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Supporting Information

Calixarenes Incorporating Sulfonamide Moieties: Versatile Ligands for Carbonic Anhydrases Inhibition

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Synthetic procedures and compounds characterization

Cone 25,26,27-tris(2-ethoxyethoxy)-28-(3-N-phthalimidopropoxy)calix[4]arene (1b): In a 2-necked round-bottom flask, 25,26,27-triethoxyethoxycalix[4]arene (1.14 g, 1.78 mmol) and NaH (55% in oil, 155 mg, 3.56 mmol) were stirred in dry DMF (30 ml) for 15 minutes at 0 °C. Then N-3-bromopropylphthalimide (0.95 g, 3.56 mmol) was added in and the mixture was stirred for 48 h at 80 °C. The reaction was monitored by TLC (Hex/AcOEt 7:3). If necessary, a catalytic amount of KI is added to favourite the alkylation. The reaction was quenched by addition of 1N HCl (20 ml) and the mixture extracted with CH_2Cl_2 (3x40 ml). The combined organic phases were hence washed with 0.5M $Na_2S_2O_3$ (1 x 20 ml), brine (3 x 40 ml), H_2O (1 x 30 ml) and eventually evaporated at rotavapor. The crude was purified by flash chromatography column (Hex/AcOEt 3:1→7:3) to get compound **1b** as a white powder (0.89 g, 1.07 mmol, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.87 (dd, *J* = 5.4, 3.0 Hz, 2H, Pht), 7.74 (dd, *J*=5.4, 3.1 Hz, 2H, Pht), 6.74–6.48 (m, 12H, Ar), 4.53 (d, *J*=13.3 Hz, 2H, ArCHH_{ax}Ar), 4.49 (d, *J*=13.3 Hz, 2H, ArCHH_{ax}Ar), 4.20–4.07 (m, 6H, ArOCH_2), 4.04 (t, *J*=7.2 Hz, 2H, CH_2CH$_2$CH$_2$N), 3.95-3.80 (m, 8H, ArOCH$_2$CH_2O and CH$_2$N), 3.60-3.47 (m, 6H, CH_2CH$_3$), 3.18 (d, *J*=13.4Hz, 2H, ArCHH_{eq}Ar), 3.16 (d, *J*=13.4 Hz, 2H, ArCHH_{eq}Ar), 2.42-2.28 (m, 2H, CH_2CH$_2$N), 1.22–1.16 ppm (m, 9H, CH$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=168.2 (CO), 156.6, 156.2, 135.2, 135.0, 134.9, 133.9, 132.2, 128.3, 128.3, 128.1, 123.2, 122.3 and 122.2 (Ar), 73.3, 73.2 and 72.5 (ArO*C*H$_2$), 69.9 and 69.7 (ArOCH$_2$*C*H$_2$O), 66.3 (*C*H$_2$CH$_3$), 35.5 (CH$_2$N), 31.0 and 30.9 (Ar*C*H$_2$Ar), 29.6 (CH$_2$*C*H$_2$CH$_2$), 15.3 ppm (CH$_3$); HRMS (ESI): *m/z* calcd for $C_{51}H_{57}NO_9$+Na$^+$: 850.3926 [*M*+Na]$^+$; found: 850.3941.

Cone 25,27-bis(2-ethoxyethoxy)-26,28-bis(3-N-phthalimidopropoxy)calix[4]arene (1d): In a 2-necked round-bottom flask, 25,27-bis(2-ethoxyethoxy)calix[4]arene (0.36 g, 0.64 mmol) and NaH (55% in oil, 112 mg, 2.56 mmol) were stirred in dry DMF (10 ml) for 15 minutes at 0 °C. Then N-3-bromopropylphthalimide (0.69 g, 2.56 mmol) was added in and the mixture was stirred for 24 h at 80 °C. The reaction was monitored by (Hex/AcOEt 3:2). The reaction was quenched with 1N HCl (5 ml) and the mixture was extracted with CH_2Cl_2 (3x30 ml). The combined organic phases were washed with H_2O (3x30 ml), brine (3x30 ml) and evaporated at reduced pressure. The crude was purified by flash chromatography column (Hex/AcOEt 3:2) to get compound **1d** as a white powder (0.19 g, 0.20 mmol, 32% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.86 (dd, *J* = 5.3, 3.1 Hz, 4H, Pht), 7.71 (dd, *J* = 5.4, 3.0 Hz, 4H, Pht), 6.74 (d, *J* = 7.2 Hz, 4H, Ar), 6.67 (t, *J* = 7.2, 7.0 Hz, 2H, Ar), 6.59–6.52 (m, 6H, Ar), 4.53 (d, *J* = 13.4 Hz, 4H, ArCHH_{ax}Ar), 4.20-4.08 (m, 8H, ArOCH$_2$), 3.91 (t, *J* = 7.2 Hz, 4H, CH$_2$NH$_2$), 3.85 (t, *J* = 5.5 Hz, 4H, ArOCH$_2$CH_2O), 3.52 (q, *J* = 7.0 Hz, 4H, CH_2CH$_3$), 3.20 (d, *J* = 13.4 Hz, 4H, ArCHH_{eq}Ar), 2.45-2.35 (m, 4H, OCH$_2$CH_2CH$_2$NH$_2$), 1.17 (t, *J* = 7.0 Hz, 6H, CH$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=168.2 (CO), 156.6, 156.0, 135.4, 134.7, 133.9, 132.3, 128.5, 128.1, 123.2, 122.3 and 122.2 (Ar), 73.3 (O*C*H$_2$CH$_2$O), 72.6 (O*C*H$_2$CH$_2$CH$_2$NH$_2$), 69.7 (OCH$_2$*C*H$_2$OCH$_2$CH$_3$), 66.2 (OCH$_2$CH$_2$O*C*H$_2$CH$_3$), 35.5 (CH$_2$NH$_2$), 31.0 (Ar*C*H$_2$Ar), 29.7 (CH$_2$*C*H$_2$CH$_2$), 15.3 ppm (CH$_3$); HRMS (ESI): *m/z* calcd for $C_{58}H_{58}N_2O_{10}$+Na$^+$: 965.3984 [*M*+Na]$^+$; found: 965.4006.

General procedure for phthalimide removal. In a 2-necked round-bottom flask, the phtalimido derivative and NH_2NH_2·H_2O (10 eq x each phthalimide group) were stirred in EtOH for 4h-18h at reflux. The reaction was monitored by TLC (AcOEt). The reaction was quenched by solvent evaporation at rotavapor (warning: collect the condensed vapours in acidic solution to trap the excess of NH_2NH_2). The residue was suspended in 1N NaOH. The aqueous phase was extracted with CH_2Cl_2 (4x) and finally the combined organic phases were evaporated at rotavapor.

Cone 25,26,27-tris(2-ethoxyethoxy)-28-(3-aminopropoxy)calix[4]arene (2b). Compound **2b** was obtained as a colourless oil (0.71 g, 1.02 mmol, 95% yield). ^{1}H NMR (400 MHz, CD_3OD): δ=6.81–6.73 (m, 4H, Ar), 6.71–6.61 (m, 6H, Ar), 6.61–6.50 (m, 2H, Ar), 4.54 (d, *J*=13.2 Hz, 2H, ArCHH_{ax}Ar), 4.53 (d, *J*=13.2 Hz, 2H, ArCHH_{ax}Ar), 4.26–4.10 (m, 6H, ArOCH_2CH$_2$O), 4.04 (t, *J*=6.9 Hz, 2H, OCH_2CH$_2$CH$_2$N), 3.89 (q, *J*=5.6 Hz, 6H, ArOCH$_2$CH_2O), 3.65 (q, *J* = 7.2 Hz, 2H, CH_2CH$_3$), 3.59 (q, *J* = 7.2 Hz, 4H, CH_2CH$_3$), 3.22 (d, *J*=13.2 Hz, 2H, ArCHH_{eq}Ar), 3.21 (d, *J* = 13.2 Hz, 2H, ArCHH_{eq}Ar), 3.06 (t, *J* = 6.9 Hz, 2H, CH$_2$N), 2.25-2.15 (m, 2H, CH$_2$CH_2CH$_2$), 1.34–1.20 ppm (m, 9H, CH$_3$); ^{13}C NMR (100 MHz, CD_3OD): δ=157.4, 157.2, 136.5, 136.4, 136.1, 136.0, 129.6, 129.5, 129.4 and 122.7 (Ar), 75.0, 74.6 and 74.4 (ArO*C*H$_2$CH$_2$), 71.1 and 70.9 (ArOCH$_2$*C*H$_2$O), 67.6 and 67.5 (*C*H$_2$CH$_3$), 40.1 (CH$_2$N), 33.3 (CH$_2$*C*H$_2$CH$_2$), 31.7 (Ar*C*H$_2$Ar), 15.7 ppm (CH$_3$). HRMS (ESI): *m/z* calcd for $C_{43}H_{55}NO_7$+H$^+$: 698.4051 [*M*+H]$^+$; found: 698.4028.

Cone 25,27-bis(2-ethoxyethoxy)-26,28-bis(3-aminopropoxy)calix[4]arene (2d). Compound **2d** was obtained as a yellow oil (0.12 g, 0.18 mmol, 87% yield). ^{1}H NMR (400 MHz, CD_3OD) of the protonated form: δ=6.83 (d, *J* = 6.9 Hz, 4H, Ar), 6.71 (t, *J* = 6.9 Hz, 2H, Ar), 6.53-6.48 (m, 6H, Ar), 4.46 (d, *J*=13.0 Hz, 4H, ArCH*H*$_{ax}$Ar), 4.19 (t, *J*=5.6 Hz, 4H, ArOC*H*$_2$CH$_2$O), 4.03 (t, *J*=6.9 Hz, 4H, OC*H*$_2$CH$_2$CH$_2$NH$_2$), 3.82 (t, *J*=5.6 Hz, 4H, ArOCH$_2$C*H*$_2$O), 3.55 (q, *J*=6.9 Hz, 4H, C*H*$_2$CH$_3$), 3.23 (d, *J* = 13.0 Hz, 4H, ArCH*H*$_{eq}$Ar), 3.25-3.15 (m, 4H, C*H*$_2$NH$_2$), 2.30-2.20 (m, 4H, CH$_2$C*H*$_2$CH$_2$), 1.21 ppm (t, *J* = 6.9 Hz, 6H, CH$_3$); HRMS (ESI): *m/z* calcd for $C_{42}H_{54}N_2O_6$+H$^+$: 683.4055 [*M*+H]$^+$; found: 683.4042.

1,3-Alternate 25,26,27,28-tetrakis(3-azidopropoxy)calix[4]arene (5): In a 2-necked round-bottomed flask calix[4]arene (1.88 mmol, 0.80 g) and Cs_2CO_3 (18.85 mmol, 6.14 g) were stirred in dry DMF (10 ml) for 1 h at room temperature. Then 1-iodo-3-azidopropane (9.42 mmol, 1.98 g) was added in and the mixture was stirred for 3 days at rt, monitoring the reaction by TLC (Hex/AcOEt 4:1 and 11:1). The reaction was quenched with 1N HCl (10 ml) and the mixture was extracted with CH_2Cl_2 (3x30 ml). The combined organic phases were washed with H_2O (3x30 ml), brine (3x30 ml) and eventually the solvent was removed at rotavapor. The crude was recrystallized from CH_2Cl_2/Et_2O to get compound **5** as colourless crystals (0.46 g, 0.61 mmol, 33% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ=7.08 (d, *J* = 7.4 Hz, 8H, Ar), 6.90 (t, J = 7.4 Hz, 4H, Ar), 3.86 (s, 8H, ArC*H*$_2$Ar), 3.57 (t, *J* = 6.9 Hz, 8H, OCH$_2$), 2.99 (t, *J* = 6.9 Hz, 8H, CH$_2$N$_3$), 1.55-1.42 ppm (m, 8H, CH$_2$C*H*$_2$CH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 156.6, 134.0, 129.4 and 122.7 (Ar), 66.9 (OCH$_2$), 48.1 (CH$_2$N$_3$), 38.2 (Ar*C*H$_2$Ar), 28.8 ppm (OCH$_2$*C*H$_2$); HRMS (ESI): *m/z* calcd for $C_{40}H_{46}N_{12}O_4$+Na$^+$: 781.3657 [*M*+Na]$^+$; found: 781.3674.

1,3-Alternate 25,26,27,28-tetrakis(3-aminopropoxy)calix[4]arene (6). In a 2-necked round-bottomed flask, compound **5** (0.39 g, 0.51 mmol) and PPh_3 (1.07 g, 4.08 mmol) were stirred in dry $CHCl_3$ (20 ml) for 1 day at rt, monitoring by TLC (AcOEt/CH_3OH 95:5 + 1% NEt_3). Then H_2O (10 ml) was added in and the mixture was kept stirring for a further day at rt. The mixture was extracted with $CHCl_3$ (3x30 ml). The combined organic phases were subsequently washed with H_2O (3x30 ml) and extracted with aqueous 1N HCl (3x30 ml). Then, the aqueous phase was neutralized with NaOH and extracted with $CHCl_3$ (3x30 ml). The solvent was removed at rotavapor to get compound **6** as a white powder (0.19 g, 0.29 mmol, 57% yield with traces of PPh_3 and $OPPh_3$). Compound **6** was used for the subsequent coupling without further purification. Since a preliminary NMR analysis resulted in a complicate spectrum as frequently happens for amine containing calixarenes, the following spectra were registered after treatment of the sample with HCl in methanol solution and subsequent evaporation (2×) to obtain clearer patterns of signals. ^{1}H-NMR (400 MHz, CD_3OD) δ (ppm): 7.27 (d, *J* = 7.3 Hz, 8H, Ar), 7.15-7.05 (m, 4H, Ar), 3.93 (s, 8H, ArC*H*$_2$Ar), 3.80-3.65 (m, 8H, OCH$_2$), 3.00-2.85 (m, 8H, C*H*$_2$N), 2.00-1.83 ppm (m, 8H, CH$_2$C*H*$_2$CH$_2$); ^{13}C-NMR (100 MHz, CD_3OD) δ (ppm): 157.5, 135.9, 135.82, 135.8, 131.4, 131.36, 131.3, 125.0, 124.5 and 124.3 (Ar), 70.0, 69.5 and 69.2 (O*C*H$_2$CH$_2$), 49.8 (CH$_2$N), 38.7, 38.4 and 38.3 (Ar*C*H$_2$Ar), 28.8, 28.4, 28.1 and 26.7 ppm (CH$_2$*C*H$_2$CH$_2$); HRMS (ESI): *m/z* calcd for $C_{40}H_{54}N_4O_4$+H$^+$: 655.4218 [*M*+H]$^+$; found: 655.4193.

Mobile 5-nitro-25,26,27,28-tetramethoxycalix[4]arene (12a). In a 2-necked round-bottom flask, 95% HNO_3 (0.2 ml) was added to a stirred solution of 25,26,27,28-tetramethoxycalix[4]arene[36] (New J. Chem. 43, 8015–8023 (2019) (0.5 g, 1.03 mmol) and glacial CH_3COOH (1.82 ml) in CH_2Cl_2 (10 ml). Immediately the mixture became deep purple colored. The reaction, monitored by TLC (Hex/AcOEt 7:3), proceeded for 40 min at rt, then was quenched by adding H_2O (10 mL) and saturated $NaHCO_3$ aqueous solution (10 ml) and stirring for 30 minutes. The mixture was extracted with CH_2Cl_2 (3x30 ml) and the combined organic phases were subsequently washed with H_2O (3x30 ml). The solvent was removed by reduced pressure and the crude was purified flash chromatography column on silica gel (eluent: Hex/AcOEt 9:1) to get compound **12a** as a white powder (57 mg, 0.11 mmol, 10% yield). The compound is present in solution in different conformations that make difficult the precise assignment of all the peaks and a reasonable integration. For this reason, it is simply reported the list of the signals apart some of them for which it was easily possible an assignment. ^{1}H-NMR (400 MHz, $CDCl_3$): δ=8.23 (bs, Ar), 7.89 (bs, Ar), 7.36–6.78 (several m, Ar), 6.70-6.25 (several m, Ar), 4.41 (d, *J*=13.6 Hz, ArCH*H*$_{ax}$Ar of the cone conformer), 4.38 (d, *J*=13.6 Hz, ArCH*H*$_{ax}$Ar of the cone conformer), 4.08 (bs, ArCH*H*$_{ax}$Ar), 3.87–3.60 (m, ArOC*H*$_3$, ArC*H*$_2$Ar), 3.28 (d, *J*=13.6 Hz, ArCH*H*$_{eq}$Ar of cone conformer), 3.23 (d, *J*=13.6 Hz, ArCH*H*$_{eq}$Ar of cone conformer), 3.17–3.09 (m, ArCH*H*$_{eq}$Ar), 2.99 ppm (s, CH$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ=163.0, 162.7, 158.2, 158.0, 157.7, 157.5, 157.3, 142.9, 142.5, 137.2, 136.5, 136.3, 136.2, 135.5, 135.1, 134.4, 134.1, 133.5, 133.2, 132.4, 131.6, 131.0, 129.8, 129.2, 129.0, 128.4, 128.2, 127.8, 125.9,

124.5, 123.5, 123.3, 123.0, 122.3 and 121.8 (Ar), 62.2, 61.9, 61.3, 60.9, 59.7 and 59.2 (OCH_3), 35.82, 35.5, 31.0, 30.6, 30.5, 29.72, 29.4 ppm (Ar*C*H_2Ar); HRMS (ESI): *m/z* calcd for $C_{32}H_{31}NO_6$+Na^+: 548.2044 [*M*+Na]$^+$; found: 548.2063**.**

Mobile 5-amino-25,26,27,28-tetramethoxycalix[4]arene (13a). In a 2-necked round-bottom flask, calixarene **12a** (0.13 g, 0.25 mmol) and NH_2NH_2·H_2O (0.24 ml, 4.95 mmol) were dissolved in absolute EtOH (30 ml) and CH_2Cl_2 (5 ml). 10% Pd/C was added in catalytic amount and the mixture stirred for 18 h at reflux. After having verified the completion of the reaction by TLC (Hex/AcOEt 7:3), the solvent was removed by reduced pressure and the crude was rinsed with CH_2Cl_2 (20 ml). The catalyst was filtered off and washed with CH_2Cl_2 (3x20 ml) and EtOH (3x20 ml). The organic mixture of solvents was evaporated at rotavapor to obtain compound **13a** as a yellow powder (0.11 g, 0.22 mmol, 89% yield). The compound is present in solution in different conformations that make difficult the precise assignment of all the peaks and a reasonable integration. For this reason, it is simply reported the list of the signals apart some of them for which it was possible an assignment. ^{1}H-NMR (400 MHz, $CDCl_3$): δ=7.12–6.38 (several bm, Ar*)*, 4.41–4.31 (m, ArCHH_{ax}Ar of cone conformer), 4.10–3.39 (m, ArOCH_3, ArCHH_{ax}Ar of cone conformer), 3.30–3.00 ppm (m, ArCHH_{eq}Ar); ^{13}C NMR (100 MHz, CD_3OD): δ=159.8, 159.3, 159.0, 137.7, 136.6, 134.9, 133.8, 131.8, 130.2, 130.0, 129.6, 128.9, 124.0, 123.1 and 122.8 (Ar), 62.9, 62.6, 61.6, 61.0, 60.4 and 59.4 (OCH_3), 36.4 and 31.5 ppm (Ar*C*H_2Ar); HRMS (ESI): *m/z* calcd for $C_{32}H_{33}NO_4$+H^+: 496.2482 [*M*+H]$^+$; found: 496.2458**.**

Mobile 5-(4-N-Boc-aminobutanoylamido)-25,26,27,28-tetramethoxycalix[4]arene (14a) In a 2-necked round-bottom flask, EDC (0.16 g, 0.85 mmol) and Boc-GABA-OH (0.16 g, 0.78 mmol) were stirred in dry DMF (10 ml) for 30 minutes at room temperature. Then calixarene **13a** (0.35 g, 0.71 mmol) was added in and the mixture was kept stirring for 3 days at rt, monitoring by TLC (Hex/AcOEt 1:1). The reaction was quenched by removing the solvent at rotavapor and the residue was suspended in H_2O (20 ml). The mixture was extracted with CH_2Cl_2 (3x20 ml). The combined organic phases were washed again with H_2O (3x20 ml), evaporated at rotavapor and the crude was purified by flash chromatography column on silica gel (eluent: Hex/AcOEt 1:1) to isolate compound **14a** as a white powder (0.11 g, 0.16 mmol, 23% yield). The compound is present in solution in different conformations that make difficult the precise assignment of all the peaks and a reasonable integration. For this reason, it is simply reported the list of the signals apart some of them for which it was possible an assignment. ^{1}H-NMR (400 MHz, $CDCl_3$): δ=8.69 (bs, NH), 8.27 (bs, NH), 8.03 (bs, NH), 7.60–6.30 (several very broad signals, Ar), 5.01 (bs, 1H, NHCOO), 4.45–4.32 (m, ArCHH_{ax}Ar of the cone conformer), 3.86–3.70 (m, ArOCH_3, ArCH_2Ar), 3.35–2.91 (several very broad signals, ArCHH_{eq}Ar of cone conformer, CH_2NH), 2.32 (bs, 2H, CH_2CO), 1.86 (bs, 2H, CH_2C$H_2$$CH_2$), 1.55 ppm (s, 9H, C(C$H_3$)$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ=170.8 (CONH), 157.7 and 156.9 (Ar), 154.8 and 154.4 (OC(O)NH), 135.6, 135.5, 134.6, 134.4, 133.8, 132.6, 130.3, 129.0, 128.2, 128.0, 122.4 and 120.3 (Ar), 79.4 (*C*(CH_3)$_3$, 61.7, 61.4, 60.6 and 59.8 (OCH_3), 39.5 (CH_2NH), 36.1 (Ar*C*H_2Ar), 34.5 (CH_2CO), 30.6 (Ar*C*H_2Ar), 28.5 (C(*C*H_3)), 26.7 ppm (CH_2C$H_2$$CH_2$); HRMS (ESI): *m/z* calcd for $C_{41}H_{48}N_2O_7$+Na^+: 703.3354 [*M*+Na]$^+$; found: 703.3337**.**

Cone 5-N-(4-Boc-aminobutanamido)-25,26,27,28-tetraethoxyethoxycalix[4]arene (14b). In a 2-necked round-bottom flask EDC (0.11 g, 0.55 mmol) and Boc-Gaba-OH (0.10 g, 0.51 mmol) were stirred in dry DMF (3 ml) for 30 minutes at rt. Then a solution of calixarene **13b** (0.33 g, 0.50 mmol) in dry DMF (4 ml) was added in and the mixture was kept stirring for 18 h at 50 °C, monitoring by TLC (AcOEt). The reaction was quenched by addition of H_2O (20 ml) and the mixture was extracted with CH_2Cl_2 (3x20 ml). The combined organic phases were evaporated at rotavapor and the residude was purified by flash chromatography column on silica gel (eluent: Hex/AcOEt 1:1) to get compound **14b** as a white powder (0.11 g, 0.12 mmol, 26% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.02 (bs, 1H, NHCO), 6.85 (bs, 2H, ArN), 6.71–6.53 (m, 9H), 4.86 (s, 1H, BocN*H*), 4.52 (d, *J*=13.2 Hz, 2H, ArCHH_{ax}Ar), 4.48 (d, *J*=13.2 Hz, 2H, ArCHH_{ax}Ar), 4.18–4.07 (m, 8H, ArOC$H_2$$CH_2$), 3.92-3.80 (m, 8H, Ar$OCH_2CH_2$O), 3.56 (q, *J*=7.0 Hz, 8H, C$H_2$$CH_3$), 3.25-3.14 (m, 2H, CH_2NH), 3.17 (d, *J*=13.2 Hz, 2H, ArCHH_{eq}Ar), 3.13 (d, *J*=13.2 Hz, 2H, ArCHH_{eq}Ar), 2.30 (t, *J*=6.2 Hz, 2H, CH_2CO), 1.90-1.80 (m, 2H, CH_2C$H_2$$CH_2$), 1.47 (s, 9H, C($CH_3$)$_3$), 1.22 ppm (t, *J*=6.2 Hz, 12H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=170.5 (CONHAr), 156.9 and 156.5 (Ar), 156.3 (OCO), 153.1, 135.5, 135.3, 134.9, 134.7, 132.4, 128.2, 122.3, 121.9 and 120.1 (Ar), 79.5 (*C*(CH_3)$_3$), 73.2 (ArO*C*H_2), 73.1 (ArO*C*$H_2$$CH_2$), 73.1 (ArO*C*$H_2$), 69.7 (Ar$OCH_2CH_2$), 69.6 (Ar$OCH_2$*C*$H_2$), 66.4 (*C*$H_2$$CH_3$), 39.5 (*C*$H_2$NH), 34.5 (*C*$H_2$CO), 30.9 (Ar*C*$H_2$Ar), 30.86 (Ar*C*$H_2$Ar), 28.4 (C(*C*$H_3$)$_3$),

26.9 ($CH_2CH_2CH_2$), 15.3 (CH_3); HRMS (ESI): *m/z* calcd for $C_{53}H_{72}N_2O_{11}+Na^+$: 935.5028 [*M*+Na]$^+$; found: 935.5040**.**

Mobile 5-N-(4-aminobutanamido)-25,26,27,28-tetramethoxycalix[4]arene (15a) In a 2-necked round-bottomed flask, compound **14a** (0.11 g, 0.17 mmol) was dissolved in dry CH_2Cl_2 (10 ml). Then TES (0.53 ml, 3.35 mmol) and TFA (0.51 ml, 6.70 mmol) were added in and the mixture was kept stirring for 4 h at rt, monitoring by TLC (AcOEt). The reaction was quenched by evaporation of the solvent and the residue dissolved in CH_2Cl_2 (15 ml). The organic solution was washed with 1N NaOH (2 x 10 ml), then evaporated at rotavapor. The solid residue was triturated with hexane (2 ml) to get, upon filtration, compound **15a** as a white powder (70 mg, 0.12 mmol, 72% yield). The compound is present in solution in different conformations that make difficult the precise assignment of all the peaks and a reasonable integration. For this reason, it is simply reported the list of the signals apart some of them for which it was possible an assignment. ^{1}H-NMR (400 MHz, $CDCl_3$): δ=8.53 (s, NH), 7.80 (bs, NH), 7.45–6.40 (several very broad signals, Ar), 4.43–4.30 (bs, ArCHH_{ax}Ar of cone conformer), 4.18–3.00 (several very broad signals, ArCH_2Ar, ArOCH_3), 2.79 (bs, CH_2NH$_2$), 2.56–2.32 (2 bs, CH_2NH$_2$), 2.15–1.80 (2 bs, CH_2CO), 1.87 ppm (bs, 2H, $CH_2CH_2CH_2$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ=171.3, 170.7 and 168.6 (CO), 157.9, 155.6, 154.5, 136.6, 135.3, 133.8, 133.2, 132.2, 131.0, 129.0, 128.1, 123.5, 123.4, 122.4, 122.2, 120.4 and 119.2 (Ar), 62.9, 61.5 and 60.7 (OCH_3), 42.0 and 41.5 (CH_2NH_2), 36.1 and 35.1 (ArCH_2Ar), 32.1, 31.7, 31.6, 30.9, 30.9, 30.7 (CH_2CO, ArCH_2Ar), 26.4 ppm ($CH_2CH_2CH_2$); HRMS (ESI): *m/z* calcd for $C_{36}H_{40}N_2O_5+H^+$: 581.3015 [*M*+H]$^+$; found: 581.3004**.**

Cone 5-N-(4-aminobutanoylamido)-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene hydrochloride 15b. In a 2-necked round-bottomed flask, compound **14b** (74 mg, 0.08 mmol) was dissolved in dry CH_2Cl_2 (9.5 ml). Then TES (0.25 ml, 1.57 mmol,) and TFA (0.25 ml, 3.26 mmol) were added in and the mixture was kept under stirring for 18 h at rt, monitoring by TLC (AcOEt). The reaction was quenched by removing the solvent at rotavapor. Subsequently, the residue was stirred for 15 minutes into a 1N ethanolic solution of HCl (10 ml) and then the solvent was removed at rotavapor. This procedure was repeated 3 times after which compound **15b** was obtained as white powder (75 mg, 0.088 mmol, 99% yield). ^{1}H NMR (400 MHz, CD_3OD): δ=6.81 (s, 2H, ArN), 6.72 (t, *J*=8.1 Hz, 4H, Ar), 6.61 (t, *J*=7.4 Hz, 2H, Ar), 6.56 (d, *J*= 6.8 Hz, 2H, Ar), 6.53-6.45 (m, 1H, Ar), 4.55 (d, *J*=13.2 Hz, 4H, ArCHH_{ax}Ar), 4.15 (t, *J*=5.3 Hz, 4H, ArOCH_2CH_2), 4.12-4.05 (m, 4H, ArOCH_2CH_2), 3.94–3.85 (m, 8H, $ArOCH_2CH_2O$), 3.65–3.54 (m, 8H, CH_2CH_3), 3.15 (d, *J*=13.2 Hz, 2H, ArCHH_{eq}Ar), 3.12 (d, *J*=13.2 Hz, 2H, ArCHH_{eq}Ar), 2.99 (t, *J*=7.0 Hz, 2H, CH_2N), 2.44 (t, *J* = 7.0 Hz, 2H, $COCH_2$), 2.02-1.90 (m, 2H, $CH_2CH_2CH_2$), 1.27–1.16 ppm (m, 12H, CH_3); ^{13}C NMR (100 MHz, CD_3OD): δ=172.2 (CO), 157.9, 157.5, 154.2, 136.6, 136.4, 136.2, 136.0, 133.6, 129.5, 129.4, 129.2, 123.3, 123.1 and 121.5 (Ar), 74.6 and 74.4 (OCH_2CH_2), 71.1 (OCH_2CH_2O), 67.4 (CH_2CH_3), 40.4 (CH_2N), 34.4 ($CH_2CH_2CH_2N$), 31.9 (ArCH_2Ar), 24.3 ($CH_2CH_2CH_2N$), 15.7 ppm (CH_3); HRMS (ESI): *m/z* calcd for $C_{48}H_{64}N_2O_9+H^+$: 813.4690 [*M*+H]$^+$; found: 813.4671**.**

General procedure for the coupling between the amino derivatives and 4-sulfamoylbenzoic acid. In a 2-necked round-bottom flask, 4-sulfamoylbenzoic acid (2eq x each amine unit) and DIPEA (2.4eq x reactive unit) were stirred in dry CH_2Cl_2 for 10 minutes at room temperature. Then EDC was added and the resulting mixture was stirred for 10 minutes. This mixture was hence dropped in a solution in dry CH_2Cl_2 or dry DMF of the amino derivative and the reaction was stirred for 4-18h at rt if not otherwise specified. To obtain compounds **3b** and **3c**, the reaction was performed in a microwave reactor (2 cycles, T = 80 °C, ramp time = 3 minutes, hold time = 2h, P = 200 psi, potency = 200 W).

Cone 25-(4-sulfamoylbenzenamido)propoxy-26,27,28-tripropoxycalix[4]arene (3a). Following the general procedure, the reaction was performed on calixarene **2a** (20.0 mg, 3.40 μmol) dissolved in dry CH_2Cl_2 (10 ml). The mixture was stirred for 18 h and monitored by TLC (AcOEt/CH_3OH 4:1). The reaction was quenched with 1N HCl (5 ml) and the organic phase separated. The aqueous phase was extracted with CH_2Cl_2 (3x30 ml). The combined organic phases were washed with H_2O (2x20 ml) and finally evaporated at rotavapor to get compound **3a** as a white powder (11.0 mg, 13.9 μmol, 41% yield). ^{1}H NMR (400 MHz, CD_3OD): δ=8.03-7.98 (m, 4H, $ArSO_2$), 6.67–6.49 (m, 12H, Ar), 4.67 (s, 2H, ArNH_2), 4.50 (d, *J* = 13.2 Hz, 2H, ArCHH_{ax}Ar), 4.48 (d, *J* = 13.2 Hz, 2H, ArCH_{ax}HAr), 4.06 (t, *J* = 7.2 Hz, 2H, $NCH_2CH_2CH_2O$), 3.93-3.80 (m, 6H, $CH_3CH_2CH_2O$), 3.60 (t, *J* = 7.2 Hz, 2H, NCH_2), 3.16 (d, *J* = 13.2 Hz, 2H, ArCH_{eq}HAr), 3.15 (d, *J* = 13.2 Hz, 2H, ArCHH_{eq}Ar), 2.27-

2.20 (t, *J* = 7.2 Hz, 2H, $NCH_2CH_2CH_2O$), 2.05-1.90 (m, 6H, $CH_3CH_2CH_2O$), 1.10-.99 (m, 9H, CH_3); HRMS (ESI): *m/z* calcd for $C_{47}H_{54}N_2O_7S$-H^+: 789.3579 [*M*-H]$^-$; found: 789.3561.

