Electronic Supporting Information

Three in One: Prototropy-free Highly Stable AADD-type Self-Complementary Quadruple Hydrogen-Bonded Molecular Duplexes with Built-in Fluorophore

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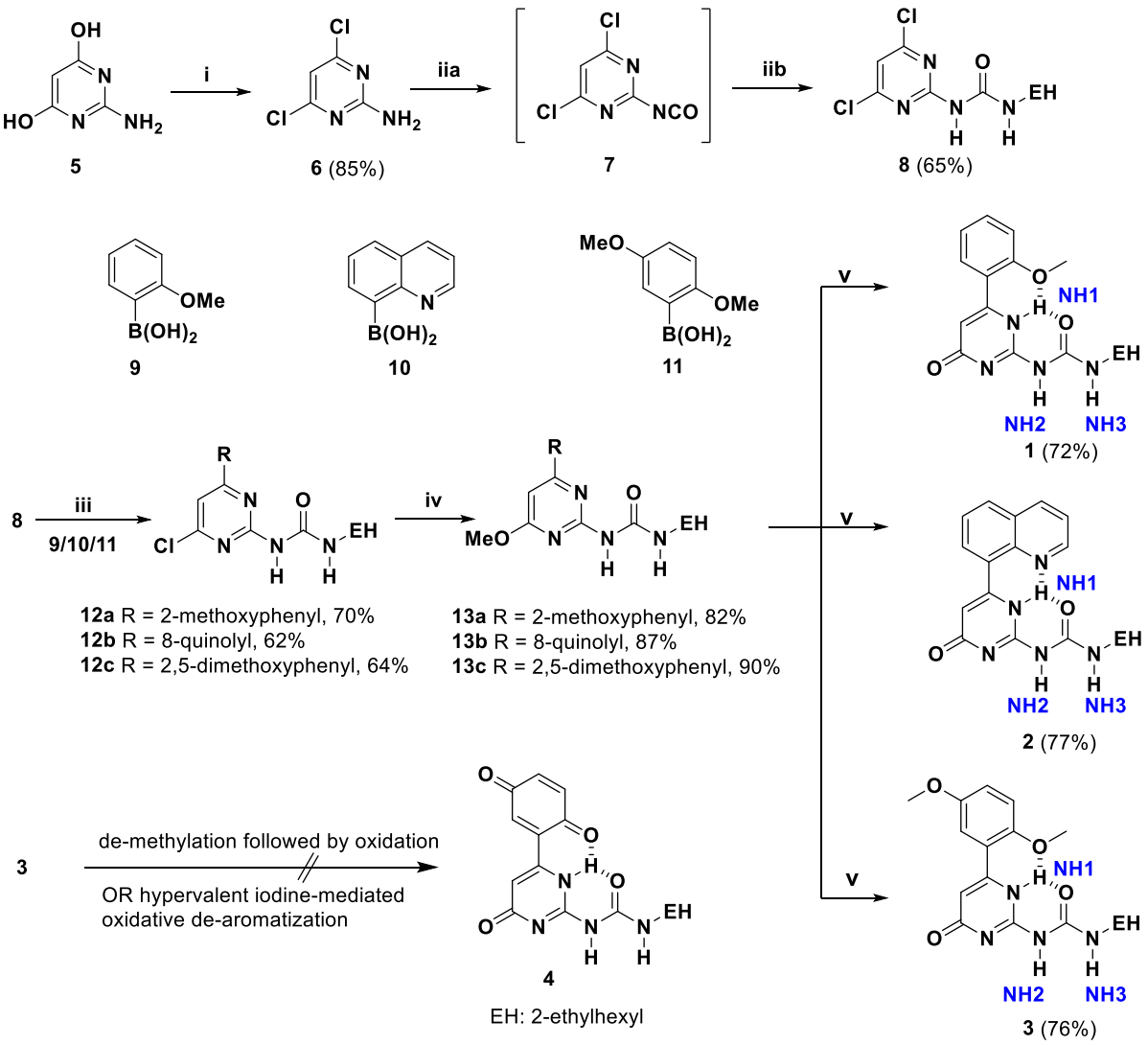
General Methods

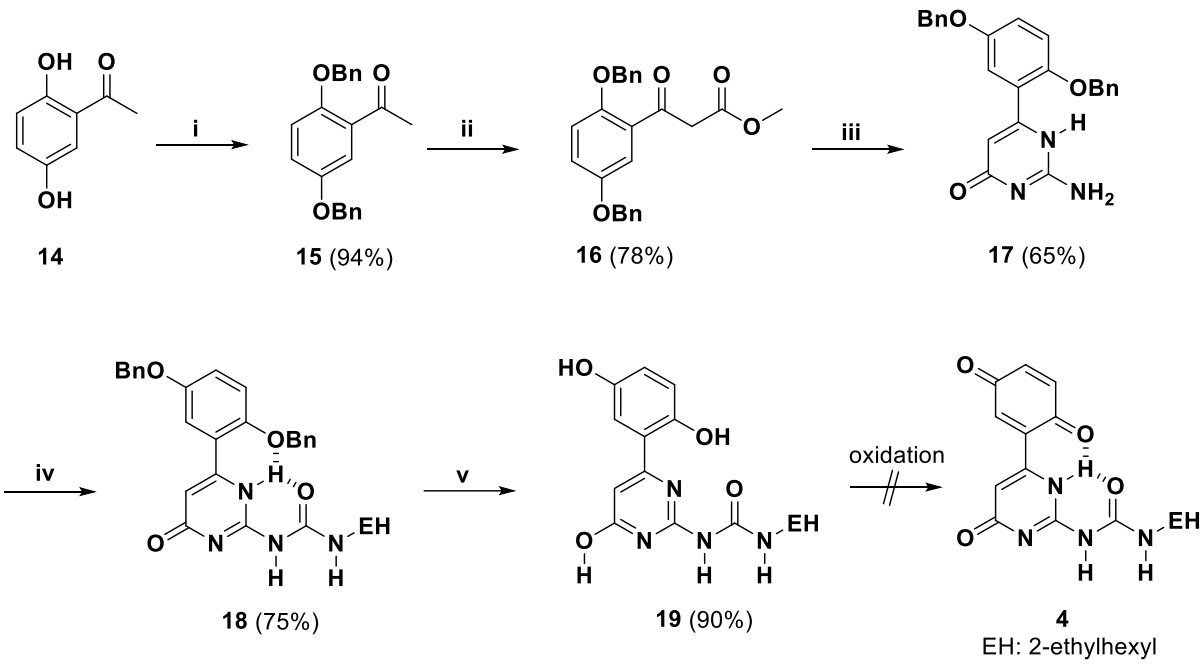
Unless otherwise stated, all the chemicals and reagents were obtained commercially. Compound **6** was synthesized as per the reported procedure.[1] Compounds **15** and **16** were synthesized as per the reported procedure.[2] Dry solvents were prepared by the standard procedures. Analytical Thin Layer Chromatography was done on precoated silica gel plates (Kieselgel $60F_{254}$, Merck). Column chromatographic purifications were done with 100-200 mesh silica gel. NMR spectra were recorded in $CDCl_3$ on AV 200 MHz, AV 400 MHz, AV 500 MHz and AV 700 MHz Bruker NMR spectrometers. All chemical shifts are reported in δ ppm downfield to TMS and peak multiplicities are referred to as singlet (s), doublet (d), quartet (q), broad singlet (bs), and multiplet (m). The titration studies were done in $CDCl_3$. Elemental analyses were performed on an Elmentar-Vario-EL (Heraeus Company Ltd., Germany). IR spectra were recorded in $CHCl_3$ using Shimadzu FTIR-8400 spectrophotometer. Melting points were determined on a Buchi Melting Point B-540. HRMS (ESI) data were recorded on a Thermo Scientific Q-Exactive, Accela 1250 pump.

Scheme 1: Synthesis of compounds **1-3**.



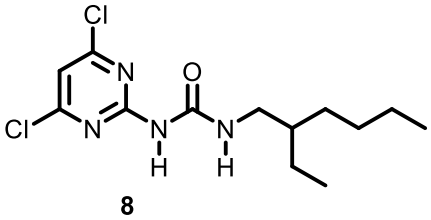
Reagents and conditions: (i) $POCl_3$, Et_3N, CH_3CN, reflux, 1 h; (ii) a) $(COCl)_2$, C_6H_6, reflux, 2 h, b) 2-ethylhexylamine, C_6H_6, rt, 18 h; (iii) $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O (3:1), 110°C, 12 h; (iv) K_2CO_3, MeOH, reflux, 6 h; (v) HBr /AcOH, reflux, 1 h.

Scheme 2: Attempted synthesis of compound **4**.



Reagents and conditions: (i) BnBr, K_2CO_3, acetone, reflux, 24 h; (ii) $(CH_3O)_2CO$, NaH, 75^{o}C, 24 h; (iii) guanidine carbonate, C_2H_5OH, reflux, 20 h; (iv) 2-ethylhexyl isocyanate, pyridine, 100^{o}C, 10 h; (v) Pd/C, THF, H_2, balloon, 24 h.

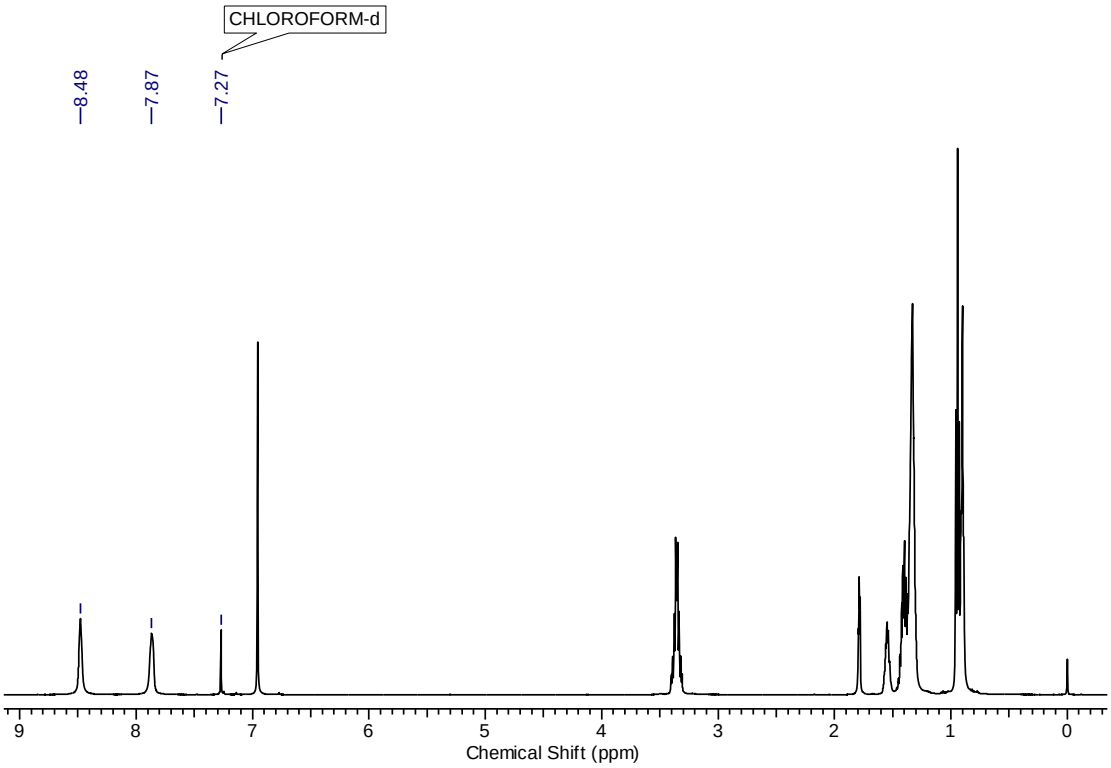
Compound 8



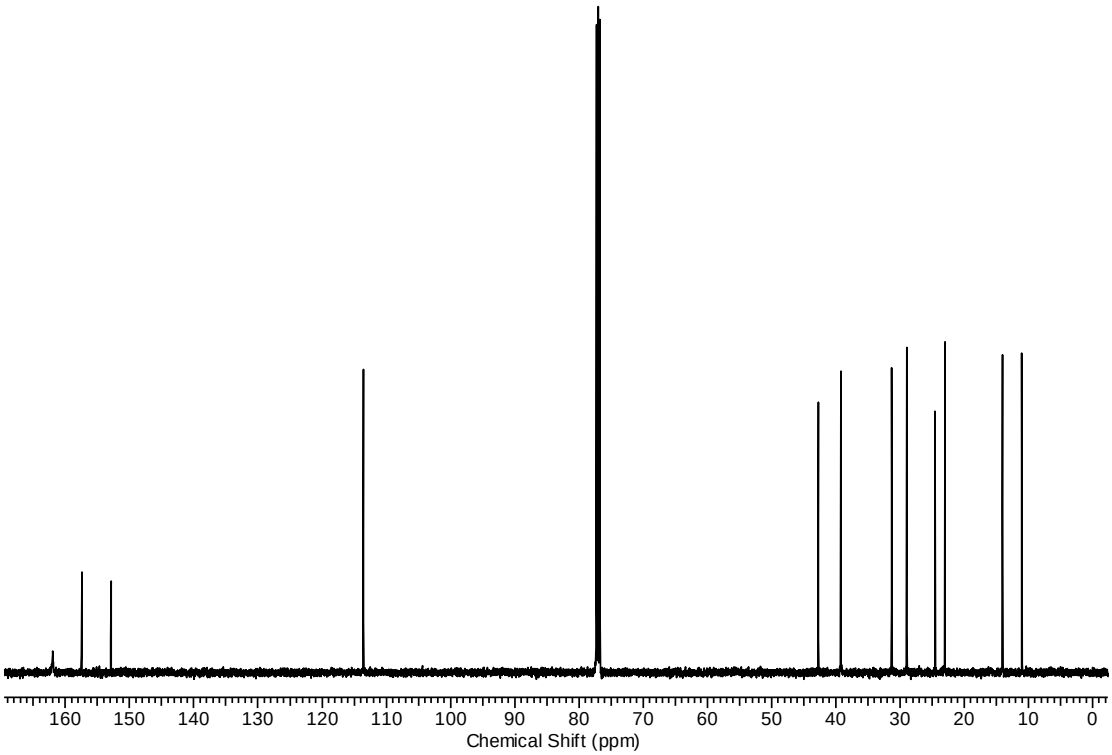
8

A solution of oxalyl chloride (5.16 mL, 60.975 mmol, 5 equiv.) in 30 mL of absolute benzene was added drop wise with stirring to a solution of **6** (2 g, 12.195 mmol, 1 equiv.) in 100 mL of absolute benzene, and the mixture was refluxed for 2 h until hydrogen chloride and carbon monoxide evolution ceased. The mixture was then cooled to 20^{o}C and filtered without access to moisture. The benzene mother liquor was evaporated under reduced pressure to give intermediate **7** (2.1 g, 90%), which was directly used without further purification. A solution of 2-ethyl-1-hexylamine (1.81 mL, 11.052 mmol, 1 equiv.) in 30 mL of absolute benzene was added to a solution of **7** (2.1 g, 11.052 mmol, 1 equiv.) in 50 mL of absolute benzene, and the

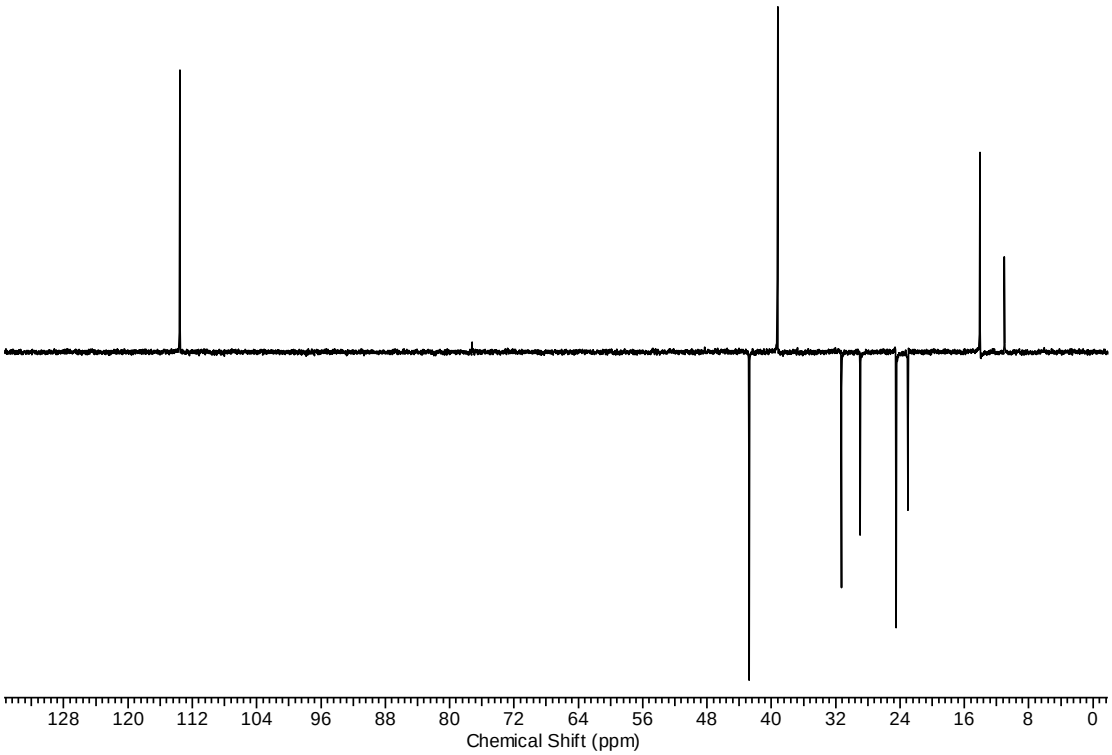
mixture was allowed to stand overnight and was concentrated under reduced pressure. Column chromatographic purification (eluent: 10% AcOEt/ pet. ether, R_f: 0.4) of the residue yielded **8** (2.3 g, 65%) as a white fluffy solid. mp: 195-197 ^oC; IR ($CHCl_3$) ν (cm^{-1}): 3413, 3319, 3019, 2927, 2867, 1697, 1544, 1219, 1105, 764; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.48 (s, 1H), 7.87 (s, 1H), 6.96 (s, 1H), 3.40-3.31 (m, 2H), 1.56-1.53 (m, 1H), 1.45-1.37 (m, 2H), 1.33-1.32 (m, 6H), 0.96-0.93 (t, J = 7.32 Hz, 3H), 0.91-0.89 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.9, 157.4, 152.8, 113.5, 42.7, 39.1, 31.2, 28.9, 24.4, 22.9, 14.0, 11.0; HRMS (ESI) calculated $[M+H]^+$ for $C_{13}H_{21}ON_4Cl_2$: 319.1080, found 319.1087, 659.1921 $[2M+Na]^+$.



^1H NMR spectrum of compound **8** ($CDCl_3$, 500 MHz, 298 K)



^{13}C NMR spectrum of compound **8** ($CDCl_3$, 125 MHz, 298 K)



DEPT-135 NMR spectrum of compound **8** ($CDCl_3$, 125 MHz, 298 K)

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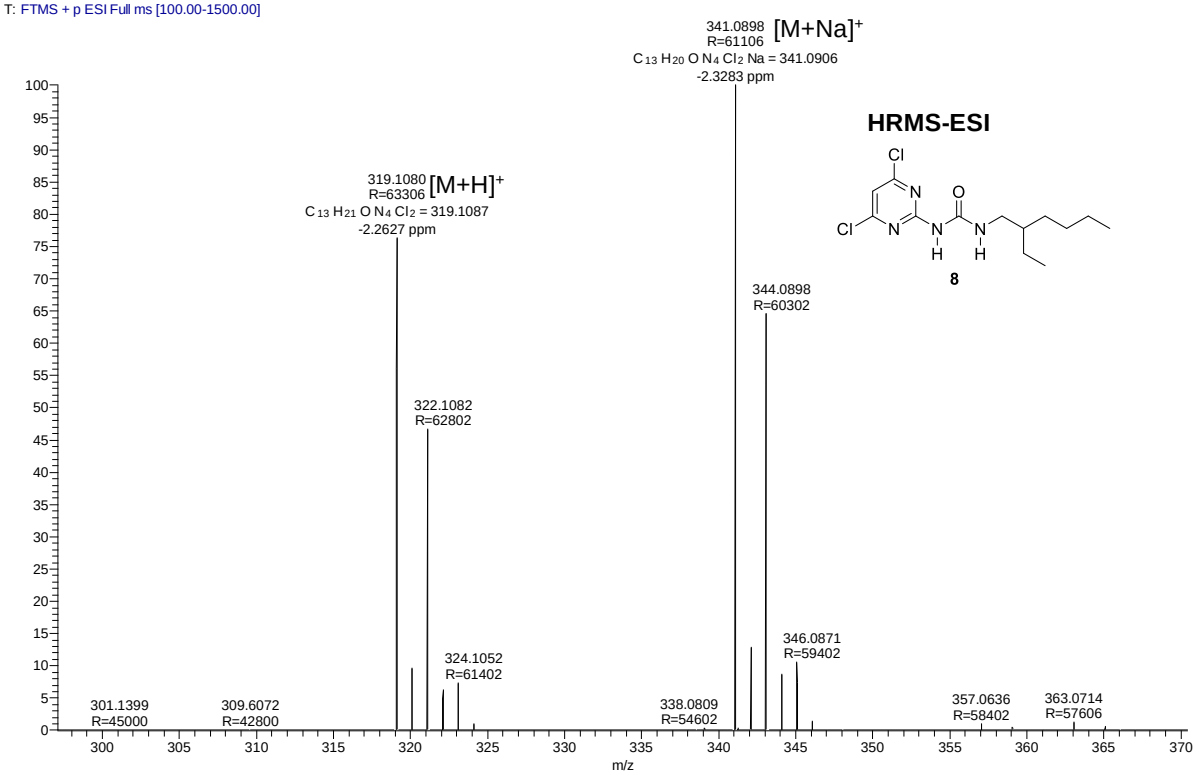
T: FTMS + p ESI Full ms [100.00-1500.00]



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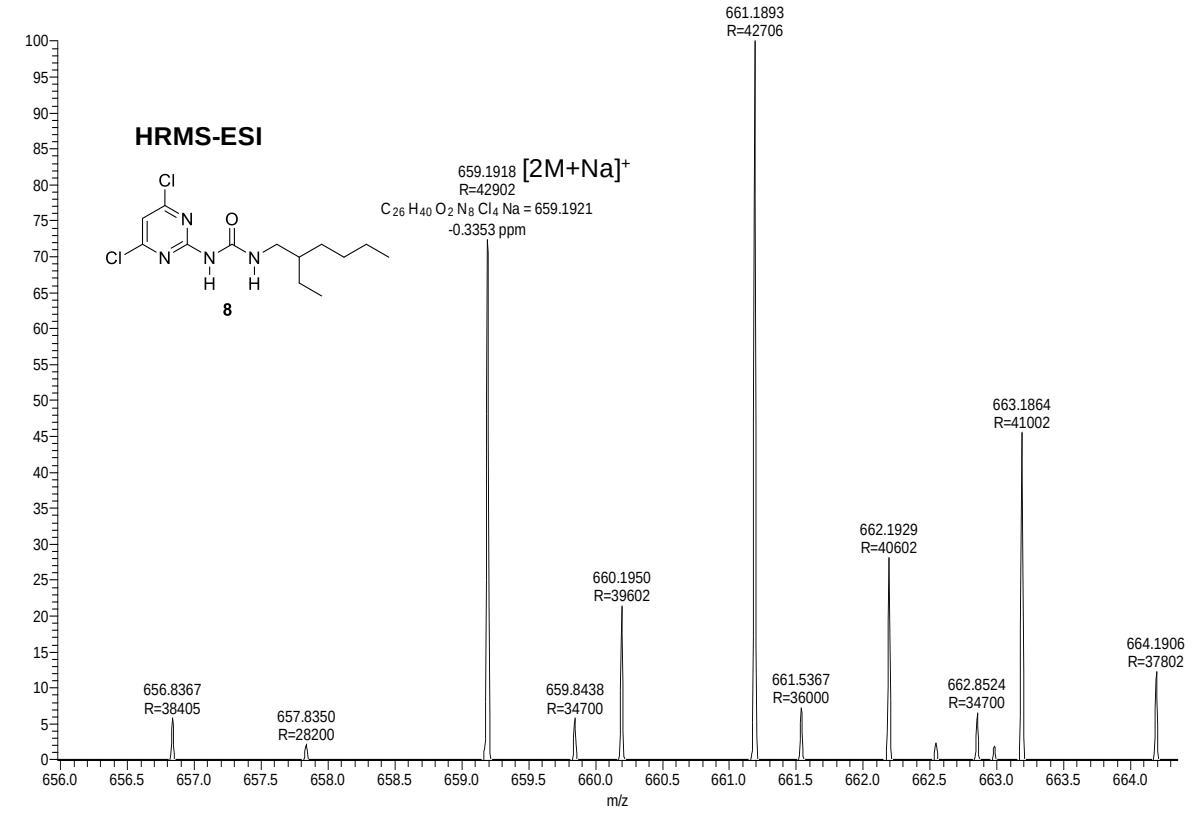
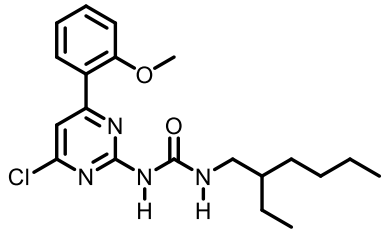
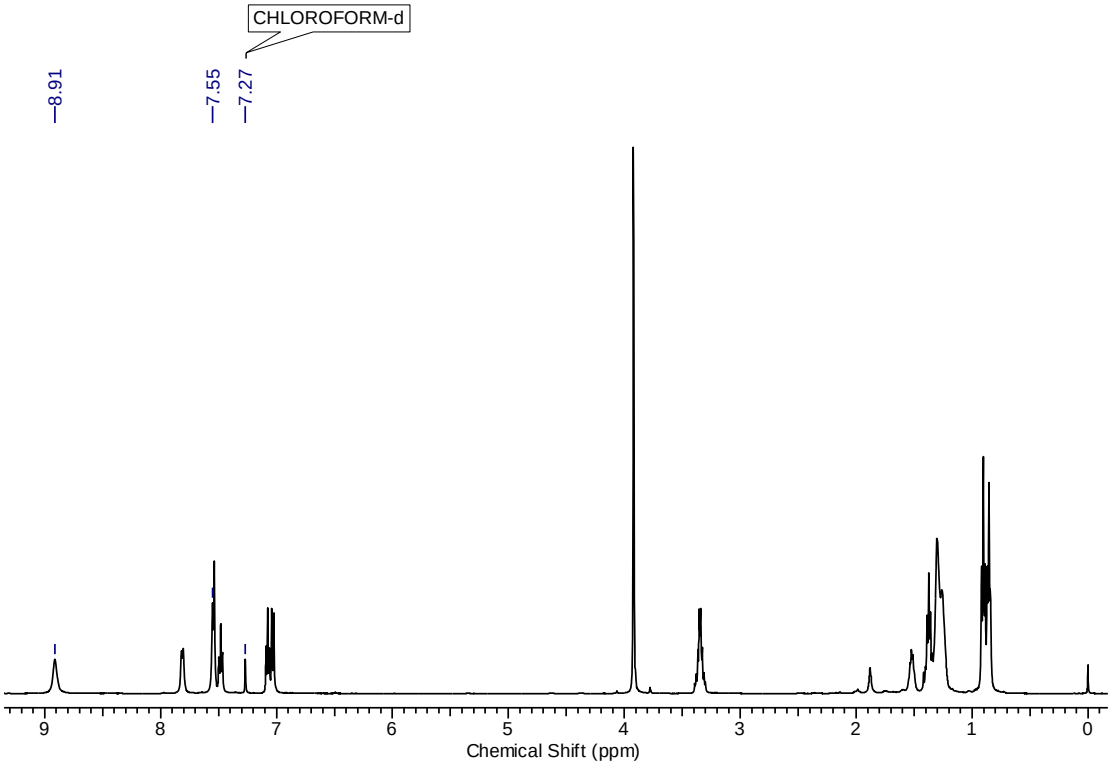


Compound 12a

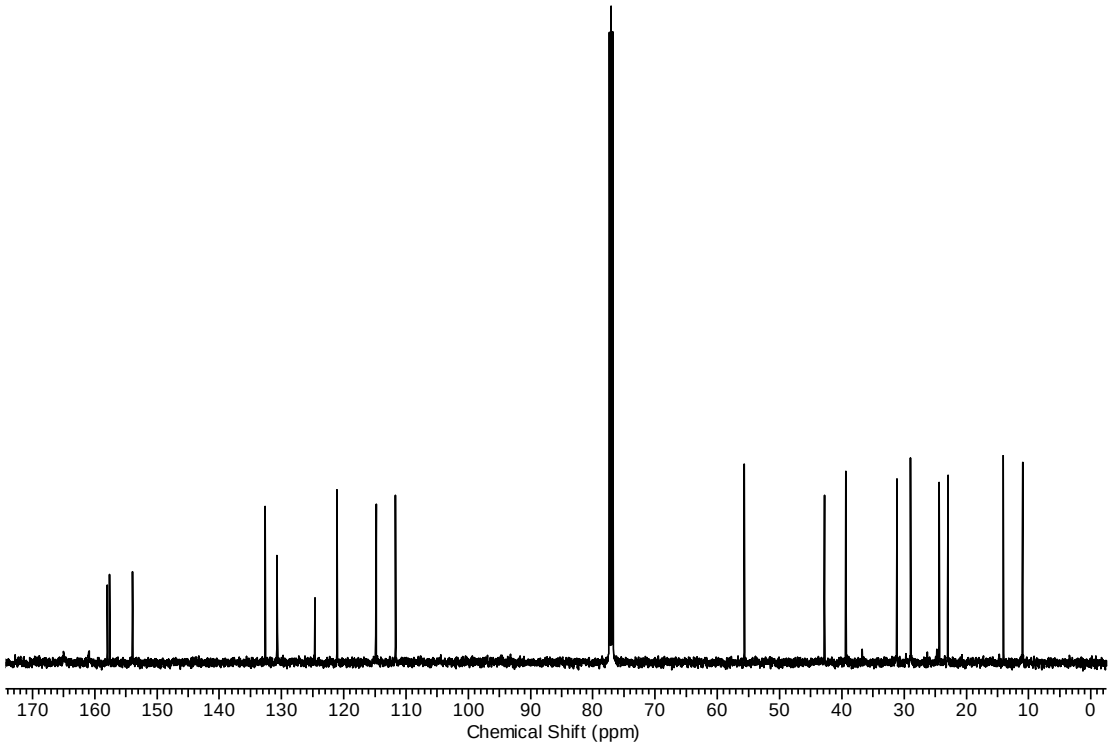


12a

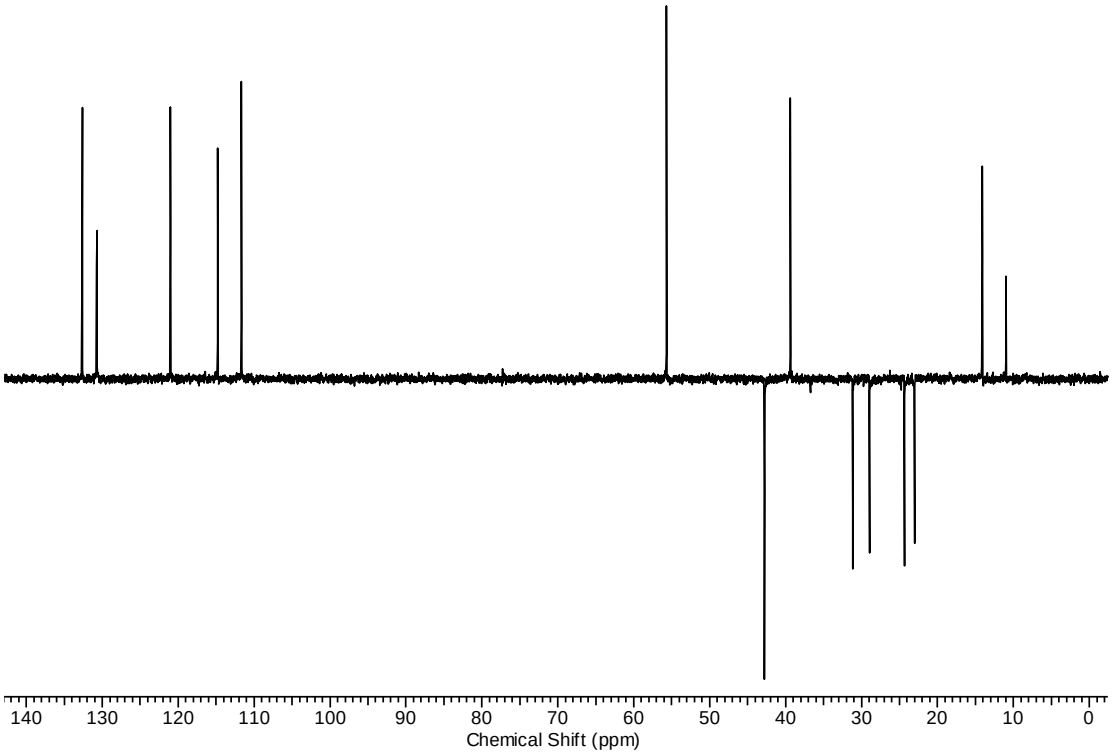
To a solution of **8** (1 g, 3.144 mmol, 1 equiv.) in 40 mL of a mixture of DME/H_2O (3:1) in schlenk tube was added 2-methoxyphenyl boronic acid (0.525 g, 3.459 mmol, 1.1 equiv.), Na_2CO_3 (0.833 g, 7.861 mmol, 2.5 equiv), $Pd(PPh_3)_4$ (0.181 g, 0.157 mmol, 0.05 equiv.) and stirred at 100^oC for 12 h. After completion of reaction, the mixture was concentrated under reduced pressure. The residue was partitioned between DCM and water. The organic layer was separated, washed with $NaHCO_3$ solution, water, brine solution, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (eluent: 30% AcOEt/ pet. ether, R_f: 0.5) afforded **12a** (0.858c g, 70%) as a fluffy white solid. mp: 69-71 ^oC; IR ($CHCl_3$) *v* (cm^{-1}): 3574, 3422, 3289, 3015, 2929, 1690, 1571, 1532, 1482, 1260, 1119, 764; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.91 (s, 1H), 7.82-7.81 (d, *J* = 6.71 Hz, 1H), 7.55 (s, 1H), 7.54 (s, 1H), 7.50-7.46 (t, *J* = 7.32 Hz, 1H), 7.09-7.06 (t, *J* = 7.63 Hz, 1H), 7.04-7.02 (d, *J* = 8.24 Hz, 1H), 3.92 (s, 3H), 3.39-3.30 (m, 2H), 1.54-1.51 (m, 1H), 1.42-1.35 (m, 2H), 1.30-1.26 (m, 6H), 0.92-0.89 (t, *J* = 7.32 Hz, 3H), 0.87-0.84 (t, *J* = 6.71 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 164.9, 160.8, 157.9, 157.5, 153.8, 132.5, 130.6, 124.5, 121.0, 114.7, 111.6, 55.6, 42.7, 39.3, 31.1, 28.9, 24.3, 22.9, 14.0, 10.9; HRMS (ESI) calculated $[M+H]^+$ for $C_{20}H_{28}O_2N_4Cl$: 391.1895, found 391.1895, 803.3537 $[2M+Na]^+$.



