Supporting Information

Water-soluble AIE-active polyvalent glycocluster: design, synthesis and the studies on carbohydrate-lectin interactions for visualization of Siglecs distributions in living cell membranes

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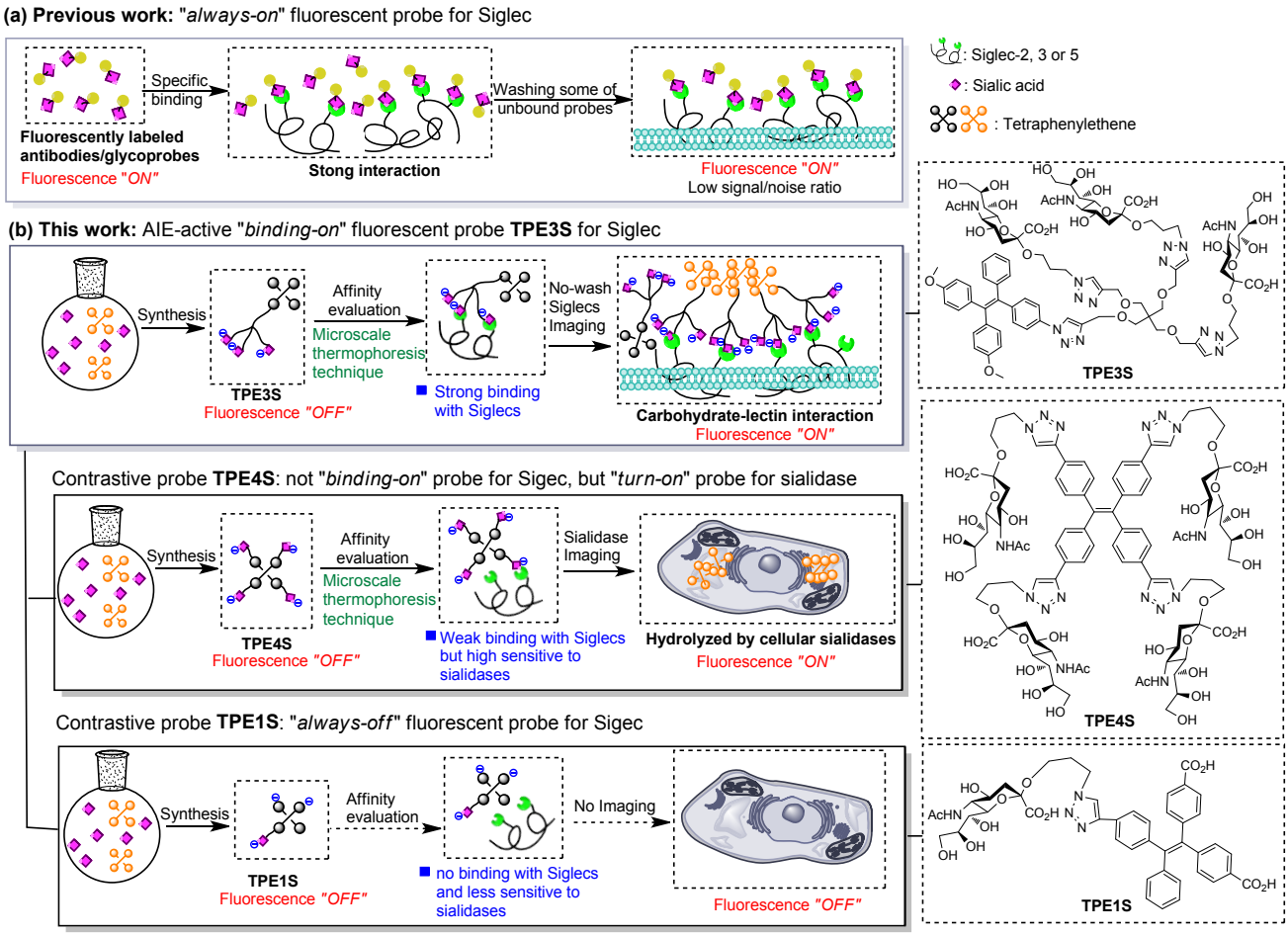
Experimental section



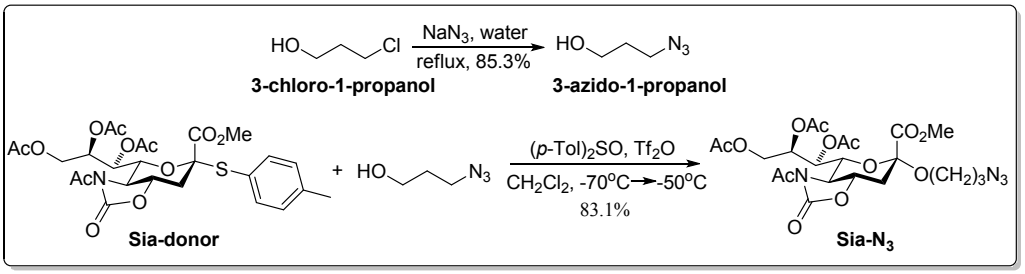
Scheme S1 (a) Schematic of common previous antibodies or glycoprobes for Siglecs imaging. (b) Schematic of tetraphenylethene-decorated sialic acid derivative TPE3S, TPE4S and TPE1S structure, measuring and imaging.