Cone 25,26,27-tris(2-ethoxyethoxy)-28-(4-sulfamoylbenzenamido)propoxycalix[4]arene (3b): The reaction was performed on calixarene **2b** (0.10 g, 0.14 mmol) in dry DMF (6 ml), following the general procedure and monitored by TLC (AcOEt/CH_3OH 9:1). The reaction was quenched with 1N HCl (5 ml) and extracted with CH_2Cl_2 (4x20 ml). The combined organic phases were washed with brine (2x20 ml) and H_2O (1x30 ml) and finally evaporated at rotavapor. The residude was purified by flash chromatography column (AcOEt/Hex 1:1) to get compound **3b** as a white powder (59.9 mg, 0.068 mmol, 48% yield). ^{1}H NMR (400 MHz, CD_3OD): δ=8.59 (t, *J* = 5.3 Hz, 1H, N*H*), 8.01 (d, *J* = 8.4 Hz, 2H, $ArSO_2$), 7.97 (d, *J* = 8.4 Hz, 2H, $ArSO_2$), 6.67–6.59 (m, 8H, Ar), 6.59–6.51 (m, 4H, Ar), 4.55 (d, *J* = 13.2 Hz, 2H, ArCHH_{ax}Ar), 4.53 (d, *J* = 13.2 Hz, 2H, ArCHH_{ax}Ar), 4.20–4.09 (m, 6H, OCH_2CH_2O), 4.06 (t, *J* = 7.2 Hz, 2H, $OCH_2CH_2CH_2N$), 3.89 (t, *J* = 4.8 Hz, 4H, OCH_2CH_2O), 3.88 (t, *J* = 4.8 Hz, 2H, OCH_2CH_2O), 3.62 (t, *J* = 5.2 Hz, 2H, CH_2N), 3.56 (q, *J* = 7.0 Hz, 6H, CH_2CH_3), 3.17 (d, *J* = 13.2 Hz, 2H, ArCHH_{eq}Ar), 3.12 (d, *J* = 13.2 Hz, 2H, ArCHH_{eq}Ar), 2.40-2.25 (m, 2H, $CH_2CH_2CH_2$), 1.19 (t, *J* = 6.0 Hz, 3H, CH_3), 1.18 (t, *J* = 6.0 Hz, 6H, CH_3). ^{13}C NMR (100 MHz, CD_3OD): δ=168.7 (CO), 157.6, 157.5, 147.6, 139.1, 136.3, 136.2, 129.4, 129.3, 129.0, 127.3 and 123.3 (Ar), 74.4, 74.3, (OCH_2CH_2O), 74.0 ($OCH_2CH_2CH_2N$), 71.2 and 71.1 (OCH_2CH_2O), 67.4 (CH_2CH_3), 38.7 (CH_2N), 31.9 ($ArCH_2Ar$), 31.3 ($CH_2CH_2CH_2$), 15.7 (CH_3). HRMS (ESI): *m/z* calcd for $C_{50}H_{60}N_2O_{10}S$-H^+: 879.3896 [*M*-H]$^-$; found: 879.3919.

Cone 25,27-dipropoxy-26,28-bis(3-(4-sulfamoylbenzamido)propoxy)calix[4]arene (3c). The reaction was performed on calixarene **2c** (64.5 mg, 0.10 mmol) dissolved in dry DMF (6 ml), following the general procedure and monitored by TLC (AcOEt). The reaction was quenched with 1N HCl (5 ml) and extracted with CH_2Cl_2 (4x20 ml). The combined organic phases were washed with brine (2x20 ml) and H_2O (1x30 ml), then evaporated at rotavapor. The residue was purified by semipreparative TLC (AcOEt/Hex 7:3) to get compound **3c** as a white powder (7.90 mg, 7.99 μmol, 8% yield). ^{1}H NMR (400 MHz, CD_3OD): δ=7.98 (s, 4H, $ArSO_2$), 6.85 (d, *J* = 7.2 Hz, 4H, Ar), 6.72 (t, *J* = 7.2 Hz, 2H, Ar), 6.37 (bs, 6H, Ar), 4.48 (d, *J* = 13.2 Hz, 4H, ArCHH_{ax}Ar), 4.31 (bs, NH_2), 4.15 (t, *J* = 7.2 Hz, 4H, $OCH_2CH_2CH_2NH$), 3.78 (t, *J* = 7.4 Hz, 4H, $OCH_2CH_2CH_3$), 3.55 (t, *J* = 7.2 Hz, 4H, $OCH_2CH_2CH_2NH$), 3.16 (d, *J* = 13.2 Hz, 4H, ArCHH_{eq}Ar), 2.45-2.30 (m, 4H, CH_2CH_2NH), 1.95-1.80 (m, 4H, CH_2CH_3), 1.01 ppm (t, *J* = 7.4 Hz, 6H, CH_3); ^{13}C NMR (100 MHz, CD_3OD): δ=168.7 (CO), 157.1, 147.8, 137.3, 135.3, 129.7, 129.0, 127.4, 123.3 and 123.0 (Ar), 78.1 ($OCH_2CH_2CH_3$), 74.0 ($OCH_2CH_2CH_2NH$), 38.5 (CH_2NH), 32.0 ($ArCH_2Ar$), 31.6 (CH_2CH_2NH), 24.6 (CH_2CH_3), 11.1 ppm (CH_3); HRMS (ESI): *m/z* calcd for $C_{54}H_{60}N_4O_{10}S_2$-H^+: 987.3678 [*M*-H]$^-$; found: 987.3701.

Cone 25,26,27,28-tetrakis(4-sulfamoylbenzenamido)propoxycalix[4]arene (**3e**). Following the general procedure, the reaction was performed on calixarene **2e** (100 mg, 0.15 mmol) dissolved in dry CH_2Cl_2/dry DMF (20 ml, 3:1) and monitored by TLC (CH_2Cl_2/CH_3OH 9:1). The reaction was quenched after 48 h with 1N HCl (5 ml) and extracted with AcOEt (3x30 ml). The combined organic phases were washed with brine (2x20 ml) and H_2O (1x30 ml), then evaporated at rotavapor. The residue was triturated with CH_2Cl_2 and then further purified by exclusion chromatography on Sephadex LH-20 column (eluent: CH_2Cl_2/CH_3OH 1:3) to obtain **3e** as white solid (42 mg, 0.03 mmol 17%). Mp: 162.7–165; ^{1}H NMR (300 MHz, CD_3OD): δ=7.90-7.76 (m, 16H, $ArSO_2$), 6.65-6.50 (m, 12H, Ar), 4.47 (d, *J* = 13.0 Hz, 4H, ArCHH_{ax}Ar), 4.03 (t, J = 6.9 Hz, 8H, OCH_2), 3.61 (t, *J* = 7.5 Hz, 8H, NCH_2), 3.16 (d, *J* = 13.0 Hz, 4H, ArCHH_{eq}Ar), 2.38-2.25 ppm (m, 8H, $CH_2CH_2CH_2$); ^{13}C NMR (100 MHz, [D6]acetone): δ=165.1 (CO), 155.2, 146.2, 138.1, 135.0, 128.2, 127.9, 126.1 and 122.1 (Ar), 71.9 (OCH_2), 38.7 (NCH_2), 30.6 ($ArCH_2Ar$), 30.3 ppm ($CH_2CH_2CH_2$); HRMS (ESI): *m/z* calcd for $C_{68}H_{72}N_8O_{16}S_4$-H^+: 1383.387 [*M*-H]$^-$; found: 1383.386.

37,38,39,40,41,42-Hexakis(4-sulfamoylbenzenamido)propoxycalix[6]arene (**3f**). Following the general procedure, the reaction was performed on calixarene **2f** (50 mg, 0.05 mmol) dissolved in dry DMF (9 ml), and monitored by TLC (CH_2Cl_2/CH_3OH 9:1). In this case the mixture was stirred at reflux 18h. The reaction was quenched with 1N HCl (5 ml) and extracted with CH_2Cl_2 (4x20 ml). The combined organic phases were washed with brine (2x20 ml) and H_2O (1x30 ml), then evaporated at rotavapor. The residue was purified by crystallization from EtOH to give **3f** as slightly brownish solid (100 mg, 0.048 mmol, 94%); m.p.: 214.8–218 °C; ^{1}H NMR (300 MHz, [D6]DMSO, 333 K): δ=8.21 (bs, 6H, NH), 7.91-7.76 (m, 24H, $ArSO_2$), 7.29 (s, 12H,

SO_2NH_2), 6.84 (bd, *J*=7.6 Hz, 12H, Ar), 6.71 (bt, *J*=7.3 Hz, 6H, Ar), 3.88 (bs, 12H, ArCH_2Ar), 3.56 (bs, 12H, OCH_2), 3.30 (bs, 12H, NCH_2), 1.69 ppm (bs, 12H, $CH_2CH_2CH_2$); HRMS (ESI): *m/z* calcd for $C_{102}H_{108}N_{12}O_{24}S_6-2H^+$: 1037.289 [*M*-2H]$^{2-}$; found: 1037.291.

N-(3-phenoxypropyl)-4-sulfamoylbenzamide (10). Following the general procedure, the reaction was performed on 1-phenoxy-3-aminopropane **8** (0.15 g, 0.69 mmol) dissolved in dry CH_2Cl_2 (30 ml), and monitored by TLC (AcOEt/CH_3OH 4:1). The mixture was stirred 18h and when the reaction resulted complete was diluted with EtOAc (20 ml) and extracted with 1M NaOH (4x15 ml). The basic aqueous solution was therefore treated with 1N HCl (70 ml) till pH=1 and the suspension formed in this way was filtered out to get compound **10** as white powder (0.19 g, 0.58 mmol, 83% yield). ^{1}H NMR (400 MHz, [D6]DMSO): δ=8.74 (t, *J* = 6.1 Hz, 1H, N*H*CO), 8.00 (d, *J* = 8.4 Hz, 2H, $ArSO_2$), 7.90 (d, *J* = 8.4 Hz, 2H, $ArSO_2$), 7.48 (s, 2H, SO_2NH_2), 7.29 (t, *J*=7.3 Hz, 2H, Ar), 6.93 (t, *J*=7.3 Hz, 3H, Ar), 4.05 (t, *J*=6.1 Hz, 2H, OCH_2), 3.50-3.40 (m, 2H, CH_2NH), 2.10-1.95 ppm (m, 2H, $CH_2CH_2CH_2$); ^{13}C NMR (100 MHz, [D6]DMSO): δ=165.7 (CO), 159.0, 146.6, 137.9, 129.9, 128.3, 126.1, 120.9 and 114.9 (Ar), 65.6 (OCH_2), 37.0 (CH_2NH), 29.3 ppm ($CH_2CH_2CH_2$); HRMS (ESI): *m/z* calcd for $C_{16}H_{18}N_2O_4S$-H^+: 333.0915 [*M*-H]$^-$; found: 333.0899.

General procedure for the coupling between the amino derivatives and 4-isothiocyanatebenzenesulfonamide. In a 2-necked round-bottom flask, 4-isothiocyanatobenzenesulfonamide (2.0 eq x amine unit) and the amino derivative are reacted in dry CH_2Cl_2 or dry DMF or in a mixture of both for 4h-4days at rt in presence of DIPEA (2eq x reactive unit).

Cone 25,26,27-tris(2-ethoxyethoxy)-28-(3-((4-benzensulfonamidyl)thioureido)propoxy)calix[4]arene (4b). Following the general procedure, the reaction was performed on calixarene **2b** (0.10 g, 0.14 mmol) dissolved in DMF/CH_2Cl_2 (7 ml, 2:5), and monitored by TLC (AcOEt/Hex 7:3). After 18 h, the reaction was quenched with 1N HCl (5 ml) and the mixture was extracted with CH_2Cl_2 (3x30 ml). The combined organic phases were evaporated at rotavapor and the crude was purified by flash chromatography column (AcOEt/Hex 7:3) to get compound **4b** as a white powder (0.10 g, 0.11 mmol, 78% yield). ^{1}H NMR (400 MHz, CD_3OD): δ=8.59 (bs, 1H, CSNH), 8.04-7.96 (m, 4H, $ArSO_2$), 6.70-6.48 (m, 12H, Ar), 4.55 (d, *J*=13.2 Hz, 2H, ArCHH_{ax}Ar), 4.52 (d, *J*=13.2 Hz, 2H, ArCHH_{ax}Ar), 4.20–4.07 (m, 6H, OCH_2CH_2O), 4.04 (t, *J*=7.0 Hz, 2H, OC$H_2CH_2CH_2$N), 3.98-3.80 (m, 6H, $ArOCH_2CH_2$O), 3.70-3.55 (m, 2H, CH_2N), 3.58 (q, *J*=7.0 Hz, 6H, CH_2CH_3), 3.18 (d, *J* = 13.2 Hz, 2H, ArCHH_{eq}Ar), 3.15 (d, *J* = 13.2 Hz, 2H, ArCHH_{eq}Ar), 2.40-2.25 (m, 2H, $OCH_2CH_2CH_2N$), 1.30–1.12 ppm (m, 9H, CH_3); ^{13}C NMR (100 MHz, CD_3OD): δ=182.5 (CS), 157.7, 157.6, 144.2, 140.0, 136.3, 136.1, 129.4, 129.36, 129.3, 128.0, 123.3 and 123.2 (Ar), 74.5 and 74.4 (OCH_2CH_2O), 73.9 ($OCH_2CH_2CH_2N$), 71.2 and 71.15 (OCH_2CH_2O), 67.44 and 67.4 (CH_2CH_3), 43.1 (CH_2N), 32.0 and 31.9 (ArCH_2Ar), 30.9 ($CH_2CH_2CH_2$), 15.7 ppm (CH_3); HRMS (ESI): *m/z* calcd for $C_{50}H_{61}N_3O_9S_2$-H^+: 910.3776 [*M*-H]$^-$; found: 910.3783.

Cone 25,27-dipropoxy-26,28-bis(3-((4-benzensulfanamidyl)thioureido)propoxy)calix[4]arene (4c): Following the general procedure, the reaction was performed on calixarene **2c** (85 mg, 0.14 mmol) dissolved in dry DMF/dry CH_2Cl_2 (8 ml, 1:1), and monitored by TLC (AcOEt/CH_3OH 9:1 + 1% NEt_3). After 18 h, the reaction was quenched with 1N HCl (5 ml) and the mixture was extracted with CH_2Cl_2 (3x30 ml). The combined organic phases were dried at rotavapor and the crude was purified by semipreparative TLC on silica gel (eluent: AcOEt + 1% NEt_3). The solid collected was hence triturated with Et_2O and, upon filtration, compound **4c** was obtained as a white powder (90 mg, 0.092 mmol, 91% yield). ^{1}H NMR (400 MHz, CD_3OD): δ=7.84 (d, *J*=8.7 Hz, 4H, $ArSO_2$), 7.66 (d, *J*=8.7 Hz, 4H, $ArSO_2$), 6.73 (d, *J*=7.4 Hz, 4H, Ar), 6.64 (t, *J*=7.4 Hz, 2H, Ar), 6.56–6.44 (m, 6H, Ar), 4.61 (bs, 4H, NH_2) 4.50 (d, *J*=13.2 Hz, 4H, ArCHH_{ax}Ar), 4.07 (t, *J*=7.2 Hz, 4H, OC$H_2CH_2CH_2NH$), 3.86 (t, *J*=7.2 Hz, 4H, OC$H_2CH_2CH_3$), 3.88-3.78 (m, 4H, $OCH_2CH_2CH_2NH$), 3.17 (d, *J*=13.2 Hz, 4H, ArCHH_{eq}Ar), 2.40-2.27 (m, 4H, $CH_2CH_2CH_2$), 2.04-1.90 (m, 4H, CH_2CH_3), 1.04 ppm (t, *J* = 7.2 Hz, 6H, CH_3). ^{13}C NMR (100 MHz, CD_3OD): δ=182.4 (CS), 157.9, 157.5, 144.2, 140.0, 136.7, 135.8, 129.5, 129.2, 128.0, 123.5, 123.3 and 123.0 (Ar), 78.2 ($OCH_2CH_2CH_3$), 73.8 ($OCH_2CH_2CH_2N$), 43.0 (CH_2N), 32.0 (ArCH_2Ar), 31.0 ($CH_2CH_2CH_2$), 24.6 (CH_2CH_3), 11.2 ppm (CH_3); HRMS (ESI): *m/z* calcd for $C_{54}H_{62}N_6O_8S_4$-H^+: 1049.344 [*M*-H]$^-$; found: 1049.343.

Cone 25,27-bis(2-ethoxyethoxy)-26,28-bis(3-((4-benzensulfonamidyl)thioureido)propoxy)calix[4]arene (4d): Following the general procedure, the reaction was performed on calixarene **2d** (30 mg, 0.044 mmol) dissolved in dry DMF/dry CH_2Cl_2 (6 ml, 1:2), and monitored by TLC (AcOEt/Hex 4:1). After 18 h, the reaction

was quenched with 1N HCl (5 ml) and the mixture was extracted with CH_2Cl_2 (3x30 ml). The combined organic phases were dried at rotavapor and the crude was purified by semipreparative TLC (AcOEt/Hex 4:1) to get compound **4d** as a white powder (11 mg, 0.010 mmol, 23% yield). ^{1}H NMR (400 MHz, CD_3OD): δ=7.85 (d, *J*=8.7 Hz, 4H, $ArSO_2$), 7.65 (d, *J*=8.7 Hz, 4H, $ArSO_2$), 6.70–6.50 (m, 12H, Ar), 4.53 (d, *J*=13.3 Hz, 4H, $ArCH\mathit{H}_{ax}Ar$), 4.13 (t, *J*= .2 Hz, 4H, $OC\mathit{H}_2CH_2O$), 4.07 (t, *J*=7.2 Hz, 4H, $OC\mathit{H}_2CH_2CH_2N$), 3.89 (t, *J*=5.2 Hz, 4H, $OCH_2C\mathit{H}_2O$), 3.85 (bt, *J*=7.2 Hz, 4H, CH_2N), 3.56 (q, *J*=7.0 Hz, 4H, $C\mathit{H}_2CH_3$), 3.18 (d, *J*=13.3 Hz, 4H, $ArCH\mathit{H}_{eq}Ar$), 2.40-2.28 (m, 4H, $CH_2C\mathit{H}_2CH_2$), 1.18 ppm (t, *J*=7.0 Hz, 6H, CH_3). ^{13}C NMR (100 MHz, CD_3OD): δ=182.3 (CS), 157.7, 157.5, 144.1, 140.0, 136.2, 129.4, 128.0, 123.3 and 123.2 (Ar), 74.3 (OCH_2CH_2O), 74.0 ($OCH_2CH_2CH_2N$), 71.2 (OCH_2CH_2O), 67.4 (CH_2CH_3), 43.2 (CH_2N), 31.9 ($ArCH_2Ar$), 30.9 ($CH_2CH_2CH_2$), 15.8 ppm (CH_3); HRMS (ESI): *m/z* calcd for $C_{56}H_{66}N_6O_{10}S_4$-H^+: 1109.365 [*M*-H]$^-$; found: 1109.368.

37,38,39,40,41,42-Hexakis(4-benzensulfonamidylthioureido]propoxycalix[6]arene (4f). Following the general procedure, the reaction was performed on calixarene **2f** (50 mg, 0.051 mmol) dissolved in dry DMF (6 ml), and monitored by TLC (AcOEt/CH_2Cl_2 1:2). In this case the mixture was stirred at reflux. After 24 h, the reaction was quenched by evaporation of the solvent and addition of 1N HCl (5 ml). The precipitate is filtered on a büchner and triturated with EtOH to obtain **4f** as a slight brownish solid (78 mg, 74% yield). ^{1}H NMR (300 MHz, [D6]DMSO, 353 K): δ=9.58 (bs, 6H, CSNH), 7.71-7.63 (m, 24H, $ArSO_2$), 6.97 (s, 12H, SO_2NH_2), 6.92 (d, *J*=6.9 Hz, 12H, Ar), 6.82 (t, *J*=6.9 Hz, 6H, Ar), 3.93 (bs, 12H, $ArC\mathit{H}_2Ar$), 3.55 (bs, 12H, OCH_2), 3.42 (bs, 12H, NCH_2), 1.63 ppm (bs, 12H, $CH_2C\mathit{H}_2CH_2$); HRMS (ESI): *m/z* calcd for $C_{102}H_{114}N_{18}O_8S_{12}$-$2H^+$: 1130.253 [*M*-2H]$^{2-}$; found: 1130.251.

1,3-Alternate 25,26,27,28-tetrakis(4-benzensulfonamidylthioureido)propoxycalix[4]arene (7). Following the general procedure, the reaction was performed on calixarene **6** (0.19 g, 0.29 mmol) dissolved in dry DMF (20 ml), and monitored by TLC (CH_2Cl_2/CH3OH 9:1). After 18 h, the mixture was concentrated at rotavapor (10 ml) and 1N HCl (10 ml) was added to the residue. The precipitate was filtered on a büchner and the solid recrystallized from acetone/Et_2O to get compound **7** as a brownish powder (0.22 g, 0.15 mmol, 50% yield). ^{1}H-NMR (400 MHz, [D6]acetone): δ=7.95-7.75 (m, 16H, $ArSO_2$), 7.16 (d, *J*=7.3 Hz, 8H, Ar), 6.95 (t, *J*=7.3 Hz, 4H, Ar), 6.51 (s, 8H, SO_2NH_2), 3.92 (bs, 8H, $ArC\mathit{H}_2Ar$), 3.48-3.44 (m, 16H, CH_2N and OCH_2), 1.67–1.61 ppm (m, 8H, $CH_2C\mathit{H}_2CH_2$); ^{13}C-NMR (100 MHz, [D6]acetone) δ (ppm): 181.0 (CS), 156.7, 143.2, 134.4, 129.8, 126.6 and 121.9 (Ar), 68.1 (OCH_2), 41.4 (CH_2N), 37.7 ($ArCH_2Ar$), 28.0 ppm ($CH_2CH_2CH_2$); HRMS (ESI): *m/z* calcd for $C_{68}H_{78}N_{12}O_{12}S_8$-$2H^+$: 754.1741 [*M*-2H]$^{2-}$; found: 754.1724.

4-(3-phenoxypropyl)thioureido)benzenesulfonamide (9). Following the general procedure, the reaction was performed on 1-phenoxy-3-aminopropane (0.11 g, 0.69 mmol) dissolved in dry CH_2Cl_2 (5 ml), and monitored by TLC (AcOEt/CH_3OH 4:1). After 24 h, the reaction was quenched by addition of 1N HCl (5 ml), then the organic phase was separated and evaporated at rotavapor to get compound **9** as a light yellow powder (0.18 g, 0.50 mmol, 80% yield). ^{1}H NMR (400 MHz, CD_3OD): δ=7.85 (d, *J*=8.3 Hz, 2H, $ArSO_2$), 7.63 (d, *J*=8.3 Hz, 2H, $ArSO_2$), 7.27 (t, *J*=7.5 Hz, 2H, Ar), 6.94 (t, *J*=7.5 Hz, 3H, Ar), 4.09 (t, *J*=7.0 Hz, 2H, OCH_2), 3.82 (bs, 2H, CH_2N), 2.20-2.08 ppm (m, 2H, $CH_2C\mathit{H}_2CH_2$); ^{13}C NMR (100 MHz, [D6]DMSO): δ=180.9 (CS), 159.0, 138.9, 129.9, 126.7, 122.1, 121.0 and 114.9 (Ar), 65.8 (OCH_2), 41.6 (CH_2N) 28.6 ppm ($CH_2CH_2CH_2$); HRMS (ESI): *m/z* calcd for $C_{16}H_{19}N_3O_3S_2$-H^+: 364.0795 [*M*-H]$^-$; found: 364.0817**.**

bis[(2-(3'-(p-benzensolfonamidyl)thioureido)propoxy-3-methyl)phenyl]methane (11). Following the general procedure, the reaction was performed on bis[(2-(3'-amino)propoxy-3-methyl)phenyl]methane (0.10 g, 0.30 mmol) dissolved in dry DMF (5 ml), and monitored by TLC (AcOEt). After 4 h, the reaction was quenched by removing the solvent at rotavapor and the resulting crude was purified by flash chromatography column (eluent: AcOEt/Hex 9:1) to get compound **11** as a light yellow powder (0.12 g, 0.16 mmol, 52% yield). Mp**:** 130-134 °C; ^{1}H NMR (400 MHz, [D6]acetone): δ=9.20 (bs, 2H, $CSN\mathit{H}Ar$), 7.79 (d, *J*=8.7 Hz, 4H, $ArSO_2$), 7.67 (d, *J*=8.7 Hz, 4H, $ArSO_2$), 7.66-7.65 (bs, 2H, $CH_2N\mathit{H}CS$), 7.06 (d, *J*=7.3 Hz, 2H, Ar), 6.94 (t, *J*=7.3 Hz, 2H, Ar), 6.88 (d, *J*=7.3 Hz, 2H, Ar), 6.51 (s, 4H, NH_2), 4.08 (s, 2H, $ArC\mathit{H}_2Ar$), 3.94-3.86 (m, 8H, $C\mathit{H}_2N$, OCH_2), 2.29 (s, 6H, $ArC\mathit{H}_3$), 2.22-2.10 ppm (m, 4H, $CH_2C\mathit{H}_2CH_2$); ^{13}C NMR (100 MHz, CD_3OD): δ=182.1 (CS), 156.8, 143.9, 140.1, 135.1, 132.2, 130.5, 129.7, 128.0, 125.1 and 123.6 (Ar), 72.2 (OCH_2), 44.0 (CH_2N), 30.8 ($ArCH_2Ar$), 30.5 ($CH_2CH_2CH_2$), 16.6 ppm (CH_3); HRMS (ESI): *m/z* calcd for $C_{35}H_{42}N_6O_6S_4$-H^+: 769.1976 [*M*-H]$^-$; found: 769.1961**.**

Mobile 5-N-(4-(4-benzensolfonamidyl)thioureido-butanamido)-25,26,27,28-tetramethoxycalix[4]arene (16a). Following the general procedure for the coupling with 4-isothiocyanatebenzenesulfonamide, the reaction was performed on calixarene **15a** (70 mg, 0.12 mmol) dissolved in dry CH_2Cl_2/dry DMF (16 ml, 15:1). The reaction was stirred for 4 days and monitored by TLC (CH_2Cl_2/CH_3OH 9:1). Then it was quenched by removing the solvent at rotavapor and the residue was treated with 1N HCl (ml). The precipitate was collected by filtration on a büchner and purified by flash chromatography column on silica gel (eluent: CH_2Cl_2/CH_3OH 96:4) to isolate compound **16a** as slightly brownish powder (25 mg, 0.032 mmol, 26% yield). The compound is present in solution in different conformations that make difficult the precise assignment of all the peaks and a reasonable integration. For this reason, it is simply reported the list of the signals apart some of them for which it was easily possible an assignment. ^{1}H-NMR (400 MHz, $CDCl_3$): δ=8.72 (bs, NHCS), 7.55, 7.30, 6.90, 6.72, 6.63, 6.50 (several very broad signals, Ar), 5.40 (bs, SO_2NH_2), 4.34 (bs, ArCHH_{ax}Ar of cone conformer), 4.15-3.00 (several very broad signals, ArCH_2Ar, OCH_3, CH_2NH), 2.50-1.65 ppm (several very broad signals, $CH_2CH_2CH_2$, CH_2CO); ^{13}C-NMR (100 MHz, $CDCl_3$): δ=180.3 (CS), 171.6 (CO), 157.8, 155.0, 135.4, 134.8, 128.2, 127.2, 122.8 (Ar) 61.4 (CH_3), 32.0, 30.8, 30.6, 30.4, 30.2, 30.0, 29.8, 29.7, 29.4, 22.7, 14.2 ppm (ArCH_2Ar, $COCH_2$, CH_2NH);); HRMS (ESI): *m/z* calcd for $C_{43}H_{46}N_4O_7S_2$-H^+: 793.2735 [*M*-H]$^-$; found: 793.2751**.**

Cone 5-N-((4-(4-benzensulfanamidyl)thioureido)butanoylamido)-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (16b): Following the general procedure for the coupling with 4-isothiocyanatebenzenesulfonamide, the reaction was performed on calixarene **15b** (74 mg, 0.09 mmol) dissolved in dry DMF (5 ml), and monitored by TLC (AcOEt/Hex 9:1). After 18 h, the reaction was quenched by evaporation of the solvent at rotavapor and the crude was purified by flash chromatography column on silica gel (eluent: AcOEt/Hex 3:1) to get compound **16b** as a white powder (7.0 mg, 0.006 mmol, 8% yield). ^{1}H NMR (300 MHz, [D6]acetone): δ=9.37 (bs, CSNH), 8.91 (bs, CSNH), 7.90-7.70 (m, 5H, $ArSO_2$ and CONH), 7.01 (s, 2H, SO_2NH_2), 6.75-6.45 (m, 11H, Ar), 4.58 (d, *J*=13.2 Hz, 2H, ArCHH_{ax}Ar), 4.55 (d, *J*=13.2 Hz, 2H, ArCHH_{ax}Ar), 4.25-4.15 (m, 8H, OCH_2CH_2O), 3.95-3.80 (m, 8H, OCH_2CH_2O), 3.66 (bs, 2H, CH_2N) 3.60-3.52 (m, 8H, CH_2CH_3), 3.16 (d, *J*=13.2 Hz, 2H, ArCHH_{eq}Ar), 3.10 (d, *J*=13.2 Hz, 2H, ArCHH_{eq}Ar), 2.41 (bt, *J*=7.2 Hz, 2H, $COCH_2$), 2.01-1.90 (m, 2H, $CH_2CH_2CH_2$), 1.19 ppm (t, *J* = 7.2 Hz, 9H, CH_3); ^{13}C NMR (75 MHz, [D6]acetone): δ=181.2 (CS), 170.2 (CO), 156.7, 156.4, 152.7, 143.1, 139.0, 135.2, 135.0, 134.7, 133.5, 128.1, 126.7, 123.1, 122.0 and 119.6 (*C*Ar), 73.5, (OCH_2CH_2), 69.7 (OCH_2CH_2O), 65.9 (CH_2CH_3), 43.9 ($CH_2CH_2CH_2NH$), 34.2 ($CH_2CH_2CH_2NH$), 30.7 and 30.6 (ArCH_2Ar), 24.6 (CH_2CH_2N), 14.8 ppm (CH_3); HRMS (ESI): *m/z* calcd for $C_{55}H_{70}N_4O_{11}S_2$-H^+: 1025.440 [*M*-H]$^-$; found: 1025.441**.**

