Oligothiophene-based Photosensitizers with Tunable Push-Pull Architectures: Design, Synthesis and Characterization

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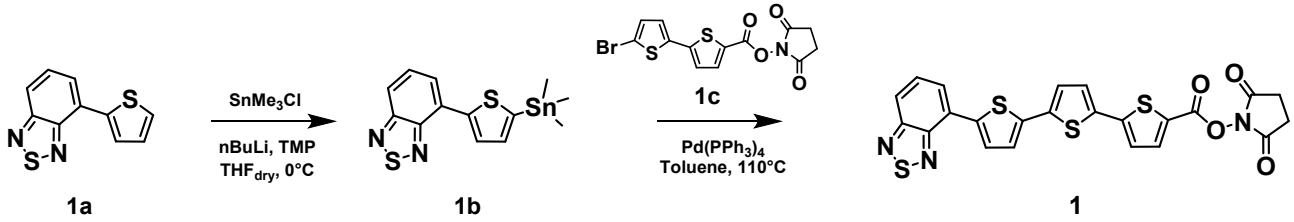
I. Synthesis of the compounds 1, 3, 4

General.

Microwave experiments were carried out in a CEM discover microwave. All ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury-400MHz spectrometer and on a Agilent Technologies 500MHz. Mass spectra were collected on a Thermos Scientific ISDQ 7610 Single quadrupole mass spectrometer. UV-Vis spectra were recorded using a Agilent Technologies Cary100 UV-Vis spectrometer. Photoluminescence spectra were recorded using a Perkin Elmer LS50B spectrofluorometer. Cyclic voltammetries (CVs) were performed in solution 1 mmol L^{-1} purged with Ar, by using AMEL model 5000 Electrochemical System at scan rates from 0.10 to 0.01 V s^{-1} in a three compartment glass electrochemical cell with Pt disk working electrode (diameter 1 mm) and Pt wire auxiliary electrode. The electrolytic solution contains 0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ (Sigma-Aldrich for electrochemical analysis ≥ 99.0% stored under reduced pressure in a dryer filled with $CaCl_2$) in CH_2Cl_2 (Sigma-Aldrich for HPLC ≥ 99.8%, distilled over P_2O_5 and stored in the dark under Ar pressure).

Materials and Synthesis. All solvents were purchased from Sigma Aldrich and used without further purification. Dry THF was prepared by distillation on $LiAlH_4$ and drying over 3Å molecular sieves; dry dioxane was prepared by refluxing on CaH_2 and drying over 3Å molecular sieves. TLC were carried out with 0.2 mm thick of silica gel 60 F254 (Merck). Preparative column chromatographies were performed on glass columns of different sizes hand packed with silica gel 60 (particle sizes 0.040-0.063 mm, Merck). [1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) ($Pd(dppf)Cl_2$), n Butyllithium solution 2.5 M in hexanes, Trimethyltin chloride, Tetrakis(trifenilfosfina)palladio(0) ($Pd(PPh_3)_4$), Hydrogen peroxide solution 30 wt%, Bis(triphenylphosphine)palladium(II) dichloride ($Pd(PPh_3)Cl_2$), Tributyltin chloride, 2,2':5',2"-terthiophene, Hydrochloric acid 37%, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) and 1-hydroxypyrrolidine-2,5-dione were purchased from Sigma Aldrich. Tetramethylpiperidine (TMP) was purchased from Alfa Aesar. 2-thienyl bromide, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[c][1,2,5]thiadiazole, 2-([2,2'-bithiophen]-5-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) and 7-bromobenzo[c][1,2,5]thiadiazole-4-carbaldehyde were purchased from Fluorochem. Potassium phosphate monobasic (KH_2PO_4), Sodium

carbonate (Na_2CO_3), Sodium bicarbonate ($NaHCO_3$) and Sodium Chlorite ($NaClO_2$) were purchased from Fluka. Sodium Chlorite ($NaClO_2$) was purchased from Carlo Erba.

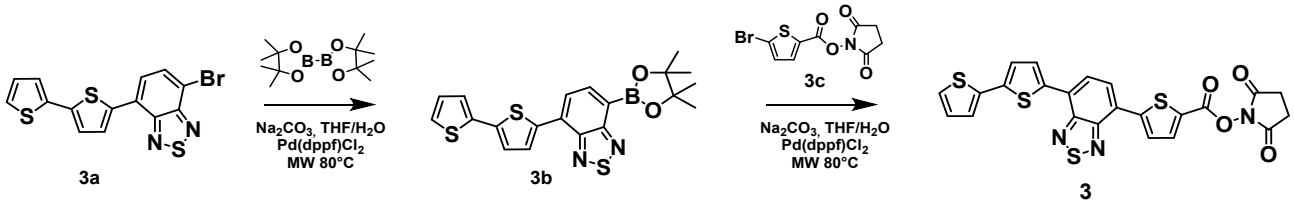


Scheme S1

4-(thiophen-2-yl)benzo[c][1,2,5]thiadiazole (1a) → 1 mmol of 2-thienyl bromide, 1.1 mmol of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[c][1,2,5]thiadiazole, 3 mmol $NaHCO_3$, 0.05 mmol of Pd(dppf)Cl_2 were dissolved in a mixture of THF/H_2O (2/1, ≈10^{-1} M) and irradiated with microwave at 80°C for 30 min. After returning to room temperature the mixture was extracted with H_2O/CH_2Cl_2 and the combined organic phases were evaporated under reduced pressure. The residue was purified by flash chromatography (Cyclohexane/CH_2Cl_2, 70:30). Yellow crystals. Yield: 50%. EI-MS m/z 218 (M^+). ^{1}H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 8.10 (d, J = 3.6 Hz, 1H), 7.90 (d, J = 8,8 Hz, 1H), 7.82 (d, J = 7.2 Hz, 1H), 7.59 (dd, ^{3}J = 8,8 Hz, ^{3}J = 7,2 Hz, 1H), 7.45 (d, J = 5.2 Hz, 1H), 7.20 (dd, ^{3}J = 5.2 Hz, ^{3}J = 3.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, TMS/ppm): δ 155.5, 152.2, 139.2, 129.6, 127.9, 127.8, 127.6, 126.8, 125.4, 120.0.

4-(5-(trimethylstannyl)thiophen-2-yl)benzo[c][1,2,5]thiadiazole (1b) → To a stirring solution of 2,2,6,6-tetramethylpiperidine (1.3 mmol) in dry THF (10^{-1} M) at 0°C, nBuli (1.1 mmol) was added dropwise under inert atmosphere (N_2). The resulting mixture was stirred for 15 minutes and, then, transferred dropwise by syringe into an already prepared $THF_{(dry)}$ solution of 4-(thiophen-2-yl)benzo[c][1,2,5]thiadiazole (1 mmol) and trimethyltin chloride (1.3 mmol) at 0°C and then stirred overnight. The reaction mixture was extracted with diethyl ether/H_2O, the organic phases were combined and evaporated under reduced pressure. The crude product was used without further purifications. Brown oil. Yield: 46%. EI-MS m/z 382 (M^+). ^{1}H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 8.18 (d, J = 3.6 Hz, 1H), 7.86 (d, J = 8,4 Hz, 1H), 7.81 (d, J = 6.4 Hz, 1H), 7.59 (dd, ^{3}J = 8,4 Hz, ^{3}J = 6,4 Hz, 1H), 7.20 (dd, ^{3}J = 5.2 Hz, ^{3}J = 3.6 Hz, 1H), 0.45 (s, 9H).

2,5-dioxopyrrolidin-1-yl 5''-(benzo[c][1,2,5]thiadiazol-4-yl)-[2,2':5',2''-terthiophene]-5-carboxylate (1) → 1 mmol of 2,5-dioxopyrrolidin-1-yl 5'-bromo-[2,2'-bithiophene]-5-carboxylate (1c), 1 mmol of 4-(5-(trimethylstannyl)thiophen-2-yl)benzo[c][1,2,5]thiadiazole (1b) and 0.05 mmol of $Pd(PPh_3)_4$ were refluxed overnight in toluene at 110°C under nitrogen atmosphere. The reaction mixture was evaporated, extracted twice with CH_2Cl_2/H_2O. The dried residue was purified by flash chromatography (Cyclohexane/CH_2Cl_2/AcOEt, 40:40:20) and precipitation from cold pentane. Dark red solid. Yield: 40%. EI-MS m/z 523 (M^+). ^1H NMR (500 MHz, $CDCl_3$, TMS/ppm): δ 8.05 (d, J = 4 Hz, 1H), 7.95 (m, 2H), 7.86 (d, J = 7.2 Hz, 1H), 7.64 (dd, ^3J = 8,8 Hz, ^3J = 7,2 Hz, 1H), 7.32 (d, J = 4 Hz, 1H), 7.30 (d, J = 3.6 Hz, 1H), 7.25 (m, 1H), 7.23 (d, J = 4 Hz, 1H), 2.91 (s, 4H).



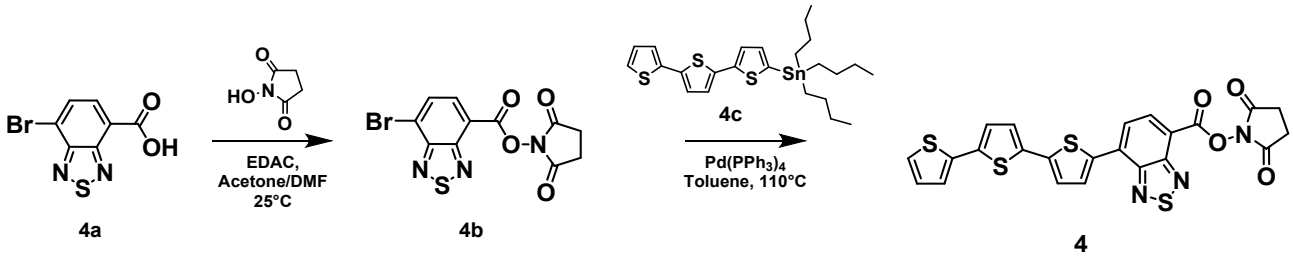
Scheme S2.

4-([2,2'-bithiophen]-5-yl)-7-bromobenzo[c][1,2,5]thiadiazole (3a) → 1 mmol of 2-([2,2'-bithiophen]-5-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 1 mmol of 4,7-dibromobenzo[c][1,2,5]thiadiazole, 3 mmol Na_2CO_3, 0.05 mmol of $Pd(dppf)Cl_2$ were dissolved in a mixture of THF/H_2O (2/1, ≈10^{-1} M) and irradiated with microwave at 80°C for 30 min. After returning to room temperature the mixture was extracted with H_2O/CH_2Cl_2 and the combined organic phases were evaporated under reduced pressure. The residue was purified by flash chromatography (Cyclohexane/CH_2Cl_2, 80:20). The final residue is then crystallized from cold pentane. Dark red powder. Yield: 45%. EI-MS m/z 380 (M^+). ^1H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 7.97 (d, J = 4 Hz, 1H), 7.90 (d, J = 8,4 Hz, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.28-7.25 (m, 2H), 7.21 (d, J = 4 Hz, 1H), 7.05 (dd, ^3J = 4.8 Hz, ^3J = 4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, TMS/ppm): δ 153.7, 151.6, 139.2, 137.0, 136.9, 132.2, 128.8, 127.9, 126.7, 125.1, 125.0, 124.5, 124.2, 112.2.

