# Supporting Information

## A supramolecular assembly of a disulfide linked adamantane-curcumin conjugate with amphiphilic cyclodextrin with redox-responsive potential

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## Materials and Methods

### General Information

Curcumin from *Curcuma longa* (C1386, purity 65%, Sigma-Aldrich) was purified by column chromatography on silica gel using $CHCl_3$/*n*-hexane 9:1 as the eluent; other commercial reagents and solvents were used without further purification. The reactions were monitored by TLC (precoated aluminium plates of silica gel 60 $F_{254}$) and the products were visualized with vanillin [1 g dissolved in MeOH (60 mL) and conc. $H_2SO_4$ (0.6 mL)] and/or by a UV lamp. Column chromatography was performed using Silica gel 60. $^1H$ and $^{13}C$ NMR spectra were recorded in $CDCl_3$ with a Varian 500 spectrometer (at 500 MHz for $^1H$ and 125 MHz for $^{13}C$). Chemical shifts are given in parts per million (ppm) referenced to the residual protons in $CDCl_3$ ( $\delta$= 7.26 ppm for $^1H$ NMR and $\delta$ = 77.16 ppm for $^{13}C$ NMR) solvents. NMR peak assignments are supported by homonuclear (COSY, correlation spectroscopy) and heteronuclear correlation $^1H$–$^{13}C$ spectroscopy (HSQCAD). Coupling constants (J) are given in Hertz. Numbering of atoms of curcumin-based compounds is reported in the spectra. Melting points were determined using Kofler hot-stage apparatus and are uncorrected. Combustion analyses were carried out on a FISONS EA1108 elemental analyzer.

**Synthesis of compound 1**

To a solution of Curcumin (1.0 g, 2.71 mmol) in dry DCM (100 mL) 4-dimethylaminopyridine (34.4 mg, 0.28 mmol), *N,N'*-dicyclohexylcarbodiimide (669.1 mg, 3.24 mmol) and 4-(bromomethyl) benzoic acid (592 mg, 2.75 mmol) were added. The reaction mixture was stirred under inert atmophere and at r. t. for 48 h, and monitored by TLC ($CHCl_3$/MeCN 95:5) the mixture was filtered and solvent was removed reduced pressure. The unreacted 4-(bromomethyl) benzoic acid was removed by chromatographic column (Silica gel, $CHCl_3$/*n*-Hexane 8:2 to $CHCl_3$/MeCN 95:5). The crude was further purificated by a second by chromatographic column (Silica gel, *n*-Hexane/Ethyl Acetate 8:2 to 6:4), obtaining the desired compound **1** as orange powder (610 mg, 43%). m.p. 174–176 °C. $R_f$ 0.6 ($CHCl_3$/MeCN 95:5. $^1$H-NMR ($CDCl_3$, 500 MHz) δ: 8.21-8.16 (m, 2H) and 7.55-7.52 (m, 2H) [2x H-13 and 2x H-14]; 7.63, 7.61, 6.58 and 6.50 (4x d, *J*= 16.0 Hz, 4H) [H-3, H-3', H-4 and H-4']; 7.23-7.16 (m, 3H) [H-6; H-9 and H-10]; 7.13( dd; *J*=8.2 Hz, J=2.0 Hz, 1H) [H-10']; 7.06 (d; *J*= 2.0 Hz, 1H) [H-6']; 6.94 (d, *J*=8.2 Hz, 1H) [H-9']; 5.89 (bs, 1H) [OH]; 5.85 (s, 1H) [H-1]; 4.54 (s, 2H) [H-16]; 3.95 and 3.87 (2x s, 6H) [2x -OMe]. $^{13}$C-NMR ($CDCl_3$, 125 MHz) δ: 184.7 and 182.0 [C-2 and C-2']; 164.2, 151.7, 148.1,147.0, 143.6 and 141.5 [Cq]; 141.3, 139.6, 124.5 and 122.0 [C-3, C-3', C-4 and C-4']; 131.0 and 129.4 [C-13 and C-14]; 134.4, 129.2 and 127.7 [Cq]; 123.5, 121.2 and 111.7 [C-6', C-9' and C-10']; 123.2 [C-10]; 115.0 [C-9]; 109.8 [C-6]; 101.7 [C-1]; 56.1 [2x -OMe]; 32.6 [C-16]. Anal. calcd for $C_{29}H_{25}BrO_7$: C, 61.60; H, 4.46; Br, 14.13; O, 19.81. Found: C, 61.73; H, 4.44.

Compound **2** was obtained by the same column chromatography in 35% yield as a yellow solid, m.p. 178–180 °C. $R_f$ 0.8 ($CHCl_3$/MeCN 95:5).