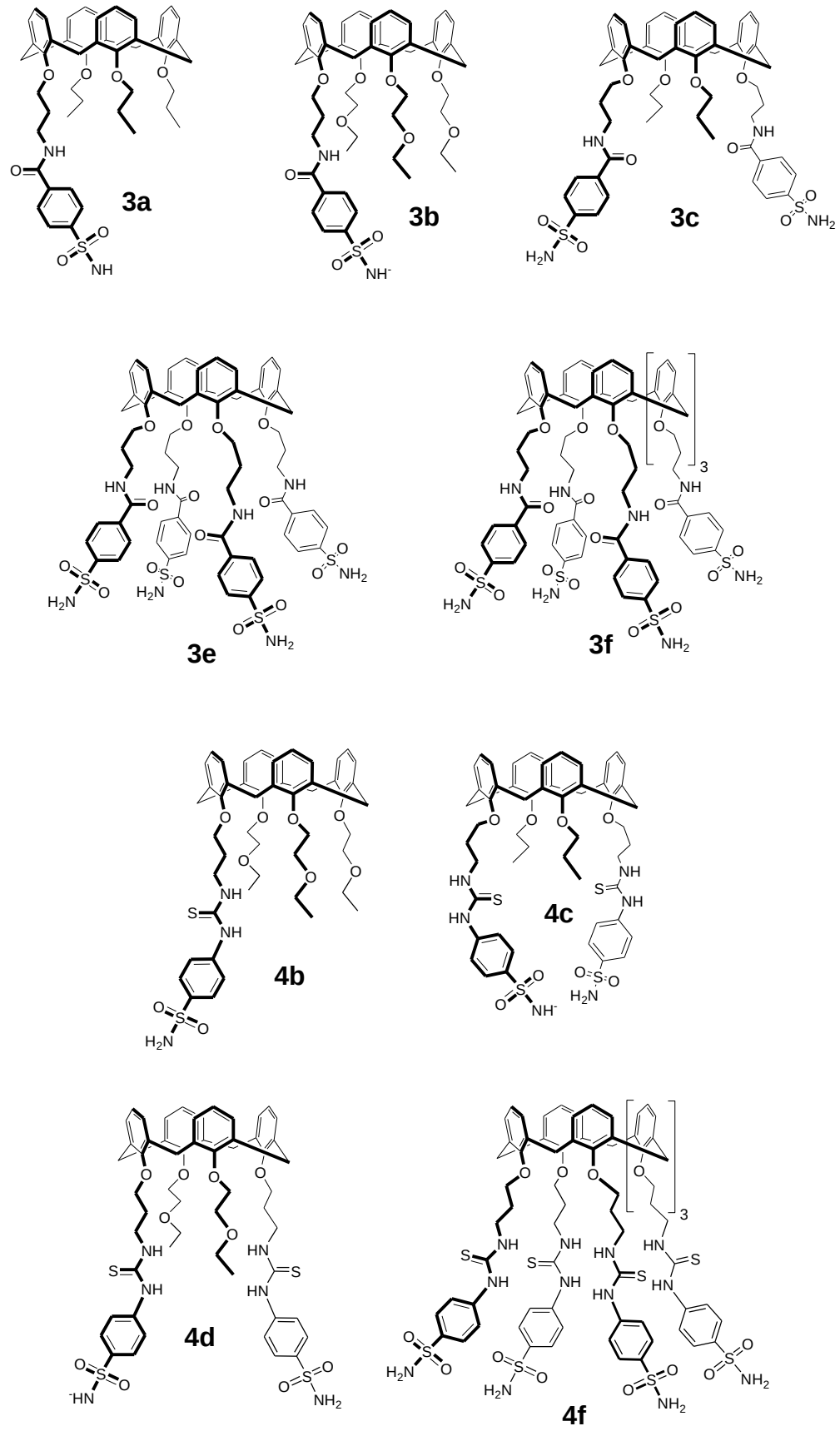


Figure S1. Structures of calix[4]arenes blocked in cone geometry and calix[6]arenes functionalized with benzensulfonamide units, obtained through the synthetic pathways reported in Scheme 1

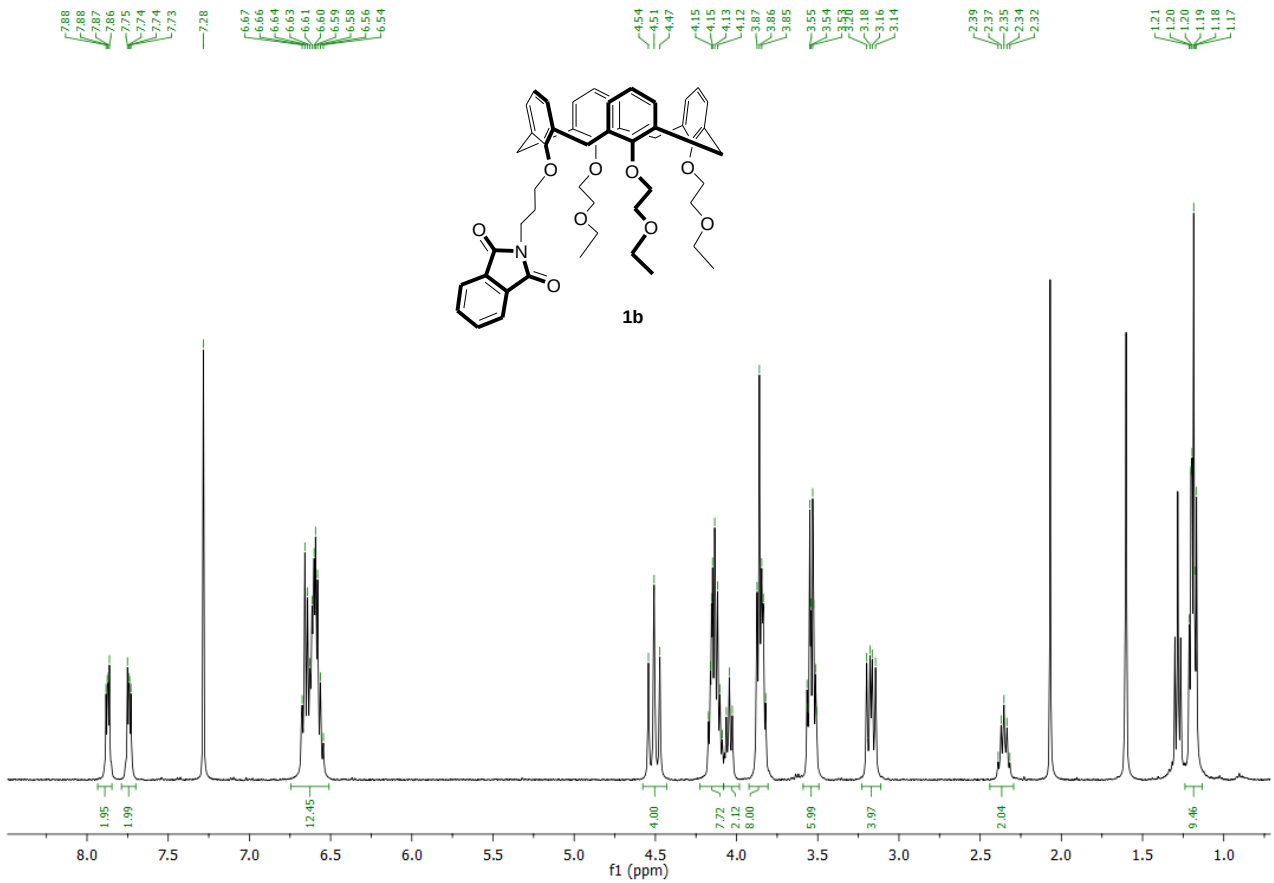


Figure S2. ^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) spectrum of compound **1b**

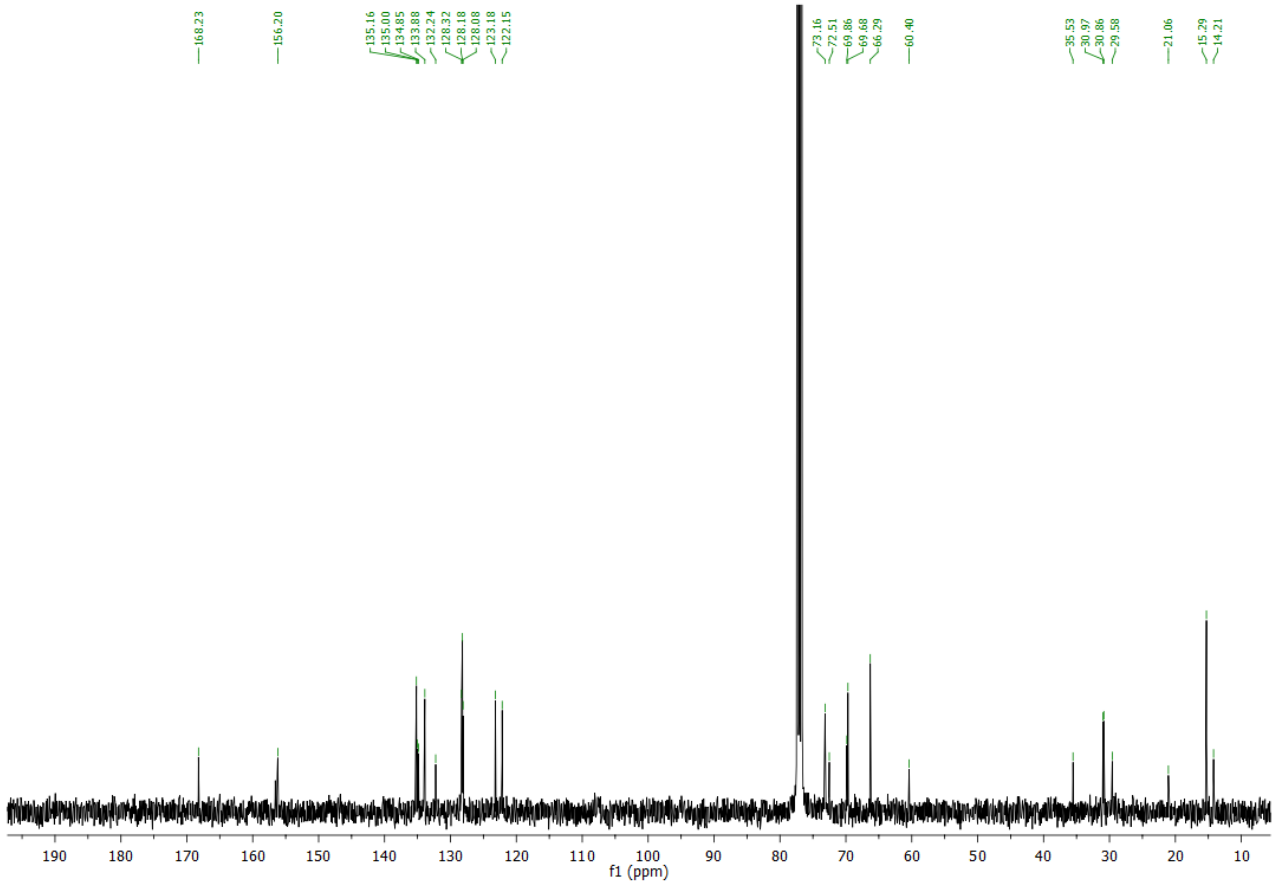


Figure S3. ^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) spectrum of compound **1b**

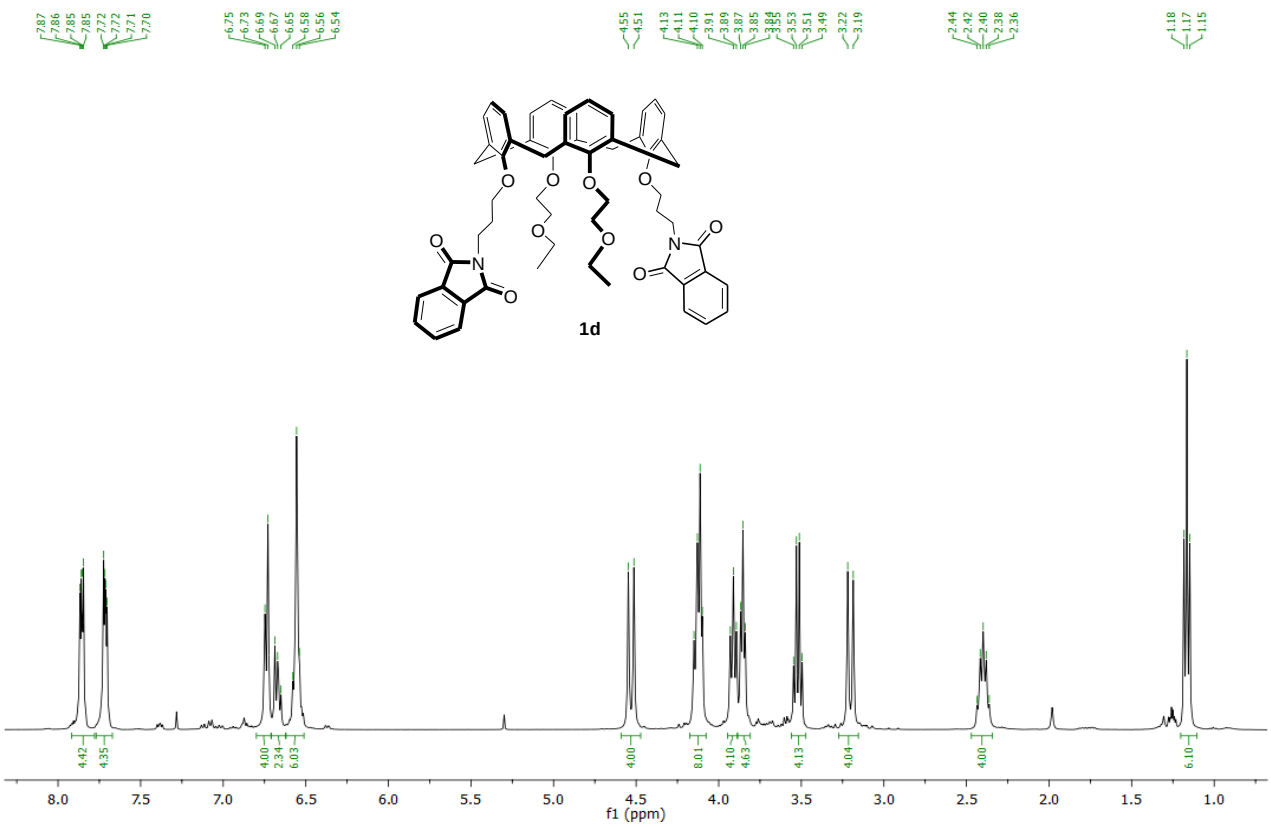


Figure S4. ^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) spectrum of compound **1d**

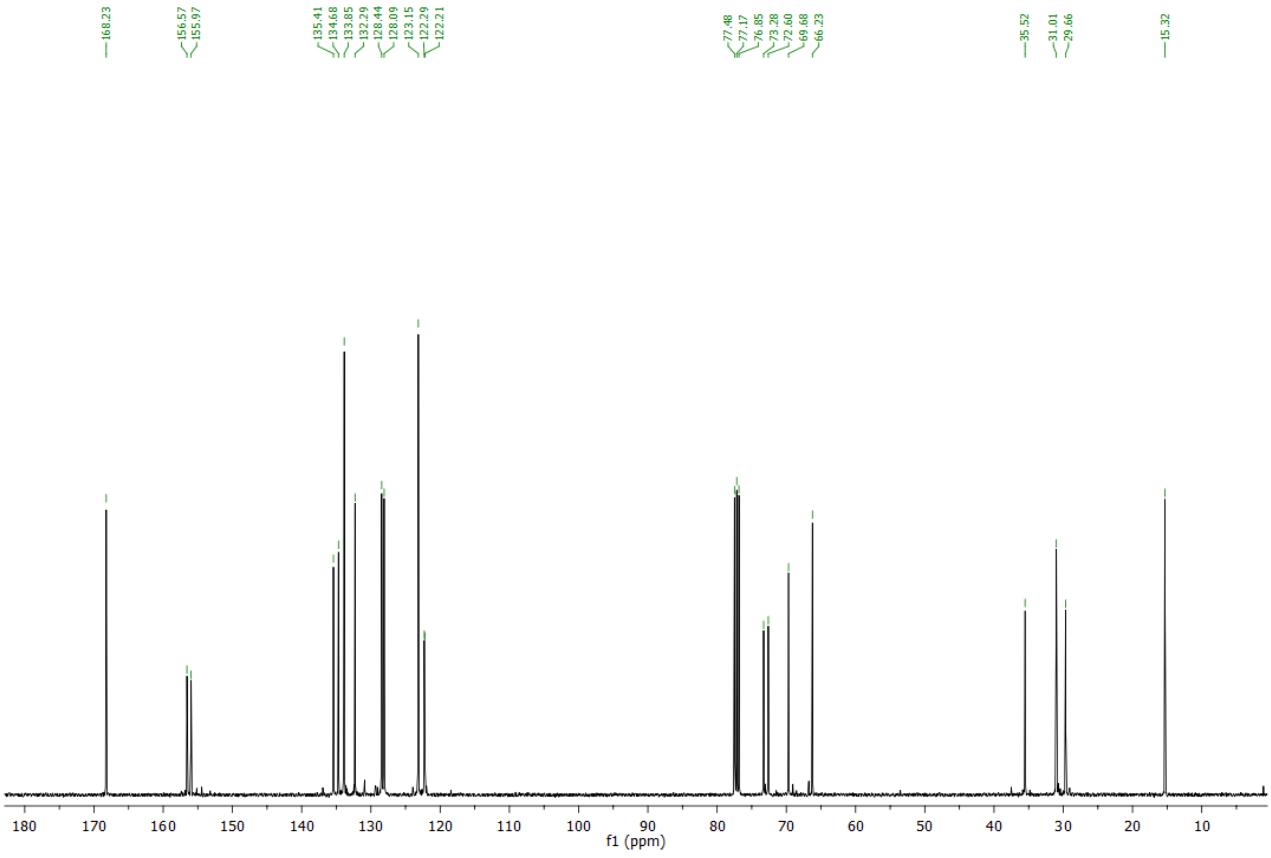


Figure S5. ^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) spectrum of compound **1d**