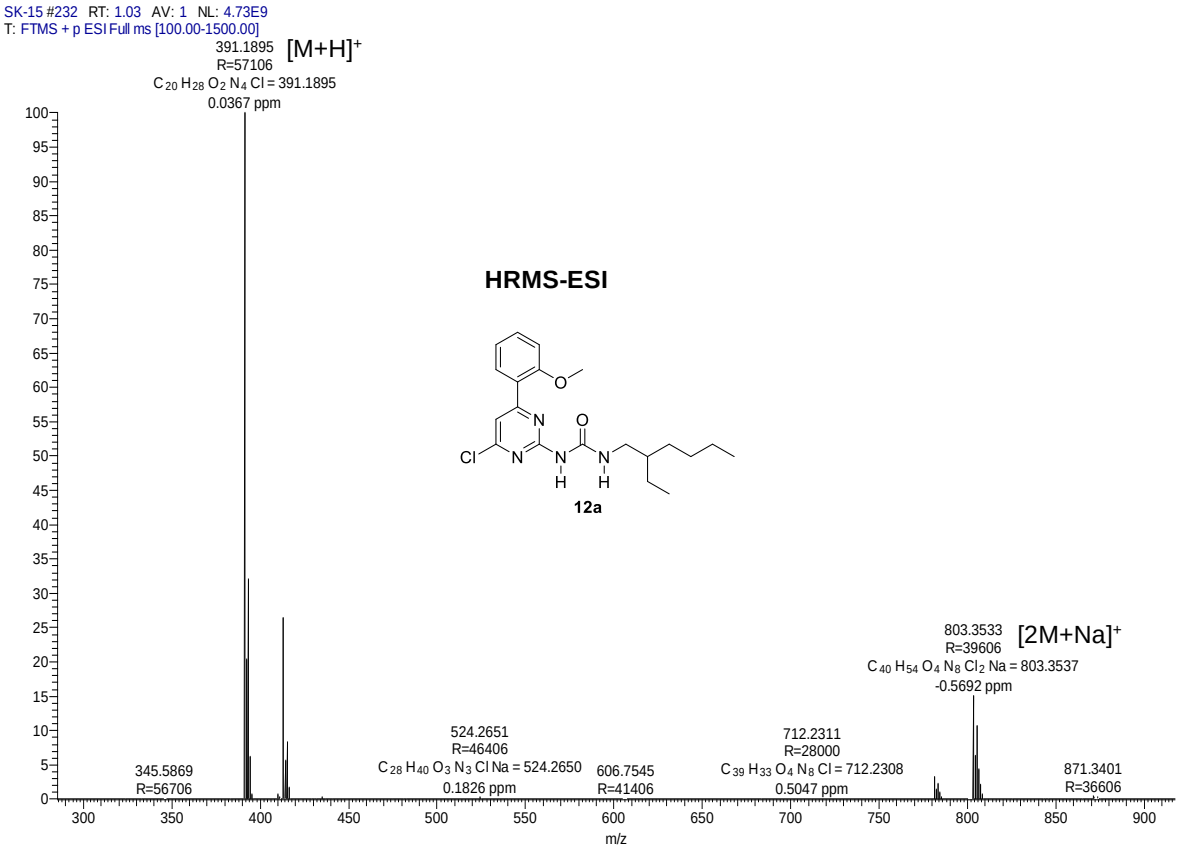
^{1}H NMR spectrum of compound **12a** ($CDCl_3$, 500 MHz, 298 K)



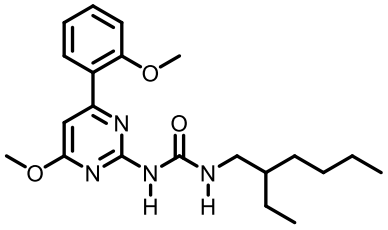
^{13}C NMR spectrum of compound **12a** ($CDCl_3$, 125 MHz, 298 K)



DEPT-135 NMR spectrum of compound **12a** ($CDCl_3$, 125 MHz, 298 K)

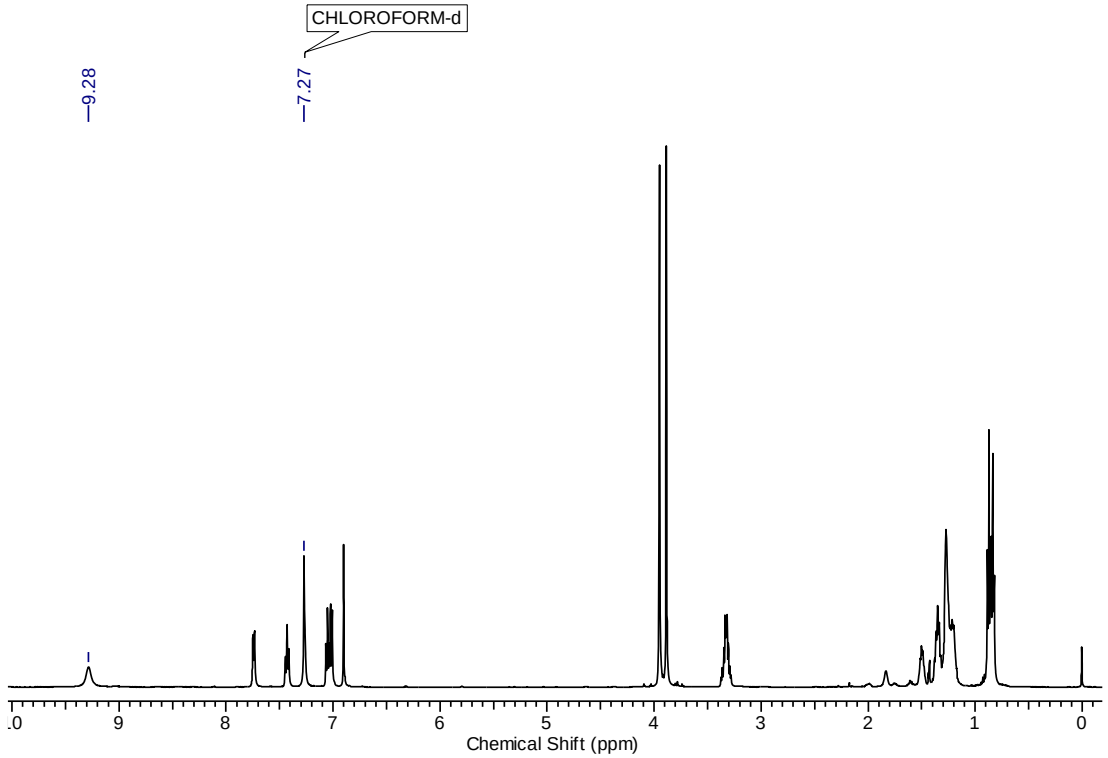


Compound 13a

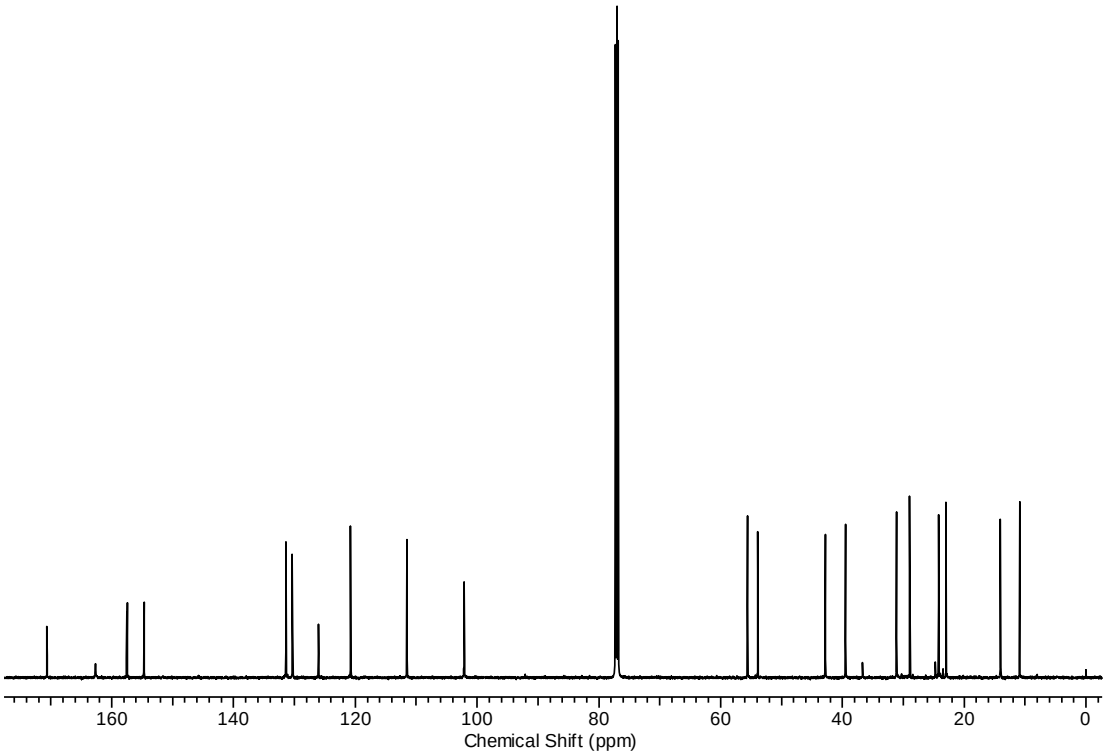


13a

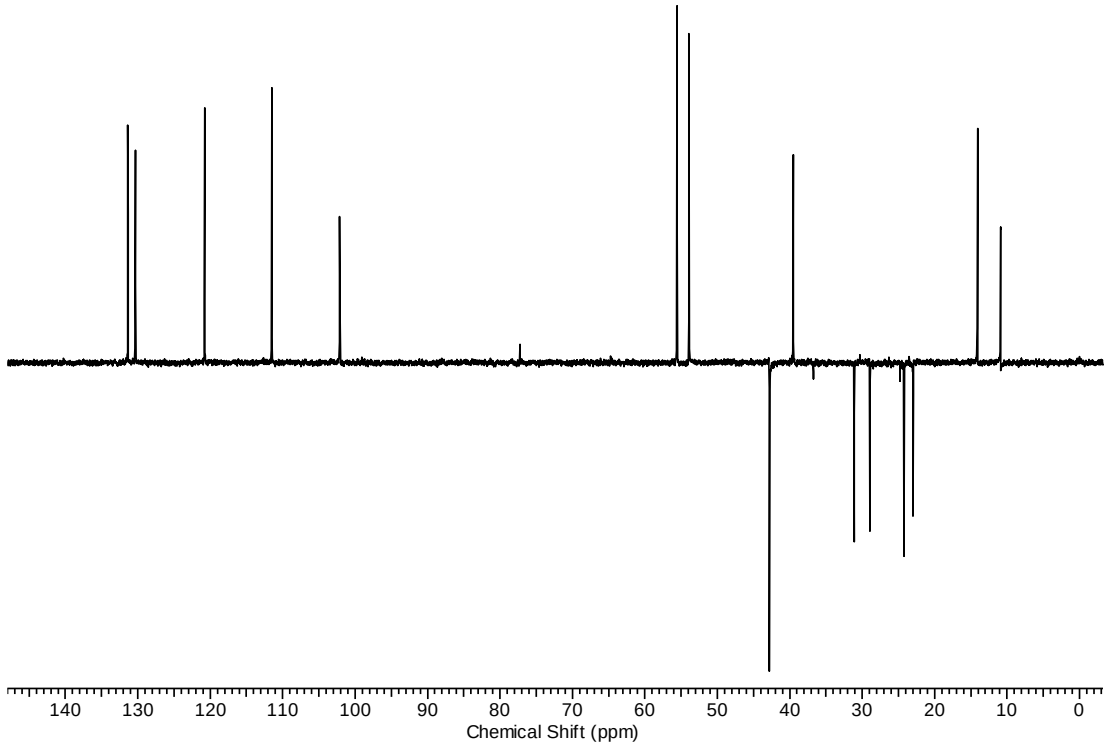
To a solution containing **12a** (0.5 g, 1.282 mmol, 1 equiv.), K_2CO_3 (0.531 g, 3.846 mmol, 3 equiv.) in 20 mL MeOH was refluxed for 6 h. After completion of reaction, the mixture was concentrated under reduced pressure. The residue was partitioned between DCM and water. The organic layer was separated, washed with water, brine solution, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (eluent: 40% AcOEt/ pet. ether, R_f: 0.4) afforded **13a** (0.405 g, 82%) as a fluffy white solid. mp: 73-75 ^oC; IR ($CHCl_3$) ν (cm^{-1}): 3431, 3249, 3012, 2930, 2868, 1684, 1596, 1545, 1453, 1370, 1272, 1124, 1019, 761; ^1H NMR (500 MHz, $CDCl_3$) δ: 9.28 (s, 1H), 7.75-7.73 (d, *J* = 7.02 Hz, 1H), 7.45-7.41 (t, *J* = 8.54 Hz, 1H), 7.27 (s, 1H), 7.07-7.04 (t, *J* = 7.63 Hz, 1H), 7.02-7.01 (d, *J* = 8.54 Hz, 1H), 6.90 (s, 1H), 3.95 (s, 1H), 3.89 (s, 1H), 3.37-3.28 (m, 2H), 1.51-1.48 (m, 1H), 1.38-1.34 (m, 2H), 1.27-1.17 (m, 6H), 0.89-0.86 (t, *J* = 7.63 Hz, 3H), 0.85-0.82 (t, *J* = 7.02 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.5,162.5, 157.5, 157.3, 154.6, 131.3, 130.2, 126.0, 120.7, 111.4, 102.0, 55.5, 53.8, 42.7, 39.4, 31.0, 28.9, 24.1, 22.9, 14.0, 10.8; HRMS (ESI) calculated $[M+H]^+$ for $C_{21}H_{31}O_3N_4$: 387.2391, found 387.2391, 795.4528 $[2M+Na]^+$.



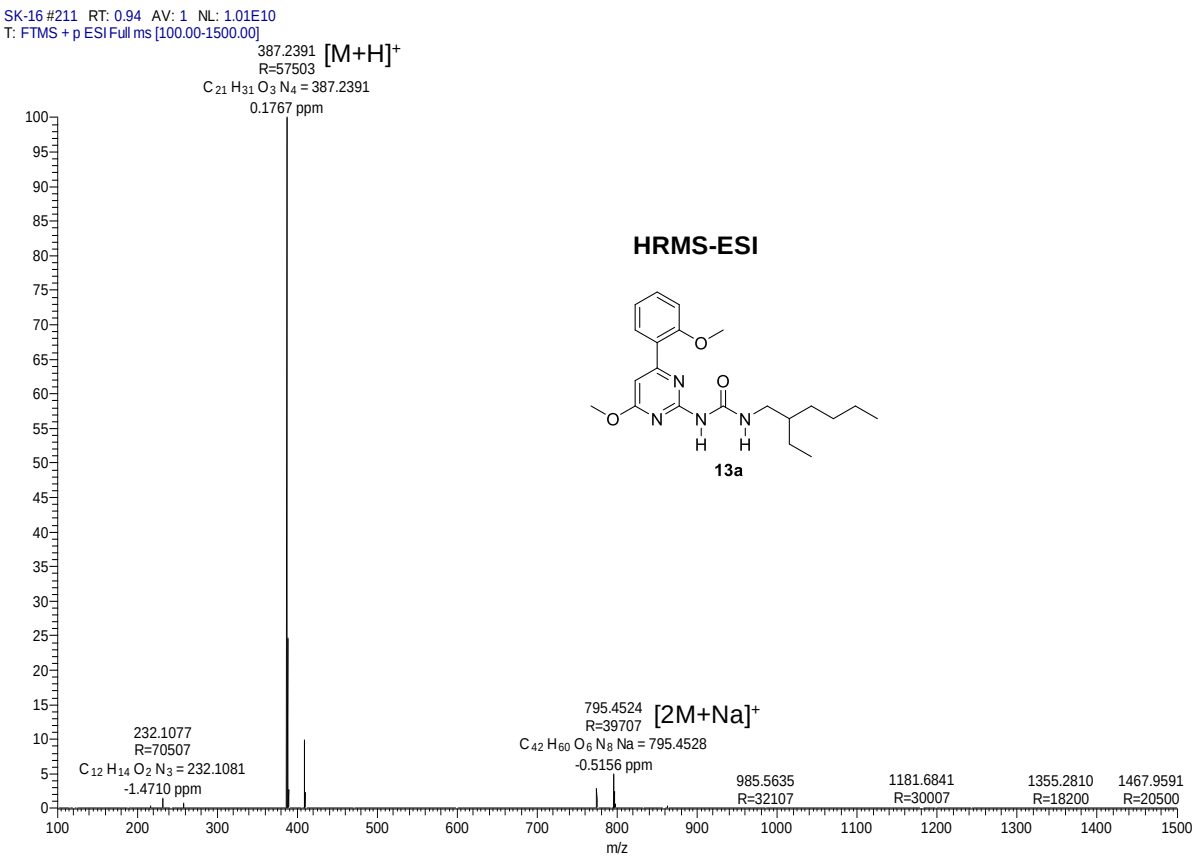
^{1}H NMR spectrum of compound **13a** ($CDCl_3$, 500 MHz, 298 K)



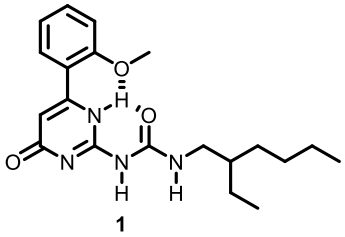
^{13}C NMR spectrum of compound **13a** ($CDCl_3$, 125 MHz, 298 K)



DEPT-135 NMR spectrum of compound **13a** ($CDCl_3$, 125 MHz, 298 K)

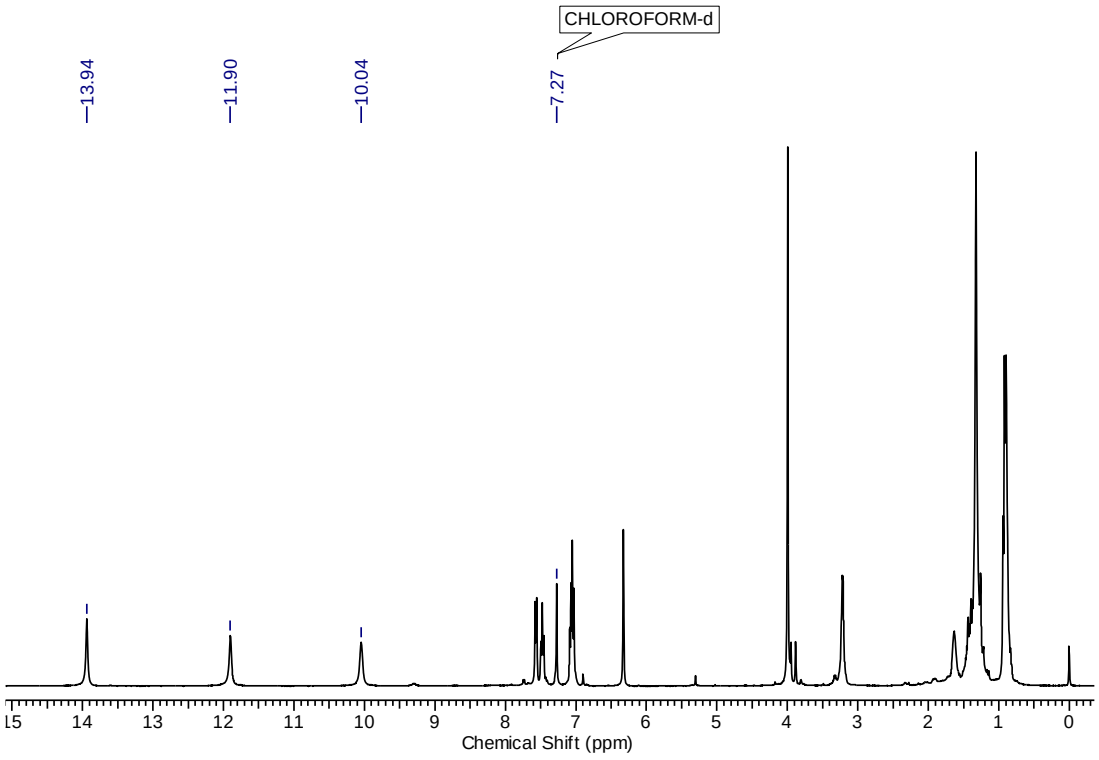


Compound 1

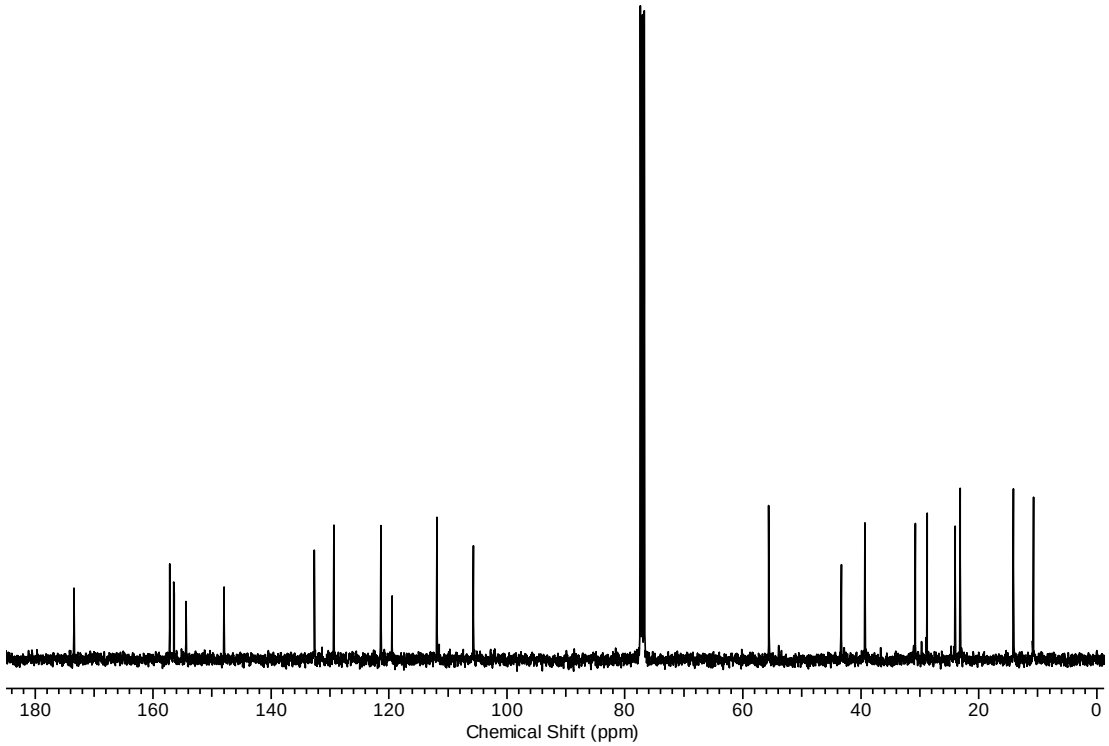


1

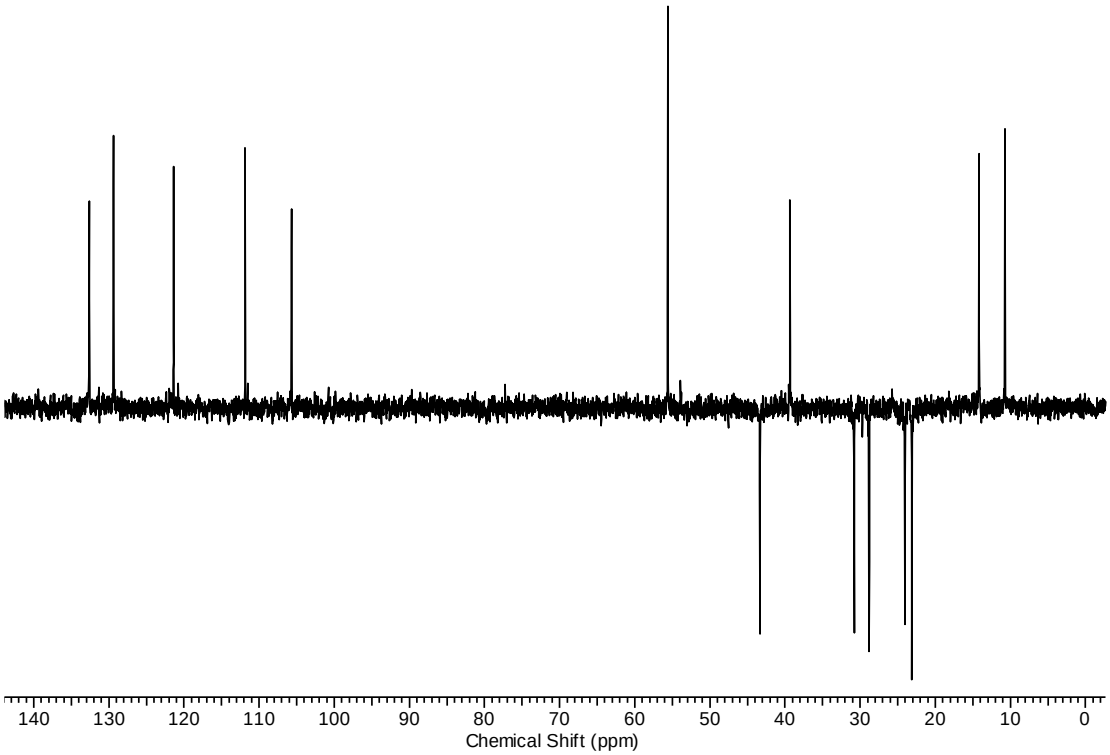
To a solution of **13a** (0.2 g, 0.518 mmol, 1 equiv.) in 4 mL of HBr in AcOH was refluxed for 1 h. The mixture was cooled to room temperature. and poured into ice water. The resulting solid was filtered and rinsed with cold water and dried under vacuum. Column chromatographic purification (eluent: 40% AcOEt/ pet. ether, R_f: 0.3) of the residue yielded **1** (0.138 g, 72%) as a white fluffy solid. mp: 130-132 ºC; IR ($CHCl_3$) *v* (cm^{-1}): 3685, 3216, 3022, 2964, 2869, 2403, 1696, 1638, 1575, 1527, 1259, 1217, 1066, 929, 772; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.94 (s, 1H), 11.90 (s, 1H), 10.04 (s, 1H), 7.58-7.56 (d, *J* = 7.58 Hz, 1H), 7.49-7.45 (t, *J* = 7.83 Hz, 1H), 7.09-7.03 (m, 2H), 6.33 (s, 1H), 3.99 (s, 3H), 3.22-3.21 (m, 2H), 1.69-1.58 (m, 1H), 1.47-1.37 (m, 2H), 1.32-1.26 (m, 6H), 0.94-0.83 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.3, 157.1, 156.4, 154.3, 147.9, 132.5, 129.3, 121.3, 119.4, 111.8, 105.6, 55.5, 43.2, 39.3, 30.7, 28.7, 24.0, 23.0, 14.1, 10.7; HRMS (ESI) calculated $[M+H]^+$ for $C_{20}H_{29}O_3N_4$: 373.2231, found 373.2234, 745.4396 $[2M+H]^+$.



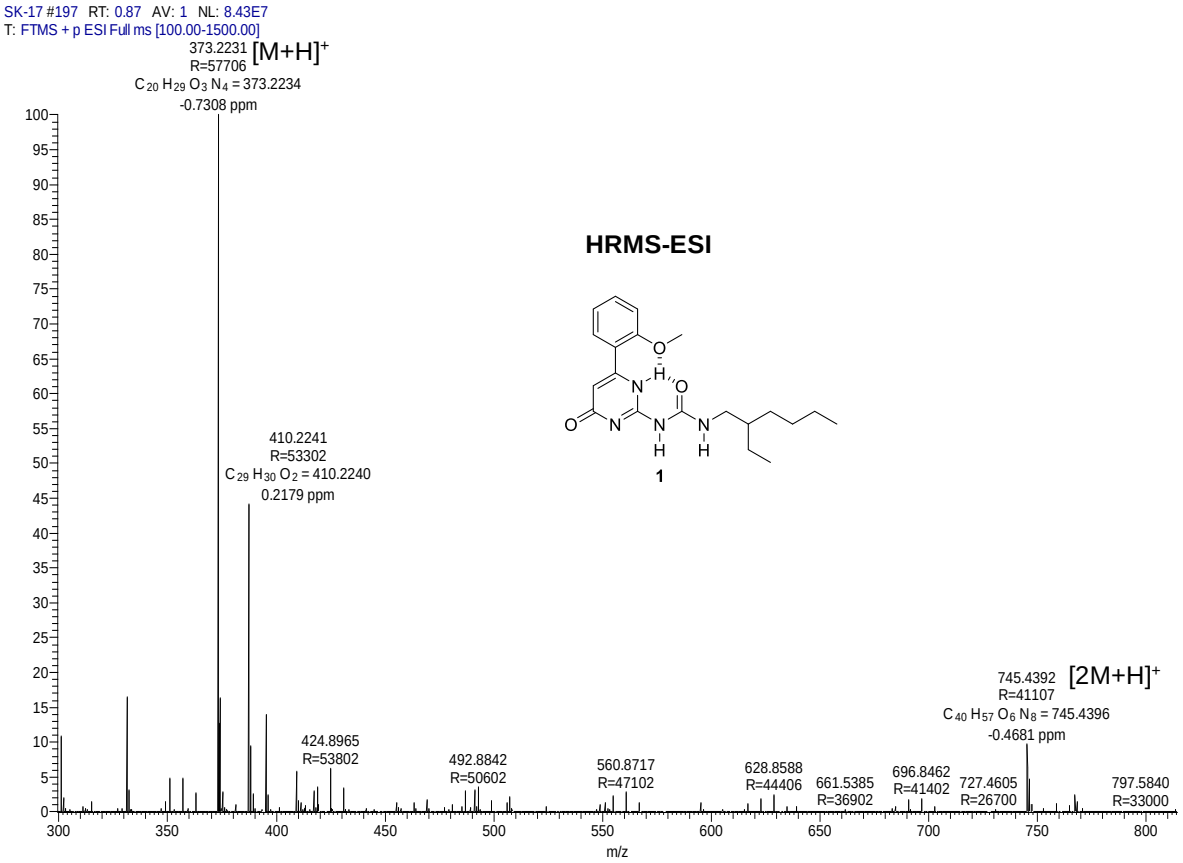
^{1}H NMR spectrum of compound **1** ($CDCl_3$, 400 MHz, 298 K)



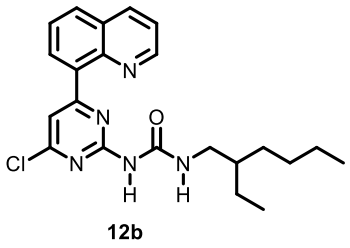
^{13}C NMR spectrum of compound **1** ($CDCl_3$, 100 MHz, 298 K)



DEPT-135 NMR spectrum of compound **1** ($CDCl_3$, 100 MHz, 298 K)

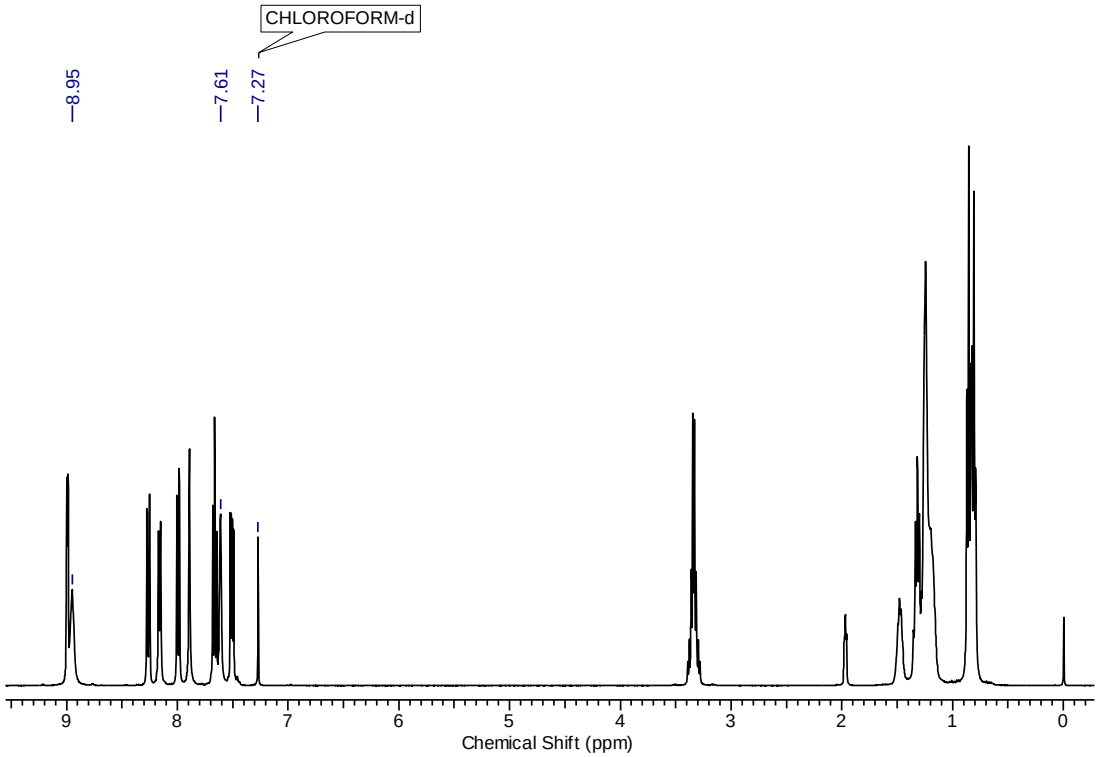


Compound 12b

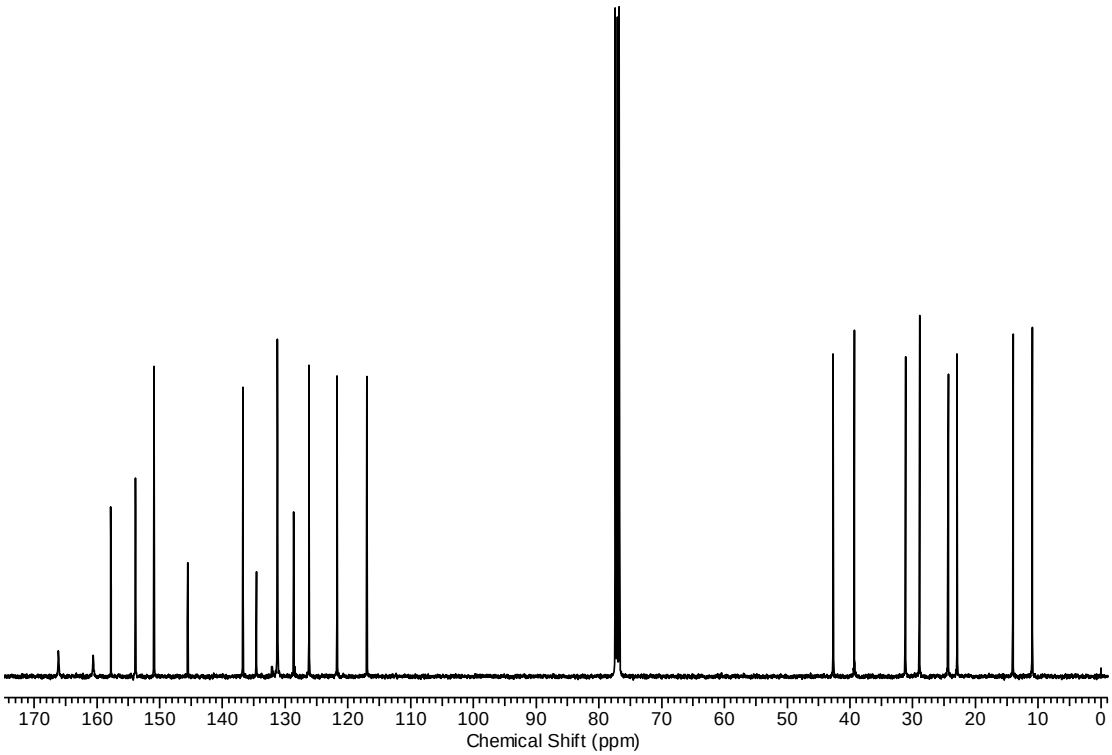


Following the same procedure for synthesis of compound **12a** and using 8-quinolinylboronic acid, **12b** was synthesized. Purification was effected by column chromatography (eluent: 30% AcOEt/ pet. ether, R_f: 0.5) to yield **12b** (62%) as a white fluffy solid. mp: 62-64 ^oC; IR ($CHCl_3$) ν (cm^{-1}): 3674, 3575, 3422, 3295, 3019, 2928, 1691, 1569, 1232, 1262, 764; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.00-8.98 (dd, J = 4.03 Hz, J = 1.47 Hz, 1H), 8.95 (s, IH), 8.27-8.25 (dd, J = 8.44 Hz, J = 1.83 Hz, 1H), 8.17-8.15 (d, J = 7.34 Hz, 1H), 8.00-7.98 (d, J = 8.07 Hz, 1H), 7.89 (s, 1H), 7.68-7.64 (t, J = 8.07 Hz, 1H), 7.61 (s, 1H), 7.52-7.49 (dd, J = 8.44 Hz, J = 4.40 Hz, 1H), 3.39-3.28 (m, 2H), 1.49-1.47 (m, 1H), 1.35-1.30 (m, 2H), 1.28-1.20 (m, 6H), 0.87-0.83 (t, J = 7.34 Hz, 3H), 0.83-0.79 (t, J = 6.97 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.0, 160.5, 157.7, 153.7, 150.8, 145.4, 136.6, 134.5, 131.2, 131.1, 128.6, 126.1,