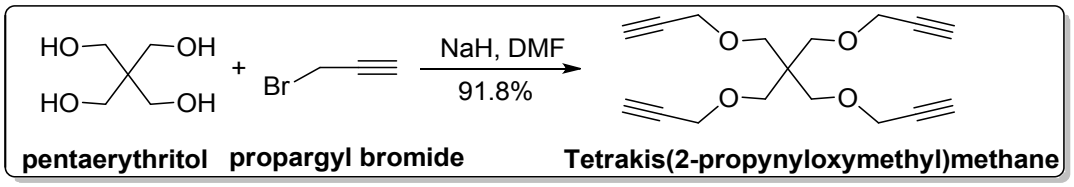
1. Materials and Instruments.

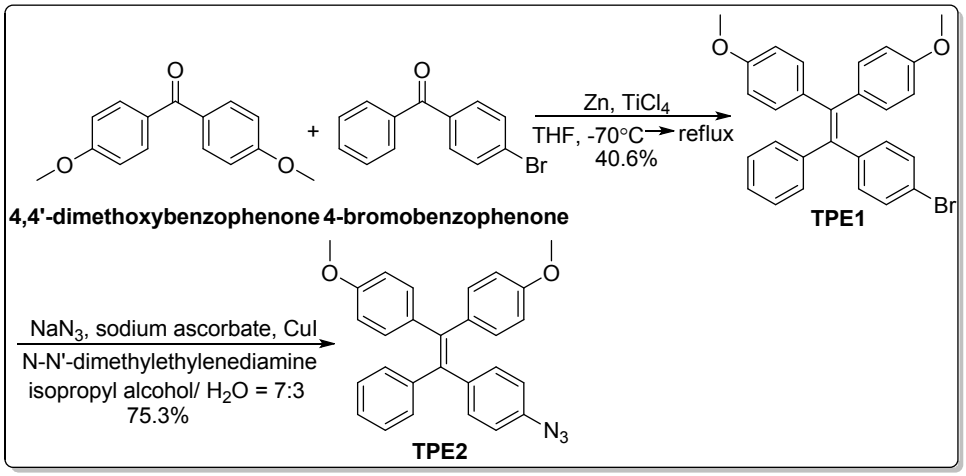
All chemicals and solvents purchased from commercial sources were of analytical grade or better and used without further purification unless otherwise noted below. Sialidase from *Clostridium perfringens* (type V, Sigma product number: N2876, 15.3 U/mg solid) was purchased from Sigma. Siglec-2, Siglec-3 and Siglec-5 were purchased from Sino Biological Inc. Chemical reactions were monitored by analytical thin-layer chromatography (TLC) on silica gel F254 glass plates and revealed by UV light (254 nm or 365 nm) or iodine or heating after dipping in EtOH-H_2SO_4(4%) . Flash column chromatography was performed on silica gel (200-300 mesh) or BioGel P-2 fine resins (Bio-Rad, Hercules, CA).

