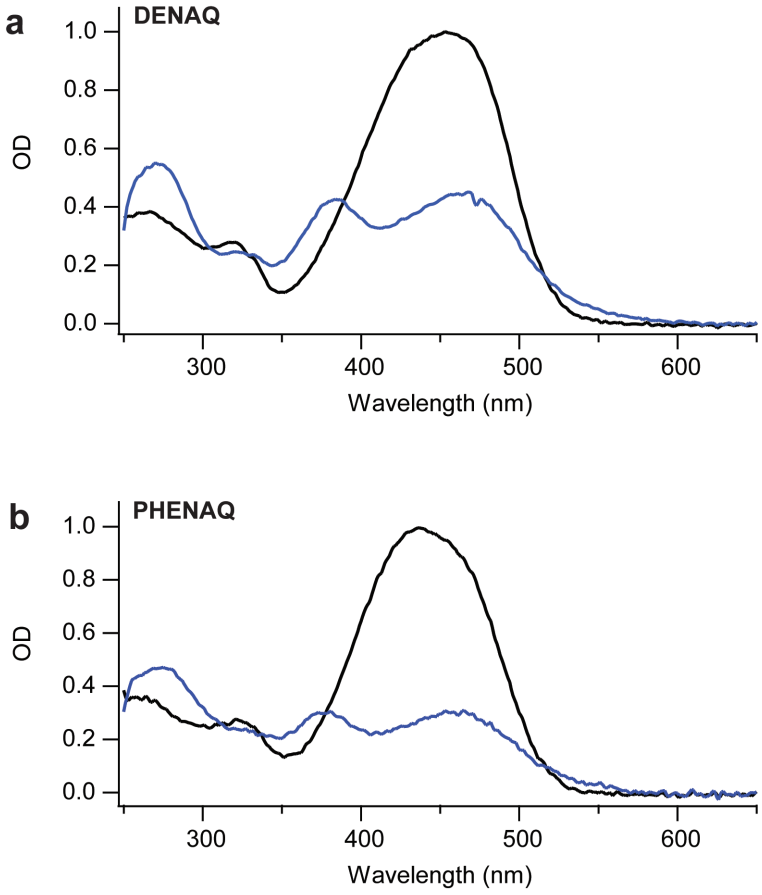
Tuning Photochromic Ion Channel Blockers

Alexandre Mourot, Michael A. Kienzler,* Matthew R. Banghart, Timm Fehrentz,*

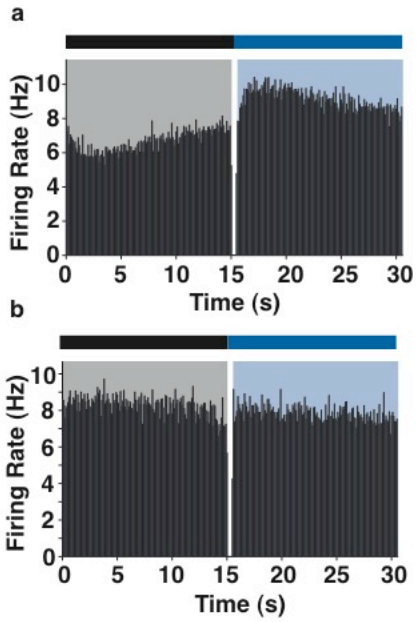
Florian M. E. Huber, Marco Stein, Richard H. Kramer and Dirk Trauner

Supplementary Information

Supplementary Figure 1: Photoisomerization of **DENAQ** and **PhENAQ** in DMSO solution. UV/VIS spectra of a) **DENAQ** and b) **PhENAQ** in the dark adapted, presumably all *trans* state (black) and at the photostationary states produced under constant 473 nm laser illumination (blue).



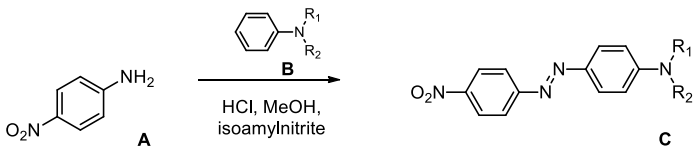
Supplementary Figure 2: Multi-electrode array recording from acute rat cerebellar slices. Condensed firing rate (average firing rate calculated in 100 ms time bins, 10 cycles of 480 nm / dark averaged) for a) a slice treated with 50 μM **PhENAQ** (same slice as in Figure 5d) and b) a control slice.



General Experimental Details. Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification. Unless otherwise noted, all reaction mixtures were magnetically stirred in oven-dried glassware under a nitrogen atmosphere. External bath temperatures were used to record all reaction mixture temperatures. Analytical thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} TLC plates. TLC visualization was accomplished using 254 nm UV light, 0.1% HCl in MeOH or a charring solution of cerric ammonium molybdenate. All aqueous solutions were saturated unless otherwise noted. Normal phase flash chromatography was performed on Dynamic Adsorbents Silica Gel (40–63 μm particle size) using a forced flow of eluant at 1.3–1.5 bar pressure. Reverse-phase chromatography was carried out

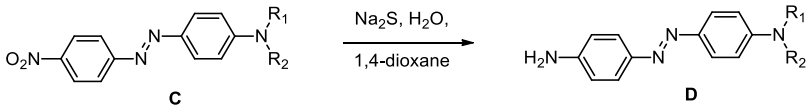
with Waters Preparative C18 Silica Gel WAT010001 125 Å. Yields refer to chromatographically and spectroscopically (^{1}H NMR and ^{13}C NMR) homogenous material. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on Varian ARX 200, AC 300, WH 400, or AMX 600 instruments. Chemical shifts (δ) are reported in ppm with the solvent resonance employed as the internal standard ($CDCl_3$ at δ = 7.26 and 77.0 ppm; DMSO-d_6 at δ = 2.50 and 39.5 ppm; Acetone-d_6 at δ = 2.05 and 206.3/29.8 ppm; Methanol-d_4 at δ = 3.31 and 49.0 ppm; THF-d_8 at δ = 3.58/1.73 and 67.4/25.2 ppm; D_2O at δ = 4.80/4.81 ppm). Chemical shifts of ^{19}F NMR spectra were calibrated to δ = 0 ppm using $CFCl_3$ as internal standard. The following abbreviations are used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Coupling constants (J_H and J_F) are reported in Hz. Infrared spectra were recorded in the of 4000–400 cm^{-1} on a Perkin Elmer BY FT-IR spectrometer using a Smiths Detection Dura Sample IR II Diamond ATR sensor for detection. Samples were prepared as neat film. UV/Visible spectra were recorded on a Varian Cary 50 Bio UV-Visible Spectrophotometer using Starna 29/B/12 quartz cuvettes with 10 mm section thickness. High resolution mass spectroscopy (HRMS) data was recorded using a Varian MAT 711 instrument by electrospray ionization (ESI) or using a Varian MAT CH 7A by electron impact (EI) techniques.

Experimental Procedures.



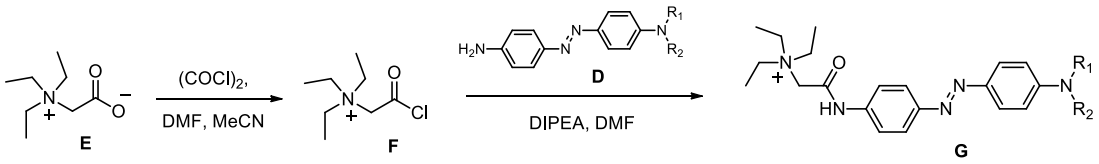
Standard Procedure 1: Azo coupling of a *p*-nitroaniline (**A**) with an aniline (**B**) to form the corresponding azobenzene derivatives (**C**).

1.0 Eq. of *p*-nitroaniline (**A**) was dissolved in methanol and cooled in an ice bath to 0 °C, at which point 12M HCl was added. The resulting solution was stirred for five minutes, and then 1.0 eq. of isoamyl nitrite was added over a period of five minutes. The reaction was stirred for one hour at 0 °C. Meanwhile, 1.0 eq. of aniline **B** was dissolved in water or methanol, depending on solubility, and 12M HCl and cooled to 0 °C. To this solution, the diazonium salt was added over a period of ten minutes. The solution turned deep red and was stirred for another 1.5 hours at 0 °C. Once all starting material was consumed as gauged by TLC, the reaction mixture was poured on an ice-cooled saturated solution of $NaHCO_3$. The crude product was extracted three times with ethyl acetate and, if red/orange color remained in the aqueous layer, twice with dichloromethane. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*.



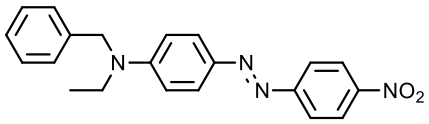
Standard Procedure 2: Reduction of an aromatic nitro group to the corresponding amine in the presence of an azobenzene group using sodium sulfide.

1.0 Eq. of *p*-nitroazobenzene **C** was dissolved in a 10:1 mixture of 1,4-dioxane and water. To this solution 1.0 eq. of sodium sulfide was added, and the reaction mixture was heated to 90 °C for one hour. The reaction was monitored by TLC, and after every hour that starting material **C** was observed, another eq. of sodium sulfide was added. When all starting material was consumed, the solution was poured on a saturated solution of $NaHCO_3$. The crude product was extracted three times with dichloromethane and the combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*.



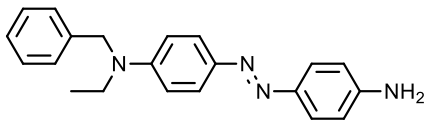
Standard Procedure 3: Amide bond formation between an azoaniline (**D**) and betaine (**E**) to form the corresponding azobenzene quaternary ammonium derivatives (**G**).

According to Fortin et al.[1] 5.0 eq. of betaine **E** were dissolved in acetonitrile. To this solution, 3 drops of DMF and 5.05 eq. of oxalyl chloride were added and the solution was stirred for 45 minutes at room temperature. The solvent then was removed *in vacuo* until all residual HCl had faded. The acid chloride **F** thus generated was partially dissolved in a 1:1 mixture of acetonitrile and DMF. This suspension was then was added dropwise to a flask containing 1.0 eq. of azoaniline **D** and 5.0 eq. of DIPEA in DMF at 0 °C over a period of ten minutes. The reaction was warmed to room temperature, stirred overnight, and then concentrated *in vacuo*.



S1

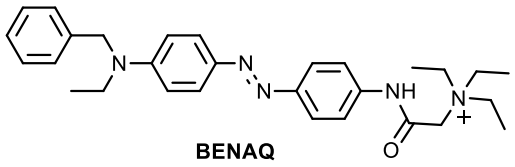
Nitroazobenzene S1. Following **standard procedure 1**, 1.38 g (10 mmol) of *p*-nitroaniline dissolved in 20 mL of MeOH and 5 mL of 12M HCl first reacted with 1.4 mL (10 mmol) of isoamylnitrite to form a diazonium salt, which then further reacted with 2.07 mL (10 mmol) of *N*-benzyl-*N*-ethylaniline dissolved in 20 mL of MeOH and 5 mL of 12M HCl. Column chromatography on silica gel (hexanes/EtOAc, 20:1 then 9:1) gave 1.45 g (87%) of nitroazobenzene **S1** as a deep red, glassy solid. R_f(EtOAc/hexanes, 3:10) = 0.7; ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 8.31 (d, ^3J = 9.0 Hz, 2H), 7.92–7.85 (m 4H), 7.35 (t, ^3J = 7.5 Hz, 2H), 7.30–7.25 (m, 2H), 7.23 (d, ^3J = 7.6 Hz, 2H), 6.78 (d, ^3J = 9.1 Hz, 2H), 4.67 (s, 2H), 3.61 (q, ^3J = 7.1 Hz, 2H), 1.31 ppm (t, ^3J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz): δ = 156.8, 152.0, 147.4, 143.8, 137.5, 131.5, 128.8, 124.9, 124.0, 123.3, 122.6, 111.7, 53.8, 45.8, 12.3 ppm; IR: 3427, 3049, 2971, 2924, 2254, 1734, 1598, 1512, 1451, 1423, 1392, 1357, 1339, 1281, 1246, 1197, 1177, 1156, 1140, 1104 cm^{-1}; HRMS (ESI), *m/z* calcd. for $C_{21}H_{21}N_4O_2$ $[MH]^+$: 361.1659, found $[MH]^+$: 361.1649.



