

Supporting Information for
**Fluorescent Thienothiophene-Containing Squaraine Dyes and Threaded Supramolecular Complexes
with Tunable Wavelengths between 600-800 nm**

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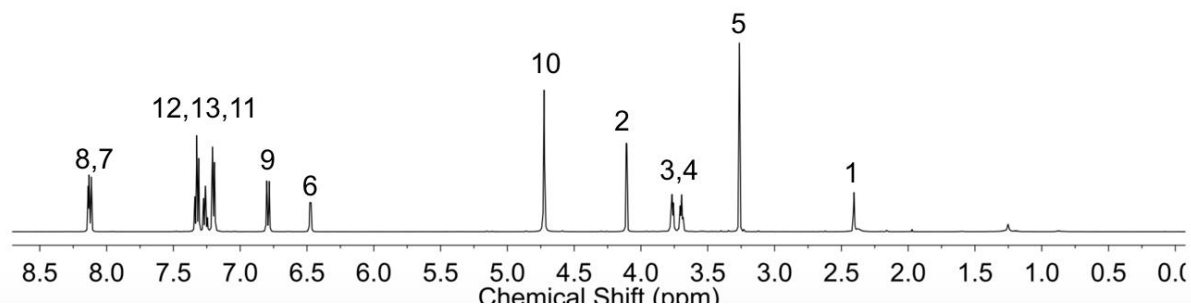
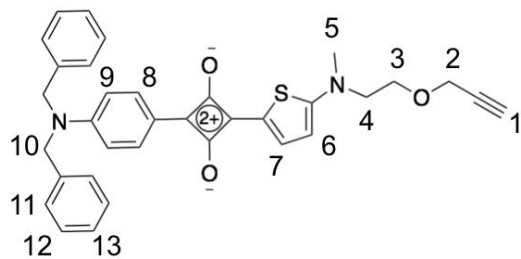
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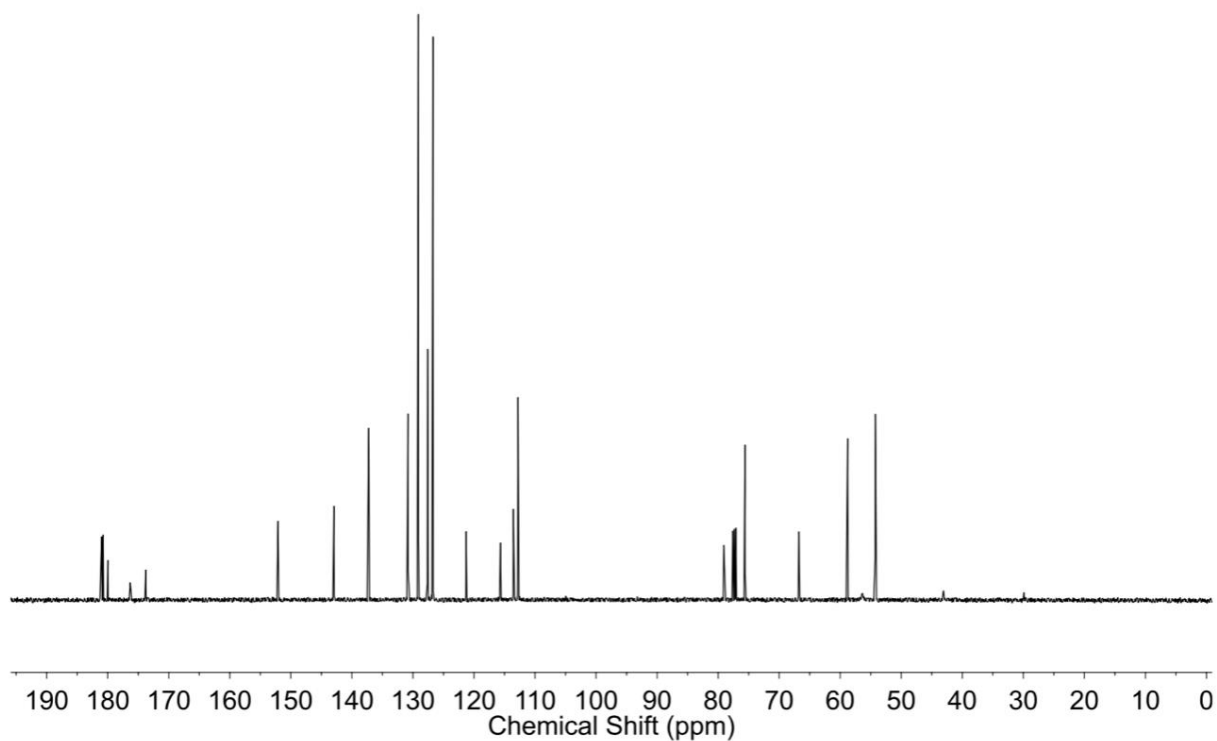
- A. Compound Characterization**
- B. Titration Data**
- C. Photophysical Data**
- D. Chemical Stability Data**
- E. Fluorescence Quantum Yields**

A. Compound Characterization

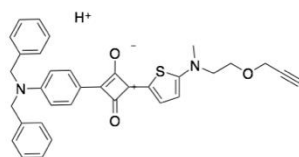
The peaks assignments below are based on precedent and logic. They have not been confirmed by correlation methods.



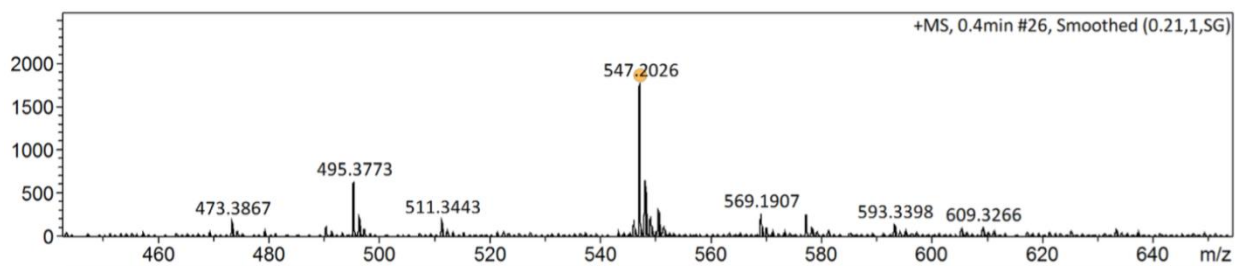
¹H NMR (500 MHz, CDCl₃) of **S1**.



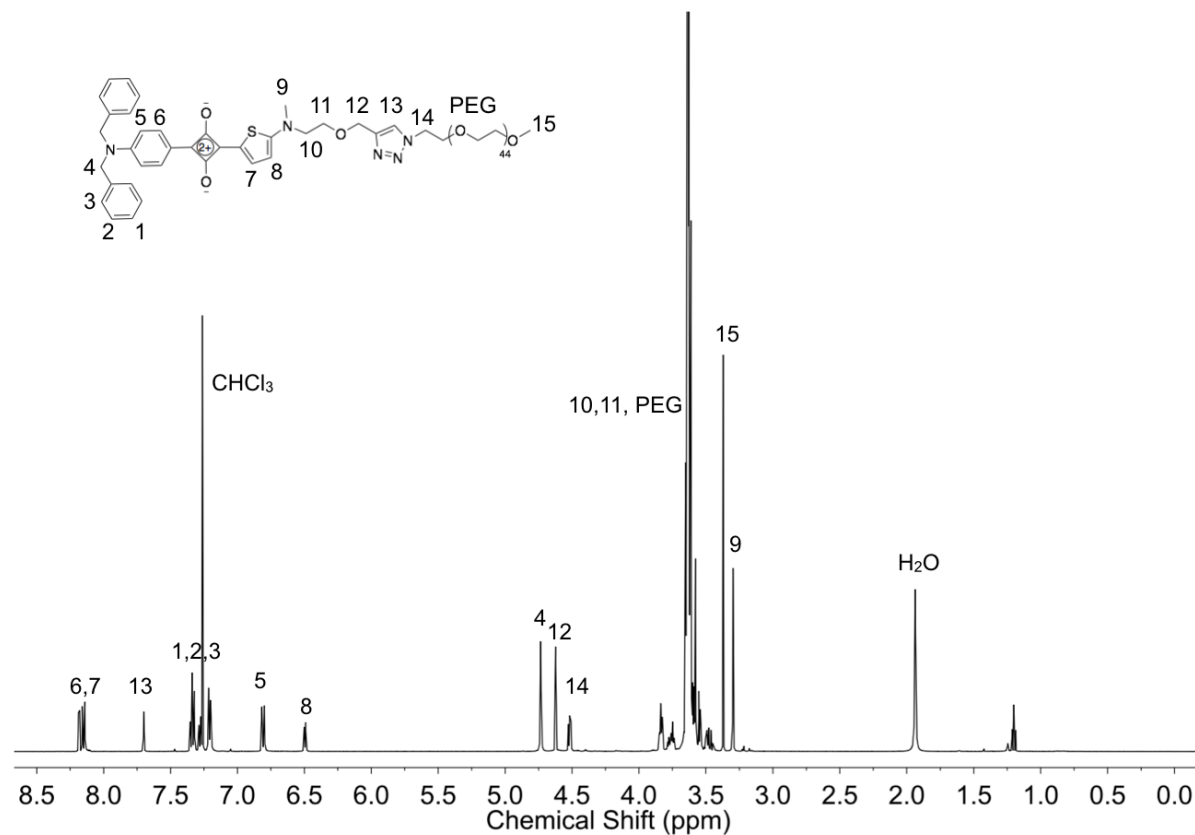
¹³C NMR (126 MHz, CDCl₃) of **S1**.