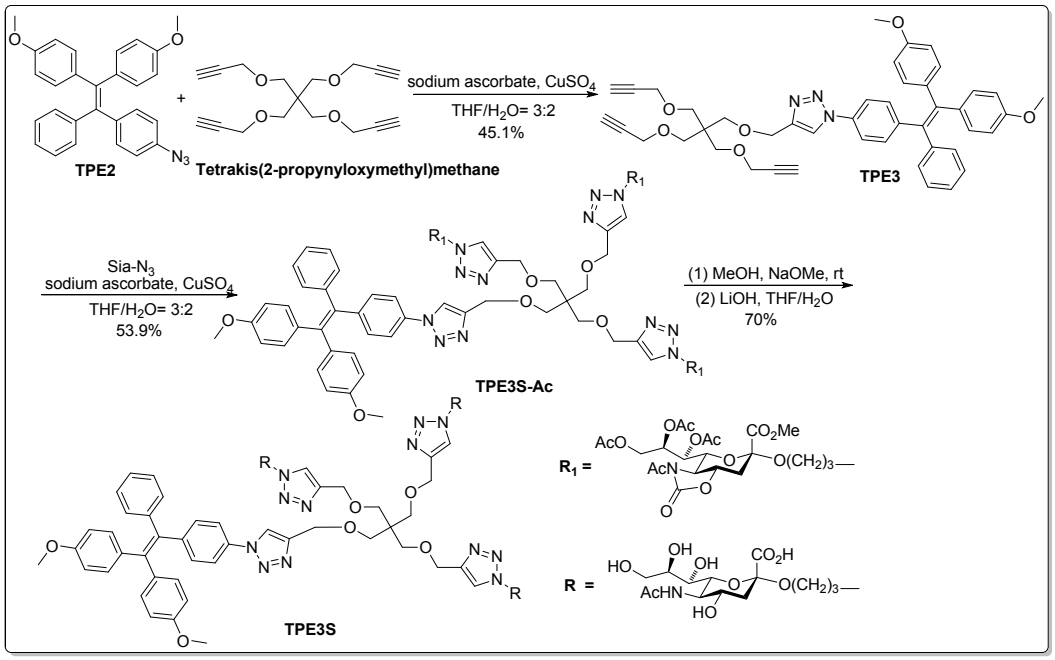
Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded at room temperature with a Bruker Avanced III 400 MHz spectrometer or JEOL's NMR (400 or 600 MHz) spectrometer in the indicated solvent. Chemical shifts (δ) were reported in units per million (ppm) and coupling constants (J) in Hz. High-resolution electrospray ionization mass spectra (HRMS-ESI) were obtained on a Waters LCT Premier XEmass spectrometer. ESI TOF Mass spectra were measured with Waters LCT PremierTM XE. Fluorescence emission spectra were recorded on a FS5 spectrometer (Edinburgh Instruments) with samples contained in quartz cells. MST assays were carried out on Monolith NT.115 instrument purchased from NanoTemper. Sialidase assays in this article were conducted according to our previous literature.[1]

2. Synthesis of TPE3S









Scheme S2 Synthetic routes to probe **TPE3S**.

3-azido-1-propanol. The synthesis was conducted according to the previous literature.[1]

Sia-donor. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 7.9 Hz, 2H), 5.54 (dd, *J* = 5.9, 1.3 Hz, 1H), 5.35 (td, *J* = 6.4, 2.8 Hz, 1H), 4.44 (dd, *J* = 12.2, 2.8 Hz, 1H), 4.37 – 4.28 (m, 1H), 4.21 (dd, *J* = 12.2, 6.7 Hz, 1H), 3.95 (td, *J* = 12.6, 3.6 Hz, 1H), 3.63 (s, 3H), 3.61 – 3.54 (m, 1H), 3.08 (dd, *J* = 12.1, 3.6 Hz, 1H), 2.46 (s, 3H), 2.37 (s, 3H), 2.17 (s, 3H), 2.13 – 2.05 (m, 7H).

Sia-N_3. This compound was synthesized according to the previously published procedure.[2] **Sia-donor** (136.4 mg, 0.235 mmol), (*p*-Tol)$_2$SO (108.1 mg, 0.469 mmol), activated 3Å powdered sieves and dry CH_2Cl_2 (8 mL) were added to a flame-dried glass vessel. This mixture was cooled to -70°C, and trifluoromethanesulfonic anhydride (47 uL) was added via syringe. After stirred for 30 min, a solution of 3-azido-1-propanol (35.6 mg, 0.352 mmol) in dry CH_2Cl_2 (2 mL) was added at -70°C under argon atmosphere. The reaction mixture was stirred at -70°C for 2h and then continually stirred at -50°C for additional 2 h. Finally, the reaction mixture was quenched with Et_3N (0.2 mL), diluted with CH_2Cl_2, filtered through Celite, washed with saturated brine and dried over anhydrous $MgSO_4$. The dried extract was then filtrated and concentrated using rotary evaporation under reduced pressure. The residue was purified by silica gel column chromatography eluting with petroleum/EtOAc to give 108.8 mg (83.1%, α/β = 8.5:1, The α/β ratios were determined by ^{1}H NMR analysis.) of **Sia-N_3** (R_f = 0.46, petroleum : EtOAc = 1:1). (α-isomer) ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.55 (d, *J* = 8.1 Hz, 1H), 5.40 (dd, *J* = 9.8, 4.7 Hz, 1H), 4.60 (d, *J* = 9.2 Hz, 1H), 4.34 (dd, *J* = 12.3, 2.2 Hz, 1H), 4.06 – 3.93 (m, 2H), 3.84 – 3.74 (m, 4H), 3.67 (t, *J* = 10.2 Hz, 1H), 3.39 – 3.34 (m, 3H), 2.81 (dd, *J* = 12.0, 3.1 Hz, 1H), 2.44 (s, 3H), 2.11 – 2.04 (m, 7H), 1.99 (s, 3H), 1.86 – 1.75 (m, 2H).

