Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Protein Recognition by Bivalent, 'Turn-On' Fluorescent Molecular Probes

*Linor Unger-Angel, Bhimsen Rout, Tal Ilani, Miriam Eisenstein, Leila Motiei, and David Margulies**

david.margulies@weizmann.ac.il

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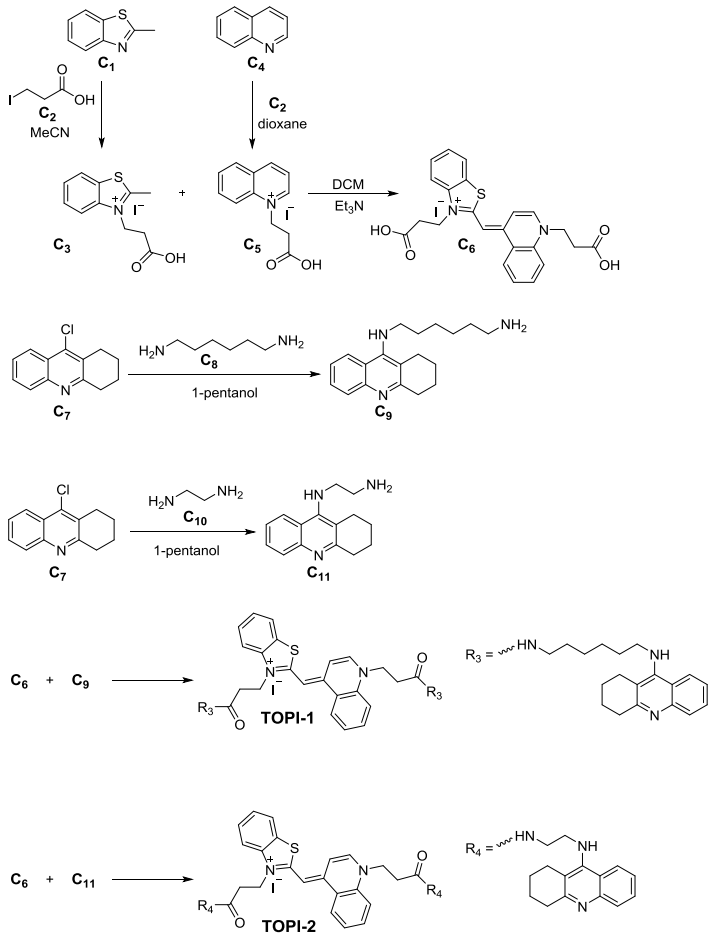
1. Materials and Methods

All reagents and solvents were obtained from commercial suppliers and used without further purification. Recombinant human GST isoforms A1-1, A2-2, M1-1, and P1-1 were obtained from the Israel Structural Proteomics Center (Weizmann Institute of Science, Rehovot, Israel). GST T1-1, GST O1-1, GST Z1-1, GST K1-1, transferrin, Fibroblast growth factor 21 (FGF-21), and platelet-derived growth factor-BB (PDGF-BB) were purchased from ProSpec-Tany TechnoGene Ltd. (Ness Ziona, Israel). Torpedo californica acetylcholinesterase was a gift from Prof. Israel Silman Lab (Weizmann Institute of Science, Rehovot, Israel). Biotin $(PEG)_3$ amine (**C_{24}**) and biotinyl ethylamine (**C_{25}**) were purchased from Chem-Impex International (Wood Dale, IL). Lysozyme (from chicken egg white), human serum albumin, and immunoglobulin G (IgG) were purchased from Sigma-Aldrich. Immunoglobulin A (IgA) and fibrinogen were obtained from Merck Millipore. Ethacrynic acid, aspirin, and 7-ethyl-10-hydroxy-camptothecin (SN-38) were purchased from Tokyo Chemical Industry Co., Ltd., Santa Cruz Biotechnology, Inc. and AK Scientific, Inc., respectively. Histamine dihydrochloride (ceplene), amikacin hydrate, quabain octahydrate, erythromycin, epinephrine, dopamine hydrochloride, azithromycin, digitoxin, roxithromycin, L-glutathione reduced, S-hexylglutathione, salicylic acid, 4-formyl benzoic acid, hexamethylenediamine, 1,2-diaminoethane and 9-chloro-1,2,3,4-tetrahydroacridine were obtained from Sigma-Aldrich. Aluminum-backed silica plates (Merck silica gel 60 F254) were used for thin layer chromatography (TLC) to monitor solution-phase reactions. TLC visualization was carried out using short wavelength ultraviolet (UV) light at 254 nm. The ^{1}H NMR spectra were recorded using 300 MHz, 400 MHz or 500 MHz Bruker Avance NMR spectrometer. The ^{13}C NMR spectra were recorded using a 400 MHz Bruker Avance NMR spectrometer. Chemical shifts are reported in ppm on the δ scale down field from TMS as the internal standard. The following abbreviations were used to describe the peaks: s-singlet, d-doublet, dd-double doublet, t-triplet, q-quartet, quin-quintet, m-multiplet, and br-broad. Electronspray mass spectrometry was performed with a Micromass Platform LCZ-4000 instrument at the Weizmann Institute of Science mass spectrometry facility. Preparative HPLC purifications were done on a Thermo Separation instrument **(**P200 pump, UV 100 detector**)** and a pre-packed Vydac C_{18} column. Analytical purity of TOPIs **1-6** was confirmed by an Agilent 1260 infinity quaternary pump LC system, maximum pressure 400 bar, equipped with a diode-

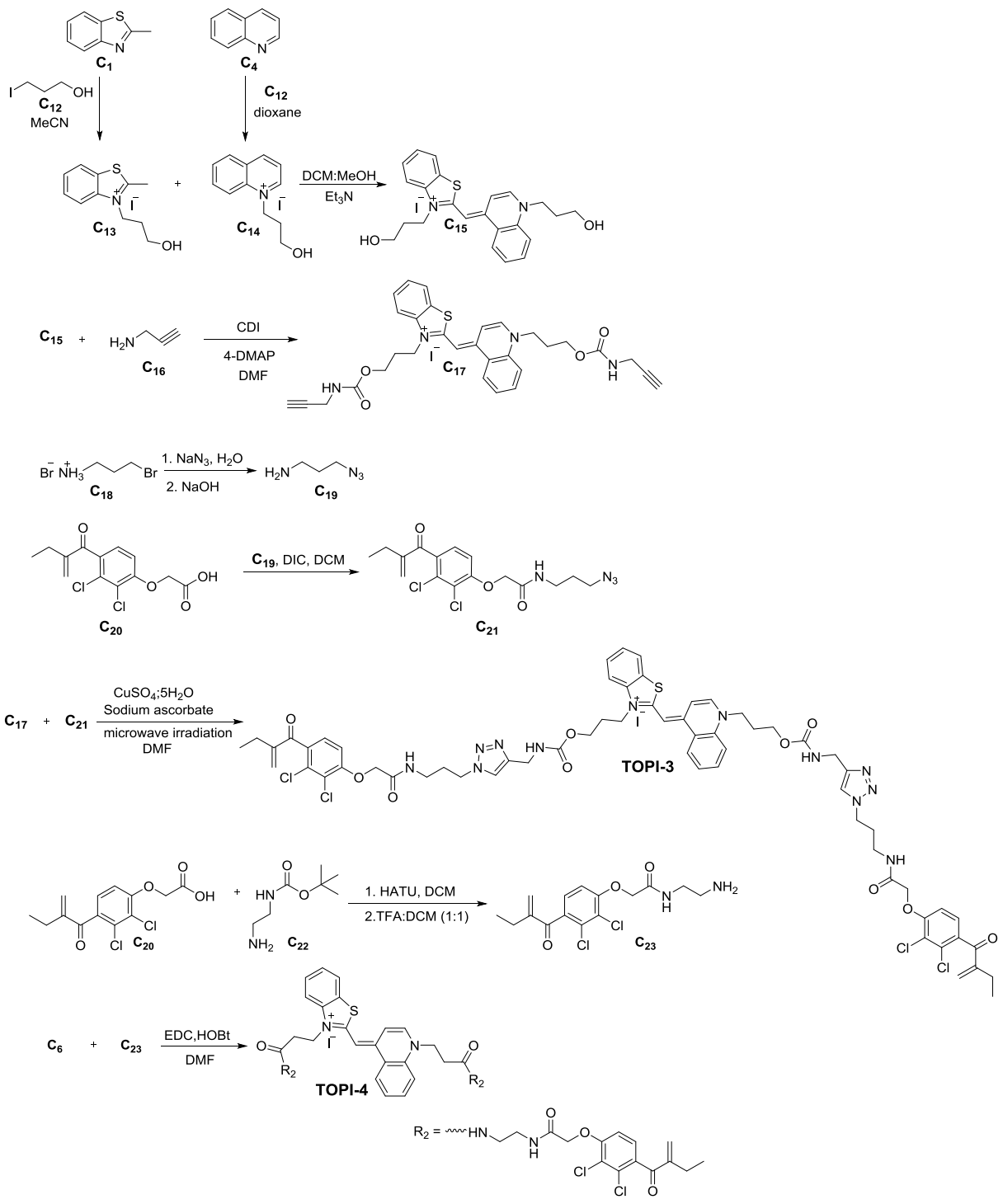
array detector with max-light high-sensitivity cartridge cell. Analysis was performed using 0.1% TFA water/acetontitrile as eluent and detecting the absorbance at 220 and 480 nm (section 8). The absorption and excitation spectra of TOPIs **1-6** in DMSO were acquired using a BioTek synergy H4 hybrid multiwell plate reader (Fig. S9), showing characteristic spectra of TO dyes with a good spectral overlap between the absorption and excitation spectra. Enzymatic assays were carried out using a BioTek synergy H4 hybrid multiwell plate reader in clear flat-bottom polystyrene 384 well microplates (Corning). Fluorescence measurements were carried out using a BioTek synergy H4 hybrid multiwell plate reader in clear and black flat-bottom polystyrene NBS 384 well microplates (Corning).

2. Synthetic Schemes and Procedures

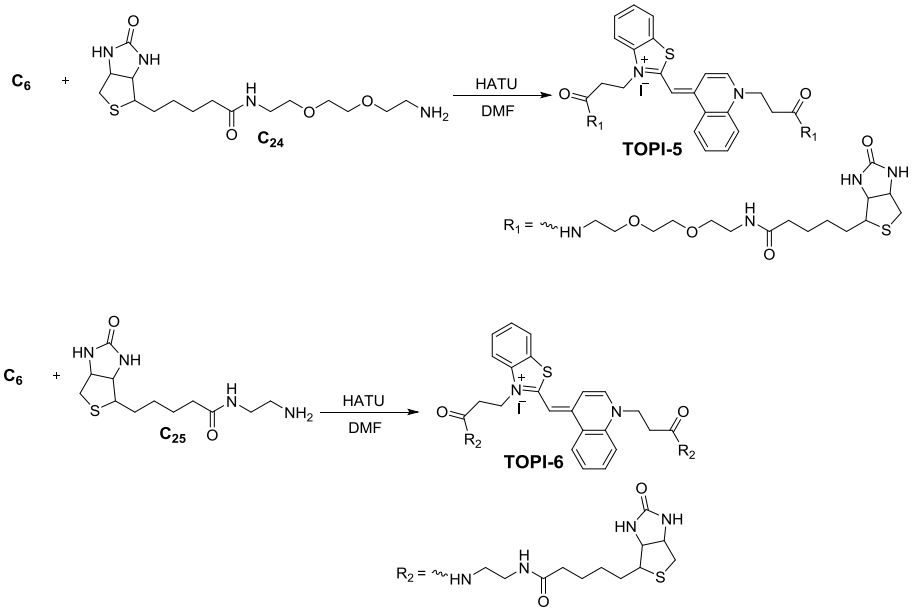
2.1. Synthetic Scheme for TOPI-1 and TOPI-2.



2.2. Synthetic Scheme for TOPI-3 and TOPI-4.



2.3. Synthetic Scheme for TOPI-5 and TOPI-6.



2.4. Synthesis of TOPI-1 and TOPI-2

3-(2-carboxyethyl)-2-methylbenzo[d]thiazol-3-ium iodide (C_3). 2-methylbenzothiazole (636 µL, 5 mmol) and 3-iodopropionic acid (2.29 g, 15 mmol) were mixed together under nitrogen. The mixture was stirred and heated at 110 °C overnight. The solid was suspended in methanol:Et_2O solution (1:2, total 60 mL solution). The precipitate was filtered and washed with Et_2O, then dried under reduced pressure to yield a white solid (1.2 g, 68%).^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.98 (t, *J* = 6 Hz, 2H), 3.26 (s, 3H), 4.89 (t, *J* = 6 Hz, 2H), 7.77-7.91 (m, 2H), 8.35-8.46 (m, 2H), 12.73 (br-s, 1H). MS-ESI (*m/z*): calcd. for $C_{11}H_{12}NO_2S$ [M-I] 222.05; found 221.96.

1-(2-carboxyethyl)quinolin-1-ium iodide (C_5). 3-iodopropionic acid (5.56 g, 27.9 mmol) was added to a solution of quinoline (2.74 mL, 23.23 mmol) in 30 mL of dioxane. The solution was stirred under reflux for 23h. After cooling to room temperature, the solvent was removed and the precipitate was washed twice with hexane and six times with acetone yielding a bright yellow solid (5.6 g, 73% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.08 (t, *J* = 6 Hz, 2H), 5.26 (t, *J* = 6 Hz, 2H), 8.06 (t, *J* = 6 Hz, 1H), 8.17-8.31 (m, 2H), 8.50 (d, *J* = 6 Hz, 1H), 8.63 (d, *J* = 9 Hz, 1H), 9.29 (d, *J* = 9 Hz, 1H), 9.58

(d, *J* = 6 Hz, 1H), 12.75 (br-s, 1H). MS-ESI (*m/z*): calcd. for $C_{12}H_{12}NO_2$ [M-I] 202.08; found 201.92.

(E)-3-(2-carboxyethyl)-2-((1-(2-carboxyethyl)quinolin-4-(1H)ylidene)methyl) benzothiazol-3-ium iodide (C_6). Triethylamine (2.12 mL, 15.2 mmol) was added to a suspension of **C_5** (0.5 g, 1.52 mmol) and **C_3** (0.53 g, 1.52 mmol) in 6 mL dry CH_2Cl_2. A deep red color was immediately formed. The reaction mixture was stirred at room temperature overnight. Then the solvent was evaporated and re-dissolved in 1:1 mixture of MeOH:ethyl acetate (total 100 mL). After reducing the volume to half by evaporation under vacuum, the residue was kept overnight at room temperature until a solid is formed. The precipitate was collected and washed with methanol, then dried under reduced pressure to yield a red solid material (190 mg, 23% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ =2.41 (t, *J* = 8 Hz, 2H), 2.46 (t, *J* = 8 Hz, 2H), 3.97 (m, 4H), 5.96 (s, 1H), 6.24 (d, *J* = 4 Hz, 1H), 6.37 (t, *J* = 8 Hz, 1H), 6.68-6.78 (m, 2H), 6.84 (d, *J* = 8 Hz, 1H), 7.22 (d, *J* = 8 Hz, 1H), 7.35 (t, *J* = 8 Hz, 1H), 7.50 (t, *J* = 8 Hz, 1H), 7.63 (d, *J* = 8 Hz, 1H), 7.81 (d, *J* = 8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 35.1, 36.5, 43.6, 51.5, 87.2, 107.6, 111.5, 116.9, 121.8, 123.4, 123.5, 124.2, 124.7, 126.9, 127.6, 133.0, 136.0, 138.2, 142.6, 146.9, 158.3, 177.8, 178.0. HRMS-ESI (*m/z*): calcd. for $C_{23}H_{21}N_2O_4S$ [M-I] 421.1217; found, 421.1220.

N-(1,2,3,4-tetrahydroacridin-9-yl)hexane-1,6-diamine (C_9). **C_7** (200 mg, 0.918 mmol) and hexamethylenediamine, **C_8**, (359.84 µL, 2.75 mmol) were refluxed in 1 mL pentanol for 16h. Then, the reaction mixture was cooled to room temperature and evaporated under reduced pressure. The crude was purified by column chromatography (9:1:1, DCM:MeOH:NH_3) to yield a brown oil (115.26 mg, 42%). ^{1}HNMR (400 MHz, $CDCl_3$): δ = 1.34-1.45 (m, 6H), 1.64 (quin, *J* = 8 Hz, 2H), 1.91 (m, 4H), 2.64-2.70 (m, 4H), 3.05 (m, 2H), 3.47 (t, *J* = 8 Hz, 2H), 3.94 (br-s, 1H), 7.33 (t, *J* = 8 Hz, 1H), 7.53 (t, *J* = 8 Hz, 1H), 7.88-7.95 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.9, 23.1, 24.9, 26.7, 26.9, 31.8, 33.6, 34.1, 42.1, 49.5, 116.0, 120.3, 122.9, 123.7, 128.4, 128.8, 147.5, 150.9, 158.5. HRMS-ESI (*m/z*): calcd. for $C_{19}H_{27}N_3$ [M+H] 298.2283; found, 298.2267.