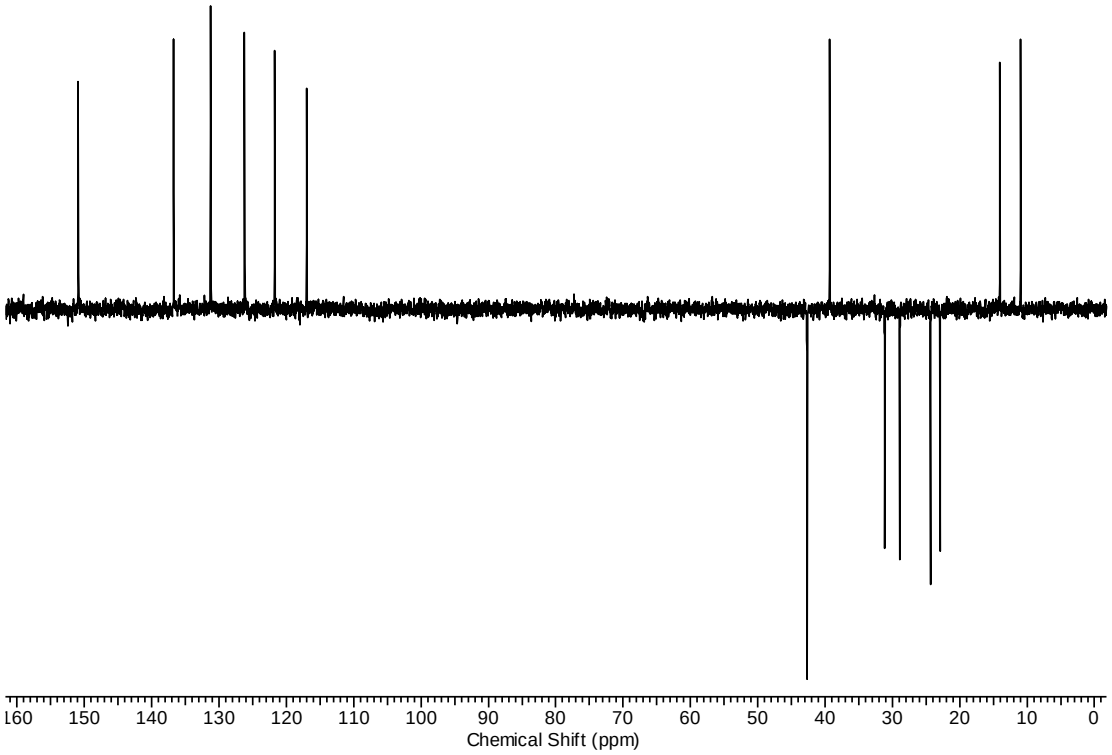
121.6, 116.9, 42.6, 39.2, 31.0, 28.8, 24.2, 22.8, 13.9, 10.9; HRMS (ESI) calculated $[M+H]^+$ for $C_{22}H_{27}ON_5Cl$: 412.1901, found 412.1899, 845.3544 $[2M+Na]^+$.



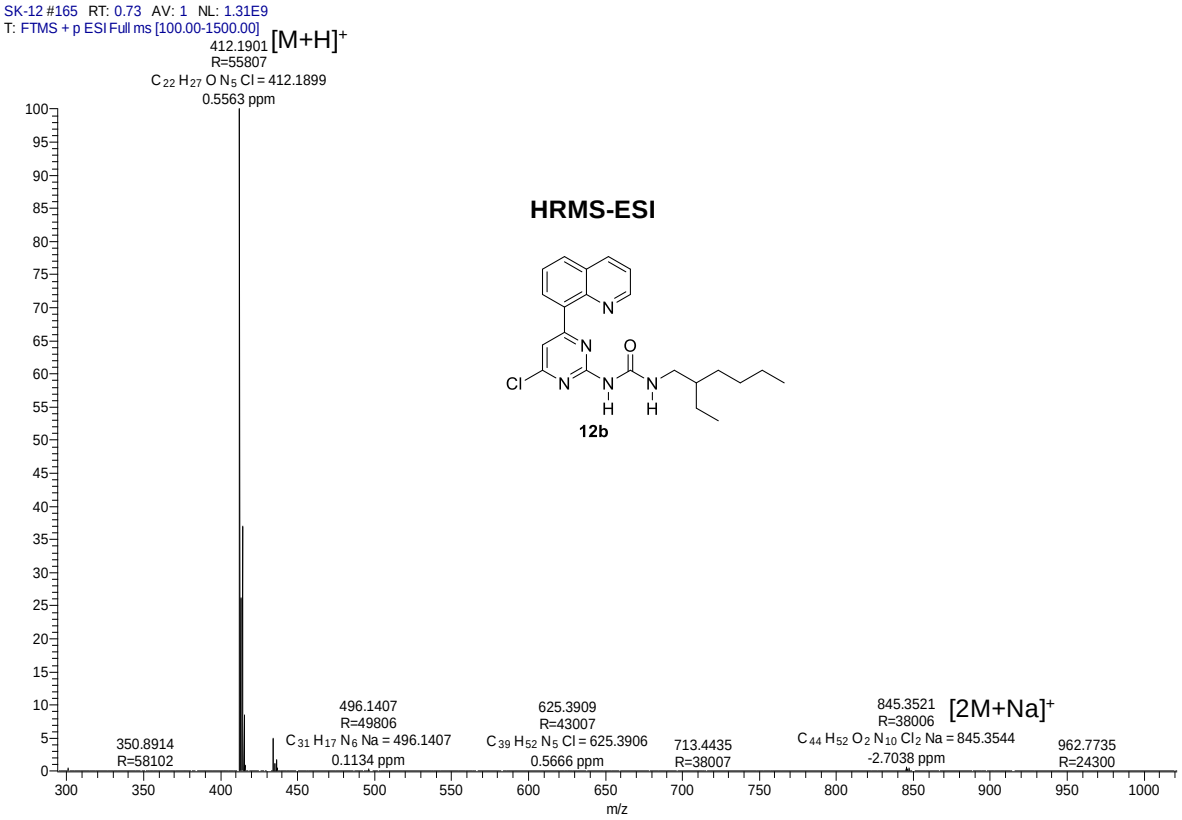
^{1}H NMR spectrum of compound **12b** ($CDCl_3$, 400 MHz, 298 K)



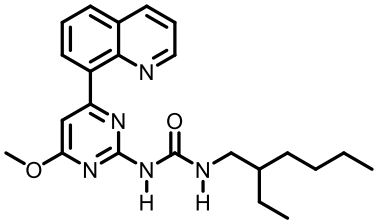
^{13}C NMR spectrum of compound **12b** ($CDCl_3$, 100 MHz, 298 K)



DEPT-135 NMR spectrum of compound **12b** ($CDCl_3$, 100 MHz, 298 K)

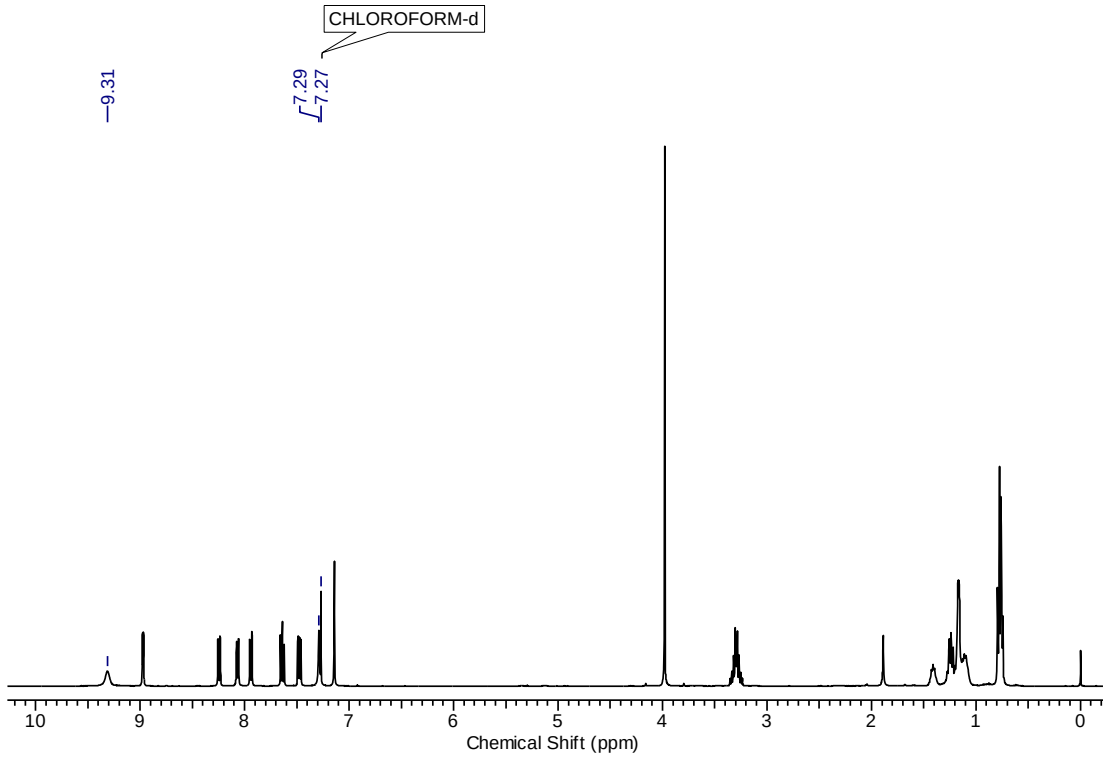


Compound 13b

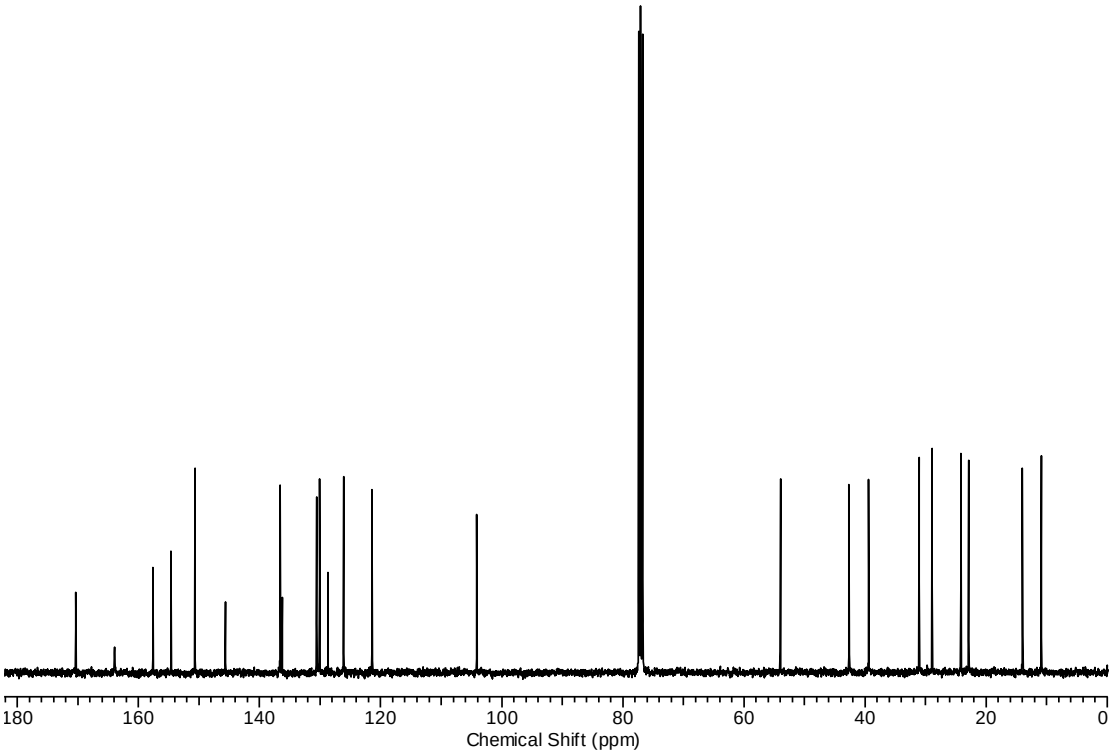


13b

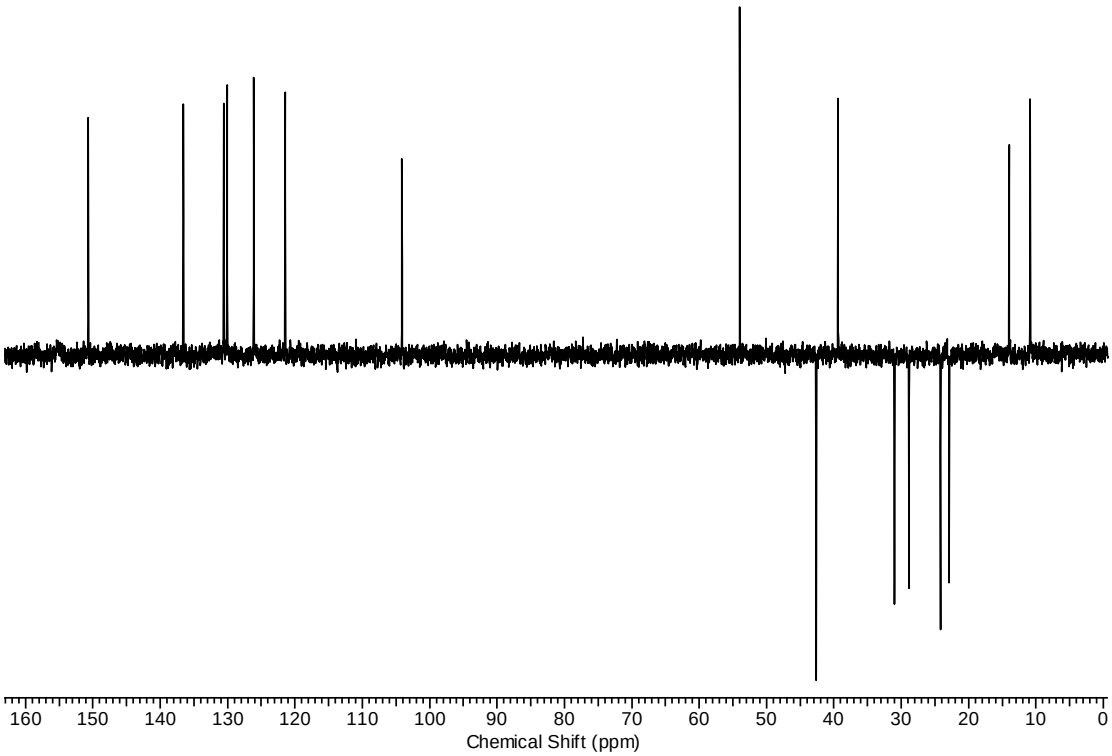
Following the same procedure for synthesis of compound **13a** and using 8-quinolinylboronic acid, **13b** was synthesized. Purification was effected by column chromatography (eluent: 40% AcOEt/ pet. ether, R_f: 0.4) to yield **13b** (87%) as a liquid. IR ($CHCl_3$) ν (cm^{-1}): 3431, 3247, 3013, 2961, 2867, 1685, 1593, 1543, 1450, 1253, 1138, 765; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.31 (s, 1H), 8.98-8.97 (dd, J = 4.16 Hz, J = 1.71 Hz, 1H), 8.26-8.23 (dd, J = 8.31 Hz, J = 1.71 Hz, 1H), 8.08-8.06 (dd, J = 7.34 Hz, J = 1.47 Hz, 1H), 7.95-7.93 (dd, J = 8.31 Hz, J = 1.47 Hz, 1H), 7.66-7.62 (t, J = 7.58 Hz, 1H), 7.49-7.46 (dd, J = 8.31 Hz, J = 4.16 Hz, 1H), 7.29 (s, 1H), 7.14 (s, 1H), 3.98 (s, 3H), 3.35-3.24 (m, 2H), 1.43-1.40 (m, 1H), 1.27-1.20 (m, 2H), 1.17-1.10 (m, 6H), 0.79-0.74 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.3, 163.8, 157.5, 154.5, 150.6, 145.6, 136. 5, 136.2, 130.5, 130.0, 128.6, 126.0, 121.3, 104.0, 53.9, 42.5, 39.3, 30.9, 28.8, 24.1, 22.8, 13.9, 10.8; HRMS (ESI) calculated $[M+H]^+$ for $C_{23}H_{30}O_2N_5$: 408.2392, found 408.2394, 837.4535 $[2M+Na]^+$.



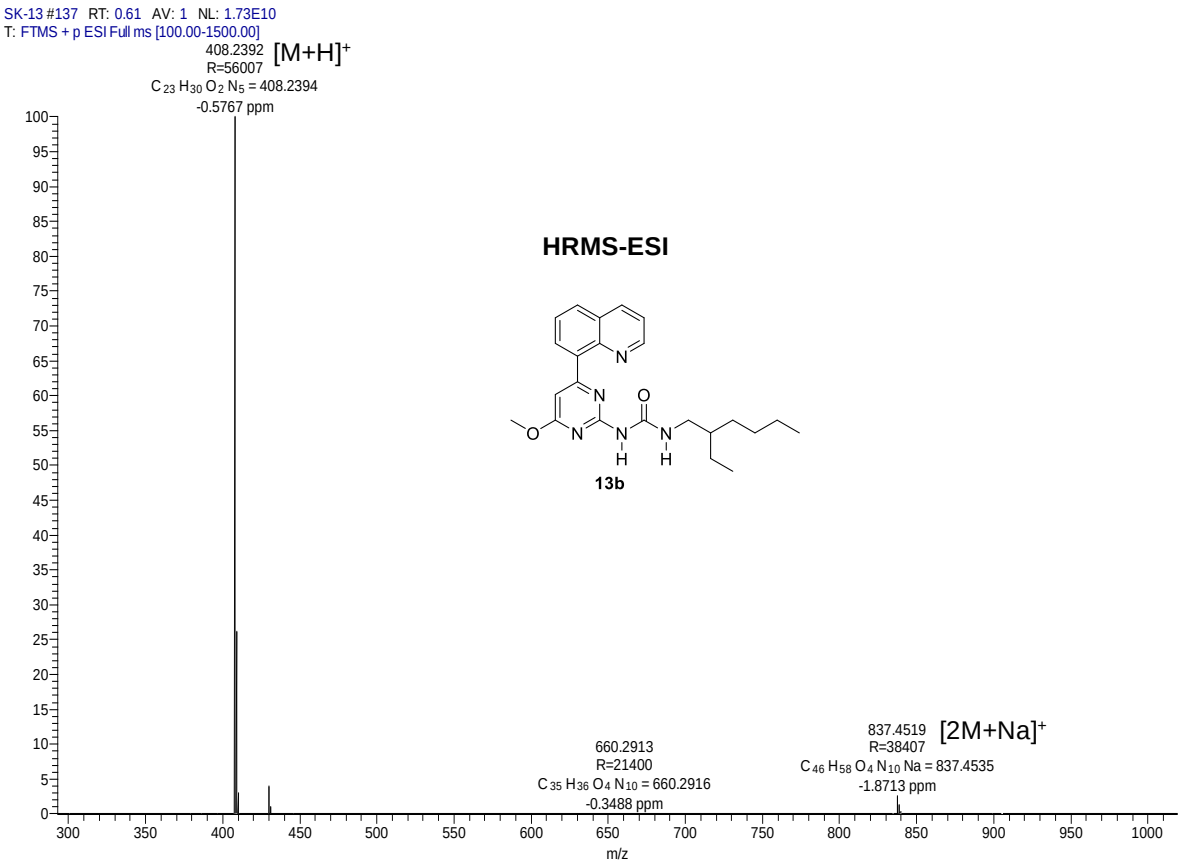
^1H NMR spectrum of compound **13b** ($CDCl_3$, 400 MHz, 298 K)



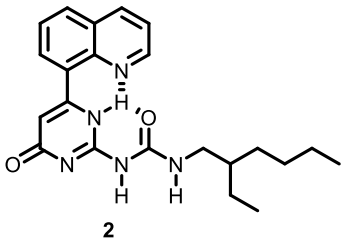
^{13}C NMR spectrum of compound **13b** ($CDCl_3$, 100 MHz, 298 K)



DEPT-135 NMR spectrum of compound **13b** ($CDCl_3$, 100 MHz, 298 K)

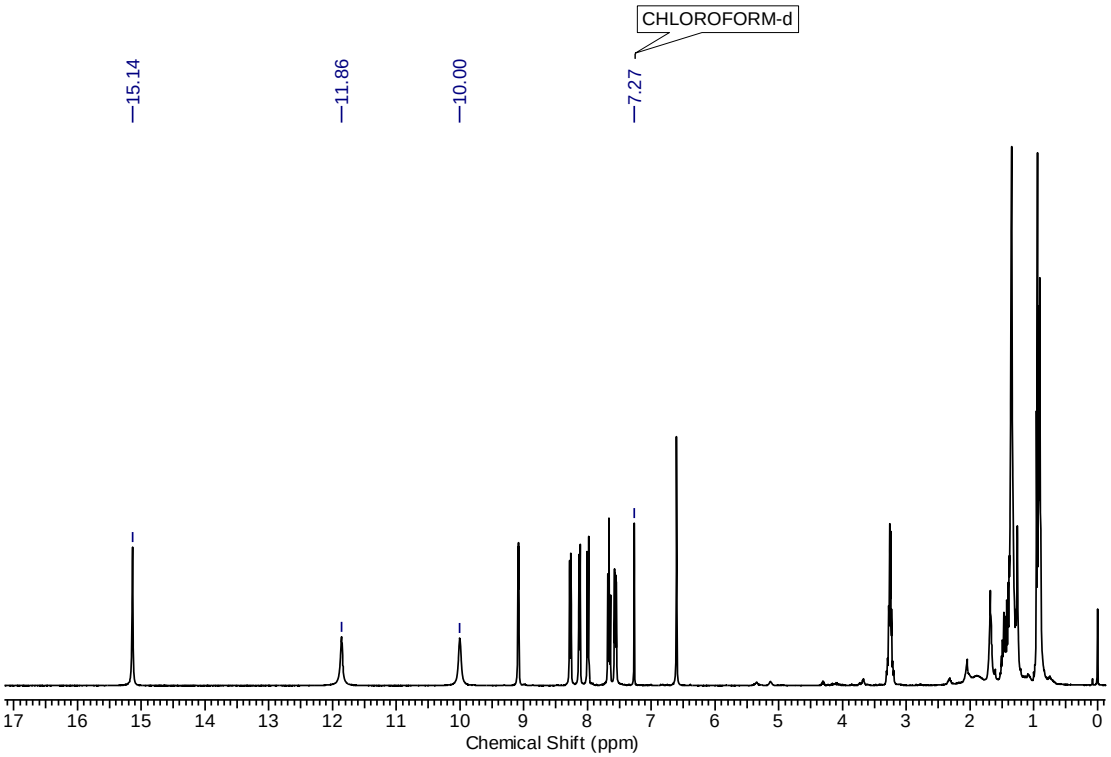


Compound 2

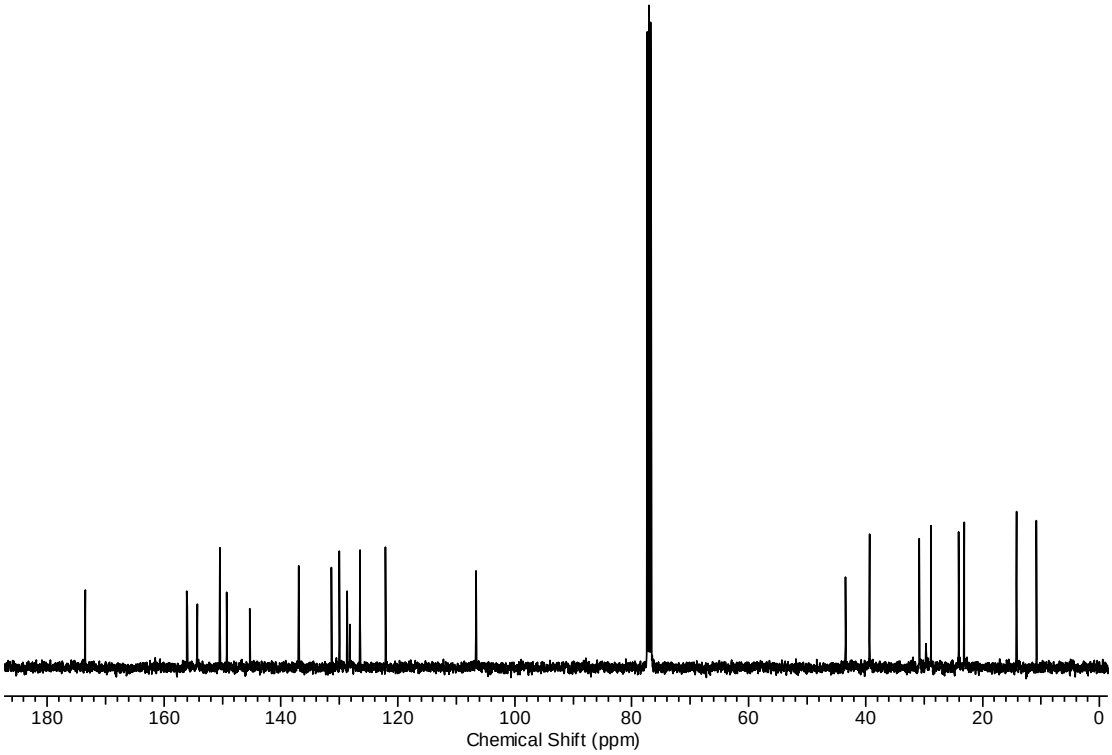


Following the same procedure for synthesis of compound **1** and using 8-quinolinylboronic acid, **2** was synthesized. Purification was effected by column chromatography (eluent: 40% AcOEt/ pet. ether, R_f: 0.3) to yield **2** (77%) as a white fluffy solid. mp: 183-185 ^oC; IR ($CHCl_3$) ν (cm^{-1}): 3199, 3016, 2958, 2863, 1703, 1634, 1573, 1530, 1256, 1032, 763; ^1H NMR (400 MHz, $CDCl_3$) δ: 15.14 (s, 1H), 11.86 (s, 1H), 10.00 (s, 1H), 9.09-9.07 (dd, J = 4.40 Hz, J = 1.83 Hz, 1H), 8.28-8.26 (dd, J = 8.44 Hz, J = 1.47 Hz, 1H), 8.13-8.12 (dd, J = 7.70 Hz, J = 1.10 Hz, 1H), 8.00-7.98 (d, J = 8.07 Hz, 1H), 7.68-7.64 (t, J = 7.70 Hz, 1H), 7.58-7.55 (dd, J = 8.44 Hz, J = 4.40 Hz, 1H), 6.60 (s, 1H), 3.31-3.20 (m, 2H), 1.68-1.61 (m, 1H), 1.51-1.38 (m, 2H), 1.35-1.26 (m, 6H), 0.96-0.89 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.4, 156.0, 154.2, 150.3, 149.2, 145.2, 136.8, 131.3, 129.9, 128.6, 128.1, 126.4, 122.0,

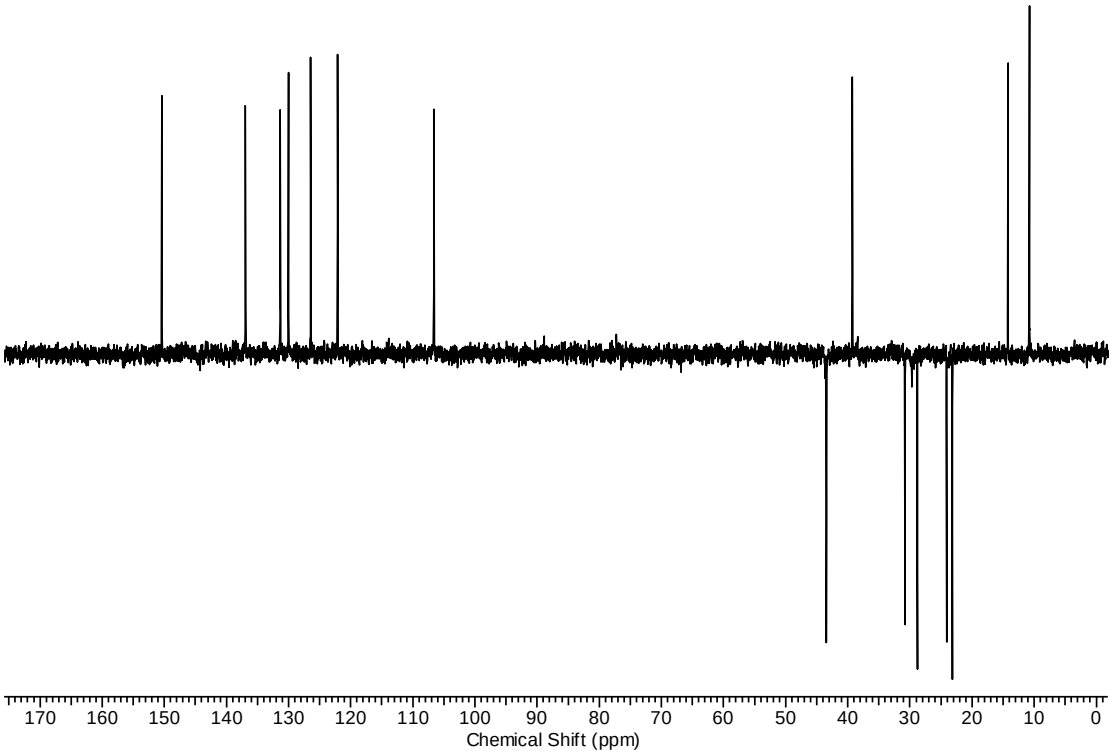
106.5, 43.3, 39.2, 30.7, 28.7, 24.0, 23.1, 14.1, 10.7; HRMS (ESI) calculated $[M+H]^+$ for $C_{22}H_{28}O_2N_5$: 394.2235, found 394.2238, 787.4402 $[2M+H]^+$.