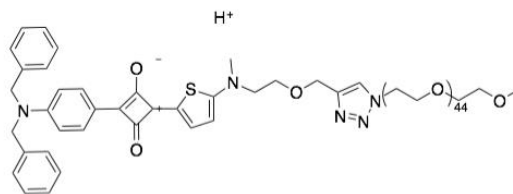
Chemical Formula: $C_{33}H_{31}N_2O_3S^+$
 Exact Mass: 547.2050
 Molecular Weight: 547.6925
 m/z: 547.2050 (100.0%), 548.2083 (36.8%), 549.2008 (4.5%),
 549.2117 (3.9%), 549.2117 (2.7%), 550.2041 (1.7%)
 Elemental Analysis: C, 74.56; H, 5.71; N, 5.11; O, 8.76; S, 5.85



HRMS-ESI of **S1**.



1H NMR (500 MHz, $CDCl_3$) of **S1PEG**.

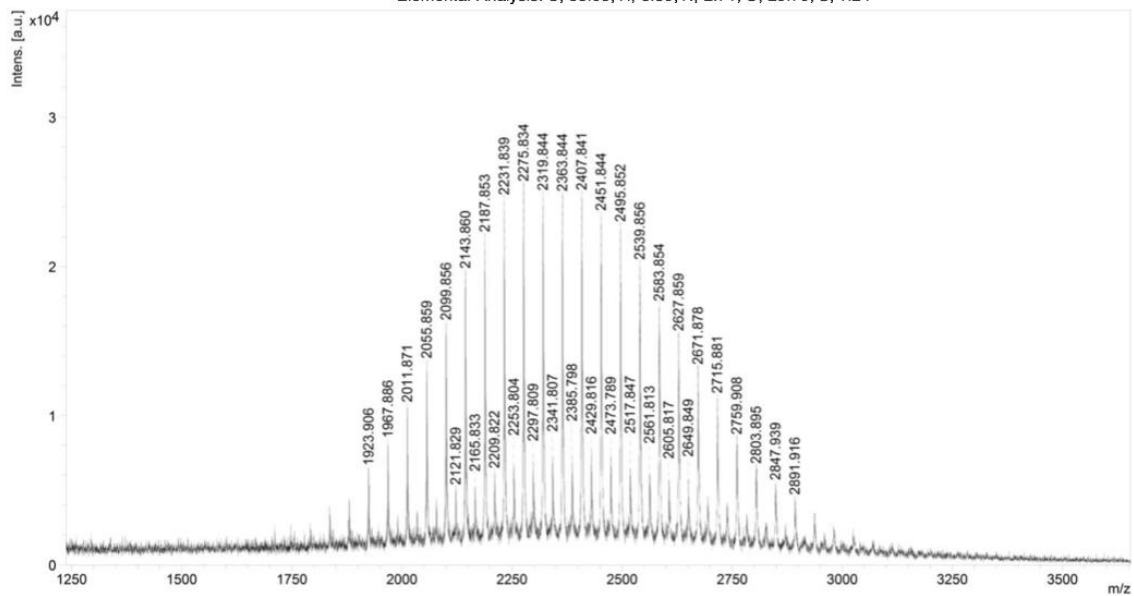


Chemical Formula: C₁₂₅H₂₁₃N₅O₄₆S

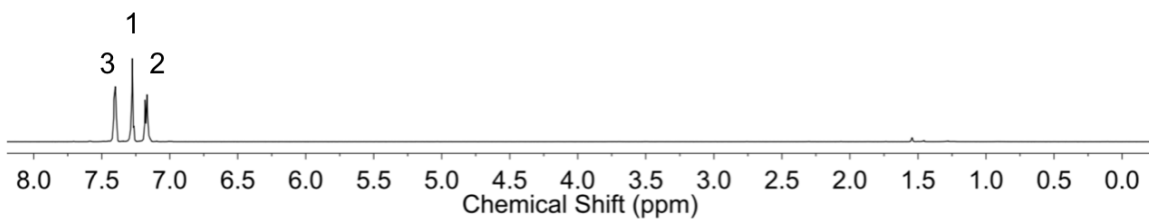
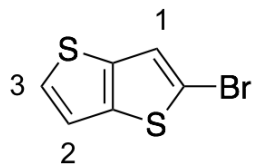
Exact Mass: 2584.4101

Molecular Weight: 2586.1260

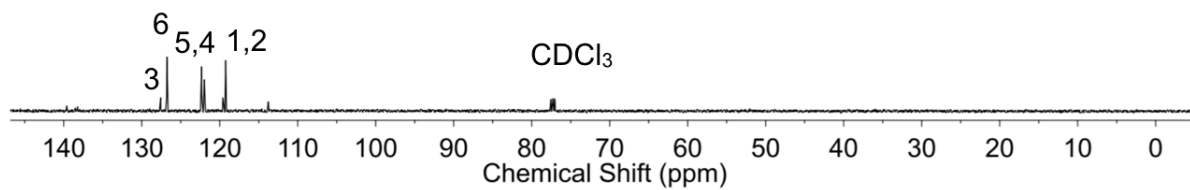
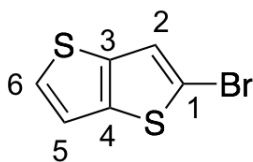
m/z: 2585.4134 (100.0%), 2584.4101 (74.0%), 2586.4168 (35.4%), 2586.4168 (31.6%), 2587.4201 (22.9%), 2587.4177 (9.9%), 2588.4235 (8.9%), 2586.4143 (7.3%), 2587.4201 (6.8%), 2587.4092 (4.5%), 2588.4210 (3.5%), 2586.4059 (3.3%), 2588.4210 (3.1%), 2589.4268 (2.5%), 2586.4197 (2.4%), 2589.4244 (2.3%), 2586.4105 (1.8%), 2586.4176 (1.8%), 2585.4164 (1.8%), 2588.4126 (1.6%), 2588.4126 (1.4%), 2585.4071 (1.4%), 2585.4143 (1.4%), 2587.4138 (1.2%), 2589.4159 (1.0%)
 Elemental Analysis: C, 58.05; H, 8.30; N, 2.71; O, 29.70; S, 1.24



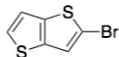
MS-MALDI (DHBA as matrix) of **S1PEG**. A set of peaks around 2363 reflect the polydispersity of the PEG₄₅ chains.



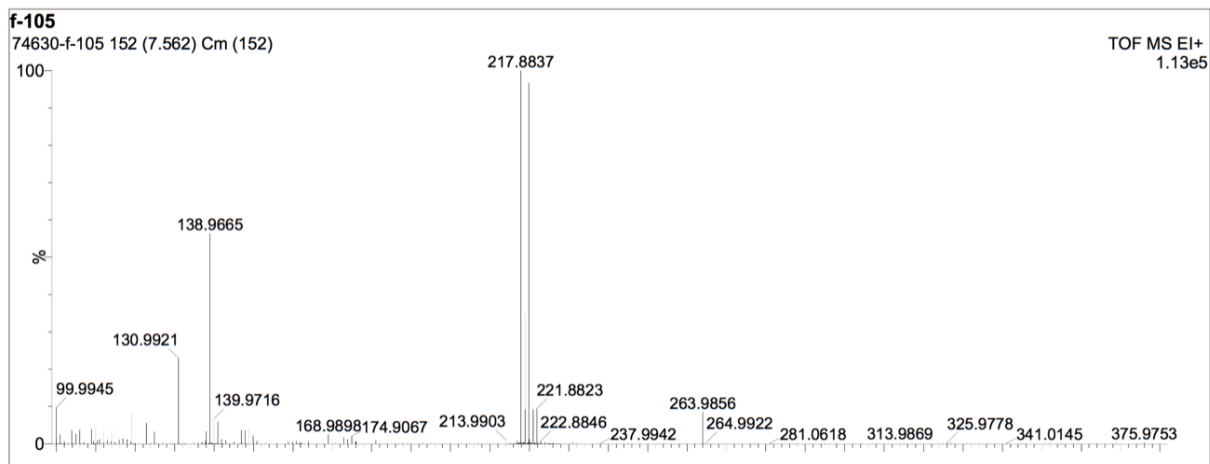
^1H NMR (500 MHz, CDCl_3) of **4**.