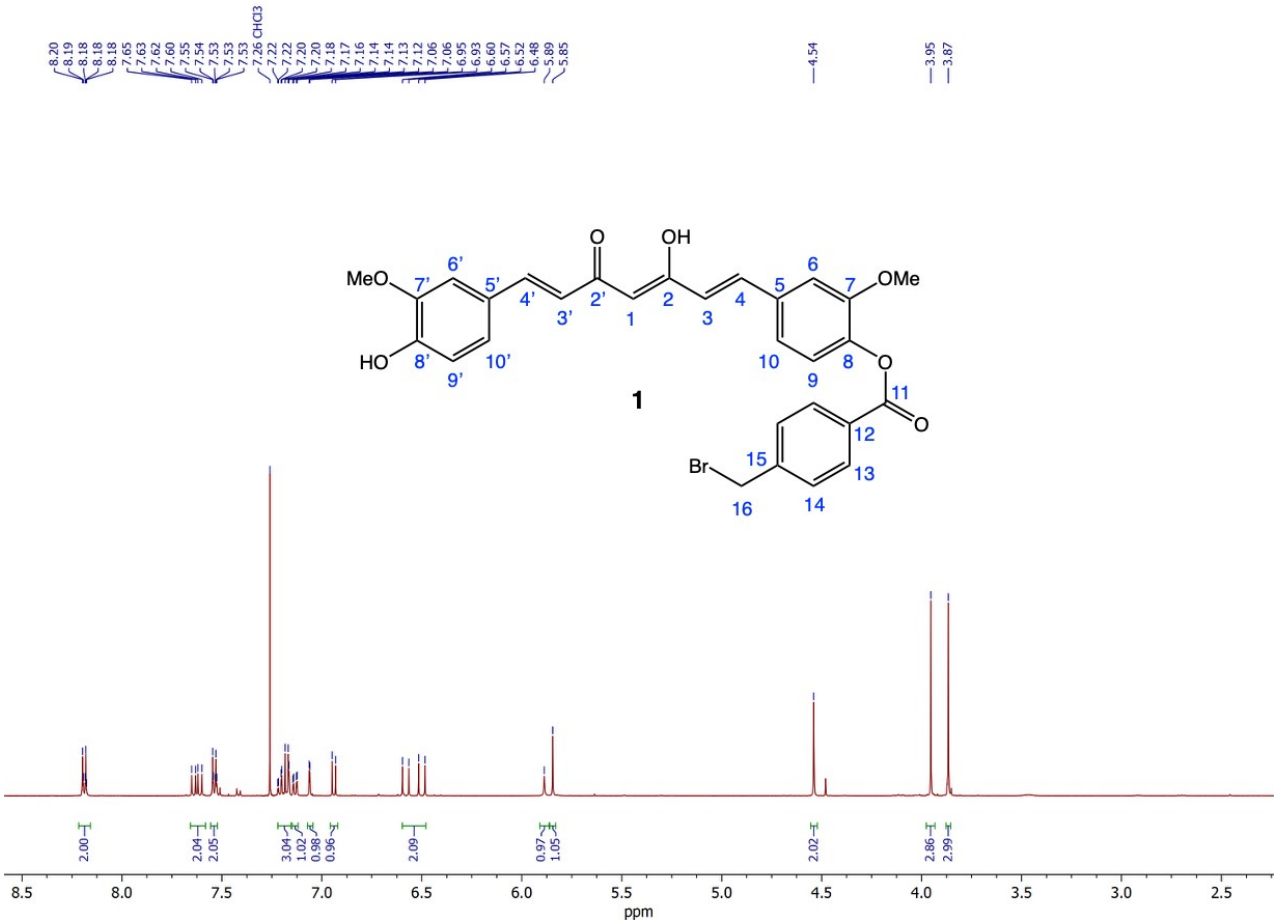


Figure S1. $^1$H-NMR of compound **1** in $CDCl_3$

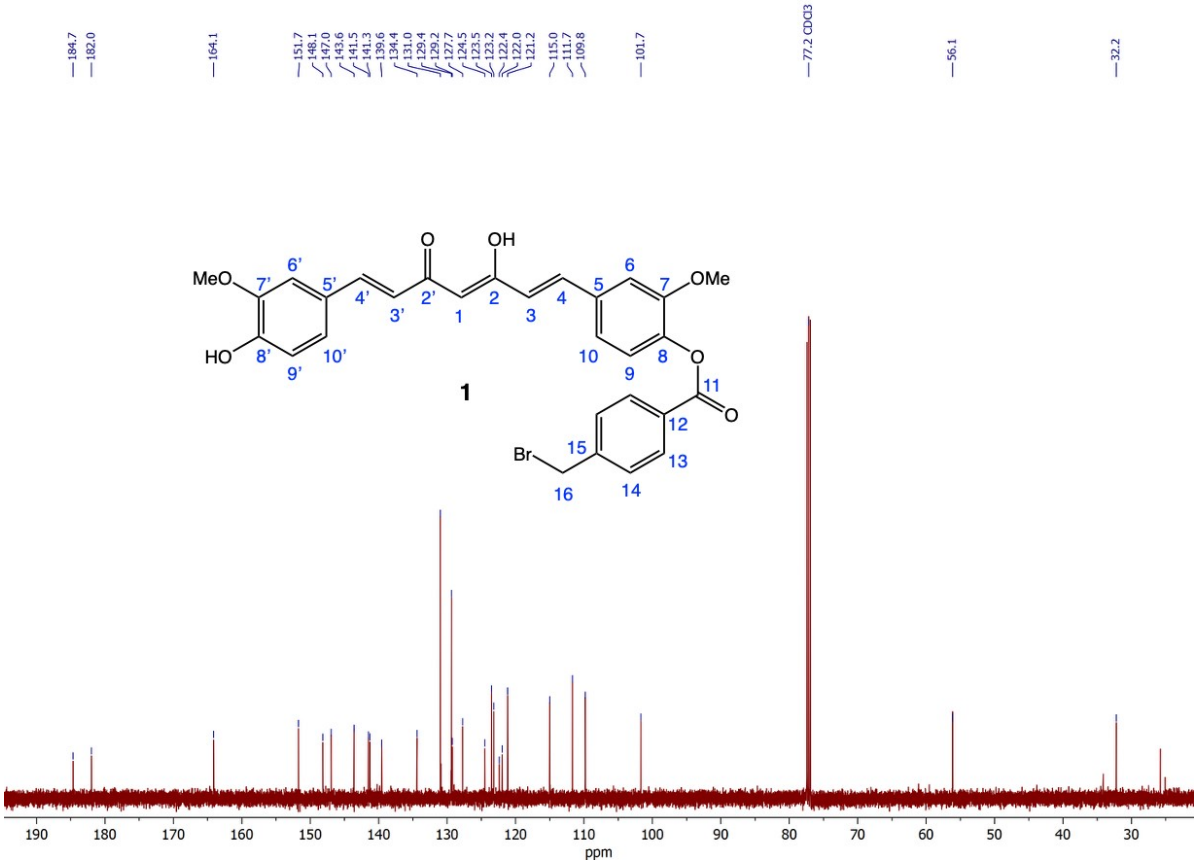


Figure S2. $^{13}$C-NMR of compound **1** in $CDCl_3$

**Synthesis of compound 4**

To a solution of compound **1** (640 mg, 1.13 mmol, 1 eq) in 120 mL of Ethyl acetate/$CHCl_3$ (2:1), 76.2 mg of thiourea (1.13 mmol, 1 eq) was added. The mixture was stirred at 50°C for 24 h in the dark and under inert atmosphere, until the complete formation of thiouronium salt **3** (TLC *n*-hexane/ Ethyl Acetate 5:5). Solvents were removed under reduced pressure, and the compound was dissolved in 80 mL of $H_2O/CH_2Cl_2$ (1:2); $Na_2S_2O_5$ (216 mg, 1.13 mmol, 1 eq.) was added and the mixture was refluxed for 6 h until the disappearance of compound **3** (TLC *n*-hexane/ Ethyl Acetate 6:4). The two solvent layers were separated, and the aqueous phase was further extracted with $CHCl_3$(3x 10 mL) the combined organic phases were dried over $Na_2SO_4$, filtered and solvent was removed under reduced pressure. The crude was purified by column chromatography ($SiO_2$, eluant: *n*-hexane/ Ethyl Acetate 6:4 to 5:5) to obtain compound **4** as a yellow solid (339.8 mg, 58%). m.p. 123–125 °C, $R_f$ 0.7 (*n*-hexane/ Ethyl Acetate 6:4); $^1$H-NMR ($CDCl_3$, 500 MHz) δ: 8.20-8.11 and 7.51-7.41 (2x d, *J*=8.0 Hz, 4H) [2x H-13 and 2x H-14]; 7.64, 7.61, 6.58 and 6.50 (4x d, *J*= 16.0 Hz, 4H) [H-3, H-3', H-4 and H-4']; 7.22-7.12 (m, 3H) [H-6; H-9 and H-10]; 7.14( dd; *J*=8.2 Hz, J=2.0 Hz, 1H) [H-10']; 7.06 (d; *J*= 2.0 Hz, 1H) [H-6']; 6.94 (d, *J*=8.2 Hz, 1H) [H-9']; 5.88 (bs, 1H) [OH]; 5.85 (s, 1H) [H-1]; 3.96 and 3.87 (2x s, 6H) [2x -OMe]; 3.82 (d, *J*= 7.8 Hz, 2H) [H-16']; 1.87 (t, *J* = 7.8 Hz, 1H) [-SH]. $^{13}$C-NMR ($CDCl_3$, 125 MHz) δ: 184.6 and 182.0 [C-2 and C-2']; 164.4, 151.8, 148.1,147.3,147.0 and 141.6 [Cq]; 141.3, 139.6, 124.4 and 122.0 [C-3, C-3', C-4 and C-4']; 131.0 and 129.4 [C-13 and C-14]; 134.3, 128.1 and 127.7 [Cq]; 123.6, 121.2 and 111.7 [C-6', C-9' and C-10']; 123.2 [C-10]; 115.0 [C-9]; 109.8 [C-6]; 101.7 [C-1]; 56.1 [2x -OMe]; 34.1 [C-16]. Anal. calcd for $C_{29}H_{26}O_7S$: C,67.17; H, 5.05; O, 21.60; S, 6.18. Found: C, 67.22; H, 5.06.

