Supporting Information

Tri-Substituted Imidazoles as *Mycobacterium tuberculosis* Glutamine Synthetase Inhibitors

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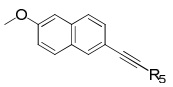
General information and instrumentation

All reactions utilizing microwave heating were performed in sealed reaction vials dedicated for microwave processing, under air and with magnetic stirring. The microwave experiments were conducted using a Smith Synthesizer™ single mode cavity, producing controlled irradiation at 2450 MHz (Biotage AB, Uppsala, Sweden). The temperature of the reaction mixture was measured using a built-in, on-line infrared temperature sensor. For flash chromatography, commercially available silica gel 60 (particle size: 0.040-0.063 mm) was used. NMR spectra were recorded on a Varian Mercury plus at 25 °C for ^{1}H at 399.9 MHz and for ^{13}C NMR at 100.5 MHz. The chemical shifts (δ) are reported in ppm and ^{1}H NMR and ^{13}C NMR are referenced to TMS via the residual solvent signals (^{1}H, $CDCl_3$ at 7.26 ppm, CD_3OD at 3.31 ppm, $(CD_3)_2SO$ at 2.50 ppm, $(CD_3)_2NCDO$ at 2.75 ppm; ^{13}C, $CDCl_3$ at 77.16 ppm, CD_3OD at 49.0 ppm, $(CD_3)_2SO$ at 39.52 ppm, $(CD_3)_2NCDO$ at 29.76 ppm). Analytical RPLC-MS analysis of reaction mixtures and pure products were performed using a Gilson HPLC system with a Chromolith SpeedROD RP-18e column (50×4.6 mm) and a Finnigan AQA quadrupole mass spectrometer using a 4 mL/min CH_3CN/H_2O gradient (0.05% HCOOH) and detection by UV (DAD) or ELSD and MS (ESI+). Preparative RP-HPLC was performed on a Gilson-Finnigan ThermoQuest AQA system equipped with a C8 (Zorbax SB-C8, 5 μm, 21.2×150 mm) column, using a CH_3CN/H_2O gradient (0.05% HCOOH) as mobile phase at a flow rate of 15 mL/min with UV detection (254 nm). GC-MS analyses were performed on a Varian 3900, equipped with a CP-SIL 5 CB Low Bleed (30 m×0.25 mm) capillary column using a 40-300 °C temperature gradient and EI ionization (70 eV).

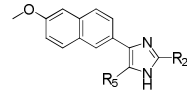
Due to the tautomerism of 2,4,5-trisubstitued imidazoles, all carbon signals could not always be detected by ^{13}C-NMR.[1]All compounds were determined to be >95% pure by HPLC-UV at 254nM.

General procedure for preparation of disubstituted ethyns 6b-d. Method A.



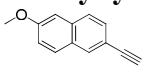
A 2-5 mL microwave vial was charged with 2-ethynyl-6-methoxynaphthalene **5** (1 equiv), aryl/heteroaryl bromide (2-4 equiv), dichlorobis(triphenylphosphine)-palladium (0.05 equiv), copper iodide (0.2 equiv), acetonitrile (2.0 mL) and diethylamine (2.0 mL). The vial was capped under air and heated by microwaves at 80-120 °C for 15 min. Purification by silica column flash chromatography yielded pure products **6b-d**.

General procedure for preparation of trisubstituted imidazoles 7a-m. Method B.



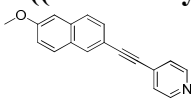
The starting material **6a-d** (1 equiv) was dissolved in 20 mL acetone and 10 mL 0.1 M phosphate buffer (pH = 5). $KMnO_4$ (2-3 equiv) was added, after which the reaction was stirred at rt. for 3 h. Water (50 mL) and brine (50 mL) were added and the resulting mixture was extracted with EtOAc (3x25 mL). The combined organic layers were washed with water (1x50 mL), dried with $MgSO_4$ and evaporated. The residue was dissolved in 1-butanol (10 mL) followed by addition of aldehyde (3 equiv) and ammonium acetate (15 equiv). The reactions were heated in a heating block at the stated temperatures (50-65 °C) and denoted times (0.5-5 h) to attain full consumption of the starting material.

2-Ethynyl-6-methoxynaphthalene (5).[2]



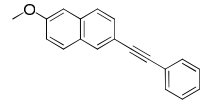
A 2-5 mL microwave vial was charged with 2-bromo-6-methoxynaphthalene (976 mg, 4.11 mmol), ethynyltrimethylsilane (2.31 mL, 16.5 mmol), dichlorobis(triphenylphosphine)-palladium (145 mg, 0.21 mmol), copper iodide (175 mg, 0.92 mmol), acetonitrile (1.0 mL) and diethylamine (1.0 mL). The vial was sealed under air and irradiated by microwaves at 120 °C for 15 min. After cooling to room temperature the solvent was evaporated; the solid residue was suspended in methanol saturated with K_2CO_3 (20 mL) and stirred for 2 h at room temperature. The suspension was filtrated through a plug of Celite and washed with methanol (10 ml). The solvent was evaporated and the residue was purified by silica column flash chromatography using EtOAc:iso-hexane (1:100 to 4:100) as eluent. The product was isolated in 85% yield (635 mg) as a pale orange solid.

4-((6-Methoxynaphthalen-2-yl)ethynyl)pyridine (6a).



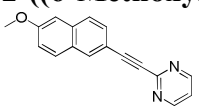
A 10-20 mL microwave vial was charged with **5** (979 mg, 5.37 mmol), 4-bromopyridine as the HCl salt (2.08 g, 10.7 mmol), dichlorobis(triphenylphosphine)-palladium (190 mg, 0.27 mmol), copper iodide (207 mg, 1.09 mmol), acetonitrile (10.0 mL) and diethylamine (10.0 mL). The vial was capped and heated by microwaves at 80 °C for 15 min. Purification by silica column flash chromatography using EtOAc:DCM (1:9 to 2:8) yielded 654 mg pure product as a white solid (47% yield). ^{1}H NMR ($CDCl_3$) δ 8.61 (m, 2H), 8.02 (m, 1H), 7.73 (dm, *J* = 8.9 Hz, 1H), 7.72 (dm, *J* = 8.5 Hz, 1H), 7.55 (dm, *J* = 8.5 Hz, 1H), 7.41 (m, 2H), 7.19 (dm, *J* = 8.9 Hz, 1H), 7.13 (m, 1H), 3.94 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.7, 149.8, 134.6, 132.0, 131.6, 129.5, 128.8, 128.4, 127.0, 125.5, 119.7, 116.9, 105.9, 94.8, 86.5, 55.3. GC-MS (*m/z)* 260 ($M + H^+$). HRMS ($M + H^+$): 260.1073, $C_{18}H_{13}NO$ requires 260.1075.

2-Methoxy-6-(phenylethynyl)naphthalene (6b).



The title compound was prepared according to method A using **5** (100 mg, 0.55 mmol), bromobenzene (116 µL, 1.10 mmol), dichlorobis(triphenylphosphine)-palladium (21 mg, 0.03 mmol), copper iodide (22 mg, 0.12 mmol) and a temperature of 120 °C. Purification by silica column flash chromatography, eluent petroleum ether:diethyl ether (100:1 to 100:3), yielded 31 mg pure product as a white solid (22% yield). ^{1}H NMR ($CDCl_3$) δ 7.99 (m, 1H), 7.72 (dm, *J* = 8.9 Hz, 1H), 7.70 (dm, *J* = 8.5 Hz, 1H), 7.60-7.56 (m, 2H), 7.55 (dm, *J* = 8.5 Hz, 1H), 7.40-7.31 (m, 3H), 7.17 (dm, *J* = 8.9 Hz, 1H), 7.12 (m, 1H), 3.94 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 157.5, 133.3, 130.7, 130.4, 128.5 128.2, 127.7, 127.5, 127.3, 126.0, 122.6, 188.6, 117.3, 105.0, 89.1, 88.2, 54.5. GC-MS (*m/z)* 258 (M^+). Anal. Calcd for $C_{19}H_{14}O$: C, 88.34; H, 5.46. Found: C, 88.64; H, 5.74

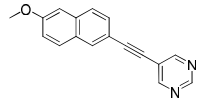
2-((6-Methoxynaphthalen-2-yl)ethynyl)pyrimidine (6c).



The title compound was prepared according to method A using **5** (400 mg, 2.20 mmol), 2-bromopyrimidine (1.40 g, 8.80 mmol), dichlorobis(triphenylphosphine)-palladium (77 mg, 0.11 mmol), copper iodide (84 mg, 0.44 mmol) and a temperature of 80 °C. After cooling to room temperature the suspension was filtrated through celite and the solvent was evaporated.

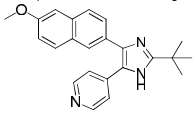
Purification by silica column flash chromatography, eluent toluene:EtOAc (8:2), yielded 311 mg pure product as a yellow solid (54% yield). ^{1}H NMR ($CDCl_3$) δ 8.68 (d, *J* = 4.9 Hz, 2H), 8.07 (br s, 1H), 7.66 (m, 1H), 7.64 (m, 1H), 7.57 (m, 1H), 7.16 (dm, *J*= 4.9 Hz, 1H), 7.10 (dm, *J* = 2.6 Hz, 1H), 7.04 (d, *J*= 2.6 Hz, 1H), 3.85 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 159.1, 157.5, 153.7, 135.2, 133.5, 129.9, 129.4, 128.5, 127.2, 119.9, 119.7, 116.3, 106.1, 89.3, 88.0, 55.6. ESI-MS (*m/z)* 261 (M + H^+). HRMS (M + H^+): 261.1033, $C_{17}H_{13}N_2O$ requires 261.1028.

5-((6-Methoxynaphthalen-2-yl)ethynyl)pyrimidine (6d).



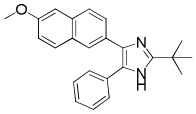
The title compound was prepared according to method A using **5** (300 mg, 1.65 mmol), 5-bromopyrimidine (1.05 g, 6.60 mmol), dichlorobis(triphenylphosphine)-palladium (56 mg, 0.08 mmol), copper iodide (63 mg, 0.33 mmol) and a temperature of 80 °C. After cooling to room temperature the suspension was filtrated through Celite and the solvent was evaporated. Purification by silica column flash chromatography, eluent iso-hexane:EtOAc (8:2), yielded 270 mg pure product as a yellow solid (63% yield). ^{1}H NMR ($CDCl_3$) δ 9.08 (s, 1H), 8.82 (s, 2H), 7.94 (br s, 1H), 7.67 (m, 1H), 7.65 (m, 1H), 7.47 (m, 1H), 7.11 (dm, *J* = 2.4 Hz, 1H), 7.05 (dm, *J* = 2.4 Hz, 1H), 3.86 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 157.8, 157.5, 155.5, 133.7, 130.9, 128.5, 127.6, 127.3, 126.1, 118.8, 115.5, 104.9, 96.1, 81.0, 54.4. ESI-MS (*m/z)* 261 (M + H^+). HRMS (M + H^+): 261.1030, $C_{17}H_{13}N_2O$ requires 261.1028.

4-(2-*tert*-Butyl-4-(6-methoxynaphthalen-2-yl)-1*H*-imidazol-5-yl)pyridine (7a).



The title compound was prepared according to method B using **6a** (35 mg, 0.12 mmol), $KMnO_4$ (57 mg, 0.36 mmol), pivalaldehyde (39 µL, 0.36 mmol), ammonium acetate (139 mg, 1.80 mmol) and 50 °C heating for 3 h. Purification by silica column flash chromatography, eluent EtOAc:iso-hexane:e (1:1), gave **7a** (25 mg) in 58% yield as a pale yellow solid.
^{1}H NMR ($CDCl_3$) δ 8.32 (m, 2H), 7.82 (br s, 1H), 7.70 (d, *J* = 8.6 Hz, 1H), 7.68 (d, *J* = 8.6 Hz, 1H), 7.49-7.37 (m, 3H), 7.17-7.10 (m, 2H), 3.91 (s, 3H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$:CD_3OD, 5:1) δ 158.5, 156.9, 149.4, 134.5, 129.7, 129.1, 127.5, 127.4, 127.0, 121.7, 119.5, 106.2, 55.4, 20.5, 12.3, (3 carbon signals were not detected). ESI-MS (*m/z)* 358 (M + H^+). HRMS (M + H^+): 358.1922, $C_{23}H_{23}N_3O$ requires 358.1919.

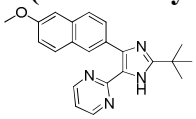
2-*tert*-Butyl-4-(6-methoxynaphthalen-2-yl)-5-phenyl-1*H*-imidazole (7b).



The title compound was prepared according to method B using **6b** (72 mg, 0.28 mmol), $KMnO_4$ (132 mg, 0.84 mmol), pivalaldehyde (92 µL, 0.84 mmol), ammonium acetate (323 mg, 4.19 mmol) and 60 °C heating for 5 h. The reaction mixture was evaporated and water (20 mL) was added followed by extraction to DCM (2x20 mL). The organic layer was dried with $MgSO_4$ and evaporated. Purification by silica column flash chromatography, eluent EtOAc:iso-hexane:diethylmethylamine (10:90:2 to 20:80:2), gave **7b** (52 mg) in 52% yield as a white solid. ^{1}H NMR (CD_3OD) δ 7.84 (br s, 1H), 7.65 (m, 1H), 7.64 (m, 1H), 7.46-7.36 (m, 3H), 7.31-7.21 (m, 3H), 7.19 (dm, *J* = 2.5 Hz, 1H), 7.09 (dm, *J* = 2.5 Hz, 1H), 3.89 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (CD_3OD) δ 159.3, 157.7, 135.2, 130.4, 130.3, 129.5, 129.4, 128.2,

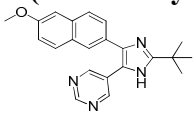
128.1, 128.0, 127.8, 127.7, 120.0, 106.7, 55.8, 34.1, 30.0, (3 carbon signals were not detected). ESI-MS (*m/z)* 357 (M + H^+). HRMS (M + H^+): 357.1955, $C_{24}H_{24}N_2O$ requires 357.1967.

2-(2-*tert*-Butyl-4-(6-methoxynaphthalen-2-yl)-1*H*-imidazol-5-yl)pyrimidine (7c).



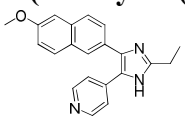
The title compound was prepared according to method using **6c** (115 mg, 0.44 mmol), $KMnO_4$ (140 mg, 0.88 mmol), pivalaldehyde (145 µL, 1.32 mmol), ammonium acetate (511 mg, 6.63 mmol) and 50 °C heating for 1.5 h. The reaction mixture was evaporated and water (20 mL) was added followed by extraction to DCM (2x20 mL). The organic layer was dried with $MgSO_4$ and evaporated. Purification by silica column flash chromatography, eluent iso-hexane:EtOAc (1:1), gave **7c** (97 mg) in 61% yield as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 8.62 (m, 2H). 8.43 (s, 1H), 8.06 (m, 1H), 7.78 (dm, *J* = 8.9 Hz, 1H), 7.73 (m, 1H), 7.14 (dm, *J* = 2.5 Hz, 1H), 7.12 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.01 (m, 1H), 3.93 (s, 3H), 1.52 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 159.5, 157.9, 157.2, 144.9, 134.5, 130.3, 129.0, 128.7, 128.6, 126.0, 118.6, 118.0, 105.9, 55.5, 33.2, 29.7, (3 carbon signals were not detected). ESI-MS (*m/z)* 359 (M + H^+). HRMS (M + H^+): 359.1869, $C_{22}H_{23}N_4O$ requires 359.1872.

5-(2-*tert*-Butyl-4-(6-methoxynaphthalen-2-yl)-1*H*-imidazol-5-yl)pyrimidine (7d).



The title compound was prepared according to method B using **6d** (80 mg, 0.31 mmol), $KMnO_4$ (147 mg, 0.93 mmol), pivalaldehyde (103 µL, 0.93 mmol), ammonium acetate (358 mg, 4.65 mmol) and 50 °C heating for 1.5 h. The reaction mixture was evaporated and water (20 mL) was added followed by extraction to DCM (2x20 mL). The organic layer was dried with $MgSO_4$ and evaporated. Purification by preparative silica TLC, eluent iso-hexane:EtOAc (1:1), gave **7d** (11 mg) in 10% yield as a pale yellow solid. ^{1}H NMR (CD_3OD) δ 8.95 (s, 1H), 8.82 (s, 2H), 7.86 (m, 1H), 7.80 (m, 1H), 7.73 (dm, *J* = 8.9 Hz, 1H), 7.40 (m, 1H), 7.26 (d, *J*= 2.5, 1H), 7.15 (dd, *J* = 8.9, 2.5 Hz, 1H), 3.92 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (CD_3OD) δ 158.7, 158.2, 155.5, 155.2, 134.7, 130.5, 129.4, 129.2, 127.5, 127.4, 126.5, 105.6, 54.7, 33.0, 28.7, (4 carbon signals were not detected). ESI-MS (*m/z)* 359 (M + H^+). HRMS (M + H^+): 359.1865, $C_{22}H_{23}N_4O$ requires 359.1872.

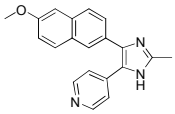
4-(2-Ethyl-4-(6-methoxynaphthalen-2-yl)-1*H*-imidazol-5-yl)pyridine (7e).



The title compound was prepared according to method B using **6a** (50 mg, 0.19 mmol), $KMnO_4$ (92 mg, 0.58 mmol), propionaldehyde (42 µl, 0.58 mmol), ammonium acetate (228 mg, 2.96 mmol) and 50 °C heating for 3 h. Purification by RP-silica column, eluent acetonitrile:water:formic acid (5:95:1 to 25:75:1), gave **7e** (34 mg) in 53% yield as a pale yellow solid. ^{1}H NMR (CD_3OD) δ 8.39 (br s, 2H), 7.89 (dm, *J* = 1.8 Hz, 1H), 7.83 (dm, *J* = 8.5 Hz, 1H), 7.75 (dm, *J* = 9.0 Hz, 1H), 7.53 (m, 2H), 7.45 (ddm, *J* = 8.5, 1.8 Hz, 1H), 7.29 (dm, *J* = 2.5 Hz, 1H), 7.17 (ddm, *J* = 9.0, 2.5 Hz, 1H), 3.94 (s, 3H), 2.85 (q, *J* = 7.6 Hz, 2H), 1.40 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$:CD_3OD, 5:2) δ 158.9, 151.5, 146.7, 135.0, 129.8, 128.8, 128.1, 128.0, 125.9, 123.3, 121.8, 120.1, 105.8, 55.4, 20.5, 12.3, (3 carbon signals

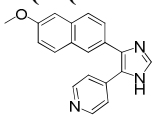
were not detected). ESI-MS (*m/z)* 330 (M + H^+). HRMS (M + H^+): 330.1614, $C_{21}H_{19}N_3O$ requires 330.1606.

4-(4-(6-Methoxynaphthalen-2-yl)-2-methyl-1*H*-imidazol-5-yl)pyridine (7f).



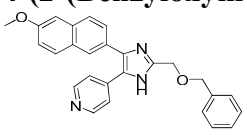
The title compound was prepared according to method B using **6a** (52 mg, 0.20 mmol), $KMnO_4$ (96 mg, 0.61 mmol), acetaldehyde (34 µl, 0.61 mmol), ammonium acetate (230 mg, 2.98 mmol) and 40 °C heating for 4 h. Purification by RP-silica column, eluent acetonitrile:water:formic acid (5:95:1 to 20:80:1), gave **7f** (40 mg) in 63% yield as a pale yellow solid. ^{1}H NMR (CD_3OD) δ 8.37 (m, 2H), 7.87 (dm, *J* = 1.8 Hz, 1H), 7.82 (dm, *J* = 8.5 Hz, 1H), 7.74 (dm, *J* = 8.9 Hz, 1H), 7.49 (m, 2H), 7.45 (ddm, *J* = 8.5, 1.8 Hz, 1H), 7.28 (dm, *J* = 2.6 Hz, 1H), 7.17 (ddm, *J* = 8.9, 2.6Hz, 1H), 3.93 (s, 3H), 2.49 (s, 3H); ^{13}C NMR (CD_3OD) δ 159.9, 150.0, 147.5, 136.0, 130.6, 130.4, 128.6, 128.3, 127.7, 122.8, 120.5, 106.8, 55.9, 13.5, (4 carbon signals were not detected). ESI-MS (*m/z)* 316 (M + H^+). HRMS (M + H^+): 336.1460, $C_{20}H_{17}N_3O$ requires 316.1450.

4-(4-(6-Methoxynaphthalen-2-yl)-1*H*-imidazol-5-yl)pyridine (7g).



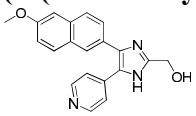
The title compound was prepared according to method B using **6a** (52 mg, 0.20 mmol), $KMnO_4$ (92 mg, 0.58 mmol), paraformaldehyde (19 mg, 0.61 mmol), ammonium acetate (234 mg, 3.04 mmol) and 40 °C for 6 h. Purification by RP-silica column, eluent acetonitrile:water:formic acid (5:95:1 to 20:80:1), gave **7g** (24 mg) in 40% yield as a pale yellow solid. ^{1}H NMR (CD_3OD) δ 8.40 (m, 2H), 7.91-7.89 (m, 2H), 7.87 (dm, *J* = 8.4 Hz, 1H), 7.76 (dm, *J* = 8.9 Hz, 1H), 7.54 (m, 2H), 7.47 (dm, *J* = 8.4 Hz, 1H), 7.30 (dm, *J* = 2.5 Hz, 1H), 7.18 (ddm, *J* = 8.9, 2.5 Hz, 1H), 3.94 (s, 3H); ^{13}C NMR (CD_3OD:$CDCl_3$, 5:2) δ 158.8, 136.6, 134.9, 129.9, 129.2, 127.9, 127.8, 126.8, 126.1, 122.1, 119.9, 106.1, 55.6, (4 carbon signals were not detected). ESI-MS (*m/z)* 302 (M + H^+). HRMS (M + H^+): 302.1286, $C_{19}H_{15}N_3O$ requires 302.1293.

4-(2-(Benzyloxymethyl)-4-(6-methoxynaphthalen-2-yl)-1*H*-imidazol-5-yl)pyridine (7h).



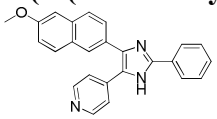
The title compound was prepared according to method B using **6a** (132 mg, 0.51 mmol), $KMnO_4$ (242 mg, 1.53 mmol), 2-(benzyloxy)acetaldehyde (214 µl, 1.53 mmol), ammonium acetate (588 mg, 7.63 mmol) and 65 °C heating for 2 h. Purification by RP-HPLC, eluent acetonitrile:water:formic acid (20:80:0.05 to 45:55:0.05), gave **7h** (112 mg) in 52% yield as a white solid. ^{1}H NMR (CD_3OD:$CDCl_3$, 5:1) δ 8.33 (m, 2H), 8.84 (m, 1H), 7.76 (dm, *J* = 8.5 Hz, 1H), 7.69 (dm, *J* = 8.9 Hz 1H), 7.47 (m, 2H), 7.42 (dm, *J* = 8.5 Hz, 1H), 7.39-7.23 (m, 5H), 7.19 (m, 1H), 7.14 (dm, 8.9 Hz, 1H), 4.66 (br s, 2H), 4.65 (br s, 2H), 3.91 (s, 3H); ^{13}C NMR (CD_3OD :$CDCl_3$, 5:1) δ 159.2, 149.5, 146.9, 143.4, 138.3, 135.3, 130.2, 129.7, 129.0, 128.7, 128.5, 128.2, 128.1, 127.3, 122.5, 120.1, 106.4, 73.6, 65.4, 55.7, (3 carbon signals were not detected). ESI-MS (*m/z)* 422 (M + H^+). HRMS (M + H^+): 422.1872, $C_{27}H_{23}N_3O_2$ requires 422.1869.

(4-(6-Methoxynaphthalen-2-yl)-5-(pyridin-4-yl)-1*H*-imidazol-2-yl)methanol (7i).



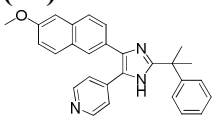
The title compound was prepared according to method B using **6a** (55 mg, 0.21 mmol), $KMnO_4$ (101 mg, 0.64 mmol), 2-(*tert*-butyldimethylsilyloxy)acetaldehyde (135 µl, 0.64 mmol), ammonium acetate (245 mg, 3.18 mmol) and 65 °C heating for 3 h. Tetrabutylammonium fluoride 1.0 M in THF (2.12 mL, 2.12 mmol) was added and the reaction mixture was stirred for 1 h at rt. before addition of water (50 mL). The resulting mixture was extracted with EtOAc (3x50 mL), dried and evaporated. Purification by RP-HPLC, eluent acetonitrile:water:formic acid (10:90:0.05 to 40:60:0.05), gave **7i** (14 mg) in 21% yield as a white solid. ^{1}H NMR ($(CD_3)_2SO$:CD_3OD, 5:1) δ 8.40 (m, 2H), 7.93 (m 1H), 7.83 (d, *J* = 8.5 Hz, 1H), 7.79 (d, *J* = 8.9 Hz, 1H), 7.46 (dm, *J* = 8.5 Hz, 1H), 7.43 (m, 2H), 7.33 (m, 1H) 7.17 (dm, *J* = 8.9 Hz, 1H), 4.55 (s, 2H), 3.89 (s, 3H); ^{13}C NMR ($(CD_3)_2SO$) δ 157.7, 149.6, 141.3, 133.7, 129.5, 128.4, 127.1, 127.0, 126.9, 120.6, 119.1, 106.0, 56.8, 55.3, (4 carbon signals were not detected). ESI-MS (*m/z)* 332 (M + H^+). HRMS (M + H^+): 332.1394, $C_{20}H_{17}N_3O_2$ requires 332.1399.

4-(4-(6-Methoxynaphthalen-2-yl)-2-phenyl-1*H*-imidazol-5-yl)pyridine (7j).



The title compound was prepared according to method B using **6a** (50 mg, 0.19 mmol), $KMnO_4$ (91 mg, 0.58 mmol), benzaldehyde (58 µl, 0.57 mmol), ammonium acetate (222 mg, 2.88 mmol) and 65 °C heating for 3 h. Purification by RP-HPLC, eluent acetonitrile:water:formic acid (20:80:0.05 to 45:55:0.05), gave **7j** (19 mg) in 26% yield as a pale yellow solid. ^{1}H NMR ($(CD_3)_2SO$:CD_3OD, 5:1) δ 8.19 (m, 2H), 8.06 (m, 2H) 7.89 (dm, *J* = 1.8 Hz, 1H), 7.70 (dm, *J* = 8.5 Hz, 1H), 7.69 (dm, *J* = 9.0 Hz, 1H), 7.55 (ddm, *J* = 8.5, 1.8 Hz, 1H), 7.44 (m, 2H), 7.30-7.23 (m, 3H), 7.10 (m, 1H), 7.09 (dm, *J* = 9.0 Hz, 1H), 3.87 (s, 3H); ^{13}C NMR ($(CD_3)_2SO$:CD_3OD, 5:1) δ 167.2, 157.3, 154.9, 148.8, 146.1, 142.1, 137.5, 134.5, 134.4, 133.2, 129.5, 129.3, 128.7, 128.1, 126.4, 126.3, 125.4, 121.1, 118.6, 106.2, 55.3. ESI-MS (*m/z)* 378 (M + H^+). HRMS (M + H^+): 378.1599, $C_{25}H_{19}N_3O$ requires 378.1606.

4-(4-(6-Methoxynaphthalen-2-yl)-2-(2-phenylpropan-2-yl)-1*H*-imidazol-5-yl)pyridine (7k).

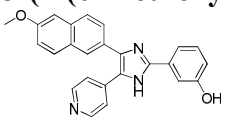


The starting material 2-methyl-2-phenylpropanoic acid (205 mg, 1.25 mmol) was dissolved in dry THF (10 mL) and cooled to -78 °C. A solution of $LiAlH_4$ (1.0 M in THF (1.54 mL, 1.54 mmol) diluted with dry THF (10 mL)) was slowly added drop wise to the reaction under N_2. The dry ice/acetone bath was exchanged to water/ice and the mixture was stirred at 0 °C for 1.5 h and then quenched by adding water (70 µL) followed by 15 % NaOH in water (70 µL). Water (20 mL) and brine (10 mL) were added and the resulting mixture was extracted to EtOAc (2x50 mL). The combined organic layers were dried with $MgSO_4$ and evaporated. The residue was dissolved in DCM (10 mL) and added drop wise to a solution of Dess-Martin periodinane (474 mg, 1.12 mmol) in DCM (10 mL). The reaction was stirred at r.t. for 1 h

before addition of 1.0 M sodium thiosulphate (aq.) (20 mL) and saturated $NaHCO_3$ (aq.) (20 mL). The layers were separated and the aqueous phase extracted with DCM (1x20 mL). The combined organic layers were washed with water (1x20 mL), dried with $MgSO_4$ and evaporated. The 2-methyl-2-phenylpropanal was used in the synthesis of **7k** without further purification.

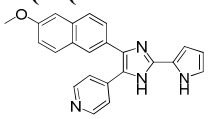
Compound **7k** was prepared according to method B using **6a** (52 mg, 0.20 mmol), $KMnO_4$ (95 mg, 0.60 mmol), crude 2-methyl-2-phenylpropanal, ammonium acetate (229 mg, 2.96 mmol) and 65 °C heating for 2.5 h. Purification by RP-HPLC, eluent acetonitrile:water:formic acid (20:80:0.05 to 45:55:0.05), gave **7k** (13 mg) in 16% yield as a pale yellow solid. ^{1}H NMR (CD_3OD:$CDCl_3$, 2:1) δ 8.31 (m, 2H), 7.82 (m, 1H), 7.73 (d, *J* = 8.5 Hz, 1H), 7.68 (d, *J* = 8.9 Hz, 1H), 7.49 (m, 2H), 7.41 (dm, *J* = 8.5 Hz, 1H), 7.31-7.28, (m, 4H), 7.21-7.16 (m, 2H), 7.13 (dm, *J* = 8.9, Hz, 1H), 3.91 (s, 3H), 1.83 (br s, 6H); ^{13}C NMR (CD_3OD:$CDCl_3$, 2:1) δ 159.0, 157.0, 149.2, 148.4, 135.1, 130.1, 129.6, 128.9, 128.3, 127.8, 127.7, 127.0, 126.6, 122.8, 120.0, 106.4, 55.7, 41.6, 29.2, (4 carbon signals were not detected). ESI-MS (*m/z)* 420 (M + H^+). HRMS (M + H^+): 420.2087, $C_{28}H_{25}N_3O$ requires 420.2076.

3-(4-(6-Methoxynaphthalen-2-yl)-5-(pyridin-4-yl)-1*H*-imidazol-2-yl)phenol (7l).



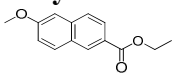
The title compound was prepared according to method B using **6a** (48 mg, 0.19 mmol), $KMnO_4$ (90 mg, 0.57 mmol), 3-hydroxibenzaldehyde (68 mg, 0.56 mmol), ammonium acetate (212 mg, 2.75 mmol) and 65 °C heating for 3 h. Purification by RP-HPLC, eluent acetonitrile:water:formic acid (10:90:0.05 to 40:60:0.05), gave **7l** (27 mg) in 37% yield as a pale yellow solid. ^{1}H NMR ((CD_3)$_2$NCDO:CD_3OD, 5:1) δ 8.67, (m, 2H), 8.17 (m, 1H), 8.02-7.92 (m, 3H), 7.93 (d, *J* = 8.9 Hz, 1H), 7.75, (m, 1H), 7.71-7.66 (m, 2H), 7.47 (d, *J* = 2.6 Hz, 1H), 7.36, (dm, *J* = 8.1 Hz, 1H) 7.27, (dd, *J* = 8.9, 2.6 Hz, 1H), 6.96 (dm, *J* = 8.1 Hz, 1H), 3.99 (s, 3H); ^{13}C NMR ((CD_3)$_2$NCDO) 163.4, 159.8, 159.7, 148.8, 146.2, 135.9, 132.4, 131.0, 130.0, 129.3, 128.6, 128.3, 122.9, 120.6, 117.7, 117.5, 113.9, 107.3, 56.3, (4 carbons were not detected). ESI-MS (*m/z)* 394 (M + H^+). HRMS (M + H^+): 394.1554, $C_{25}H_{19}N_3O_2$ requires 394.1556.

4-(4-(6-Methoxynaphthalen-2-yl)-2-(1*H*-pyrrol-2-yl)-1*H*-imidazol-5-yl)pyridine (7m).



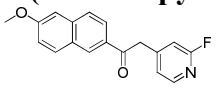
The title compound was prepared according to method B using **6a** (51 mg, 0.20 mmol), $KMnO_4$ (95 mg, 0.60 mmol), 1*H*-pyrrole-2-carbaldehyde (56 mg, 0.59 mmol), ammonium acetate (227 mg, 2.95 mmol) and 65 °C heating for 3 h. Purification by RP-HPLC, eluent acetonitrile:water:formic acid (15:85:0.05 to 40:60:0.05), gave **7m** (12 mg) in 17% yield as a pale yellow solid. ^{1}H NMR (CD_3OD:$CDCl_3$, 1:1) δ 8.35 (m, 2H), 7.91 (m 1H), 7.77 (dm, *J* = 8.5 Hz, 1H), 7.71 (dm, *J* = 8.9 Hz, 1H), 7.51 (m, 2H), 7.48 (dm, *J* = 8.5 HZ, 1H), 7.19 (m, 1H), 7.16 (dm, *J* = 8.9 Hz, 1H), 6.91 (dd, *J* = 2.6, 1.4Hz, 1H), 6.81 (dd, *J* = 3.6, 1.4 Hz, 1H), 6.24 (dd, *J* = 3.6, 2.6 Hz, 1H), 3.93 (s, 3H); ^{13}C NMR (CD_3OD:$CDCl_3$, 1:1) δ 158.9, 149.1, 143.4, 142.9, 134.9, 130.0, 129.5, 127.9, 127.2, 126.8, 122.3, 122.1, 120.8, 119.9, 109.9, 108.7, 106.3, 55.7, (3 carbon signals were not detected). ESI-MS (*m/z)* 367 (M + H^+). HRMS (M + H^+): 367.1550, $C_{23}H_{18}N_4O$ requires 367.1559.

Ethyl 6-methoxy-2-naphthoate (8).[3]



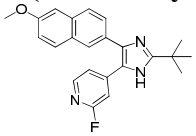
To a microwave vial (10-20 mL) equipped with a teflon coated stirring bar were added 2-bromo-6-methoxynaphthalene (1.19 g, 5.00 mmol), $Pd(OAc)_2$ (28 mg, 0.125 mmol), xantphos (144 mg, 0.25 mmol), diaza(1,3)bicyclo[5.4.0]undecane (2.24 g, 15 mmol), $Mo(CO)_6$ (1.32 g, 5.0 mmol) and EtOH (10 mL). The vial was then sealed under air and heated at 120 °C by microwave irradiation for 30 min using a fixed hold time. After cooling, the mixture was diluted with EtOAc (20 mL) and brine (20 mL) and the two layers separated. The aqueous layer was washed twice with EtOAc (20 mL) and the combined organic phases were concentrated *in vacuo*. The crude product was thereafter purified by silica column flash chromatography eluting with iso-hexane:EtOAc (10:1). Yield: 93%, 1.07 g as white solid. ^{1}H-NMR ($CDCl_3$) δ 8.52 (d, J = 1.9 Hz, 1H), 8.04 (dd, J = 8.6, 1.9 Hz, 1H), 7.83 (d, J = 8.6 Hz, 1H), 7.74 (d, J = 8.9 Hz, 1H), 7.17 (dd, J = 8.9, 2.5 Hz, 1H), 7.13 (d, J = 2.5 Hz, 1H), 4.36 (q, J = 7.2 Hz, 2H), 3.92 (s, 3H), 1.43 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 167.1, 159.7, 137.3, 131.1, 130.9, 128.1, 127.0, 126.2, 125.8, 119.8, 105.9, 61.1, 55.6, 14.6.

2-(2-Fluoropyridin-4-yl)-1-(6-methoxynaphthalen-2-yl)ethanone (9).



To a chilled (0 °C) solution of 2-fluoro-4-methylpyridine (55 mg, 0.50 mmol) in dry THF (2 mL) was added dropwise NaHMDS (1 M in THF, 1.1 mL, 1.1 mmol). After 15 min a solution of **5** (115 mg, 0.5 mmol) in THF (2 mL) was added and the reaction mixture stirred at 0 °C for 2 h. The mixture was then quenched with brine (10 mL) and diluted with EtOAc (20 mL). The aqueous phase was separated, extracted twice with EtOAc (20 mL) and the combined organic phases were concentrated *in vacuo*. The crude product was thereafter purified by silica column flash chromatography eluting with iso-hexane:EtOAc (2.5:1). Yield: 64%, 94 mg as white solid 64%. ^{1}H NMR ($CDCl_3$) δ 8.44 (d, J = 2.0 Hz, 1H), 8.19 (d, J = 5.3 Hz, 1H), 8.01 (dd, J = 8.7, 2.0 Hz, 1H), 7.87 (d, J = 8.9 Hz, 1H), 7.80 (d, J = 8.7 Hz, 1H), 7.23 (dd, J = 8.9, 2.6 Hz, 1H), 7.16 (d, J = 2.6 Hz, 1H), 7.14 (ddm J = 5.3, 1.5 Hz, 1H), 6.90 (br s, 1H), 4.43 (s, 2H), 3.96 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 195.0, 164.6 (d, J_{CF} = 240.3 Hz), 160.4, 149.6 (d, J_{CF} = 7.6 Hz), 147.8 (d, J_{CF} = 15.5 Hz), 137.8, 131.6, 131.5, 130.4, 128.0, 127.8, 124.9, 122.9 (d, J_{CF} = 3.5 Hz), 120.3, 110.8 (d, J_{CF} = 38.0 Hz), 106.1, 55.7, 44.3. ESI-MS (*m/z)* 296 ($M + H^+$). HRMS ($M + H^+$): 296.1092, $C_{18}H_{14}FNO_2$ requires 296.1087.