TOPI-1. EDCI (8.39 mg, 0.044 mmol) and HOBT (5.93 mg, 0.0438 mmol) were added to a solution of **C_6** (10 mg, 0.018 mmol) in dry DMF (1 mL) at 0°C. This mixture was

kept at 0°C for 15 min. Then, **C_9** (11.93 mg, 0.04 mmol) was added and the reaction mixture was stirred at room temperature overnight and monitored by HPLC. The solvent was evaporated under reduced pressure and the residue was purified by reversed-phase HPLC (3.21 mg, 15%).^{1}HNMR (400 MHz, DMSO-d_6): δ = 1.12-1.32 (m, 12H), 1.60-1.69 (m, 4H), 1.83 (m, 8H), 2.62 (m, 4H), 2.68-2.77 (m, 4H), 2.93-3.0 (m, 8H), 3.77 (q, *J* = 8 Hz, 4H), 4.74-4.83 (m, 4H), 7.03 (s, 1H), 7.33-7.40 (m, 2H), 7.51-7.59 (m, 2H), 7.63-7.70 (m, 2H), 7.75 (t, *J* = 8 Hz, 1H), 7.80-7.85 (m, 4H), 7.94-8.05 (m, 3H), 8.13 (d, *J* = 8 Hz, 1H), 8.33 (t, *J* = 8 Hz, 1H), 8.54 (d, *J* = 8 Hz, 1H), 8.75 (d, *J* = 8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 20.1, 21.2, 23.6, 25.5, 25.7, 27.7, 28.6, 29.6, 33.3, 34.3, 38.4, 42.7, 47.1, 50.7, 88.1, 107.6, 111.0, 112.8, 115.3, 117.8, 118.9, 122.5, 123.7, 124.2, 124.3, 124.7, 124.8, 125.6, 126.6, 127.9, 132.5, 133.1, 136.8, 137.6, 139.6, 144.8, 148.7, 150.4, 155.5, 159.4, 168.5, 169.1. HRMS-ESI (*m/z*): calcd. for $C_{61}H_{71}N_8O_2S$ [M-I] 979.5415; found, 979.5419.

N-(1,2,3,4-tetrahydroacridin-9-yl)ethane-1,2-diamine (C_{11}). **C_7** (200 mg, 0.918 mmol) and 1,2-diaminoethane, **C_{10}**, (184.1 µL, 2.75 mmol) were refluxed in 1 mL pentanol for 16h. Then, the reaction mixture was cooled to room temperature and evaporated under reduced pressure. The crude was purified by column chromatography (9:1:1 DCM:MeOH:NH_3) to yield a brown oil (70.45 mg, 32%).^{1}HNMR (300 MHz, $CDCl_3$): δ = 1.92 (m, 4H), 2.75 (m, 2H), 2.99 (t, *J* = 6 Hz, 2H), 3.12 (m, 2H), 3.60 (t, *J* = 6 Hz, 2H), 7.36 (t, *J* = 9 Hz, 1H), 7.58 (t, *J* = 9 Hz, 1H), 8.06 (d, *J* = 9 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.9, 23.1, 24.9, 34.0, 42.5, 51.1, 116.6, 120.5, 122.9, 123.8, 128.4, 128.7, 147.4, 151.1, 158.5. HRMS-ESI (*m/z*): calcd. for $C_{15}H_{19}N_3$ [M+H] 242.1657; found, 242.1660.

TOPI-2. EDCI (8.39 mg, 0.0438 mmol) and HOBT (5.93 mg, 0.044 mmol) were added to a solution of **C_6** (10 mg, 0.0182 mmol) in dry DMF (1 mL) at 0°C. The reaction mixture was kept at 0°C for 15 min. Then, **C_{11}** (9.67 mg, 0.04 mmol) was added and the reaction mixture was stirred at room temperature overnight and monitored by HPLC. The solvent was evaporated under reduced pressure and the residue was purified by reversed-phase HPLC (2.85 mg, 16%).^{1}HNMR (400 MHz, DMSO-d_6): δ = 1.75-1.82 (m, 8H), 2.44 (m, 4H), 2.74-2.80 (m, 4H), 2.90-2.93 (m, 4H), 3.38-3.43 (m, 4H), 3.75-3.85 (m,

4H), 4.71-4.76 (m, 4H), 5.06 (t, *J* = 8 Hz, 1H), 5.31 (t, *J* = 8 Hz, 1H), 6.86 (s, 1H), 7.04 (d, *J* = 8 Hz, 1H), 7.32-7.47 (m, 3H), 7.57-7.78 (m, 7H), 7.82-7.93 (m, 2H), 7.97 (d, *J* = 12 Hz, 1H), 8.08-8.10 (m, 1H), 8.27-8.31 (m, 1H), 8.9 (d, *J* = 8 Hz, 1H), 8.55 (t, *J* = 8 Hz, 1H), 8.61-8.63 (m, 1H). HRMS-ESI (*m/z*): calcd. for $C_{53}H_{55}N_8O_2S$ [M-I] 867.4163; found, 867.4166.

2.5. Synthesis of TOPI-3 and TOPI-4.

3-iodo-1-propanol (**C_{12}**) was prepared according to a published procedure.[1]

3-(3-hydroxypropyl)-2-methylbenzothiazol-3-ium (**C_{13}**). 2-methylbenzothiazole (1.37 mL, 10.75 mmol) and 3-iodo-1-propanol (1 g, 5.38 mmol) were dissolved in 3 mL acetonitrile under N_2. The mixture was stirred under reflux for 91 h, then cooled to room temperature and stored in the fridge overnight. The precipitate was collected and washed three times with 1.5 mL cold Et_2O, then dried under reduced pressure, yielding a white solid (1.62 g, 90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.04 (quin, *J* = 6 Hz, 2H), 3.23 (s, 3H), 3.53 (t, *J* = 6 Hz, 2H), 4.78 (t, *J* = 6 Hz, 2H), 7.78 (t, *J* = 9 Hz, 1H), 7.9 (t, *J* = 9 Hz, 1H), 8.32 (d, *J* = 9 Hz, 1H), 8.70 (d, *J* = 9 Hz, 1H). MS-ESI (*m/z*): calcd. for $C_{11}H_{14}NOS$ [M-I] 208.07; found 208.96.

1-(3-hydroxypropyl)quinolin-1-ium iodide (C$_{14}$). 3-iodo-1-propanol (2.45 mL, 25.5 mmol) was added to a solution of quinoline (1.01 mL, 8.5 mmol) in 9 mL of dioxane and the solution was stirred under reflux for 2.5 h. After cooling to room temperature, the product was precipitated by addition of acetone (6 mL). The residue was separated by filtration and then washed with acetone to yield a yellow solid compound (2.1 g, 80%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.14 (quin, *J* = 6 Hz, 2H), 3.54 (t, *J* = 6 Hz, 2H), 4.80 (br-s, 1H, OH), 5.13 (t, *J* = 6 Hz, 2H), 8.06 (t, *J* = 7.5 Hz, 1H), 8.19 (dd, *J* = 6 Hz, *J* = 3 Hz, 1H), 8.29 (t, *J* = 9 Hz, 1H), 8.50 (d, *J* = 6 Hz, 1H), 8.60 (d, *J* = 9 Hz, 1H), 9.30 (d, *J* = 9 Hz, 1H), 9.54 (d, *J* = 6 Hz, 1H). MS-ESI (*m/z*): calcd. for $C_{12}H_{14}NO$ [M-I] 188.10; found 188.03.

3-(3-hydroxypropyl)-2-((1-(3-hydroxypropyl)quinolin-4(1H) ylidene) methyl) benzo thiazol-3-ium iodide (C_{15}). **C_{13}** (1.5 g, 4.76 mmol) and **C_{14}** (1.91 g, 5.71 mmol) were dissolved in 28 mL CH_2Cl_2/MeOH (1:1, v:v) and then Et_3N (1.63 mL, 11.7 mmol) was added. A deep red color immediately appeared. The reaction mixture was stirred under reflux overnight, and then cooled to room temperature. The reaction mixture was evaporated and washed with diethyl ether and ethyl acetate. The product was precipitated by addition of water. The residue was separated by filtration to yield a red solid material 0.91 g (39%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.00 (quin, *J* = 6 Hz, 2H), 3.50-3.60 (m, 4H), 4.62 (quin, *J* = 6 Hz, 2H), 4.80 (t, *J* = 6 Hz, 2H), 5.04 (t, *J* = 6 Hz, 2H), 7.02 (s, 1H), 7.34-7.43 (m, 2H), 7.59 (t, *J* = 6 Hz, 1H), 7.70-7.77 (m, 2H), 7.95-8.14 (m, 3H), 8.58 (d, *J* = 9 Hz, 1H), 8.71 (d, *J* = 6 Hz, 1H). MS-ESI (*m/z*): calcd. for $C_{23}H_{25}N_2O_2S$ [M-I] 393.16; found 393.18.

3-(3-((prop-2-yn-1-ylcarbamoyl)oxy)propyl)-2-((1-(3-((prop-2-yn-1-ylcarbamoyl) oxy) propyl)quinolin-4(1H)-ylidene)methyl)benzothiazol-3-ium iodide (C_{17}). N,N'-carbonyldiimidazole (CDI) (0.343 g, 2.11 mmol) and 4-Dimethylaminopyridine (4-DMAP) (0.00035 g, 2.88 ×10^{-3} mmol) were added to a solution of **C_{15}** (0.5 g, 0.96 mmol) in dry DMF (4.6 mL) and dry DCM (1 mL). The reaction mixture was stirred at room temperature for 4 h. Then, triethylamine (402 µL, 2.88 mmol) was added, the reaction mixture was cooled to 0 °C, and propargylamine (135.4 µL, 2.11 mmol) was added dropwise. The mixture was stirred at room temperature for 48 h and monitored by TLC (10% DCM:MeOH) and HPLC. The reaction mixture was evaporated and purified by column chromatography (7-10% MeOH:DCM) to yield a dark red solid material (0.22 g, 33%). ^{1}H NMR (400 MHz, DMSO-d_6): δ =2.14-2.21 (m, 4H), 3.06-3.07 (m, 2H), 3.77-3.80 (m, 4H), 4.08-4.15 (m, 4H), 4.68 (m, 4H), 6.94 (s, 1H), 7.42-7.45 (m, 2H), 7.60-7.62 (t, *J* = 8 Hz, 1H), 7.74-7.81 (m, 2H), 7.99-8.06 (m, 2H), 8.12 (d, *J* = 8 Hz, 1H), 8.60 (d, *J* = 8 Hz, 1H), 8.72 (d, *J* = 12 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 26.4, 28.2, 29.7, 42.7, 51.1, 61.0, 61.1, 72.8, 87.6, 108.1, 112.6, 117.7, 121.6, 122.8, 123.8, 124.2, 124.4, 125.6, 126.8, 128.1, 133.2, 136.9, 139.7, 144.2, 148.9, 155.6, 159.5. HRMS-ESI (*m/z*): calcd. for $C_{31}H_{31}N_4O_4S$ [M-I] 555.2061; found, 555.2072.

3-azidopropylamine **(C_{19})** was prepared according to a published procedure.[2]

N-(3-azidopropyl)-2-(2,3-dichloro-4-(2-methylenebutanoyl)phenoxy)acetamide (C_{21}). A solution of ethacrynic acid (0.744 g, 2.45 mmol) in dry DCM (10 mL) was cooled to 0 °C for 10 min. Then, DIC (380.1 µl, 2.45 mmol) and 3-azidopropylamine (0.27 g, 2.7 mmol) were added to the reaction mixture and the mixture was stirred at room temperature overnight and monitored by TLC (5% MeOH:DCM). The organic layer was washed three times with water, brine, and dried over sodium sulfate. The crude mixture was further purified by column chromatography (0-1% MeOH:DCM) to yield a clear oil (0.8 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.14 (t, *J* = 6 Hz, 3H), 1.86 (quin, *J* = 6 Hz, 2H), 2.46 (q, *J* = 6 Hz, 2H), 3.42 (t, *J* = 6 Hz, 2H), 3.49 (q, *J* = 6 Hz, 2H), 4.57 (s, 2H), 5.58 (s, 1H), 5.95 (s, 1H), 6.85 (d, *J* = 6 Hz, 1H), 6.97 (br-s, 1H), 7.18 (d, *J* = 6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.5, 23.5, 28.8, 37.0, 49.6, 68.3, 111.0, 123.1, 127.4, 128.9, 131.7, 134.4, 150.3, 154.6, 167.0, 195.7. HRMS-ESI (*m/z*): calcd. for $C_{16}H_{18}Cl_2N_4NaO_3$ [M+Na] 407.0654, found: 407.0671.

TOPI-3. A solution of **C_{17}** (5 mg, 7.33×10^{-3} mmol) in DMF (300 µL) was degassed by bubbling argon gas. After addition of sodium ascorbate (2.9 mg, 0.0146 mmol in 100 µL water), the reaction mixture was degassed again for 30 seconds. Then, copper sulfate pentahydrate (3.66 mg, 0.0146 mmol in 100 µL water) was added and the mixture was flushed with argon. After adding **C_{21}** (8.44 mg, 0.0219 mmol), the reaction mixture was exposed to microwave irradiation for 3 min at 100 °C. The solvent was evaporated under reduced pressure and the residue was purified by reversed-phase HPLC (5.46 mg, 51%). ^{1}HNMR (500 MHz, DMSO-d_6): δ = 1.05 (t, *J* = 5 Hz, 6H), 1.96 (q, *J* = 5 Hz, 4H), 2.16 (m, 4H), 2.34 (m, 4H), 3.13 (m, 4H), 4.06 (m, 2H), 4.20 (dd, *J* = 5 Hz, *J* = 15 Hz, 4H), 4.32 (q, *J* = 5 Hz, 4H), 4.66 (m, 4H), 4.72 (s, 4H), 5.53 (s, 2H), 6.04 (s, 2H), 6.93 (s, 1H), 7.07 (d, *J* = 10 Hz, 2H), 7.32 (d, *J* = 10 Hz, 2H), 7.40-7.44 (m, 2H), 7.58 (t, *J* = 10 Hz, 1H), 7.70-7.78 (m, 4H), 7.93-7.96 (m, 3H), 8.05 (d, *J* = 10 Hz, 1H), 8.11-8.15 (m, 3H), 8.60 (d, *J* = 5 Hz, 1H), 8.73 (d, *J* = 5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 12.2, 22.7, 26.5, 28.2, 28.8, 29.6, 35.6, 42.7, 46.9, 51.1, 60.7, 60.9, 67.9, 87.6, 108.1, 111.9, 112.6, 117.2, 117.7, 121.2, 122.5, 122.7, 123.8, 124.2, 124.4, 125.6, 126.7, 127.3, 128.1, 128.9, 129.3, 132.4, 133.1, 136.9, 139.7, 144.2, 148.8, 149.3, 155.4, 158.1, 159.5, 166.6, 194.8. HRMS-ESI (*m/z*): calcd. for $C_{63}H_{67}Cl_4N_{12}O_{10}S$ [M-I] 1323.3578, found: 1323.3574.

N-(2-aminoethyl)-2-(2,3-dichloro-4-(2-methylenebutanoyl)phenoxy)acetamide(C_{23}). Ethacrynic acid (500 mg, 1.65 mmol) was dissolved in 5 mL dry DCM, the reaction mixture was cooled to 0° C for 15 min, and HATU (0.75 g, 19.8 mmol), tert-butyl (2-aminoethyl)carbamate (313.3 µL, 1.98 mmol) and DIPEA (344.8 µL, 1.98 mmol) were added. The reaction mixture was stirred at room temperature overnight and monitored by HPLC and TLC (10% DCM:MeOH). The organic layer was washed four times with water and brine, and then dried over sodium sulfate. The crude mixture was further purified by column chromatography (2% MeOH:DCM) to yield a clear oil (0.35 g, 48%).^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.14 (t, *J* = 8 Hz, 3H), 1.41 (s, 9H), 2.46 (q, *J* = 8 Hz, 2H), 3.31 (q, *J* = 8 Hz, 2H), 3.50 (q, *J* = 8 Hz, 2H), 4.58 (s, 2H), 5.58 (s, 1H), 5.95 (s, 1H), 6.86 (d, *J* = 8 Hz, 1H), 7.18 (d, *J* = 8 Hz, 1H). MS-ESI (*m/z*): calcd. for $C_{20}H_{26}Cl_2N_2O_5$ [M+Na] 467.12; found 467.18.

Trifluoroacetic acid (1 mL) was added to a solution of the crude (90 mg, 0.2 mmol) in DCM (1 mL) and the reaction was stirred at room temperature for 2h. After completion of the reaction, the solvent was evaporated and the mixture was washed 6 times with DCM and evaporated for 2h under high vacuum. This compound was used for the next step without further purification.

TOPI-4. EDCI (41.95 mg, 0.22 mmol) and HOBT (29.57 mg, 0.22 mmol) were added to a solution of **C_6** (50 mg, 0.09 mmol) in dry DMF (1 mL) at 0°C. This mixture was kept at 0°C for 15 min. Then, **C_{23}**, (69 mg, 0.200 mmol) was added and the solution was basified to pH~7 with DIPEA (95 µL, 0.547 mmol), the reaction mixture was stirred at room temperature overnight and monitored by HPLC. The crude was purified by reversed-phase HPLC (20.64 mg, 18%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.06 (t, *J* = 8 Hz, 6H), 2.35 (q, *J* = 8 Hz, 4H), 2.70 (m, 4H), 3.11 (s, 8H), 4.65 (d, *J* = 12 Hz, 4H), 4.80 (m, 4H), 5.53 (s, 2H), 6.03 (s, 2H), 7.03 (s, 1H), 7.06-7.09 (m, 2H), 7.28-7.35 (m, 3H), 7.41 (t, *J* = 8 Hz, 1H), 7.60 (t, *J* = 8 Hz, 1H), 7.75 (m, 2H), 7.96-8.06 (m, 4H), 8.13-8.20 (m, 3H), 8.57 (d, *J* = 8 Hz, 1H), 8.75 (d, *J* = 8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 12.3, 22.9, 33.2, 34.3, 38.1, 42.5, 50.7, 67.8, 88.1, 107.8, 111.9 , 112.9, 117.9, 121.1, 122.8, 123.9, 124.3, 124.5, 125.8, 126.8, 127.4, 128.1, 129.3, 132.48, 132.49, 133.2,

136.9, 139.8, 145.0, 148.9, 149.3, 155.3, 159.5, 166.8, 169.2, 169.7, 195.1. HRMS-ESI (*m/z*): calcd. for $C_{53}H_{53}Cl_4N_6O_8S$ [M-I] 1073.2400; found 1073.2407.