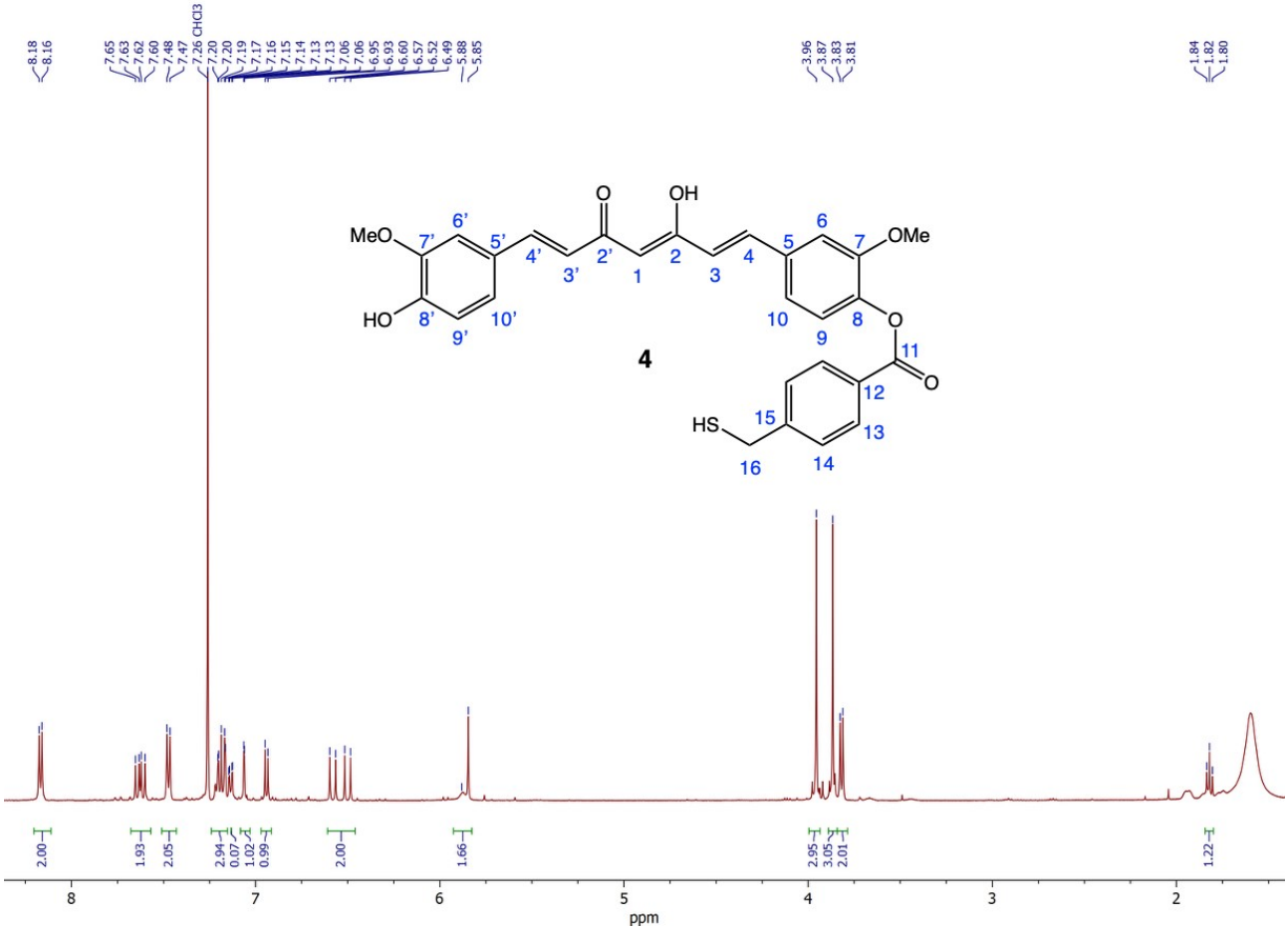


Figure S3.$^1$H-NMR of compound **4** in $CDCl_3$

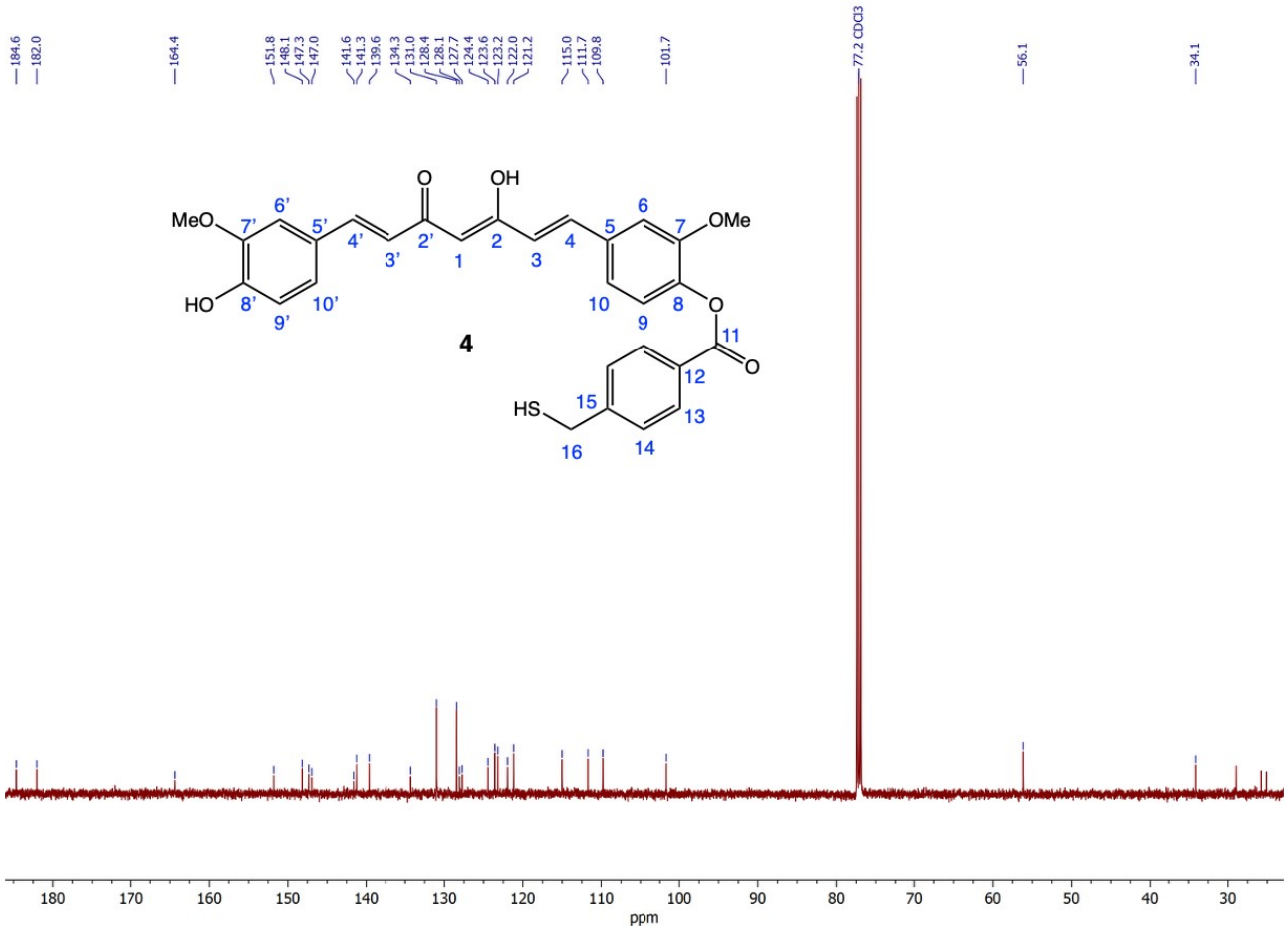


Figure S4.$^{13}$C-NMR of compound **4** in $CDCl_3$

**Synthesis of compound 5**

To a solution of compound **4** (150 mg, 0.297 mmol) in anhydrous THF (5 mL) at -78°C Triton B (40 wt% solution in MeOH, 16.2 µL, 0.060 mmol,0.2 eq.) was added and the mixture was stirred under inert atmosphere for 10 min, then methyl acrylate (54 µL, 0.594 mmol, 2 eq.) was added. The mixture was allowed to reach r.t. and stirred at r.t. for 6 h; the solvent was removed under reduced pressure, and the crude was purified by column chromatography ($SiO_2$, eluant: *n*-hexane/ Ethyl Acetate 6:4) to obtain compound **5** as a yellow solid (107.7 mg, 60%). m.p.128-130°C. $R_f$ 0.6 (*n*-hexane/ Ethyl Acetate 6:4). $^1$H-NMR ($CDCl_3$, 500 MHz) δ: 8.16 and 7.47 (2x d, *J*=8.1 