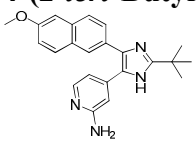
4-(2-*tert*-Butyl-4-(6-methoxynaphthalen-2-yl)-1*H*-imidazol-5-yl)-2-fluoropyridine (10).



To a solution of **9** (370 mg, 1.25 mmol) in DMSO (16 mL) was added HBr (48 % in H_2O, 1.04 mL) and the reaction mixture stirred at 70 °C for 2 h. After cooling, the mixture was made basic with saturated $NaHCO_3$ (20 mL) and EtOAc was added (50 mL). The aqueous phase was separated, washed twice with EtOAc (50 mL) and the combined organic phases were concentrated *in vacuo*. The residue was taken up in *n*-BuOH (20 mL) and pivalaldehyde (322 mg, 3.75 mmol), ammonium acetate (1.44 g, 18.75 mmol) were added and the mixture heated at 50 °C for 2 h. After cooling, the mixture was diluted with EtOAc and saturated $NaHCO_3$ (20 mL each) and the two layers separated. The aqueous layer was washed twice with EtOAc (20 mL) and the combined organic phases were concentrated *in vacuo*. The crude product was thereafter purified by silica column flash chromatography eluting with iso-hexane:EtOAc (2.5:1). Yield: 71%, 330 mg as white solid. ^{1}H NMR ($CDCl_3$) δ 7.88 (d, J =

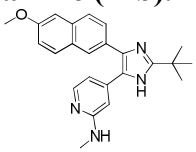
5.2 Hz, 1H), 7.78 (br s, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.67 (d, J = 9.0 Hz, 1H), 7.39 (dm, J = 8.4 Hz, 1H), 7.29 (br s, 1H), 7.21 (br s, 1H), 7.18 (d, J = 2.5 Hz, 1H), 7.14 (dd, J = 7.2, 2.5 Hz, 1H), 3.94 (s, 3H) 1.98 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.0, 164.3 (d, J_{CF} = 237.6 Hz), 158.6, 156.8 (br). 148.8 (br), 147.1 (d, J_{CF} = 15.9 Hz), 134.6, 132.4 (br), 129.7, 129.0, 127.8, 127.5, 127.0. 125.8 (br), 119.9, 119.2 (d, J_{CF} = 3.8 Hz), 106.5 (d, J_{CF} = 37.6 Hz), 106.0, 55.6, 33.2, 29.8. ESI-MS (*m/z)* 376 (M + H^+). HRMS (M + H^+): 376.1825, $C_{23}H_{22}FN_3O$ requires 376.1819.

4-(2-*tert*-Butyl-4-(6-methoxynaphthalen-2-yl)-1*H*-imidazol-5-yl)pyridin-2-amine (11a).



To a microwave vial (2-5 mL) equipped with a teflon coated stirring bar was added **10** (50 mg, 0.13 mmol), diphenylmethanamine (1.5 mL) and dioxane (1.5 mL). The vial was then sealed under air and heated at 200 °C by microwave irradiation for 10 h using a fixed hold time. After cooling, the mixture was filtered through a plug of silica, eluted with EtOAc:iso-hexane (1:1) and the filtrate concentrated *in vacuo*. The crude mixture was then taken up in MeOH (2.0 mL) and transferred to a microwave transparent vial (2-5 mL) containing a teflon coated stirring bar and Pd/C (10%, 5 mg) and ammonium acetate (100 mg, 1.3 mmol) were added. The vial was then sealed under air and heated at 120 °C by microwave irradiation for 2 h using a fixed hold time. After cooling, Pd/C (10%, 10 mg) and ammonium acetate (200 mg, 2.6 mmol) were added and the mixture heated by microwave irradiation for a further 20 min at 140 °C. After cooling, the mixture was diluted with EtOAc and saturated $NaHCO_3$ (10 mL each) and the two layers separated. The aqueous layer was washed twice with EtOAc (10 mL) and the combined organic phases were concentrated *in vacuo*. The crude product was thereafter purified by silica column flash chromatography eluting with EtOAc:methanol:triethylamine (1:0.05:0.01). Yield: 28%, 14 mg as pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 7.81 (d, J = 1.9 Hz, 1H), 7.72 (d, J = 8.6 Hz, 1H), 7.69 (m, 1H), 7.51 (dm, J = 6.1 Hz, 1H), 7.40 (dd, J = 8.6, 1.9 Hz, 1H), 7.16 (m, 1H), 7.14 (m, 1H), 6.91 (br s, 1H), 6.65 (dm, J = 6.1 Hz, 1H) 3.91 (s, 3H), 1.43, (s, 9H); ^{13}C NMR ($CDCl_3$:CD_3OD, 5:1) δ 170.3, 158.6, 157.4, 156.9, 146.6, 140.5, 134.5, 129.7, 128.9, 127.8, 127.6, 127.2, 126.4, 119.8, 111.7, 107.5, 106.0, 55.6, 33.2, 29.6, (1 carbon signal was not detected). ESI-MS (*m/z)* 373 (M + H^+). HRMS (M + H^+): 373.2037, $C_{23}H_{24}N_4O$ requires 373.2028.

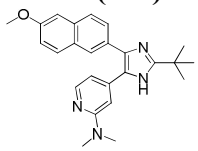
4-(2-*tert*-Butyl-4-(6-methoxynaphthalen-2-yl)-1*H*-imidazol-5-yl)-N-methylpyridin-2-amine (11b).



To a microwave vial (2-5 mL) equipped with a teflon coated stirring bar was added **10** (44 mg, 0.117 mmol) and 2.5 mL of methylamine (2.0 M solution in THF). The vial was capped and irradiated with microwaves to 150 °C for 17 h. Purification by RP-HPLC, eluent acetonitrile:water:formic acid (10:90:0.05 to 50:50:0.05), gave **11b** (8 mg) in 18% yield as a pale yellow solid. ^{1}H NMR (CD_3OD) δ 7.90 (s, 1H), 7.85 (m, 1H), 7.78 (d, J = 8.9 Hz, 1H), 7.64 (m, 1H), 7.46 (m, 1H), 7.29 (d, J = 2.1 Hz, 1H), 7.18 (dd, J = 8.9, 2.1 Hz, 1H), 6.97 (m, 1H), 6.81 (m, 1H), 3.93 (s, 3H), 2.84 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (CD_3OD) δ 158.9, 157.9, 155.7, 147.1, 138.0, 134.9, 134.1, 130.0, 129.5, 129.1, 128.1, 127.4, 127.0, 126.0,

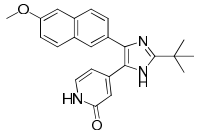
119.4, 110.9, 106.8, 105.6, 54.7, 33.0, 28.6, 27.5. MS (*m/z)* 387 (M + H^+). HRMS (M + H^+): 387.2180, $C_{24}H_{26}N_4O$ requires 387.2185.

4-(2-*tert*-Butyl-4-(6-methoxynaphthalen-2-yl)-1*H*-imidazol-5-yl)-*N*,*N*-dimethylpyridin-2-amine (11c).



To a microwave vial (2-5 mL) equipped with a teflon coated stirring bar was added **10** (20 mg, 0.053 mmol), ammonia (25% in H_2O, 0.5 mL) and DMF (2.0 mL). The vial was then sealed under air and heated at 150 °C by microwave irradiation for 12 h using a fixed hold time. After cooling, the mixture was diluted with EtOAc and saturated $NaHCO_3$ (10 mL each) and the two layers separated. The aqueous layer was washed twice with EtOAc (10 mL) and the combined organic phases were concentrated *in vacuo*. The crude product was thereafter purified by silica column flash chromatography eluting with EtOAc:iso-hexane:triethylamine (1:1:0.05). Yield: 85%, 18 mg as pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 8.01 (m, 1H), 7.90 (m, 1H), 7.70-7.65 (m, 2H), 7.52 (m, 1H), 7.16-7.14 (m, 2H), 6.78 (m, 1H), 6.68 (m, 1H), 3.91 (s, 3H), 2.97 (s, 6H), 1.48, (s, 9H); ^{13}C NMR ($CDCl_3$) δ 159.4, 158.2, 156.4, 147.2, 134.2, 129.7, 129.1, 127.3, 127.2, 127.0, 119.4, 110.5, 105.9, 104.2, 55.6, 38.5, 33.2, 29.9, (4 carbon signals were not detected). ESI-MS (*m/z)* 401 (M + H^+). HRMS (M + H^+): 401.2331, $C_{25}H_{28}N_4O$ requires 401.2341.

4-(2-*tert*-Butyl-4-(6-methoxynaphthalen-2-yl)-1*H*-imidazol-5-yl)pyridin-2(5*H*)-one (11d).



To a microwave vial (2-5 mL) equipped with a teflon coated stirring bar was added 10 (21mg, 0.056 mmol), AcOH (2 mL) and water (0.2 mL). The vial was capped and irradiated with microwaves to 190 °C for 2 h. The solvent was removed to give pure product **11d** (19 mg) in 91% yield as a white solid. ^{1}H NMR (CD_3OD) δ 7.87 (br s, 1H). 7.81 (d, *J* = 8.5 Hz, 1H). 7.76 (d, *J* = 9.0 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.26 (d, *J* = 2.4 Hz, 2H), 7.16 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.67 (m, 1H), 6.47 (m, 1H), 3.92 (s, 3H), 1.46 (s, 9H); ^{13}C NMR (CD_3OD) δ 158.7, 157.6, 147.7, 134.8, 134.0, 129.5, 129.1, 128.1, 127.3, 127.1, 126.4, 119.3, 115.4, 107.3, 105.6, 54.7, 33.0, 28.6, (3 carbon signals were not detected). ESI-MS (*m/z)* 374 (M + H^+). HRMS (M + H^+): 374.1867, $C_{23}H_{23}N_3O_2$ requires 374.1869.

Protein expression, purification and activity/inhibition studies

Escherichia coli strain GJ4745, lacking adenylyltransferase, was the kind gift of Dr. J. Gowrishankar (Centre for Cellular and Molecular Biology, Hyderabad, India). Protein was over-expressed and purified in this strain, using the plasmid and methods described previously,[4] to provide unadenylylated *Mt*GS. Compounds were dissolved in DMSO to prepare stock solutions (1 and 10 mM) that were stored frozen at 4 °C. Inhibition screening assays and IC_{50} determination were performed essentially as described earlier.[5] A typical 100 µL reaction contained 50 mM HEPES-HCl (pH 7.5), 25 mM $MgCl_2$, 1 mM ATP, 30 mM NH_4Cl, 30 mM L-glutamate, and 7 nM of *Mt*GS (subunit concentration). For inhibition screening and IC_{50} determinations, DMSO concentrations were 2.5% (v/v) and 2% (v/v), respectively. Compound concentration during inhibition screening was 25 µM; for IC_{50} determination, compound was prepared in an appropriate concentration range (generally comprised of 9 points) by 2-fold serial dilutions in DMSO. The incubation was carried out for 1 h at room temperature, after which released inorganic phosphate was detected using a P_iColorlock Gold reagent kit purchased from Innova Biosciences, UK. IC_{50} values were determined by nonlinear regression of $Y=Lo+[(Hi-Lo)/(1+X/IC_{50})]$ as described earlier.[5] The reported IC_{50} values are an average from three separate experiments, and reported together with the standard deviation.

Structural work

L-Methionine-*S*-sulfoximine phosphate MSO-P synthesis was described in Nilsson et al. 2009.[6] Crystals of the *Mt*GS/MSO-P/MgP_i/**3** complex were obtained under conditions similar to those used for the *Mt*GS/MSO-P/MgADP structure solved earlier.[4] Equal volumes of reservoir [0.1 M MES, pH 6.8, 0.2 M $MgCl_2$, 35% (v/v) PEG 400] and *Mt*GS protein [13 mg/mL in 0.1 M Tris–HCl, pH 7.5, 125 mM NaCl, 4 mM $MgCl_2$, 4% (v/v) DMSO, 4 mM MSO-P, 1 mM compound **3**] solutions were equilibrated by hanging-drop vapour diffusion for 1 day at 20 °C prior to streak-seeding; crystals appeared within 1 day after seeding. Crystallization conditions for the complex with compound **11a** were the same, except that the PEG400 concentration was 45%, and drops were not streak-seeded. For X-ray data collection, crystals were directly flash-cooled in liquid nitrogen. Data sets extending to 2.15 (compound **3**) and 2.26 (compound **11a**) Å resolution were collected at the European Synchrotron Radiation Facility (ESRF), Grenoble. Diffraction data were merged and scaled using XDS and XSCALE.[7] The *Mt*GS hexamer from PDB[8] entry 2BVC[4], with water molecules and ligands removed, was used as the starting point for the structure with compound **3**; rigid-body refinement was followed by rounds of refinement with non-crystallographic symmetry restraints. A similar procedure for the complex with **11a** utilized the complex with **3** as the starting model. Models were rebuilt using O[9] and Coot,[10] and refinement was carried out with REFMAC5.[11] Water molecules and other ligands were added in averaged $|F_o|-|F_c|$ maps calculated in O. Coordinates and structure libraries for the ligands **3** and **11a** were created in Prodrg.[12] Due to crystal contacts, there is a breakdown in the NCS at a helical section in the E chain between residues 403-415 in both structures. This section was therefore omitted from the non-crystallographic symmetry restraints during the last cycles of refinement. The final structures were validated using O, MOLEMAN2[13] and Molprobity.[14] Statistics for data collection and refinement are summarized in Table 1. Averaged omit map electron density ($|F_o|-|F_c|$) and averaged electron density ($2|F_o|-|F_c|$) in the final map for **3** and **11a** is shown in Figure 1S and 2S. Omit map were created by removing the ligand from the final structure then performing multiple rounds of Refmac5 refinements until Rfree stabilizes. Maps were six-fold NCS averaged in Coot using the A-chain of the final structure as reference. Figures were prepared using the program Pymol (http://pymol.sourceforge.net/).

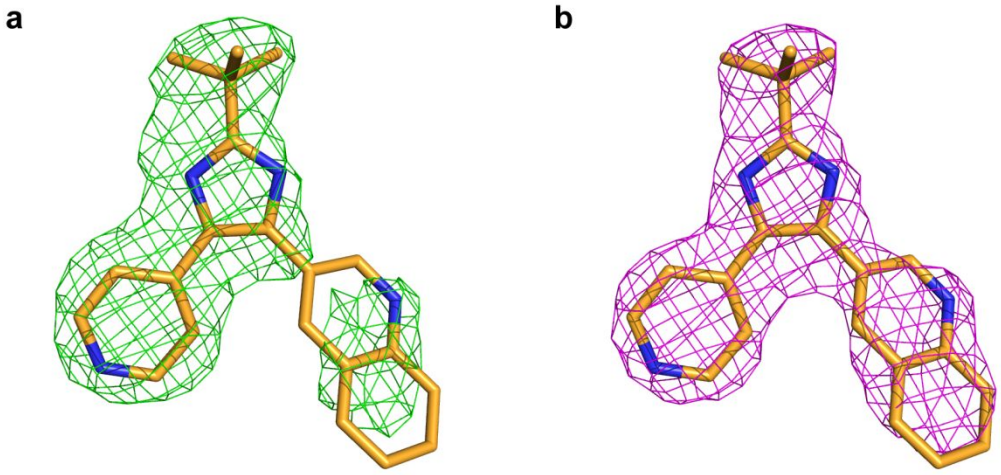


Figure 1. a) Six-fold averaged electron density ($|F_o|-|F_c|$ map) obtained after 40 rounds of refmac5 refinement using the final structure with **3** removed, contoured at 3.5 σ (0.07 e/Å^3). **b)** Six-fold averaged electron density ($2|F_o|-|F_c|$ map) of final structure (containing **3**), contoured at 1 σ (0.18 e/Å^3). NCS-average maps are obtained from Coot using the final structure (3ZXR) with the A molecule as reference together with respective output mtz file from Refmac5.

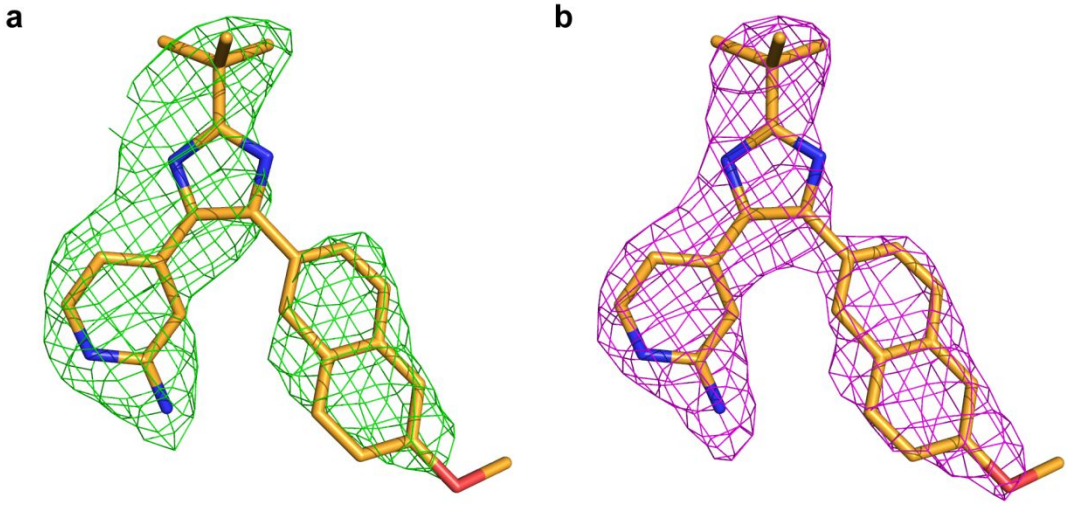


Figure 2. a) Six-fold averaged electron density ($|F_o|-|F_c|$ map) obtained after 20 rounds of refmac5 refinement using the final structure with **11a** removed, contoured at 3.5 σ (0.08 e/Å^3). **b)** Six-fold averaged electron density ($2|F_o|-|F_c|$ map) of final structure (containing **11a**), contoured at 1 σ (0.19 e/Å^3). NCS-average maps are obtained from Coot using the final structure (3ZXV) with the A molecule as reference together with respective output mtz file from Refmac5.

Table 1. Statistics for data collection and refinement.

PDB entry code	3ZXR	3ZXV
Inhibitor	**3**	**11a**
Data collection[a]		
Beamline	ID14:4	ID23:1
Matthews coefficient, Vm / solvent content	2.35 / 47.6 %	2.32 / 47.0 %
Cell axial lengths (Å)	133.260 227.470 200.590	132.59 227.15 201.62
Space group	$C222_1$	$C222_1$
Molecules in asymmetric unit	6	6
Resolution range (Å)	20–2.15 (2.25–2.15)	30–2.26 (2.36–2.26)
No. of reflections measured	575,370 (72,200)	549,995 (57,355)
No. of unique reflections	149,481 (19,416)	140,955 (16,964)
Average multiplicity	3.8 (3.7)	3.9 (3.4)
Completeness (%)	90.9 (93.5)	99.5 (99.0)
R_{meas}[b]	9.0 (33.4)	9.1 (69.8)
$\langle I/\sigma (I) \rangle$	12.4 (4.8)	16.2 (2.3)
Wilson B-factor	33.6	39.0
Refinement		
Resolution range (Å)	19.95–2.15	29.73–2.26
No. of reflections used in working set	142,000	133,842
No. of reflections for Rfree calculation	7480	7,110
R-value, Rfree (%)	22.5, 24.5	19.5, 21.1
No. of non-hydrogen atoms	24,276	23,988
No. of solvent molecules	1,410	1,104
Mean B-factor, protein atoms ($Å^2$)	35.3	33.2
Mean B-factor, compound atoms ($Å^2$)	68.6	66.1
Mean B-factor, MSO-P ($Å^2$)	27.8	26.5
Mean B-factor, phosphate atoms ($Å^2$)	36.4	28.7
Mean B-factor, Mg^{2+} ions ($Å^2$)	29.7	26.0
Mean B-factor, solvent atoms ($Å^2$)	34.4	33.5
Mean B-factor, chloride ions ($Å^2$)	39.7	37.2
Ramachandran plot outliers (%)[c]	1.5	0.9
r.m.s. deviation from ideal bond length (Å)[d]	0.011	0.011
r.m.s. deviation from ideal bond angle (°)[d]	1.22	1.26

[a] Values in parentheses are for the highest resolution shell.
[b] The multiplicity-weighted value, as defined by Diederichs and Karplus.[15]
[c] Calculated using a strict-boundary Ramachandran plot.[16]
[d] Using the parameters of Engh & Huber (1991).[17]

Molecular docking

Library preparation. A focused library of virtual compounds was created based on the final step in the synthesis leading up to compound **7a**, were aldehydes are used as reagents (see Scheme 1 in the main text). Aldehydes found in-house and in the ACD database were used.
The library, consisting of synthetically feasible compounds similar to **7a**, was created using the software Legion.[18] LigPrep[19] was used to prepare the compounds before docking; creating 3D conformations, tautomers and stereoisomers. The Epik module was used to generate different ionization states of the ligands.
Docking. Chain A and B of the co-complexed structure of **3** (PDB:3ZXR) was prepared for docking using the protein preparation tool from Schrödinger, as implemented within the Schrödinger 2008 suite (Schrödinger, LLC New York, 2008). All waters were deleted together with the phosphate coordinating with the metal. Hydrogens were added and a +2 formal charge was added to the metal. The H-bonding pattern was optimized and the structure

was minimized within the OPLS2001 force field, allowing a maximum RMSD deviation of 0.30 Å. Compound **3** was identified as the ligand, and the grid box for docking was centered upon it. The grid was calculated with default settings. Glide[20] (Grid-based Ligand Docking from Energetics) docking was performed employing a vdW scaling of 0.80, collecting at most 10 poses per compound. Both the Standard Precision (SP) and Extra Precision (XP) algorithm for docking was used. The suggested setup was validated by successful retrospective docking of **3**. In order to reduce the number of erroneous poses to manually process, a core restraint was used during the docking. The core restraint consisted of all non-hydrogen atoms that were common for all the docked compounds. The top scoring compounds were visually inspected and a number of possible binders were suggested of which compounds **7j-m** were synthesized.

Resazurin based microtiter assay for MIC determination

M. tuberculosis H37Rv ATCC 27294 was grown in roller bottles at 37 °C for 7 to 10 days in Middlebrook 7H9 broth supplemented with 0.2% glycerol, 0.05% Tween 80 and 10% albumin dextrose catalase. The cells were harvested by centrifugation, washed twice in 7H9 broth, resuspended in fresh 7H9 broth. Aliquots of 0.5 mL were dispensed and the seed-lot suspensions were stored at -70 °C. Representative samples were thawed and the number of CFU/mL enumerated for every batch. Antimicrobial susceptibility testing was performed against *M. tuberculosis* in 7H9 broth by resazurin microtitre assay (REMA)[21] with minor modifications.

Briefly, 2 fold serial dilutions of the test compound in DMSO were prepared in a 96-well microtiter plates (Greinier V bottom plates). An aliquot (4 µL) of each of the concentrations was transferred to a new 96 well (catalog no. 900196; Tarsons, Kolkata, India) assay plate in which all the peripheral wells were filled with sterile distilled water. *M. tuberculosis* seed lots were thawed and diluted in Middlebrook 7H9 broth supplemented with 0.2% glycerol, 0.05% Tween 80. 0.1% casitone and 10% albumin dextrose catalase (final CFU ~$3x10^5$/mL) and added into each of the wells in a volume of 200 µl. The plates were packed in gas permeable polythene bags and incubated at 37°C for 5 days. Following incubation, 40 µl of a freshly prepared 1:1 mixture of Resazurin (20 mg/100mL water) and 10% Tween 80 was added to all the wells. The plates were re-incubated for an additional 24 hours at 37 °C, and the ratio of the absorbance measured at 575 nm and 610 nm with Spectramax (Molecular devices) was used to calculate the % inhibition at each concentration. Growth controls with no inhibitor and sterility control with only media (equivalent to 100% inhibition) was always included in the same plate. Minimum Inhibitory Concentration (MIC) was defined as the lowest drug concentration, which prevented a colour change, from blue to pink or in quantitative terms, yields > 80% inhibition. Isoniazid was used as positive control for the assay.

Cytotoxicity evaluation on A549 cells

A549 cells (human lung carcinoma cell line, ATCC CCL 185) in complete RPMI media (Sigma #R7509) were plated at 1000 cells per well of a 384-well culture plate (Corning #3570) containing serially diluted (100µM - 0.195µM, 2-fold serial dilution) **11a**. The media only, cells without the compounds and reference compounds (Amphotericin B and Mitomycin C) were used as controls. The final concentration of DMSO in each wells was 1% in the 50 µl assay volume per well. After incubation for 72 h in a CO_2 incubator, resazurin (Sigma) dye[22] was added to each well to the final concentration of 10 µM followed by incubation for additionally 2 h. The resultant fluorescence was measured using Safire 2 fluorescence reader at $535_{Ex}/590_{Em}$. The background subtracted relative fluorescence units (RFU) of each well

were converted to % inhibition using the RFU of the culture control without compounds as 100% activity. A 10-point dose response curve was generated by plotting the % inhibition (Y-axis) against compound concentration (X-axis) using XLfit™ program for IC_{50} determination (Figure 3).

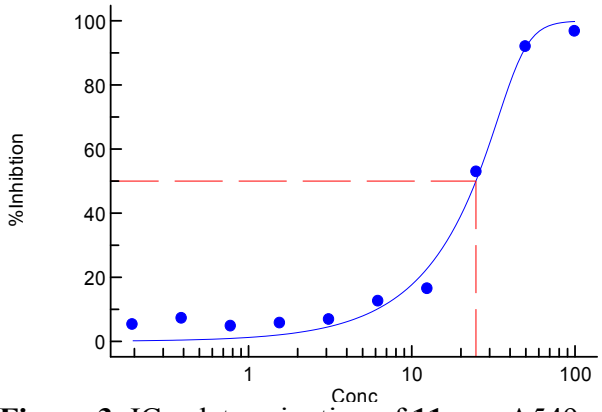


Figure 3. IC_{50} determination of **11a** on A549 cells.

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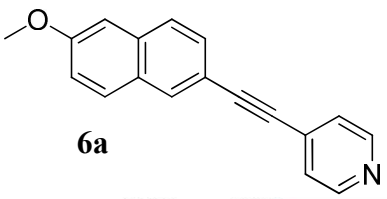

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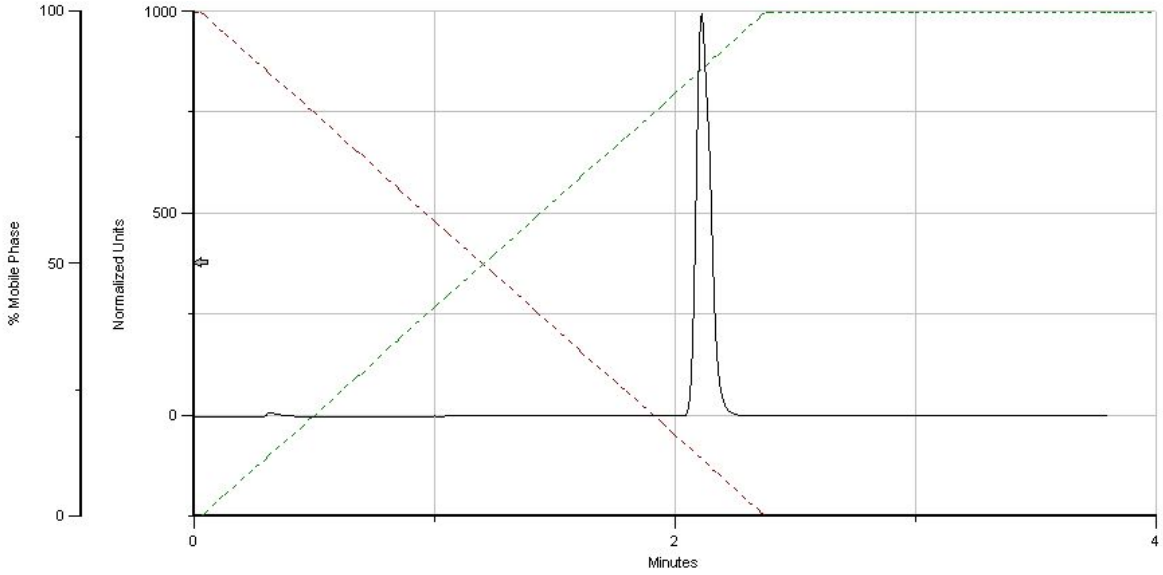
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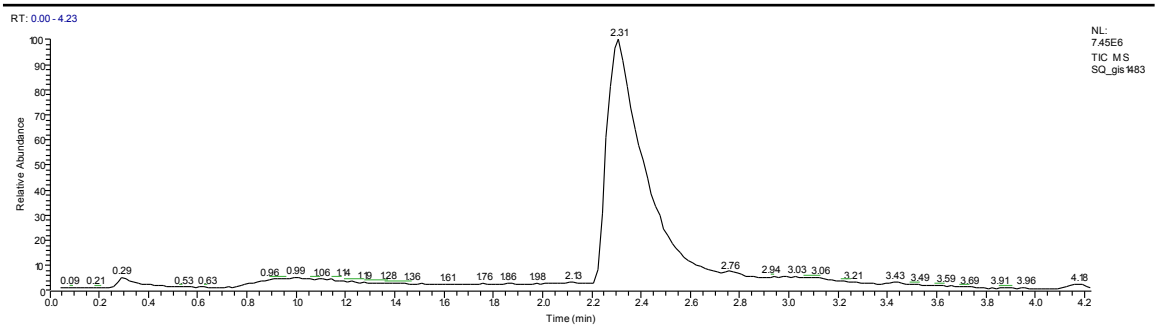
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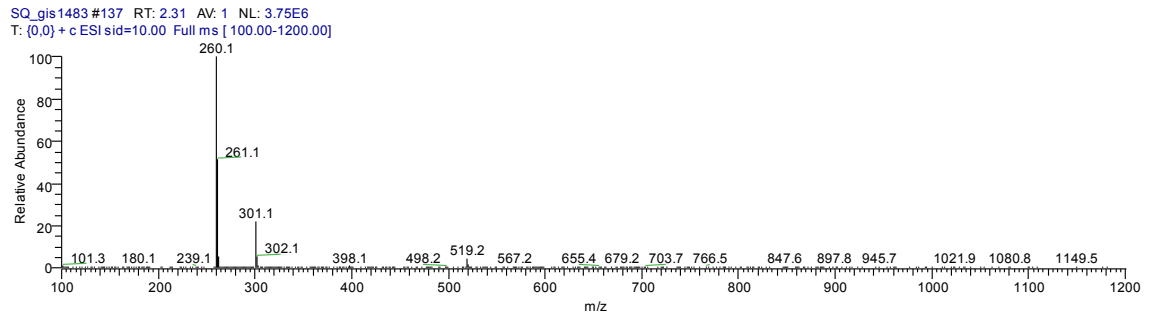
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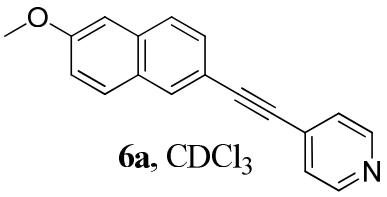
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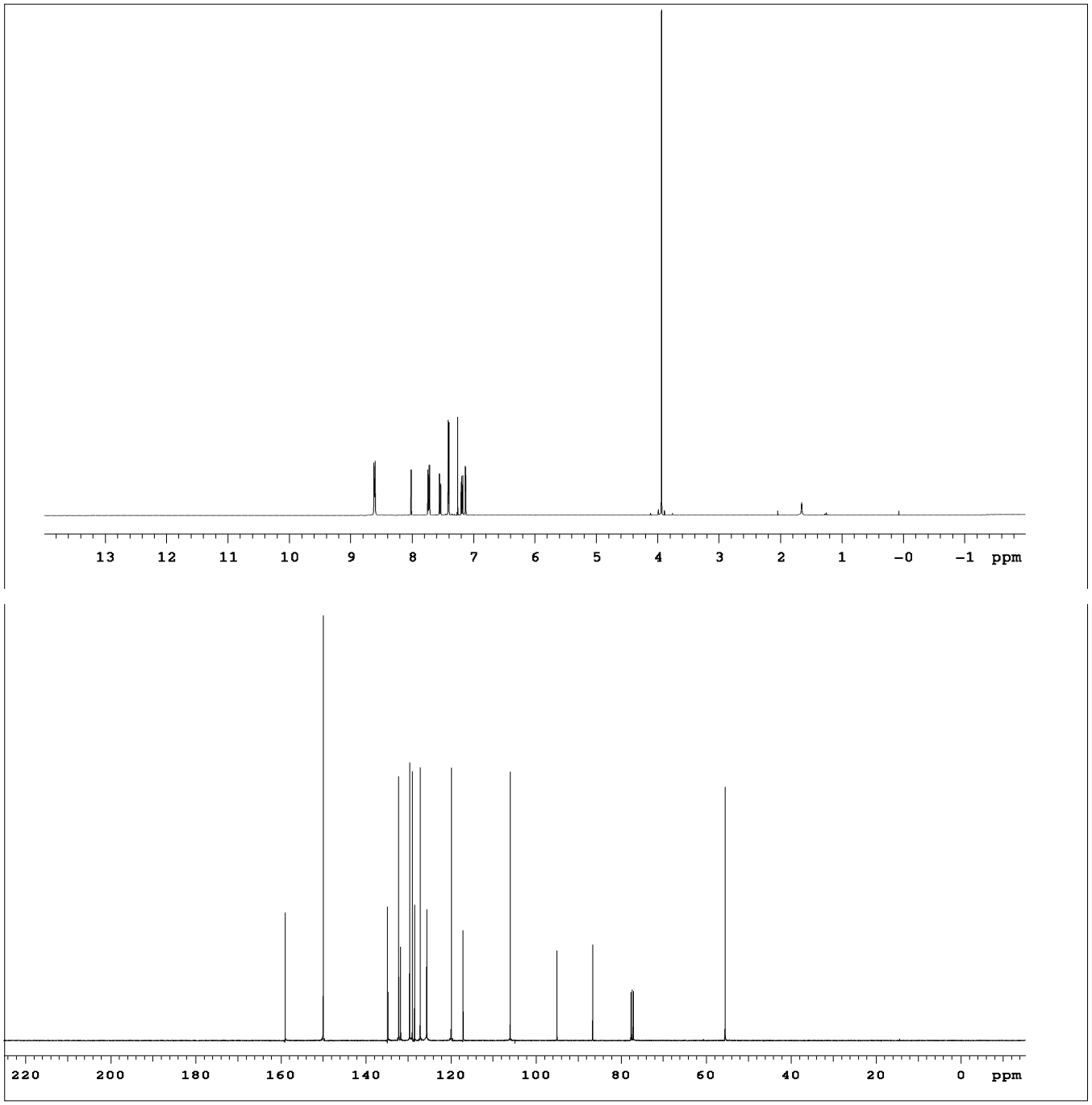
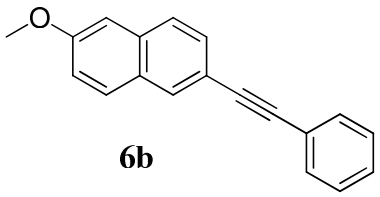
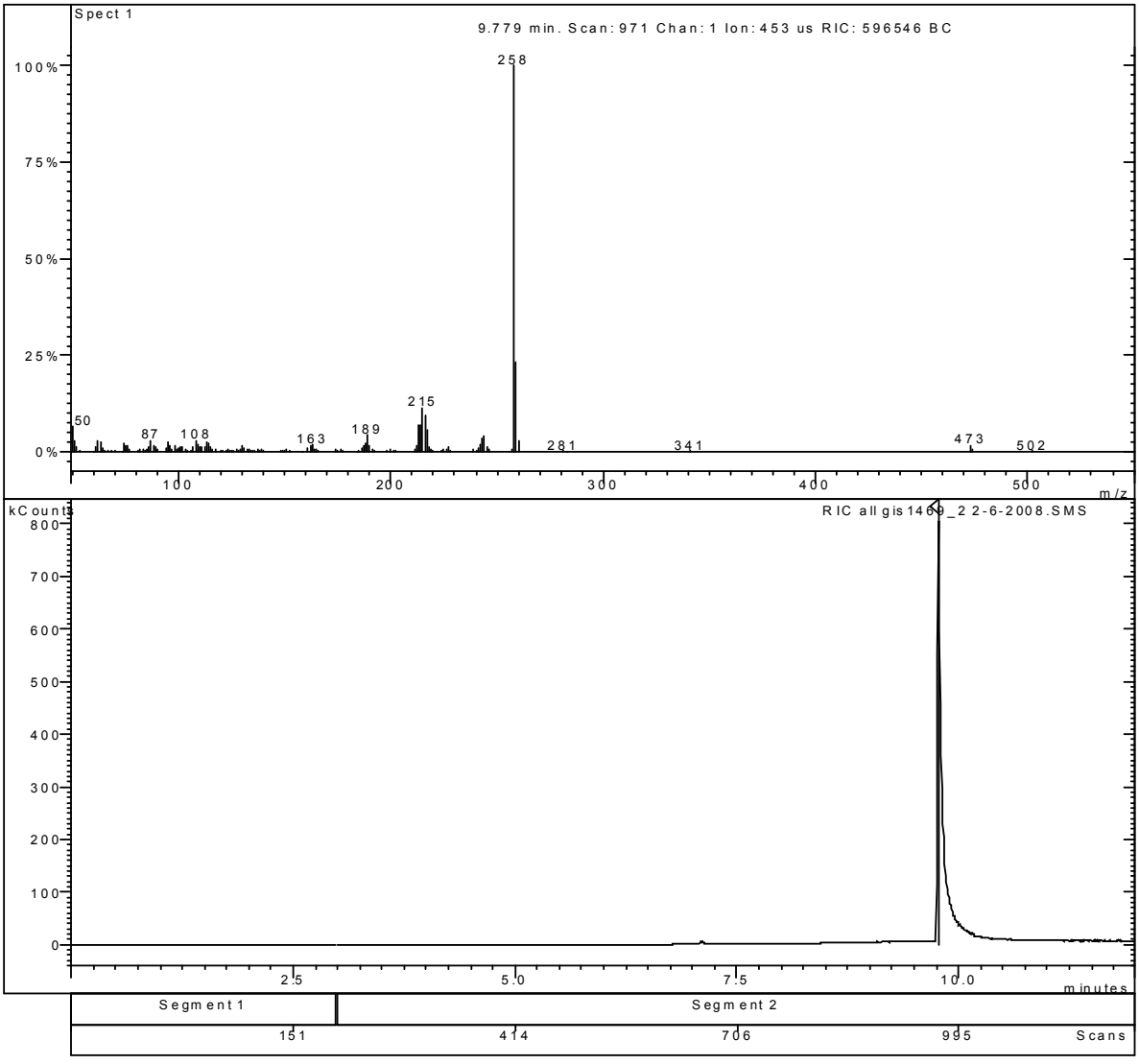
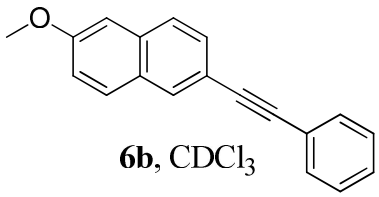

