Electronic Supplementary Material (ESI) for Chemical Science.

Thiophene bridged aldehydes (TBAs) image ALDH activity in cells via modulation of intramolecular charge transfer

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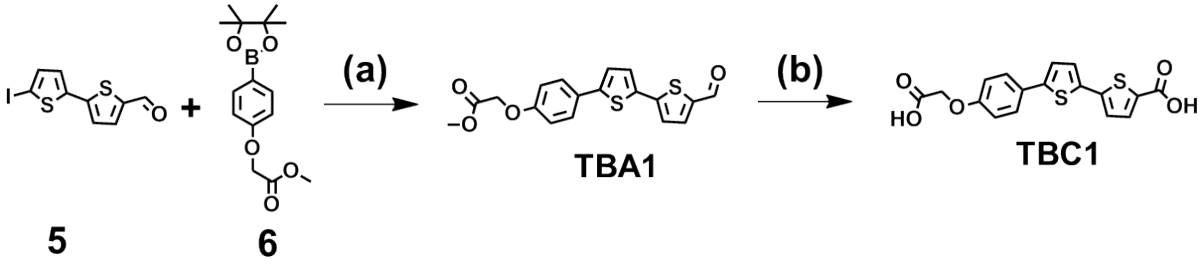
Table of contents

Materials and Methods: All the chemicals used in the synthesis of TBA were purchased from Sigma-Aldrich. 0.1 M phosphate buffered saline (PBS), syringes, needles, pipette tips, eppendorf tubes, and nuclear magnetic resonance (NMR) tubes were purchased from VWR. ^{1}H-NMR spectra were recorded in $CDCl_3$, Acetone-d_6 and DMSO-d_6 in a Bruker 300 MHz and 400 MHz spectrometer at 300K. TMS (δ (ppm) H = 0.00) was used as the internal reference. ^{13}C-NMR spectra were recorded in $CDCl_3$, Acetone-d_6 and DMSO-d_6 at a 100MHz on a bruker 900 MHz spectrometer, using the central resonances of $CDCl_3$ (δ (ppm) C = 77.23). Chemical shifts are reported in ppm and multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), and m (multiplet). Coupling constants, J, are reported in hertz (Hz). High-resolution mass spectra (HRMS) were obtained on an AB SCIEX TOF/TOF 5800 system and are reported as m/z (relative intensity). Accurate masses are reported for the molecular ion (M^+) or a suitable fragment ion. Chemicals were purchased from Aldrich or VWR and used without further purification. All solvents were purified using standard methods. Flash chromatography was carried out using silica gel (230-400 mesh). All reactions were performed under anhydrous conditions under N_2 or Argon and monitored by TLC on Kieselgel 60 F254 plates (Merck). Detection was accomplished by examination under UV light (254 nm). Recombinant ALDH1A1, ALDH1A3, ALDH2, ALDH3A1, ALDH3A2, ALDH4A1, ALDH5A1 and ALDH7A1 were prepared following literature procedures.[1-5] A549 cells were obtained from the UCB Cell Culture Facility, which is supported by The University of California Berkeley. A 1460 large covalent fragment library was purchased from Enamine. A LSM 710 was used for laser scanning confocal microscopy of A549 cells and imaging of histological sections. Fluorescent images were analyzed by ImageJ. A BD LSRFORTESSA X-20 was used for the flow cytometry experiments. Flow cytometry results were analyzed by flowing software 2.5.1. Female 6-8 weeks old C57BL6/J mice were purchased from Charles River. All animal experiments were performed in compliance with the relevant laws and institutional guidelines. An animal protocol has been submitted to the institutional committee that has approved the experiments. The glioblastoma tumor initiating cells (TICs) were obtained from Mcknight Brain Institute, Department of Neurosurgery, University of Florida, Gainesville, FL 32610, USA.

A. Synthesis of TBAs and TBCs

*A1. Synthesis of **TBA1***

In an oven dried two neck round bottom flask was added **5** (100 mg, 0.312 mmol), **6** (96 mg, 0.327 mmol) and triphenylphosphine (62 mg, 0.125 mmol). The resulting reaction mixture was dissolved into degassed and dry DMF (20 mL). Pd_2dba_3 (16 mg, 5 mol%) and cesium fluoride (141 mg, 0.936 mmol) were sequentially added to the



Scheme S1: **Synthesis of TBA1 and TBC1.** (a) $Pd_2(dba)_3$ (10 mol%), PPh_3, CsF, DMF, 60 °C, 18h, 82%. (b) $AgNO_3$, NaOH, rt, 4h, 67%.

reaction mixture. The reaction mixture was degassed and refilled with nitrogen five times and heated to 85 °C for 16h. The reaction mixture was poured into a large excess of water (120 mL) and extracted with ethyl acetate (3 x 35 mL). The organic layers were collected together and washed with brine (3 x 20 mL) and dried over anhydrous Na_2SO_4. The ethyl acetate was removed under reduced pressure to afford a yellow oil. The crude product was purified by silica gel chromatography to afford a solid product. TBA1 was purified by silica gel column chromatography (Toluene/DCM (8/2) to afford TBA1 (92 mg, 82%) as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.90 (s, 1H), 7.71 (d, 1H, *J* = 4.0 Hz), 7.61 (d, 2H, *J* = 8.0 Hz), 7.35 (d, 1H, *J* = 4.0 Hz), 7.29 (d, 1H, *J* = 4.0 Hz), 7.28 (d, 1H, *J* = 4.0 Hz), 7.20 (d, 1H, *J* = 4.0 Hz), 6.99 (d, 2H, *J* = 8.0 Hz), 4.72 (s, 2H), 3.87 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 183.4 (s), 168.8 (s), 157.7 (s), 145.4 (s), 144.8 (s), 140.9 (s), 138.8 (s), 133.2 (s), 127.9 (s), 126.7 (d), 126.1 (s), 124.6 (s), 124.0 (s), 115.2 (s), 64.6 (s), 51.6 (s); HRMS (EI, +ve): Calculated for $C_{18}H_{15}O_4S_2$: 359.0334, found: 359.0351.

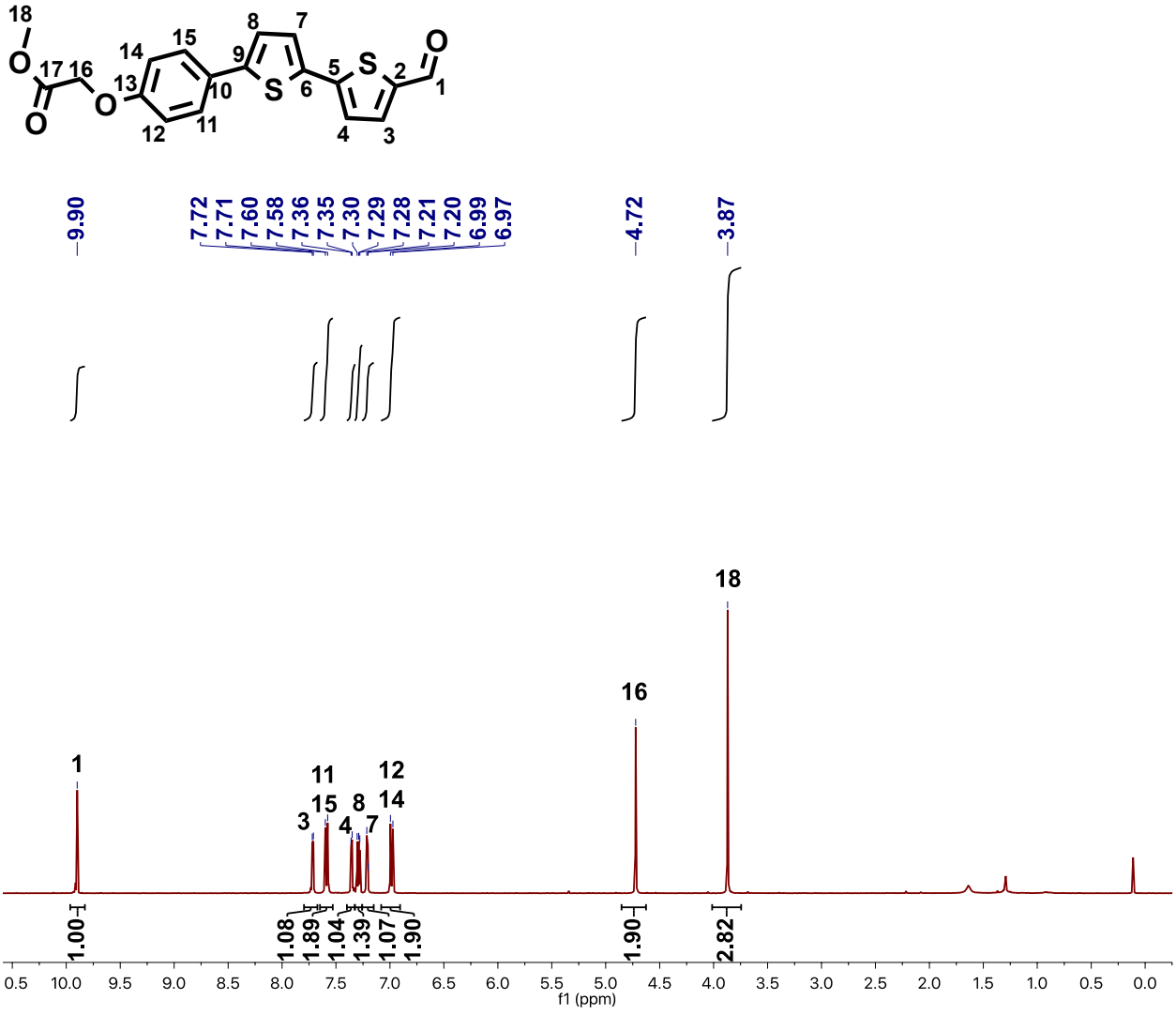


Figure S1: ^{1}H NMR spectra of TBA1.

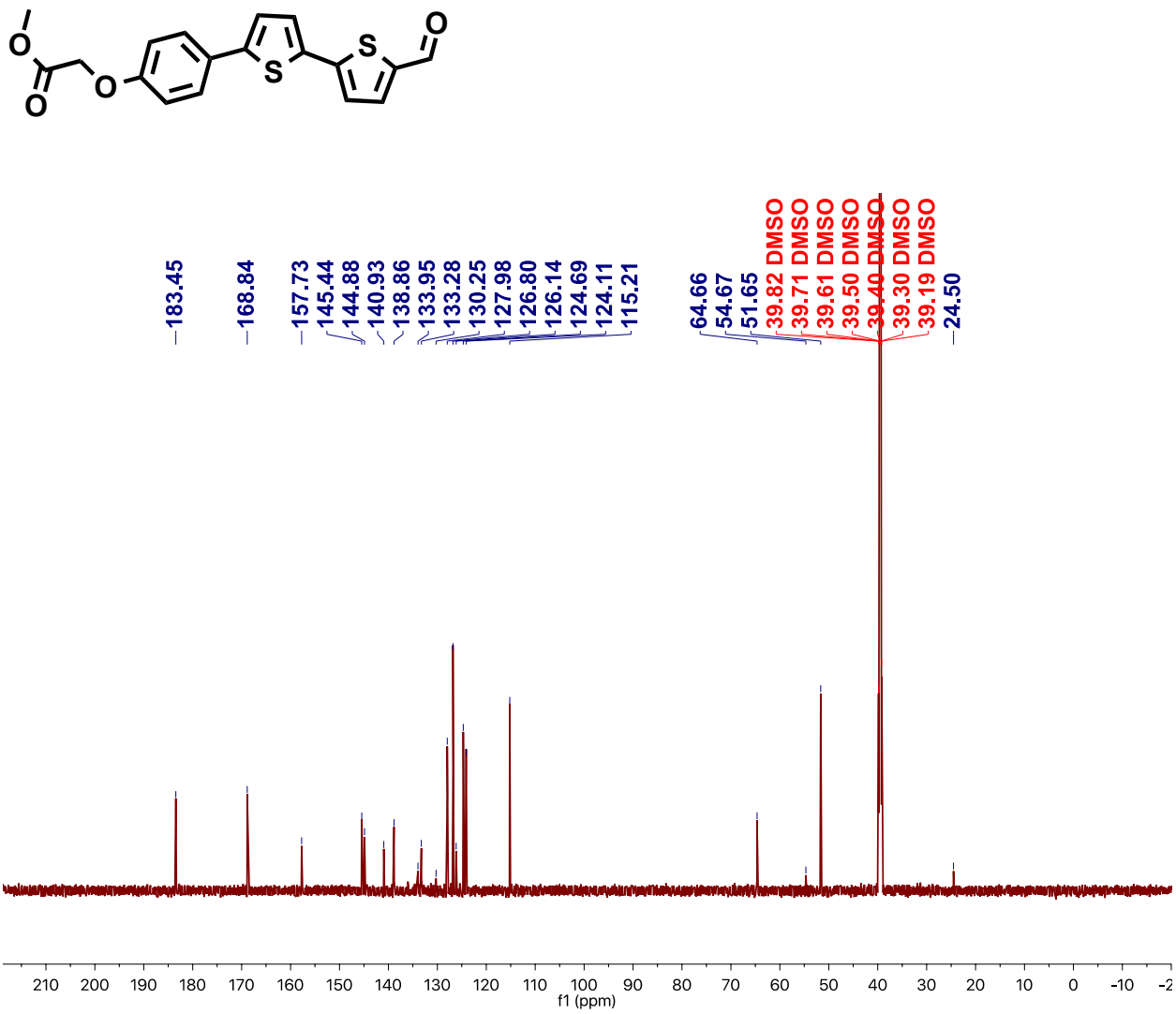


Figure S2: ^{13}C NMR of TBA1.