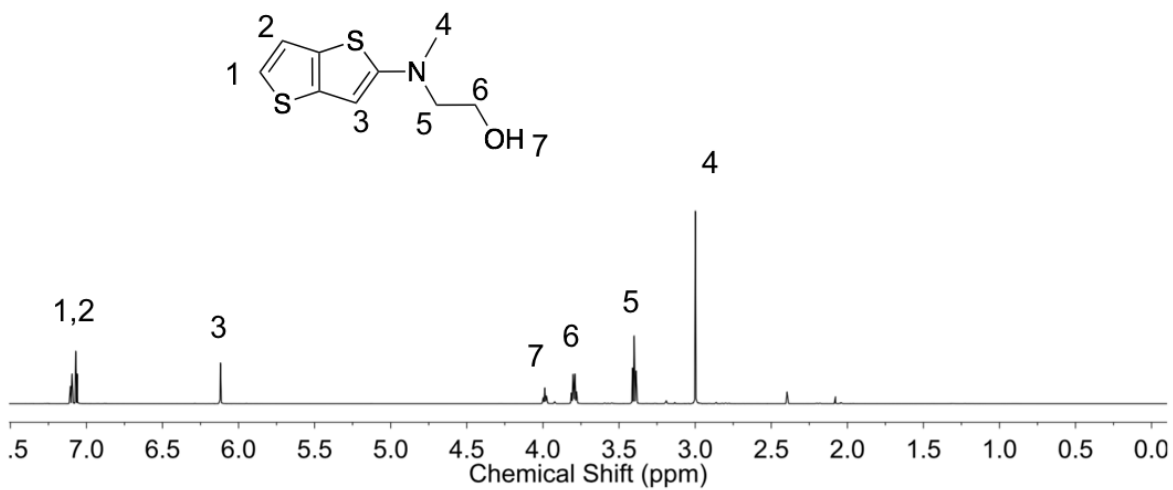
^{13}C NMR (126 MHz, CDCl_3) of **4**.



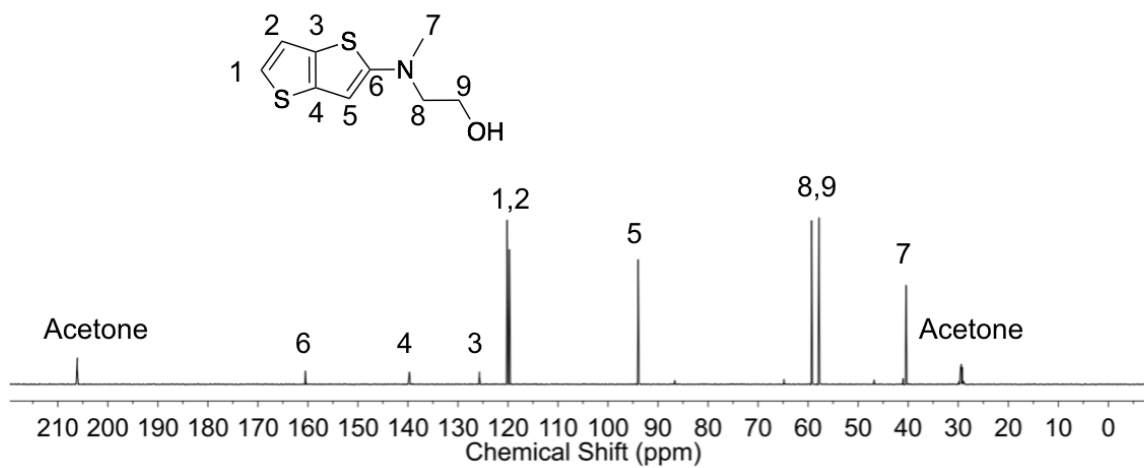
Chemical Formula: $C_6H_3BrS_2$
Exact Mass: 217.8860
Molecular Weight: 219.1140
m/z: 217.8860 (100.0%), 219.8839 (97.3%), 219.8818 (9.0%),
221.8797 (8.8%), 218.8893 (6.5%), 220.8873 (6.3%), 218.8853
(1.6%), 220.8833 (1.6%)
Elemental Analysis: C, 32.89; H, 1.38; Br, 36.47; S, 29.26



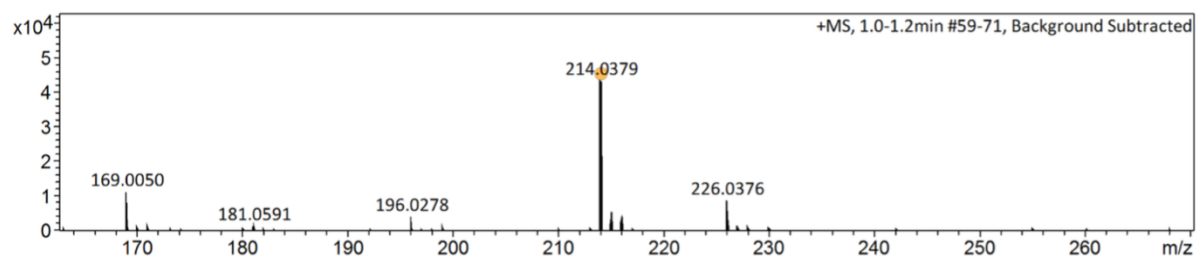
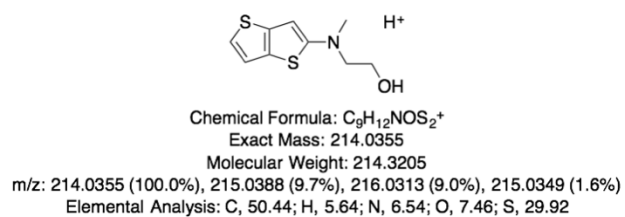
HRMS-ESI of **4**.



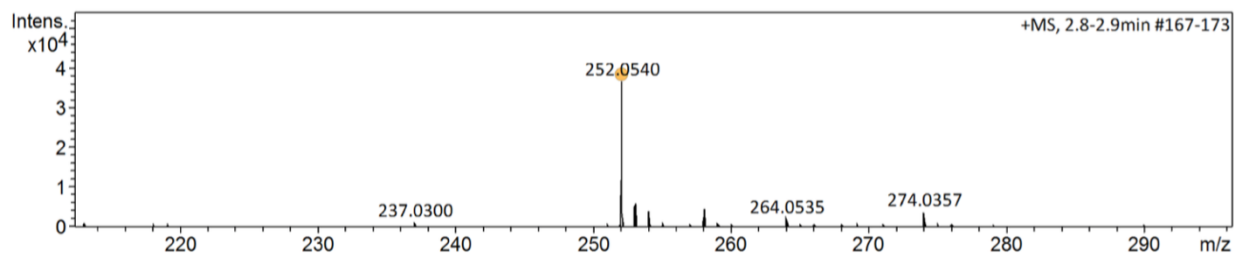
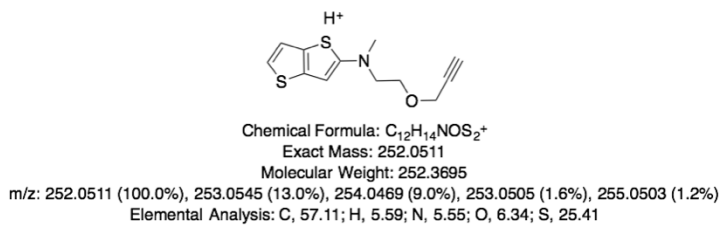
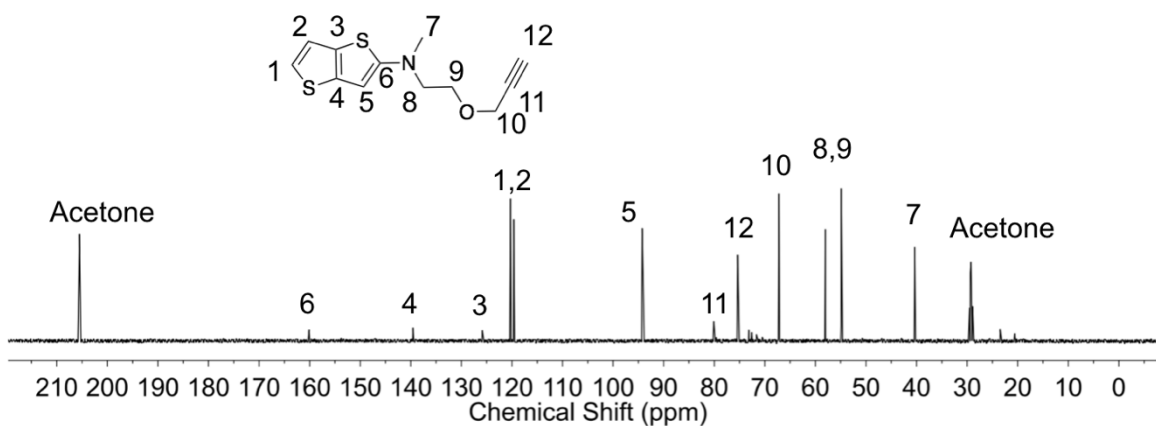
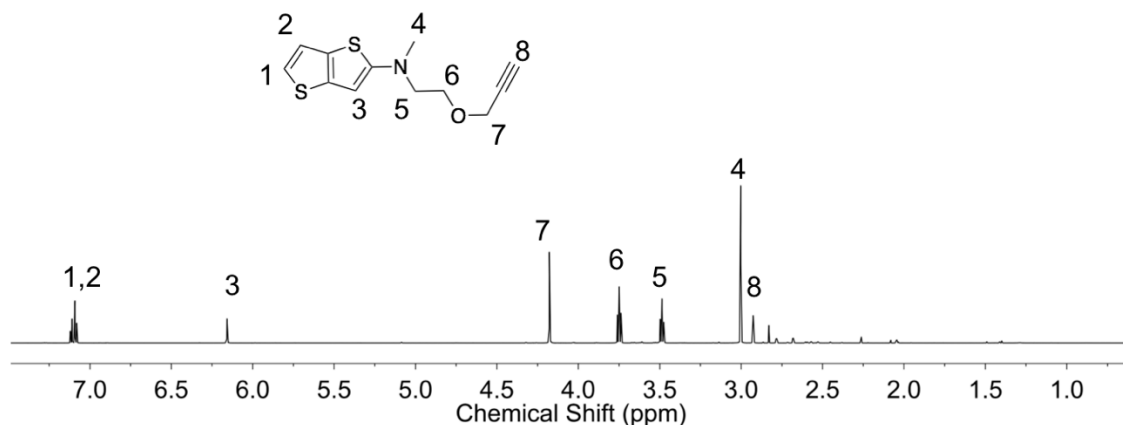
1H NMR (500 MHz, Acetone- d_6) of **5**.