13 12 11 10 9 8 7 6 5 4 3 2 1 −0 −1 ppm
220 200 180 160 140 120 100 80 60 40 20 0 ppm

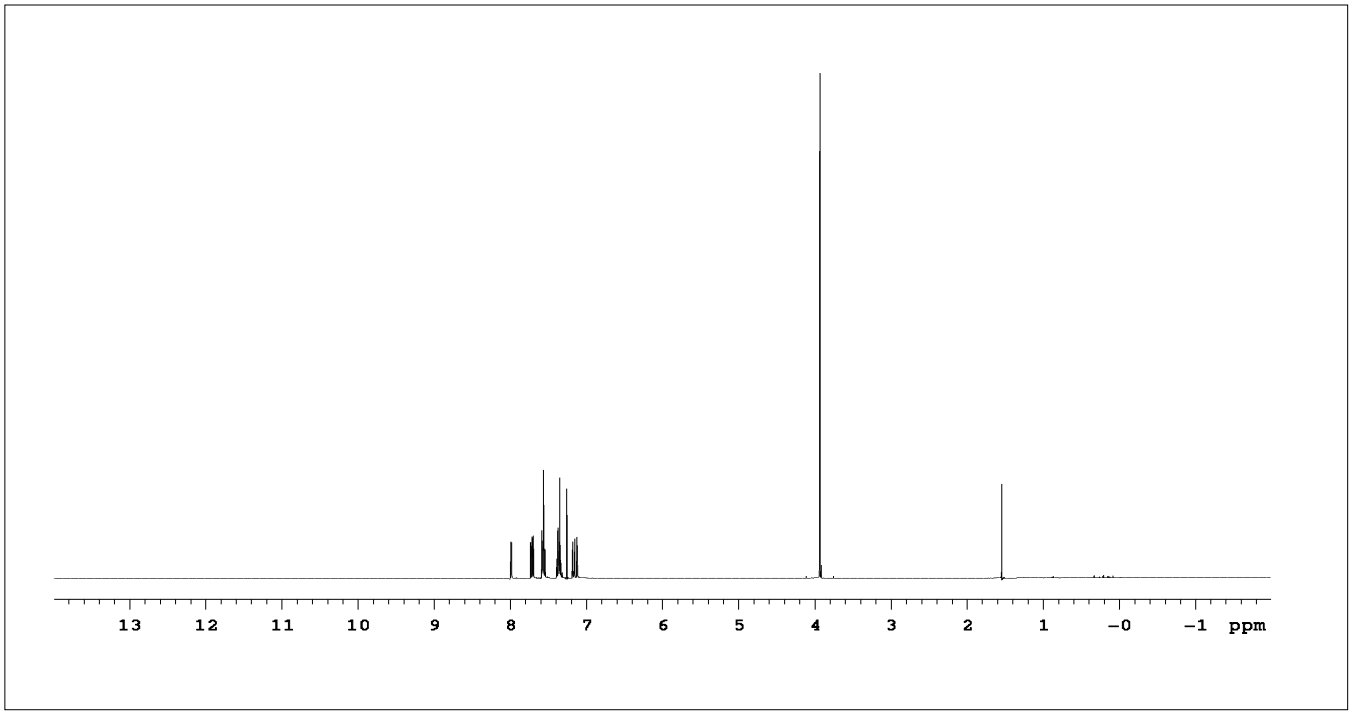


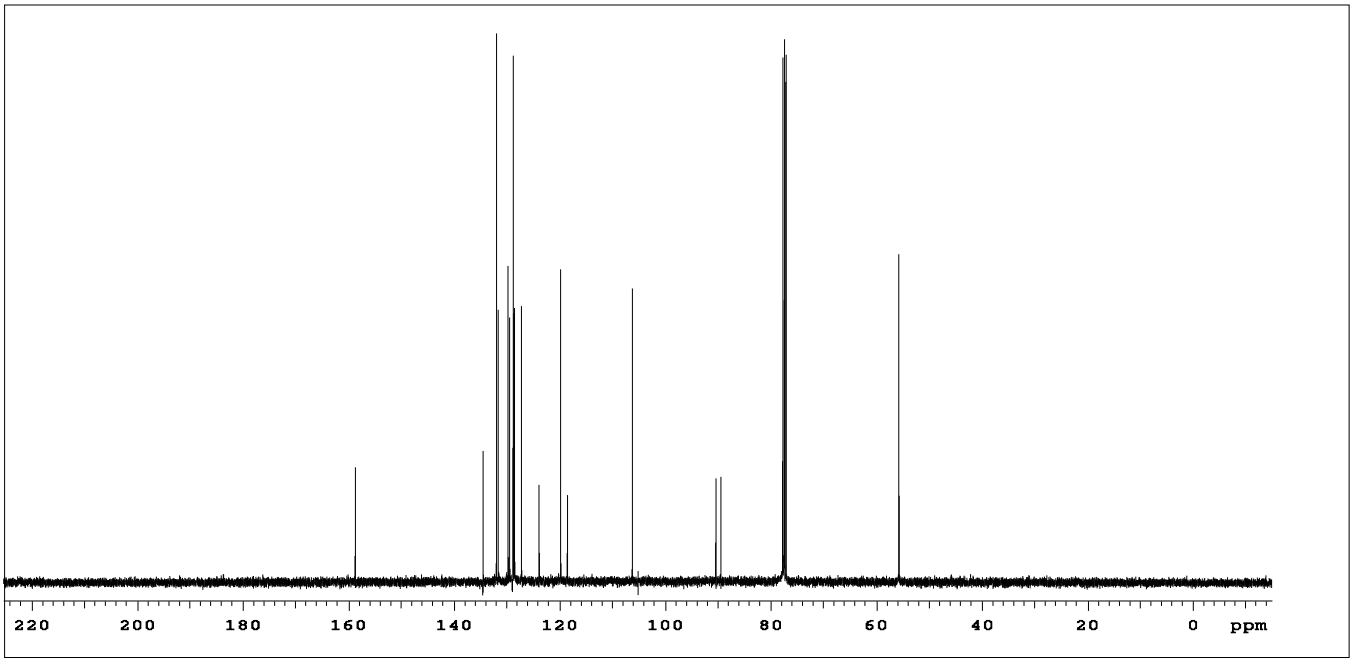
6b

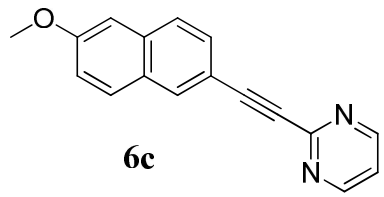

Spect 1
9.779 min. Scan: 971 Chan: 1 Ion: 453 us RIC: 596546 BC
100%
75%
50%
25%
0%
258
215
50
87
108
163
189
281
341
473
502
100
200
300
400
500
m/z
kCounts
800
700
600
500
400
300
200
100
0
RIC allgis1469_22-6-2008.SMS
2.5
5.0
7.5
10.0
minutes
Segment 1
Segment 2
151
414
706
995
Scans



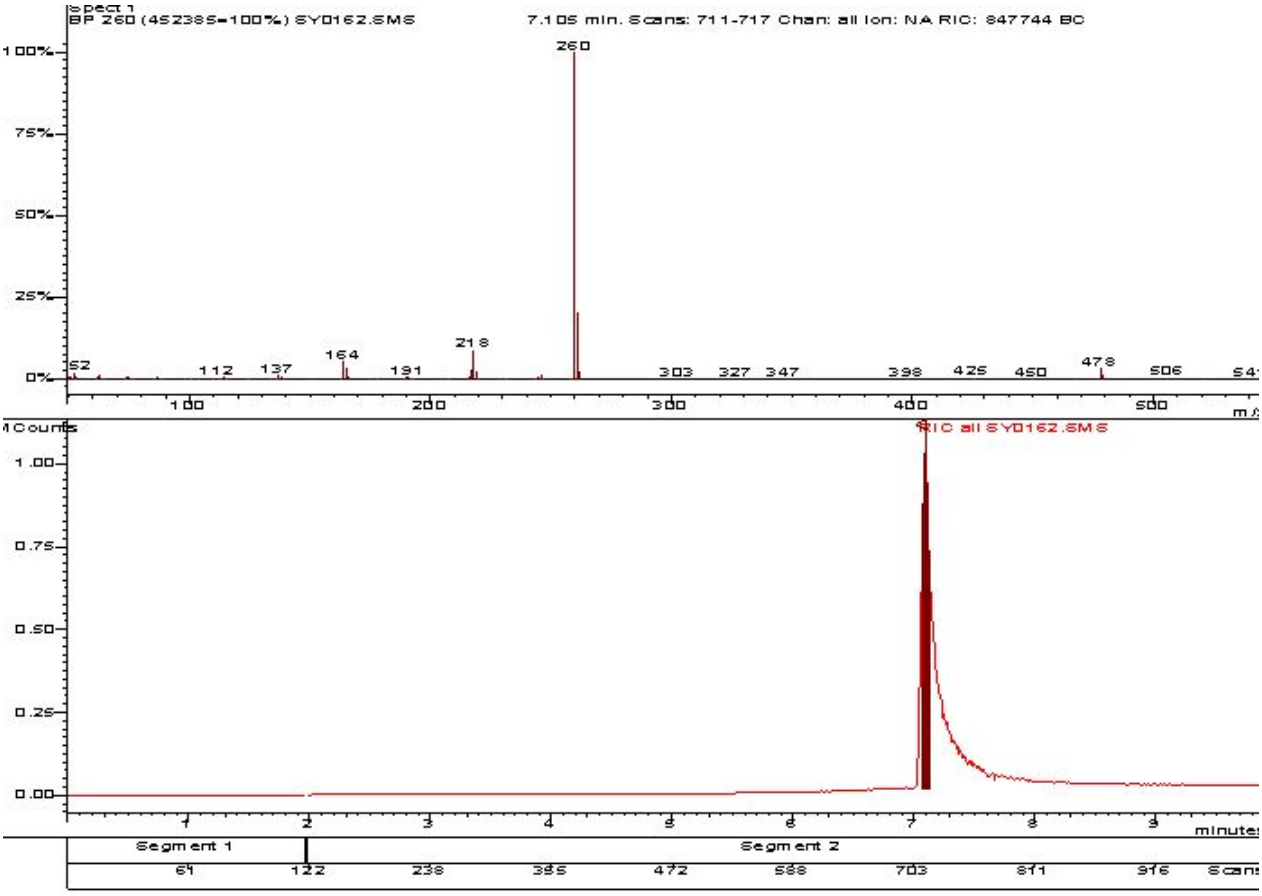
6b, $CDCl_3$

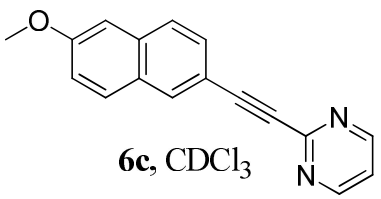

13
12
11
10
9
8
7
6
5
4
3
2
1
−0
−1
ppm


220
200
180
160
140
120
100
80
60
40
20
0
ppm

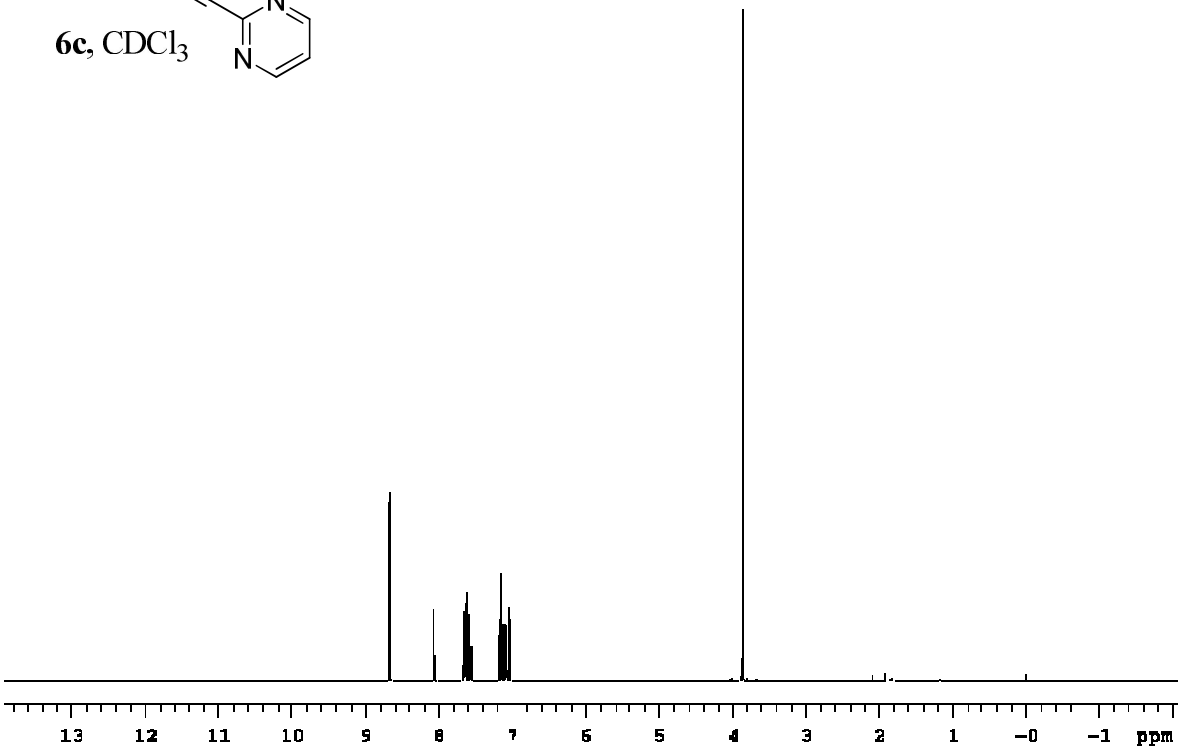

O
N
N

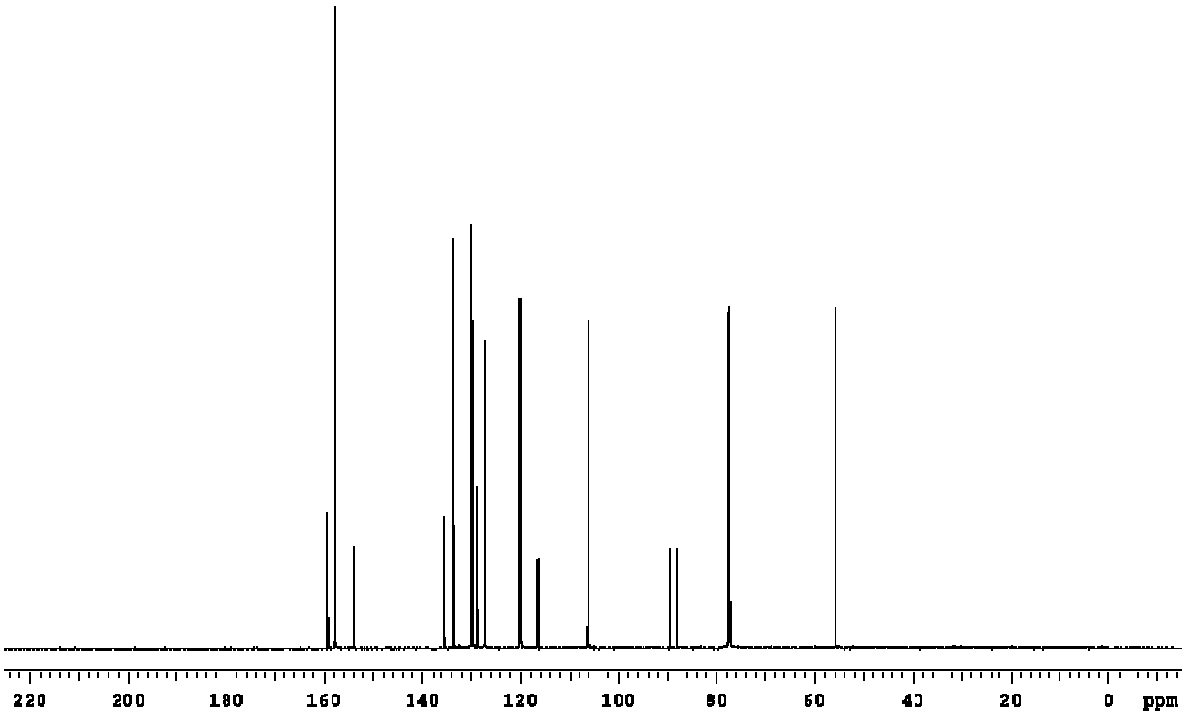
6c

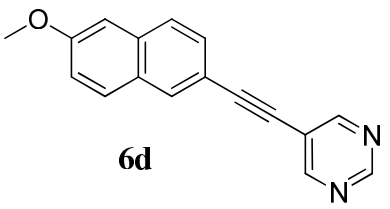

Spect 1
BP 260 (452385=100%) SY0162.SMS
7.105 min. Scans: 711-717 Chan: all Ion: NA RIC: 847744 BC
260
218
164
152
112
137
191
303
327
347
398
425
450
478
506
100%
75%
50%
25%
0%
100
200
300
400
500
m/z
Counts
RIC all SY0162.SMS
1.00
0.75
0.50
0.25
0.00
minutes
Segment 1
Segment 2
64
122
238
385
472
588
703
811
916
Scans


O
N
N

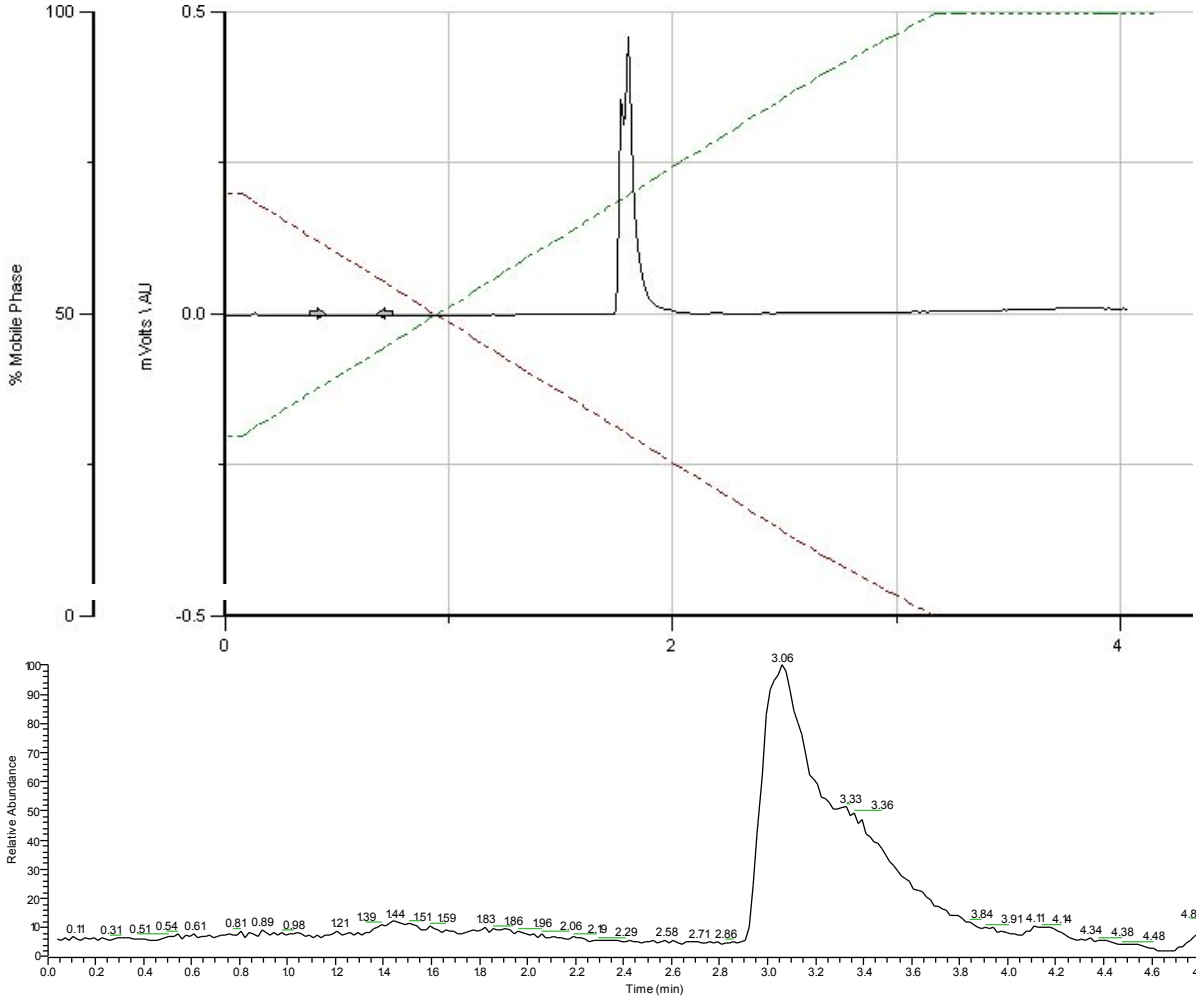
6c, $CDCl_3$


13
12
11
10
9
8
7
6
5
4
3
2
1
−0
−1
ppm

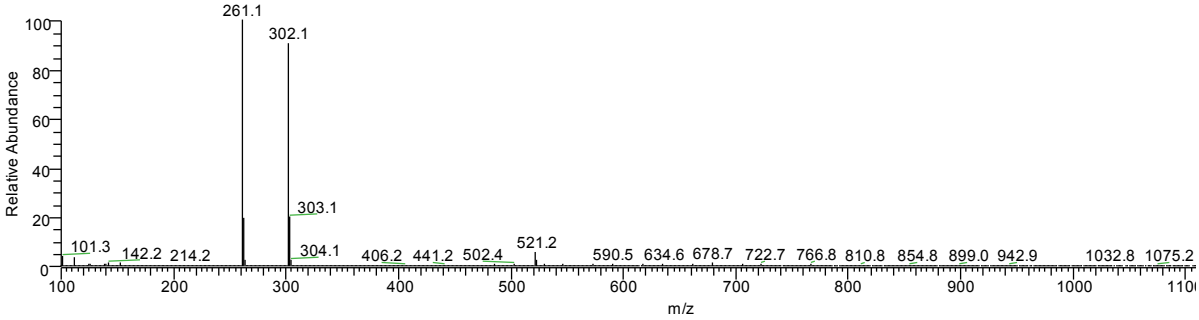

220
200
180
160
140
120
100
80
60
40
20
0
ppm

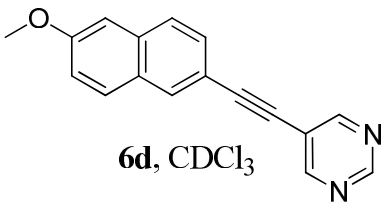


6d



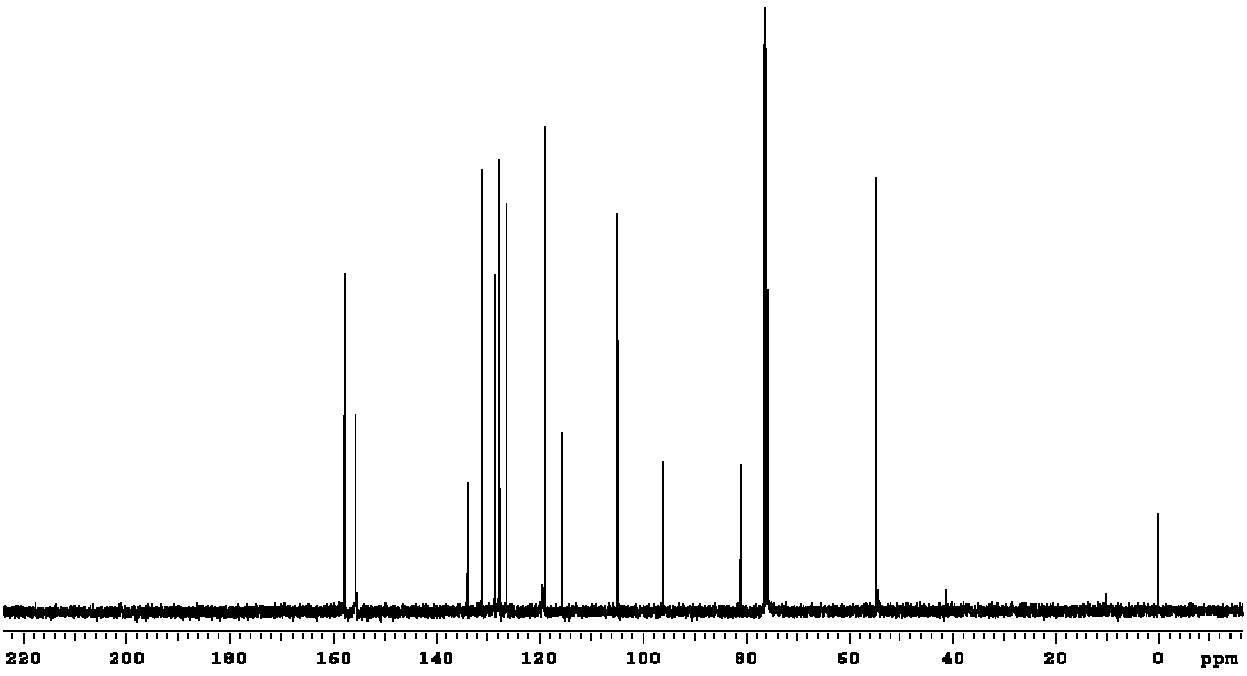
SQ_SY0069_pure_30_90 #178-210 RT: 2.99-3.53 AV: 33 NL: 4.36E5
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]

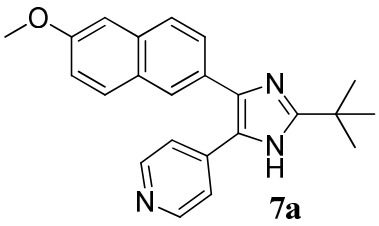



O
N
N

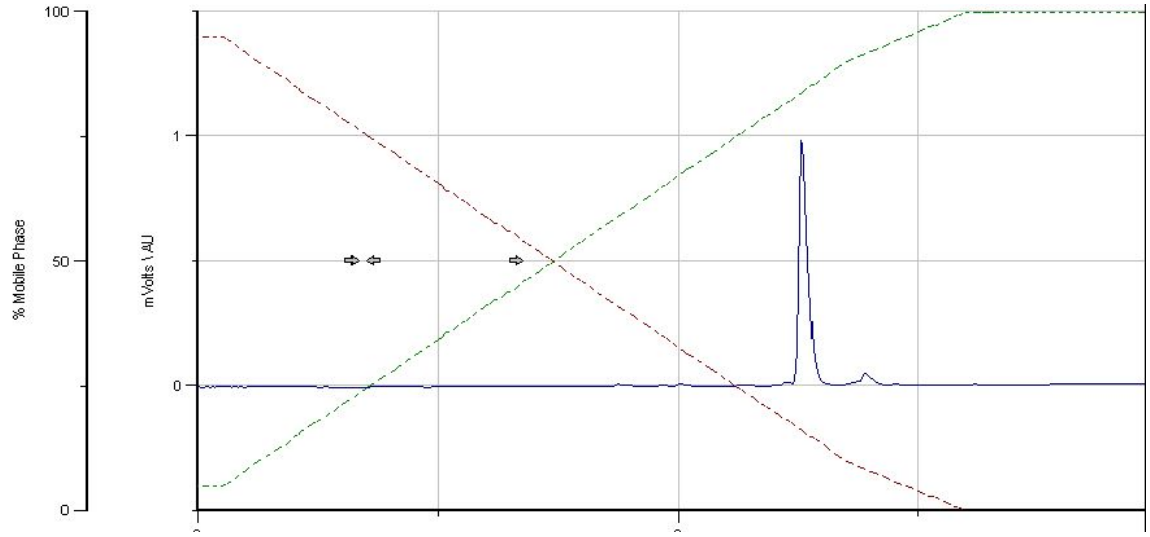
6d, $CDCl_3$

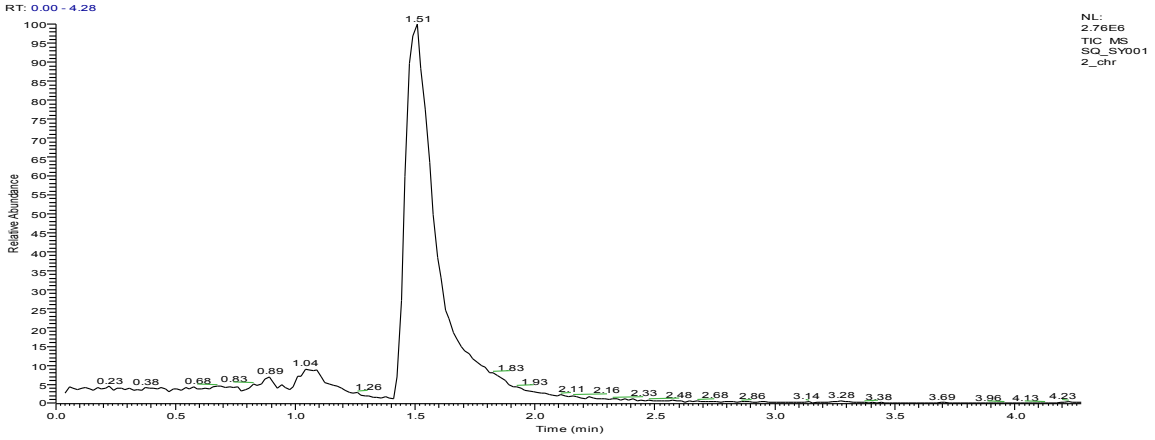
13
12
11
10
9
8
7
6
5
4
3
2
1
-0
-1
ppm

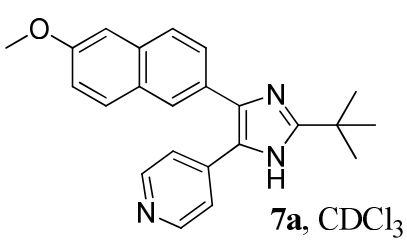

220
200
180
160
140
120
100
80
60
40
20
0
ppm



7a

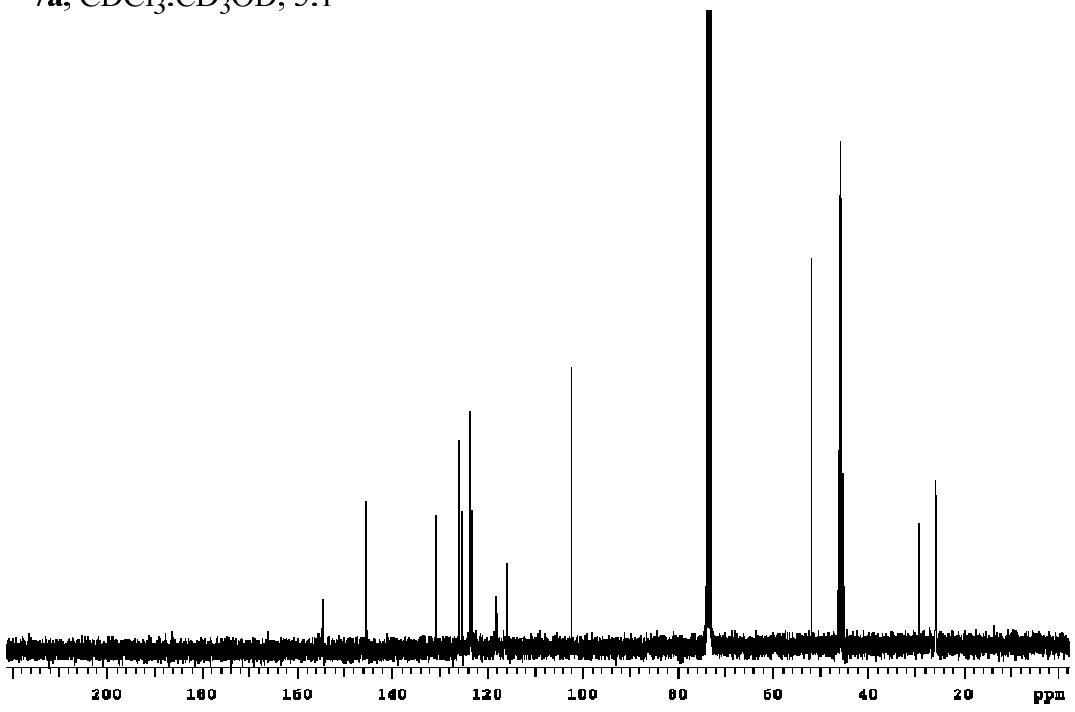

100
50
0
% Mobile Phase
1
0
mVolts \ AU

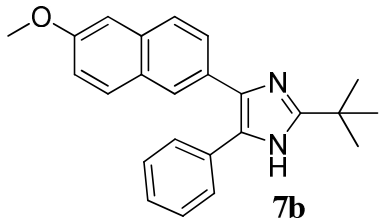

RT: 0.00 - 4.28
NL:
2.76E6
TIC MS
SQ_SY001
2_chr
Relative Abundance
Time (min)
1.51
0.23
0.38
0.68
0.83
0.89
1.04
1.26
1.83
1.93
2.11
2.16
2.33
2.48
2.68
2.86
3.14
3.28
3.38
3.69
3.96
4.13
4.23



