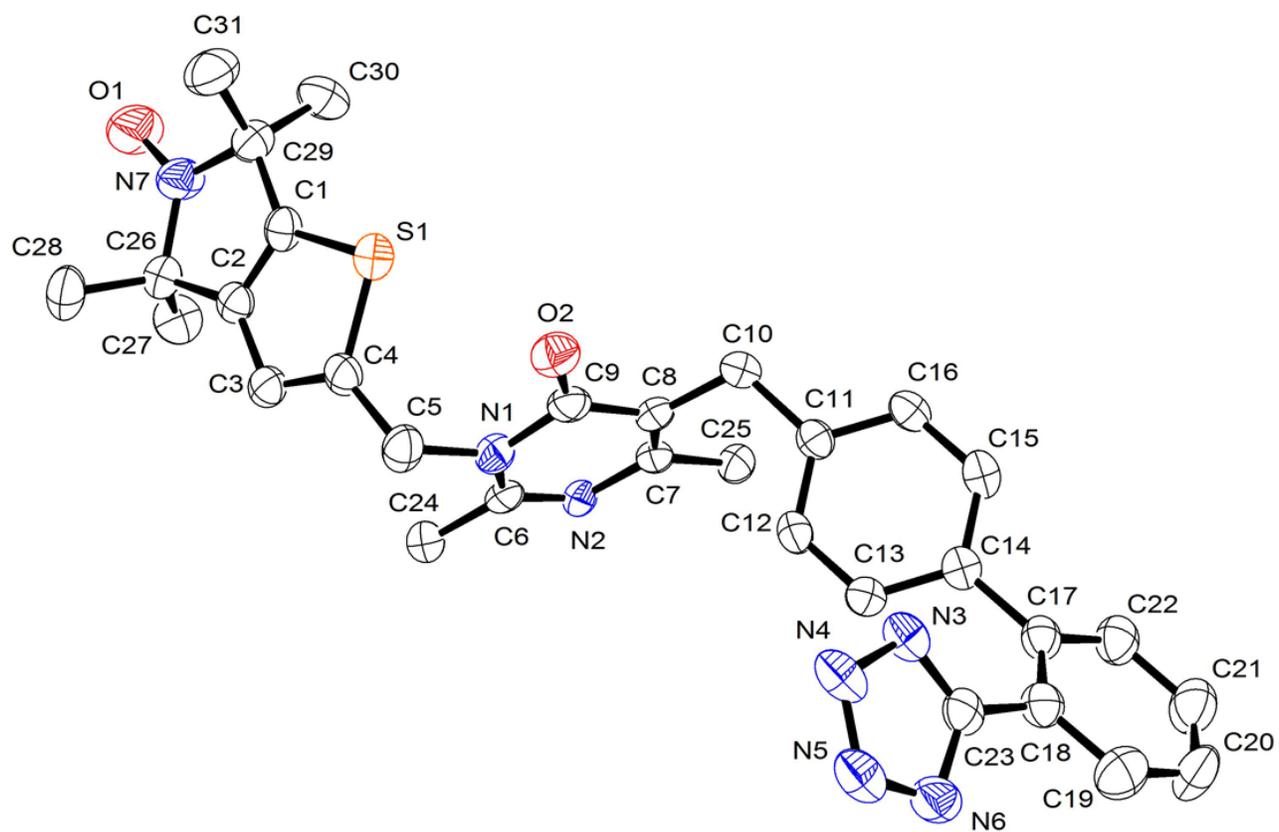


# Novel Paramagnetic AT<sub>1</sub> Receptor Antagonists

Nichole P. H. Tan,<sup>a,b</sup> Michelle K. Taylor,<sup>a,b,c</sup> Steven E. Bottle,<sup>a,d</sup> Christine E. Wright,<sup>a,e</sup>  
James Ziogas,<sup>a,e</sup> Jonathan M. White,<sup>b</sup> Carl H. Schiesser\*<sup>a,b</sup> and Nitya V. Jani\*<sup>a,e</sup>

## Electronic Supplementary Information

Figure S1, experimental details for the preparation of new compounds; HPLC traces for compounds **3**, **4**; EPR spectrum of compound **4** (R = Bu), <sup>1</sup>H and <sup>13</sup>C NMR spectra for compounds **9**, lucigenin assay protocol (28 pages).



**Fig. 1.** Perspective diagram of **4** (R = Me) with compound numbering.

### Compound 3 (R = Bu)

To a stirred solution of sodium hydride (50 % dispersion in mineral oil) (0.21 g, 5.34 mmol) in anhydrous DMF (12 mL) at 0 °C was added a solution of 6-butyl-2-methyl-5-[[2'-[1-(triphenylmethyl)-1*H*-tetrazol-5-yl][1,1'-biphenyl]-4-yl]methyl]pyrimidin-4(1*H*)-one (**5**, R = Bu) ( 2.00 g, 3.10 mmol) in anhydrous DMF (12 mL). After stirring for 10 min., lithium bromide (0.91 g, 5.34 mmol) in anhydrous DMF (20 mL) was added. After another 10 min., crude 3-bromomethyl-2,2,5,5-tetramethyl-2,5-dihydropyrrole-1-oxyl (**6**) (1.00 g, 4.28 mmol) in DMF (20 mL) was added to the reaction mixture. The ice-bath was then removed and the mixture was stirred for 2 hr at r.t. The mixture was then poured into a vigorously stirred ice-water slurry. The pH was adjusted to 5 with 5 % aqueous AcOH and the precipitate was filtered off and washed with H<sub>2</sub>O. Further purification of the crude via column chromatography (EtOAc: Pet. spirit, 3:1) yielded the crude tritylated product (1.23 g, 49.8 %) which was then dissolved in MeOH ( 70 mL) and refluxed overnight. The MeOH was removed *in vacuo* and the residue was purified by column chromatography ( EtOAc:MeOH, 3:1) to give **3** (R = Bu) as pale yellow foam (0.56 g, 33 %). IR  $\nu_{\max}$  (neat): 1554, 1643, 2972, 3330 cm<sup>-1</sup>. MS (ESI) *m/z* 575.3 [M+Na]<sup>+</sup>. HRMS calcd. for C<sub>32</sub>H<sub>38</sub>N<sub>7</sub>O<sub>2</sub> [M+Na]<sup>+</sup> 575.29792; found,575.29790. HPLC purity analysis showed that compound **3** (R = Bu) is 98.5 % pure.

### Compound 4 (R = Bu)

To a stirred solution of NaH (60 % dispersion in mineral oil) (0.052 g, 0.777 mmol) in anhydrous DMF (3.1 mL) at 0°C was added a solution of 6-butyl-2-methyl-5-[[2'-[1-(triphenylmethyl)-1*H*-tetrazol-5-yl][1,1'-biphenyl]-4-yl]methyl]pyrimidin-4(1*H*)-one (**5**, R = Bu) (0.500 g, 0.777 mmol) in anhydrous DMF (6.2 mL). After 10 min, LiBr (0.230 g, 2.64 mmol) dissolved in anhydrous DMF (6.2 mL) was added. After stirring for 10 min, 2-(bromomethyl)-4,4,6,6-tetramethyl-4,6-dihydro-5*H*-thieno[2,3-*c*]pyrrol-5-yloxyl (**7**) (0.310 g, 1.08 mmol) in DMF (6.2 mL) was added and the cold bath was removed and the mixture stirred at r.t. for 2 hr. The mixture was then poured into a vigorously stirred ice-water slurry. The pH was adjusted to 5 with 5 % aq. AcOH. The precipitate was filtered off and washed with H<sub>2</sub>O. The crude material was purified by flash chromatography (Pet. spirit:EOAc, 2:1, followed by Pet. spirit:EtOAc, 1:1) to furnish the title compound as slight pale yellow foam which was dissolved in MeOH (35 mL) and refluxed overnight. The MeOH was then removed *in vacuo* and the residue was purified by flash chromatography (straight Et<sub>2</sub>O, followed by EtOAc:MeOH, 6:1) to afford compound **4** (R = Bu) ( 0.31 g, 42 %) as pale yellow foam. IR  $\nu_{\max}$  (neat): 747, 1542, 1651, 2972 cm<sup>-1</sup> The ESR

spectrum displayed a triplet with hyperfine coupling constant  $A_N = 15.0805$  and  $g = 2.00375$ . HRMS (ESI) calcd. for  $C_{34}H_{38}N_7O_2 [M+Na]^+$  631.26999, found, 631.26996. Anal. calcd. for  $C_{34}H_{38}N_7O_2S$ : C 67.08; H 6.29; N 16.11; O 5.26; S 5.27 %, found: C 67.03; H 6.39; N 16.23; O 5.3; S 5.05 %. HPLC purity analysis showed that compound **4** (R = Bu) is 98.4 % pure.

**Compound 3 (R = Me)** was isolated as pale yellow foam (200mg, 49 % over two steps) following the procedure described above, using 2,6-dimethyl-5-[[2'-[1-(triphenylmethyl)-1*H*-tetrazol-5-yl][1,1'-biphenyl]-4-yl]methyl]pyrimidin-4(1*H*)-one (**5**, R = Me) (298 mg, 1.28 mmol) and 3-bromomethyl-2,2,5,5-tetramethyl-2,5-dihydropyrrole-1-oxyl (**6**) (555 mg, 0.93 mmol). IR  $\nu_{max}$  (neat): 756, 1246, 1538, 1658, 1733, 2976  $cm^{-1}$ . MS (ESI)  $m/z$  511.3 4  $[M+H]^+$ . HRMS (ESI) calcd. for  $C_{29}H_{32}N_7O_2 [M+Na]^+$  533.25097; found, 533.25073 HPLC purity analysis showed that compound **3** (R = Me) is over 99 % pure.

**Compound 4 (R = Me)** was prepared as pale yellow foam (200mg, 46 % over two steps) following the procedure described above, using 2,6-dimethyl-5-[[2'-[1-(triphenylmethyl)-1*H*-tetrazol-5-yl][1,1'-biphenyl]-4-yl]methyl]pyrimidin-4(1*H*)-one (**5**, R = Me) (0.52 g, 0.874 mmol) and 2-(bromomethyl)-4,4,6,6-tetramethyl-4,6-dihydro-5*H*-thieno[2,3-*c*]pyrrol-5-yloxy (**7**) (0.350 mg, 1.21 mmol). IR  $\nu_{max}$  (neat): 761, 1241, 1541, 1651, 2976  $cm^{-1}$ . MS (ESI)  $m/z$  567.2  $[M+H]^+$ . HRMS(ESI) calcd. for  $C_{31}H_{32}N_7O_2S [M+Na]^+$ , 589.22304; found, 589.22272. HPLC purity analysis showed that compound **4** (R = Me) is over 99 % pure.

**Compound 3 (R = Et)** was prepared as pale yellow foam (168 mg, 38 % over two steps) following the procedure described above, using 6-ethyl-2-methyl-5-[[2'-[1-(triphenylmethyl)-1*H*-tetrazol-5-yl][1,1'-biphenyl]-4-yl]methyl]pyrimidin-4(1*H*)-one (**5**, R = Et) (640 mg, 1.04 mmol) and 3-bromomethyl-2,2,5,5-tetramethyl-2,5-dihydropyrrole-1-oxyl (**6**) (360 mg, 1.56 mmol). IR  $\nu_{max}$  (neat): 1544, 1652, 2976  $cm^{-1}$ . MS (ESI)  $m/z$  525.3  $[M+H]^+$ . HRMS calcd. for  $C_{30}H_{34}N_7O_2 [M+Na]^+$ , 547.26662; found, 547.26648. HPLC purity analysis showed that compound **3** (R = Et) is over 99 % pure.