2.6. Synthesis of TOPI-5 and TOPI-6.

TOPI-5. $\mathbf{C_6}$ (20 mg, 0.036 mmol) was dissolved in 500 µL dry DMF. Then, HATU (31 mg, 0.8 mmol), $\mathbf{C_{24}}$ (30.05 mg, 0.8 mmol) and DIPEA (14 µl, 0.08 mmol) were added. The reaction mixture was stirred at room temperature overnight and monitored by HPLC. The solvent was evaporated under reduced pressure and the residue was purified by reversed-phase HPLC (5.13 mg, 11%). ^{1}HNMR (400 MHz, DMSO-d_6): δ = 1.24-1.64 (m, 12H), 2.04 (t, *J* = 8 Hz, 4H), 2.60 (d, *J* = 12 Hz, 2H), 2.74-2.84 (m, 6H), 3.06-3.11 (m, 2H), 3.12-3.20 (m, 8H), 3.24-3.4 (m, 12H), 3.45 (s, 4H), 4.11-4.14 (m, 2H), 4.29-4.32 (m, 2H), 4.82 (q, *J* = 8 Hz, 4H), 6.30 (m, 4H), 7.06 (s, 1H), 7.37-7.45 (m, 2H), 7.60-7.80 (m, 5H), 7.99-8.05 (m, 3H), 8.13-8.15 (m, 2H), 8.55 (d, *J* = 8 Hz, 1H), 8.76 (d, *J* = 8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 25.0, 27.8, 27.9, 33.2, 34.2, 34.9, 38.2, 38.6, 42.6, 50.6, 55.1, 59.1, 60.9, 68.7, 69.2, 88.1, 107.7, 112.88, 117.8, 122.6, 123.8, 124.2, 124.4, 125.6, 126.6, 127.9, 133.1, 136.8, 139.7, 144.8, 148.7, 159.5, 162.5, 168.8, 169.5, 171.9. HRMS-ESI (*m/z*): calcd. for $C_{55}H_{77}N_{10}O_{10}S_3$ [M-I] 1133.4981; found, 1133.4970.

TOPI-6. $\mathbf{C_6}$ (20 mg, 0.036 mmol) was dissolved in 500 µl dry DMF. Then HATU (31 mg, 0.8 mmol), $\mathbf{C_{25}}$ (23 mg, 0.8 mmol) and DIPEA (14 µL, 0.08 mmol) were added. The reaction mixture was stirred at room temperature overnight and monitored by HPLC. The solvent was then evaporated under reduced pressure and the residue was purified by reversed-phase HPLC (6 mg, 15%). ^{1}HNMR (400 MHz, DMSO-d_6): δ = 1.25-1.61 (m, 12H), 1.98-2.04 (m, 4H), 2.26-2.34 (m, 4H), 2.56-2.59 (m, 2H), 2.66-2.82 (m, 6H), 2.99-3.07 (m, 10H), 4.10-4.13 (m, 2H), 4.28-4.31 (m, 2H), 4.83 (q, *J* = 8 Hz, 4H), 6.27,6.29 (s, 2H), 6.38 (br-s, 2H), 7.06 (s, 1H), 7.39-7.45 (m, 2H), 7.60-7.67 (m, 2H), 7.74-7.80 (m, 2H),7.97-8.05 (m, 3H), 8.15 (d, *J* = 8 Hz, 1H), 8.57 (d, *J* = 8 Hz, 1H), 8.77 (d, *J* = 8 Hz, 1H). HRMS-ESI (*m/z*): calcd. for $C_{23}H_{61}N_2O_4S$ [M-I] 957.3932; found, 957.3923.

3. Inhibition and Dissociation Constants

3.1 GST Kinetic Measurements and Inhibitory Constants

Inhibition of GST-A1-1, GST-M1-1, GST P1-1, and GST-A2-2 activity by TOPI-**3** and TOPI-**4** was tested using a method developed by Habig *et al*.[3] (Fig. 5 and S5). The concentrations of different GSTs, GSH, and CDNB were 20 nM, 350 µM, and 700 µM, respectively. The GST activity was measured spectrophotometrically using chloro-2,4-dinitrobenzene (CDNB) and GSH as substrates, in phosphate buffer (10 mM, pH 6.5). In a typical experiment, GST and TOPI-**3** or TOPI-**4** were incubated for 10 min at 25°C and then GSH and CDNB were subsequently added. The formation of S-(2,4-dinitrophenyl)-glutathione was monitored using the microplate reader at λ = 340 nm. In order to obtain the inhibition constant, we performed the enzymatic assays using three different concentrations of GSTs (20, 60, and 100 nM) and TOPI-**3** or TOPI-**4** (10 nM-5 µM). The concentrations of GSH and CDNB were 3 mM and 1.5 mM, respectively. The K_m values were determined using 20 nM of each GST isoform, 3 mM GSH, and variable CDNB concentrations (50 µM-2 mM). Data were fit to the Michaelis-Menten equation using Sigmaplot version 12.0 statistical software (Systat) to obtain the K_m values. The obtained K_m values were 350 µM, 600 µM, 375 µM, and 450 µM for GST-M1-1, GST-P1-1, GST-A1-1, and GST-A2-2, respectively.

The data were analyzed using Graphpad Prism 6.0 and fitted to the Morrison equation for tight binding substrates[4] (Fig. S4b, S4c and S6).

$$Y = \frac{V_0\,(1-(E+X+Q)-\{(E+X+Q)^2-4\cdot E\cdot X\}^{1/2})}{2\cdot E} \quad , \quad Q = K_i\cdot\left(1+\frac{S}{K_m}\right) \quad .$$

Where Y is the enzyme activity, X is different concentrations of inhibitor (TOPI-**3** or TOPI-**4**), E is the enzyme concentration, S is the concentration of substrate, K_m is the Michaelis-Menten constant determined in an experiment without inhibitor, and V_0 is the initial velocity. The inhibition constant obtained for GST isoforms (GST M1-1, GST P1-1, GST A1-1, and GST A2-2) are listed in Table S1 for TOPI-**3**.

3.2 Dissociation Constants

The approximate dissociation constants were determined for GST-M1-1 and GST-P1-1. For these experiments, the concentration of the probe was chosen according to the enzymatic assays (K_i values, Fig. S4). It was chosen to be lower than the K_i (expected K_d), but such that would also generate a detectable and reproducible emission signal in the plate reader. For GST-M1-1, TOPI-**3** (5 nM), and various concentrations of GST M1-1 (0, 2, 3, 4, 5, 6, 7.5, 20, 25, 40, 45, 50, and 60 nM) were incubated in phosphate buffer (5 mM, pH=6.5) and the fluorescent intensities were recorded at λ_{ex}=500 nm. Similarly, for GST-P1-1, TOPI-**4** (15 nM) and various concentrations of GST P1-1 (0, 5, 7.5, 10, 15, 20, 25, 30, 50, 100, 150, and 200 nM) were incubated in phosphate buffer (5 mM, pH=6.5) for 30 min and the fluorescent intensities were recorded at λ_{ex}=500 nm (Fig. S3). These experiments were performed in triplicate. The data were analyzed according to one-site binding model using Graphpad Prism 6.0 and fitted to the equation: $I_F = I_{Fmax} \cdot L/(k_d+L)$, where I_F is the fluorescence intensity at a relative protein concentration (L), I_{Fmax} is the maximum fluorescence intensity at the saturated point and L is the concentration of the protein. The approximate dissociation constants (K_d) for the TOPI-**3**-GST-M1-1 and the TOPI-**4**-GST-P1-1 interaction were found to be 16.4 ± 2.14 nM (R^2=0.99), and 28.97 ± 5.21 nM (R^2=0.98), respectively.

3.3 AChE Kinetic Measurements and Inhibitory Constants

Inhibition of AChE activity by TOPI-**1** and TOPI-**2** was tested according to a published method by Ellman *et al.*[5] The AChE activity was measured spectrophotometrically using 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and acetylthiocholine iodide as substrates, in phosphate buffer (50 mM, pH 8.0). In a typical experiment, AChE and TOPI-**1** (50 nM) or TOPI-**2** (2 µM) were incubated for 10 min at 25°C and then DTNB and acetylthiocholine iodide were subsequently added. The final concentrations of AChE, DTNB, and acetylthiocholine iodide were 8 pM, 0.4 mM, and 0.4 mM, respectively. The AChE activity was monitored using a microplate reader at λ = 410 nm (Fig. 5). TOPI-**1** demonstrated full inhibition at low nanomolar concentration, while TOPI-**2** inhibited the AChE activity only at 2 µM. For the control assays, the activity of AChE was evaluated using 50 nM of 9-chloro-1,2,3,4-tetrahydroacridine (tacrine), a known AChE inhibitor,

and using a 10-fold excess (5 μM) of a control TO derivative **C_6**, which lacks the AChE inhibitors. To obtain K_i value, the initial velocities were measured for six different concentrations of TOPI-**1,** ranging from 0 to 2 nM and for six different concentrations of acetylthiocholine iodide (25-300 μM). The initial velocities were fitted into an equation that corresponds to complete competitive inhibition: $v = \frac{V_{max}}{[1+(\frac{K_m}{S})(1+\frac{I}{K_i})]}$ using Sigmaplot 12.0 statistical software (Systat). An inhibition constant of K_i = 0.31 nM ± 0.04 was obtained for TOPI-**1**.

4. Fluorescence Measurements

4.1 GST Sensing

Based on the obtained binding constants (Fig. S3), a concentration of 100 nM probe was chosen for GST sensing. This concentration should ensure strong binding and a high S/N ratio. The fluorescence intensity of TOPI-**3** or TOPI-**4** (100 nM) in phosphate buffer (5 mM, pH=6.5) was recorded before and after the addition of 90 nM of different GST isoforms (GST A1-1, GST A2-2, GST P1-1, GST M1-1, GST O1-1, GST K1-1, GST Z1-1, and GST T1-1) using a microplate reader and an excitation wavelength of 500 nm. For TOPI-**3**, fluorescence values were recorded immediately after addition of the GSTs (Fig. 3b and 4c). The emission of TOPI-**4** was recorded after 30 min incubation (Fig. 3c and 4c).

4.2 GST M1-1 Sensing in the Presence of Other GST Isoforms

We tested the ability of TOPI-**3** to selectively bind to GST M1-1 in the presence of all other GST isoforms. Two different mixtures of GST isoforms were prepared: one that contained all the GST isoforms (20 nM of each GST, in total 160 nM) (A1-1, A2-2, P1-1, M1-1, T1-1, O1-1, Z1-1, and K1-1) and the second mixture contained all the GST isoforms except GST-M1-1. The fluorescence of TOPI-**3** (100 nM) in phosphate buffer (5 mM, pH=6.5) was recorded before and after the addition of each GST mixture (Fig. 10a).

4.3 Screening Assay with Different Known Drugs

Fluorescence intensities were recorded before and after the addition of known GST inhibitors or randomly selected drugs (50 μM) to a mixture containing TOPI-**3** and GST M1-1 (90 nM each) (Fig. 8).

4.4 Av and SAv Sensing

The fluorescence intensity of TOPI-**5** (100 nM) or TOPI-**6** (100 nM) in phosphate buffer (15 mM, pH=7.3) was recorded before and after the addition of 90 nM Av or SAv using a microplate reader. Excitation wavelengths for TOPI-**5** and TOPI-**6** were 495 nm and 505 nm, respectively. Fluorescence values were recorded immediately after addition of the proteins (Fig. 3d and 4b).

4.5 AChE Sensing

The fluorescence intensity of TOPI-**1** (100 nM) or TOPI-**2** (100 nM) in a phosphate buffer (20 mM, pH=8) was recorded before and after the addition of 90 nM AChE using a microplate reader. Excitation wavelengths for TOPI-**1** and TOPI-**2** were 505 nm and 510 nm, respectively. Fluorescence values were recorded immediately after addition of the proteins (Fig. 3a and 4a).

4.6 Control Experiments

For the control experiments with serum proteins (Fig. 6), 2μM of lysozyme, PDGF-BB, IgA, IgG, FGF-21, fibrinogen, and transferrin and 1μM of HSA were used. In the control experiments with dsDNA (Fig. 7e), two complementary ODNs (oligo A and oligo T, 20 bp) were incubated at 95°C for five minutes and then annealed at room temperature for 30 minutes. Fluorescence intensities of TO (100 nM) or TOPI-**1**, TOPI-**3**, TOPI-**4** and TOPI-**5** (100 nM) were recorded before and after the addition of a dsDNA (400 nM) in phosphate buffer (5 mM, pH=6.5) at λ_{ex}= 500 nm.

4.7 General Procedure for Fluorescence Titration

TOPI-**1**, TOPI-**3**, TOPI-**4**, and TOPI-**5** were excited at 500 ± 5 nm and the emission spectra were collected (Fig. S2). Increasing concentrations of the corresponding target

proteins (20 nM, 40 nM, 60 nM, 90 nM, and 120 nM) were added and the emission spectra were recorded again. As shown in Fig. S2, incremental addition of the target proteins has led to gradual increase in the fluorescence intensity of each of the TOPI probes, while the overall spectral shape remained intact.

5. GST Sensing in Urine Samples

Fresh urine samples were desalted by ultrafiltration using 3-kDa cutoff Centricon Plus-70 filters (Millipore, MA) according to manufacturer's procedure. Then GST-P1-1 (60 µg), GST M1-1 (54 µg) or combination of both were added to 150 µL of desalted urine samples and each urine sample was incubated with 50 µL of pre-washed Glutathione Sepharose™ affinity beads (GE Healthcare, UK) and gently agitated by end-over-end rotation at room temperature for 1 h. After separation of supernatant from the beads, the beads were washed with phosphate binding buffer (10 mM, pH 7.3 containing 140 mM NaCl, 2.7 mM KCl, and 1 mM dithiothreitol) to remove the non-specifically bound proteins. GSTs were then eluted from the beads using 100 µL of 10 mM reduced L-glutathione in Tris-HCl buffer (50 mM, pH = 8) and collected by centrifuging (500 × g, 1 min). Finally, the excess of GSH was removed by ultrafiltration using 3 kDa cutoff centrifugal filters (Amicon Ultra, Millipore).

For the sensing experiments, TOPI-**3** and TOPI-**4** (100 nM) were dispensed into 384-well microplates and then fluorescence intensities were recorded following excitation at 500 nm. Then, urine samples containing GST M1-1, GST P1-1 or both (final concentration of 100 nM of GST) were added to each well and the fluorescence intensity values were recorded again. While fluorescence values for urine samples containing GST-M1-1 were recorded immediately, fluorescence values for urine samples containing GST-P1-1 were recorded after 30 min incubation. These experiments were performed in triplicate.

6. Cell Imaging

MDA-MB-231 cells were maintained in RPMI supplemented with 10% FBS, L-glutamine, and antibiotics. MCF-10A cells were cultured as previously described.[6] 5×10^4

cells, of each type, were plated in 24-well culture dish and allowed to adhere for 24 hours before the experiment. Cells were then rinsed twice with PBS (10 mM, pH = 7.4) and were incubated with 2 μM of TOPI-**4** in PBS for 15 minutes at 37°C. Similarly, control experiments were performed following incubation of MDA-MB-231 cells with TO (2 μM) as well as with TOPI-**4** (2 μM) and an excess of EA (50 μM in PBS). Cells were then washed twice with PBS, and imaged using an Olympus IX51 fluorescent microscope equipped with U-MNIBA3 Fluorescence filter cube (excitation 470-495, emission 510-550). Cell images were analyzed using imageJ.

7. Figures and Tables

Table S1. Approximate inhibition constants of different GST isoforms by TOPI-**3**.

GST isoform	K_i **[nM]**
GST P1-1	48.45 ± 5.05
GST A1-1	453.8 ± 51.84
GST A2-2	19.56 ± 2.76
GST M1-1	4.27 ± 0.57

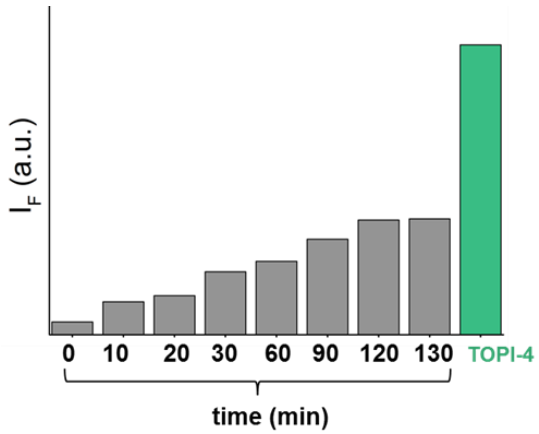


Fig. S1. Grey bars: Fluorescence of TOPI-**3** (100 nM) in the presence of 90 nM of GST P1-1 over a period of 130 min. Green bar: the emission intensity of TOPI-**4** (100 nM) in the presence of 90 nM of GST P1-1.

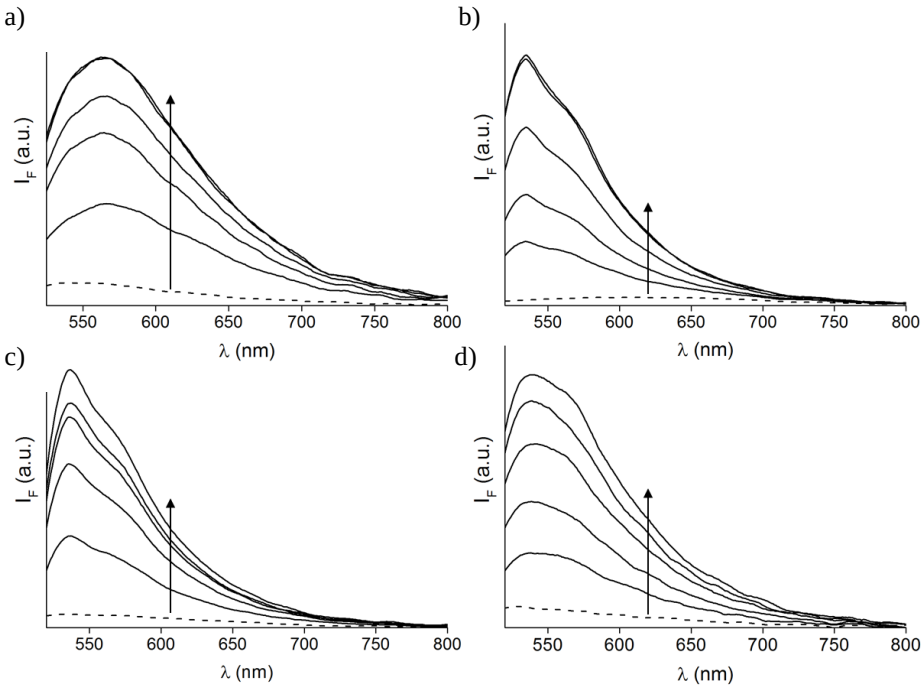


Fig. S2. Changes in the fluorescence signal of the most efficient TOPI probes: a) TOPI-**1**, b) TOPI-**3**, c) TOPI-**4**, and d) TOPI-**5** (100 nM each) upon incremental addition of 20, 40, 60, 90 and 120 nM of AChE, GST-M1-1, GST-P1-1, and Av, respectively. Conditions: a) λ_{ex} = 505 nm, PBS buffer (20 mM, pH=8), b and c) λ_{ex} = 500 nm, PBS buffer (5 mM, pH=6.5), d) λ_{ex} = 495 nm, PBS buffer (15 mM, pH=7.3).