Tetrakis(2-propynyloxymethyl)methane. Sodium hydride (120 mmol, 60% w/w in mineral oil) was added to the round-bottom flask containing a solution of pentaerythritol (1.3615 g, 10 mmol) in *N*,*N*-dimethylformamide (DMF, 35 mL) and the mixture was stirred at 0°C for 30 min. Propargyl bromide (6 mL) was slowly added into the above solution over a 30 min-period and the solution was heated to 40°C. After 2.5 h, an additional 3 mL of propargyl bromide was added to the mixture. The reaction was stirred at 50°C for 16 h. Water was added to quench the reaction after cooling and the mixture was extracted with ethyl acetate. The organic phase was dried over anhydrous $NaSO_4$ and the solvent was evaporated in vacuo. Then the raw product was applied to silica gel column chromatography to afford the desired compound tetrakis(2-propynyloxymethyl)methane (2.747 g, 91.8%, R_f = 0.71, petroleum : EtOAc = 3:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (d, *J* = 2.3 Hz, 8H, 4-CH_2-), 3.53 (s, 8H, 4-CH_2-), 2.40 (t, *J* = 2.4 Hz, 4H, 4 —C≡CH).

1-(4-Bromophenyl)-2,2-bis(4-methoxyphenyl)-1-phenylethene (TPE1). The synthesis of **TPE1** was conducted according to the previously published literature[3]. ^{1}H NMR (400 MHz) δ 7.24 – 7.17 (m, 2H), 7.14 – 7.06 (m, 3H), 7.04 – 6.98 (m, 2H), 6.97 – 6.84 (m, 6H), 6.74 – 6.57 (m, 4H), 3.75 (d, *J* = 11.0 Hz, 6H). ^{13}C NMR (101 MHz) δ 158.40, 158.32, 143.93, 143.44, 140.91, 138.03, 136.15, 136.07, 133.18, 132.69, 132.66, 131.48, 130.99, 127.95, 126.43, 120.15, 113.33, 113.15, 55.26, 55.23. MS (MALDI-TOF): calcd for $C_{28}H_{24}BrO_2$ $[M+ H]^+$ 471.10, found 470.41

1-(4-Azidophenyl)-2,2-bis(4-methoxyphenyl)-1-phenylethene (TPE2): Compound **TPE1** (528 mg, 1.12 mmol), sodium azide (291 mg, 4.48 mmol), sodium ascorbate (33.3 mg, 0.168 mmol), copper(I) iodide (64 mg, 0.336 mmol) and *N*,*N*'-dimethylethylenediamine (44.4 mg, 0.5 mmol) were dissolved in argon degassed isopropyl alcohol-water (7:3, 40 mL) solution. The mixture was further degassed with argon for 5 minutes and afterwards refluxed for 7 h under preclusion of light. Then the solution was allowed to cool down to room temperature, diluted with water and extracted with ethyl acetate twice. The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated via rotovap. The obtained crude product was purified by column chromatography to give the product **TPE2** (365.6 mg, 75.3%, R_f = 0.44, petroleum : dichloromethane = 7:3). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (d, *J* = 6.9 Hz, 3H), 7.06 – 6.86 (m, 8H), 6.78 (d, *J* = 8.3 Hz, 2H), 6.66 (dd, *J* = 13.8, 8.6 Hz, 4H), 3.75 (d, *J* = 10.0 Hz, 6H). ^{13}C NMR (101 MHz) δ 158.30, 158.23, 144.13, 141.35, 140.56, 138.25, 137.63, 136.27, 136.25, 132.91, 132.69, 132.67, 131.47, 127.90, 126.37, 118.49, 113.28, 113.12, 55.22, 55.19.

2,2,2-tri((prop-2-ynyloxy)methyl)propyl-1-phenylethene (TPE3). A solution of sodium ascorbate (36.6 mg) in water (4 mL) and a solution of $CuSO_4 \cdot 5H_2O$ (23.0 mg) in water (4 mL) were added to a solution of **TPE2** (200 mg, 0.461 mmol) and tetrakis(2-propynyloxymethyl)methane (1.33 g, 4.61 mmol) in THF (12 mL) under an Ar atmosphere. After stirring at 70°C for 1.5 h, the mixture was cooled to room temperature. It was then extracted with ethyl acetate and the organic layer was dried