**Compound 4 (R = Et)** was prepared as pale yellow foam (212 mg, 39 % over two steps) following the procedure described above, using 6-ethyl-2-methyl-5-[[2'-[1-(triphenylmethyl)-1*H*-tetrazol-5-

y][1,1'-biphenyl]-4-yl)methyl]pyrimidin-4(1*H*)-one (**5**, R = Et) (640 mg, 1.04 mmol) and 2-(bromomethyl)-4,4,6,6-tetramethyl-4,6-dihydro-5*H*-thieno[2,3-*c*]pyrrol-5-yloxyl (**7**) (360 mg, 1.56 mmol). IR  $\nu_{\max}$  (neat): 761, 1542, 1648, 2977  $\text{cm}^{-1}$ . MS (ESI)  $m/z$  581.3 [M+H]<sup>+</sup>. HRMS calcd. for C<sub>32</sub>H<sub>34</sub>N<sub>7</sub>O<sub>2</sub>S [M+Na]<sup>+</sup>, 603.23869; found, 603.23859. HPLC purity analysis showed that compound **4** (R = Et) is over 99 % pure.

**Compound 3 (R = Pr)** was prepared as pale yellow foam (155 mg, 47 % over two steps) following the procedure described above, using 6-propyl-2-methyl-5-[[2'-[1-(triphenylmethyl)-1*H*-tetrazol-5-y][1,1'-biphenyl]-4-yl)methyl]pyrimidin-4(1*H*)-one (**5**, R = Et) (402 mg, 0.64 mmol) and 3-bromomethyl-2,2,5,5-tetramethyl-2,5-dihydropyrrole-1-oxyl (**6**) (224 mg, 0.96 mmol). MS (ESI)  $m/z$  539.3 [M+H]<sup>+</sup>. HRMS calcd. for C<sub>31</sub>H<sub>36</sub>N<sub>7</sub>O<sub>2</sub> [M+Na]<sup>+</sup>, 561.28227; found, 561.28211. HPLC purity analysis showed that compound **3** (R = Pr) is over 99 % pure.

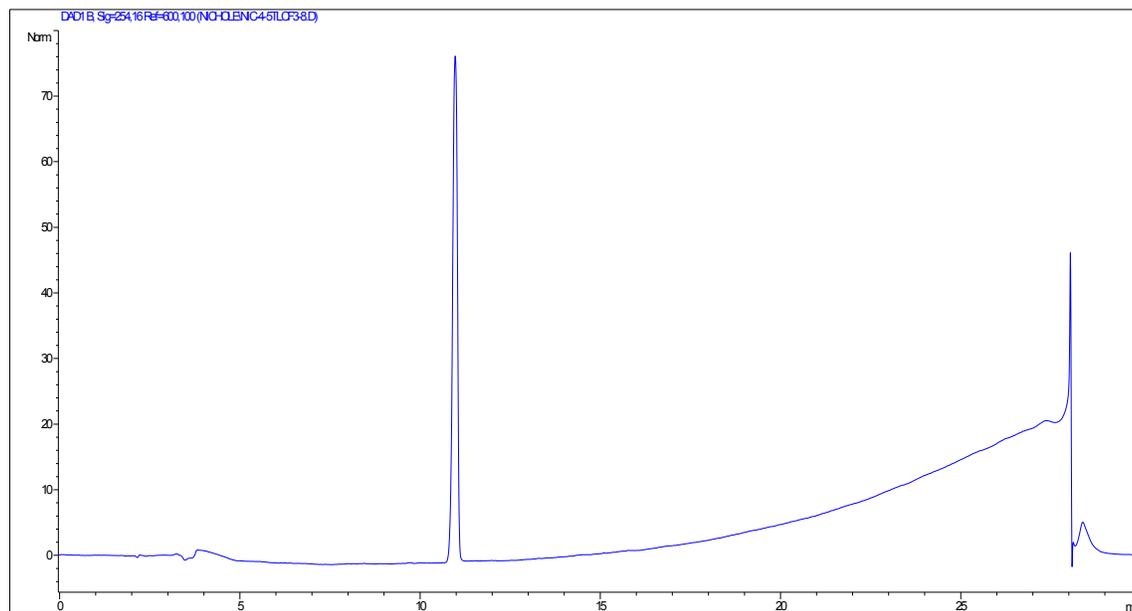
**Compound 4 (R = Pr)** was prepared as pale yellow foam (262 mg, 56 % over two steps) following the procedure described above, using 6-propyl-2-methyl-5-[[2'-[1-(triphenylmethyl)-1*H*-tetrazol-5-y][1,1'-biphenyl]-4-yl)methyl]pyrimidin-4(1*H*)-one (**5**, R = Pr) (550 mg, 0.88 mmol) and 2-(bromomethyl)-4,4,6,6-tetramethyl-4,6-dihydro-5*H*-thieno[2,3-*c*]pyrrol-5-yloxyl (**7**) (450 mg, 3.00 mmol). IR  $\nu_{\max}$  (neat): 735, 1542, 1650, 2974  $\text{cm}^{-1}$ . MS (ESI)  $m/z$  595.3 [M+H]<sup>+</sup>. HRMS calcd. for C<sub>33</sub>H<sub>36</sub>N<sub>7</sub>O<sub>2</sub>S [M+Na]<sup>+</sup>, 617.25434; found, 617.25402. HPLC purity analysis showed that compound **16** is over 99 % pure.

#### **Compound 9 (R = Bu).**

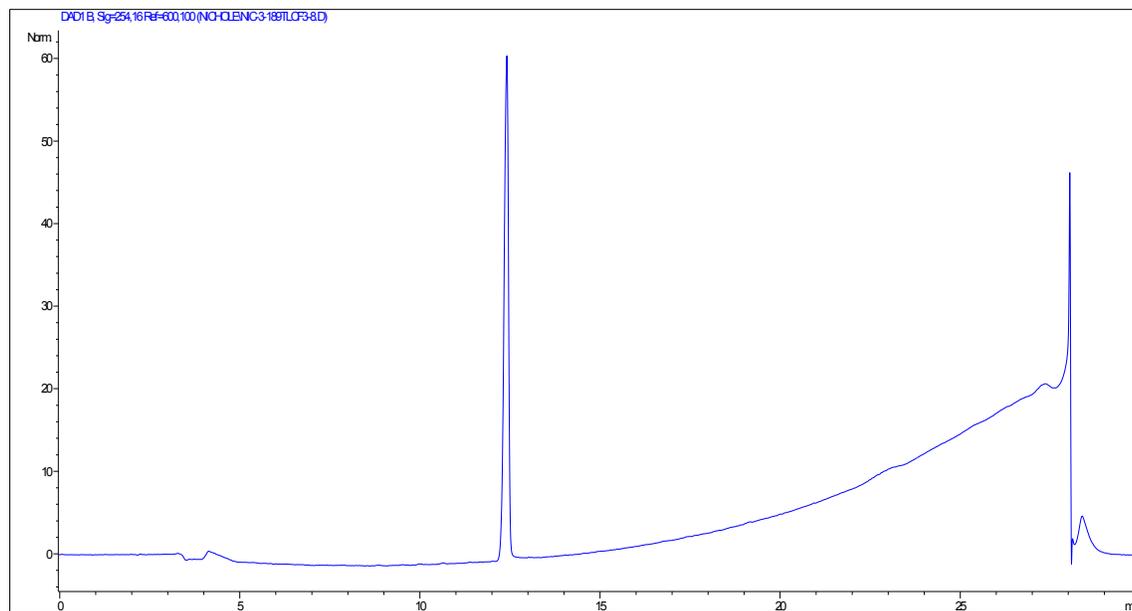
Aqueous 30% hydrogen peroxide (0.05 mL, 0.047 mmol) was added to a solution of iron (II) heptahydrate (71.0 mg, 0.26 mmol) and **4** (R = Bu) (70.0 mg, 0.13 mmol) in DMSO (7 mL). The mixture was stirred for 1h, then poured into ice-water, extracted with CHCl<sub>3</sub> (3x). The combined organic phases were washed with water (3x), brine (3x) and dried (Na<sub>2</sub>SO<sub>4</sub>). Removal of the solvent *in vacuo* yielded **9** (R = Bu) as a colourless foam (64 mg, 89%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  0.90 (t, *J* 7.5 Hz, 3H), 1.15 (s, 6H), 1.27 (2, 6H), 1.32 – 1.38 (m, 2H), 1.56 – 1.62 (m, 2H), 2.43 (s, 3H), 2.51 – 2.54 (m, 2H), 3.56 (s, 3H), 3.85 (s, 2H), 4.56 (s(br), 2H), 4.77 (s, 1H), 7.02 (d, *J* 8.0 Hz, 2H), 7.10 (d, *J* 8.0 Hz, 2H), 7.37 – 7.39 (m, 1H), 7.46 – 7.56 (m, 2H), 7.99 (d, *J* 7.5 Hz, 1H); <sup>13</sup>C HMR (CDCl<sub>3</sub>): 13.9,

22.1, 22.8, 30.7, 31.5, 34.6, 41.9, 65.2, 67.6, 69.8, 119.7, 122.6, 128.0, 128.3, 128.6, 129.0, 130.7, 131.1, 137.1, 138.5, 140.1, 141.0, 154.7, 156.6, 162.5, 163.3; MS (ESI)  $m/z$  590.5  $[M+Na]^+$ ; HRMS (ESI) calcd. for  $C_{33}H_{41}N_7O_2$  (M+H)<sup>+</sup>, 568.3394; found 568.3393.

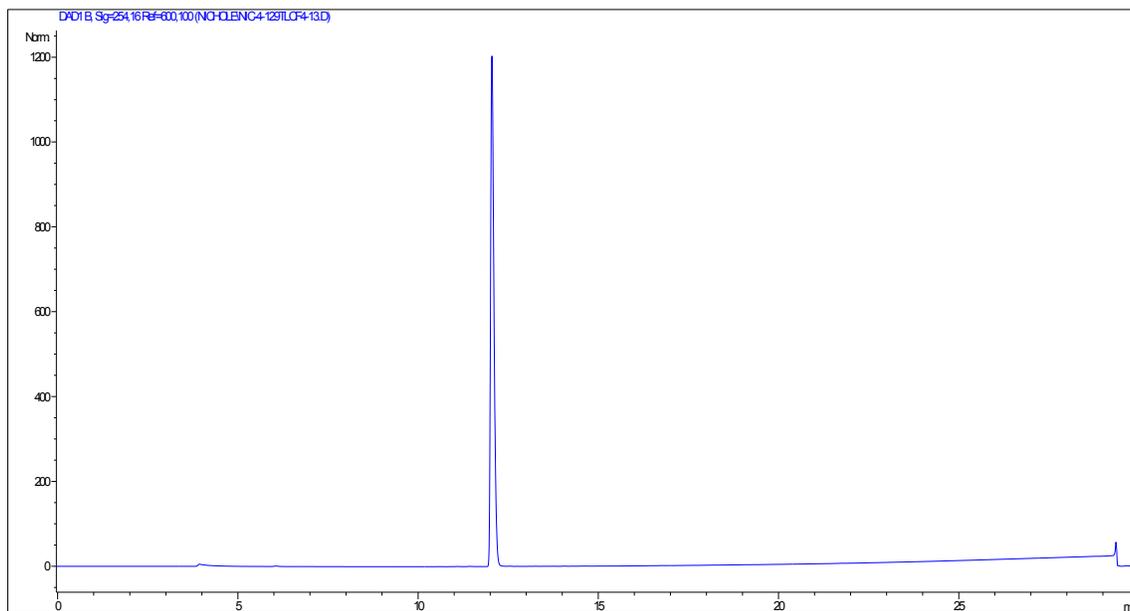
### HPLC trace for compound 3 (R = Me).