4-([2,2'-bithiophen]-5-yl)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[c][1,2,5]thiadiazole (3b) → 1 mmol of 4-([2,2'-bithiophen]-5-yl)-7-bromobenzo[c][1,2,5]thiadiazole (3a), 1 mmol of 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane), 3 mmol of NaOAc, and 0.05 mmol of $Pd(PPh_3)Cl_2$ were dissolved in

dry dioxane (≈ 10^{-2} M) and refluxed overnight at 110°C under nitrogen atmosphere. The crude product was extracted with H_2O/CH_2Cl_2, the organic phases were combined and evaporated under reduce pressure. The residue was used without further purification. Brown oil. Yield: 61%. EI-MS m/z 426 (M^+). ^{1}H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 8.18 (d, J = 7.1 Hz, 1H), 8.11 (d, J = 3.9 Hz, 1H), 7.84 (d, J = 7.1 Hz, 1H), 7.31 (dd, J = 7.3 Hz, 1H) 7.28-7.26 (m, 2H), 1.45 (s, 12H).

2,5-dioxopyrrolidin-1-yl 5-(7-([2,2'-bithiophen]-5-yl)benzo[c][1,2,5]thiadiazol-4-yl)thiophene-2-carboxylate (3)→ 1 mmol 2,5-dioxopyrrolidin-1-yl 5-bromothiophene-2-carboxylate (3c), 1 mmol of 4-([2,2'-bithiophen]-5-yl)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[c][1,2,5]-thiadiazole (3b), 3 mmol of Na_2CO_3 and 0.05 mmol of $Pd(PPh_3)_2Cl_2$ were dissolved in mixture of THF/H_2O (2/1, ≈10^{-2} M) and irradiated with microwave at 80°C for 30 min. After returning to room temperature the mixture was extracted with H_2O/CH_2Cl_2 and the combined organic phases were evaporated under reduced pressure. The residue was purified by flash chromatography (Cyclohexane/CH_2Cl_2, 50:50). Dark orange solid. Yield: 55%. EI-MS m/z 523 (M^+). ^{1}H NMR (500 MHz, DMSO-d6, TMS/ppm): δ 8.38 (d, J = 7.7 Hz, 1H), 8.26 (d, J = 4.2 Hz, 1H), 8.19-8.16 (m, 3H), 7.56 (d, J = 4.2 Hz, 1H), 7.46-7.44 (m, 2H), 7.16-7.13 (m, 1H), 2.91 (s, 4H).



Scheme S3.

7-bromobenzo[c][1,2,5]thiadiazole-4-carboxylic acid (4a) → To a solution of 1 mmol of 7-bromobenzo[c][1,2,5]thiadiazole-4-carbaldehyde in acetonitrile (≈ 0.5 M), a water solution of 0.25 mmol of KH_2PO_4 (≈ 0.5 M) and 1.1 mmol of H_2O_2 (30 wt%) were added by syringe. To the resulting mixture a water solution of 1.4 mmol of $NaClO_2$ (≈ 0.5 M) was added dropwise through a dropping funnel in about thirty minutes. The reaction was carried out until bubbling ceased. The reaction is then quenched adding a water solution of HCl (10%). The final crude product is extracted with CH_2Cl_2/H_2O, the organic phase is washed

with a solution of $NaHCO_3$ (1%) to remove the starting material, the aqueous phase is acidified with HCl and the product extracted with chloroform. Pale yellow oil. Yield: 70%. EI-MS m/z 258 (M^+). ^1H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 8.45 (d, J = 7.6 Hz, 1H), 8.07 (d, J = 7.6 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d6, TMS/ppm): δ 165.7, 153.7, 151.6, 133.6, 132.9, 132.1, 124.2, 118.7.

2,5-dioxopyrrolidin-1-yl 7-bromobenzo[c][1,2,5]thiadiazole-4-carboxylate (4b) → To a stirring solution of 1 mmol of 7-bromobenzo[c][1,2,5]thiadiazole-4-carboxylic acid and 1.1 mmol of 1-hydroxypyrrolidine-2,5-dione in acetone (≈ 10^{-2} M) under N_2 atmosphere 1.4 mmol of EDAC were added and the reaction was carried out overnight. The mixture was then extracted with H_2O/CH_2Cl_2 and the combined organic phases were evaporated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2, 100%). Pale pink solid. Yield: 75%. EI-MS m/z 357 (M^+). ^1H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 7.78 (d, J = 7.6 Hz, 1H), 7.17. (d, J = 7.6 Hz, 1H), 2.89 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$, TMS/ppm): δ 168.9, 159.2, 153.9, 151.0, 135.5, 131.2, 123.3, 117.4, 25.7, 25.7.

[2,2':5',2''-terthiophen]-5-yltributylstannane (4c) → To a stirring solution of 1 mmol of 2,2':5',2''-terthiophene in dry THF (≈ 10^{-2} M) 1.1 mmol of nBuli were added dropwise *via* a syringe at -78°C. After 1 h 1.2 mmol of tributyltin chloride were added dropwise and the reaction was carried out overnight. The mixture was then extracted with H_2O/CH_2Cl_2 and the combined organic phases were evaporated under reduced pressure. The crude product was used without further purification. Dark green solid. Yield: 80%. EI-MS m/z 538 (M^+). ^1H NMR (400 MHz, $CDCl_3$, TMS/ppm): δ 7.28 (d, J = 3.6 Hz, 1H), 7.20 (d, J = 5.2 Hz, 1H), 7.16 (d, J = 3.6 Hz, 1H), 7.08-7.06 (m, 3H), 7.01 (dd, ^3J = 5.2 Hz, ^3J = 3,6 Hz, 1H), 1.62-1.55 (m, 6H), 1.40-1.31 (m, 6H), 1.15-1.10 (m, 6H), 0.91 (t, 9H).

2,5-dioxopyrrolidin-1-yl 7-([2,2':5',2''-terthiophen]-5-yl)benzo[c][1,2,5]thiadiazole-4-carboxylate (4) → 1 mmol of 2,5-dioxopyrrolidin-1-yl 7-bromobenzo[c][1,2,5]thiadiazole-4-carboxylate (4b), 1 mmol of [2,2':5',2''-terthiophen]-5-yltributylstannane (4c) and 0.05 mmol of $Pd(PPh_3)_4$ were refluxed overnight in toluene at 110°C under nitrogen atmosphere. The reaction mixture was evaporated, extracted twice with CH_2Cl_2/H_2O. The dried residue was purified by flash chromatography (Cyclohexane/CH_2Cl_2/AcOEt, 50:40:10) and precipitation from cold pentane. Dark red solid. Yield: 45%. EI-MS m/z 523 (M^+). ^1H NMR (500 MHz, $CDCl_3$, TMS/ppm): δ 8.58 (d, J = 8 Hz, 1H), 8.24 (d, J = 4 Hz, 2H), 7.94 (d, J = 8 Hz, 1H), 7.32

(d, J = 4 Hz, 1H), 7.28-7.26 (m, 2H), 7.22(d, J = 3.6 Hz, 1H), 7.15 (d, J = 3.6 Hz, 1H), 7.05 (dd, ^{3}J = 4 Hz, ^{3}J = 3.6 Hz, 1H), 2.96 (s, 4H).

II. NMR Spectra

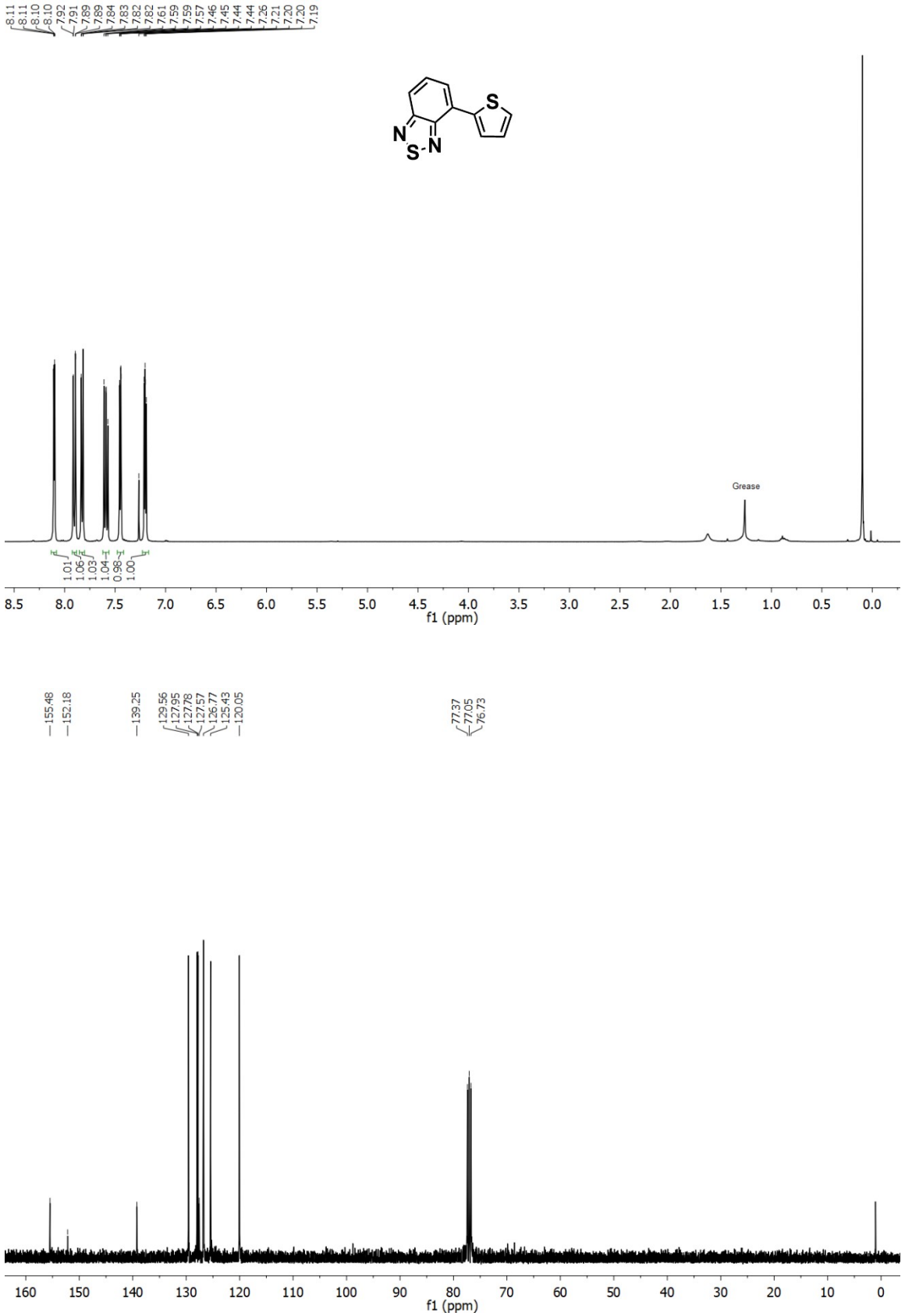


Figure S1. ^{1}H NMR and ^{13}C NMR spectra of compound **1a**.

