Electronic Supplementary Material (ESI) for Analyst.

A High-resolution Method to Assess Cell Multinucleation with Cytoplasm-localized Fluorescent Probes

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1, CLFP development

1.1 General information

General: All the solvents and chemicals were purchased from commercial sources: J&K® Chemical Corporation, Beijing Ouhe Reagents Corporation with a purity > 95%. Flash column chromatography was performed on Biotage Isolera one. ^{1}H NMR and ^{13}C NMR were recorded on Bruker AVANCEIII 400 spectrometer. Chemical shifts are referenced to the residual solvent peak and reported in ppm (δ scale) and all coupling constant (J) values are given in Hertz (Hz). The following multiplicity abbreviations are used: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet. ESI-HRMS data were measured on Thermo Exactive Orbitrap plus spectrometer. Purity was determined using HPLC, LCMS and NMR spectroscopy. All of the synthesized compounds have the purity over than 95%.

1.2 Compound information (^{1}H, ^{13}C, HRMS)

Series 1, BODIPY derivatives

We designed and synthesized 13 BODIPY derived probes, the structures of them are listed in table S1.

Table S1, The structure information of 13 BODIPY derived CLFPs.

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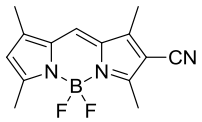
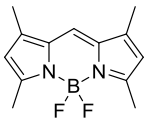
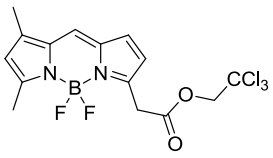
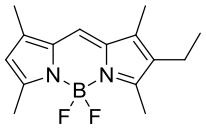
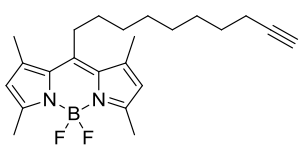
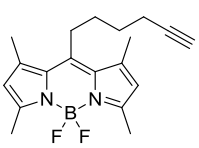
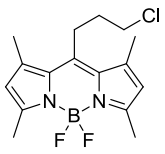
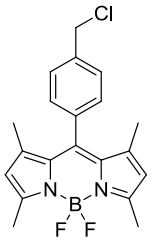
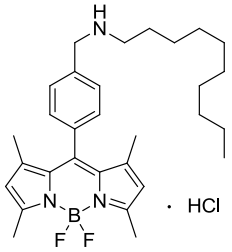
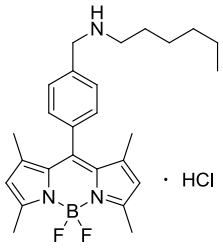
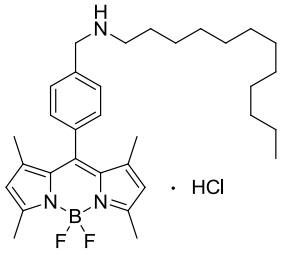
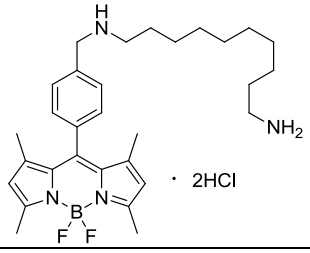
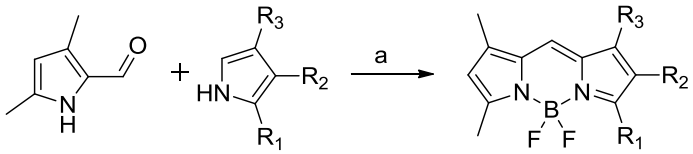
B1		B2	
B3		B4	
B5		B6	

B7		B8	
B9		B10	
B11		B12	
B13			

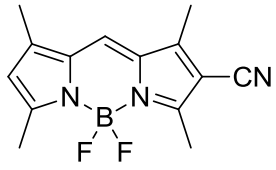
Synthesis protocol and compound characterization (^{1}H NMR, ^{13}C NMR and HRMS) [1,2]



Scheme S1, Synthesis of B1-B4. (a) $POCl_3$, DCM; TEA, BF_3 Et_2O, DCM

B1

3-Carbonitrile-4, 4-difluoro-1, 3, 5, 7-tetramethyl-4-bora-3a, 4a-diaza-s-indacene

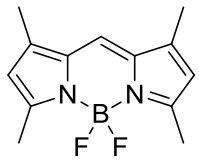


3, 5-dimethyl-pyrrole-2-carbaldehyde (100mg, 0.81mmol) and 2,4-dimethyl-pyrrole-3-carbonitrile (89mg, 0.74mmol) were dissolved in dry DCM (15 mL) , the reaction mixture cooled to 0 ℃ and stirred for 10 min under argon atmosphere, then POCl3 (124mg, 0.81mmol) was slowly added in 5 mins. The reaction mixture was stirred at 0 ℃ for 1h, then another 4h at 25 ℃. Dry TEA (750mg, 7.4 mmol) was added, and after 15 min BF_3 Ɛt_2O (0.93ml, 7.4 mmol) was added. After 2 h, the reaction mixture was evaporated in vacuum, and was extracted by EtOAc (200 mL), then washed with H_2O (3× 50 mL) and dried by Na_2SO_4. The crude product was purified by silica gel column chromatography (hexane/EtOAc 5:1) to yield 99 mg (49%) of B1 as red crystal.

^{1}H NMR (400MHz, $CDCl_3$): δ = 2.30 (s, 3H), 2.36 (s, 3H), 2.59 (s, 3H), 2.62 (s, 3H), 6.23(s, 1H), 7.13 (s, 1H). ^{13}C NMR (101MHz, $CDCl_3$): δ = 10.58, 11.49, 13.34, 15.29, 100.97, 114.89, 121.27, 122.39, 130.18, 136.81, 140.54, 146.31, 155.37, 164.63. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{14}H_{15}N_3BF_2$: 274.13216; found: 274.13153

B2

4, 4-Difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene

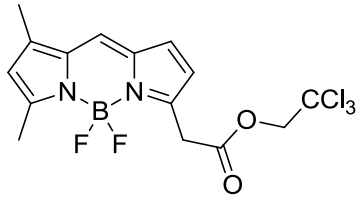


B2 (109 mg, 60%) was obtained from 3, 5-dimethyl-pyrrole-2-carbaldehyde (100mg, 0.81mmol) and 2, 4-dimethyl-pyrrole (70 mg, 0.74mmol) as red powder.

^{1}H NMR (400MHz, $CDCl_3$): δ = 2.24 (s, 6H), 2.53 (s, 6H), 6.04 (s, 2H), 7.04 (s, 1H). ^{13}C NMR (101MHz, $CDCl_3$): δ = 11.27 (2C), 14.66 (2C), 119.01 (2C), 120.08, 133.40 (2C), 141.20 (2C), 156.71 (2C). HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{13}H_{16}N_2BF_2$: 249.13691; found: 249.13643

B3

2, 2, 2-Trichloroethyl-2-(4, 4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-yl)acetate

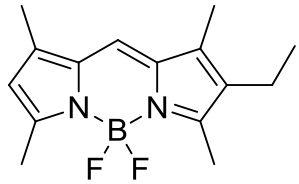


B3 (63mg, 21%) was obtained from 3, 5-dimethyl-pyrrole-2-carbaldehyde (100mg, 0.81mmol) and 2,2,2-trichloroethyl-2-(pyrrol -2-yl) acetate (188mg, 0.74mmol) as red powder.

^{1}H NMR (400MHz, $CDCl_3$): δ = 2.25 (s, 3H), 2.57 (s, 3H), 4.18 (s, 2H), 4.81 (s, 2H), 6.14 (s, 1H), 6.48 (d, J = 4.0Hz, 1H), 6.90 (d, J = 4.0Hz, 1H), 7.13 (s, 1H). ^{13}C NMR (101MHz, $CDCl_3$): δ = 11.35, 15.08, 33.86, 74.37, 94.74, 117.75, 121.12, 124.30, 127.28, 133.03, 136.01, 145.16, 146.98, 162.29, 168.12. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{15}H_{15}O_2N_2BF_2Cl_3$: 409.02547; found: 409.02542

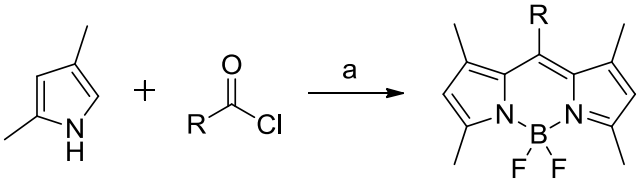
B4

2-Ethyl-4, 4-difluoro-1, 3, 5, 7-tetramethyl-4-bora-*3a*, *4a*-diaza-s-indacene



B4 (83 mg, 41%) was obtained from 3, 5-dimethyl-pyrrole-2-carbaldehyde (100 mg, 0.81 mmol) and 3-ethyl-2,4-dimethyl-pyrrole (91mg, 0.74 mmol) as green crystal.

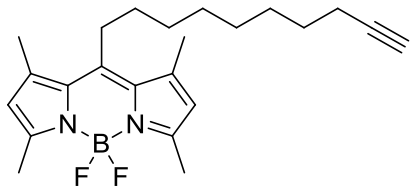
^{1}H NMR (400MHz, $CDCl_3$): δ = 1.07 (t, J = 8.0Hz, 3H), 2.17 (s, 3H), 2.23 (s, 3H), 2.39 (q, J = 8.0Hz, 2H), 2.51 (s, 6H), 6.00 (s, 1H), 6.99 (s, 1H). ^{13}C NMR (101MHz, $CDCl_3$): δ = 9.41, 11.23, 12.68, 14.50, 14.55, 17.28, 118.23, 119.26, 132.41, 132.79, 133.05, 137.64, 139.89, 155.09, 156.48. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{15}H_{20}N_2BF_2$: 277.16821; found: 277.16763



Scheme S2: Synthesis of B5-B9 (a) TEA, BF_3 ·Et_2O, DCM

B5

8-(Dec-9-yn-1-yl)-4, 4-difluoro-1, 3, 5, 7-tetramethyl-4-bora-3a, 4a-diaza-s-indacene

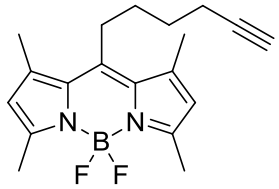


2, 4-Dimethyl-pyrrole (200 mg, 2.1 mmol) were dissolved in dry DCM (30 mL), undec-10-ynoyl chloride (210 mg, 1.05 mmol) was added under argon atmosphere at 25 ℃. Then the reaction mixture was heated at 50 ℃ and stirred for 2h, and cooled to 25 ℃. The solution was evaporated in vacuum, dry toluene (30 mL) and dry DCM (5 mL) was added to reaction mixture. After the reaction mixture was stirred for 5 min at 25 ℃, dry TEA (505mg, 5mmol) was added at 25 ℃, and after 15mins BF_3•Et_2O (760mg, 5mmol) was added by dropwise. The reaction mixture was heated to 50 ℃ for 1 h. The reaction mixture was evaporated in vacuum, and was extracted by EtOAc (300 mL), then washed with H_2O (3×50mL) and dried by Na2SO4. The crude product was purified by silica gel column chromatography (hexane/DCM 2:1) to yield 15 mg (3.7%) of B5 as red powder.

^{1}H NMR (400MHz, $CDCl_3$): δ = 1.32-1.44 (m, 6H), 1.45-1.56 (m, 4H), 1.59-1.67 (m, 2H), 1.94 (s, 1H), 2.17-2.21 (m, 2H), 2.41(s, 6H), 2.51(s, 6H), 2.91-2.95 (m, 2H), 6.05(s, 2H). ^{13}C NMR (101MHz, $CDCl_3$): δ = 14.44 (2C), 16.39 (2C), 18.37, 28.41, 28.47, 28.62, 29.02, 29.27, 30.32, 31.89, 68.17, 84.64, 121.56 (2C), 131.44, 140.27 (2C), 146.62(2C), 153.74 (2C). HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{23}H_{32}N_2BF_2$: 385.26211; found: 385.26135

B6

4, 4-Difluoro-8-(hex-5-yn-1-yl)- 1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene

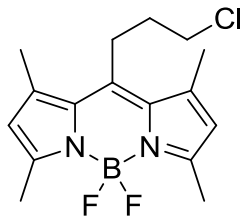


B6 (25 mg, 7.2%) was obtained from hept-6-ynoyl chloride (151mg, 1.05mmol) and 2, 4-dimethyl-pyrrole (200mg, 2.1mmol) as red powder.

^{1}H NMR (400MHz, $CDCl_3$): δ = 1.71-1.80 (m, 4H), 1.96 (s, 1H), 2.26-2.29 (m, 2H), 2.43(s, 6H), 2.52(s, 6H), 2.95-2.99(m, 2H), 6.06(s, 2H). ^{13}C NMR (101MHz, $CDCl_3$): δ = 14.46 (2C), 16.39 (2C), 18.26, 27.94, 28.96, 30.70, 69.05, 83.66, 121.67 (2C), 131.42, 140.30 (2C), 145.85(2C), 153.95 (2C). HRMS (ESI) : m/z $[M + H]^+$ calculated for $C_{19}H_{24}N_2BF_2$: 329.19951; found: 329.19904

B7

8- (Chloropropyl)-4, 4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene

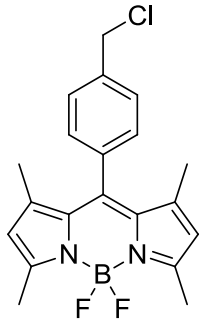


B7 (30 mg, 8.7%) was obtained from 4-chlorobutanoyl chloride (147 mg, 1.05 mmol) and 2, 4-dimethyl-pyrrole (200 mg, 2.1 mmol) as red powder.

^{1}H NMR (400MHz, $CDCl_3$): δ = 2.05-2.12 (m, 2H), 2.44 (s, 6H), 2.52 (s, 6H), 3.11-3.16 (m, 2H), 3.70 (t, J = 8.0Hz, 2H), 6.06 (s, 2H). ^{13}C NMR (101MHz, $CDCl_3$): δ = 14.48 (2C), 16.60 (2C), 25.95, 34.03, 44.75, 121.86 (2C), 131.43, 140.33 (2C), 144.43(2C), 154.37 (2C). HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{16}H_{21}N_2BClF_2$: 325.14489; found: 325.14423

B8

8-[4-(Chloromethyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene

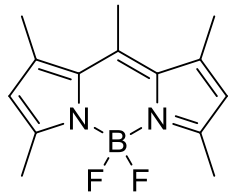


B8 (351 mg, 17.8%) was obtained from 4-(chloromethyl)benzoyl chloride (1.0 g, 5.32 mmol) and 2,4-dimethyl-pyrrole (1.01 g, 10.6 mmol) as red powder.

^{1}H NMR (400MHz, $CDCl_3$): δ = 1.38 (s, 6H), 2.55 (s, 6H), 4.66 (s, 2H), 5.98 (s, 2H), 7.29 (d, *J* = 8.0Hz, 2H), 7.52 (d, *J* = 8.0Hz, 2H). ^{13}C NMR (101MHz, $CDCl_3$): δ = 14.47 (2C), 14.59 (2C), 45.61, 121.34 (2C), 128.42 (2C), 129.26(2C), 131.32, 135.09(2C), 138.60(2C), 140.94, 143.03, 155.67 (2C). HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{20}H_{21}N_2BClF_2$: 373.14489; found: 373.14429

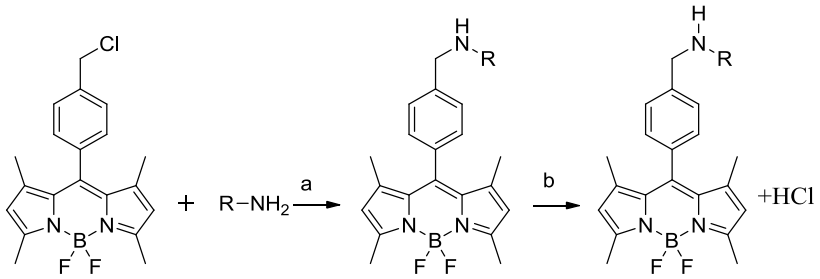
B9

4, 4-Difluoro-8-methyl-1, 3, 5, 7-tetramethyl-4-bora-3a, 4a-diaza-s-indacene



B9 (50 mg, 18.4%) was obtained from acetyl chloride (82 mg, 1.05 mmol) and 2, 4-dimethyl-pyrrole (200 mg, 2.1 mmol) as red powder.

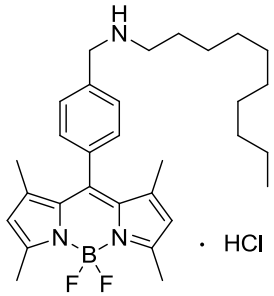
^{1}H NMR (400MHz, $CDCl_3$): δ = 2.40 (s, 6H), 2.51 (s, 6H), 2.56 (s, 3H), 6.05 (s, 2H). ^{13}C NMR (101MHz, $CDCl_3$): δ = 14.42 (2C), 16.37, 17.32 (2C), 121.22 (2C), 132.06, 140.99 (2C), 141.42 (2C), 153.60 (2C). HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{14}H_{18}N_2BF_2$: 263.15256; found: 263.15210



Scheme S3: Synthesis of B10-B13. (a) Cs_2CO_3, KI, CH_3CN; (b) HCl, DCM

B10

8-[4-((decylamino)methyl)phenyl]- 4, 4- difluoro-1, 3, 5, 7-tetramethyl- 4 -bora- *3a, 4a*-diaza-s -indacene



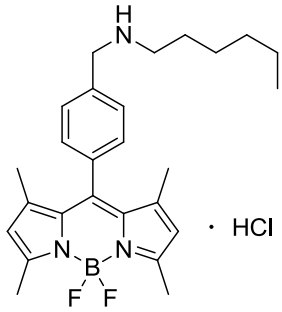
8-[4-(chloromethyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (B8) (50mg, 0.13mmol) , Cs_2CO_3 (87 mg, 0.26 mmol) and KI (45 mg, 0.26 mmol) were dissolved in dry CH_3CN (30mL) , decan-1-amine (210mg, 1.3mmol) was added under argon atmosphere at 25℃. Then the reaction mixture was heated at 80 ℃ and stirred for 2 h, and cooled to 25 ℃. The reaction mixture was evaporated in vacuum, and was extracted by EtOAc (200 mL), then washed with H_2O (3×50mL) and dried by Na_2SO_4. The crude product was purified by silica gel column chromatography (DCM/CH_3OH 50:1). The HCl salt of B10 was obtained by using a solution of HCl in DCM, and yielded 50 mg (70%) as red powder.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.86 (br, 3H), 1.22-1.27 (m, 14H), 1.35 (s, 6H), 1.89 (br, 2H), 2.55 (s, 6H), 2.76 (br, 2H), 4.29 (br, 2H), 5.97 (s, 2H), 7.38 (d, J = 4.0Hz, 2H), 7.80 (d, J = 4.0Hz, 2H), 10.14 (br, NH-HCl, 2H). ^{13}C NMR (101MHz, $CDCl_3$): δ = 14.10, 14.52 (2C), 14.62 (2C), 22.66, 26.03, 26.87, 28.98, 29.25, 29.44, 29.46, 31.86, 45.53, 49.89, 121.51 (2C), 129.24

(2C), 130.98 (2C), 131.05, 131.16 (2C), 136.60, 140.18, 142.64 (2C), 155.97 (2C). HRMS (ESI) : m/z $[M + H]^+$ calculated for $C_{30}H_{43}N_3BF_2$: 494.35126; found: 494.35129

B11

8-[4-((hexylamino)methyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indace ne

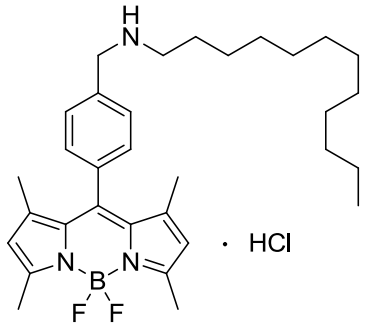


B11 (+HCl) (80 mg, 90%) was obtained from 8-[4-(chloromethyl)phenyl]-4,4-difluoro -1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (B8) (70 mg, 0.19 mmol) and hexan-1-amine (190 mg, 1.9 mmol) as red powder.

^{1}H NMR (400MHz, $CDCl_3$): δ = 0.84 (t, J = 8.0Hz, 3H), 1.26-1.30 (m, 6H), 1.35 (s, 6H), 1.90(br, 2H), 2.55 (s, 6H), 2.76 (br, 2H), 4.29 (br, 2H), 5.98 (s, 2H), 7.39 (d, J = 8.0Hz, 2H), 7.81 (d, J = 8.0Hz, 2H), 10.13 (br, NH-HCl, 2H). ^{13}C NMR (101MHz, $CDCl_3$): δ = 13.90, 14.51 (2C), 14.62 (2C), 22.41, 25.93, 26.48, 31.05, 45.52, 49.91, 121.52 (2C), 129.25 (2C), 130.97 (2C), 131.04, 131.16 (2C), 136.60, 140.18, 142.65 (2C), 155.97 (2C). HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{26}H_{35}N_3BF_2$: 438.28866; found: 438.28851

B12

8-[4-((dodecylamino)methyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-inda cene

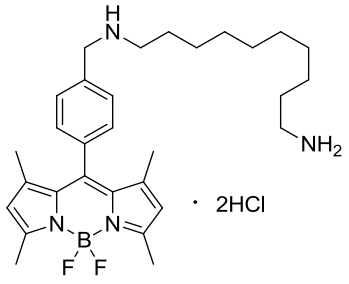


B12 (+HCl) (70 mg, 93%) was obtained from 8-[4-(chloromethyl)phenyl]-4,4-difluoro -1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (B8) (50 mg, 0.13 mmol) and dodecan-1-amine (248 mg, 1.3 mmol) as red powder.

^{1}H NMR (400MHz, $CDCl_3$): δ = 0.87 (t, J = 8.0Hz, 3H), 1.20-1.27 (m, 18H), 1.35 (s, 6H), 1.89 (br, 2H), 2.55 (s, 6H), 2.75 (br, 2H), 4.29 (br, 2H), 5.97 (s, 2H), 7.38 (d, J = 8.0Hz, 2H), 7.80 (d, J = 8.0Hz, 2H), 10.10 (br, NH-HCl, 2H). ^{13}C NMR (101MHz, $CDCl_3$): δ = 14.12, 14.50 (2C), 14.62 (2C), 22.69, 25.98, 26.86, 28.98, 29.35, 29.45, 29.52, 29.60, 29.63, 31.91, 45.52, 49.87, 121.51 (2C), 129.23 (2C), 130.97 (2C), 131.05, 131.16 (2C), 136.59, 140.19, 142.64 (2C), 155.96 (2C). HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{32}H_{47}N_3BF_2$: 522.38256; found: 522.38239

B13

8-[4-(((10-aminodecyl)amino)methyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaz a-s-indacene

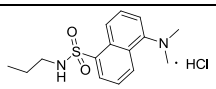
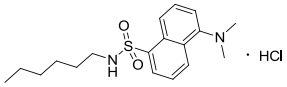
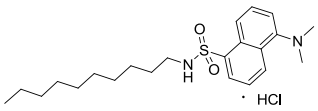
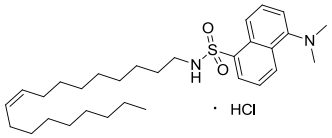
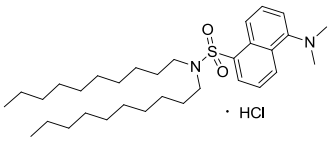
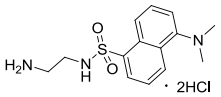
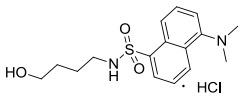
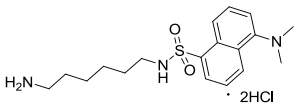
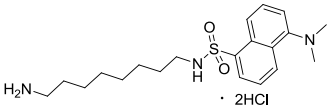
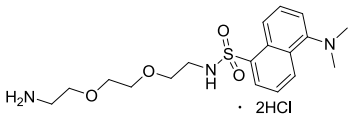
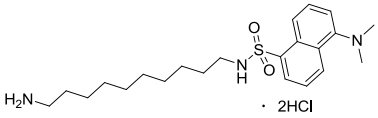
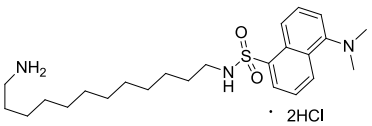
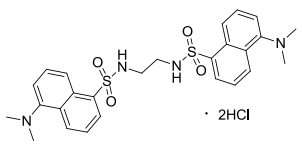
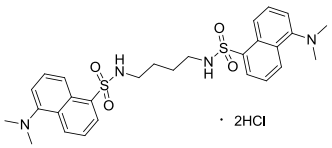


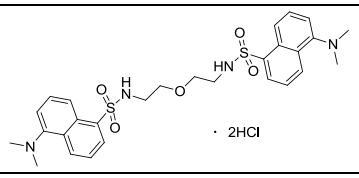
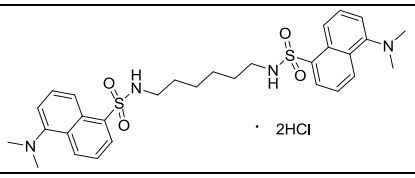
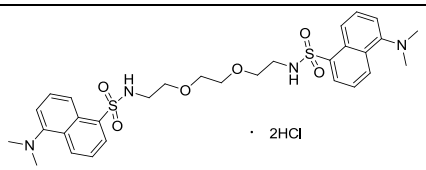
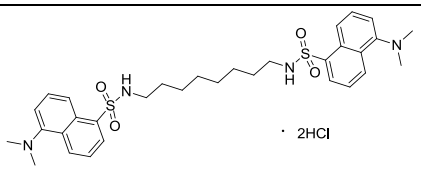
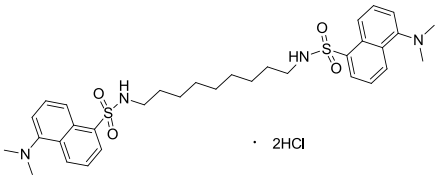
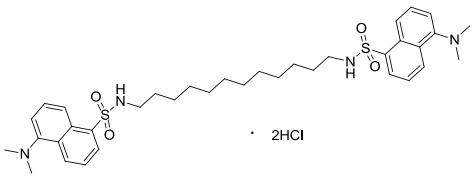
B13 (+2HCl) (80 mg, 73%) was obtained from 8-[4-(chloromethyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (B8) (70 mg, 0.19 mmol) and decane-1,10-diamine (323 mg, 1.9 mmol) as red powder.

^{1}H NMR (400MHz, CDCl3): δ = 1.30 (m, 12H), 1.35 (s, 6H), 1.86 (br, 4H), 2.55 (s, 6H), 2.79 (br, 2H), 3.06 (br, 2H), 4.34 (br, 2H), 5.98 (s, 2H), 7.37 (br, 2H), 7.81 (br, 2H), 8.28 (br, NH2-HCl, 3H), 9.88 (br, NH-HCl, 2H). ^{13}C NMR (101MHz, CDCl3): δ = 14.56 (2C), 14.63 (2C), 25.70, 25.93, 26.25, 27.15, 28.17 (3C), 29.72, 40.19, 45.77, 50.20, 121.52 (2C), 129.17 (2C), 131.12 (2C), 131.16 (3C), 136.51, 140.22, 142.66 (2C), 155.95 (2C). HRMS (ESI) : m/z $[M + H]^{+}$ calculated for $C_{30}H_{44}N_4BF_2$: 509.36216; found: 509.36160

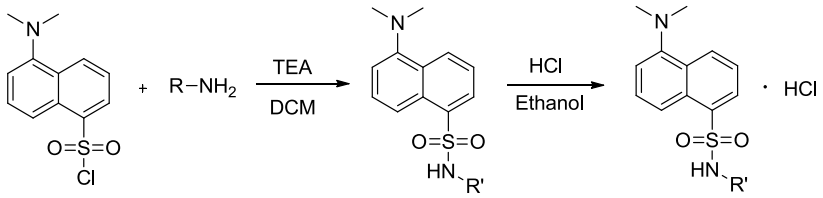
Series 2, Dansyl chloride derivatives

Table S2, The structure information of 20 DNS derived CLFPs

No	STRUCTURE	No	STRUCTURE
D1		D2	
D3		D4	
D5		D6	
D7		D8	
D9		D10	
D11		D12	
D13		D14	

D15		D16	
D17		D18	
D19		D20	

General procedure of the synthesis of 5-(dimethylamino)-naphthalene-1- sulfonamide derivatives (dansyl chloride based probes).

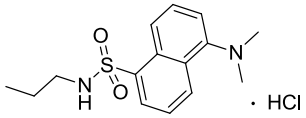


Scheme S4. Reaction conditions: a), D1 5 mmol, D2 5 mmol, TEA (10 mmol) solvent DCM (2 ml), for 2 hours; b), HCl, Ethanol (2.5 ml).

Dansyl chloride was added to a solution of alkyl amine and TEA in DCM at room temperature. The reaction mixture was kept to stir for 15h, and DCM was then evaporated. Final compound was obtained by column chromatography (MeOH/DCM). The product was kept as the HCl via re-crystalization in HCl of ethanol solution, all of the probes was produced as the form of HCl salt.