4

Aminoazobenzene 4. Following **standard procedure 2**, 350 mg (0.97 mmol) of nitrobenzene **S1** in 20 mL of 1,4-dioxane and 1 mL of water was reduced by three separate additions of 76 mg (0.97 mmol) of sodium sulfide. Purification of the crude product by column chromatography on silica gel (hexanes/EtOAc, 9:1→1:1) gave 280 mg (88%) of aminoazobenzene **4** as a deep red, glassy solid. R_f(hexanes/EtOAc, 10:3) = 0.3;

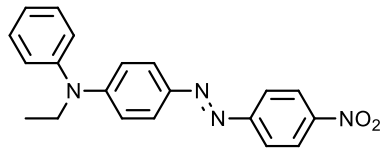
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.80–7.69 (m, 4H), 7.36–7.20 (m, 5H), 6.80–6.65 (m, 4H), 4.60 (s, 2H), 3.90 (s, 2H), 3.54 (q, ^3J = 7.1 Hz, 2H), 1.32–1.21 ppm (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 146.1, 144.2, 142.1, 139.9, 134.4, 122.4, 120.4, 120.3, 120.0, 100.9, 110.7, 107.7, 49.9, 41.6, 8.4 ppm; IR: 3452, 3378, 2965, 2923, 2853, 1618, 1595, 1511, 1450, 1394, 1355, 1293, 1275, 1242, 1198, 1178, 1151, 1125, 1073 cm^{-1}; HRMS (ESI), *m/z* calcd. for $C_{21}H_{23}N_4$ [MH]$^+$: 331.1917, found [MH]$^+$: 331.1907.



BENAQ

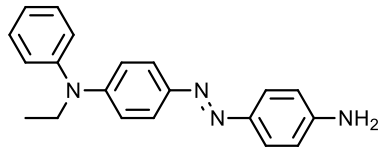
BENAQ. Following **standard procedure 3**, 96.4 mg (0.61 mmol) of betaine **E** dissolved in 5 mL of acetonitrile were reacted with 0.32 mL (2M solution in CH_2Cl_2, 0.64 mmol) of oxalyl chloride to form acid chloride **F**, which was further reacted with 100 mg (0.30 mmol) of aminoazobenzene **4** dissolved in 3 mL of DMF and 0.11 mL (3.0 mmol) of DIPEA. The crude product was purified by column chromatography on reversed-phase silica gel (0.1% formic acid in water/methanol, 1:0→2:3) to yield 105 mg (67%) **BENAQ** formate salt as a red-orange, glassy solid. ^{1}H NMR (CD_3OD, 600 MHz): δ = 8.40 (s, 1H), 7.84–7.69 (m, 6H), 7.30–7.23 (m, 2H), 7.23–7.14 (m, 3H), 6.79–6.68 (m, 2H), 4.58 (s, 2H), 4.22 (s, 2H), 3.60 (q, ^3J = 6.9 Hz, 6H), 3.53 (q, ^3J = 7.0 Hz, 2H), 1.33 (t, ^3J = 6.9Hz, 9H), 1.19 ppm (t, ^3J = 7.0 Hz, 3H); ^{13}C NMR (CD_3OD, 151 MHz): δ = 167.9, 163.2, 152.5, 151.4, 144.8, 140.1, 139.8, 129.8, 128.1, 127.7, 126.2, 124.0, 121.7, 112.9, 57.8, 55.9, 54.8, 46.8, 12.8, 8.1 ppm; IR: 3189, 3028, 2958, 2928, 2854, 2684, 1691, 1585, 1557, 1502, 1479, 1464, 1447, 1374, 1343, 1320, 1304, 1255, 1223, 1158, 1095, 1074, 1042 cm^{-1};

HRMS (ESI), *m/z* calcd. for $C_{29}H_{38}N_5O^+$ $[M]^+$: 506.2681, found: 472.3070; UV/Vis (DPBS, pH = 7.4): λ_{max} = 459 nm.



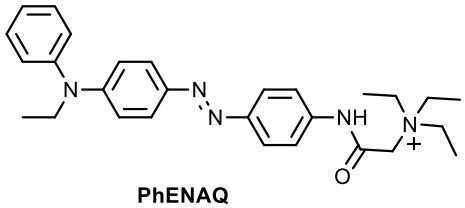
S2

Nitroazobenzene S2. Following **standard procedure 1**, 385 mg (2.8 mmol) of *p*-nitroaniline in 50 mL of MeOH and 5 mL of 12M HCl first reacted with 0.375 mL (2.8 mmol) of isoamylnitrite to form a diazonium salt, which then further reacted with 0.27 mL (2.8 mmol) of freshly prepared *N*-ethyl-*N*-phenylaniline[2] in 50 mL of MeOH and 5 mL of 12M HCl. Column chromatography on silica gel (CH_2Cl_2/hexanes, 1:1) gave 763 mg (79%) of nitroazobenzene **S2** as a red solid. R_f(hexanes/EtOAc, 5:2) = 0.9; ^{1}H NMR (acetone-d_6, 400 MHz): δ = 8.37 (d, ^3J = 9.2 Hz, 2H), 7.98 (d, ^3J = 9.2 Hz, 2H), 7.85 (d, ^3J = 9.2 Hz, 2H), 7.50 (t, ^3J = 7.2 Hz, 3H), 7.33 (t, ^3J = 8.0 Hz, 3H), 6.85 (d, ^3J = 9.2 Hz, 2H), 3.92 (q, ^3J = 7.2 Hz, 2H), 1.27 ppm (t, ^3J = 7.2 Hz, 3H); ^{13}C NMR (acetone-d_6, 100 MHz): δ = 156.6, 152.2, 147.7, 145.6, 144.5, 130.1, 127.4, 126.4, 125.6, 124.7, 122.6, 114.0, 46.8, 11.9 ppm; IR: 3454, 3016, 2969, 2361, 2338, 1738n, 1602, 1584, 1513, 1490, 1477, 1448, 1425, 1372, 1230, 1217, 1155, 1143, 1132, 1091 cm^{-1}; HRMS (ESI), *m/z* calcd. for $C_{20}H_{18}N_4O_2$ $[MH]^+$: 346.38, found $[MH]^+$: 347.1504.



5

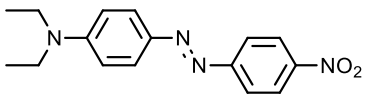
Aminoazobenzene 5. Following **standard procedure 2**, 763 mg (2.2 mmol) of nitrobenzene **S2** in 30 mL of 1,4-dioxane was reduced by two separate additions of 1.05 g (13.2 mmol) and 516 mg (6.6 mmol) of sodium sulfide. Purification of the crude product by column chromatography on silica gel (hexanes/EtOAc, 9:1→4:1) gave 291 mg (42%) of aminoazobenzene **5** as a red solid. R_f(hexanes/EtOAc, 5:1) = 0.3; ^{1}H NMR (CD_3OD, 400 MHz): δ = 7.65 (dd, ^3J = 8.6 Hz, 2H), 7.61 (dd, ^3J = 8.6 Hz, 2H), 7.38 (t, ^3J = 9.2 Hz, 2H), 7.17 (m, 3H), 6.85 (dd, ^3J = 8.6 Hz, 2H), 6.73 (dd, ^3J = 8.6 Hz, 2H), 3.84 (q, ^3J = 7.2 Hz, 2H), 1.21 ppm (m, 3H); ^{13}C NMR (CD_3OD, 100 MHz): δ = 150.8, 149.6, 146.6, 145.2, 144.6, 129.4, 125.4, 124.3, 123.9, 123.2, 115.5, 114.0, 46.3, 11.6 ppm; IR: 3464, 3379, 3213, 3037, 2974, 2536, 2361, 2339, 1618, 1599, 1586, 1504, 1493, 1372, 1375, 1347, 1269, 1245, 1151, 1132, 1090 cm^{-1}; HRMS (ESI), *m/z* calcd. for $C_{20}H_{21}N_4$ $[MH]^+$: 317.1761, found $[MH]^+$: 317.1760.



PhENAQ

PhENAQ. Following **standard procedure 3**, 73 mg (0.46 mmol) of betaine **E** in 5 mL of MeCN reacted with 0.24 mL (2M solution in DCM, 0.48 mmol) of oxalyl chloride to form acid chloride **F**, which was further reacted with 143 mg (0.45 mmol) of aminoazobenzene **5** in 10 mL of DMF and 0.1 mL (10.0 mmol) of DIPEA over the course of two days. The crude product was purified by column chromatography on reversed-phase

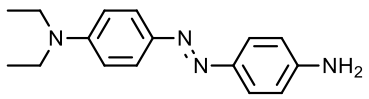
silica gel (0.1% formic acid in water/MeOH, 1:0→1:1) to yield 149 mg (66%) **PhENAQ** formate salt as a red solid. R_f(0.1% formic acid in water/MeOH, 1:1) = 0.1; ^{1}H NMR (CD_3OD, 200 MHz): δ = 8.53 (s, 1H), 7.79 (d, ^3J = 8.8 Hz, 2H), 7.72 (m, 4H), 7.40 (t, ^3J = 7.6 Hz, 2H), 7.21 (m, 3H), 6.79 (d, ^3J = 9.2 Hz, 2H), 4.19 (s, 2H), 3.83 (q, ^3J = 7.2 Hz, 2H), 3.63 (q, ^3J = 7.2 Hz 6H), 1.35 (t, ^3J = 7.2 Hz, 9H), 1.22 ppm (t, ^3J = 7.2 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz): δ = 168.3, 161.7, 150.9, 149.8, 146.1, 144.5, 138.8, 129.6, 129.4, 126.5, 125.4, 125.3, 124.2, 123.9, 122.6, 120.1, 114.4, 56.2, 54.4, 46.4, 11.5, 6.6 ppm; IR: 3500–3200, 3030, 2980, 2660, 1678, 1585, 1556, 1506, 1461, 1385, 1345, 1320, 1276, 1135, 1089, cm^{-1}; HRMS (ESI), *m/z* calcd. for $C_{28}H_{36}N_5O$ $[M]^+$: 458.2914, found $[M]^+$: 358.2912; UV/Vis (H_2O): λ_{max} = 463 nm.