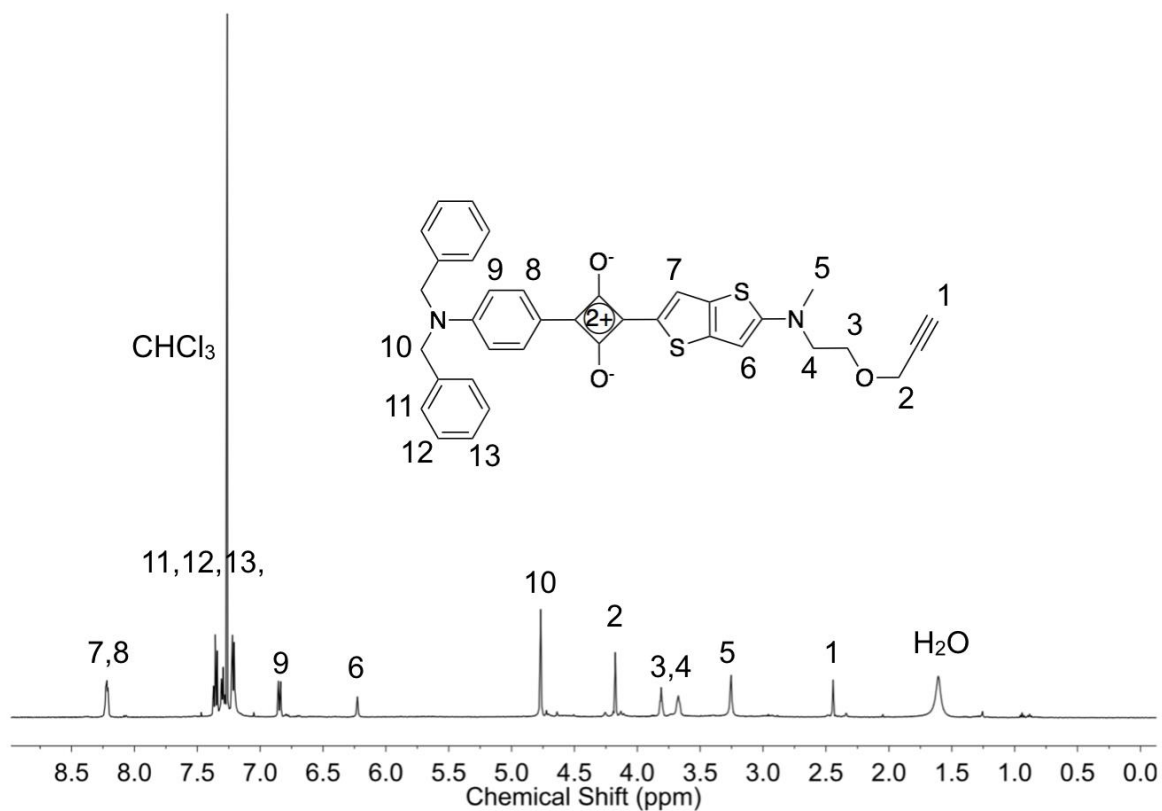


¹³C NMR (126 MHz, Acetone-d₆) of **5**.

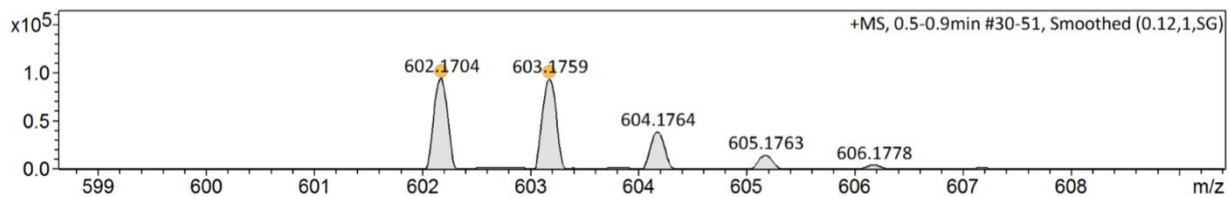
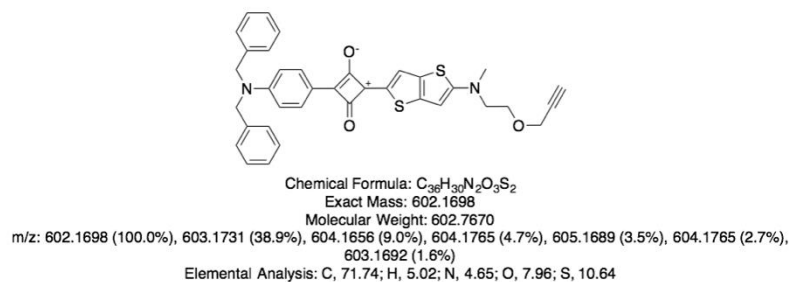


HRMS-ESI of **5**.