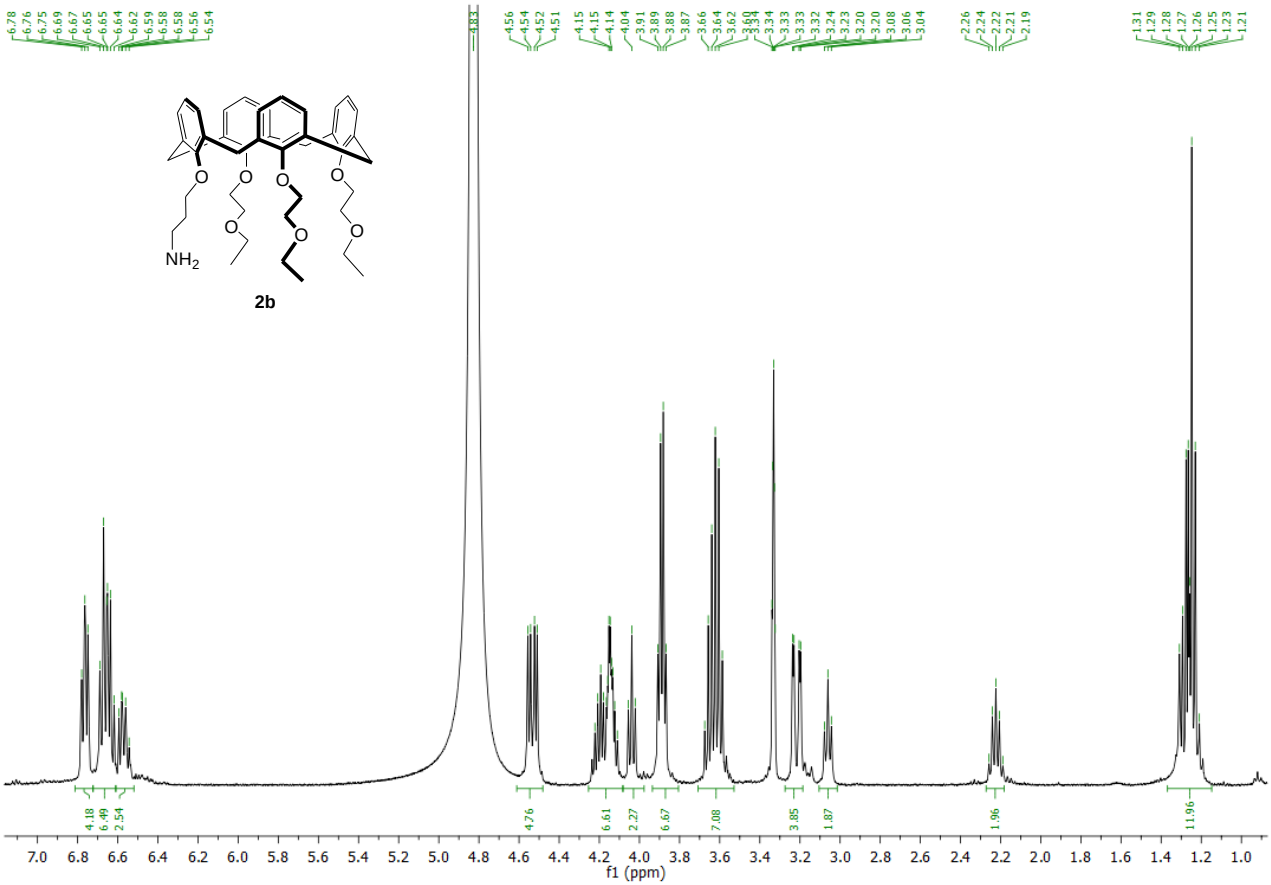


Figure S6. ^{1}H-NMR (400 MHz, CD_3OD, 298 K) spectrum of compound **2b**

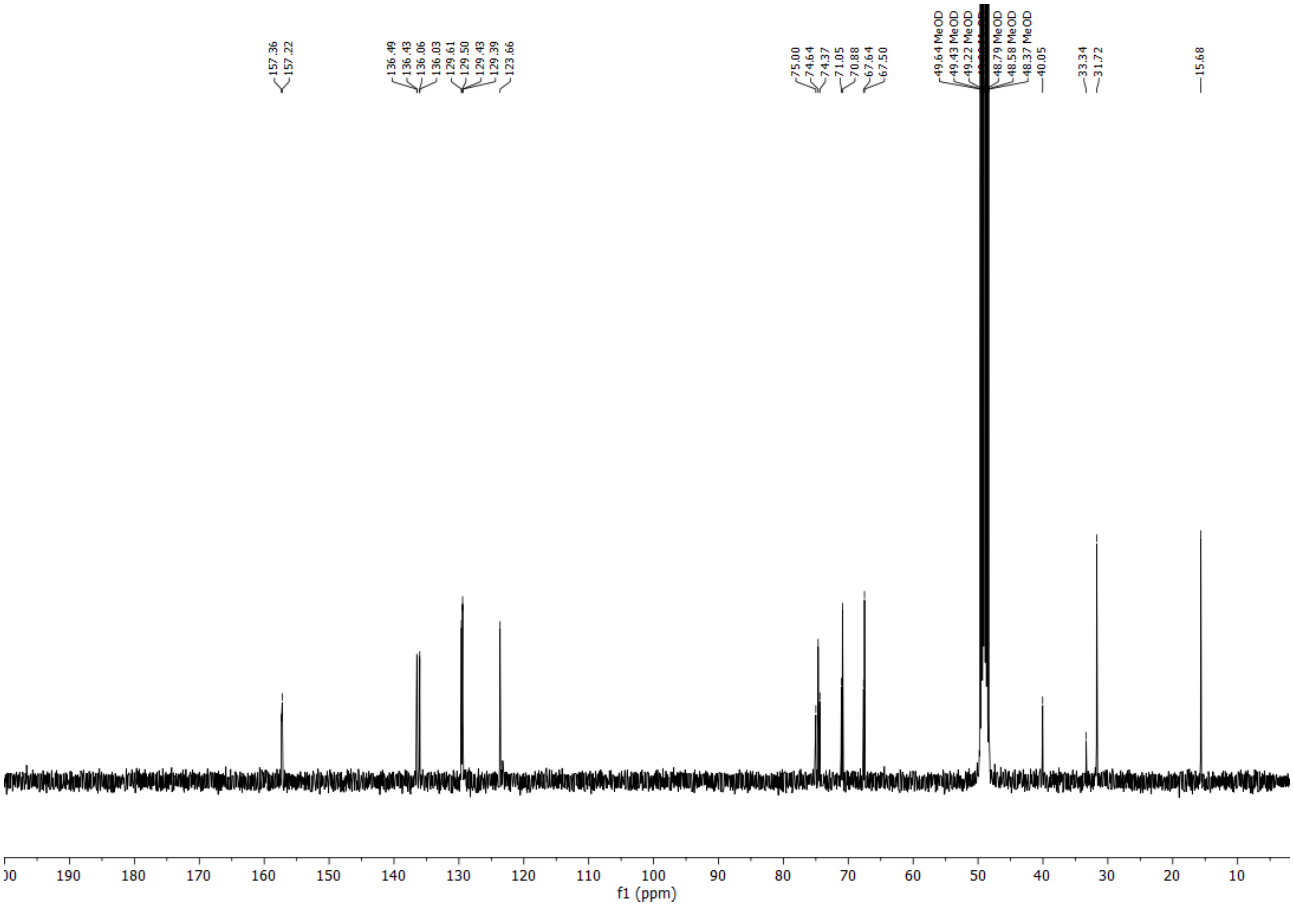


Figure S7. ^{13}C-NMR (100 MHz, CD_3OD, 298 K) spectrum of compound **2b**

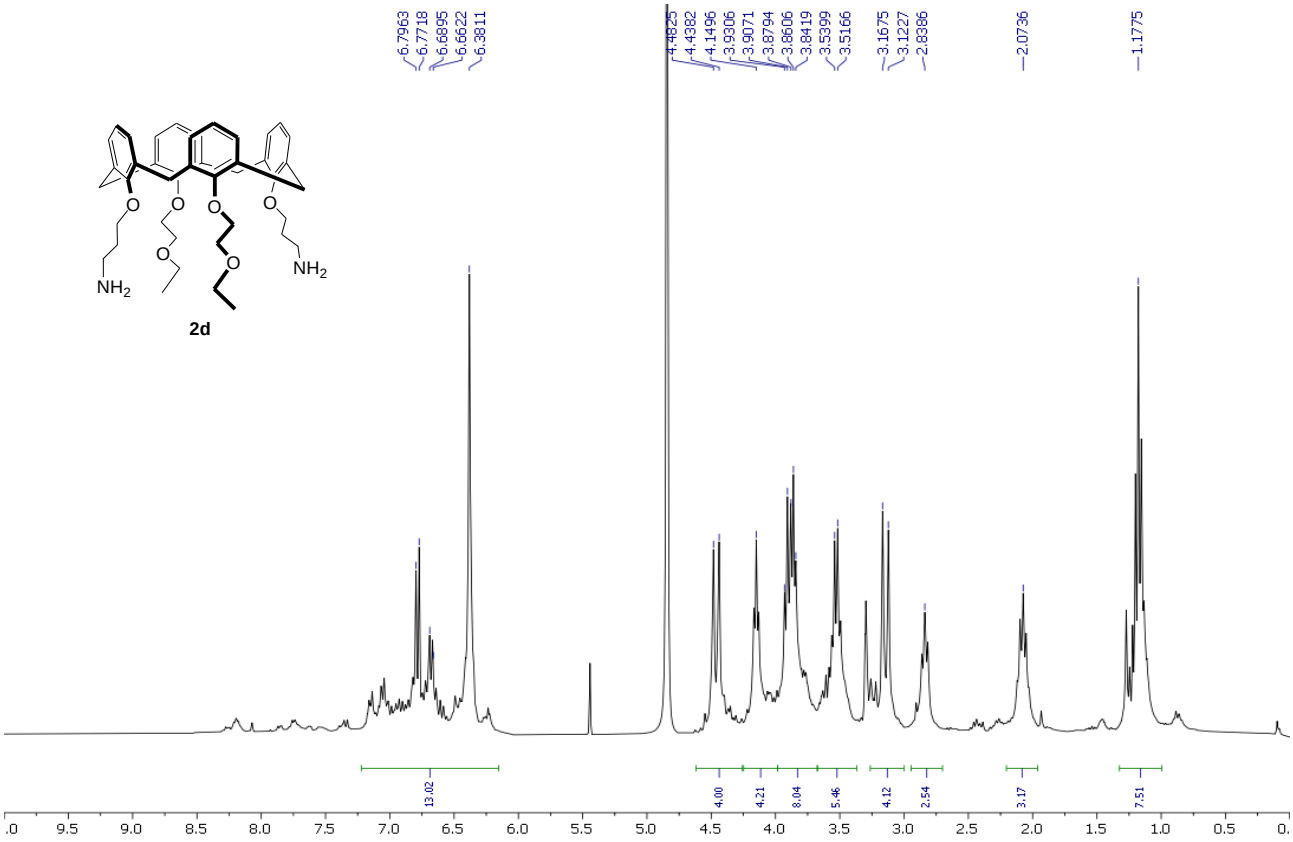


Figure S8. ^{1}H-NMR (300 MHz, CD_3OD, 298 K) spectrum of compound **2d**

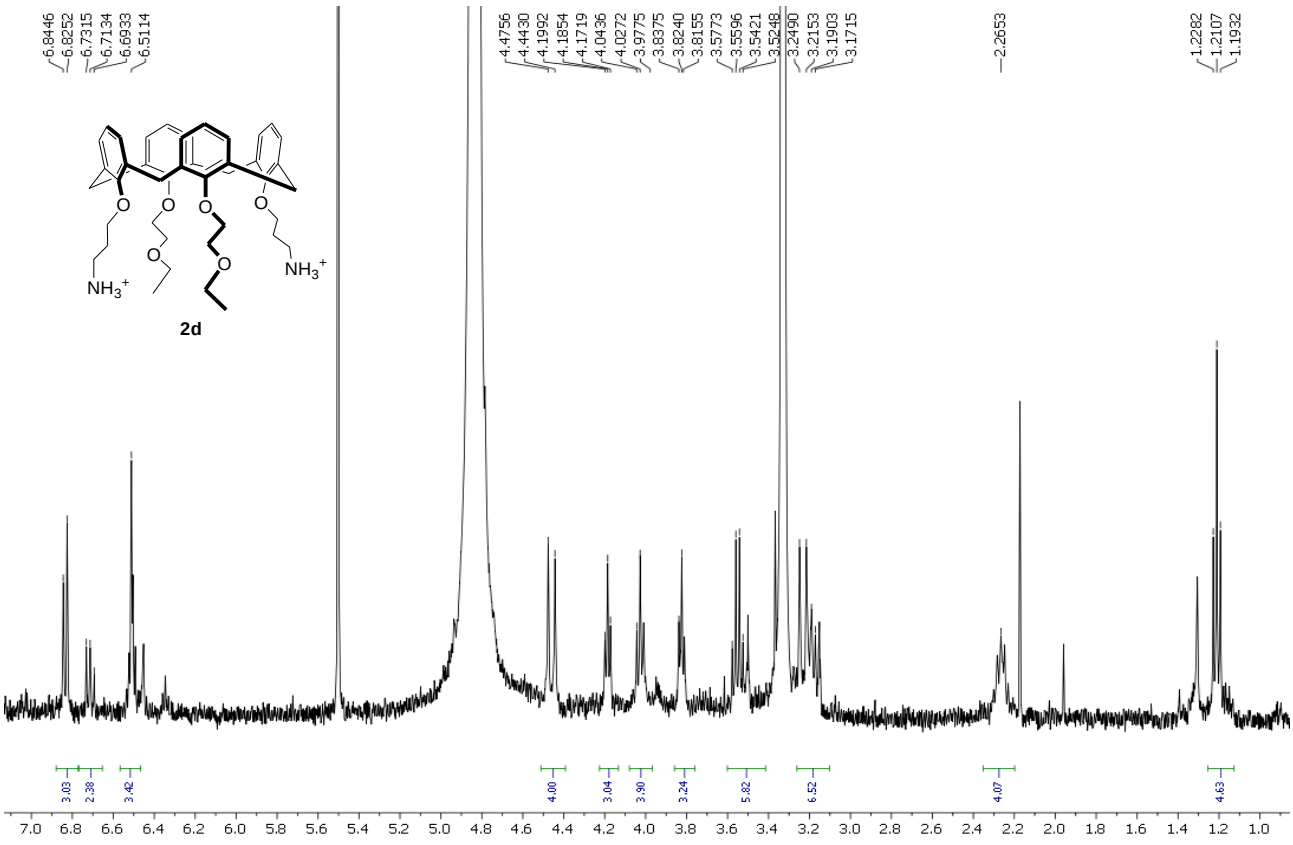


Figure S9. ^{1}H-NMR (300 MHz, CD_3OD, 298 K) spectrum of compound **2d**

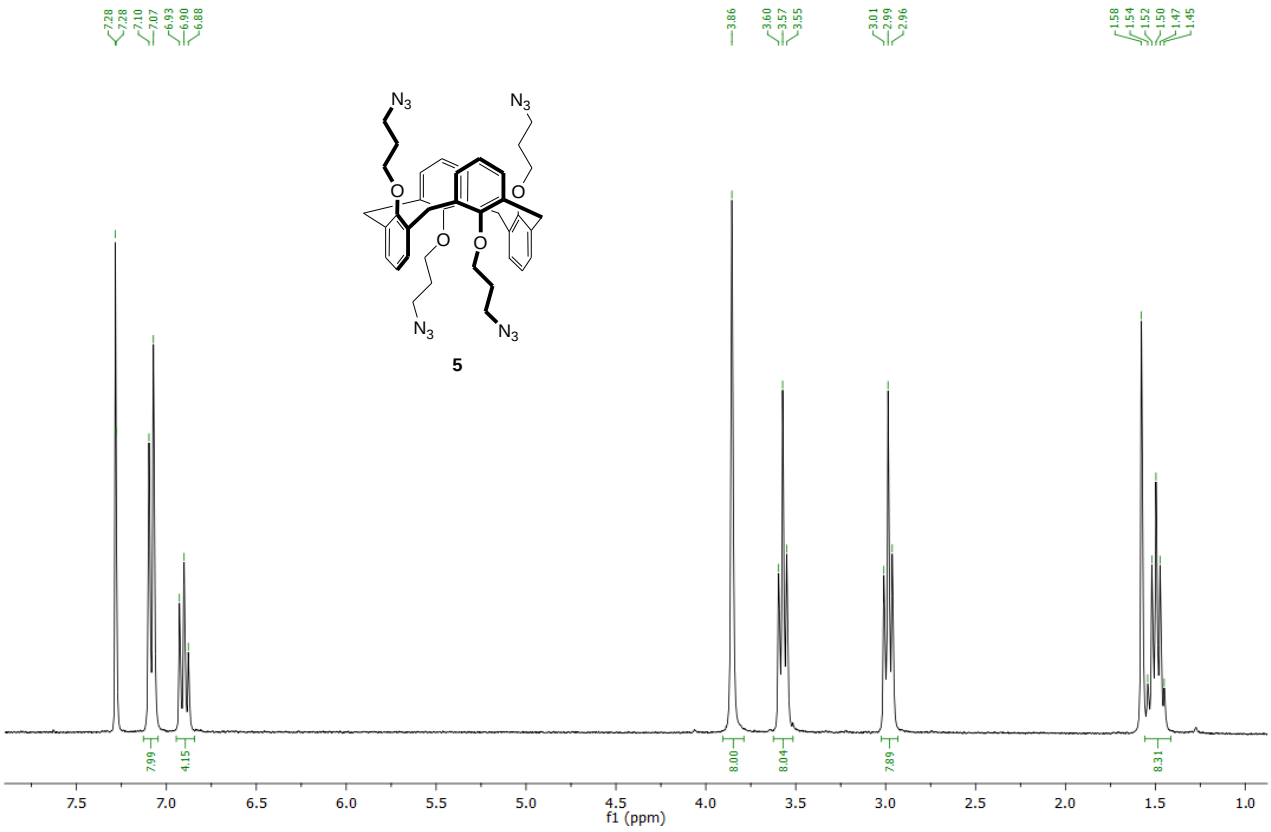


Figure S10. ^{1}H-NMR (300 MHz, $CDCl_3$, 298 K) spectrum of compound **5**

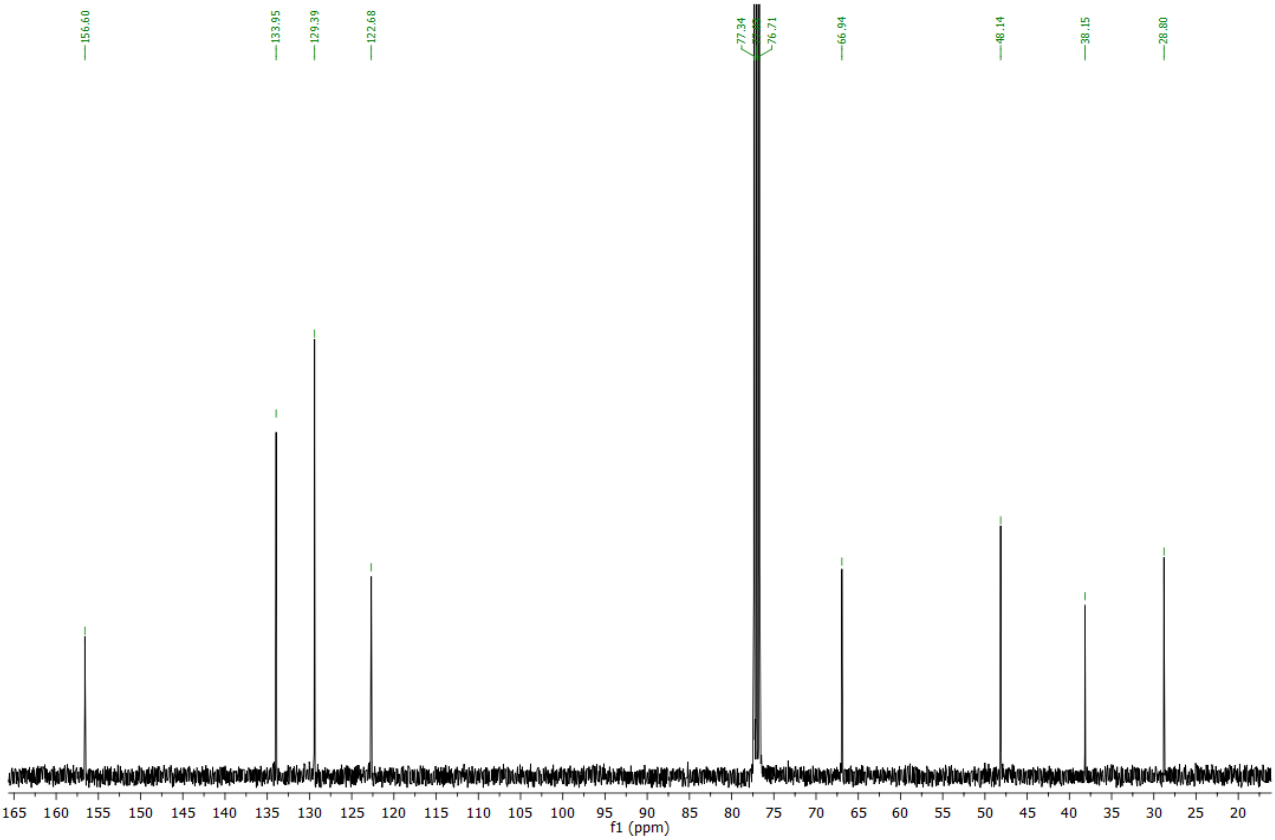


Figure S11. ^{13}C-NMR (100 MHz, $CDCl_3$, 298 K) spectrum of compound **5**

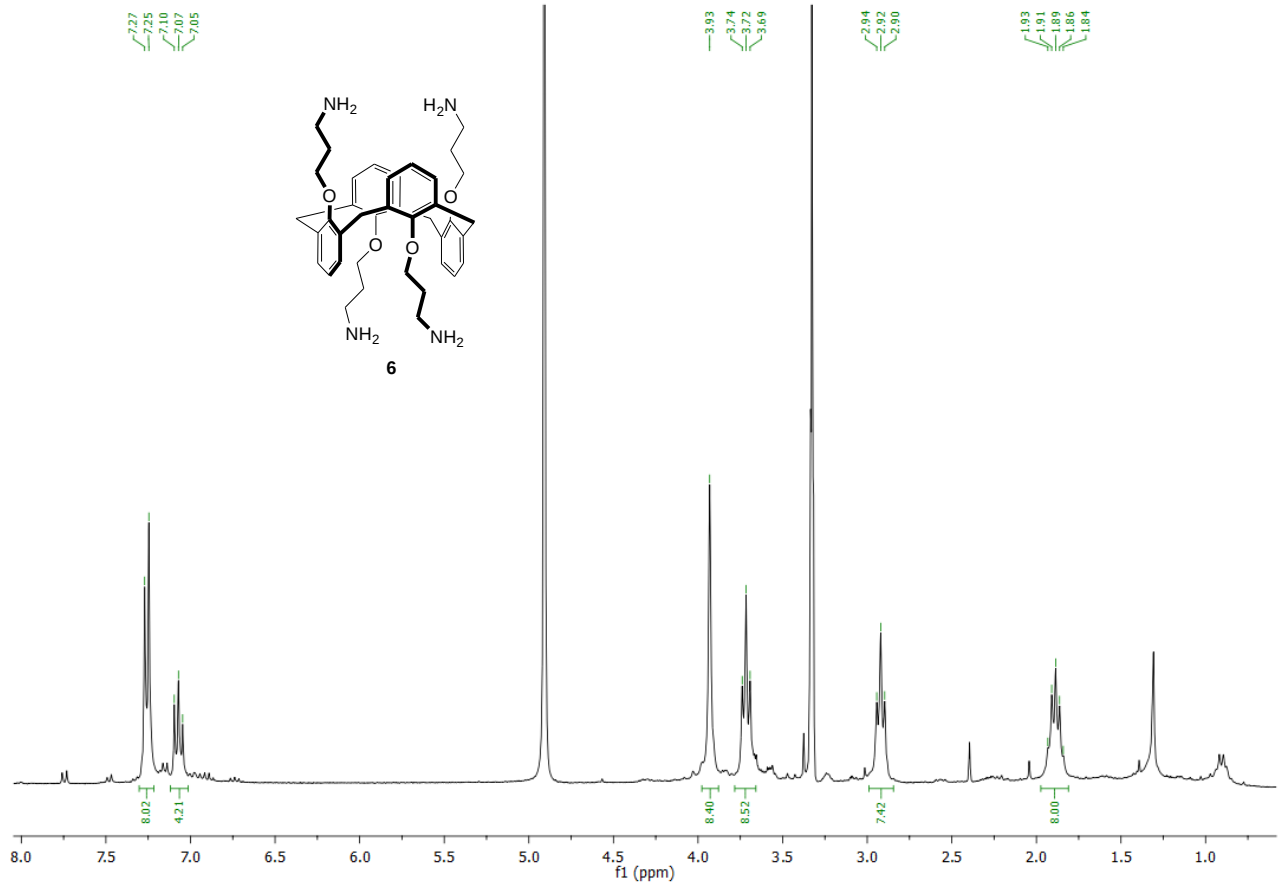


Figure S12. ^{1}H-NMR (300 MHz, $CDCl_3$, 298 K) spectrum of compound **6**

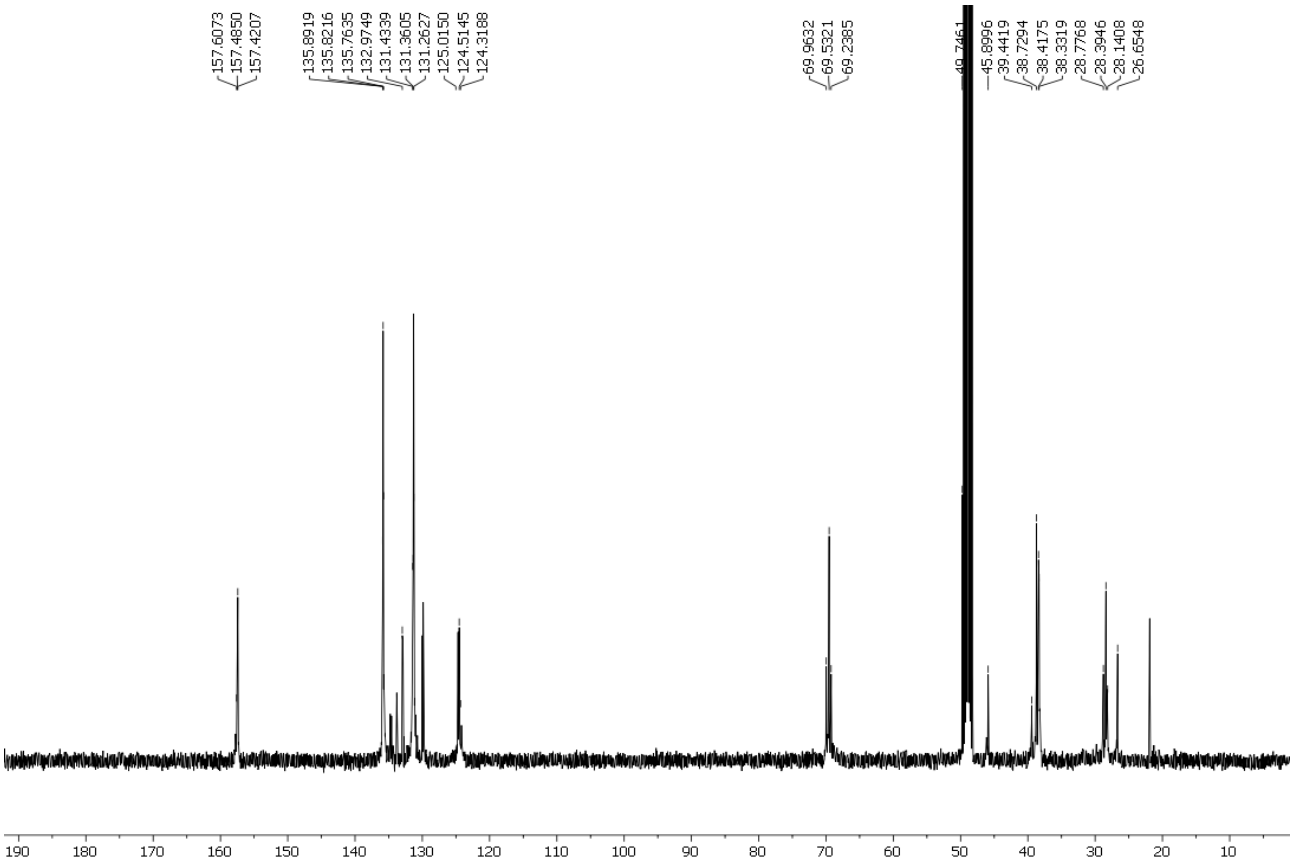


Figure S13. ^{13}C NMR (100 MHz, CD_3OD, 298 K) spectrum of compound **6**

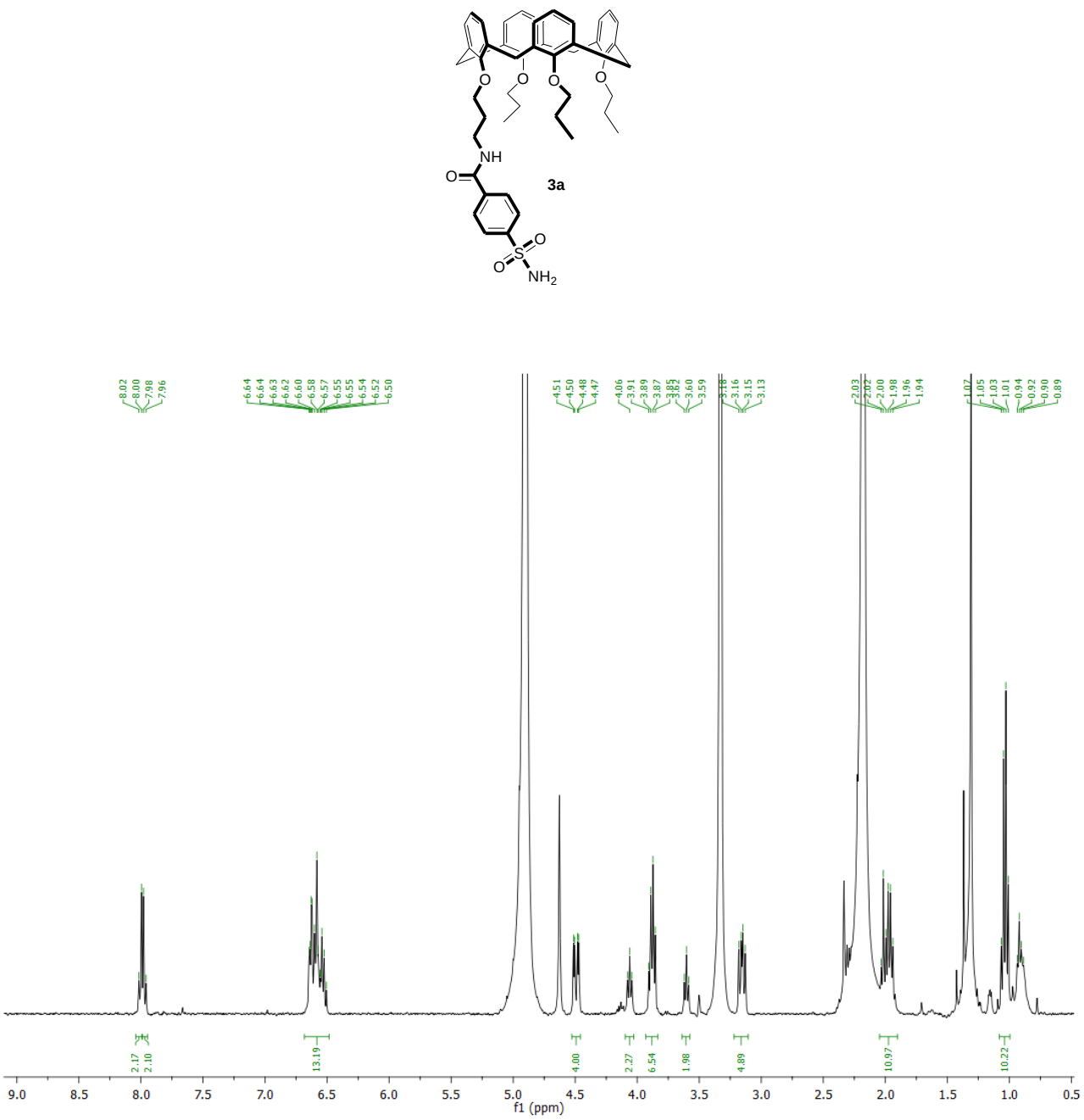


Figure S14. ^{1}H-NMR (400 MHz, CD_3OD, 298 K) spectrum of compound **3a**

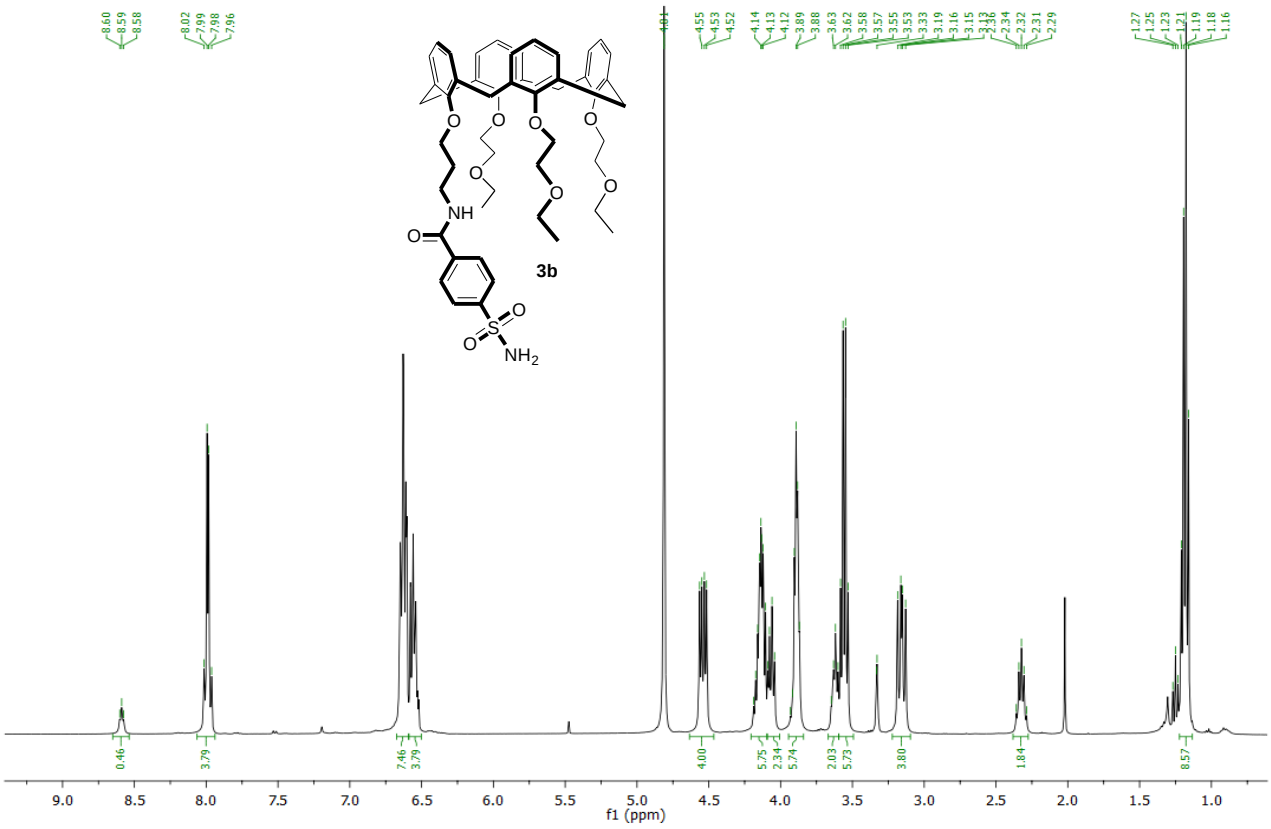


Figure S15. ^{1}H-NMR (400 MHz, CD_3OD, 298 K) spectrum of compound **3b**

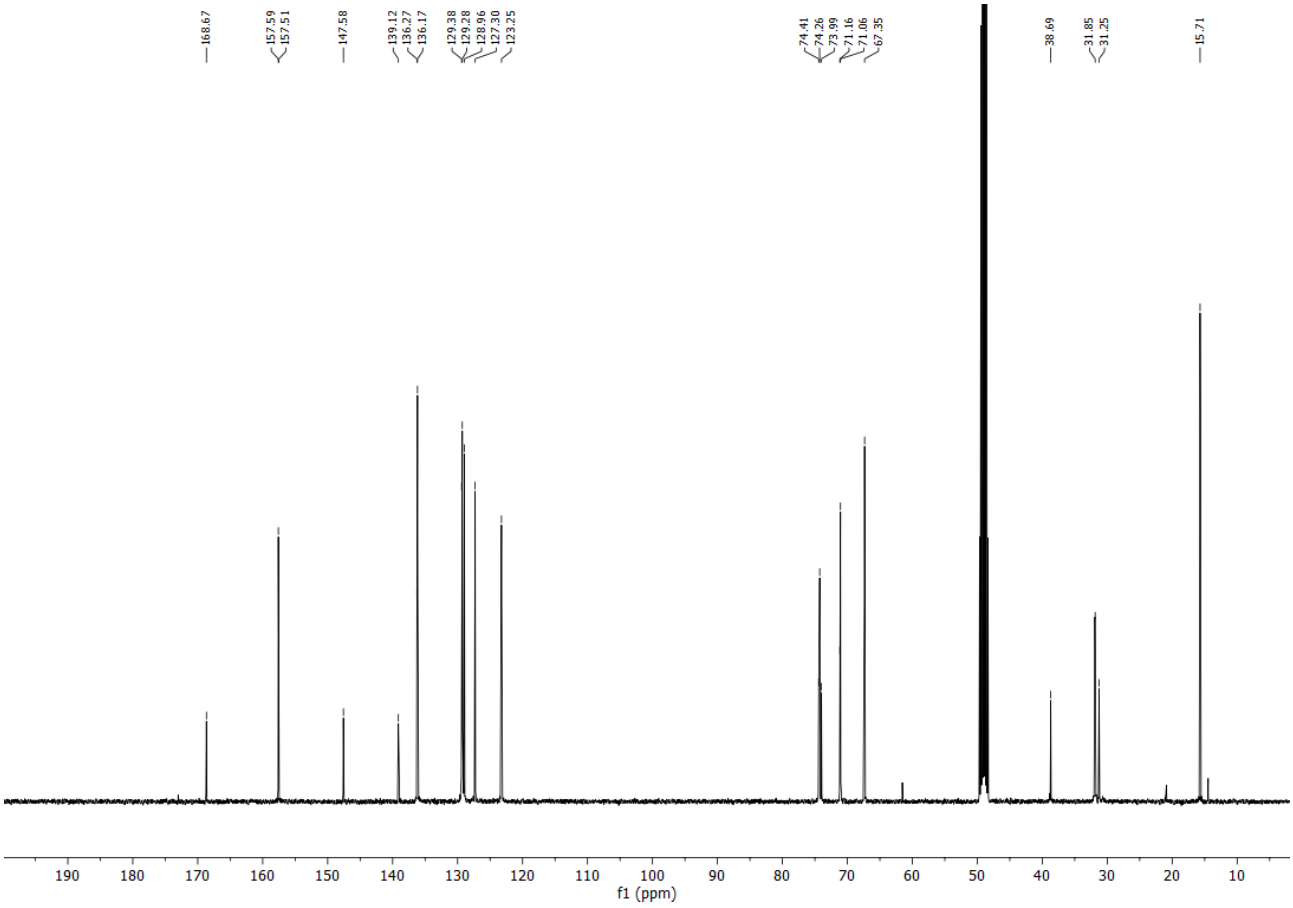


Figure S16. ^{13}C-NMR (100 MHz, CD_3OD, 298 K) spectrum of compound **3b**

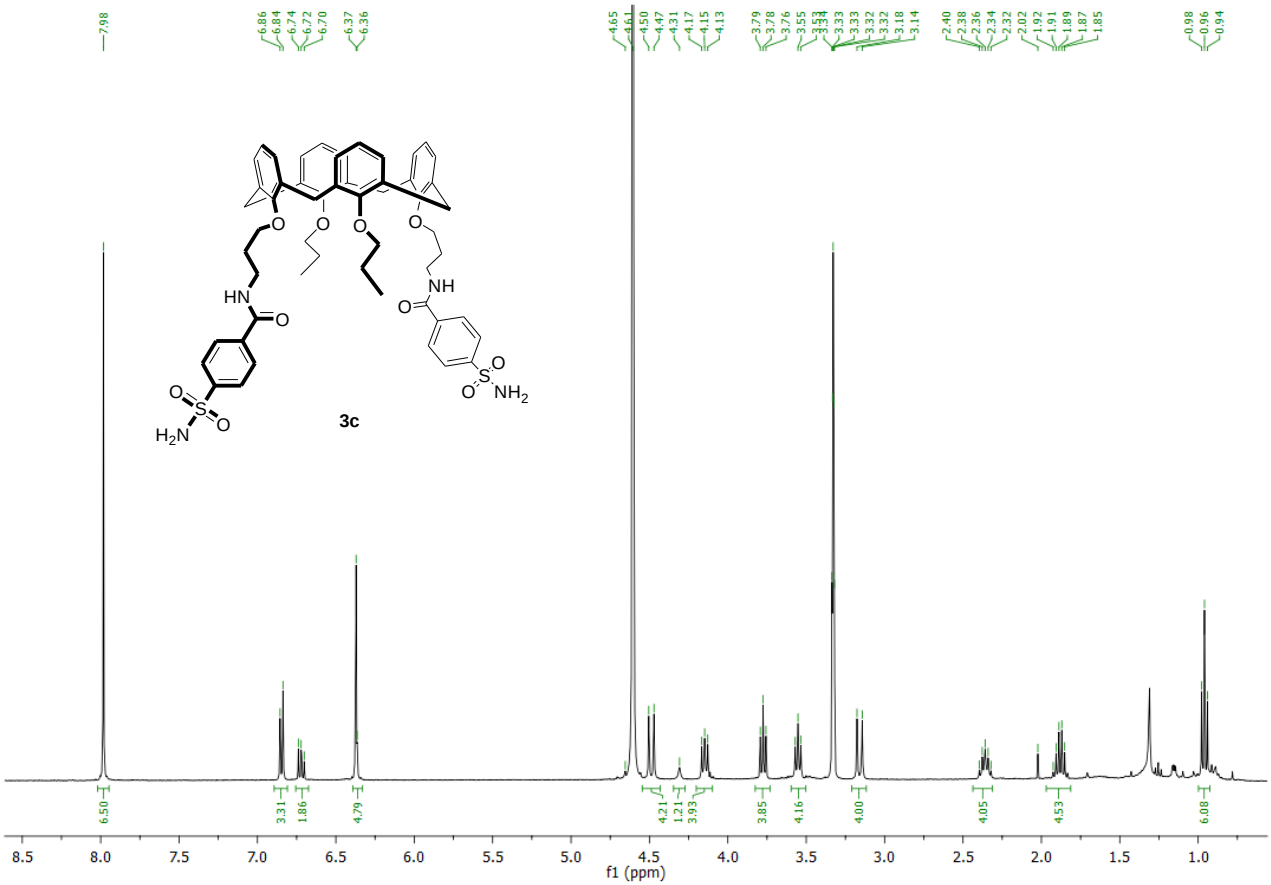


Figure S17. ^{1}H NMR (400 MHz, CD_3OD, 298 K) spectrum of compound **3c**