S3

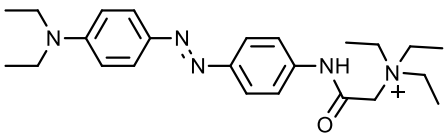
Nitroazobenzene S3. Following **standard procedure 1**, 1.38 g (10 mmol) of *p*-nitroaniline dissolved in 20 mL of MeOH and 5 mL of 12M HCl first reacted with 1.4 mL (10 mmol) of isoamylnitrite to form a diazonium salt, which then further reacted with 1.66 g (10 mmol) of diethylaniline in 20 mL of MeOH and 5 mL of 12M HCl. Column chromatography on silica gel (hexanes/EtOAc, 10:3 then 2:1) gave 1.85 g (62%) of nitroazobenzene **S3** as a red-brown solid. R_f(hexanes/EtOAc, 10:3) = 0.7; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.32–8.29 (m, 2H), 7.92–7.86 (m, 4H), 6.75–6.71 (m, 2H), 3.48 (q, ^3J = 7.1 Hz, 4H), 1.25 ppm (t, ^3J = 7.1 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 157.0, 151.3, 147.2, 143.3, 126.4, 124.7, 122.5, 111.0, 44.9, 12.7 ppm; IR: 3427, 2965, 2926, 1937, 1600, 1587, 1518, 1504, 1444, 1425, 1408, 1391, 1341, 1324, 1274, 1258, 1194,

1139, 1103, 1076 cm^{-1}; HRMS (ESI), *m/z* calcd. for $C_{16}H_{19}N_4O_2$ $[MH]^+$: 299.1503, found $[MH]^+$: 299.1492.



6

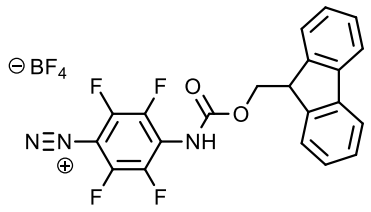
Aminoazobenzene 6. Following **standard procedure 2**, 1.8 g (6.0 mmol) of nitrobenzene **S3** in 90 mL of 1,4-dioxane and 9 mL of water was reduced by three separate additions of 0.47 g (6.0 mmol) of sodium sulfide. Purification of the crude product by column chromatography on silica gel (hexanes/EtOAc, 7:3) gave 1.41 g (88%) of aminoazobenzene **6** as a deep red, glassy solid. R_f(EtOAc/hexanes, 3:10) = 0.3; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.62–7.57 (m, 2H), 7.53–7.47 (m, 2H), 6.73–6.68 (m, 2H), 6.62–6.57 (m, 2H), 5.68 (m, 2H), 3.38 (q, ^3J = 7.0 Hz, 4H), 1.10 ppm (t, ^3J = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 162.7, 151.4, 149.1, 143.7, 142.8, 124.2, 113.9, 111.4, 44.4, 13.0 ppm; IR: 3440, 3323, 2972, 2252, 1896, 1669, 1623, 1590, 1557, 1512, 1450, 1403, 1386, 1374, 1350, 1307, 1271, 1250, 1196, 1166, 1148, 1095 cm^{-1}; HRMS (ESI), *m/z* calcd. for $C_{16}H_{21}N_4$ $[MH]^+$: 269.1761, found $[MH]^+$: 269.1751.



DENAQ

DENAQ. Following **standard procedure 3**, 478 mg (3.0 mmol) of betaine **E** dissolved in 10 mL of acetonitrile was reacted with 1.5 mL (2M solution in DCM, 3.0 mmol) of oxalyl chloride to form the acid chloride **F**, which was further reacted with 161 mg (0.6 mmol) of aniline **6** dissolved in 20 mL of DMF and 0.51 mL (3.0 mmol) of DIPEA. The crude product

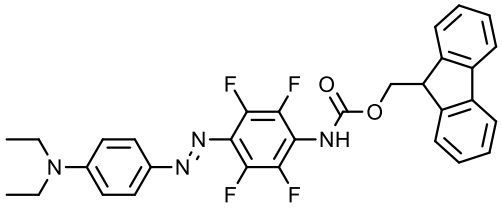
was purified by column chromatography on reversed-phase silica gel (0.1% formic acid in water/acetonitrile, 1:0→17:3) to yield 250 mg (92%) of **DENAQ** formate salt as a deep red, glassy solid. ^{1}H NMR (CD_3OD, 400 MHz): δ = 8.48 (s, 1H), 7.79–7.67 (m, 6H), 6.74–6.62 (m, 2H), 4.23–4.12 (m, 2H), 3.64–3.48 (m, 6H), 3.43–3.31 (m, 4H), 1.37–1.24 (m, 9H), 1.18–1.05 ppm (m, 6H); ^{13}C NMR (CD_3OD, 100 MHz): δ = 167.2, 161.7, 150.3, 149.9, 142.7, 138.6, 125.0, 122.4, 120.2, 110.8, 56.3, 54.4, 44.2, 11.7, 6.7 ppm; IR: 2972, 1688, 1630, 1597, 1547, 1514, 1448, 1394, 1351, 1249, 1194, 1154, 1138, 1076 cm^{-1}; HRMS (ESI), *m/z* calcd. for $C_{24}H_{36}N_5O$ $[MH]^+$: 410.2914, found $[MH]^+$: 410.2899; UV/Vis (H_2O): λ_{max} = 470 nm.



S4

Diazonium tetrafluoroborate S4. Under argon atmosphere a solution of 100 mg (0.25 mmol) of 4-{*N*-[(9-fluorenylmethoxy)carbonyl]amino}tetrafluoroaniline (synthesized following the procedures of Chehade et al.[3]) in 1 mL of dry THF was added dropwise to 47.2 μL (0.37 mmol) of boron trifluoride etherate at –40 °C. The resulting solution was stirred for 5 minutes and 41.8 μL isoamyl nitrite (0.31 mmol, 1.25 eq) was subsequently added dropwise. The reaction mixture was warmed to –5 °C over 3 hours, causing a color change to orange. The crude diazonium salt was precipitated by addition of 15 mL of cold pentane. The supernatant was transfused and when necessary centrifuged at 4 °C. The orange and solid diazonium tetrafluoroborate **S4** was dried *in vacuo* at –5 °C for one hour and employed for further reaction without additional purification. ^{1}H NMR

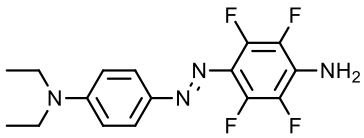
(DMSO-d_6, 200 MHz): δ = 9.96 (s, br, 1H), 7.90–7.80 (m, 2H), 7.72–7.63 (m, 2H), 7.44–7.27 (m, 4H), 4.58–4.56 (m, ^3J = 6.8 Hz, 2H), 4.35–4.25 ppm (m, 1H).



8

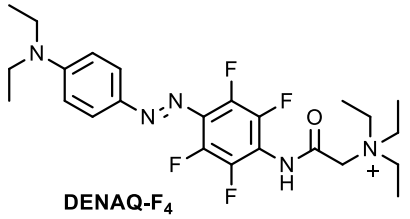
Tetrafluoroazobenzene 8. 601 mg (1.20 mmol) of freshly prepared diazonium tetrafluoroborate **S4** was dissolved in 6 mL of dry THF under argon atmosphere. To this solution, 0.23 mL (1.44 mmol) of *N,N*-diethylanilin and 98 mg (1.44 mmol) of sodium acetate were added at 0 °C. The dark red reaction mixture was allowed to warm to room temperature and stirred overnight. Then the mixture was poured into 100 mL of EtOAc and washed with saturated bicarbonate solution, water, and brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (hexanes/EtOAc = 20:1→8:1) yielded 246 mg (36%) of carbamate **8** as a red-orange solid. R_f(hexanes/EtOAc = 7:3) = 0.42. ^{1}H NMR (THF-d_8, 400 MHz): δ = 8.90 (s, br, 1H), 7.83–7.75 (m, 4H), 7.64 (d, ^3J = 7.4 Hz, 2H), 7.38–7.33 (m, 2H), 7.28 (td, ^3J = 7.4 Hz, 2H), 6.81–6.76 (m, 2H), 4.52 (d, ^3J = 6.6 Hz, 2H), 4.27 (t, ^3J = 6.6 Hz, 1H), 3.51 (q, ^3J = 7.1 Hz, 4H), 1.21 ppm (t, ^3J = 7.1 Hz, 6H). ^{13}C NMR (THF-d_8, 100 MHz): δ = 155.0, 153.4, 146.2–146.0 (m), 145.9, 145.8, 143.8–143.5 (m), 143.2, 141.3–143.0 (m), 132.9–132.7 (m), 129.2, 126.8, 127.7, 126.6, 121.5, 117.7, 112.7, 49.0, 46.3, 13.7 ppm. ^{19}F NMR (THF-d_8, 376 MHz): δ = –147.93 (dd, ^3J = 20.6 Hz, ^4J = 9.3 Hz, 2F), –153.09 ppm (dd, ^3J = 20.6 Hz, ^4J = 9.3 Hz, 2F). IR: 3255, 2976, 1703, 1602, 1527, 1498, 1473, 1406, 1383,

1353, 1305, 1273, 1231, 1195, 1144, 1104, 1077, 1023 cm^{-1}. HRMS (ESI), *m/z* calcd. for $C_{31}H_{27}F_4N_4O_2$ $[MH]^+$: 563.2065, found $[MH]^+$: 563.2061. UV/Vis (EtOH): λ_{max} = 446 nm.



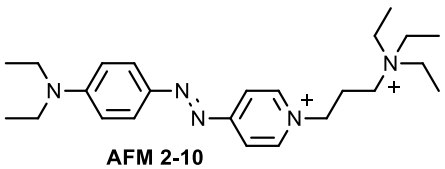
S5

Tetrafluoroazobenzene S5. 243 mg of freshly prepared FMOC-protected azobenzene **8** (0.43 mmol) was suspended in 9 mL of degassed diethyl ether under argon atmosphere and cooled to 0 °C. After addition of 0.9 mL (774 mmol) of piperidine, the mixture was warmed to room temperature, stirred for 3 hours, subsequently diluted with 100mL of diethyl ether , and washed with HCl (1M), water, and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (CH_2Cl_2/hexanes= 1:4→2:3) yielded 125 mg (85%) of tetrafluoroaniline **S5** as red crystals. R_f(hexanes/EtOAc = 7:3) = 0.64. ^{1}H NMR (Acetone-d_6, 400 MHz, 27 °C): δ = 7.77–7.70 (m, 2H), 6.84–6.78 (m, 2H), 5.72 (s, br, 2H), 3.52 (q, ^3J = 7.1 Hz, 4H), 1.21 ppm (t, ^3J = 7.1 Hz, 6H). ^{13}C NMR (Acetone-d_6, 100 MHz, 27 °C) δ = 150.6, 143.9, 143.0–142.9 (m), 140.6–140.4 (m), 137.8–137.5 (m), 135.3135.1 (m), 124.9, 110.9, 44.3, 12.0 ppm. ^{19}F NMR (Acetone-d_6, 376 MHz, 27 °C): δ = –155.12– –155.30 (m, 2F), –165.55– –165.74 ppm (m, 2F). IR: 3500, 3397, 2972, 2926, 1654, 1596, 1559, 1515, 1501, 1455, 1399, 1351, 1296, 1273, 1238, 1196, 1138, 1095, 1080, 1058, 1012, 1000 cm^{-1}. HRMS (ESI), *m/z* calcd. for $C_{16}H_{17}F_4N_4$ $[MH]^+$: 341.1384, found $[MH]^+$: 341.1381. UV/Vis (EtOH): λ_{max} = 430, 458 nm.