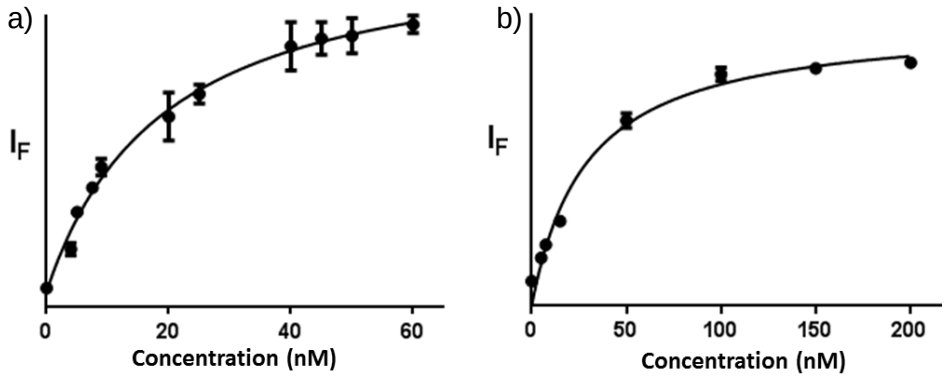


Fig. S3. Binding curves for a) GST-M1-1 and TOPI-**3** and b) GST-P1-1 and TOPI-**4** determined by monitoring the emission signal (normalized) of TOPI-**3** or TOPI-**4** upon a gradual addition of GST M1-1 or GST P1-1. The approximate dissociation constants between GST-M1-1 and TOPI-**3**, and GST-P1-1 and TOPI-**4** were found to be K_d = 16.4 ± 2.14 nM, K_d = 28.97 ± 5.21 nM, respectively.

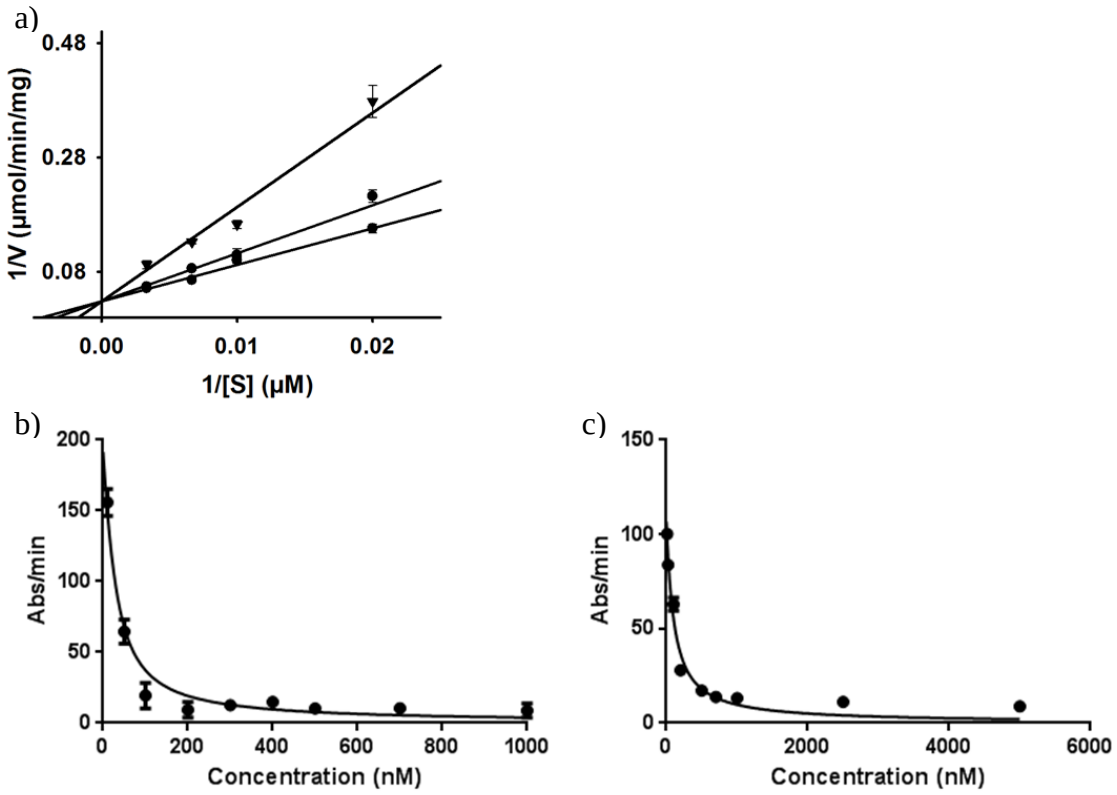


Fig. S4. a) Lineweaver-Burk plot of inhibition of AChE activity by TOPI-**1.** Acetylthiocholine iodide was used as substrate of AChE. Data points show inhibitor (TOPI-**1**) concentrations of 0 (■), 0.1 (●), and 0.5 (▼) nM**.** Inhibition of GST-M1-1 (panel b) and GST-P1-1 (panel c) by TOPI-**3** and TOPI-**4**, respectively. The GSH and CDNB concentrations were 3 mM and 1.5 mM, respectively. Additional details are given in the section 3.1 and 3.3.

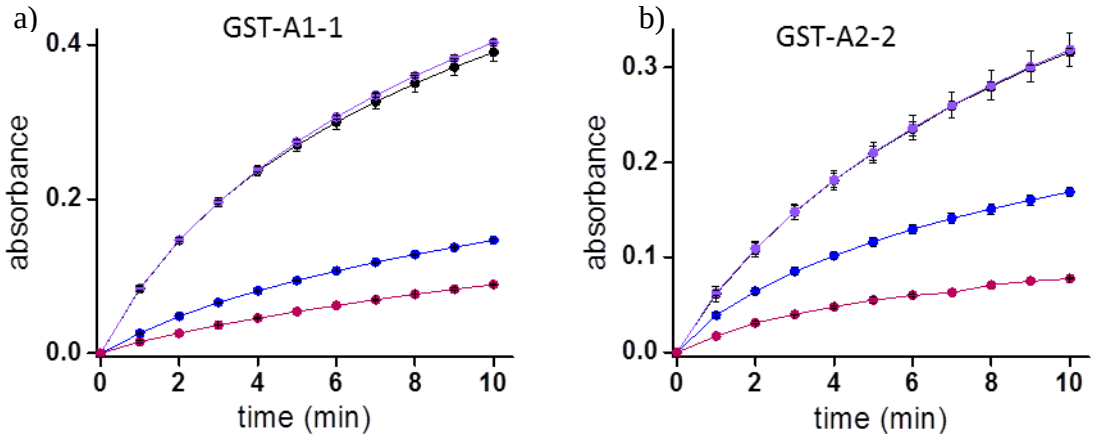


Fig. S5. Enzymatic activity of 20 nM of a) GST-A1-1, b) GST-A2-2 in the absence (—) and presence of 5 µM EA (—), 5 µM compound **C17** (—), or 500 nM TOPI-**3** (—). Enzymatic activity was monitored at 340 nm.

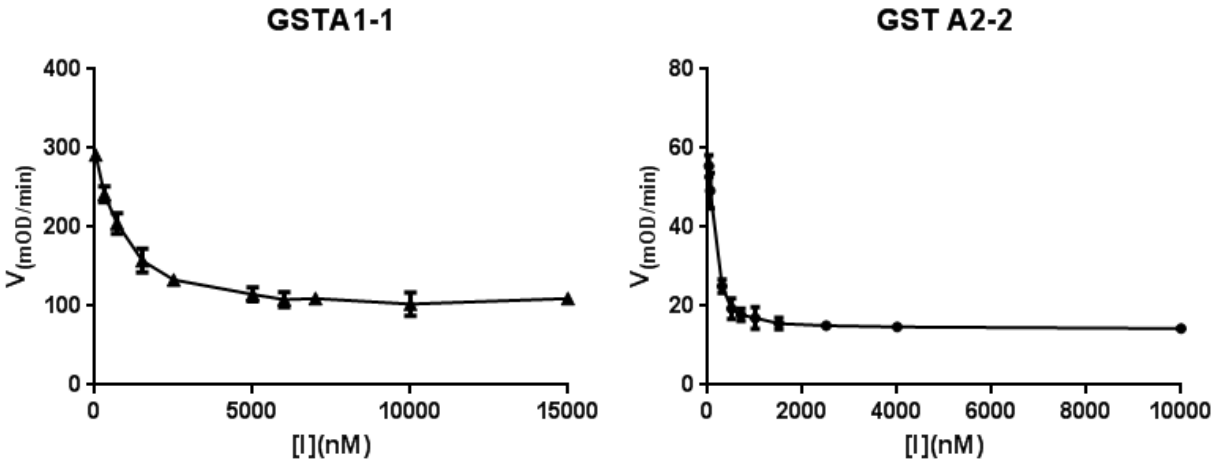


Fig. S6. Global nonlinear fit for inhibition of a) GST A1-1 (100 nM) and b) GST A2-2 (20 nM) by TOPI-**3**. The GSH and CDNB concentrations were 3 mM and 1.5 mM, respectively.

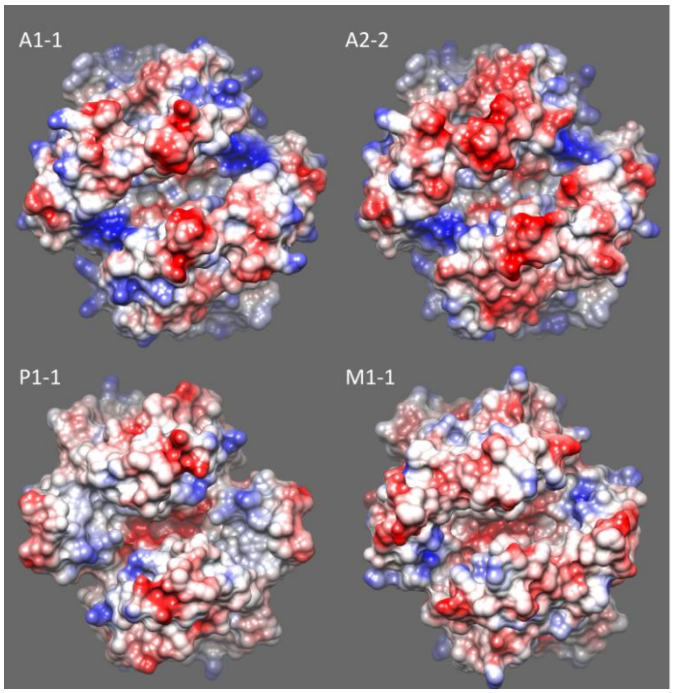


Fig. S7. The electrostatic potential on the surface of four GST variants. Blue indicates positive potential, red negative potential and white neutral potential. A quantitative estimate of the width of the crevice was obtained by calculating the average distance between the Cα atoms of residues 97, 100, 101, 104, 105 and 108 in one monomer to the corresponding Cα atoms in the other monomer; these residues line the central part of the crevice. The average distances for GST-A1-1, GST-A2-2, GST-P1-1 and GST-M1-1 are 17.3Å, 15.8Å, 11.7Å and 11.4Å, respectively.

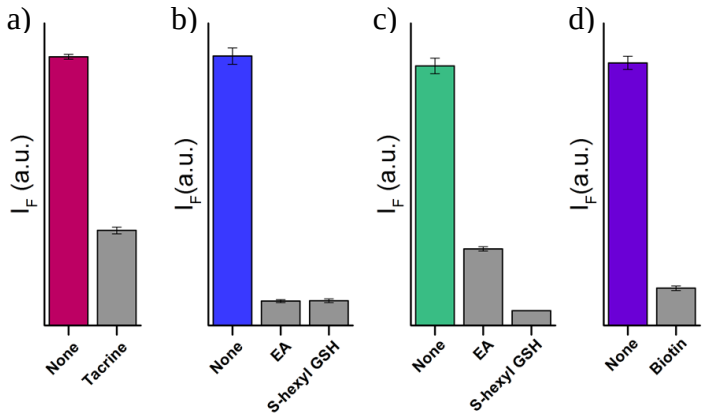


Fig. S8. Displacement assay in which a) TOPI-**1**-AChE, b) TOPI-**3**-GST M1-1, c) TOPI-**4**-GSTP1-1, and d) TOPI-**5**-Avidin complex (90 nM, colored) were treated with 50 µM tacrine, EA, S-hexyl GSH, and biotin, respectively (grey). These experiments were performed in triplicate.

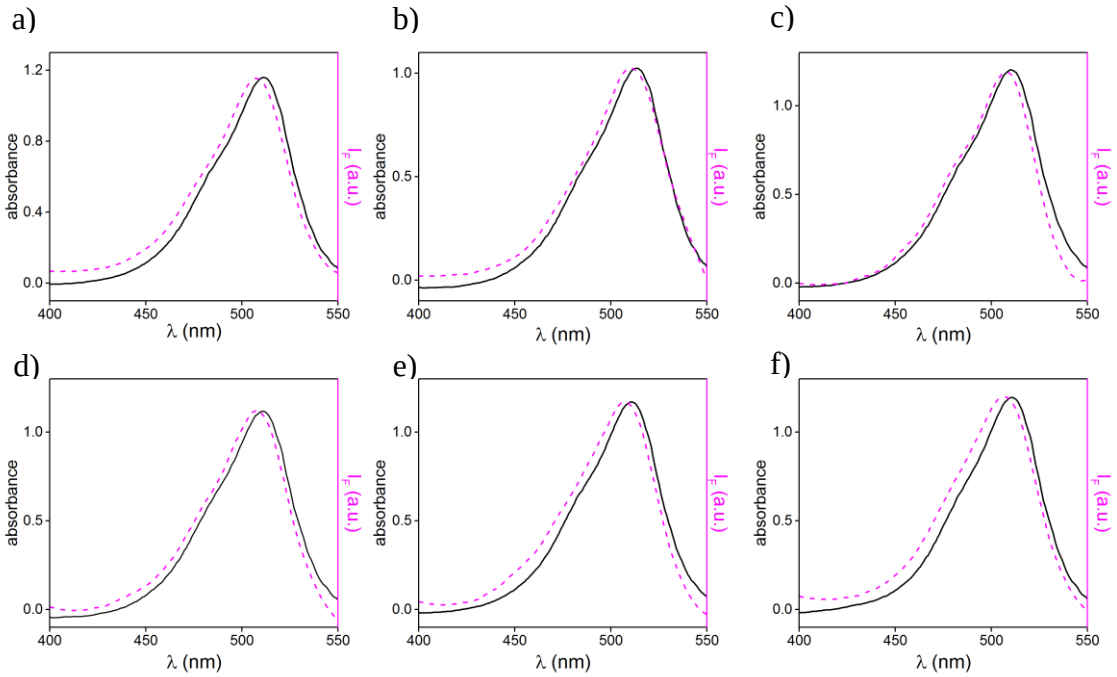
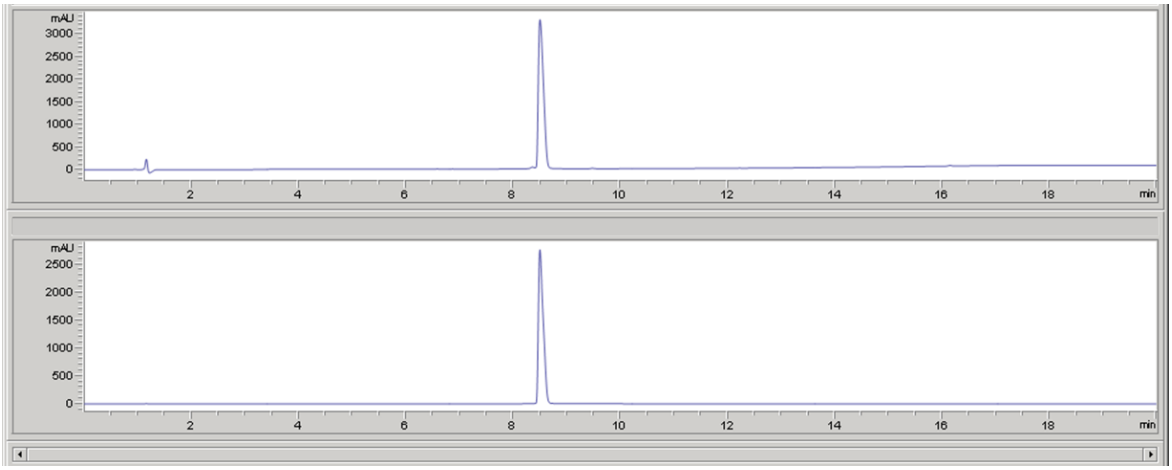


Fig. S9. Absorption (black solid line) and excitation (dashed pink line**)** spectrum of: a) TOPI-**1**, b) TOPI-**2**, c) TOPI-**3,** d) TOPI-**4**, e) TOPI-**5** and f) TOPI-**6**. The absorption and excitation spectra were recorded using 30 µM and 1 µM of TOPIs **1-6**, respectively.