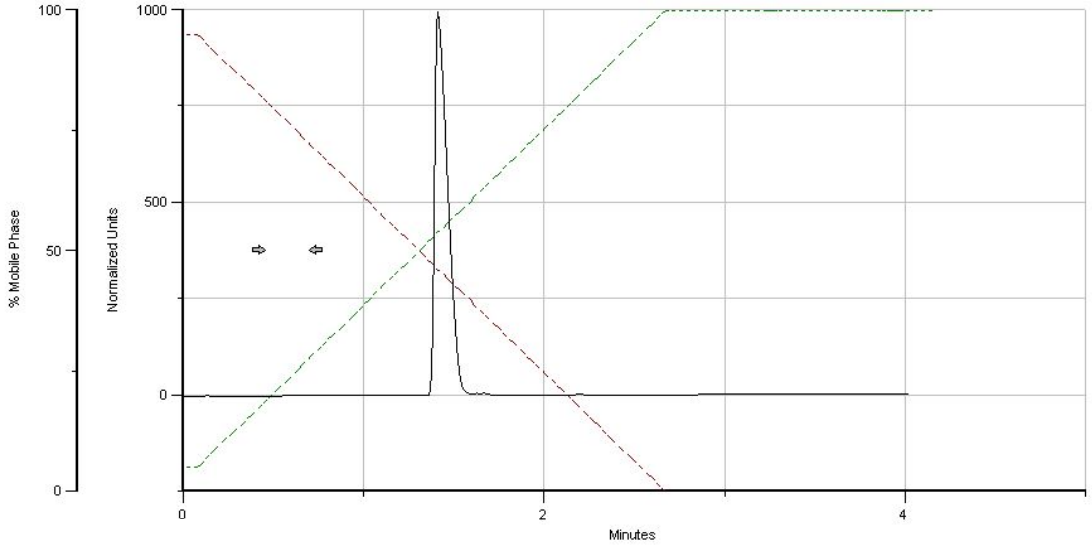
7a, $CDCl_3$

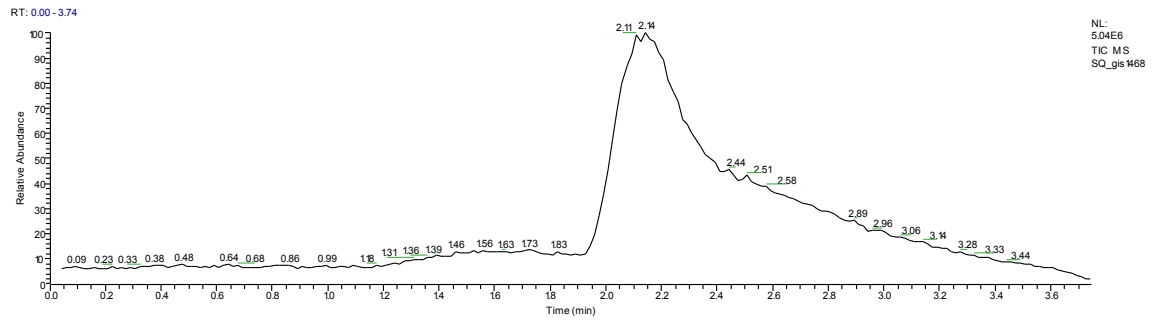
7a, $CDCl_3$:CD_3OD, 5:1

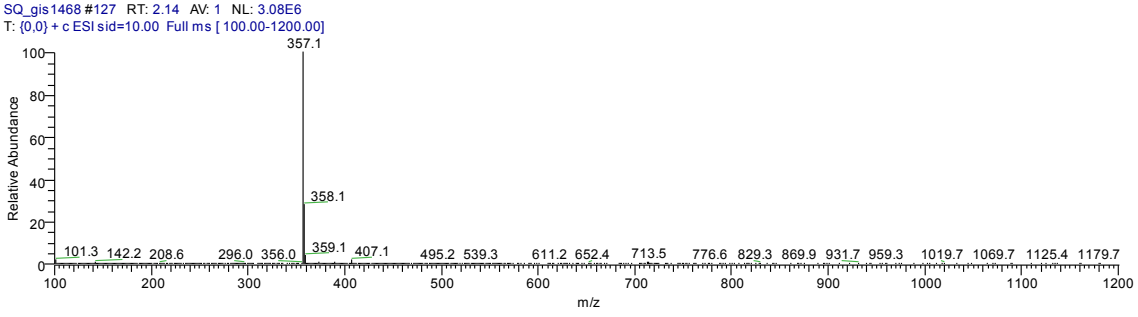


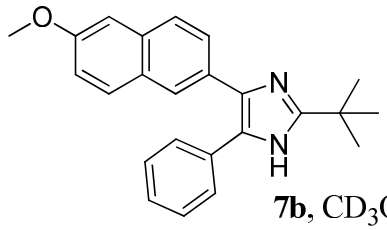

O
N
N
H

7b

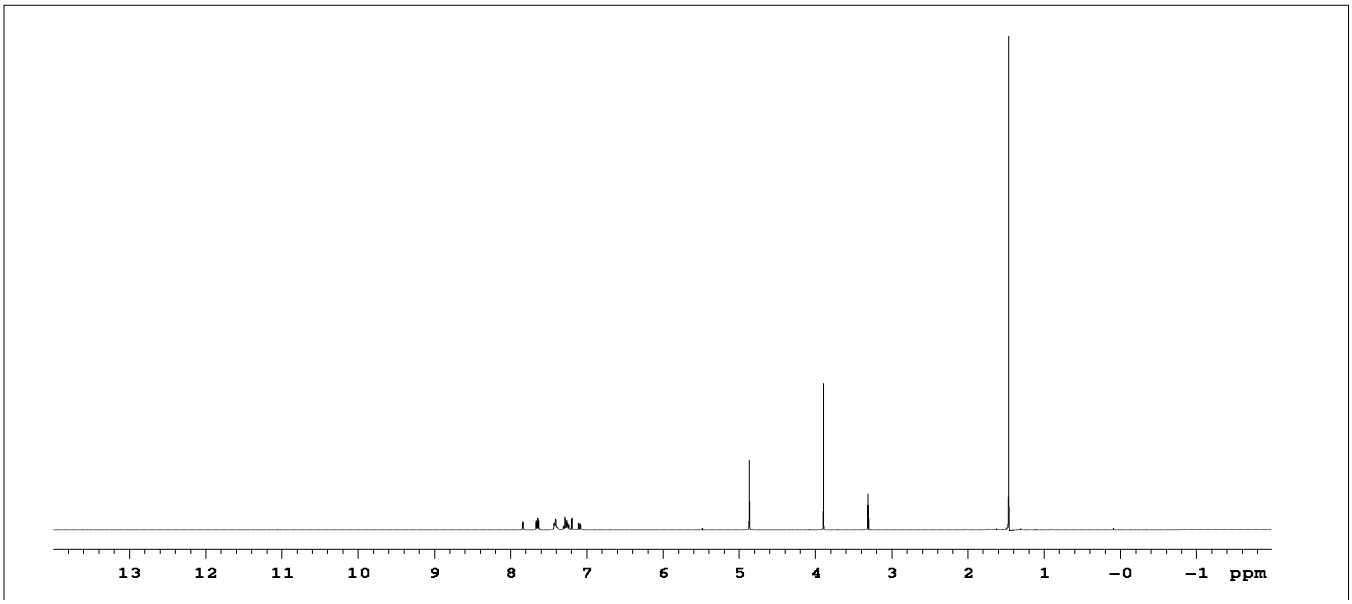

% Mobile Phase
Normalized Units
Minutes

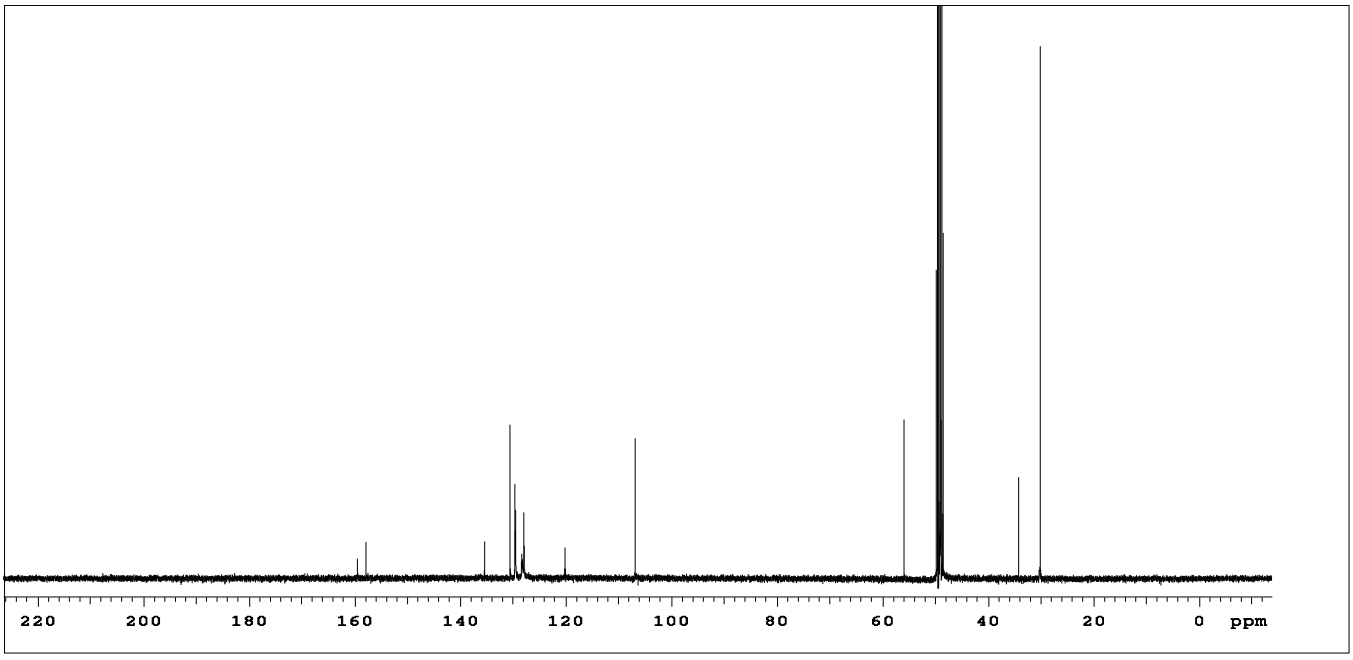

RT: 0.00 - 3.74
NL:
5.04E6
TIC MS
SQ_gis1468
Relative Abundance
Time (min)

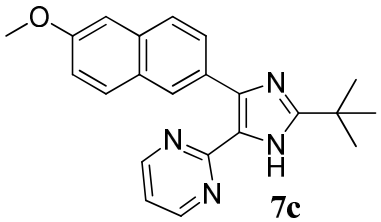

SQ_gis1468 #127 RT: 2.14 AV: 1 NL: 3.08E6
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance
m/z


O
N
N
H

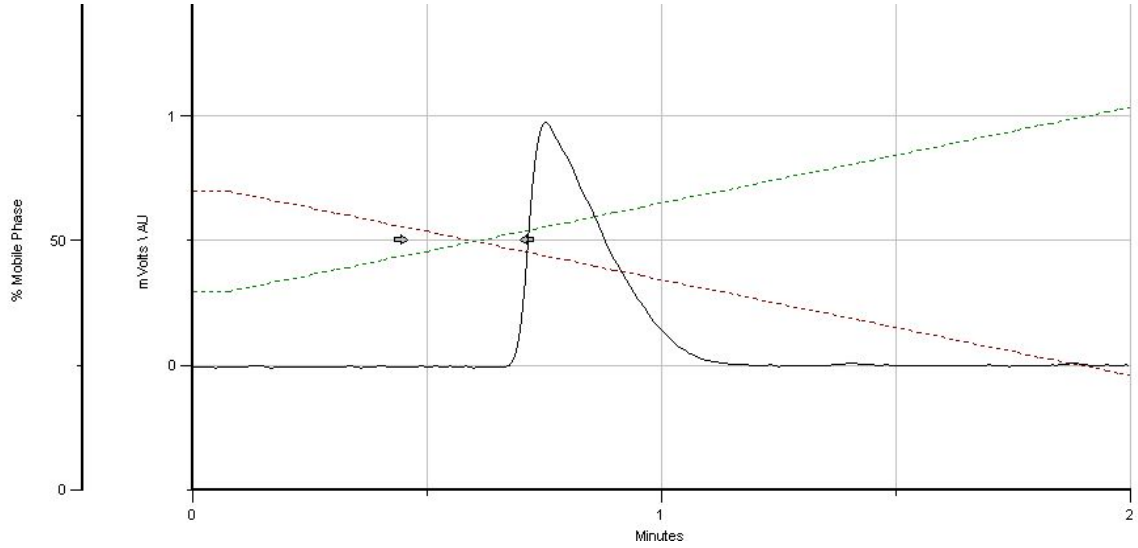
7b, CD_3OD

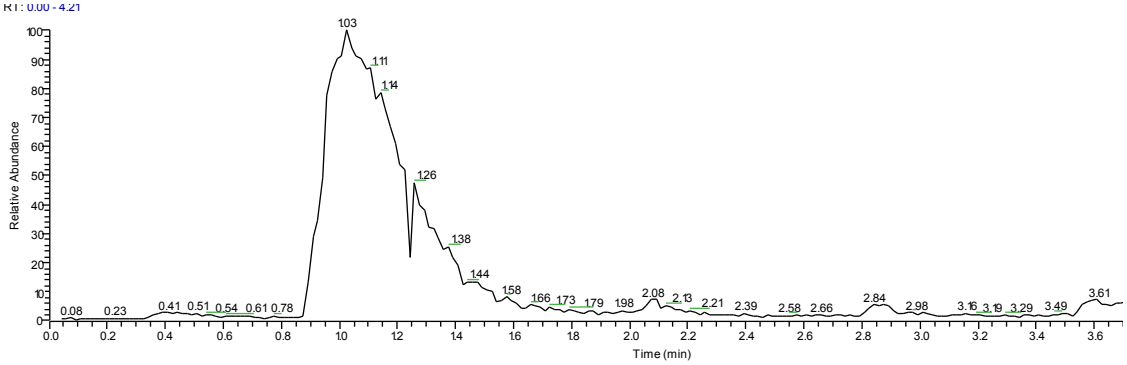

13
12
11
10
9
8
7
6
5
4
3
2
1
−0
−1
ppm

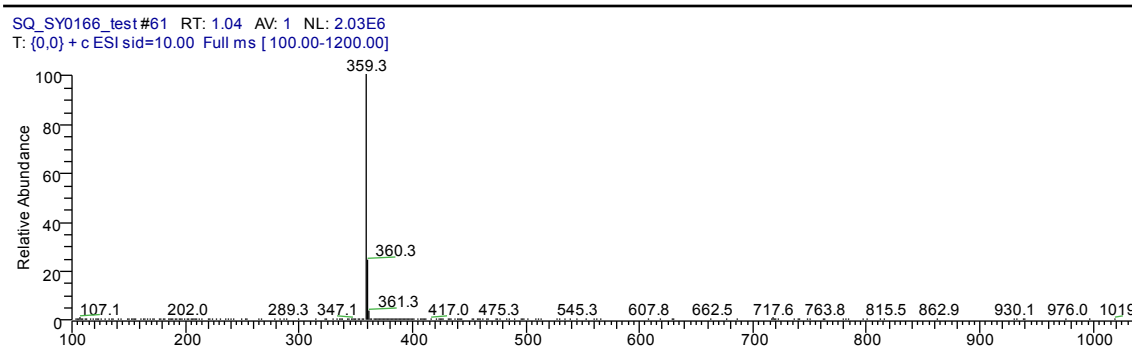

220
200
180
160
140
120
100
80
60
40
20
0
ppm

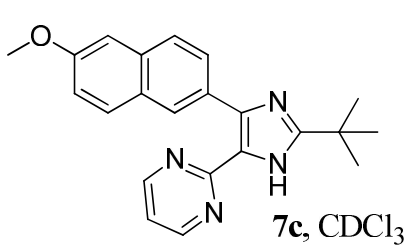


7c


% Mobile Phase
m Volts \ AU
Minutes

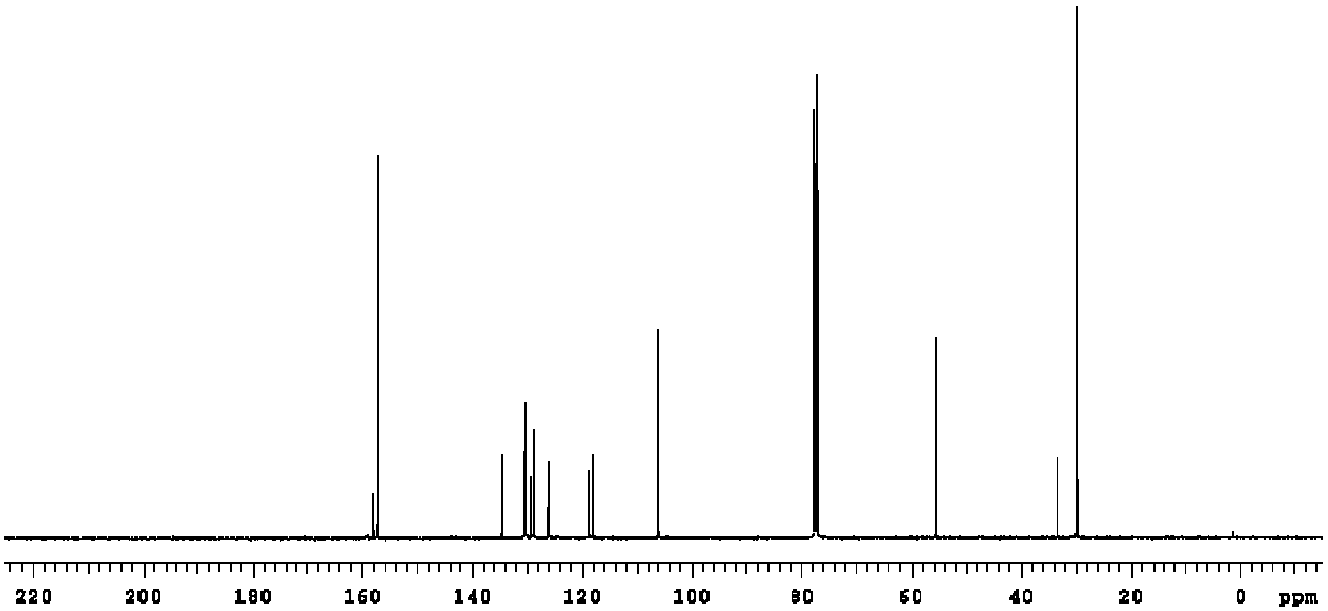

Relative Abundance
Time (min)

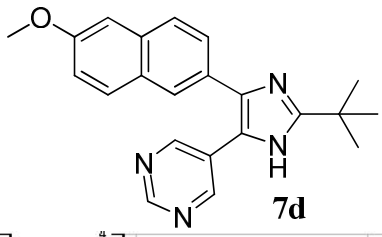

SQ_SY0166_test #61 RT: 1.04 AV: 1 NL: 2.03E6
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance


O
N
N
H
N
N

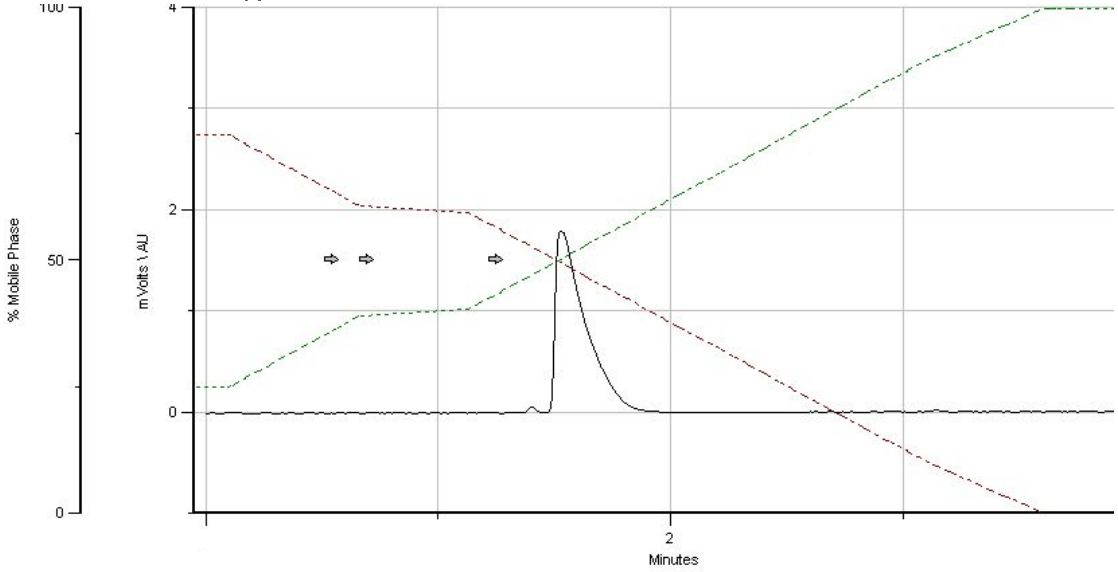
7c, $CDCl_3$

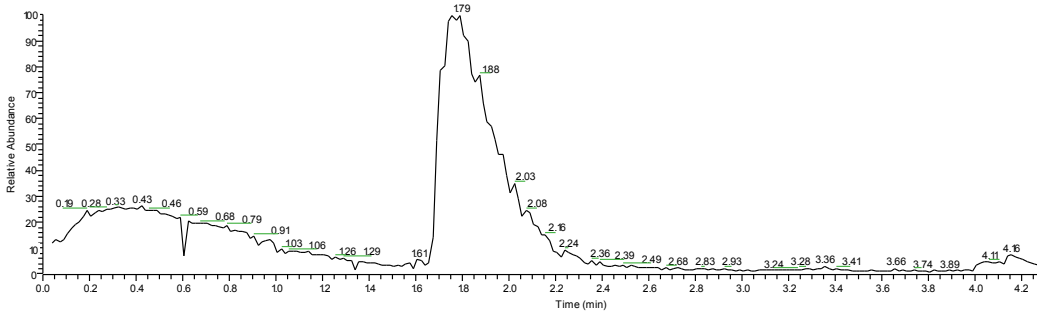
13
12
11
10
9
8
7
6
5
4
3
2
1
-0
-1
ppm


220
200
180
160
140
120
100
80
60
40
20
0
ppm

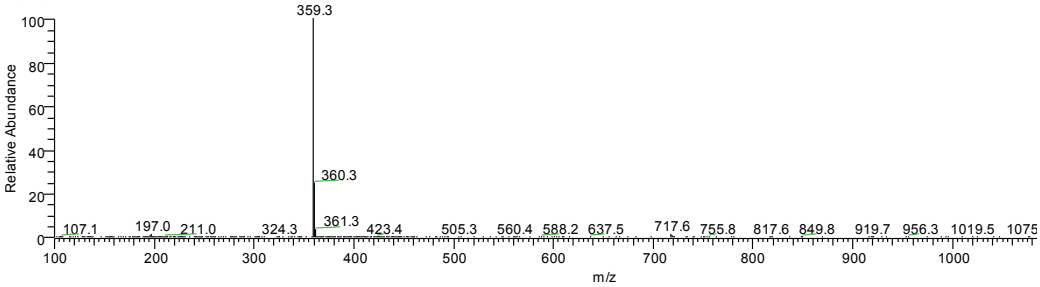


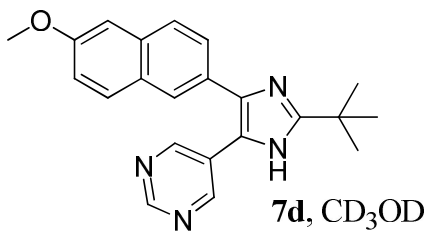
7d





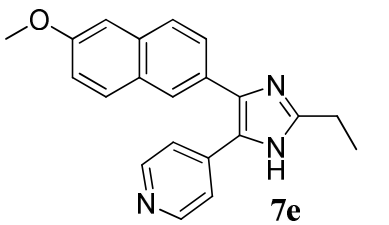
SQ_SY0165_tst #105 RT: 1.78 AV: 1 NL: 2.50E6
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]



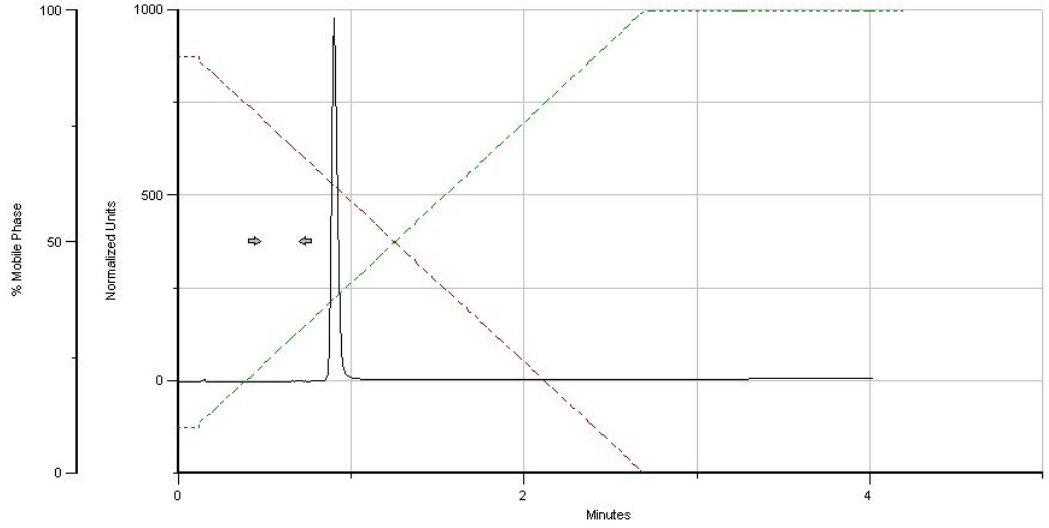

O
N
N
H
N
N

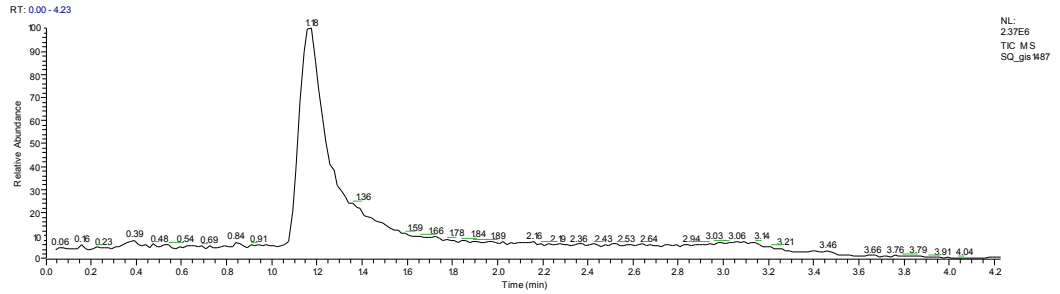
7d, CD_3OD

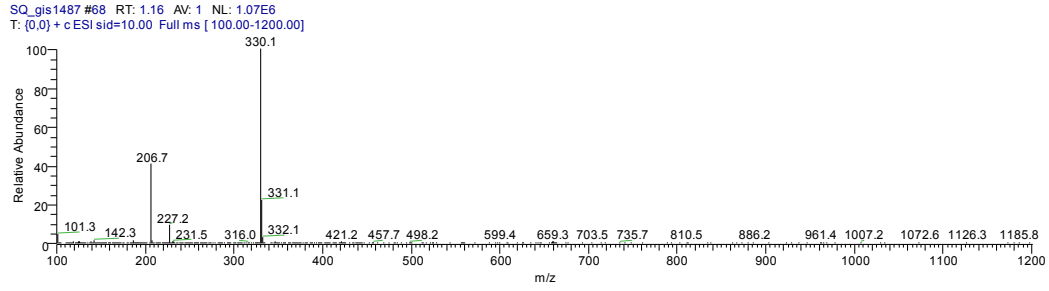
13
12
11
10
9
8
7
6
5
4
3
2
1
-0
-1
ppm
220
200
180
160
140
120
100
80
60
40
20
0
ppm

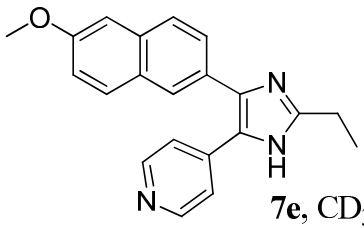

O
N
N
H
N

7e

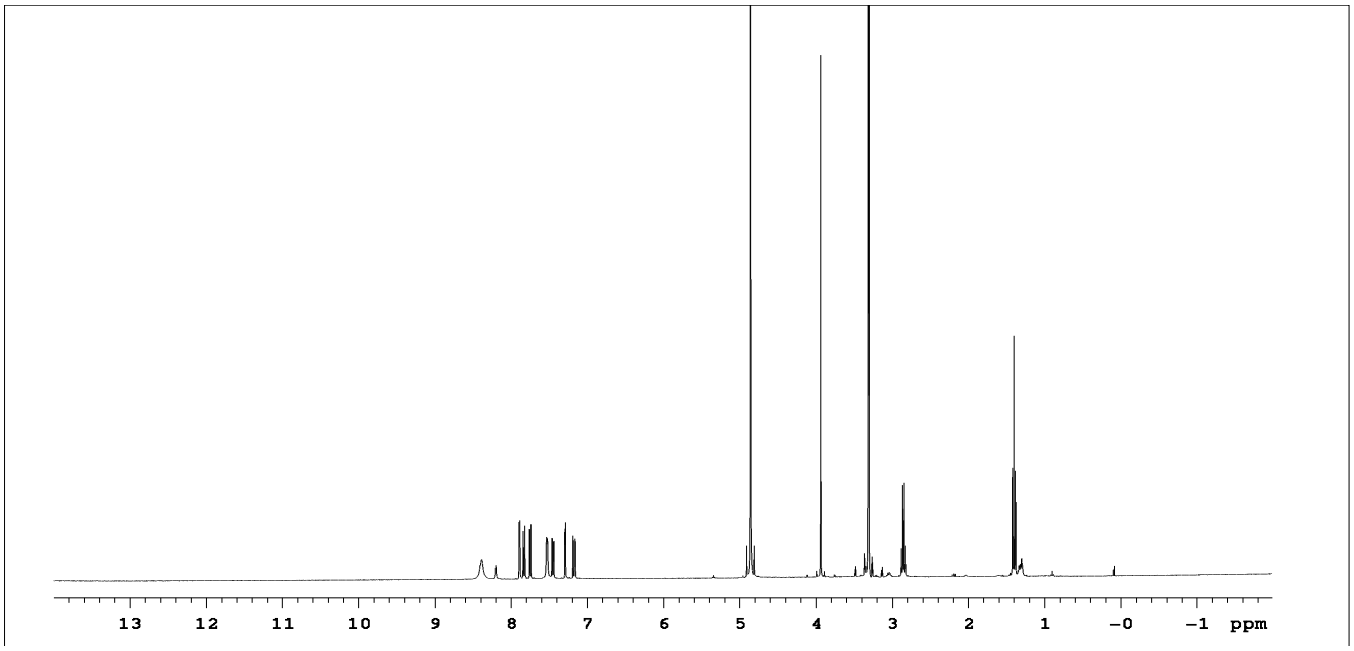

% Mobile Phase
Normalized Units
Minutes
100
50
0
1000
500
0
0
2
4


RT: 0.00 - 4.23
NL:
2.37E6
TIC MS
SQ_gis1487
Relative Abundance
Time (min)
1.18
1.36

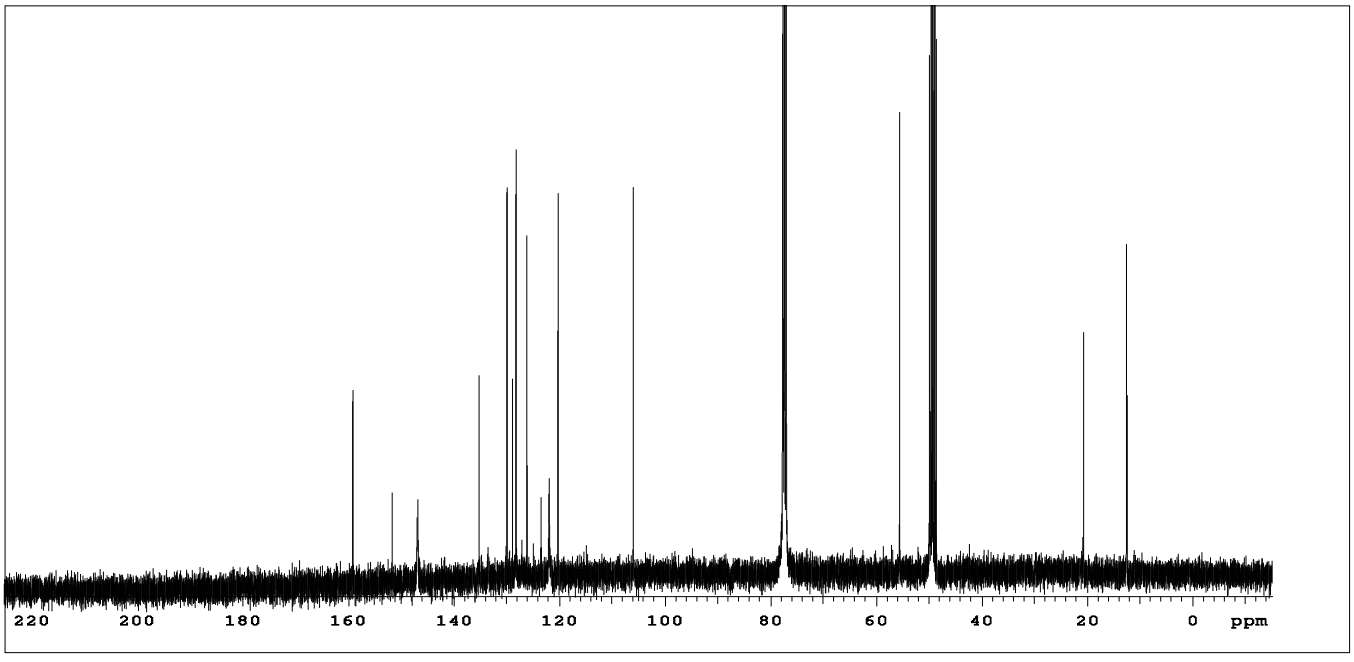

SQ_gis1487 #68 RT: 1.16 AV: 1 NL: 1.07E6
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance
m/z
330.1
206.7
331.1
227.2
101.3
142.3
231.5
316.0
332.1

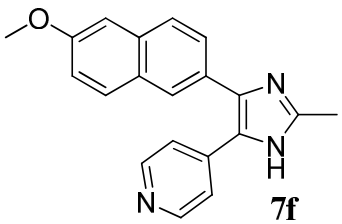


7e, CD_3OD

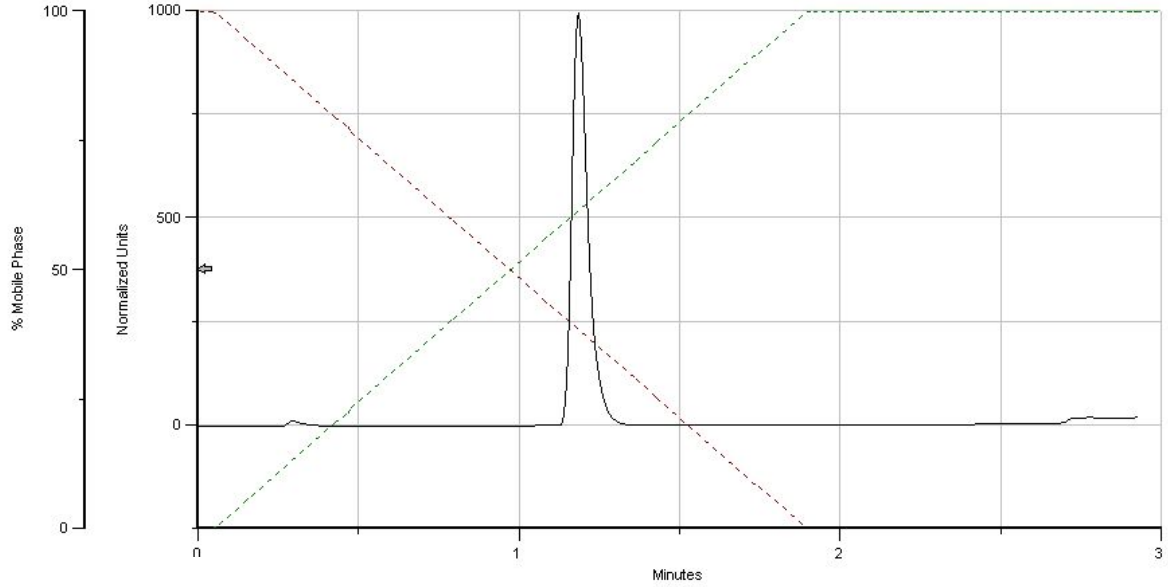


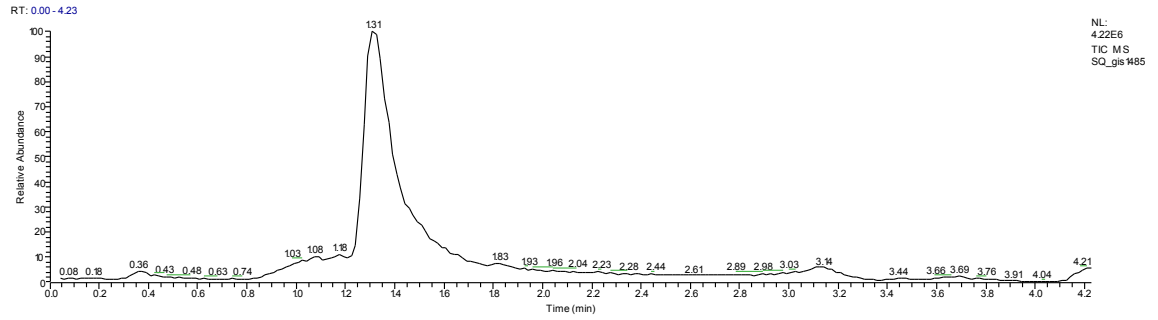
7e, $CDCl_3$:CD_3OD, 5:2

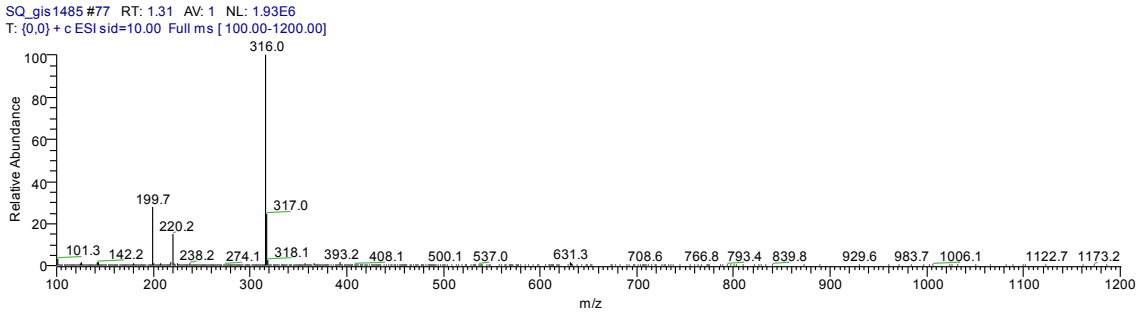


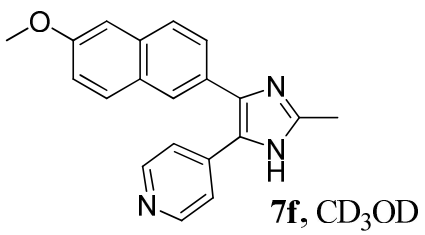

O
N
N
H
N

7f

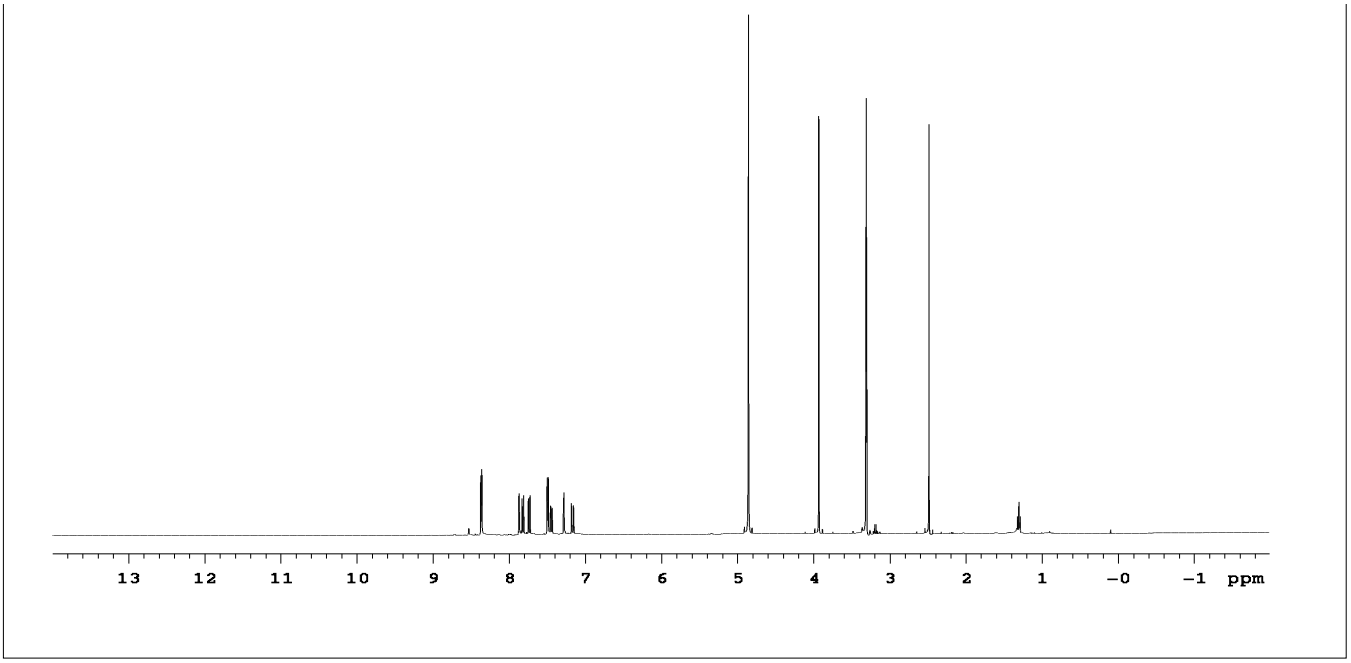

% Mobile Phase
Normalized Units
Minutes

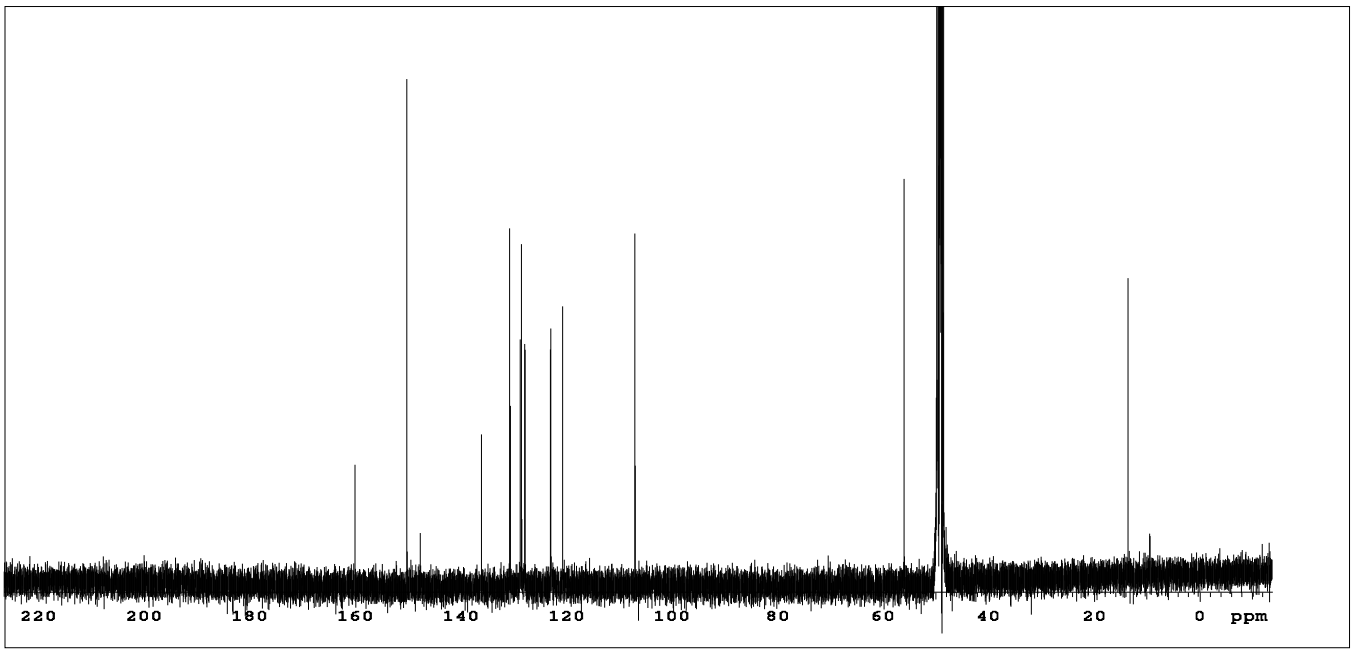

RT: 0.00 - 4.23
NL:
4.22E6
TIC MS
SQ_gis1485
Relative Abundance
Time (min)