Hz, 4H) [H-13 and H-14]; 7.63, 7.61, 6.58 and 6.50 (4x d, *J*=16 Hz, 4H) [H-3, H-4, H-3' and H-4']; 7.22-7.15 (m, 3H) [H-6, H-9 and H-10]; 7.13 (dd, *J*=8.2 Hz, *J*=2.0 Hz, 1H) [H-10']; 7.06 (d, *J*=2.0 Hz, 1H)[H-10']; 6.94 (d, *J*=8.2 Hz, 1H) [H-9']; 5.84 (s, 1H) [H-1];3.95 and 3.87 (2s, 6H) [2x -OMe]; 3.81 (s, 2H) [H-16]; 3.70 (s, 3H) [H-21]; 2.70 and 2.59 (2x t, *J*=7.0 Hz, 4H) [H-17 and H-18]. $^{13}$C-NMR ($CDCl_3$, 125 MHz) δ: 184.6 and 182.0 [C-2 and C-2']; 172.3 [C-11]; 164.4 [C-19]; 151.8, 148.1, 147.0, 144.5 and 141.6 [Cq]; 141.3, 139.6, 124.4 and 122.0 [C-3, C-4, C-3' and C-4']; 134.3, 128.1 and 127.7 [Cq]; 123.6, 121.2 and 111.6 [C-6, c-9, C-10]; 123.2 [C-6']; 115.0 [C-9']; 109.8 [C-10']; 101.7 [C-1]; 56.1 [2x -OMe]; 52.0 [C-20]; 36.3 [C-16]; 34.4 and 26.52 [C-17 and C-18]. Anal. calcd for $C_{33}H_{32}O_9S$: C, 65.55; H, 5.33; O, 23.81; S, 5.30. Found: C, 65.65; H, 5.31.