DENAQ-F$_4$

DENAQ-F$_4$. Following **Standard Procedure 3**, 125 mg (0.37 mmol) of tetrafluoroaniline **S5** and 0.32 mL (1.83 mmol) of DIPEA were dissolved in 7 mL of dry DMF. To this solution, 390 mg (1.83 mmol) of betaine acid chloride **F** dissolved in 10 mL of dry DMF was added. The resulting mixture was allowed to warm to room temperature, stirred overnight, and DMF was subsequently removed *in vacuo*. Reversed phase column chromatography (0.1% formic acid in water/methanol, 9:1→1:1) yielded 74.1 mg (38%) of **DENAQ-F$_4$** formate salt as a deep red hygroscopic solid. R_f(0.1% formic acid in water/methanol = 1:1) = 0.20. ^{1}H NMR (CD_3OD, 400 MHz): δ = 8.51 (s, 1H) 7.84–7.72 (m, 2H), 6.84–6.70 (m, 2H), 4.36 (s, 2H), 3.64 (q, ^3J = 7.2 Hz, 6H), 3.49 (q, ^3J = 7.1 Hz, 4H), 1.38 (t, ^3J = 7.2 Hz, 9H), 1.20 ppm (t, ^3J = 7.1 Hz, 6H). ^{13}C NMR (CD_3OD, 100 MHz) δ = 167.6 (br), 162.6, 152.0, 144.2–143.9 (m), 143.7, 141.8–141.4 (m), 139.3–139.1 (m), 132.1–131.9 (m), 126.0, 110.8, 55.6, 54.5, 44.4, 11.5, 6.6 ppm. ^{19}F NMR (CD_3OD, 376 MHz): δ = –148.72 (dd, ^3J = 19.2 Hz, ^4J = 8.5 Hz, 2F), –155.15 ppm (dd, ^3J = 19.2 Hz, ^4J = 8.5 Hz, 2F). IR: 3406, 3186, 2978, 1673, 1597, 1555, 1519, 1494, 1380, 1352, 1307, 1274, 1246, 1200, 1150, 1116, 1076, 1008 cm^{-1}. HRMS (ESI), *m/z* calcd. for $C_{24}H_{32}F_4N_5O$ $[M]^+$: 482.2537, found $[M]^+$: 482.2533. UV/Vis (DPBS, pH = 7.4): λ_{max} = 487 nm.