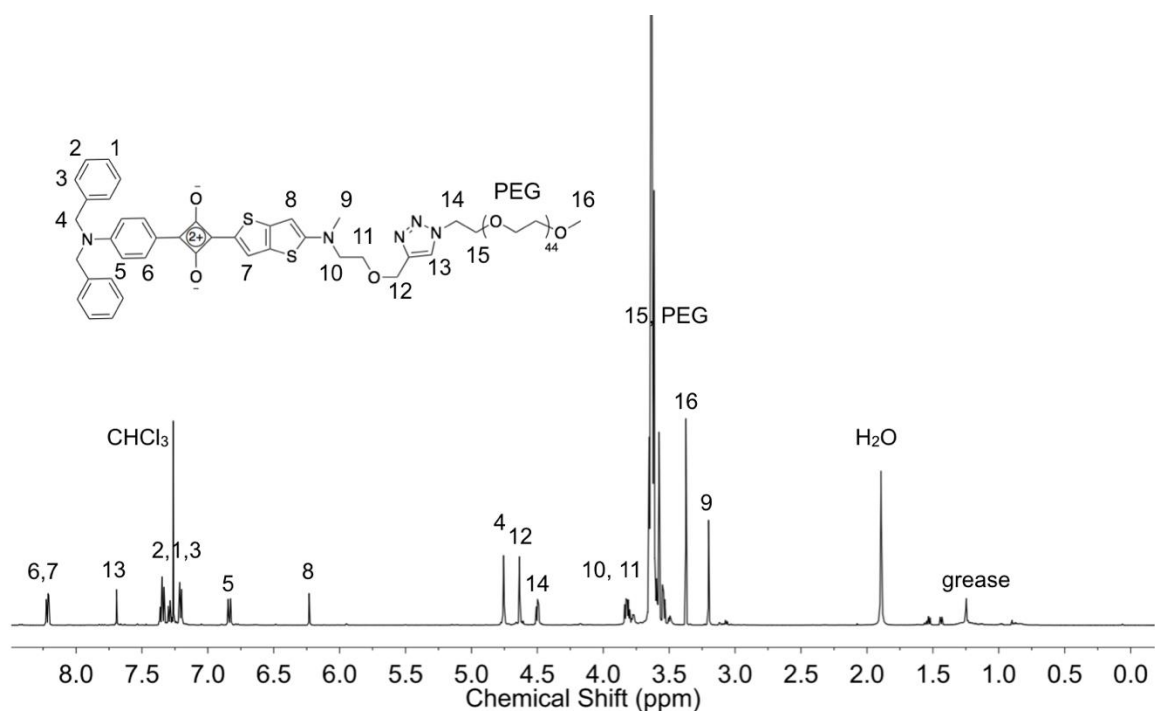




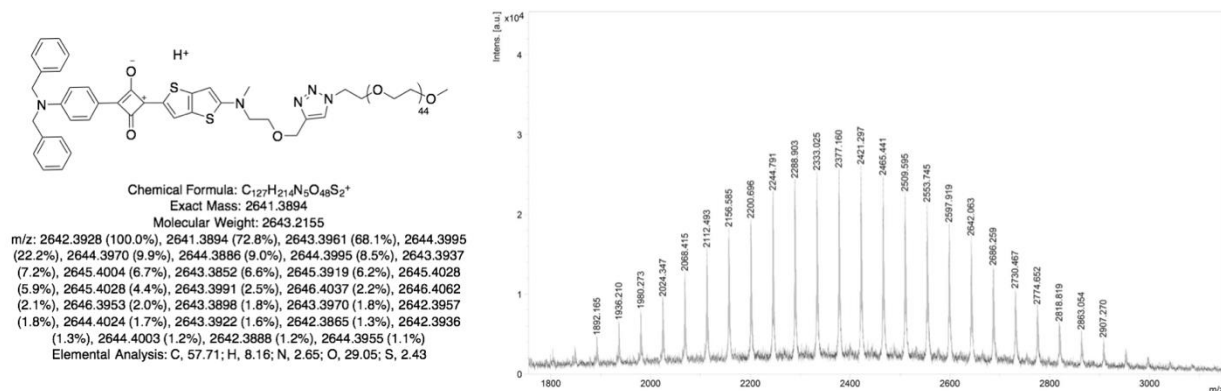
^1H NMR (500 MHz, CDCl_3) of **S2**.



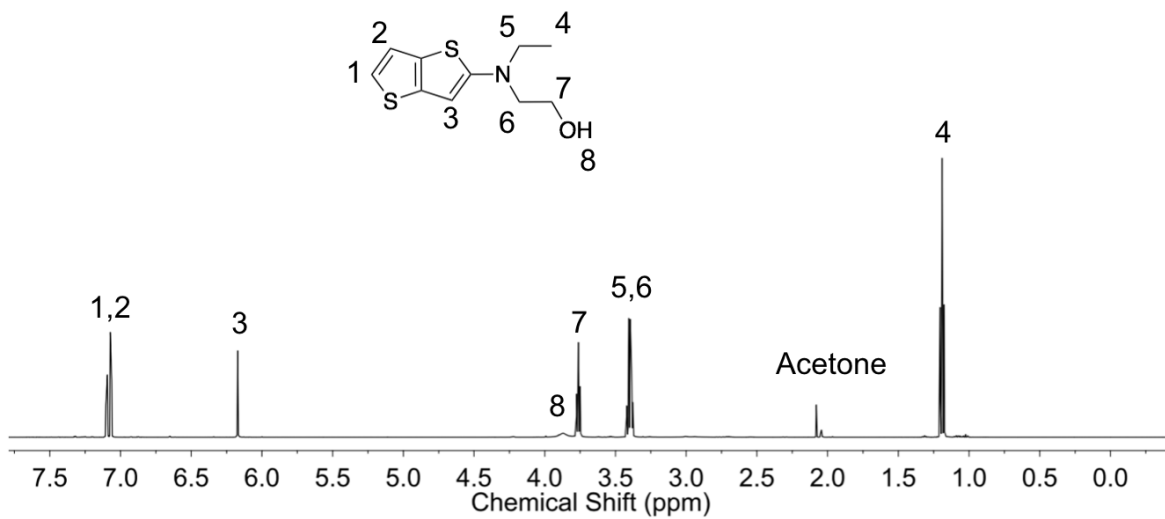
HRMS-ESI of **S2**.



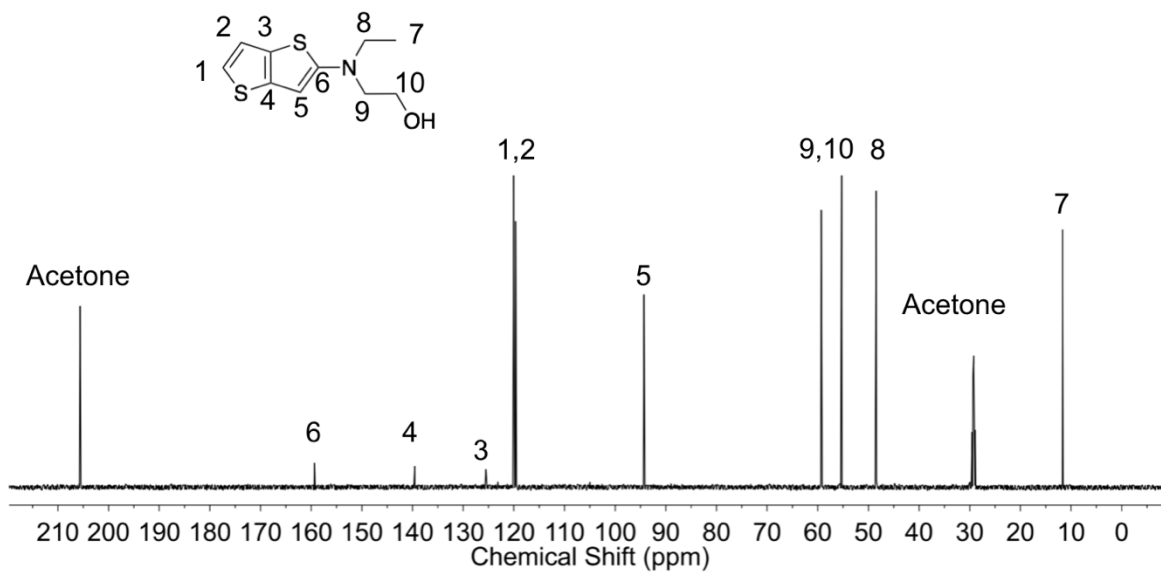
^1H NMR (500 MHz, CDCl_3) of **S2PEG**.



MS-MALDI (DHBA as matrix) of **S2PEG**. A set of peaks around 2421 reflect the polydispersity of the PEG_{45} chain.

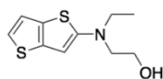


¹H NMR (500 MHz, Acetone-d₆) of 7.



¹³C NMR (126 MHz, Acetone-d₆) of 7.

H⁺



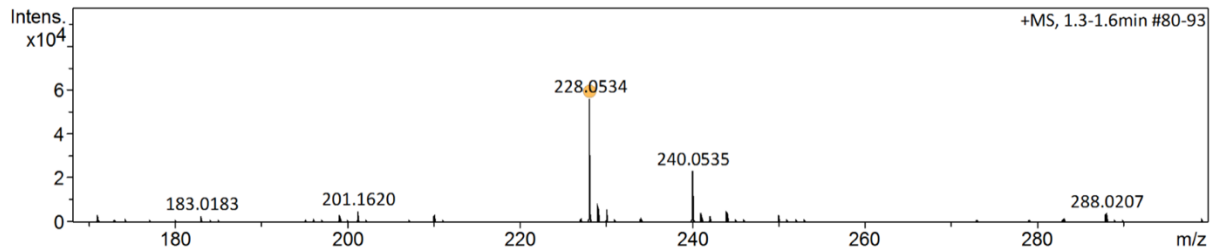
Chemical Formula: C₁₀H₁₄NOS₂⁺

Exact Mass: 228.0511

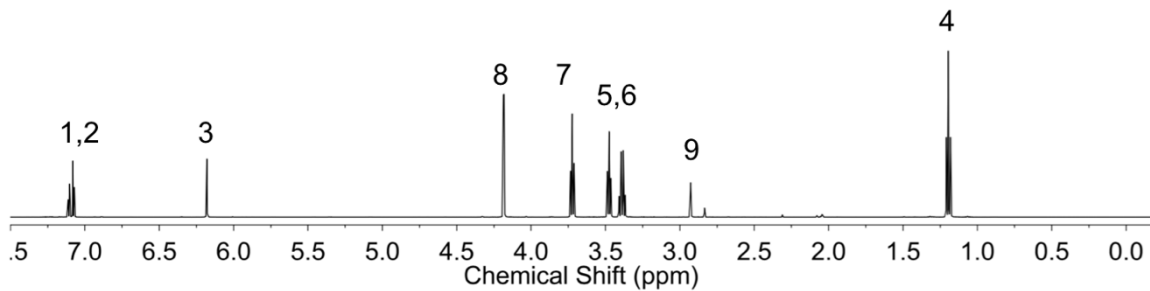
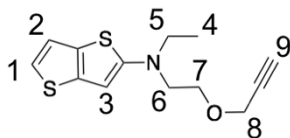
Molecular Weight: 228.3475

m/z: 228.0511 (100.0%), 229.0545 (10.8%), 230.0469 (9.0%), 229.0505 (1.6%)