D1

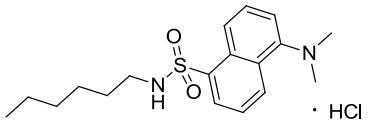
5-(Dimethylamino)-*N*-propylnaphthalene-1-sulfonamide



D1 (HCl-salt) (114 mg, 94%) was obtained from propylamine (22 mg, 0.37 mmol) and DNS-Cl (100mg, 0.37mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 0.76 (t, J = 7.4 Hz, 3H), 1.33-1.43 (m, 2H), 2.83 (t, J = 7.0 Hz, 2H), 3.51 (s, 6H), 7.85-7.93 (m, 2H), 8.13 (d, J = 7.8 Hz, 1H), 8.38 (d, J = 7.3 Hz, 1H), 8.59 (d, J = 8.7 Hz, 1H), 8.95 (d, J = 8.8 Hz, 1H). ^{13}C NMR (101MHz, CD_3OD): δ = 11.40, 24.05, 45.79, 47.81 (2C), 120.52, 126.48, 127.25, 128.01, 128.55, 128.68, 130.74, 131.10, 139.17, 140.61. HRMS (ESI): m/z $[M + H]^+$ calculated for $C_{15}H_{21}N_2O_2S$: 293.13183; found: 293.13162.

D2

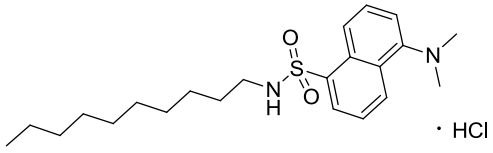
5-(Dimethylamino)-*N*-hexylnaphthalene-1-sulfonamide



D2 (HCl-salt) (124mg, 91%) was obtained from hexylamine (37mg, 0.37mmol) and DNS-Cl (100mg, 0.37mmol) as white power. ^{1}H NMR (400MHz, CD3OD): δ = 0.76 (t, J = 6.9 Hz, 3H), 1.07-1.12 (m, 6H), 1.28-1.33(dd, 2H), 2.85 (t, J = 6.9 Hz, 2H), 3.51 (s, 6H), 7.84-7.91 (m, 2H), 8.15 (d, J = 7.8 Hz, 1H), 8.36 (d, J = 7.3 Hz, 1H), 8.64 (d, J = 8.7 Hz, 1H), 8.93 (d, J = 8.8 Hz, 1H); ^{13}C NMR (101MHz, CD_3OD): δ = 14.25, 23.43, 27.17, 30.58, 32.29, 43.91, 47.88 (2C), 120.64, 126.56, 127.17, 128.04, 128.57, 128.77, 130.71, 131.18, 139.12, 140.44; HRMS (ESI) : m/z $[M + H]^+$ calculated for $C_{18}H_{27}N_2O_2S$: 335.17878; found: 335.17871.

D3

N-decyl-5-(dimethylamino) naphthalene-1-sulfonamide

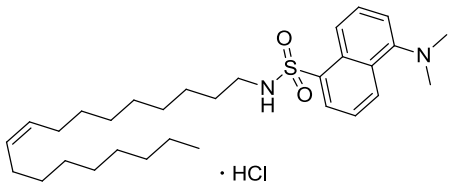


D3 (HCl-salt) (137mg, 87%) was obtained from decylamine (58mg, 0.37mmol) and DNS-Cl (100mg, 0.37mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 0.89 (t, J = 6.9 Hz, 3H), 1.12-1.36 (m, 16H), 2.87 (t, J = 7.0 Hz, 2H), 3.50 (s, 6H), 7.85-7.92 (m, 2H), 8.12 (d, J = 7.8 Hz,

1H), 8.38 (d, *J* = 7.3 Hz, 1H), 8.59 (d, *J* = 8.7 Hz, 1H), 8.94 (d, *J* = 8.7 Hz, 1H); ^{13}C NMR (101MHz, CD_3OD): δ = 14.42, 23.70, 27.54, 30.11, 30.37, 30.52, 30.57, 30.69, 33.01, 43.94, 47.76 (2C), 120.39, 126.56, 127.35, 127.93, 128.50, 128.57, 130.77, 131.13, 139.15, 140.96. HRMS (ESI): m/z [M + H] calculated for $C_{22}H_{35}N_2O_2S$: 391.24138; found: 391.24127.

D4

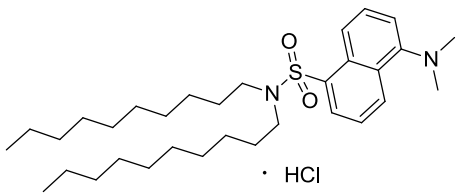
(Z)-5-(dimethylamino)-*N*-(octadec-9-en-1-yl) naphthalene-1-sulfonamide



D4 (HCl-salt) (180 mg, 91%) was obtained from octadece-9-nyl amine (110mg, 0.37mmol) and DNS-Cl (100mg, 0.37 mmol) as white wax. ^{1}H NMR (400MHz, CD_3OD): δ = 0.86 (t, *J* = 6.6 Hz, 3H), 1.11-1.26 (m, 26H), 1.94-2.00 (m, 2H), 2.84 (t, *J* = 6.9 Hz, 2H), 3.48 (s, 6H), 5.28-5.35 (m, 2H), 7.82-7.91 (m, 2H), 8.10 (d, *J* = 7.5 Hz, 1H), 8.35 (dd, J_1 = 7.5 Hz, J_2 = 0.9 Hz, 1H), 8.57 (d, *J* = 8.7 Hz, 1H), 8.92 (d, *J* = 8.7 Hz, 1H); ^{13}C NMR (101MHz, CD_3OD): δ = 14.45, 23.71, 27.55, 28.11, 30.10, 30.19, 30.32, 30.40, 30.42, 30.58, 30.71, 30.74, 30.77, 30.81, 33.03, 43.94, 47.83 (2C), 120.57, 126.56, 127.23, 128.01, 128.57, 128.73, 130.75, 130.76, 130.86, 131.13, 139.15, 140.58; HRMS (ESI) : m/z [M + H] calculated for $C_{30}H_{49}N_2O_2S$: 501.35393; found: 501.35303.

D5

N, *N'*-didecyl-5-(dimethylamino) naphthalene-1-sulfonamide

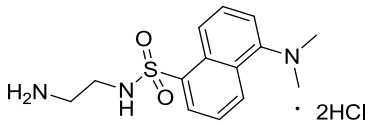


D5 (HCl-salt) (179mg, 93%) was obtained from didecylamine (100mg, 0.34mmol) and DNS-Cl (91mg, 0.34mmol) as white wax. ^{1}H NMR (400MHz, CD_3OD): δ = 0.90 (t, *J* = 6.7 Hz, 6H), 1.19-1.27 (m, 28H), 1.50 (br, 4H), 3.28 (br, 2H), 3.35 (br, 2H), 3.45 (s, 6H), 7.83-7.92 (m, 2H),

8.05 (d, *J* = 7.7 Hz, 1H), 8.33 (d, *J* = 7.2 Hz, 1H), 8.55 (d, *J* = 8.8 Hz, 1H), 8.81 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (101MHz, CD_3OD): δ = 14.43 (2C), 23.72 (2C), 27.61 (2C), 29.33 (2C), 30.19 (2C), 30.41 (4C), 30.56 (2C), 30.59 (2C), 33.04 (2C), 47.63 (2C), 120.21, 126.93, 127.76 (2C), 128.19, 128.63, 129.54, 131.22 (2C), 138.62. HRMS (ESI) : m/z [M + H] calculated for $C_{32}H_{55}N_2O_2S$: 531.39788; found: 531.39752.

D6

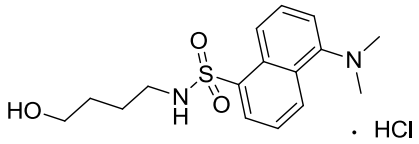
N-(2-aminoethyl)-5-(dimethylamino) naphthalene-1-sulfonamide



D6 (HCl-salt) (110 mg, 80%) was obtained from ethane-1,2-diamine (22mg, 0.37mmol) and DNS-Cl (100mg, 0.37mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 3.07-3.11 (m, 4H), 3.49 (s, 6H), 7.89-7.95 (m, 2H), 8.13 (d, *J* = 7.7 Hz, 1H), 8.41 (d, *J* = 7.3 Hz, 1H), 8.66 (d, *J* = 8.7 Hz, 1H), 8.90 (d, *J* = 8.7 Hz, 1H). ^{13}C NMR (101MHz, CD_3OD): δ = 40.81, 41.23, 47.83(2C), 120.85, 127.36, 127.40, 127.97, 128.30, 129.02, 130.55, 131.51, 137.69, 140.83. HRMS (ESI) : m/z [M + H] calculated for $C_{14}H_{20}N_3O_2S$: 294.12707; found: 294.12665.

D7

5-(Dimethylamino)-N-(4-hydroxybutyl) naphthalene-1-sulfonamide

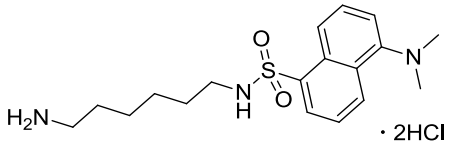


D7 (HCl-salt) (110 m g, 83%) was obtained from 4-Amino-1-butanol (33mg, 0.37mmol) and DNS-Cl (100mg, 0.37mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 1.33-1.47 (m, 4H), 2.89 (t, *J* = 6.5 Hz, 2H), 3.39 (t, *J* = 6.0 Hz, 2H), 3.51 (s, 6H), 7.85-7.92 (m, 2H), 8.13 (d, *J* = 7.8 Hz, 1H), 8.38 (d, *J* = 7.4 Hz, 1H), 8.61 (d, *J* = 8.7 Hz, 1H), 8.94 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (101MHz, CD_3OD): δ = 27.24, 30.47, 43.80, 47.83 (2C), 62.19, 120.60, 126.59, 127.21,

128.01, 128.60, 128.66, 130.69, 131.15, 139.00, 140.52; HRMS (ESI) : m/z [M + H] calculated for $C_{16}H_{23}N_2O_3S$: 323.14239; found: 323.14233.

D8

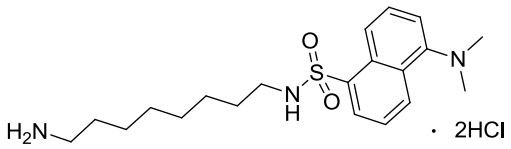
N-(6-aminohexyl)-5-(dimethylamino) naphthalene-1-sulfonamide



D8 (2HCl-salt) (142mg, 74%) was obtained from 1,6-hexanediamine (429mg, 3.7mmol) and DNS-Cl (100mg, 0.37mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 1.25 (br, 4H), 1.41 (br, 2H), 1.54 (br, 2H), 2.82-2.89(m, 4H), 3.51 (s, 6H), 7.86-7.92 (m, 2H), 8.15 (d, *J* = 7.7 Hz, 1H), 8.36 (d, *J* = 7.3 Hz, 1H), 8.67 (d, *J* = 8.6 Hz, 1H), 8.94 (d, *J* = 8.7 Hz, 1H); ^{13}C NMR (101MHz, CD_3OD): δ = 26.83, 26.96, 28.35, 30.52, 40.61, 43.72, 44.14, 47.88 (2C), 120.77, 126.75, 127.17, 128.04, 128.64, 128.79, 130.71, 131.09, 139.06, 140.36; HRMS (ESI) : m/z [M + H] calculated for $C_{18}H_{28}N_3O_2S$: 350.18967; found: 350.18900.

D9

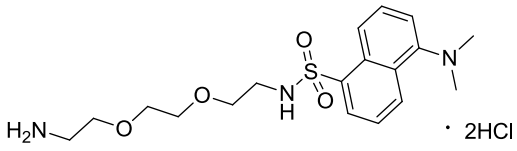
N-(8-aminooctyl)-5-(dimethylamino) naphthalene-1-sulfonamide



D9 (2HCl-salt) (113mg, 68%) was obtained from 1,8-diaminooctane (535mg, 3.7mmol) and DNS-Cl (100mg, 0.37mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 1.21-1.40 (br, 10H), 1.60-1.63 (m, 2H), 2.86-2.91 (m, 4H), 3.48(s, 6H), 7.85-7.92 (m, 2H), 8.10 (d, *J* = 7.8 Hz, 1H), 8.36 (d, *J* = 7.1 Hz, 1H), 8.62 (d, *J* = 8.8 Hz, 1H), 8.92 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (101MHz, CD_3OD): δ = 27.28, 27.32, 28.46, 29.76, 29.93, 30.60, 40.73, 43.84, 47.80 (2C), 120.60, 126.93, 127.31, 127.89, 128.48, 128.63, 130.71, 131.09, 139.00, 140.87; HRMS (ESI) : m/z [M + H] calculated for $C_{20}H_{32}N_3O_2S$: 378.22097; found: 378.22049.

D10

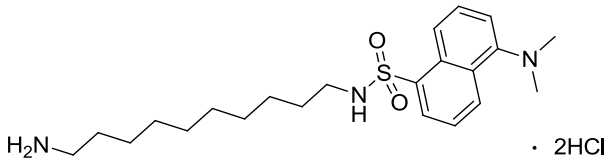
N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide



D10 (2HCl-salt) (110mg, 66%) was obtained from 1,8-diamino-3,6-dioxaoctane (551mg, 3.7mmol) and DNS-Cl (100mg, 0.37mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 3.05-3.08 (m, 4H), 3.40-3.43 (m, 4H), 3.48 (br, 8H), 3.64 (t, *J* = 4.8 Hz, 2H), 7.84-7.89 (m, 2H), 8.13 (d, *J* = 7.7 Hz, 1H), 8.36 (d, *J* = 7.3 Hz, 1H), 8.72 (d, *J* = 8.6 Hz, 1H), 8.89 (d, *J* = 8.7 Hz, 1H); ^{13}C NMR (101MHz, CD_3OD): δ = 40.63, 43.68, 47.82 (2C), 49.85, 67.74, 70.69, 71.12, 120.70, 127.12, 127.28, 127.88, 128.44, 128.70, 130.63, 130.95, 138.90, 140.82; HRMS (ESI) : m/z [M + H] calculated for $C_{18}H_{28}N_3O_4S$: 382.17950; found: 382.17908.

D11

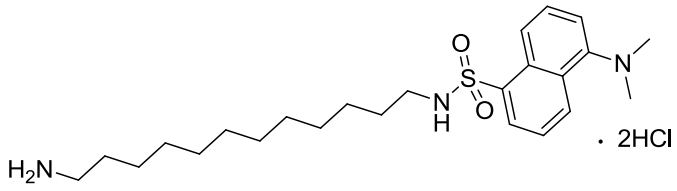
N-(10-aminodecyl)-5-(dimethylamino) naphthalene-1-sulfonamide



D11 (2HCl-salt) (99.5mg, 56%) was obtained from 1,10-Diaminodecane (640mg, 3.7mmol) and DNS-Cl (100mg, 0.37mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 1.16-1.37 (br, 14H), 1.60-1.67 (br, 2H), 2.84-2.92 (br, 4H), 3.49(s, 6H), 7.85-7.92 (m, 2H), 8.13 (d, *J* = 7.8 Hz, 1H), 8.37 (d, *J* = 7.3 Hz, 1H), 8.64 (d, *J* = 8.7 Hz, 1H), 8.94 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (101MHz, CD_3OD): δ = 27.42, 27.47, 28.55, 30.02, 30.12, 30.31, 30.36, 30.70, 40.78, 43.91, 47.81(2C), 120.52, 126.78, 127.34, 127.91, 128.52, 128.60, 130.76, 131.08, 139.09, 140.90; HRMS (ESI) : m/z [M + H] calculated for $C_{22}H_{36}N_3O_2S$: 406.25227; found: 406.25177.

D12

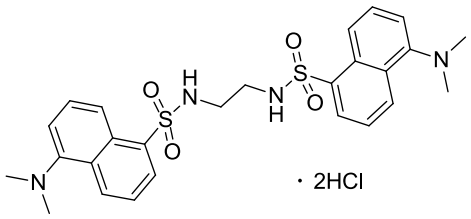
N-(12-aminododecyl)-5-(dimethylamino) naphthalene-1-sulfonamide



D12 (2HCl-salt) (200.3mg, 71%) was obtained from 1,12-diaminododecane (1.1g, 5.6mmol) and DNS-Cl (150mg, 0.56mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 1.16-1.37 (br, 18H), 1.62-1.67 (m, 2H), 2.85-2.92 (m, 4H), 3.49(s, 6H), 7.85-7.92 (m, 2H), 8.11 (br, 1H), 8.38 (d, *J* = 7.3 Hz, 1H), 8.64 (br, 1H), 8.94 (d, *J* = 8.7 Hz, 1H). ^{13}C NMR (101MHz, CD_3OD): δ = 27.44, 27.51, 28.56, 30.08, 30.19, 30.46, 30.49, 30.55(2C), 30.69, 40.77, 43.92, 47.80(2C), 120.55, 126.79, 127.31, 127.91, 128.53, 128.60, 130.73, 131.11, 139.05, 140.86. HRMS (ESI) : m/z [M + H] calculated for $C_{24}H_{40}N_3O_2S$: 434.28357; found: 434.28326.

D13

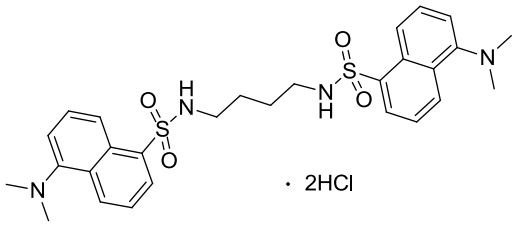
N, *N'*-(ethane-1, 2-diyl) bis(5-(dimethylamino)naphthalene-1-sulfonamide)



D13 (2HCl-salt) (99mg, 92%) was obtained from 1,2-Diaminoethane (11mg, 0.18mmol) and DNS-Cl (100mg, 0.37mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 2.87 (s, 4H), 3.50 (s, 12H), 7.88 (dd, J_1 = 17.6 Hz, J_2 =8.8 Hz, 4H), 8.12 (d, *J* = 7.7 Hz, 2H), 8.30 (d, *J* = 7.3 Hz, 2H), 8.58 (d, *J* = 8.8 Hz, 2H), 8.84 (d, *J* = 8.8 Hz, 2H). ^{13}C NMR (101MHz, CD_3OD): δ = 43.75 (2C), 47.77 (4C), 120.48 (2C), 126.70 (2C), 127.42 (2C), 127.91 (2C), 128.38 (2C), 128.74 (2C), 130.65 (2C), 131.18 (2C), 138.56 (2C), 140.89 (2C). HRMS: m/z [M + H] calculated for $C_{26}H_{31}N_4O_4S_2$: 527.17812; found: 527.17810.

D14

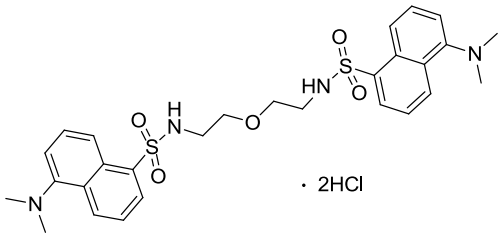
N, *N'*-(butane-1, 4-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide)



D14 (2HCl-salt) (146mg, 88%) was obtained from 1,4-diaminobutane (23mg, 0.26mmol) and DNS-Cl (150mg, 0.56mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 1.23 (br, 4H), 2.68 (br, 4H), 3.50(s, 12H), 7.87-7.89 (m, 4H), 8.12 (d, *J* = 7.1 Hz, 2H), 8.32 (d, *J* = 7.1 Hz, 2H), 8.58 (d, *J* = 8.5 Hz, 2H), 8.89 (d, *J* = 7.9 Hz, 2H). ^{13}C NMR (101MHz, CD_3OD): δ = 27.52(2C), 43.12(2C), 47.73(4C), 120.36(2C), 126.67(2C), 127.45(2C), 127.89(2C), 128.24(2C), 128.67(2C), 130.68(2C), 131.11(2C), 138.92(2C), 141.21(2C). HRMS (ESI) : m/z [M + H] calculated for $C_{28}H_{35}N_4O_4S_2$: 555.20942; found: 555.20935.

D15

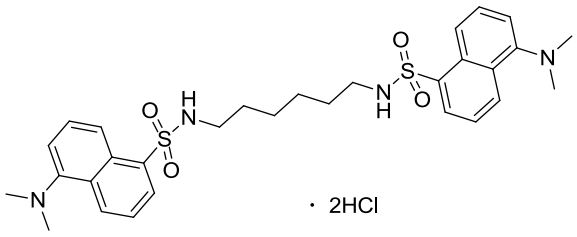
5-(Dimethylamino)-*N*-(2-(2-(8-(dimethylamino)naphthalene-2-sulfonamido)ethoxy)ethyl)naphthalene-1-sulfonamide



D15 (2HCl-salt) (91.7mg, 53%) was obtained from 2, 2'-oxybis(ethylamine) (28mg, 0.26mmol) and DNS-Cl (150mg, 0.56mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 2.83(t, *J* = 5.3 Hz, 4H), 3.04 (t, *J* = 5.3 Hz, 4H), 3.49 (s, 12H), 7.85-7.90 (m, 4H), 8.11 (d, *J* = 7.8 Hz, 2H), 8.34 (d, *J* = 7.3 Hz, 2H), 8.61 (d, *J* = 8.7 Hz, 2H), 8.88 (d, *J* = 8.8 Hz, 2H). ^{13}C NMR (101MHz, CD_3OD): 43.61(2C), 47.67(4C), 70.26(2C), 120.31(2C), 126.98(2C), 127.53(2C), 127.75(2C), 128.00(2C), 128.74(2C), 130.70(2C), 130.93(2C), 139.03(2C), 141.58(2C); HRMS (ESI) : m/z [M + H] calculated for $C_{28}H_{35}N_4O_5S_2$: 571.20434; found: 571.20422.

D16

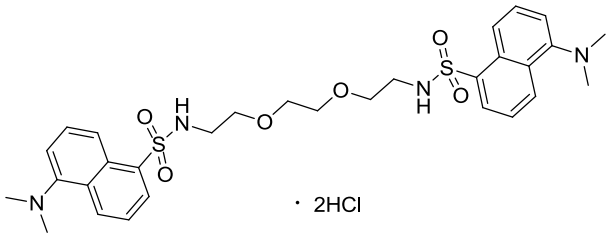
N, N'-(hexane-1, 6-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide)



D16 (2HCl-salt) (118mg, 95%) was obtained from 1,6-Hexanediamine (21mg, 0.18mmol) and DNS-Cl (100mg, 0.37mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 0.95 (br, 4H), 1.17 (br, 4H), 2.77 (t, *J* = 6.9 Hz, 4H), 3.50 (s, 12H), 7.85-7.93 (m, 4H), 8.12 (d, *J* = 7.8 Hz, 2H), 8.36 (d, *J* = 7.3 Hz, 2H), 8.59 (d, *J* = 8.7 Hz, 2H), 8.92 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (101MHz, CD_3OD): δ = 26.80 (2C), 30.44 (2C), 43.68 (2C), 47.78 (4C), 120.46 (2C), 126.66 (2C), 127.36 (2C), 127.95 (2C), 128.45 (2C), 128.63 (2C), 130.74 (2C), 131.12 (2C), 139.07 (2C), 140.97 (2C); HRMS (ESI) : m/z [M + H] calculated for $C_{30}H_{39}N_4O_4S_2$: 583.24072; found: 583.24054.

D17

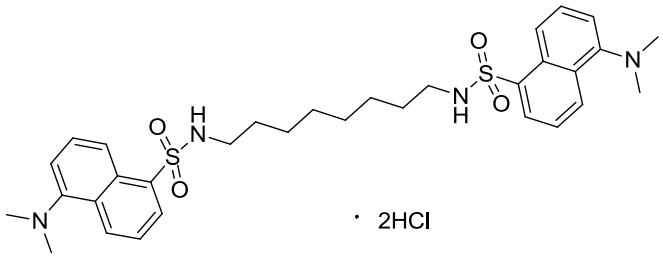
N, *N'*-((ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(5-(dimethylamino) naphthalene-1-sulfonamide)



D17 (2HCl-salt) (106mg, 62%) was obtained from 1,8-diamino-3,6-dioxaoctane (38mg, 0.25mmol) and DNS-Cl (150mg, 0.50mmol) as yellow power. ^{1}H NMR (400MHz, CD_3OD): δ = 3.05 (t, *J* = 5.4 Hz, 4H), 3.24 (s, 4H), 3.35 (t, *J* = 5.4 Hz, 4H), 3.48 (s, 12H), 7.88 (dd, J_1 = 17.5 Hz, J_2 = 8.8 Hz, 4H), 8.10 (d, *J* = 7.7 Hz, 2H), 8.39 (d, *J* = 7.3 Hz, 2H), 8.58 (d, *J* = 8.7 Hz, 2H), 8.91 (d, *J* = 8.7 Hz, 2H);^{13}C NMR (101MHz, CD_3OD): δ = 43.77 (2C), 47.85 (4C), 70.65 (2C), 71.00 (2C), 120.63 (2C), 126.75 (2C), 127.26 (2C), 128.04 (2C), 128.67 (4C), 130.71 (2C), 131.04 (2C), 139.09 (2C), 140.63 (2C); HRMS (ESI) : m/z [M + H] calculated for $C_{30}H_{39}N_4O_6S_2$: 615.23055; found: 615.22974.

D18

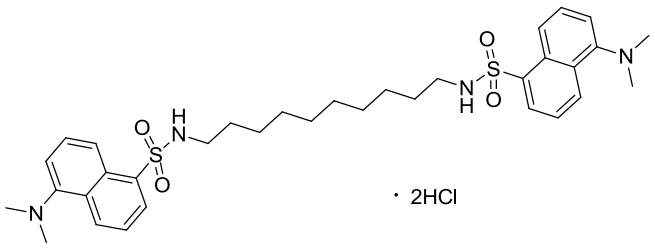
N, *N'*-(octane-1, 8-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide)



D18 (2HCl-salt) (109mg, 89%) was obtained from 1,8-Diaminooctane (26mg, 0.18mmol) and DNS-Cl (100mg, 0.37mmol) as white power. ^{1}H NMR (400MHz, $(CD_3)_2SO$): δ = 0.79 (br, 4H), 0.92 (br, 4H), 1.17-1.20 (m, 4H), 2.72-2.74 (m, 4H), 3.01 (s, 12H), 7.59 (br, 2HNH), 7.69 (dd, J_1 = 15.8 Hz, J_2 = 8.0 Hz, 4H), 7.95 (br, 2H), 8.14 (d, J = 7.2 Hz, 2H), 8.50 (d, J = 7.9 Hz, 2H), 8.68 (d, J = 8.0 Hz, 2H); ^{13}C NMR (101MHz, $(CD_3)_2SO$): δ = 25.58 (2C), 28.05 (2C), 28.82 (2C), 42.21 (2C), 45.53 (4C), 124.51 (2C), 127.51 (4C), 128.43 (4C), 128.49 (4C), 128.84 (4C), 136.47 (2C); HRMS (ESI) : m/z [M + H] calculated for $C_{32}H_{43}N_4O_4S_2$: 611.27202; found: 611.27045.

D19

N, *N'*-(decane-1,10-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide)

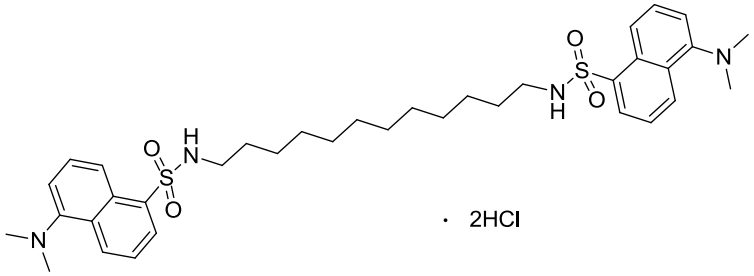


D19 (2HCl-salt) (163.5mg, 86%) was obtained from 1, 10-diaminodecane (46mg, 0.26mmol) and DNS-Cl (150mg, 0.56mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 1.05-1.23 (br, 12H), 1.30-1.36 (br, 4H), 2.86 (t, J = 6.9 Hz, 4H), 3.49(s, 12H), 7.85-7.92 (m, 4H), 8.12 (d, J = 7.8 Hz, 2H), 8.37 (d, J = 7.3 Hz, 2H), 8.58 (d, J = 8.7 Hz, 2H), 8.94 (d, J = 8.8 Hz, 2H); ^{13}C NMR (101MHz, CD_3OD): δ = 27.43(2C), 29.95(2C), 30.27(2C), 30.63(2C), 43.89(2C), 47.82(4C), 120.53(2C), 126.60(2C), 127.28(2C), 127.99(2C), 128.60(4C), 130.74(2C),

131.14(2C), 139.11(2C), 140.74(2C); HRMS (ESI) : m/z [M + H] calculated for $C_{34}H_{47}N_4O_2S_2$: 639.30332; found: 639.30310.