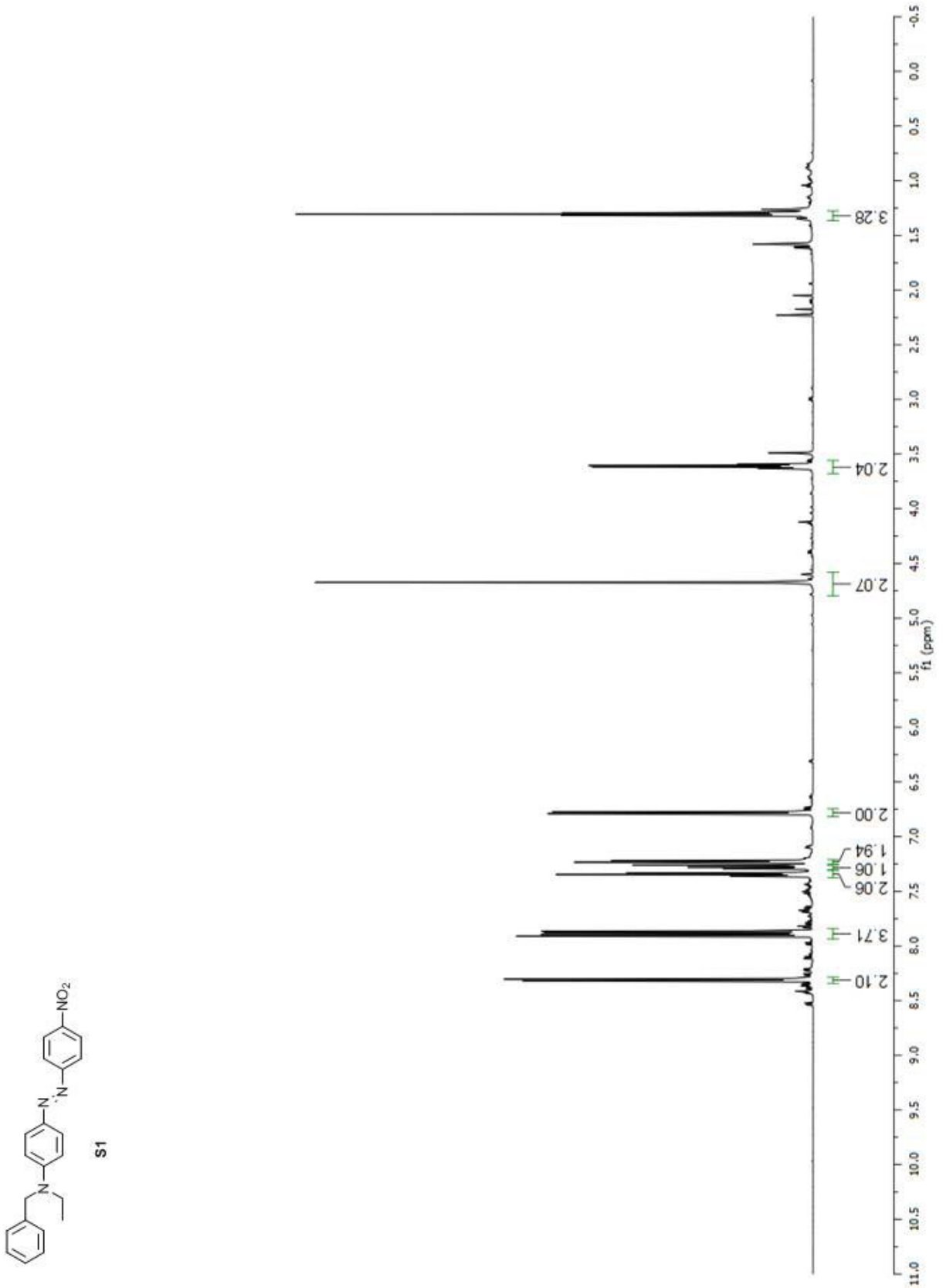


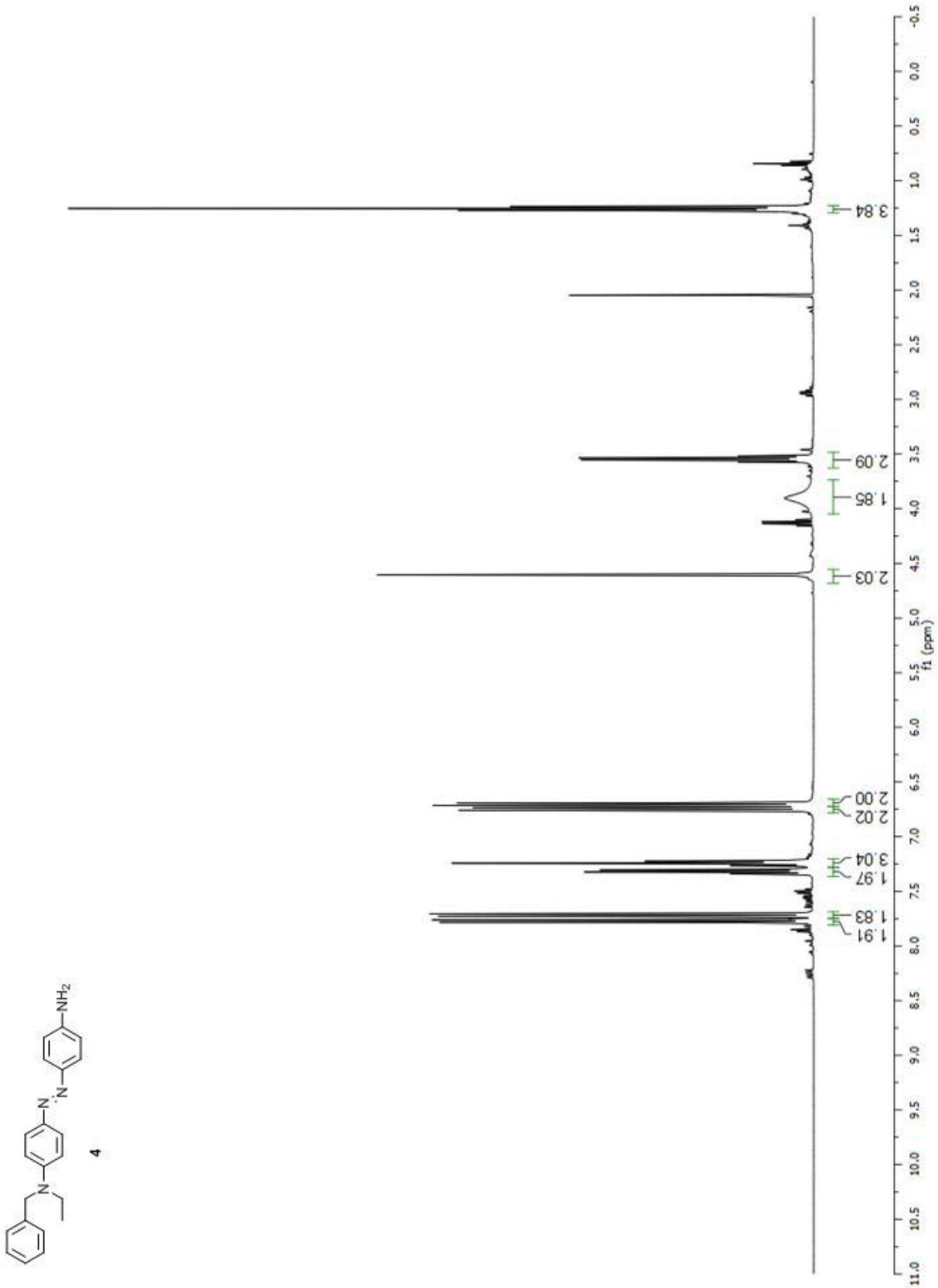
AFM 2-10

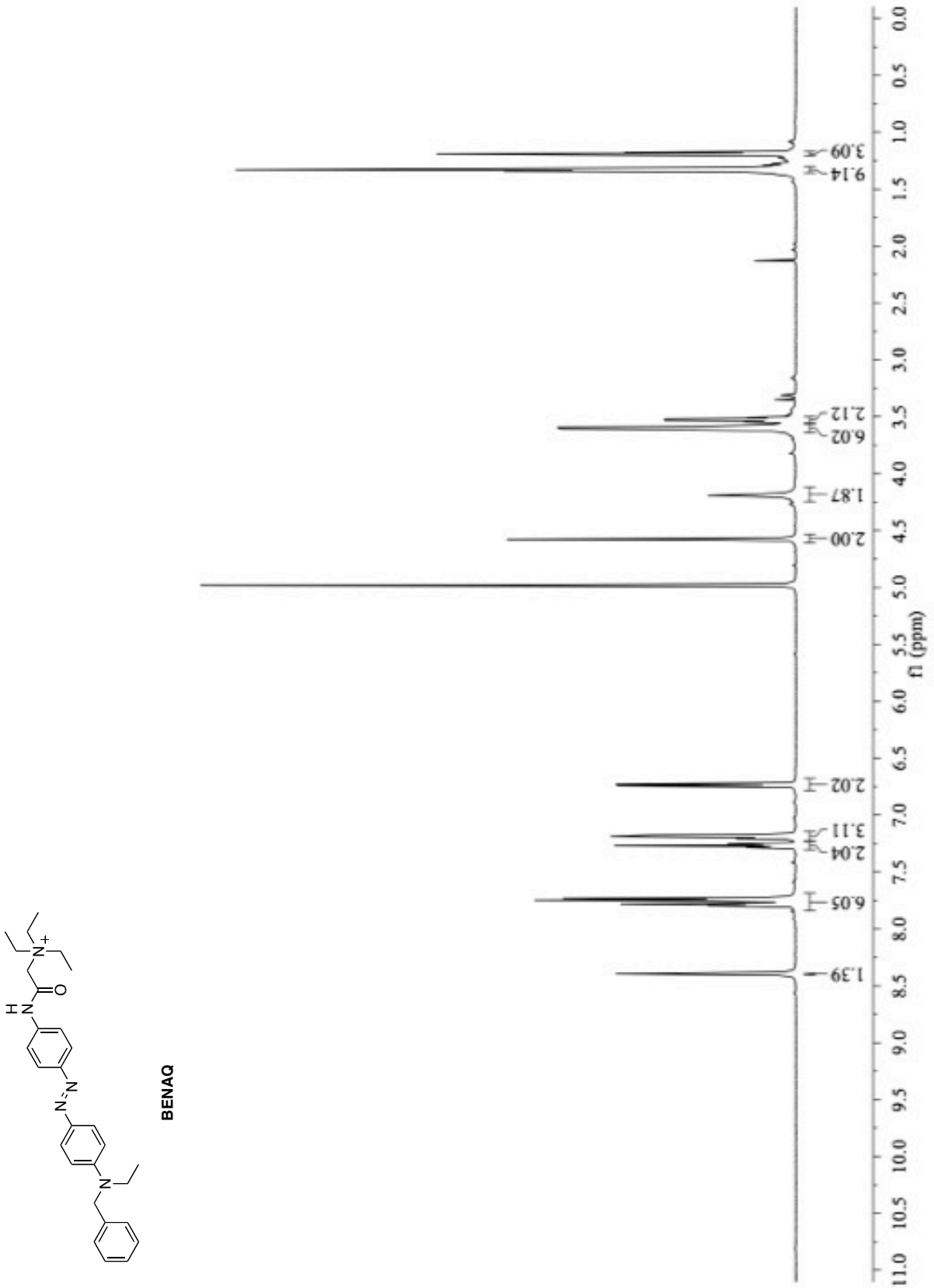
AFM 2-10. In a 10 mL Schlenk flask equipped with a reflux condenser, 100 mg (0.39 mmol) of 4-(4-diethylaminophenylazo)pyridine and 358 mg (1.18 mmol) of (3-bromopropyl)triethylammonium bromide (see Jeon et al.[4]) were dissolved in 8 mL of dry DMF under argon atmosphere. The reaction mixture was stirred at 80 °C for 30 hours, cooled to room temperature, and subsequently dried *in vacuo*. Purification by reversed phase column chromatography (0.1% formic acid in water/methanol, 1:0→50:1) had to be performed two times, yielding 180 mg (92%) **AFM 2-10** as deep purple hygroscopic solid bisformate salt. ^{1}H and ^{13}C NMR samples were taken from the corresponding trifluoroacetate salt, which was obtained by further purification using reversed phase HPLC. R_f(0.1% formic acid in water/methanol, 1:10) = 0.05. ^{1}H NMR (D_2O, 400 MHz): δ = 8.48 (d, ^{3}J = 7.4 Hz, 2H), 7.77 (m, br, 4H), 7.31 (s, br, 2H), 4.40 (t, ^{3}J = 7.6 Hz, 2H), 3.90 (q, ^{3}J = 7.2 Hz, 4H), 3.21–3.16 (m, 8H), 2.29 (m,2H), 1.29 (t, ^{3}J = 7.2 Hz, 6H), 1.13 ppm (t, ^{3}J = 7.2, 9H). ^{13}C NMR (D_2O, 100 MHz): δ = 162.7 (q), 160.2, 154.1, 144.4, 141.9, 126 (br), 126.5 (br), 116.2 (q), 112.7, 56.1, 52.9, 52.6, 49.4, 23.0, 12.8, 6.5 ppm. IR: 3390, 2977, 1650, 1634, 1593, 1543, 1513, 1502, 1476, 1418, 1395, 1370, 1343, 1318, 1300, 1266, 1189, 1164, 1115, 1071, 1025, 1006 cm^{-1}. HRMS (ESI), *m/z* calcd. for $C_{24}H_{39}N_5$ $[M]^{2+}$: 198.6597, found $[M]^{2+}$: 198.6596. UV/Vis (DPBS, pH = 7.4): λ_{max} = 580 nm.

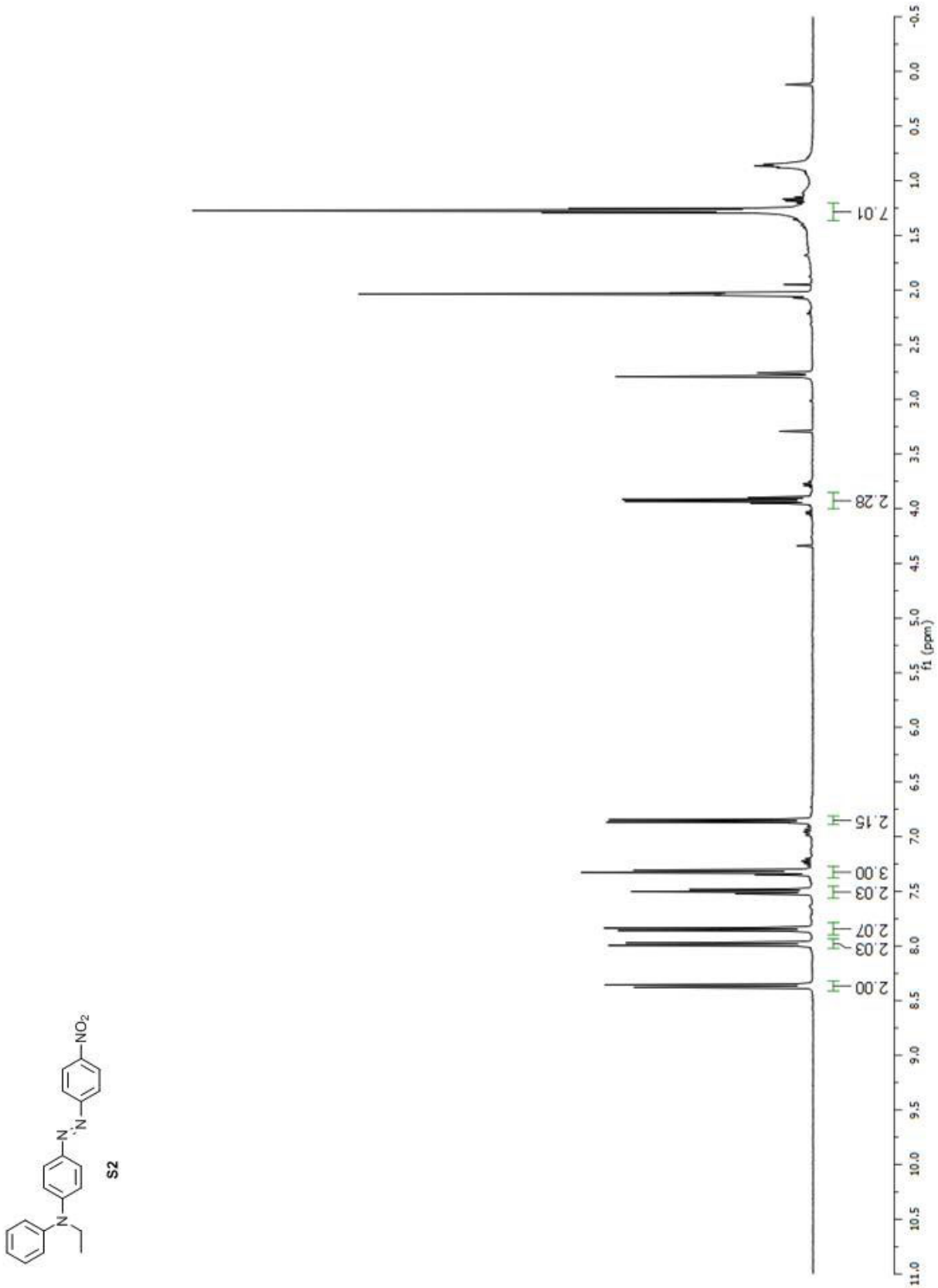
References:

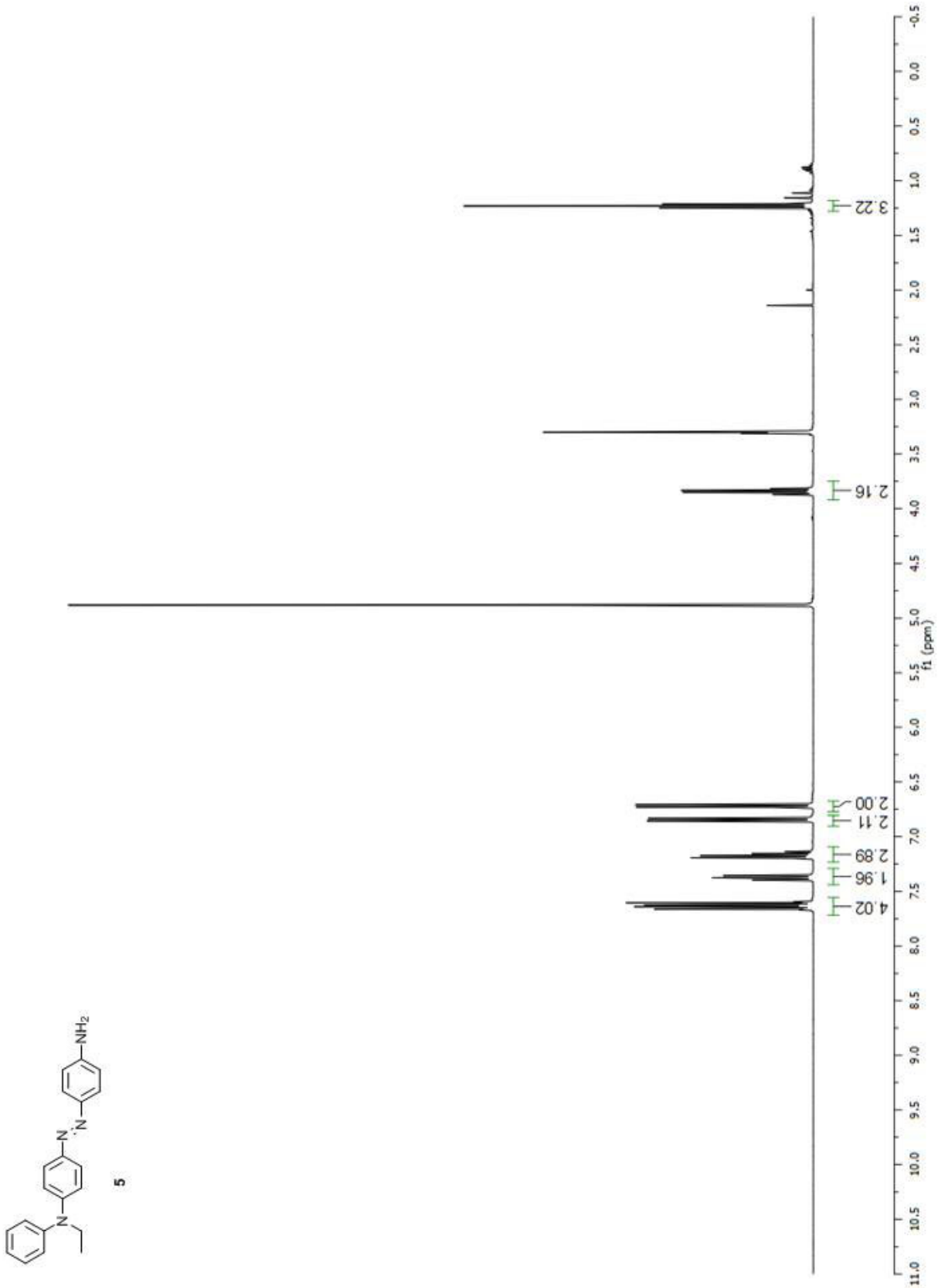
1. Fortin, D. L.; Banghart, M. R.; Dunn, T. W.; Borges, K.; Awagenaar, D.; Gaudry, Q.; Karakossian, M. H.; Otis, T. S.; Kristan, W. B.; Trauner, D.; Kramer, R. H., *Nature Methods* **2008,** *5*(4), 331–338.
2. Haga, K.; Oohashi, M.; Kaneko, R., *Bulletin of the Chemical Society of Japan* **1984,** *57*(6), 1586–1590.
3. Chehade, K. A. H.; Spielmann, H. P., *Journal of Organic Chemistry* **2000,** *65*(16), 4949–4953.
4. Jeon, W. S.; Kim, E.; Ko, Y. H.; Hwang, I. H.; Lee, J. W.; Kim, S. Y.; Kim, H. J.; Kim, K., *Angewandte Chemie International Edition* **2005,** *44*(1), 87–91.