*A2. Synthesis of **TBC1***

To a rapidly stirred solution of 10% NaOH (3 mL) was slowly added silver nitrate (1.4 g, 8.2 mmol). To this reaction mixture was added TBA1 (50 mg, 0.139 mmol), and the resulting reaction mixture was vigorously stirred for 3h. The reaction mixture was poured into a large excess of water (60 mL) and the pH was adjusted to pH=2. The acidified aqueous phase was extracted with ethyl acetate (3x 20 mL) and the combined ethyl acetate was evaporated to dryness. The solid crude product was suspended into DCM (30 mL) and filtered through Buchner to afford TBC1 (26 mg, 53%) as a bright yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.64 (d, 1H, J = 4.0 Hz), 7.60 (d, 2H, J = 8.0 Hz), 7.45 (d, 1H, J = 4.0 Hz), 7.40 (d, 1H, J = 4.0 Hz), 7.33 (d, 1H, J = 4.0 Hz), 6.94 (d, 2H, J = 8.0 Hz), 4.70 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.4 (s), 163.0 (s), 158.2 (s), 144.3 (s), 143.2 (s), 134.7 (s), 134.0 (s), 127.5 (s), 127.2 (s), 126.5 (s), 124.7 (s), 124.3 (s), 115.6 (s), 64.9 (s); HRMS (ESI, -ve): Calculated for $C_{17}H_{11}O_5S_2$: 359.0126, found: 358.0231.

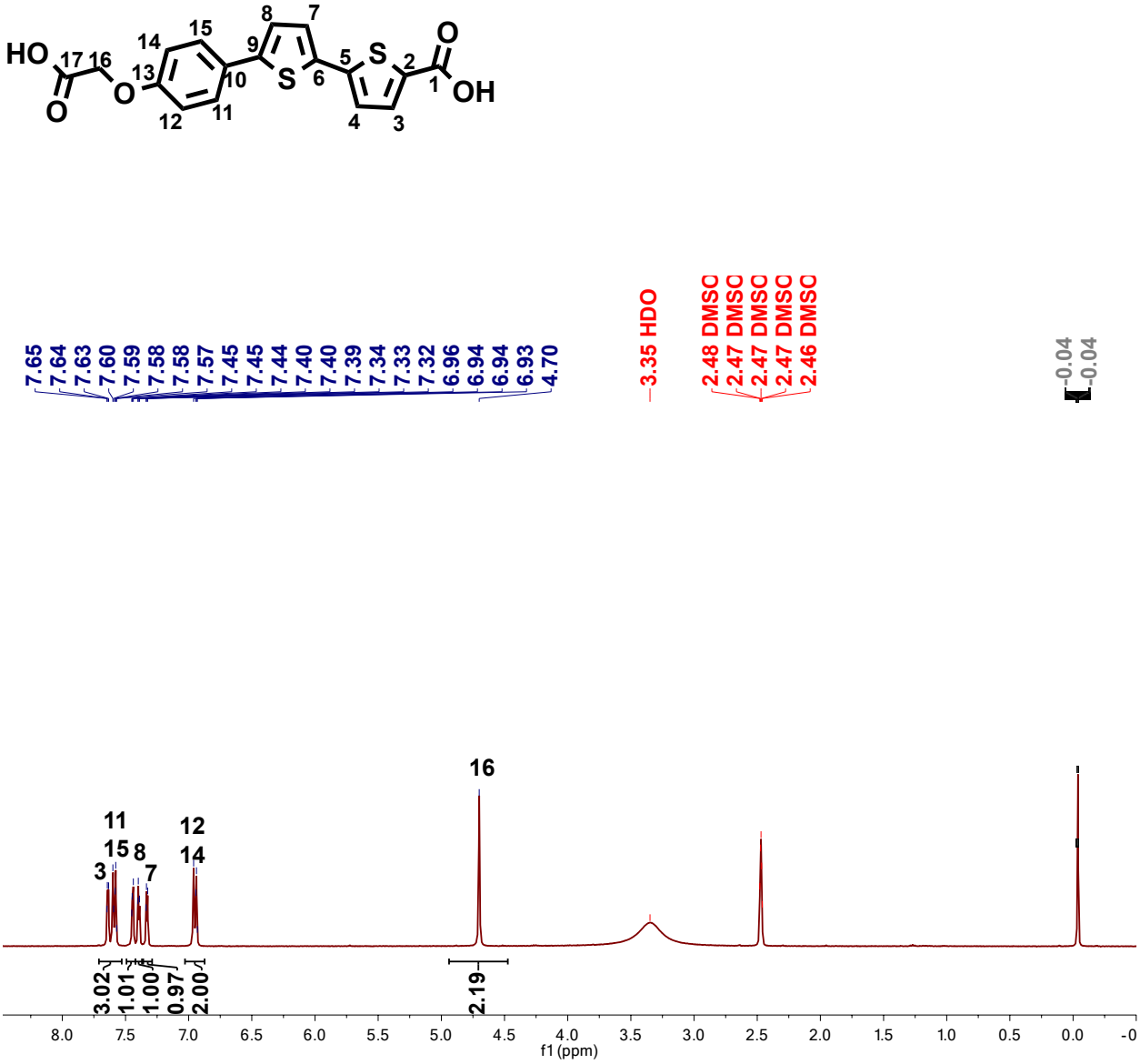


Figure S3: ^{1}H NMR of TBC1.

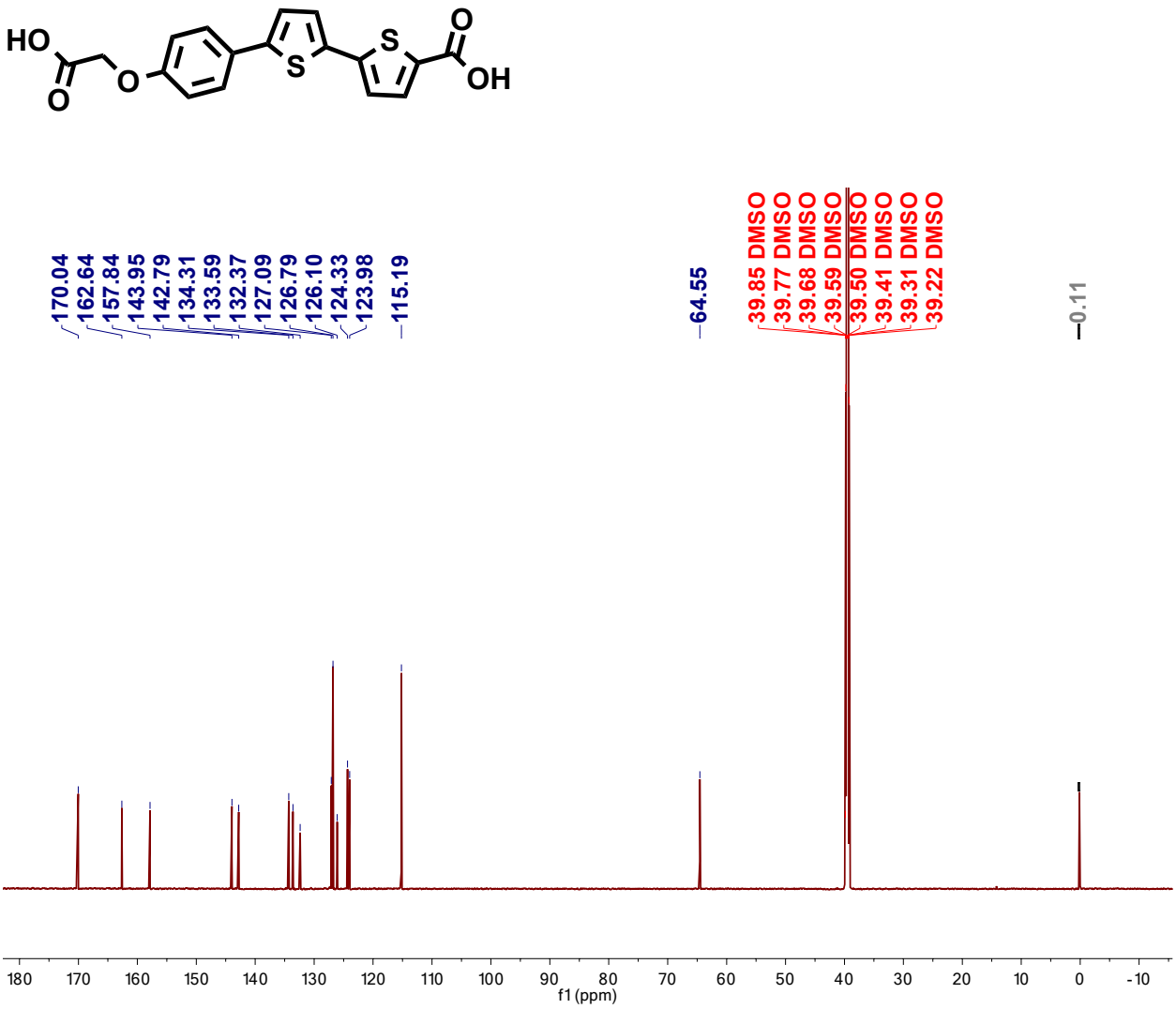
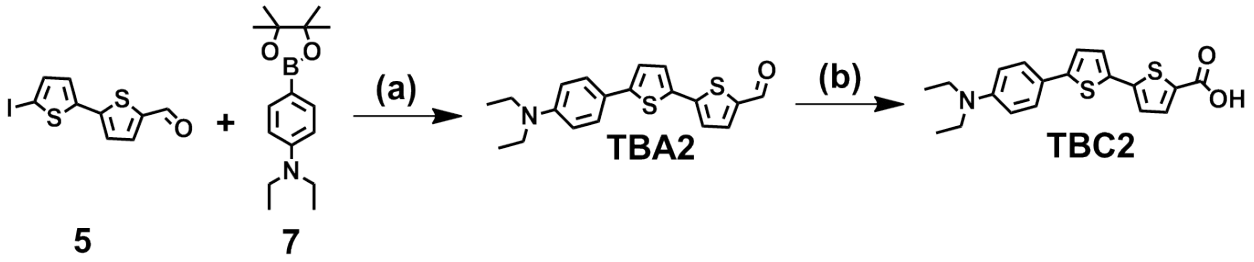


Figure S4: ^{13}C NMR spectra of TBC1.



Scheme S2: **Synthesis of TBA2 and TBC2.** (a) $Pd_2(dba)_3$ (10 mol%), PPh_3, CsF, DMF, 60 °C, 18h, 82%. (b) $AgNO_3$, NaOH, rt, 4h, 61%.

*A3. Synthesis of **TBA2***

The synthesis of TBA2 has been reported in the literature.[6] We employed a different synthetic method to make TBA2, described below. In an oven dried two neck round bottom flask was added **5** (100 mg, 0.312 mmol), **7** (103 mg, 0.315 mmol) and triphenylphosphine (62 mg, 0.125 mmol). The resulting reaction mixture was dissolved into degassed and dry DMF (20 mL). Pd_2dba_3 (16 mg, 5 mol%) and cesium fluoride (141 mg, 0.936 mmol) were sequentially added to the reaction mixture. The reaction mixture was degassed and refilled with nitrogen five times and heated to 85 °C for 16h. The reaction mixture was poured into a large excess of water (120 mL) and extracted with ethyl acetate (3 x 35 mL). The organic layers were collected together and washed with brine (3 x 20 mL) and dried over anhydrous Na_2SO_4. The ethyl acetate was removed under reduced pressure to afford a yellow oil. The crude product was purified by silica gel chromatography to afford a solid product. The crude product was purified by silica gel chromatography (DCM/hexane (9/1)) to achieve 262 mg (82%) of TBA2 as a deep red solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.83 (s, 1H), 7.66 (d, 1H, *J* = 4.0 Hz), 7.48 (d, 2H, *J* = 8.0 Hz), 7.30 (d, 1H, *J* = 4.0 Hz), 7.21 (d, 1H, *J* = 4.0 Hz), 7.09 (d, 1H, *J* = 4.0 Hz), 6.68 (d, 2H, *J* = 8.0 Hz), 3.43-3.37 (q, 4H, *J* = 6.0 Hz), 1.22-1.18 (t, 6H, *J* = 6.0 Hz, 9.0 Hz); ^{13}C (100 MHz, $CDCl_3$): δ 182.2 (s), 148.1 (s), 143.4 (s), 141.1 (s), 137.4 (s), 132.7 (s), 130.6 (s), 129.1 (s), 128.5 (s), 123.4 (s), 121.7 (s), 44.65 (s), 12.83 (s); HRMS (EI, +ve): Calculated for $C_{19}H_{19}NOS_2$: 342.0918, found: 342.0973.

*A4. Synthesis of **TBC2***

To a rapidly stirred solution of 10% NaOH (3 mL) was slowly added silver nitrate (1.4 g, 8.2 mmol). To this reaction mixture was added TBA2 (50 mg, 0.15 mmol), and the resulting reaction mixture was vigorously stirred for 3h. The reaction mixture was poured into a large excess of water (60 mL) and adjusted to pH=3. The acidified aqueous phase was extracted with ethyl acetate (3x 20 mL) and the combined organic phases were dried over Na_2SO_4. The crude product was purified over silica gel (acetonitrile/water (9/1) to afford compound TBC2 (33 mg, 61%) as a yellow solid. ^{1}H NMR (400 MHz, Acetone-d_6): δ 7.72 (d, 1H, *J* = 4.0 Hz), 7.53 (d, 2H, *J* = 8.0 Hz), 7.51 (d, 1H, *J* = 4.0 Hz), 7.39 (d, 1H, J = 4.0 Hz), 7.24 (d, 1H, J = 4.0 Hz), 6.77 (d, 2H, *J* = 8.0 Hz) 3.49-3.44 (q, 4H, *J* = 8.0 Hz), 1.21-1.08 (t, 6H, *J* = 8.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.6 (s), 147.3 (s), 145.6 (s), 143.3 (s), 134.3 (s), 131.6 (s), 127.0 (s), 126.6 (s), 123.6 (s), 121.6 (s), 119.6 (s), 111.5 (s), 43.2 (s), 12.4 (s); HRMS (ESI, -Ve): Calculated for $C_{19}H_{18}NO_2S_2$: 356.0857, found: 356.0789.