over anhydrous sodium sulfate. After filtration, the solvent was removed under reduced pressure and the residue was purified by flash column chromatography to obtain the coupling product **TPE3** (150.2 mg, 45.1%, R_f = 0.41, petroleum : ethyl acetate = 7:3). ^{1}H NMR (600 MHz) δ 7.95 (s, 1H), 7.49 (d, *J* = 8.6 Hz, 2H), 7.18 – 7.08 (m, 5H), 7.03 (d, *J* = 7.9 Hz, 2H), 7.00 – 6.89 (m, 4H), 6.65 (dd, *J* = 12.9, 8.8 Hz, 4H), 4.71 (s, 2H), 4.12 – 4.09 (m, 6H), 3.74 (d, *J* = 3.0 Hz, 6H), 3.54 (s, 2H), 3.53 (s, 6H), 2.42 – 2.32 (m, 3H). ^{13}C NMR (151 MHz) δ 158.50, 158.42, 146.47, 145.22, 143.80, 141.53, 137.78, 135.96, 135.94, 134.93, 132.73, 132.66, 131.47, 128.03, 126.57, 120.37, 119.90, 113.41, 113.19, 80.14, 74.31, 69.38, 69.09, 65.32, 58.84, 55.22, 45.07. HRMS calcd for $C_{45}H_{43}N_3O_6Na$ $[M + Na]^+$: 744.3044, found: 744.3048.

TPE3S-Ac. Sia-N_3 (134.6 mg, 0.241 mmol) and TPE3 (43.5 mg, 0.06 mmol) was added in a reaction vessel. After the system was evacuated and refilled with argon three times, THF (6 mL) was injected to the mixture. Then the stock solutions of sodium ascorbate (9.5 mg) and $CuSO_4 \cdot 5H_2O$ (6.0mg) were added twice subsequently within 5 hours. The THF/H_2O ratio was 3:2 (v/v). After stirred at 60°C for 36 h, the resultant mixture was diluted with saturated NH_4Cl solution and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered and concentrated, then the crude product was purified by a silica gel column. The product TPE3S-Ac was obtained in 53.9% yield (77.8 mg). ^{1}H NMR (600 MHz) δ 7.97 (s, 1H), 7.64 (s, 3H), 7.49 (d, J = 8.0 Hz, 2H), 7.16 (d, J = 8.1 Hz, 2H), 7.14 – 7.07 (m, 3H), 7.02 (d, J = 7.5 Hz, 2H), 6.94 (dd, J = 19.0, 8.2 Hz, 4H), 6.64 (dd, J = 12.0, 8.5 Hz, 4H), 5.58 (d, J = 8.3 Hz, 3H), 5.47 – 5.26 (m, 3H), 4.65 – 4.34 (m, 20H), 4.13 – 3.96 (m, 6H), 3.91 – 3.83 (m, 3H), 3.78 (s, 9H), 3.73 (s, 9H), 3.55 – 3.42 (m, 8H), 3.40 – 3.30 (m, 3H), 2.83 (dd, J = 12.0, 3.1 Hz, 3H), 2.47 (s, 9H), 2.20 – 2.04 (m, 27H), 2.00 (s, 9H). ^{13}C NMR (101 MHz) δ 172.12, 170.82, 170.29, 170.15, 168.80, 158.51, 158.42, 153.74, 145.92, 145.25, 143.83, 141.56, 137.72, 135.93, 134.80, 132.77, 132.73, 132.65, 131.46, 130.19, 128.02, 126.54, 122.97, 120.78, 119.78, 113.40, 113.18, 99.17, 75.63, 74.96, 71.82, 69.34, 68.89, 65.14, 63.34, 62.45, 59.13, 55.22, 53.21, 47.34, 45.49, 36.61, 30.54, 29.80, 24.79, 21.24, 21.06, 20.89. HRMS calcd for $C_{111}H_{134}N_{15}O_{45}$ $[M + H]^+$: 2397.8686, found: 2397.9267.