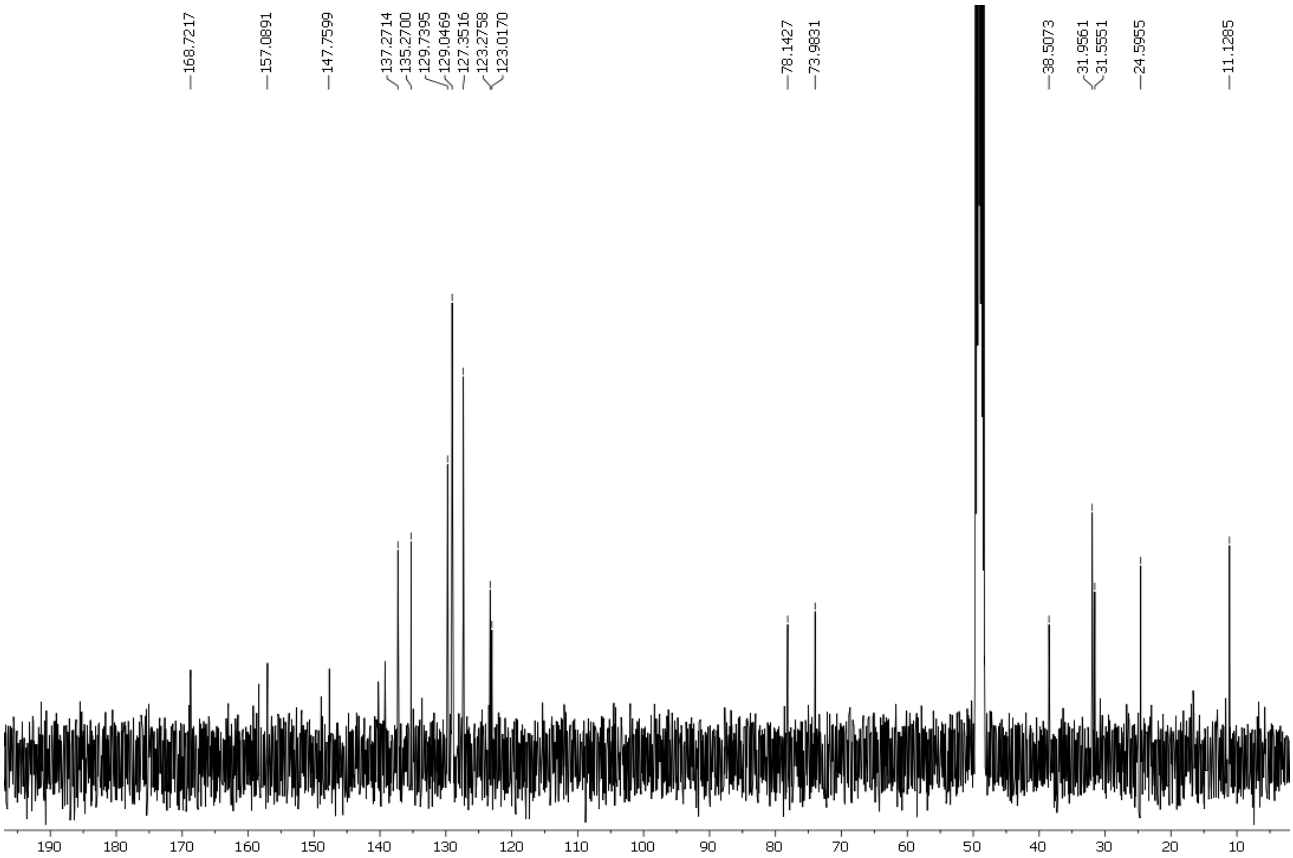


Figure S18. ^{13}C NMR (100 MHz, CD_3OD, 298 K) spectrum of compound **3c**

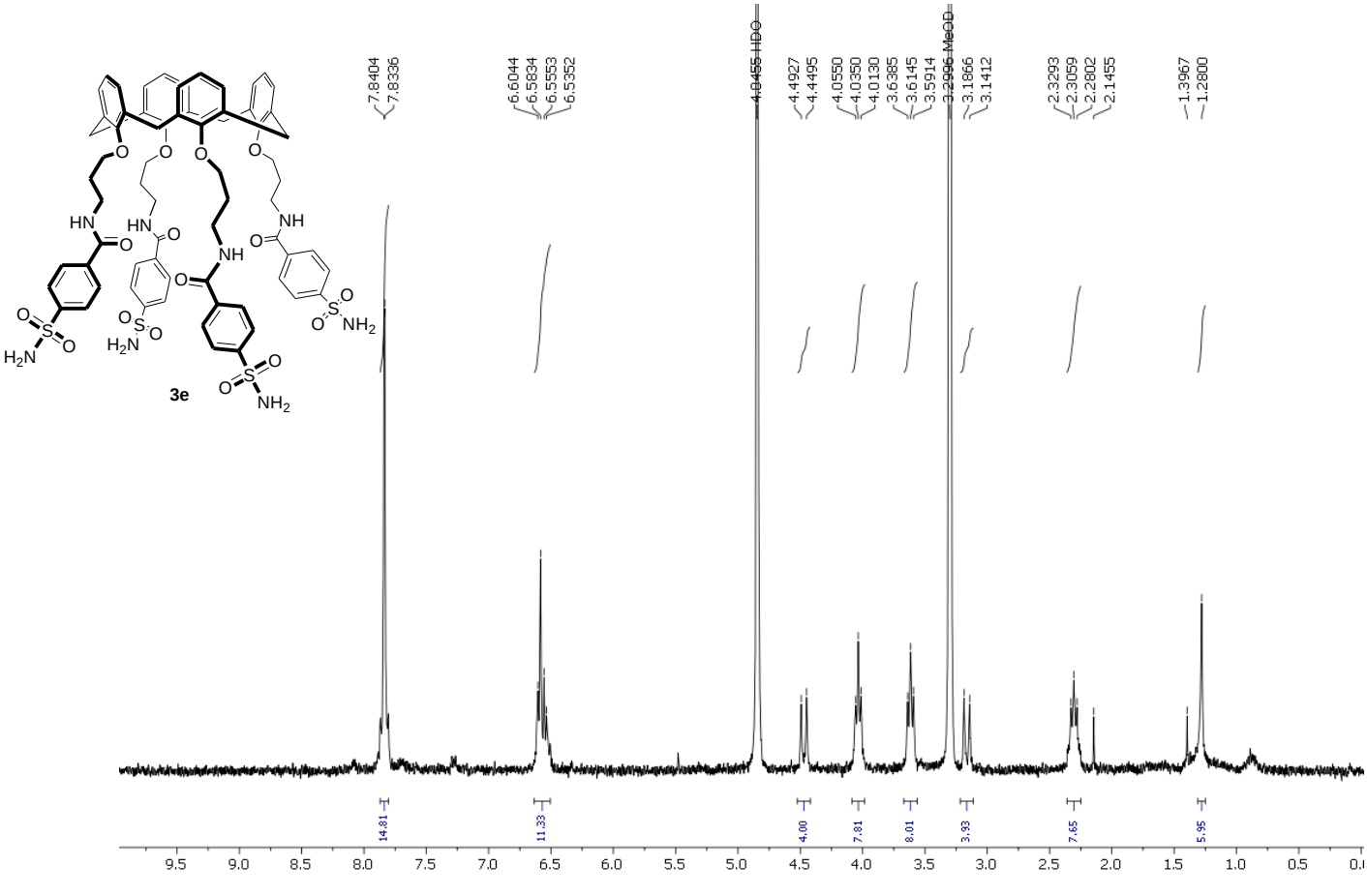


Figure S19. ^{1}H-NMR (300 MHz, CD_3OD, 298 K) spectrum of compound **3e**

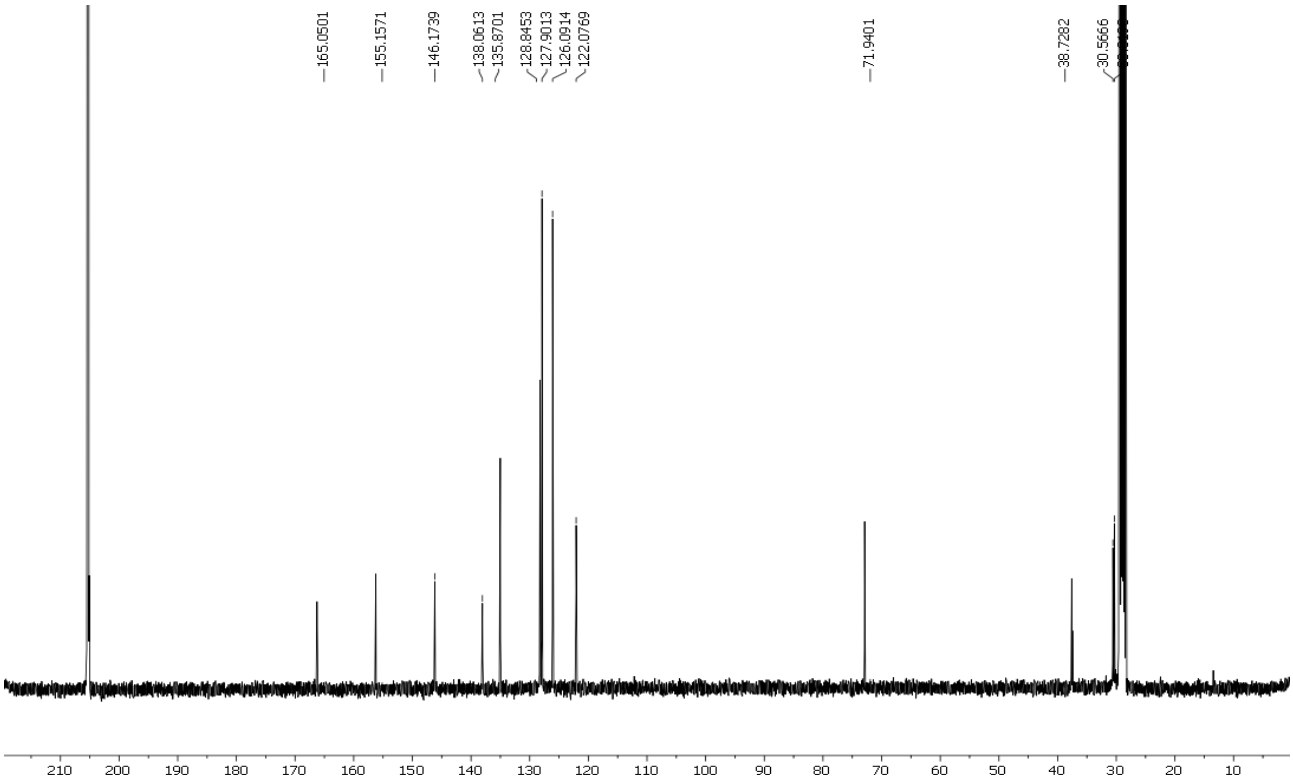


Figure S20. ^{13}C NMR (100 MHz, acetone-d6, 298 K) spectrum of compound **3e**

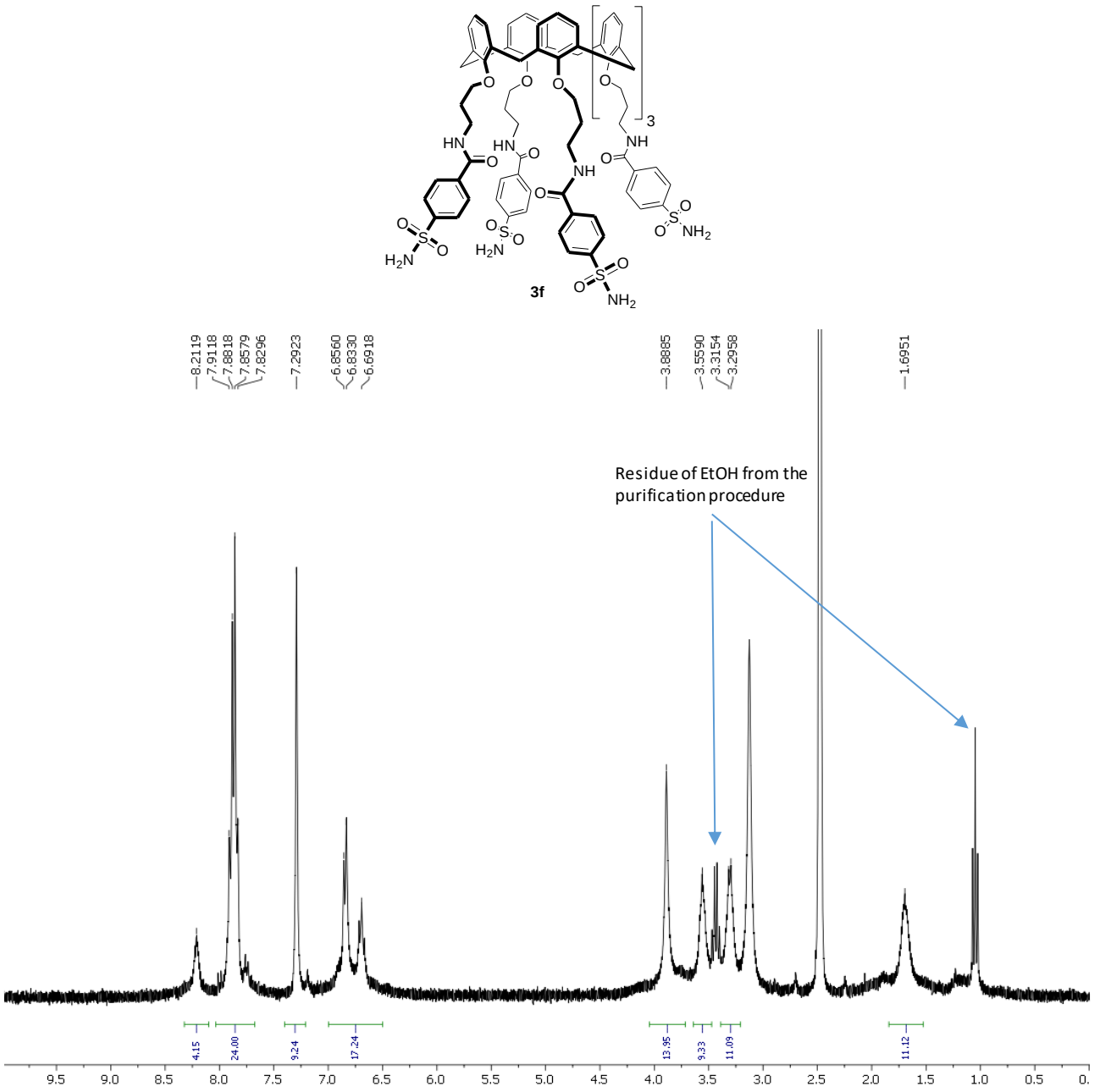


Figure S21. ^{1}H NMR (300 MHz, DMSO-d6, 333 K) spectrum of compound **3f**

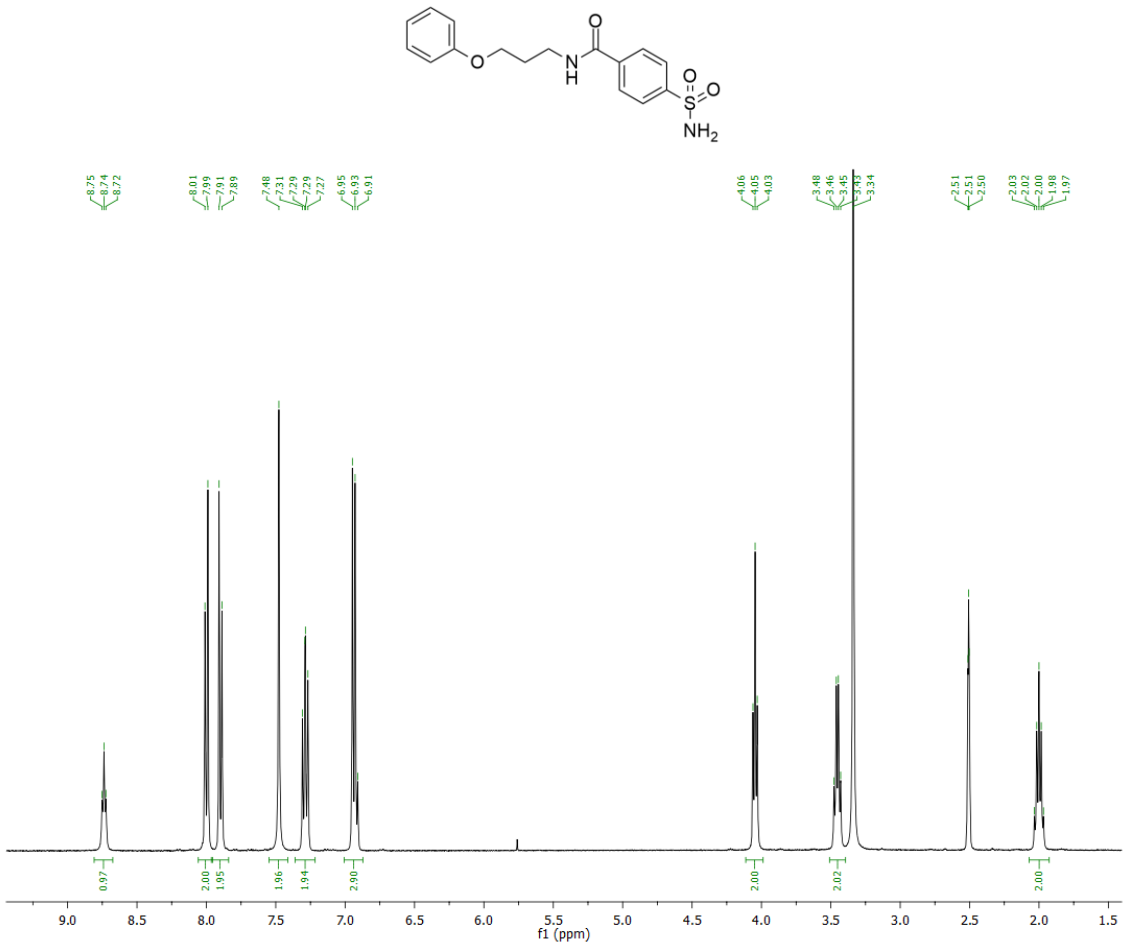


Figure S22. ^{1}H-NMR (400 MHz, DMSO-d_6, 298 K) spectrum of compound **10**

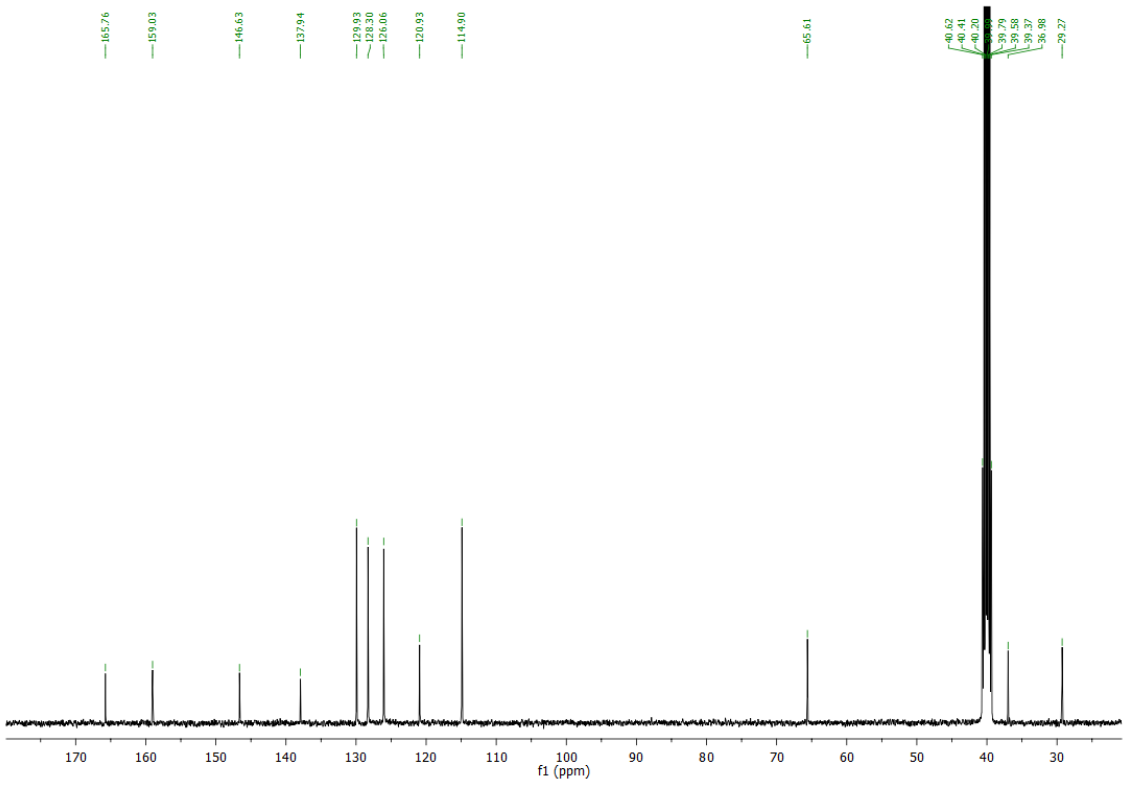


Figure S23. ^{13}C-NMR (100 MHz, DMSO-d_6, 298 K) spectrum of compound **10**

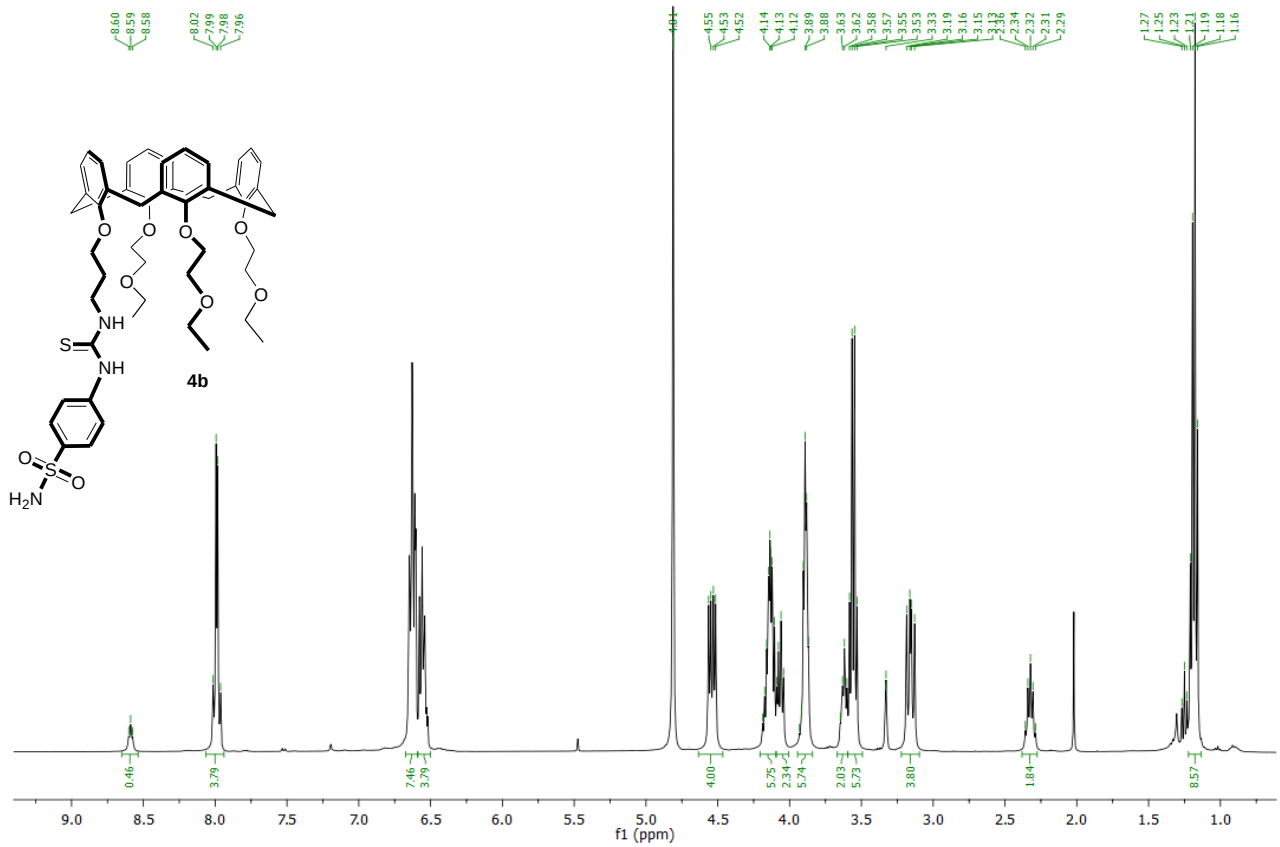


Figure S24. ^{1}H-NMR (400 MHz, CD_3OD, 298 K) spectrum of compound **4b**

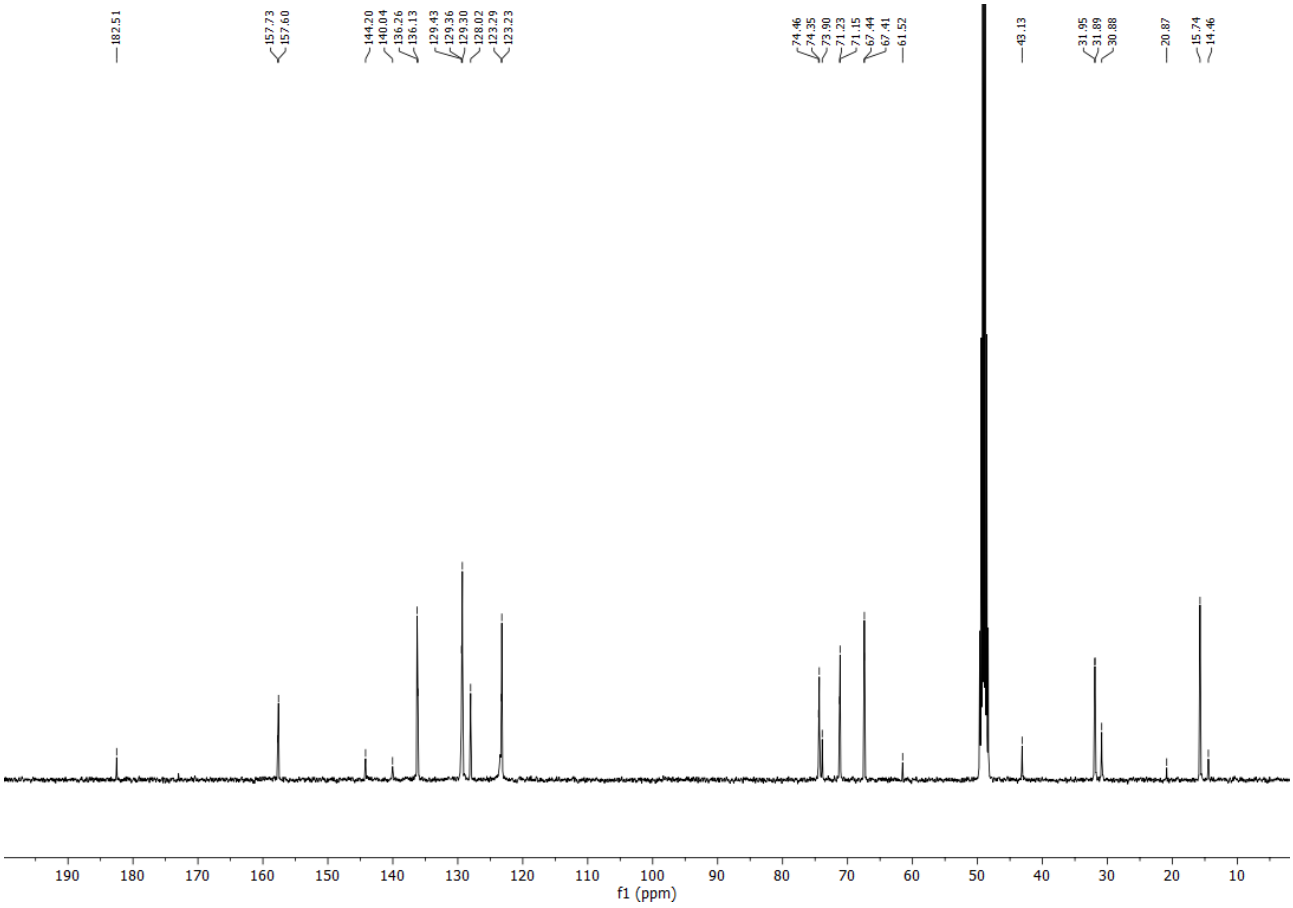


Figure S25. ^{13}C-NMR (100 MHz, CD_3OD, 298 K) spectrum of compound **4b**

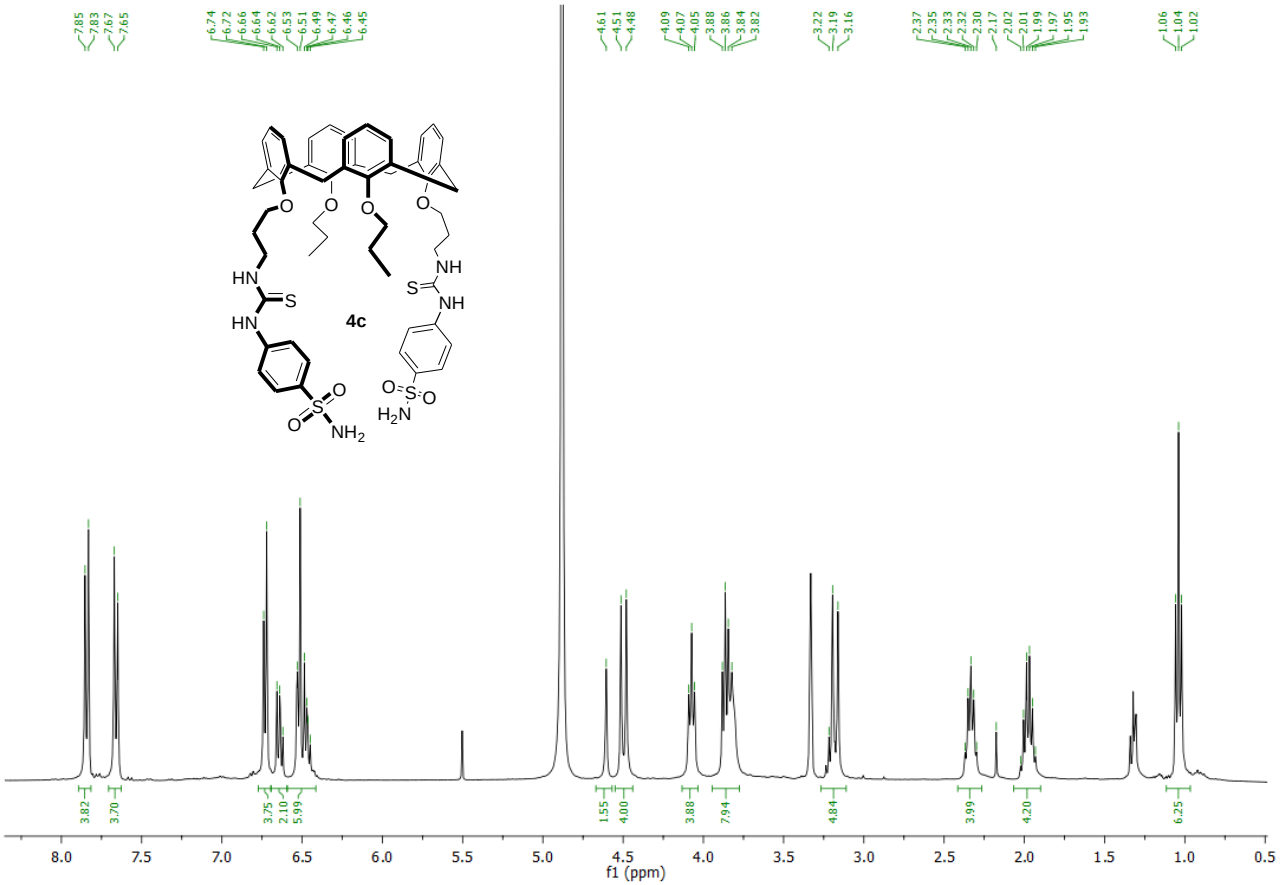


Figure S26. ^{1}H-NMR (400 MHz, CD_3OD, 298 K) spectrum of compound **4c**

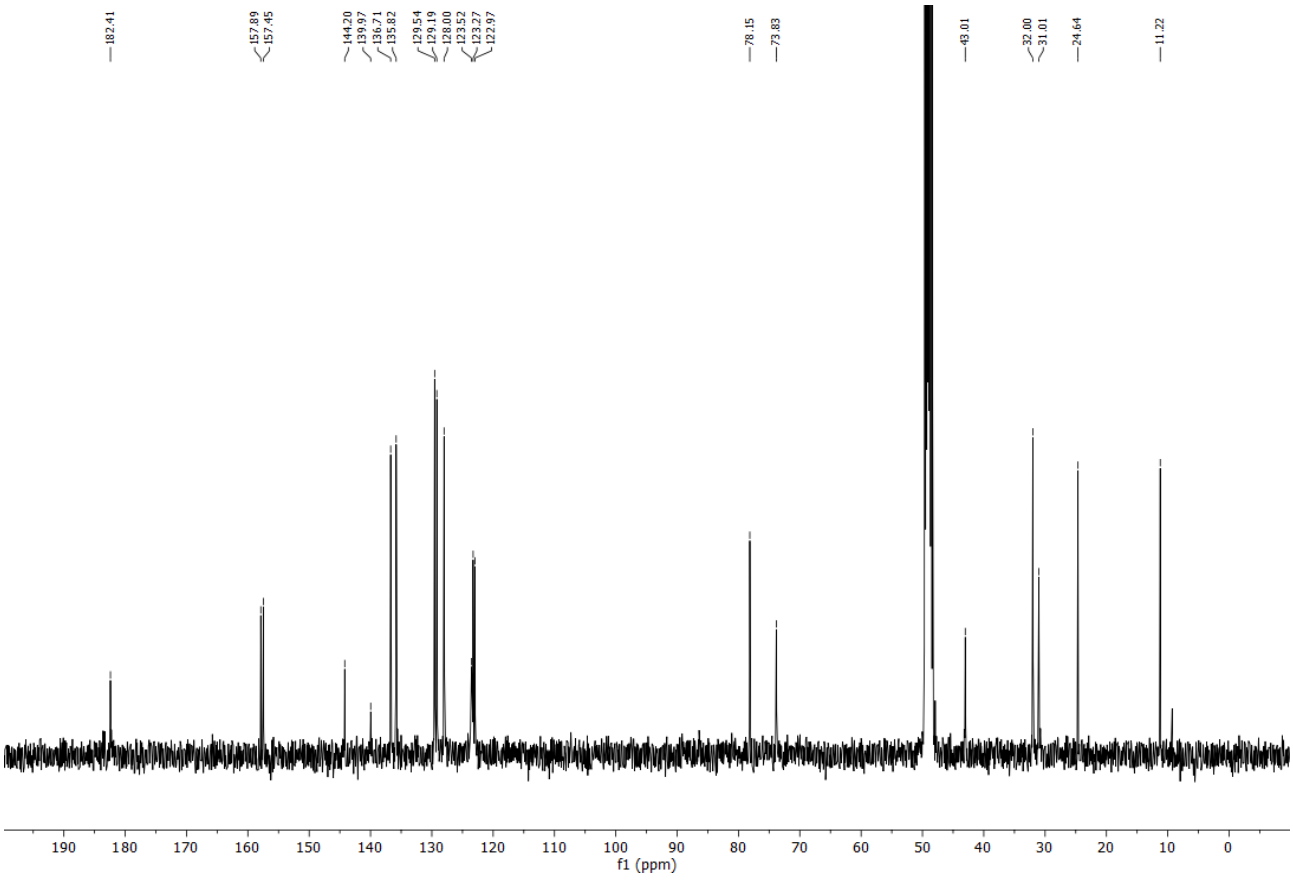


Figure S27. ^{13}C-NMR (100 MHz, CD_3OD, 298 K) spectrum of compound **4c**

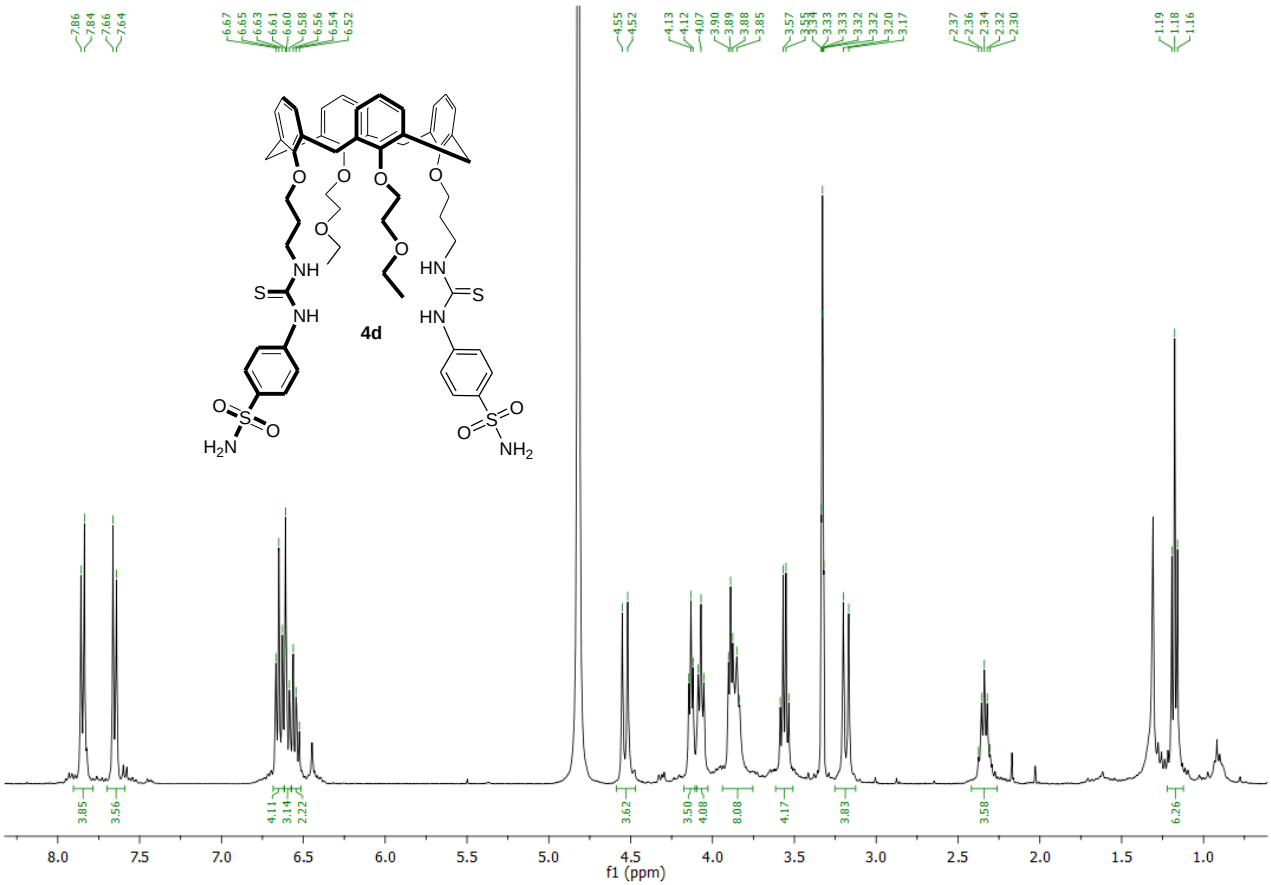


Figure S28. ^{1}H-NMR (400 MHz, CD_3OD, 298 K) spectrum of compound **4d**

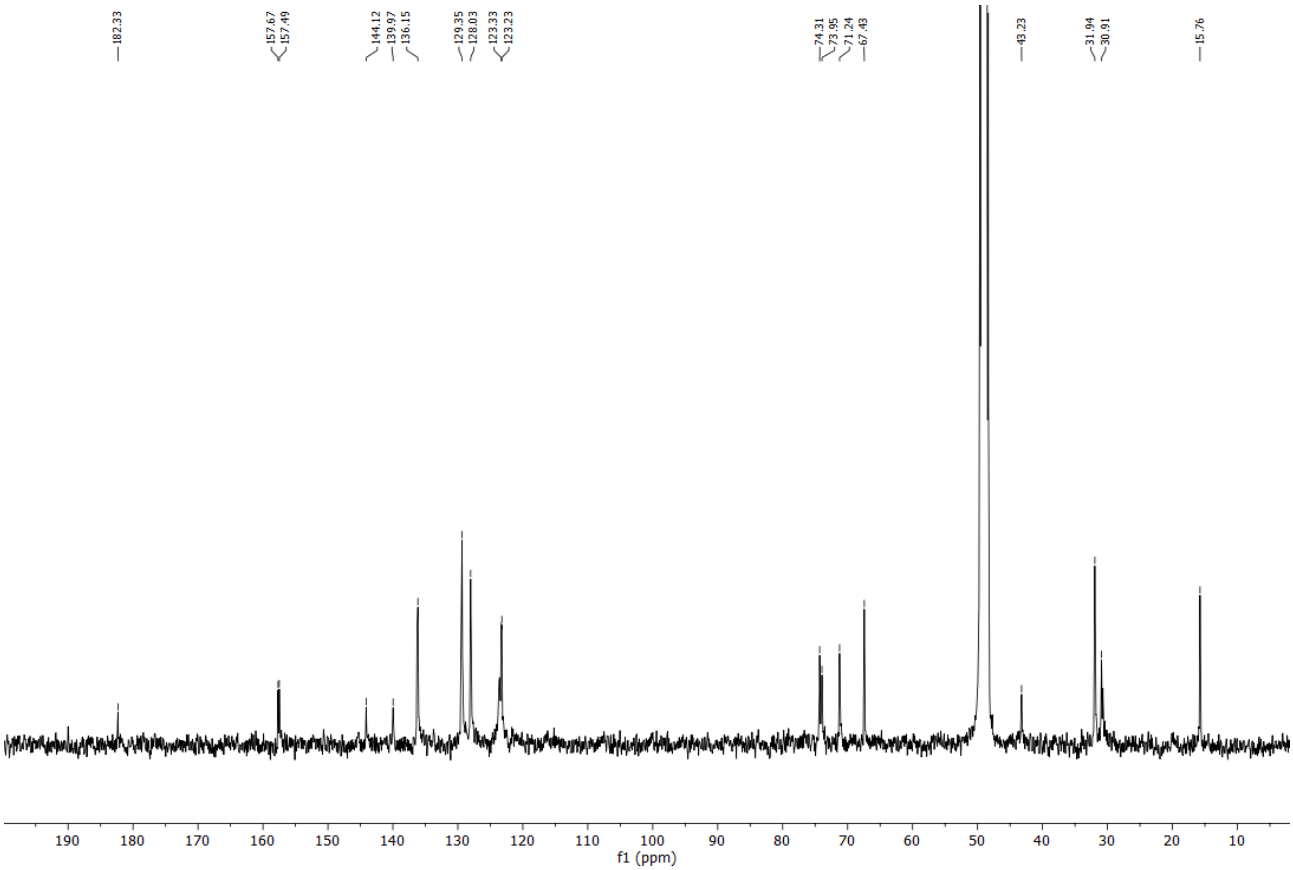
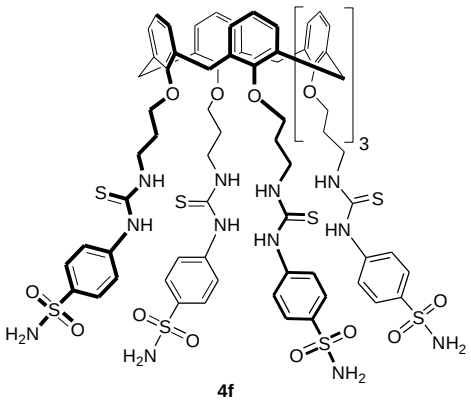