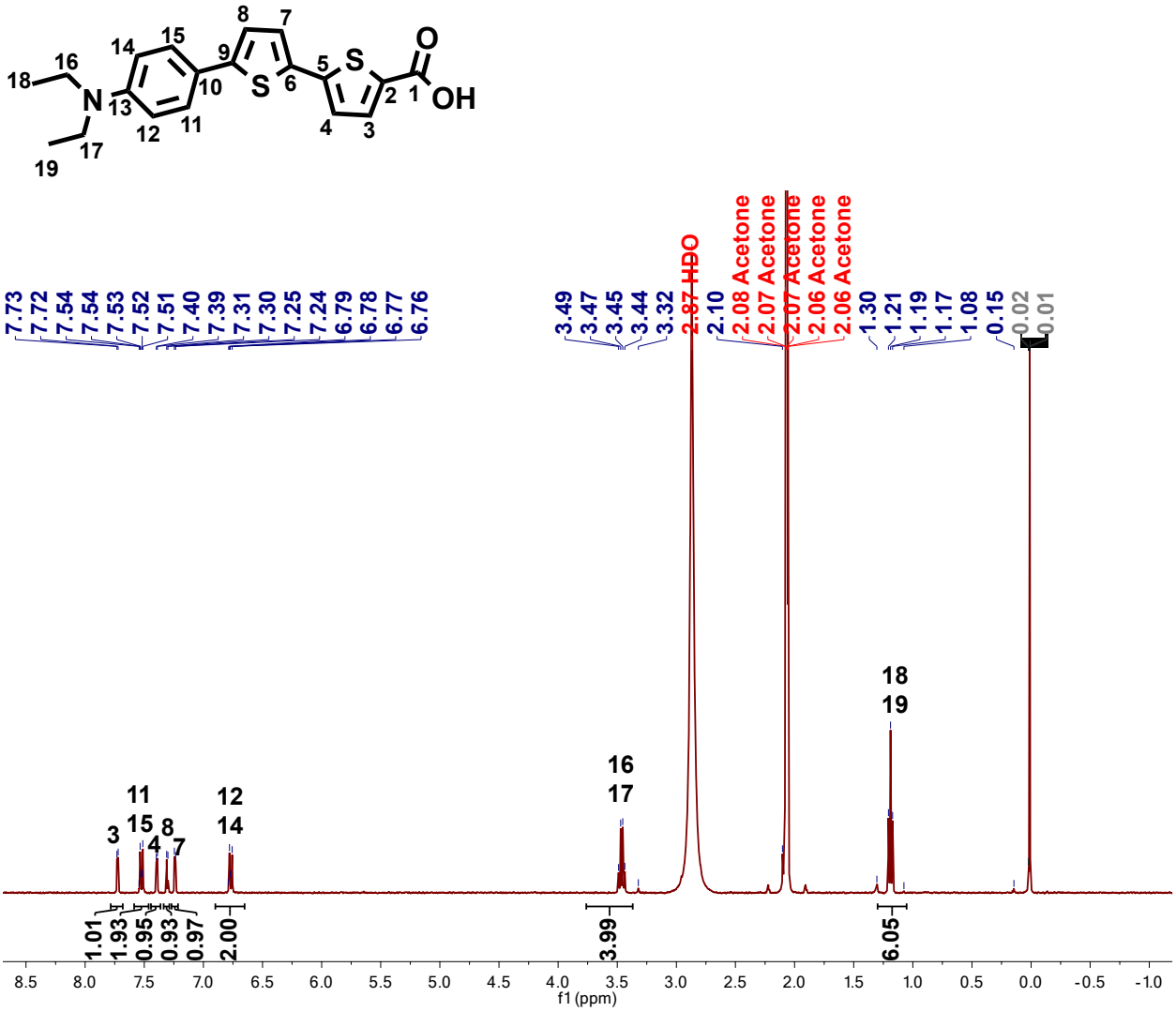


Figure S5: ^{1}H NMR of TBC2.

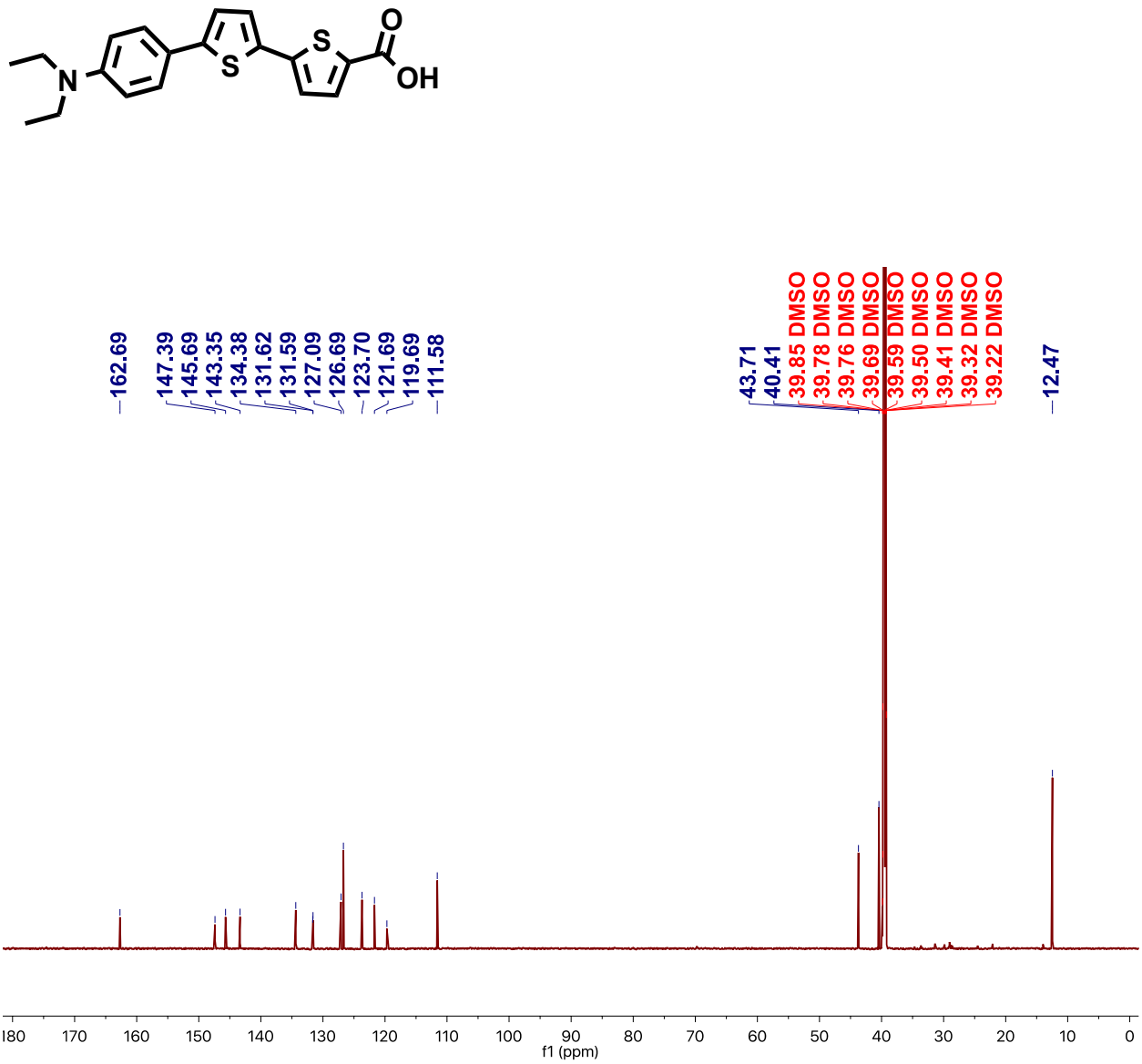
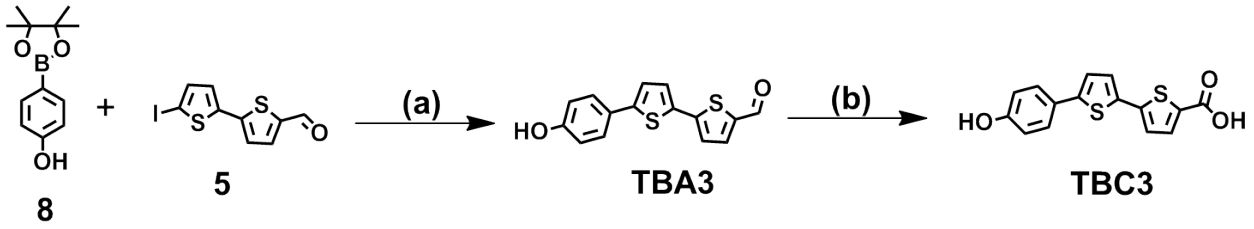


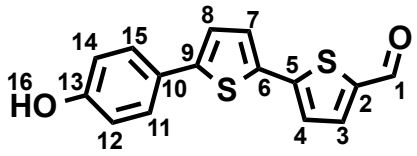
Figure S6: ^{13}C NMR spectra of TBC2.



Scheme S3: **Synthesis of TBA3 and TBC3.** (a) $Pd_2(dba)_3$ (10 mol%), PPh_3, CsF, DMF, 60 °C, 18h, 52%. (d) $AgNO_3$, NaOH, rt, 4h, 11%.

*A5. Synthesis of **TBA3***

Synthesis of TBA3 is reported in the literature.[7] However, we have employed different synthetic protocol. In an oven dried two neck round bottom flask was added **5** (100 mg, 0.312 mmol), **8** (96 mg, 0.327 mmol) and triphenylphosphine (62 mg, 0.125 mmol). The resulting reaction mixture was dissolved into degassed and dry DMF (20 mL). Pd_2dba_3 (16 mg, 5 mol%) and cesium fluoride (141 mg, 0.936 mmol) were sequentially added to the reaction mixture. The reaction mixture was degassed and refilled with nitrogen five times and heated to 85 °C for 16h. The reaction mixture was poured into a large excess of water (120 mL) and extracted with ethyl acetate (3 x 35 mL). The organic layers were collected together and washed with brine (3 x 20 mL) and dried over anhydrous Na_2SO_4. The ethyl acetate was removed under reduced pressure to afford a yellow oil. The crude product was purified by silica gel chromatography (toluene/acetone (19/5)) to achieve TBA3 (73 mg, 82%) as a deep yellow solid. ^{1}H NMR (400 MHz, Acetone-d_6): δ 9.87 (S, 2H), 7.99 (d, 1H, *J* = 4.0 Hz), 7.57-7.51 (m, 4H), 7.38 (d, 1H, *J* = 4.0 Hz), 6.85 (d, 2H, *J* = 8.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 183.7 (s), 157.9 (s), 145.9 (s), 145.8 (s), 140.8 (s), 139.1 (s), 132.5 (s), 128.2 (s), 127.0 (s), 124.6 (s), 123.9 (s), 123.3 (s), 116.0 (s); HRMS (EI, +ve): Calculated for $C_{15}H_{11}O_2S_2$: 287.0122, found: 287.0131.



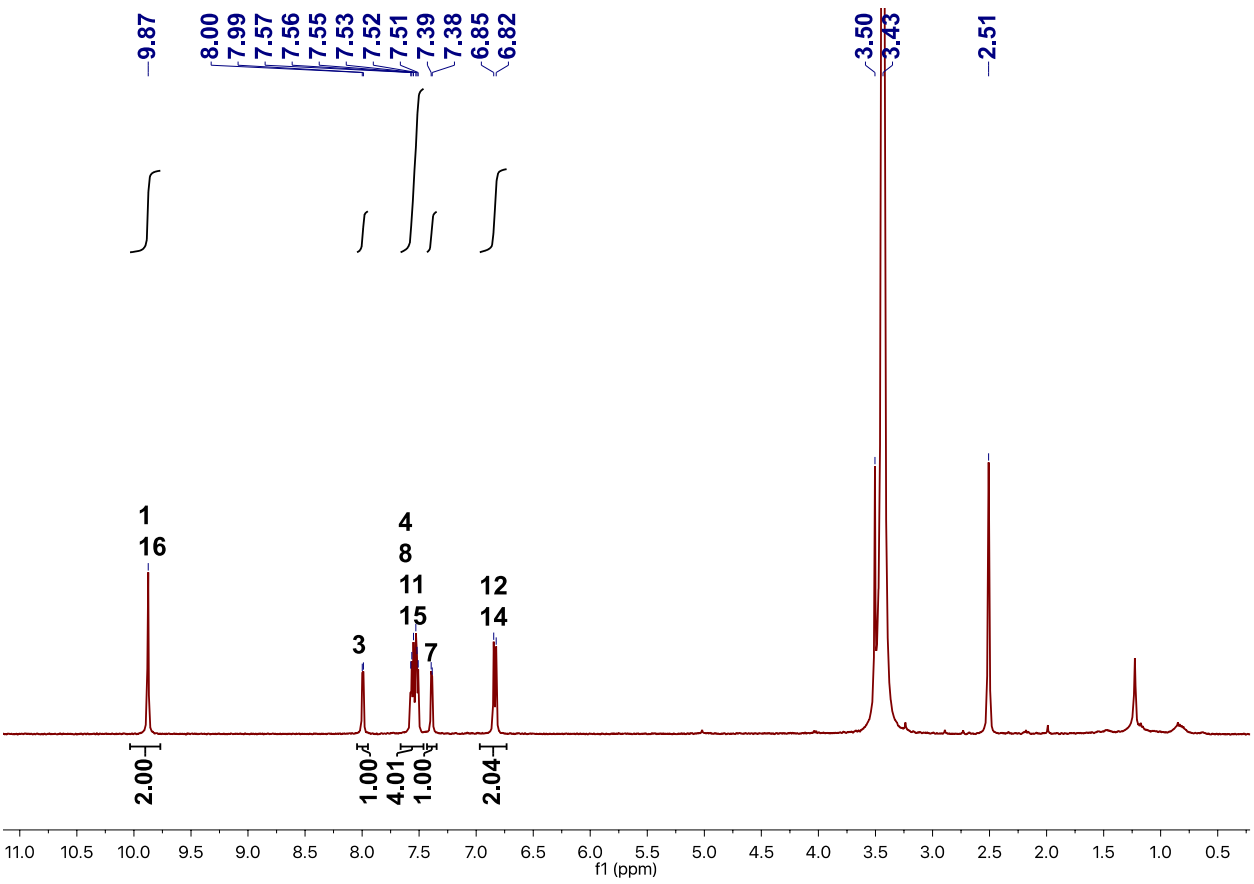


Figure S7: ^{1}H NMR spectra of TBA3.