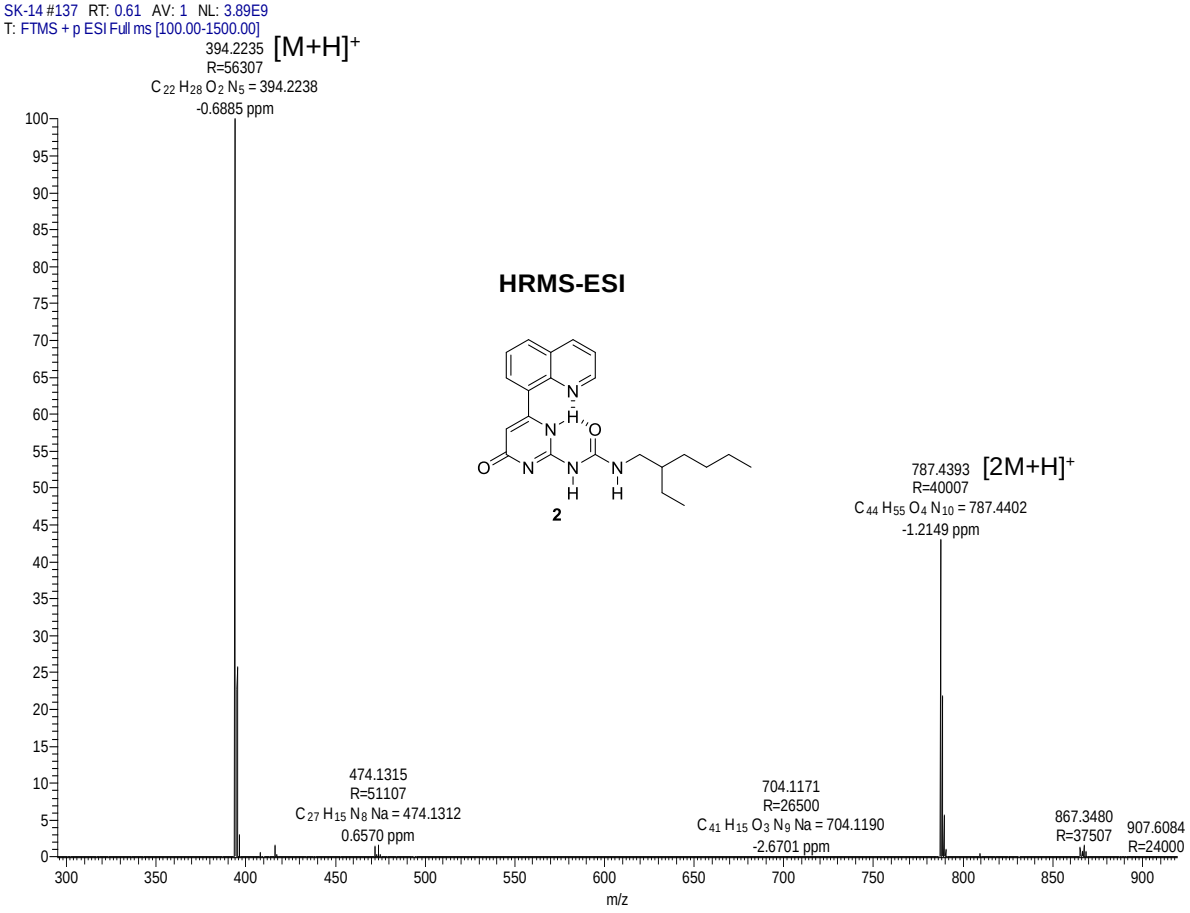
^{1}H NMR spectrum of compound **2** ($CDCl_3$, 400 MHz, 298 K)



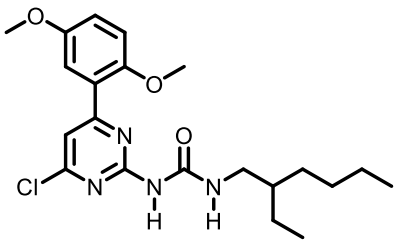
^{13}C NMR spectrum of compound **2** ($CDCl_3$, 100 MHz, 298 K)



DEPT-135 NMR spectrum of compound **2** ($CDCl_3$, 100 MHz, 298 K)

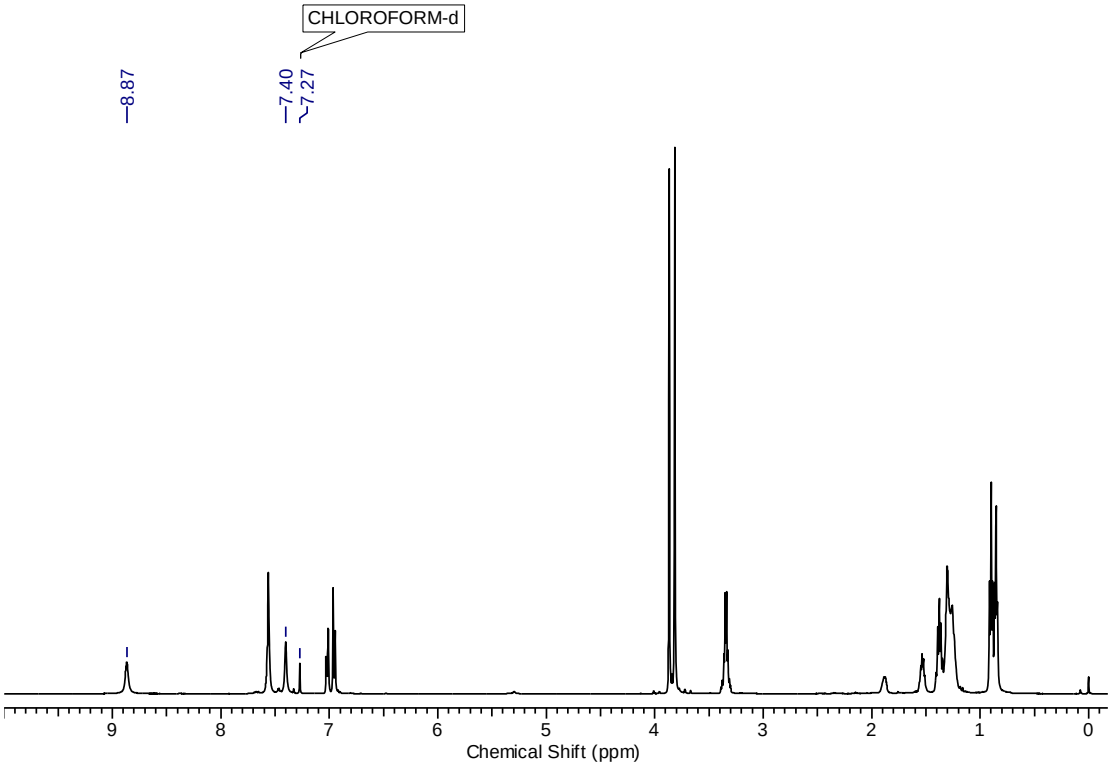


Compound 12c

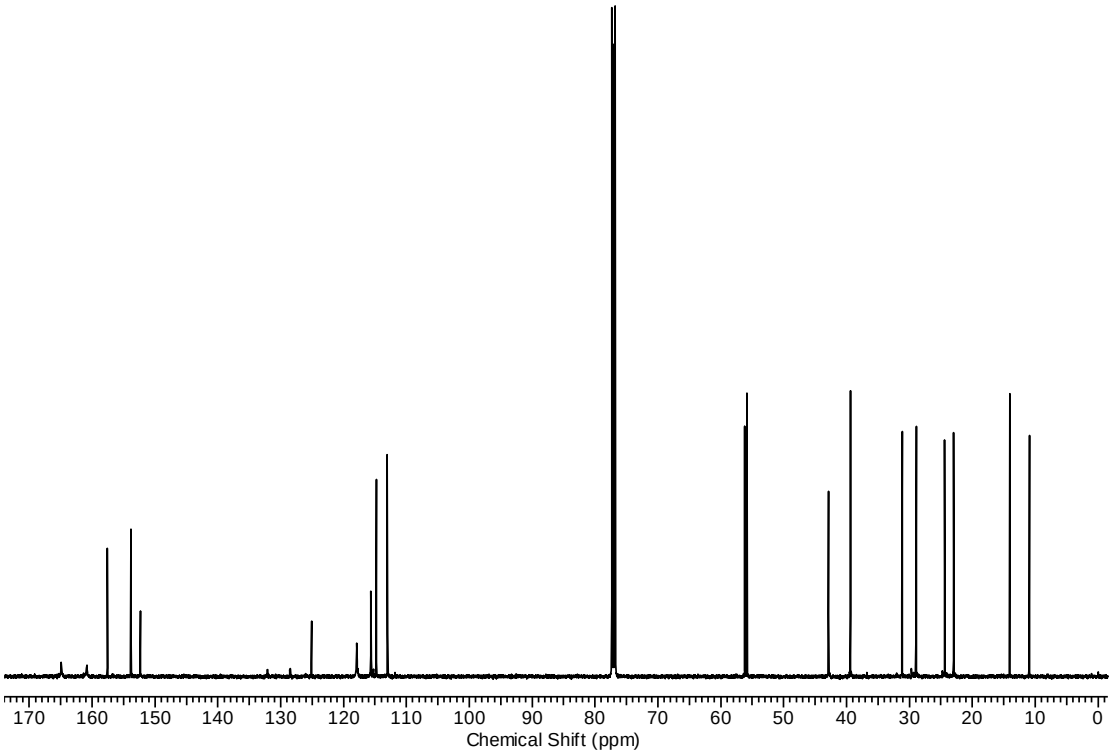


12c

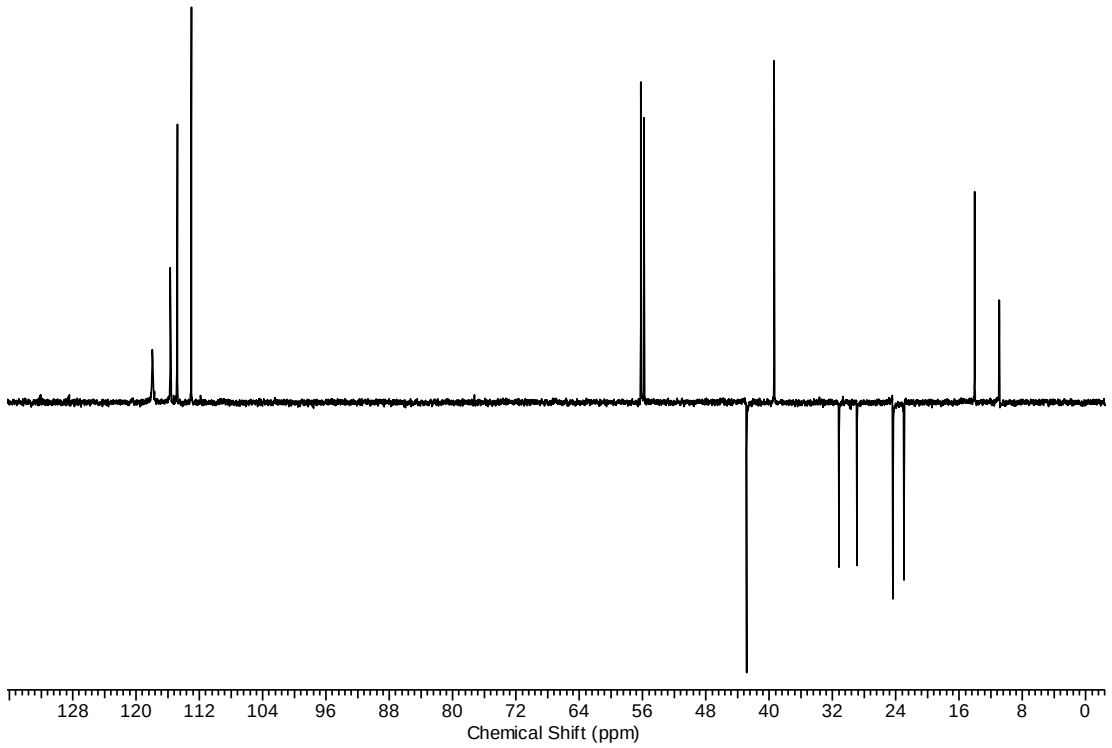
Following the same procedure for synthesis of compound **12a** and using 2,5-dimethoxyphenyl boronic acid, **12c** was synthesized. Purification was effected by column chromatography (eluent: 30% AcOEt/ pet. ether, R_f: 0.5) to yield **12c** (64%) as a white fluffy solid. mp: 76-78 ^oC; IR ($CHCl_3$) *v* (cm^{-1}): 3423, 3287, 3144, 3008, 2927, 2866, 1689, 1571, 1434, 1263, 1111, 759; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.87 (s, 1H), 7.56 (s, 2H), 7.40 (s, 1H), 7.03-7.01 (dd, *J* = 9.16 Hz, *J* = 3.05 Hz, 1H), 6.96-6.94 (d, *J* = 9.16 Hz, 1H), 3.87 (s, 3H), 3.82 (s, 3H), 3.39-3.30 (m, 2H), 1.56-1.51 (m, 1H), 1.40-1.35 (m, 2H), 1.31-1.24 (m, 6H), 0.91-0.89 (t, *J* = 7.32 Hz, 3H), 0.87-0.84 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 164.8, 160.8, 157.5, 153.8, 153.7, 152.3, 125.0, 117.8, 115.6, 114.7, 112.9, 56.1, 55.7, 42.8, 39.3, 31.1, 28.8, 24.3, 22.9, 13.9, 10.8; HRMS (ESI) calculated $[M+H]^+$ for $C_{21}H_{30}O_3N_4Cl$: 421.2001, found 421.2001, 863.3749 $[2M+Na]^+$.



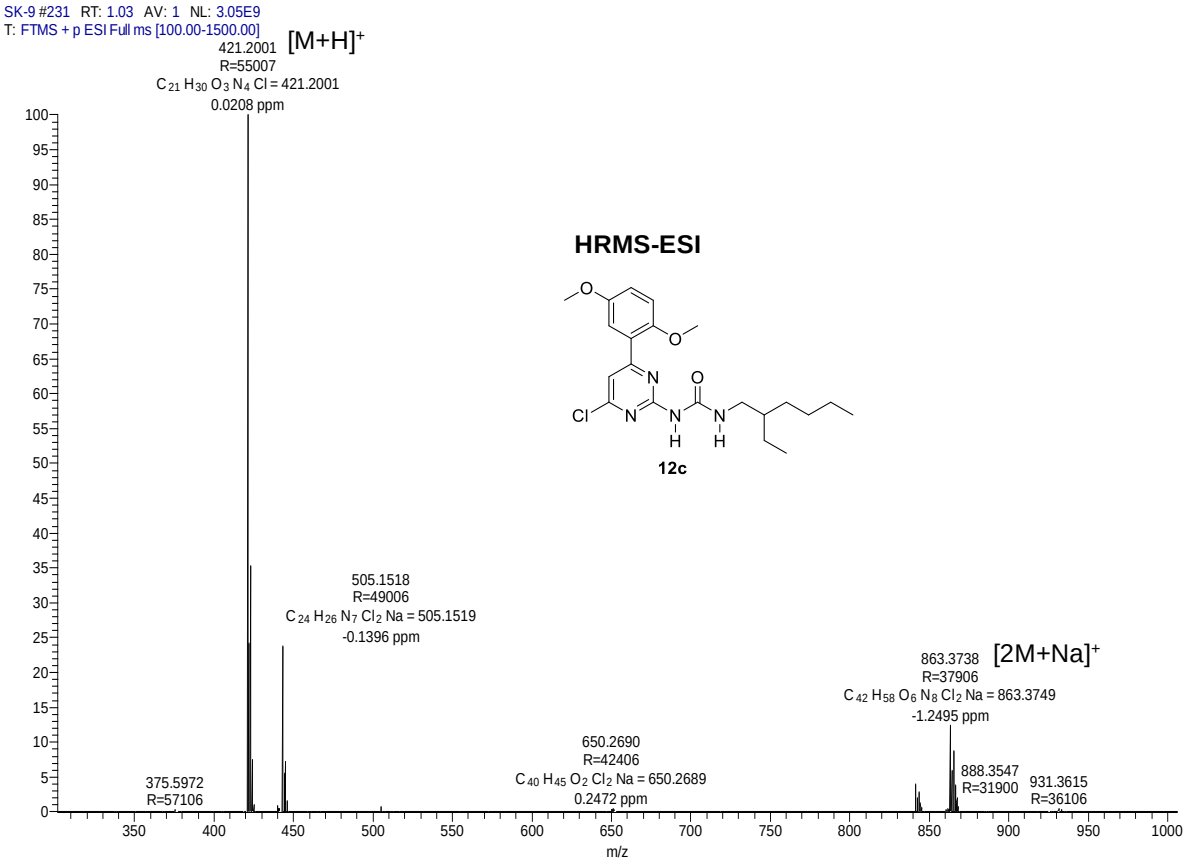
^1H NMR spectrum of compound **12c** ($CDCl_3$, 500 MHz, 298 K)



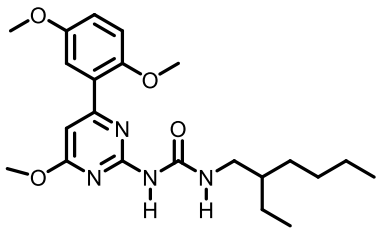
^{13}C NMR spectrum of compound **12c** ($CDCl_3$, 125 MHz, 298 K)



DEPT-135 NMR spectrum of compound **12c** ($CDCl_3$, 125 MHz, 298 K)

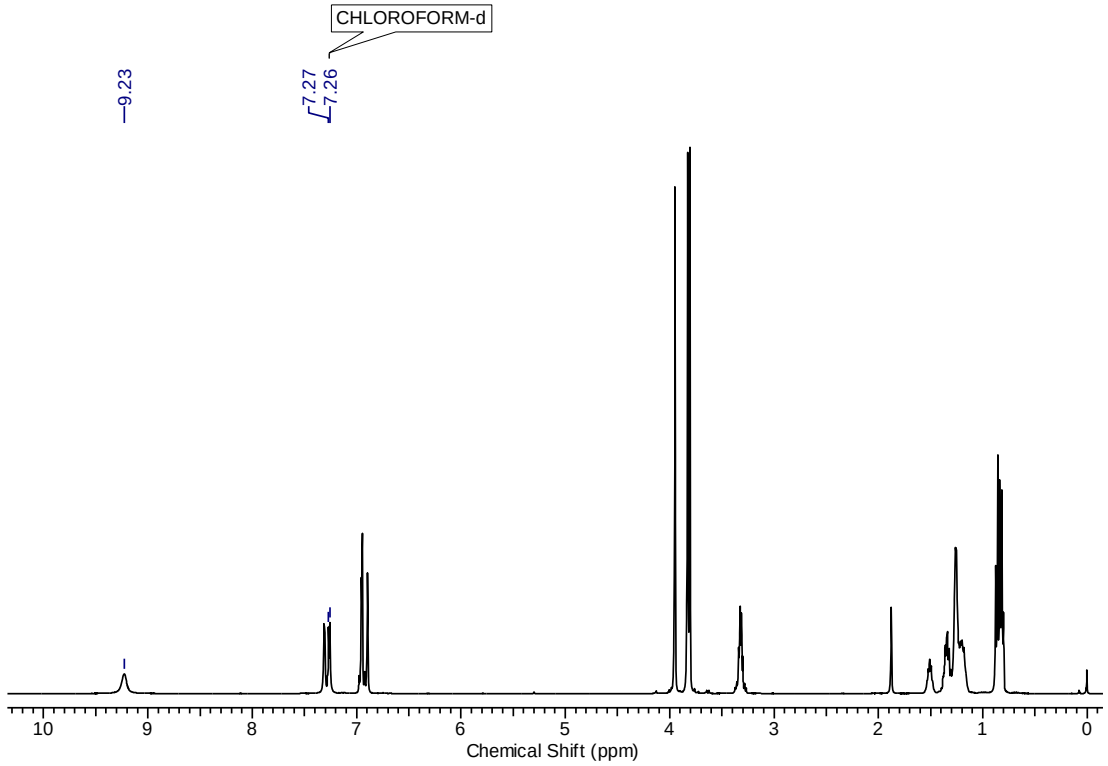


Compound 13c

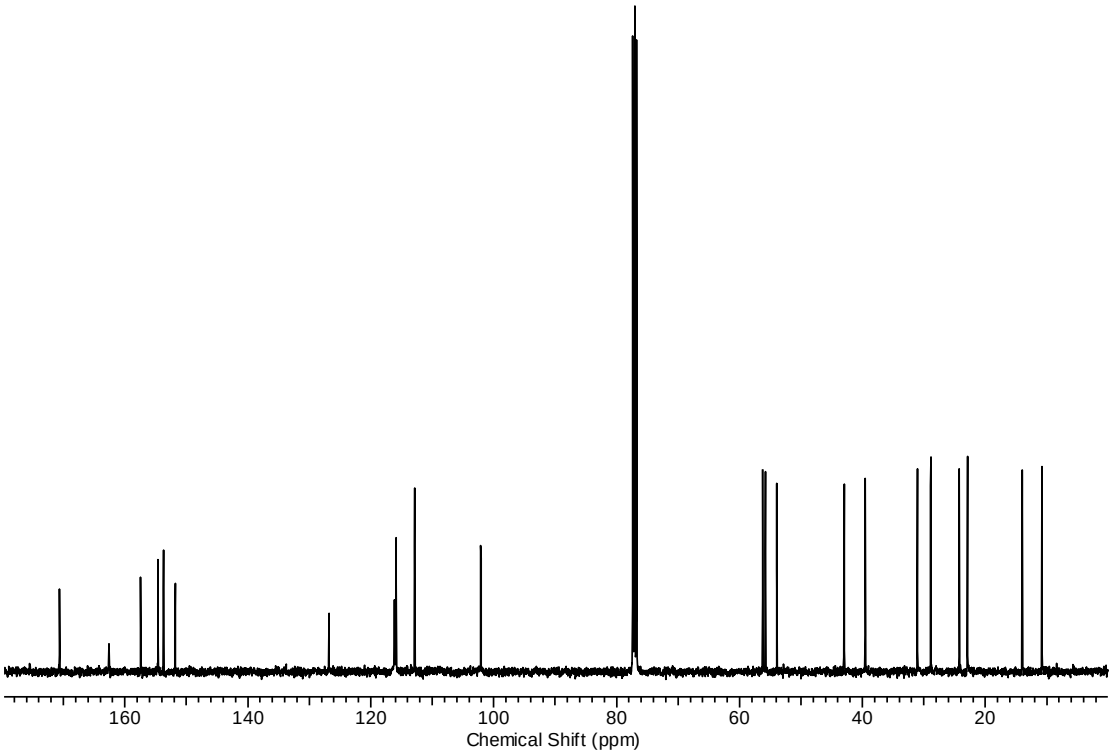


13c

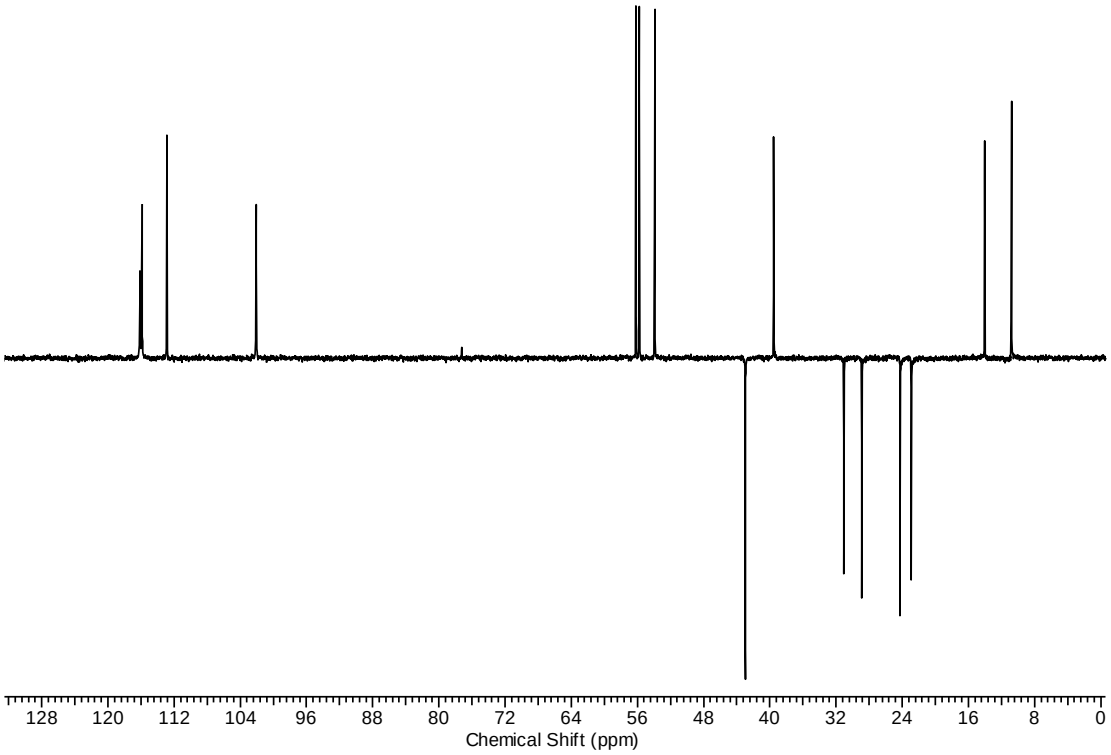
Following the same procedure for synthesis of compound **13a** and using 2,5-dimethoxyphenyl boronic acid, **13c** was synthesized. Purification was effected by column chromatography (eluent: 30% AcOEt/ pet. ether, R_f: 0.4) to yield **13c** (90%) as a fluffy white solid. mp: 48-50 ^oC; IR ($CHCl_3$) ν (cm^{-1}): 3431, 3249, 3010, 2952, 2868, 1685, 1589, 1546, 1361, 1220, 762; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.23 (s, 1H), 7.31 (s, 1H), 7.26 (s,1H), 6.95-6.94 (m, 2H), 6.89 (s, 1H), 3.95 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.37-3.27 (m, 2H), 1.54-1.48 (m, 1H), 1.38-1.30 (m, 2H), 1.26-1.18 (m, 6H), 0.87-0.80 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.5, 162.5, 157.3, 154.5, 153.6, 151.7, 126.7, 116.0, 115.8, 112.8, 102.0, 56.1, 55.7, 53.8, 42.9, 39.5, 30.9, 28.8, 24.2, 22.8, 13.9, 10.7; HRMS (ESI) calculated $[M+H]^+$ for $C_{22}H_{33}O_4N_4$: 417.2491, found 417.2496, 855.4739 $[2M+Na]^+$.



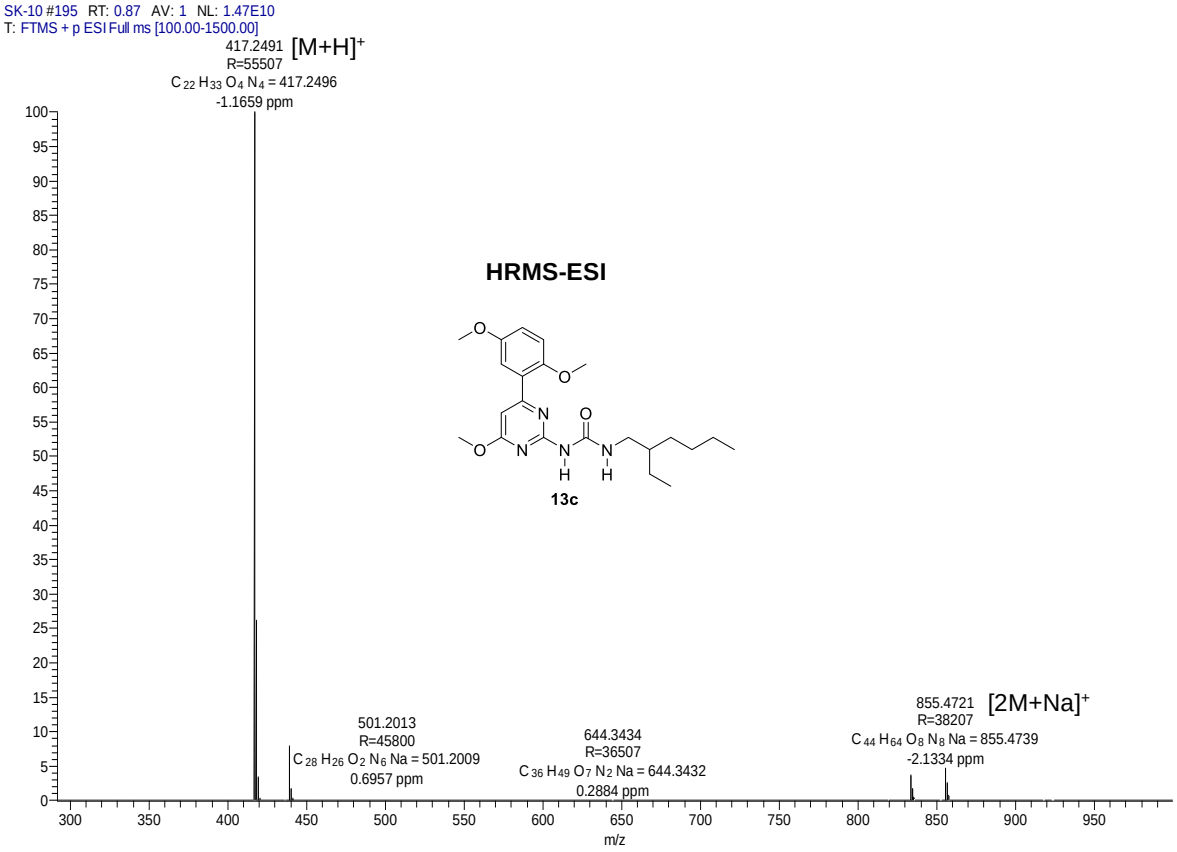
^{1}H NMR spectrum of compound **13c** ($CDCl_3$, 400 MHz, 298 K)



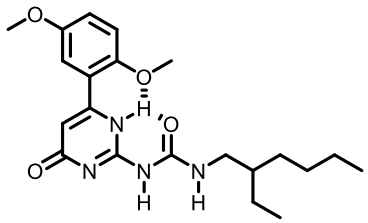
^{13}C NMR spectrum of compound **13c** ($CDCl_3$, 100 MHz, 298 K)



DEPT-135 NMR spectrum of compound **13c** ($CDCl_3$, 100 MHz, 298 K)

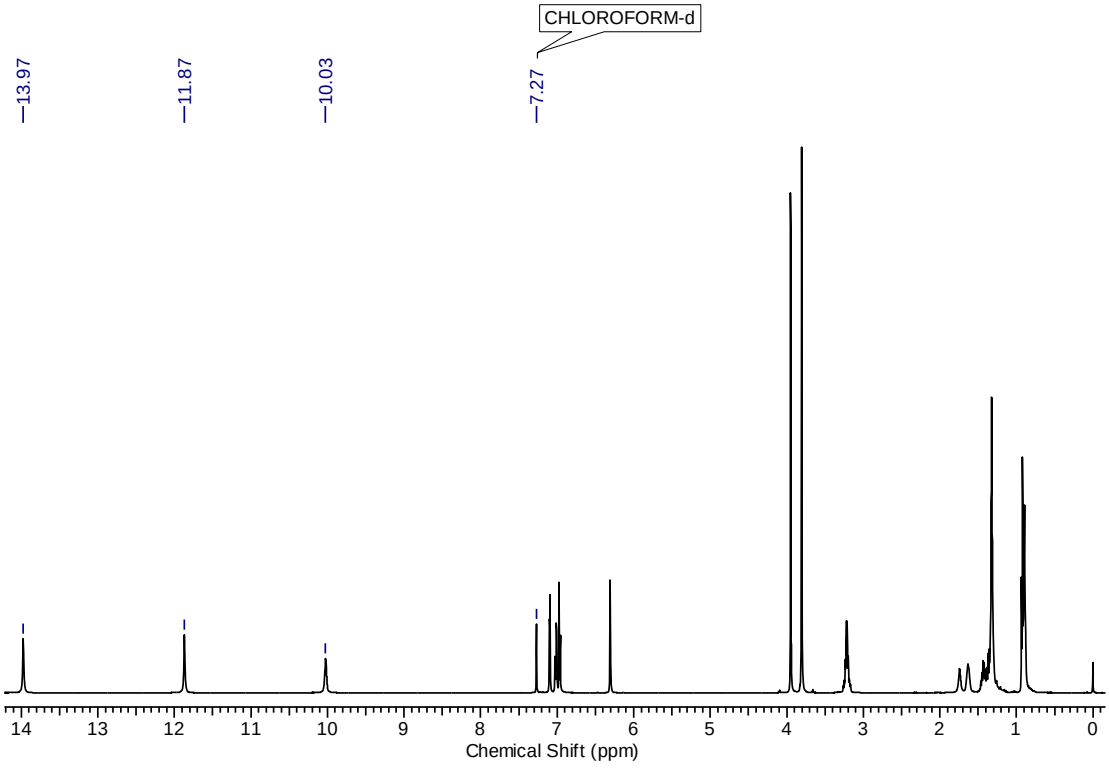


Compound 3

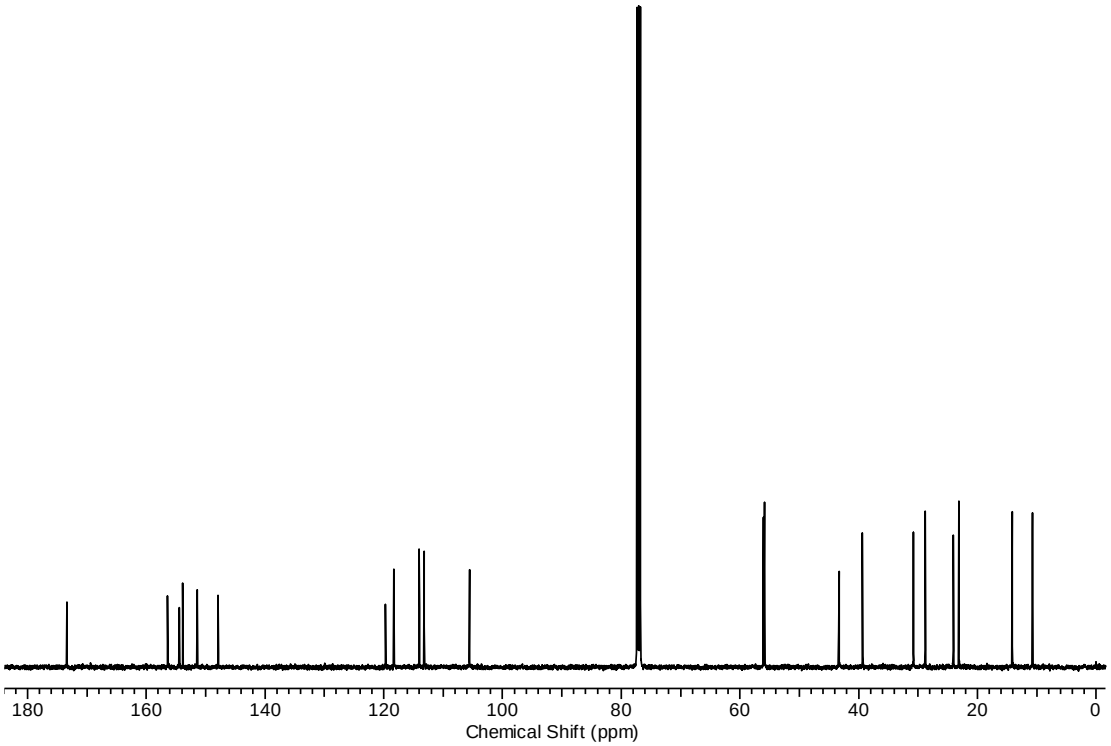


3

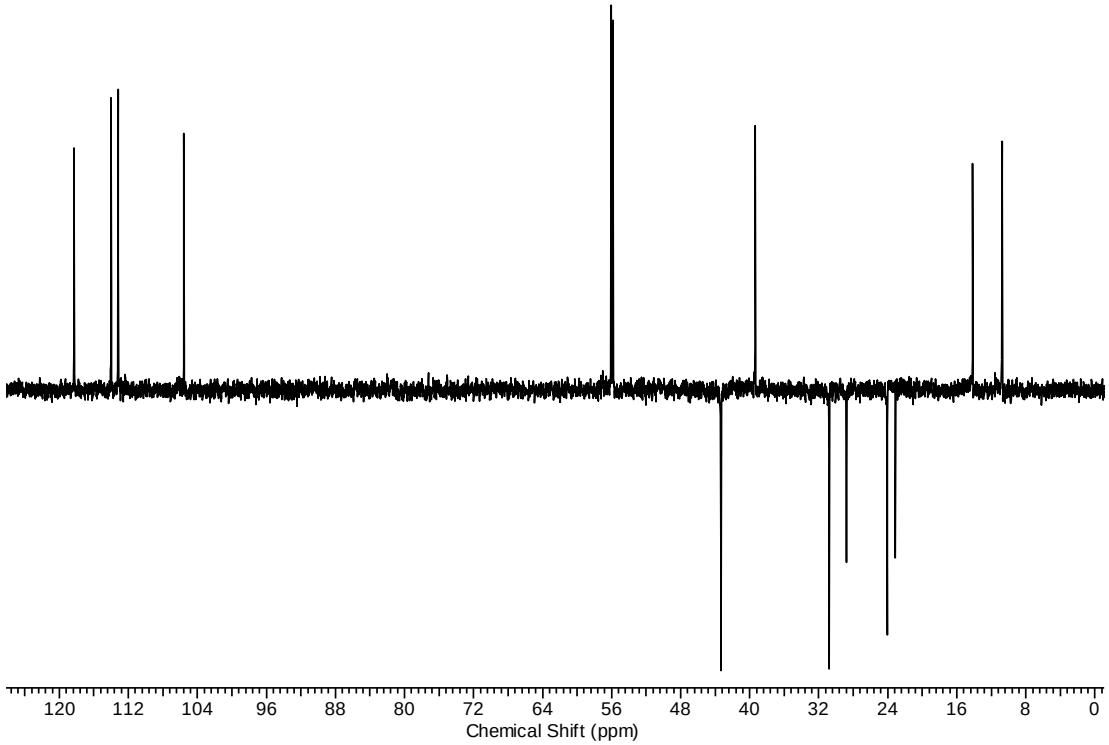
Following the same procedure for synthesis of compound **1** and using 2,5-dimethoxyphenyl boronic acid, **3** was synthesized. Purification was effected by column chromatography (eluent: 30% AcOEt/ pet. ether, R_f: 0.3) to yield **3** (76%) as a white fluffy solid. mp: 146-148 ^oC; IR ($CHCl_3$) ν (cm^{-1}): 3683, 3577, 3022, 1693, 1636, 1580, 1432, 1218, 768; ^1H NMR (500 MHz, $CDCl_3$) δ: 13.97 (s, 1H), 11.87 (s, 1H), 10.03 (s, 1H), 7.10-7.09 (d, J = 2.75 Hz, 1H), 7.03-7.01 (dd, J = 8.85 Hz, J = 2.75 Hz, 1H), 6.97-6.96 (d, J = 9.16 Hz, 1H), 6.31 (s, 1H), 3.95 (s, 3H), 3.81 (s, 3H), 3.26-3.17 (m, 2H), 1.46-1.41 (m, 1H), 1.39-1.32 (m, 8H), 0.93-0.89 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.3, 156.3, 154.3, 153.7, 151.3, 147.8, 119.6, 118.2, 113.9, 113.1, 105.1, 56.0, 55.8, 43.2, 39.3, 30.7, 28.7, 24.0, 23.0, 14.1. 10.7; HRMS (ESI) calculated $[M+H]^+$ for $C_{21}H_{31}O_4N_4$: 403.2331, found 403.2340, 805.4607 $[2M+H]^+$.