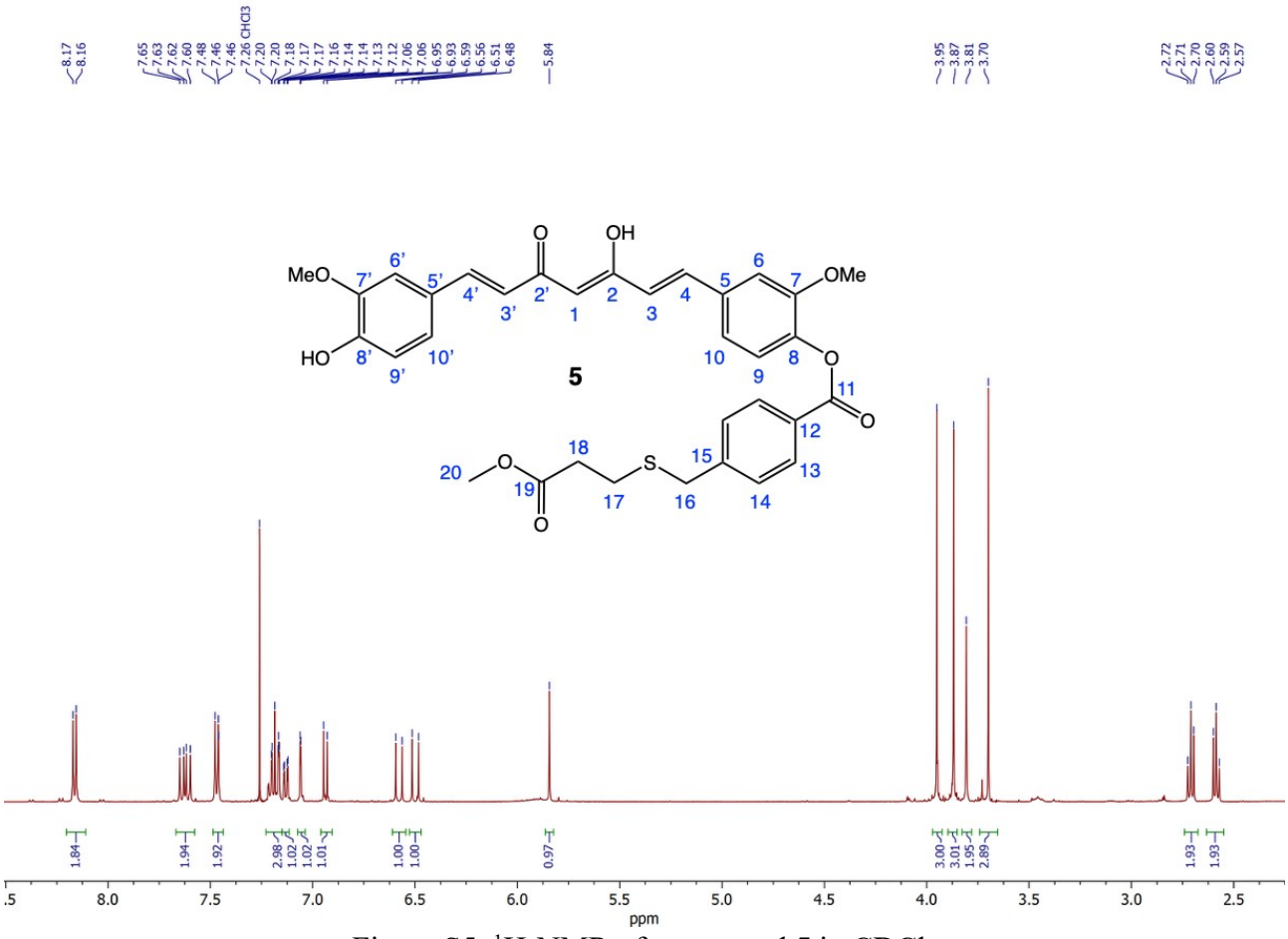


Figure S5. $^1$H-NMR of compound **5** in $CDCl_3$

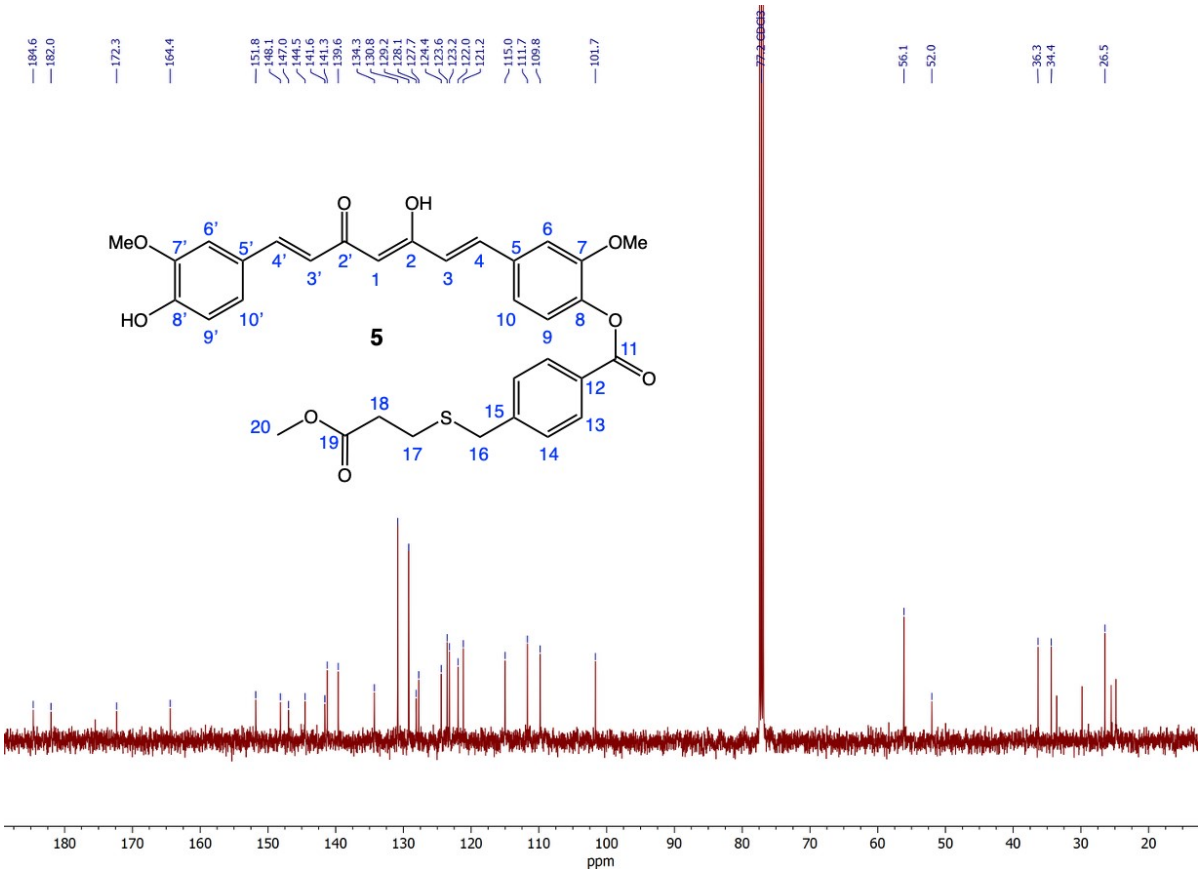


Figure S6.$^{13}$C-NMR of compound **5** in $CDCl_3$

**Synthesis of compound 6**

m-CPBA (20 mg, 0.115 mmol was dissolved in DCM (4 mL) and added dropwise to the thioether solution (68 mg, 0.115 mmol) in DCM (4 mL) and stirred at -78°C. The reaction appeared to be completed (TLC n-hexane/ Ethyl Acetate 6:4) shortly after the addition of the oxidant, and $Na_2S_2O_3$ (10% in water,10 mL) was then added. The organic layer was separated, washed twice with saturated solution of $NaHCO_3$ and twice with brine. The combined organic phases were dried over $Na_2SO_4$, filtered and solvent was removed under reduced pressure. The crude was purified by column chromatography ($SiO_2$, EtOAc) to give sulfoxide **7** as yellow solid (63 mg, 90% yield), m.p. 165–168 °C $R_f$ 0.1 (n-hexane/ Ethyl Acetate 6:4) $^1$H-NMR ($CDCl_3$, 500 MHz) δ: 8.23 and 7.47 (2x d, *J*= 8.2 Hz, 4H) [H-12 and H-13]; 7.63, 7.61, 6.58 and 6.50 (4x d, *J*=16.0 Hz, 4H) [H-3, H-4, H-3' and H-4']; 7.23-7.16 (m, 3H) [H-6, H-9 and H-10]; 7.13 (dd, *J*=8.2 Hz, *J*=2.0 Hz, 1H) [H-10']; 7.06 (d, *J*=2.0 Hz, 1H) [H-6']; 6.94 (d, *J*= 8.2 Hz, 1H) [H-9']; 5.92 (s, 1H) [OH]; 5.84 (s, 1H) [H-1]; 4.13-4.04 (m, 2H) [H-16]; 3.95 and 3.87 (2x s, 6H) [2x -OMe]; 3.73 (s, 3H) [H-20]; 3.07-2.77 (m, 4H) [H-17 and H-18]. $^{13}$C-NMR ($CDCl_3$, 125 MHz) δ: 184.7 and 182.0 [C-2 and C-2']; 171.8 [C-19]; 164.2 [C-11]; 151.7, 148.2, 147.0 and 141.5 [Cq]; 141.3, 139.6, 124.5 and 122.0 [C-3, C-4, C-3' and C-4']; 135.6, 134.4, 129.5 and 127.7 [Cq]; 123.5, 121.2 and 111.7 [C-6, C-9 and C-10]; 123.2 [C-6']; 115.0 [C-9']; 109.8 [C-10']; 101.7 [C-1]; 58.4 [C-16]; 56.1 [-OMe]; 52.4 [C-20]; 46.1 and 26.9 [C-17 and C-18]. Anal. calcd for $C_{33}H_{32}O_{10}S$: C, 63.86; H, 5.20; O 25.78, S, 5.17. Found: C, 63.99; H, 5.19.