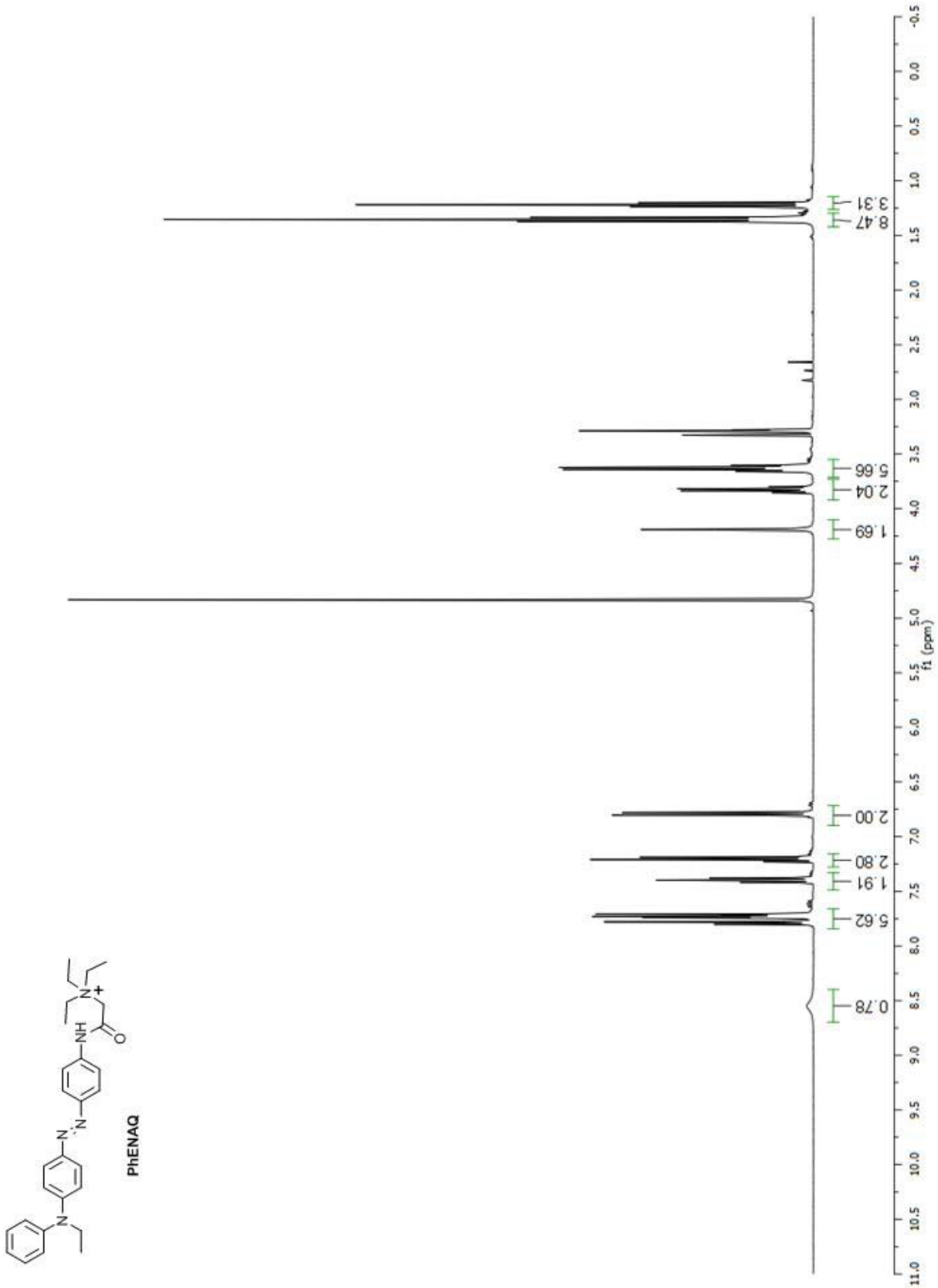

NO2
N
N
N
S1
3.28
2.04
2.07
2.00
1.94
1.06
2.06
3.71
2.10
f1 (ppm)

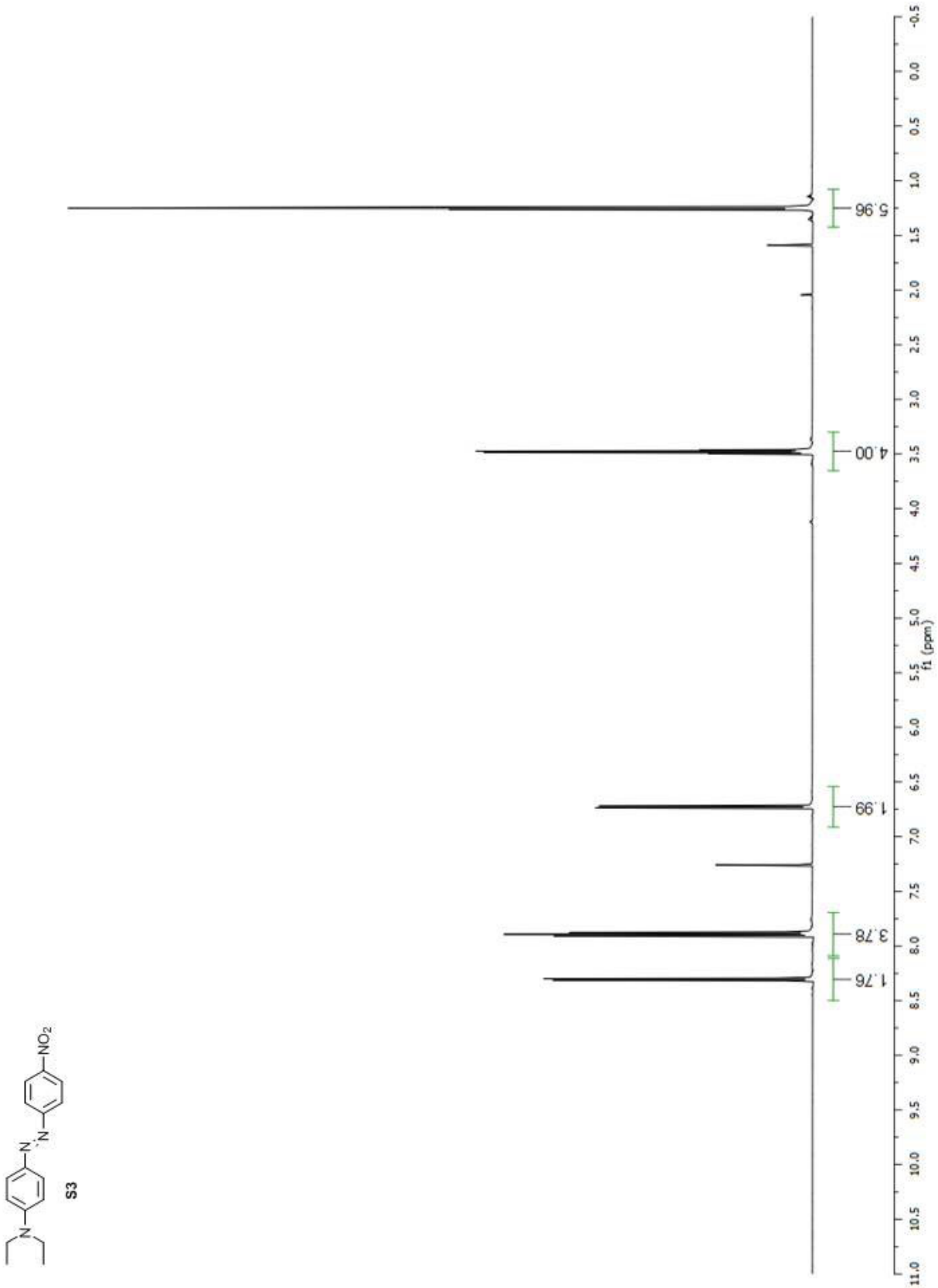

NH2
N
N
N
4
3.84
2.09
1.85
2.03
2.00
2.02
3.04
1.97
1.83
1.91
f1 (ppm)