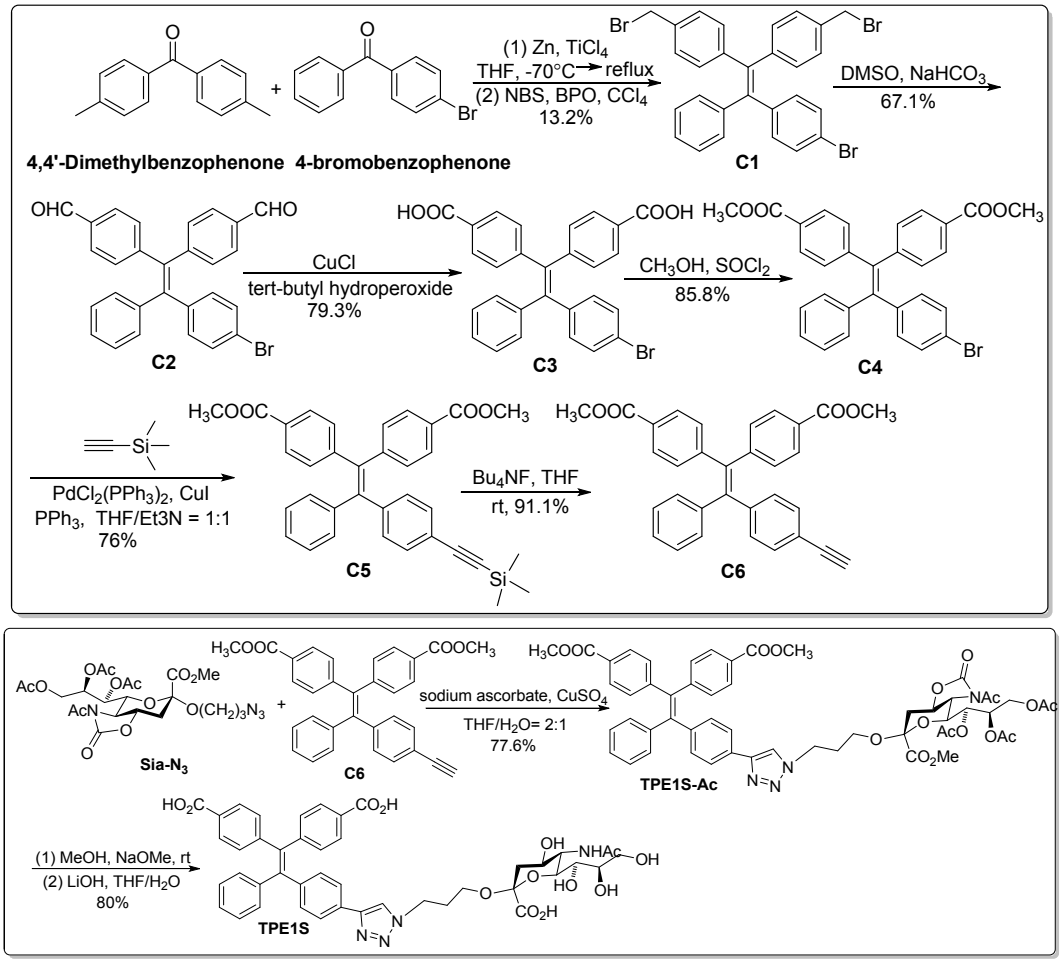
TPE3S. Compound TPE3S-Ac (77.8 mg, 0.0325 mmol) was dissolved in methanol (3 mL). Sodium methoxide in methanol (5.4 M, 0.1 mL) was added slowly and the reaction mixture was stirred at room temperature for 2 h. Neutralization with Amberlite IR-120 (H^+ form) followed by filtration and evaporation of the solvents afforded the partially deprotected compound. Then a mixture of THF/H_2O solution (v/v = 2:1, 3 mL) and $LiOH \cdot H_2O$ (26 mg) were successively added to the reaction flask and the mixture was stirred at room temperature overnight. After neutralization using Dowex-H^+ ion-exchange resin, filtration and evaporation of solvents, the final product was purified using size exclusion chromatography (eluent system: 50 mM ammonium formate aqueous solution). Product-containing fractions were collected, concentrated and freeze-dried to afford the probe TPE3S (43.1 mg, 70%). ^{1}H

NMR (600 MHz) δ 7.85 (s, 4H), 7.19 (s, 2H), 7.00 – 6.23 (m, 15H), 4.50 – 4.20 (m, 13H), 4.09 – 2.81 (m, 48H), 2.68 (s, 3H), 2.15 – 1.85 (m, 15H), 1.61 (s, 3H). HRMS (ESI) calcd for $C_{87}H_{116}N_{15}O_{33}$ $[M+H]^+$: 1898.7854, found: 1898.7951.

3. Synthesis of TPE4S

TPE4S was synthesized according to the previously published procedures.[1]

4. Synthesis of TPE1S



Scheme S3 Synthetic routes to probe **TPE1S**.

C1. In analogy to literature, 4-bromobenzophenone (1.43 g, 5.48 mmol), 4,4'-dimethylbenzophenone (772 mg, 3.67 mmol) and Zn power (1.82 g, 27.8 mmol) were added to a pre-dried two necked round-bottom flask with a magnetic stirrer and a water condenser. The flask was evacuated and flushed with argon repeatedly. After anhydrous THF (35 mL) was injected into the flask, titanium tetrachloride ($TiCl_4$, 1.5 mL) was subsequently added at -50°C and the mixture was then stirred at room temperature for 30 min. Afterwards, the dark reaction mixture was placed in a 70°C oil bath to stir for 15 h. Then the reaction