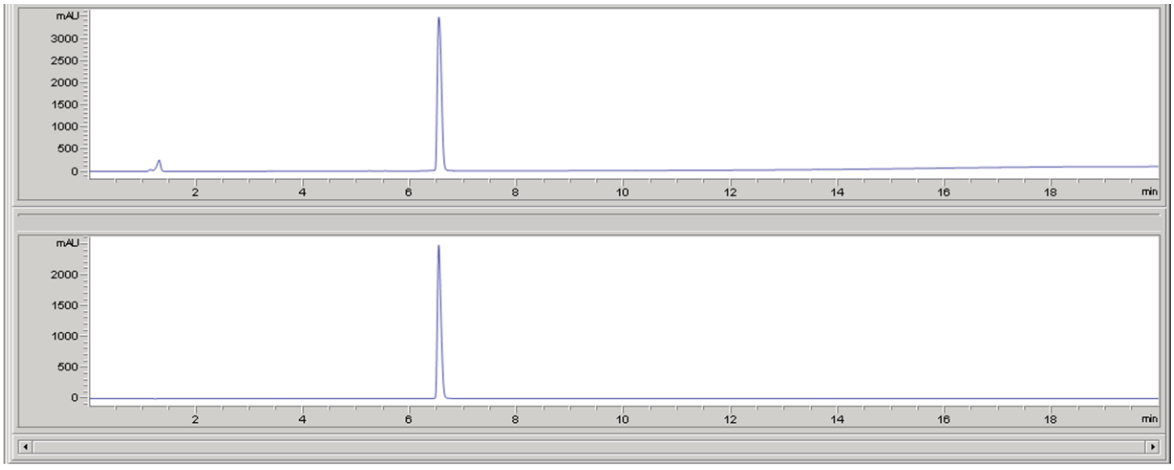
8. HPLC Chromatograms of TOPIs 1-6

Detection wavelengths: 220 nm (top) and 480 nm (bottom).