D20

N, *N'*-(dodecane-1, 12-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide)



D20 (2HCl-salt) (180.5mg, 92%) was obtained from 1,12-diaminododecane (53mg, 0.26mmol) and DNS-Cl (150mg, 0.56mmol) as white power. ^{1}H NMR (400MHz, CD_3OD): δ = 1.11 (br, 16H), 1.33-1.36 (m, 4H), 2.86 (t, *J* = 6.9 Hz, 4H), 3.49(s, 12H), 7.85-7.92 (m, 4H), 8.12 (d, *J* = 7.8 Hz, 2H), 8.37 (d, *J* = 7.3 Hz, 2H), 8.59 (d, *J* = 8.7 Hz, 2H), 8.94 (d, *J* = 8.7 Hz, 2H); ^{13}C NMR (101MHz, $(CD_3)_2SO$): δ = 25.63(2C), 28.18(2C), 28.57(2C), 28.59(2C), 28.81(2C), 42.13(2C), 45.70(4C), 118.00(2C), 123.56(2C), 124.97(2C), 126.87(2C), 127.32(4C), 127.92(2C), 128.55(2C), 128.61(2C), 136.51(2C); HRMS (ESI) : m/z [M + H] calculated for $C_{36}H_{51}N_4O_4S_2$: 667.33462; found: 667.33411.

1.3. Probe characterization (photophysical parameters, cell cytoxicity, cell distribution, incubation concentration)

General information: The fluorescence microscopy was performed with fluorescence microscopy Olympus IX71. Cancer cell lines, HepG2, MCF-7 and A2780 were obtained from cell center of Chinese Academy of Medical Sciences & Peking Union Medical College. They were cultured in DMEM medium (Invitrogen) with 10% fetal bovine serum (Gibco) at 37 ℃ with 5% CO_2.

Screening of photophysical parameters (λex, λem) for all CLFP probes.

Fluorescence excitation and emission spectra were measured and calculated with HITACHI F-7000 Fluorescence Spectrophotometer. Each compound was dissolved in PBS buffer, pH 7.4 with the concentration of 10 μM.

Table S3, The photophysical parameters (λ_{ex}, λ_{em}) for all of the CLFP probe in this study.

Cmpd	λ_{ex} (nm)	λ_{em} (nm)
B1	482	504
B2	502	508
B3	502	510
B4	514	522
B5	500	508
B6	492	504
B7	496	506
B8	498	510
B9	490	504
B10	498	510
B11	500	510
B12	500	510
B13	500	510
D1	330	558
D2	336	544
D3	370	498
D4	350	498
D5	350	494
D6	330	564
D7	332	556
D8	332	554
D9	334	550
D10	330	560
D11	332	546
D12	348	530
D13	352	502
D14	352	504
D15	356	506
D16	352	500
D17	330	508
D18	350	502
D19	350	500
D20	352	502

Cell Cytoxicity Assay

Probe B10 (0.5 μM) and D13 (25 μM) were incubated with three cell line HepG2, A2780 and MCF-7 for 1 hour, which is similar to the staining condition in this study. After that, the dyes were washed away using PBS buffer and cells were kept culturing under regular condition, and we did not observe cell toxicity for all three cell lines.

Nevertheless, we also evaluated cell cytoxicities for all 33 fluorescent probes using MTT method. Most of the compounds have the IC_{50} over than 50 μM for all tested cancer cell lines, but a few of them do show some cell cytotoxicities.

Table S4: Evaluation of cell cyto-toxicity of CLFP compounds

Cmpd	IC_{50} (μM)		
	HepG2	A2780	MCF-7
B1	>50	>50	>50
B2	>50	>50	>50
B3	>50	>50	>50
B4	>50	>50	>50
B5	>50	>50	>50
B6	>50	>50	>50
B7	>50	>50	>50
B8	>50	>50	>50
B9	>50	>50	>50
B10	37	32	34
B11	24	15	13
B12	43	35	26
B13	>50	46	41
D1	>50	>50	>50
D2	>50	>50	>50
D3	>50	46	>50
D4	>50	>50	>50
D5	>50	>50	>50
D6	>50	>50	>50
D7	>50	>50	>50
D8	>50	>50	>50
D9	>50	>50	>50
D10	>50	>50	>50
D11	26	29	26
D12	27	29	31

D13	>50	48	>50
D14	>50	>50	>50
D15	>50	>50	>50
D16	>50	>50	>50
D17	>50	>50	>50
D18	>50	>50	>50
D19	>50	>50	>50
D20	>50	>50	>50

Screening of fluorescence intensities of the probes in living cells

HepG2 cells were seeded in cell culture plates, and when the cells reached 50% confluence, two series of fluorescent probes (BODIPY and DNS) were separately added into the each well with the final concentration of 0.5μM, 1μM (BODIPY), 10μM, 25μM (DNS). After incubation for 10 to 30 min, the fluorescent probes were washed away with PBS buffer. HepG2 cells were imaged under fluorescent microscopy. The relative fluorescence intensities of all probes were compared to choose the feasible CLFP probes (Figure S1-S4).

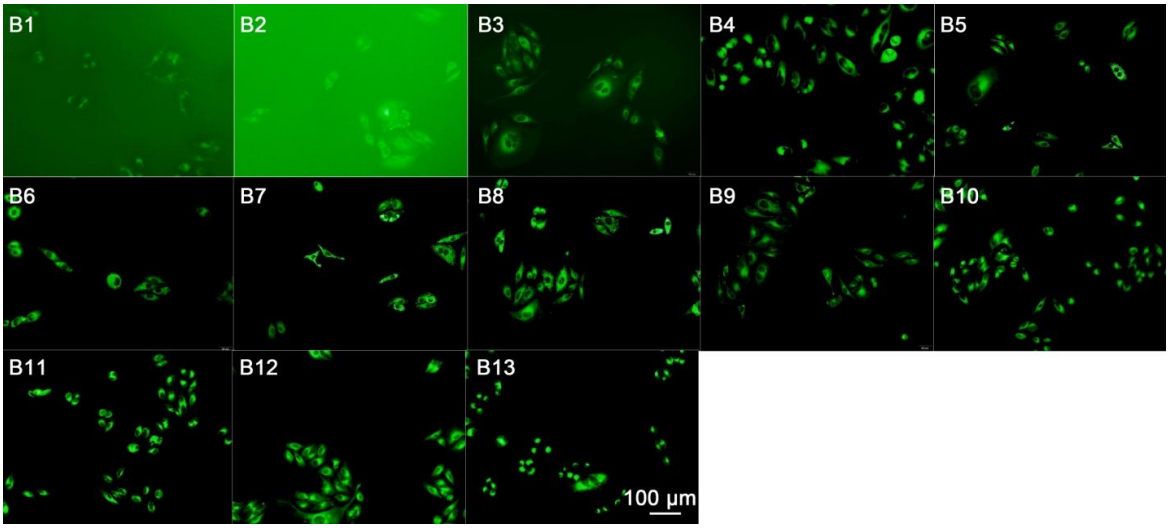


Figure S1. Screening of cellular distribution of fluorescent probes B1 to B13 in HepG2 cells at 0.5 μM after 30min of incubation.

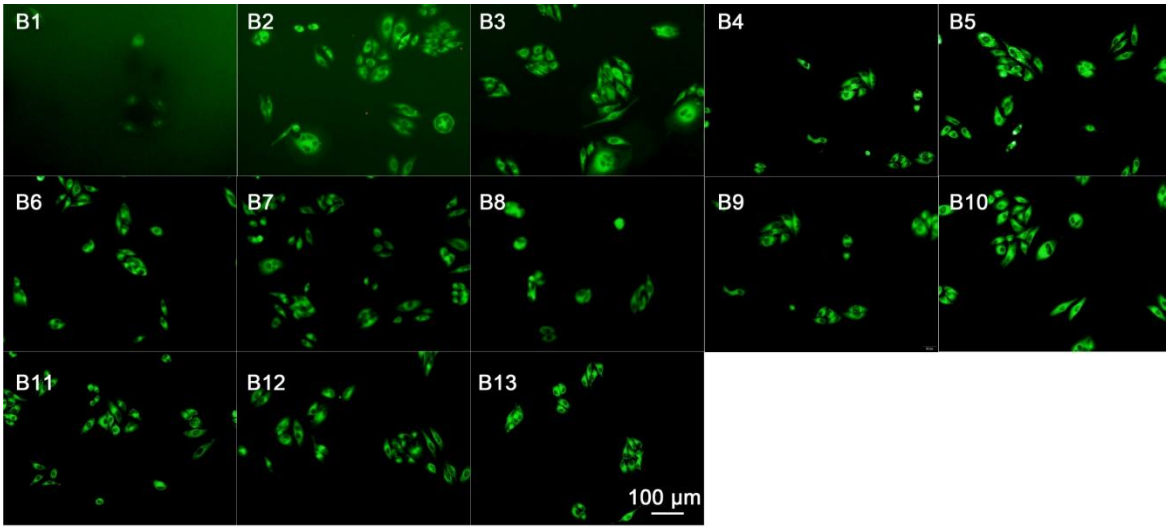


Figure S2. Screening of cellular distribution of fluorescent probes B1 to B13 in HepG2 cells at 1 μM after 30 min of incubation.

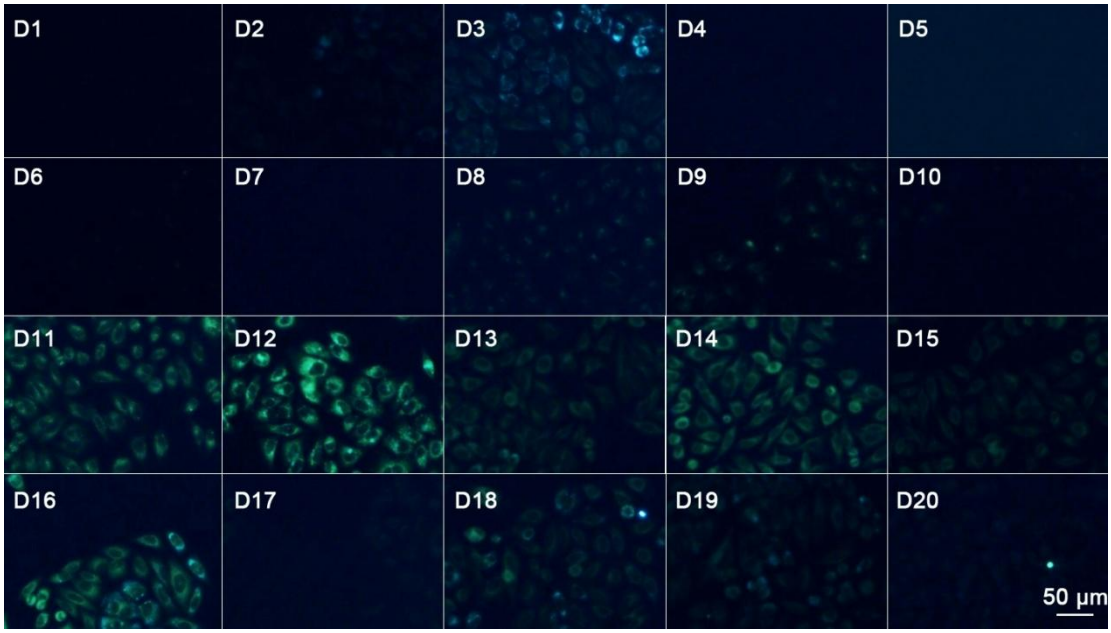


Figure S3. Screening of cellular distribution of fluorescent probes D1 to D20 in HepG2 cells at 10 μM after 30 min of incubation.

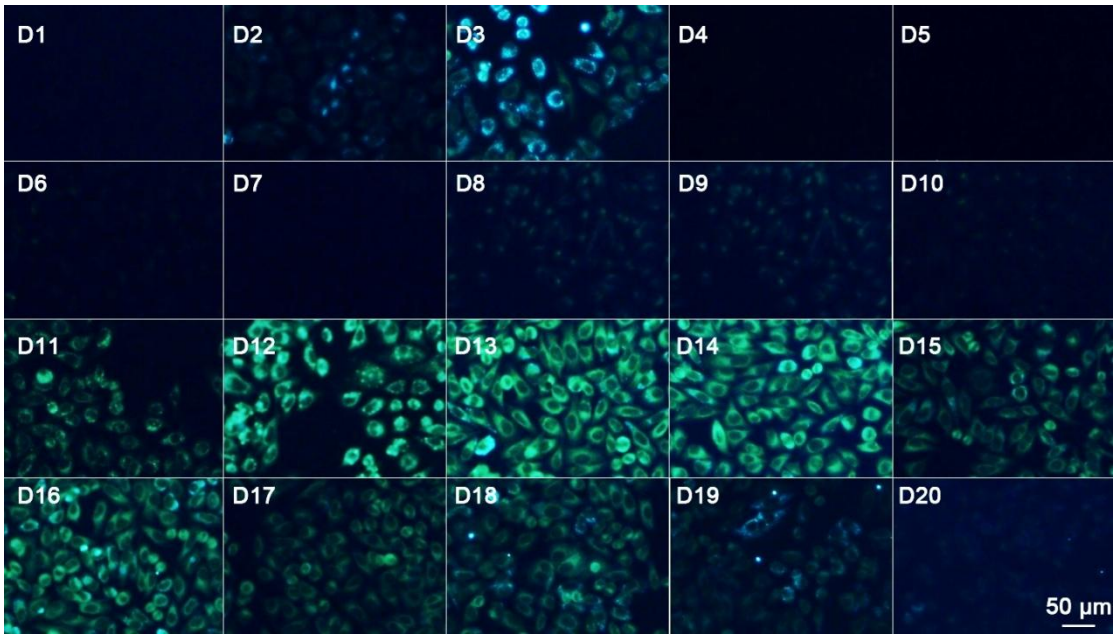


Figure S4. Screening of cellular distribution of fluorescent probes D1 to D20 in HepG2 cells at 25 μM after 30min of incubation.

For BODIPY derivatives, at a concentration of 0.5 and 1 μM, the fluorescent intensities of many probes in HepG2 cells were sufficiently bright (Figure S1, S2) after 10 to 30 min incubation. For DNS derivatives, at a concentration of 10 μM, the fluorescent intensities of many probes in HepG2 cells were sufficiently bright (Figure S3, S4) after 10 to 30 min incubation. Generally, at the same molar concentration, probes with two DNS moieties exhibited stronger fluorescent intensity than probes with a single DNS moiety.

We finally chose probe B10 (0.5 μM) and D13 (25 μM) as the CLFP for this study, considering the sub-cellular distribution and fluorescence intensity.

Sub-cellular localization of fluorescent probes in various cell lines

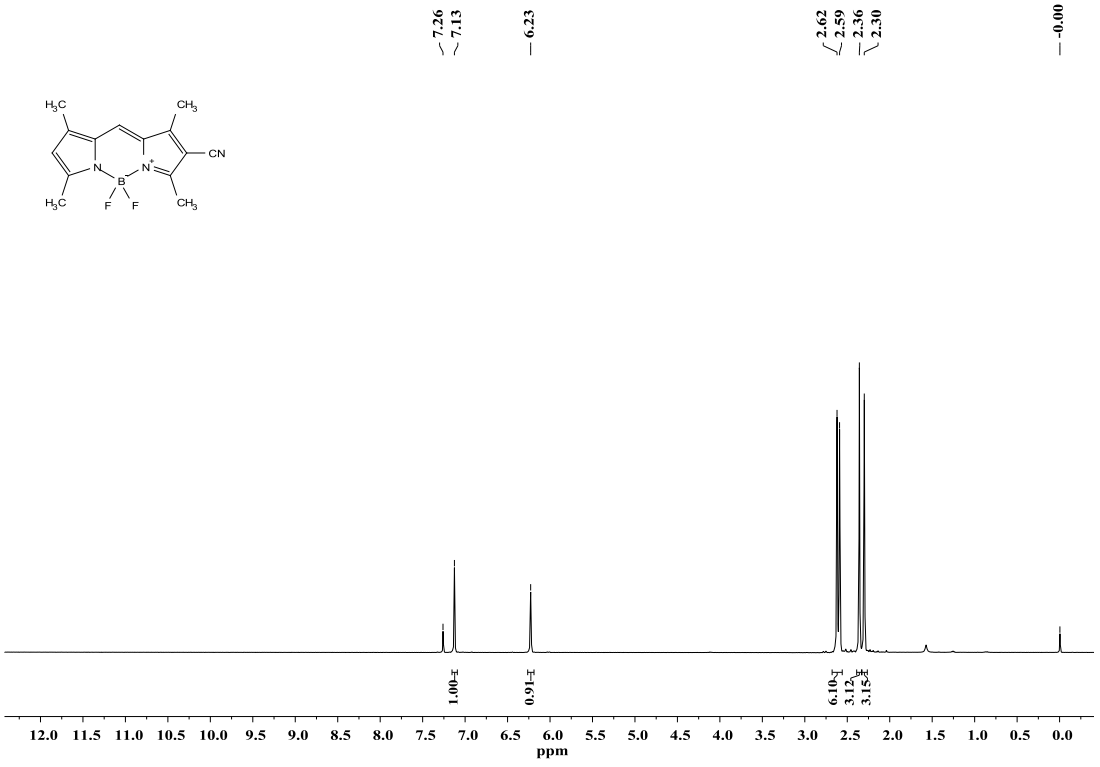
According to fluorescence intensities and the physical chemistry properties, probe B10 and D13 was selected to further detect the sub-cellular localization in various cancer cell lines. Cells were cultured in 24-well plates with low density. Notably, in the imaging experiment, probes D13 was only needed to incubate with cells for 0.5 hour, but in this sub-cellular localization experiment, we decided to incubate the probe with cells for at least 3 hours to evaluate the localization of these probes. So this make sure that probe D13 will not stain cell nuclei after long period of

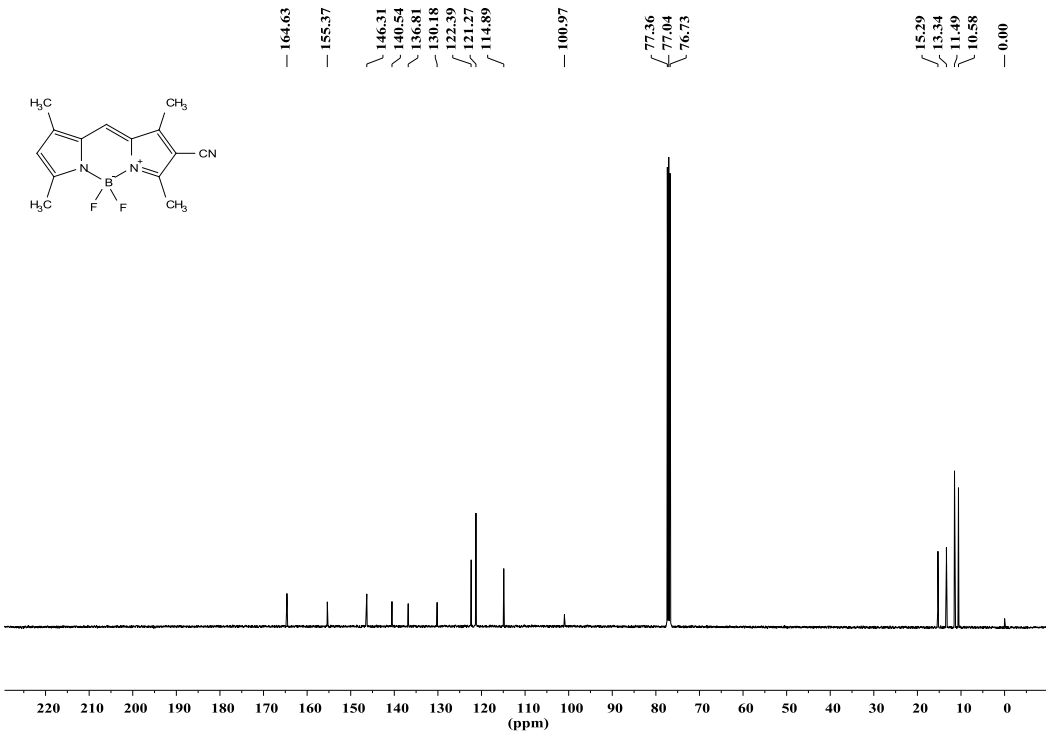
incubation, which is important to maintain the reliability of CLFP assay of this study. After incubation, the medium was washed away with PBS buffer, HepG2 cells were imaged under confocal microscopy.

Copy of NMR spectra of all compounds

B1

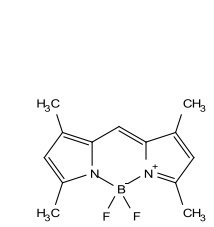
3-carbonitrile-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene

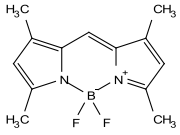




B2

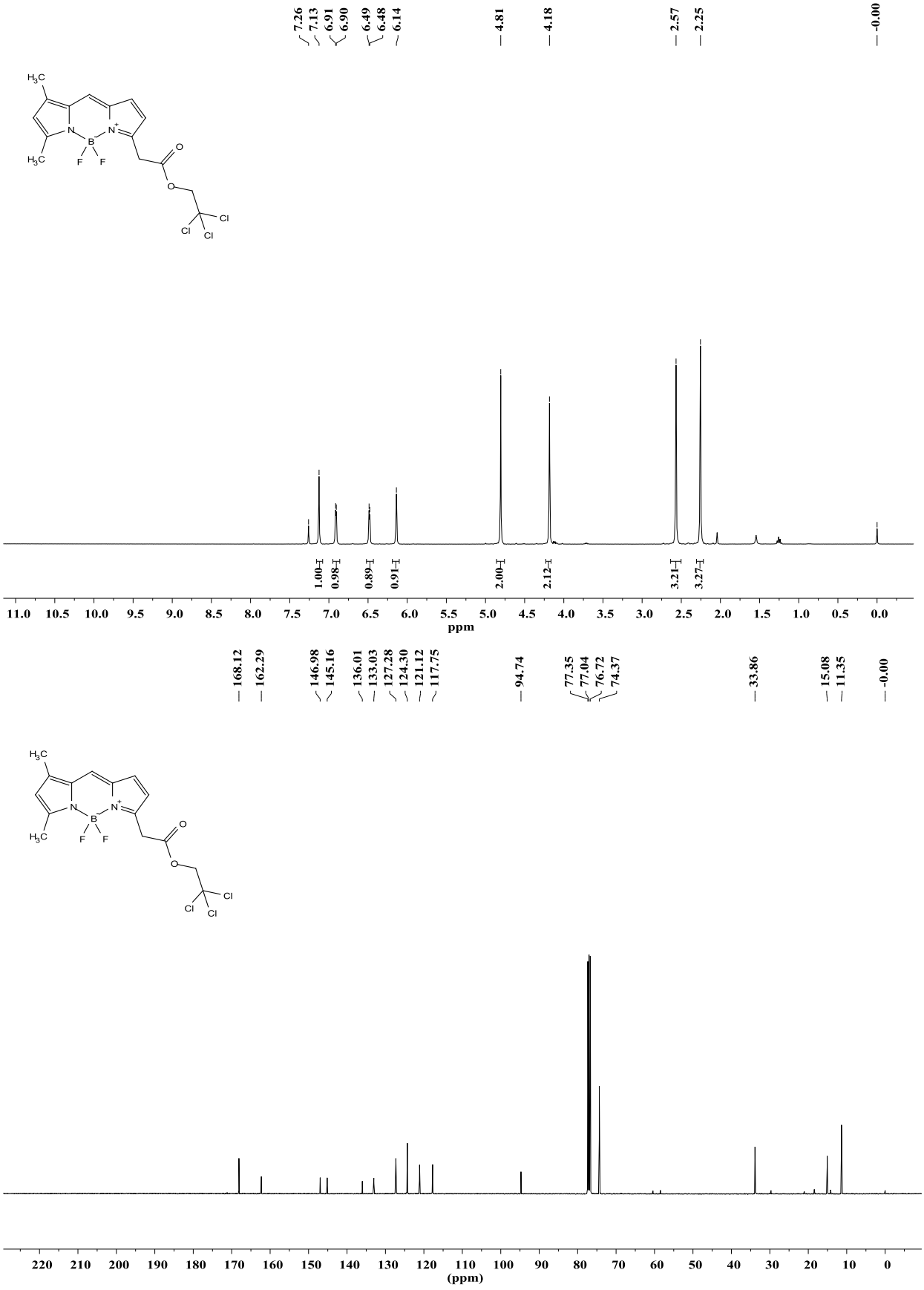
4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene





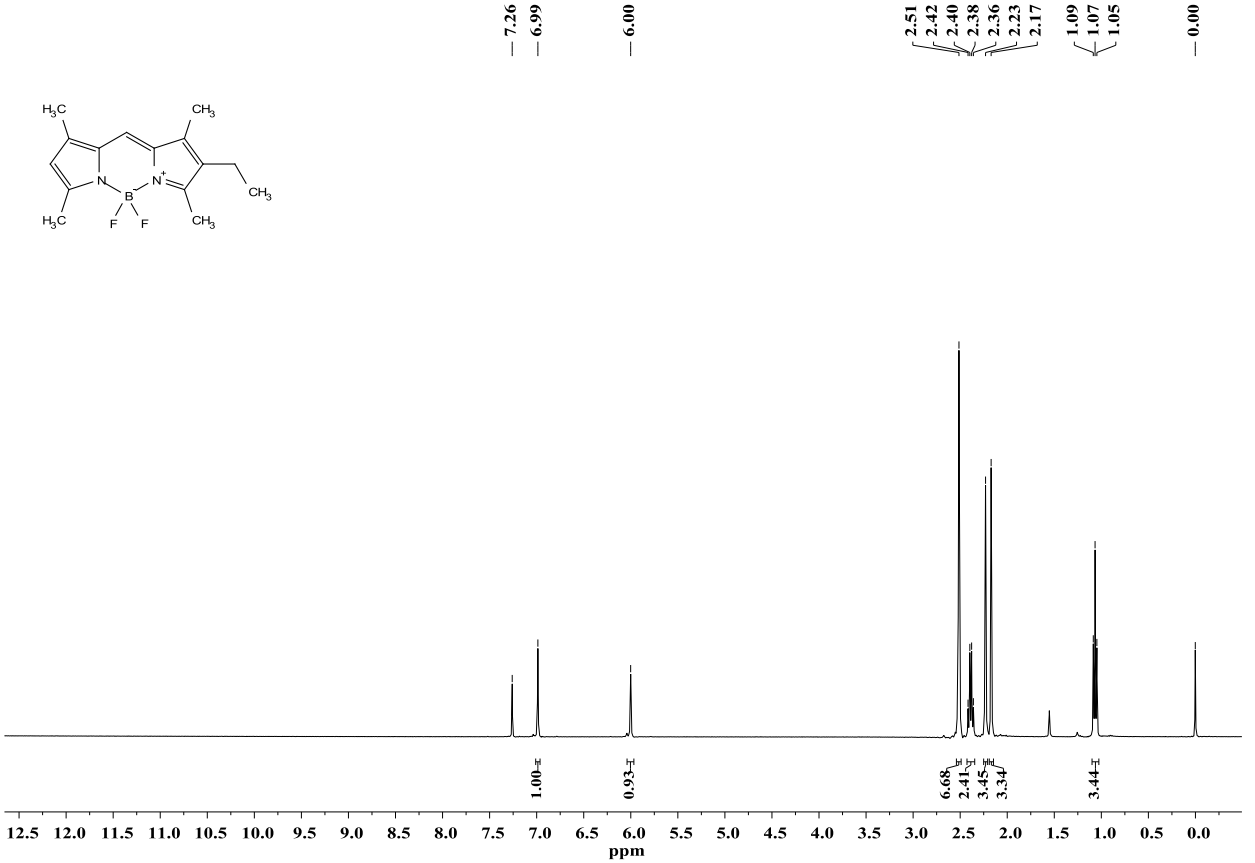
B3

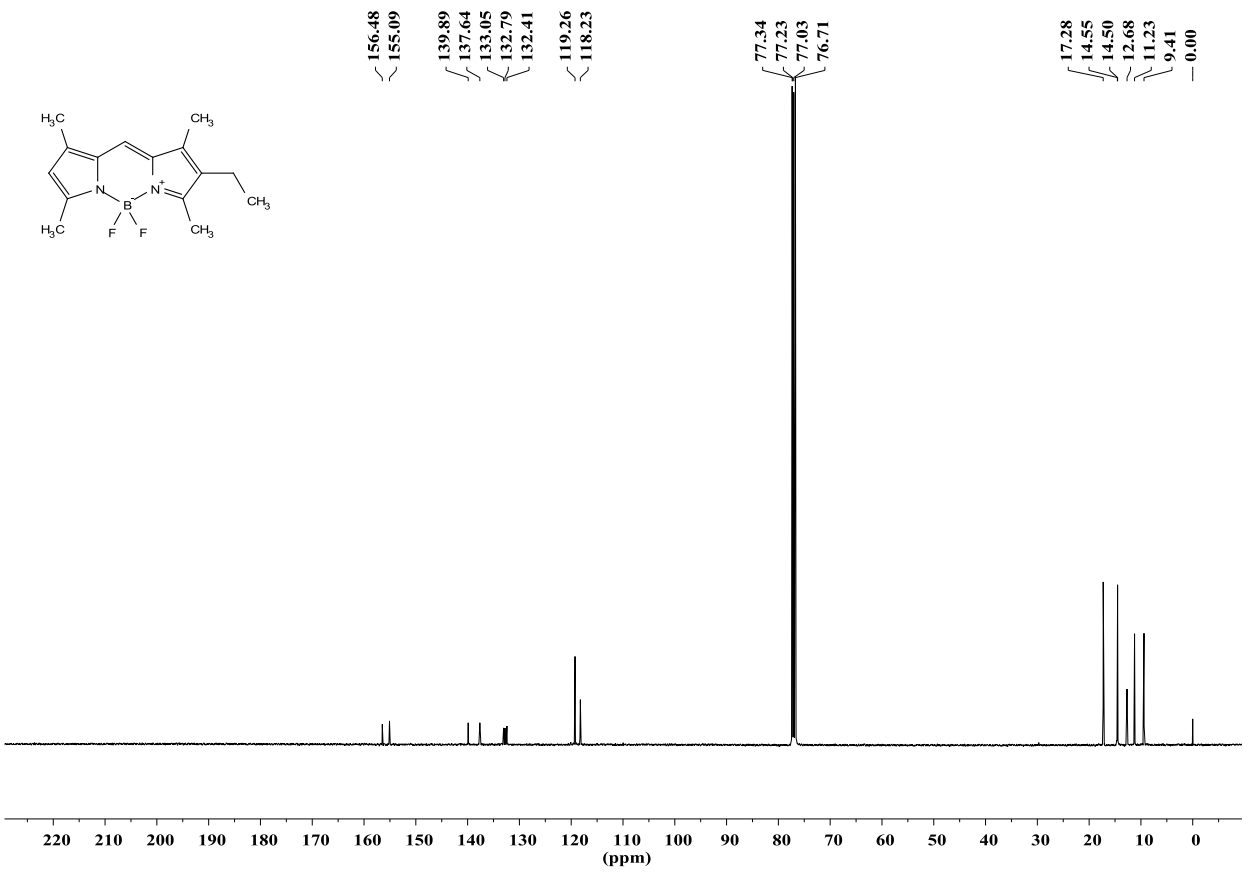
2,2,2-trichloroethyl-2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-yl)acetate