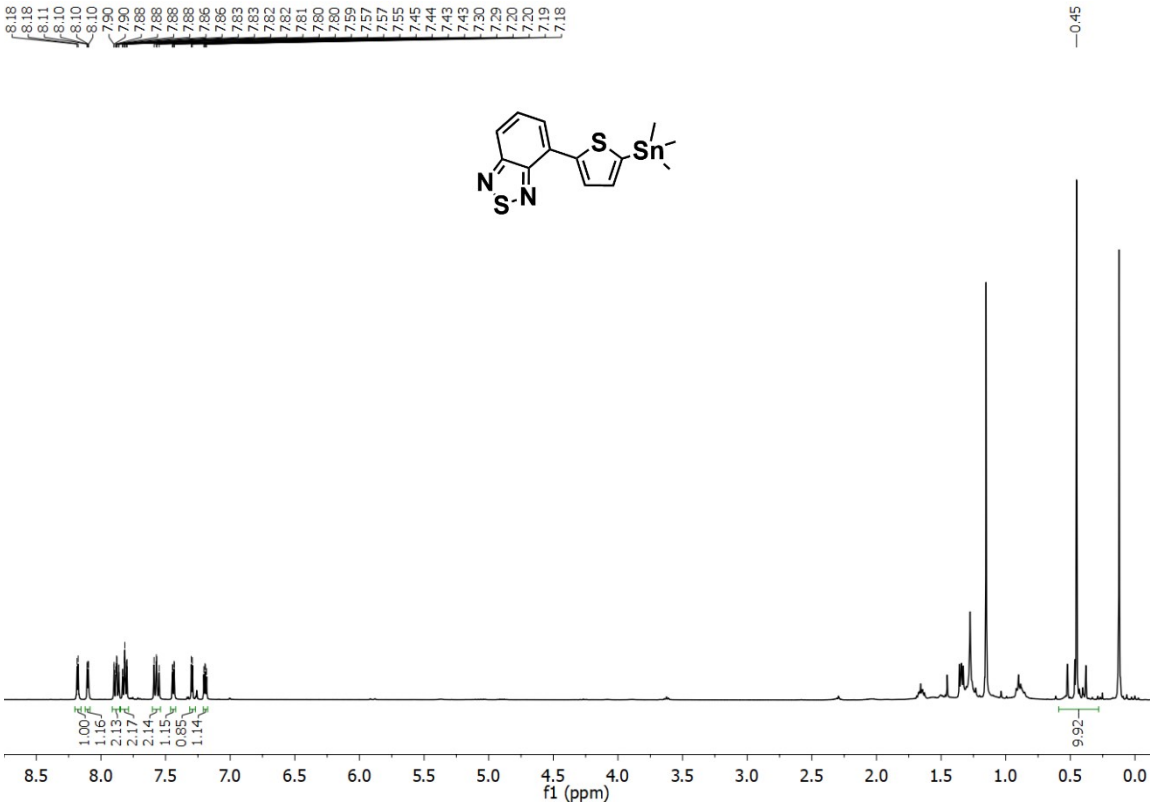
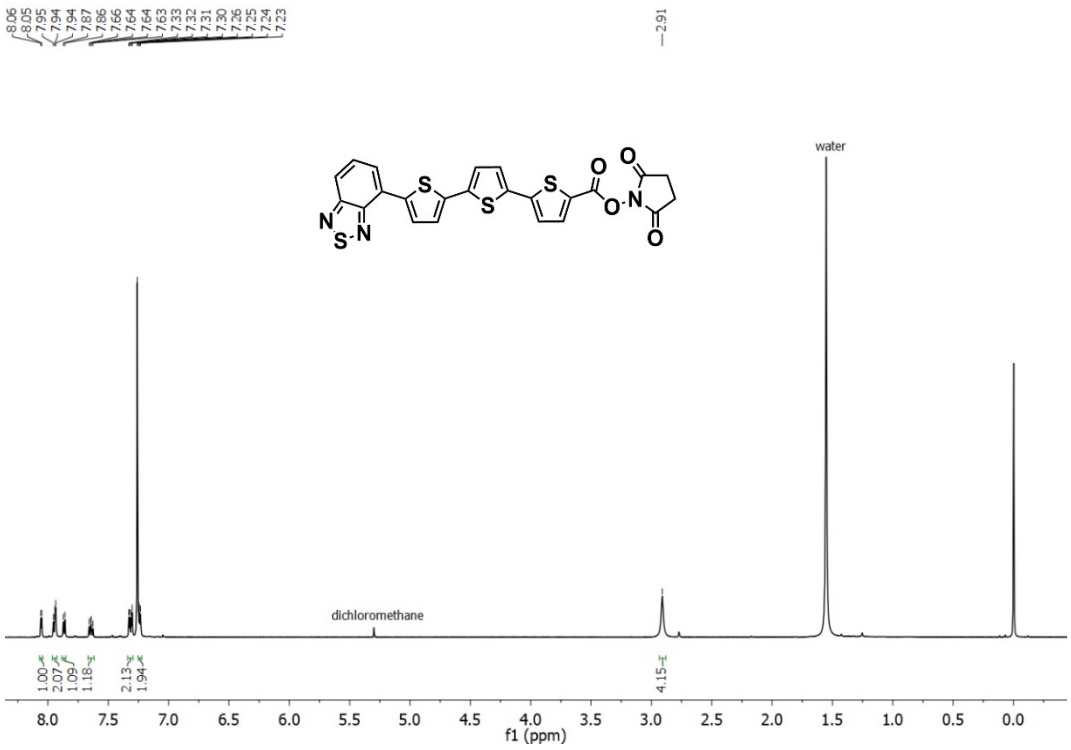


Figure S2. ^{1}H NMR spectrum of compound **1b**.



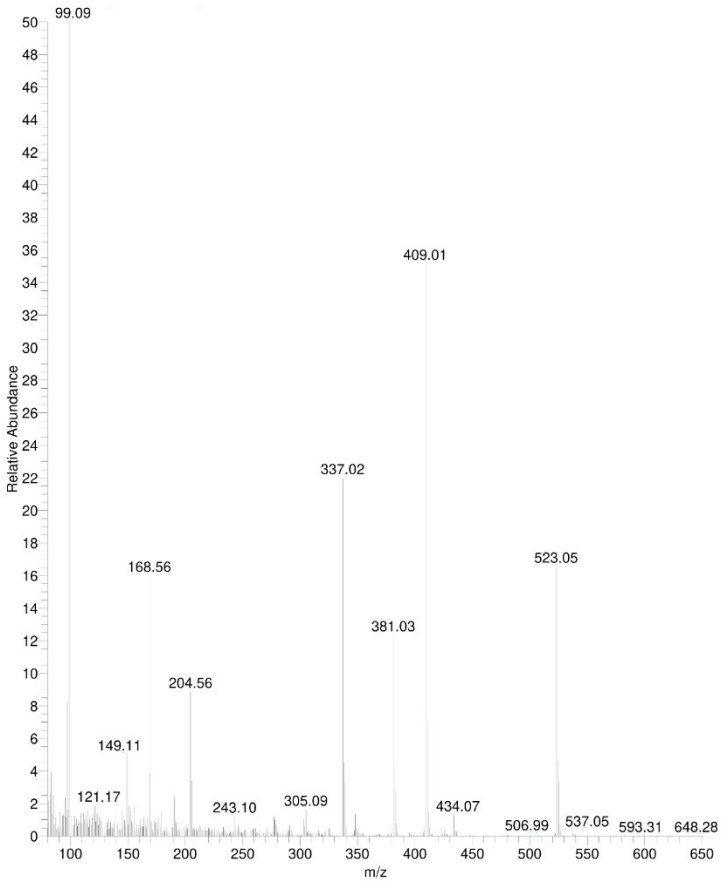


Figure S3. [1]H NMR and mass spectra of compound **1**.

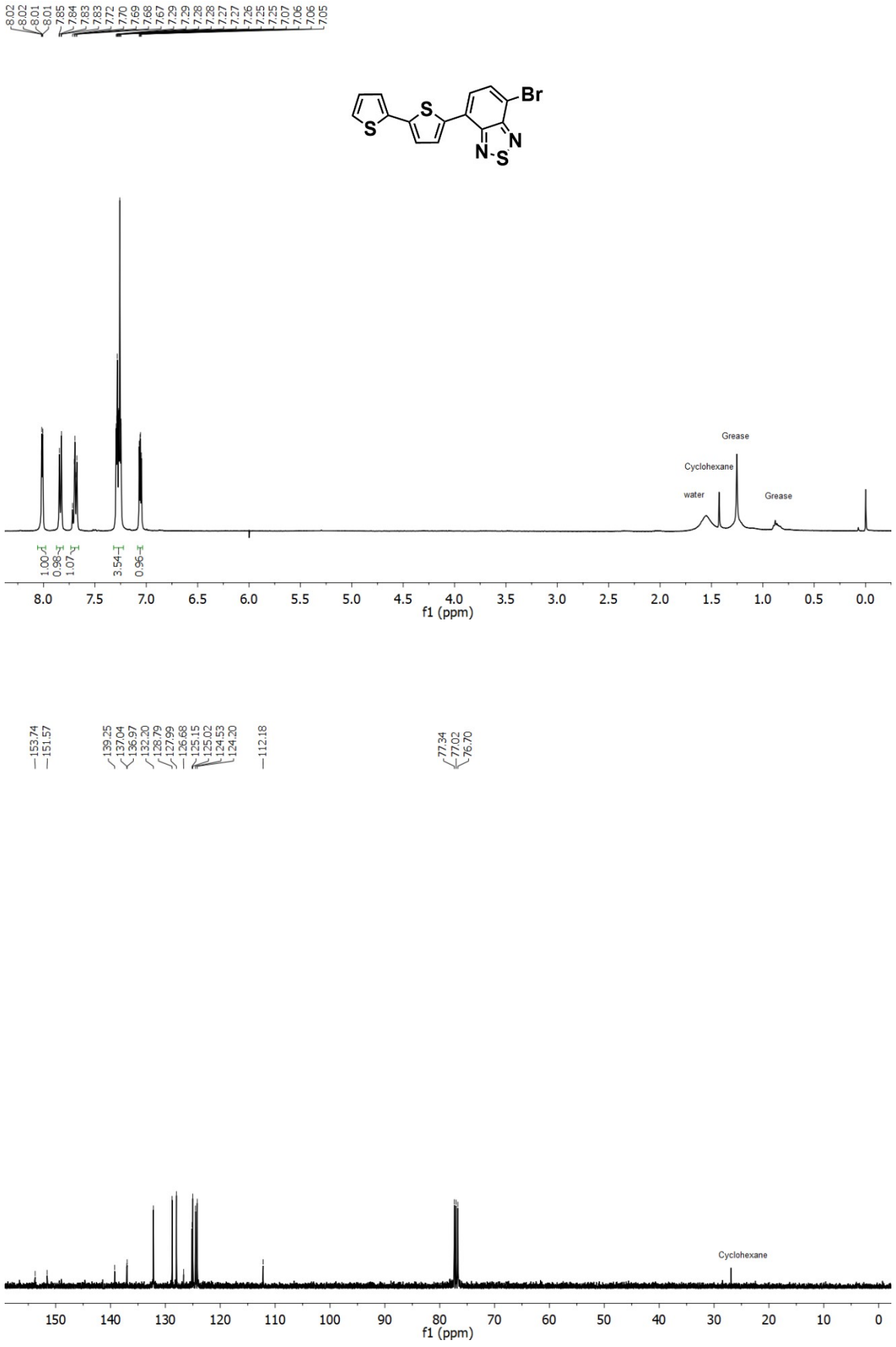


Figure S4. [1]H NMR and [1]C NMR spectra of compound **3a**.