Figure S29. ^{13}C-NMR (100 MHz, CD_3OD, 298 K) spectrum of compound **4d**



4f

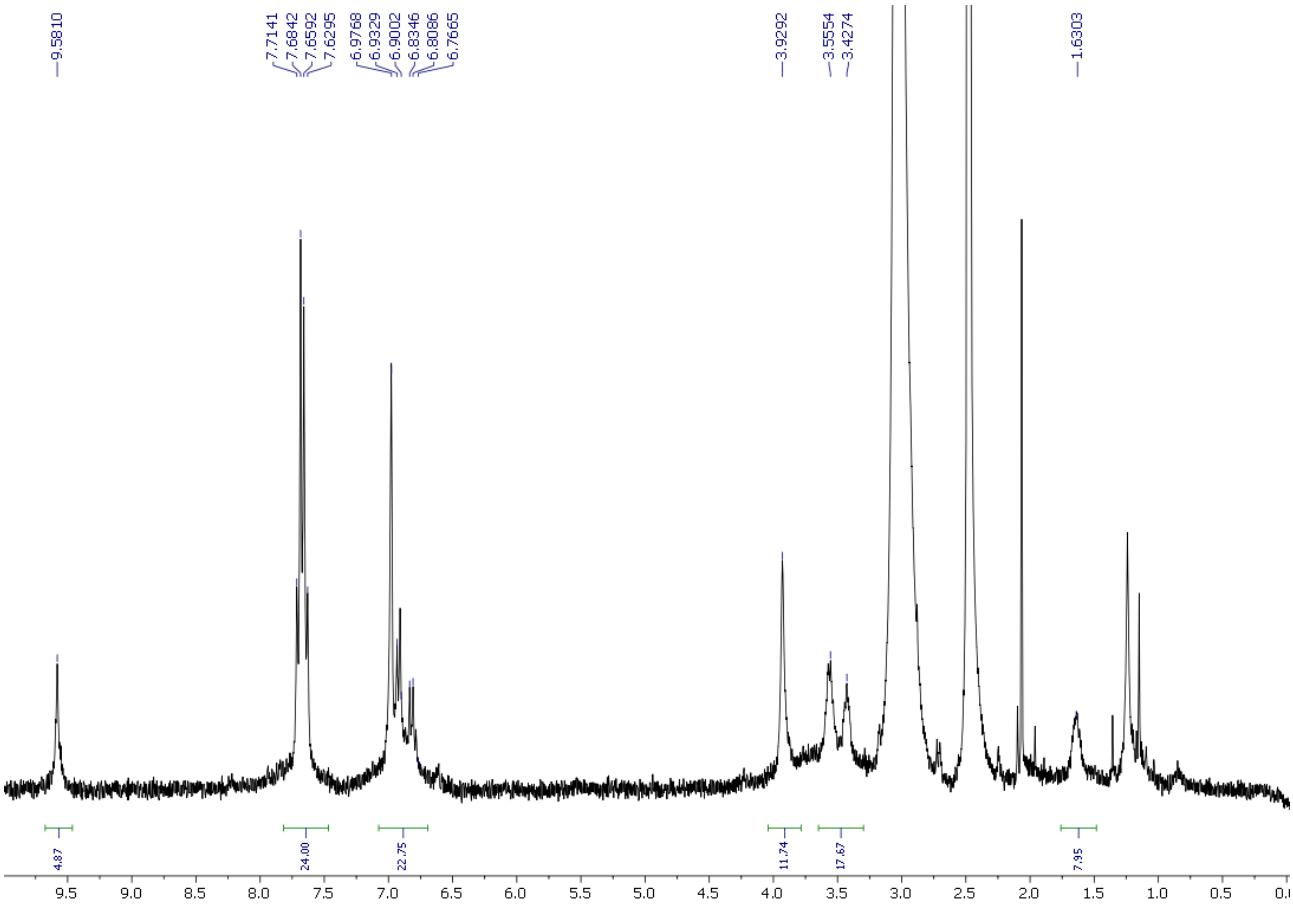


Figure S30. ^{1}H NMR (300 MHz, acetone-d6, 353 K) spectrum of compound **4f**

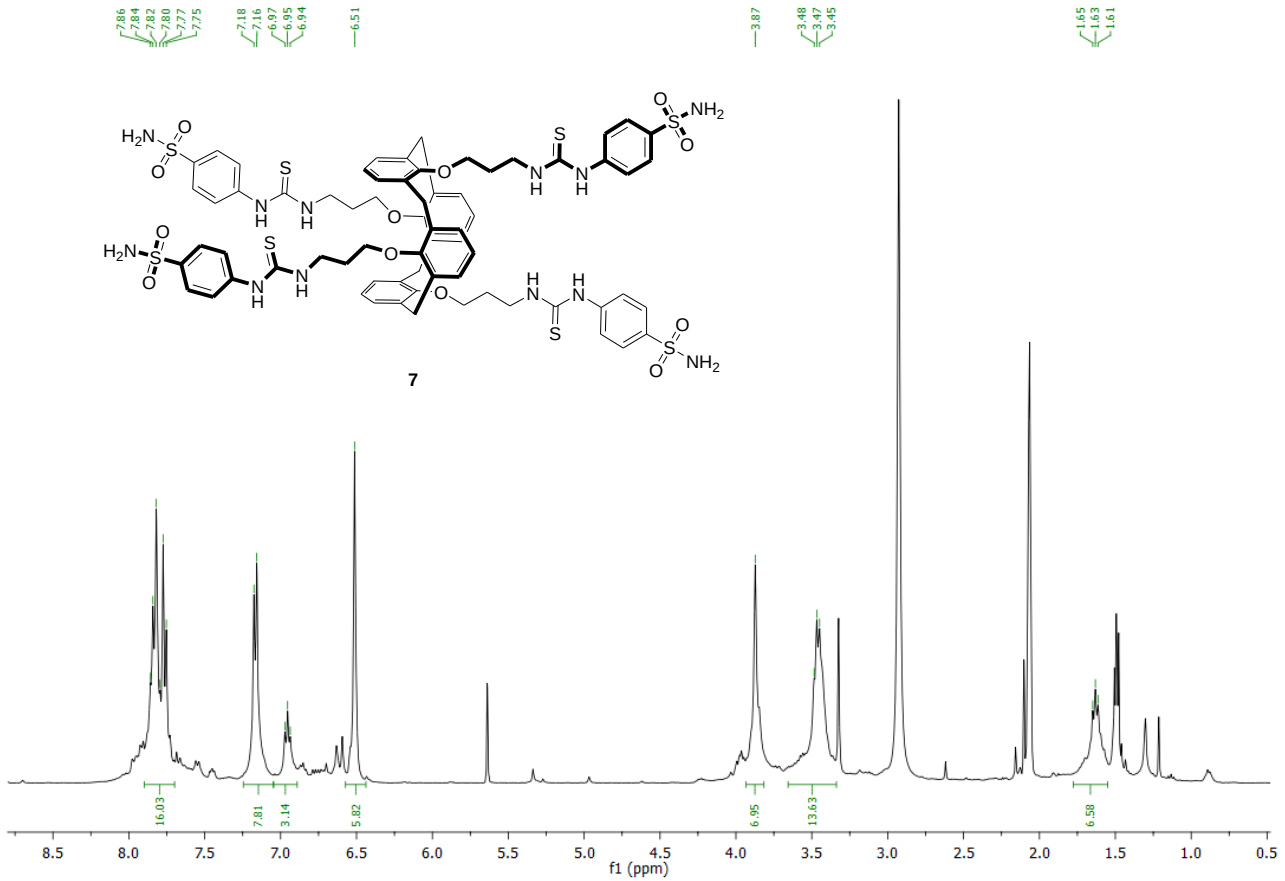


Figure S31. ^{1}H-NMR (400 MHz, acetone-d6, 298 K) spectrum of compound **7**

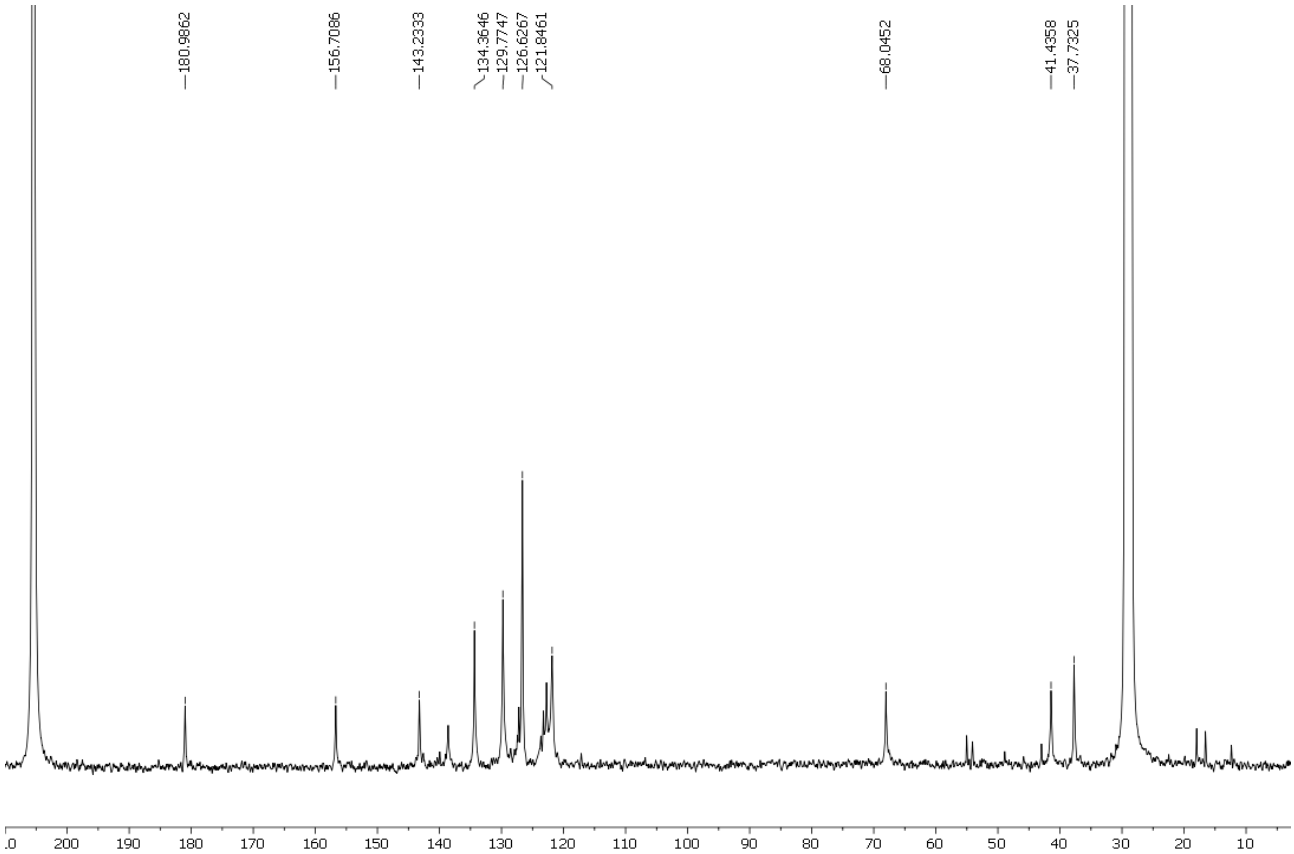


Figure S32. ^{13}C NMR (100 MHz, acetone-d6, 298 K) spectrum of compound **7**

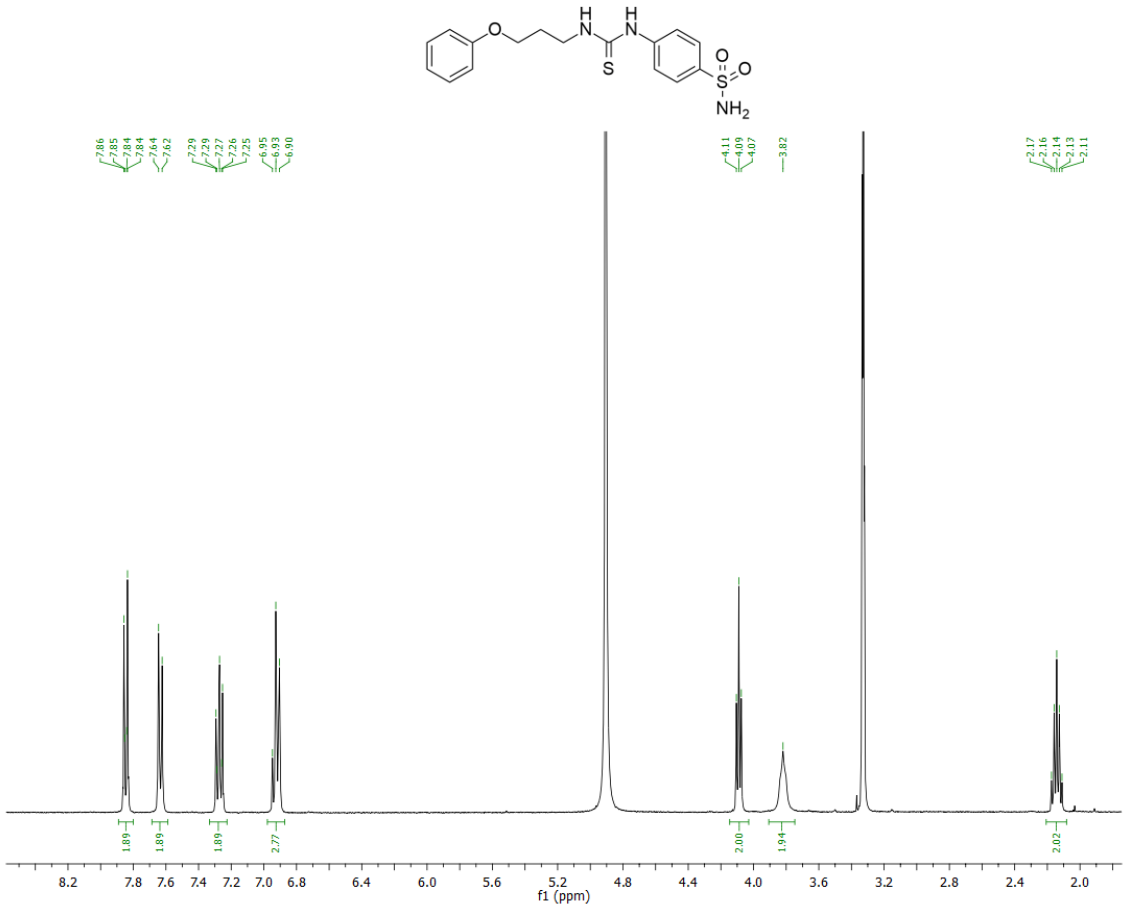


Figure S33. ^{1}H-NMR (400 MHz, CD_3OD, 298 K) spectrum of compound **9**

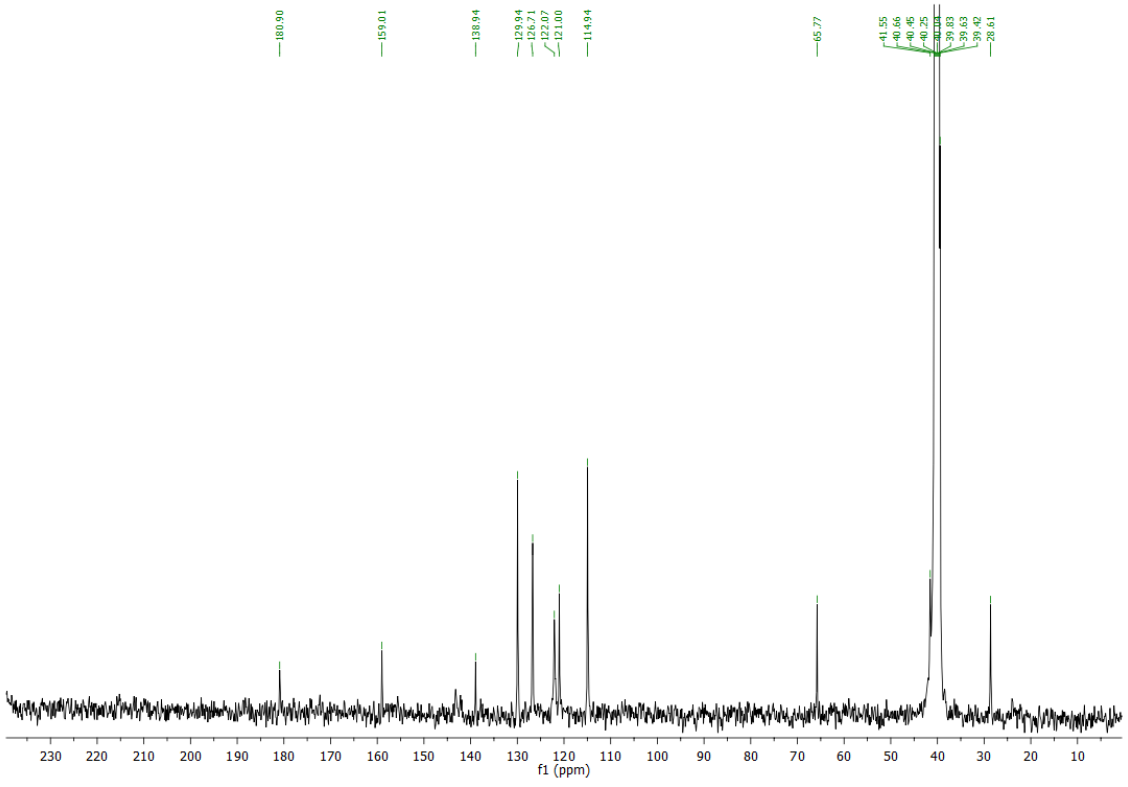


Figure S34. ^{13}C-NMR (100 MHz, DMSO-d_6, 298 K) spectrum of compound **9**

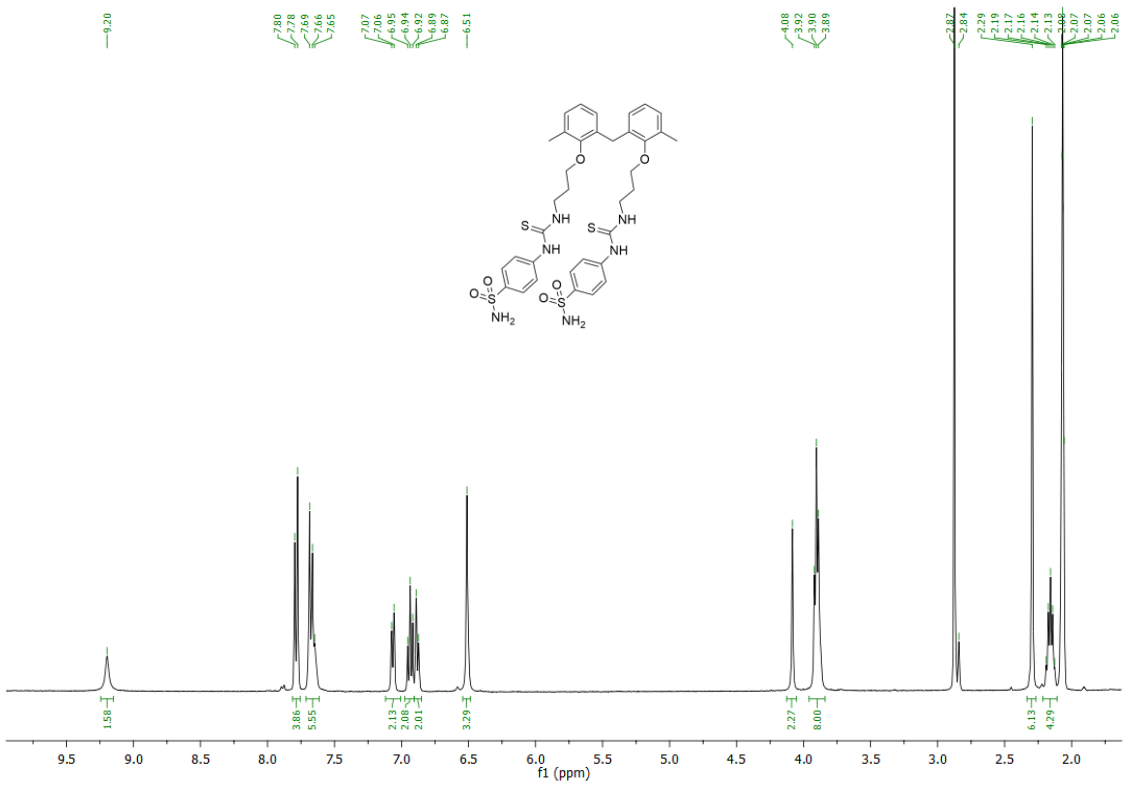


Figure S35. ^{1}H-NMR (400 MHz, acetone-d_6, 298 K) spectrum of compound **11**

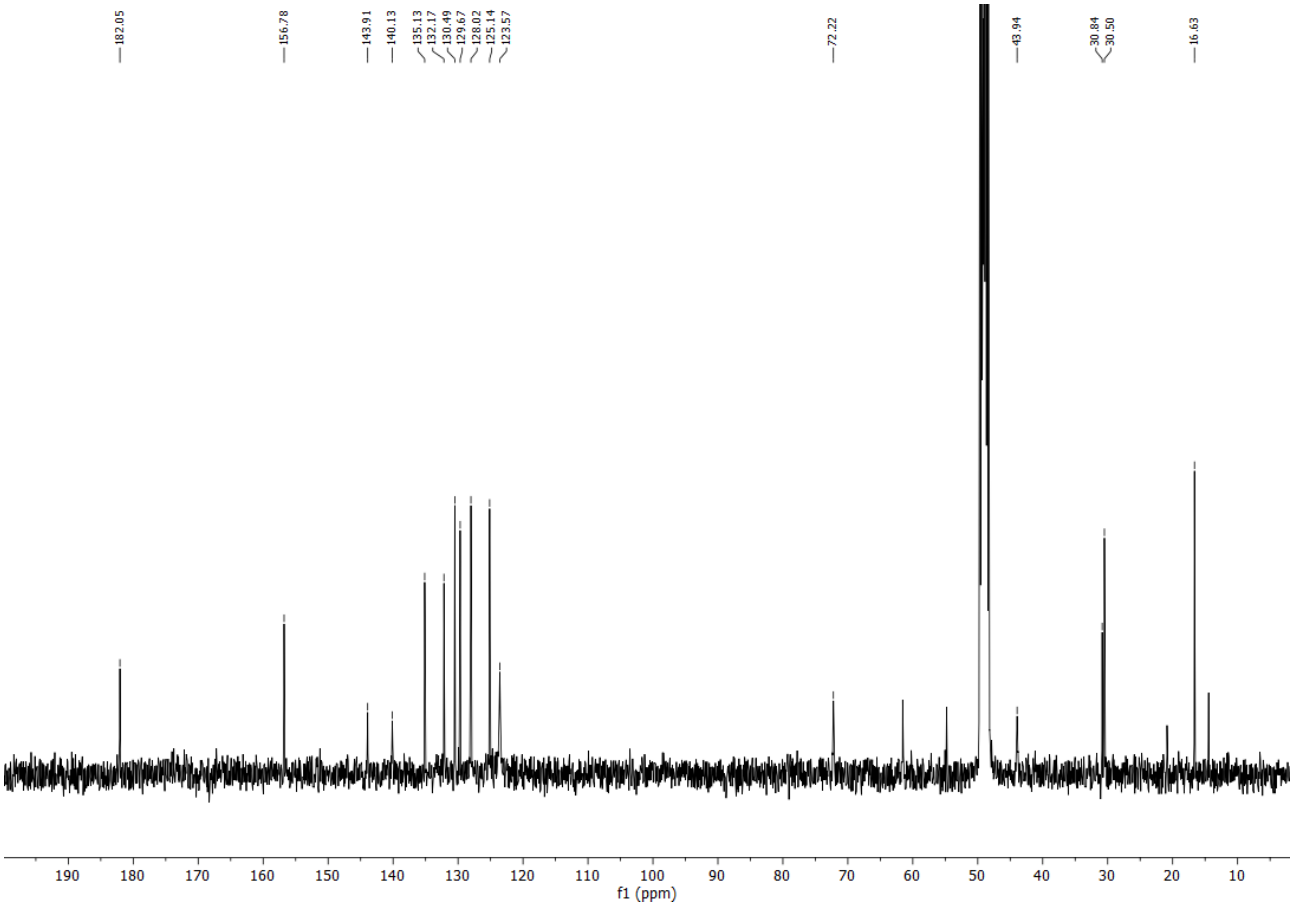


Figure S36. ^{13}C-NMR (100 MHz, CD_3OD, 298 K) spectrum of compound **11**

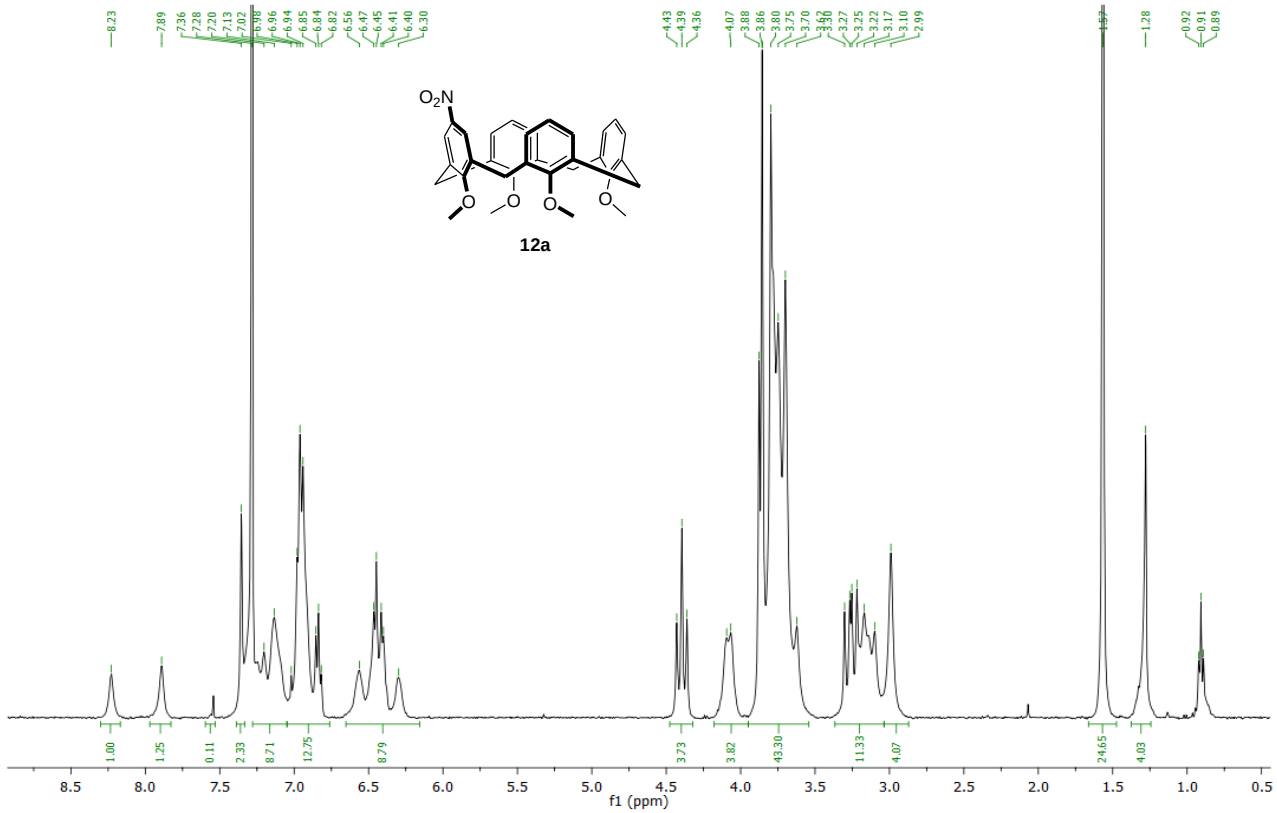


Figure S37. ^{1}H-NMR (400 MHz, $CDCl_3$, 298 K) spectrum of compound **12a**