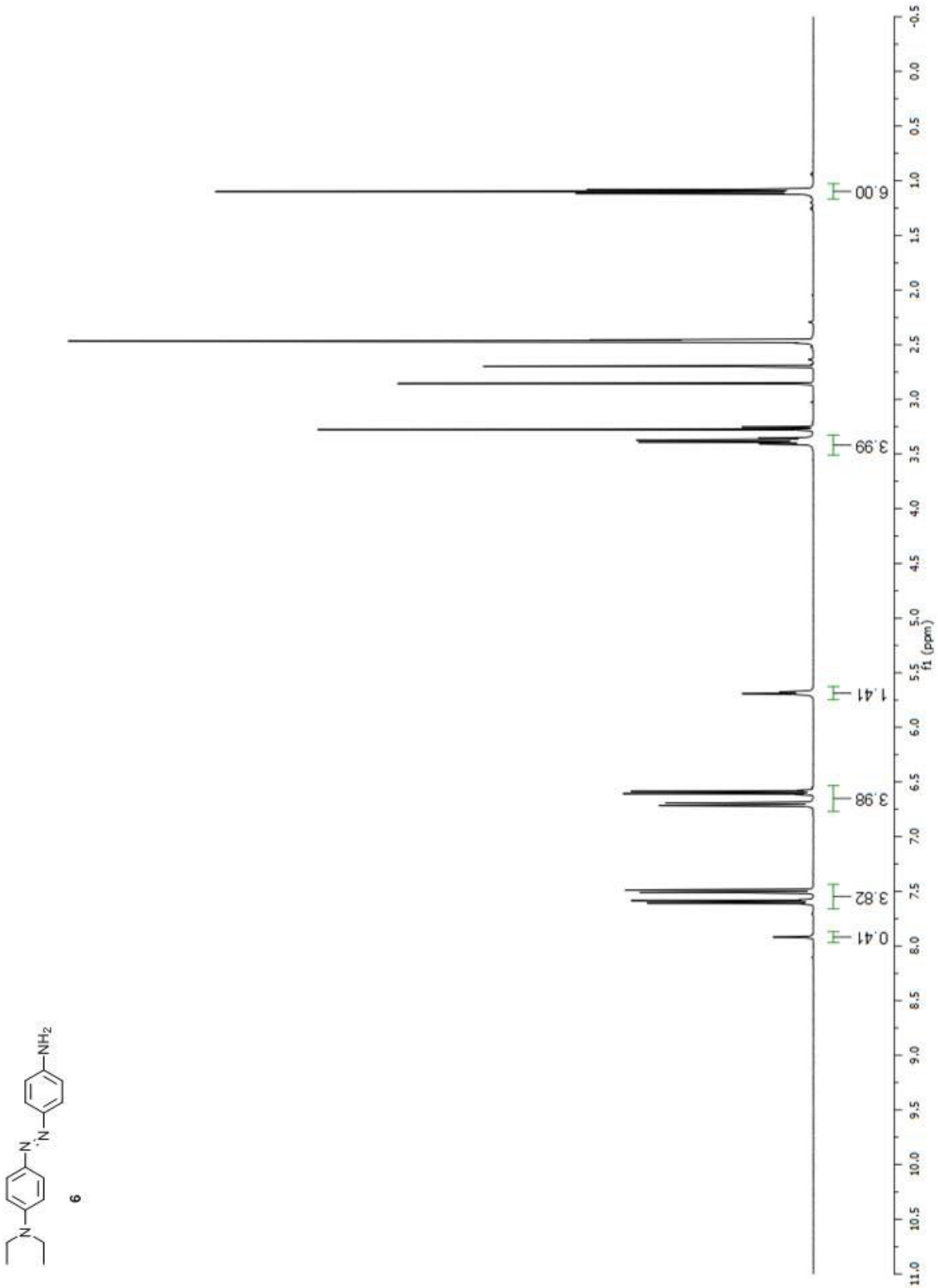

BENAQ
3.09
9.14
2.12
6.02
1.87
2.00
2.02
3.11
2.04
6.05
1.39
f1 (ppm)


NO2
N
N
N
S2
7.01
2.28
2.15
3.00
2.03
2.07
2.03
2.00
f1 (ppm)

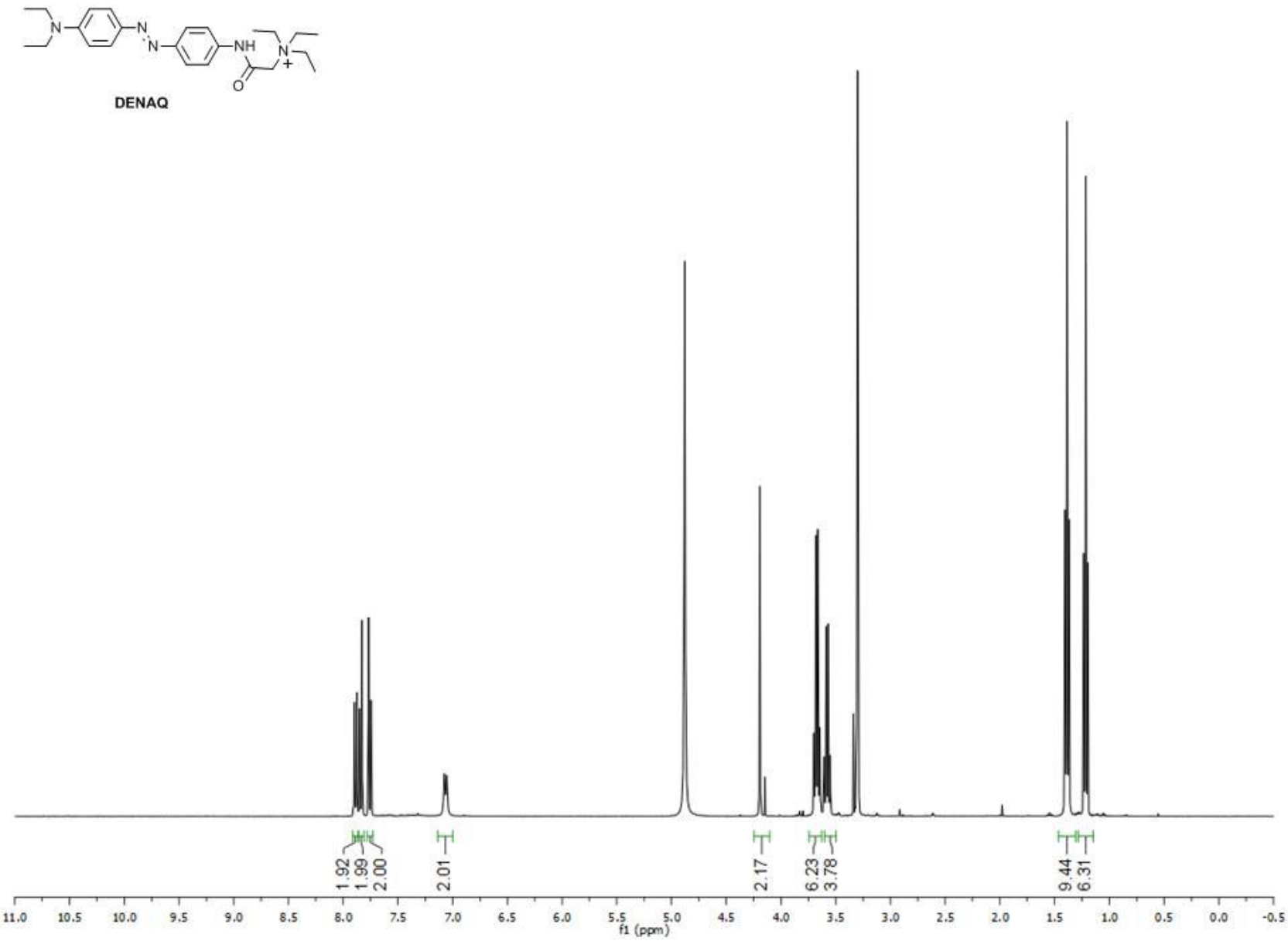

NH2
N
N
N
5
3.22
2.16
2.00
2.11
2.89
1.96
4.02
f1 (ppm)

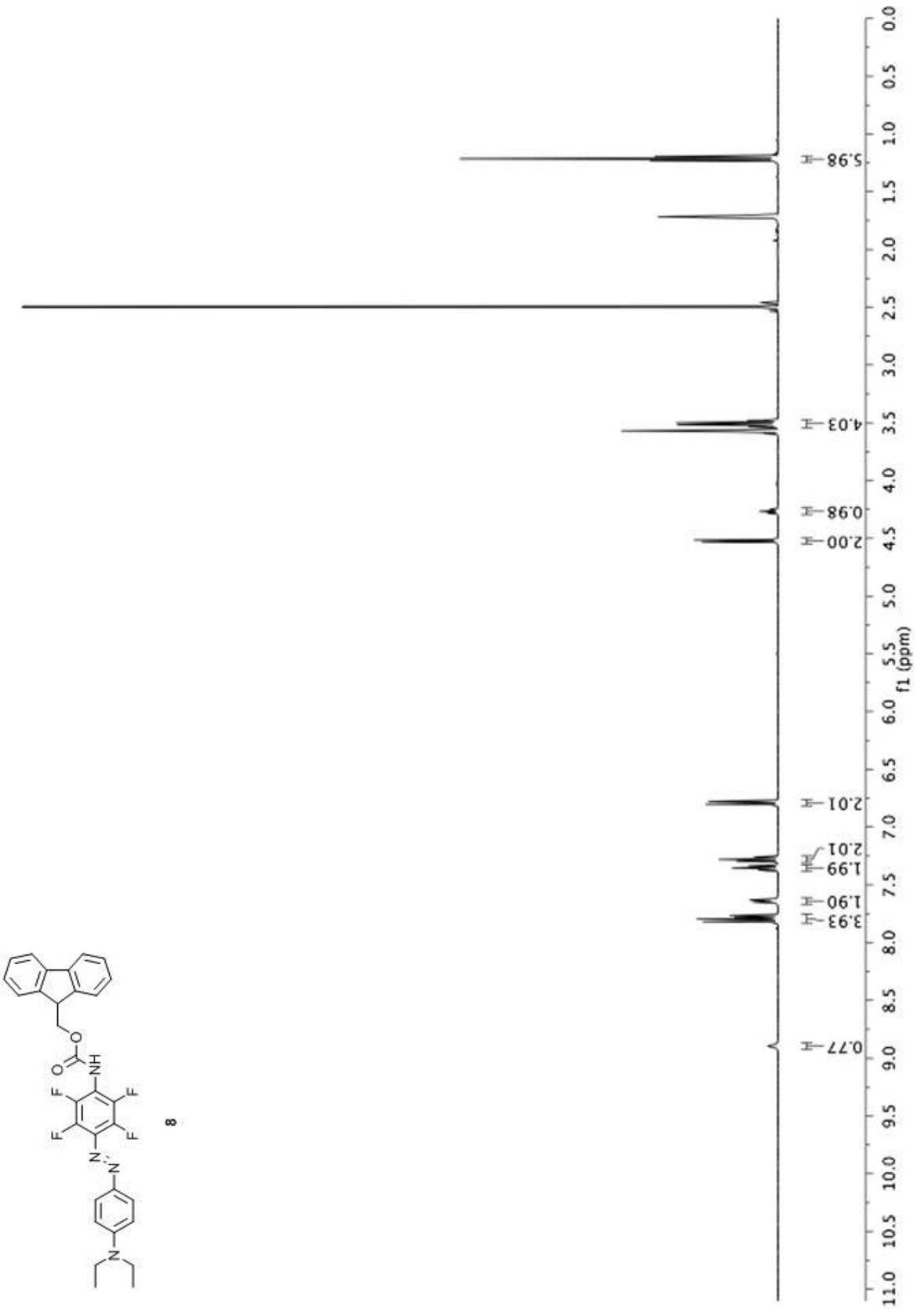

PhENAQ
3.31
8.47
5.66
2.04
1.69
2.00
2.80
1.91
5.62
0.78
f1 (ppm)