B4

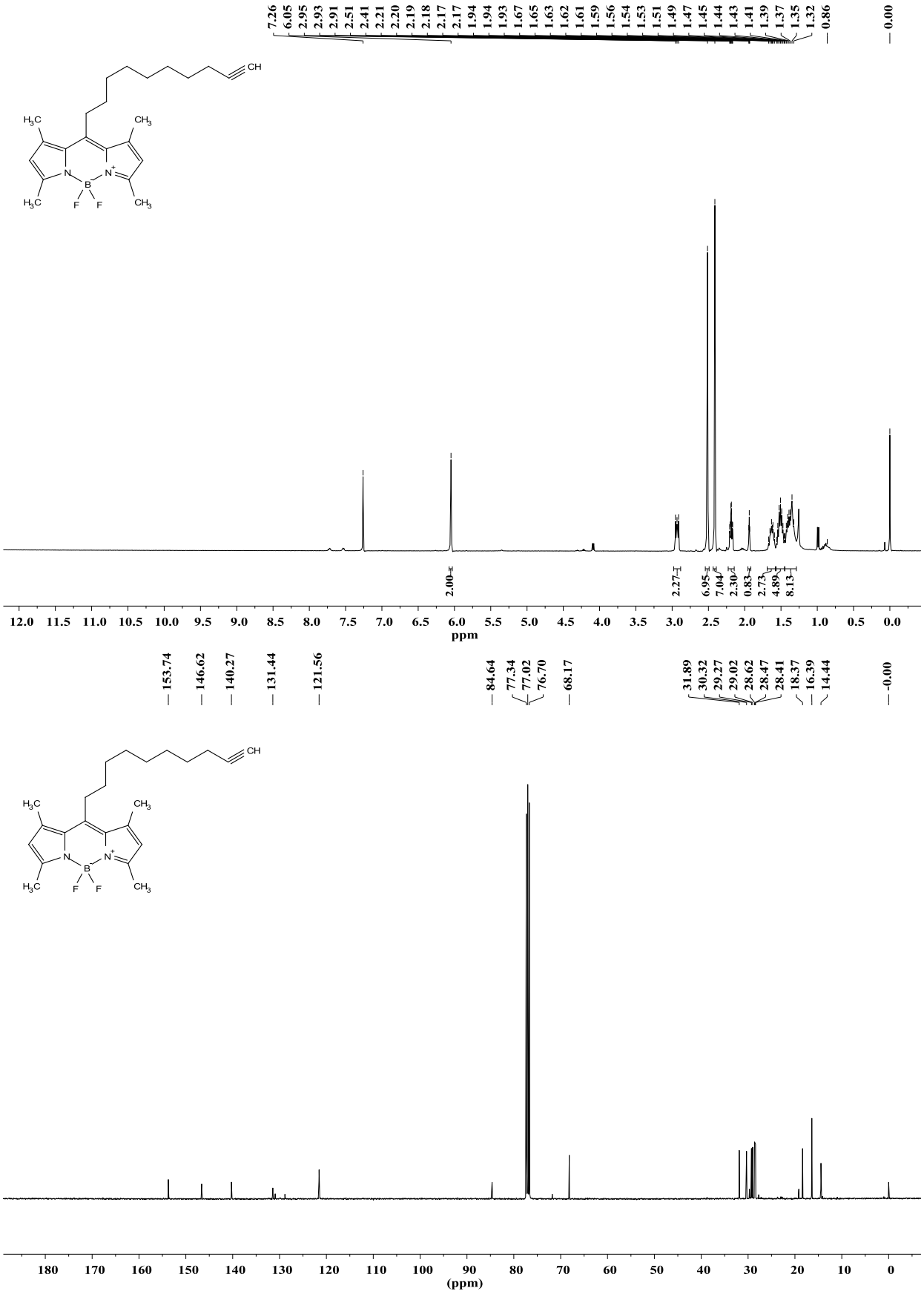
2-ethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene





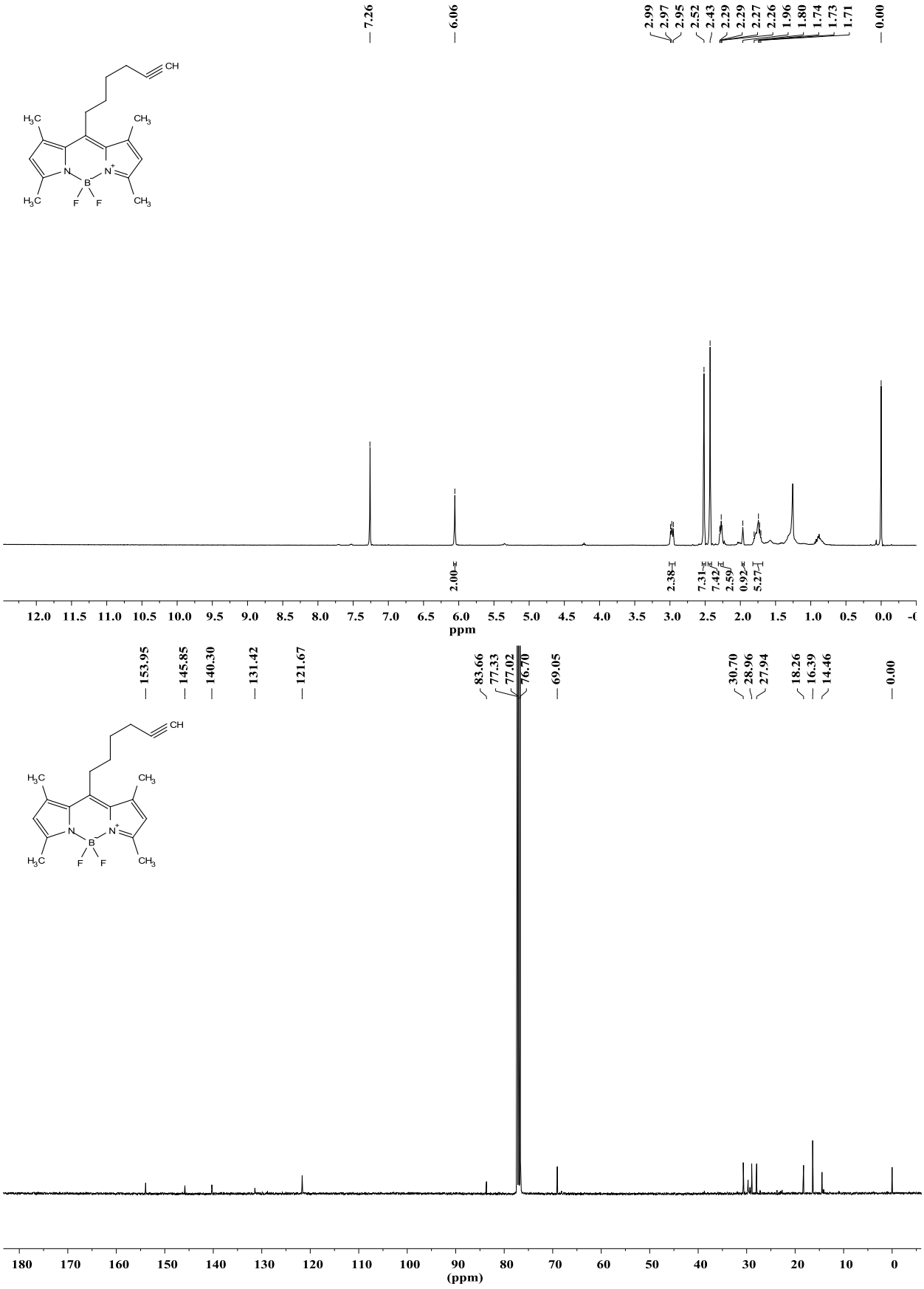
B5

8-(dec-9-yn-1-yl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene



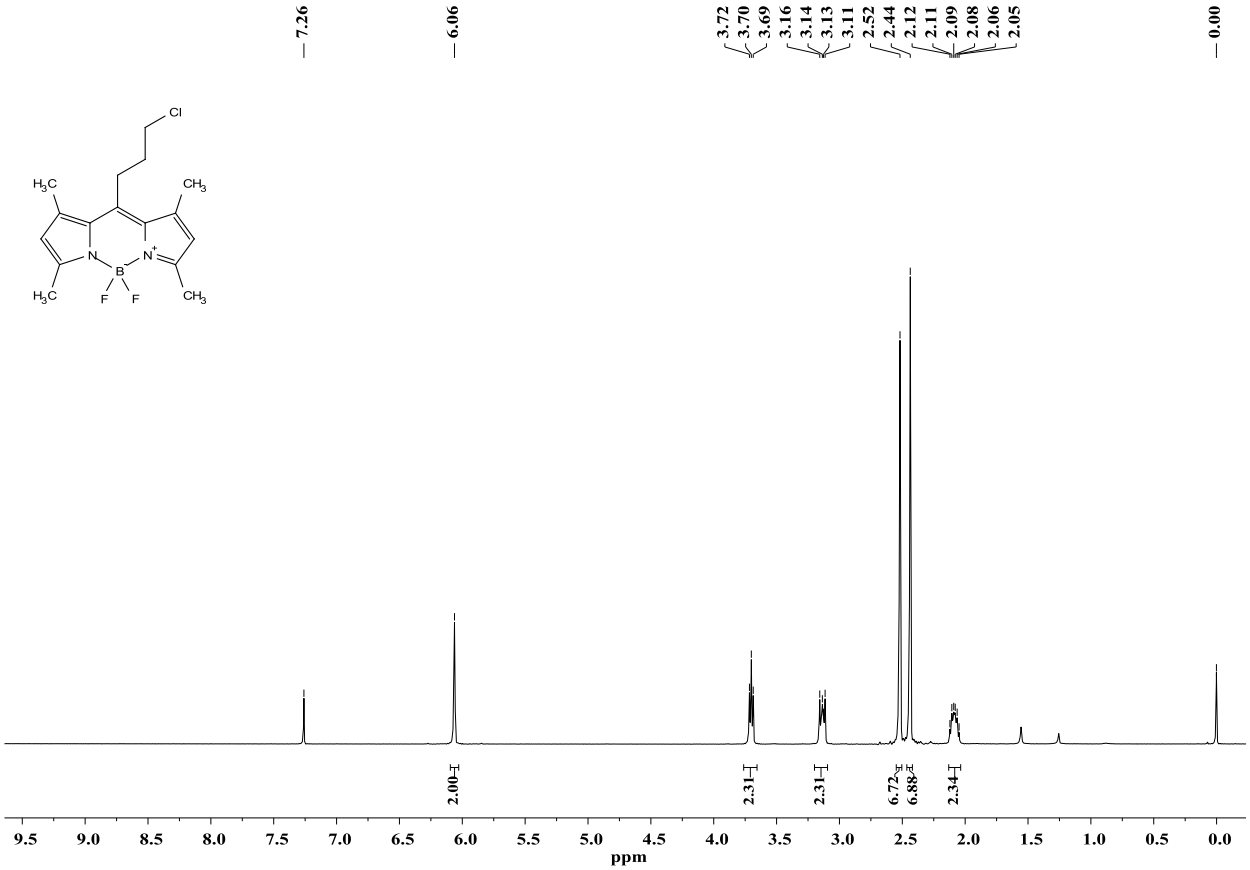
B6

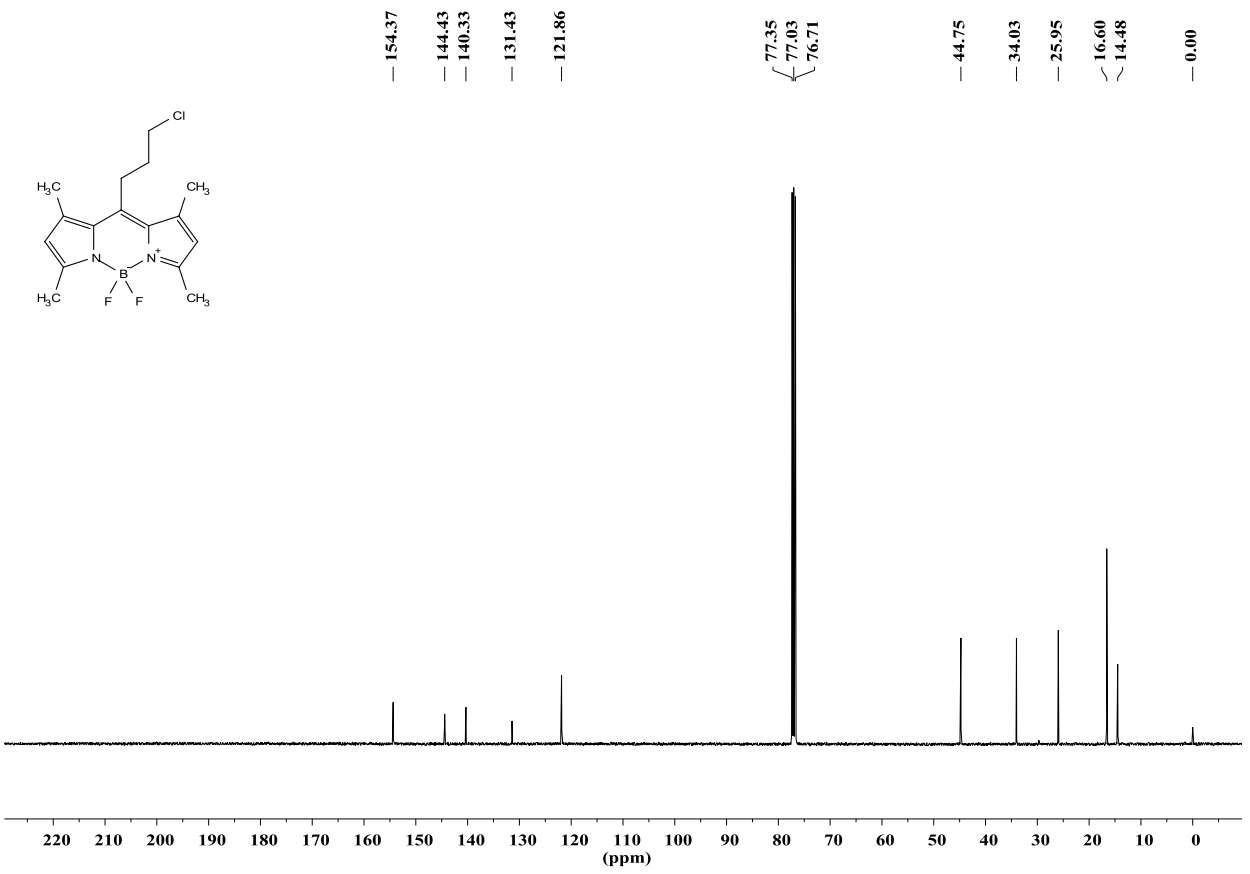
4,4-difluoro-8-(hex-5-yn-1-yl)- 1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene



B7

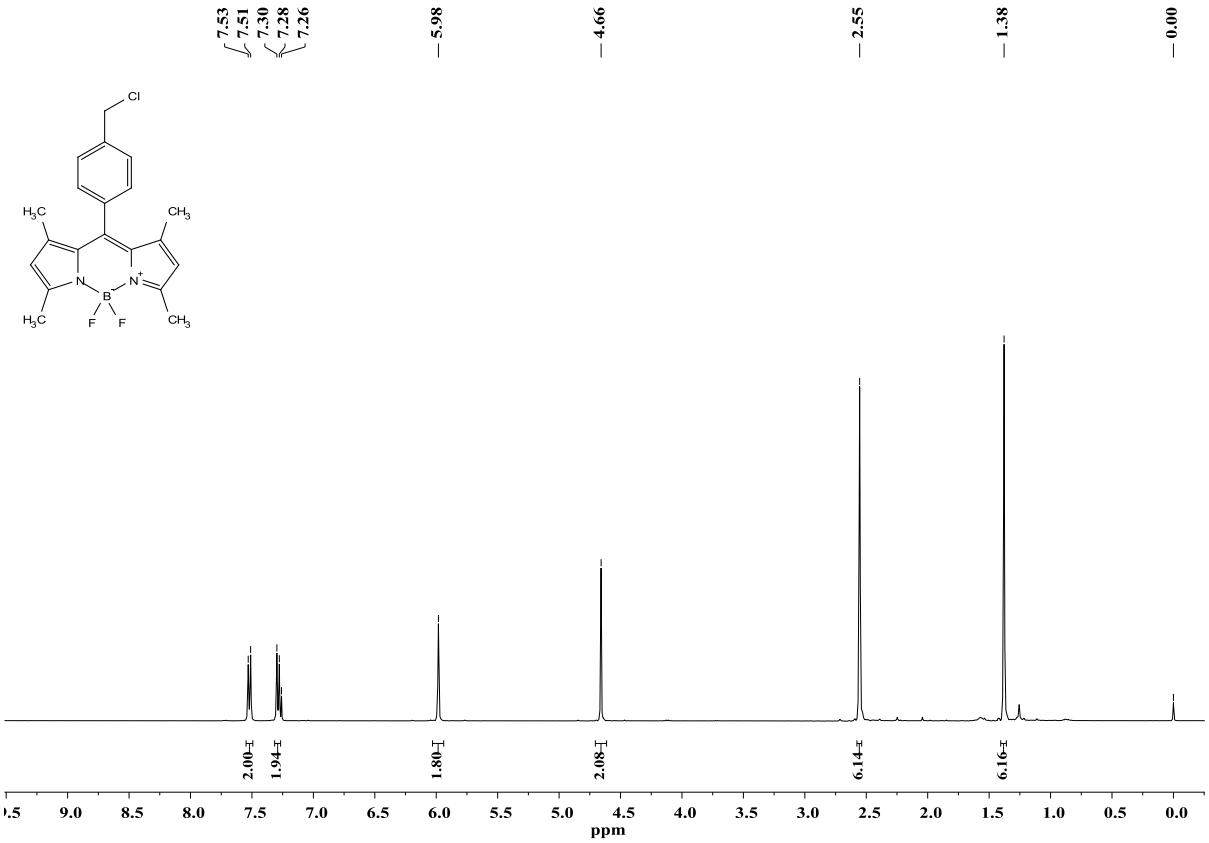
8- (chloropropyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene

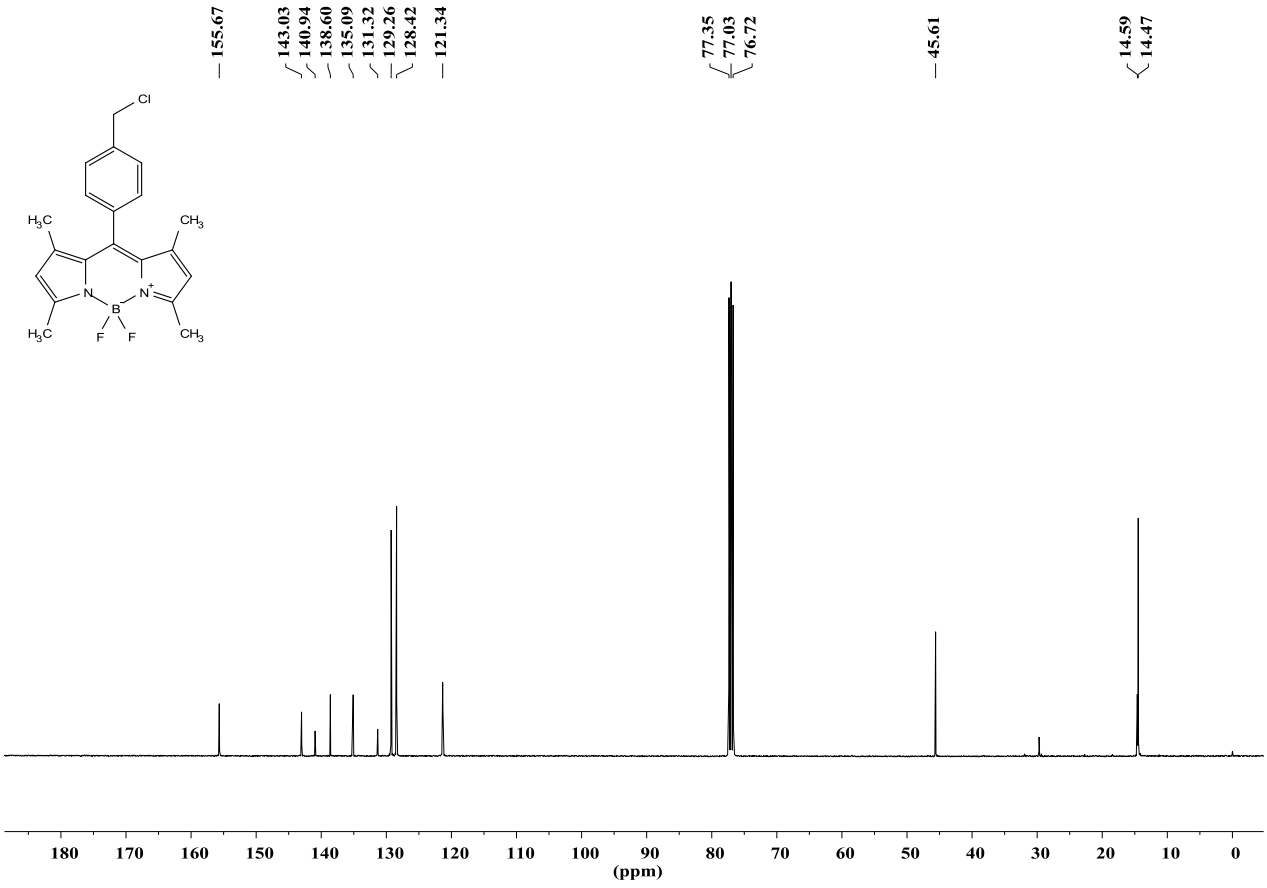




B8

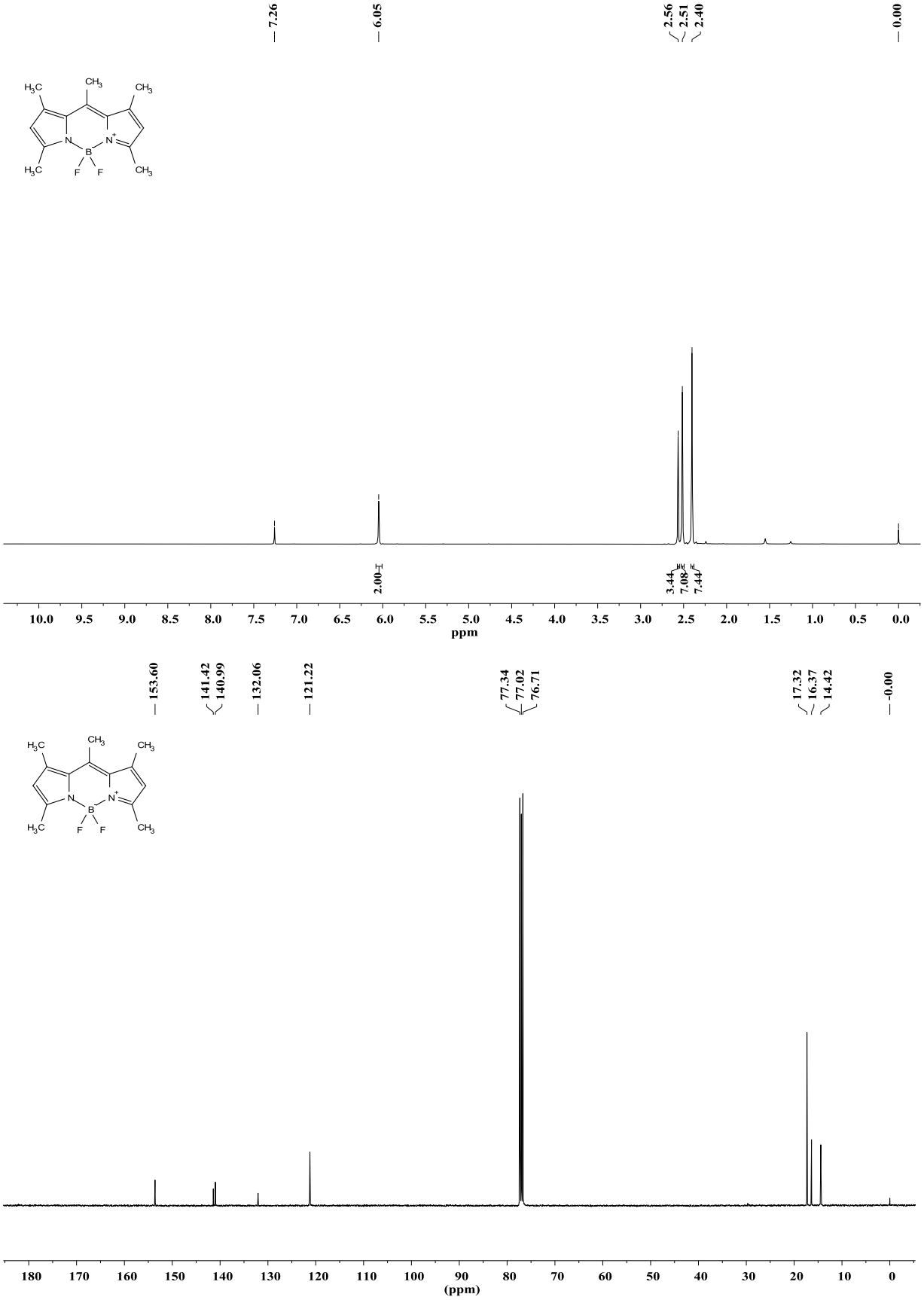
8-[4-(chloromethyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene