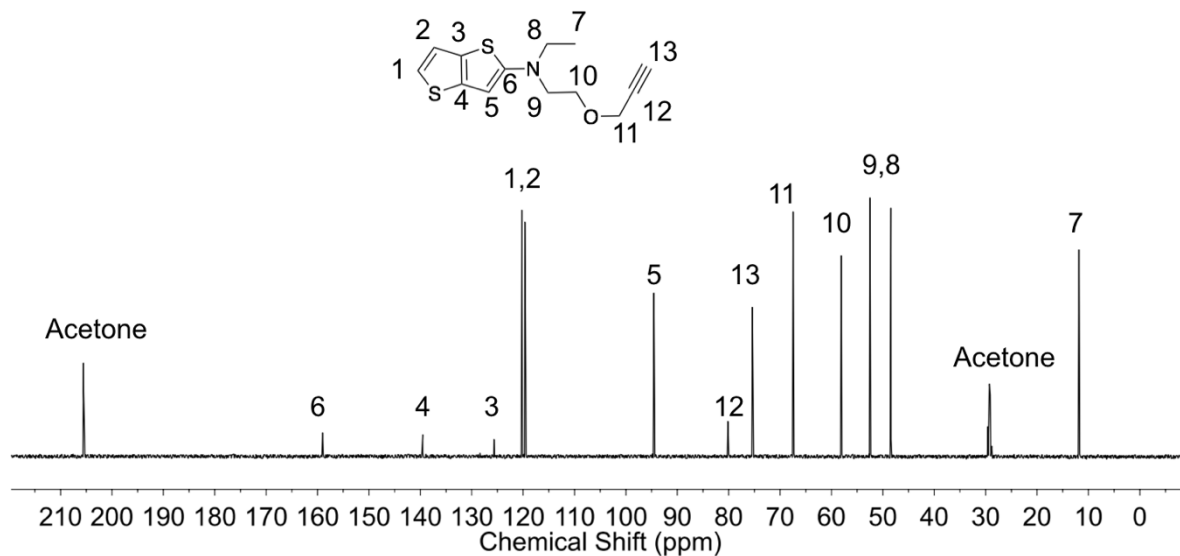
Elemental Analysis: C, 52.60; H, 6.18; N, 6.13; O, 7.01; S, 28.08



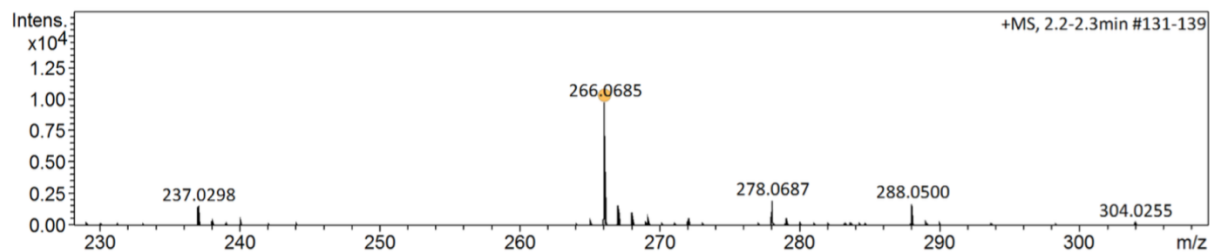
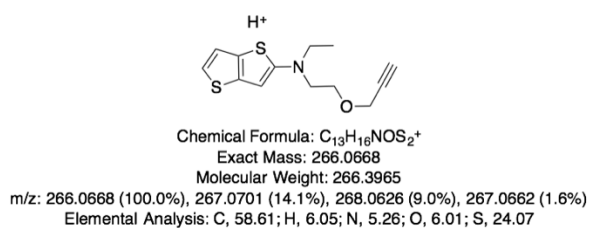
HRMS of 7.



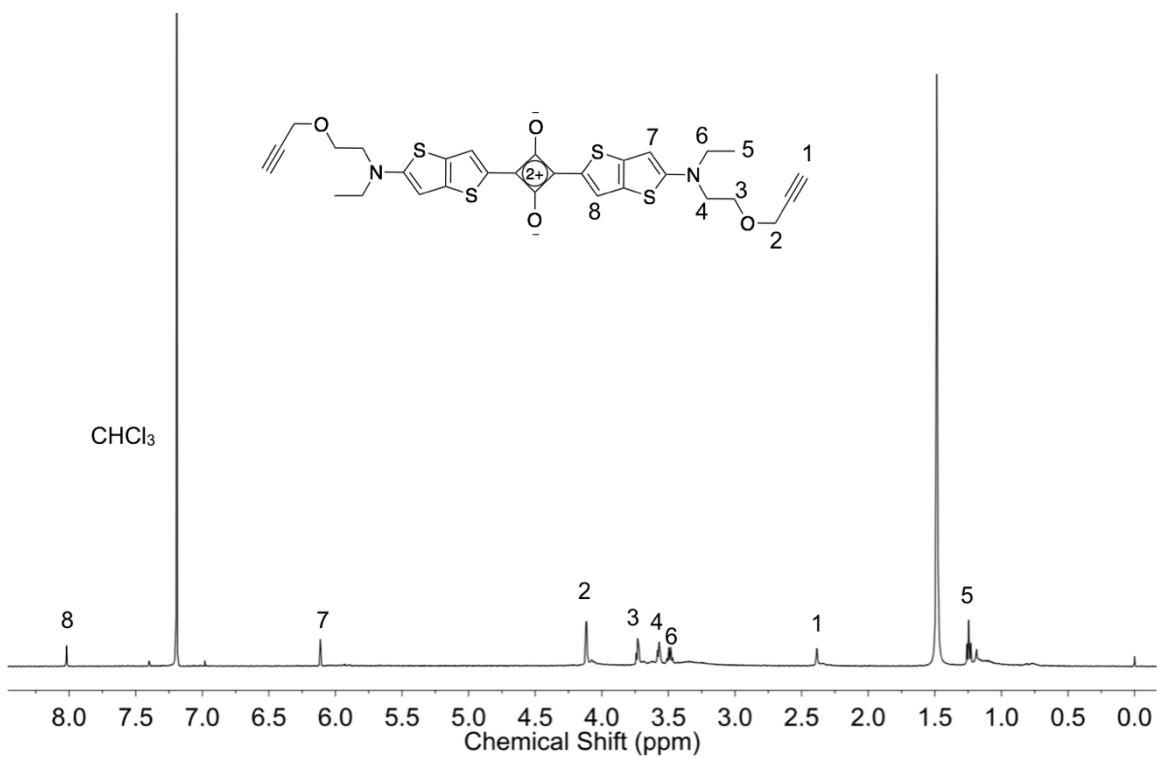
¹H NMR (500 MHz, Acetone-d₆) of 8.



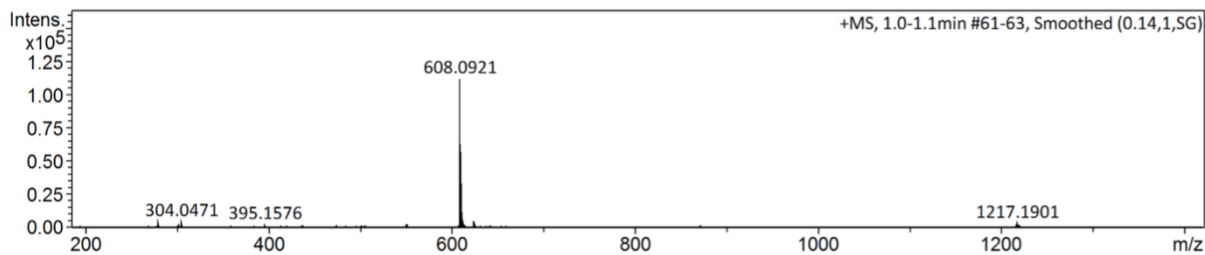
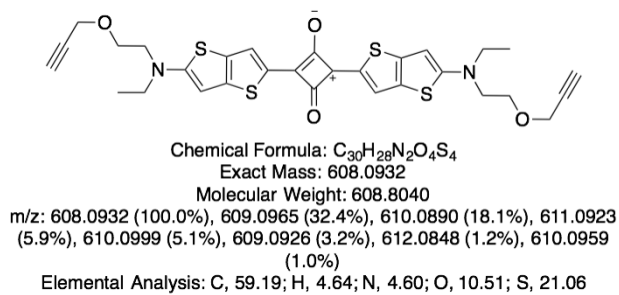
^{13}C NMR (126 MHz, Acetone- d_6) of **8**.