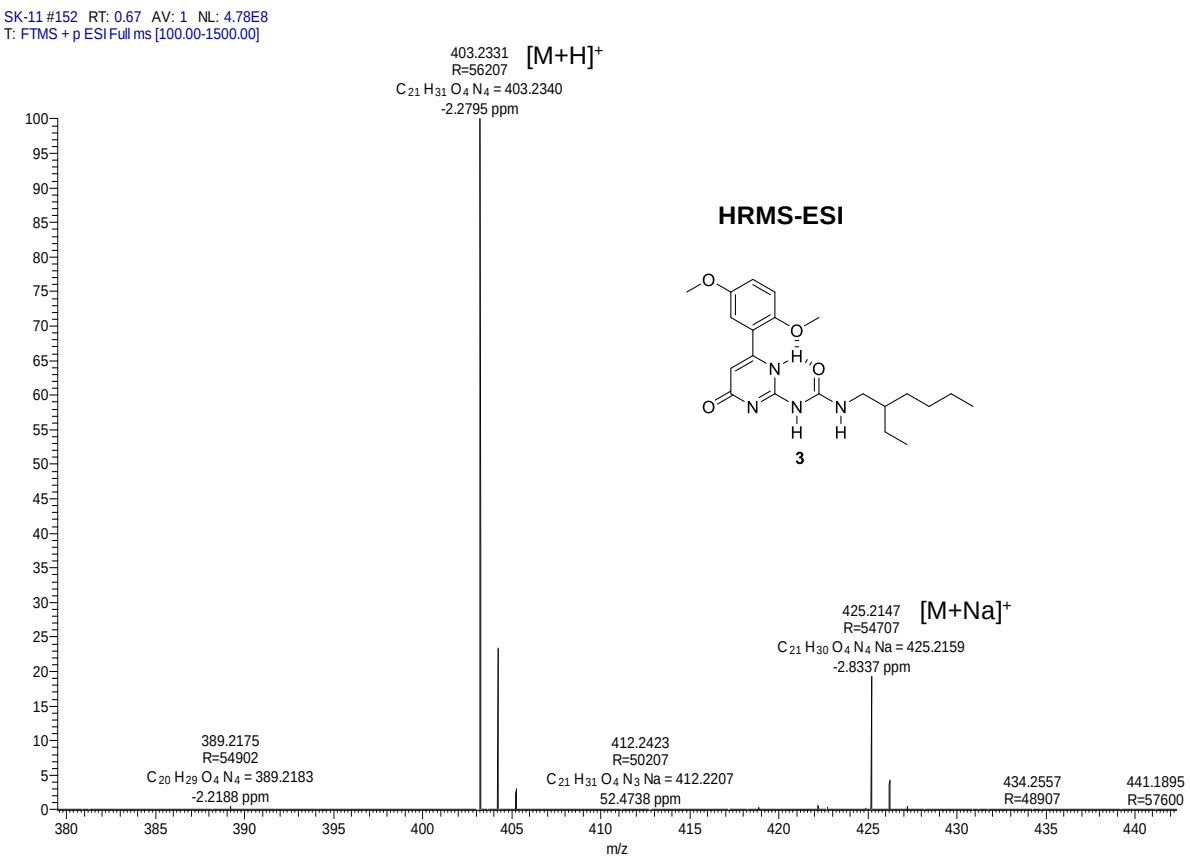
^1H NMR spectrum of compound **3** ($CDCl_3$, 500 MHz, 298 K)

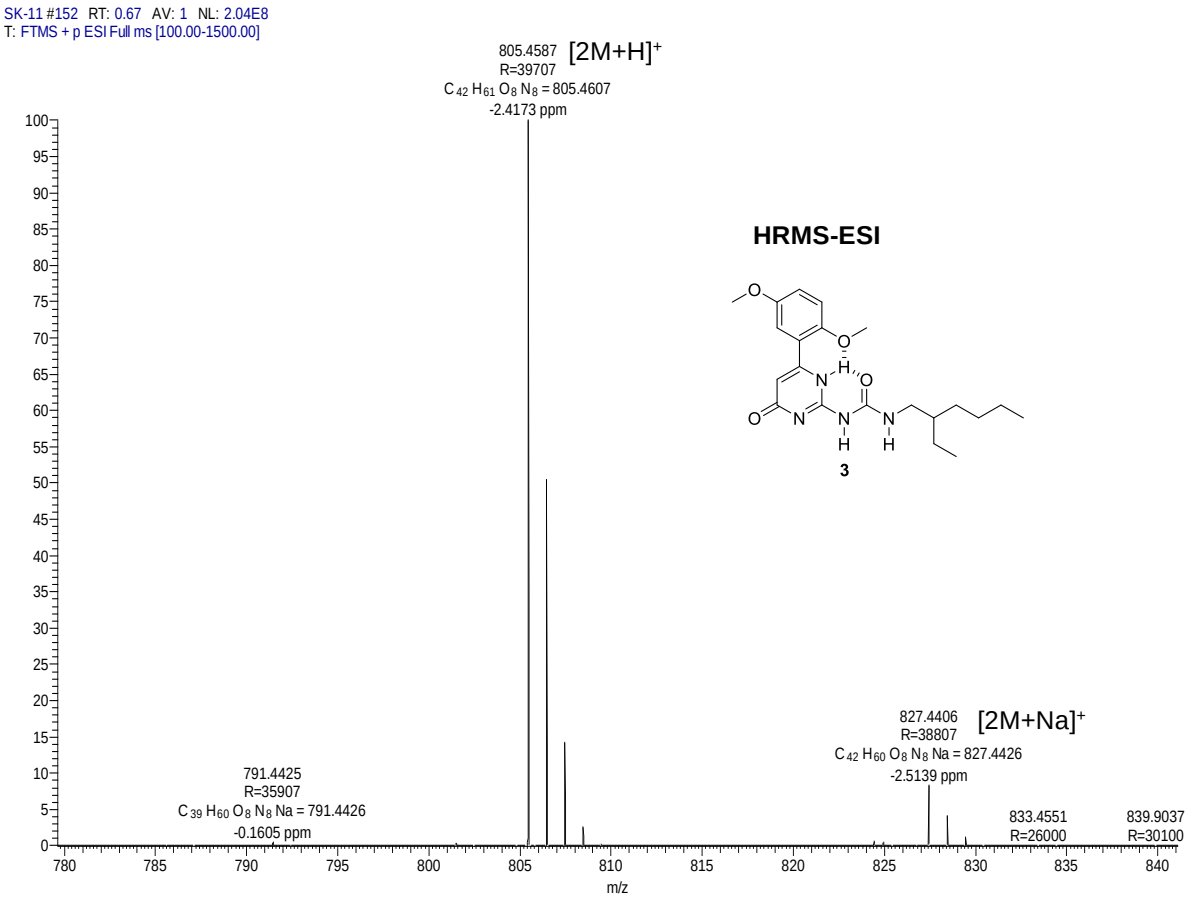


^{13}C NMR spectrum of compound **3** ($CDCl_3$, 125 MHz, 298 K)

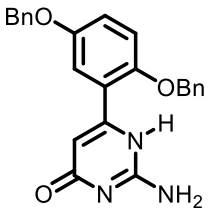


DEPT-135 NMR spectrum of compound **3** ($CDCl_3$, 125 MHz, 298 K)


SK-11 #152 RT: 0.67 AV: 1 NL: 4.78E8
T: FTMS + p ESI Full ms [100.00-1500.00]
403.2331 [M+H]+
R=56207
C21 H31 O4 N4 = 403.2340
-2.2795 ppm
HRMS-ESI
3
425.2147 [M+Na]+
R=54707
C21 H30 O4 N4 Na = 425.2159
-2.8337 ppm
389.2175
R=54902
C20 H29 O4 N4 = 389.2183
-2.2188 ppm
412.2423
R=50207
C21 H31 O4 N3 Na = 412.2207
52.4738 ppm
434.2557
R=48907
441.1895
R=57600
m/z

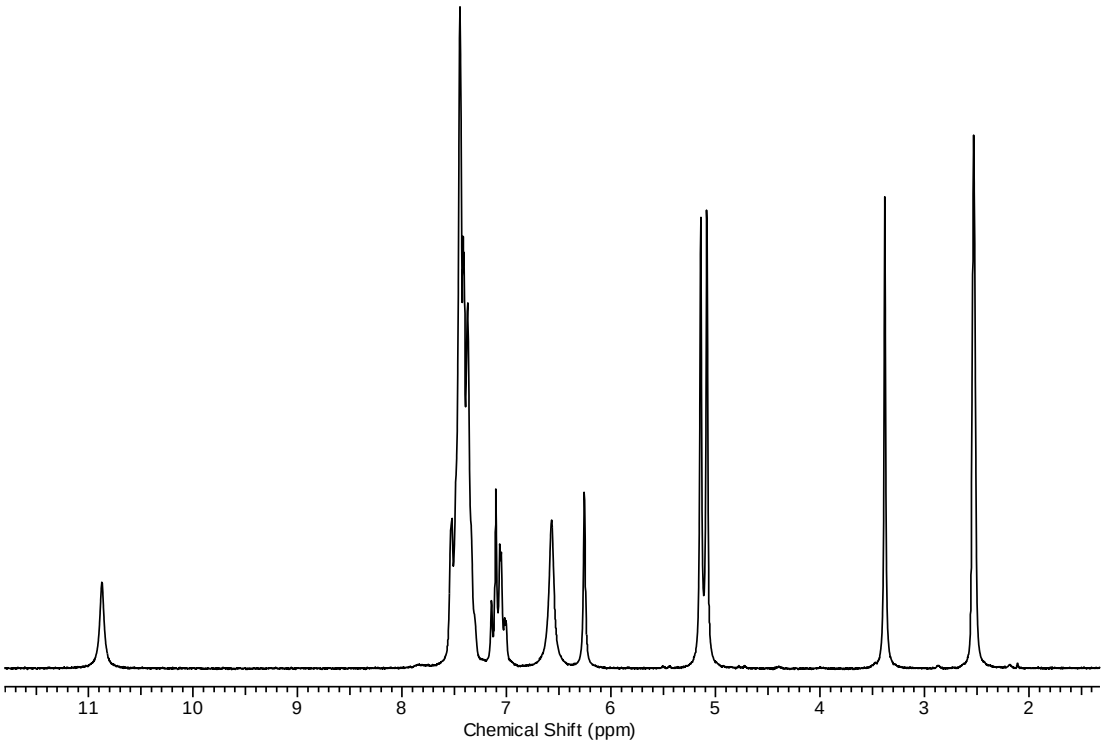

SK-11 #152 RT: 0.67 AV: 1 NL: 2.04E8
T: FTMS + p ESI Full ms [100.00-1500.00]
805.4587 [2M+H]+
R=39707
C42 H61 O8 N8 = 805.4607
-2.4173 ppm
HRMS-ESI
3
827.4406 [2M+Na]+
R=38807
C42 H60 O8 N8 Na = 827.4426
-2.5139 ppm
791.4425
R=35907
C39 H60 O8 N8 Na = 791.4426
-0.1605 ppm
833.4551
R=26000
839.9037
R=30100
m/z

Compound 17

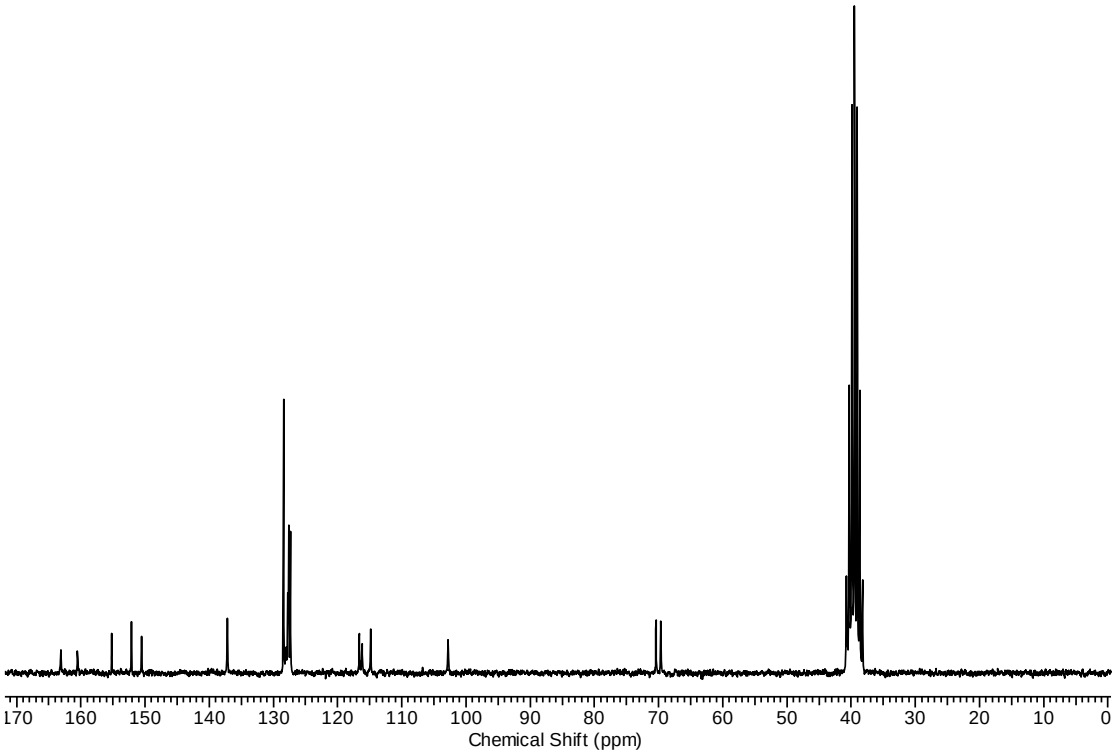


17

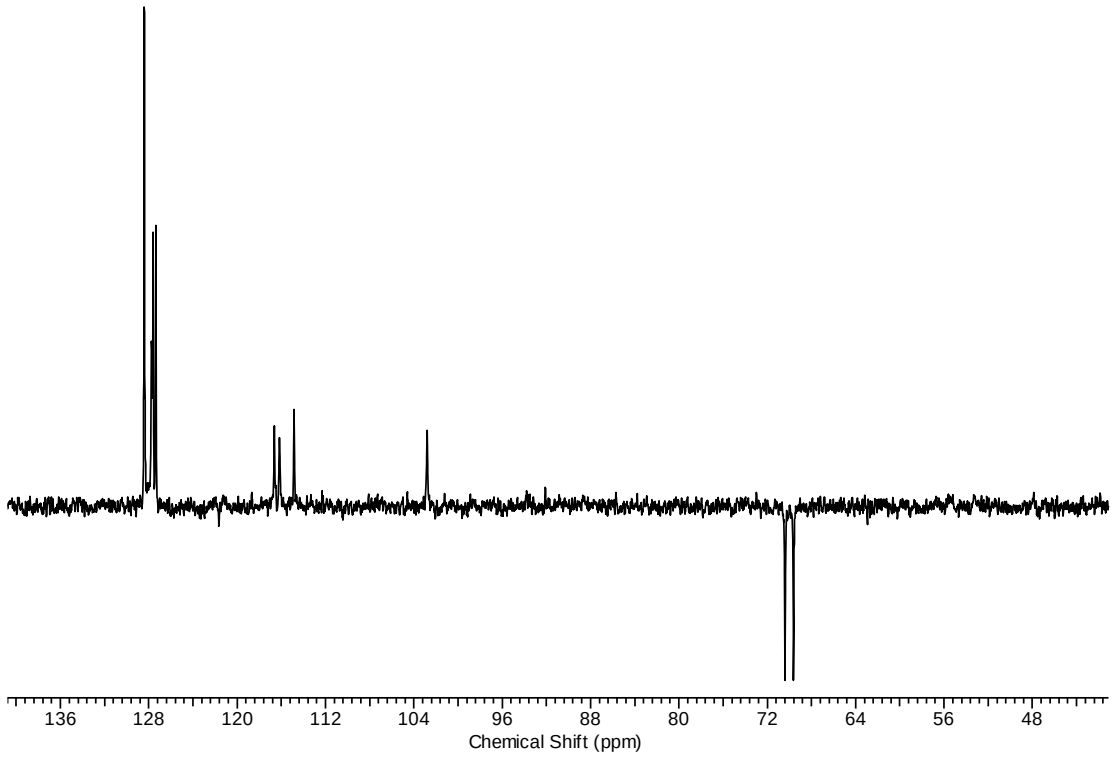
The β-keto ester **16** (1 g, 2.56 mmol, 1 equiv.) in ethanol (20 mL) was heated under reflux with guanidinium carbonate (0.277 g, 1.53 mmol, 0.6 equiv.) overnight. The resulting clear solution was partially evaporated, and then cooled inducing precipitation of a white solid. The resulting precipitate was filtered off, washed thoroughly with chilled ethanol, and dried under vacuum gave **17** (0.670 g, 65%) as a white fluffy solid. mp: 246-248 ºC; IR ($CHCl_3$) *v* (cm^{-1}): 3274, 3073, 2954, 2403, 1654, 1600, 1480, 1146, 1032, 958, 749; ^{1}H NMR (200 MHz, DMSO-d_6) δ: 10.84 (s, 1H), 7.49 (m, 1H), 7.42-7.34 (m, 10H), 7.12-6.98 (m, 2H), 6.54 (bs, 2H), 6.23 (s, 1H), 5.11 (s, 2H), 5.06 (s, 2H); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 163.1, 160.5, 155.2, 152.1, 150.6, 137.2, 137.1, 128.4, 128.0, 127.7, 127.6, 127.3, 116.6, 116.2, 114.9, 102.8, 70.4, 69.6; HRMS (ESI) calculated $[M+H]^+$ for $C_{24}H_{22}O_3N_3$: 400.1656, found 400.1656, 799.3239 $[2M+H]^+$.



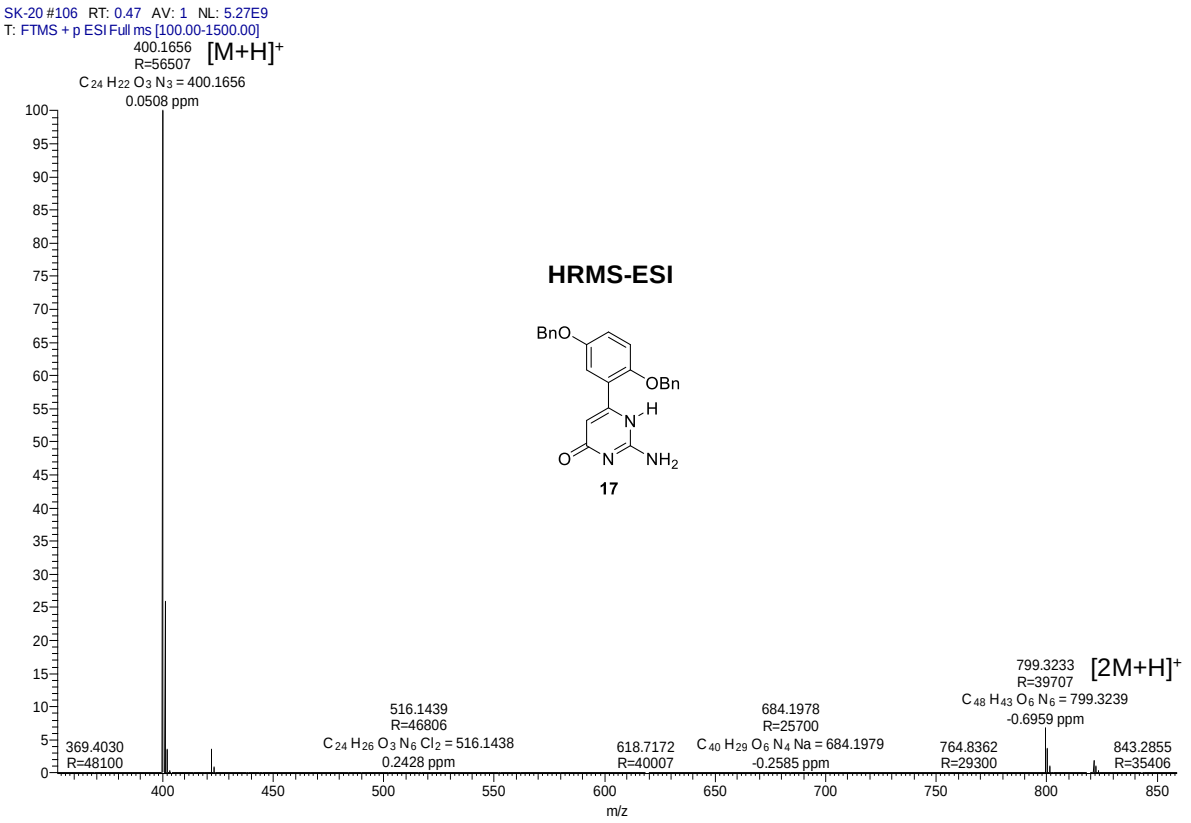
^{1}H NMR spectrum of compound **17** (DMSO-d_6, 200 MHz, 298 K)



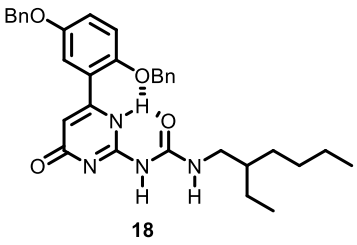
^{13}C NMR spectrum of compound **17** (DMSO-d_6, 50 MHz, 298 K)



DEPT-135 NMR spectrum of compound **17** (DMSO-d_6, 50 MHz, 298 K)

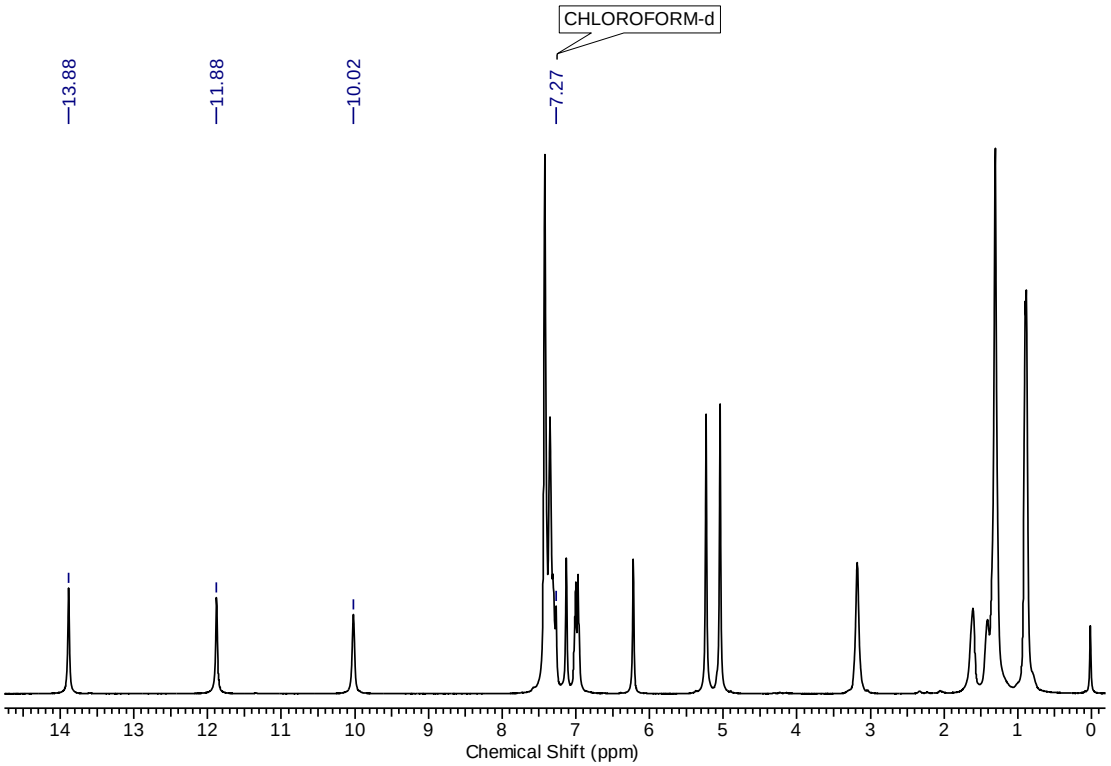


Compound 18

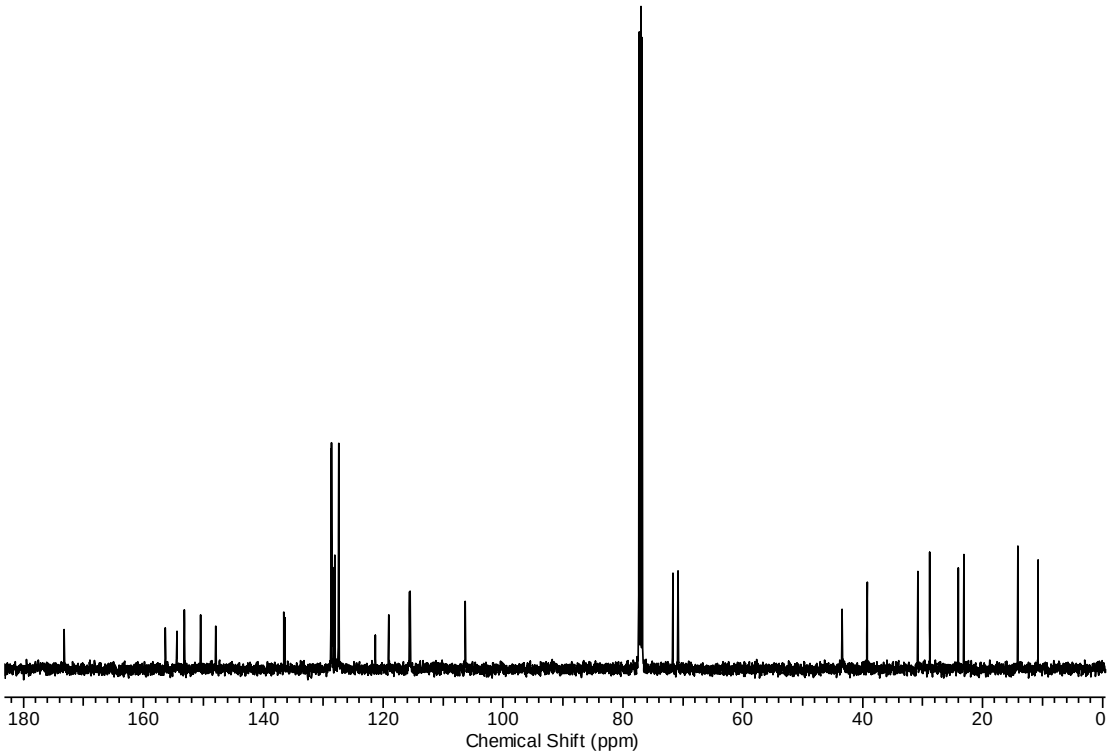


A solution of **17** (0.5 g, 1.25 mmol, 1 equiv.) and 2-ethylhexyl isocyanate (0.877 mL, 5.01 mmol, 4 equiv.) in dry pyridine was heated at 100^{o} C for 10 h. The resulting suspension was evaporated to dryness and column chromatographic purification (eluent: 40% AcOEt/ pet. ether, R_f: 0.3) of the residue yielded **18** (0.520 g, 75%) as a white fluffy solid. mp: 146-148 ^{o}C; IR ($CHCl_3$) ν (cm^{-1}): 3681, 3216, 3022, 2963, 2871, 2403, 1696, 1639, 1577, 1526, 1457, 1382, 1220, 1019, 766; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 13.88 (s, 1H), 11.88 (s, 1H), 10.02 (s, 1H), 7.42-7.31 (m, 10H), 7.13 (s, 1H), 7.00-6.96 (m, 2H), 6.22 (s, 1H), 5.23 (s, 2H), 5.04 (s, 2H), 3.18 (m, 2H), 1.61 (m, 1H), 1.42-1.41 (m, 2H), 1.30 (m, 6H), 0.90-0.88 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.1, 156.3, 154.4, 153.1, 150.4, 147.8, 136.5, 136.3, 128.6, 128.5, 128.1, 128.0, 127.4, 121.2, 119.0, 115.5, 115.4, 106.2, 71.6, 70.7, 43.3, 39.2, 30.7,

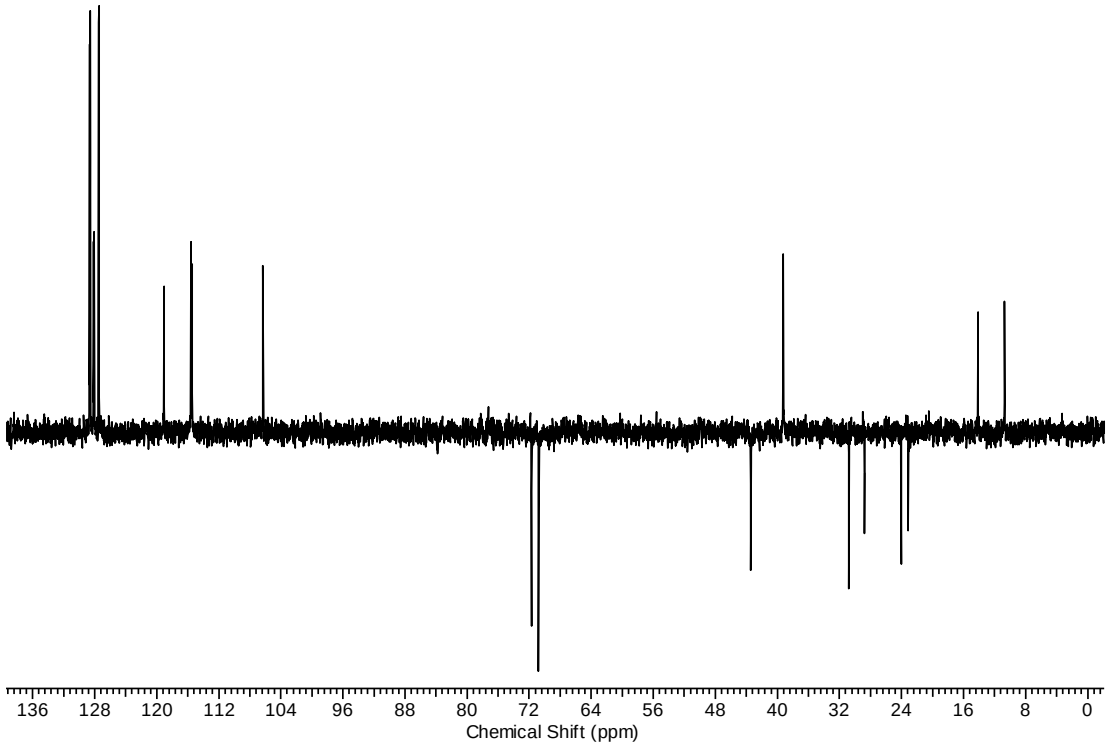
28.7, 24.0, 23.1, 14.1, 10.7; HRMS (ESI) calculated $[M+H]^+$ for $C_{33}H_{39}O_4N_4$: 555.2964, found 555.2966, 1109.5859 $[2M+H]^+$.