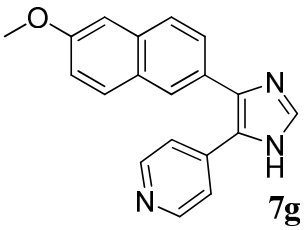

SQ_gis1485 #77 RT: 1.31 AV: 1 NL: 1.93E6
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance
m/z


O
N
N
H
N

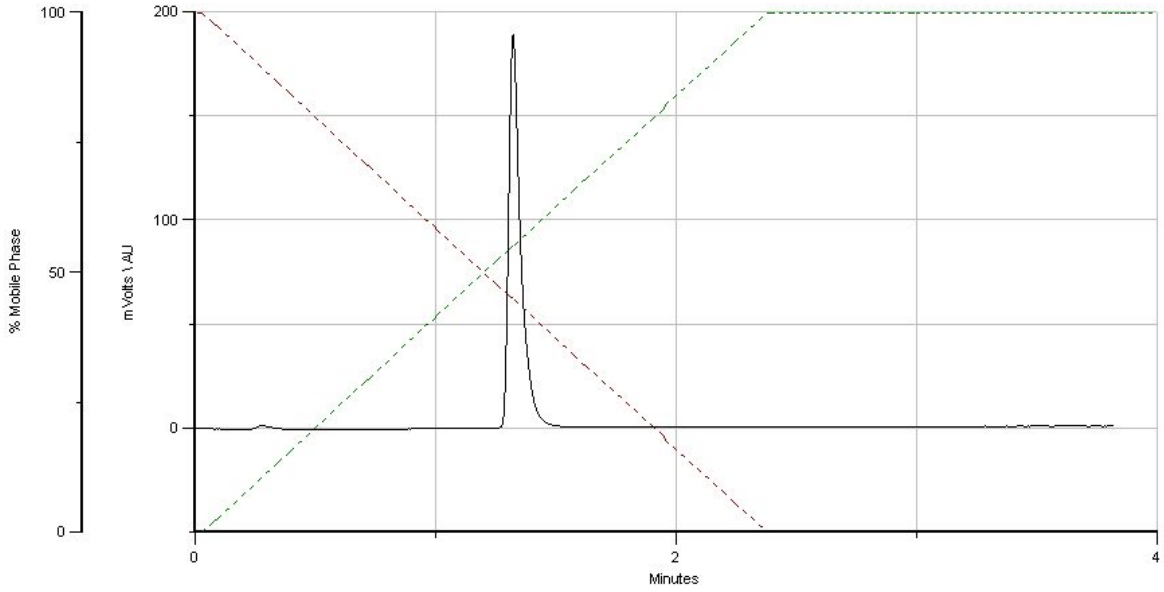
7f, CD_3OD

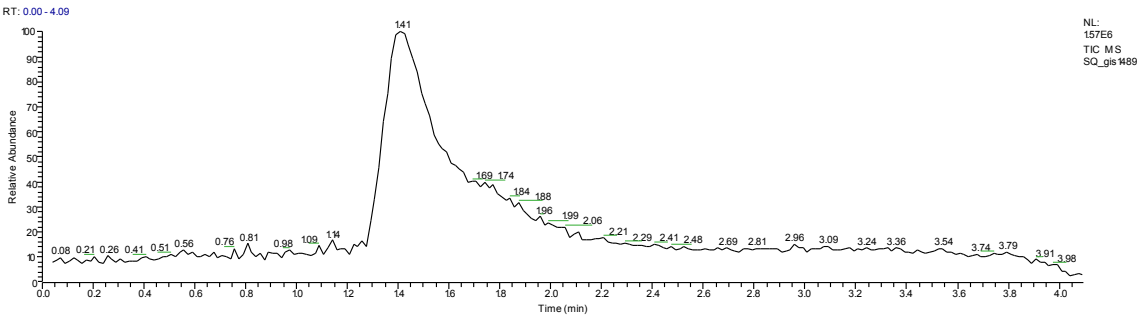

13
12
11
10
9
8
7
6
5
4
3
2
1
−0
−1
ppm

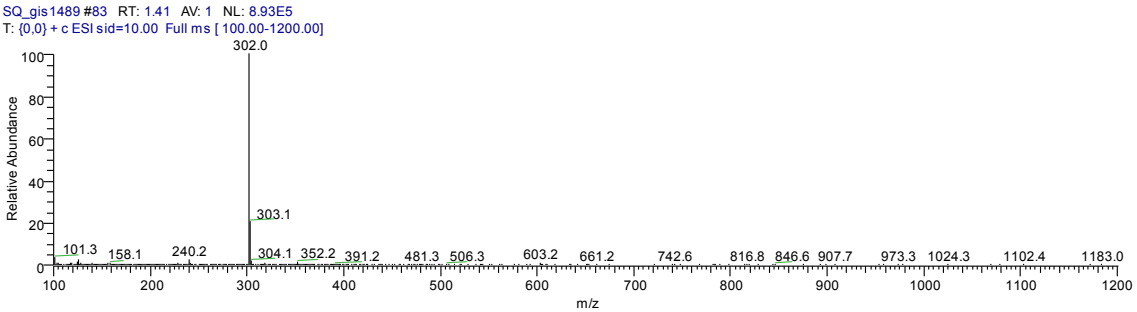

220
200
180
160
140
120
100
80
60
40
20
0
ppm

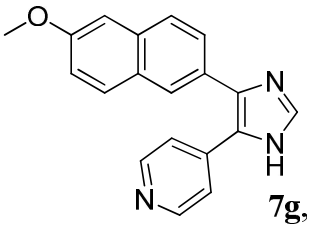


7g

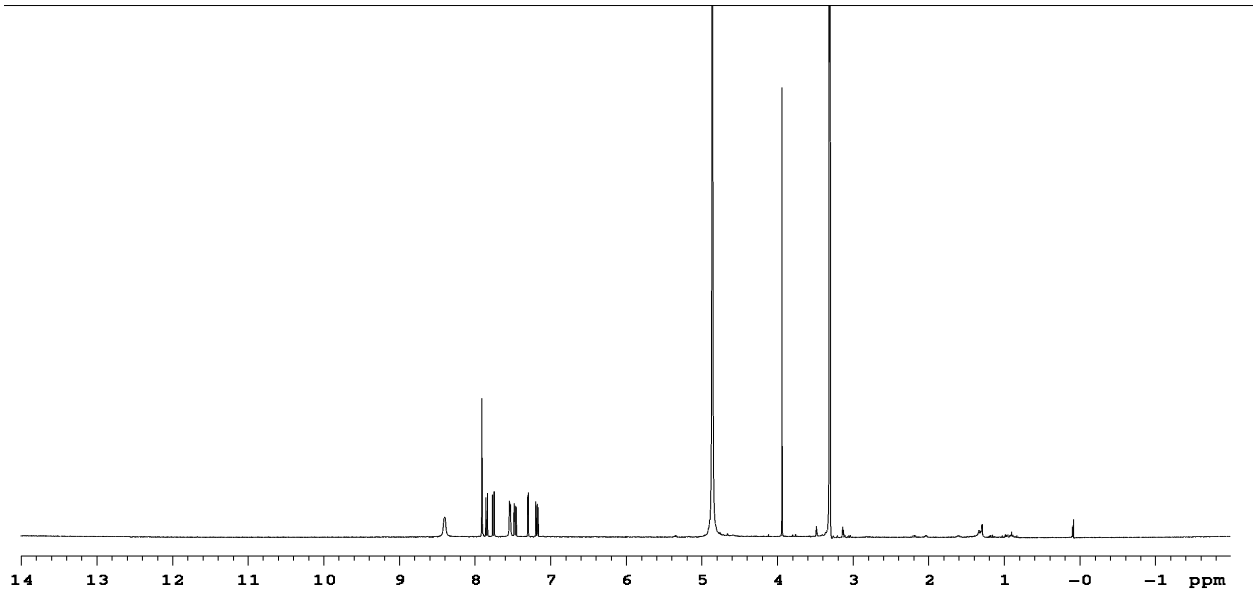

% Mobile Phase
mVolts \ AU
Minutes


RT: 0.00 - 4.09
NL:
1.57E6
TIC MS
SQ_gis1489
Relative Abundance
Time (min)

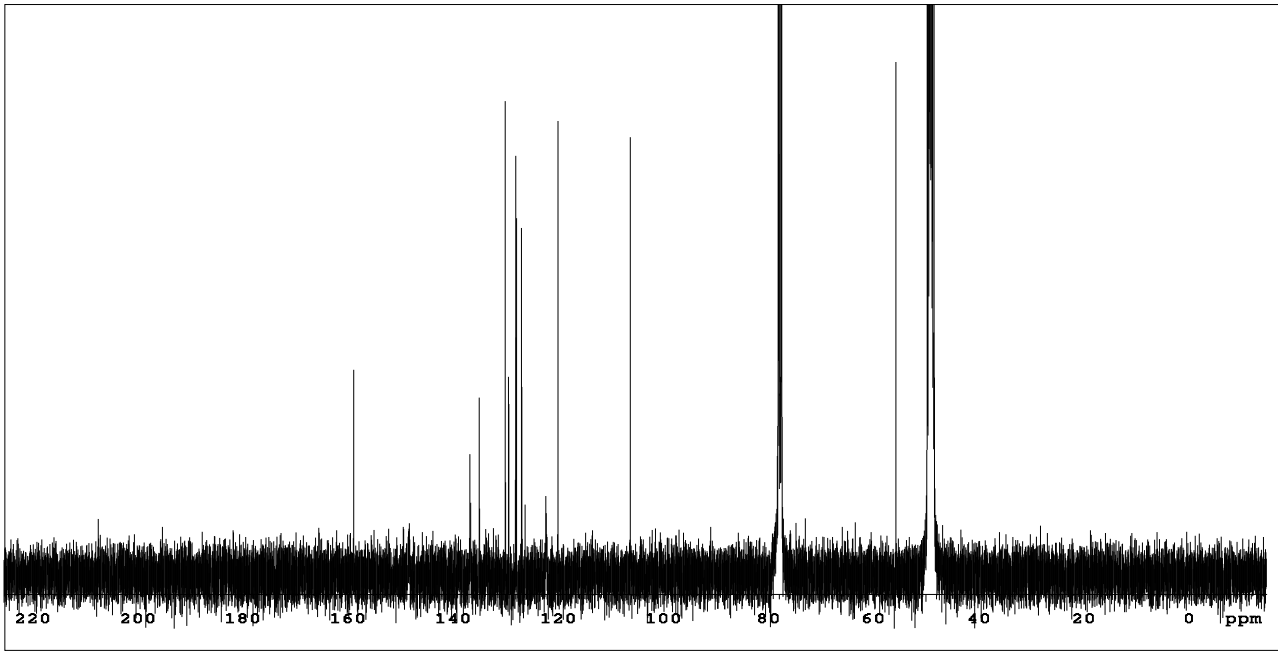

SQ_gis1489 #83 RT: 1.41 AV: 1 NL: 8.93E5
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance
m/z

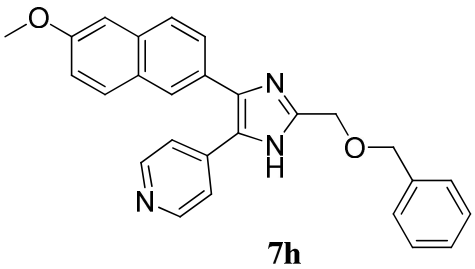


7g, CD_3OD

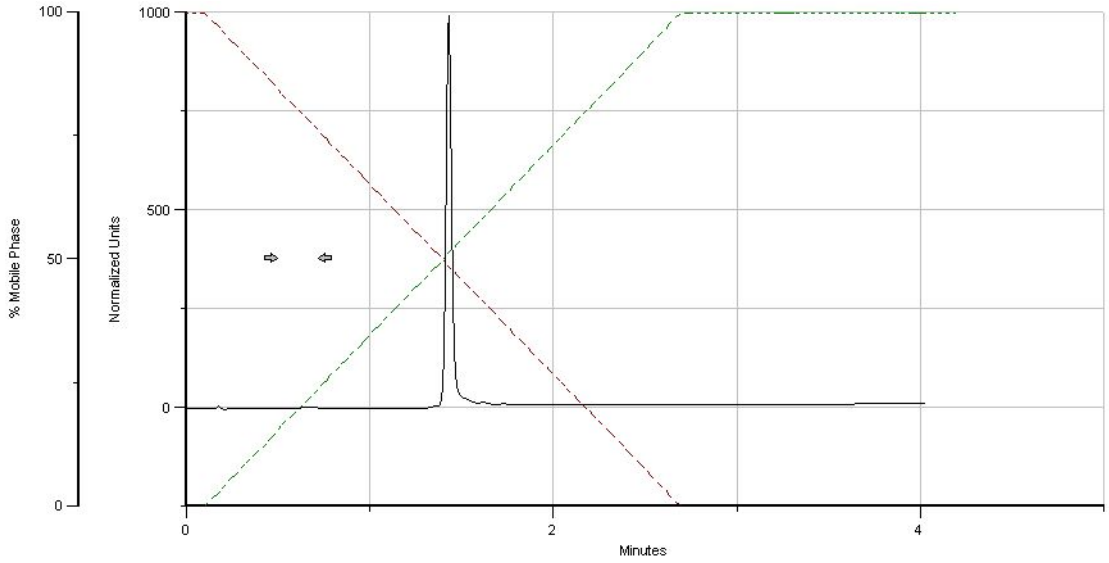


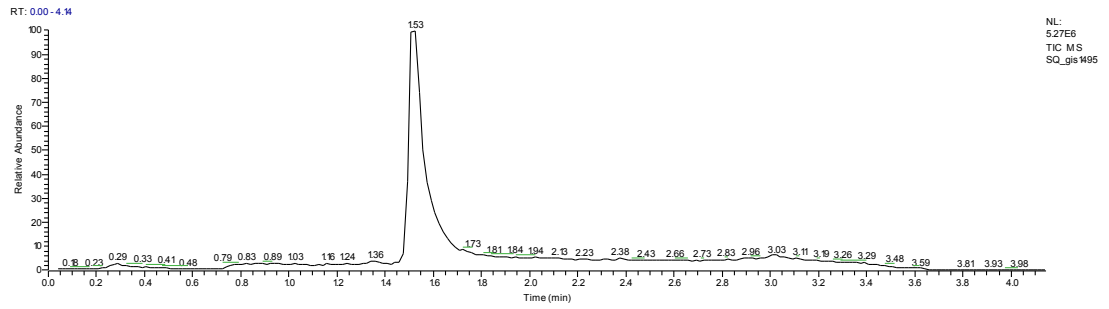
7g, $CD_3OD:CDCl_3$, 5:2

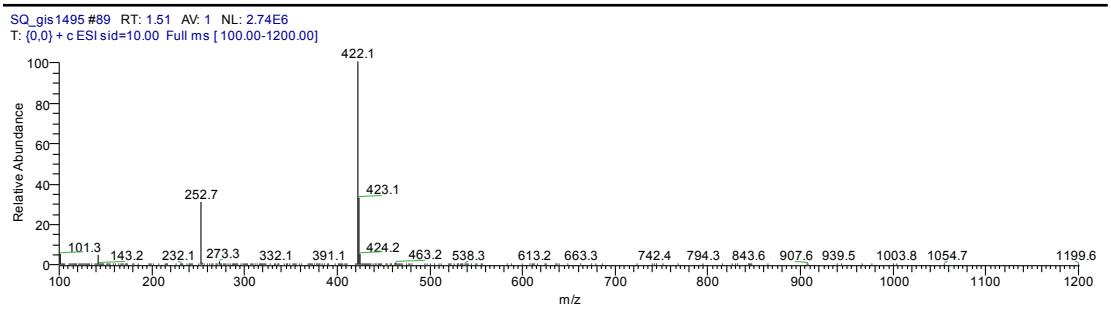


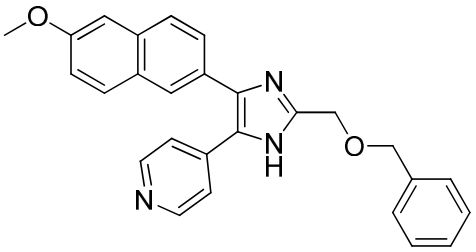


7h

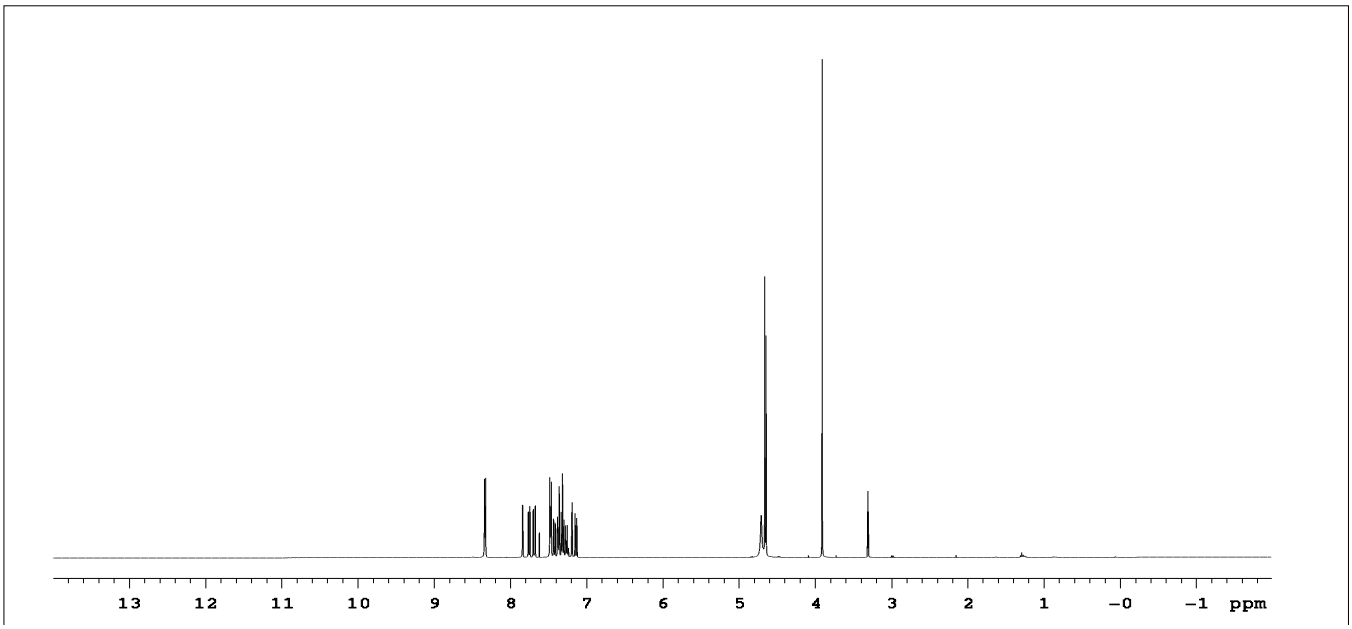

% Mobile Phase
Normalized Units
Minutes

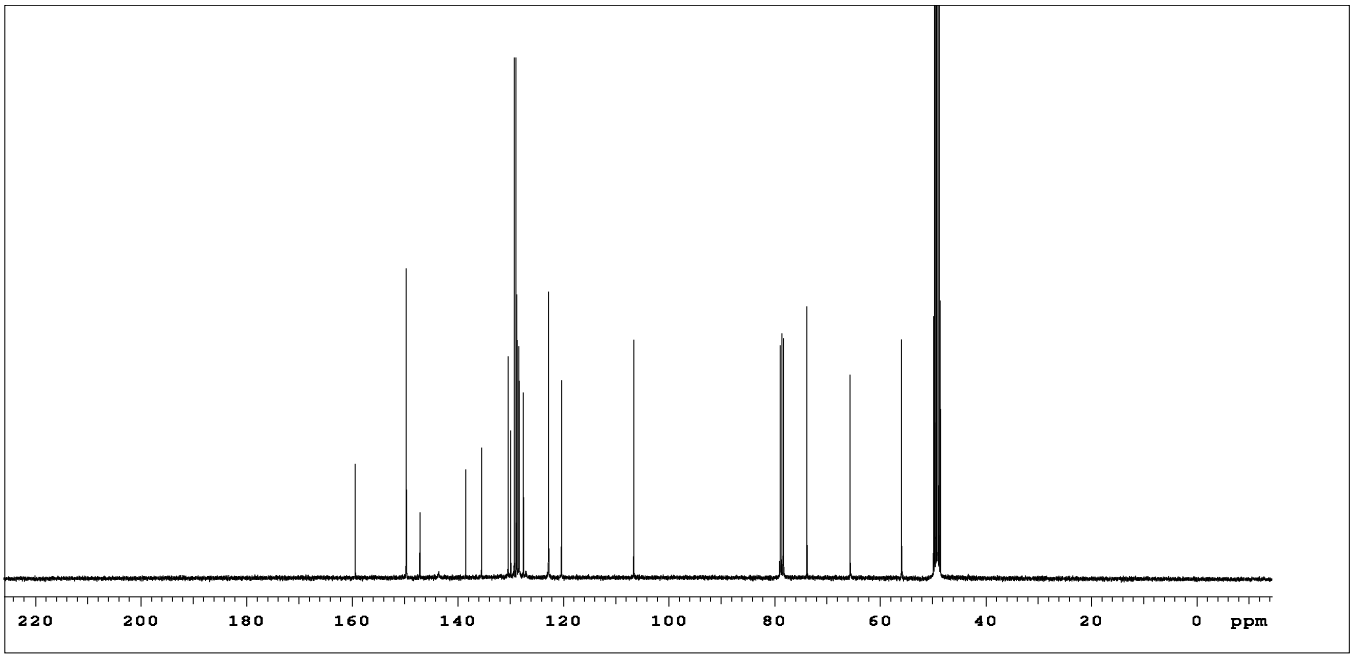

RT: 0.00 - 4.14
NL:
5.27E6
TIC MS
SQ_gis1495
Relative Abundance
Time (min)

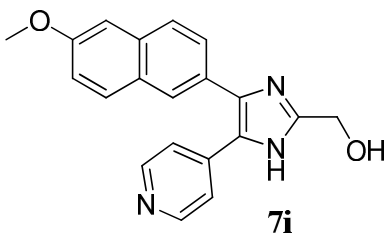

SQ_gis1495 #89 RT: 1.51 AV: 1 NL: 2.74E6
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance
m/z



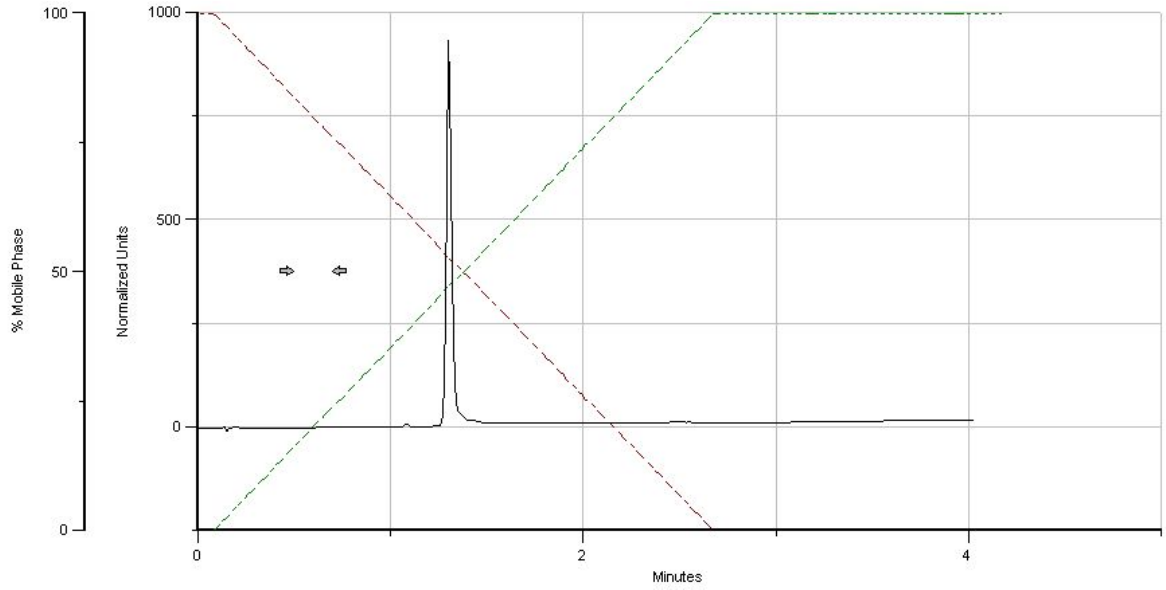
7h, CD_3OD:$CDCl_3$, 5:1

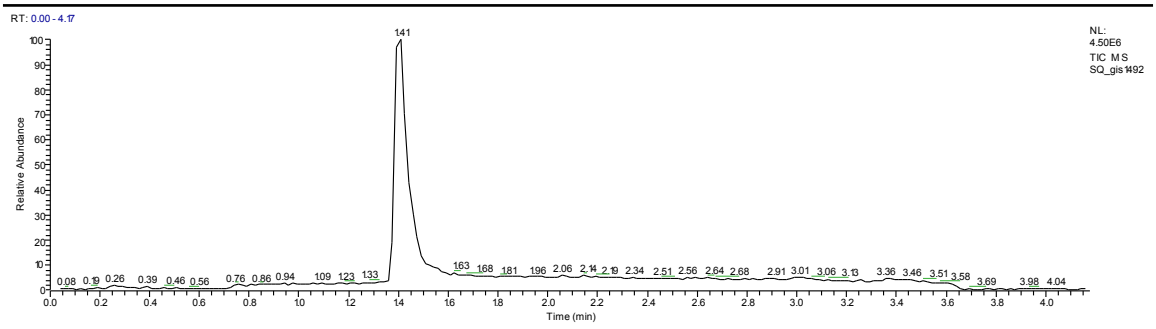


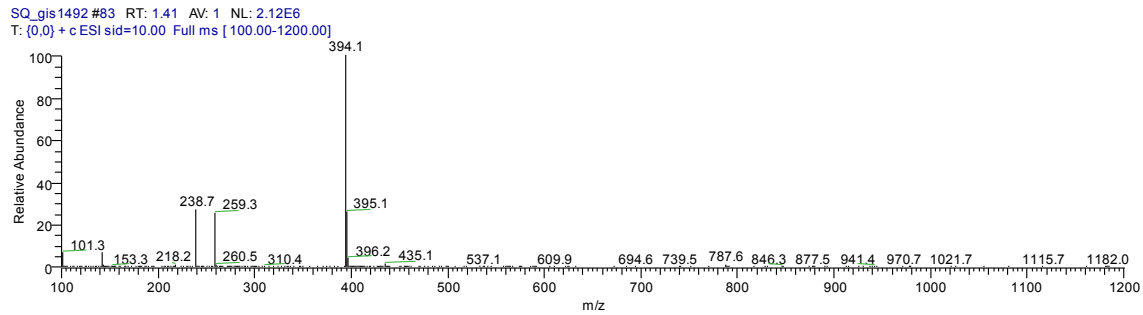


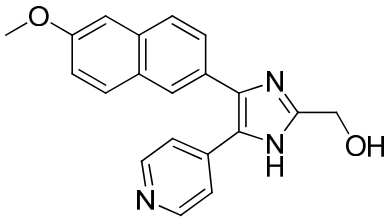

O
N
N
H
OH
N

7i

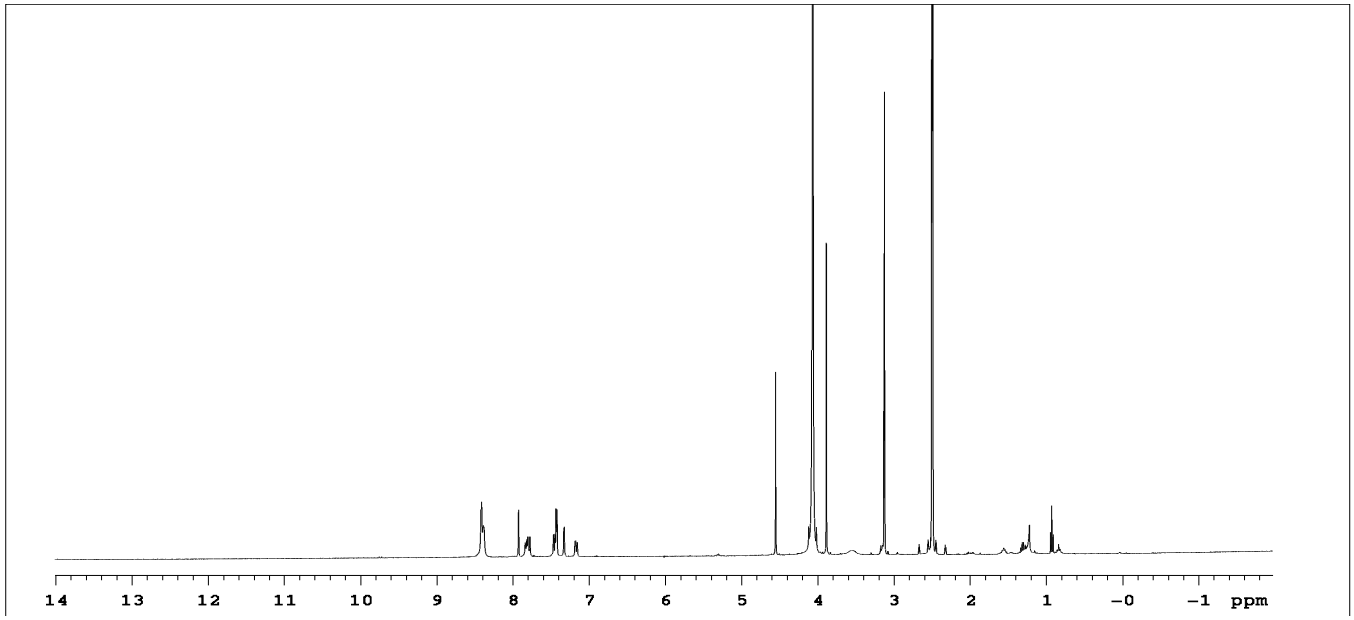

100
50
0
% Mobile Phase
1000
500
0
Normalized Units
0
2
4
Minutes


RT: 0.00 - 4.17
NL:
4.50E6
TIC MS
SQ_gis1492
Relative Abundance
141
Time (min)

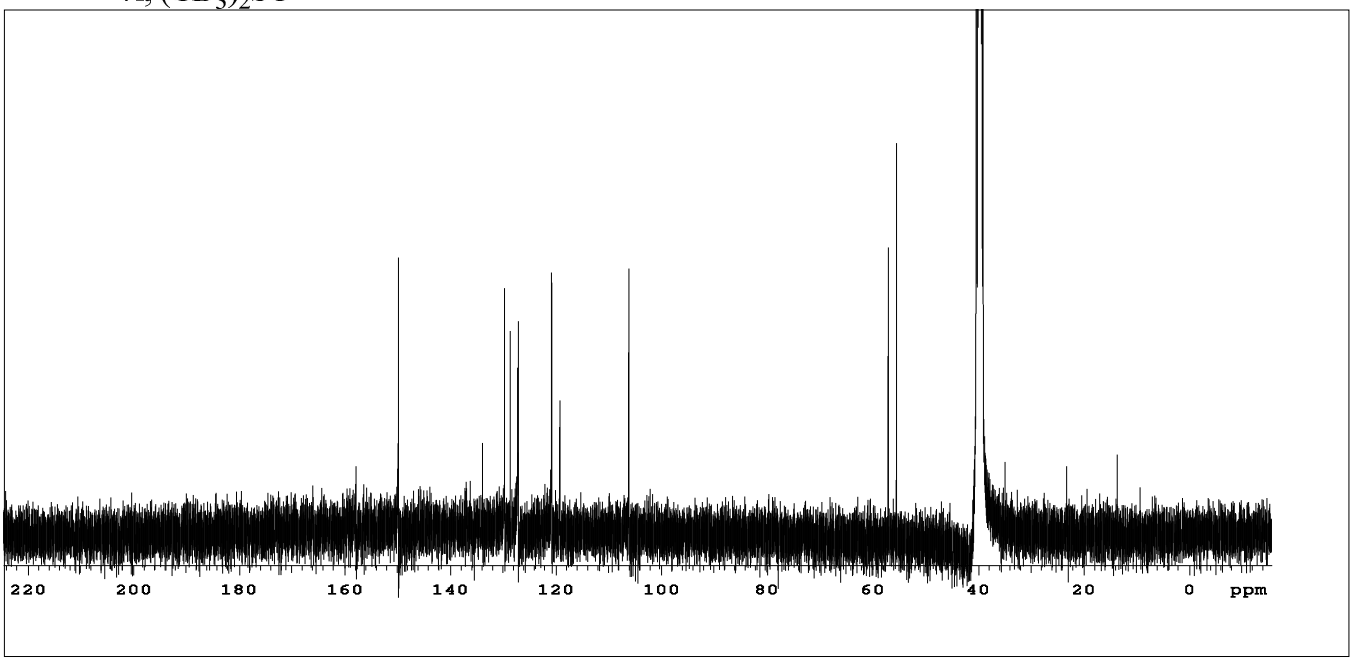

SQ_gis1492 #83 RT: 1.41 AV: 1 NL: 2.12E6
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance
394.1
238.7
259.3
395.1
101.3
153.3
218.2
260.5
310.4
396.2
435.1
537.1
609.9
694.6
739.5
787.6
846.3
877.5
941.4
970.7
1021.7
1115.7
1182.0
m/z