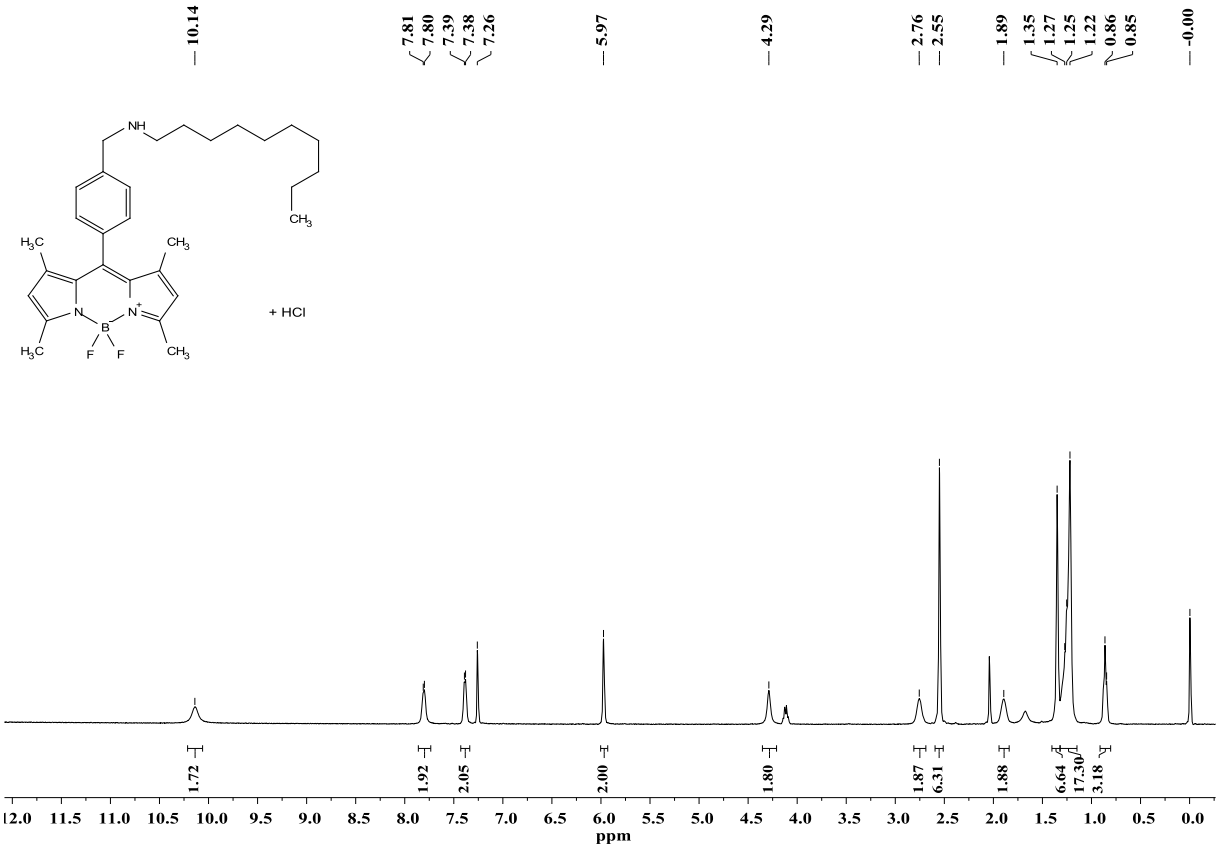
B9

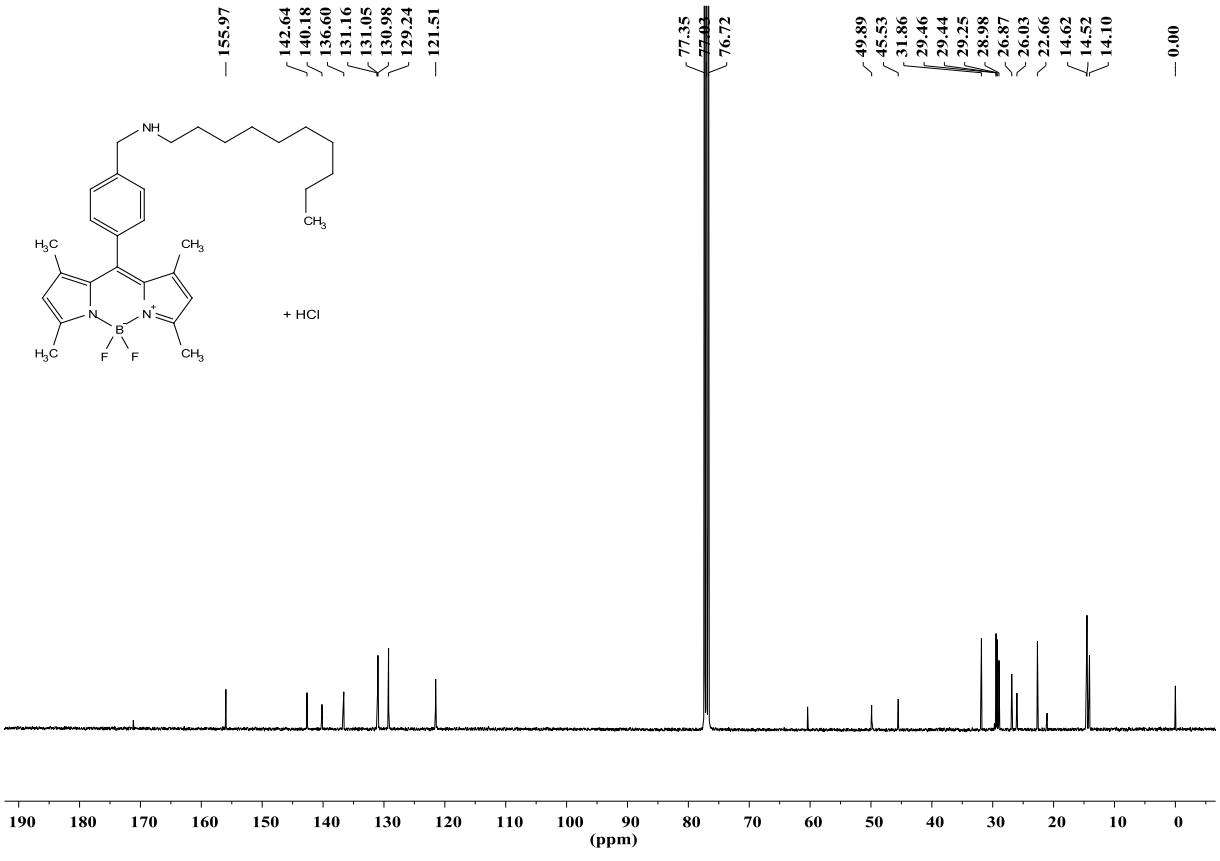
4,4-difluoro-8-methyl-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene



B10

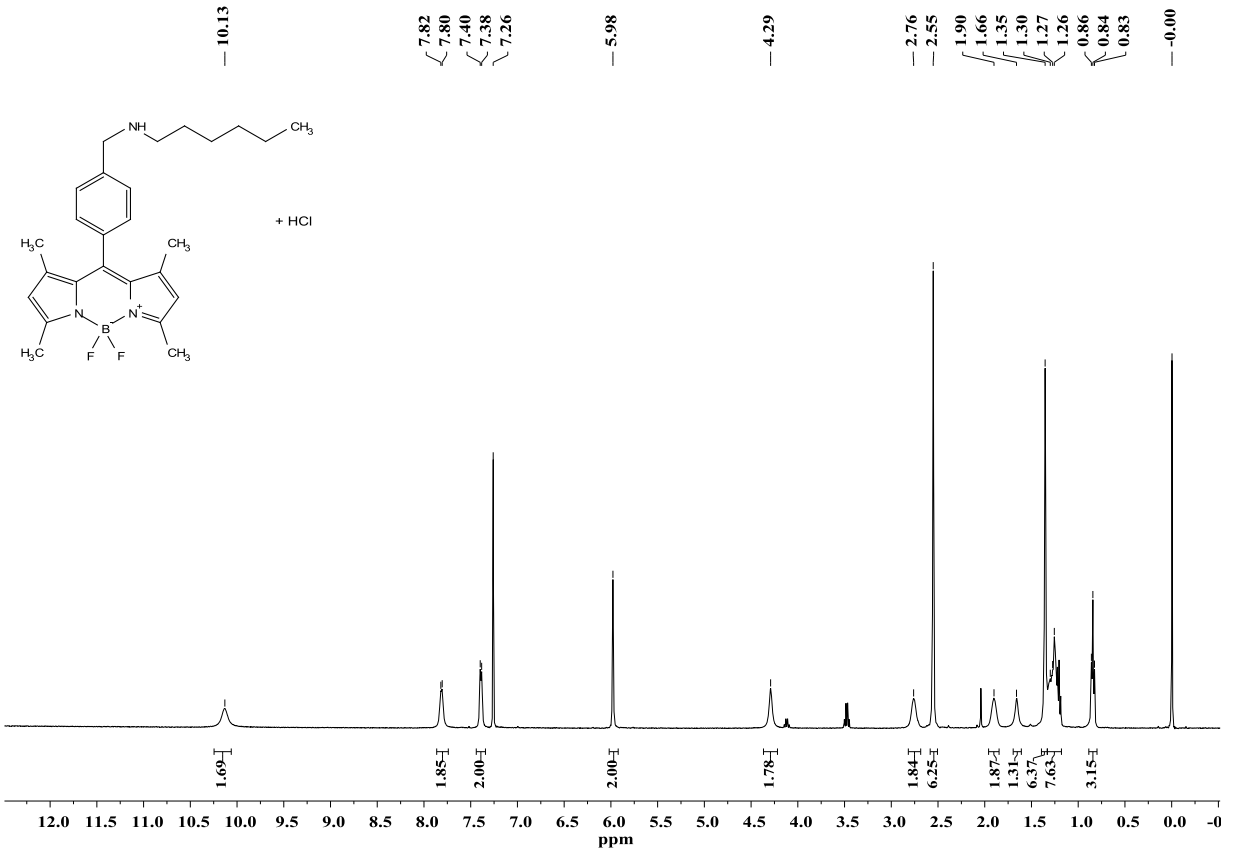
8-[4-((decylamino)methyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene

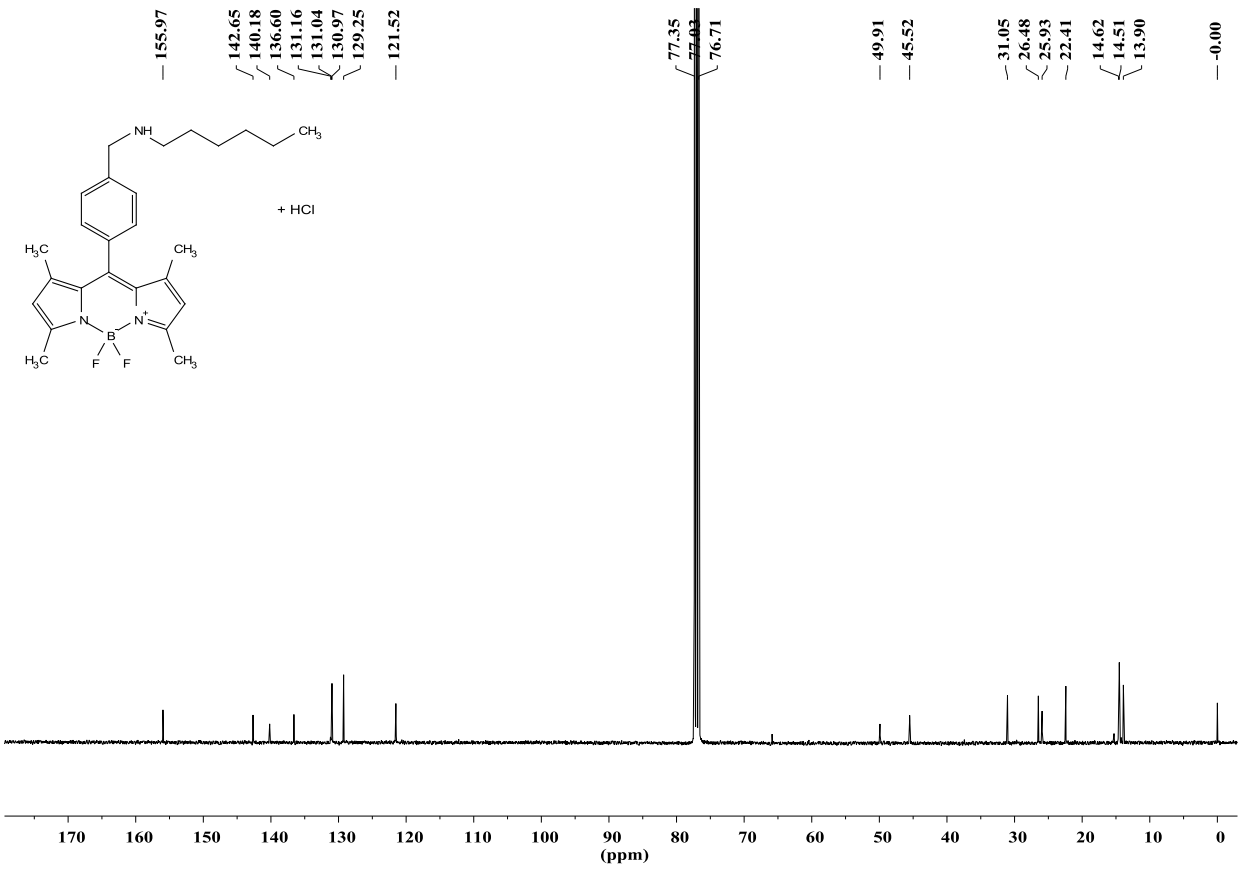




B11

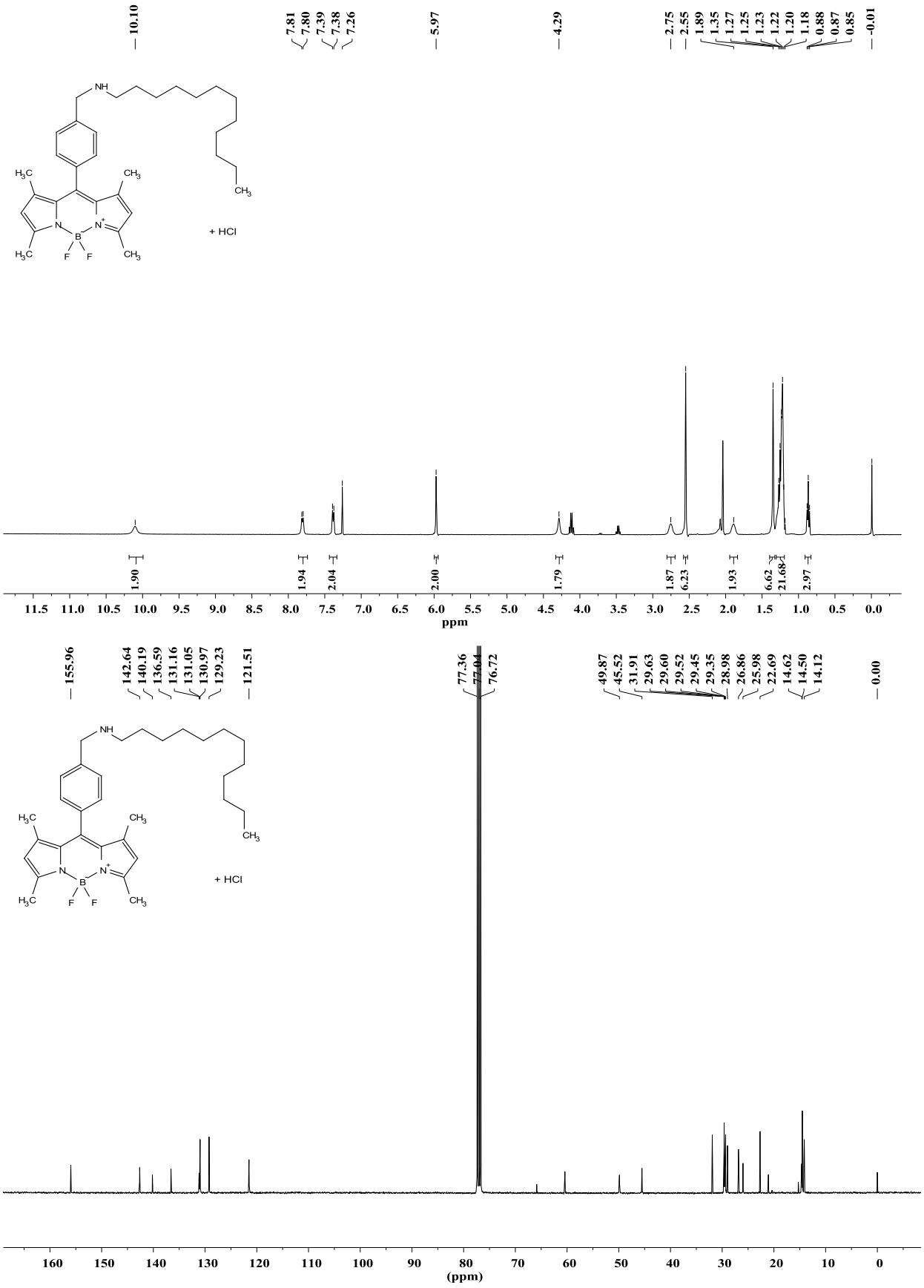
8-[4-((hexylamino)methyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene





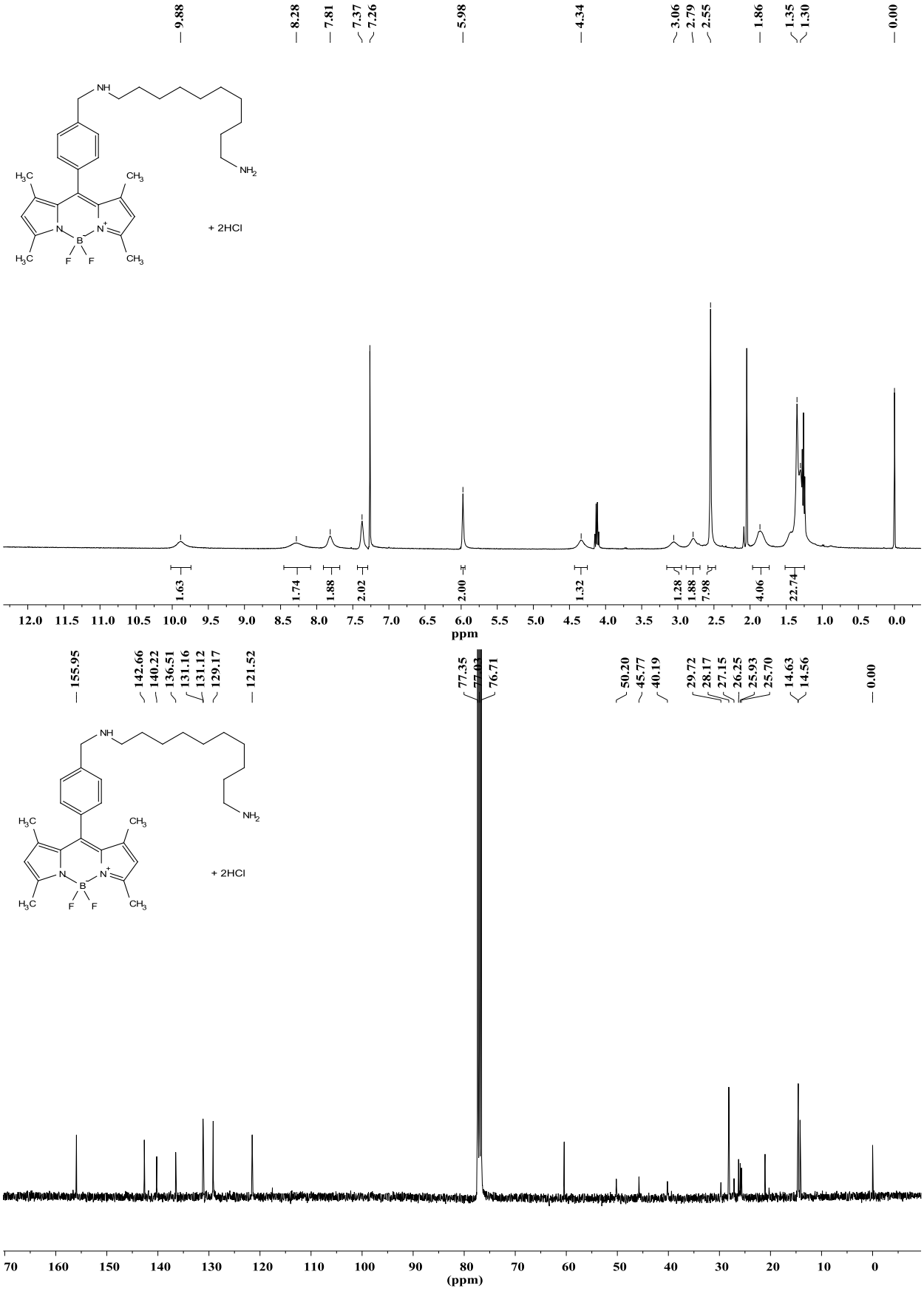
B12

8-[4-((dodecylamino)methyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene



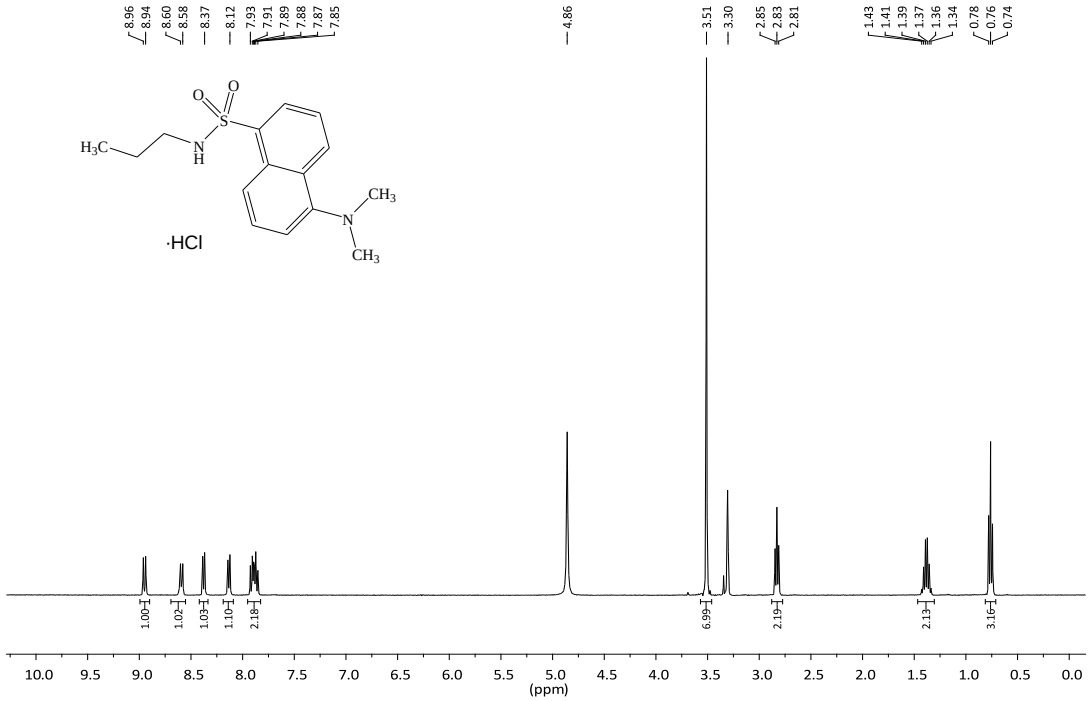
B13

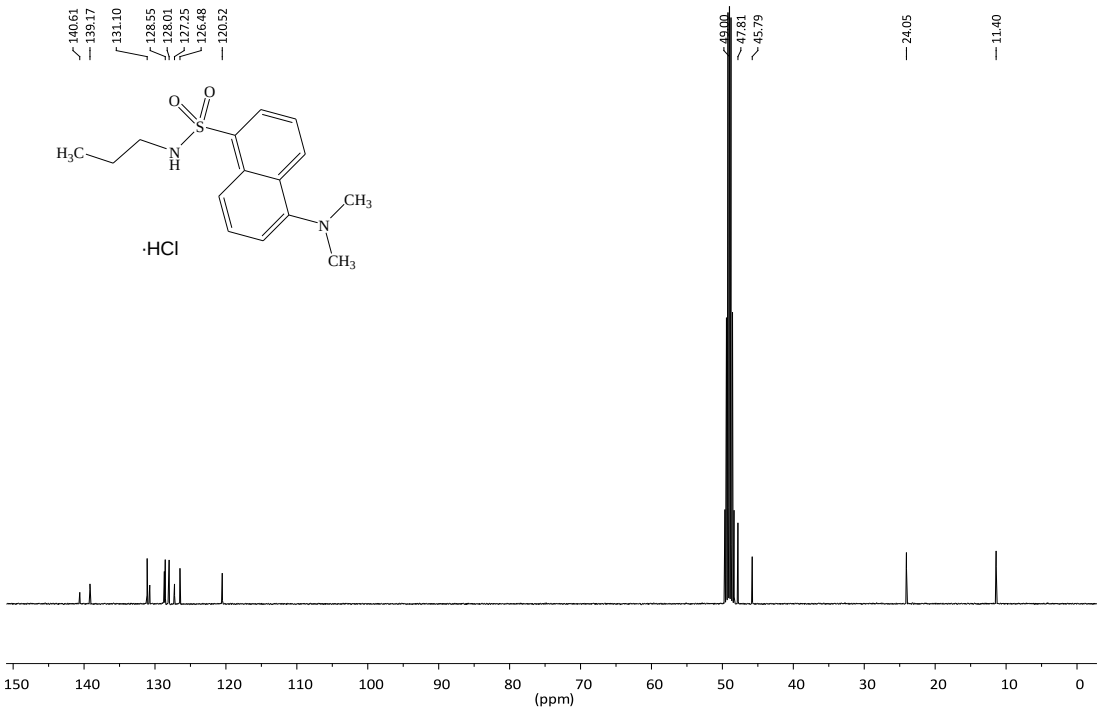
8-[4-(((10-aminodecyl)amino)methyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene



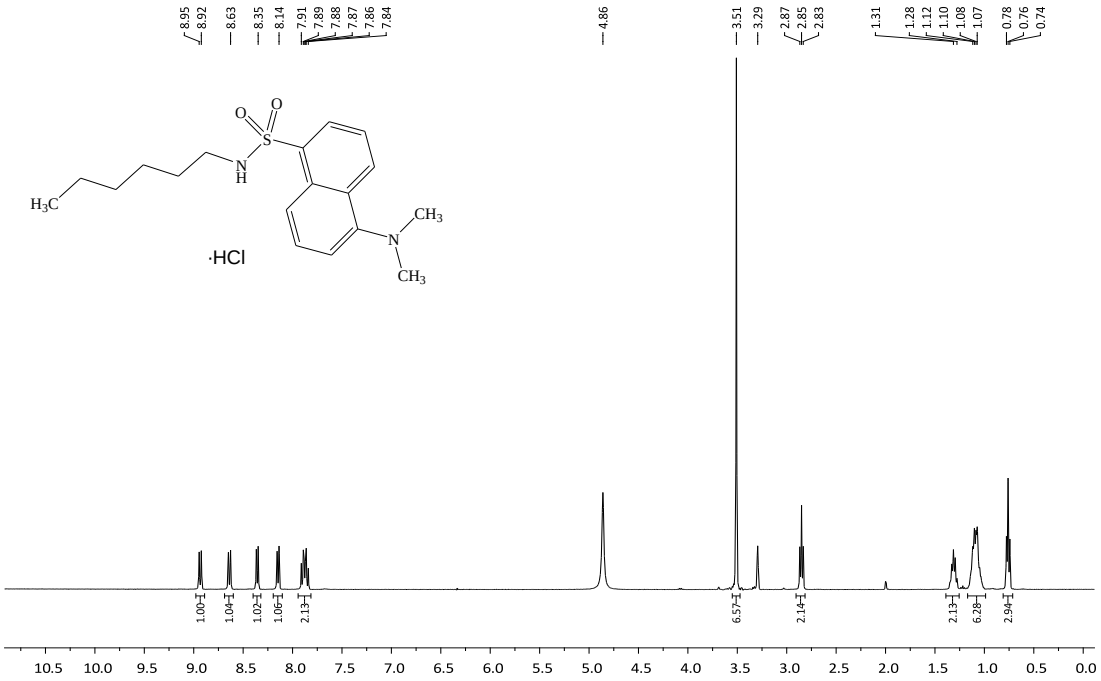
Copy of NMR spectra of series 2

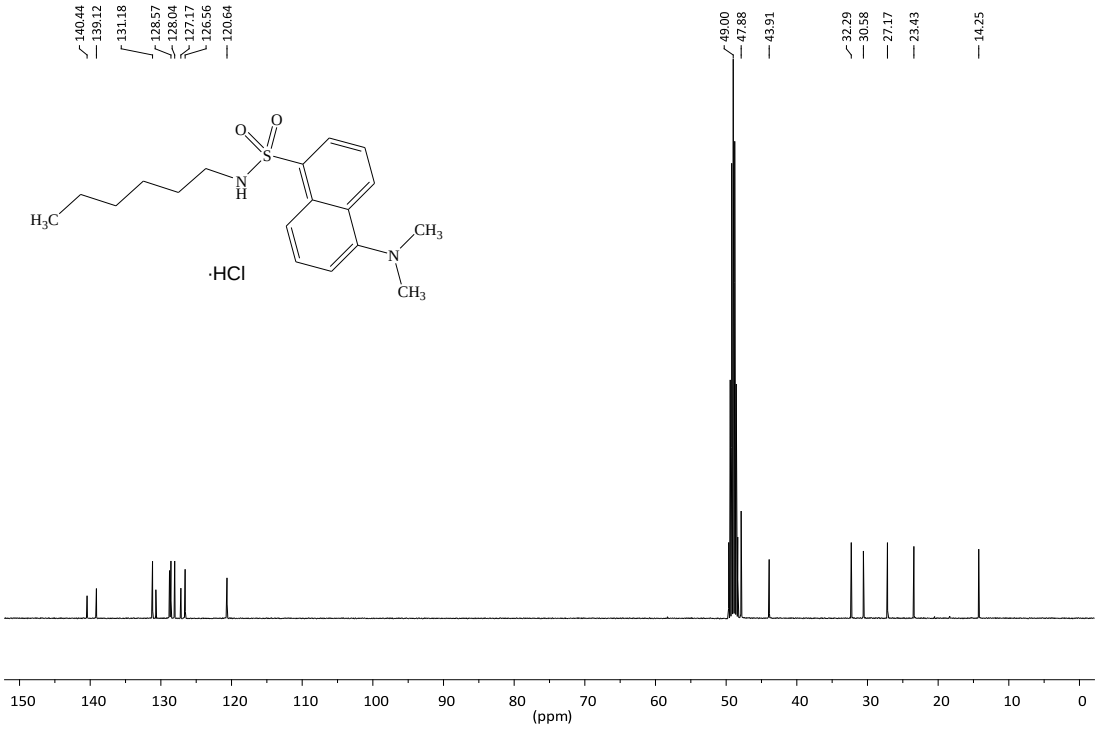
5-(Dimethylamino)-*N*-propylnaphthalene-1-sulfonamide (D1)