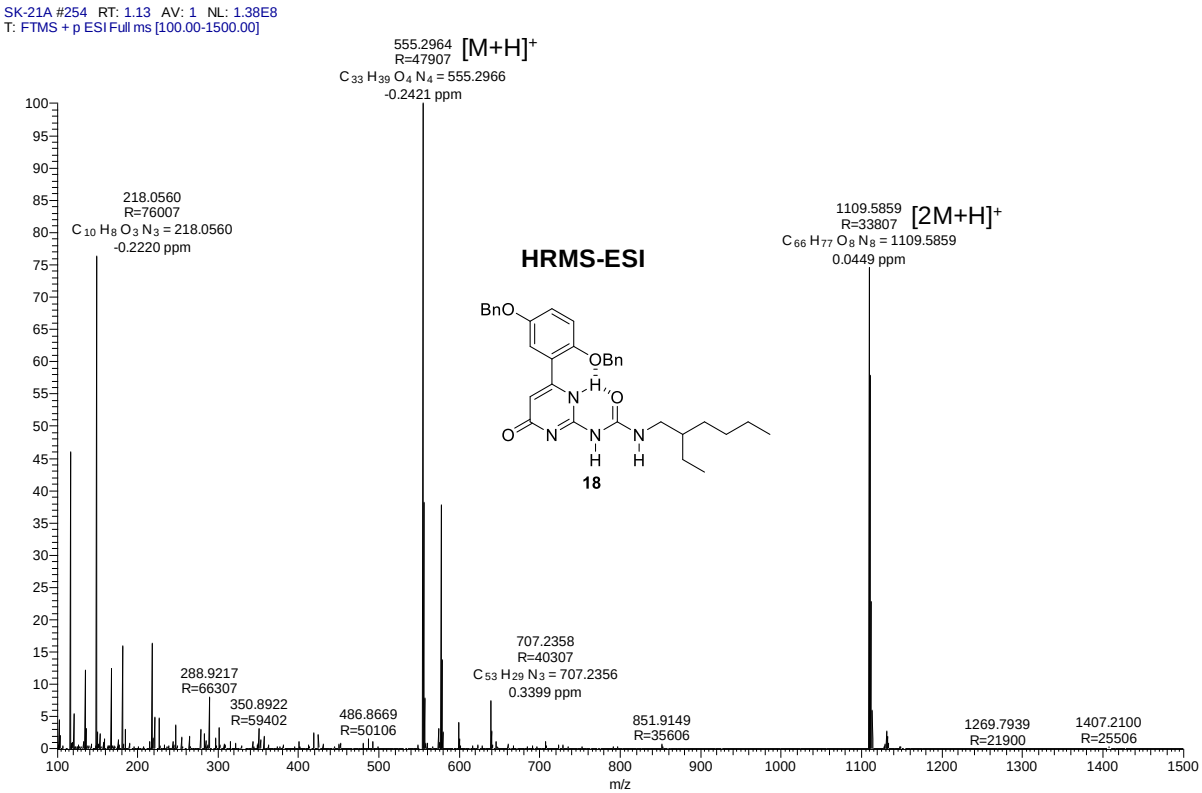
^{1}H NMR spectrum of compound **18** ($CDCl_3$, 500 MHz, 298 K)



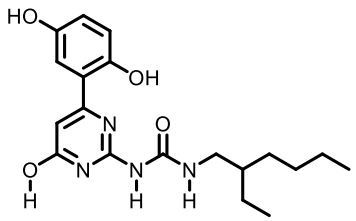
^{13}C NMR spectrum of compound **18** ($CDCl_3$, 125 MHz, 298 K)



DEPT-135 NMR spectrum of compound **18** ($CDCl_3$, 125 MHz, 298 K)

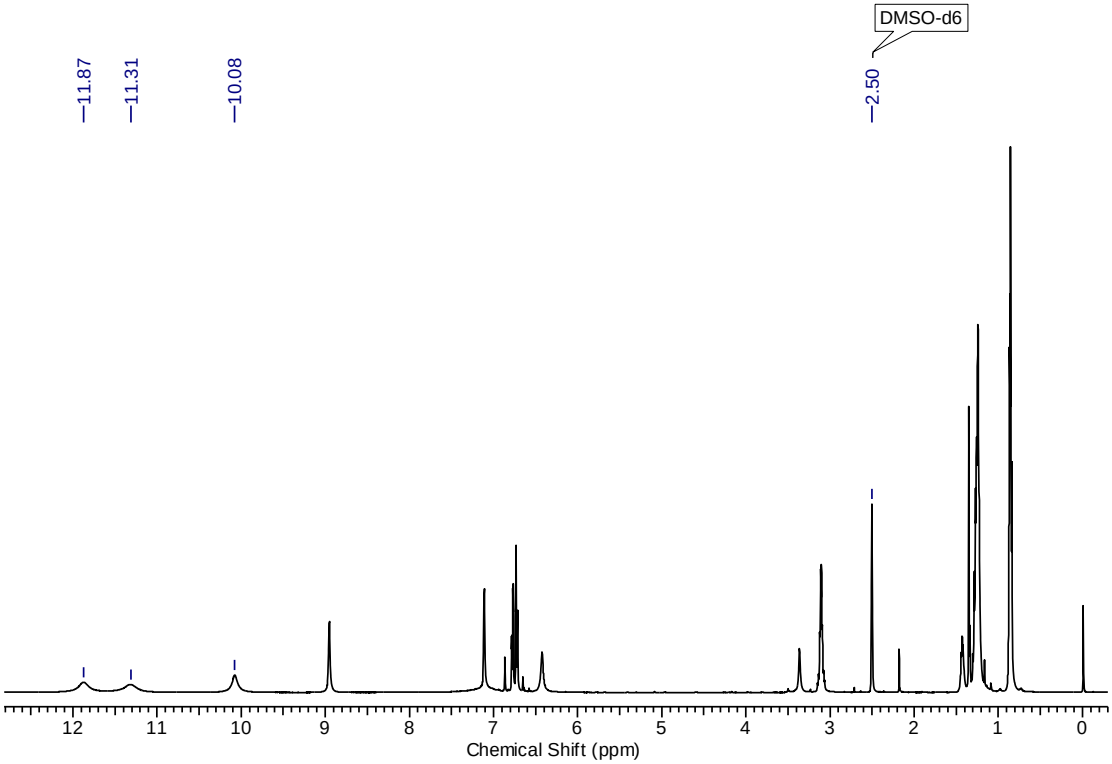


Compound 19

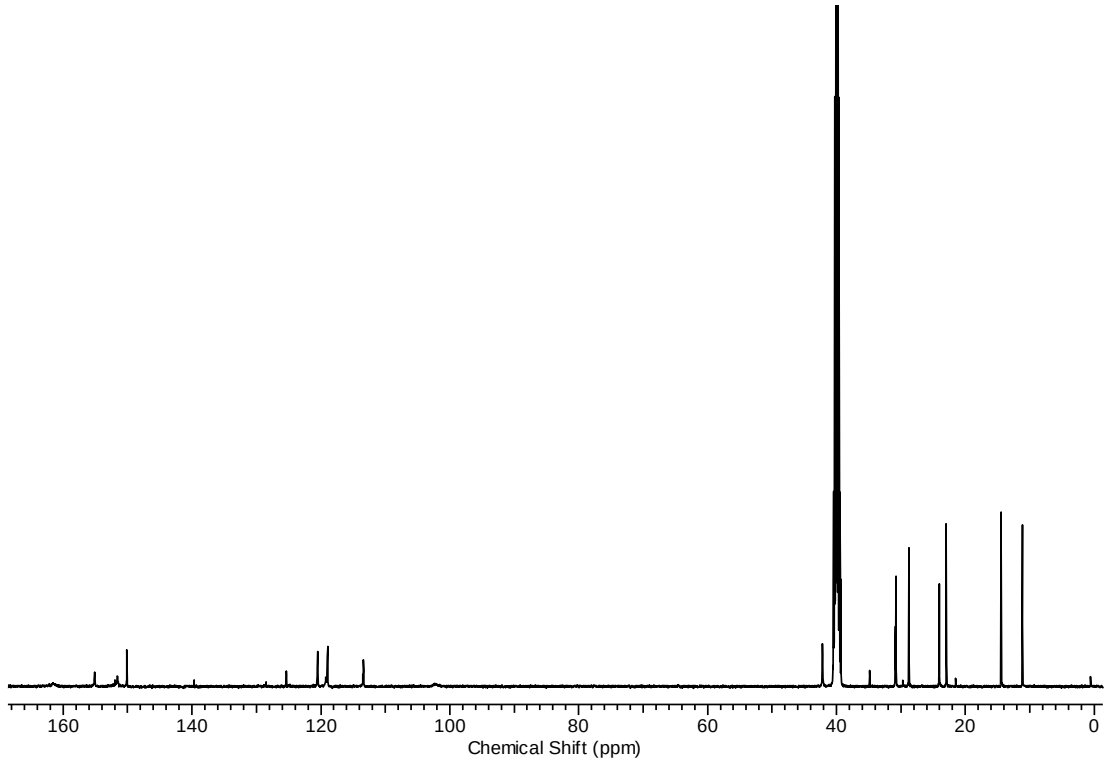


19

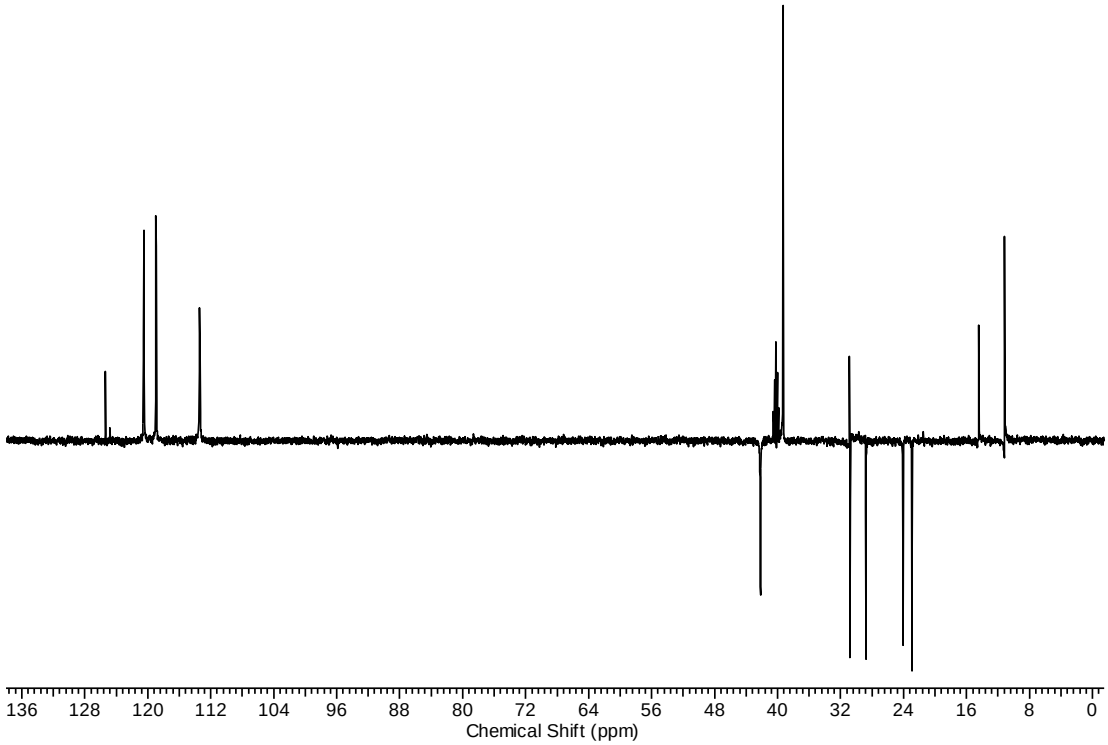
Pd/C (20% of the weight of the substrate) was added to a solution of **18** (0.2 g) in 5 mL of THF and the reaction mixture was stirred under hydrogen atmosphere (balloon) at room temperature for 24 h. The mixture was then filtered over celite and was concentrated under reduced pressure to give **19** (0.122 g, 90%) as a yellow solid. mp: > 285 ^{o}C; IR ($CHCl_3$) *v* (cm^{-1}): 3640, 3356, 3024, 2925, 2867, 1711, 1662, 1559, 1368, 1031, 778; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.87 (bs, 1H), 11.32 (bs, 1H), 10.08 (s, 1H), 8.95 (s, 1H), 7.11 (s, 2H), 6.79-6.76 (dd, *J* = 8.77 Hz, *J* = 2.67 Hz, 1H), 6.73-6.71 (d, *J* = 8.77 Hz, 1H), 6.43 (s, 1H), 3.15-3.06 (m, 2H), 1.44-1.42 (m, 1H), 1.35-1.34 (m, 2H), 1.30-1.24 (m, 6H), 0.87-0.84 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 154.6, 151.5, 151.1, 149.6, 139.2, 128.07, 124.9, 120.0, 118.7, 118.5, 112.9, 41.7, 38.87, 30.3, 28.3, 23.6, 22.5, 13.9, 10.7; HRMS (ESI) calculated $[M+H]^+$ for $C_{19}H_{27}O_4N_4$: 375.2025, found 375.2027, 749.3981 $[2M+H]^+$.



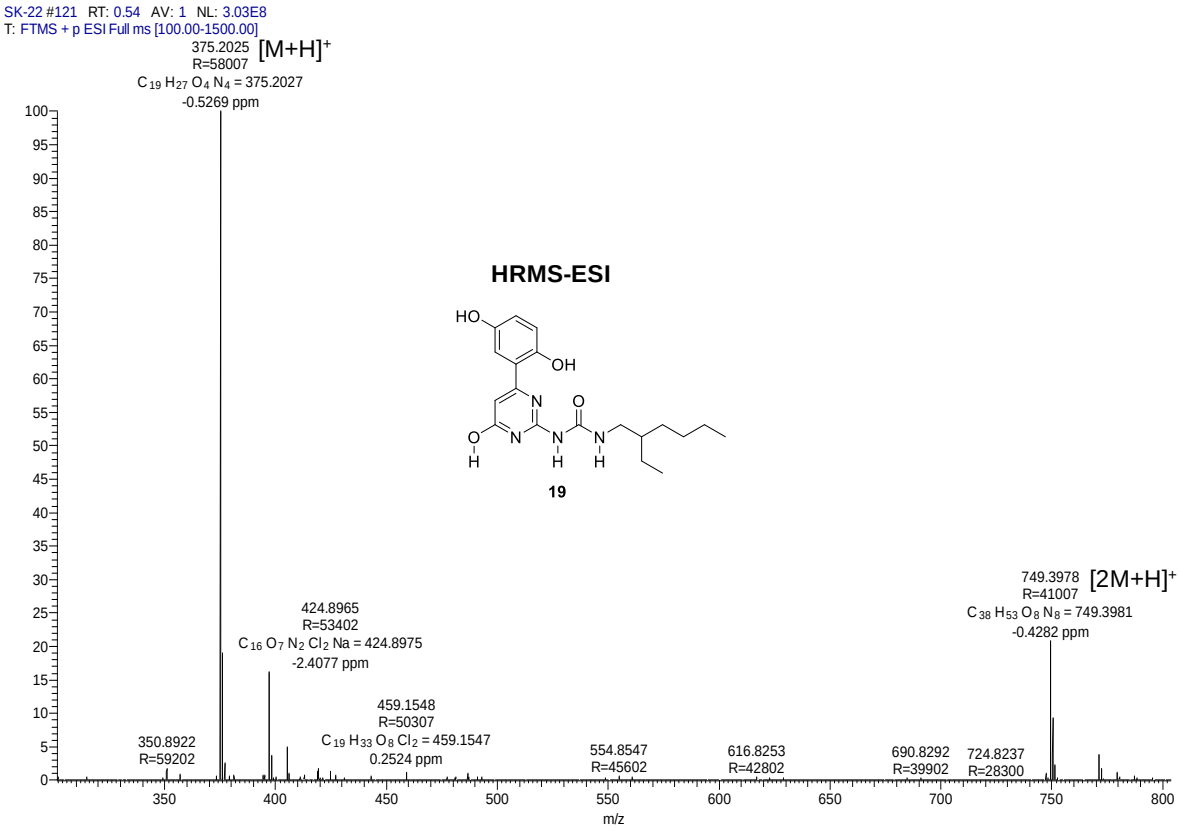
^{1}H NMR spectrum of compound **19** (DMSO-$d_6^{\#}$, 500 MHz, 298 K). $^{\#}$*Note*: Owing to poor solubility, NMR could not be taken in $CDCl_3$.



^{13}C NMR spectrum of compound **19** (DMSO-d_6[#], 125 MHz, 298 K). [#]*Note*: Owing to poor solubility, NMR could not be taken in $CDCl_3$.



DEPT-135 NMR spectrum of compound **19** (DMSO-d_6[#], 125 MHz, 298 K). [#]*Note*: Owing to poor solubility, NMR could not be taken in $CDCl_3$.


SK-22 #121 RT: 0.54 AV: 1 NL: 3.03E8
T: FTMS + p ESI Full ms [100.00-1500.00]
375.2025 [M+H]+
R=58007
C19 H27 O4 N4 = 375.2027
-0.5269 ppm
HRMS-ESI
HO
OH
N
O
O
H
N
N
H
N
H
19
749.3978 [2M+H]+
R=41007
C38 H53 O8 N8 = 749.3981
-0.4282 ppm
424.8965
R=53402
C16 O7 N2 Cl2 Na = 424.8975
-2.4077 ppm
459.1548
R=50307
C19 H33 O8 Cl2 = 459.1547
0.2524 ppm
350.8922
R=59202
554.8547
R=45602
616.8253
R=42802
690.8292
R=39902
724.8237
R=28300
m/z

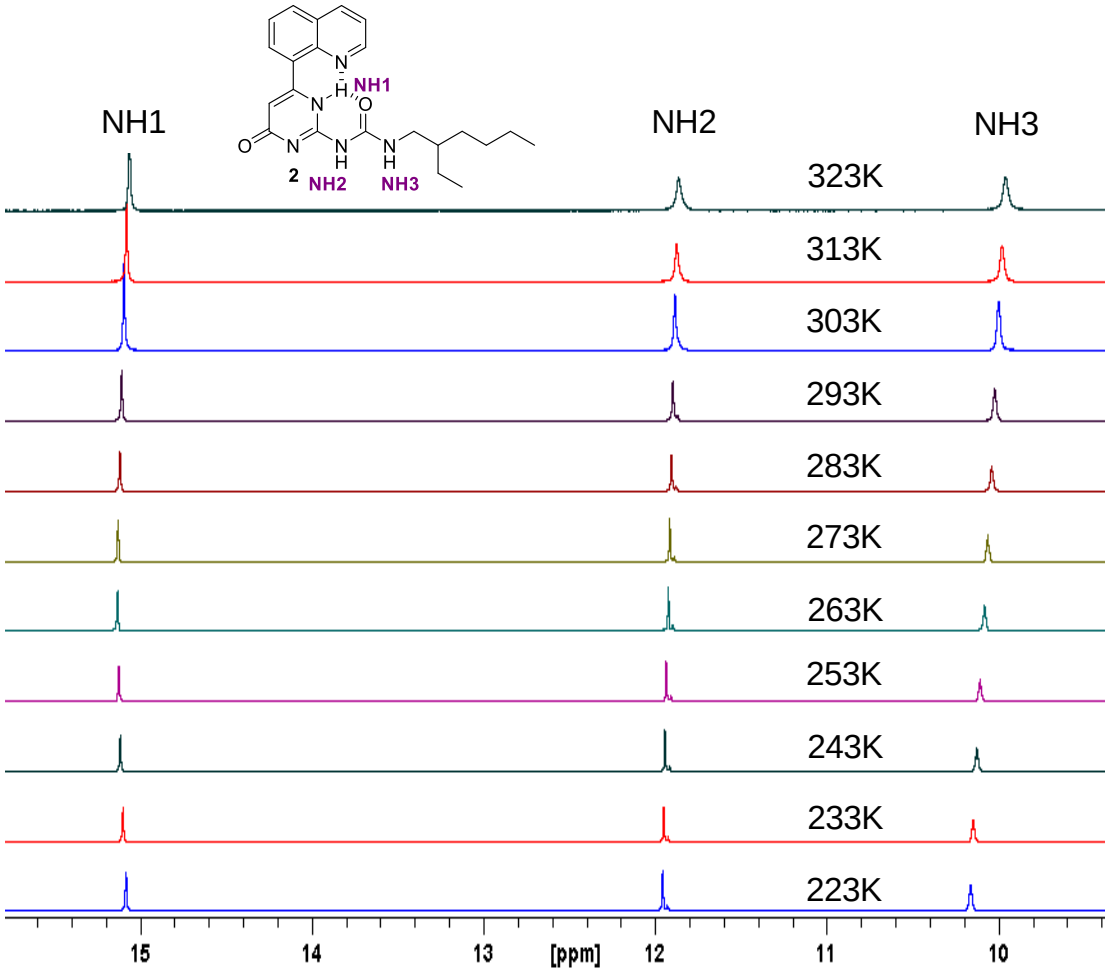


Figure S1. Stacked partial ^{1}H NMR spectra (700 MHz) of **2** at different temperatures, (10 mM in $CDCl_3$), showing the temperature-dependent changes of NH1, NH2 and NH3.

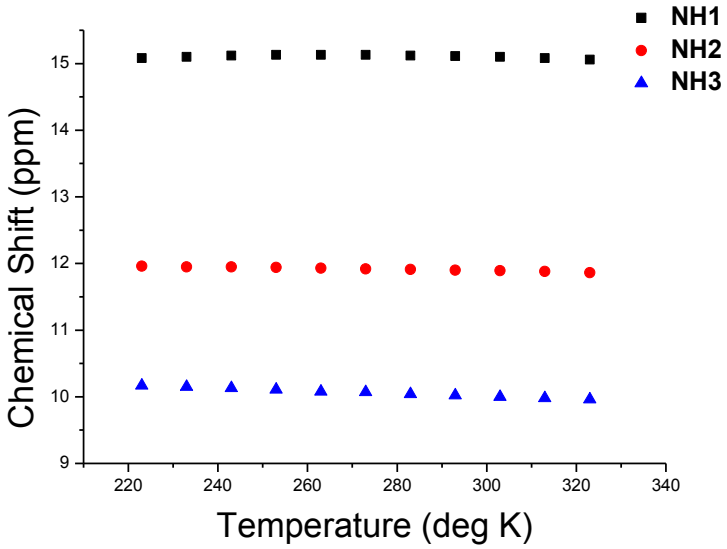


Figure S2. Plot of the chemical shifts of NH1, NH2 and NH3 of **2** at different temperatures, (10 mM in $CDCl_3$, 700MHz).

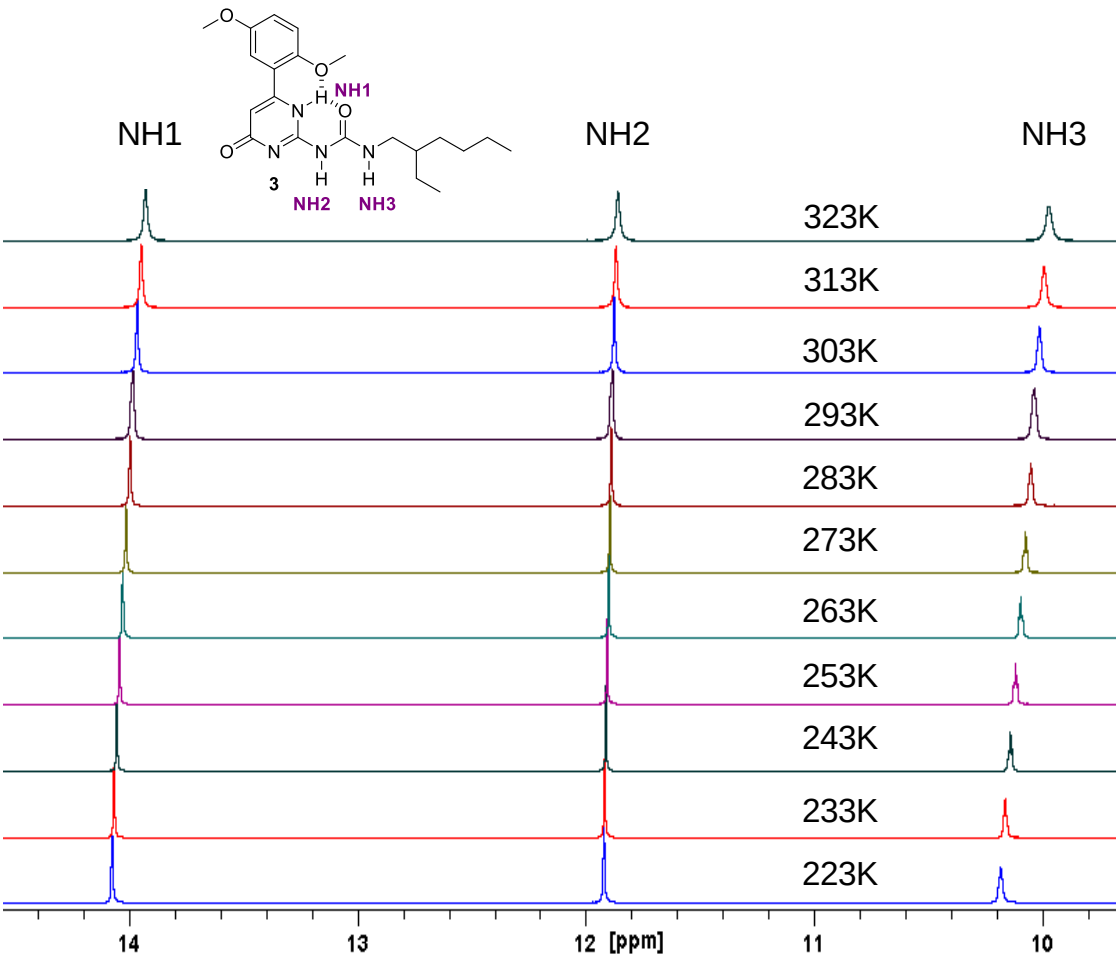


Figure S3. Stacked partial ^{1}H NMR spectra (700 MHz) of **3** at different temperatures, (10 mM in $CDCl_3$), showing the temperature-dependent changes of NH1, NH2 and NH3.

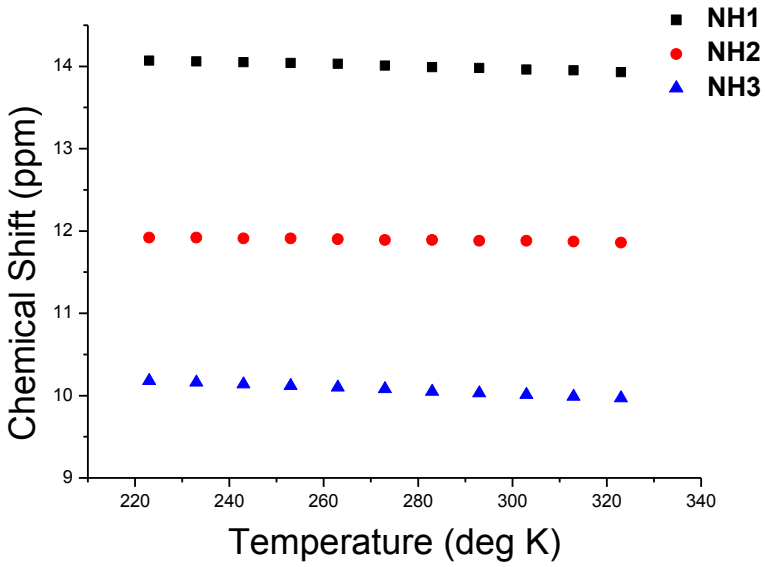


Figure S4. Plot of the chemical shifts of NH1, NH2 and NH3 of **3** at different temperatures, (10 mM in $CDCl_3$, 700MHz).

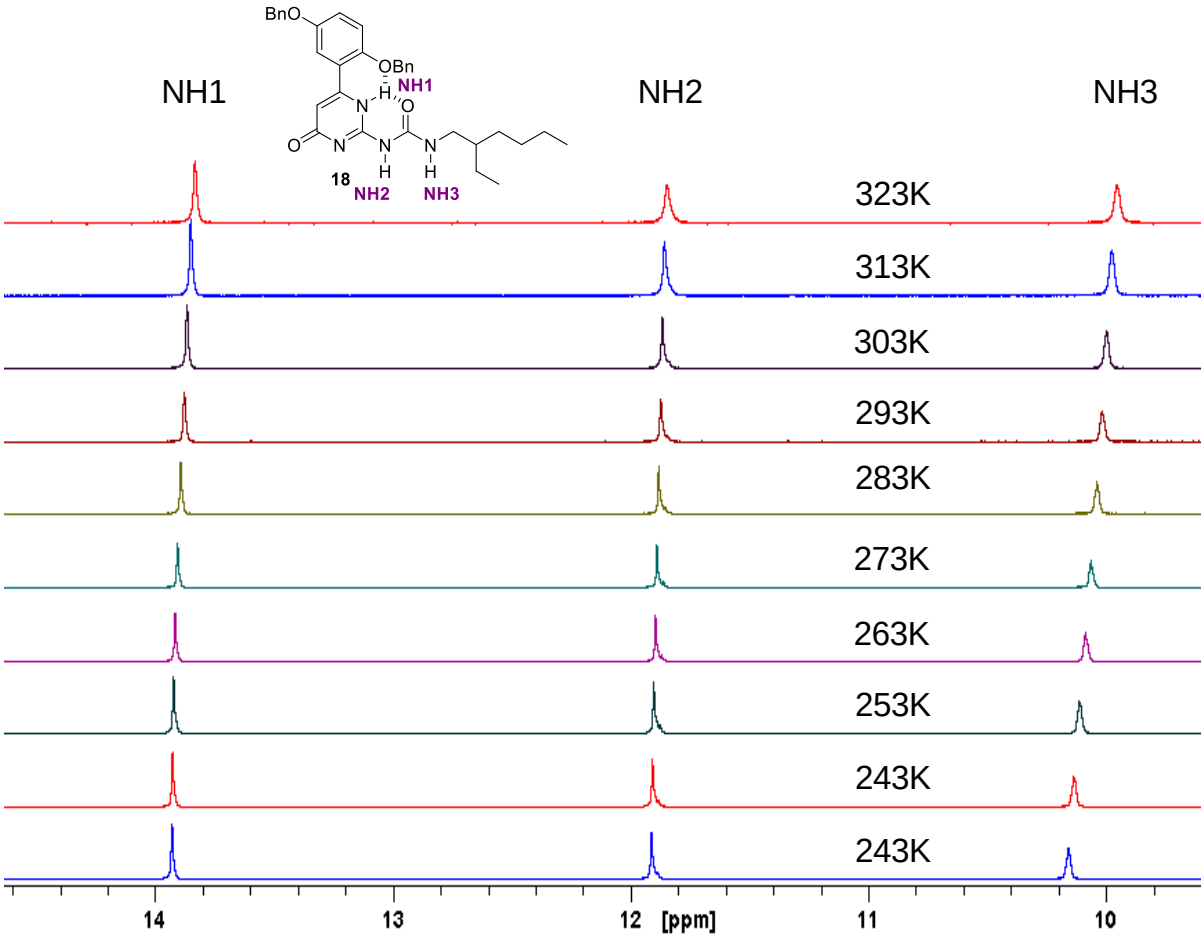


Figure S5. Stacked partial ^{1}H NMR spectra (700 MHz) of **18** at different temperatures, (10 mM in $CDCl_3$), showing the temperature-dependent changes of NH1, NH2 and NH3.

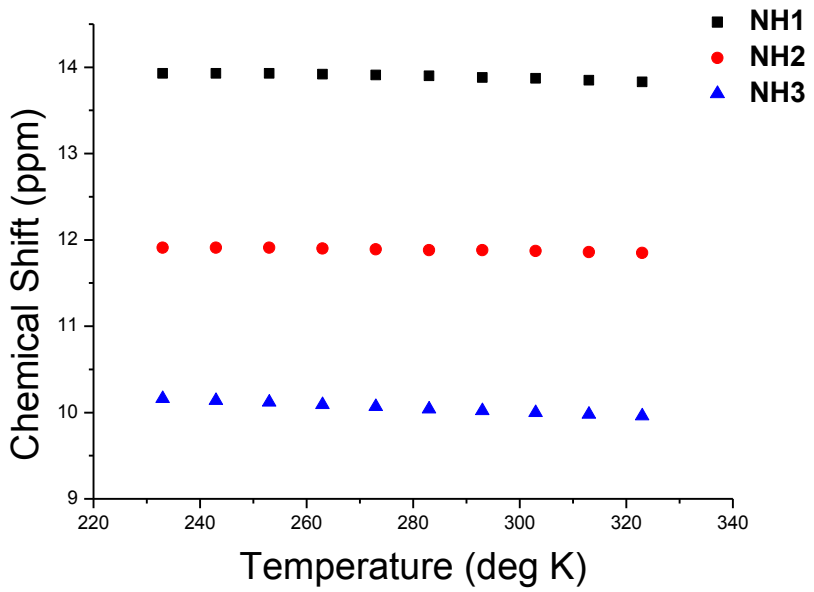


Figure S6. Plot of the chemical shifts of NH1, NH2 and NH3 of **18** at different temperatures, (10 mM in $CDCl_3$, 700MHz).

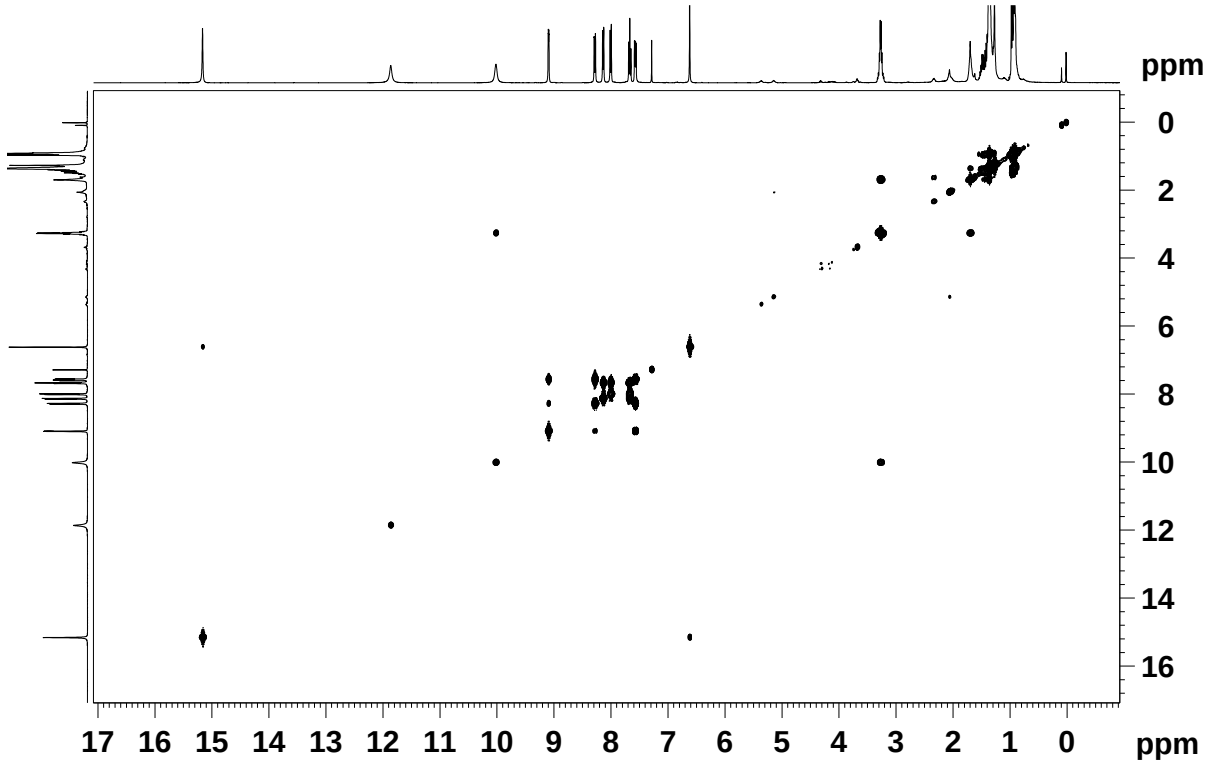


Figure S7. 2D COSY spectra of **2** (400 MHz, $CDCl_3$).