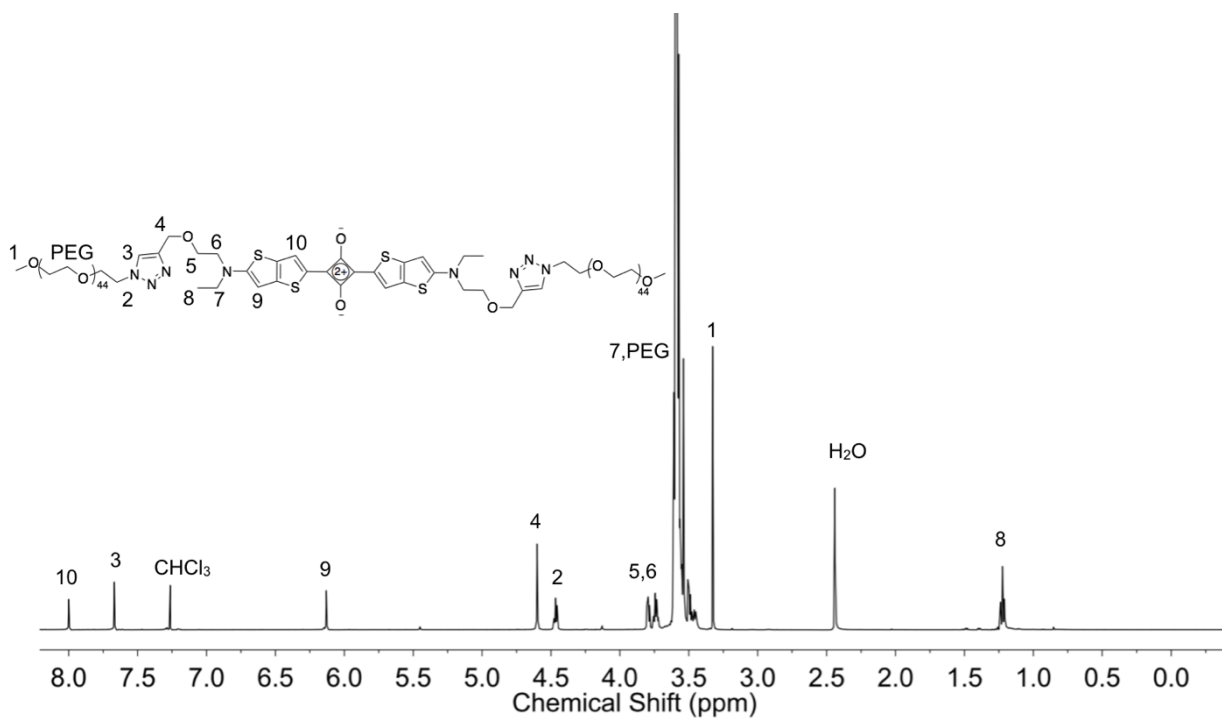
HRMS of **8**.



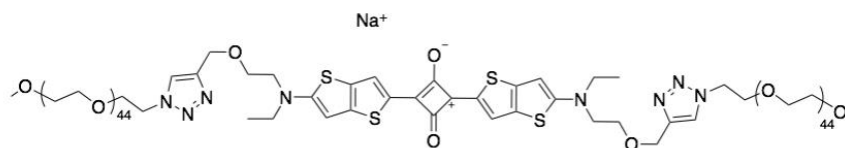
^1H NMR (500 MHz, CDCl_3) of **S3**.



HRMS-ESI of **S3**.



¹H NMR (500 MHz, CDCl₃) of **S3PEG**.



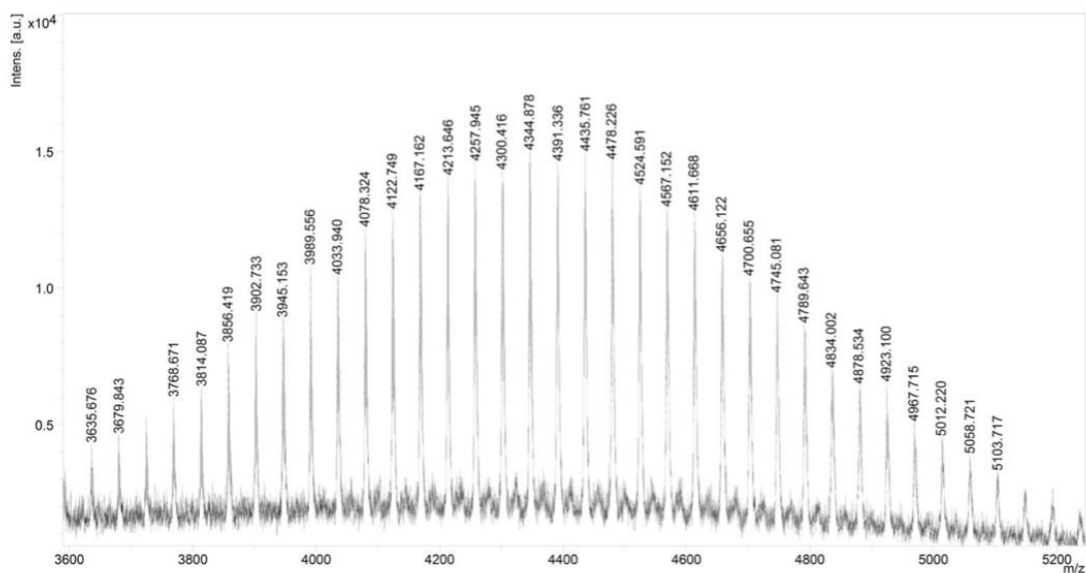
Chemical Formula: $C_{212}H_{394}N_8NaO_{94}S_4^+$

Exact Mass: 4707.5071

Molecular Weight: 4710.6752

m/z: 4709.5138 (100.0%), 4708.5105 (87.6%), 4710.5172 (75.7%), 4707.5071 (38.2%), 4711.5206 (28.4%), 4711.5181 (19.3%), 4712.5239 (18.1%), 4711.5096 (18.1%), 4710.5147 (16.9%), 4710.5063 (15.8%), 4711.5206 (14.0%), 4712.5130 (13.7%), 4712.5214 (12.2%), 4709.5114 (7.4%), 4709.5029 (6.9%), 4713.5248 (5.5%), 4713.5163 (5.1%), 4713.5139 (3.5%), 4714.5197 (3.3%), 4710.5132 (3.2%), 4713.5273 (3.2%), 4712.5105 (3.1%), 4710.5109 (3.0%), 4709.5099 (2.8%), 4709.5075 (2.6%), 4713.5163 (2.5%), 4711.5166 (2.4%), 4711.5142 (2.2%), 4714.5172 (2.2%), 4713.5273 (1.7%), 4711.5072 (1.3%), 4713.5054 (1.2%), 4708.5065 (1.2%), 4708.5042 (1.1%), 4712.5021 (1.1%)

Elemental Analysis: C, 54.05; H, 8.43; N, 2.38; Na, 0.49; O, 31.93; S, 2.72



MS-MALDI (DHBA as matrix) of **S3PEG**. A set of peaks around 4391 reflect the polydispersity of the PEG₄₅ chains.

B. Titration Data

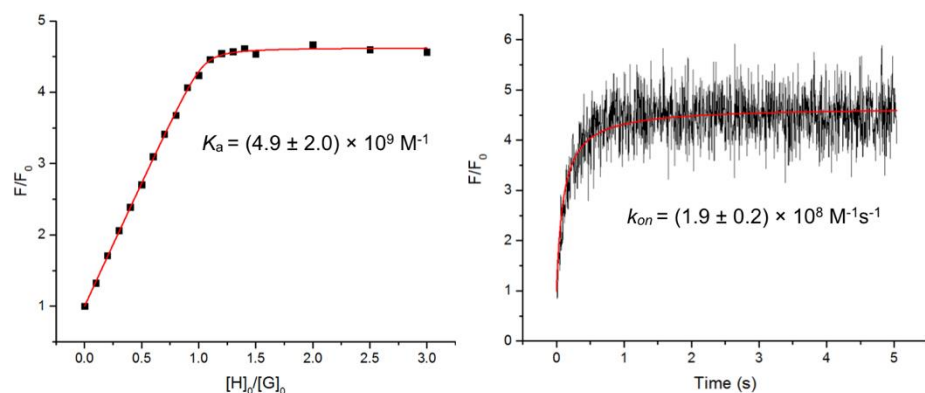


Figure S1. (*left*) Fluorescence (ex: 630 nm, em: 700 nm, slit 3 nm) titration isotherm for incremental addition of **M2** to a solution of **S1PEG** (250 nM) in water. (*right*) Threading kinetic profile generated by mixing equal molar concentration (50 nM each) of **S1PEG** and **M2** in a stopped flow device (ex: 630 nm, em: 700 nm, slit 3 nm). The red lines are computer fits of experimental data to 1:1 binding model or second order kinetic model, respectively.

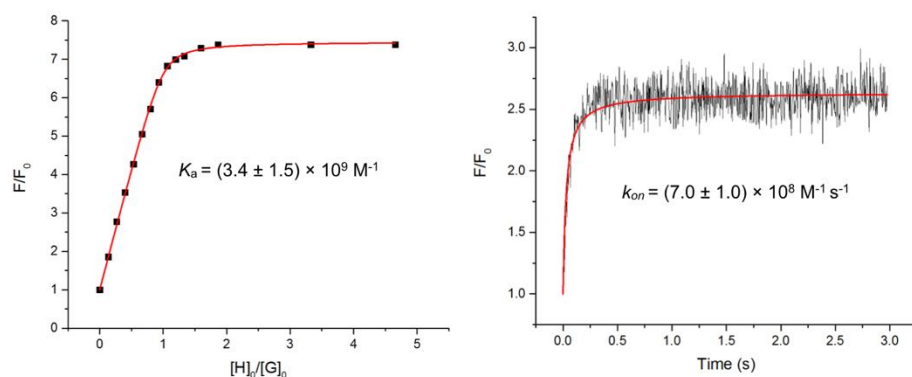


Figure S2. (*left*) Fluorescence (ex: 723 nm, em: 753 nm, slit 5 nm) titration isotherm for incremental addition of **M2** to a solution of **S2PEG** (250 nM) in water. (*right*) Threading kinetic profile generated by mixing equal molar concentration (50 nM each) of **S2PEG** and **M2** in a stopped flow device (ex: 723 nm, em: 753 nm, slit 3 nm). The red lines are computer fits of experimental data to 1:1 binding model or second order kinetic model, respectively.