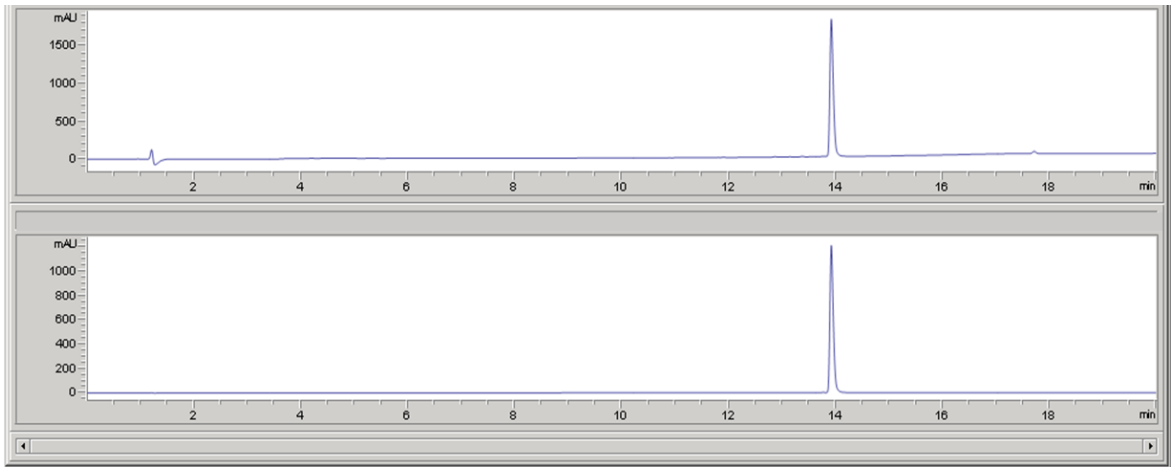
a) TOPI-**1**



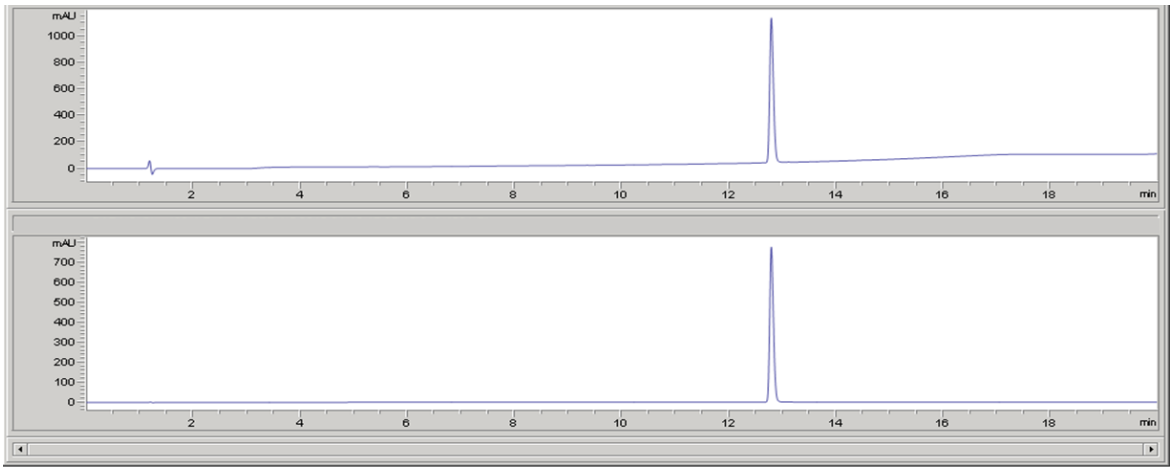
b) TOPI-**2**



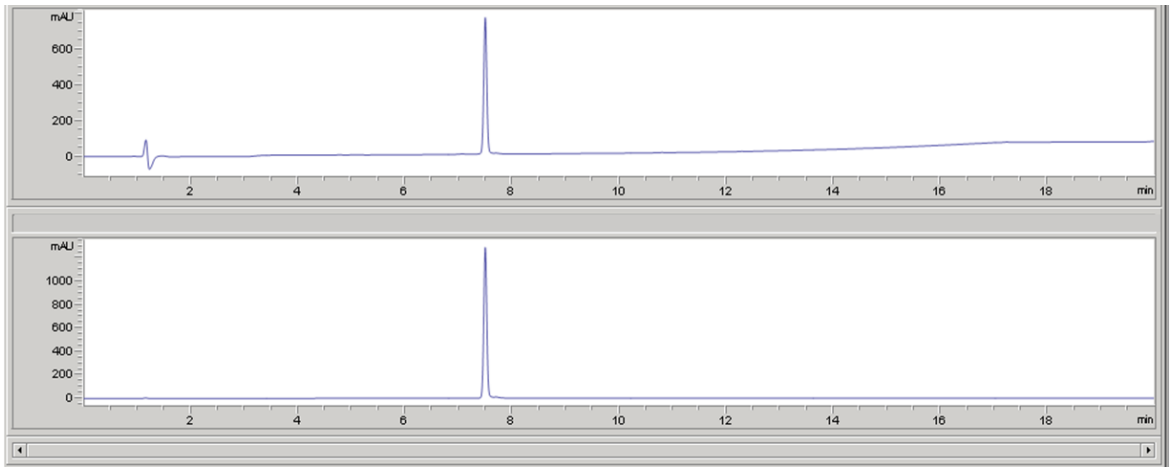
c) TOPI-**3**



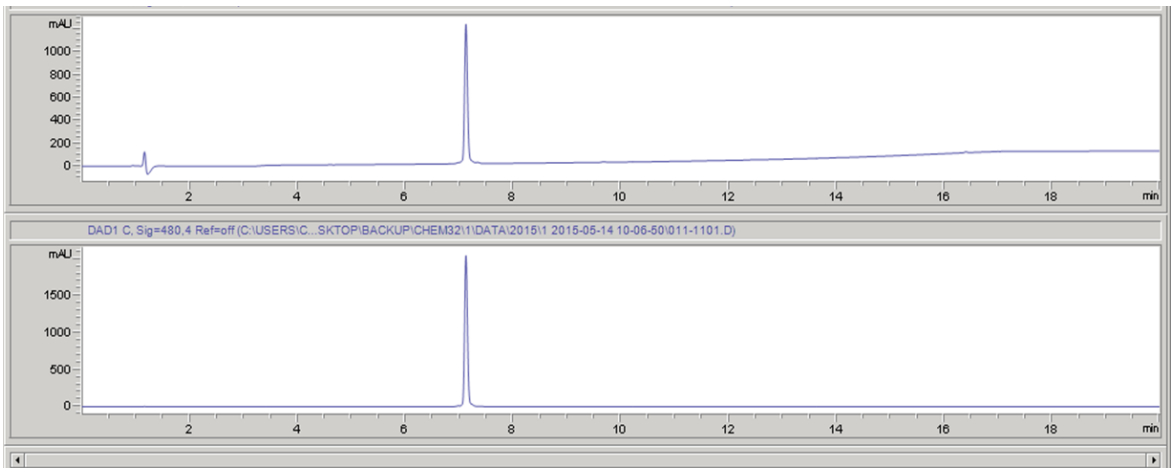
d) TOPI-**4**



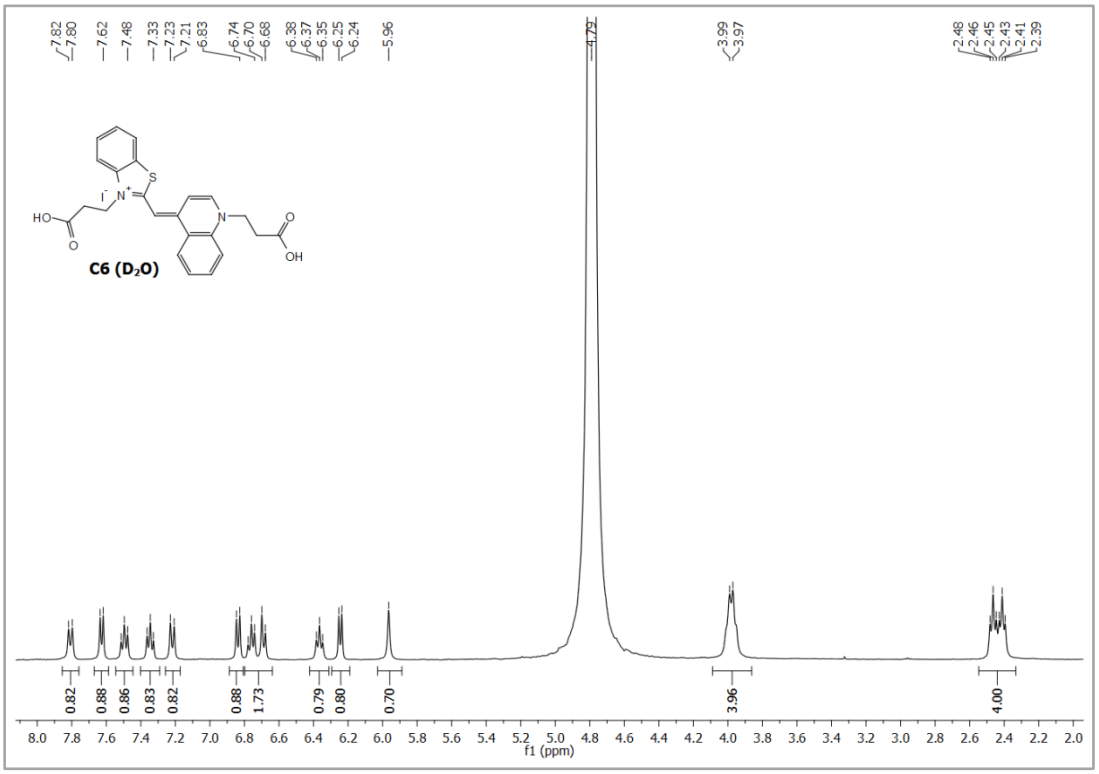
e) TOPI-**5**

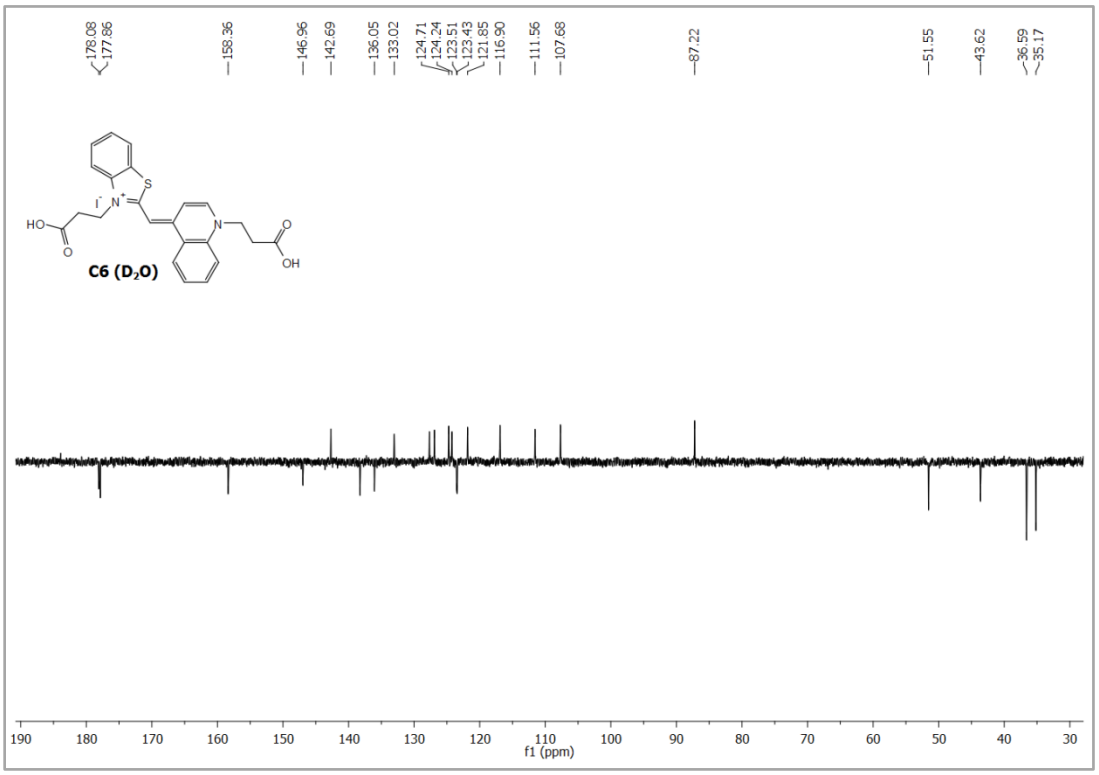


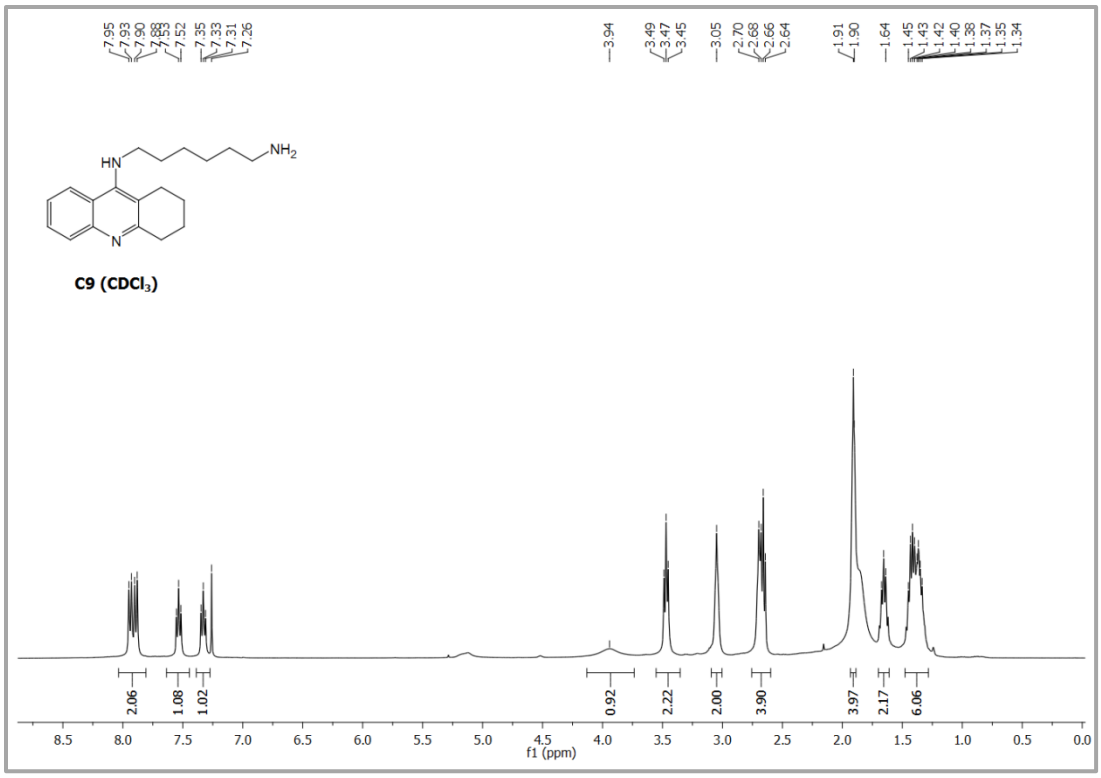
f) TOPI-**6**

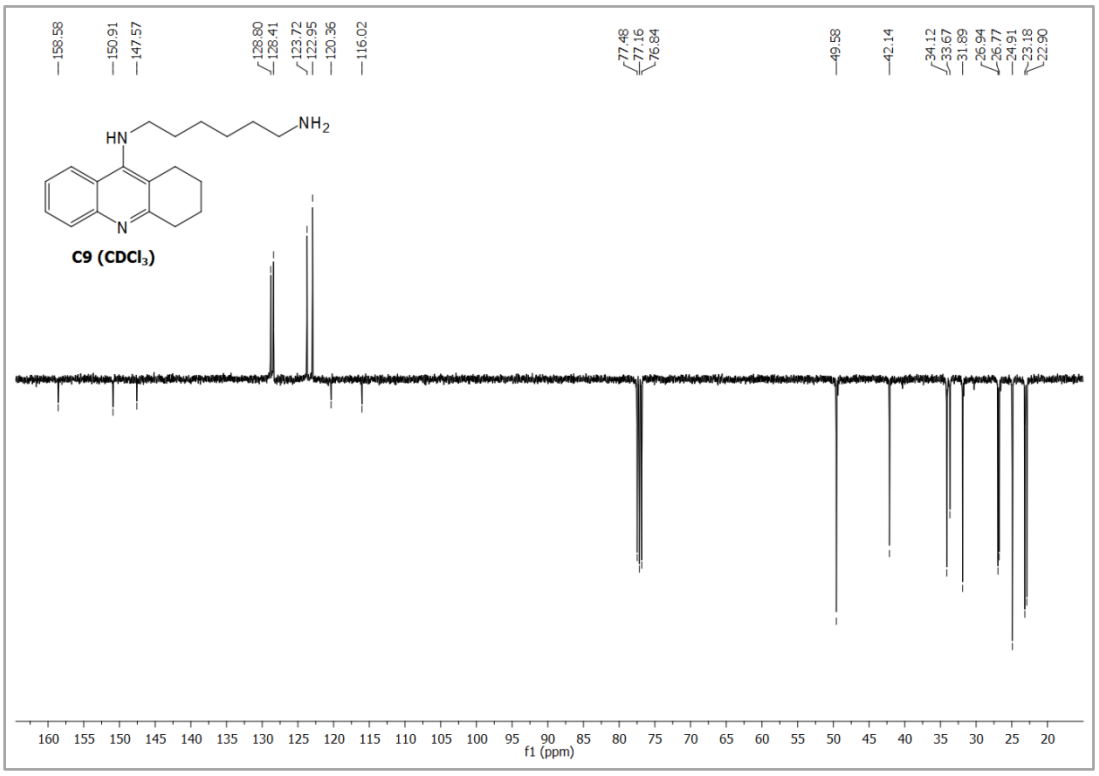


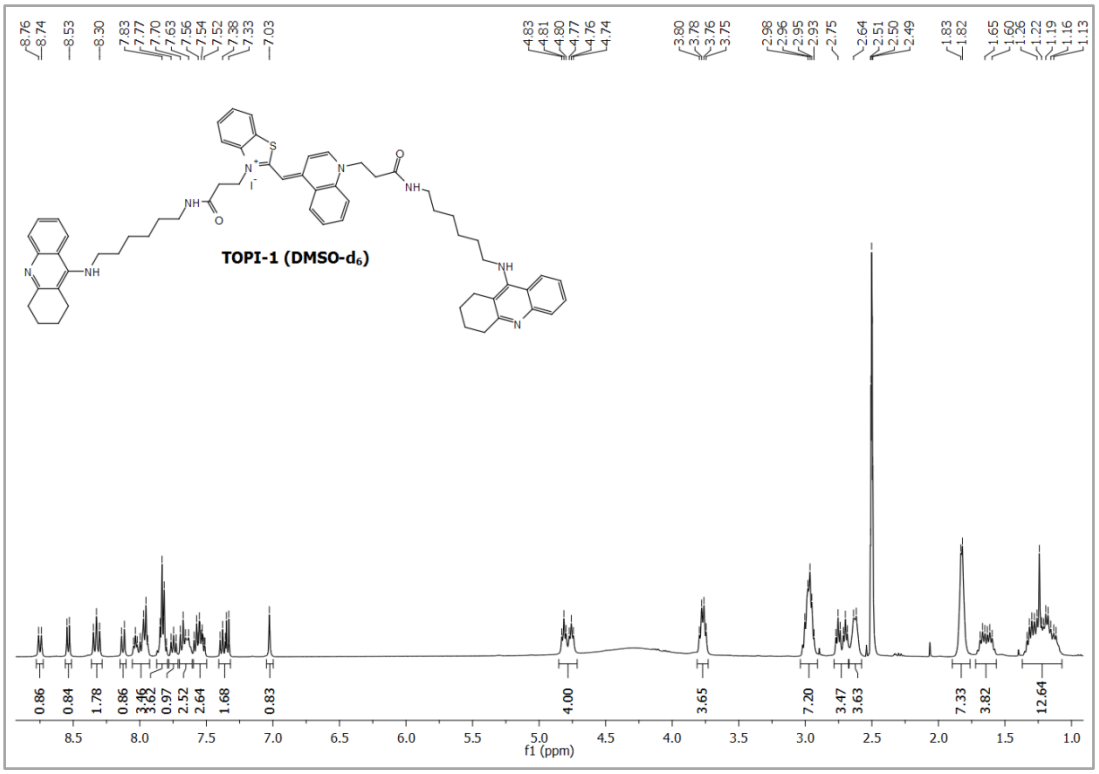
9. ^{1}HNMR and ^{13}CNMR Spectra

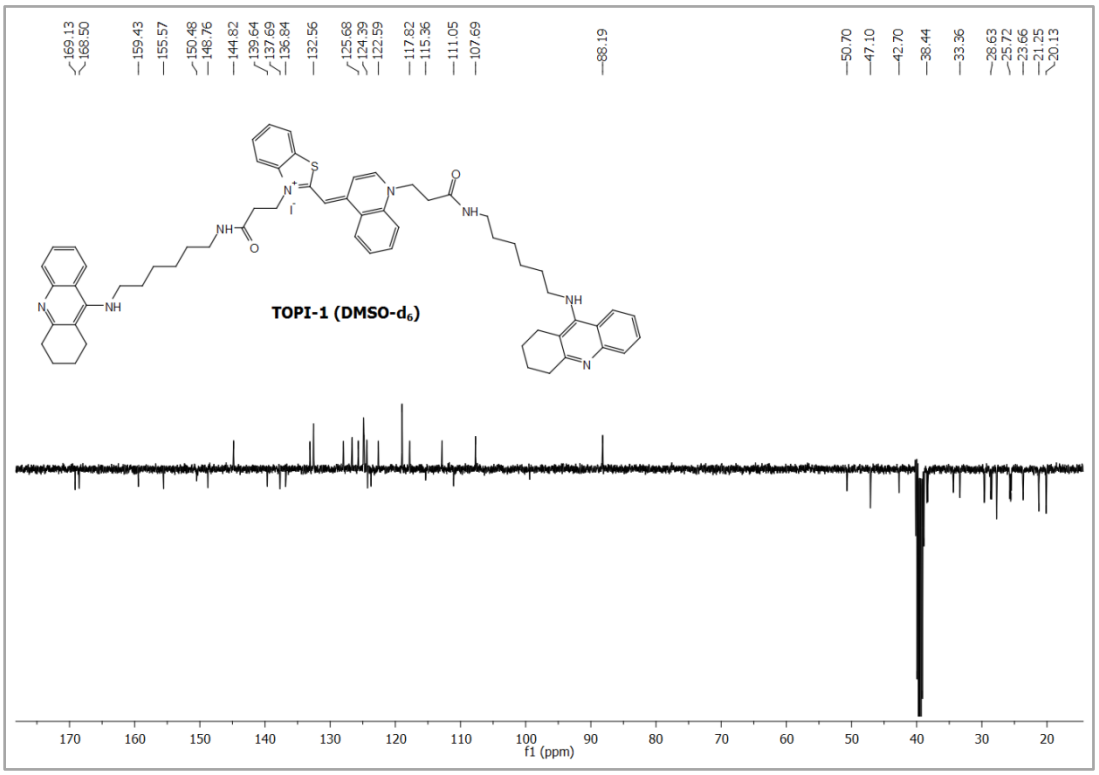


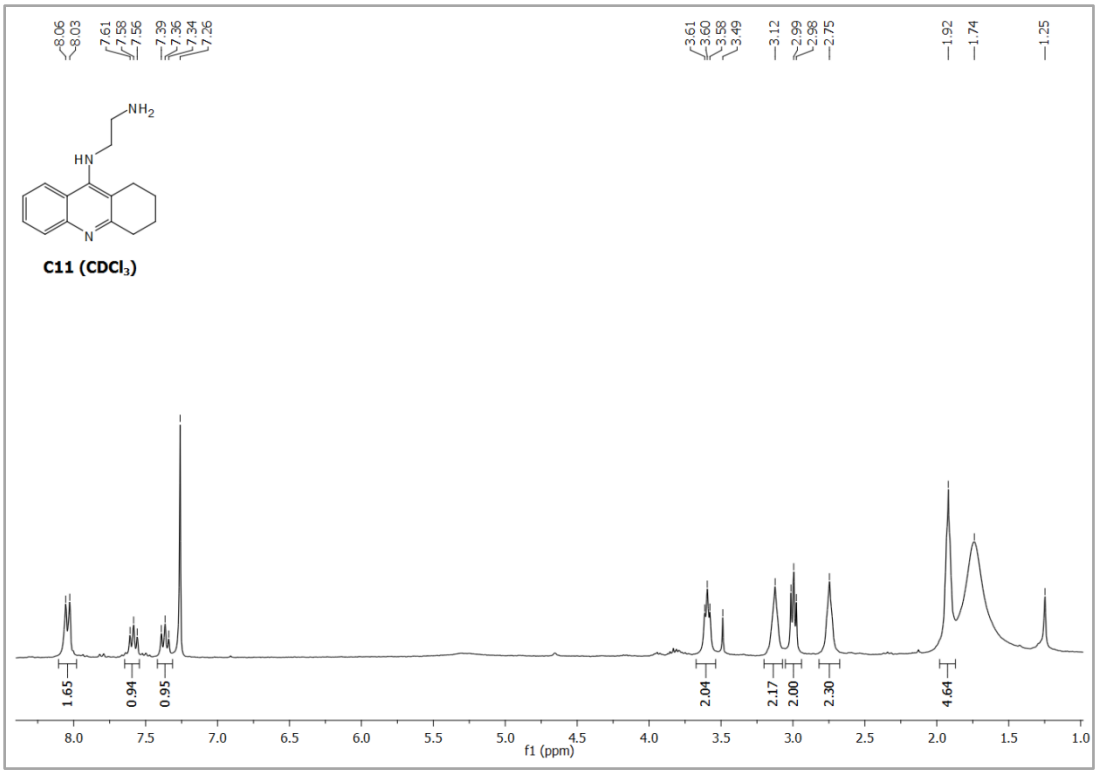


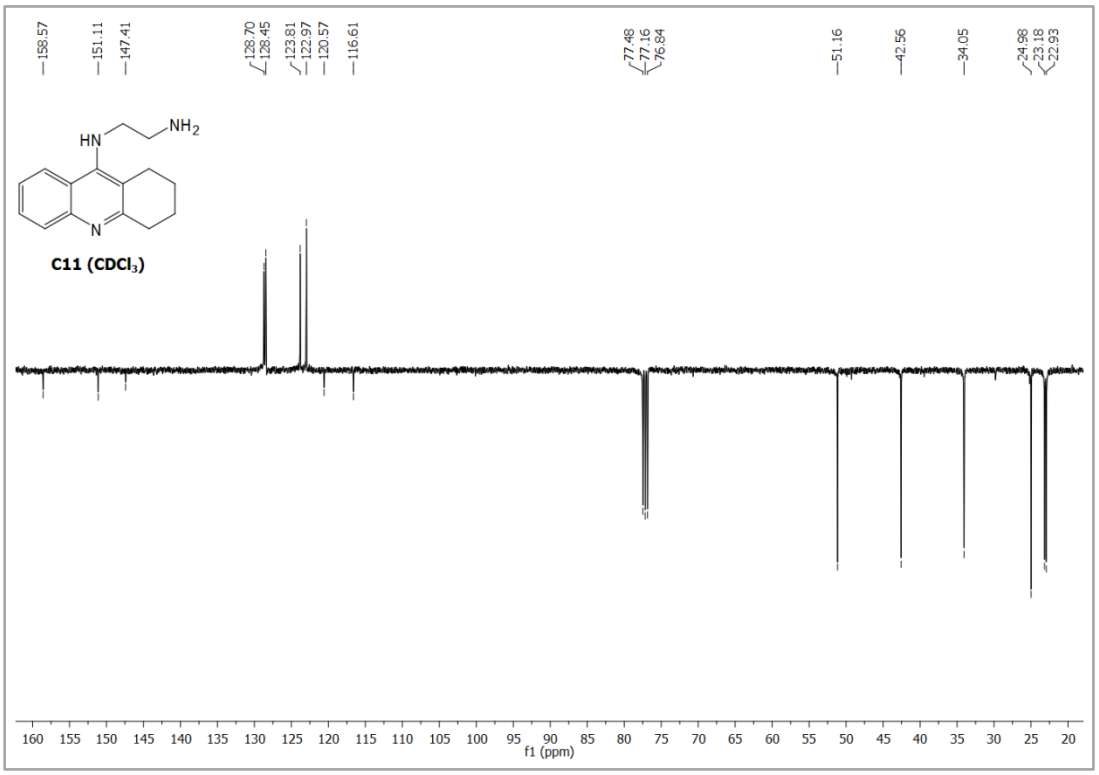

7.95
7.93
7.90
7.88
7.55
7.52
7.35
7.33
7.31
7.26
3.94
3.49
3.47
3.45
3.05
2.70
2.68
2.66
2.64
1.91
1.90
1.64
1.45
1.43
1.42
1.40
1.38
1.37
1.35
1.34
HN
NH2
N
C9 (CDCl3)
2.06
1.08
1.02
0.92
2.22
2.00
3.90
3.97
2.17
6.06
f1 (ppm)

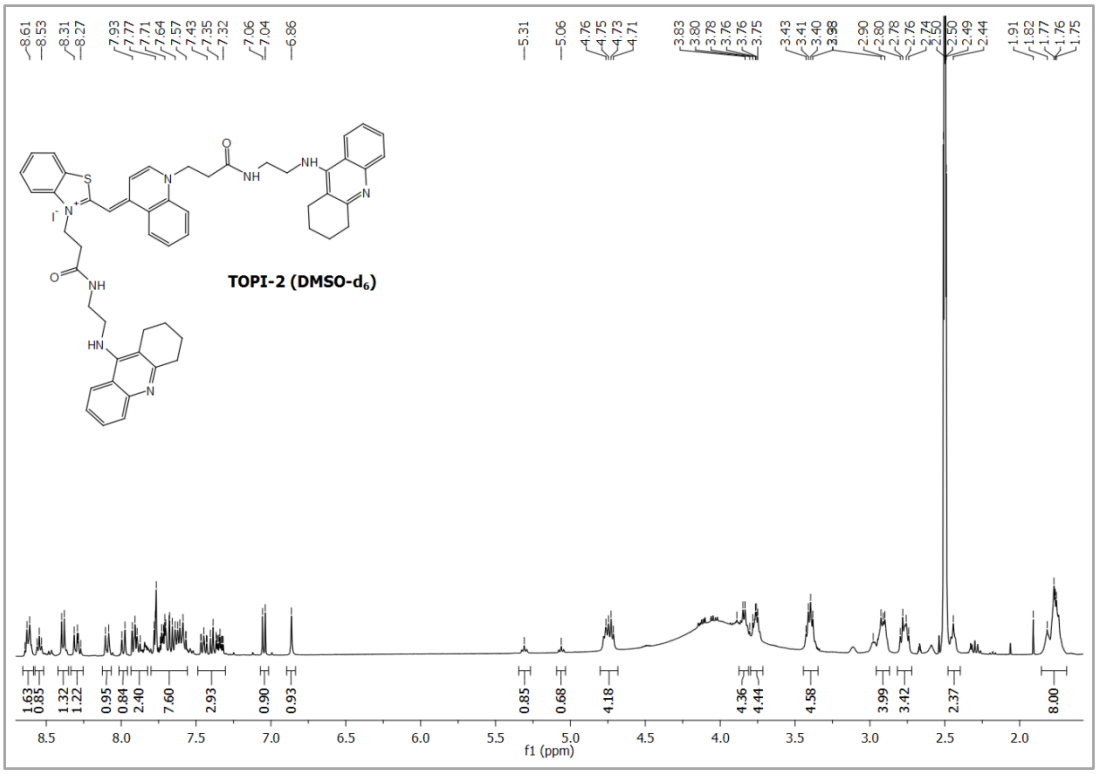

158.58
150.91
147.57
128.80
128.41
123.72
122.95
120.36
116.02
77.48
77.16
76.84
49.58
42.14
34.12
33.67
31.89
26.94
26.77
24.91
23.18
22.90
HN
NH2
N
C9 (CDCl3)
f1 (ppm)

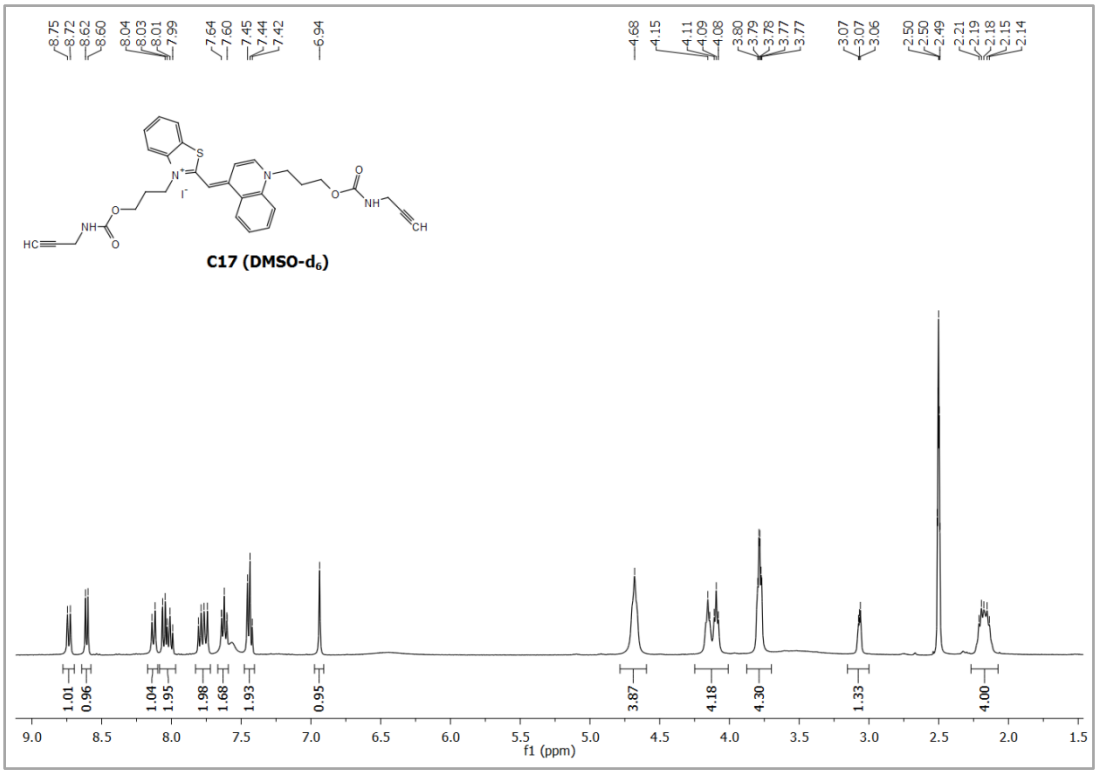

TOPI-1 (DMSO-d6)
f1 (ppm)