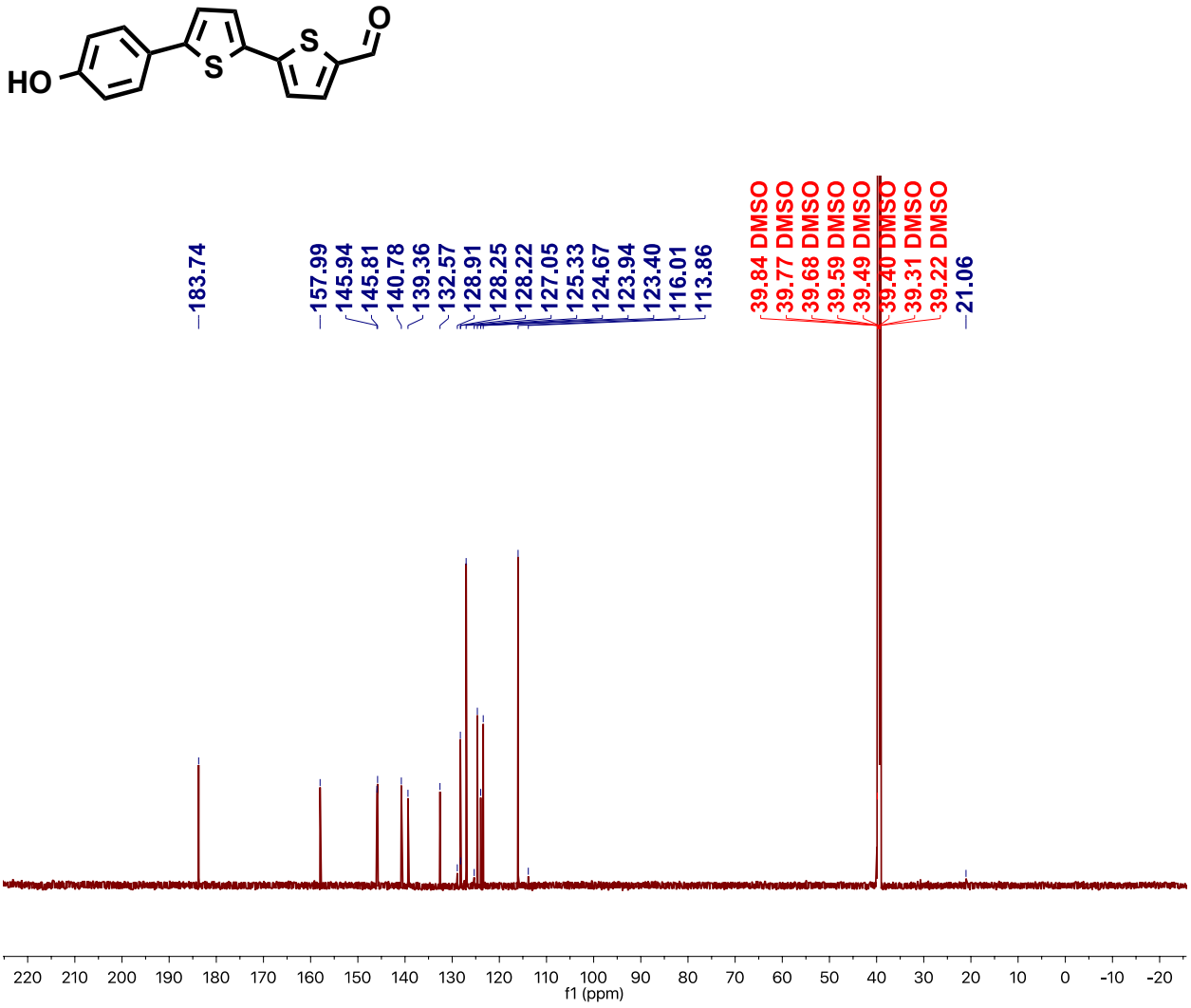


Figure S8: ^{13}C NMR spectra of TBA3.

*A6. Synthesis of **TBC3***

To a rapidly stirred solution of 10% NaOH (3 mL) was slowly added silver nitrate (1.4 g, 8.2 mmol). To this reaction mixture was added TBA3 (35 mg, 0.12 mmol), and the resulting reaction mixture was vigorously stirred for 3h. The reaction mixture was poured into a large excess of water (60 mL) and adjusted to pH=3. The acidified aqueous phase was extracted with ethyl acetate (3x 20 mL) and the combined organic phases were dried over Na_2SO_4. The crude product was recrystallized from DCM-acetone (1:1) to afford TBC3 (10 mg, 11%) as yellow solid. ^1H NMR (400 MHz, Acetone-d_6): 7.72 (d, 1H, *J* = 4.0 Hz), 7.58 (d, 2H, J = 8.0 Hz), 7.55 (d, 1H, J = 4.0 Hz), 7.41-7.32 (m, 2H), 6.92 (d, 2H, *J* = 4.0 Hz); ^{13}C NMR (125 MHz, Acetone-d_6): 163.1 (s), 158.8 (s), 146.29 (s), 144.7 (s), 135.2 (s), 134.6 (s), 132.9 (s), 127.9 (s), 127.4 (s),

126.0 (s), 124.6 (s), 123.7 (s), 116.8 (s); HRMS (ESI, -Ve): Calculated for $C_{15}H_{10}O_3S_2$: 301.0071, found: 301.0182.

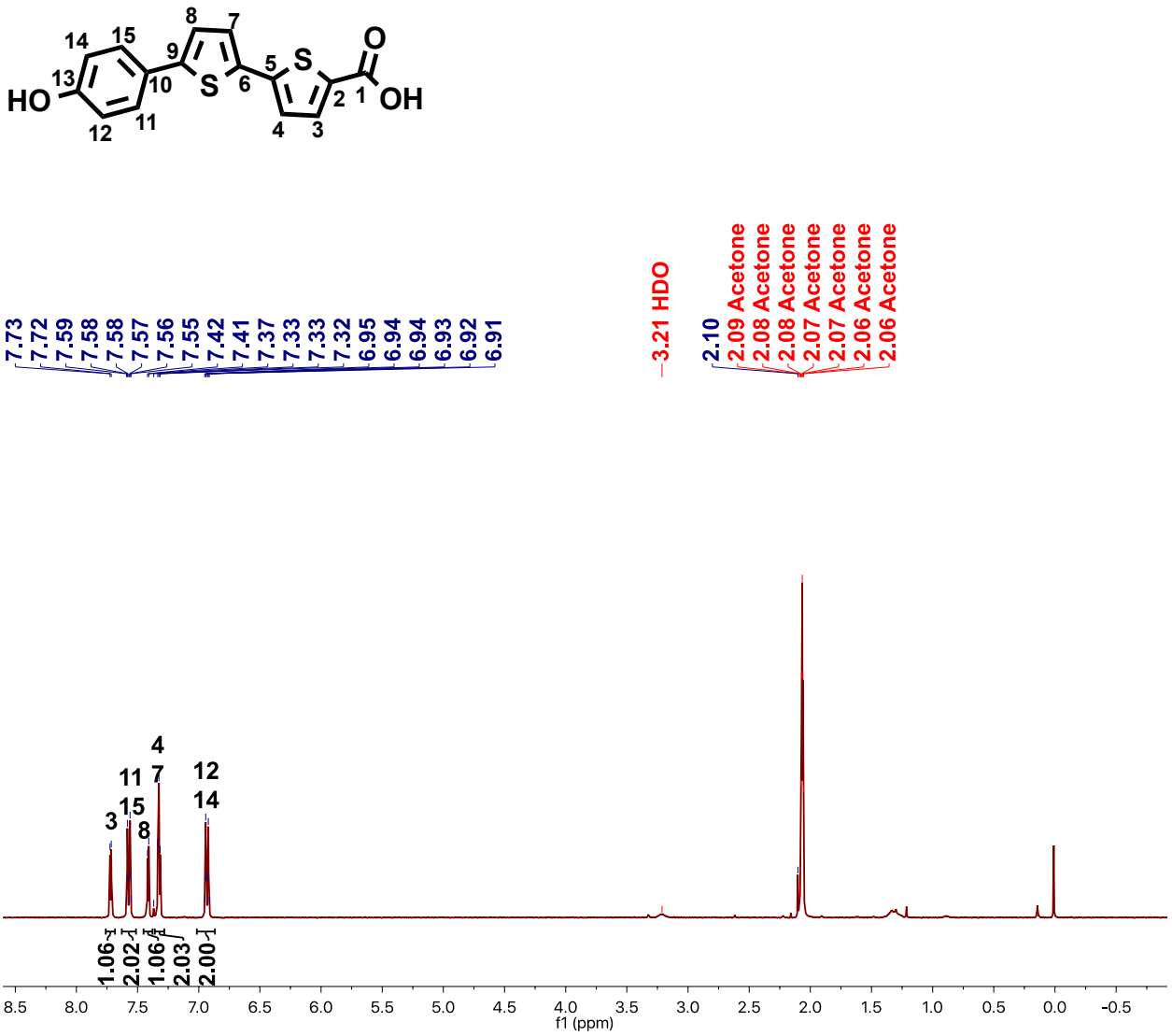
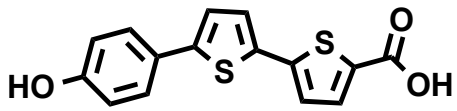


Figure S9: ^{1}H NMR of TBC3.



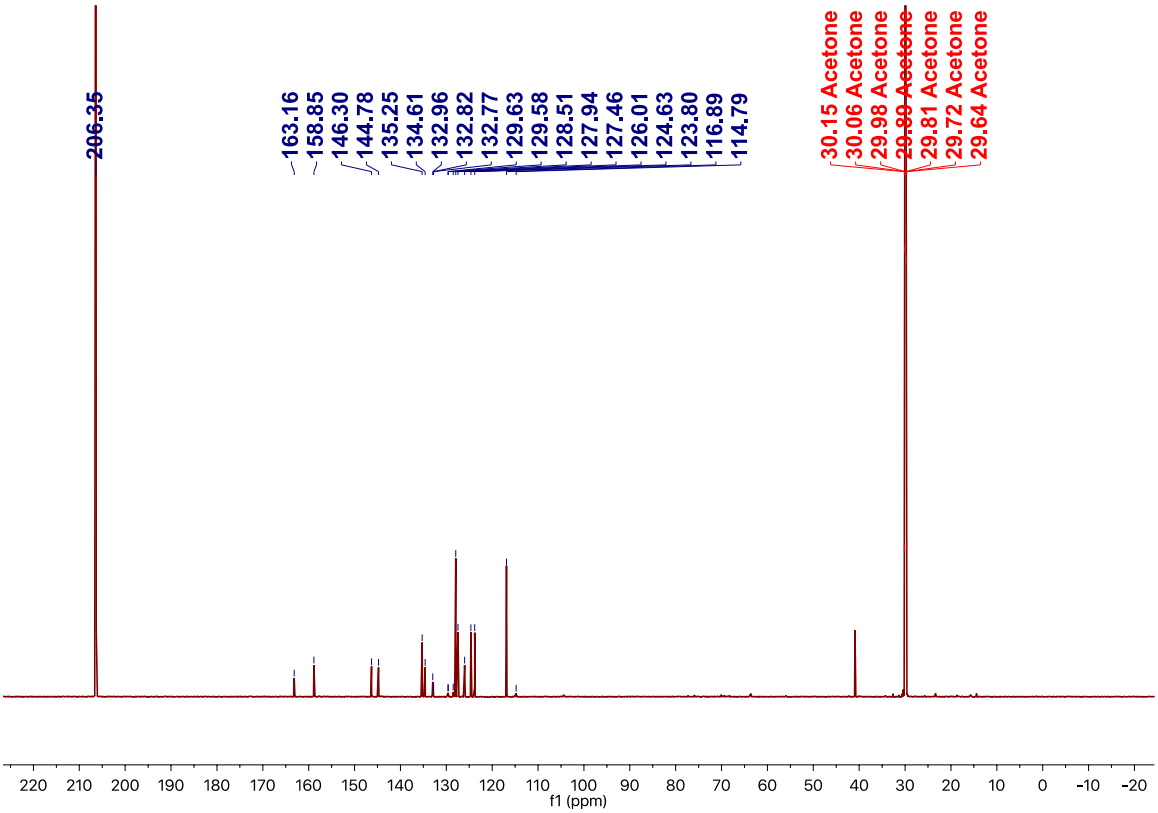
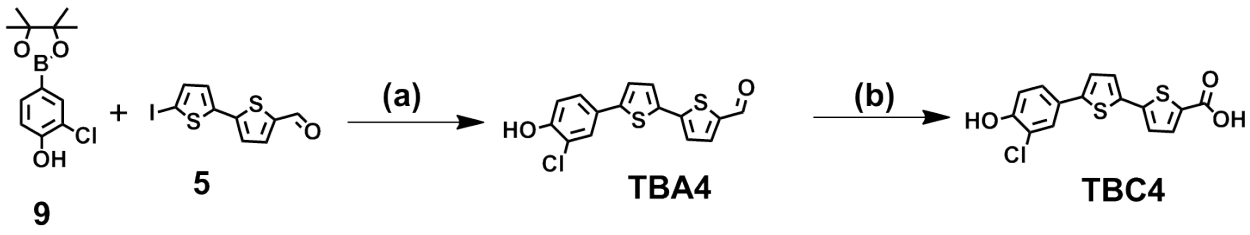


Figure S10: ^{13}C NMR spectra of TBC3.