was quenched with a 10% K_2CO_3 aqueous solution (10 mL). After filtration, the filtrate was diluted with ethyl acetate and NaCl (aq.). The organic layer was separated and the aqueous phase was further extracted with ethyl acetate twice. The combined organic layers were dried over magnesium sulfate, filtrate through paper and finally evaporated to dryness. The resulting residue was roughly purified by chromatography on a silica gel column. Subsequently, the obtained solid (1 g), *N*-bromosuccinimide (NBS, 931 mg, 5.23 mmol) and benzoyl peroxide (BPO, 20 mg) were added into a 100-mL round-bottom flask fitted with a condenser. The flask was evacuated and flushed with argon repeatedly. Then CCl_4 (25 mL) was added and the mixture was stirred for 15 h at 70°C to complete the reaction. Then the formed solid was removed by filtration and the filtrate was concentrated by a rotary evaporator. The crude product was purified by a silica gel column chromatography using petroleum ether / dichloromethane mixture as eluent. White solid of **C1** was obtained (809.4 mg). ^{1}H NMR (600 MHz) δ 7.24 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 8.0 Hz, 2H), 7.15 – 7.07 (m, 5H), 7.05 – 6.93 (m, 6H), 6.88 (d, J = 8.4 Hz, 2H), 4.42 (d, J = 16.7 Hz, 4H). ^{13}C NMR (151 MHz) δ 143.46, 143.34, 142.94, 142.40, 140.91, 140.25, 136.39, 136.22, 133.02, 131.72, 131.35, 131.11, 128.84, 128.65, 128.03, 127.09, 120.93, 33.54, 33.48. MS (MALDI-TOF): calibrated for $C_{28}H_{22}Br_3$ $[M+ H]^+$ 596.92, found 597.38.

C2. The procedure was modified according to the previous literature. To a DMSO solution of compound **C1** (500 mg, 0.842 mmol) was added $NaHCO_3$ (282.9 mg, 3.367 mmol). After stirring the reaction mixture at 90°C for 4 h, it was cooled to room temperature and diluted with saturated brine solution. The aqueous layer was extracted with ethyl acetate three times and the combined organic layers were dried over anhydrous sodium sulfate. After removing the solvent by a rotary evaporator, the crude product was purified by silica gel column chromatography using petroleum/ethyl acetate mixture as the eluent to yield **C2** (263.2 mg, 67.1%, R_f = 0.71, petroleum : EtOAc = 3:1). ^{1}H NMR (600 MHz) δ 9.95 (s, 1H), 9.92 (s, 1H), 7.68 (d, J = 8.3 Hz, 2H), 7.64 (d, J = 8.3 Hz, 2H), 7.29 – 7.25 (m, 2H), 7.21 – 7.12 (m, 7H), 7.00 (dd, J = 8.1, 1.4 Hz, 2H), 6.89 (d, J = 8.5 Hz, 2H). ^{13}C NMR (151 MHz) δ 191.83, 149.34, 149.23, 143.83, 141.99, 141.45, 139.19, 134.94, 134.83, 132.95, 131.98, 131.42, 131.31, 129.70, 129.54, 128.32, 127.90, 121.86. MS (MALDI-TOF): calibrated for $C_{28}H_{20}BrO_2$ $[M+ H]^+$ 469.06, found 469.45

C3. Compound **C2** (150 mg, 0.321 mmol) was reacted with tert-butyl hydroperoxide (70% aqueous solution, 260 uL) and CuCl (19 mg, 0.192 mmol) in CH_3CN (5 mL) at room temperature for 20 h. After completion of the reaction, the resulting mixture was diluted with saturated $NaHSO_3$ (aq.), extracted using ethyl acetate and the organic layer was dried over anhydrous sodium sulfate. After removing solvent by a rotary evaporator, the crude product was chromatographed on a silica-gel column using petroleum/ethyl acetate mixture (containing 1‰ acetic acid) as eluent. After recrystallization and dried in vacuum, the compound **C3** (127.1 mg, R_f = 0.46, petroleum : EtOAc : acetic acid = 3:7: 0.02) was obtained in 79.3% yield. HRMS (ESI) calcd for $C_{28}H_{18}BrO_4$ $[M - H]^-$: 497.0383, found: 497.0387.