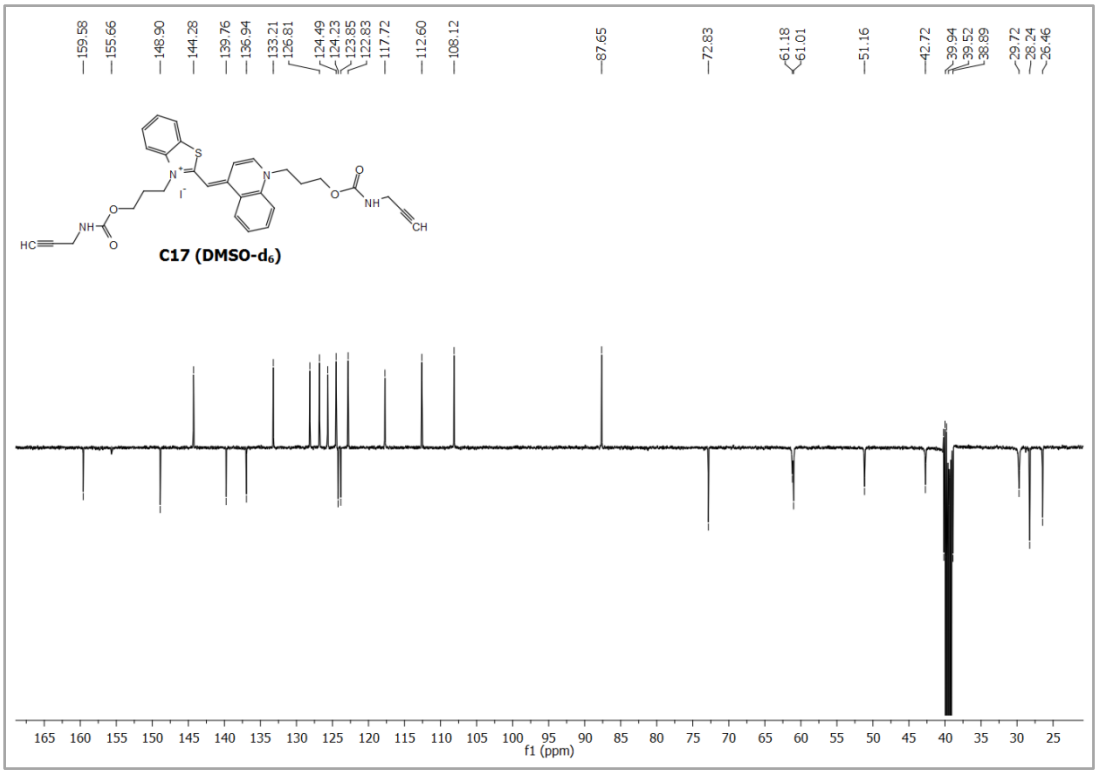

TOPI-1 (DMSO-d6)
f1 (ppm)

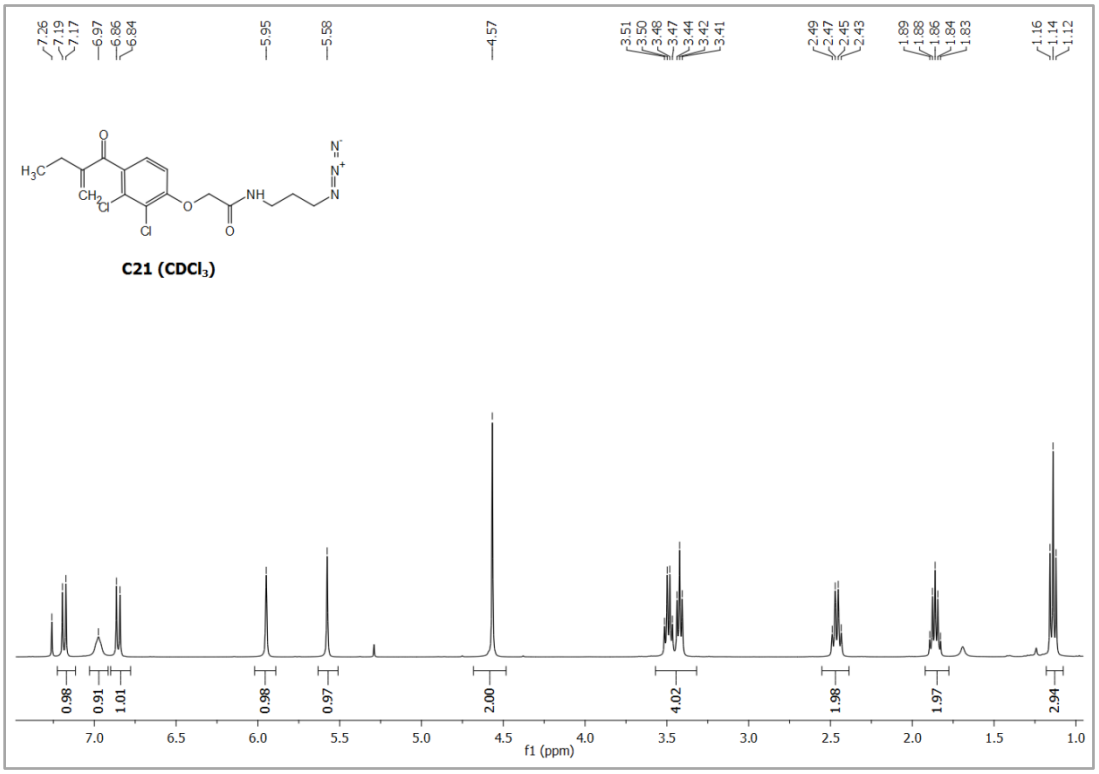

8.06
8.03
7.61
7.58
7.56
7.39
7.36
7.34
7.26
3.61
3.60
3.58
3.49
3.12
2.99
2.98
2.75
1.92
1.74
1.25
NH2
HN
N
C11 (CDCl3)
1.65
0.94
0.95
2.04
2.17
2.00
2.30
4.64
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

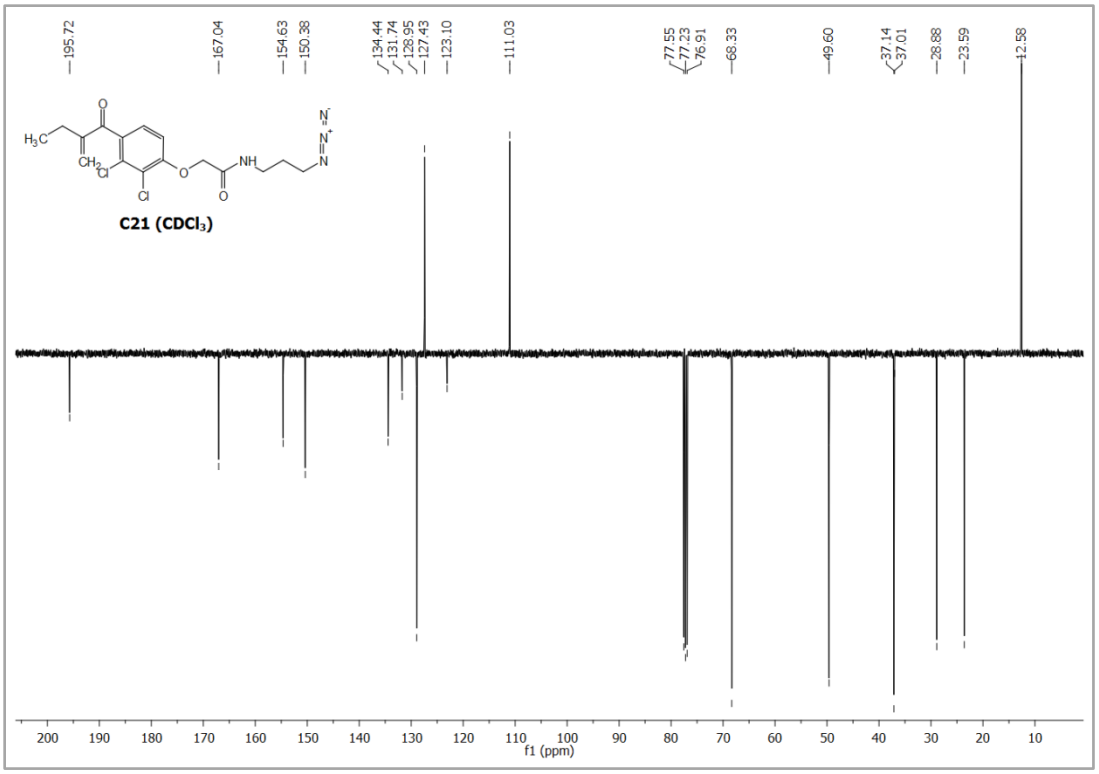

158.57
151.11
147.41
128.70
128.45
123.81
122.97
120.57
116.61
77.48
77.16
76.84
51.16
42.56
34.05
24.98
23.18
22.93
HN
NH2
N
C11 (CDCl3)
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
f1 (ppm)

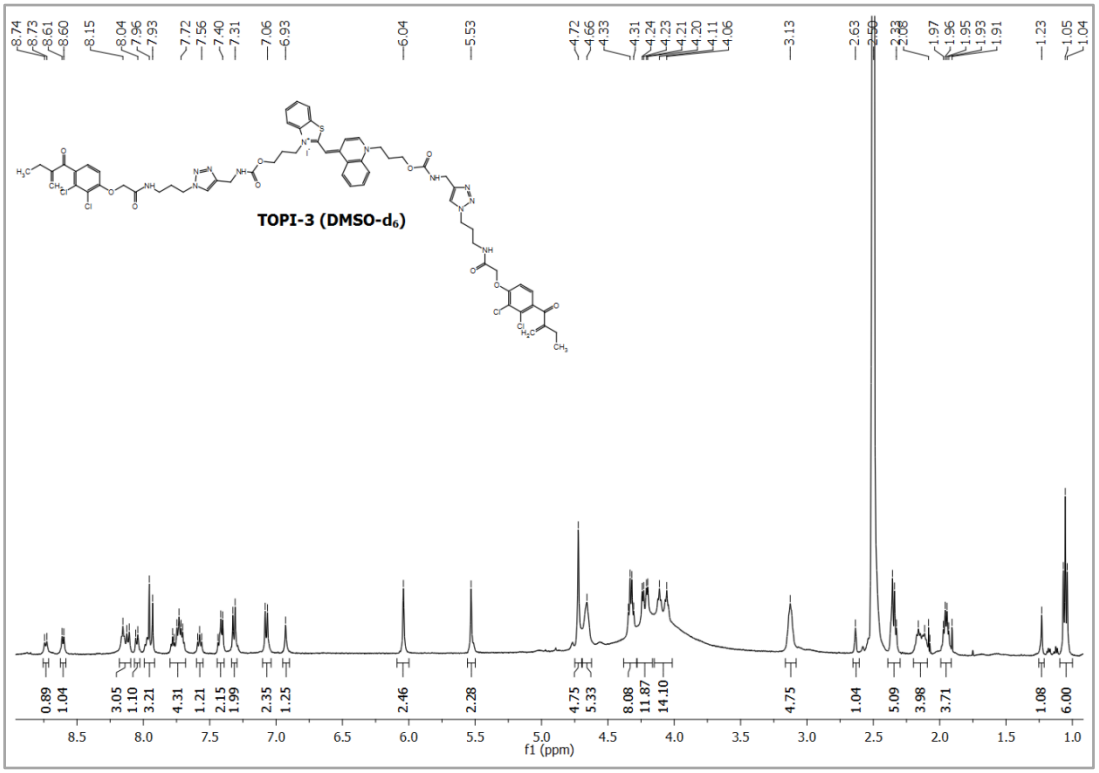

TOPI-2 (DMSO-d6)
f1 (ppm)

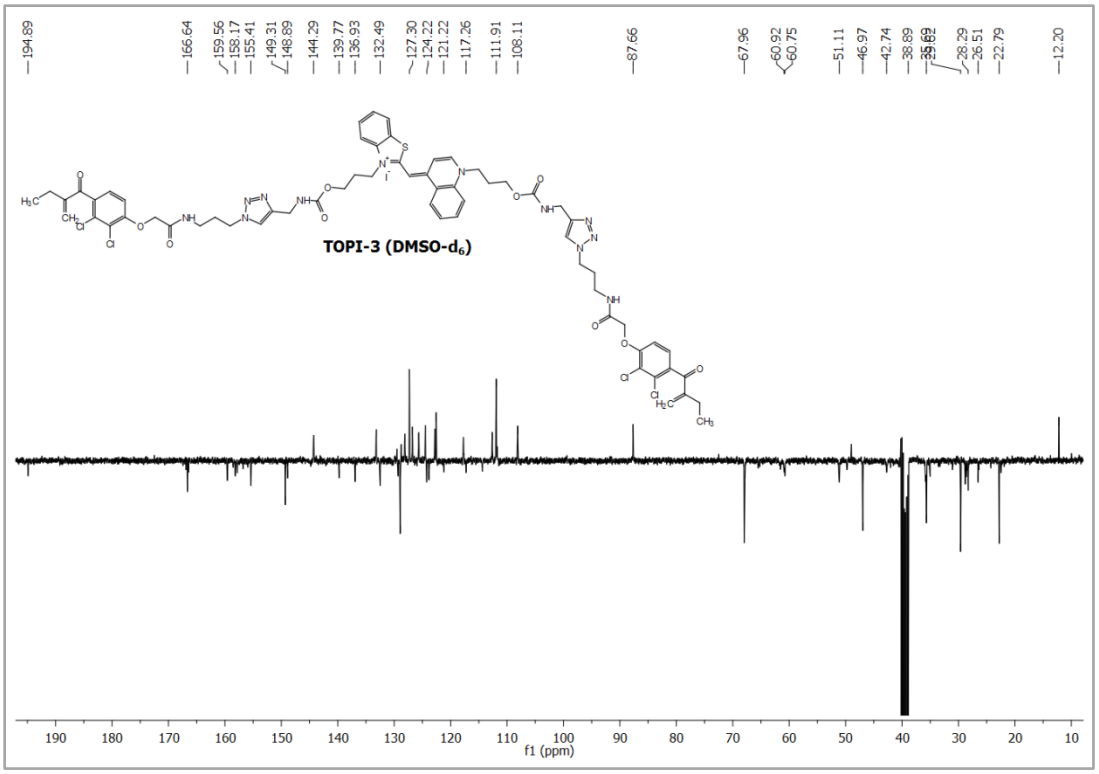

C17 (DMSO-d6)
f1 (ppm)

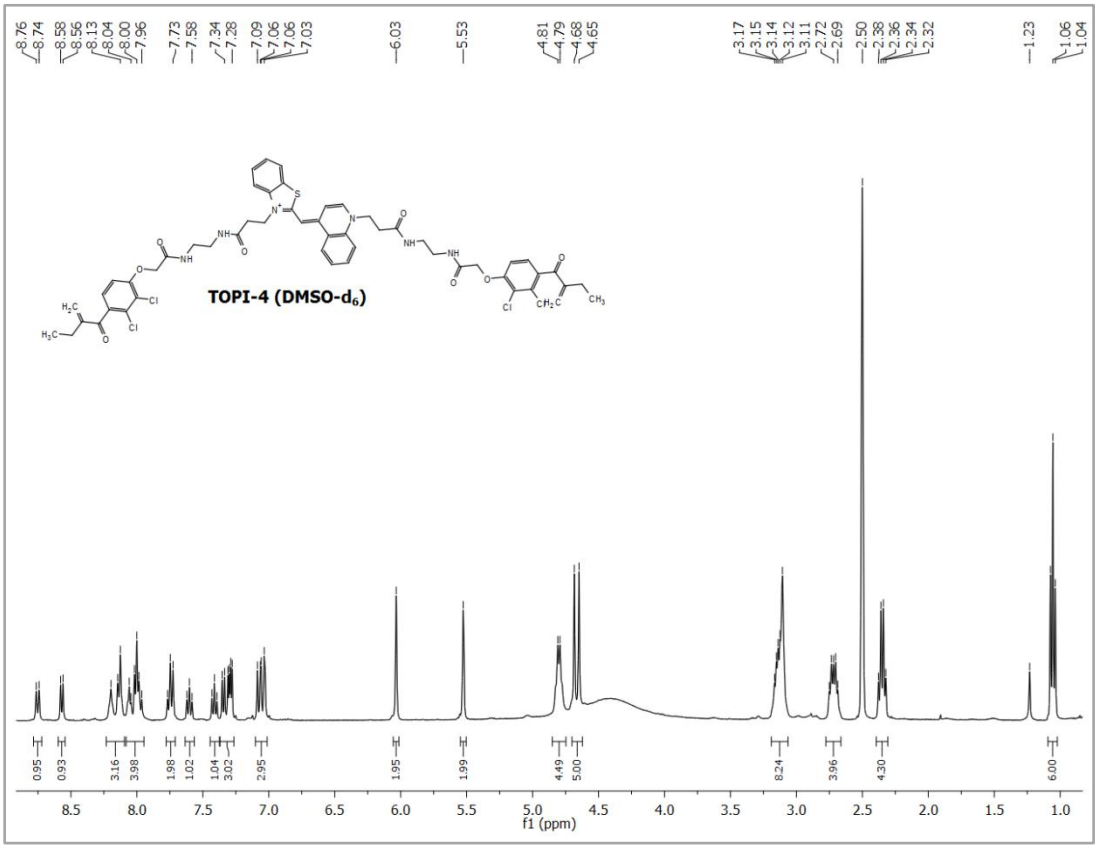

159.58
155.66
148.90
144.28
139.76
136.94
133.21
126.81
124.49
124.23
123.85
122.83
117.72
112.60
108.12
87.65
72.83
61.18
61.01
51.16
42.72
39.94
39.52
38.89
29.72
28.24
26.46
C17 (DMSO-d6)
f1 (ppm)