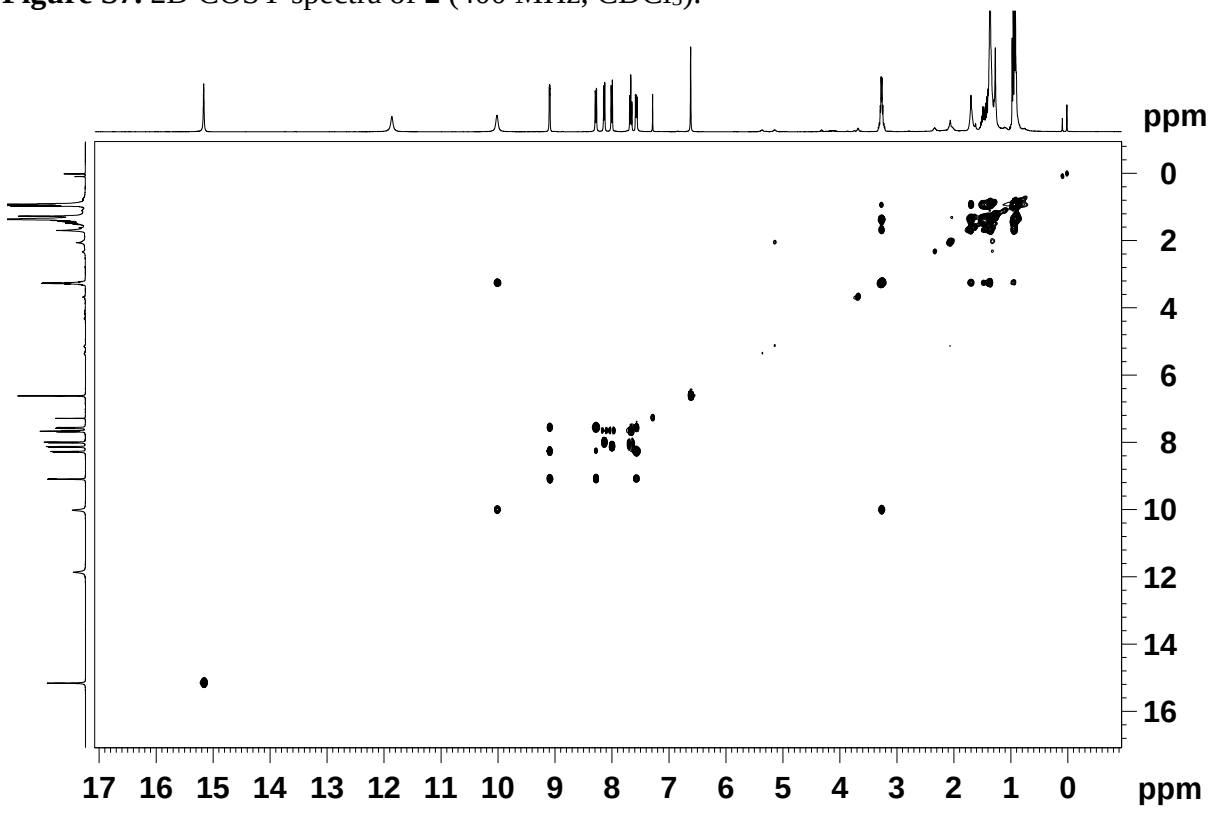


Figure S8. 2D TOCSY spectra of **2** (400 MHz, $CDCl_3$).

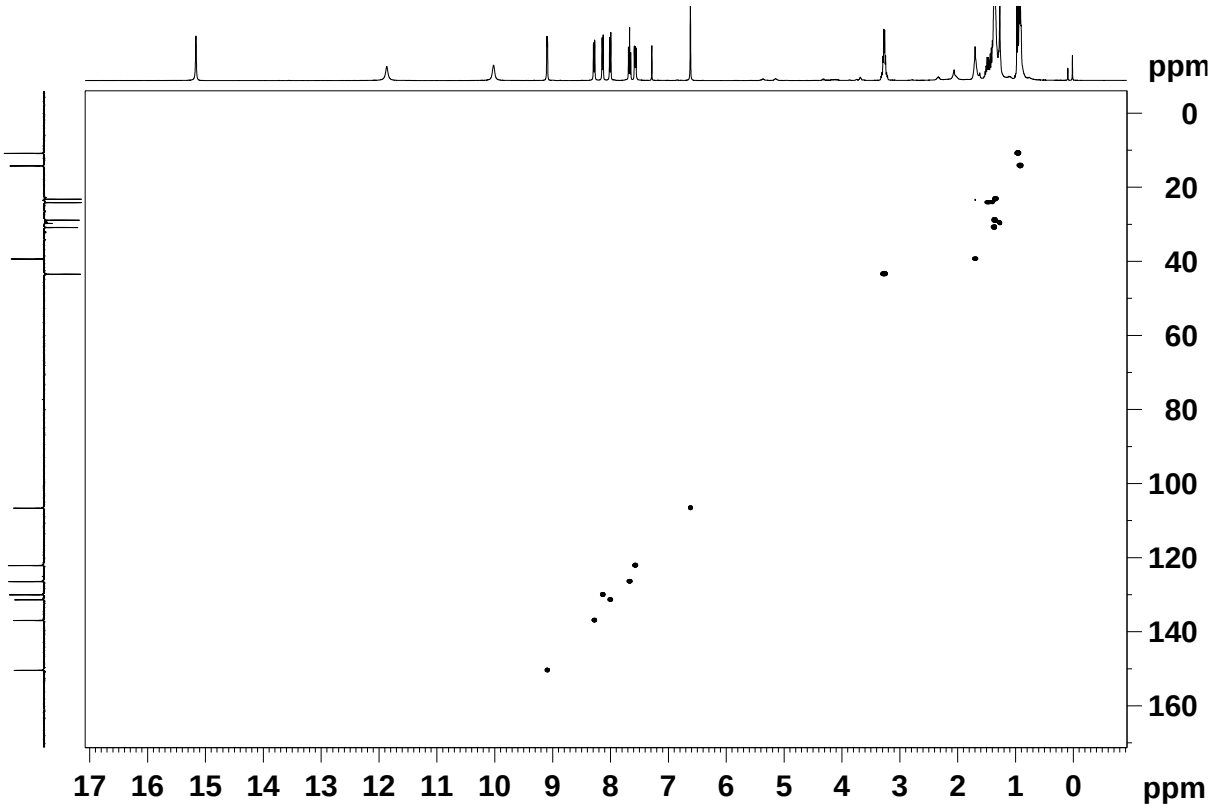


Figure S9. 2D HSQC spectra of **2** (400 MHz, $CDCl_3$).

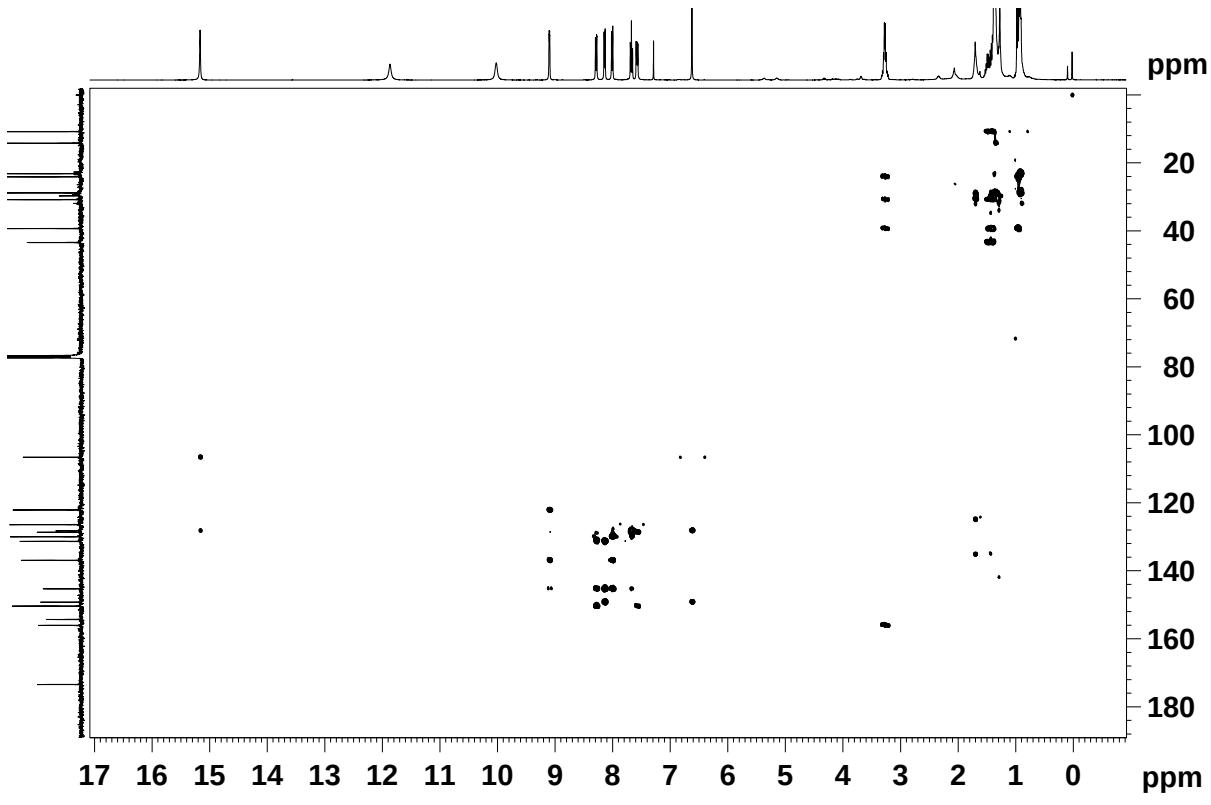


Figure S10. 2D HMBC spectra of **2** (400 MHz, $CDCl_3$).

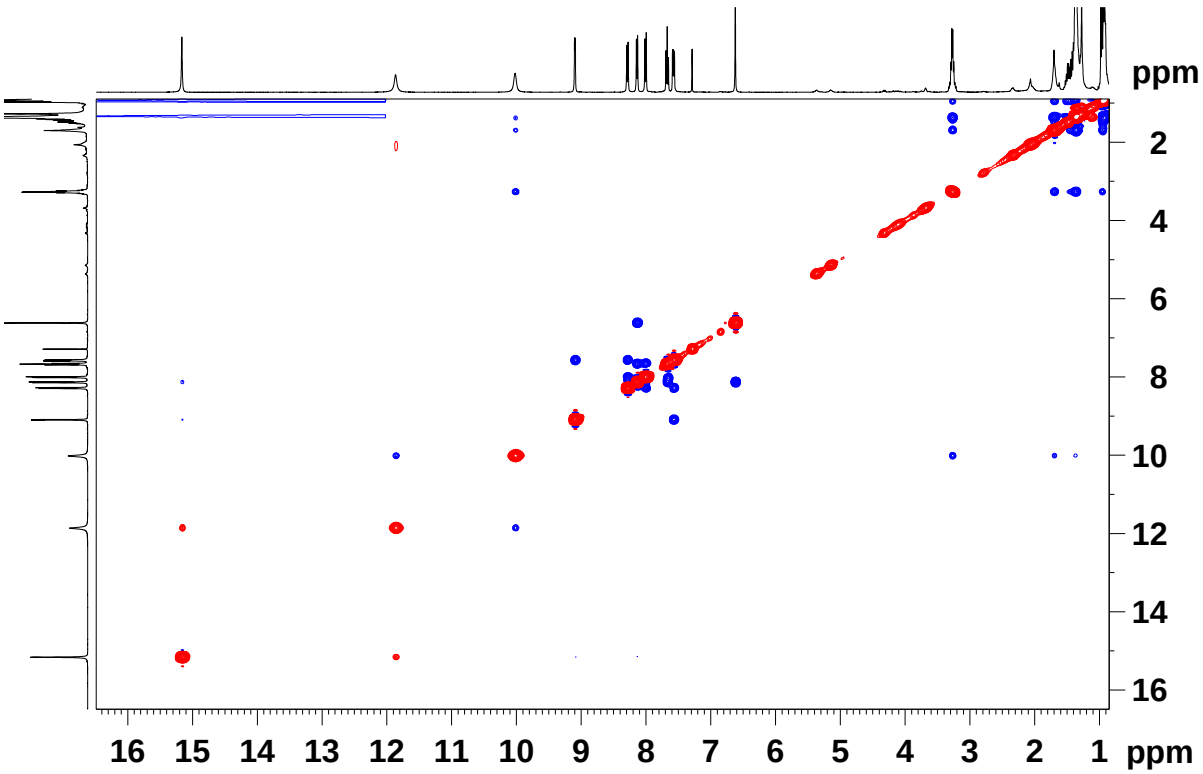


Figure S11. 2D NOESY spectra of **2** (400 MHz, $CDCl_3$).

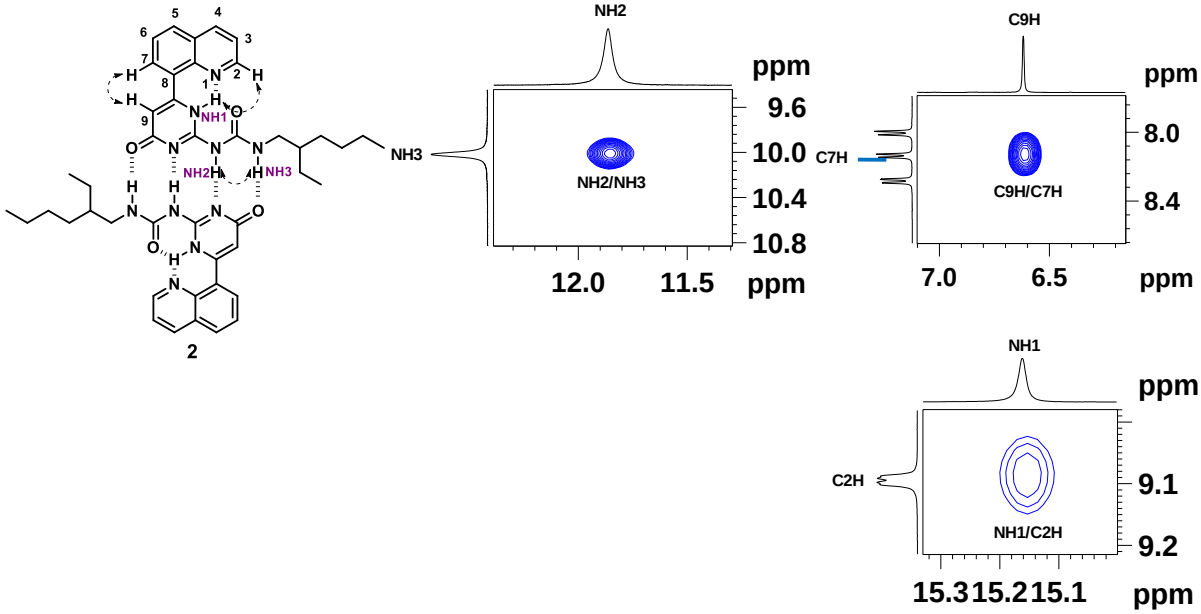


Figure S12. 2D extracts of **2** (400 MHz, $CDCl_3$).

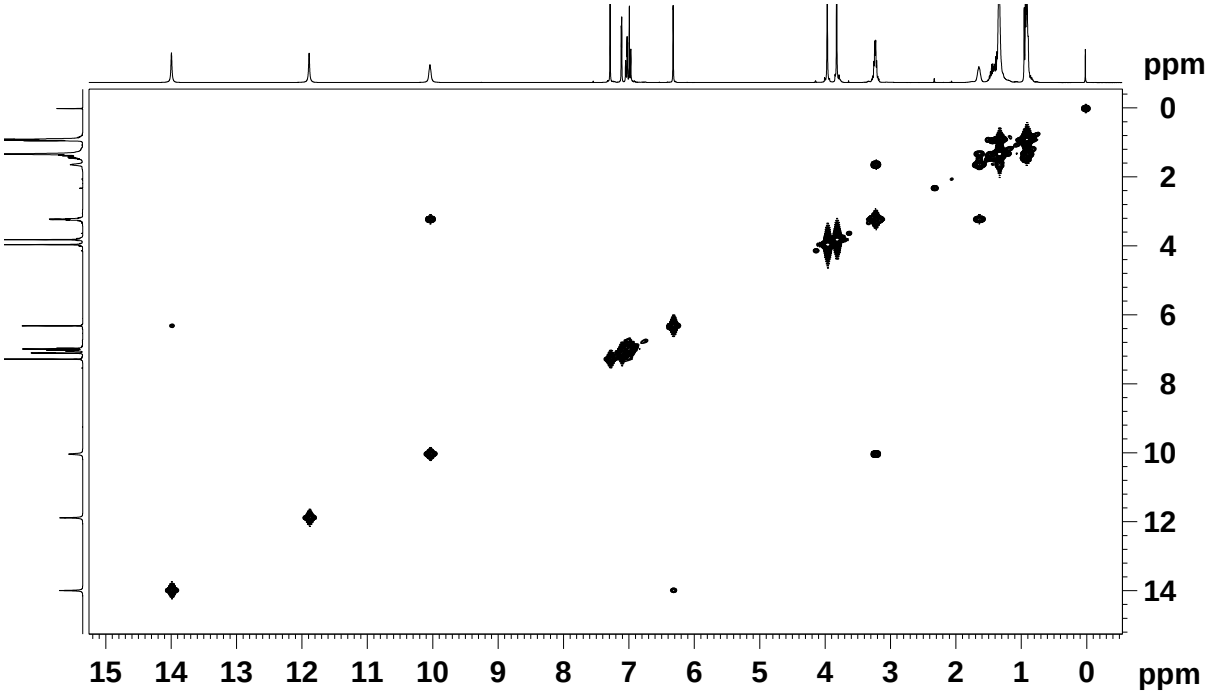


Figure S13. 2D COSY spectra of **3** (400 MHz, $CDCl_3$).

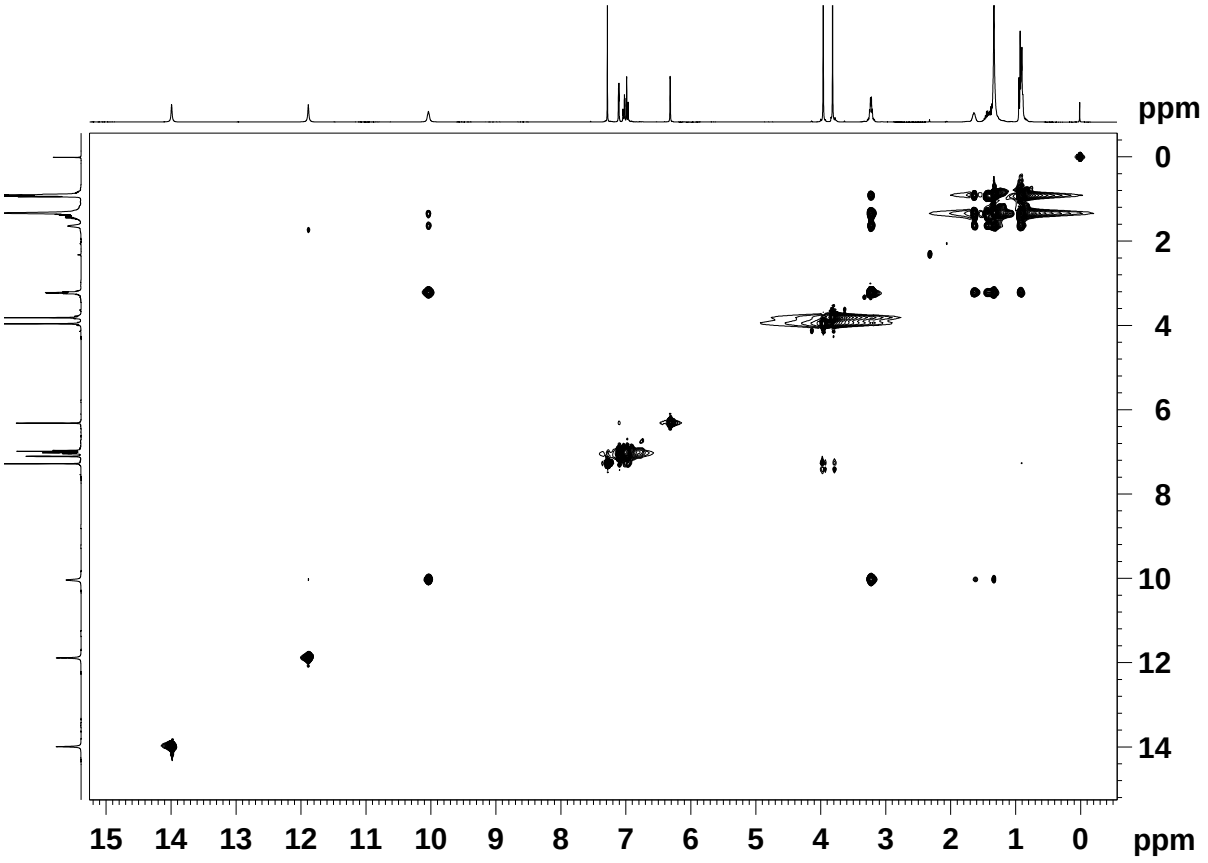


Figure S14. 2D TOCSY spectra of **3** (400 MHz, $CDCl_3$).

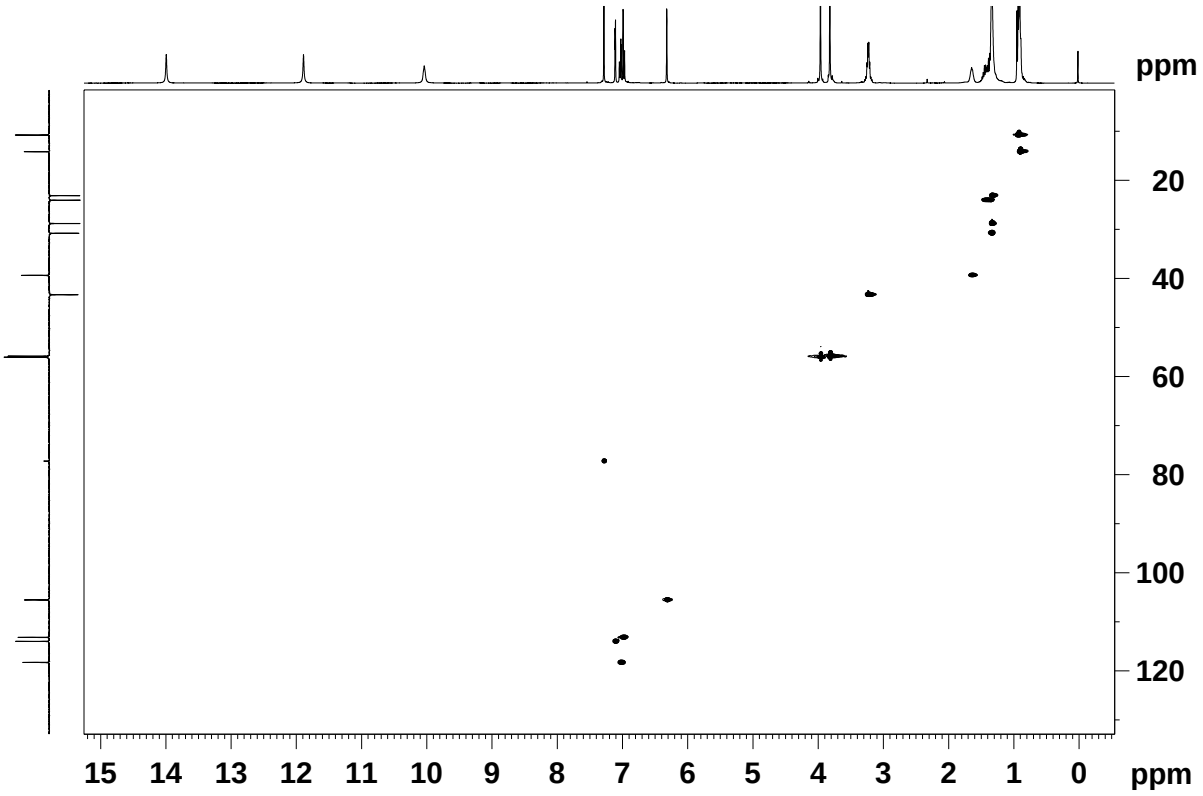


Figure S15. 2D HSQC spectra of **3** (400 MHz, $CDCl_3$).

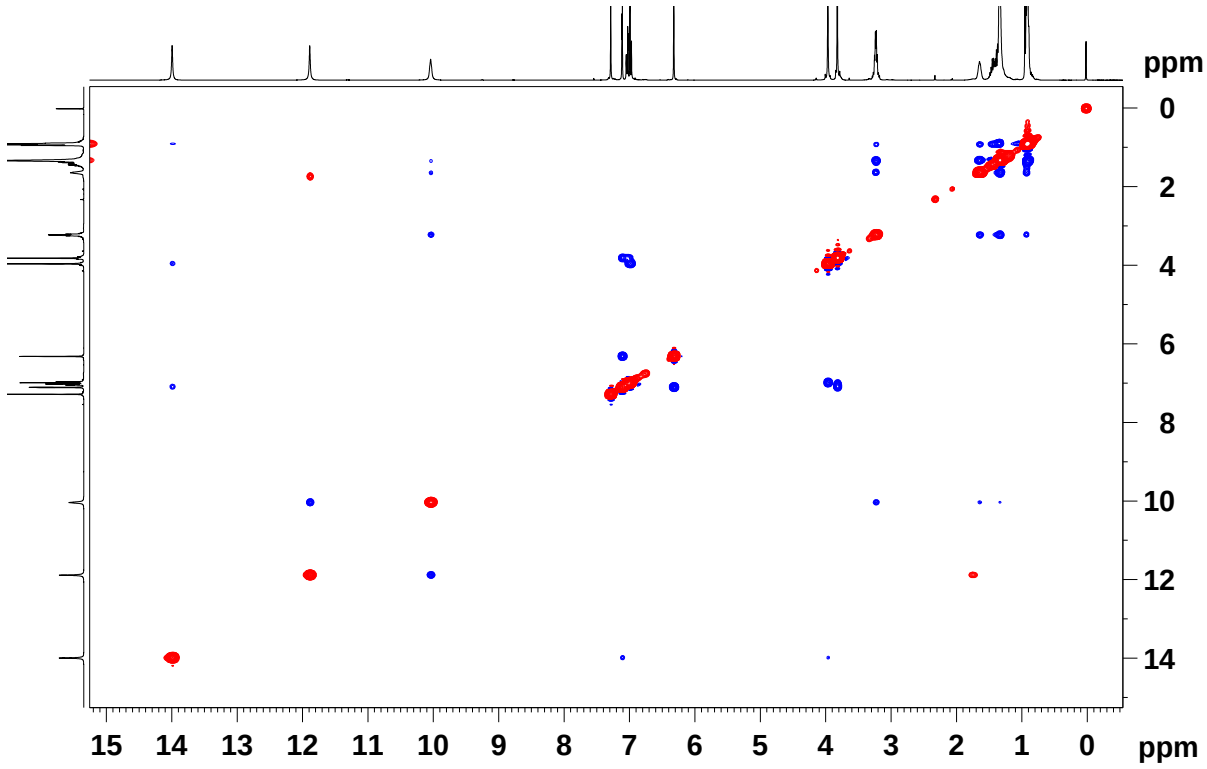


Figure S16. 2D NOESY spectra of **3** (400 MHz, $CDCl_3$).

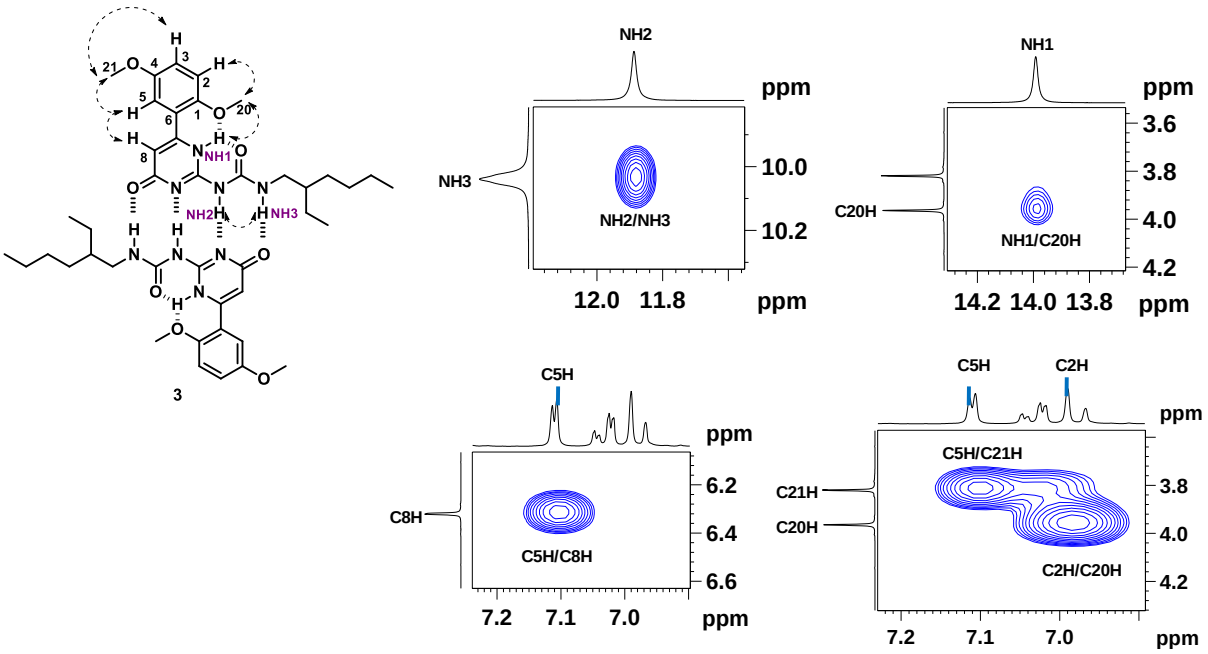


Figure S17. 2D extracts of **3** (400 MHz, $CDCl_3$).

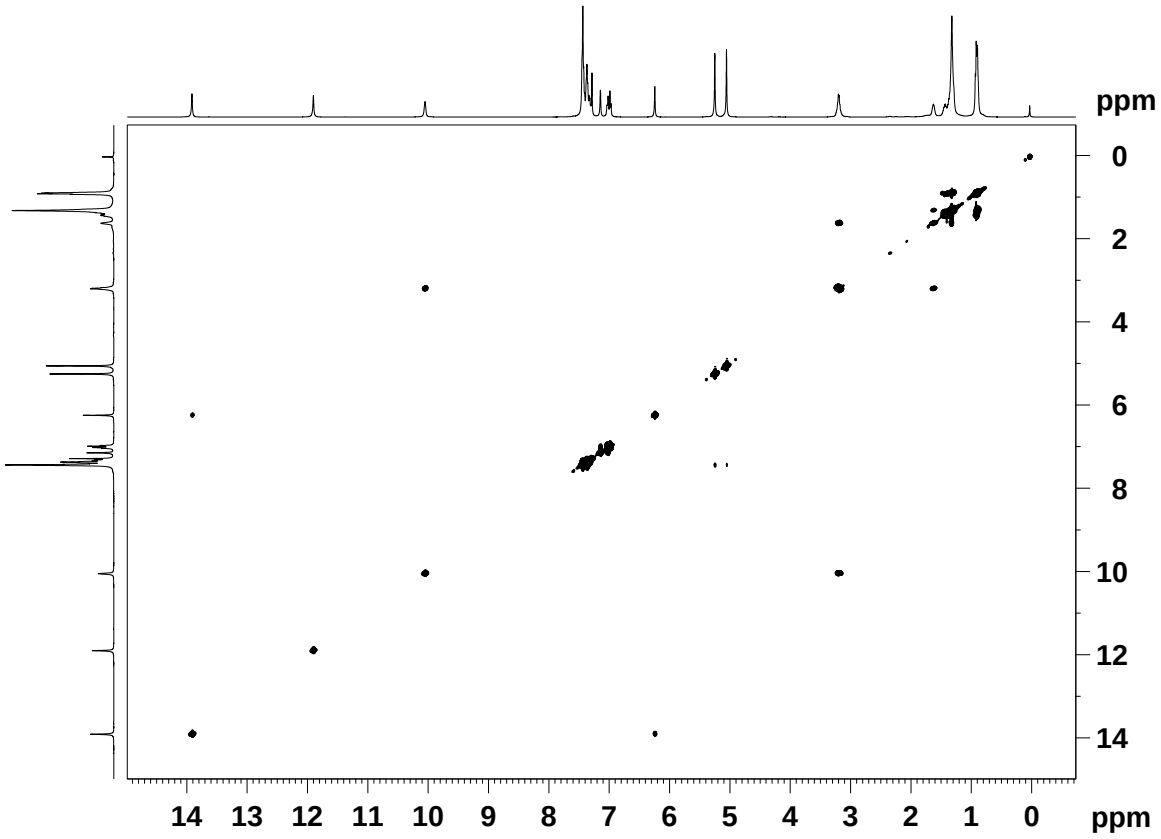


Figure S18. 2D COSY spectra of **18** (500 MHz, $CDCl_3$).

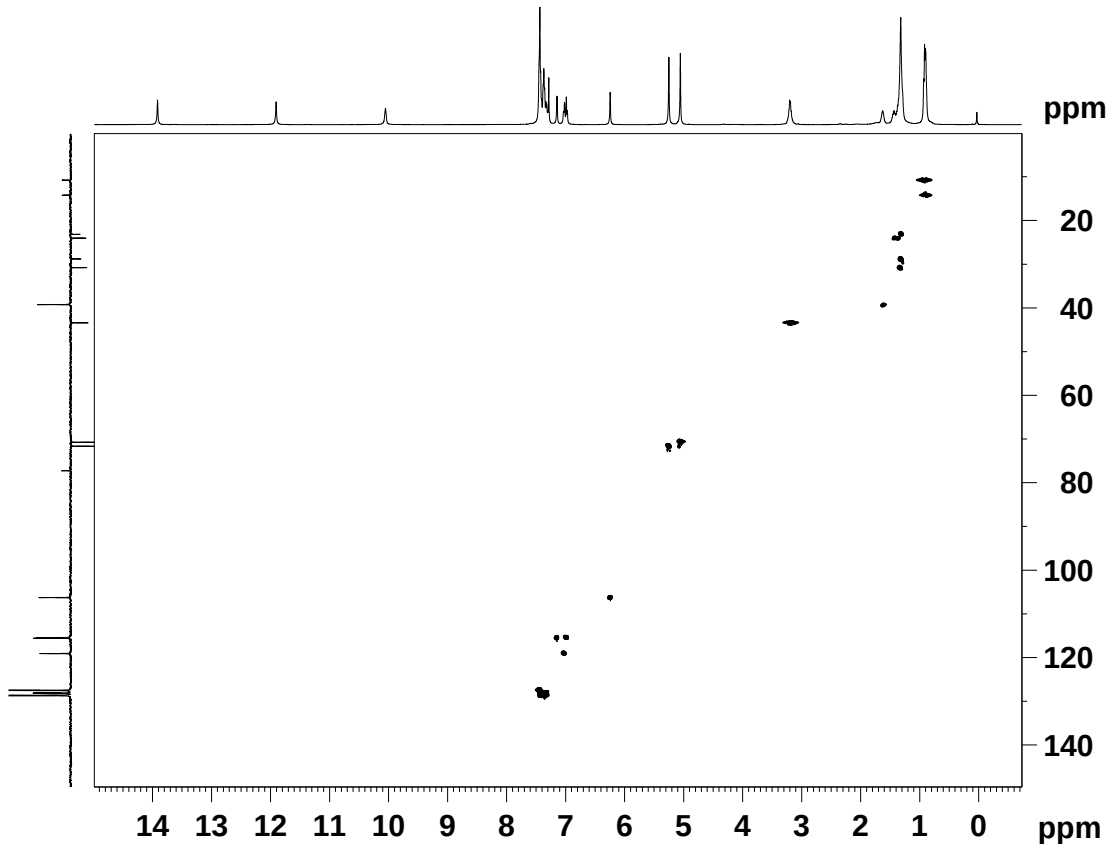


Figure S19. 2D HSQC spectra of **18** (500 MHz, $CDCl_3$).