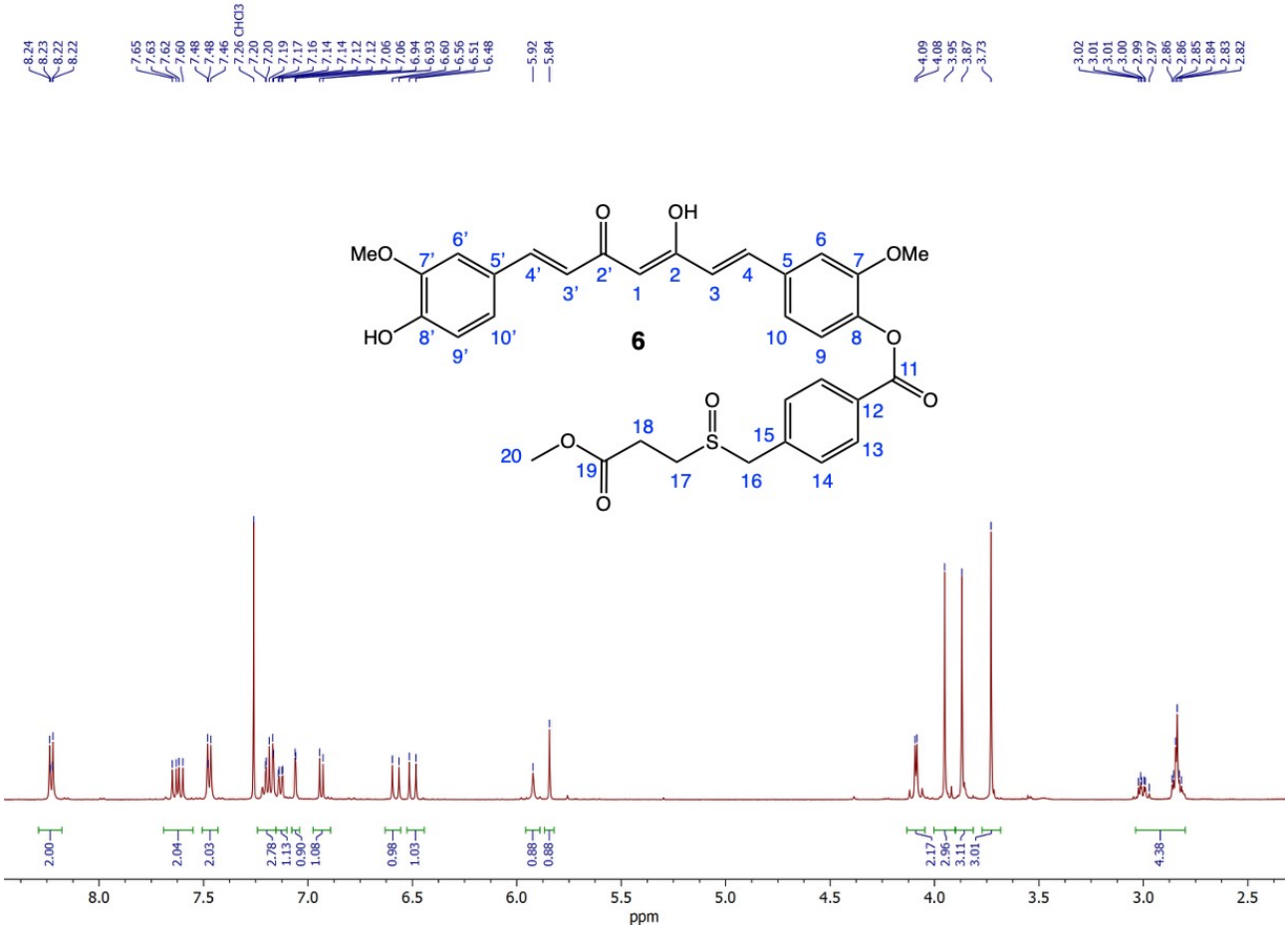


Figure S7. $^1$H-NMR of compound **6** in $CDCl_3$

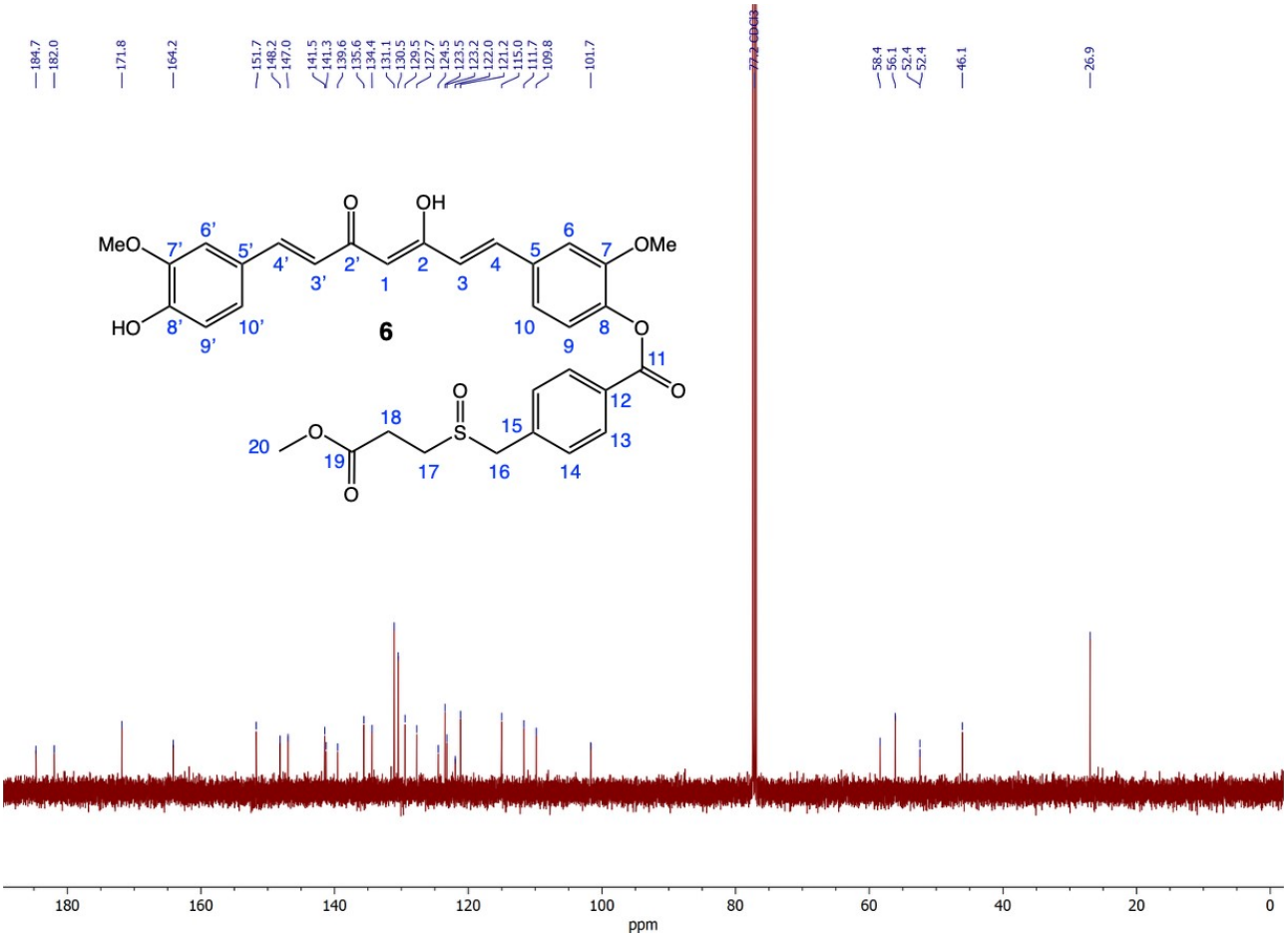


Figure S8. $^{13}$C-NMR of compound **6** in $CDCl_3$

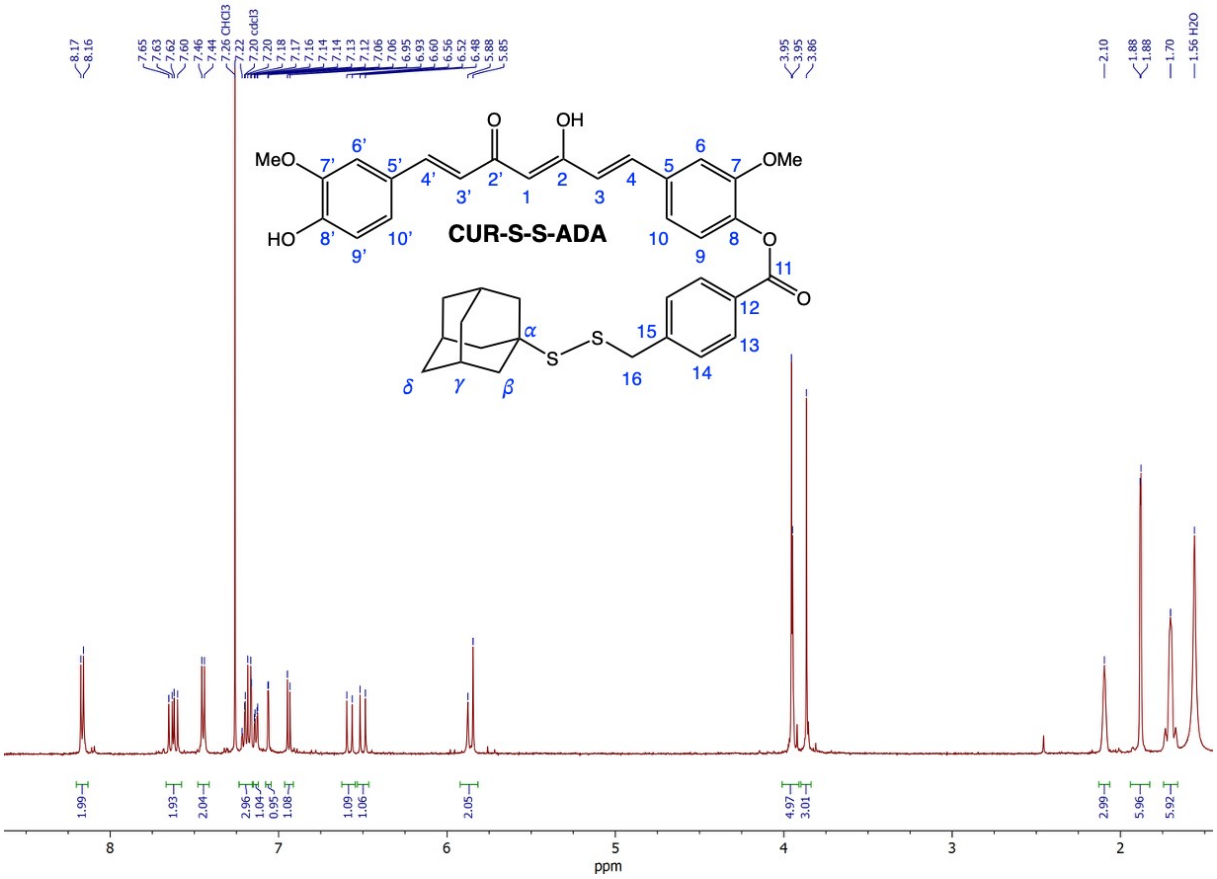


Figure S9.[1]H-NMR of compound **CUR-S-S-ADA** in $CDCl_3$

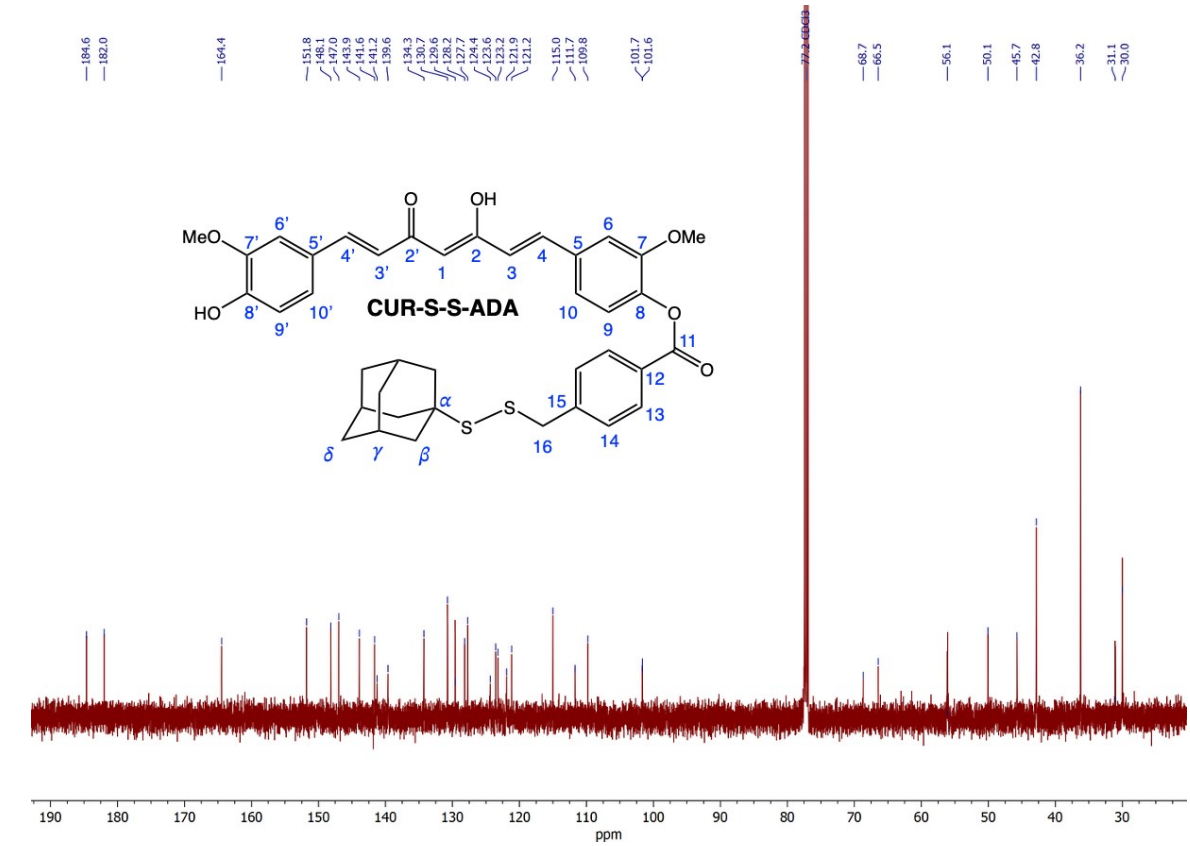


Figure S10. [13]C-NMR of compound **CUR-S-S-ADA** in $CDCl_3$

## Cell culture

The murine fibroblast NIH-3T3 cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were maintained at 37°C in a humidified atmosphere containing 5% $CO_2$ and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) heat-inactivated bovine serum, penicillin G, and streptomycin (100 mg/L). Prior to the formation of a confluent monolayer, cells were detached from the culture flask surface using a 0.25% trypsin solution and routinely subcultured two to three times per week.