Scheme S4: **Synthesis of TBA4 and TBC4.** (a) $Pd_2(dba)_3$ (10 mol%), PPh_3, CsF, DMF, 60 °C, 18h, 72%. (d) $AgNO_3$, NaOH, rt, 4h, 41%.

*A7. Synthesis of **TBA4**:*

In an oven dried two neck round bottom flask was added **5** (100 mg, 0.312 mmol), **9** (80 mg, 0.327 mmol) and triphenylphosphine (62 mg, 0.125 mmol). The resulting reaction mixture was dissolved into degassed and dry DMF (20 mL). Pd_2dba_3 (16 mg, 5 mol%) and cesium fluoride (141 mg, 0.936 mmol) were sequentially added to the reaction mixture. The reaction mixture was degassed and refilled with nitrogen five times and heated to 85 °C for 16h. The reaction mixture was poured into a large excess of water (120 mL) and extracted with ethyl acetate (3 x 35 mL). The organic layers were collected together and washed with brine (3 x 20 mL) and dried over anhydrous Na_2SO_4. The ethyl acetate was removed under reduced pressure to afford a yellow oil. The crude product was purified by silica gel column chromatography (toluene/acetone=19/1) to afford TBA4 (73 mg, 72%) as a yellow solid. 1H NMR (400 MHz, Acetone-d_6): δ 10.65 (brs, 1H), 9.88 (s, 1H), 7.98 (d, 1H, J = 4.0 Hz), 7.71 (s, 1H), 7.71-7.47 (m, 4H), 7.02 (d, 1H, J = 8.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 183.3 (s), 153.3 (s), 145.3 (s), 143.9 (s), 140.9 (s), 138.7 (s), 133.2 (s), 127.8 (s), 126.5 (s), 125.3 (s), 124.6 (s), 124.1 (s), 120.0 (s), 117.1 (s); (HRMS (ESI, +ve): Calculated for $C_{15}H_{10}ClO_2S_2$: 320.9732, found: 320.0103.

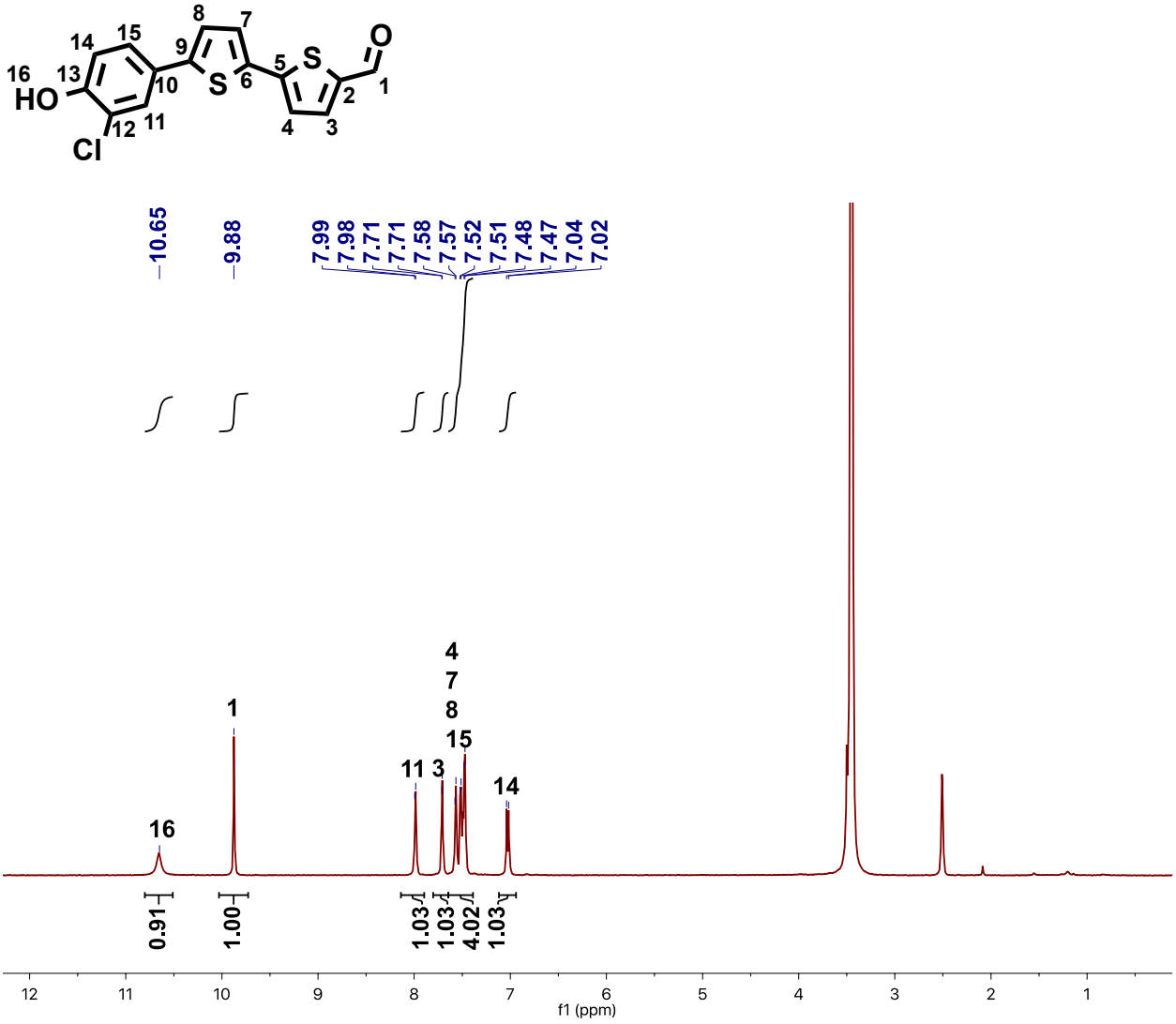
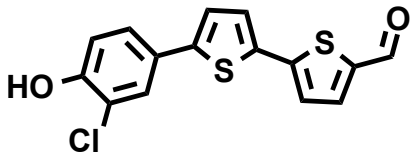


Figure S11: 1H NMR spectra of TBA4.



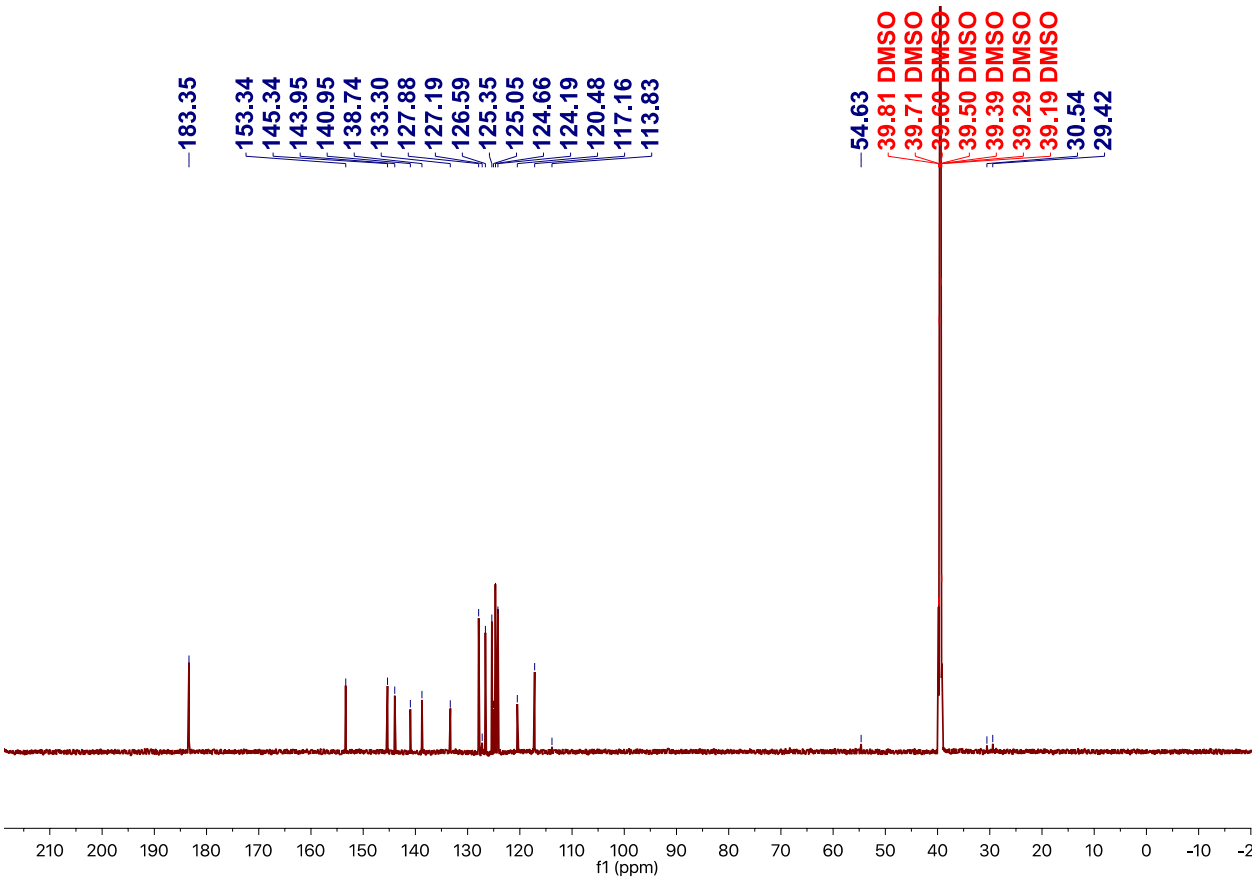
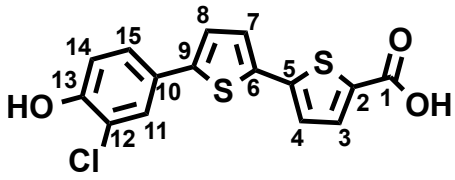


Figure S12: ^{13}C NMR spectra of TBA4.

*A8. Synthesis of **TBC4**:*

To a rapidly stirred solution of 10% NaOH (3 mL) was slowly added silver nitrate (1.4 g, 8.2 mmol). To this reaction mixture was added TBA4 (38 mg, 0.12 mmol), and the resulting reaction mixture was vigorously stirred for 3h. The reaction mixture was poured into a large excess of water (60 mL) and adjusted to pH=3. The acidified aqueous phase was extracted with ethyl acetate (3x 20 mL) and the combined organic phases were dried over Na_2SO_4. The crude product was purified by silica gel column chromatography (acetonitrile/water=4/1) to afford TBC4 (17 mg, 41%) as a yellow solid. ^1H NMR (400 MHz, Acetone-d_6): δ 7.74 (d, 1H, J = 4.0 Hz), 7.71 (d, 1H, J = 4.0 Hz), 7.54-7.51 (dd, 1H, J = 4.0 Hz), 7.44 (d, 1H, J = 4.0 Hz), 7.41 (d, 1H, J = 4.0 Hz), 7.36 (d, 1H, J = 4.0 Hz), 7.10 (d, 1H, J = 8.0 Hz); ^{13}C NMR (125 MHz, Acetone-d_6): δ 162.0 (s), 153.1 (s), 143.5 (s), 143.4 (s), 134.5 (s), 134.4 (s), 132.1 (s), 126.8 (s), 126.6 (s), 126.5 (d), 124.0, 123.9, 120.9, 117.2; HRMS (ESI, -ve): Calculated for $C_{15}H_8ClO_3S_2$:, 334.9682, found: 334.9769.