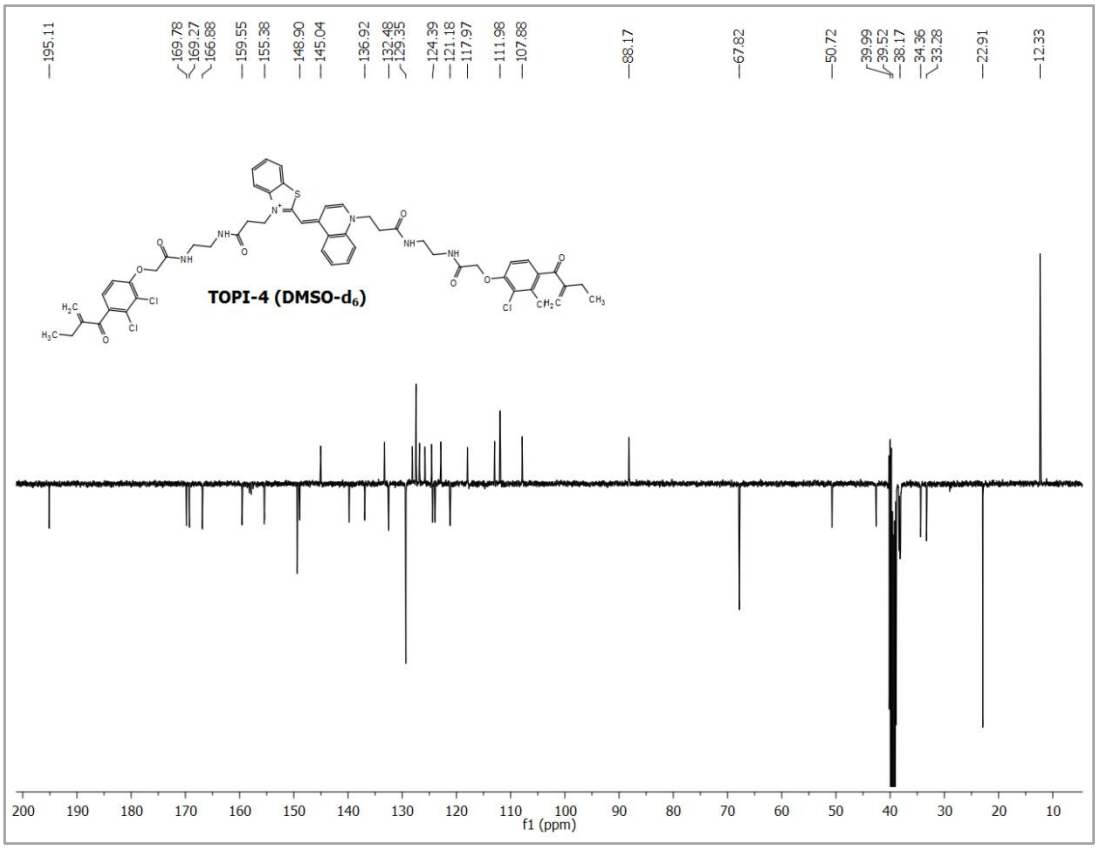

7.26
7.19
7.17
6.97
6.86
6.84
5.95
5.58
4.57
3.51
3.50
3.48
3.47
3.44
3.42
3.41
2.49
2.47
2.45
2.43
1.89
1.88
1.86
1.84
1.83
1.16
1.14
1.12
C21 (CDCl3)
0.98
0.91
1.01
0.98
0.97
2.00
4.02
1.98
1.97
2.94
f1 (ppm)

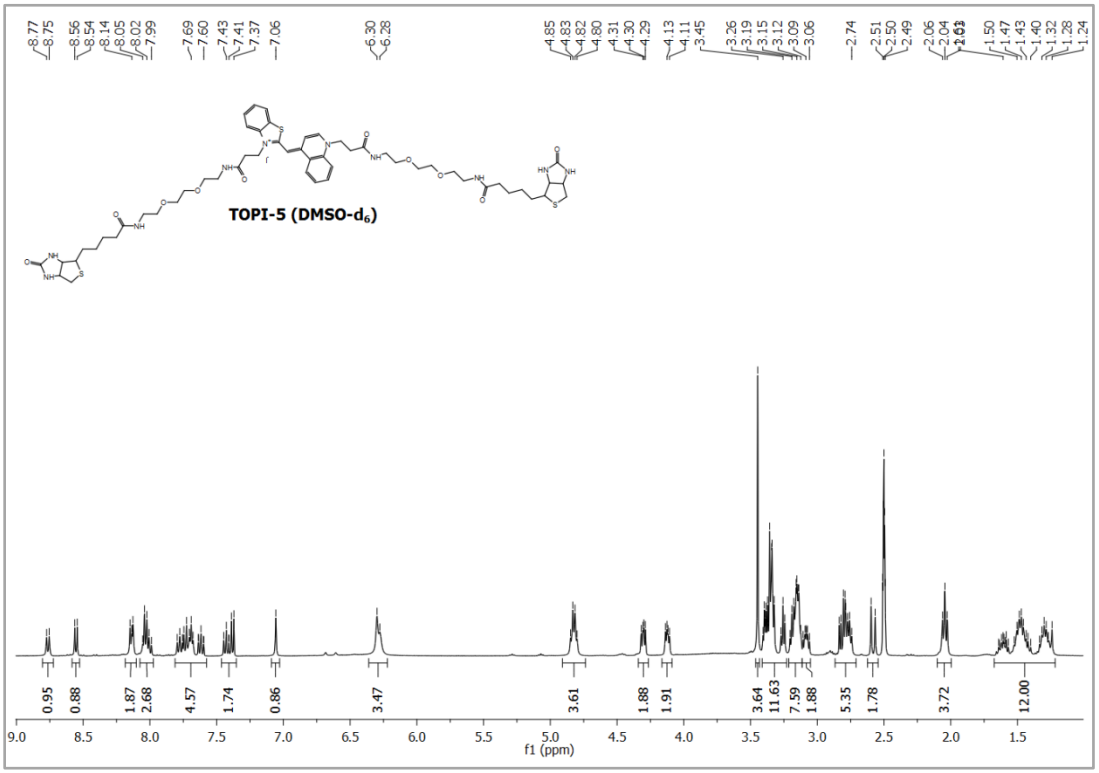

195.72
167.04
154.63
150.38
134.44
131.74
128.95
127.43
123.10
111.03
77.55
77.23
76.91
68.33
49.60
37.14
37.01
28.88
23.59
12.58
C21 (CDCl3)
f1 (ppm)

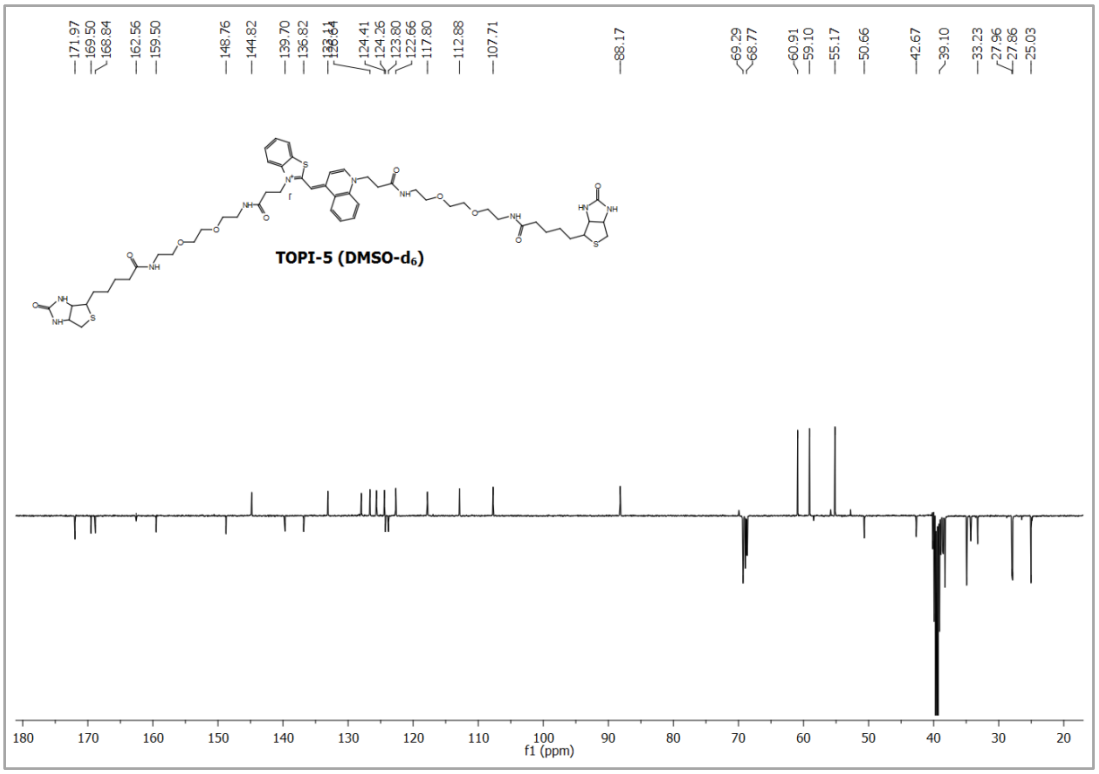

8.74
8.73
8.61
8.60
8.15
8.04
7.96
7.93
7.72
7.56
7.40
7.31
7.06
6.93
6.04
5.53
4.72
4.66
4.33
4.31
4.24
4.23
4.21
4.20
4.11
4.06
3.13
2.63
2.50
2.33
2.08
1.97
1.96
1.95
1.93
1.91
1.23
1.05
1.04
TOPI-3 (DMSO-d6)
0.89
1.04
3.05
1.10
3.21
4.31
1.21
2.15
1.99
2.35
1.25
2.46
2.28
4.75
5.33
8.08
11.87
14.10
4.75
1.04
5.09
3.98
3.71
1.08
6.00
f1 (ppm)

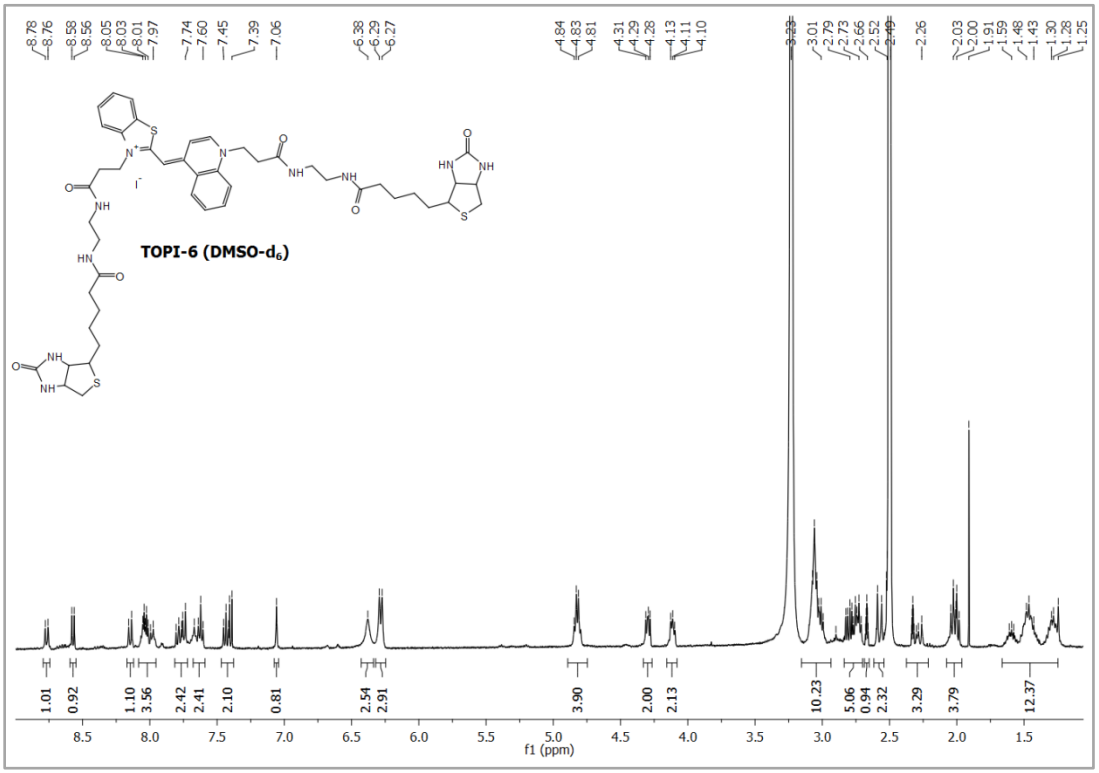

194.89
166.64
159.56
158.17
155.41
149.31
148.89
144.29
139.77
136.93
132.49
127.30
124.22
121.22
117.26
111.91
108.11
87.66
67.96
60.92
60.75
51.11
46.97
42.74
38.89
28.29
26.51
22.79
12.20
TOPI-3 (DMSO-d6)
f1 (ppm)


8.76
8.74
8.58
8.56
8.13
8.04
8.00
7.96
7.73
7.58
7.34
7.28
7.09
7.06
7.06
7.03
6.03
5.53
4.81
4.79
4.68
4.65
3.17
3.15
3.14
3.12
3.11
2.72
2.69
2.50
2.38
2.36
2.34
2.32
1.23
1.06
1.04
TOPI-4 (DMSO-d6)
0.95
0.93
3.16
3.98
1.98
1.02
1.04
3.02
2.95
1.95
1.99
4.49
5.00
8.24
3.96
4.30
6.00
f1 (ppm)


195.11
169.78
169.27
166.88
159.55
155.38
148.90
145.04
136.92
132.48
129.35
124.39
121.18
117.97
111.98
107.88
88.17
67.82
50.72
39.99
39.52
38.17
34.36
33.28
22.91
12.33
TOPI-4 (DMSO-d6)
f1 (ppm)


8.77
8.75
8.56
8.54
8.14
8.05
8.02
7.99
7.69
7.60
7.43
7.41
7.37
7.06
6.30
6.28
4.85
4.83
4.82
4.80
4.31
4.30
4.29
4.13
4.11
3.45
3.26
3.19
3.15
3.12
3.09
3.06
2.74
2.51
2.50
2.49
2.06
2.04
1.50
1.47
1.43
1.40
1.32
1.28
1.24
TOPI-5 (DMSO-d6)
0.95
0.88
1.87
2.68
4.57
1.74
0.86
3.47
3.61
1.88
1.91
3.64
11.63
7.59
1.88
5.35
1.78
3.72
12.00
f1 (ppm)


171.97
169.50
168.84
162.56
159.50
148.76
144.82
139.70
136.82
124.41
124.26
123.80
122.66
117.80
112.88
107.71
88.17
69.29
68.77
60.91
59.10
55.17
50.66
42.67
39.10
33.23
27.96
27.86
25.03
TOPI-5 (DMSO-d6)
f1 (ppm)


8.78
8.76
8.58
8.56
8.05
8.03
8.01
7.97
7.74
7.60
7.45
7.39
7.06
6.38
6.29
6.27
4.84
4.83
4.81
4.31
4.29
4.28
4.13
4.11
4.10
3.33
3.01
2.79
2.73
2.66
2.52
2.49
2.26
2.03
2.00
1.91
1.59
1.48
1.43
1.30
1.28
1.25
TOPI-6 (DMSO-d6)
1.01
0.92
1.10
3.56
2.42
2.41
2.10
0.81
2.54
2.91
3.90
2.00
2.13
10.23
5.06
0.94
2.32
3.29
3.79
12.37
f1 (ppm)

10. References

1. D. Arian, L. Kovbasyuk and A. Mokhir, *J. Am. Chem. Soc.*, 2011, **133**, 3972-3980.

2. T. Mayer and M. E. Maier, *Eur. J. Org. Chem.*, 2007, **2007**, 4711-4720.

3. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. Biol. Chem.*, 1974, **249**, 7130-7139.

4. (a) S. S. Mahajan, L. Hou, C. Doneanu, R. Paranji, D. Maeda, J. Zebala and W. M. Atkins, *J. Am. Chem. Soc.*, 2006, **128**, 8615-8625; (b) R. P. Lyon, J. J. Hill and W. M. Atkins, *Biochemistry*, 2003, **42**, 10418-10428.

5. G. L. Ellman, K. D. Courtney, V. Andres jr and R. M. Featherstone, *Biochem. Pharmacol.*, 1961, **7**, 88-95.

6. G. Tarcic, R. Avraham, G. Pines, I. Amit, T. Shay, Y. Lu, Y. Zwang, M. Katz, N. Ben-Chetrit, J. Jacob-Hirsch, L. Virgilio, G. Rechavi, G. Mavrothalassitis, G. B. Mills, E. Domany and Y. Yarden, *FASEB J.*, 2012, **26**, 1582-1592.