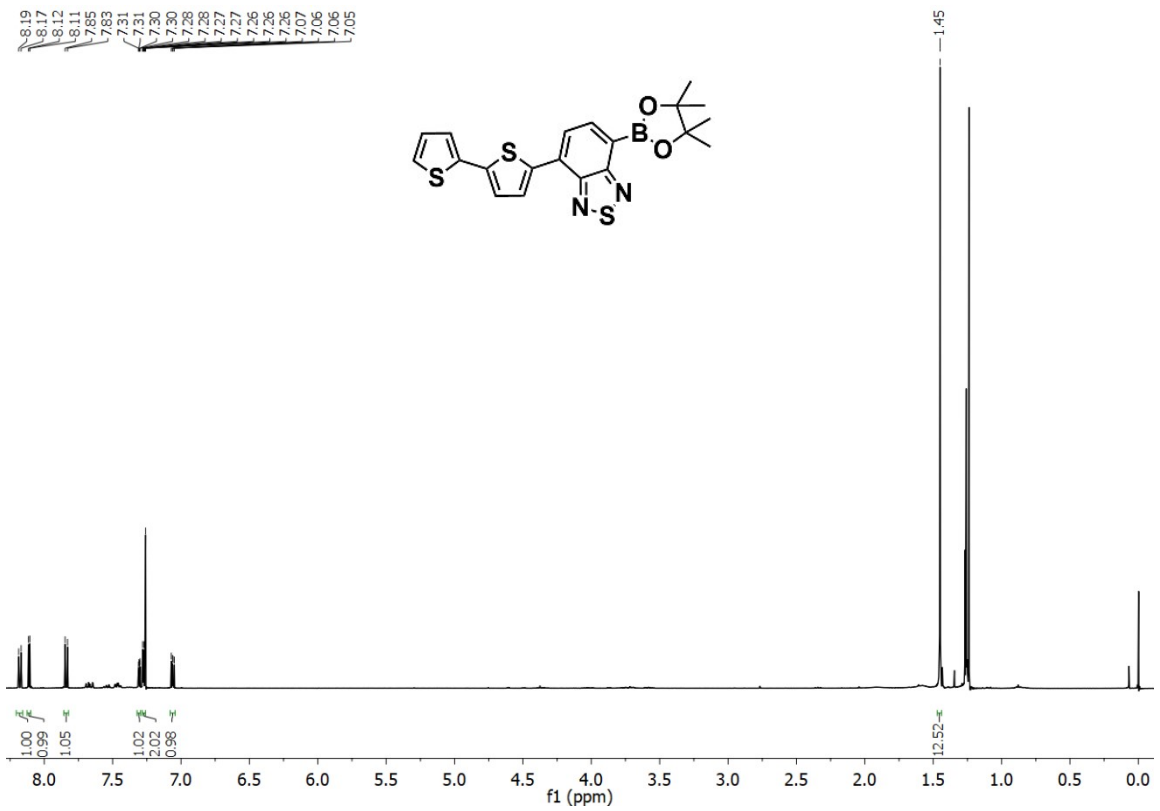


Figure S5. ^{1}H NMR spectrum of compound **3b**.

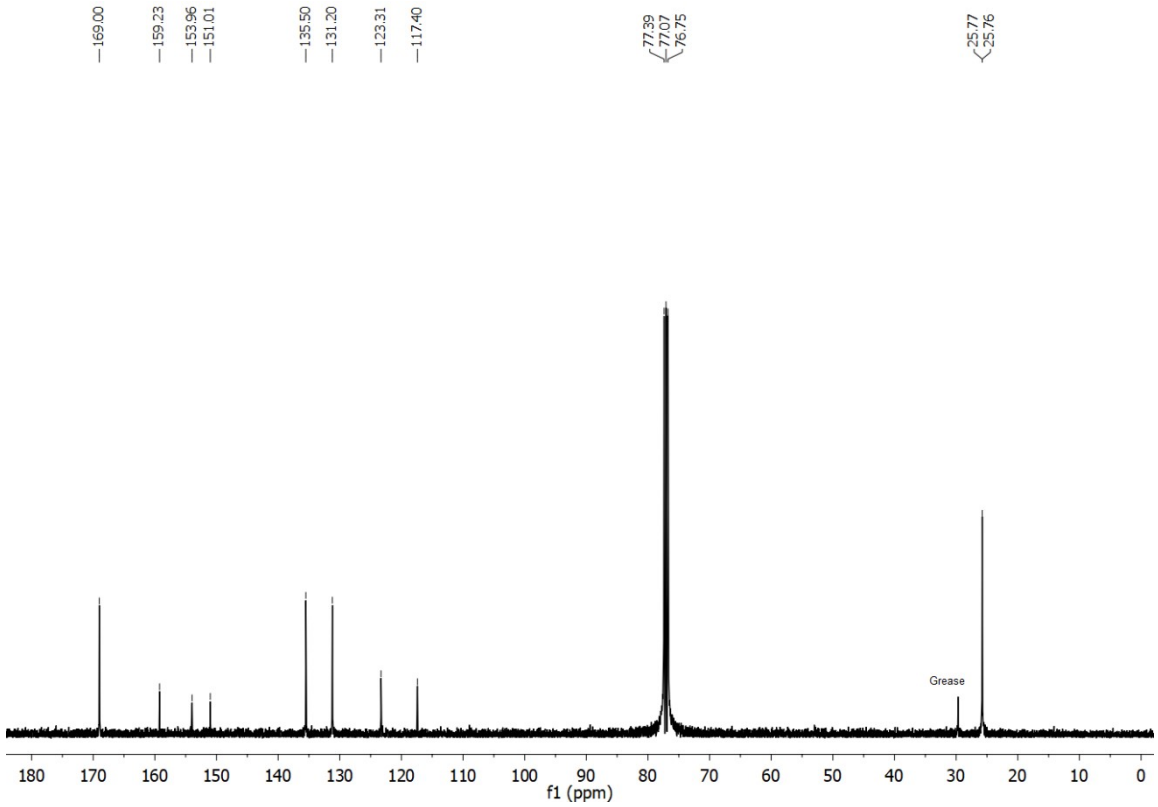


Figure S6. ^{1}H NMR and ^{13}C NMR spectra of compound **3c**.