## Cytotoxicity assay

NIH-3T3 cells (200 µL) were seeded into 96-well plates at a density of $1 \times 10^4$ cells per well. After 24 h, allowing for cell attachment, the cells were treated with increasing concentrations (0.02–100.00 µg/mL) of the tested compound. The plates were then incubated for 24 h at 37°C in a humidified atmosphere containing 5% $CO_2$. Following the incubation period, the culture medium was carefully aspirated, and the cells were washed three times with 200 µL of PBS. Subsequently, the cells were incubated for an additional 2 h in serum-free DMEM containing the tetrazolium salt thiazolyl blue tetrazolium bromide (MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) at a final concentration of 0.01%. At the end of the incubation, the medium was removed, and the resulting formazan crystals were solubilized by adding 100 µL of DMSO. The plate was gently agitated to ensure complete dissolution of the crystals, and the absorbance was measured at 570 nm using a microplate reader. The cytotoxicity assay was performed in quintuplicate, and the results are expressed as mean ± standard error (SE).

## Statistical analysis

Data are presented as means ± standard error (SE). Data were analyzed by one-way analysis of variance (ANOVA). The significance of the difference from the respective controls for each experimental test condition was assayed by using Dunnet for each paired experiment. A $p<0.05$ was regarded as indicating a significant difference.

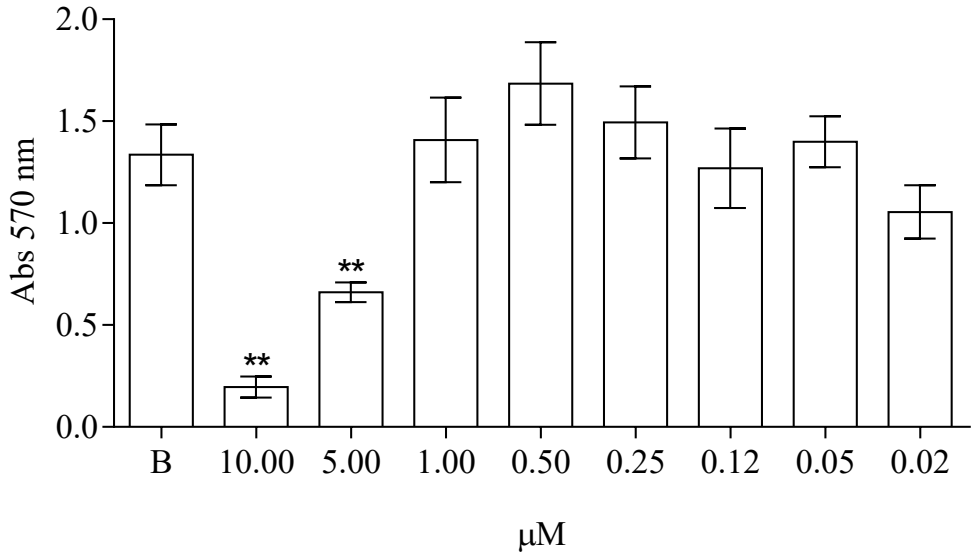


Figure S11. Viability of NIH-3T3 cells after 24 h incubation with different concentrations of **CUR-S-S-ADA**. Cell viability was determined using MTT assay. The data are expressed as mean±SE. B) indicate the control without tested compound. The asterisks (**) indicate a significant statistical difference at $p<0.05$.

**Stability studies**

The stability studies of CUR-S-S-ADA@SC6OH was evaluated by UV/Vis spectroscopy. An aqueous dispersion of the nanoassemblies was diluted 1:10 in PBS buffer 10 mM, pH 7.4, and the resulting sample was monitored over a 24 h period at 25 °C.

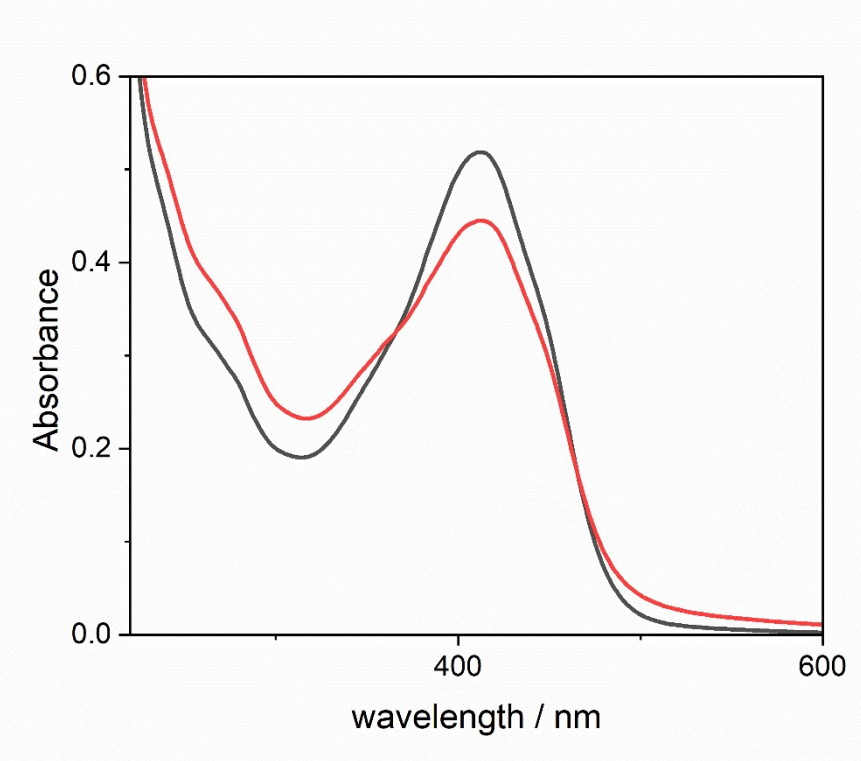


Figure S12. Stability studies as visualized by UV/Vis spectra of **CUR–S–S–ADA@SC6OH** nanoassemblies in PBS (10 mM, pH= 7.4) freshly prepared ( black trace) and upon storage for 24 h (red trace) at T= 25°C ([CUR-SS-ADA] = 20 μM, [SC6OH] = 40 μM, path length 1 cm).