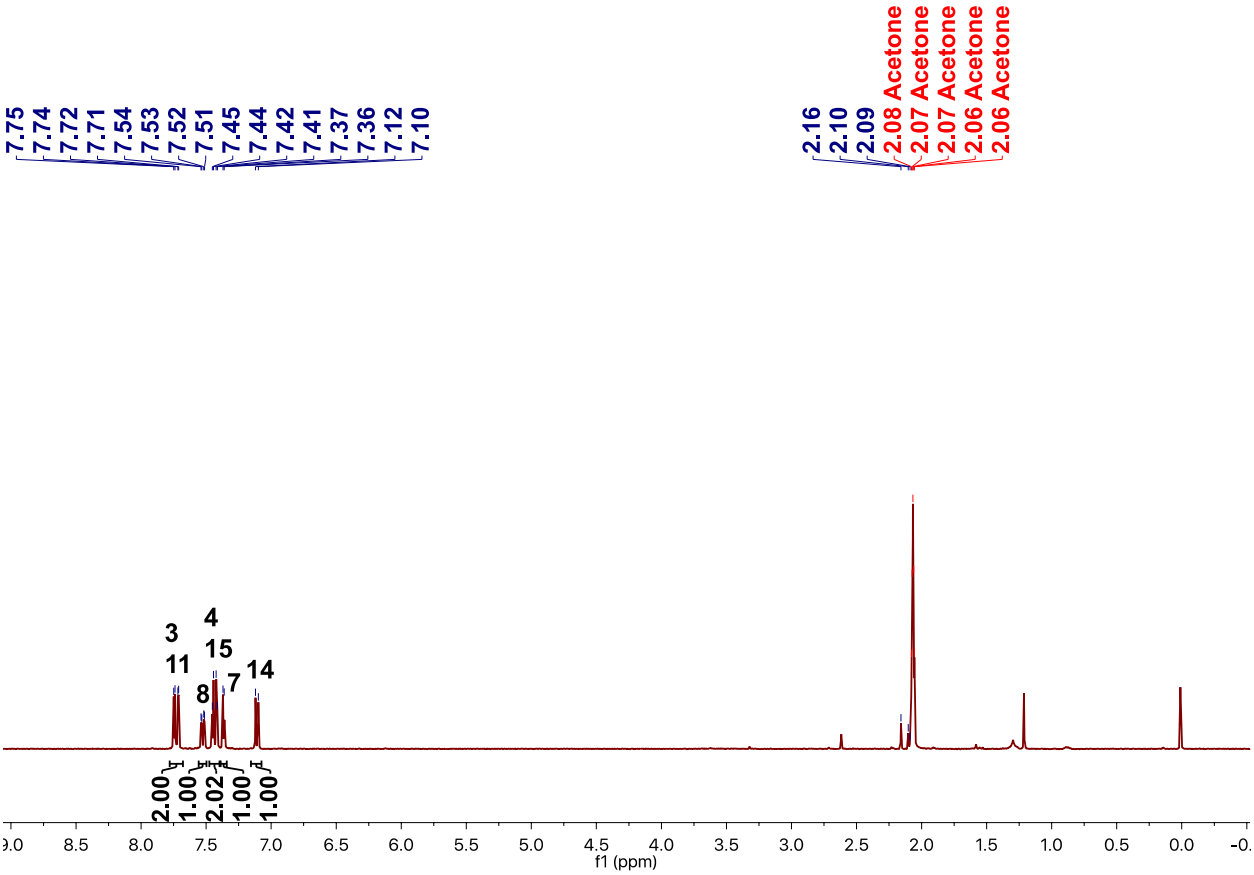
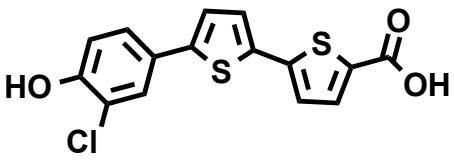


Figure S13: ^{1}H NMR spectra of TBC4.



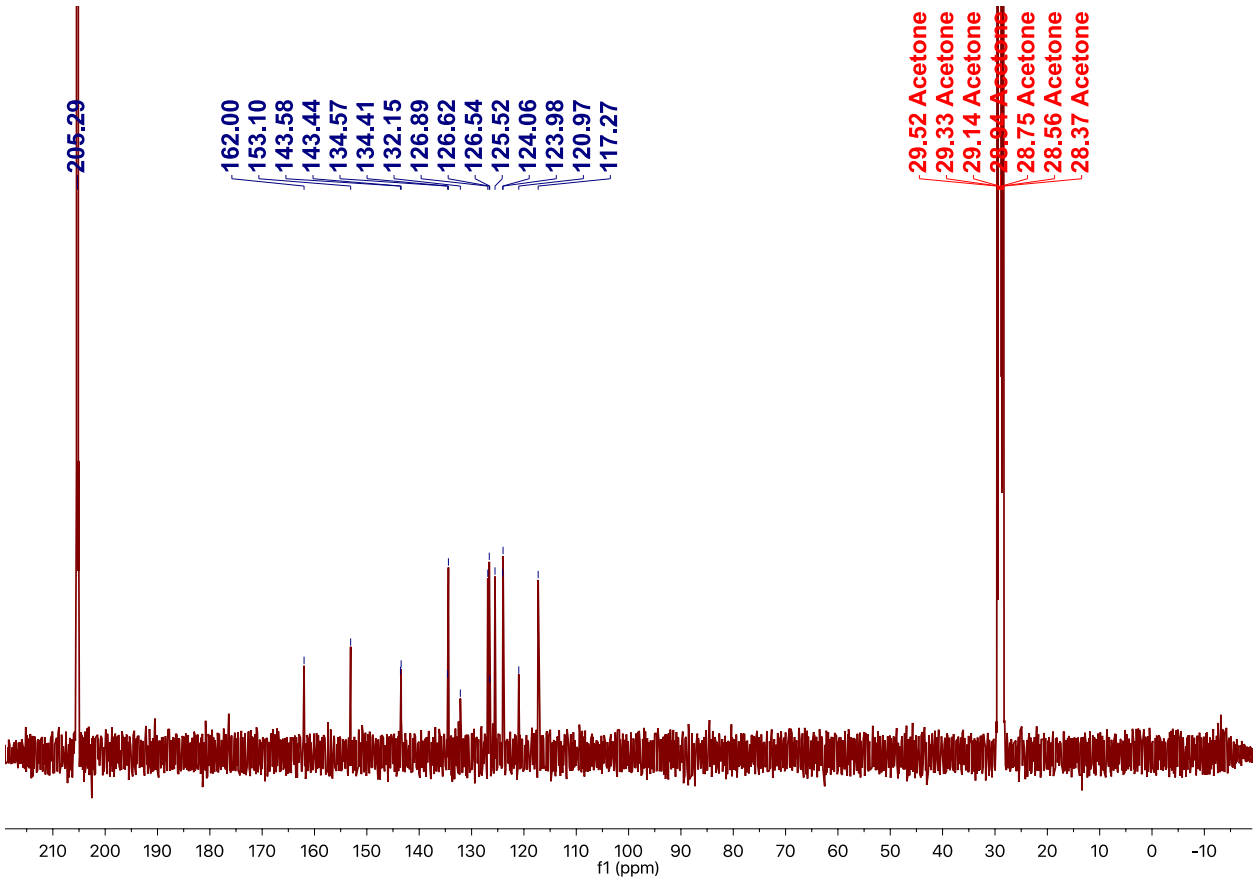


Figure S14: ^{13}C NMR spectra of TBC4.

B. Absorption and fluorescence spectra of the TBAs and the TBCs

TBAs and TBCs were dissolved into DMSO (2 mM) and were diluted to a 120 μM concentration in PBS containing 50 mM methyl-β-cyclodextrin (100 μL). The absorbance and fluorescence spectra of the TBAs and TBCs were measured in a

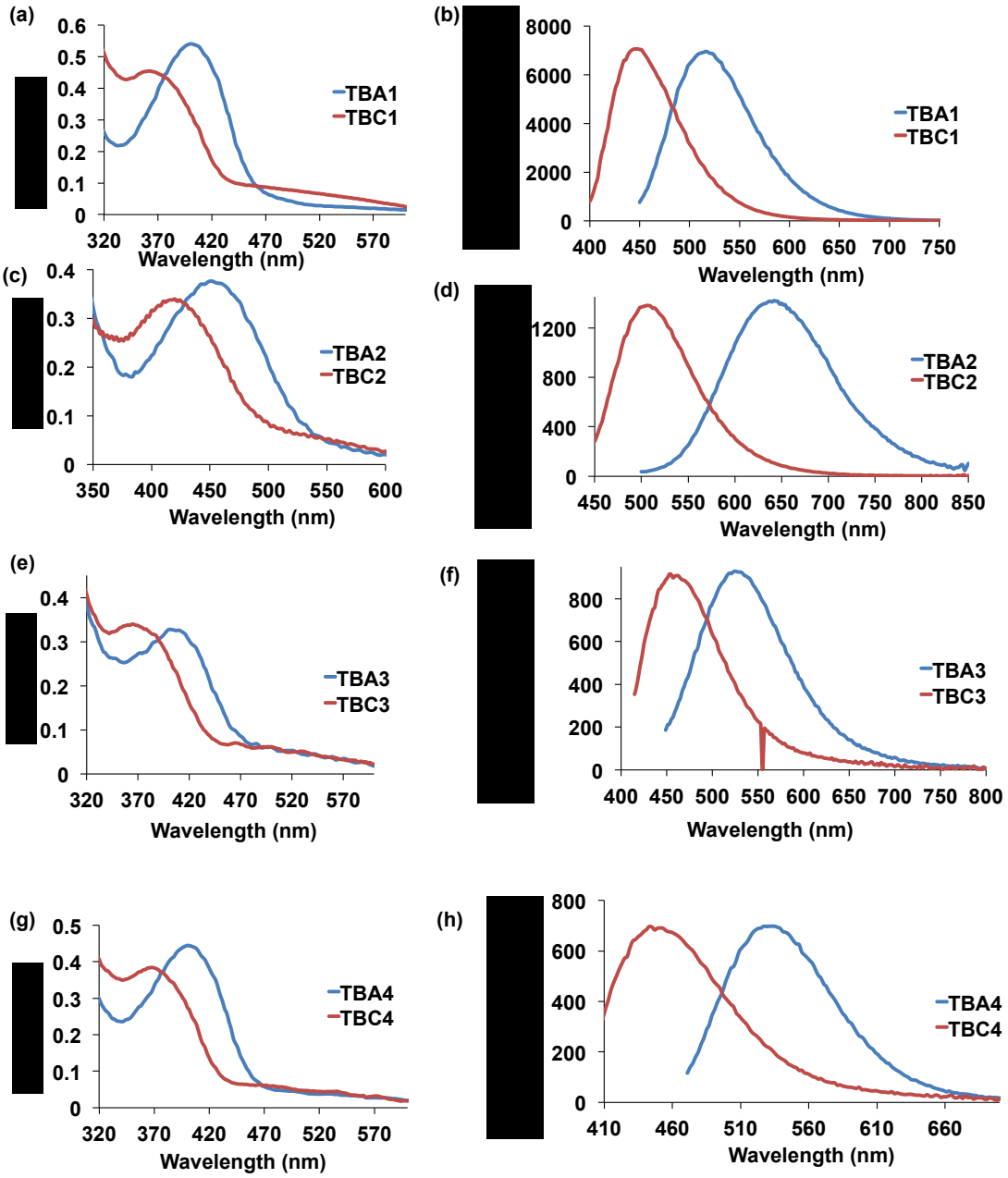


Figure S15: Absorption and fluorescence spectra of TBAs and TBCs. (a, c, e, g) shows the absorption spectra of TBA1, TBA2, TBA3 and TBA4 respectively; (b, d, f, h) shows fluorescence spectra of TBA1, TBA2, TBA3 and TBA4.

Tecan plate reader.

C. Fluorescence quantum yield of TBA2 and TBC2

The fluorescence of 10 μM solutions of either TBA2 or TBC2 was measured at either 650 nm or 520 nm, while exciting at 405 nm. Fluoroscein isothiocyate (for TBC2) or Alexa 647 (for TBA2) was used as standards to calculate the quantum yields.

Table S1: Fluorescent quantum yield of TBA2 and TBC2

Solvent	TBA2 (% ϕ)[a]	TBC2 (% ϕ)[b]
Acetone	23	14
DCM	31	-
DMSO	47	27
DMF	33	17
MeOH	11	19
Water	-	9

a: Fluorescence emission was measured at 650 nm while it was excited at 405 nm and Alexa 647 was used as standard; b: fluorescence emission maximum was measured at 520 nm while the probe was excited at 405 nm and fluorescein isothiocyanate used standard.

D. Density functional theory calculations on TBA2 and TBC2: All theoretical calculations were done with gaussview 5.0.8,[8] which is a graphical user interface for Gaussian 09. We have used 6-31G(D,P) as a basis set for these calculations. The frequency and energy minimization calculations were done with B3LYP/6-31G.

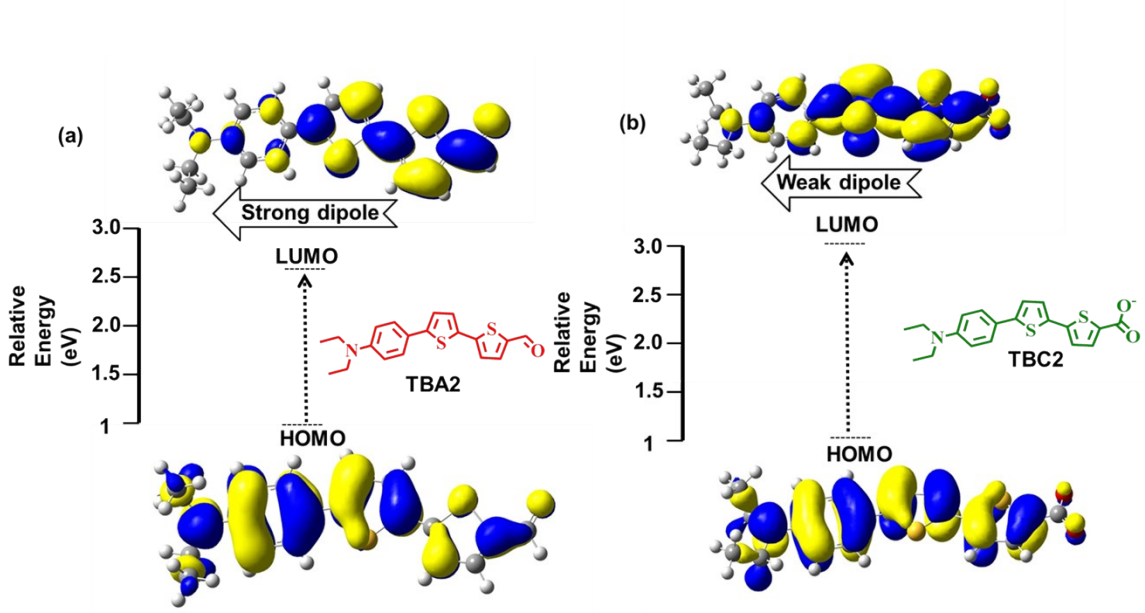


Figure S16: Density functional calculation of the HOMO-LUMO of TBA2 and TBC2: (a) TBA2 has a low energy LUMO due to its electron withdrawing aldehyde. (b) TBC2 has a high energy LUMO due to the weak electron withdrawing ability of its carboxylate.