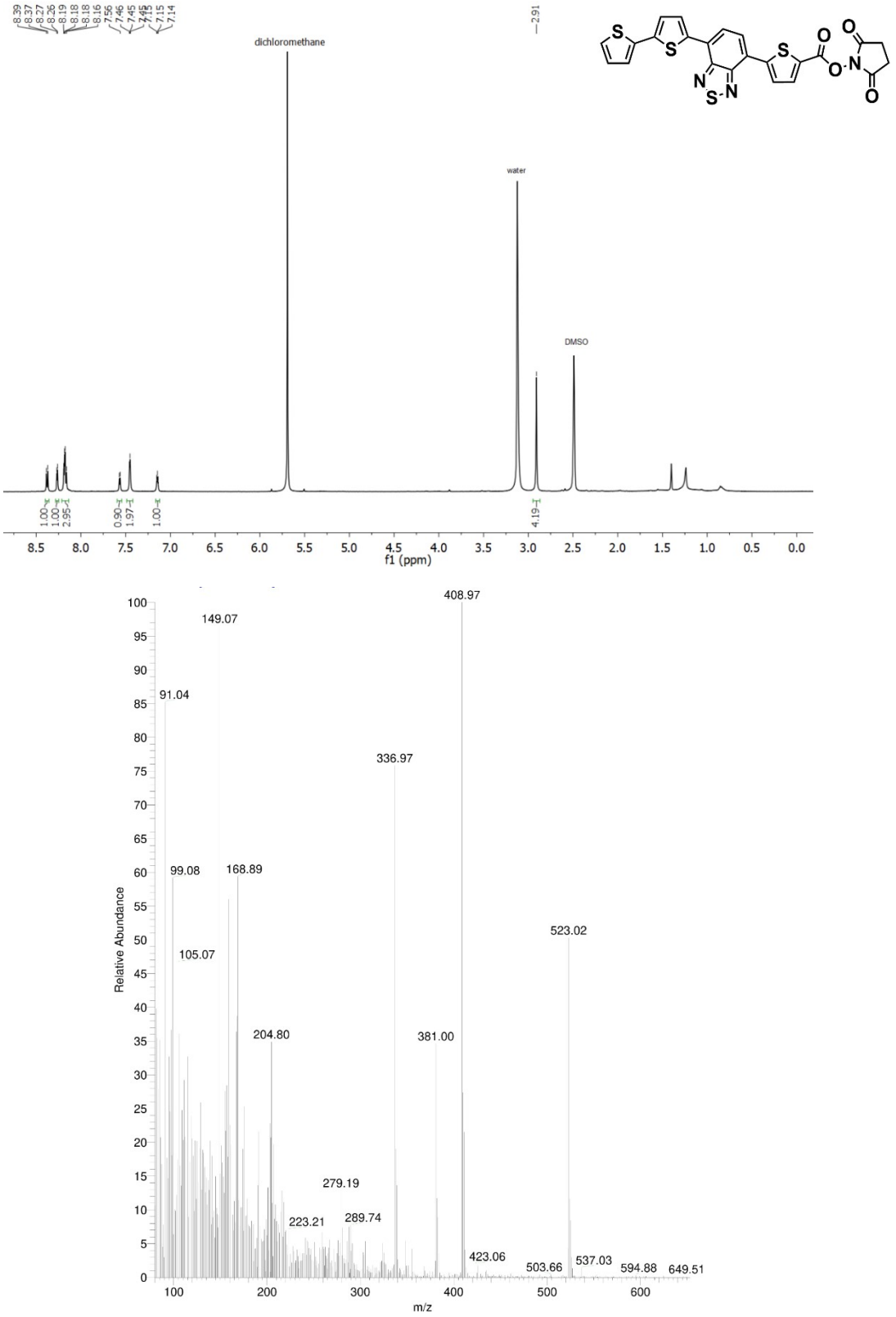


Figure S7. ^{1}H NMR and mass spectra of compound **3.**

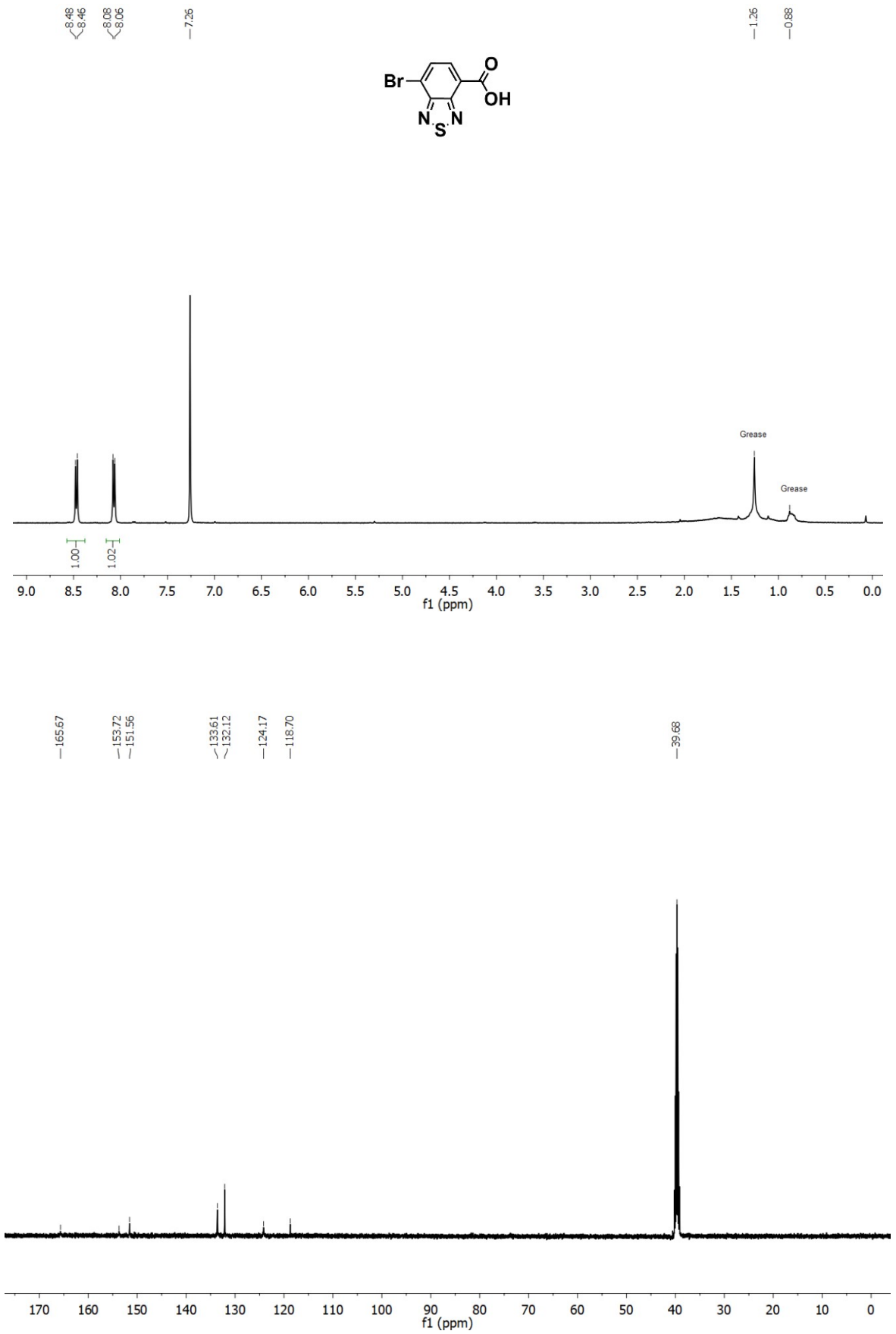


Figure S8. ^{1}H NMR and ^{13}C NMR spectra of compound **4a**.

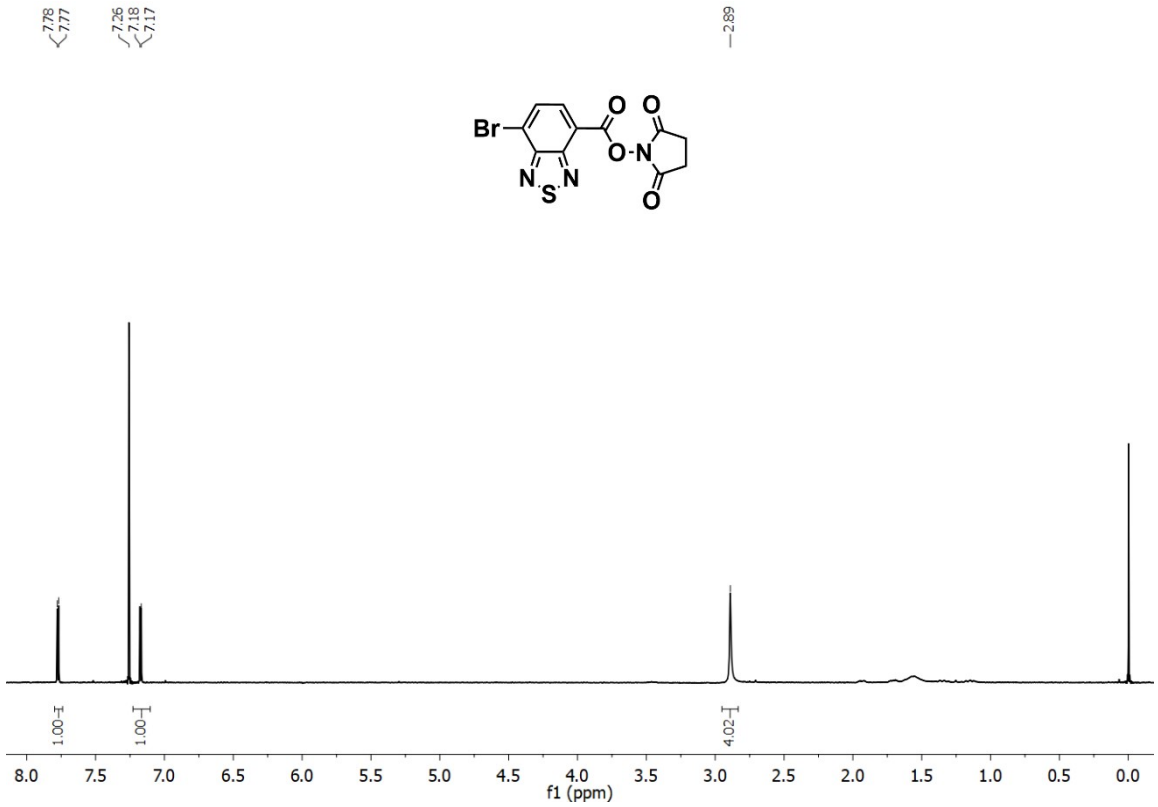


Figure S9. [1]H NMR spectrum of compound **4b**.

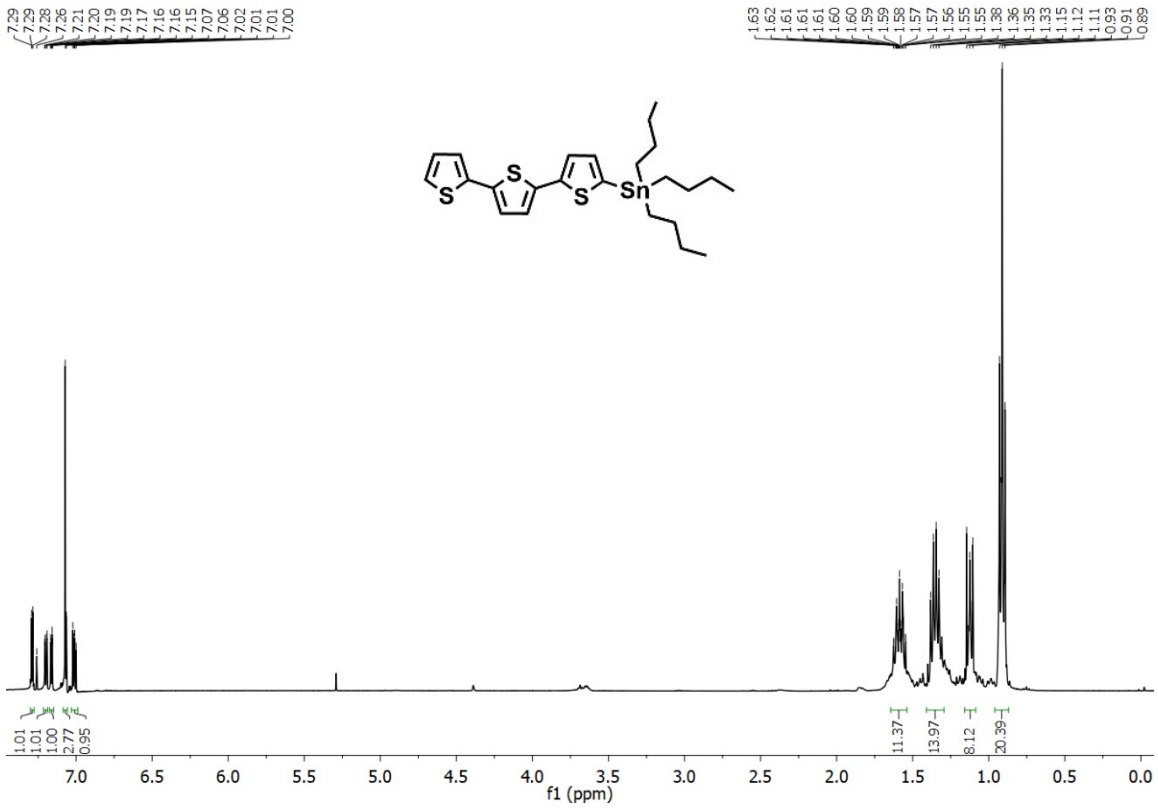
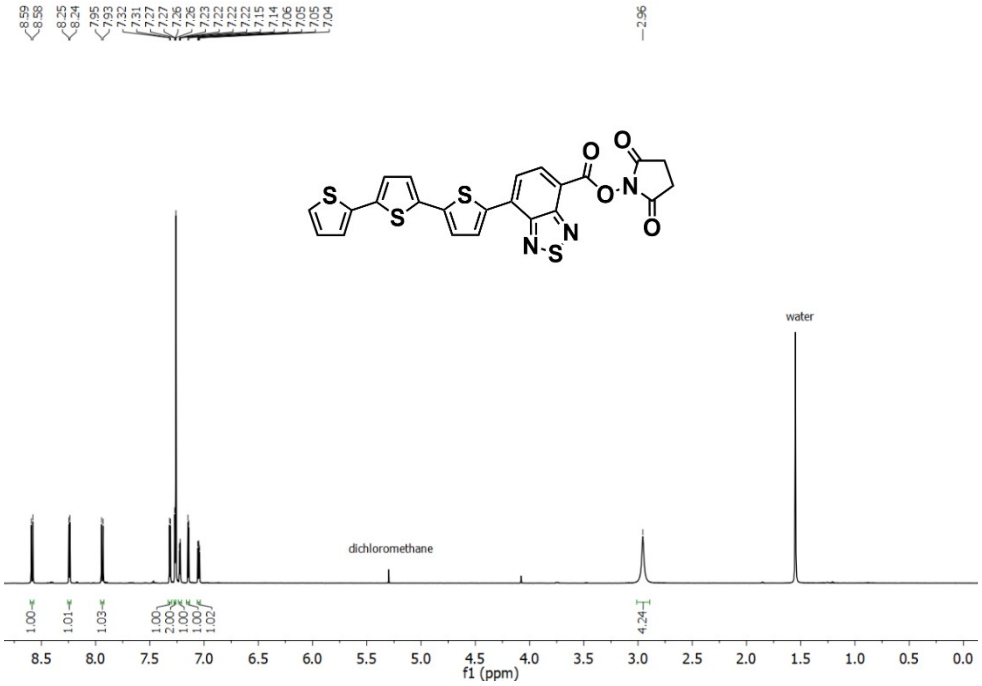


Figure S10. [1]H NMR spectrum of compound **4c**.