C. Photostability Data

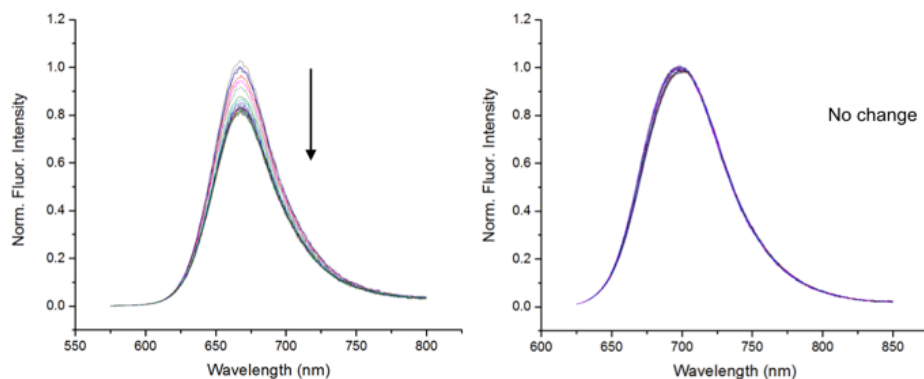


Figure S3. Photostability of free **S1PEG** (5 μM, *left*) and **M2 S1PEG** (5 μM, *right*) in H₂O with continuous irradiation at 550 nm over 15 h (fluorescence spectrum was collected every 30 min).

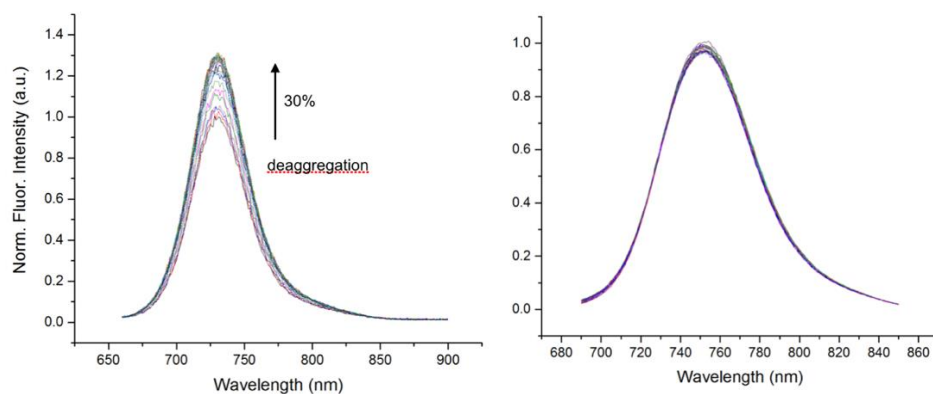


Figure S4. Photostability test of free **S2PEG** (2 μM, *left*) and **M2 S3PEG** (2 μM, *right*) in H₂O with continuous irradiation at 650 nm over 15 h (fluorescence spectrum was collected every 30 min). Note: slight increase in fluorescence over time for free **S2PEG** is attributed to slow deaggregation.

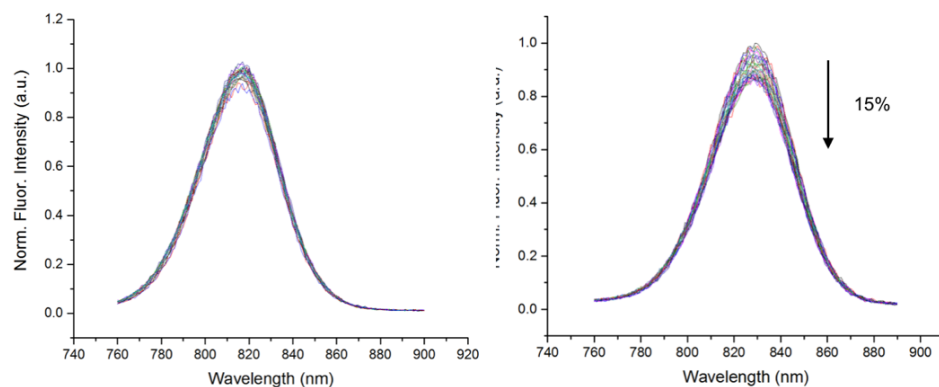


Figure S5. Photo stability test of the **S3PEG** (2 μM, *left*) and **M2 S3PEG** (2 μM, *right*) in H₂O with continuous irradiation at 750 nm over 15 h (fluorescence spectrum was collected every 30 min).

D. Chemical Stability Data

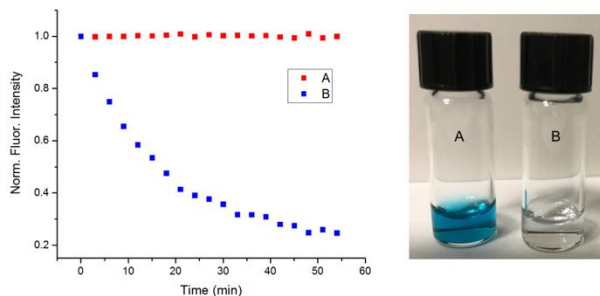


Figure S6. Chemical stability test of **S1PEG** and **M2** \supset **S1PEG**. (left) Change in fluorescent maxima band for solutions of (A) **M2** \supset **S1PEG** (5.0 μ M) or (B) free **S1PEG** (5 μ M), in the presence of excess nucleophile Na_2S (5 mM) in water at 20°C. (right) Photograph of samples containing, (A) **M2** \supset **S1PEG** (80 μ M) or (B) free **S1PEG** (80 μ M), after sitting in the presence of excess nucleophile Na_2S (100 mM) in water at 20 °C.

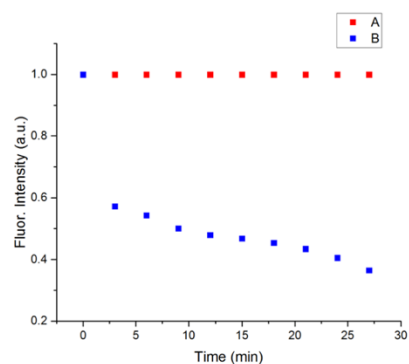


Figure S7. Chemical stability test of **S2PEG** and **M2** \supset **S2PEG** by monitoring the change in fluorescent maxima band for solutions of (A) **M2** \supset **S2PEG** (5.0 μ M) or (B) free **S2PEG** (5 μ M) over time in the presence of excess nucleophile Na_2S (5 mM) in water at 20 °C.

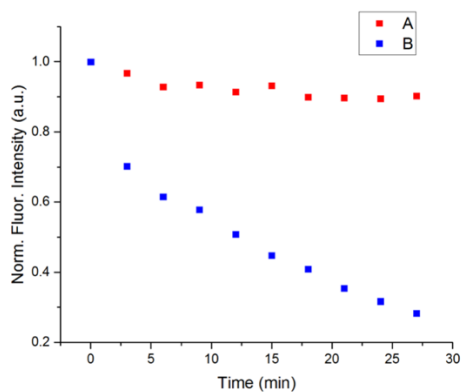


Figure S8. Chemical stability test of **S3PEG** and **M2** \supset **S3PEG** by monitoring change in fluorescent maxima band for solutions of (A) **M2** \supset **S3PEG** (1.0 μ M) or (B) free **S3PEG** (1 μ M), in the presence of excess nucleophile Na_2S (1 mM) in water at 20 °C.

E. Fluorescence Quantum Yields

Table S1. Integrated fluorescent and quantum yield of **ICG**, **S3PEG**, and **M2** \supset **S3PEG** in H₂O.

| | ICG | S3PEG | M2 \supset S3PEG |
|-------------------------|------------------------------|------------------------------|----------------------------------|
| Integrated fluorescence | 2.2 \times 10 ⁶ | 2.9 \times 10 ⁶ | 4.4 \times 10 ⁶ |
| Quantum yield % | 5.3 | 7.0 | 10.6 |

Table S2. Photophysical properties of squaraines and their complexes with **M1** in CHCl₃.

| | S1 | M1 \supset S1 | S2 | M1 \supset S2 | S3 | M1 \supset S3 |
|--|-----------|-------------------------------|-----------|-------------------------------|-----------|-------------------------------|
| Abs (nm) | 627 | 650 | 692 | 717 | 767 | 783 |
| Em (nm) | 651 | 685 | 715 | 749 | 792 | 820 |
| log ϵ (M ⁻¹ cm ⁻¹) | 5.39 | 4.68 | 5.48 | 4.84 | 5.59 | 5.02 |