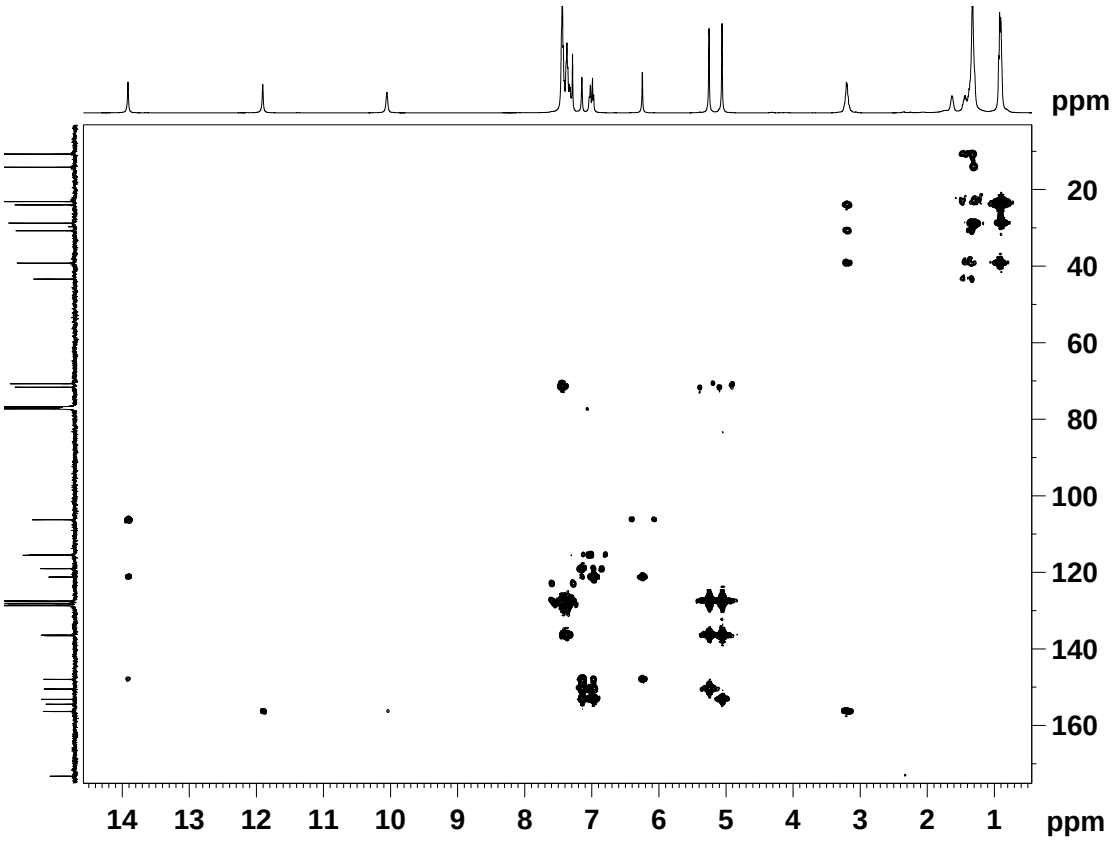


Figure S20. 2D HMBC spectra of **18** (500 MHz, $CDCl_3$).

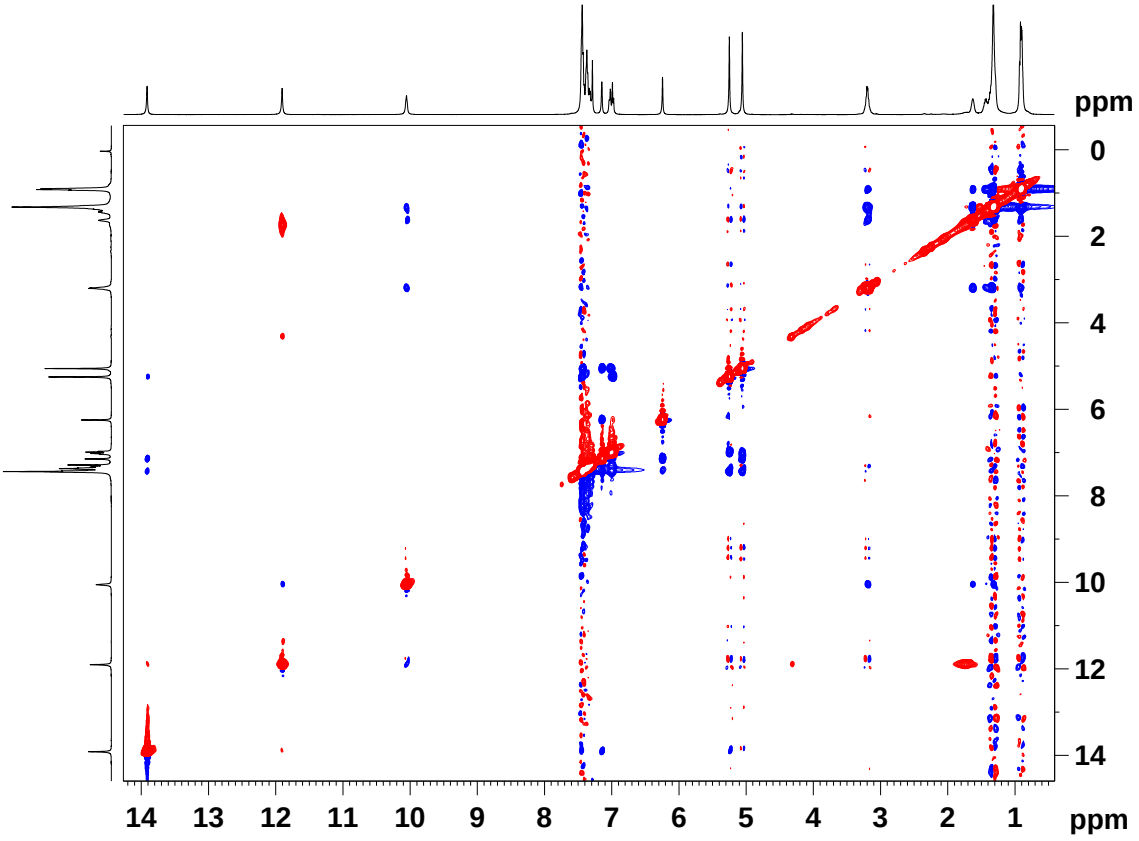


Figure S21. 2D NOESY spectra of **18** (500 MHz, $CDCl_3$).

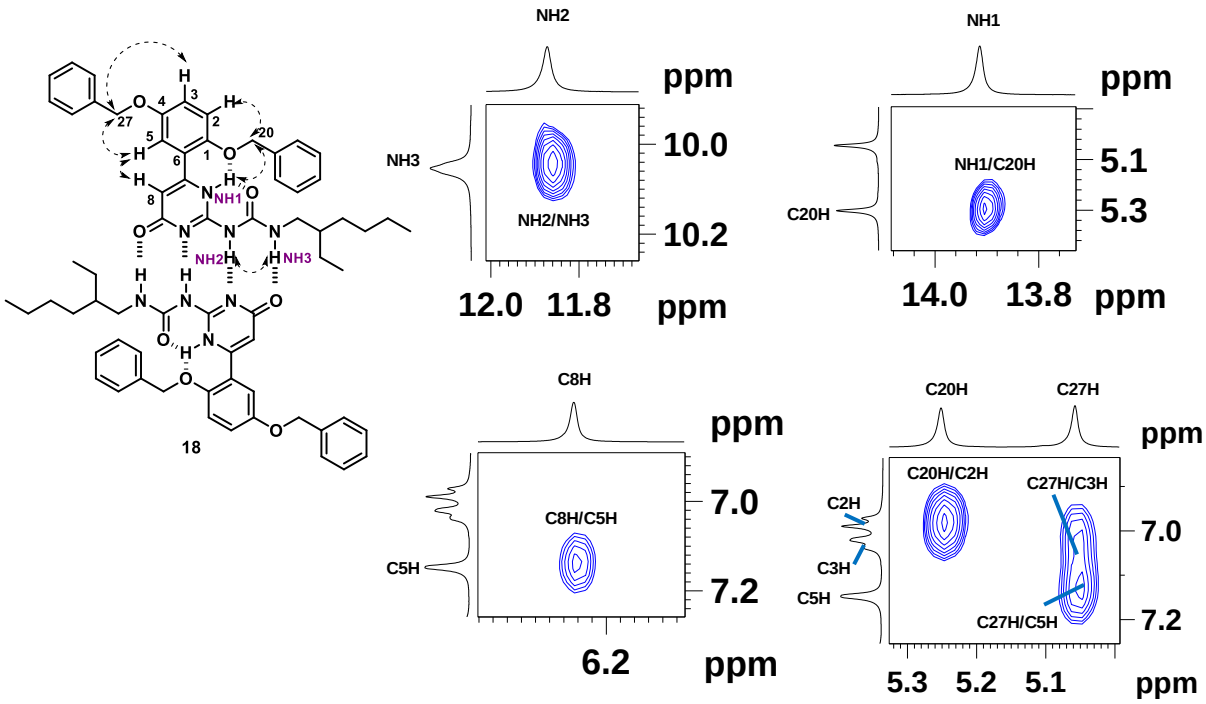
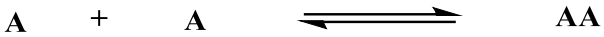


Figure S22. 2D extracts of **18** (500 MHz, $CDCl_3$).

Nonlinear regression analysis is based on the following equation[3]:



$$K_{\mathrm{d}} = \frac{[AA]}{[A]2}$$

$$[\mathrm{A}] = [\mathrm{A}]_0 - 2[\mathrm{AA}]$$

$$AA = \frac{4K_{\mathrm{d}}[\mathrm{A}]_0 + 1 - \sqrt{8K_{\mathrm{d}}[\mathrm{A}]_0 + 1}}{8K_d}$$

$$\delta_{\mathrm{obs}} = \frac{2[AA]}{[A]_0}\delta_{\mathrm{d}} + \frac{[A]}{[A]_0}\delta_{\mathrm{f}}$$

$$= \delta_{\mathrm{f}} + (\delta_{\mathrm{d}} - \delta_{\mathrm{f}})\,\frac{4K_{\mathrm{d}}[\mathrm{A}]_0 + 1 - \sqrt{8K_{\mathrm{d}}[\mathrm{A}]_0 + 1}}{4K_{\mathrm{d}}[\mathrm{A}]_0}$$

[A] : the concentration of unbound free species

$[\mathrm{A}]_0$: the total concentration

[AA] : the concentration of dimer

K_{d} : the dimerization constant

δ_{d} : the limiting bound chemical shift of the dimer

δ_{f}: the free chemical shift

δ_{obs} : chemical shift measured by experiment

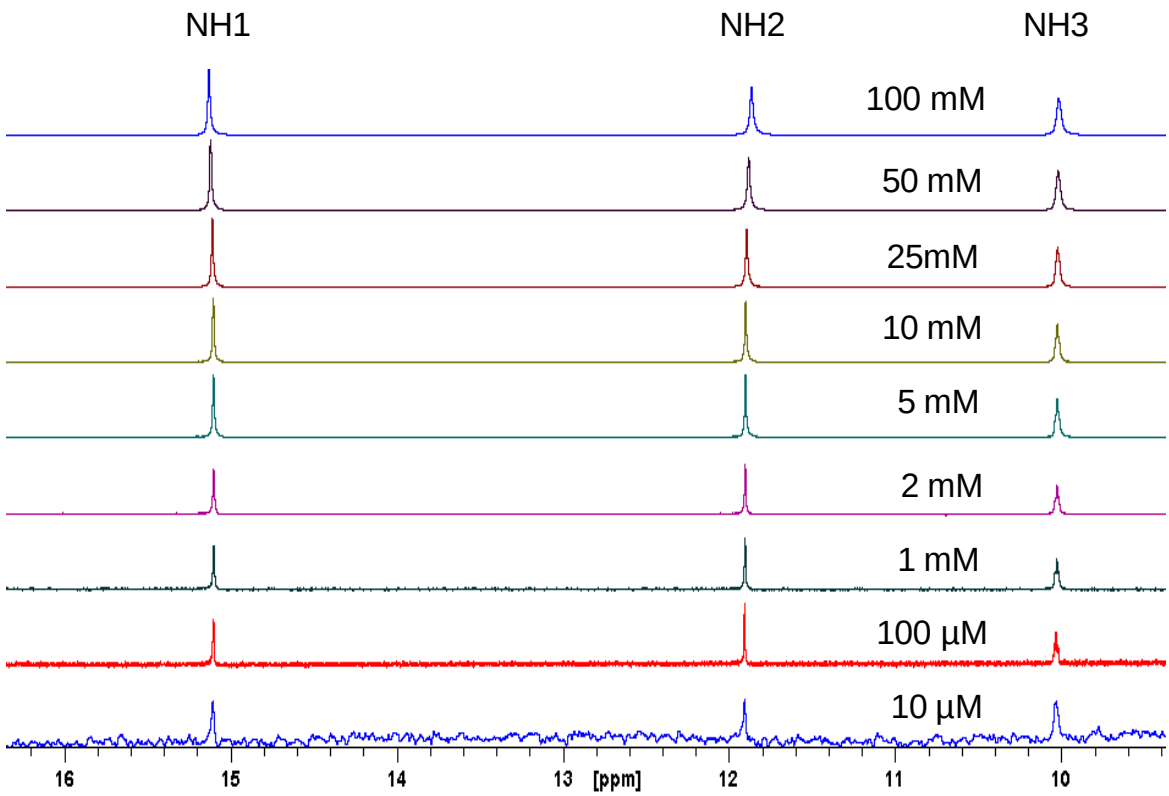


Figure S23. Stacked partial ^{1}H NMR spectra of compound **2** at different concentration in $CDCl_3$ (500 MHz, 298K), showing no chemical shift change of protons NH1, NH2 and NH3.

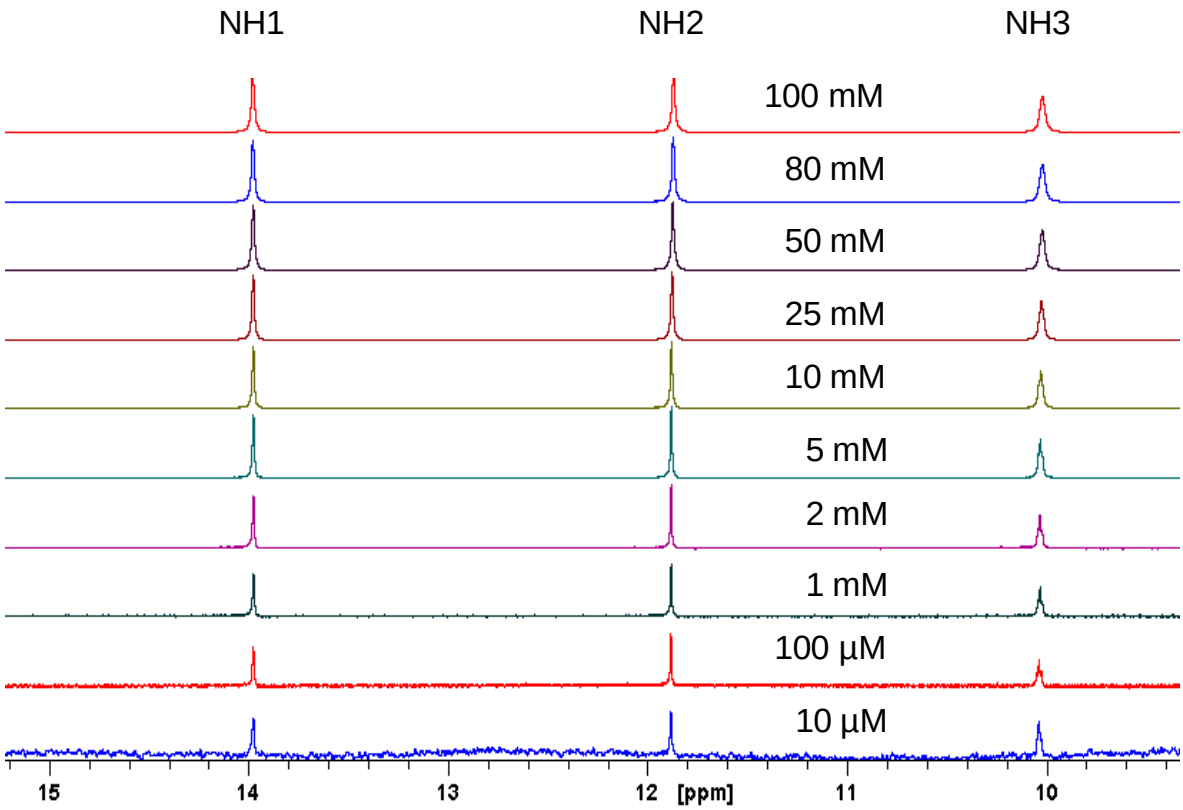


Figure S24. Stacked partial ^{1}H NMR spectra of compound **3** at different concentration in $CDCl_3$ (500 MHz, 298K), showing no chemical shift change of protons NH1, NH2 and NH3.

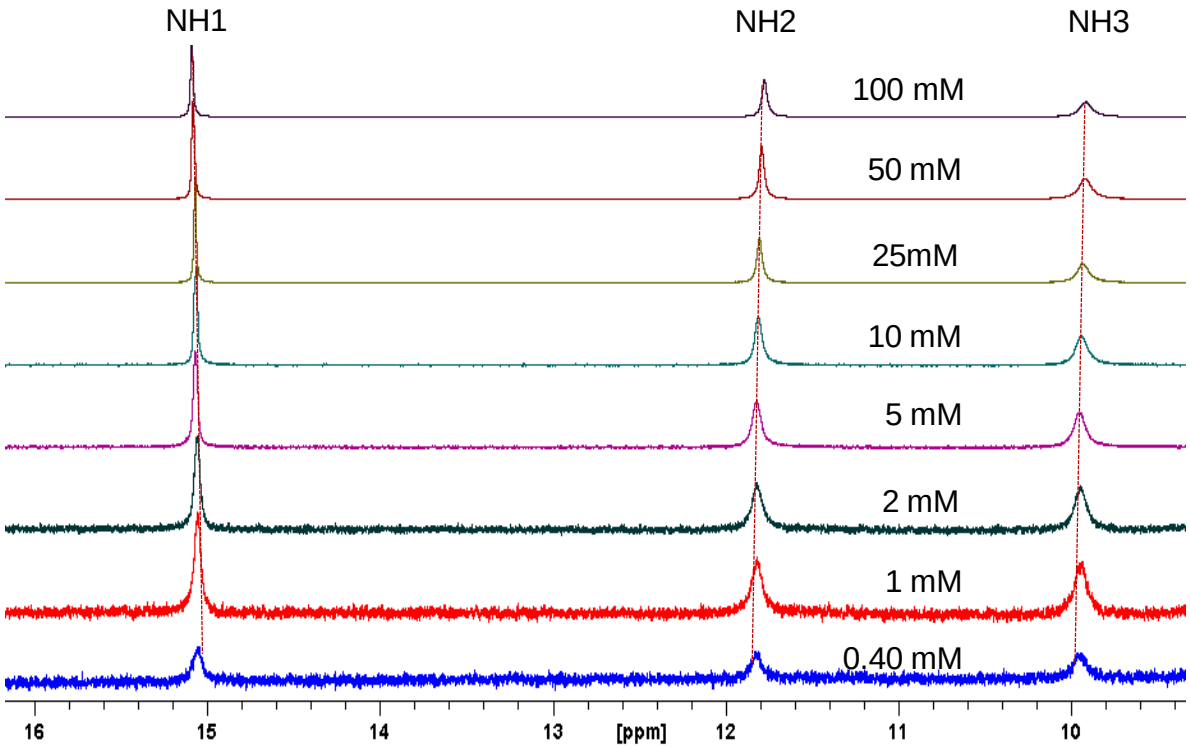


Figure S25. Stacked partial ^{1}H NMR spectra of compound **2** at different concentration in 5% DMSO-d_6/$CDCl_3$ (500 MHz, 298K).

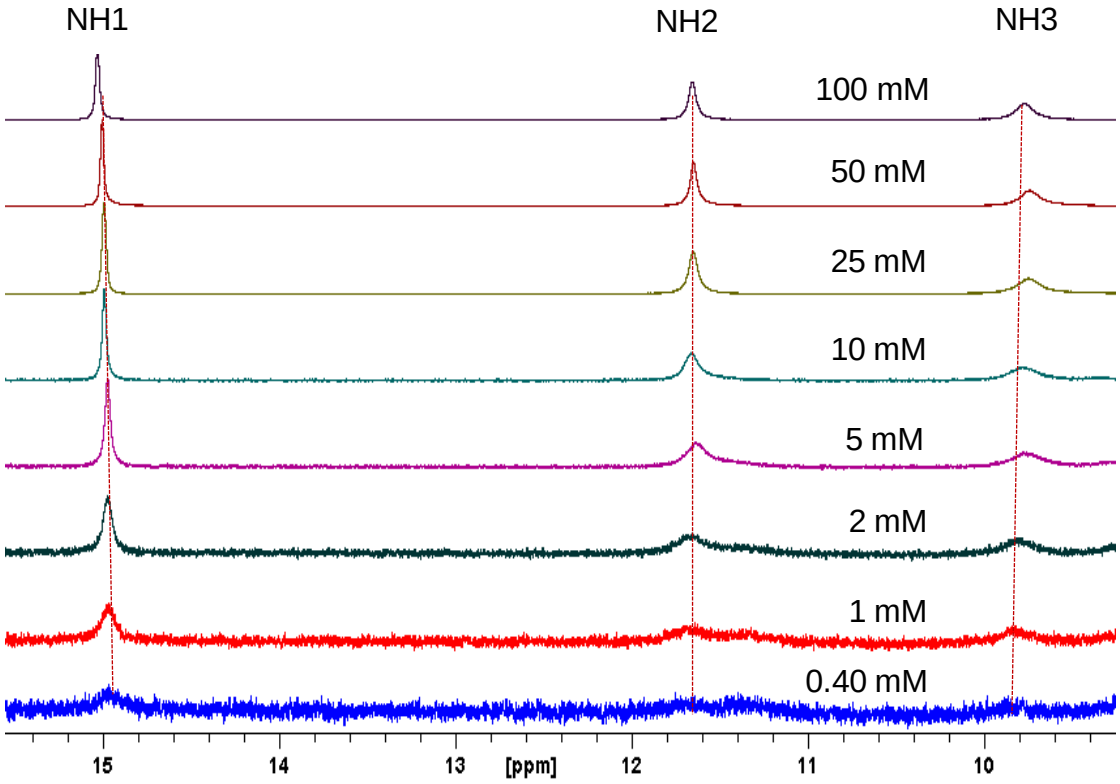


Figure S26. Stacked partial ^{1}H NMR spectra of compound **2** at different concentration in 10% DMSO-d_6/$CDCl_3$ (500 MHz, 298K).

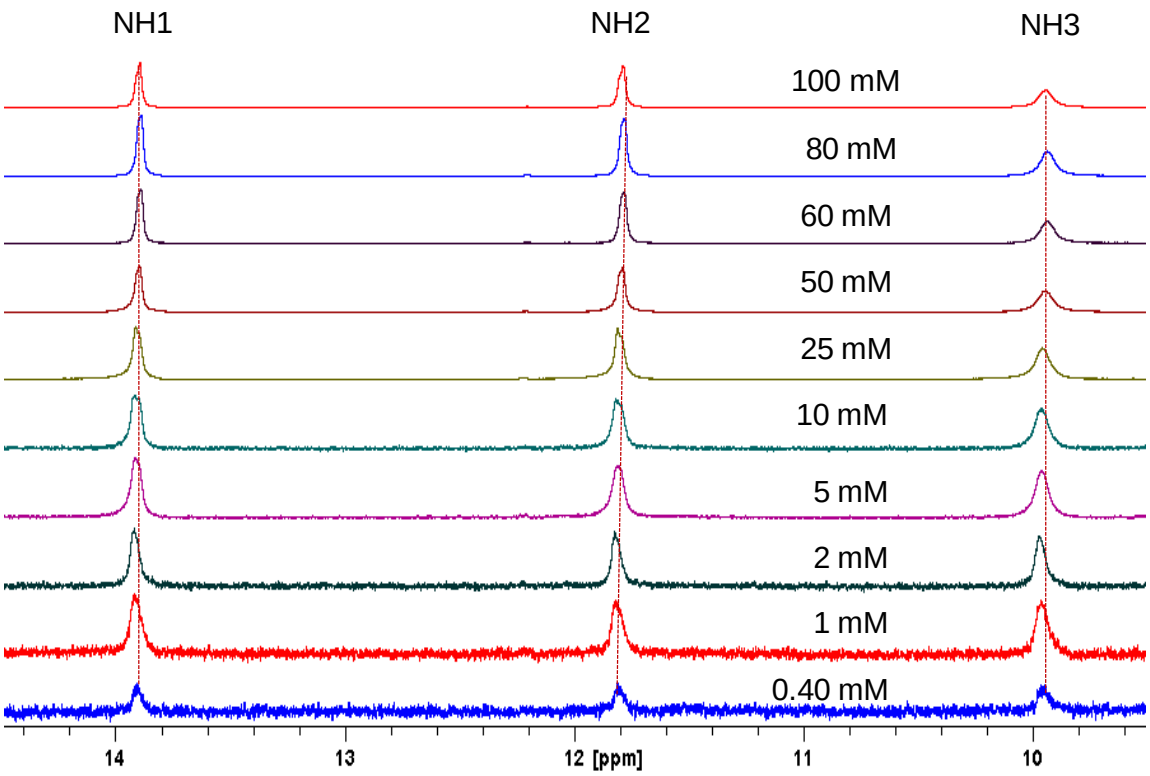


Figure S27. Stacked partial ^{1}H NMR spectra of compound **3** at different concentration in 5% DMSO-d_6/$CDCl_3$ (500 MHz, 298K).

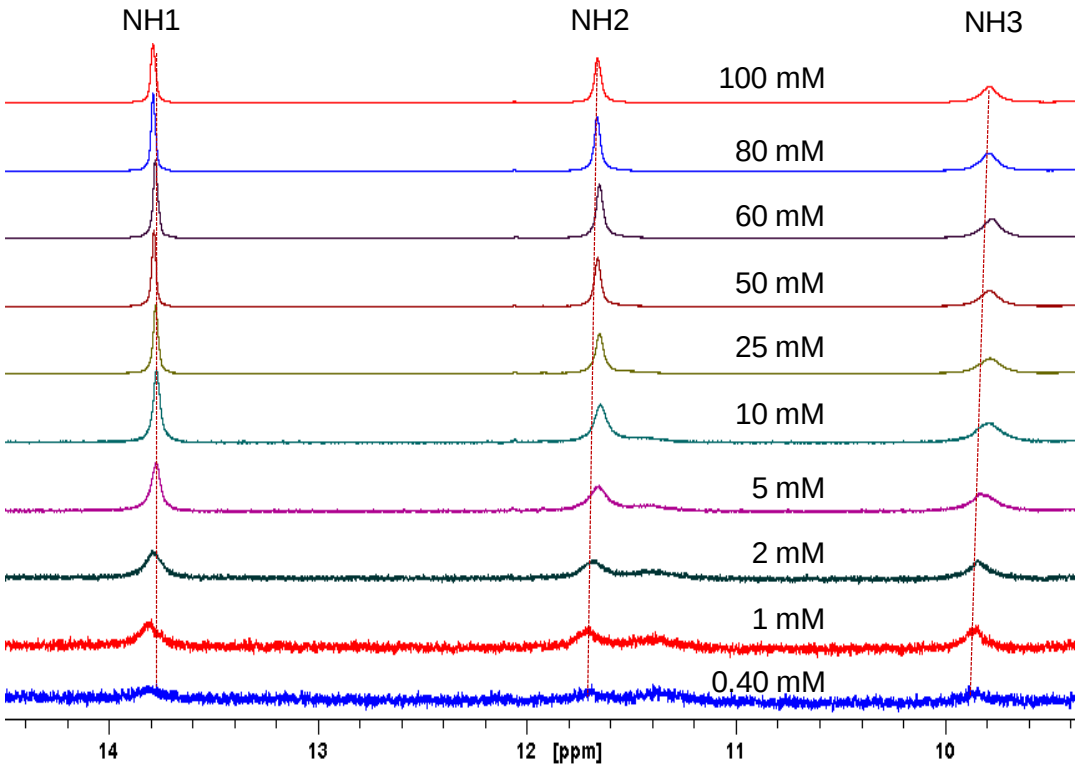
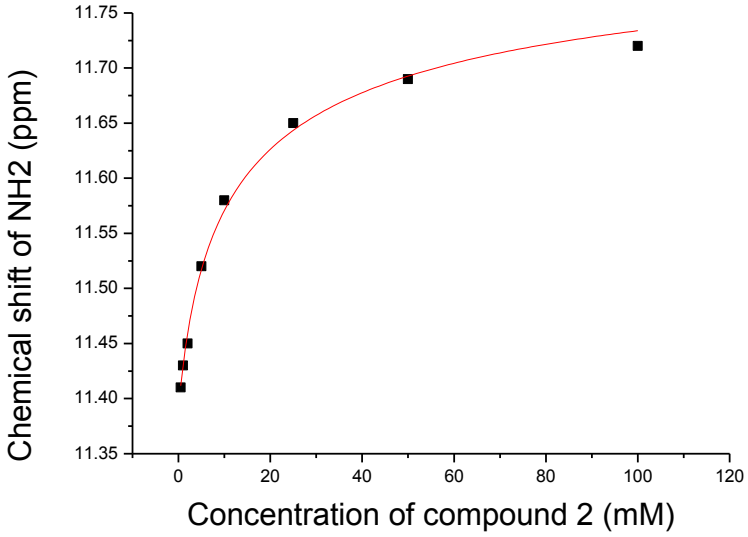
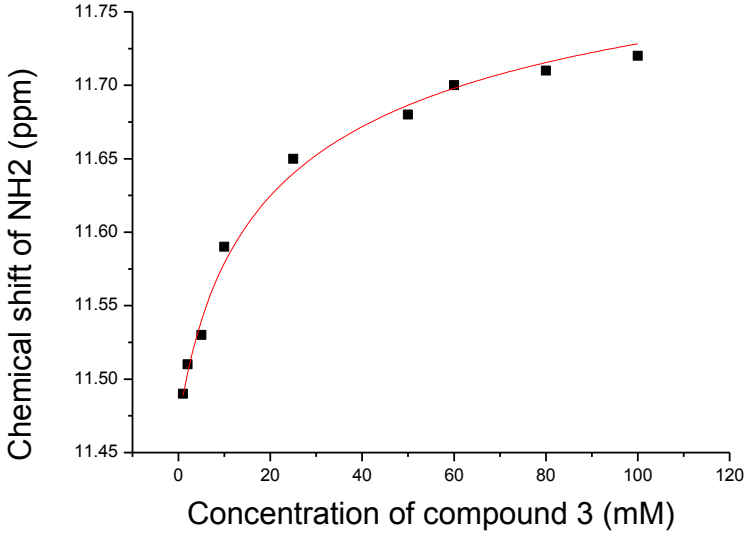


Figure S28. Stacked partial ^{1}H NMR spectra of compound **3** at different concentration in 10% DMSO-d_6/$CDCl_3$ (500 MHz, 298K).



K_{dim} = (5.08 ± 0.002) x 10 M^{-1} R^2 = 0.9959

Figure S29. Determination of the dimerization constant of **2·2** in 20% DMSO-d_6/$CDCl_3$ at 298K. Fitting result based on NH2.



K_{dim} = (2.57 ± 0.001) x 10 M^{-1} R^2 = 0.9925

Fig. S30. Determination of the dimerization constant of **3·3** in 20% DMSO-d_6/$CDCl_3$ at 298K. Fitting result based on NH2.

Fluorescence experiments and emission spectra of 3 at various concentrations

Fluorescence emission spectra were measured with a HORIBA Fluoromax-4 spectrometer set to an excitation wavelength of 340 nm. The chloroform used for fluorescence spectroscopy was of spectrophotometric grade and was used as received. For determination of the K_{dim} of **3,** fluorescence spectra were measured in the concentration range from 10^{-9} to 10^{-6} M in chloroform. The excimer band (λ_{max} = 400 nm) was integrated from 360 to 520 nm. The data was fitted into a dimerization equation described by Wilcox.[3a]

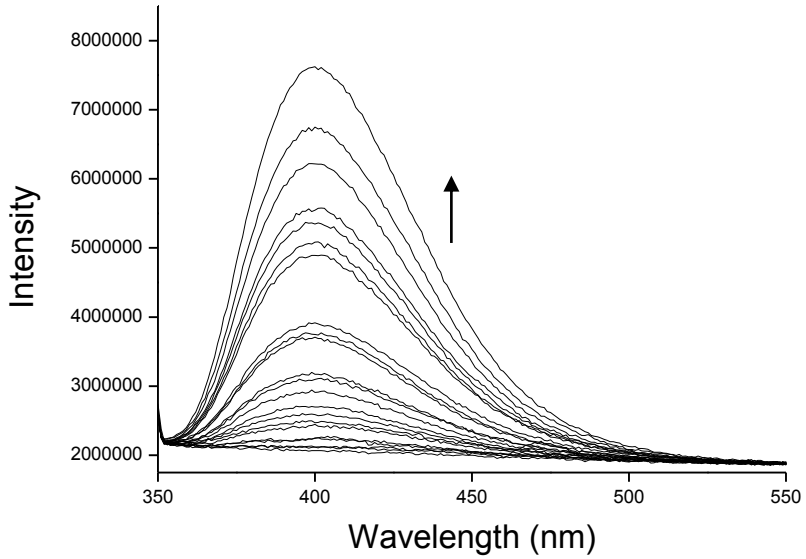
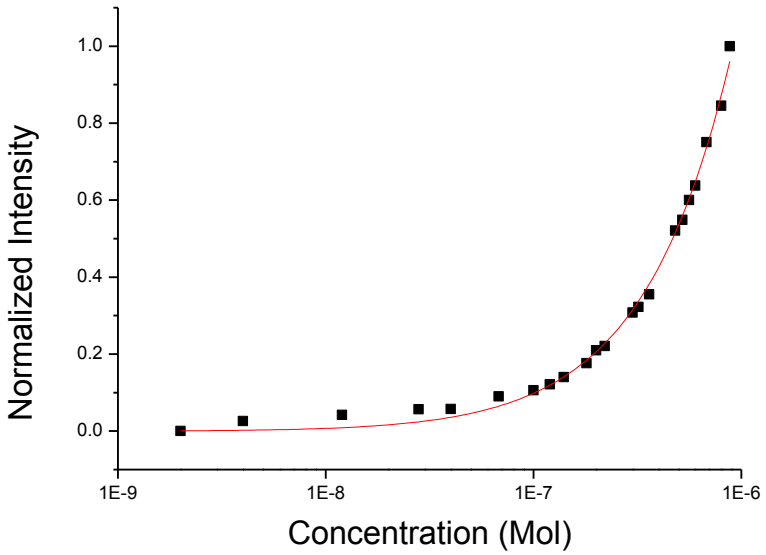


Figure S31. Fluorescence emission spectra (λ_{ex} = 340 nm) of the **3** dimer in the concentration range of 10^{-9} to 10^{-6} M in chloroform.



K_{dim} = (2.02 ± 2.42) x 10^8 M^{-1} R^2 = 0.99558

Figure S32. Plot of the normalized intensity of **3** vs concentration, measured in chloroform, curve is derived from the nonlinear curve fit.

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