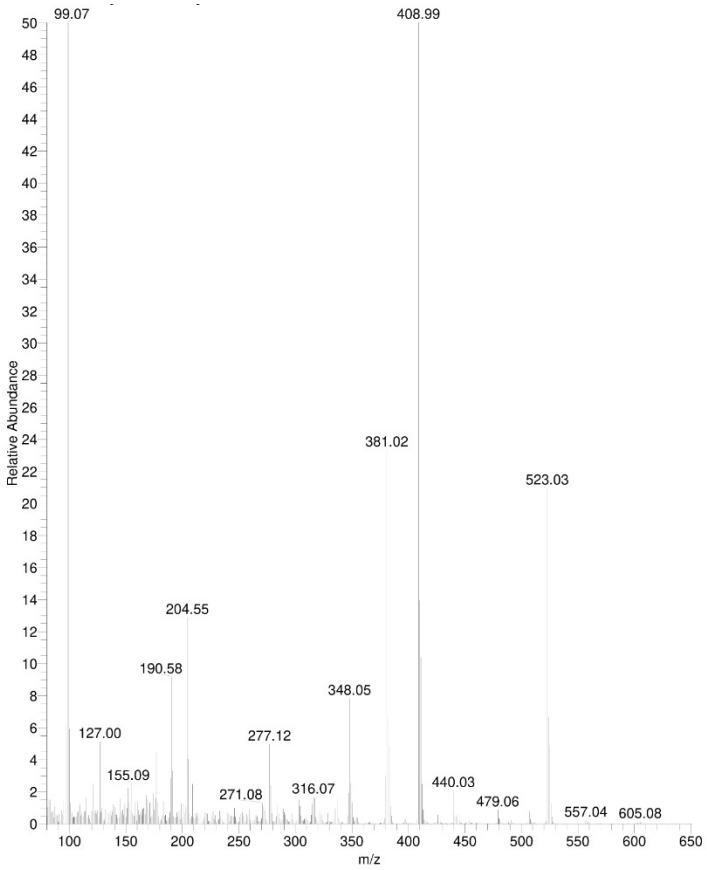


Figure S11. [1]H NMR and mass spectra of compound **4**.

Table S1. Absorption and emission maxima, Stokes shift, molar extinction coefficients (ε) and fluorescence quantum yields (φ_{em}) of compounds **1-4**.

Compound	λ_{max}^{abs} (nm)	λ_{max}^{em} (nm)	Stokes shift	Molar extinction coefficients (ε)	φ_{em}
1	445	606	161	30250	0.099
2	476	611	135	26240	0.34
3	489	648	159	22020	0.24
4	492	680	188	22400	0.015

Computational details

All computations were carried out using Gaussian16 suites of program.[1] The S_0, S_1, and T1 molecular structures of oligothiophenes **1-4** were optimized using the DFT long-range corrected hybrid functional CAM-B3LYP and 6-31+G* basis set, a well-tested combination of DFT functional and basis-set, commonly used for the description of the electronic properties of this kind of molecules.[2] Frequency calculations were carried out on the DMF optimized structures to check the nature of the critical points.

A full conformational analysis via dihedral scanning was carried out for the **1-4** compounds. The solvent effect was taken into account using the IEF-PCM solvation model,[3] with DMF as solvent. TD-DFT calculations were carried out to simulate the UV-Vis spectrum of the OT. Spin–orbit coupling (SOC) were calculated between singlet (S^i) and triplet (T_j) states by the ORCA 5.0.4 package[4] using the same level of theory of the previous calculations.

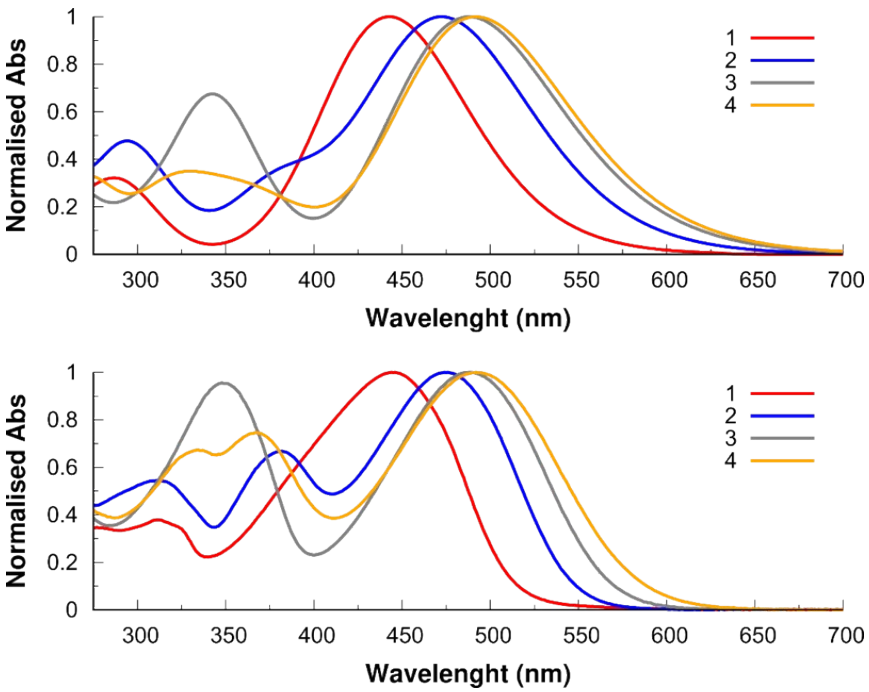
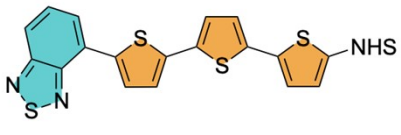


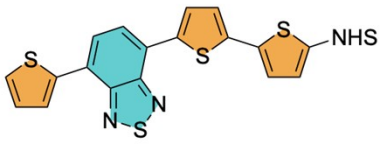
Figure S12. UV-Vis absorption spectra of the compounds **1-4** in DMF. On top, the simulated spectra. On bottom, the spectra acquired experimentally by UV-Vis spectrophotometer.

1



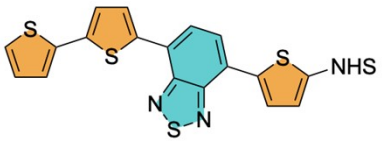
	P1	P2	P3	P4	NHS
$q(S_0)$	-0.04	0.05	0.03	0.00	-0.04
$q(S_1)$	-0.10	0.16	0.13	-0.08	-0.1
Δq	**-0.06**	0.11	0.10	-0.08	-0.06

2



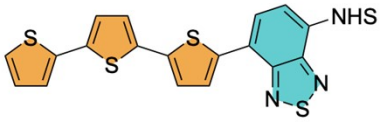
	P1	P2	P3	P4	NHS
$q(S_0)$	-0.01	-0.04	0.05	0.02	-0.02
$q(S_1)$	0.11	-0.18	0.14	-0.03	-0.04
Δq	0.12	**-0.14**	0.09	-0.05	-0.02

3



	P1	P2	P3	P4	NHS
$q(S_0)$	0.05	0.02	-0.08	0.02	-0.01
$q(S_1)$	0.22	0.22	-0.27	-0.09	-0.08
Δq	0.17	0.20	**-0.19**	-0.11	-0.07

4



	P1	P2	P3	P4	NHS
$q(S_0)$	0.05	0.00	0.05	-0.17	0.07
$q(S_1)$	0.17	0.23	0.14	-0.49	-0.05
Δq	0.12	0.23	0.09	**-0.32**	-0.12

Figure S13. ESP charges grouped into molecular fragments (benzo-dithiazole, thiophenes and, succinimide residues) and analyzed for the S_0 and S_1 states.

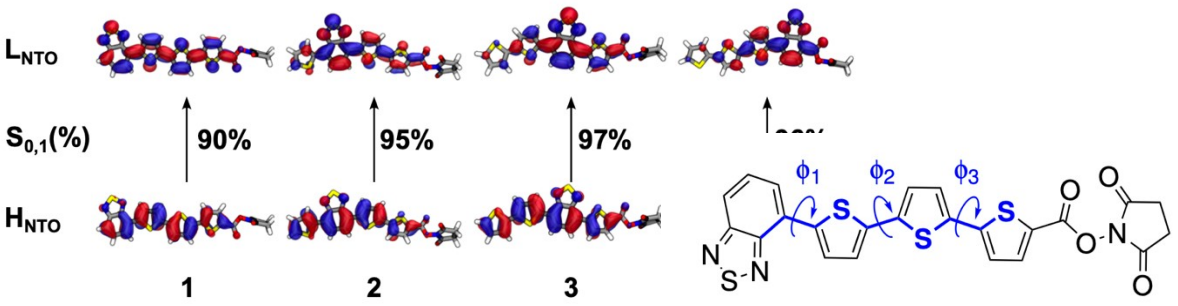


Figure S14. Calculated natural transition orbitals (NTO) that characterize the $S_0 \rightarrow S_1$ transition in compounds **1**-**4**.

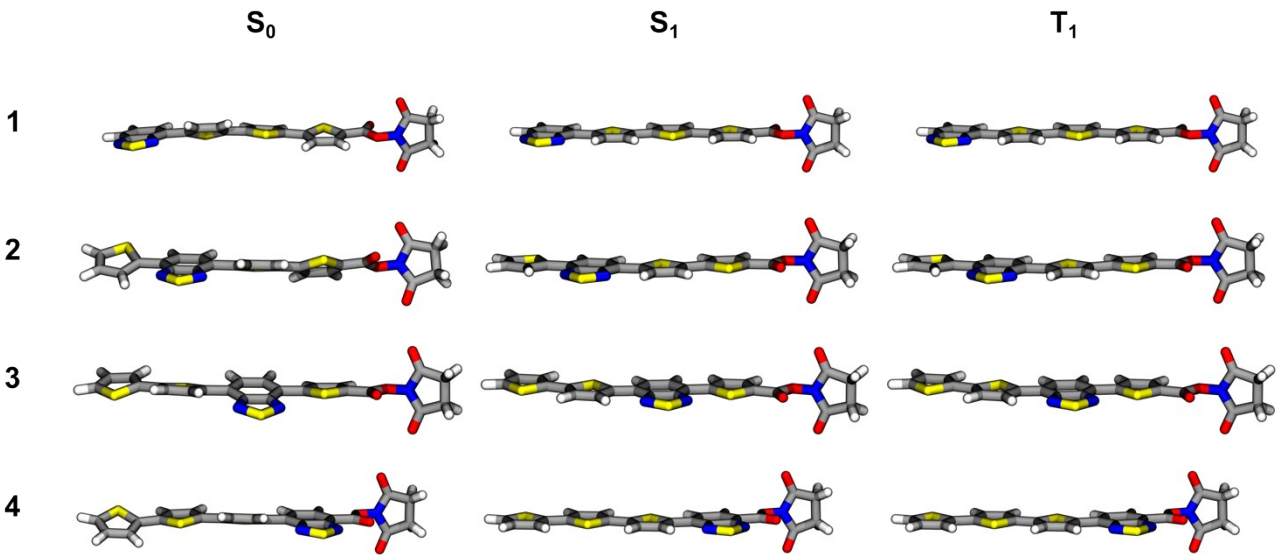


Figure S15. Optimized geometries of the ground state S_0, the first excited singlet state S_1 and the first excited triplet state T_1 for the compounds **1**-**4**.