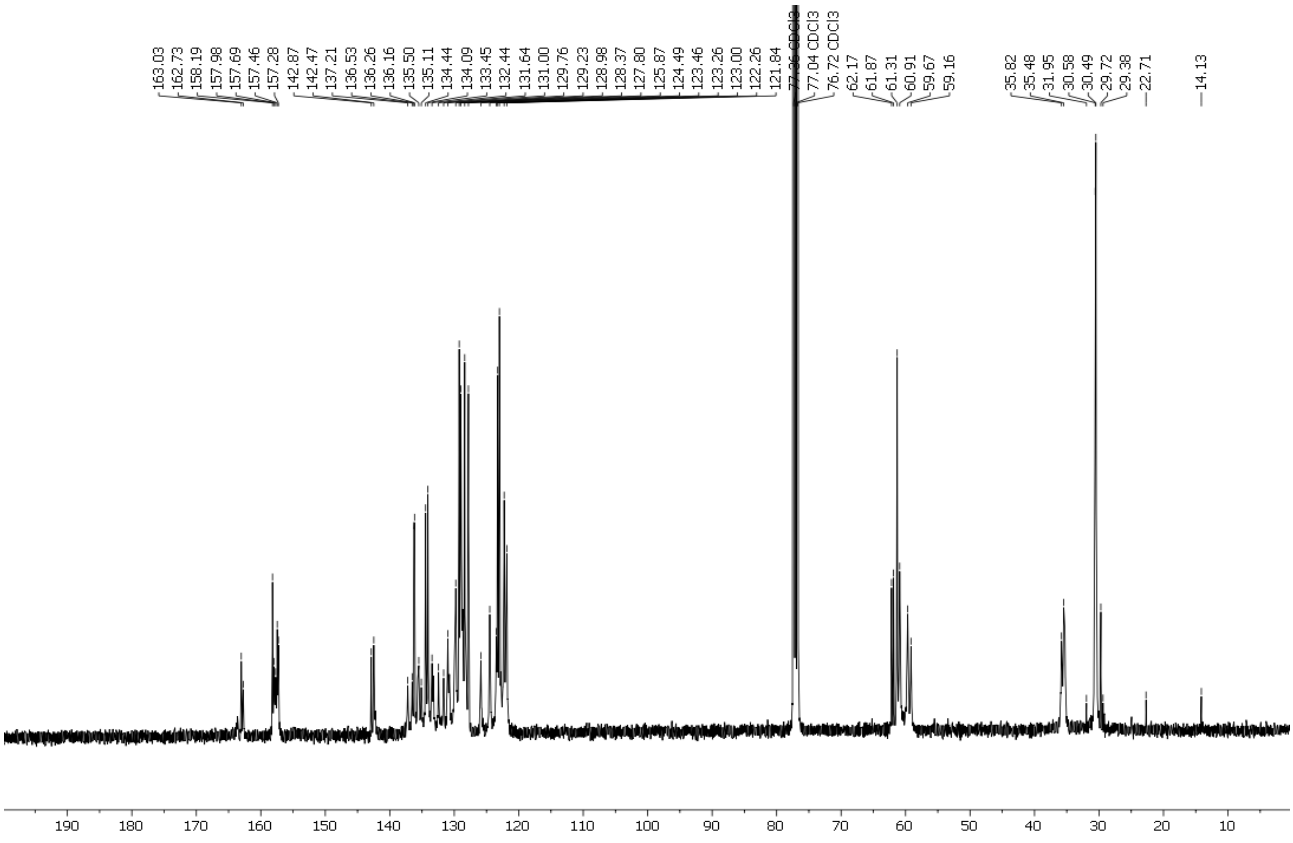


Figure S38. ^{13}C NMR (100 MHz, $CDCl_3$, 298 K) spectrum of compound **12a**

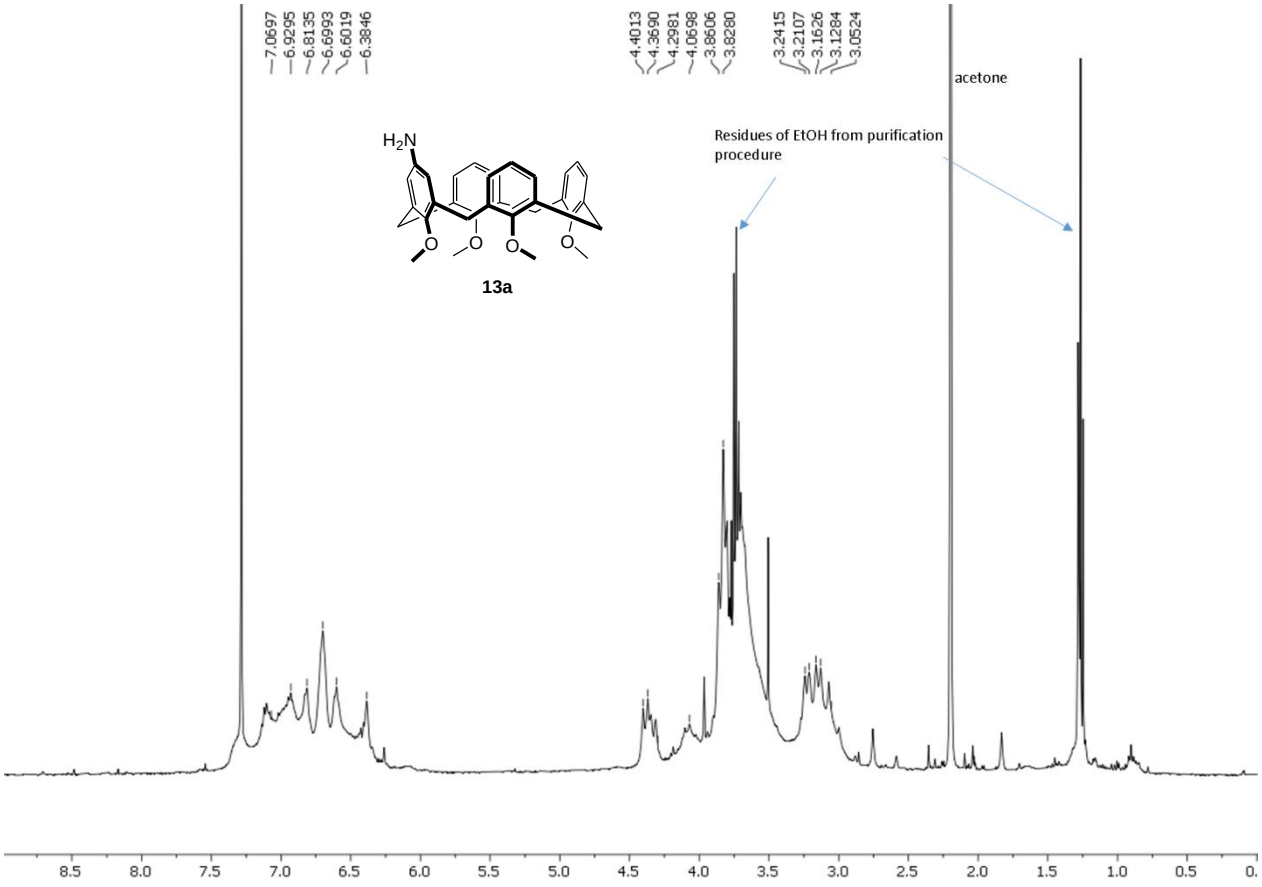


Figure S39. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum of compound **13a**

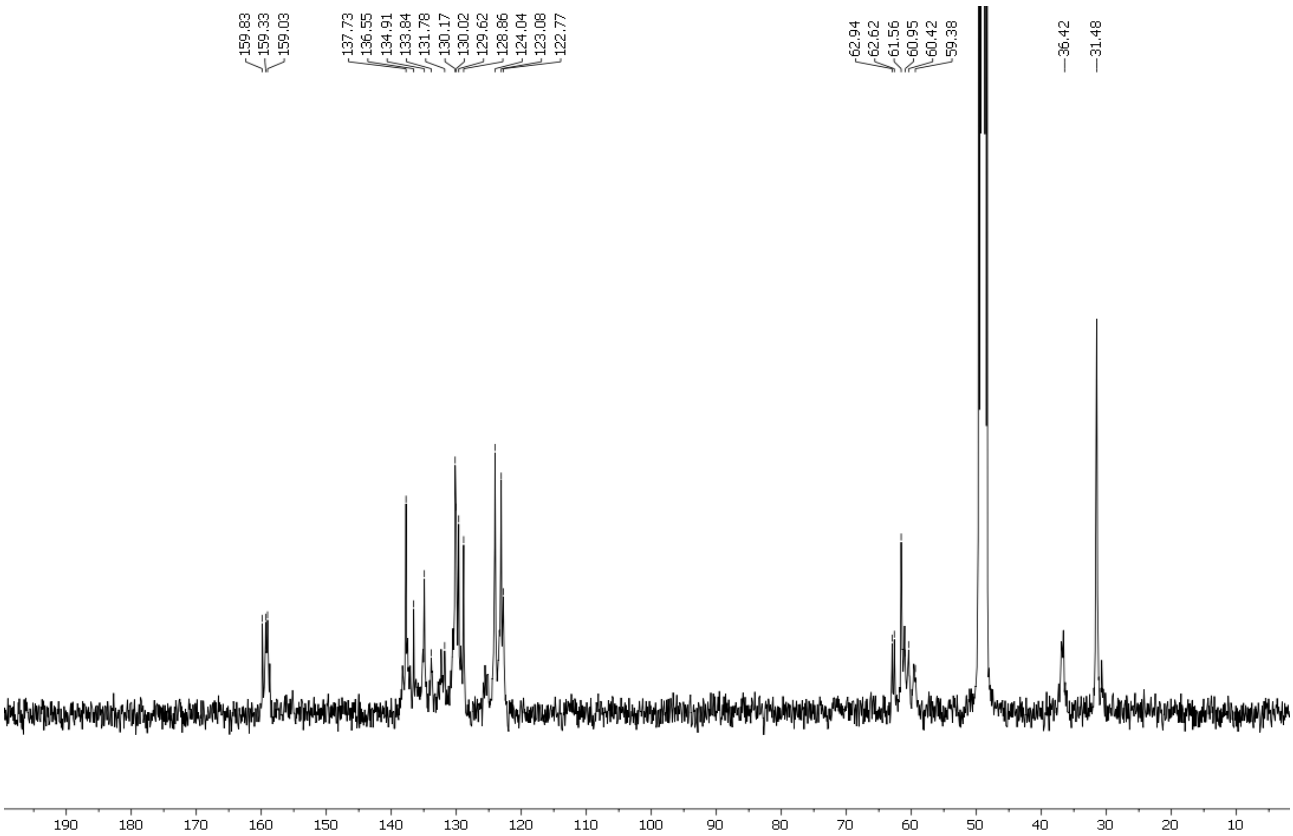


Figure S40. ^{13}C NMR (100 MHz, CD_3OD, 298 K) spectrum of compound **13a**

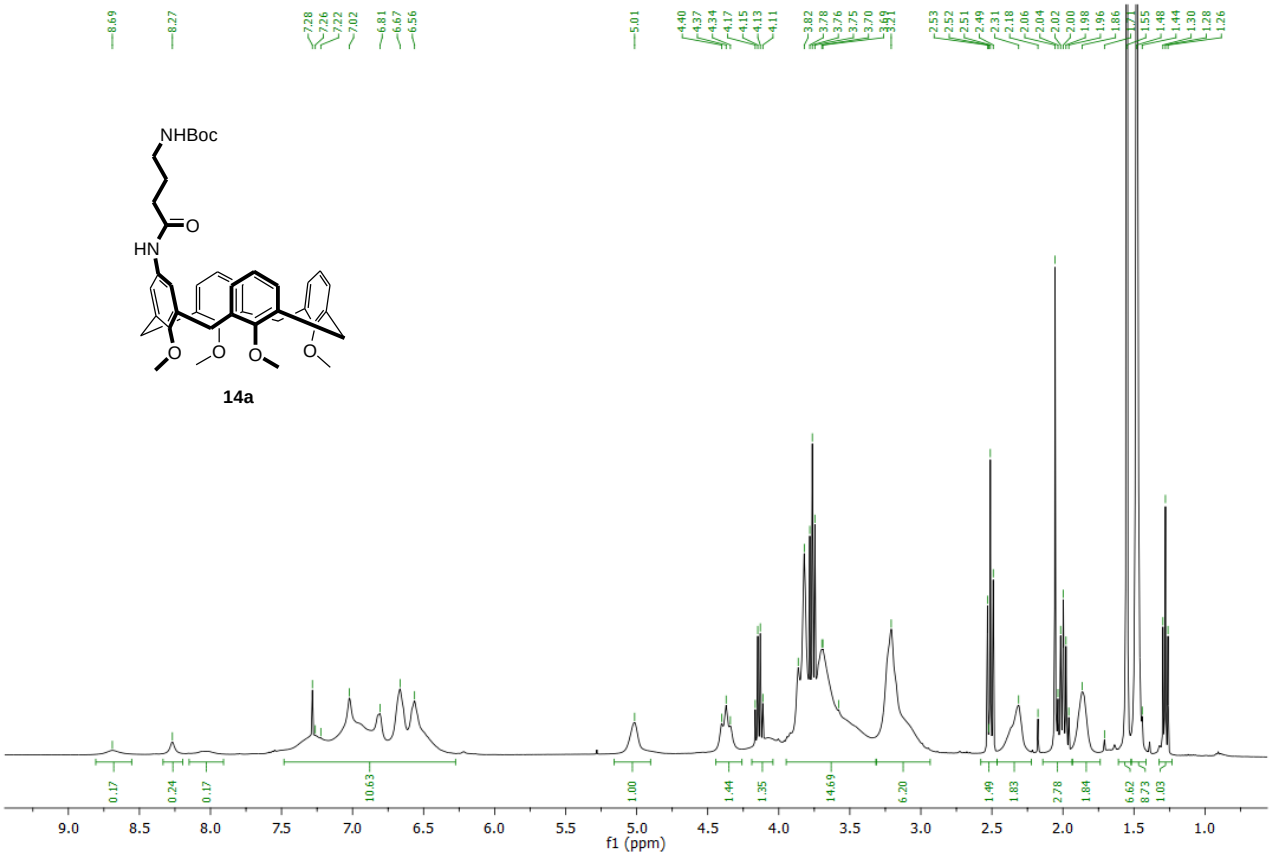


Figure S41. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum of compound **14a**

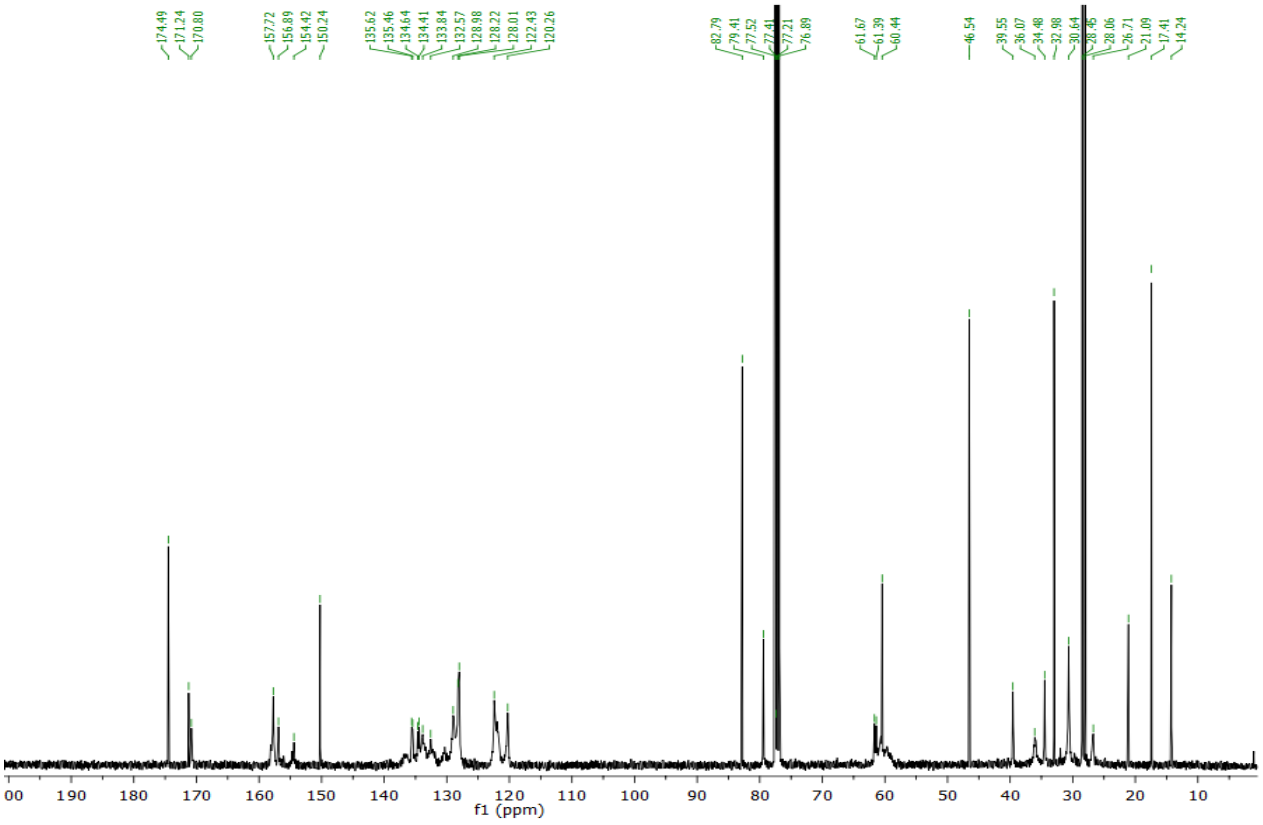


Figure S42. ^{13}C NMR (100 MHz, $CDCl_3$, 298 K) spectrum of compound **14a**

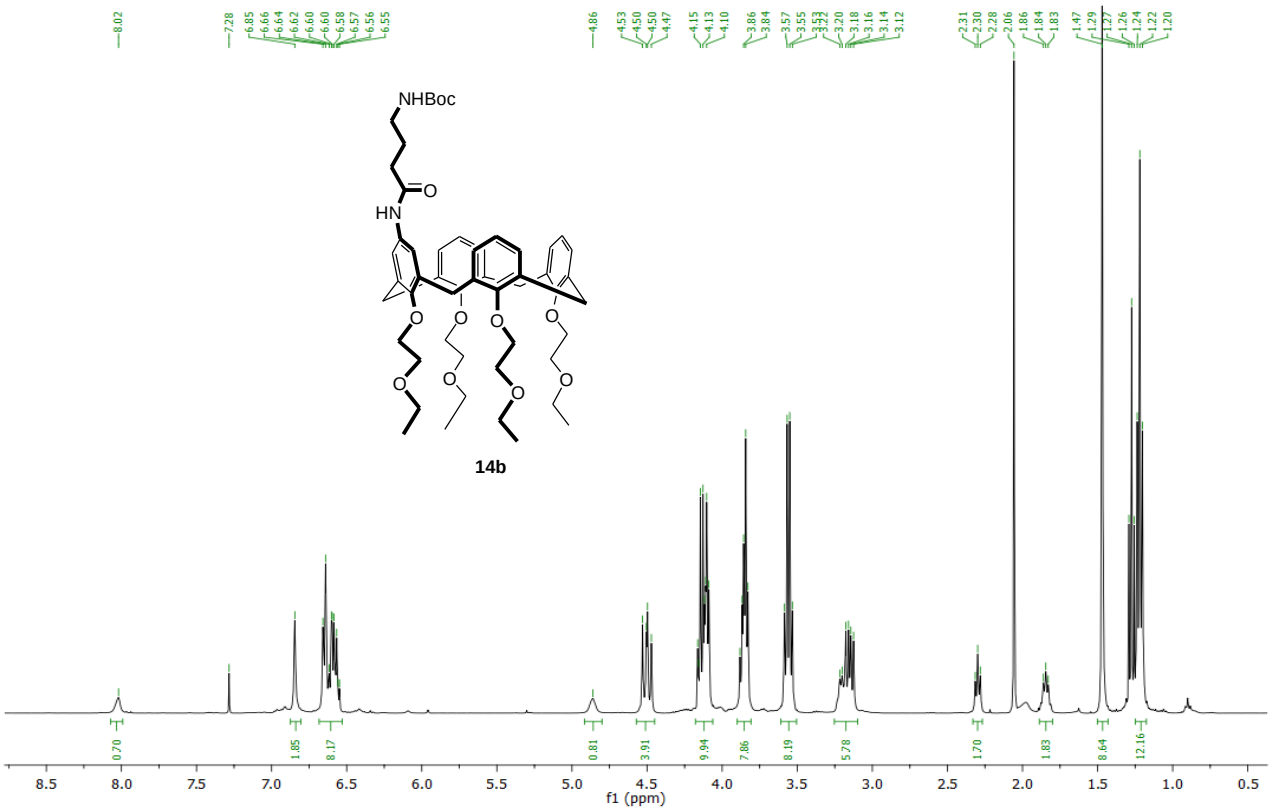


Figure S43. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum of compound **14b**

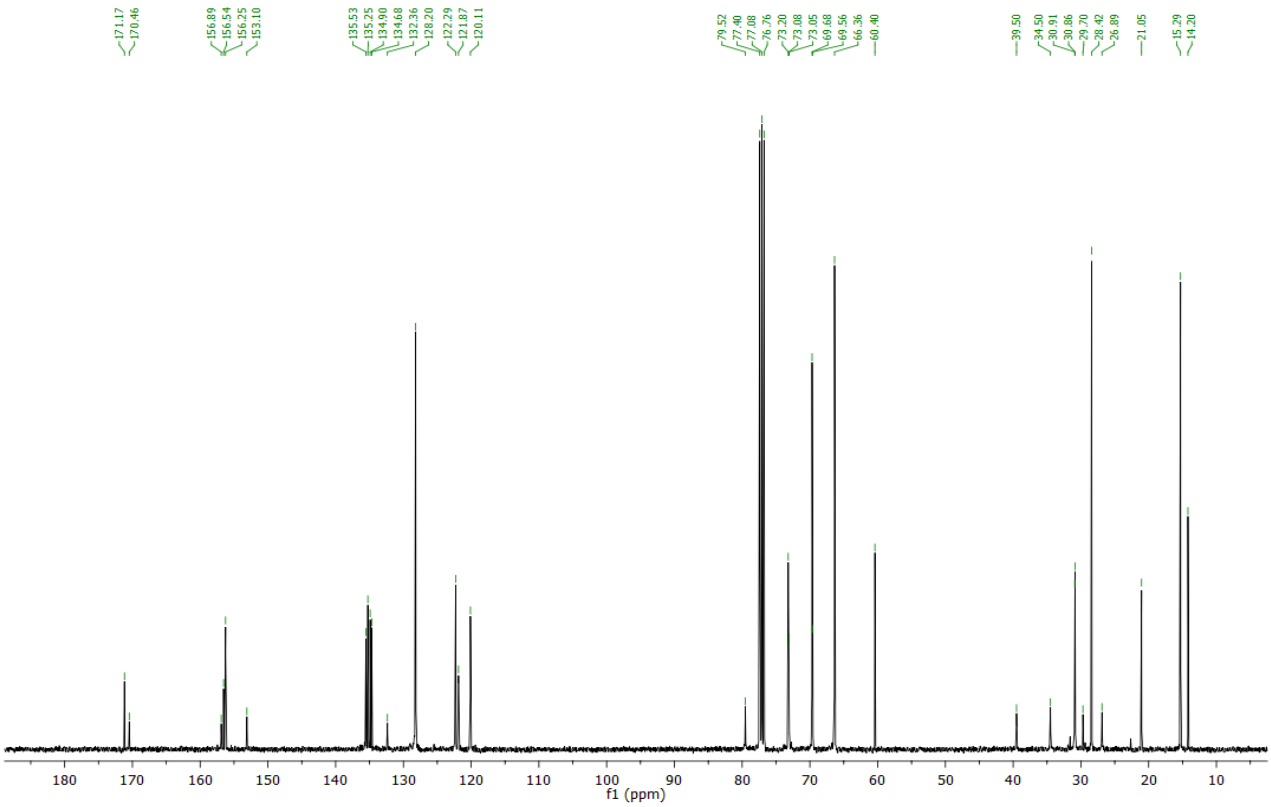


Figure S44. ^{13}C NMR (100 MHz, $CDCl_3$, 298 K) spectrum of compound **14b**

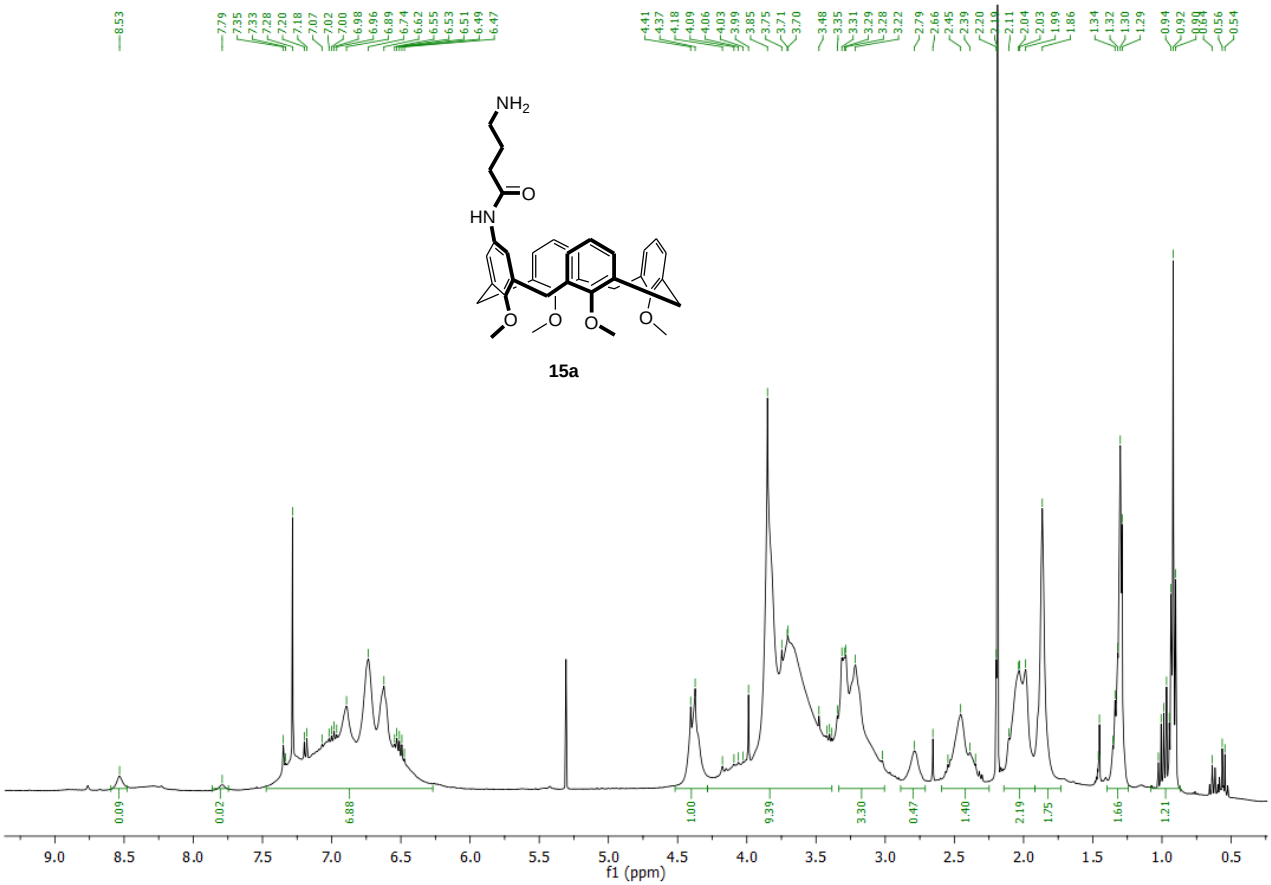


Figure S45. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum of compound **15a**