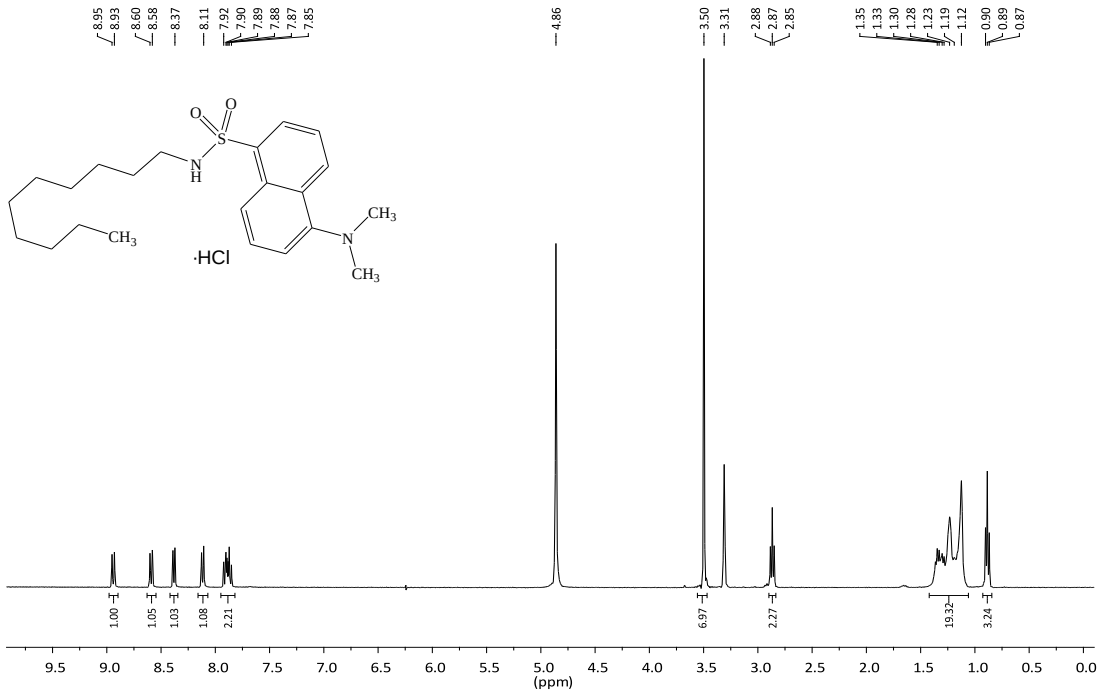


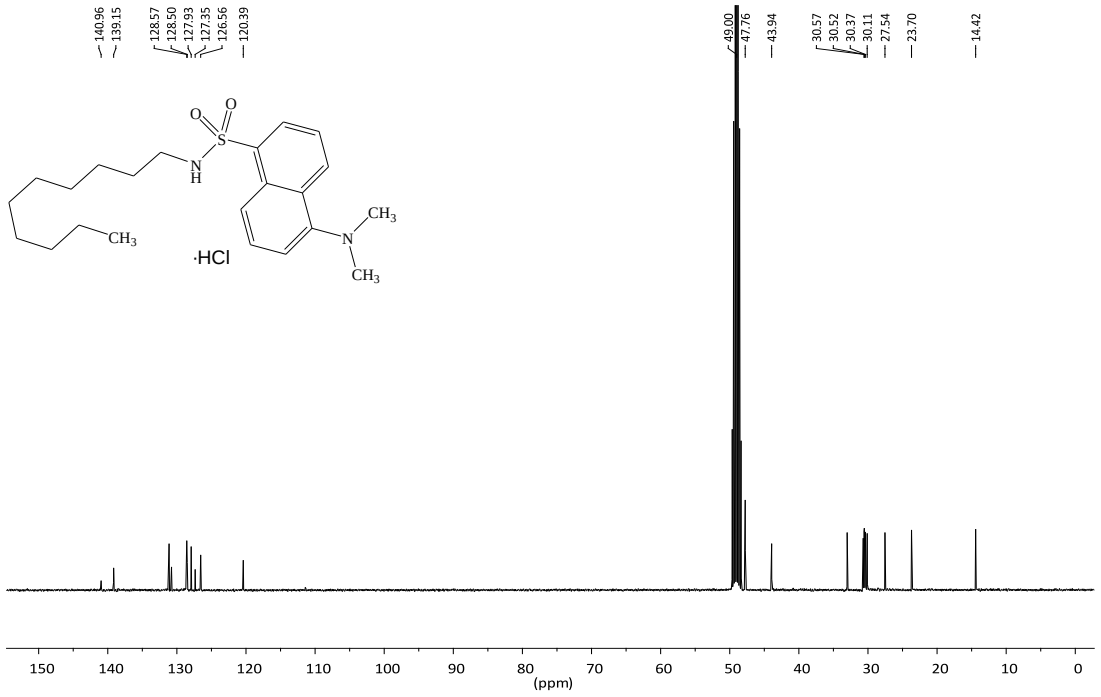
5-(Dimethylamino)-*N*-hexylnaphthalene-1-sulfonamide (D2)



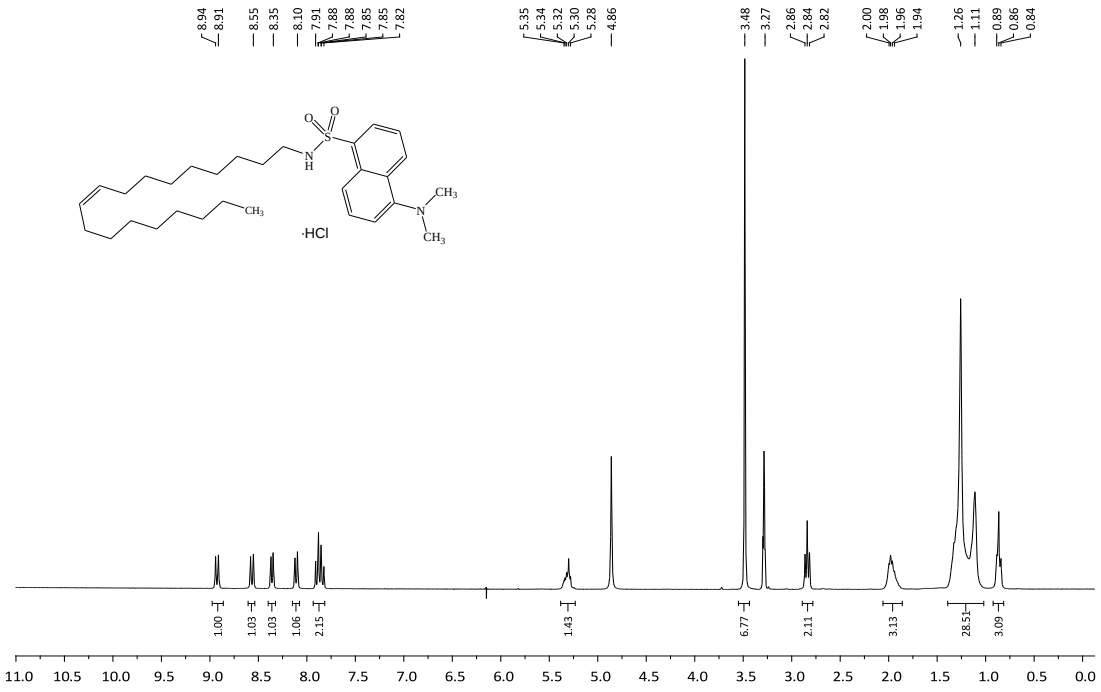


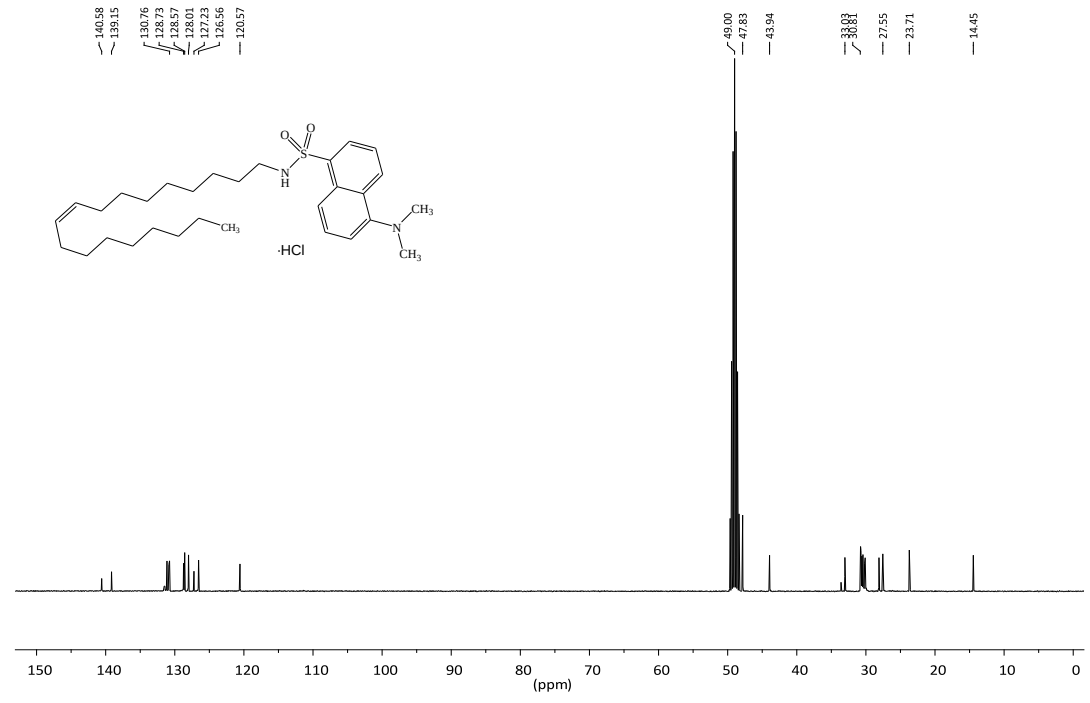
N-decyl-5-(dimethylamino) naphthalene-1-sulfonamide (D3)



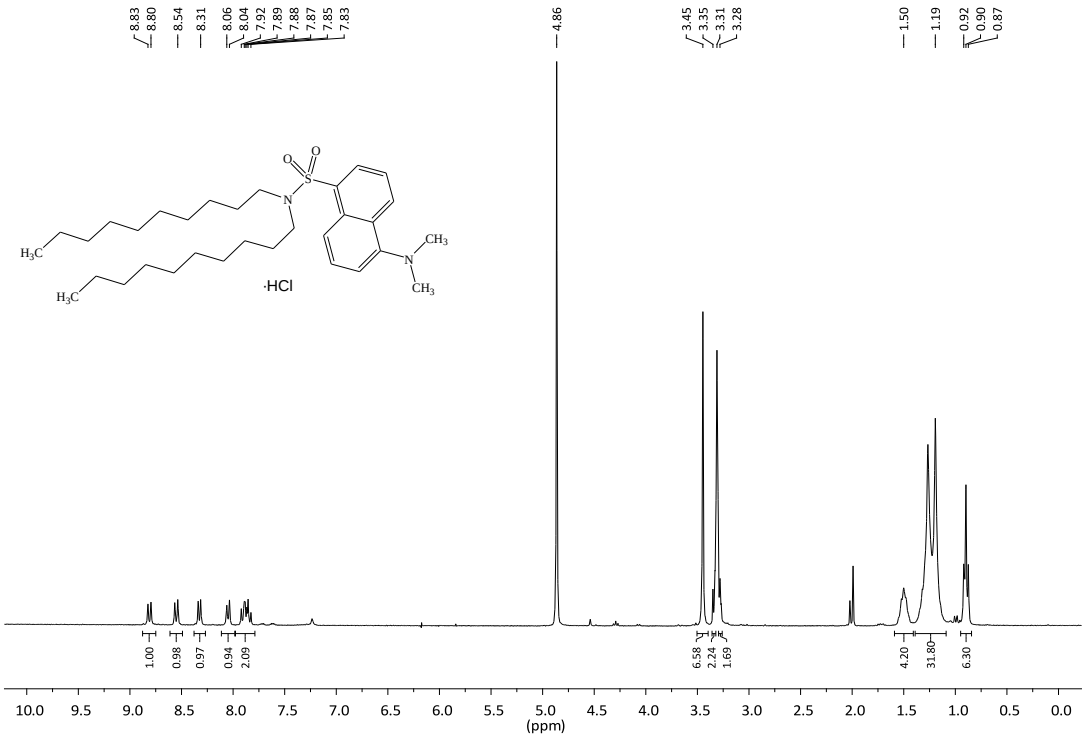


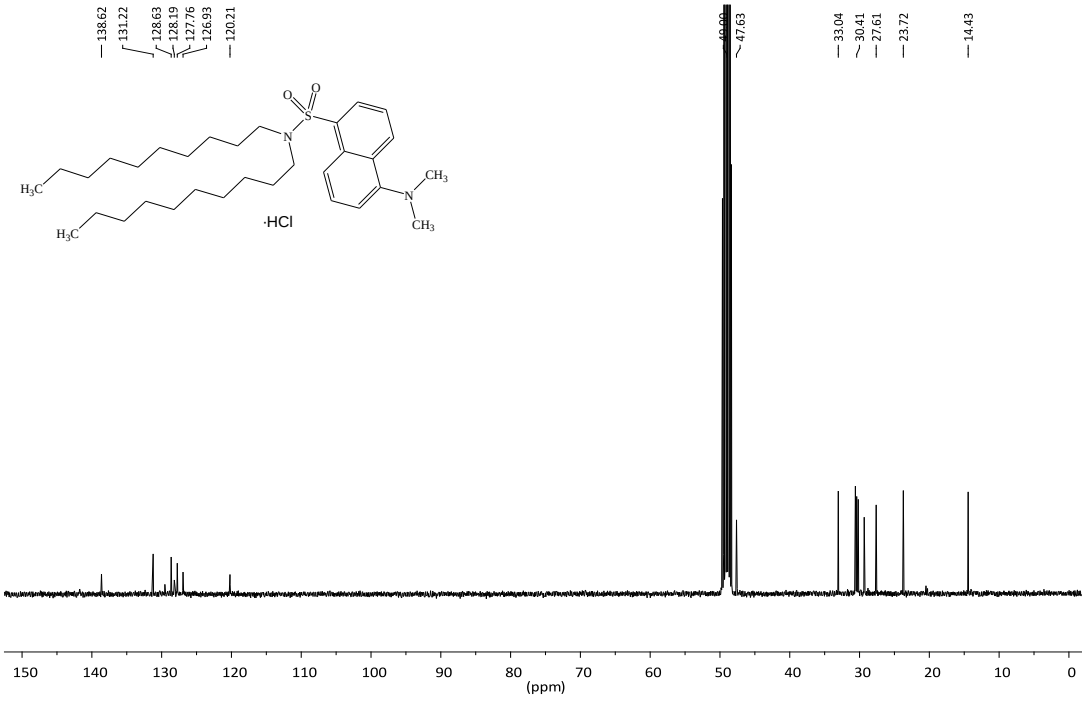
(Z)-5-(dimethylamino)-*N*-(octadec-9-en-1-yl) naphthalene-1-sulfonamide (D4)



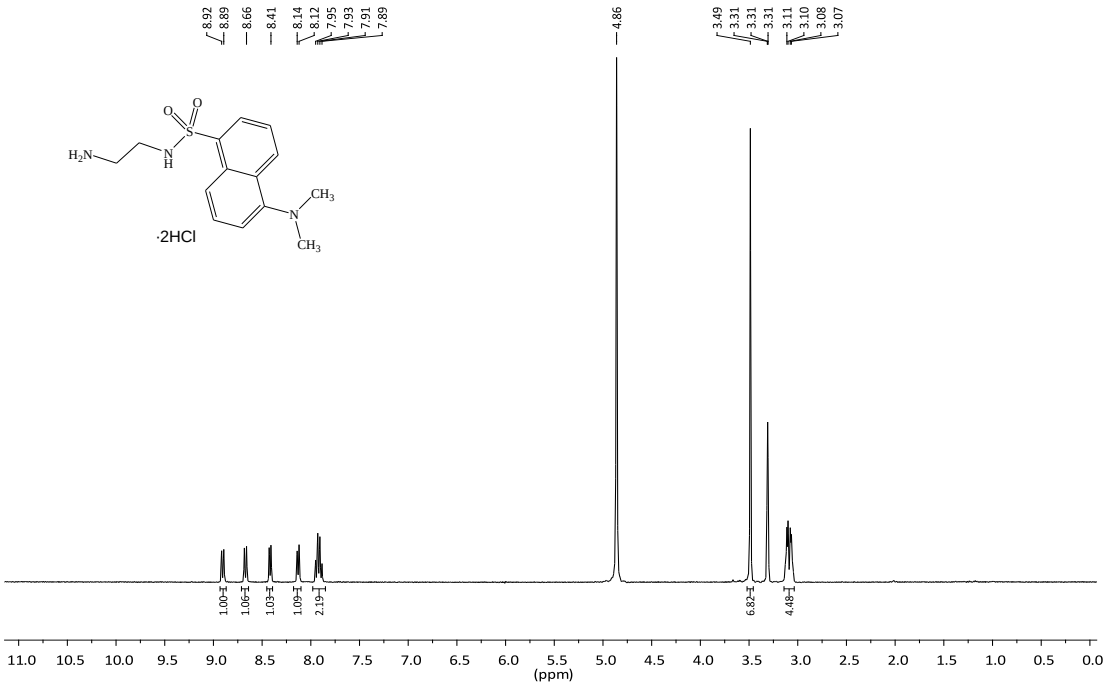


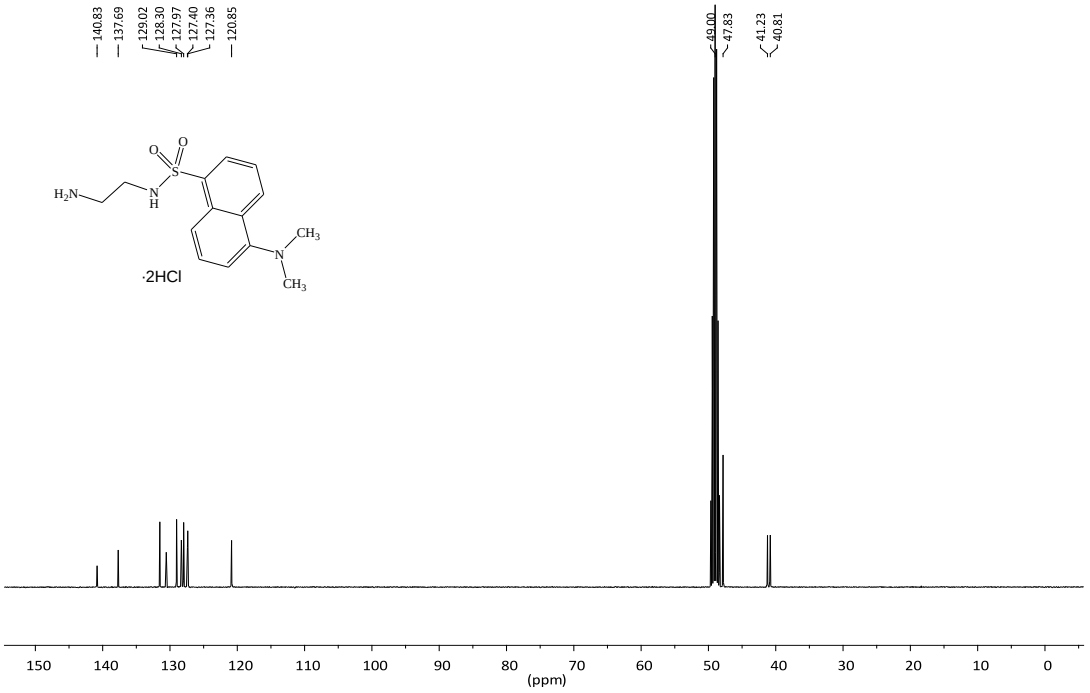
N, *N'*-didecyl-5-(dimethylamino) naphthalene-1-sulfonamide (D5)



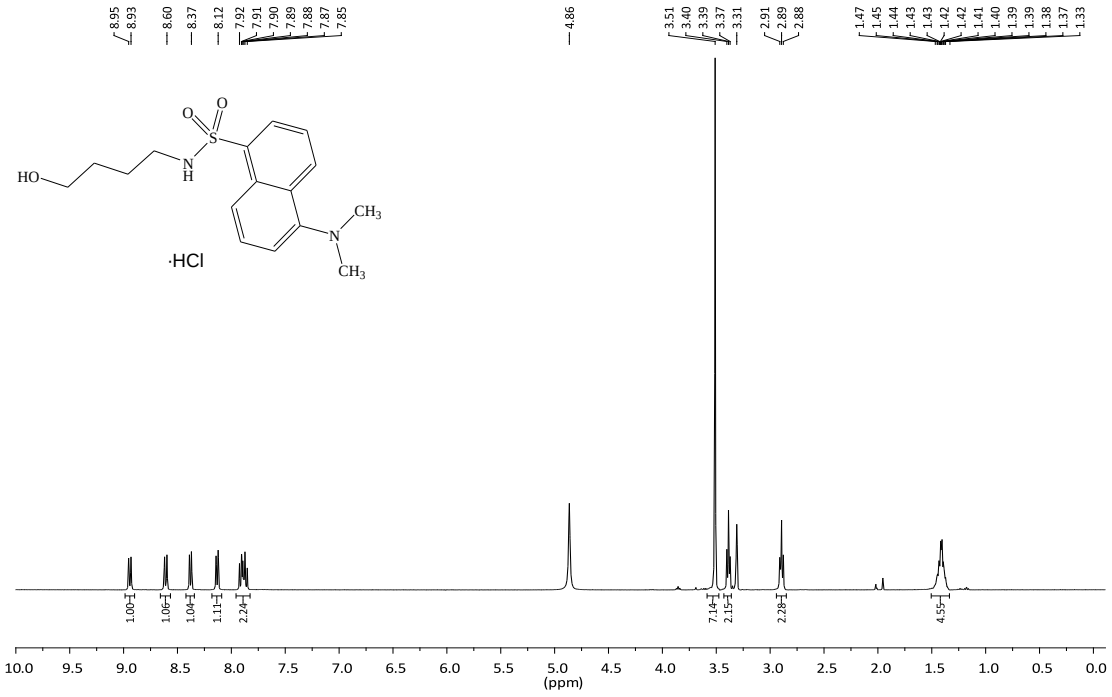


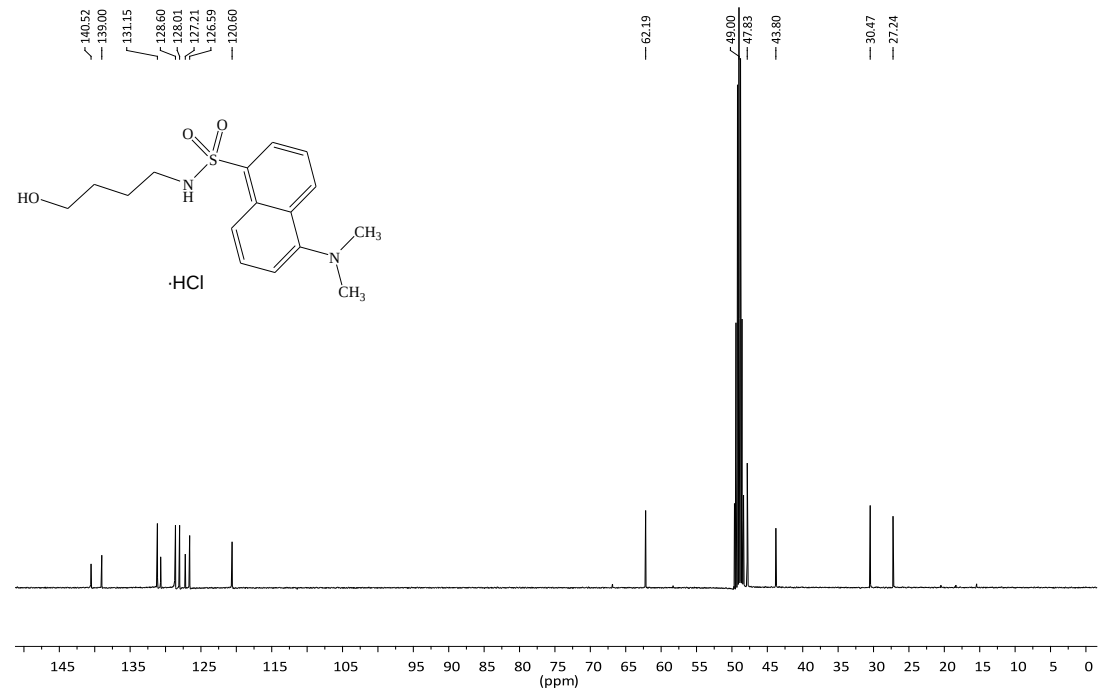
N-(2-aminoethyl)-5-(dimethylamino) naphthalene-1-sulfonamide (D6)



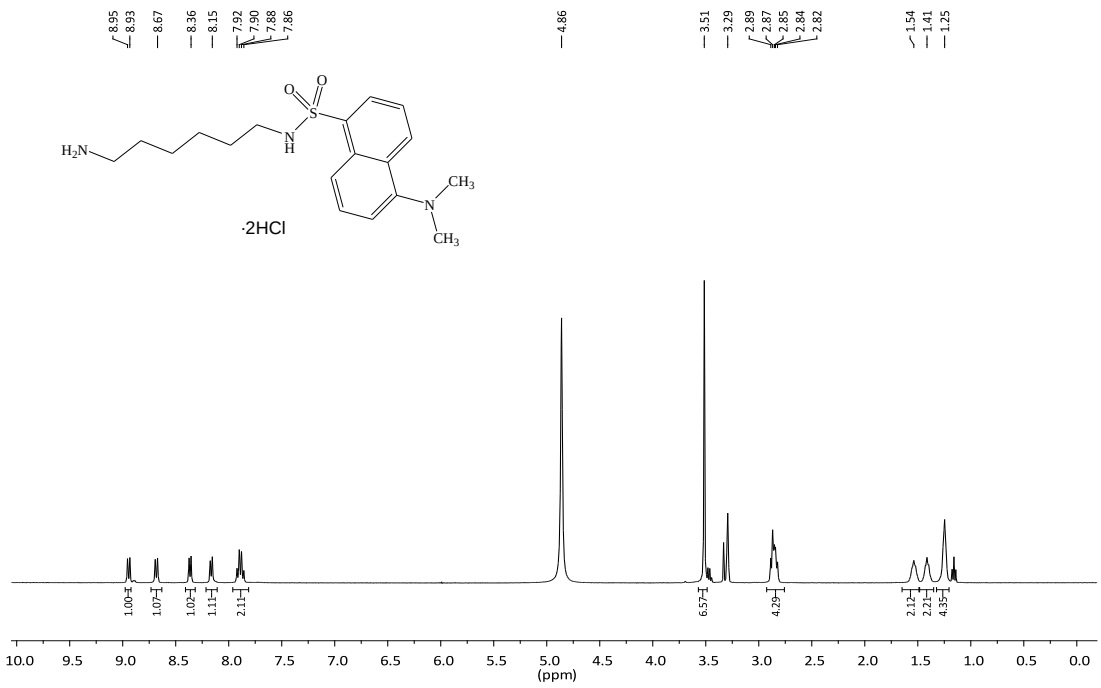


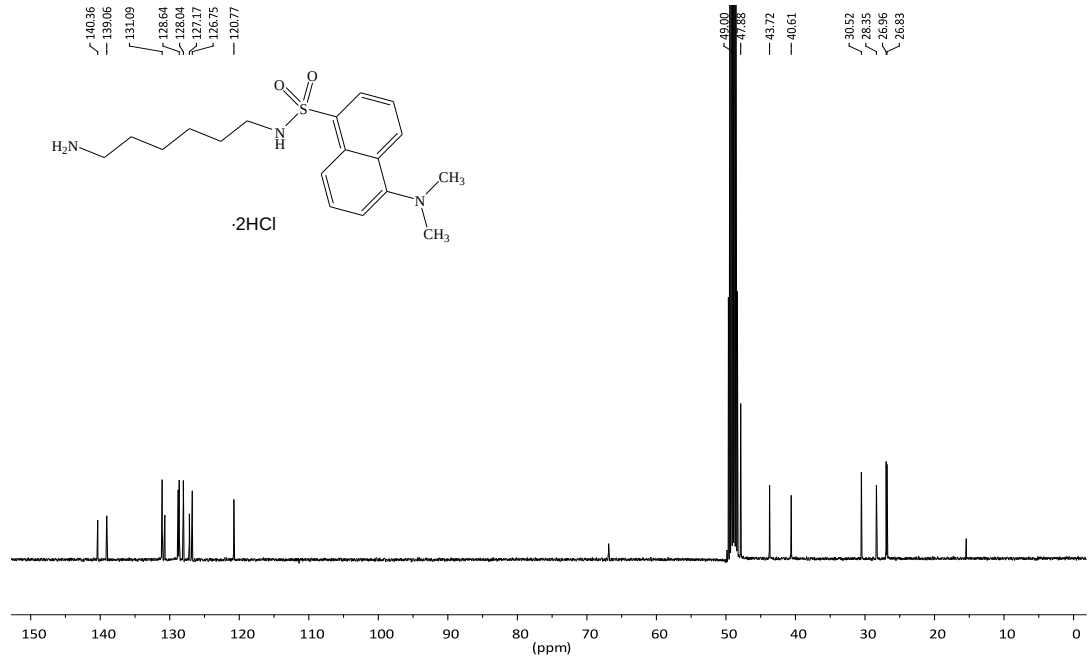
5-(Dimethylamino)-N-(4-hydroxybutyl) naphthalene-1-sulfonamide (D7)