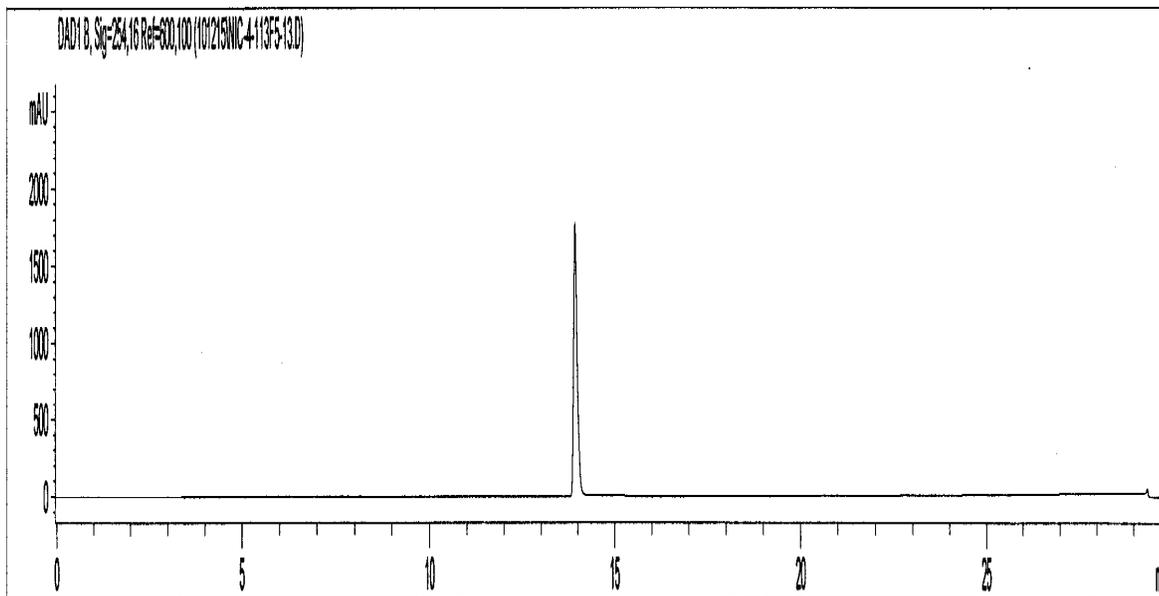
### HPLC trace for compound 4 (R = Me).



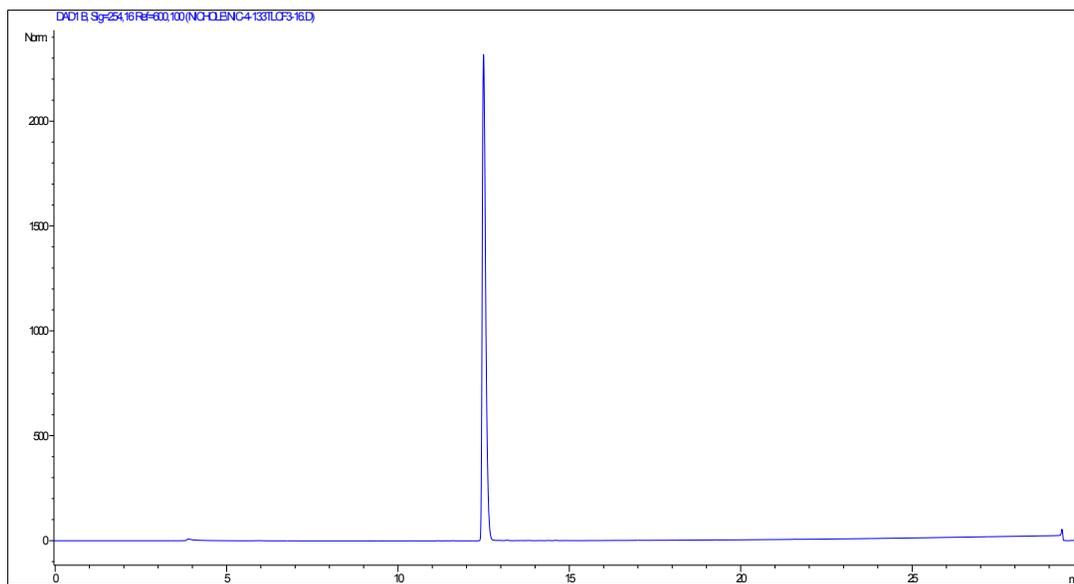
### HPLC trace for compound 3 (R = Et).



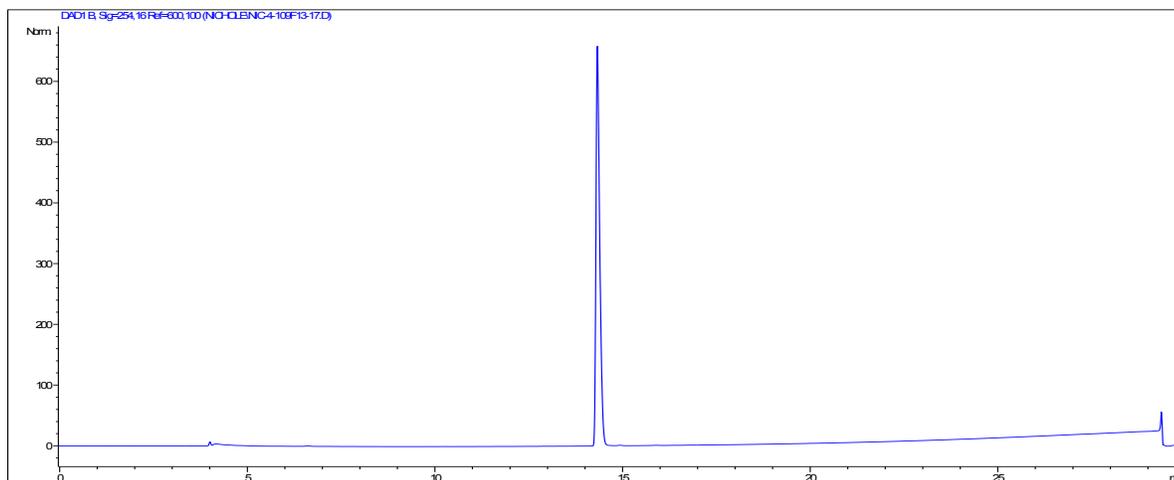
### HPLC trace for compound 4 (R = Et).



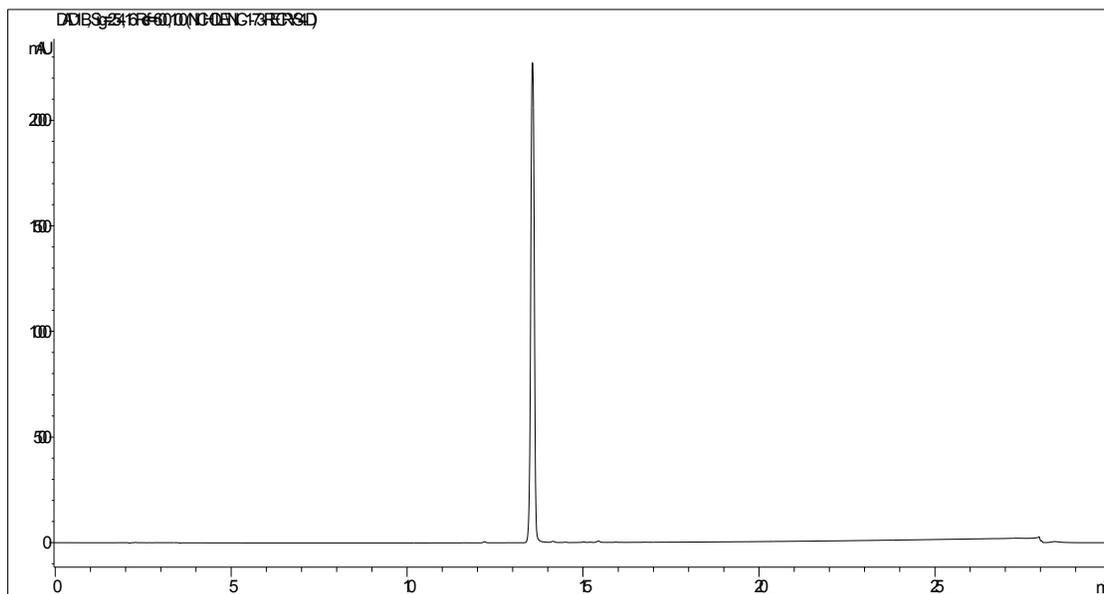
### HPLC trace for compound 3 (R = Pr).



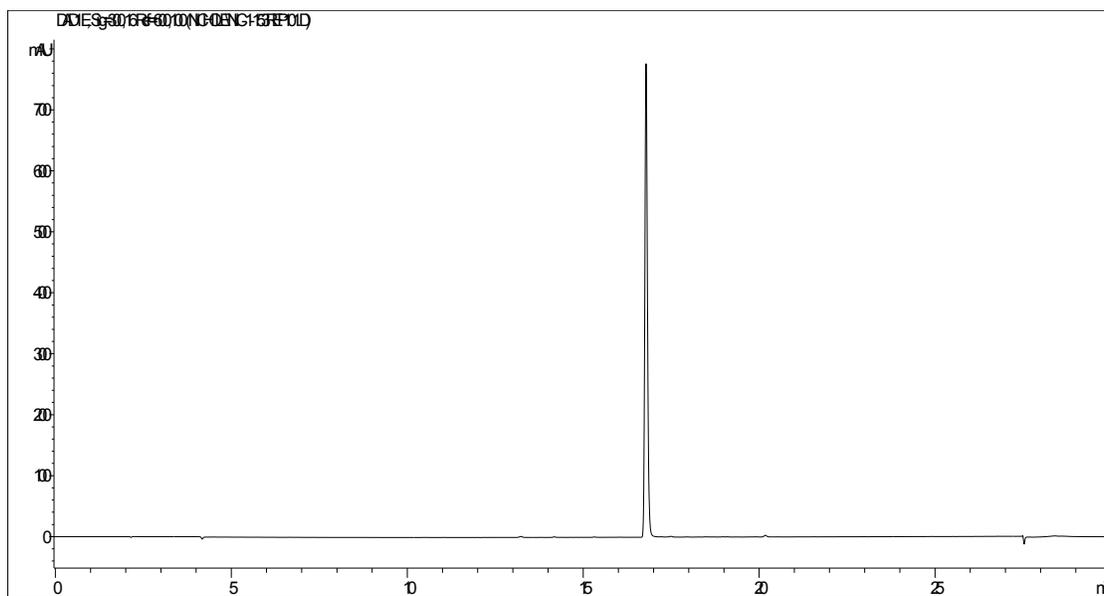
### HPLC trace for compound 4 (R = Pr).



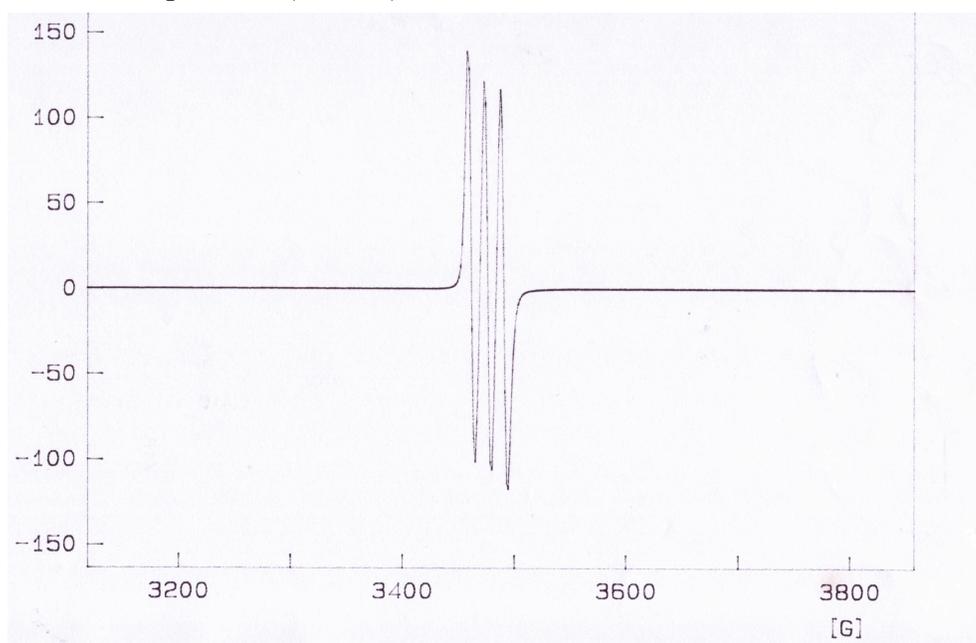
### HPLC trace for compound 3 (R = Bu).