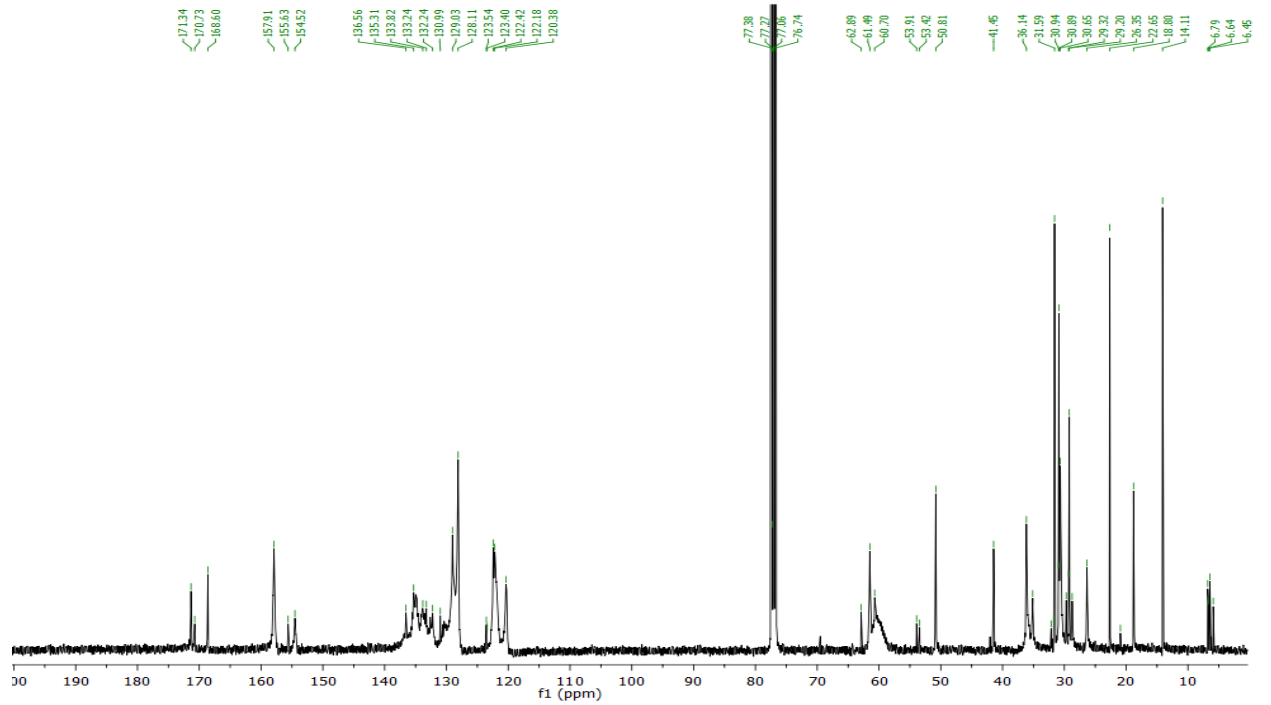


Figure S46. ^{13}C NMR (100 MHz, $CDCl_3$, 298 K) spectrum of compound **15a**

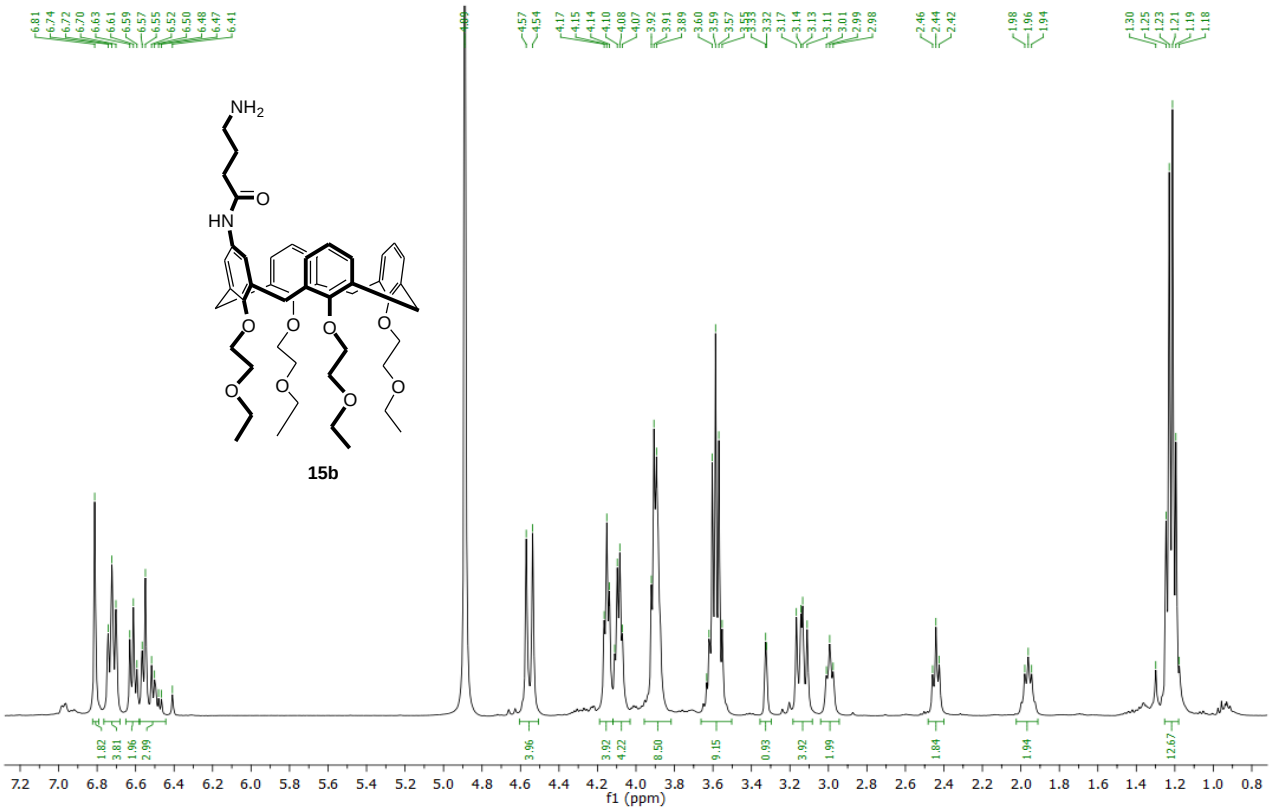


Figure S47. ^{1}H NMR (400 MHz, CD_3OD, 298 K) spectrum of compound **15b**

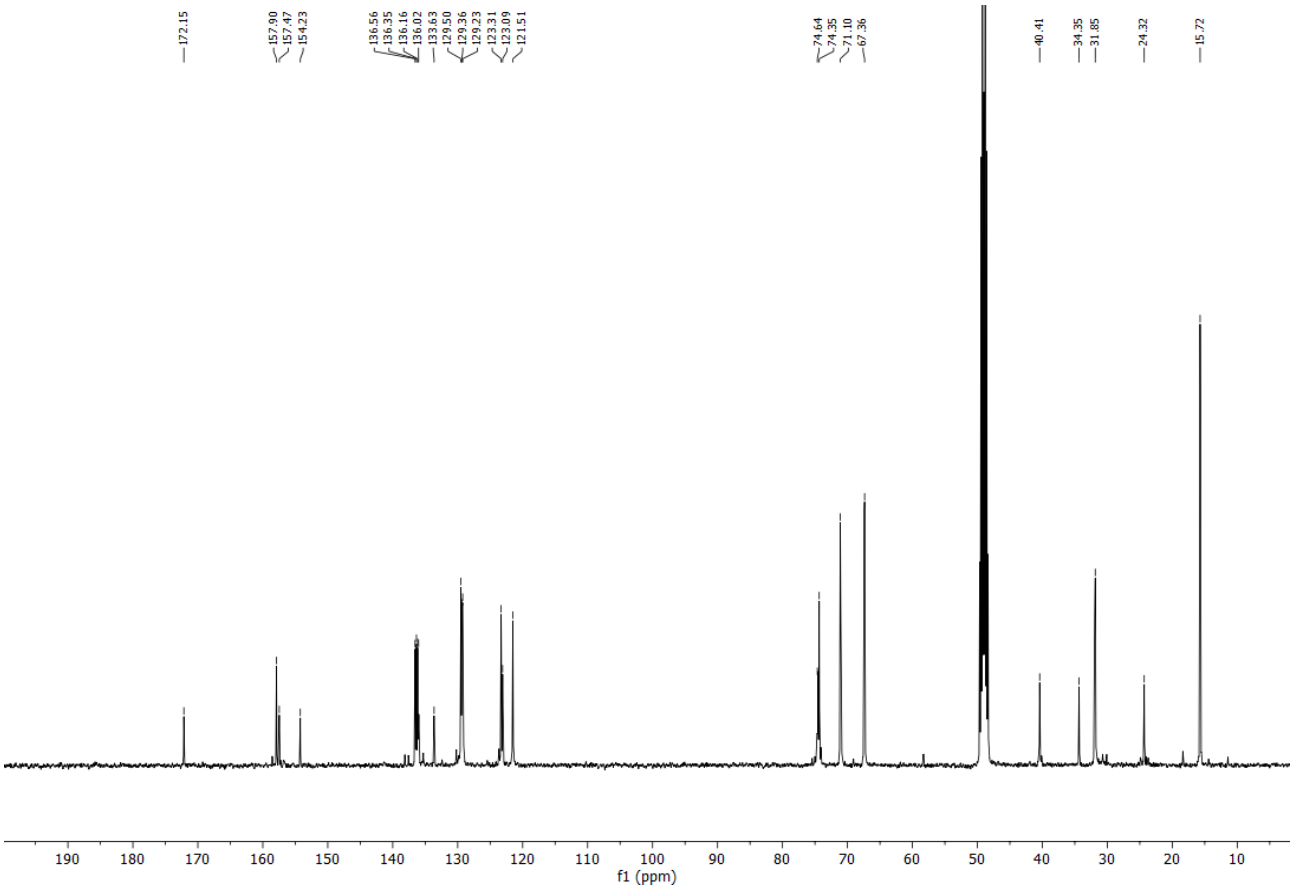


Figure S48 ^{13}C-NMR (100 MHz, CD_3OD, 298 K) spectrum of compound **15b**

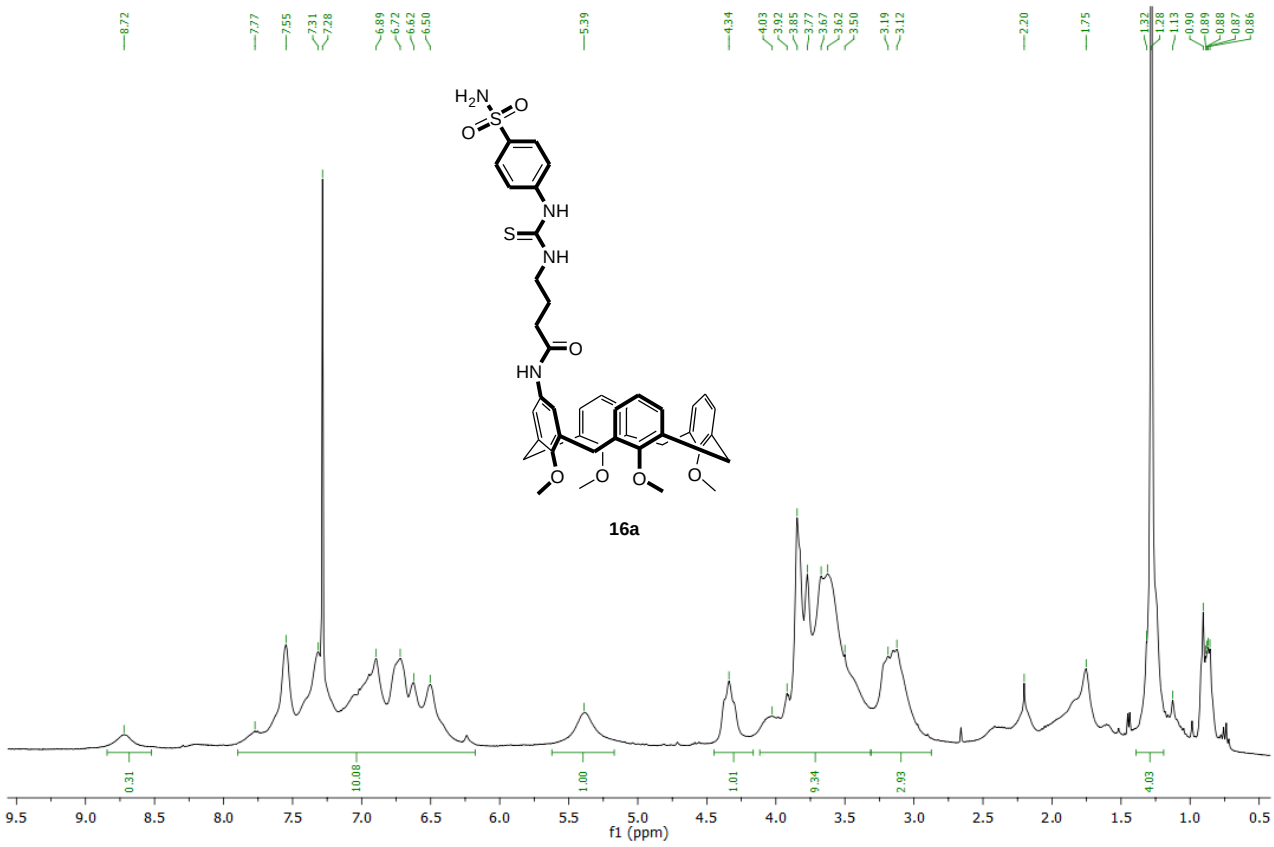


Figure S49. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum of compound **16a**

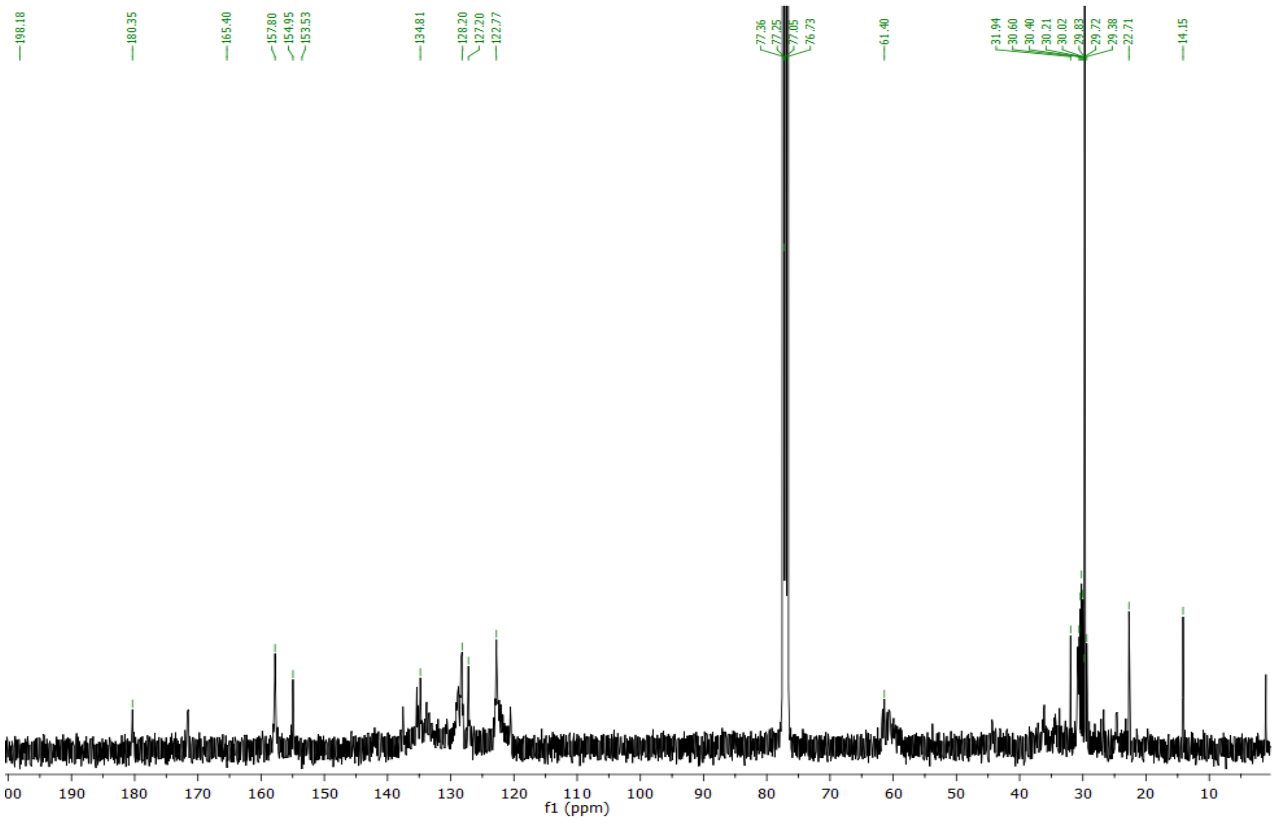


Figure50. ^{13}C NMR (100 MHz, $CDCl_3$, 298 K) spectrum of compound **16a**

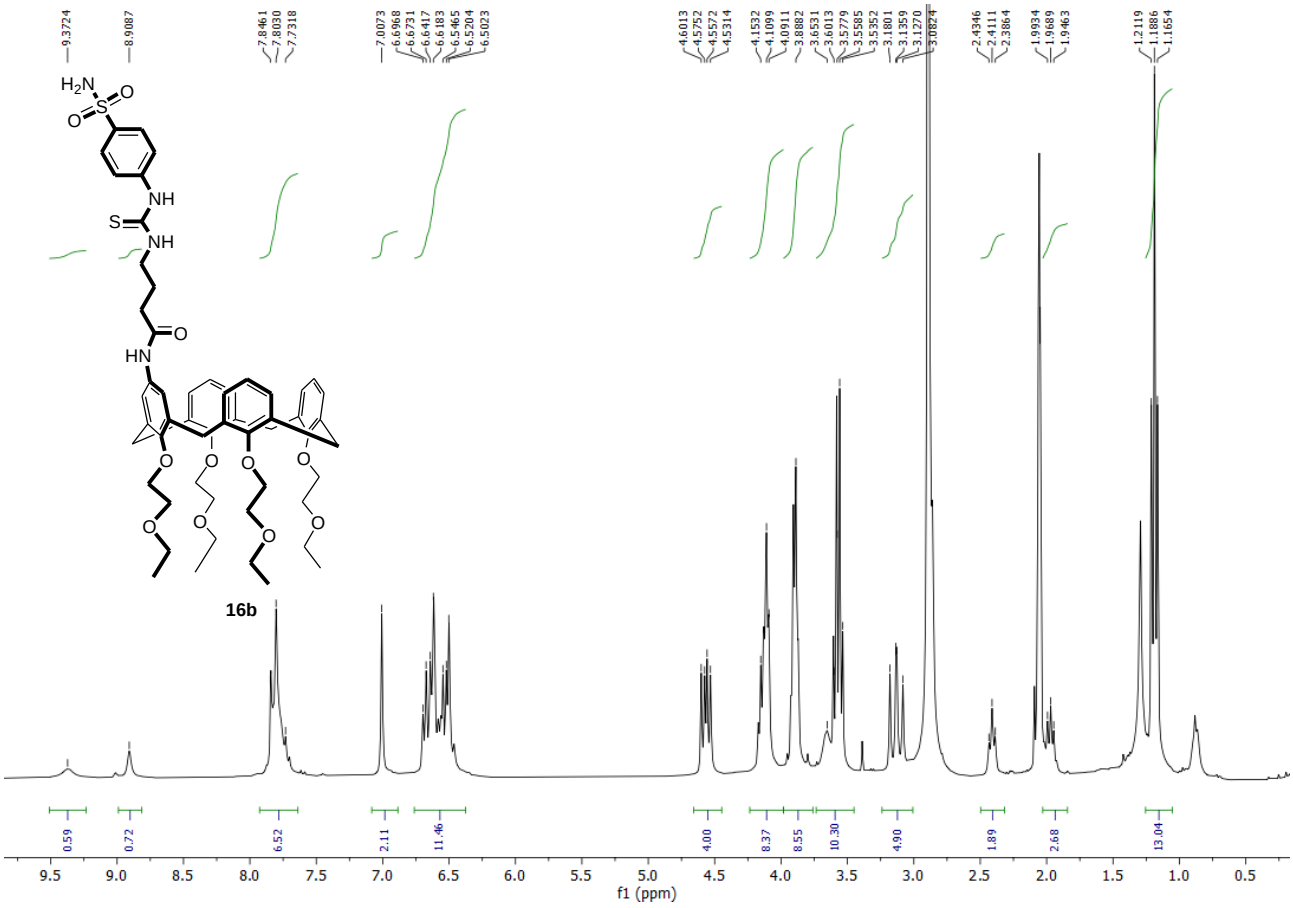


Figure S51. ^{1}H-NMR (400 MHz, acetone-d6, 298 K) spectrum of compound **16b**

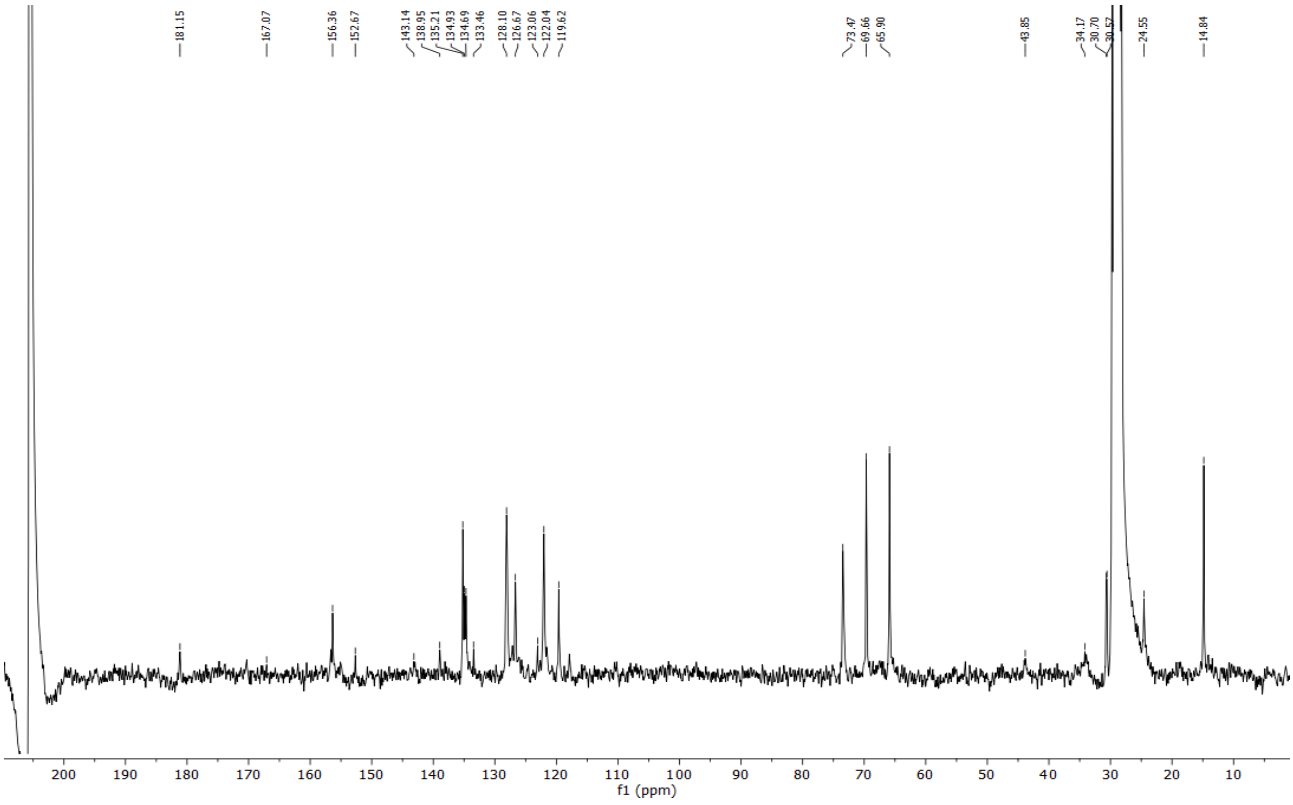


Figure S52. ^{13}C NMR (100 MHz, acetone-d6, 298 K) spectrum of compound **16b**

Table S1. Summary of Data Collection and Atomic Model Refinement Statistics. Values in parentheses are for the highest resolution shell.

PDB ID	7A6V
Wavelength (Å)	1.54184
Space Group	P21
Unit cell (a, b, c, α, β, γ) (Å,°)	42.18;41.21;72.12; 90.00; 104.41; 90.00
Limiting resolution (Å)	10.0-2.0 (3.0-2.0)
Unique reflections	16419 (11479)
R sym (%)	17.9 (42.2)
R meas (%)	20.5 (48.5)
Redundancy	4.19 (4.16)
Completeness overall (%)	99.6 (99.6)
<I/σ(I)>	8.1 (3.6)
CC (1/2)	98.9 (89.5)
Refinement statistics	
Resolution range (Å)	10.0-2.0
Unique reflections, working/free	15589, 14521
R factor (%)	20.9
R free (%)	25.81
r.m.s.d. bonds(Å)	0.0077
r.m.s.d. angles (°)	1.5611
Ramachandran statistics (%)	
Most favored	96.9
additionally allowed	3.1
outlier regions	0.0
Average B factor (Å2)	
All atoms	18.87
inhibitors	38.84
solvent	15.40

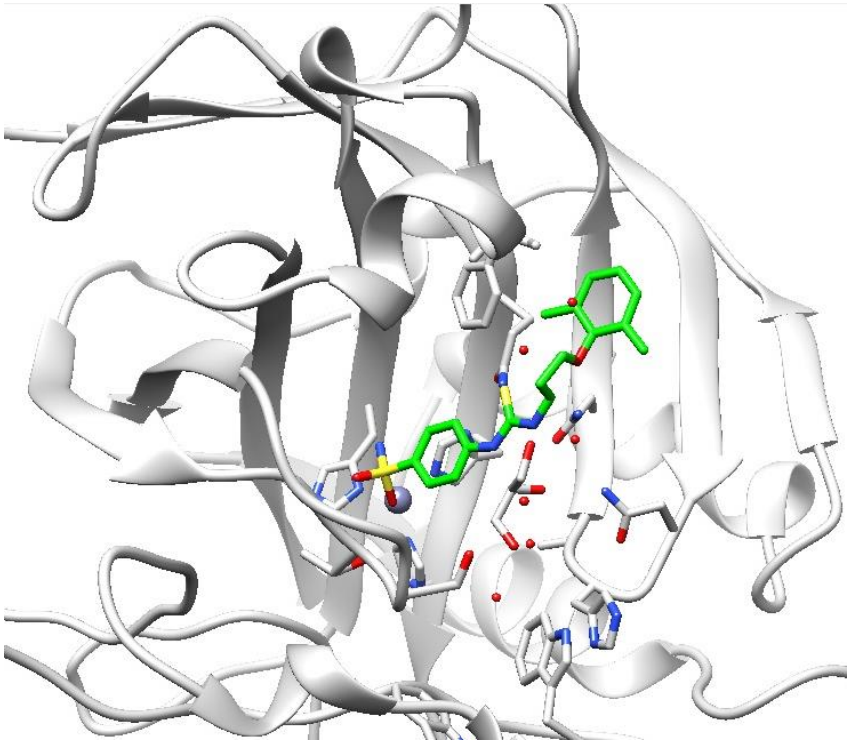


Figure S53. Crystal structure of hCAII-**4b** complex. For the ligand, only the atomic positions of the arm containing the benzensulfonamide moiety and the calixarene phenolic unit linked to (in green) could be accurately determined.

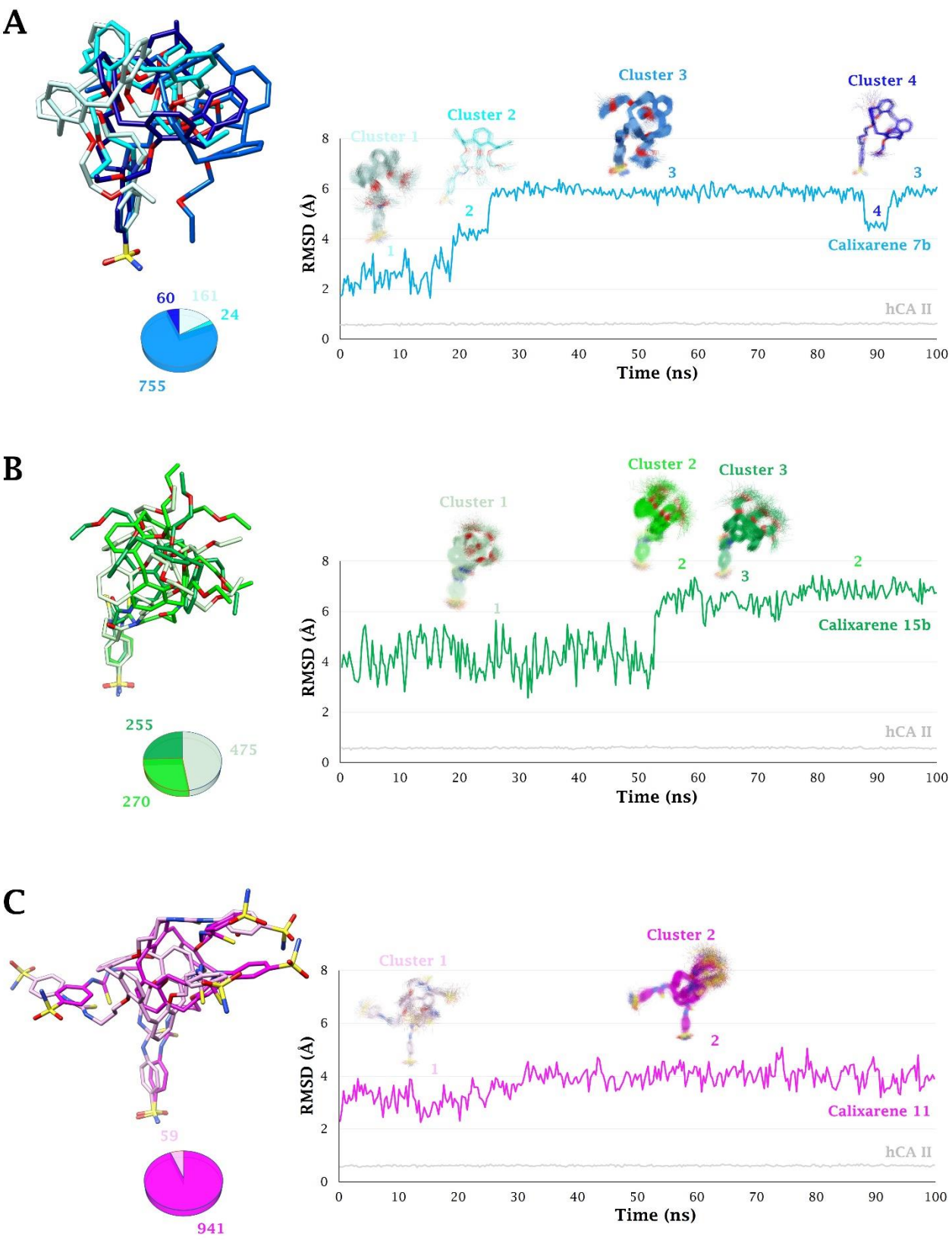


Figure S54_MM1. Average RMSD (Å) from 100 ns long MD simulations performed in triplicate. A) **7b**-hCA II, B) **15b**-hCA II and C) **11**-hCA II adducts. The representative conformers per cluster are depicted on the left together with the pie representation of their respective abundancies.