E. ALDH catalyzed oxidation of TBAs

E 1 General procedure for ALDH oxidation studies

ALDH catalysis experiments were done in a 50 μL volume in HEPES (50 mM), which contained 2% acetonitrile, methyl-β-cyclodextrin (50 mM), NAD (500 μM), EDTA (1 mM), TBAs (2 mM) and aldehyde dehydrogenases (ALDH1A1, ALDH1A3, ALDH2, ALDH3A1, ALDH3A2, ALDH4A1, ALDH5A1 and ALDH7A1) (4 μg). The reactions were performed in a 96 well plate at 37 °C. The progress of the reactions were followed, every 1 min over 20 minutes, using a plate reader (Tecan's infinite M2000 plate reader), λ_{ex} = 405 nm, λ_{em} = 520 nm (for the oxidation of TBA2) and λ_{ex} = 405 nm, λ_{em} = 450 nm (for the oxidation of TBA1, TBA3 and TBA4).

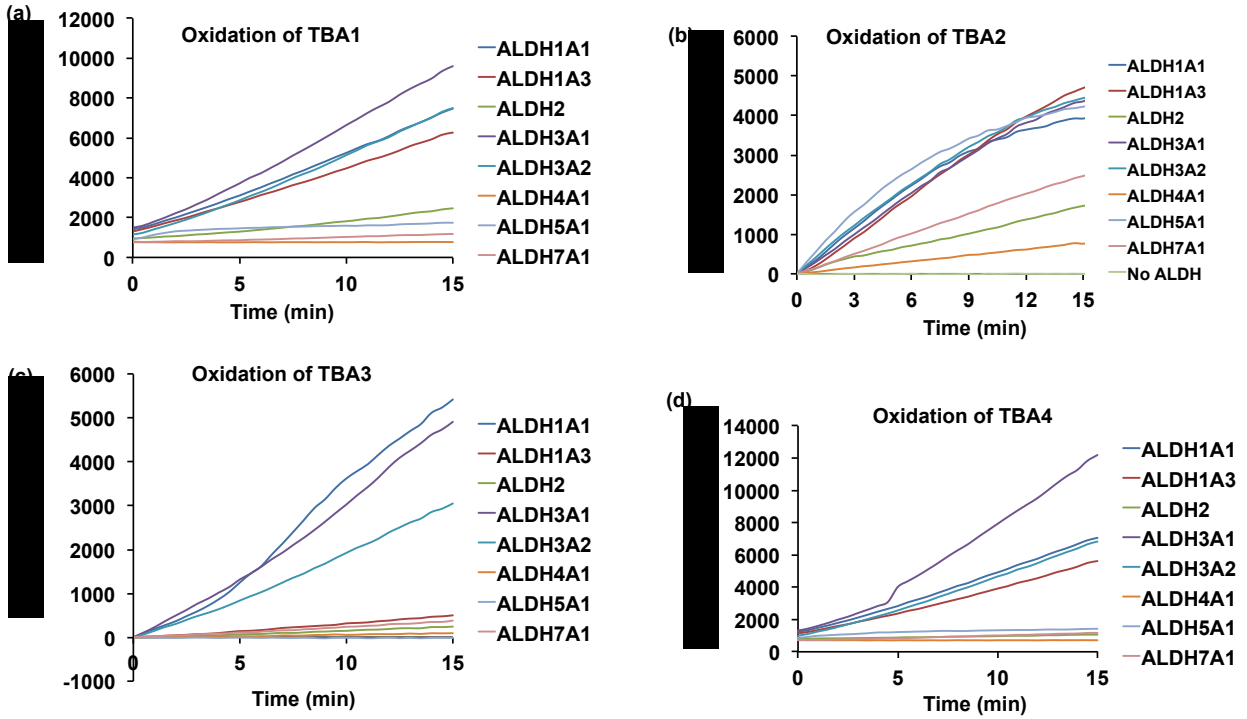


Figure S17: Recombinant ALDHs oxidize TBAs. TBA1 (a), TBA2 (b), TBA3 (c) and TBA4 (d) are substrates for ALDHs. The rate of TBA oxidations were determined by measuring the fluorescence at 520 nm. (TBA2 oxidation) and 450 nm (TBA1, TBA3, TBA4)

F. Determination of k_{cat} and K_m for ALDH catalyzed oxidation of TBA2

ALDH catalysis experiments were done in a 50 μL volume in HEPES (50 mM), which contained 2% acetonitrile, methyl-β-cyclodextrin (50 mM), NAD (2.5 mM), EDTA (1 mM), TBA, aldehyde dehydrogenases (ALDH1A1, ALDH1A3, ALDH2, ALDH3A1, ALDH3A2, ALDH4A1, ALDH5A1 and ALDH7A1 (4 μg), 500 μM resazurin and 0.25 units diaphorase. The reactions were performed in a 96 well plate at 37 °C. Five different TBA2 concentrations were used for these studies, 2 mM, 1 mM, 500 μM, 250 μM and 100 μM. The progress of the reactions were followed, every 2 min over 30 minutes, using a plate reader (Tecan's infinite M2000 plate reader), λ_{ex} = 550 nm, λ_{ex} = 580 nm.

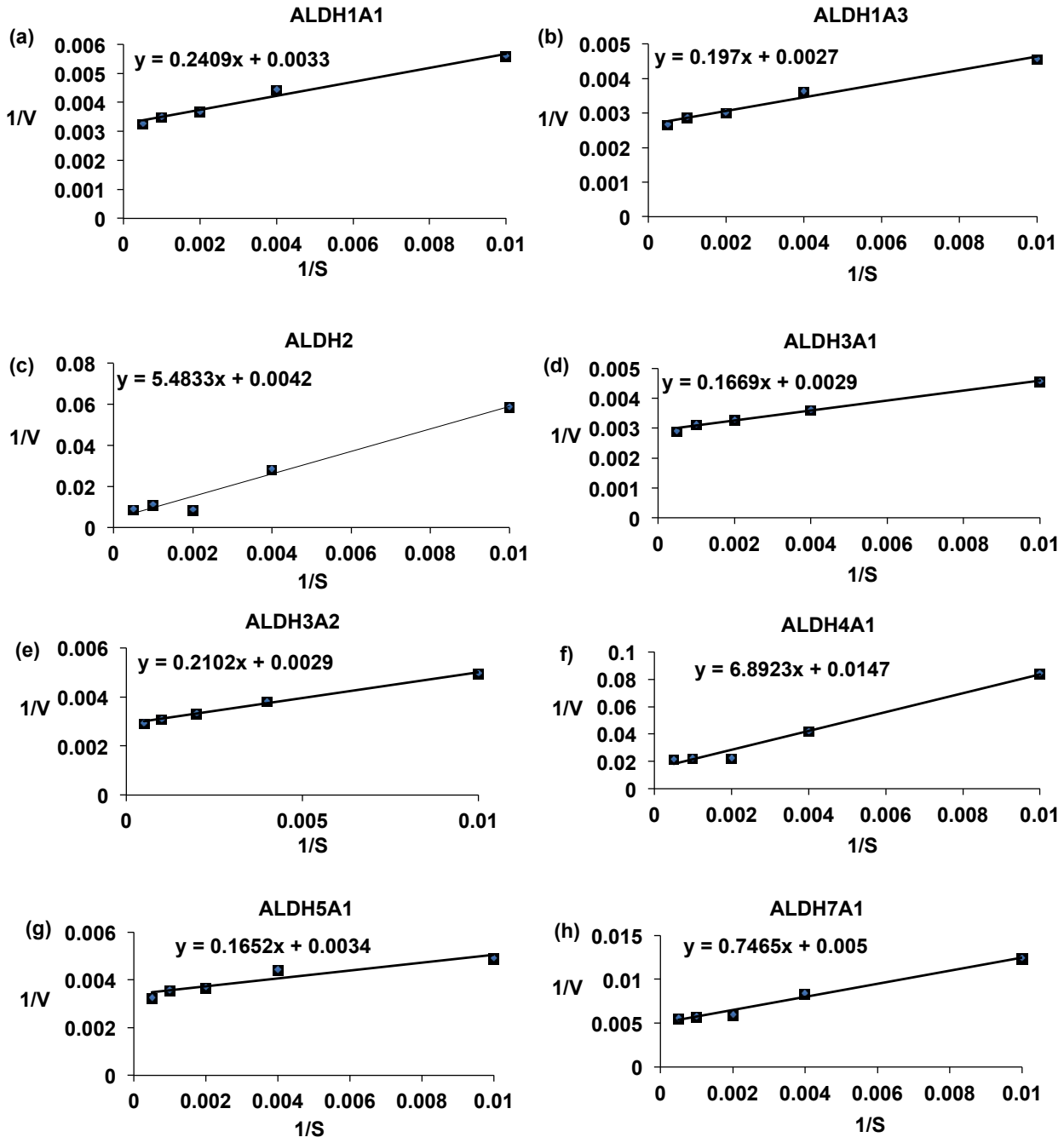


Figure S18: Lineweaver-Burk plot of recombinant ALDHs catalyzed TBA2 oxidation. (a) ALDH1A1; (b) ALDH1A3; (c) ALDH2; (d) ALDH3A1; (e) ALDH3A2; (f) ALDH4A1; (g) ALDH5A1; (h) ALDH7A1.

G. Oxidation of TBA2 by A549 cell lysates

G 1 Preparation of A549 cell lysates

The human lung carcinoma cell line, A549, were seeded at $1x10^5$ per well in a six well plate. The cells were cultured in Dulbecco's modified eagle medium (DMEM) containing

10% FBS and 1% penicillin-streptomycin (PS). The cells were grown to 60-80% confluency at 37 °C in the presence of 5% CO_2. The Media was aspirated and the cells were washed with PBS and incubated with a 0.25% trypsin-EDTA at 37 °C for 7 min. The trypsin was neutralized with trypsin neutralizer and the detached cells were collected via centrifugation (1000 rpm, 5 min, 4 °C). The Trypsin-EDTA was aspirated and the cells were washed with ice-cold PBS, and treated with 1 mL hypotonic lysis buffer (purchased from amresco) at 0 °C for 30 min. The lysate was collected via centrifugation at 12,000 rpm for 10 min at 4 °C.

Table S2: K_m, k_{cat} and catalytic efficiency of ALDH catalyzed oxidation of TBA2

	K_m (μM)[a]	k_{cat} (min^{-1})[b]	k_{cat}/K_m ($min^{-1}M^{-1}$)[c]
ALDH1A1	73.0	3276.0	44.87×10^6
ALDH1A3	72.96	4004.0	54.87×10^6
ALDH2	1305.54	2574.0	1.97×10^6
ALDH3A1	57.55	3727.86	64.77×10^6
ALDH3A2	72.72	3727.86	51.26×10^6
ALDH4A1	468.84	735.42	1.56×10^6
ALDH5A1	48.58	3179.65	65.44×10^6
ALDH7A1	1276.35	2002.0	1.56×10^6

[a]Michaelis-Menton constant calculated from the slope of Lineweaver-Burk plot, [b]rate constant of ALDH mediated oxidation of TBA2 into TBC2, [c]catalytic efficiency of ALDH catalyzed oxidation of TBA2.

G 2 Oxidation of TBA2 with A549 cell lysates

10 μL of A549 cell lysate was treated with methyl-β-cyclodextrin (50 mM), NAD (500 μM), EDTA (1 mM), HEPES (50 mM), 2% acetonitrile and TBA2 (20 μM). The reaction mixture was incubated at 37 °C for 25 min. The reaction was monitored with a Tecan's infinite M2000 plate reader at λ_{ex} = 405 nm, λ_{em} = 520 nm. The progress of the reactions were followed, every 5 min over 2 hours, using a plate reader (Tecan's infinite M2000 plate reader), λ_{ex} = 405 nm, λ_{ex} = 520 nm. ALDH experiments containing the ALDH inhibitor disulfiram were done at a 100 μM disulfiram concentration.

H. Quantification of ALDH catalyzed oxidation of TBA2

HPLC analysis was performed on the reaction of TBA2 with cell lysates to quantify the oxidation TBA2 into TBC2. All HPLC analysis was performed using a Shimadzu HPLC system (serial no. L203047), which was equipped with an auto sampler, column oven, an X-bridge C-18 column 5 μm pore size (4.6 × 150 mm), diode array detector, and a fraction collector.