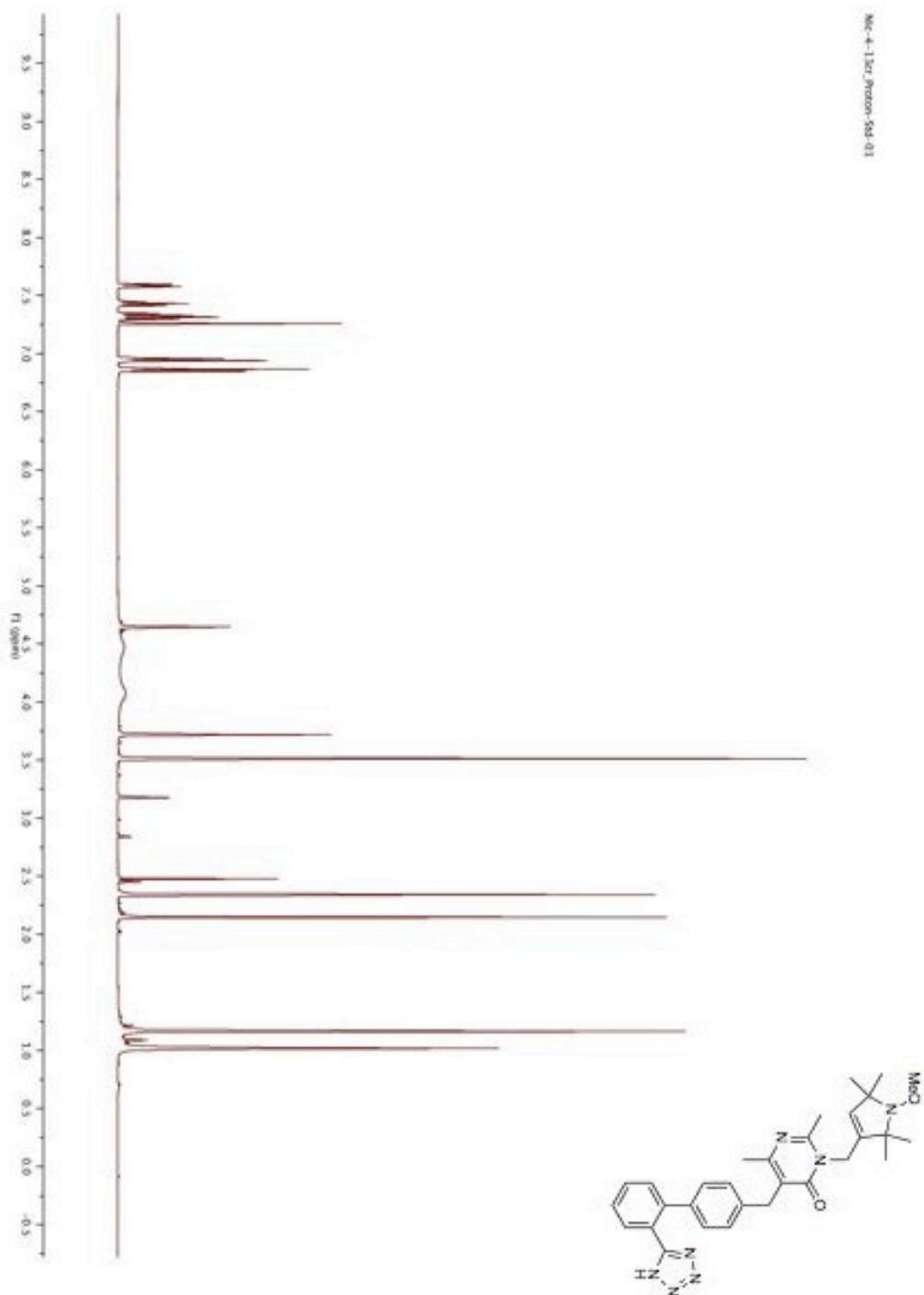


### HPLC trace for compound 4 (R = Bu).

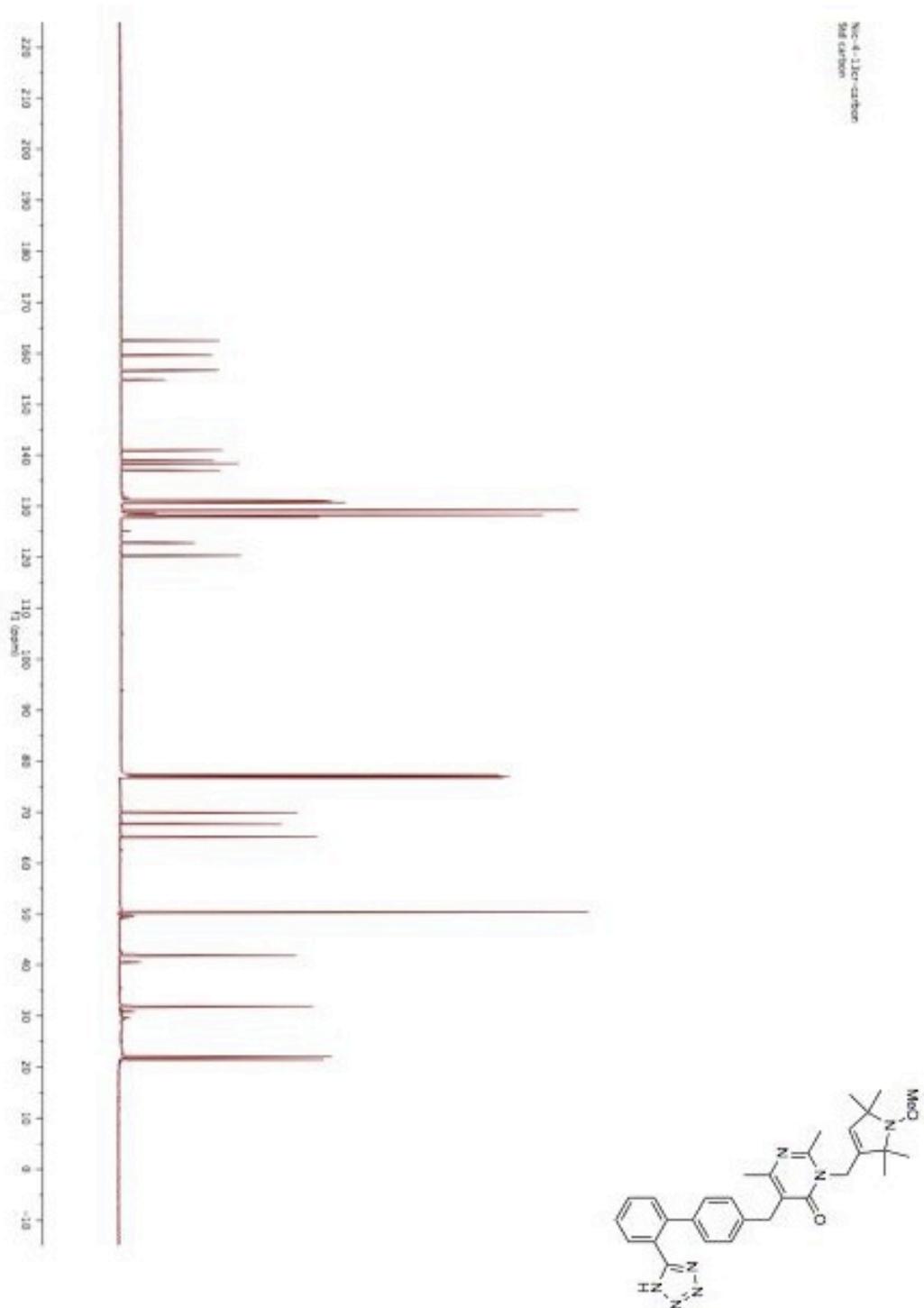


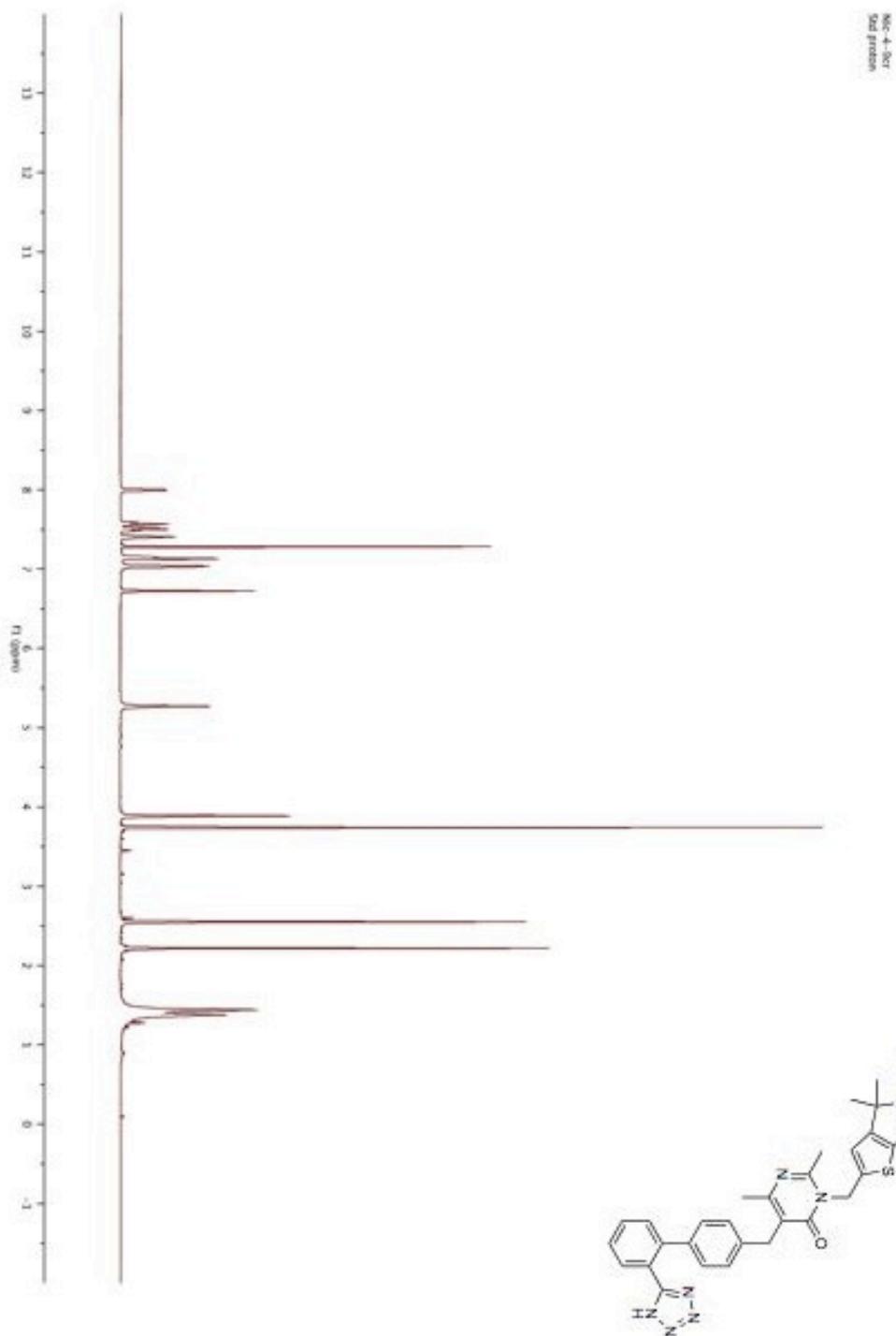
**EPR spectrum for compound 4 (R = Bu).**