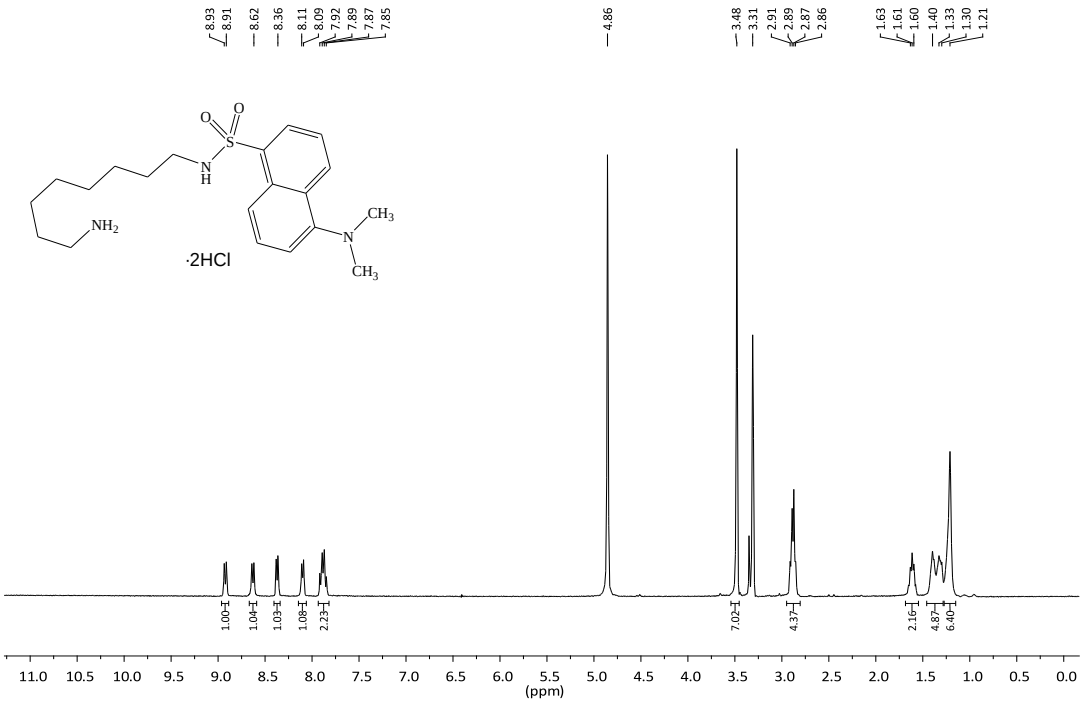


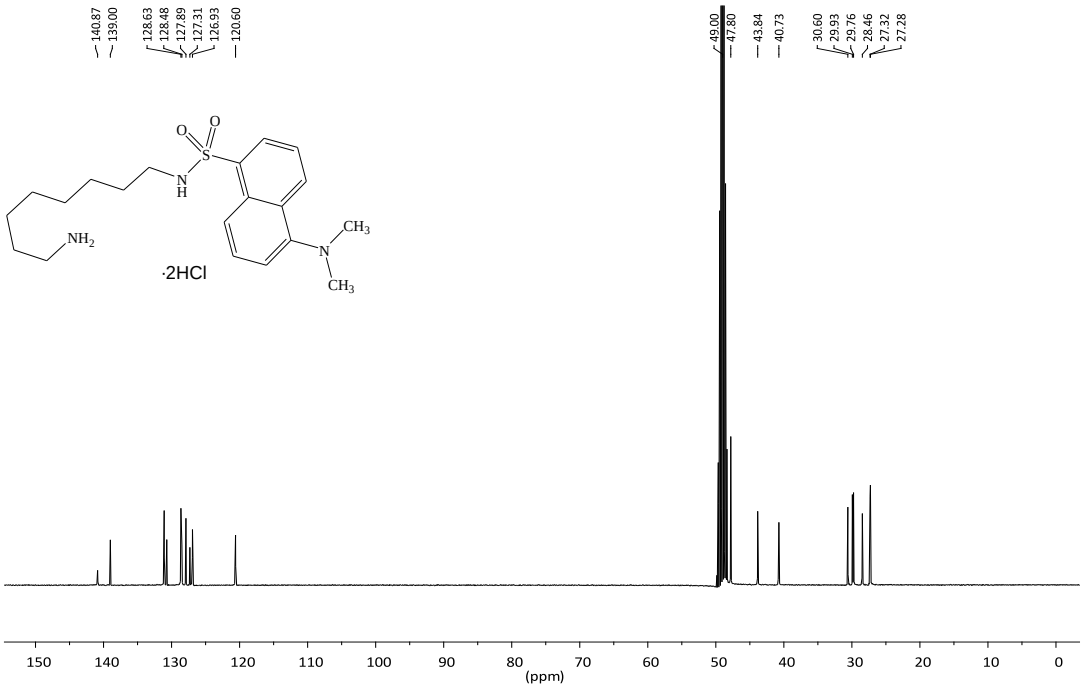
N-(6-aminohexyl)-5-(dimethylamino) naphthalene-1-sulfonamide (D8)



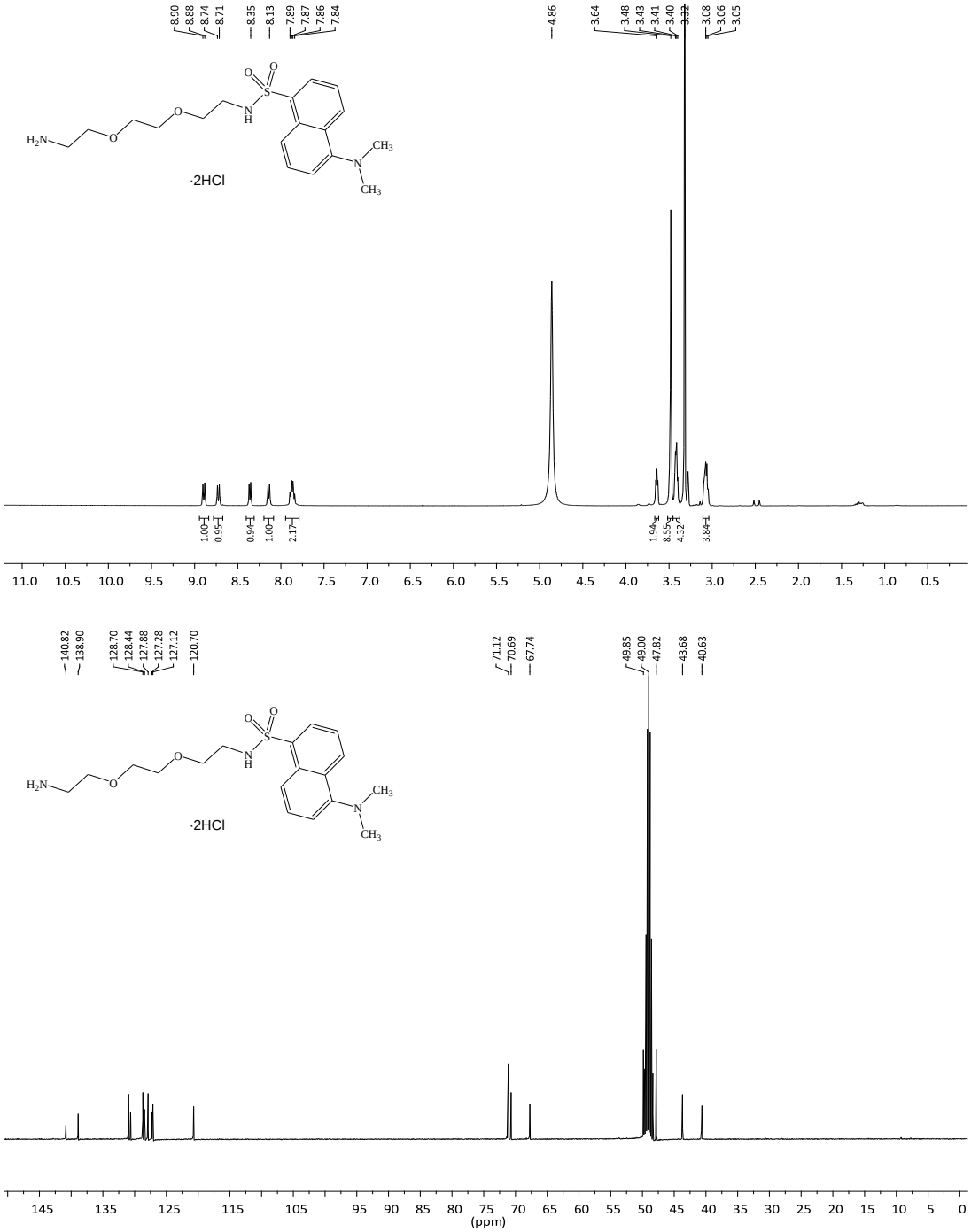


N-(8-aminooctyl)-5-(dimethylamino) naphthalene-1-sulfonamide (D9)

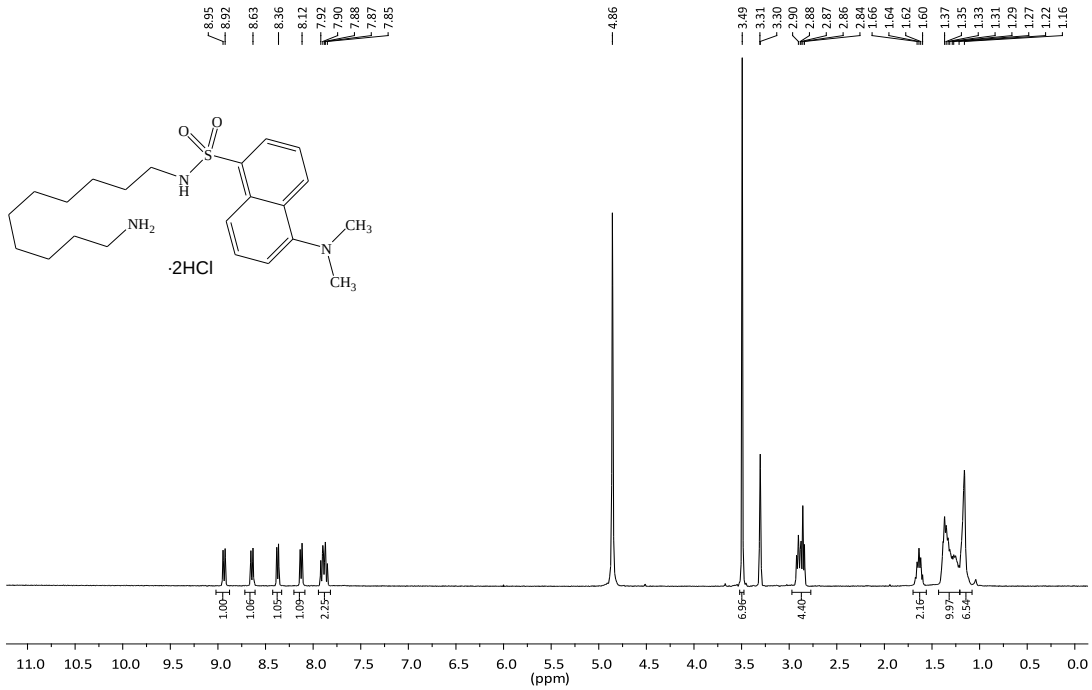


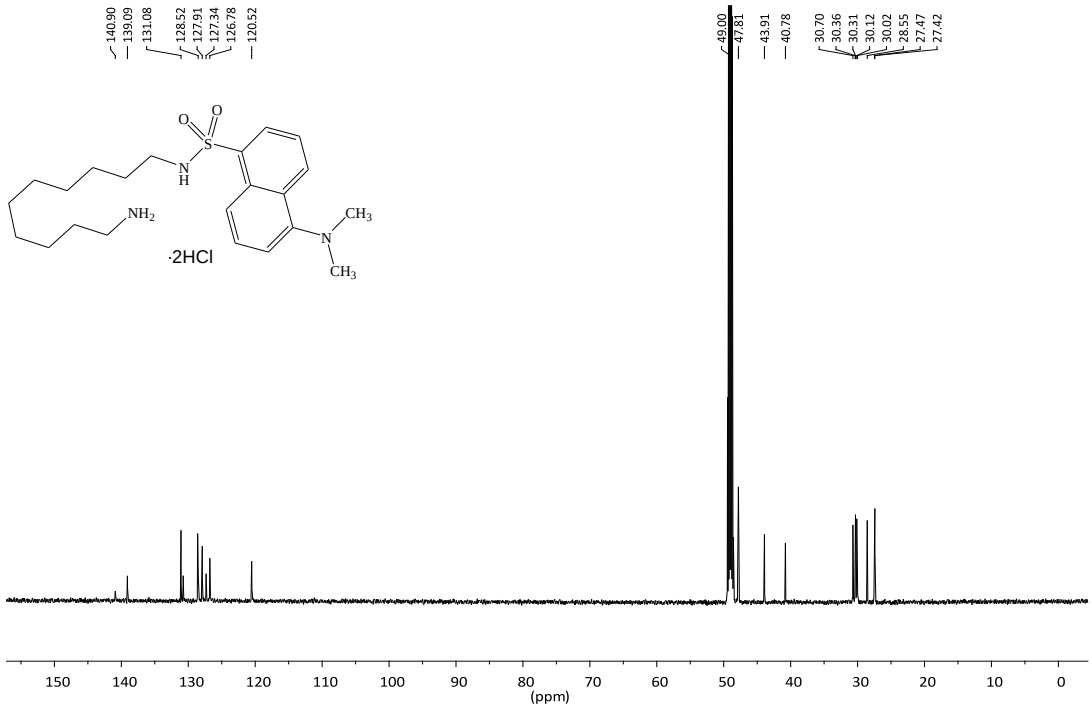


N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide (D10)

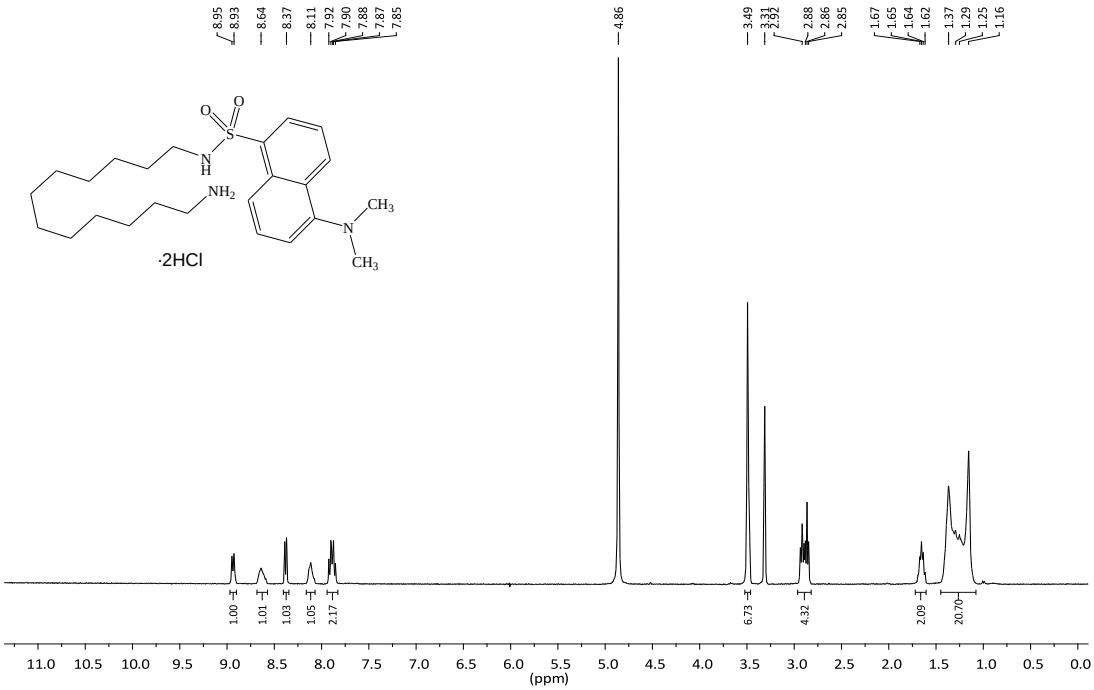


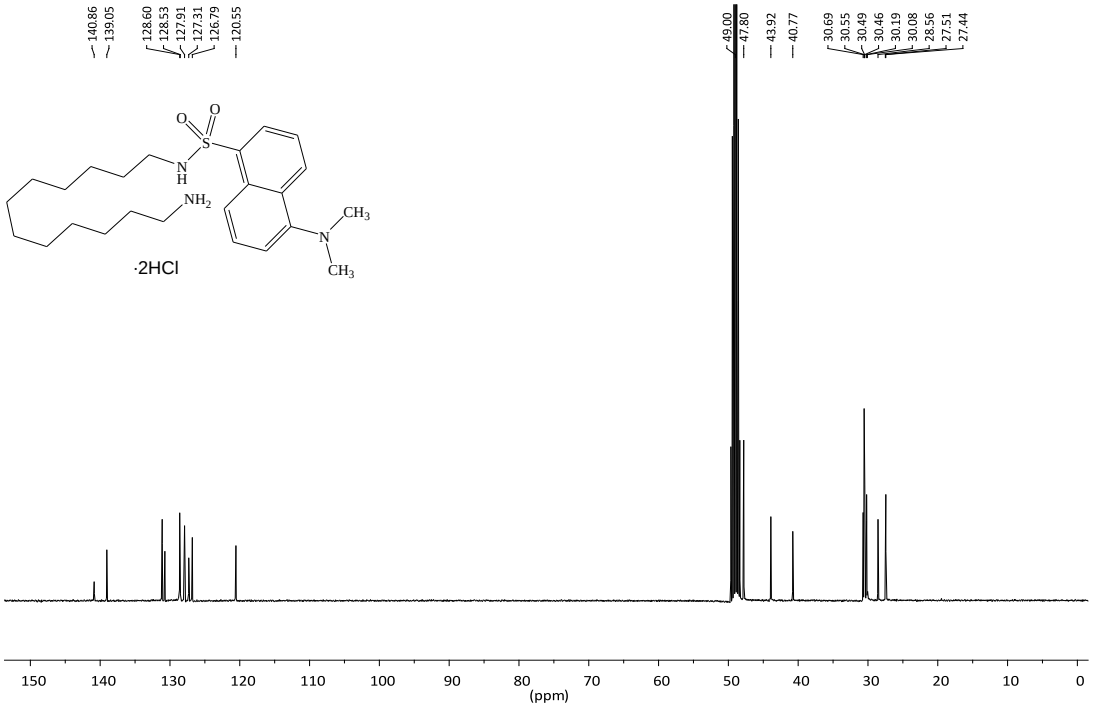
N-(10-aminodecyl)-5-(dimethylamino) naphthalene-1-sulfonamide (D11)



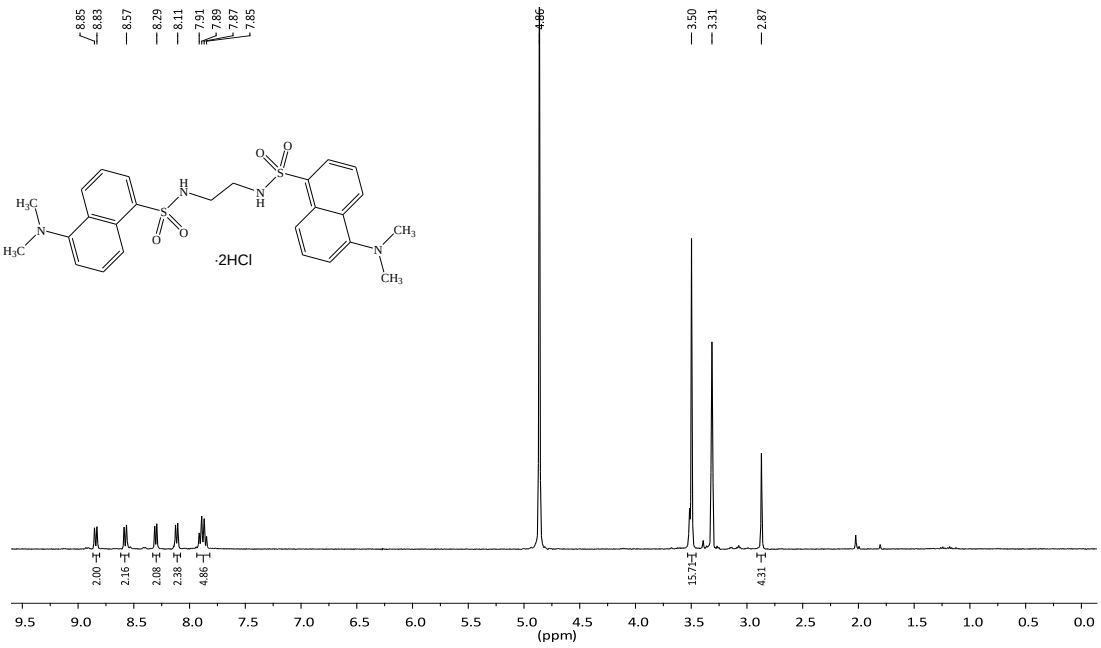


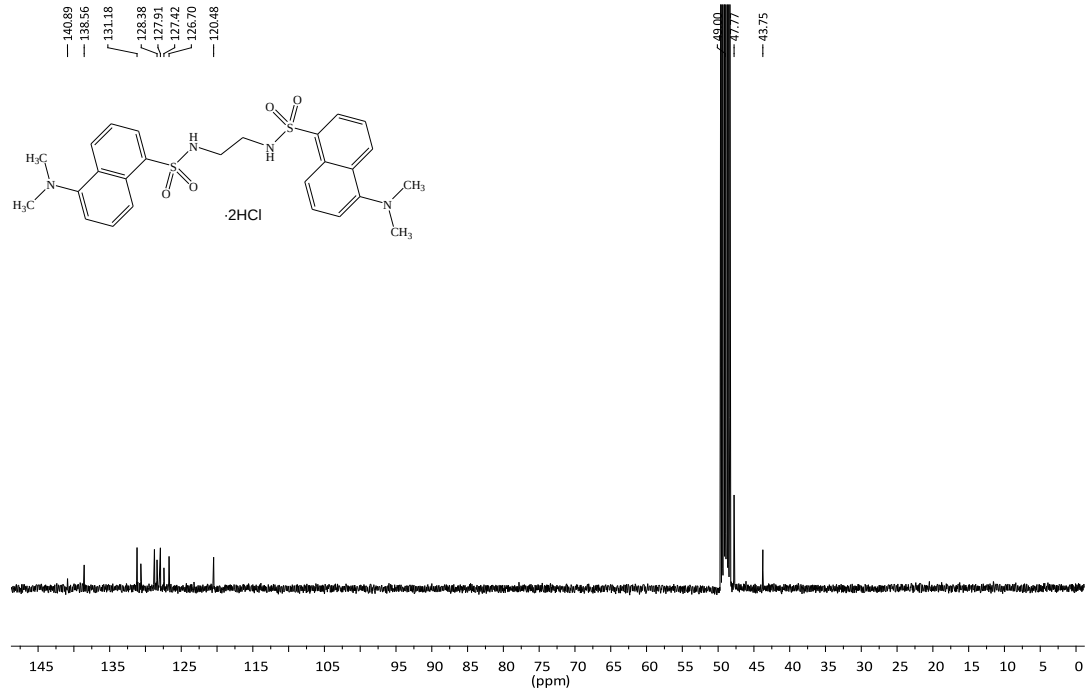
N-(12-aminododecyl)-5-(dimethylamino) naphthalene-1-sulfonamide (D12)



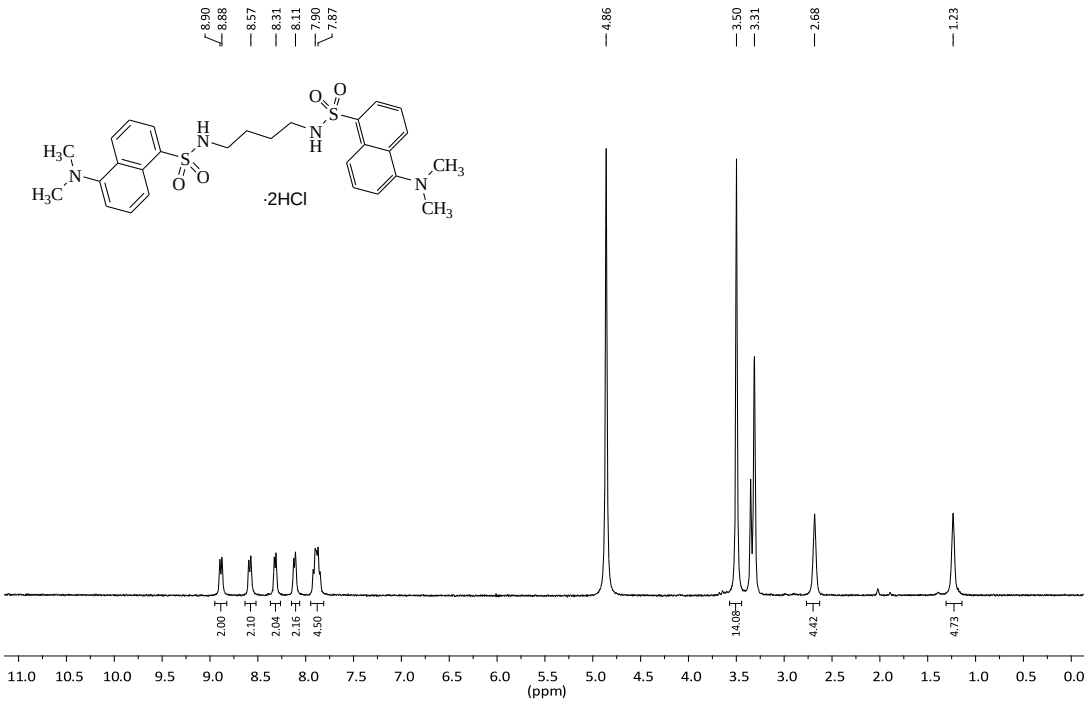


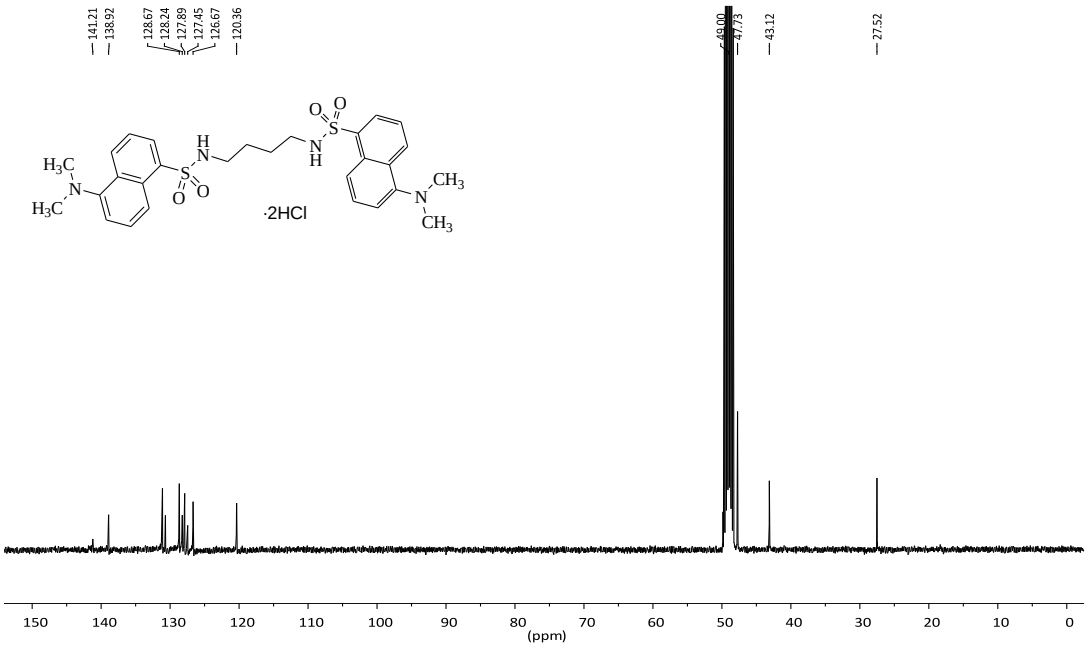
N, *N'*-(ethane-1, 2-diyl) bis(5-(dimethylamino)naphthalene-1-sulfonamide) (D13)