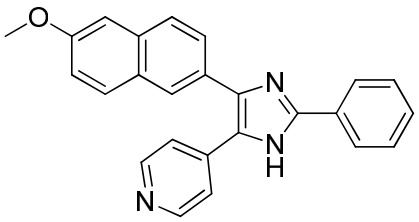


7i, $(CD_3)_2SO:CD_3OD$, 5:1

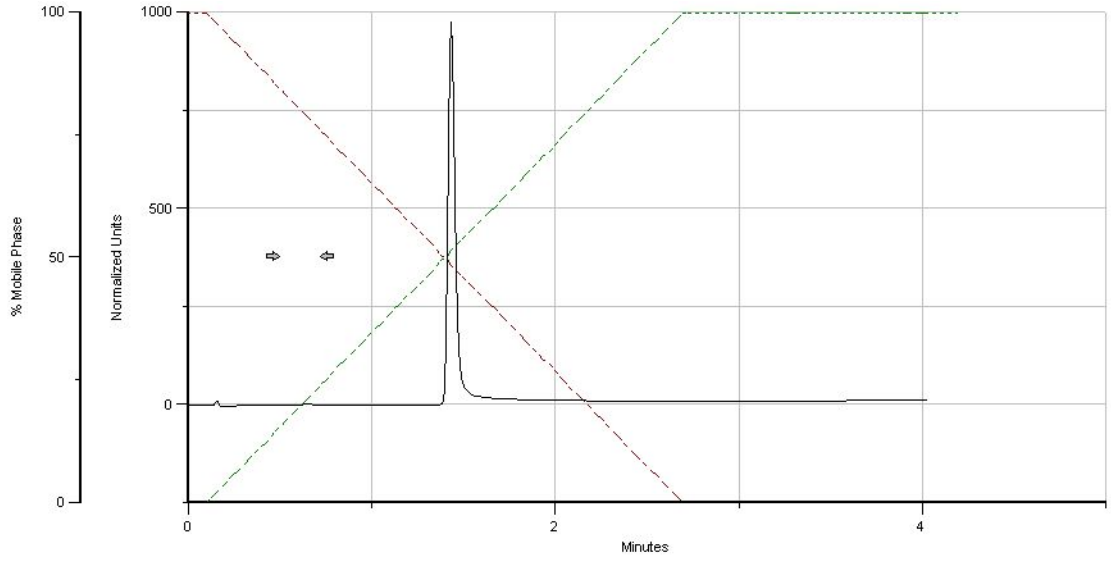


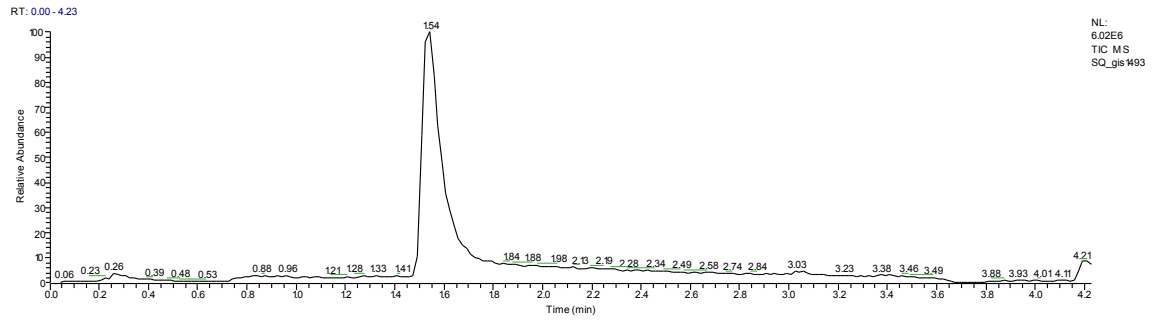
7i, $(CD_3)_2SO$

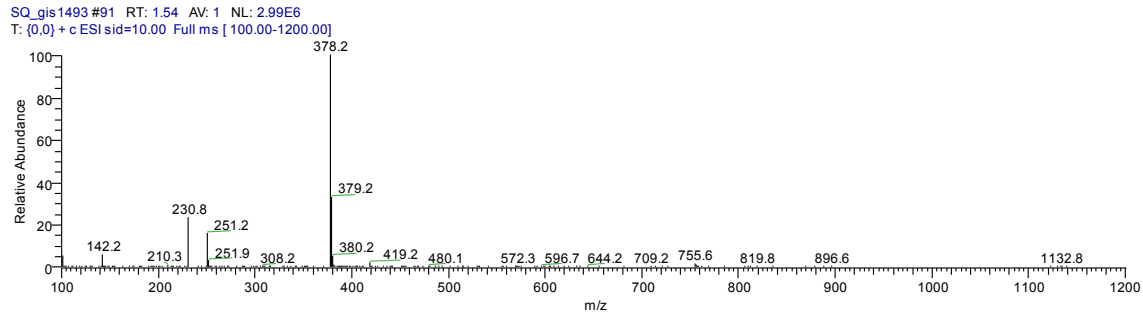


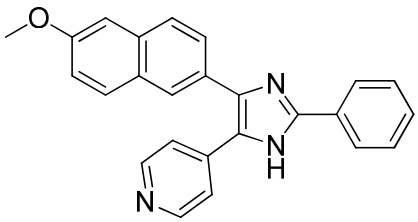


7j

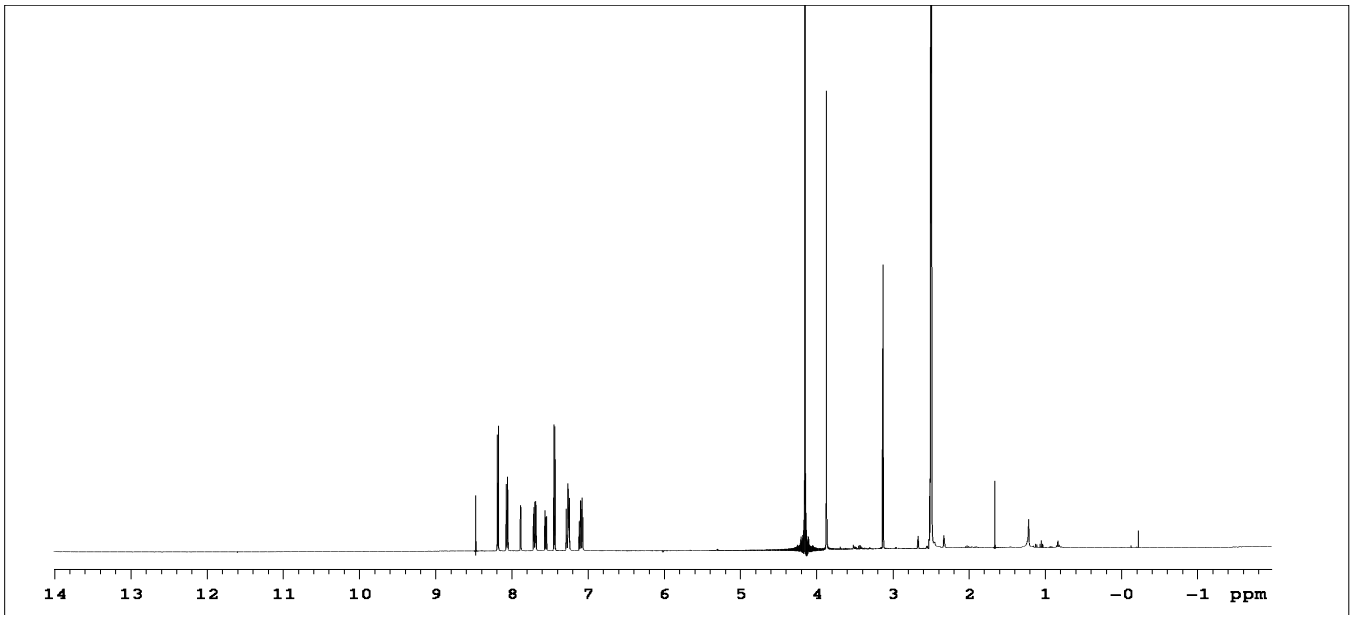

% Mobile Phase
Normalized Units
Minutes

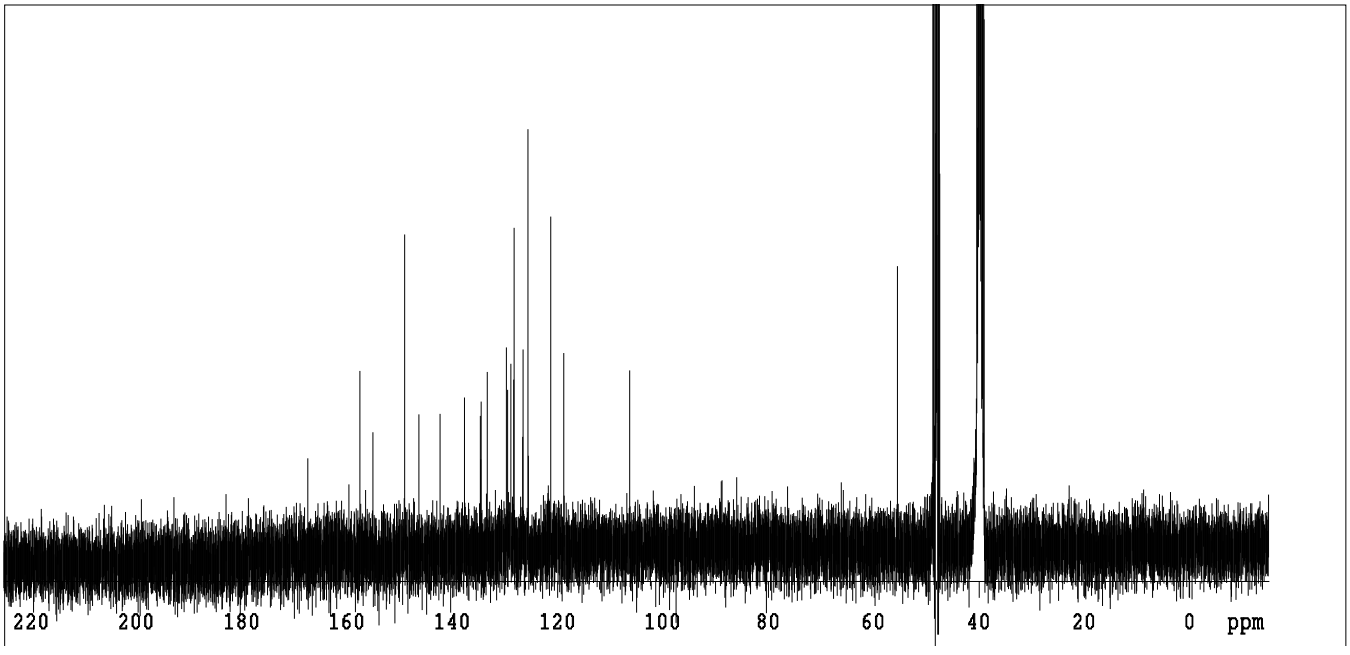

RT: 0.00 - 4.23
NL:
6.02E6
TIC MS
SQ_gis1493
Relative Abundance
Time (min)

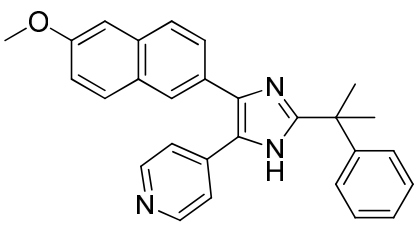

SQ_gis1493 #91 RT: 1.54 AV: 1 NL: 2.99E6
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance
m/z



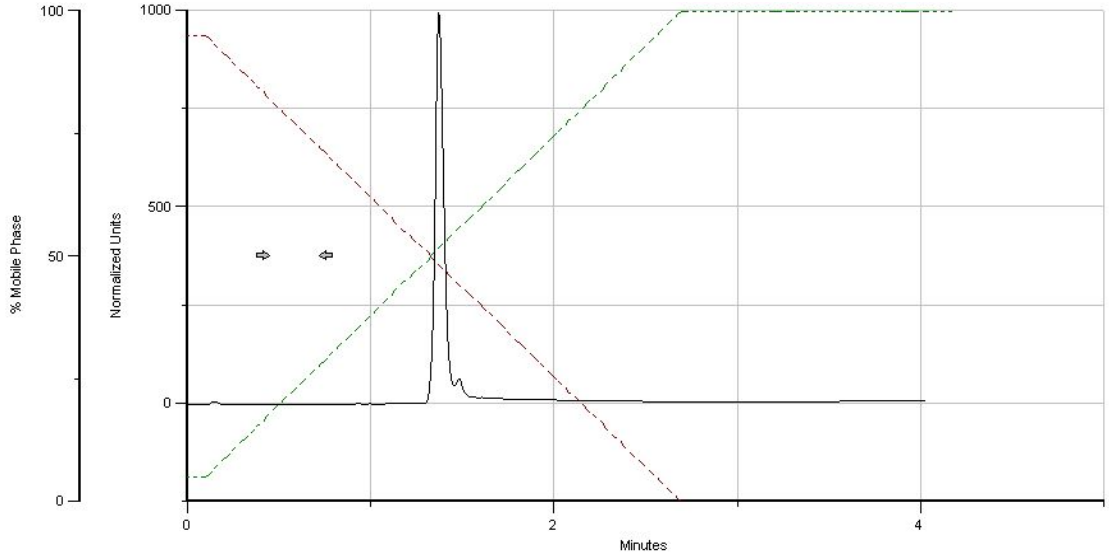
7j, $(CD_3)_2SO$:CD_3OD, 5:1

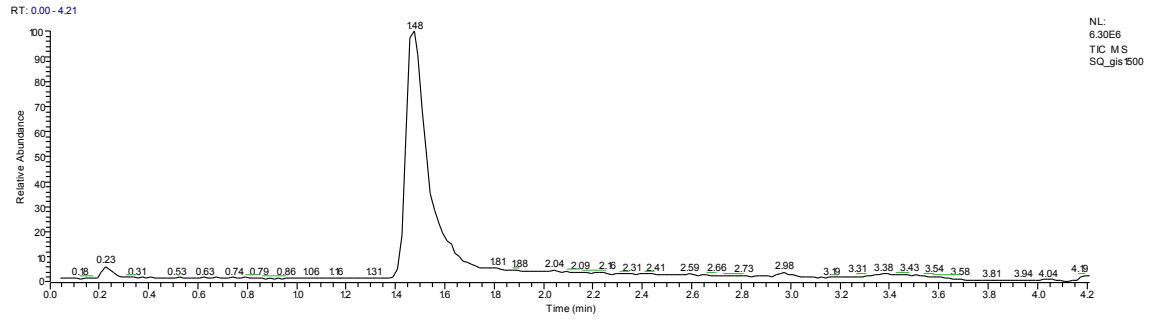


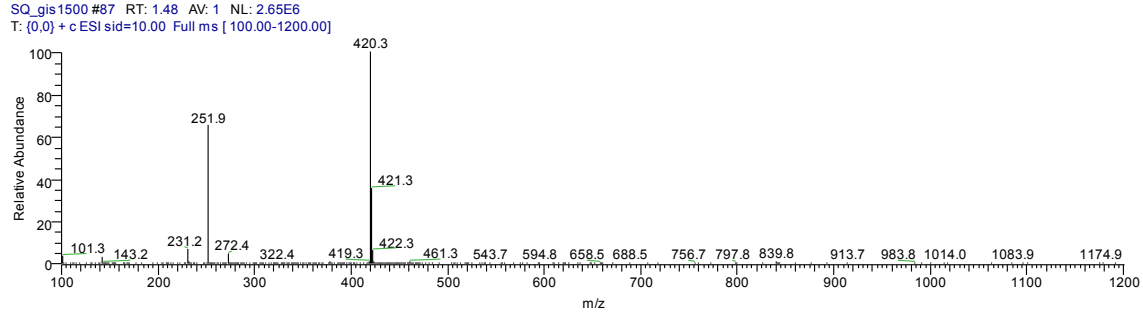


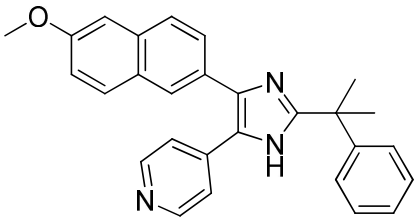


7k

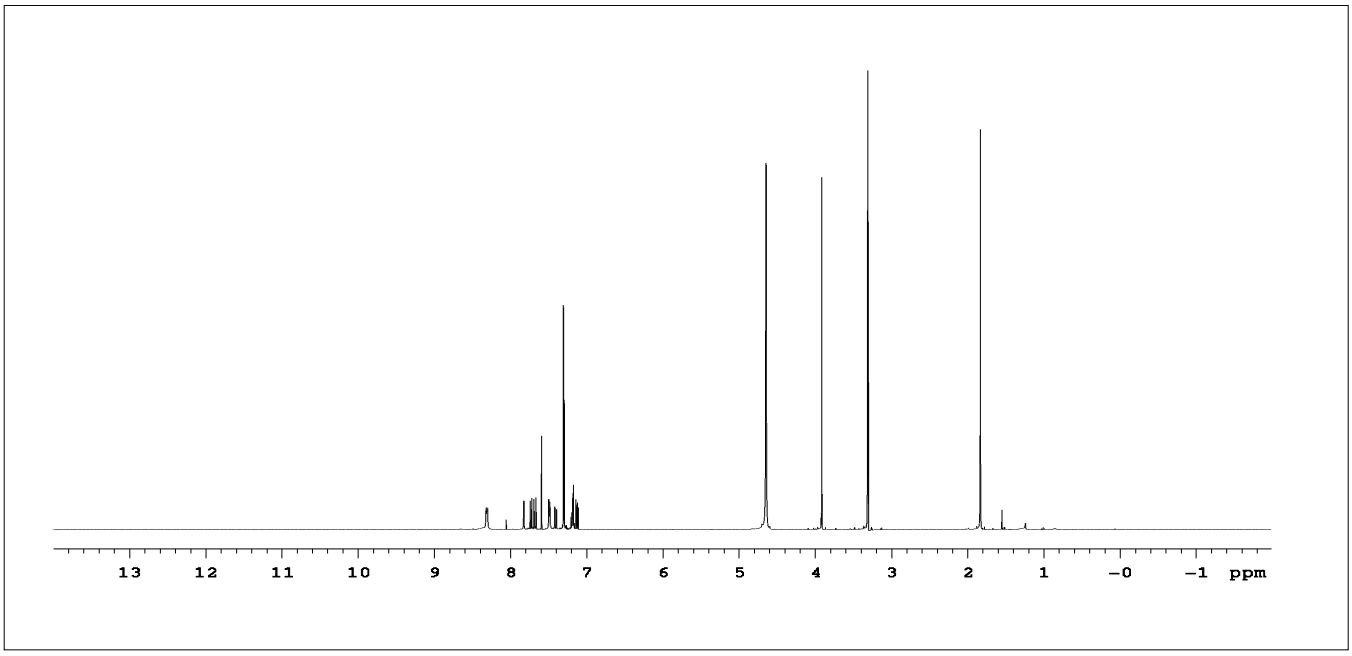

% Mobile Phase
Normalized Units
Minutes

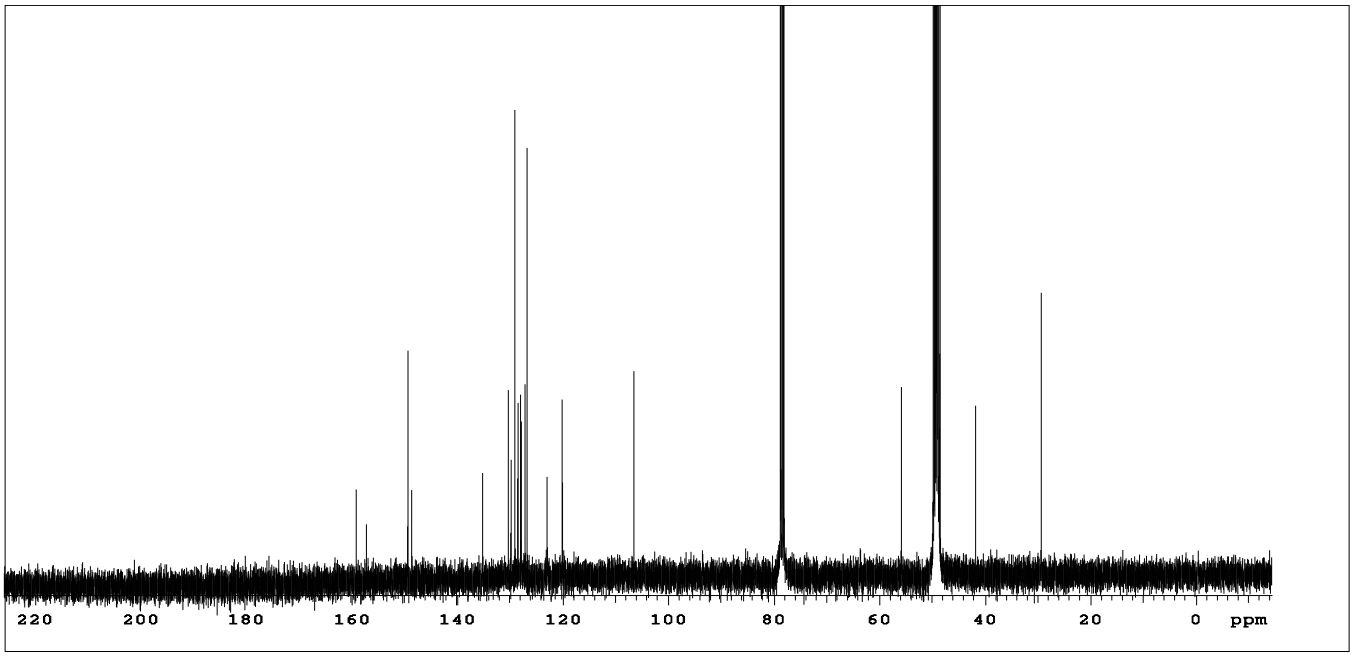

RT: 0.00 - 4.21
NL:
6.30E6
TIC MS
SQ_gis1500
Relative Abundance
Time (min)

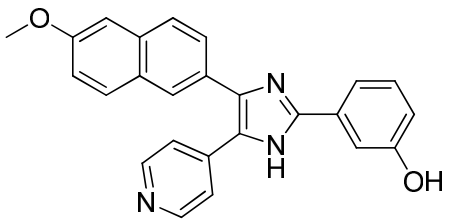

SQ_gis1500 #87 RT: 1.48 AV: 1 NL: 2.65E6
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance
m/z



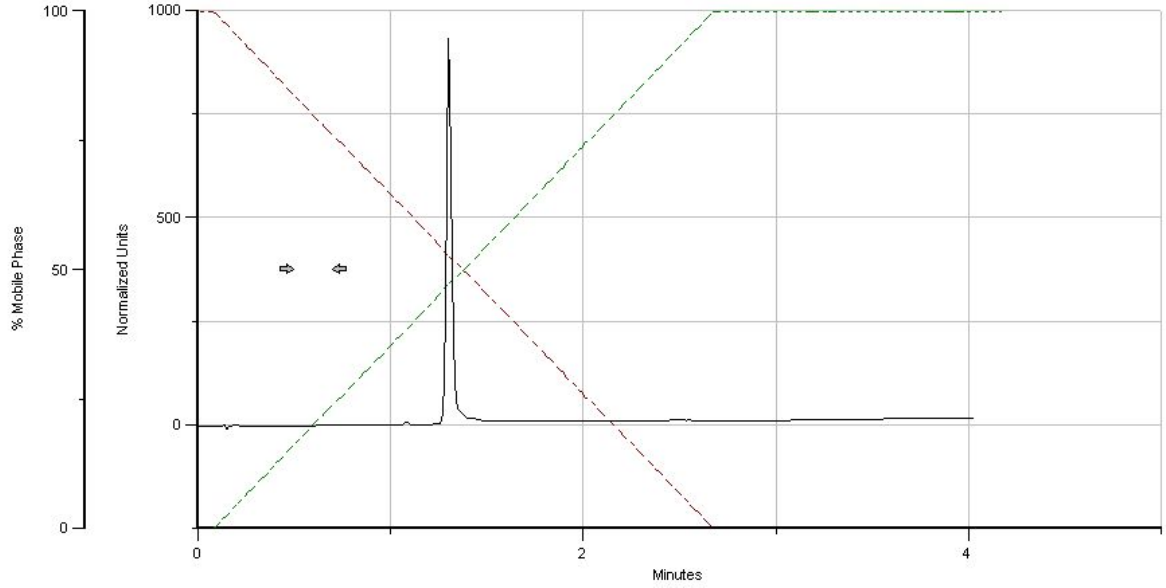
7k, $CD_3OD:CDCl_3$, 2:1

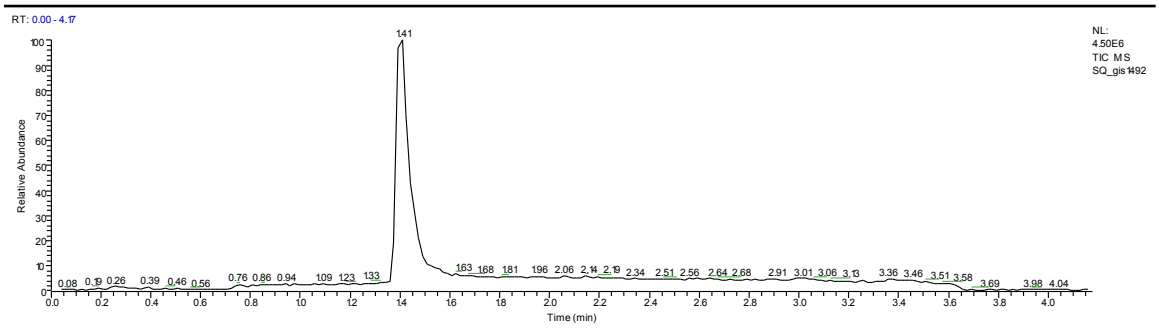


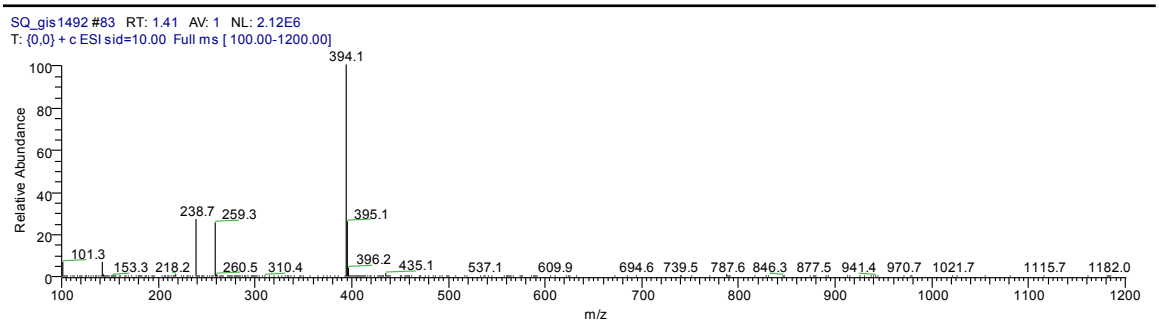


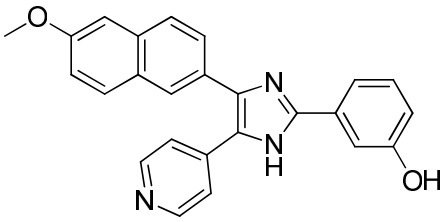

O
N
N
H
OH
N

7l

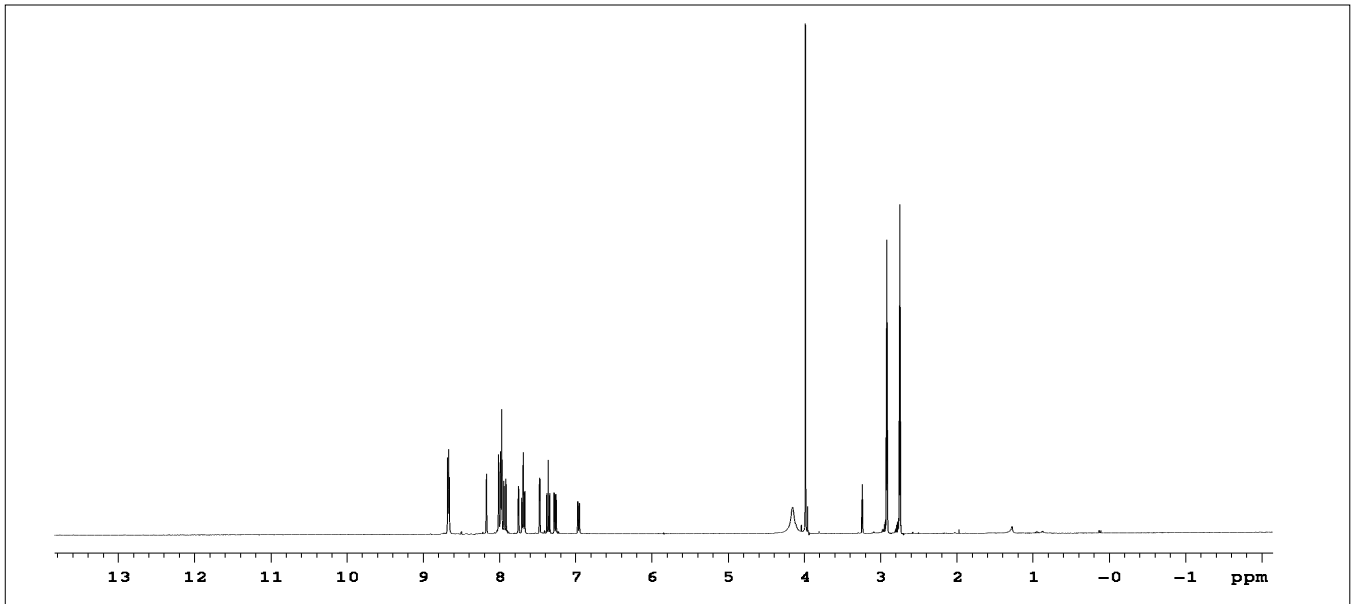

100
50
0
% Mobile Phase
1000
500
0
Normalized Units
0
2
4
Minutes


RT: 0.00 - 4.17
NL:
4.50E6
TIC MS
SQ_gis1492
Relative Abundance
141
Time (min)

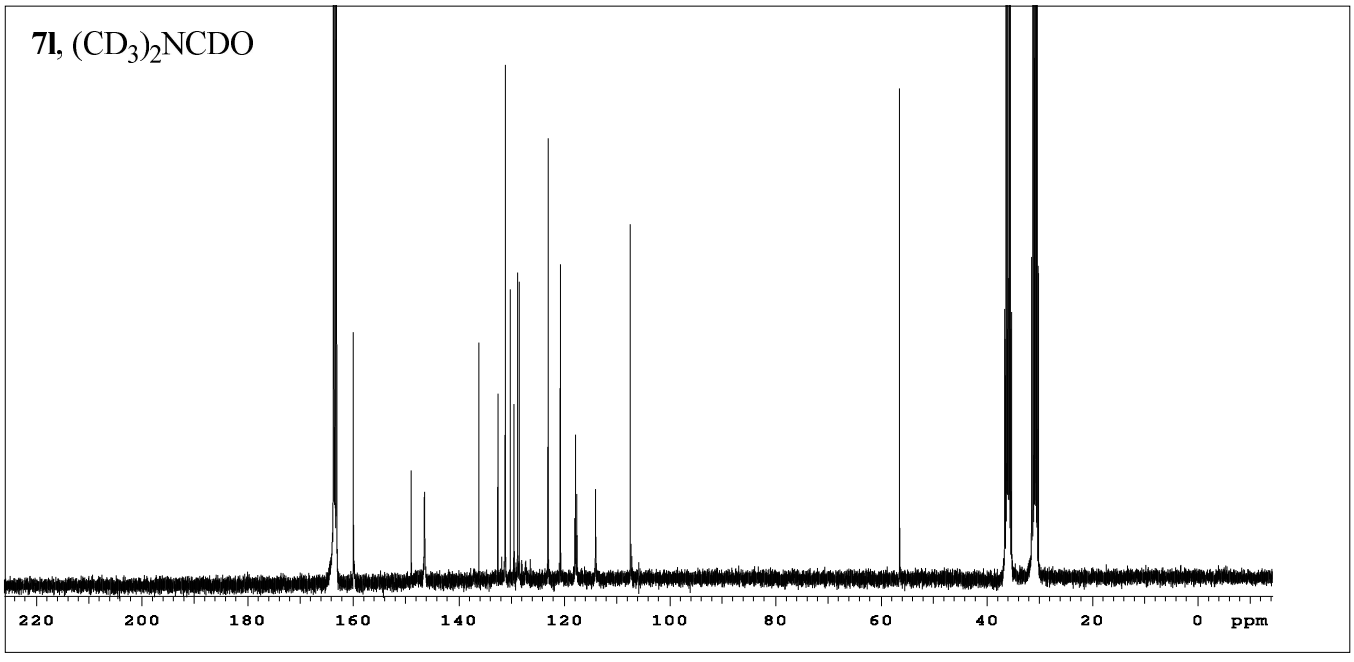

SQ_gis1492 #83 RT: 1.41 AV: 1 NL: 2.12E6
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance
394.1
395.1
238.7
259.3
101.3
396.2
m/z