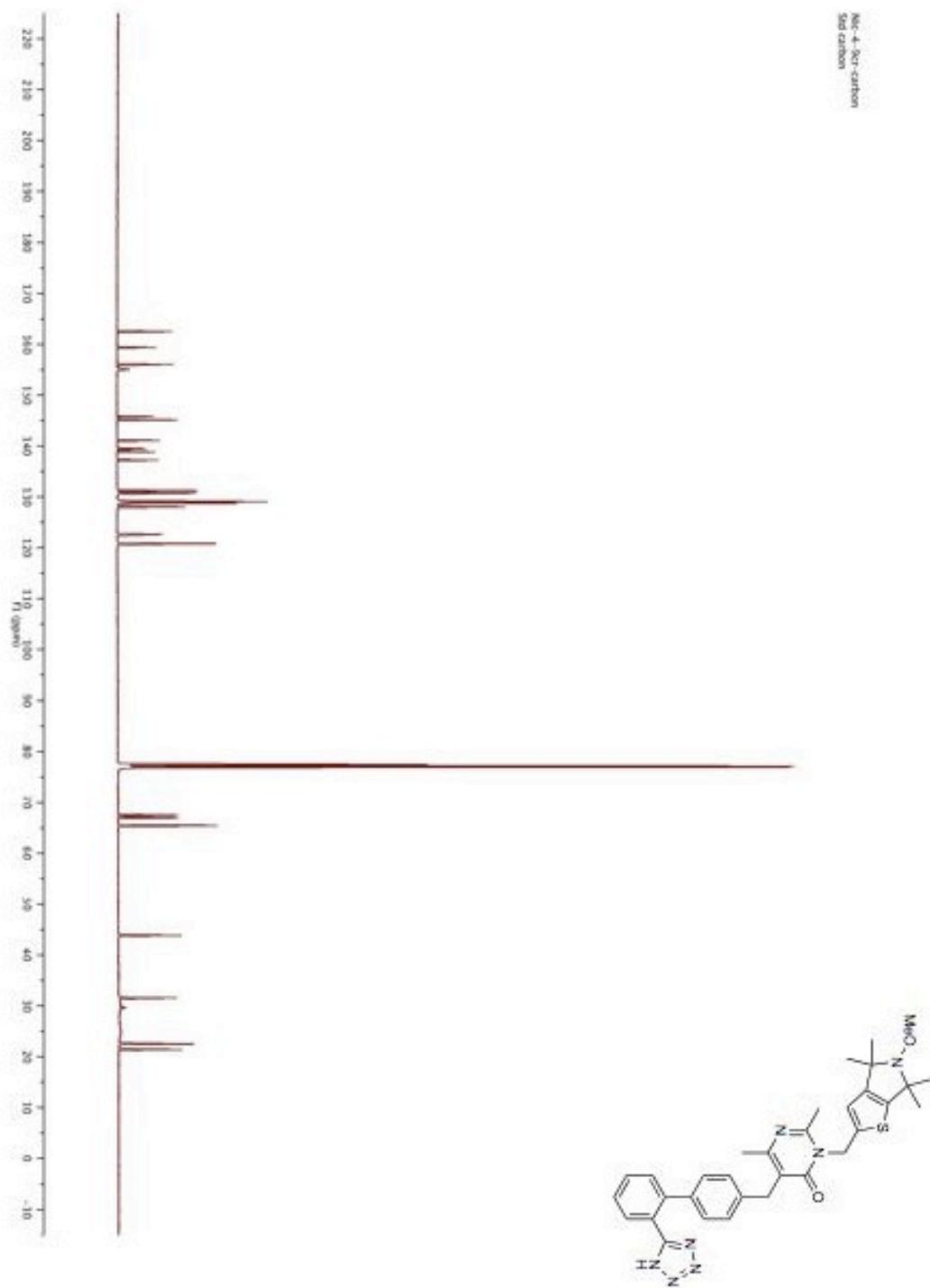


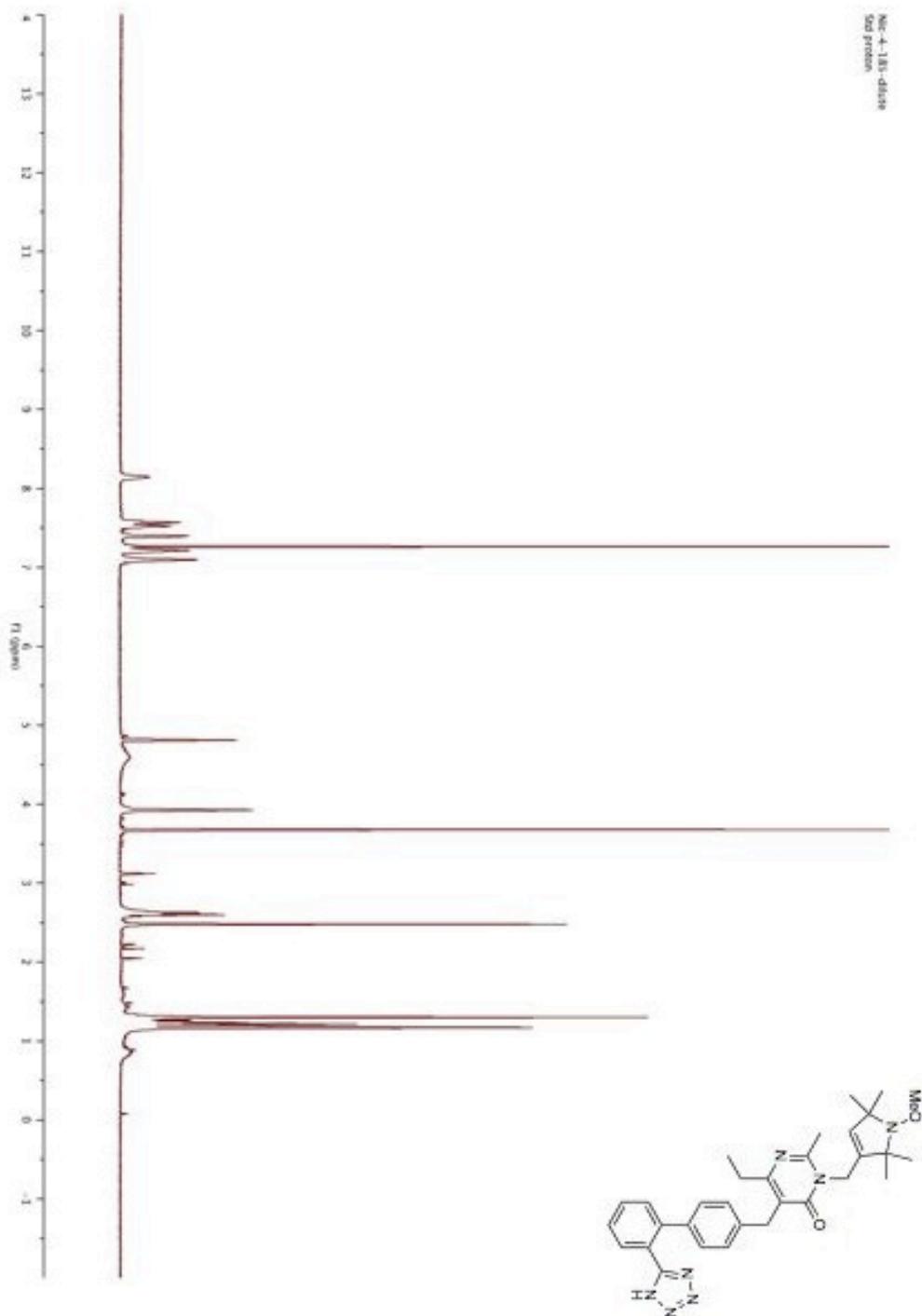
MeO-4-13c7\_00000-584-011

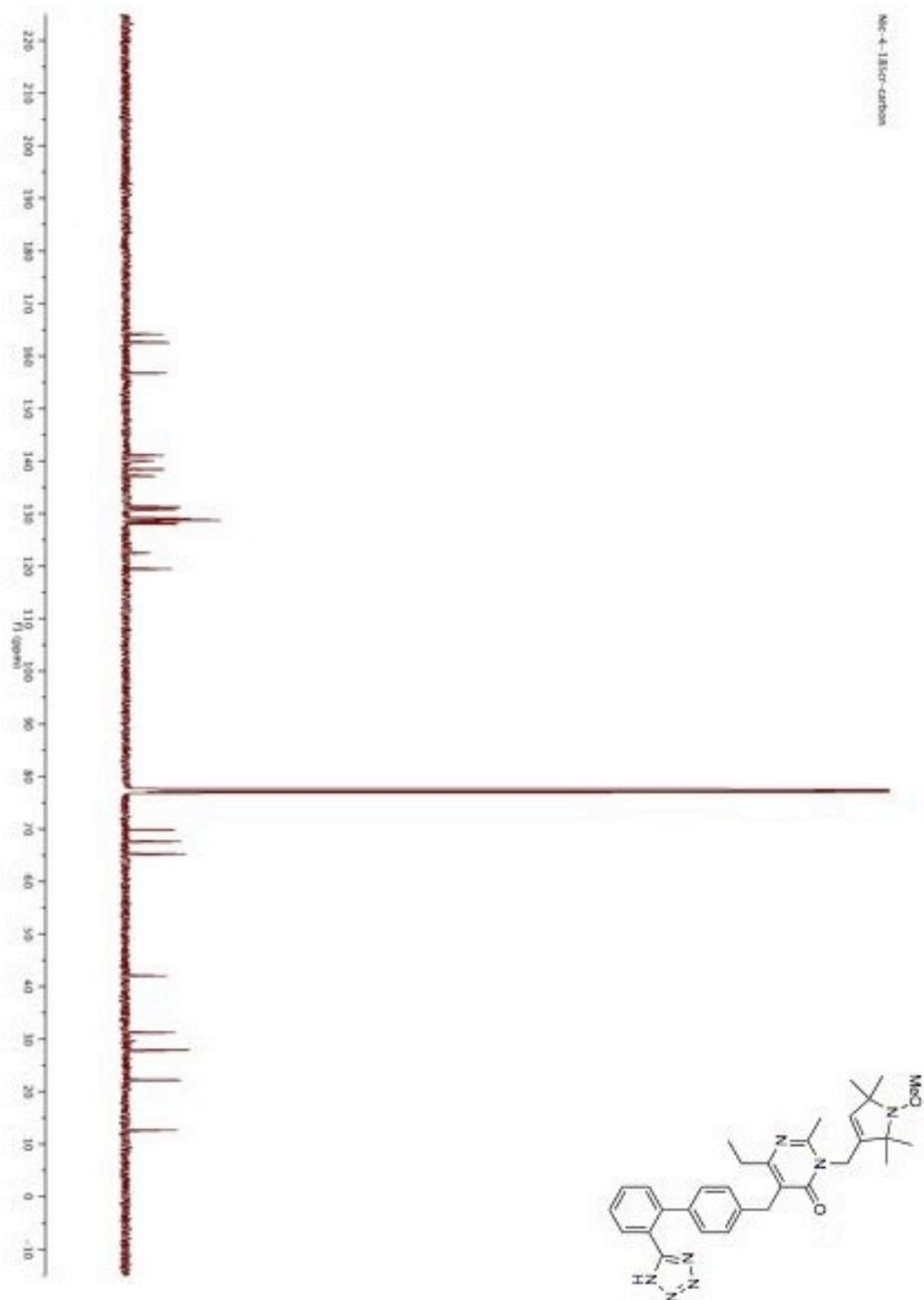


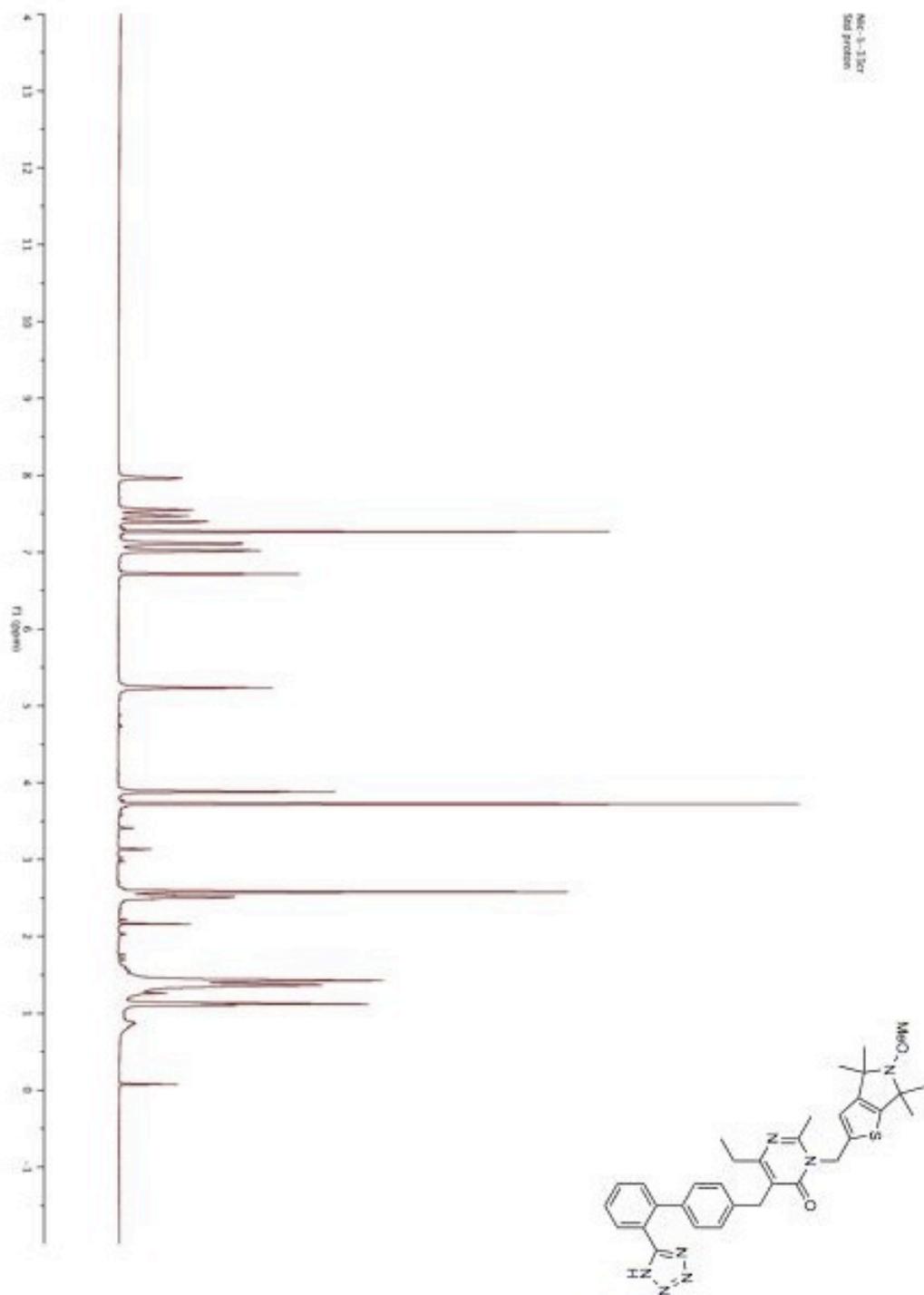


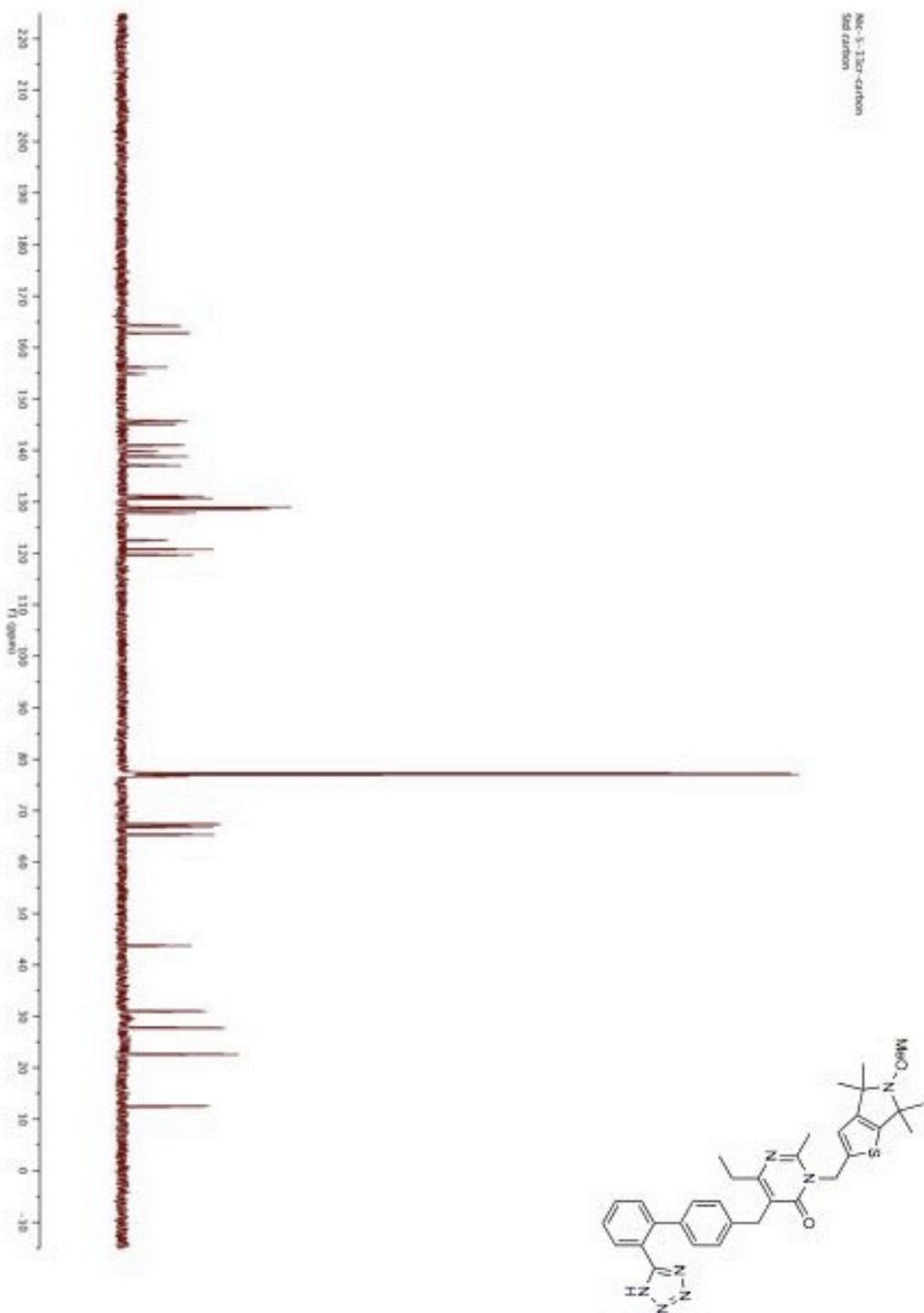
100 + 307  
100 + 307  
100 + 307

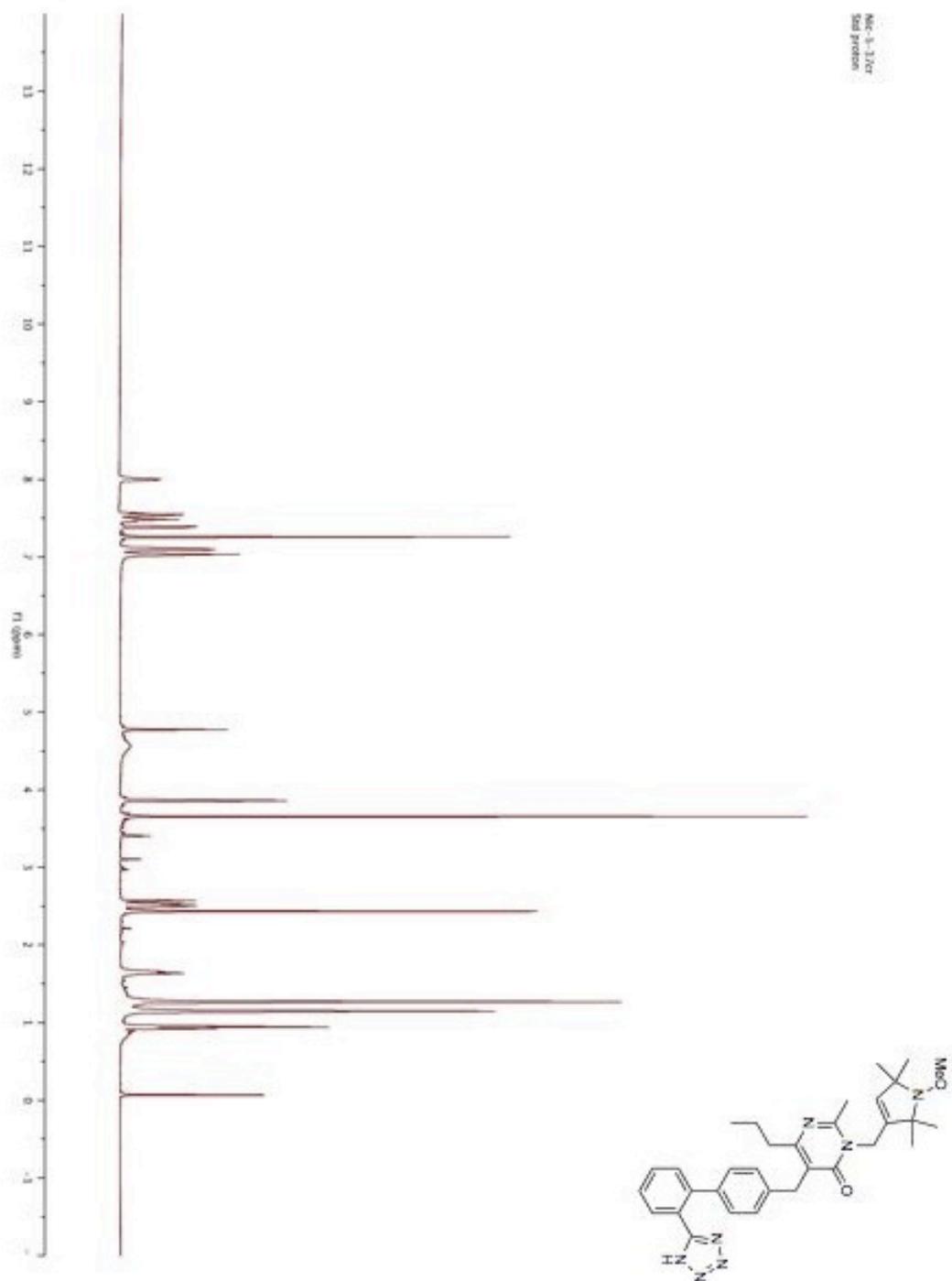


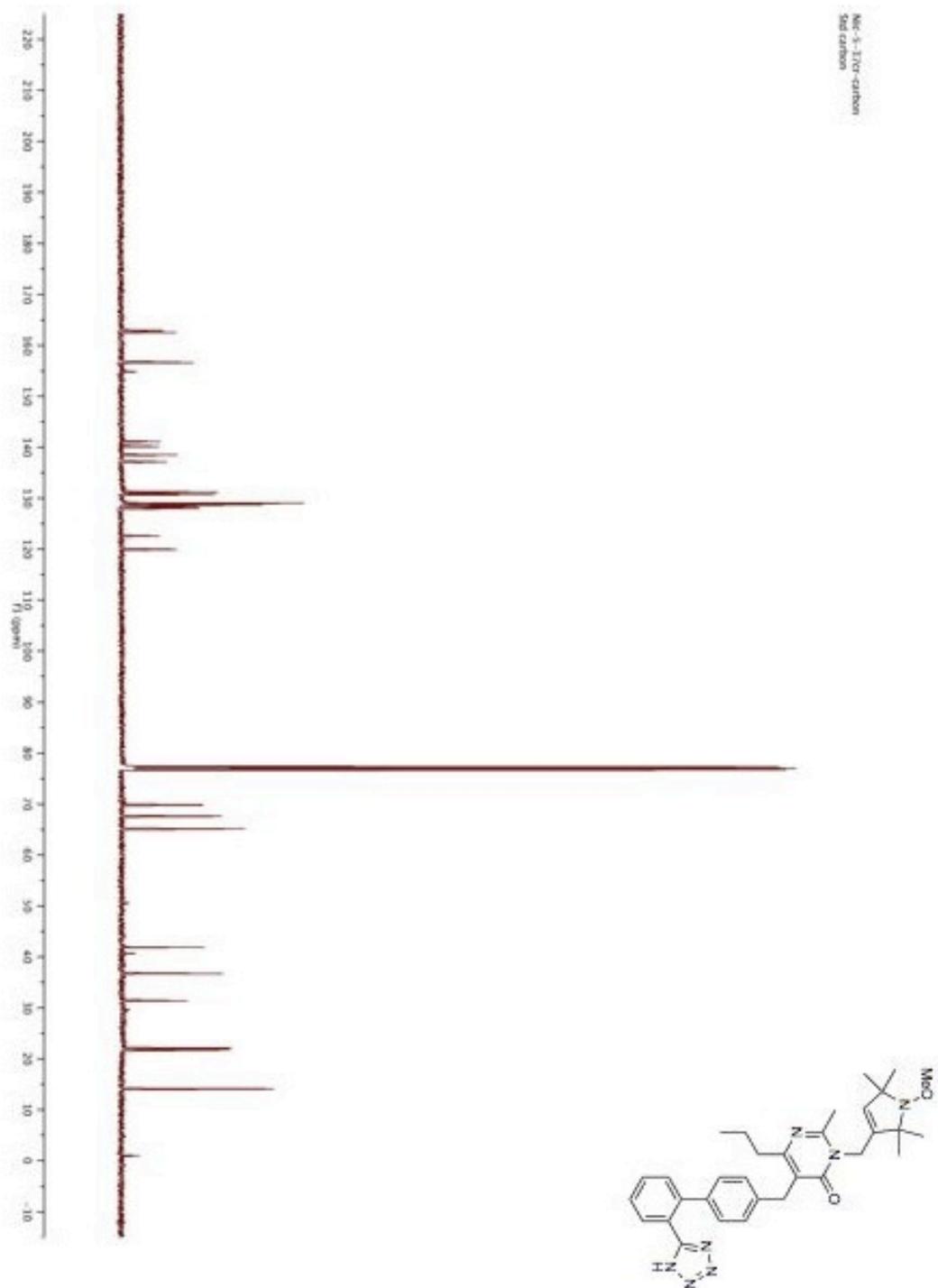




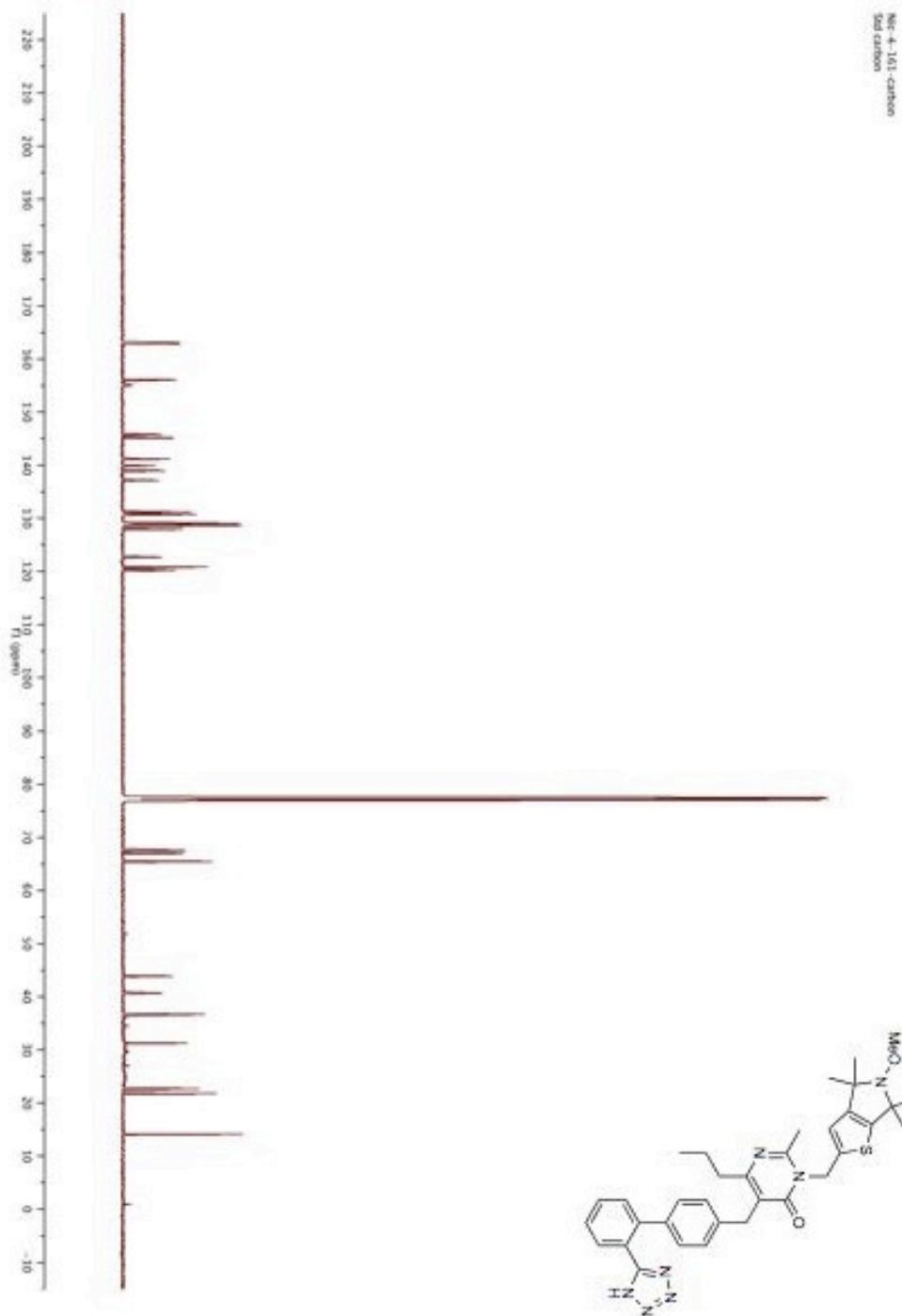


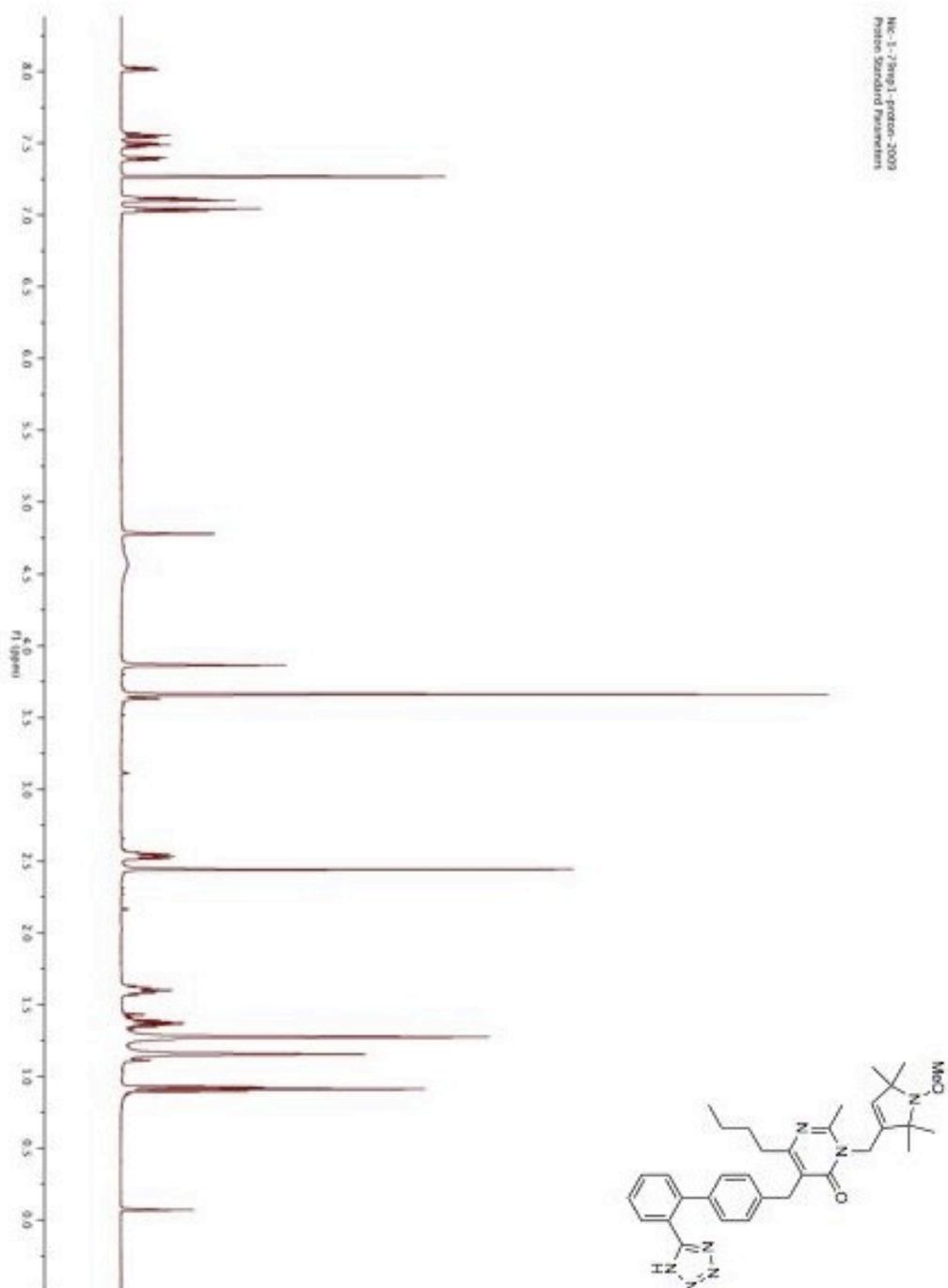


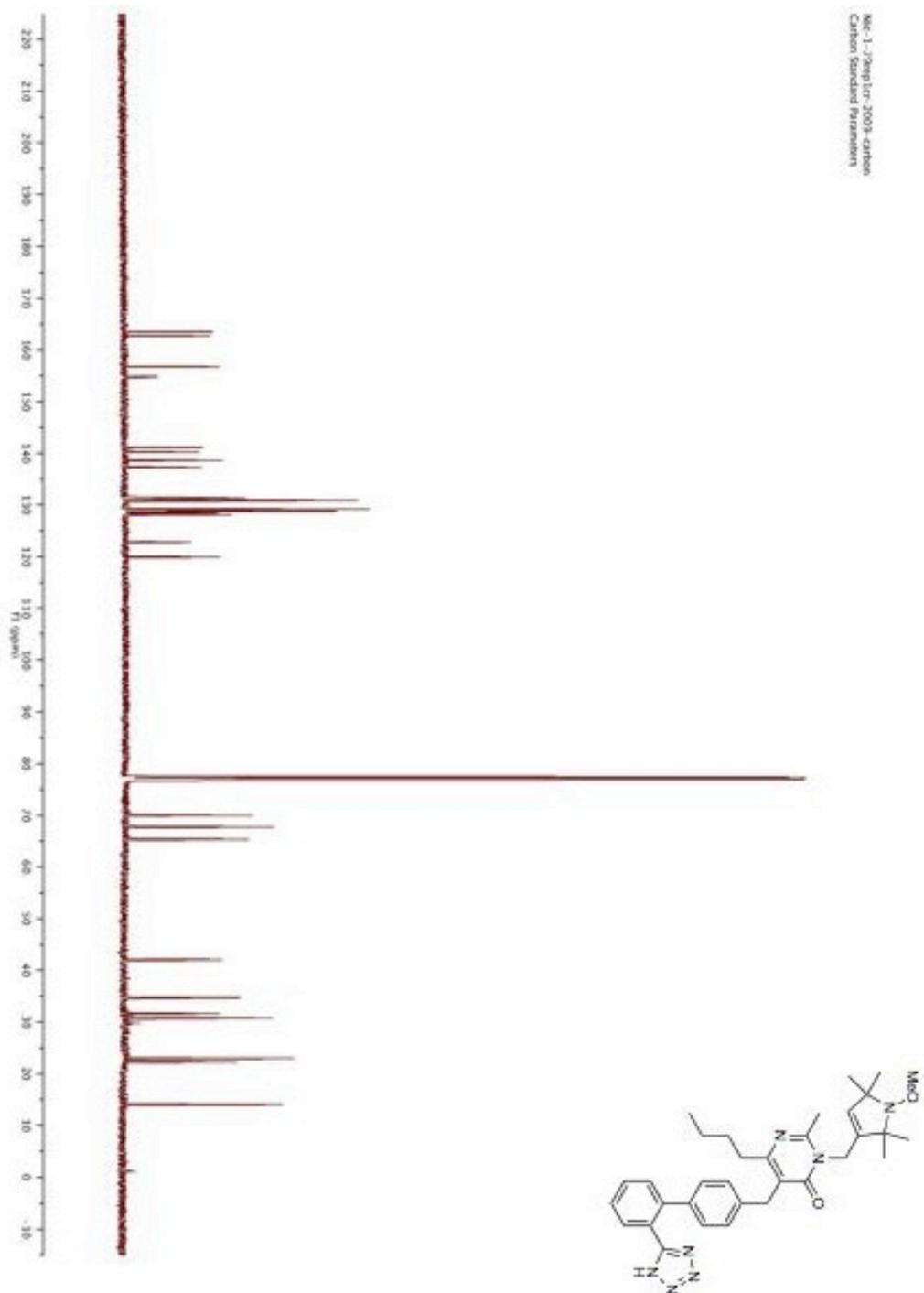