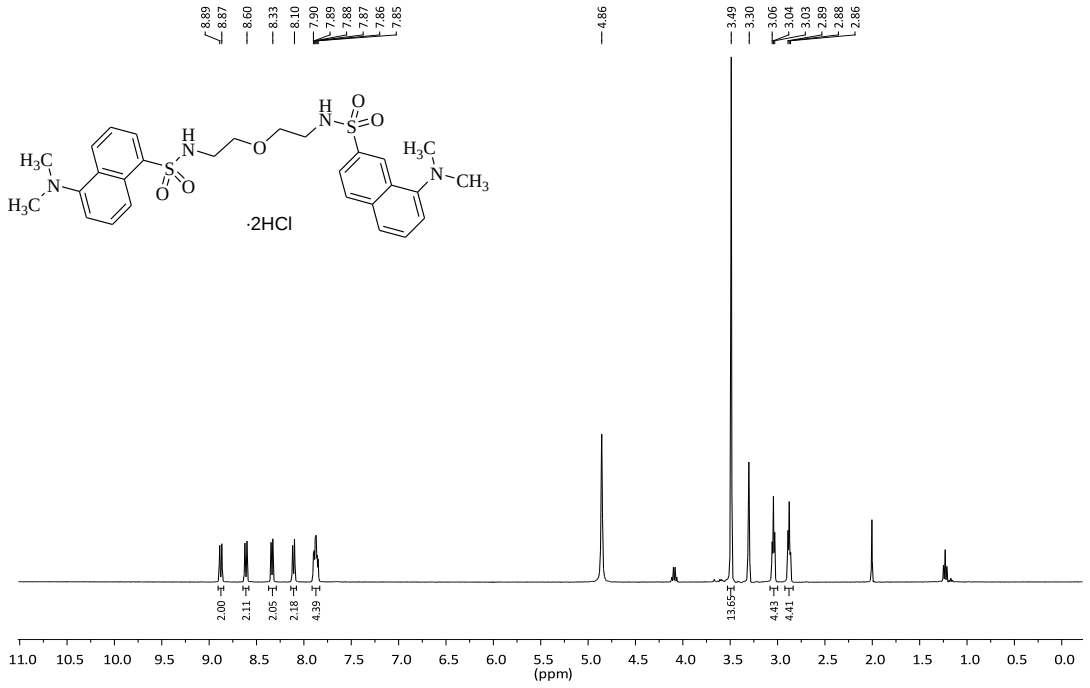


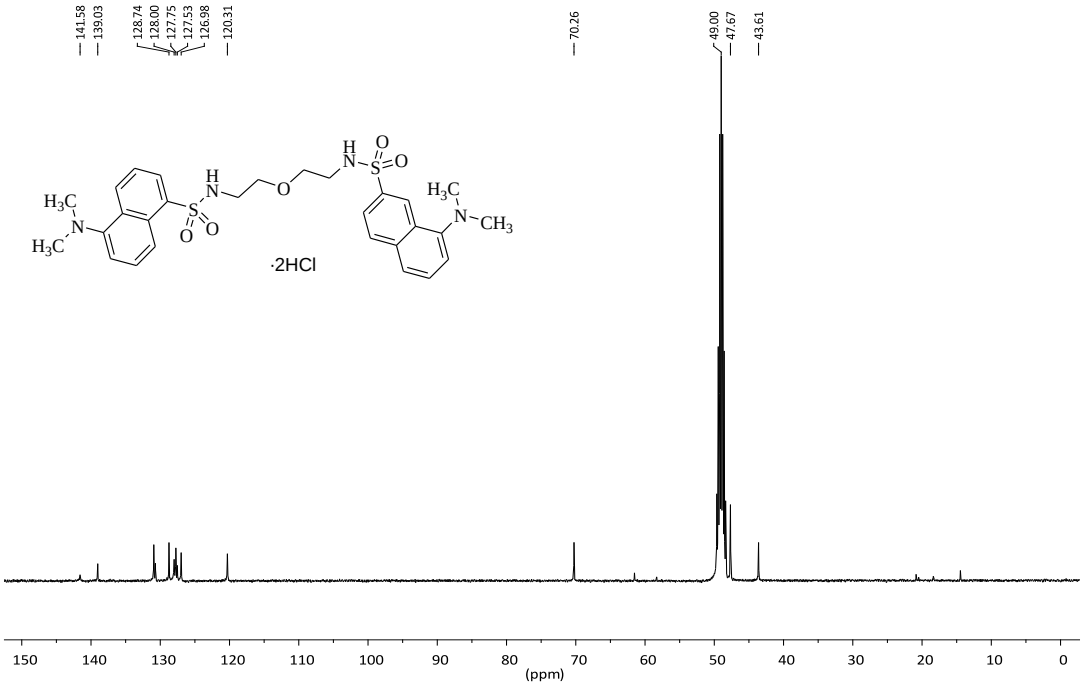
N, *N'*-(butane-1, 4-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide) (D14)



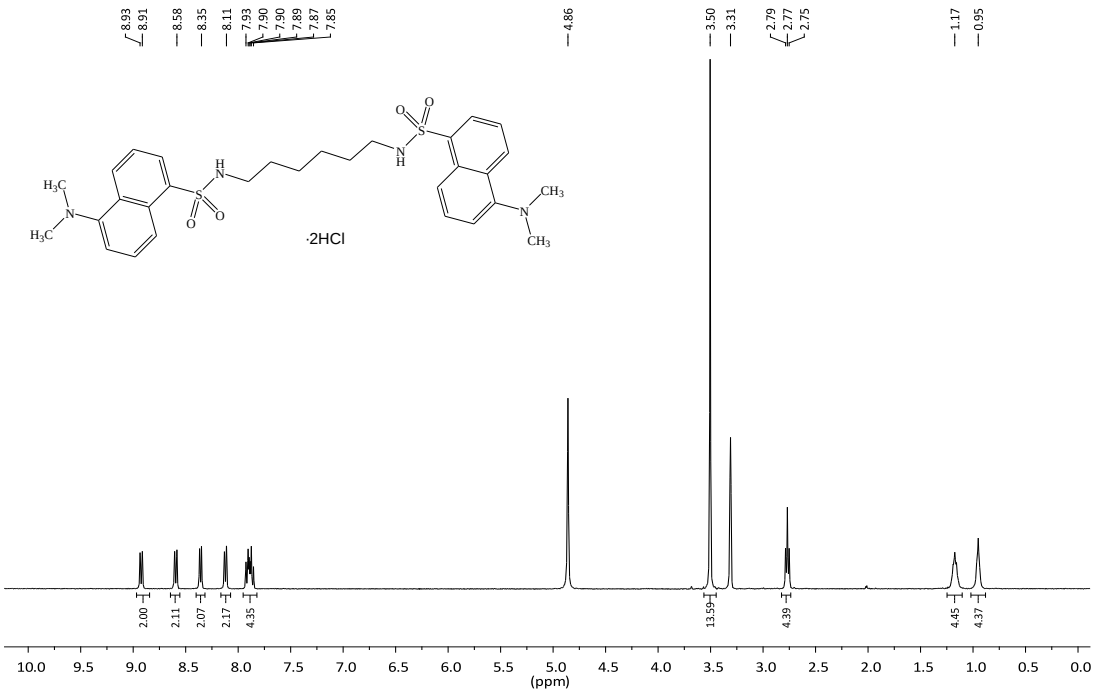


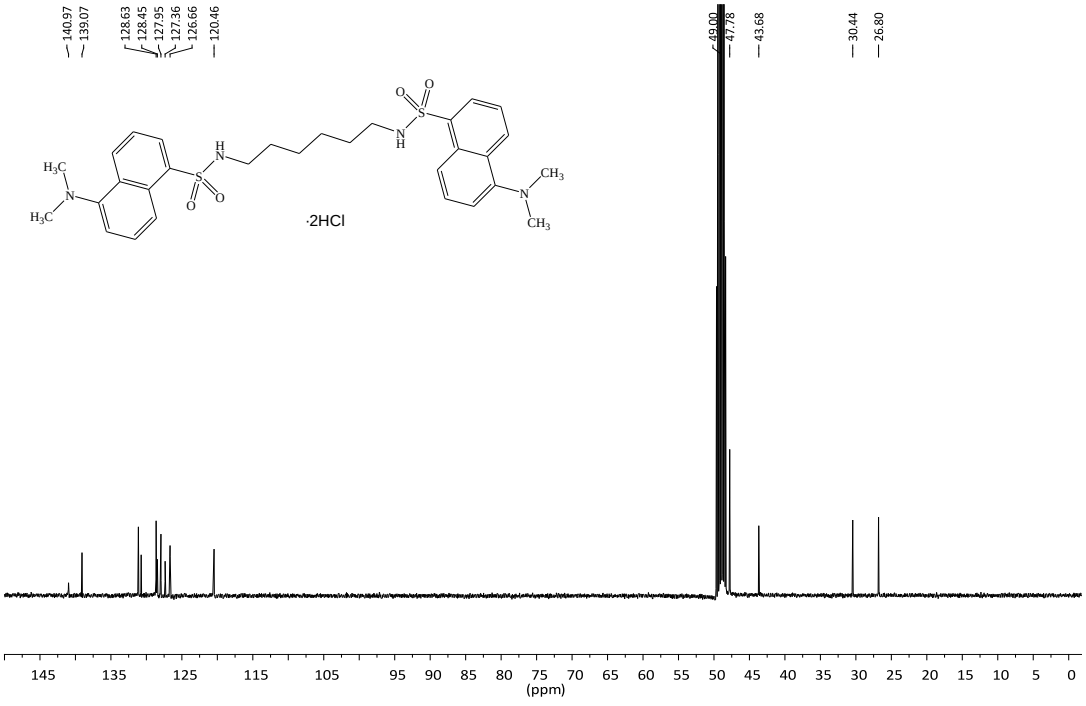
5-(Dimethylamino)-*N*-(2-(2-(8-(dimethylamino)naphthalene-2-sulfonamido)ethoxy)ethyl)naphthalene-1-sulfonamide (D15)



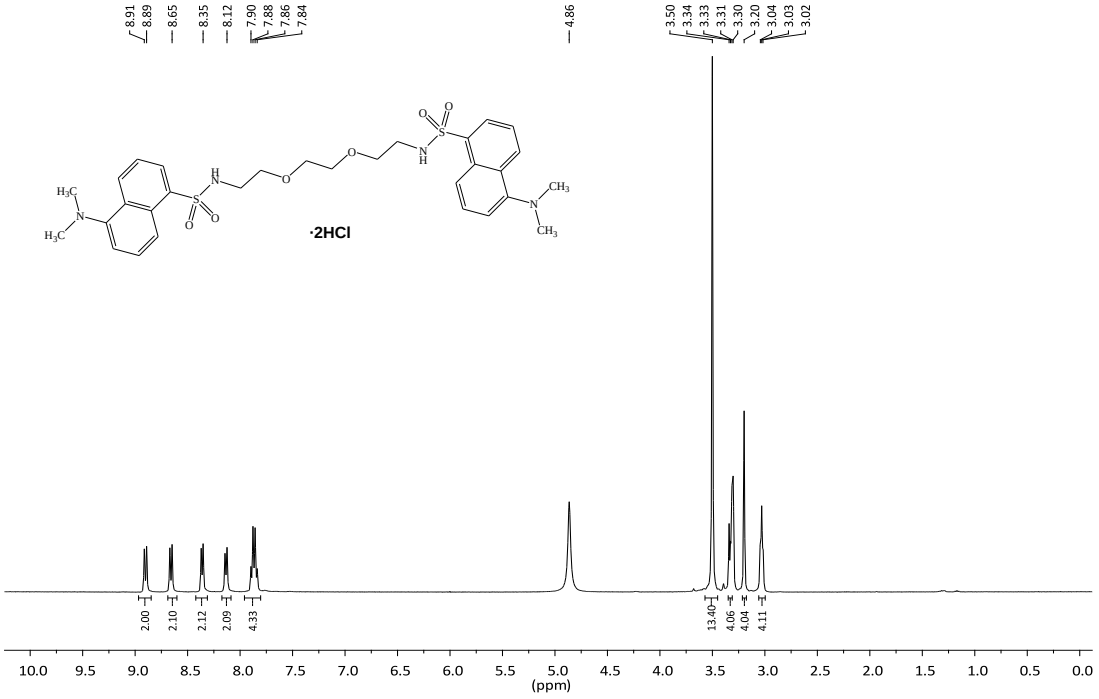


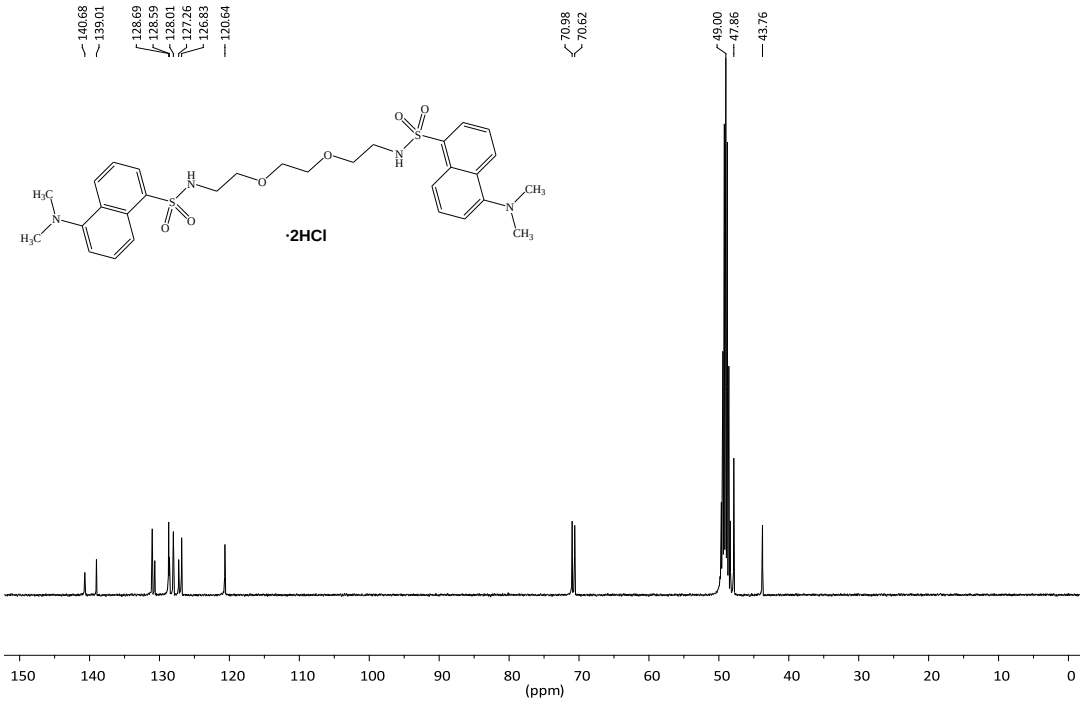
N, *N'*-(hexane-1, 6-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide) (D16)



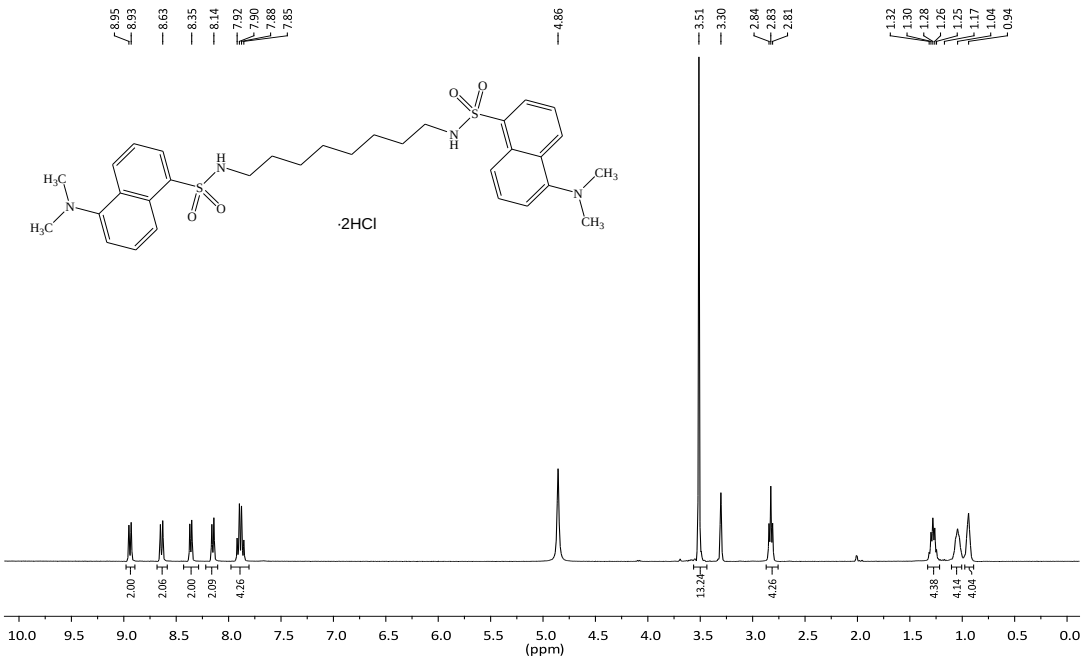


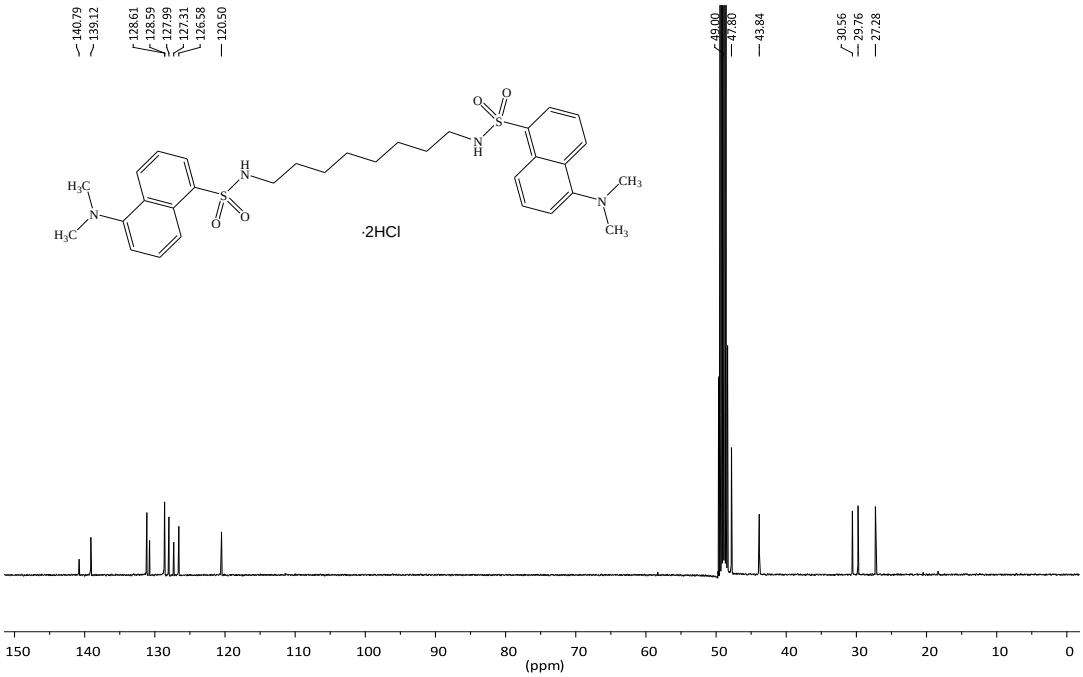
N, *N'*-((ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(5-(dimethylamino) naphthalene-1-sulfonamide) (D17)



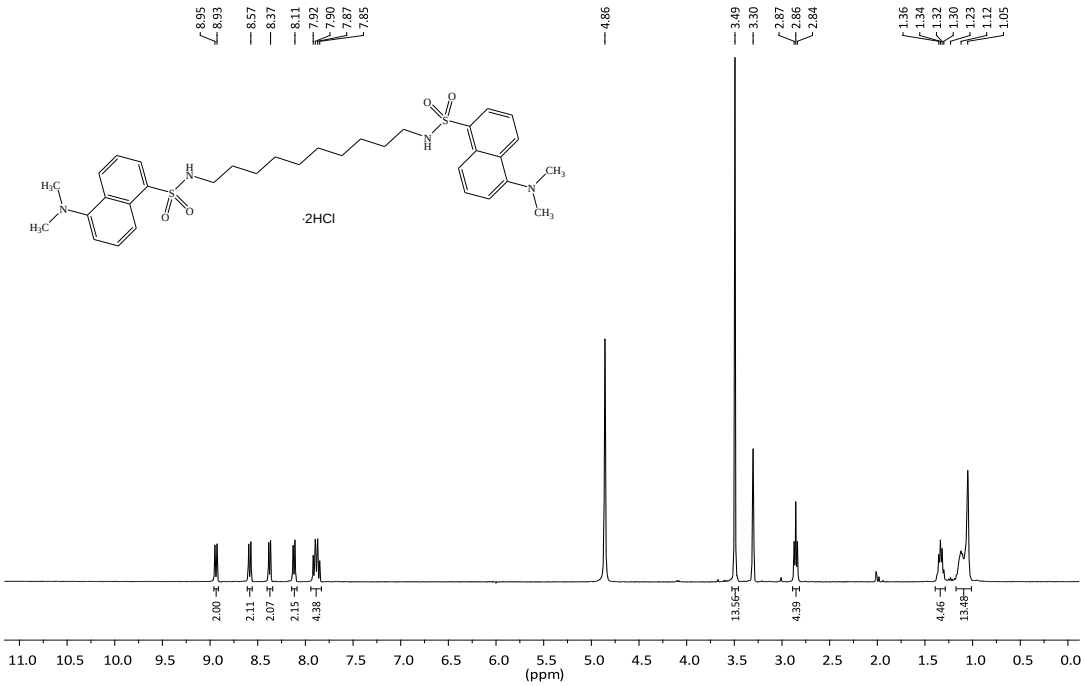


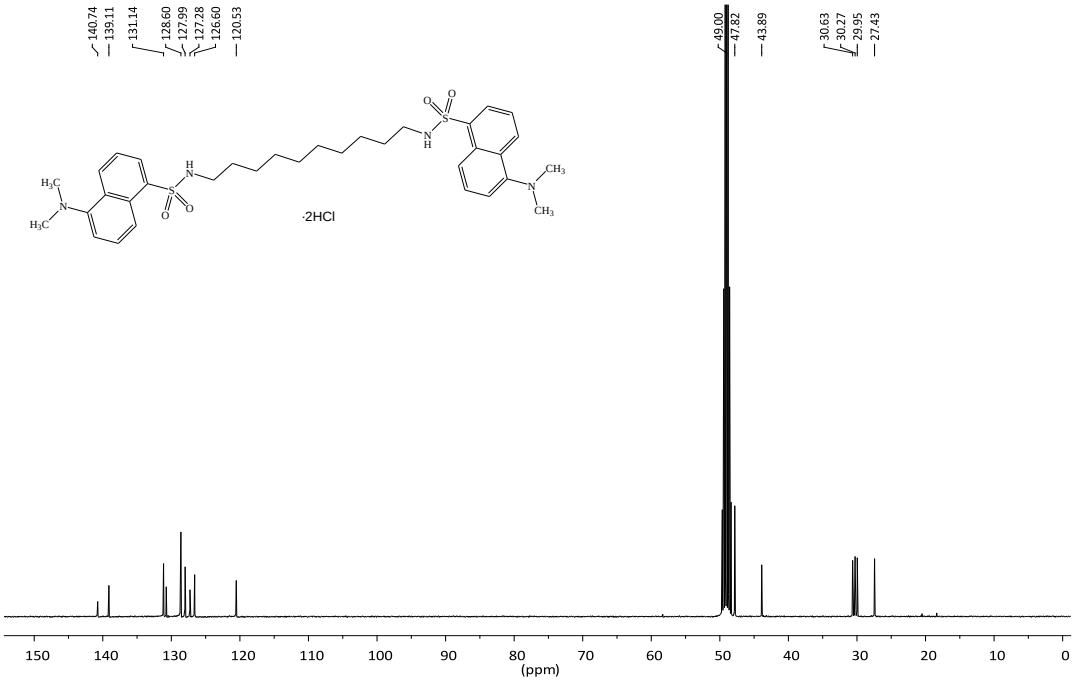
N, *N'*-(octane-1, 8-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide) (D18)



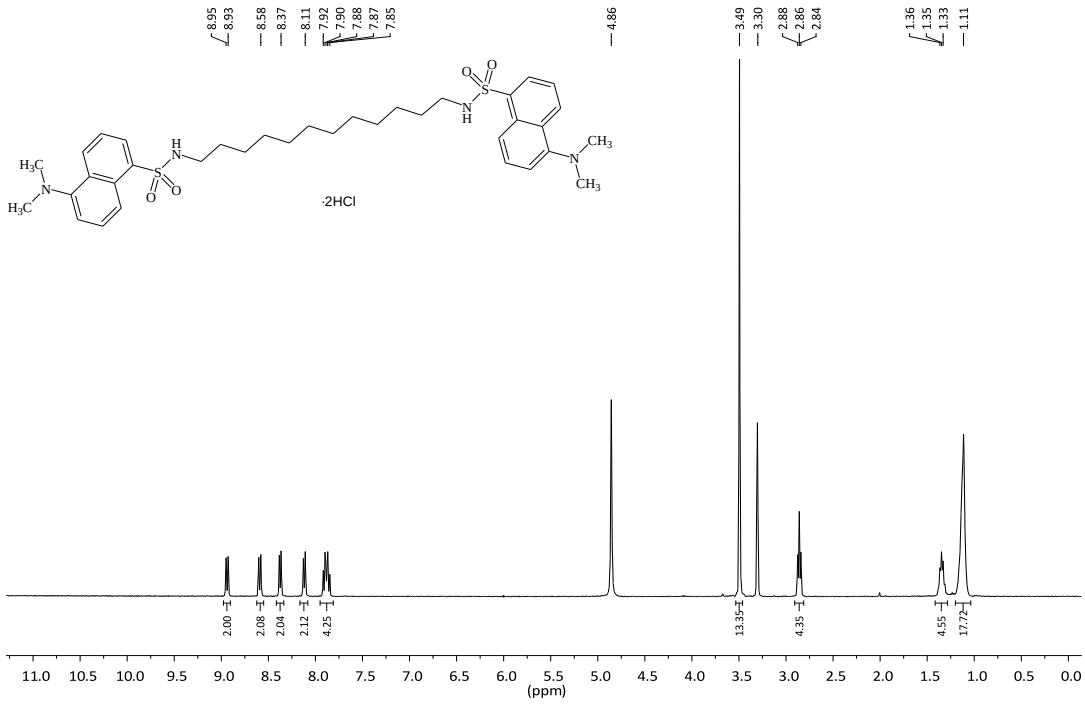


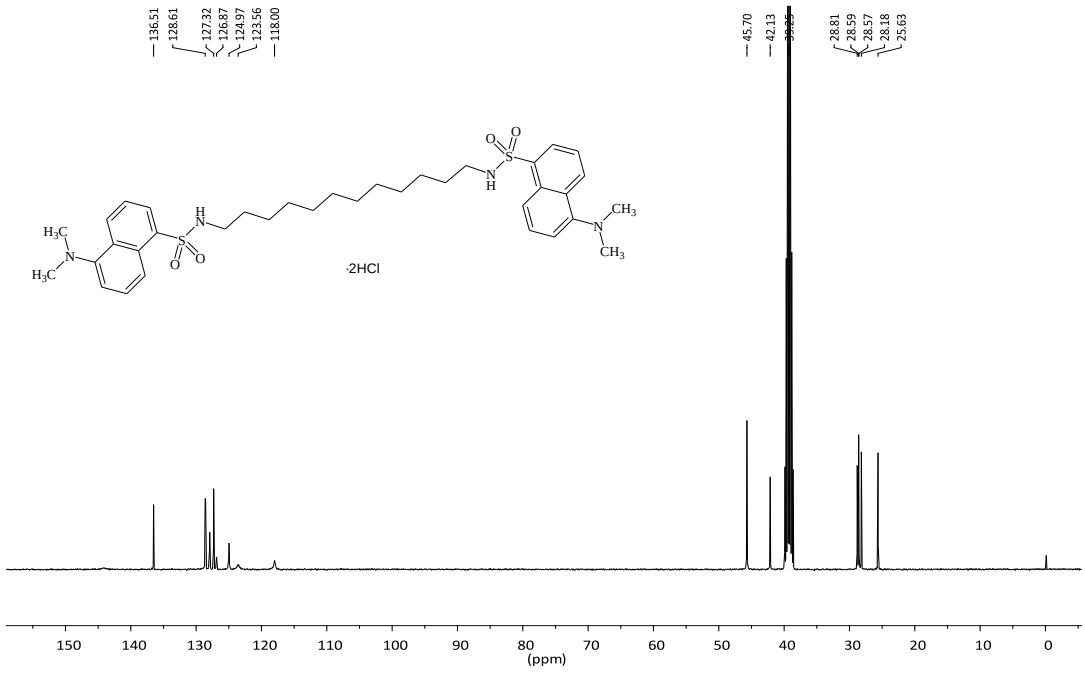
N, *N'*-(decane-1,10-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide) (D19)





N, *N'*-(dodecane-1, 12-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide) (D20)





1. (a) Lee, J. S.; Kang, N. Y.; Kim, Y. K.; Samanta, A.; Feng, S.; Kim, H. K.; Vendrell, M.; Park, J. H.; Chang, Y. T., Synthesis of a BODIPY library and its application to the development of live cell glucagon imaging probe. *Journal of the American Chemical Society* **2009,** *131* (29), 10077-82;
2. (b) Michel, B. W.; Lippert, A. R.; Chang, C. J., A reaction-based fluorescent probe for selective imaging of carbon monoxide in living cells using a palladium-mediated carbonylation. *Journal of the American Chemical Society* **2012,** *134* (38), 15668-71.