figure S20.**  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{COCD}_3$ ) spectrum of (E)-tert-butyl 2-(N-(2-(((9H-fluoren-9-yl)methoxy)carbonylamino)ethyl)-2-(5-(3-(2-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino)ethylamino)-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido) acetate (**10**).

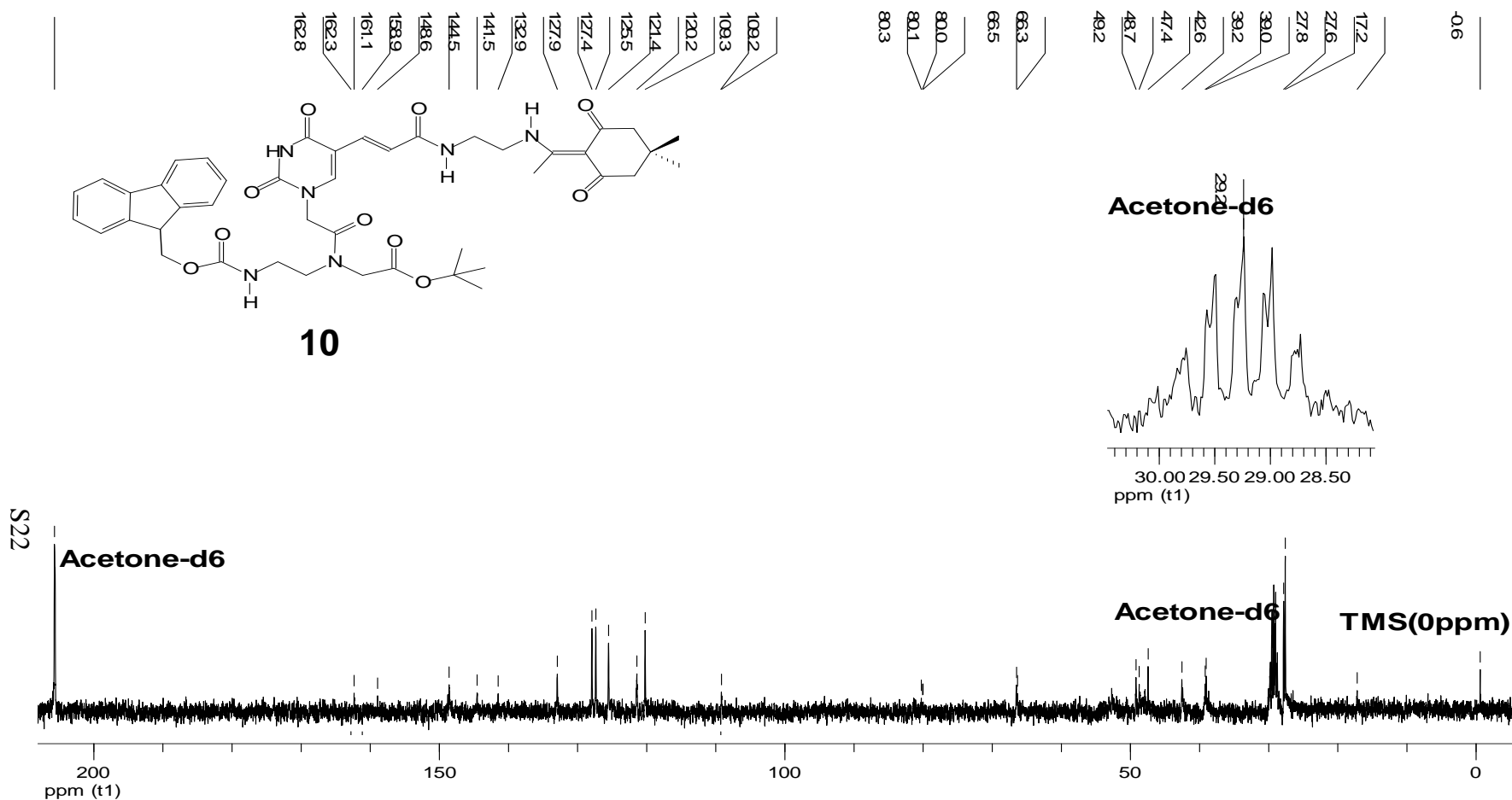
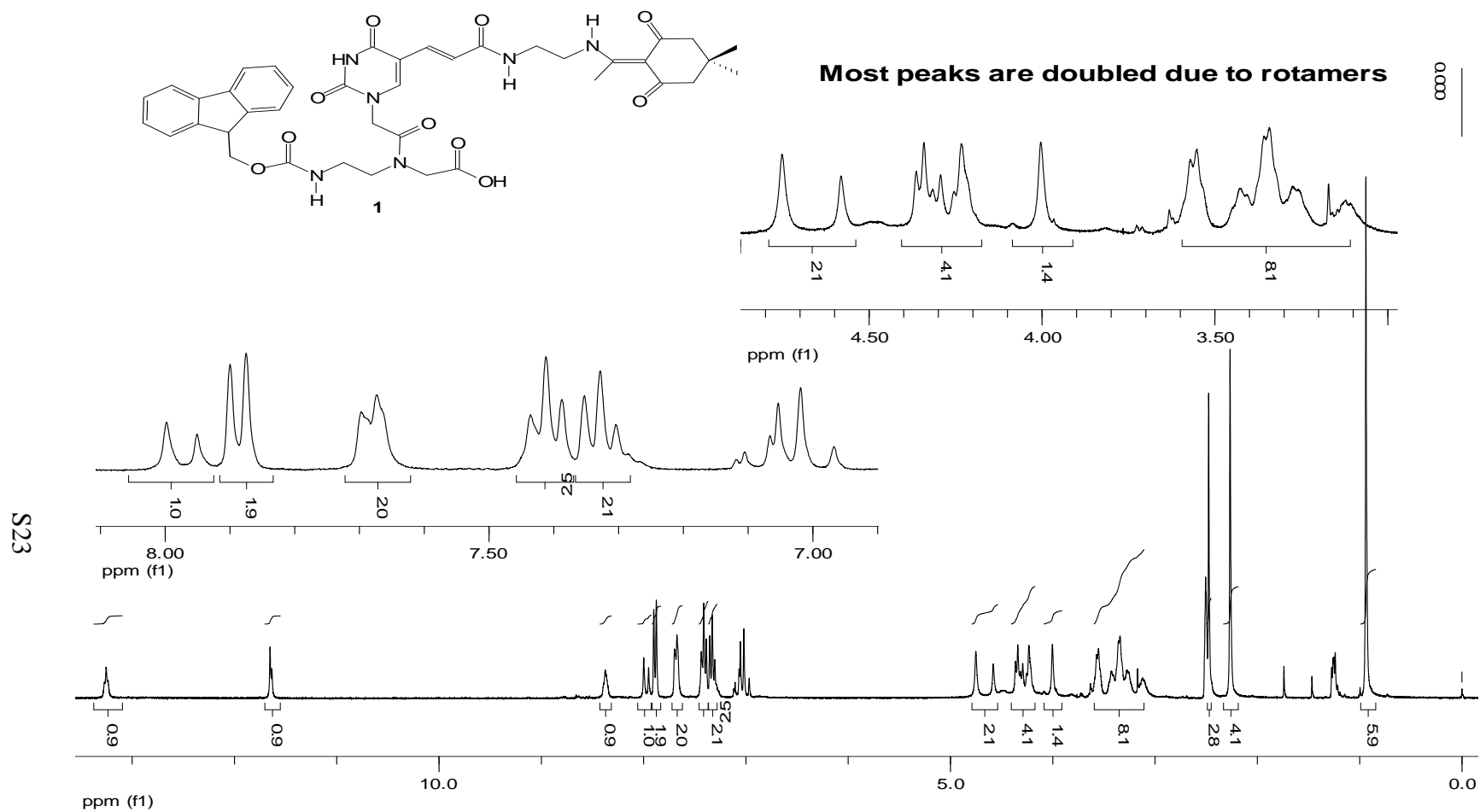
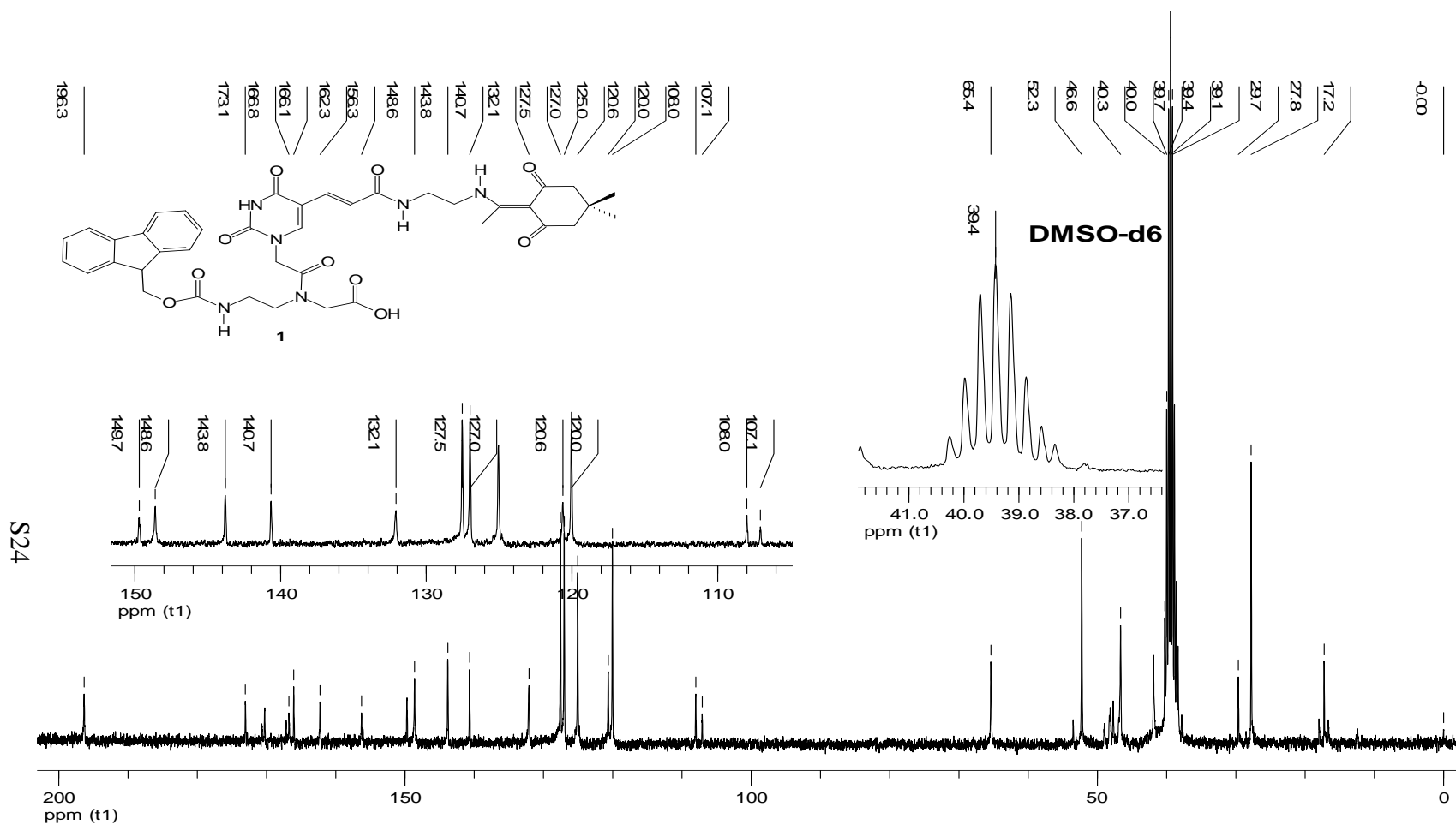


Figure S21.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{COCD}_3$ ) spectrum of (E)-tert-butyl 2-(N-(2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-2-(5-(3-(2-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino)ethylamino)-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido) acetate (**10**).



**Figure S22.** <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) spectrum of (E)-2-(N-(2-(((9H-fluoren-9-yl)methoxy)carbonylamino)ethyl)-2-(5-(3-(2-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino)ethylamino)-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)acetic acid; Fmoc-U' (Dde)-OH (**1**).



**Figure S23.** <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>) spectrum of (E)-2-(N-(2-(((9H-fluoren-9-yl)methoxy)carbonylamino)ethyl)-2-(5-(3-(2-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethylamino)ethylamino)-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)acetic acid; Fmoc-U' (Dde)-OH (1).