Table S2. Dihedral angles $\phi_{1\text{-}3}$ (degree) of compounds **1-4** in the S_0 S_1 and T_1 minima, calculated in DMF

OT	State	ϕ_1	ϕ_2	ϕ_3
1	S_0	157.6	160.2	164.9
	S_1	179.8	179.9	180.0
	T_1	179.9	179.9	179.4
2	S_0	158.7	161.3	165.3
	S_1	179.9	179.8	179.3
	T_1	179.9	179.8	178.5
3	S_0	158.1	168.8	163.1

	S_1	179.5	179.9	179.7
	T_1	179.3	180.0	179.6
4	S_0	157.7	163.2	164.7
	S_1	179.9	180.0	179.2
	T_1	180.0	180.0	179.4

Table S3. Redox potentials and reduction peak voltage (in V vs. SCE at 0.1 Vs^{-1}) of compounds **1**-**4**.

Sample	**E°_{ox}/V V vs. SCE**	**E°_{red1}/V V vs. SCE**	**V_{red1}/V**	**V_{red2}/V**
1	1.16	-1.31	-1.29	-1.35
2	1.18*	-1.20	-1.27	-
3	1.09	-1.02	-1.11	-1.28
4	1.07	-0.83	-0.97	-1.09

*half wave potential

Synthesis and characterization of HSA-OT bioconjugates

Materials

Human Serum Albumin (HSA) fatty acid free (Cat. No. A3782), 10-Acetyl-3,7-dihydroxyphenoxazine (Amplex Red) (Cat. No. 90101), Type VI-A Peroxidase from horseradish lyophilized powder (HRP) (Cat. no. P6782), Hydrogen Peroxide Solution 30% (w/w) (Cat. No. 31642-M), 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABMDMA) (Cat. No. 75068), Agarose for Molecular Biology (Cat. No. A9539), Trizma® base (Cat. No. T6066), Glycine (Cat. No. G8898), Glycerol (Cat. No. G2025), Comassie Brilliant Blue G-250 (Cat. No. 27815), Sodium dodecyl sulfate (SDS) (Cat. No. 74255), DL-Dithiothreitol (DTT) (Cat. No. D5545), Bromophenol blue (Cat. No. 114391), Acrylamide/Bis-acrylamide 40% solution (Cat. No. A7802)**,** Deuterium Oxide (Cat. No. 151882), Dimethyl Sulfoxide (DMSO) (Cat. No. 472301), N,N-dimethylformamide (DMF) (Cat. No. 33120-M), Acetonitrile (Cat. No. 34851), Acetic acid (Cat. No. 33209-M), Sodium Chloride (Cat. No. S9888-M), Potassium phosphate monobasic (Cat. No. P0662-M), Sodium phosphate dibasic (Cat. No. S0876), Potassium chloride (Cat. No. P3911-M), Sodium bicarbonate (Cat. No. 31437-M), Sodium carbonate (Cat. No. 223530), 14 kDa dialysis tubing cellulose membrane (Cat. No. D9652) were purchased from Sigma Aldrich (Merck). Milli-Q water was used for the preparation of all the aqueous solutions.

Synthesis and Purification of the HSA–OT bioconjugates

The oligothiophene derivatives **1-4** were dissolved in dimethylformamide (DMF) at a 1 mM concentration. 180 μL of these solutions were added dropwise to 1.8 mL of HSA 10 μM (10:1 dye:protein ratio) in sodium carbonate buffer 100 mM pH 9. The reaction was incubated overnight in the dark, under mild stirring conditions (700 rpm) at 25°C (ThermoMixer HC, S8012-0000; STARLAB). The samples were centrifuged at 14000 g for 10 min to remove the insoluble excess of non-conjugated oligothiophene derivatives, and then extensively dialyzed against Milli-Q water, using cellulose membrane dialysis tubes with a 14 kDa cutoff, to remove the water-soluble byproducts generated during the coupling procedure. An additional centrifuge cycle of 10 min at 14000 g was performed at the end of the purification process.

Characterization of the HSA–OT bioconjugates

Photophysical Characterization

UV-vis absorption spectra were recorded using a Cary 60 UV-Vis spectrophotometer (Agilent).

UPLC-MS

Mass spectra were acquired using ACQUITY UPLC equipped with a BEH300 C4 column (2.5 mm, 4.6 x150 mm, Waters Corporation, Milford, Worcester, MA, USA) and coupled with an ESI-QTOF mass spectrometer (Waters Corporation, Milford, Worcester, MA, USA). The concentration of the protein bioconjugates was 5 μM. The samples were prepared by adding 5% ACN. They were injected in H2O with 0.1% FA at a flow rate of 0.2 mL/min and eluted by a gradient elution (phase A: 0.1% formic acid in H2O, phase B: 0.1% formic acid in ACN; 0 min 80%A 20%B, 30 min 30%A 70%B, 31 min 80%A 20%B, 50 min 80%A 20%B; column temperature 40 °C). Positive-ion ESI mass spectra were acquired using a capillary voltage of 3 kV, a sample cone of 45V and a desolvation gas flow of 800 l/h. The source and desolvation temperatures were 150 and 350 °C, respectively. All spectra were acquired in the range of m/z 50–5000. Raw data were background-subtracted and deconvoluted using Unidec software in the range of m/z 1000–4500.

Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS) measurements were performed using a Malvern Instruments DLS ZetaSizer Nano-ZS. DLS measurements were carried out on 1 μM solutions of protein or bioconjugates in Milli-Q water at room temperature.

Electrophoresis

Agarose gel electrophoresis. Native agarose gel electrophoresis was performed on an Owl Easycast B-Series Horizontal Gel Systems Model B2. A 1% *w/v* agarose gel was prepared, dissolving the powder in a tris-glycine buffer (25 mM trizma and 192 mM glycine, pH = 7.4 adjusted with acetic acid). To 25 μL of protein samples, 5 μL of glycerol was added, and 12 μL of the mixture was loaded into each well. A 9 μM solution of HSA was used as a control. The run was performed by applying potential of 100 V for 15 min, using tris-glycine pH 7.4 as running buffer. A Coomassie Blue staining was performed to visualize the protein spots. Images of the gel before and after the Coomassie Blue staining were obtained employing a ChemiDoc™ MP Imaging System.

SDS-PAGE electrophoresis. SDS-Polyacrylamide gel electrophoresis (PAGE) was performed on a Mini-PROTEAN Tetra electrophoresis system (Bio-Rad). 20 μL of each sample were diluted with 10 μL of a Laemmli buffer (187.5 mM Tris-HCl pH 6.8, 6% w/v SDS, 30% v/v glycerol, 0.03% w/v bromophenol blue, 125 mM DTT) and incubated at 95°C for 5 minutes (ThermoMixer HC, S8012-0000; STARLAB), obtaining protein denaturation. 12 μL of each mixture was loaded in a 10% acrylamide gel, together with a protein ladder (PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, Thermo Fischer). The run was performed applying a potential of 130 V for 70 minutes, using a tris-glycine-SDS buffer (25 mM trizma, 192 mM glycine and 0.1% w/v SDS) as running buffer. Protein bands were visualized using a Coomassie Blue staining. Images of the gel before and after the staining were obtained employing a ChemiDoc™ MP Imaging System.

ROS detection

Amplex Red Assay. The generation of peroxides, upon irradiation with visible light, was quantified by the Amplex Red assay. 10-Acetyl-3,7-dihydroxyphenoxazine (Amplex Red), through HRP catalysis, reacts with peroxides to form the pink-colored resorufin. 10 μl of Amplex Red 50 mM in DMSO was added to 1 ml of phosphate buffer 50 mM pH 7.4 (PB). Then, 10 μl of HRP 0.4 mg/ml in PB was added to the Amplex Red solution to obtain the final working solution (WS). In a 96-multiwell plate, 90 μl of the samples under investigation were loaded, containing different concentrations of HSA-oligothiophenes (0 μM, 0.1 μM, 0.3 μM, 1 μM, calculated as concentration of the dyes). The plate was irradiated for 30 min with a white led (Valex

30 W lamp, irradiation power density on the cell plate = 24 mW/cm^2; measured with the photo-radiometer Delta Ohm LP 471 RAD), while an identical plate was kept in the dark. 10 µl of WS was added to each sample in both plates. After 30 min of incubation at room temperature, the absorbance of the samples is read at 560 nm. A calibration curve generated using standard solutions of H_2O_2 was used to interpolate the absorbance signal and convert it to the concentration of peroxides generated upon irradiation. The resorufin produced by the reference samples, which were kept in the dark, was respectively subtracted from the resorufin generated from the irradiated ones. Absorbance measurements were carried out using a Perkin Elmer EnSpire® Multimode Plate Reader.

ABMDMA Assay. 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABMDMA) was used as a $^1O_2$ selective detector. The ABMDMA reacts with $^1O_2$ to form the relative endoperoxide, visualized by a decline of the absorbance at 401 nm. 97 µL of HSA-oligothiophenes (0 µM, 0.1 µM, 0.3 µM, 1 µM) were prepared in deuterated PBS 10mM pH 7.4 and loaded in a 96-multiwell plate. 3 µL of ABMDMA 5 mM stock solution in DMSO was added to each well. The samples were irradiated in the same conditions used in the Amplex Red assay, and the bleaching of the absorption band of ABMDMA at 401 nm was monitored using a Perkin Elmer EnSpire® Multimode Plate Reader.

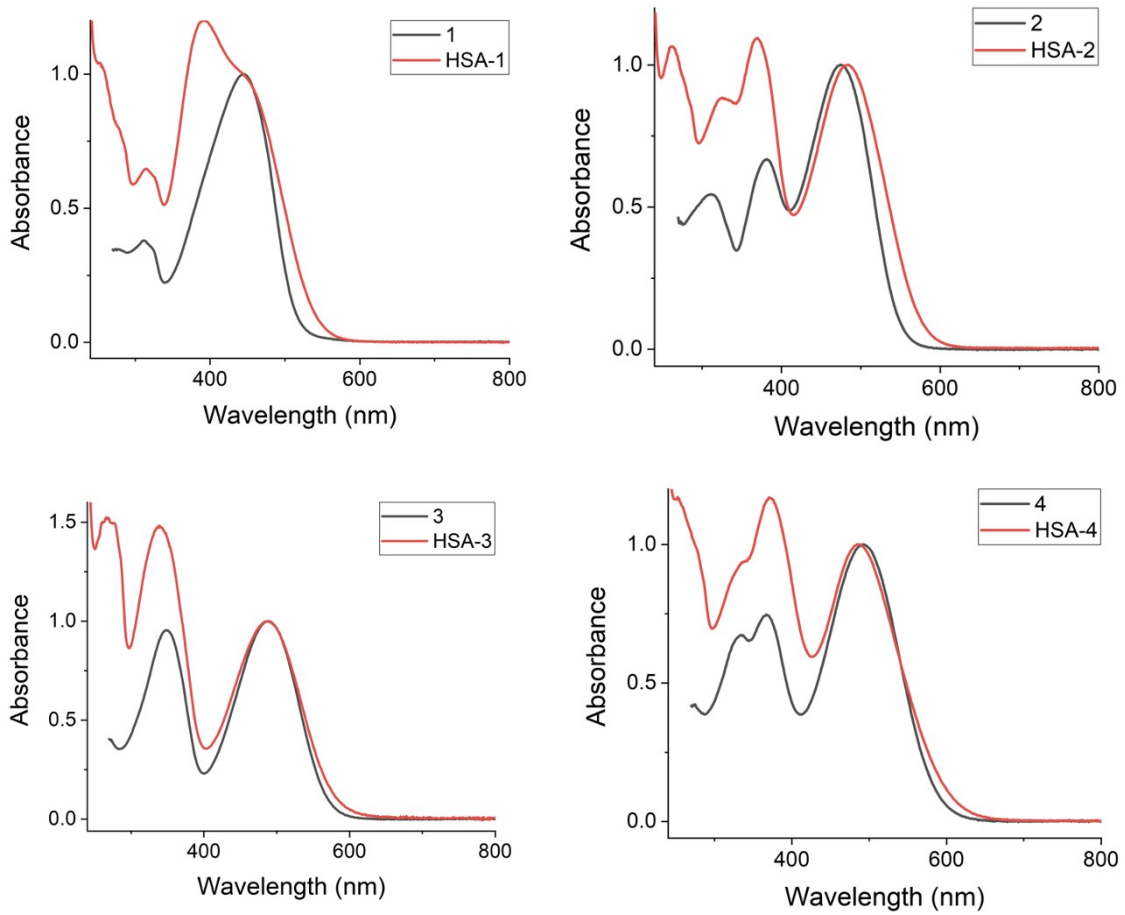


Figure S16. UV-Vis absorption spectra of the compounds **1-4** in DMF (black lines) and their corresponding HSA-OT bioconjugates in PBS (red lines).