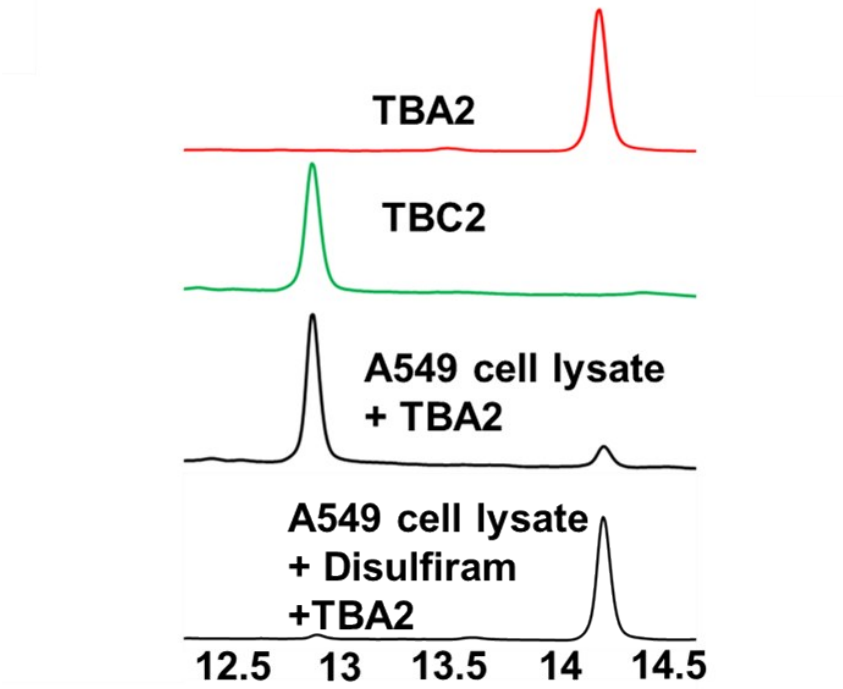


Figure S19: ALDH catalyzed oxidation of TBA2 was measured by HPLC and the rate of oxidations were quantified by peak height.

H1 General method of sample preparation for HPLC analysis of TBA2 oxidation.

The human lung carcinoma cell line, A549, were seeded at $1x10^5$ per well in a six well plate. The cells were cultured in Dulbecco's modified eagle medium (DMEM) containing 10% FBS and 1% penicillin-streptomycin (PS). The cells were grown to 60-80% confluency at 37 °C in the presence of 5% CO_2. The Media was aspirated and the cells were washed with PBS and incubated with TBA2 (5 μM) for 2 hours. As a control experiment the A549 cells were initially incubated with the ALDH inhibitor disulfiram (100 μM) for 2 hours then with TBA2 (5 μM) for 2 hours. The media was aspirated and washed three times with PBS. Then it was treated with a 0.25% trypsin-EDTA at 37 °C for 7 min. The trypsin was neutralized with trypsin neutralizer and the detached cells were collected via centrifugation (1000 rpm, 5 min, 4 °C). The Trypsin-EDTA was aspirated and the cells were washed with ice-cold PBS, and treated with 1 mL hypotonic lysis buffer (purchased from amresco) at 0 °C for 30 min. The lysate was collected via centrifugation at 12,000 rpm for 10 min at 4 °C. The cell lysate was acidified to pH=2 and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and the organic solvent was removed under reduced pressure. The crude product was dissolved into water/acetonitrile (9/1) and injected into the HPLC.

Table S3: HPLC analysis of ALDH2 catalyzed oxidation of TBA2

Experiment Number	Condition[a]	% TBA2[b]	% TBC2[c]
1	TBA2 + ALDH2	15	85
2	TBA2 + ALDH2 + disulfiram	93	7
3	TBA2+ A549 cell lysate	2	98
4	TBA2 + A549 cell lysate + disulfiram	89	11

[a]TBA2 was either treated with ALDH2 or ALDH2 + disulfiram and A549 cell lysate or A549 cell lysate + disulfiram. Conversion of TBA2 to TBC2 was measured by HPLC. [b]represents %TBA2 present in the reaction mixture. [c]represents %TBC2 present in the reaction mixture.

I. Fluorescence microscopy and flow cytometry experiments with A549 cells and TBA2 or ALDEFLUOR

I 1. General cell culture

A549 cells were seeded at a density of 1 x 10^4 into a 60 x 15 mm round Petri dish and were cultured to 70% confluency in DMEM containing heat-inactivated 10% calf serum and 1% penicillin-streptomycin (PS). The following experiments are performed using these cultures.

I 1.1 Preparation of A549 cells for flow cytometry with TBA2

A549 cells were incubated with 5 μM TBA2 for 4 hours. In a control experiment the cells were incubated with disulfiram (100 μM) for 2 hours then with TBA2 (5 μM) for 4 hours. The media was aspirated and washed with PBS for three times. Subsequently cells were treated with 0.05% trypsin-EDTA (1 mL) for 15 min and diluted with trypsin neutralizer (2 mL). The unattached cells were collected via centrifuge (1000 rpm, 5 min, 4 °C). The cell palate was suspended into ALDEFLUOR assay buffer (Stemcell Technologies).

I 1.2 Preparation of A549 cells for flow cytometry with ALDEFLUOR

The cells were suspended into a ALDEFLUOR assay buffer (Stemcell Technologies). Incubated with either ALDEFLUOR (1.5 μM) or with ALDEFLUOR + DEAB (100 μM) for 1 h. Then cells were collected via centrifugation (1000 rpm, 5 min, 4 °C). Further cells were suspended into ALDEFLUOR assay buffer and kept under ice.

I 1.3 Preparation of A549 cells for fluorescent microscopy with TBA2 or ALDEFLUOR

A549 cells were incubated with 5 μM of TBA2 or ALDEFLUOR. In a control experiment A549 cells were incubated with disulfiram (100 μM) for 2 h and then with TBA2 or ALDEFLUOR (5 μM) for 4 hours and 1 hour respectively at 37 °C with 5% CO_2 in a serum free media. The media was aspirated and wash with PBS for three times and observed under the microscope (Zeiss 710LSM).

J. *In vivo* detection of ALDH activity with TBA2

C57Bl6/j mice 6-8 weeks of age, weighing 20 ± 2 g, were obtained from OLAC Approved Vendors, USA. All animal studies were carried out in accordance with the "Guidelines for Animal Experimentation" by the OLAC of UC Berkeley. C57Bl6/j mice were quarantined for a minimum of 5 days in the SPF grade Animal House under 12 h light/dark cycles at 24−25 °C with a relative humidity of 50−55%. TBA2 was dissolved in DMSO at a 10 mg/mL concentration. C57Bl6/j mice (n = 4) were randomly separated into three groups, and received intraperitoneal injections of the following (1) DMSO control group (0.1 mL), (2) TBA2 treated (0.1 mL, 1 mg/kg) group, (3) TBA2 (0.1 mL, 1 mg/kg) and disulfiram (0.1 mL, 400 mg/kg) group. Inhibitor was administrated via intraperitoneal injection, followed by the intraperitoneal injection of TBA2 after 3h. The mice were sacrificed 15 hours after the TBA2 injection. Various organs, including brain, heart, liver, spleen, lung, and kidney were harvested and washed with saline. 3 mm sections of each organ were placed into cassettes and were immersed into a vial containing ¾ of 4% paraformaldehyde and stored at room temperature for 48h. The fixed tissues were sequentially dipped into 70%, 85%, 95% and 100% isopropyl alcohol for 1h each. Isopropanol was discarded and the tissues were immersed into a vial ¾ filled with melted paraffin and equilibrated for 1h at 58 °C. This process was repeated and the paraffin equilibrated tissues were transferred into a mold containing melted paraffin and allowed to solidify at room temperature. Tissues were sectioned by microtome and then observed with a Laser Scanning Confocal Microscopy (Zeiss LSM710, Germany).

K. TBA2 based plate reader based assay for ALDH inhibitor identification

10^3 A549 cells/ well was seeded into a 96 well plate and grown to 60% confluency. A DMSO solution of disulfiram (100 μM final cellular concentration) was added into each well and incubated at 37 °C for 2.5 hours. Subsequently, TBA2 (10 μM final cellular concentration) was added into each well and incubated for another 2.5 hours. The increase in fluorescence at E_{ex} = 405 nm and E_{em} = 520 nm was measured in a plate reader, and compared to cells treated with just TBA (10 μM final cellular concentration).

L. High-throughput screening of electrophile fragment library

10^3 A549 cells/well were seeded into a 96 well plate and the cells were grown to 60% confluency. Each compound (1 mg) of the library was dissolved into 200 μL of DMSO. A 1 μL DMSO solution of each compound was sequentially added into each well of the 96 well plate (n=1). The plates were incubated for 2.5 hours and subsequently, a 10 μM final concentration of TBA2 was added and further incubated for 2.5 hours. The increase in fluorescence at 520 nm was measured. Similarly, in a separate positive control experiment, cells were only treated with 1 μL of DMSO and incubate at 37 °C for 2.5 hours, and a 10 μM final concentration of TBA2 was added and incubated further for another 2.5 hours. The increase in fluorescence at 520 nm was measured. Compounds

that generated a 60% inhibition of relative fluorescence (compared to DMSO treatment) were considered positive hits. Positive hits were revalidated with n = 12 wells.

M. Glioblastoma multiforme Cell Culture

Primary GBM TIC line, L0, was collected in a previous study after informed consent from male patients who underwent surgical treatment and Institutional Review Board approval.[9] The TIC neurospheres were propagated in neurosphere assay growth condition with serum-free medium (Neurocult NS-A Proliferation kit, Stem Cell Technologies, Vancouver, Canada) supplemented with epidermal growth factor (20 ng/mL, R&D Systems, Minneapolis, Minnesota), basic fibroblast growth factor (R&D Systems) and 2 mg/mL heparin (Sigma-Aldrich, St. Louis MO) The gliomaspheres were serially passaged every 5–7 days, when the spheres reached a diameter of ~150 mm. Gliomaspheres were dissociated with trypsin/ethylenediaminetetraacetic acid (0.05%) for 2 min and then replated in fresh media with the addition of epidermal growth factor, basic fibroblast growth factor, and heparin.

Limited Dilution Assay

For the TMZ and inhibitor experiments, TICs were first dissociated into a single cell suspension. TICs were then diluted in media containing the appropriate concentration of inhibitor, TMZ or DMSO control to a concentration of 200 cells per milliliter. For each experimental condition, 100 µl of cell suspension, containing an average of 20 cells, were added to a well of a 96 well plate. At least three wells were plated for per experimental condition. Cells were allowed to grow for 10 days to form spheres and the spheres were then imaged. Cells with a diameter larger than 40 microns were classified as a sphere.

N. Cell viability (MTT) assay

A549 cells were plated in a 96 well plate at the density of 10^4 cells per well. The cells were allowed to attach overnight in the media with phenol red. The following day, the old media was changed to media without phenol red after a quick 1X PBS wash. TBA2 dye was added to the wells at increasing concentrations from 0-70 µM and incubated for 2 hour. The cells were then allowed to recover from the treatment for 24 hours. The wells were washed 3 times with 1X PBS and kept in 100 µL PBS. The MTT reagent (12 mM) was added to each well (10 µL) and incubated for 2 hours at 37 °C. SDS-HCl solution was added later to permeabilize the cells and incubated for 2 hours at 37 °C. The 96-well plate was finally inserted into a plate reader to read absorbance at 570 nm. Absorbance values were recorded and analyzed in Microsoft Excel.

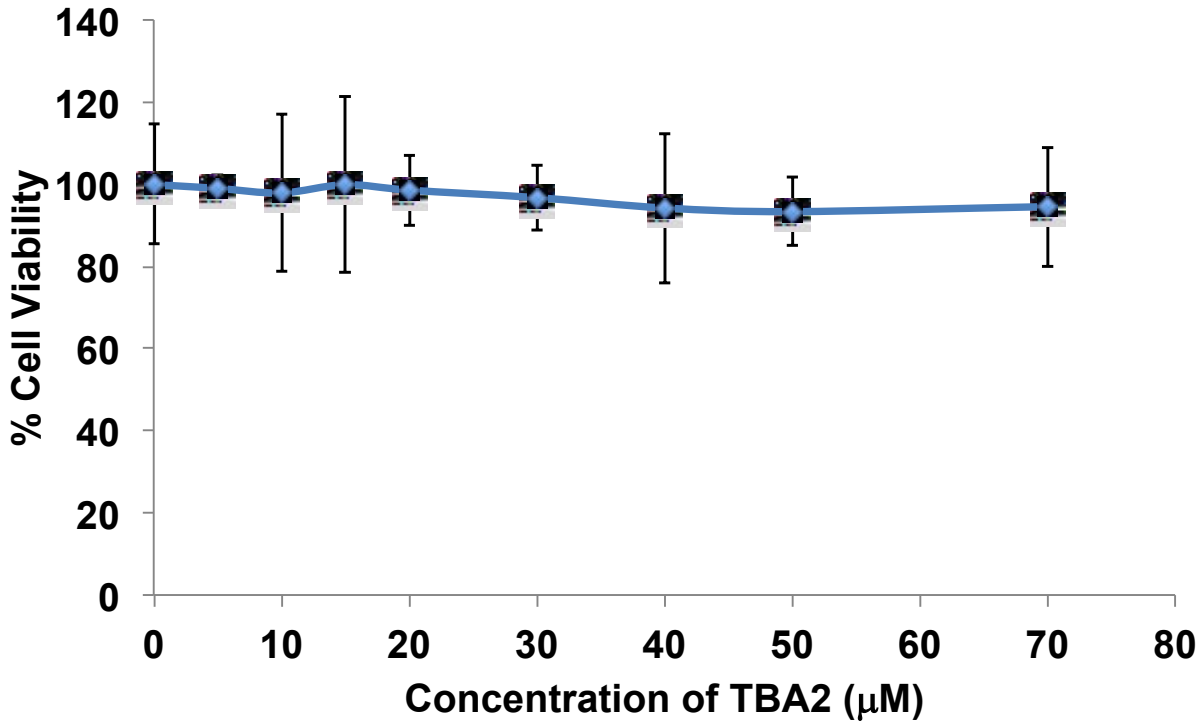


Figure S20: A549 cell viability study with different concentration of TBA2

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