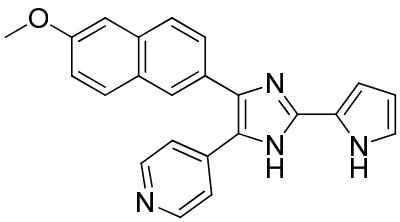


7l, $(CD_3)_2NCDO:CD_3OD$, 5:1

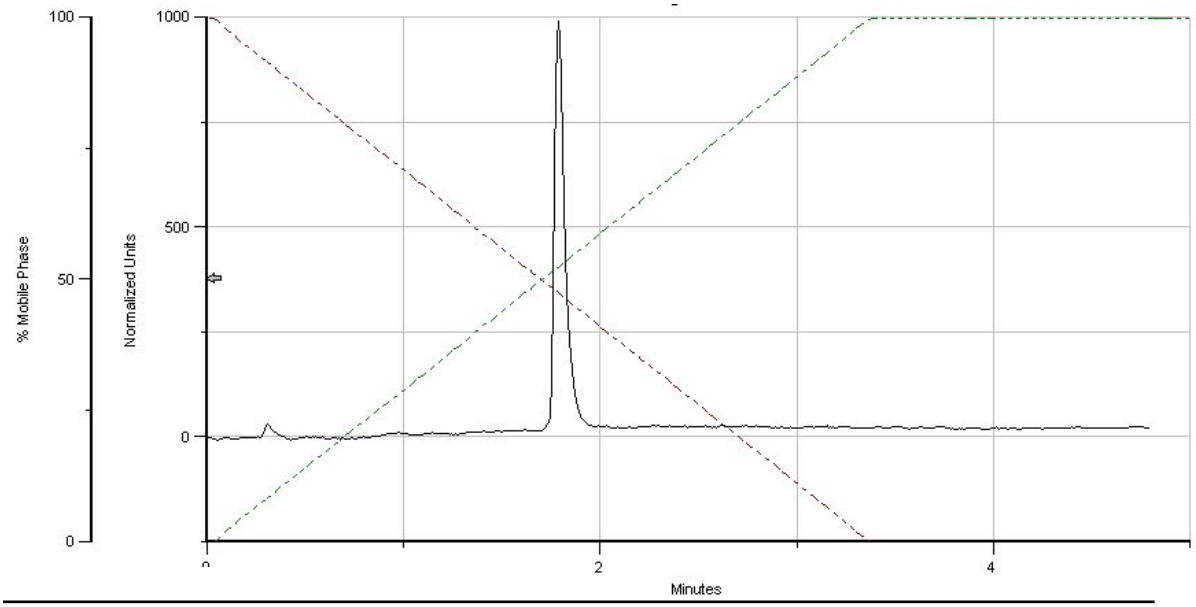


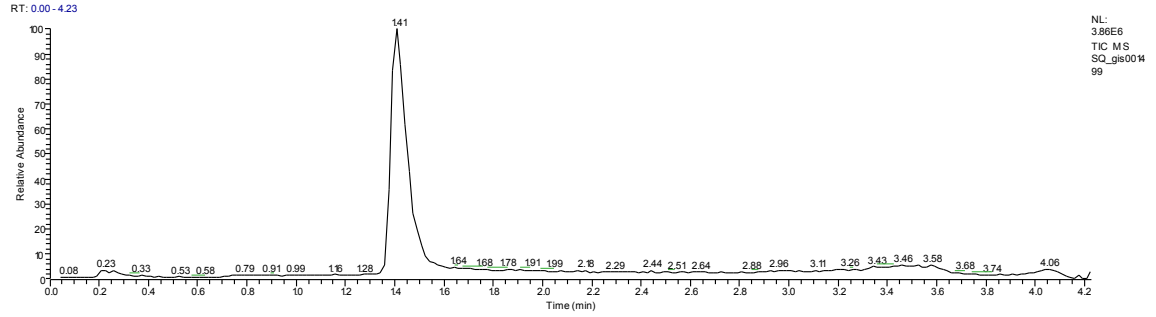
7l, $(CD_3)_2NCDO$

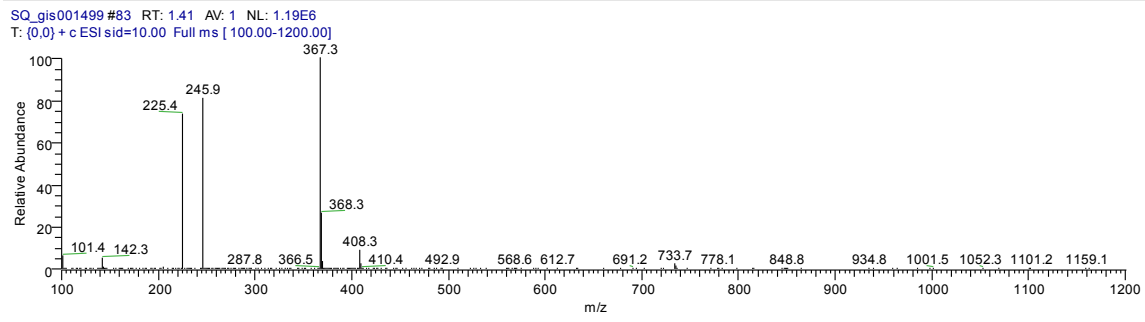


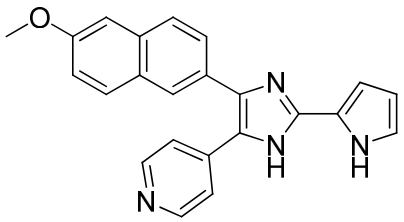


7m

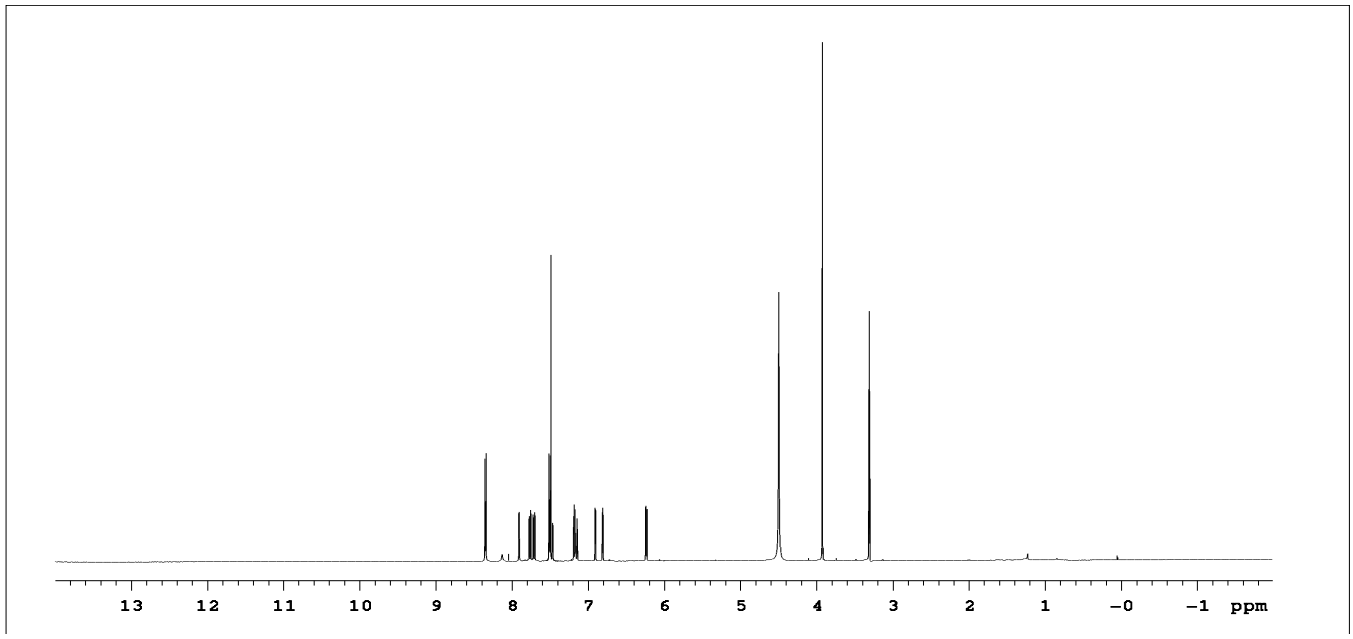

% Mobile Phase
Normalized Units
Minutes

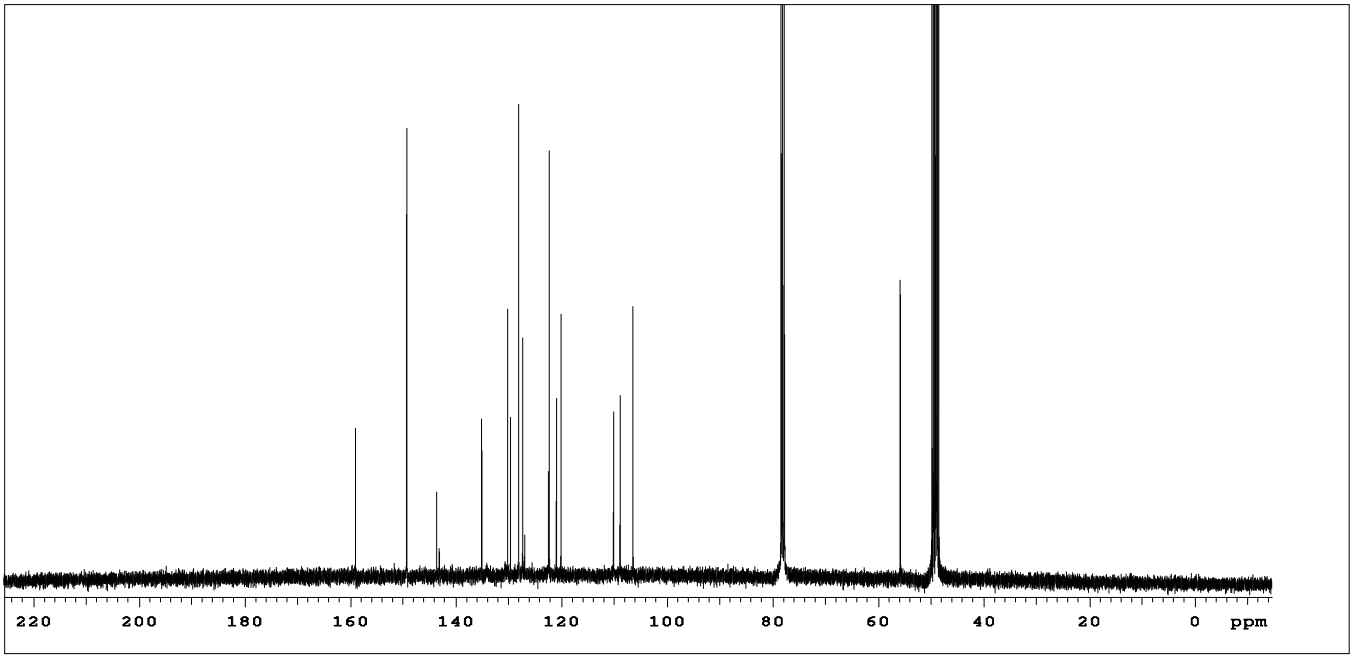

RT: 0.00 - 4.23
NL:
3.86E6
TIC MS
SQ_gis0014
99
Relative Abundance
Time (min)

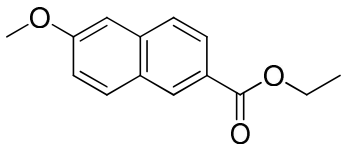

SQ_gis001499 #83 RT: 1.41 AV: 1 NL: 1.19E6
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance
m/z



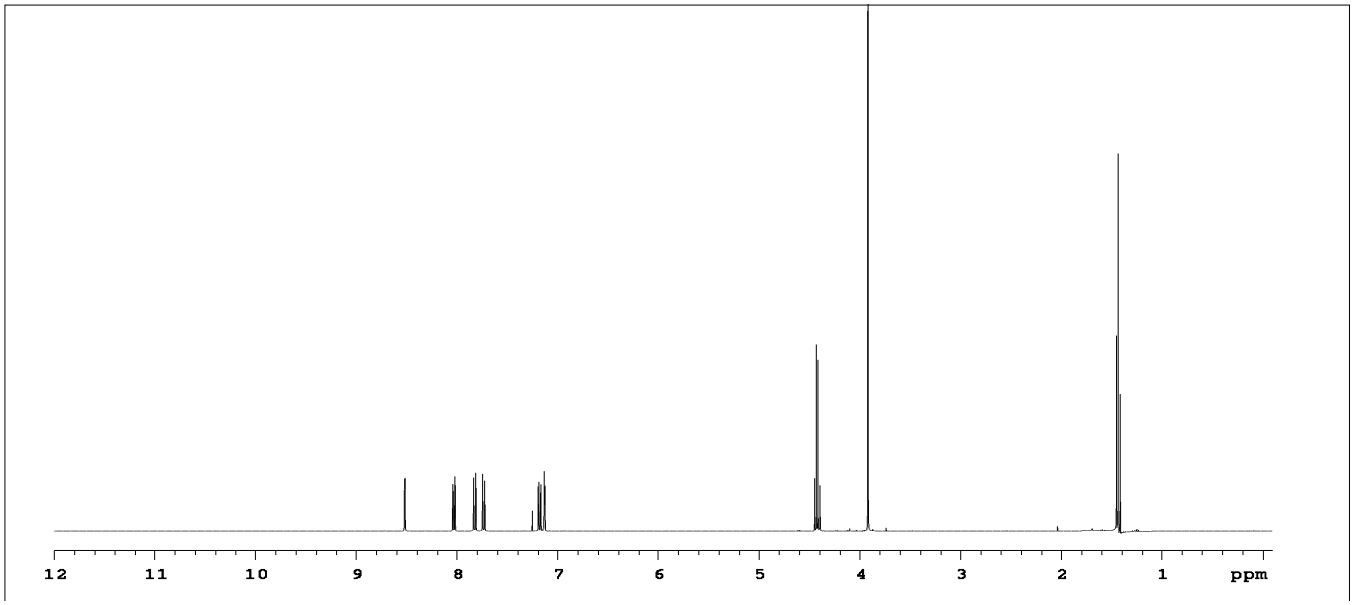
7m, CD_3OD:$CDCl_3$, 1:1

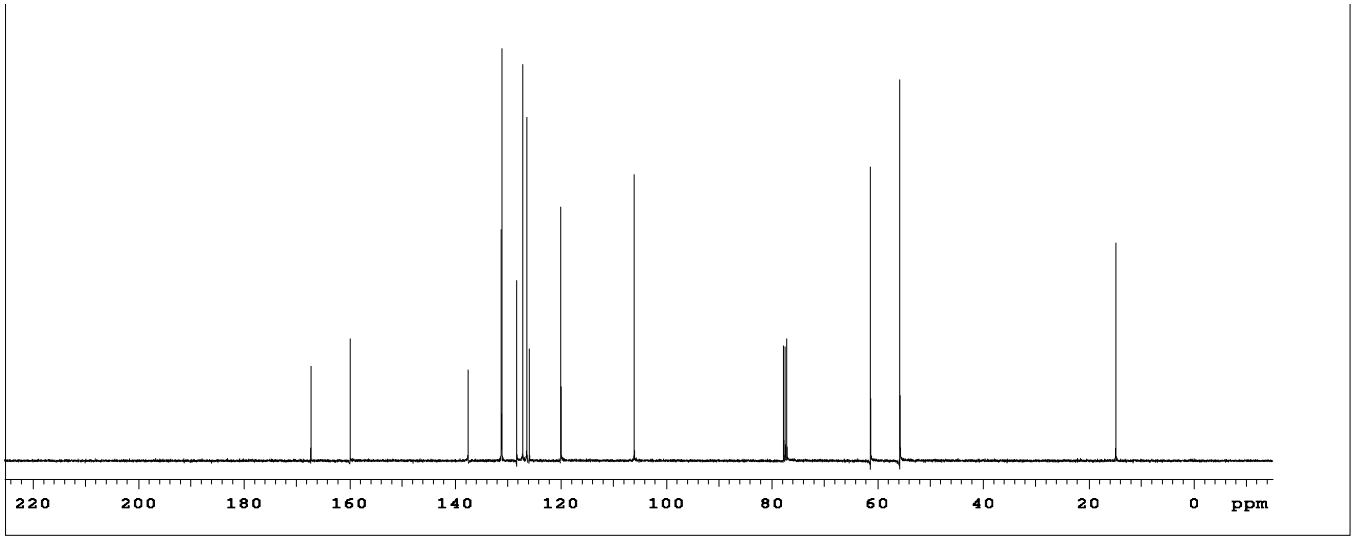


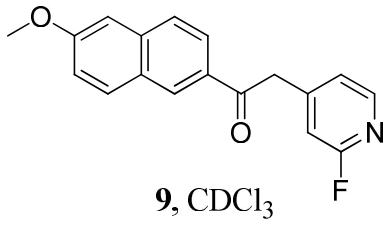




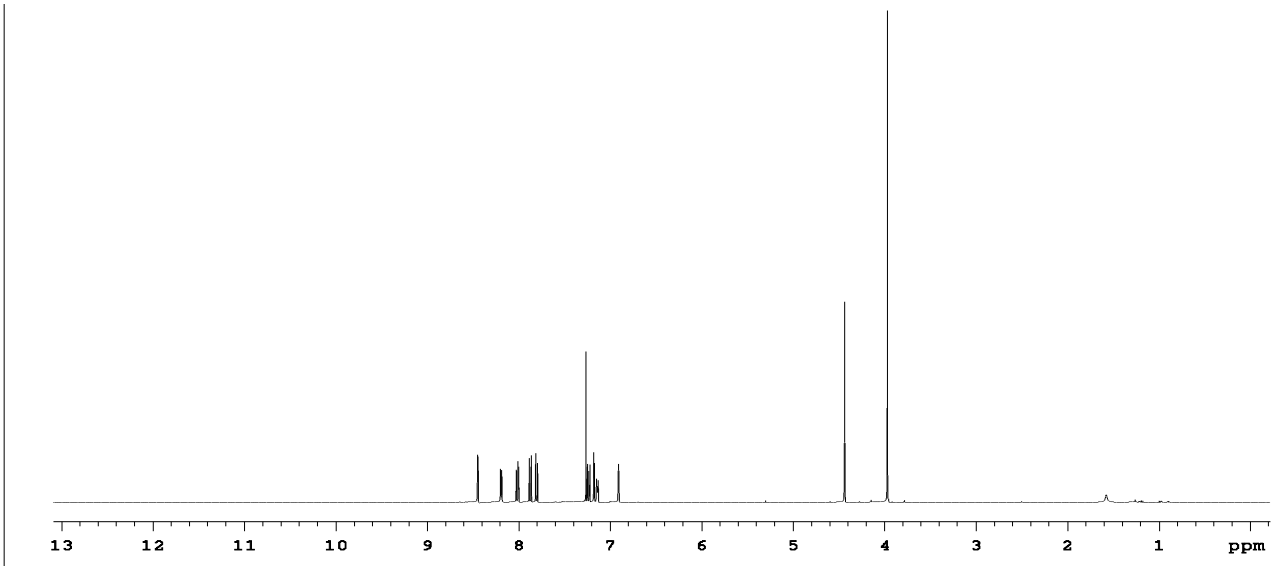
8, $CDCl_3$

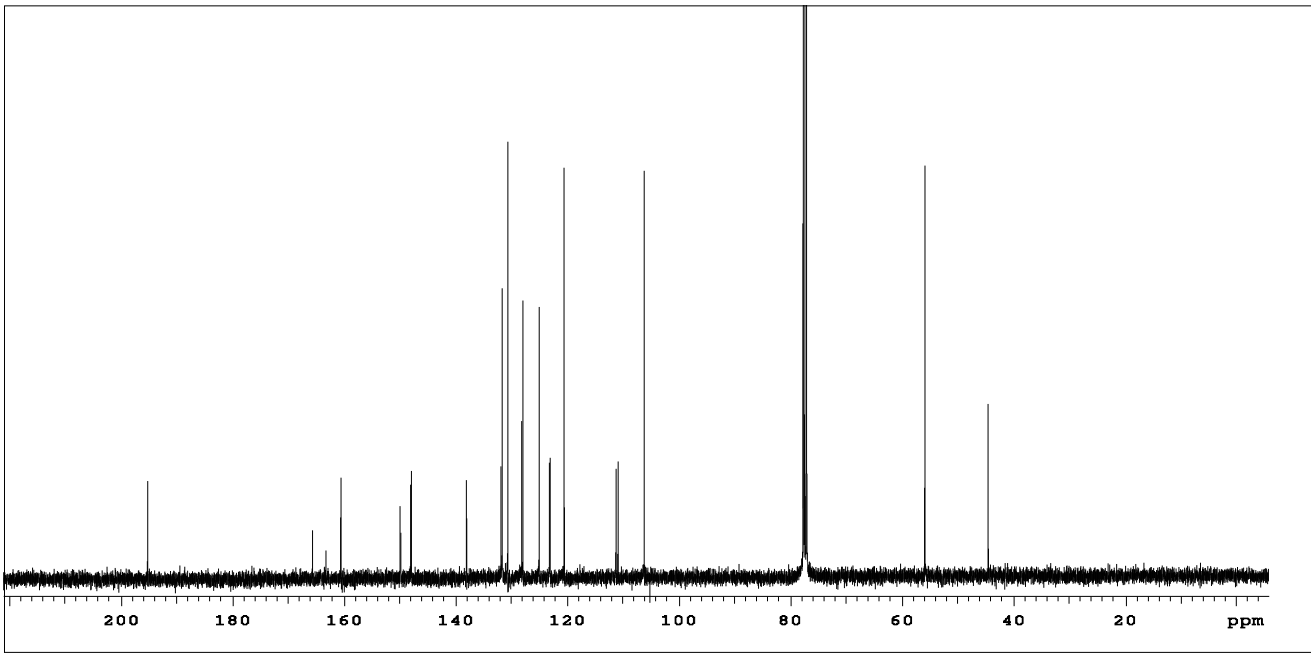

12
11
10
9
8
7
6
5
4
3
2
1
ppm

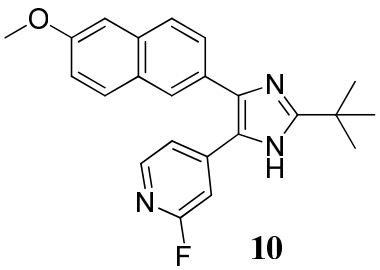

220
200
180
160
140
120
100
80
60
40
20
0
ppm


O
O
N
F

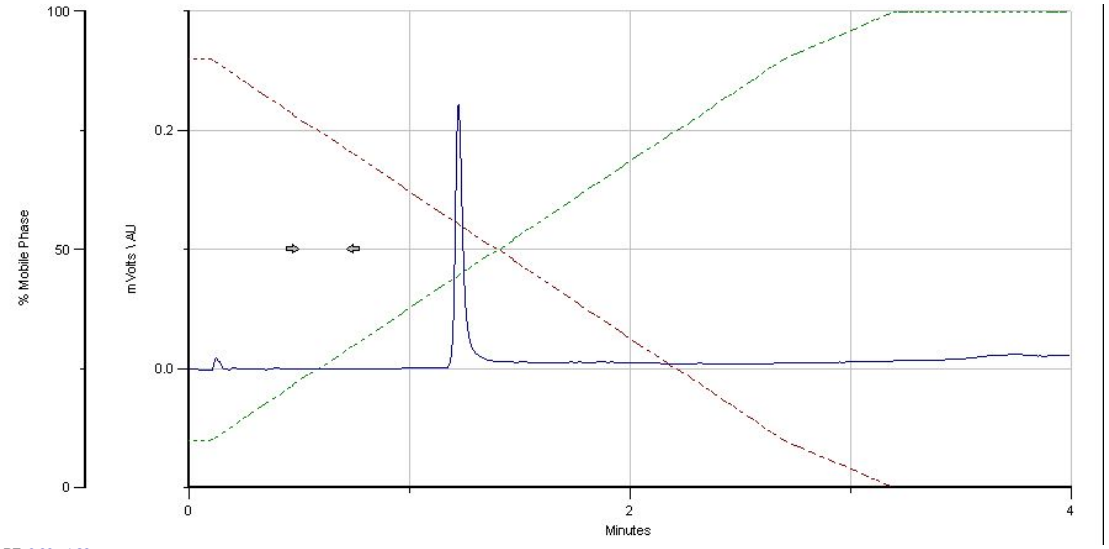
9, $CDCl_3$

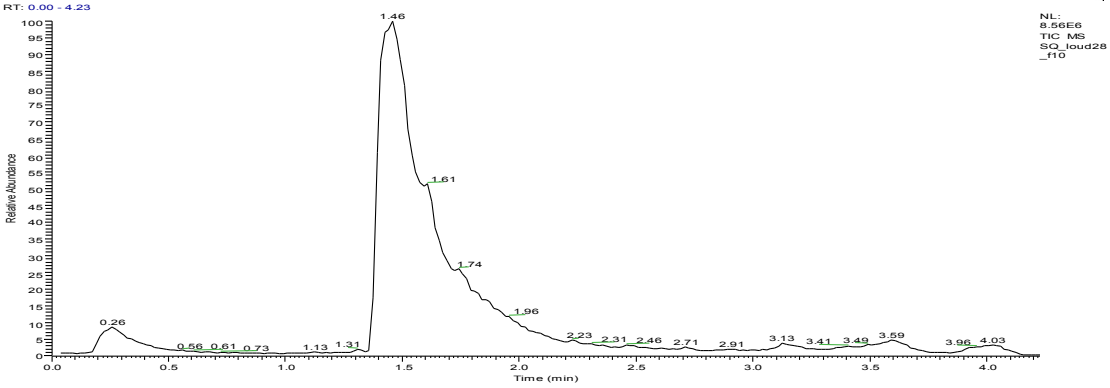

13
12
11
10
9
8
7
6
5
4
3
2
1
ppm

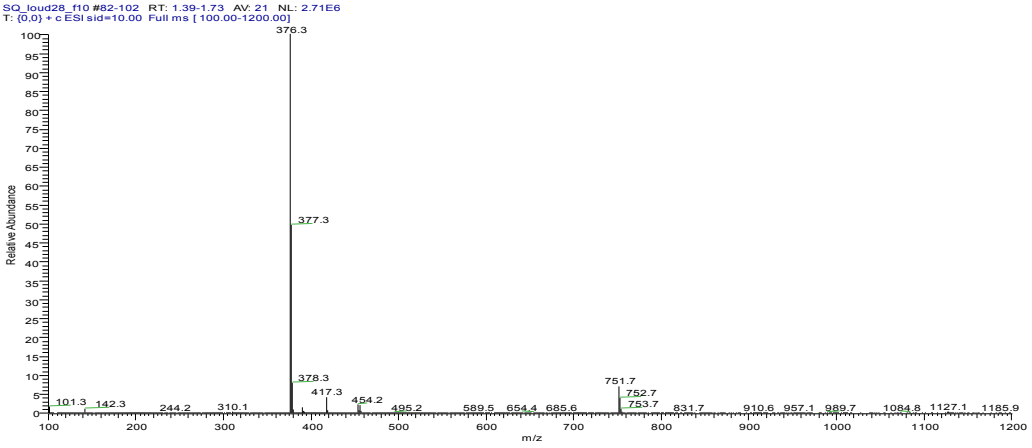

200
180
160
140
120
100
80
60
40
20
ppm

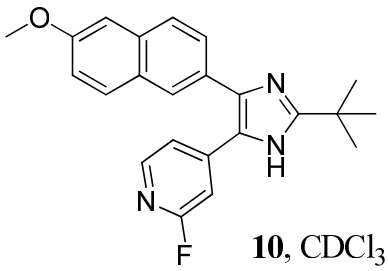


10

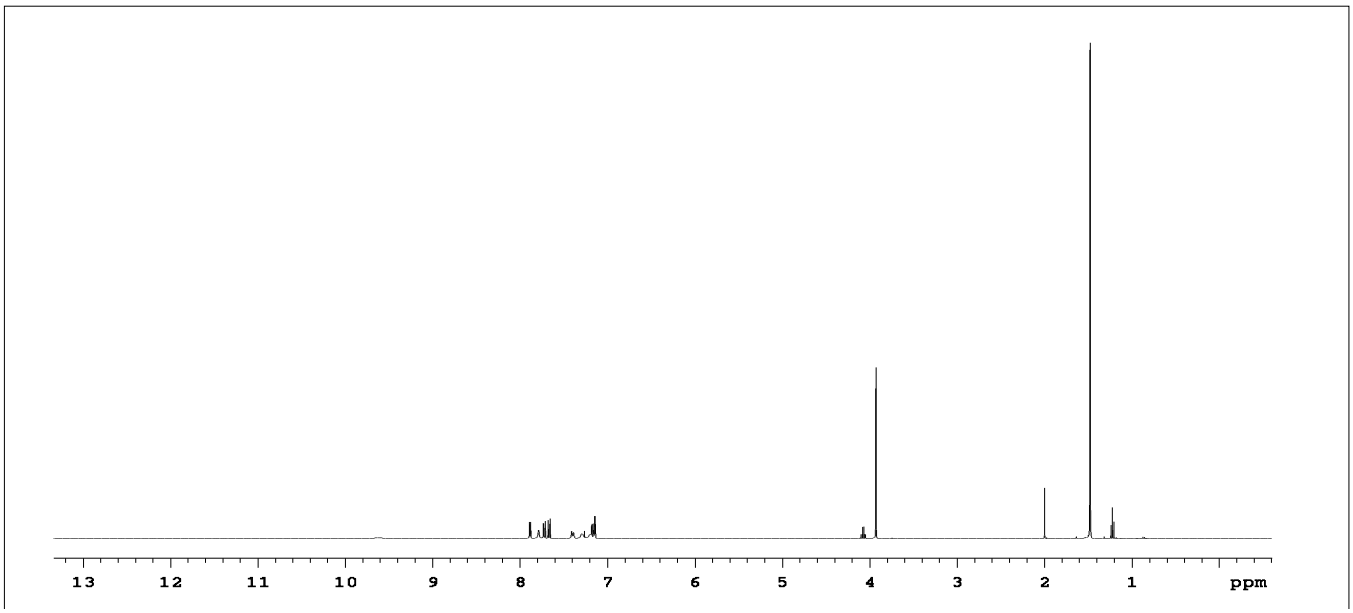

100
50
0
% Mobile Phase
0.2
0.0
m Volts \.AU
0
2
4
Minutes

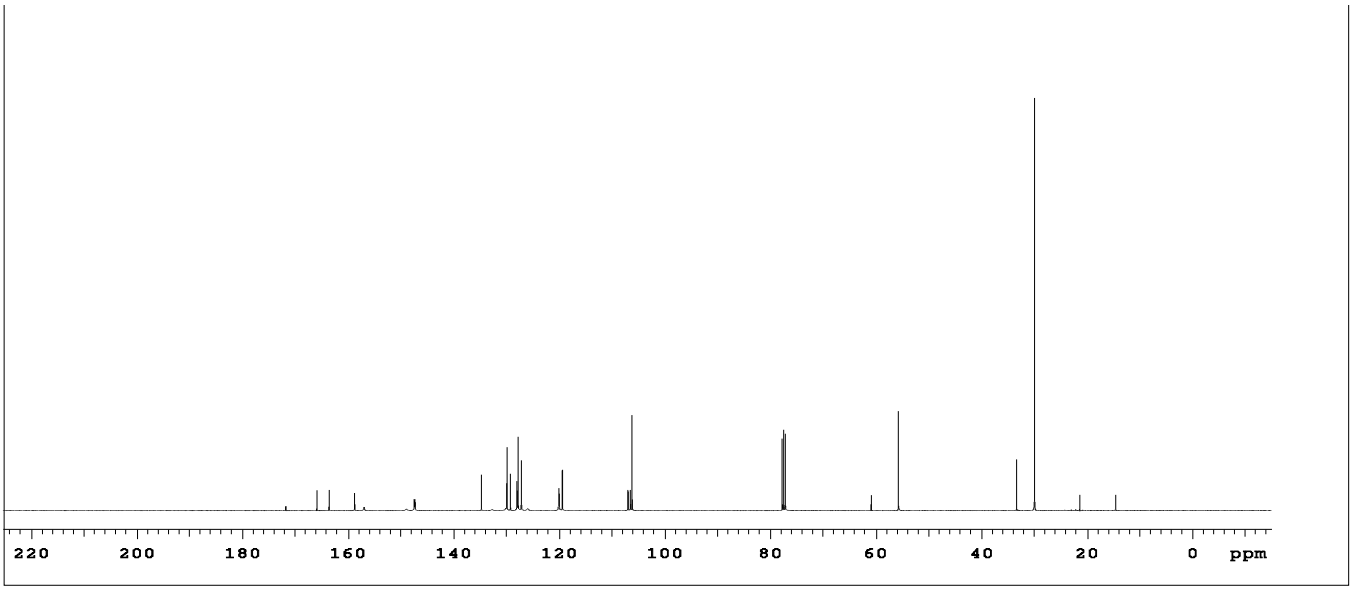

RT: 0.00 - 4.23
NL:
8.56E6
TIC MS
SQ_loud28
_f10
Relative Abundance
Time (min)
1.46
1.61
1.74
1.96
0.26

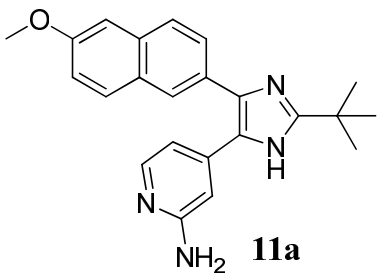

SQ_loud28_f10 #82-102 RT: 1.39-1.73 AV: 21 NL: 2.71E6
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance
m/z
376.3
377.3
378.3
751.7
752.7


O
N
N
H
N
F

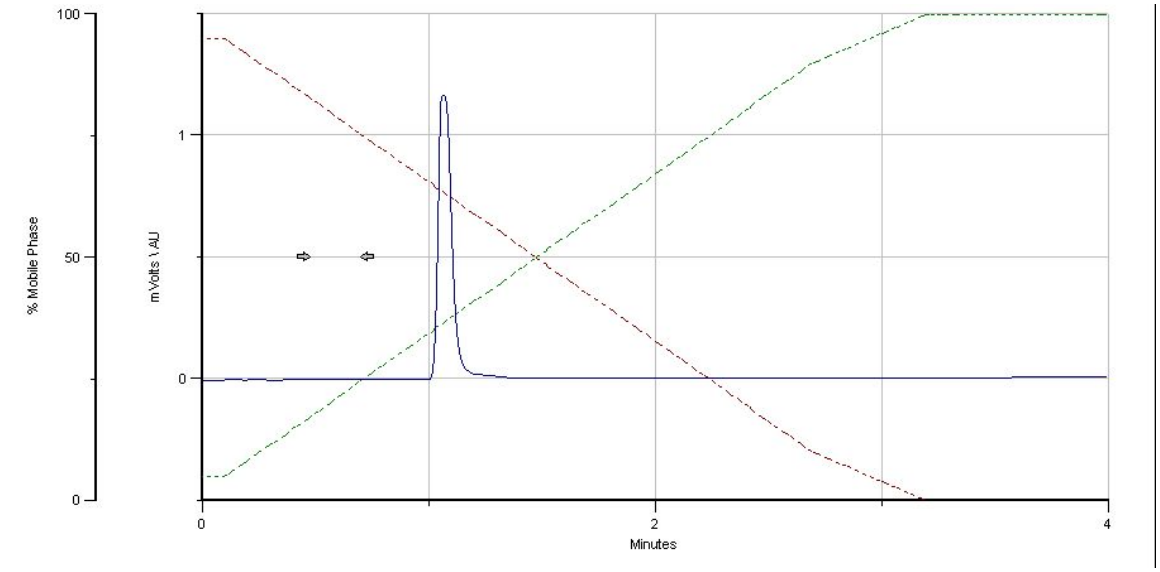
10, $CDCl_3$

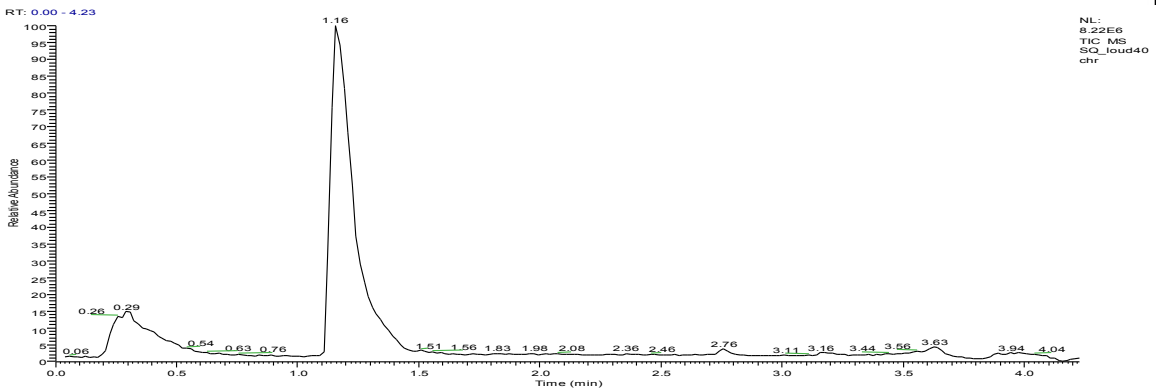

13
12
11
10
9
8
7
6
5
4
3
2
1
ppm

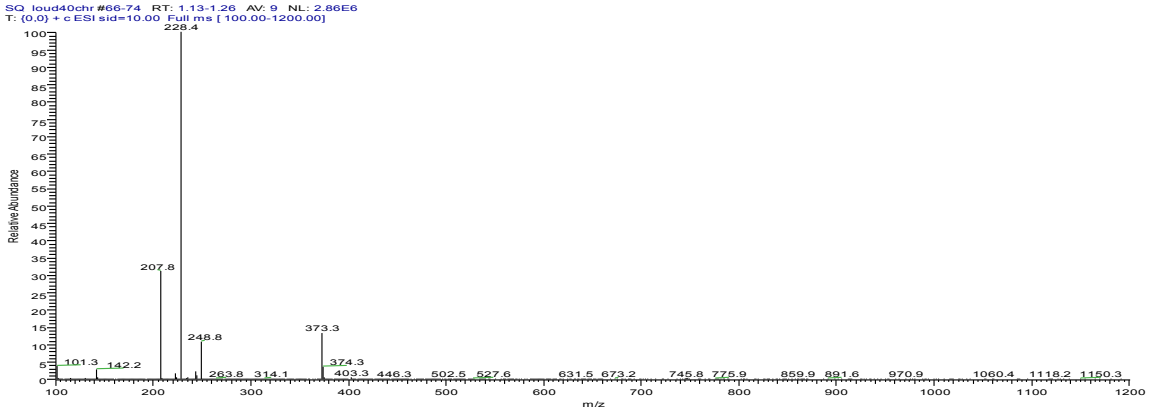

220
200
180
160
140
120
100
80
60
40
20
0
ppm

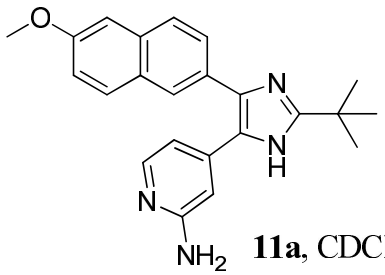

O
N
N
H
N
NH2

11a

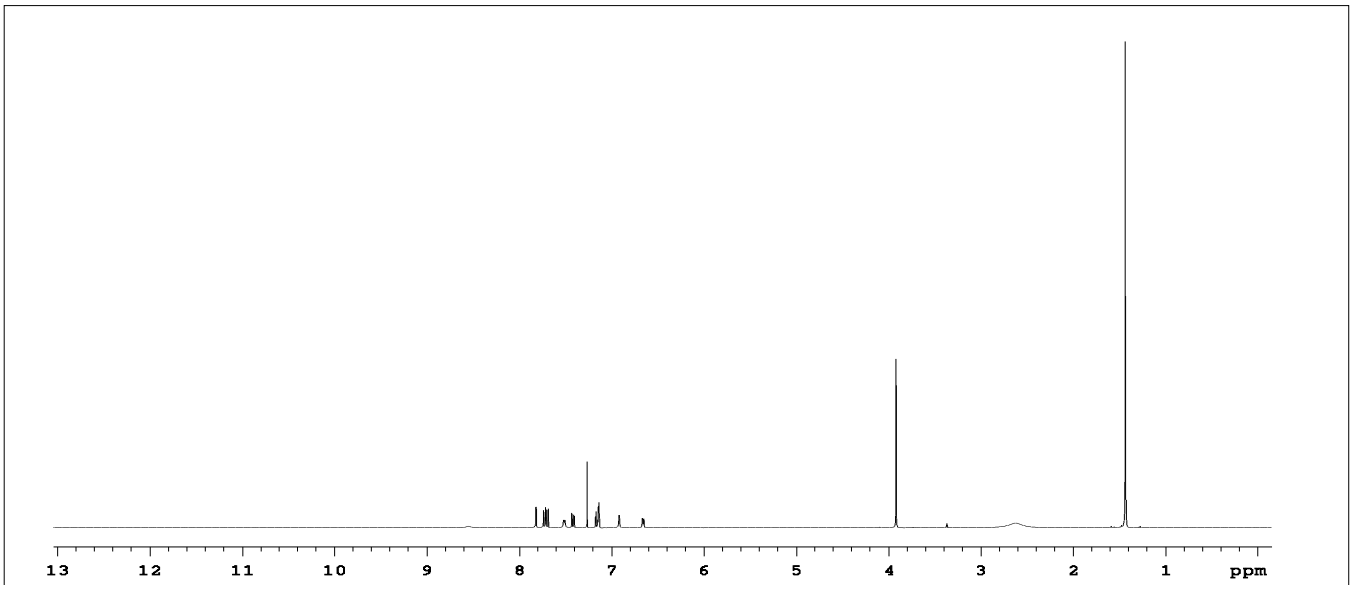

100
50
0
% Mobile Phase
1
0
mVolts \ AU
0
2
4
Minutes

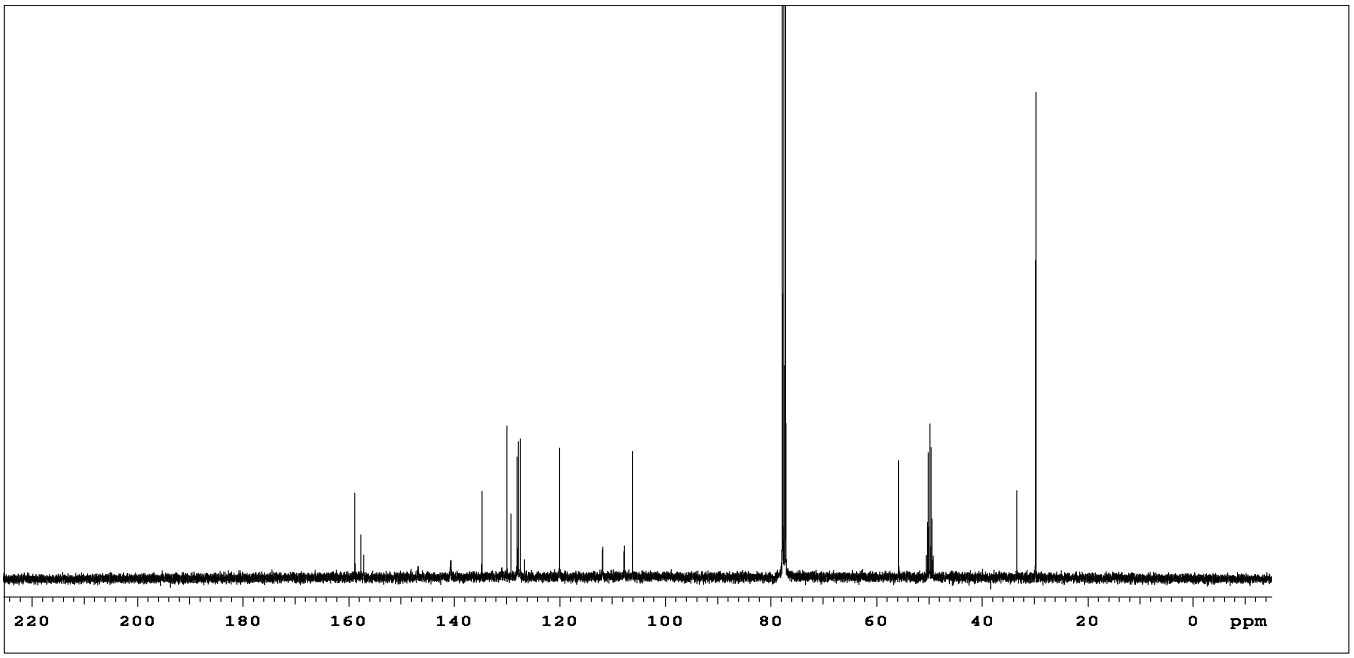

RT: 0.00 - 4.23
NL:
8.22E6
TIC MS
SQ_loud40
chr
Relative Abundance
1.16
0.26
0.29
0.06
0.54
0.63
0.76
1.51
1.56
1.83
1.98
2.08
2.36
2.46
2.76
3.11
3.16
3.44
3.56
3.63
3.94
4.04
Time (min)