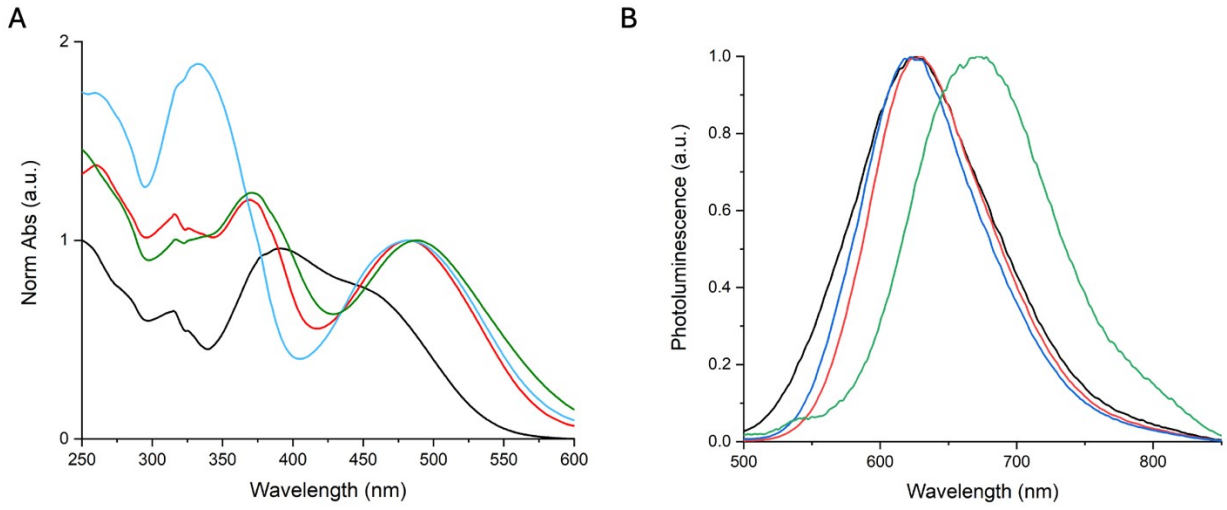


Figure S17. A) UV-Vis absorption and B) emission spectra of compounds HSA-**1** (black line), HSA-**2** (red line), HSA-**3** (cyan line) and HSA-**4** (green line) in PBS.

Table S4. Relative Quantum Yields (φ_{em}) in water PBS solutions calculated using $[Ru(bpy)_3]Cl_2$ as reference (φ_{em}=0,04 in water)

	φ_{em}
HSA-**1**	0.028
HSA-**2**	0.071
HSA-**3**	0.068
HSA-**4**	0.013

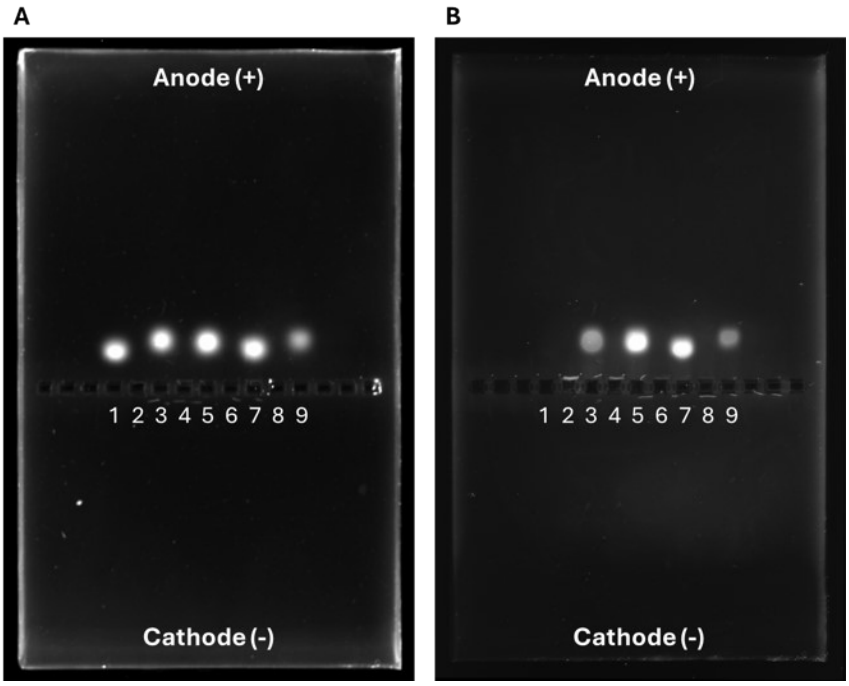


Figure S18. Coomassie blue stained (A) and stain-free fluorescence imaging (B) of agarose gel loaded with unconjugated OT (compounds **1**-**4**) and their corresponding bioconjugates (HSA-**1**, HSA-**2**, HSA-**3**, HSA-4). List of samples:

1) HSA,

2) **1,**

3) HSA-**1**,

4) **2**,

5) HSA-**2**,

6) **3**,

7) HSA-**3**,

8) **4**,

9) HSA-**4**.

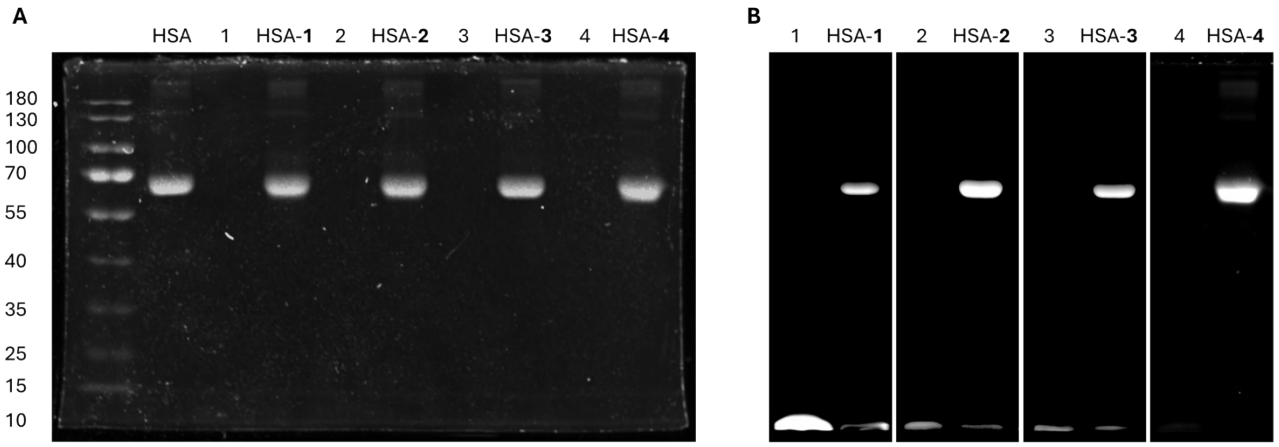


Figure S19. Coomassie blue stained (A) and stain-free fluorescence imaging (B) of SDS-PAGE gel loaded with unconjugated OT (compounds **1**-**4**) and their corresponding bioconjugates (HSA-**1**, HSA-**2,** HSA-**3**, HSA-**4**), and HSA as a reference. According to the absorption/emission spectra of the compounds, different combination of excitation/emission filters were used for the acquisition of **1**, HSA-**1** (Alexa488), **2**, HSA-**2**, **3**, HSA-**3** (Alexa 546), 4, HSA-**4** (Alexa 647), then the images were assembled (B).

Cancer cell line

The A431 human epidermoid carcinoma cell line was cultured in RPMI 1640 medium (Euroclone, Italy), enriched with 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin–streptomycin (final concentration 100 U/mL), and 1% L-glutamine (200 mM). Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

Confocal microscopy of A431 cells treated with HSA-OT

A431 were plated onto round glass coverslips in 6-well plates (Corning) and allowed to adhere overnight. The following day, cells were treated for 45 minutes with complete RPMI medium containing HSA-OP at the final concentration of 1 µM. After three PBS washes, cells were stained with Hoechst 33342 (1 µg/mL, Invitrogen) for 15 minutes and the stained coverslip was then transferred into an Attofluor chamber (Invitrogen, USA) and overlaid with 1 mL of phenol red-free RPMI medium supplemented with 10% FBS, 1% PenStrep, and 1% L-glutamine. Imaging was acquired using a NIKON A1R confocal microscope under fixed laser settings. Membrane blebbing pictures were acquired as just described but after 10 minutes of irradiation with white LED lamp.

Flow Cytometry analysis on cancer cell incubated with HSA-OT.

Cellular uptake of HSA-OT bioconjugates was assessed on A431 cell line by incubating 500,000 adherent cells with 1 µM of HSA-OTs for 45 minutes. Unbound material was removed with three PBS washes, and cells were detached with 1× trypsin. Trypsin activity was neutralized using complete RPMI, and cells were then washed twice, resuspended in 1 mL PBS, and analyzed with a CytoFLEX S cytometer (Beckman Coulter). A minimum of 10,000 events were recorded. Data processing was performed using CytExpert (Beckman Coulter) and FlowJo™ software.

Photodynamic therapy of HSA-OT bioconjugates on cancer cell

20 000 A431 cells seeded in 96-well plates were exposed to increasing concentrations of each 3 HSA-OT bioconjugates for 45 minutes. Post-incubation, cells were washed twice with PBS and irradiated with LED light for 10 minutes. Following illumination, PBS was removed, and cells were incubated in complete RPMI

for 24 hours. Cell viability was determined using the MTT assay, and absorbance was measured at 570 and 690 nm using an EnSpire multimode plate reader (PerkinElmer, USA). Statistical analyses and IC_{50} values were calculated using GraphPad Prism 8.0 (GraphPad Software, California, USA).

Measurement of extracellular ATP levels

A431 cells have been treated with HSA-**1** in medium containing 2% FBS, to detect extracellular ATP according to the method previously described.[5] Supernatants were collected and transferred to a white 96-well plate. CellTiter-Glo® Luminescent Cell Viability Assay was used to detect extracellular ATP following the manufacturer instructions. Briefly, 100 µL of reconstituted reagent was added to 100 µL supernatant. The samples were mixed in an orbital shaker for 2 minutes and then incubated at room temperature for 10 minutes. Luminescence was recorded using the multimodal microplate reader Victor X3 (PerkinElmer, Waltham, MA, USA). ATP has been expressed as fold-increase compared to untreated cells.

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