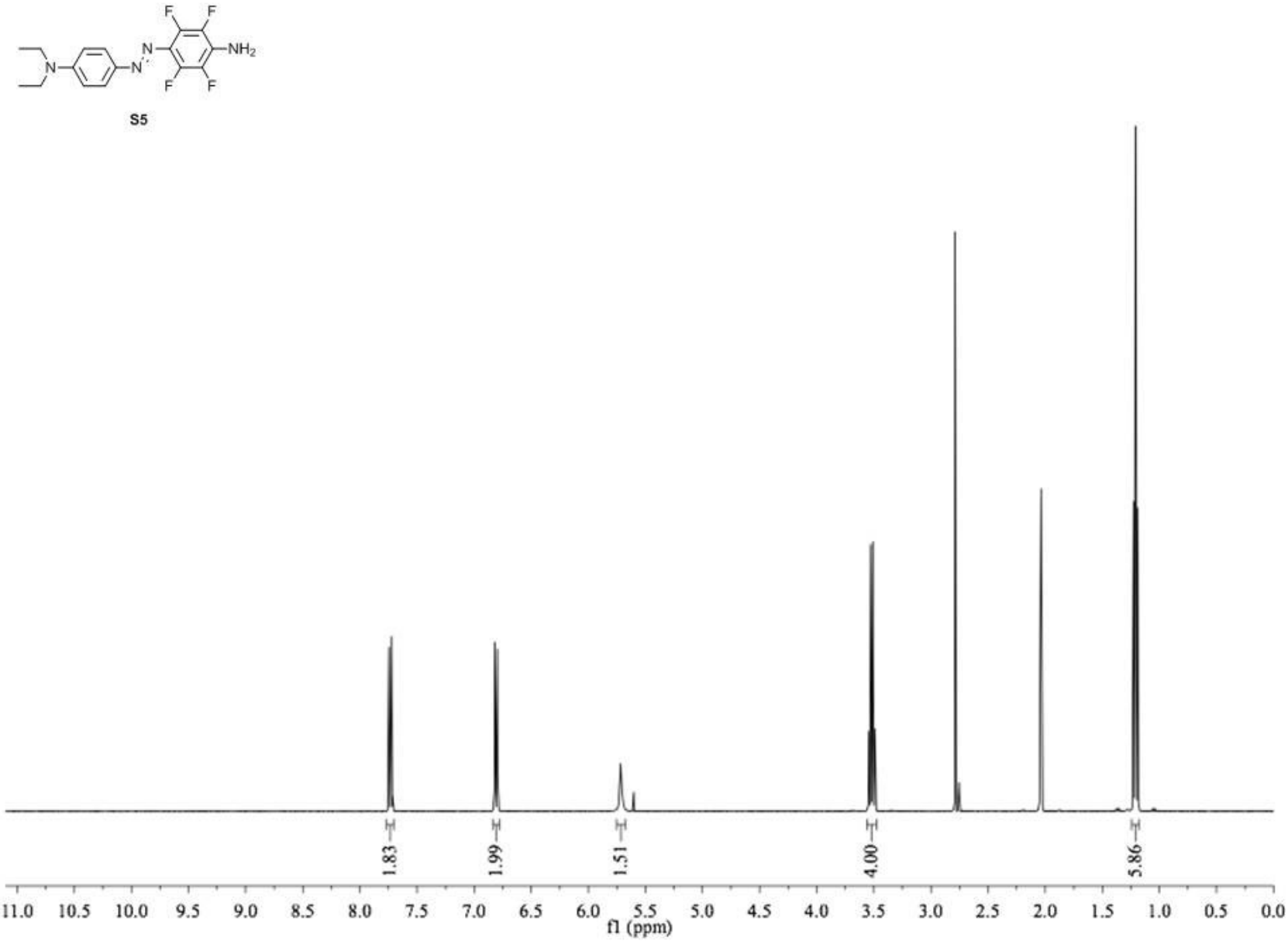

S3
5.96
4.00
1.99
3.78
1.76
f1 (ppm)

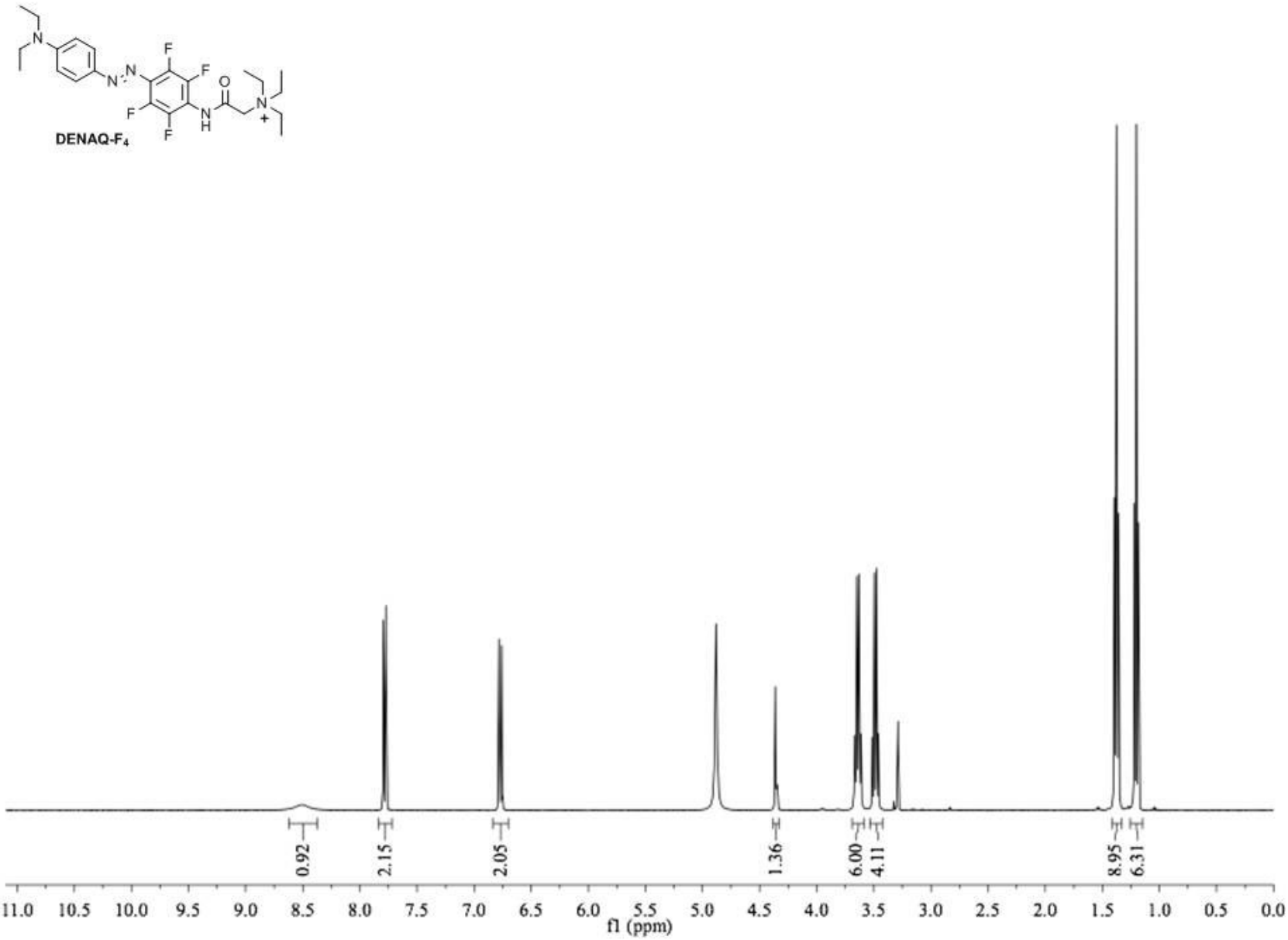


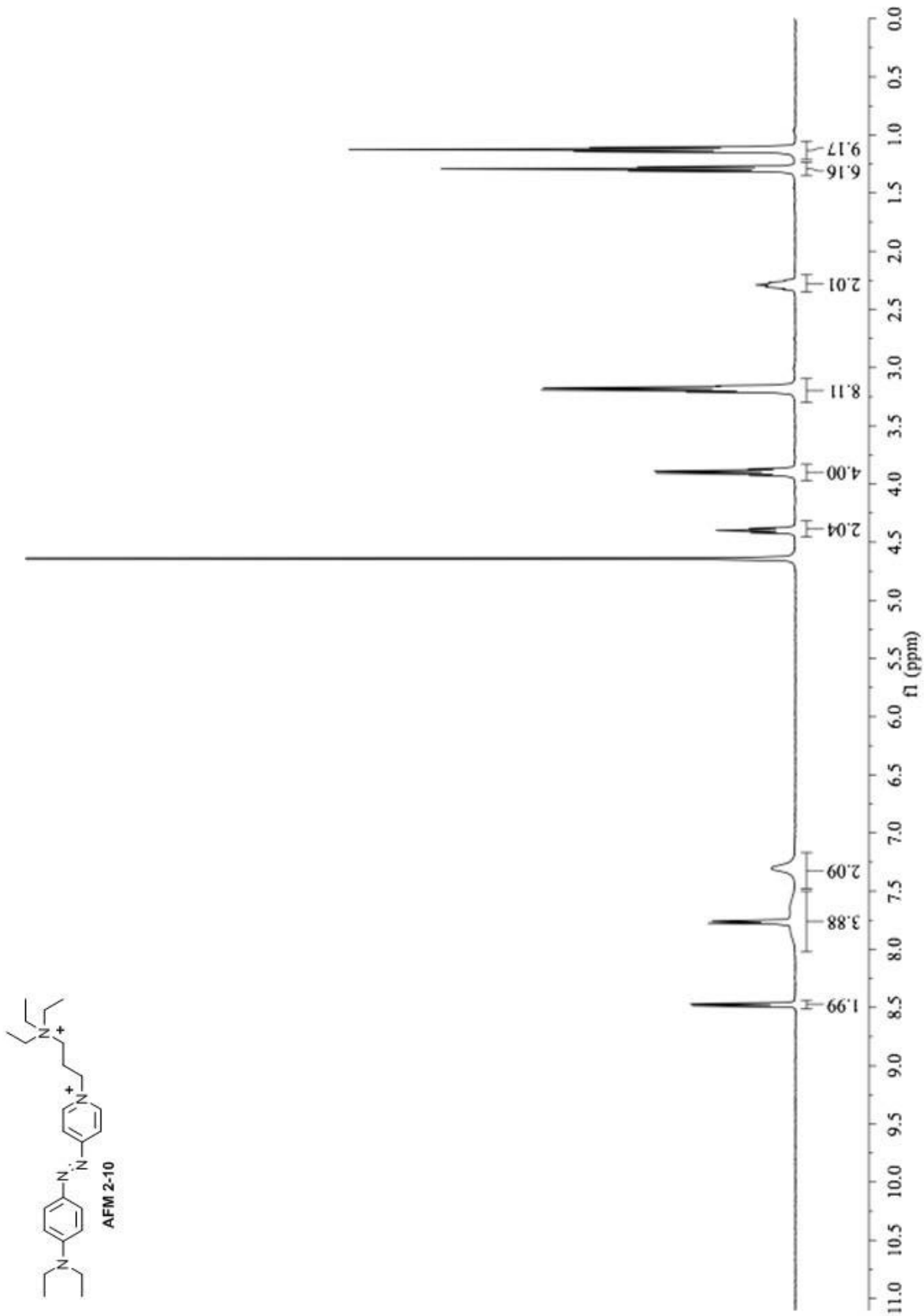
6


DENAQ
1.92
1.99
2.00
2.01
2.17
6.23
3.78
9.44
6.31
f1 (ppm)


8
5.98
4.03
0.98
2.00
2.01
2.01
1.99
1.90
3.93
0.77
f1 (ppm)


F
F
N
NH2
N
N
F
F
S5
1.83
1.99
1.51
4.00
5.86
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


DENAQ-F4
0.92
2.15
2.05
1.36
6.00
4.11
8.95
6.31
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


AFM 2-10
9.17
6.16
2.01
8.11
4.00
2.04
2.09
3.88
1.99
f1 (ppm)