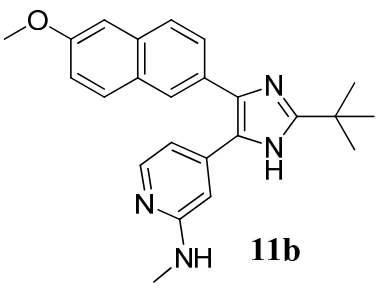

SQ loud40chr #66-74 RT: 1.13-1.26 AV: 9 NL: 2.86E6
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance
228.4
207.8
248.8
373.3
374.3
101.3
142.2
263.8
314.1
403.3
446.3
502.5
527.6
631.5
673.2
745.8
775.9
859.9
891.6
970.9
1060.4
1118.2
1150.3
m/z


O
N
N
H
N
NH2

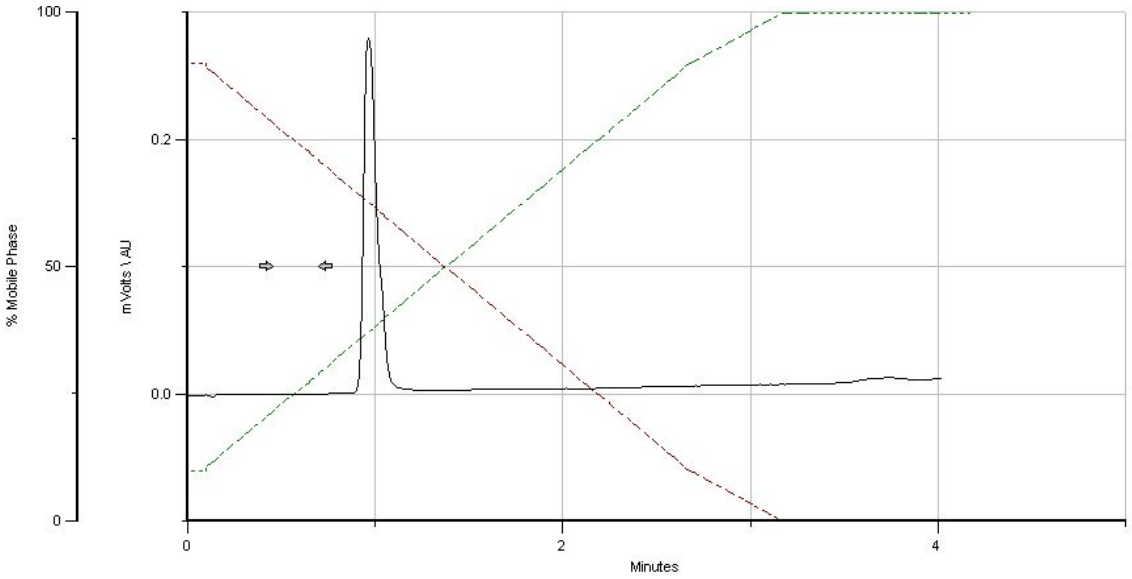
11a, $CDCl_3$

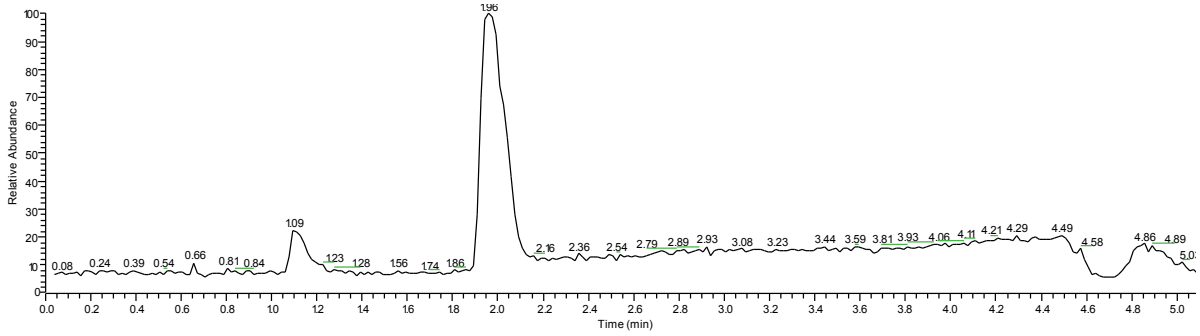

13
12
11
10
9
8
7
6
5
4
3
2
1
ppm


220
200
180
160
140
120
100
80
60
40
20
0
ppm

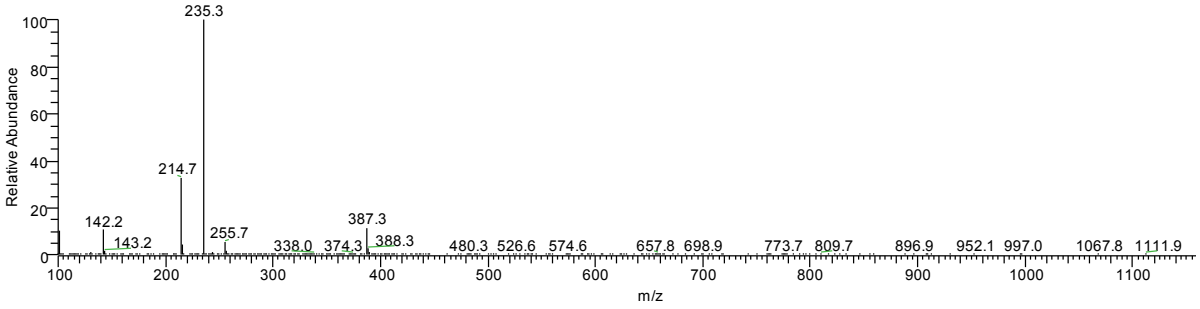


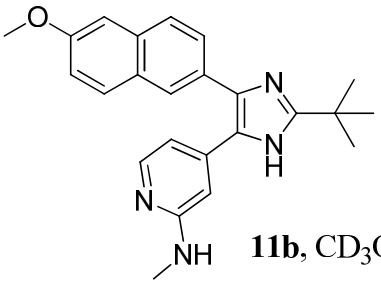
11b



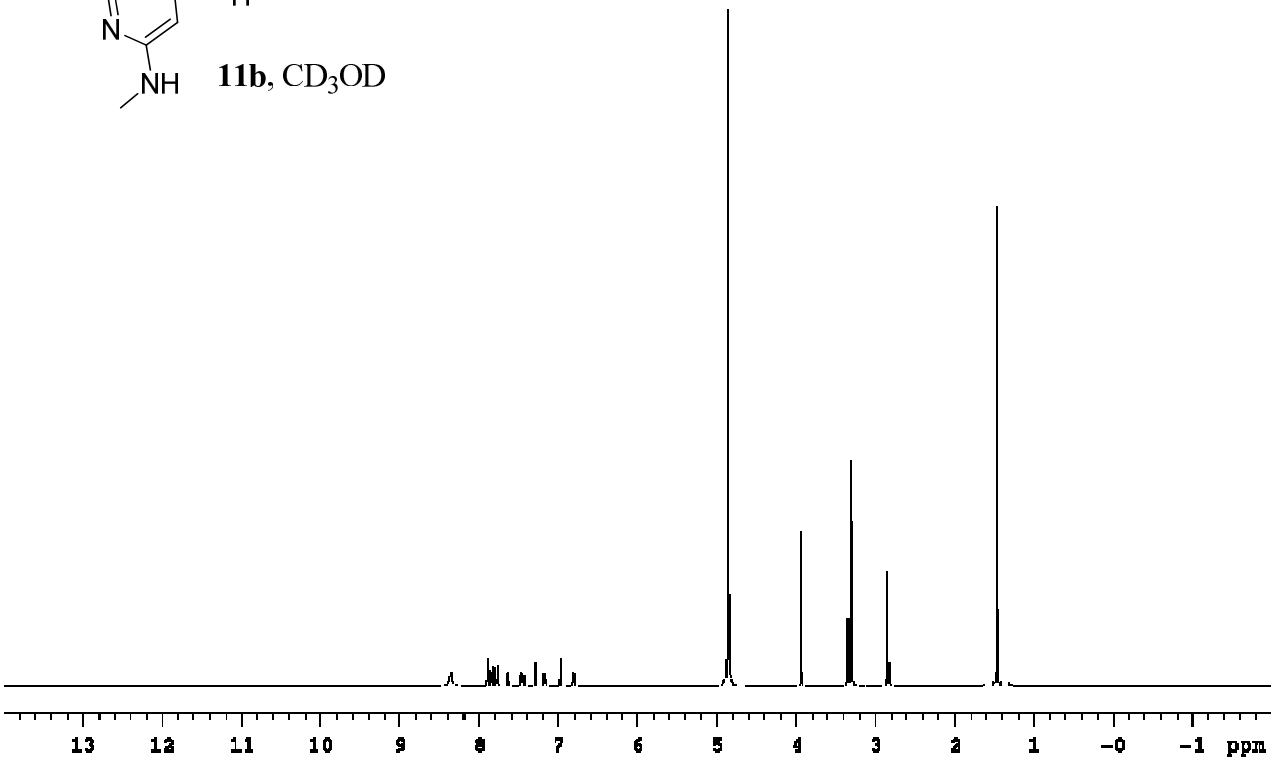


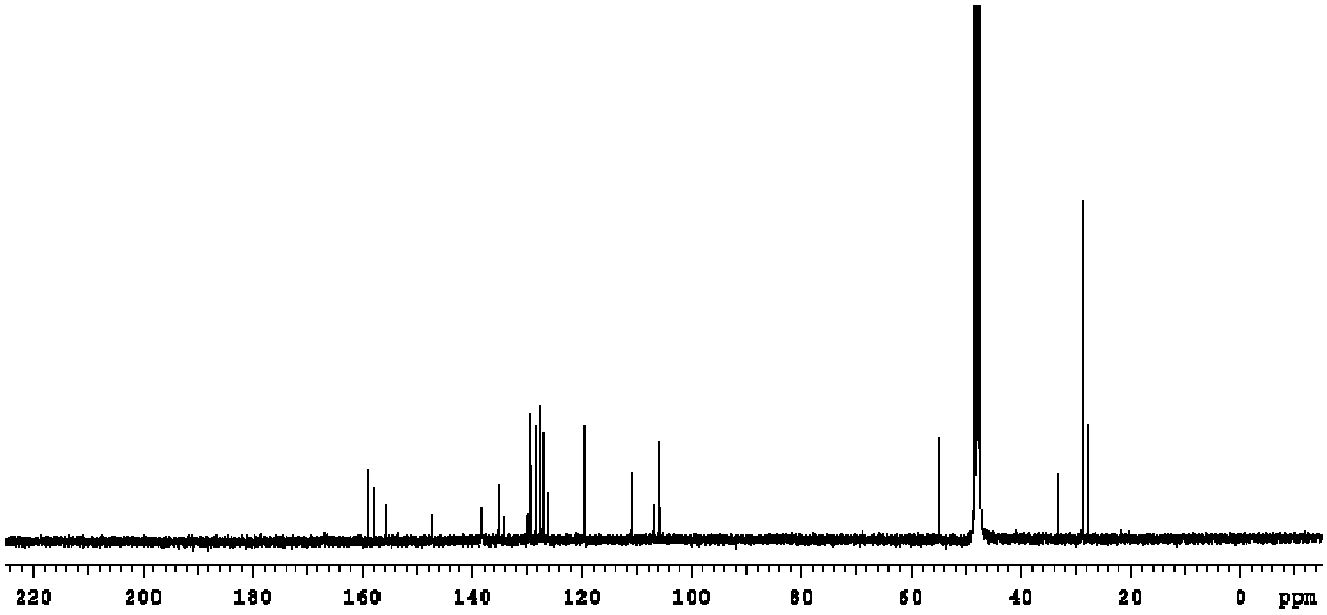
SQ_SY0159_Pure #116 RT: 1.96 AV: 1 NL: 9.93E5
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]

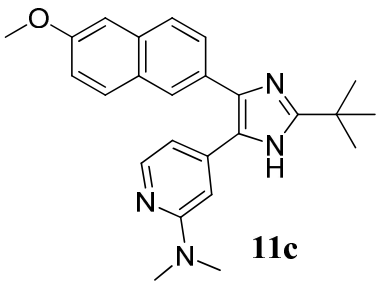



O
N
N
H
N
NH

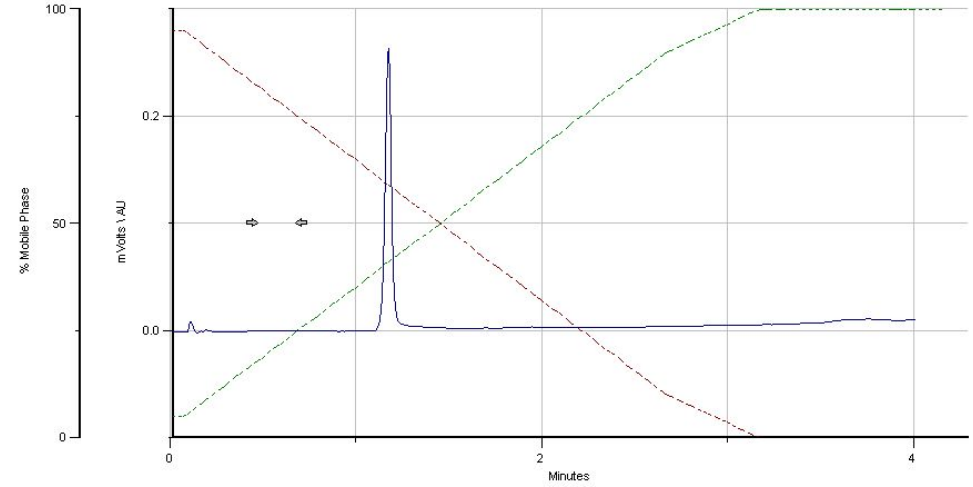
11b, CD_3OD

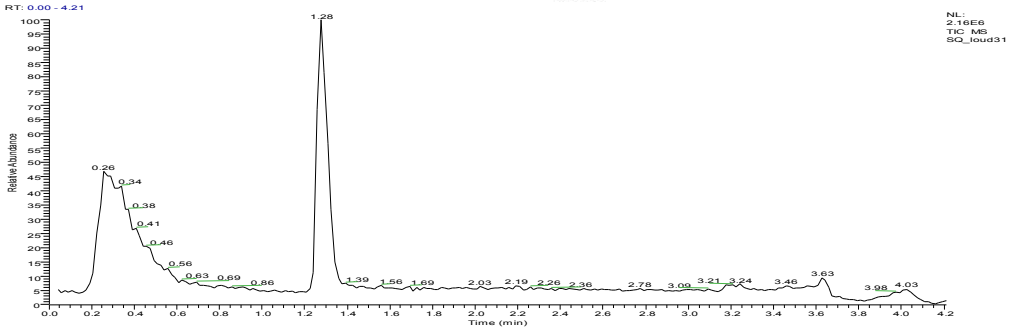

13
12
11
10
9
8
7
6
5
4
3
2
1
-0
-1
ppm

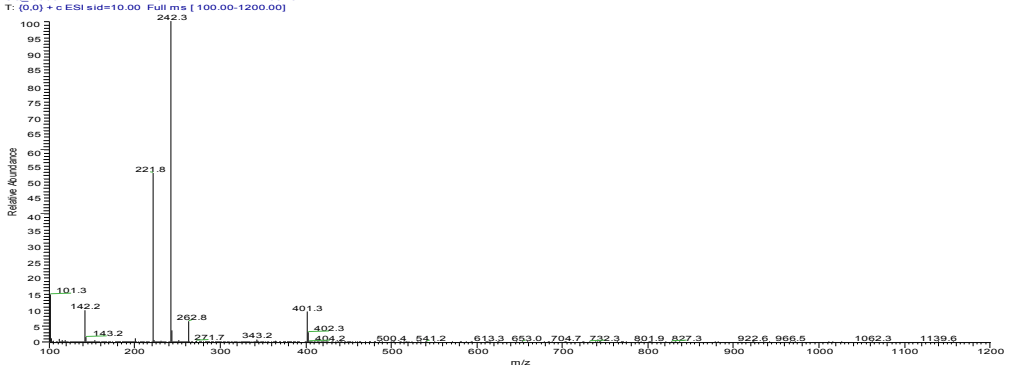

220
200
180
160
140
120
100
80
60
40
20
0
ppm

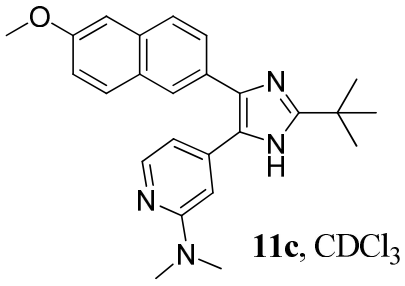


11c

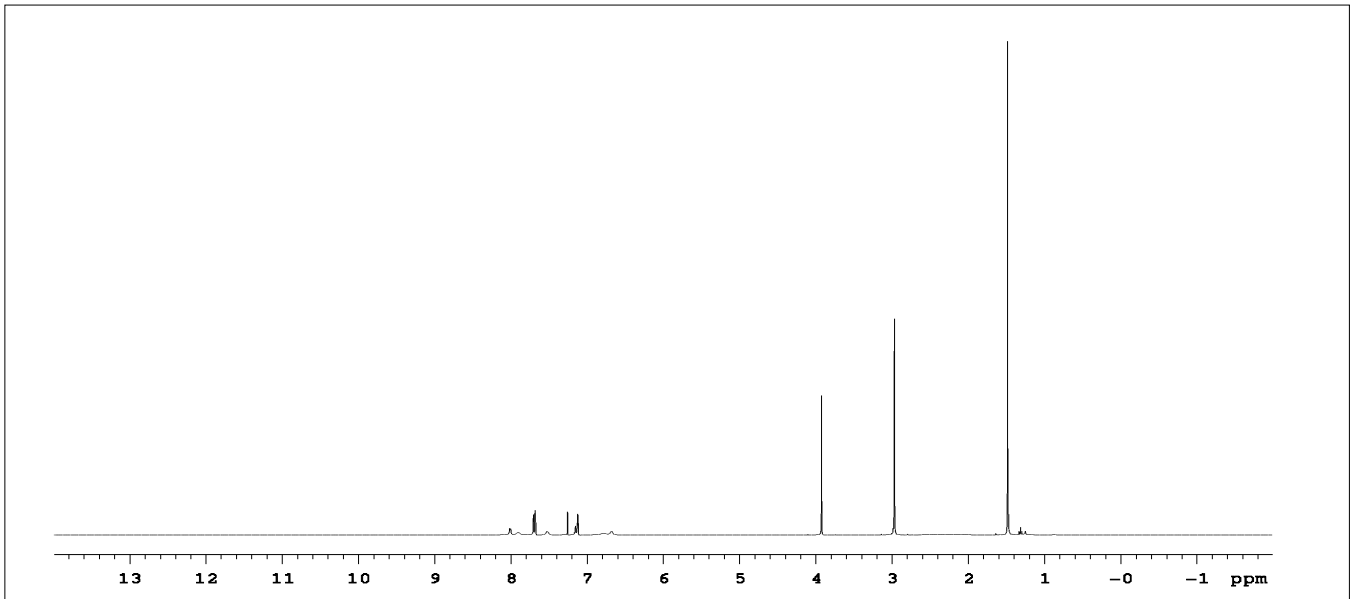

% Mobile Phase
mVolts \ AU
Minutes

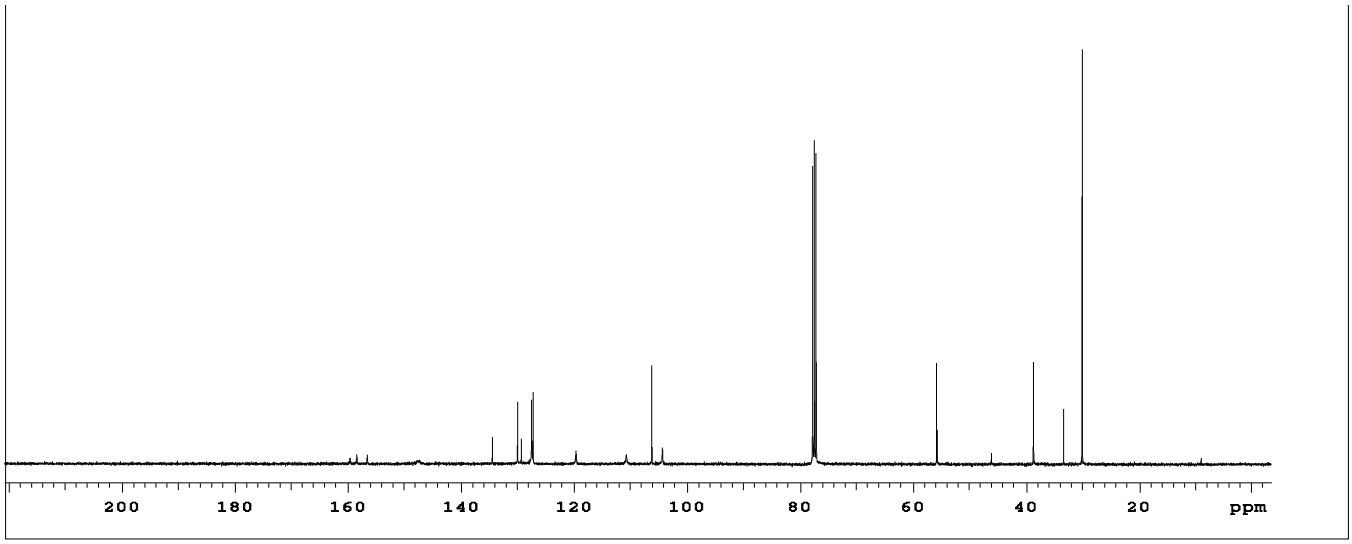

RT: 0.00 - 4.21
Relative Abundance
Time (min)
NL:
2.16E6
TIC MS
SQ_loud31

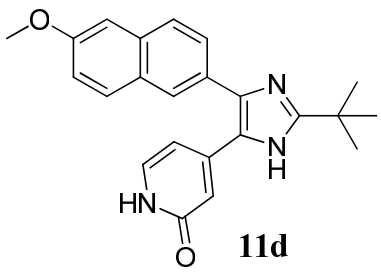

SQ_loud31 #74-77 RT: 1.26-1.31 AV: 4 NL: 7.58E5
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance
m/z



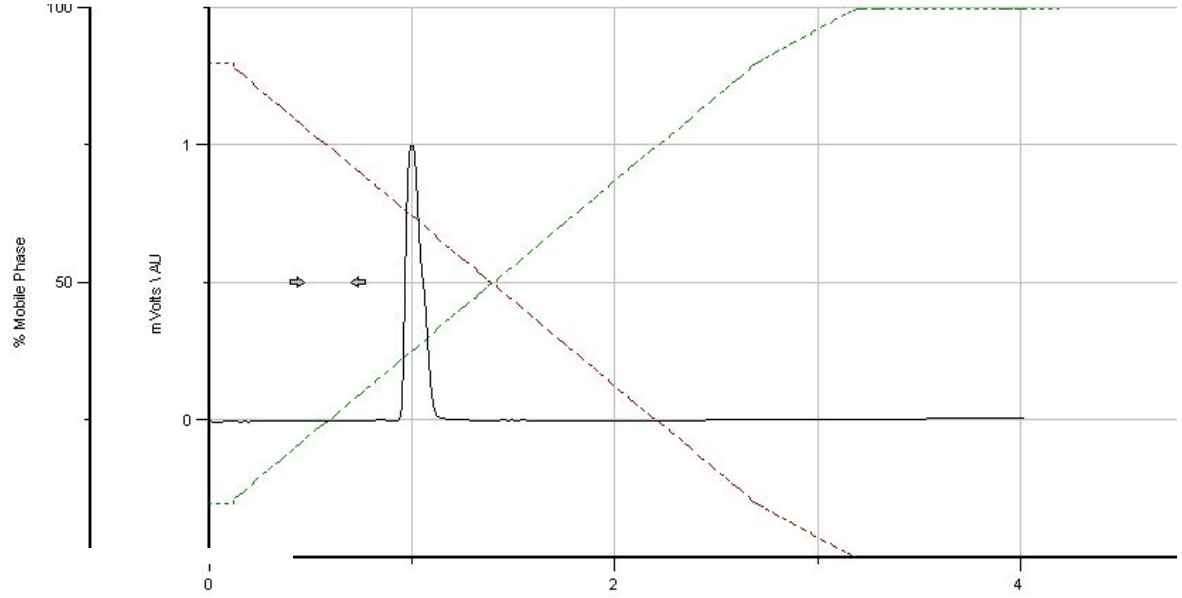
11c, $CDCl_3$

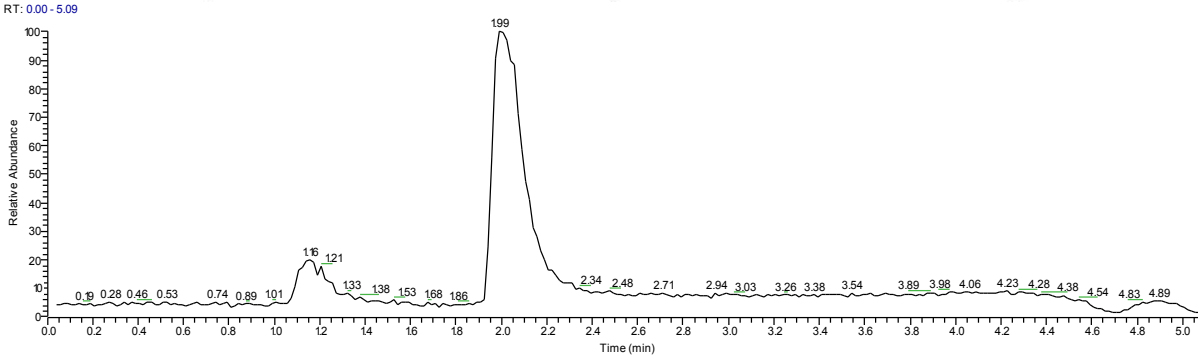

13
12
11
10
9
8
7
6
5
4
3
2
1
−0
−1
ppm

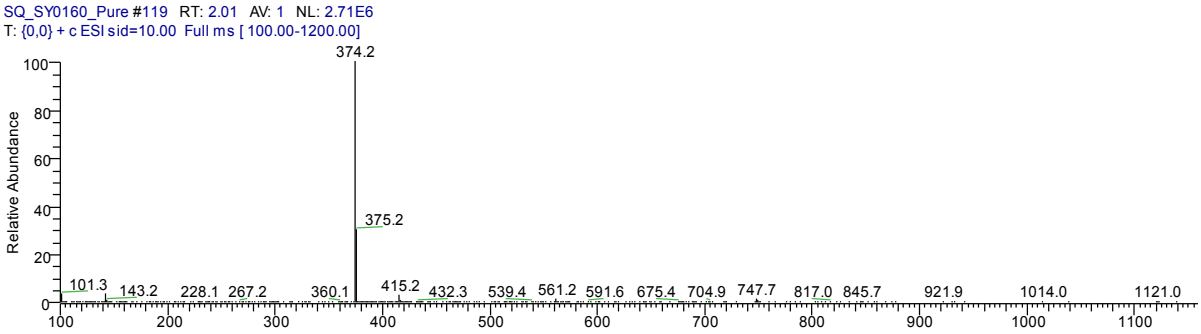

200
180
160
140
120
100
80
60
40
20
ppm

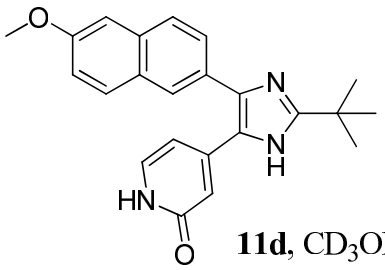

O
N
N
H
HN
O

11d

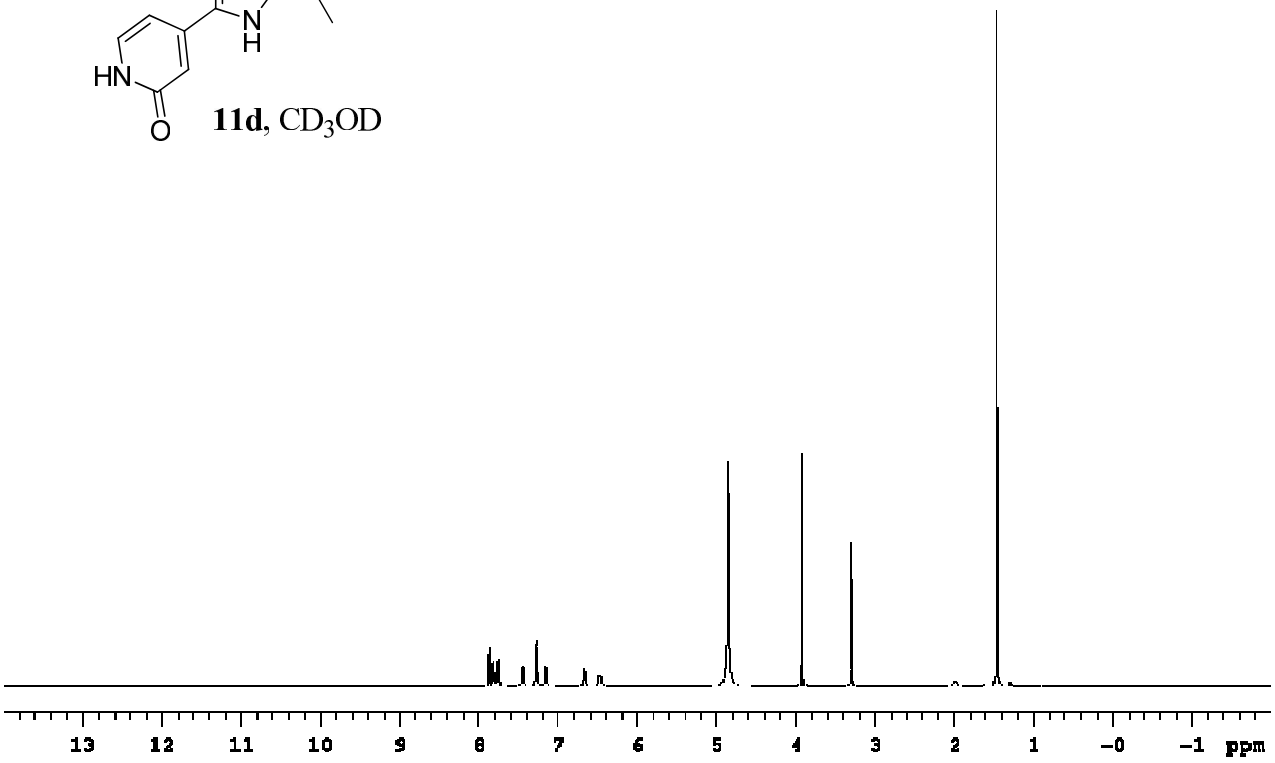

% Mobile Phase
mVolts \ AU
100
50
0
1
0
2
4

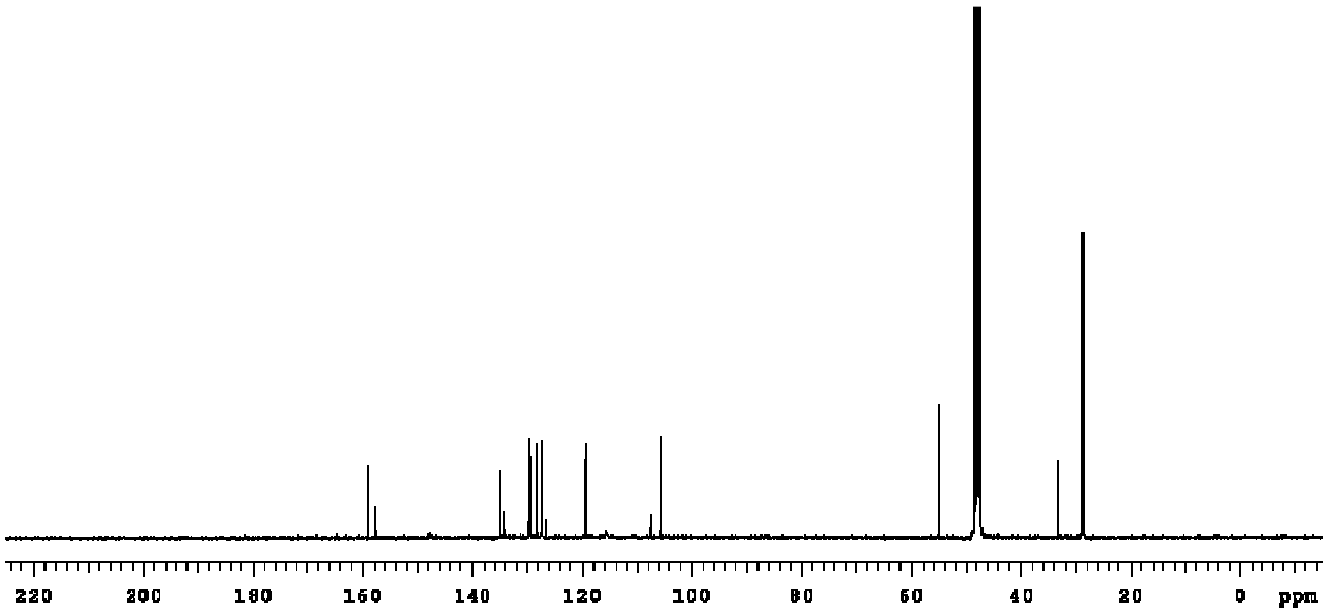

RT: 0.00 - 5.09
Relative Abundance
Time (min)
199
116
121
133
138
153
168
186


SQ_SY0160_Pure #119 RT: 2.01 AV: 1 NL: 2.71E6
T: {0,0} + c ESI sid=10.00 Full ms [100.00-1200.00]
Relative Abundance
374.2
375.2
101.3
143.2
228.1
267.2
360.1
415.2
432.3
539.4
561.2
591.6
675.4
704.9
747.7
817.0
845.7
921.9
1014.0
1121.0


O
N
N
H
HN
O

11d, CD_3OD


13
12
11
10
9
8
7
6
5
4
3
2
1
−0
−1
ppm


220
200
180
160
140
120
100
80
60
40
20
0
ppm