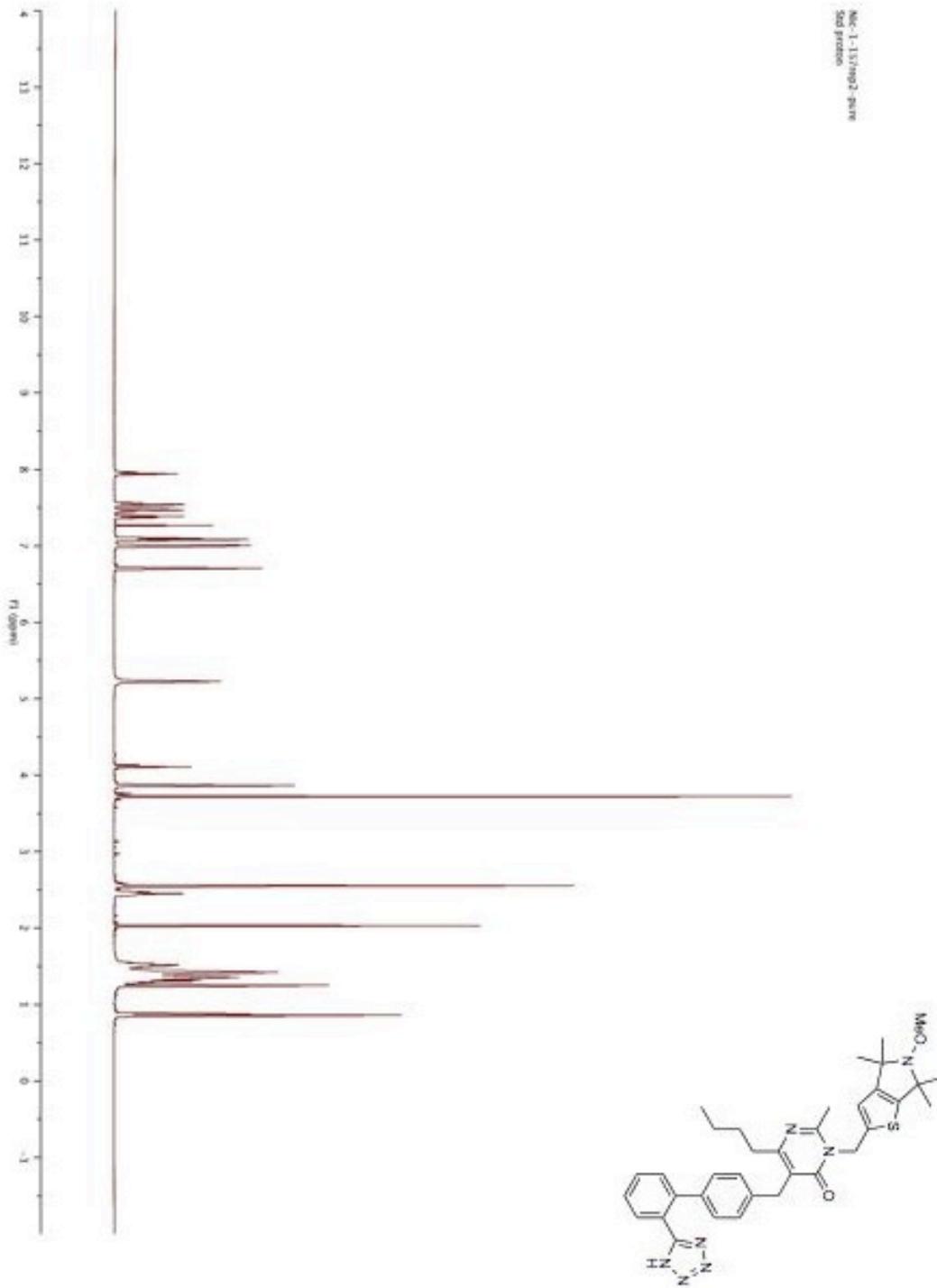


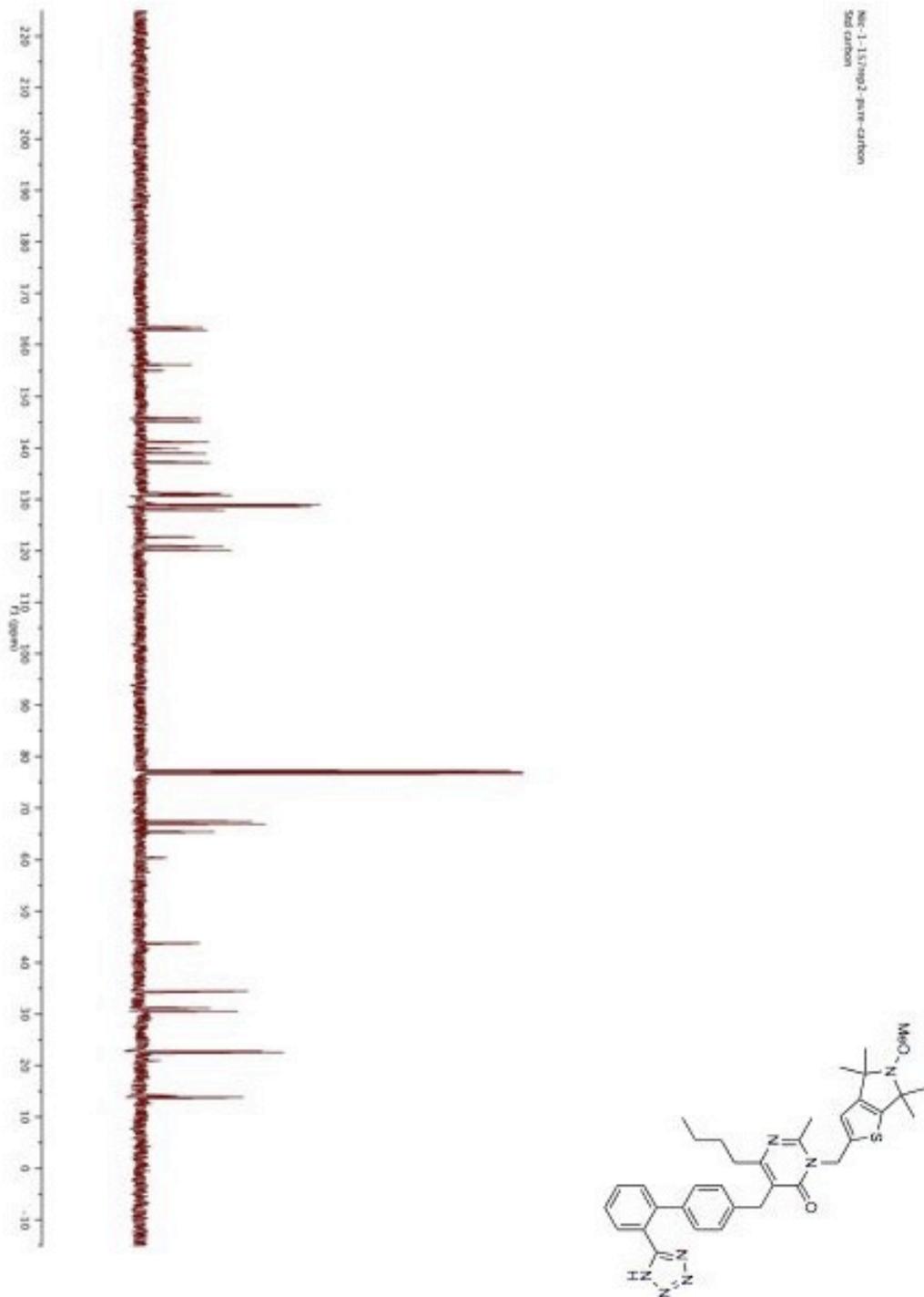












### **Lucigenin Protocol details**

Male Sprague Dawley rats were deeply anaesthetised using spontaneous inhalation of halothane (5% in O<sub>2</sub>/room air) and killed by cervical dislocation. Segments of thoracic aortae (3 mm long) were dissected out in Krebs-Hepes solution (in mM: NaCl 99.0; KCl 4.7; CaCl<sub>2</sub> 1.9; MgSO<sub>4</sub> 1.2; K<sub>2</sub>HPO<sub>4</sub> 1.0; NaHCO<sub>3</sub> 25.0; HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) 20.0; and glucose 11.1; pH 7.4) and incubated at 37°C in 6 well plates containing Krebs-HEPES buffer solution, NADPH (100 µM), DETCA (diethylthiocarbamate, 300 µM) and compound 10 or 11 (1, 3 or 10 µM, each) for 45 min in a 37°C oven (Forma Scientific, Marietta, OH, USA). After a 45 min incubation period, aorta segments were placed in a 96 well Optiplate (Perkin Elmer, Downers Grove, IL, USA) containing NADPH (100 µM), lucigenin (5 µM) and Compound 10 or Compound 11 (1, 3 or 10 µM, each). Photon emission was detected by a luminescence counter (Perkin Elmer) which read each well 12 times over 25 min. Background photon emission counts of the 96 well Optiplate were also taken prior to addition of the tissue segments. Vessel segments were then dried in a 65°C oven (Daihan Scientific, Seoul, Korea) overnight in order to determine the dry weight of the tissue. Luminescent counts were expressed as average counts per mg of dry tissue weight (references 14 and 15).