C4. To a suspension of **C3** (450 mg, 0.9 mmol) in methanol (5.4 mL) was slowly added thionyl chloride (1 mL) at 0°C. After stirring at 55°C for ca. 15 h, the solvent was removed under reduced pressure. Then EtOAc was added to the flask and the mixture was washed with brine, sat. $NaHCO_3$ (aq.) and brine successively. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The compound **C4** (407.8 mg, R_f = 0.71, petroleum : EtOAc = 7:3) was obtained in 85.8% chemical yield after column chromatography purification. ^{1}H NMR (600 MHz) δ 7.80 (dd, *J* = 23.6, 8.4 Hz, 4H), 7.24 (d, *J* = 8.4 Hz, 2H), 7.15 – 7.09 (m, 3H), 7.06 (dd, *J* = 13.6, 8.4 Hz, 4H), 6.98 (dd, *J* = 8.0, 1.5 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 2H), 3.88 (d, *J* = 13.0 Hz, 6H). ^{13}C NMR (151 MHz) δ 166.96, 166.92, 147.86, 147.77, 142.71, 142.31, 141.77, 139.63, 132.97, 131.35, 131.30, 129.50, 129.32, 128.67, 128.52, 128.20, 127.58, 121.50, 52.26, 52.22. HRMS (ESI), calcd for $C_{30}H_{24}BrO_4$ $[M + H]^+$: 527.0852, found: 527.0855.

C5: To a mixture of **C4** (342.8 mg, 0.65 mmol), copper(I) iodide (9.9 mg, 0.052 mmol), $PdCl_2(PPh_3)_2$ (18.2 mg, 0.026 mmol) and PPh_3 (20.4 mg, 0.078 mmol) in degassed THF-Et_3N (1:1, 8 mL) was added TMS-acetylene (0.17 mL, 1.23 mmol). The resulting solution was stirred at 50°C for 24 h under preclusion of light and then cooled to room temperature. After the addition of diethyl ether, the mixture was filtered and the filtrate was concentrated via rotovap. Final purification was achieved by column chromatography to give the product **C5** (269 mg, 76%).

C6: To a solution of **C5** (108.9 mg, 0.2 mmol) in THF (1.5 mL) was added tetrabutylammonium fluoride (TBAF, 0.4 mmol) slowly. After 10 hours at room temperature, the resulting solution was quenched by addition of saturated aq. NH_4Cl and extracted twice with $CHCl_3$. The organic extract was dried with $MgSO_4$ and the solvents were evaporated. The crude product was purified by column chromatography to give **C6** (86 mg, 91.1%). ^{1}H NMR (600 MHz) δ 7.79 (dd, *J* = 13.5, 8.3 Hz, 4H), 7.24 (d, *J* = 8.2 Hz, 2H), 7.14 – 7.10 (m, 3H), 7.07 (dd, *J* = 8.1, 7.0 Hz, 4H), 7.00 – 6.94 (m, 4H), 3.87 (d, *J* = 8.9 Hz, 6H), 3.06 (s, 1H). ^{13}C NMR (151 MHz) δ 166.91, 166.87, 147.85, 147.75, 143.45, 143.12, 142.32, 139.78, 131.82, 131.34, 131.30, 129.42, 129.29, 128.64, 128.50, 128.16, 127.52, 120.89, 83.57, 77.93, 52.20, 52.18. HRMS (ESI), calcd for $C_{32}H_{25}O_4$ $[M + H]^+$: 473.1747, found: 473.1740.

TPE1S-Ac. Sia-N_3 (185 mg, 0.331 mmol) and **C6** (313.2 mg, 0.662 mmol) were dissolved in THF (6 mL) under argon atmosphere. Then the freshly prepared aqueous solutions of sodium ascorbate (13.1 mg) and $CuSO_4 \cdot 5H_2O$ (8.3 mg) were added subsequently under vigorous stirring. 3 hours later, another addition of sodium ascorbate (13.1 mg) and $CuSO_4 \cdot 5H_2O$ (8.3 mg) was conducted. After stirring at 60°C for 36 h under Ar atmosphere, the reaction mixture was diluted with aqueous solution of NH_4Cl and extracted with dichloromethane twice. The combined organic phases were dried over anhydrous sodium

sulfate. After filtration and solvent evaporation, the residue was purified on a silica-gel column to give title compound **TPE1S-Ac** (264.9 mg, 77.6%, R_f = 0.23, petroleum : EtOAc = 2:3).

TPE1S. The peracetylated compound **TPE1S-Ac** (177 mg, 0.172 mmol) was dissolved in methanol (4.5 mL), and NaOMe (5.4 M in methanol, 0.15 mL) was added. The reaction mixture was left stirring for 3 h at room temperature and neutralized using Dowex-H^+ ion-exchange resin. After filtration, the solvent was removed under reduced pressure and the residue was then dissolved in a mixture of THF:water (2 : 1, 4.6 mL). $LiOH \cdot H_2O$ (39 mg) was added to the mixture and the mixture was stirred at room temperature overnight. Then Amberlite IR-120 (H^+ form) was added to neutralize the reaction mixture. After filtration and solvent evaporation, the crude material was purified using size exclusion chromatography (P2). Product-containing fractions were collected, concentrated and freeze-dried to afford the probe **TPE1S** (114.9 mg, 80%).

Microscale thermophoresis measurements (MST).

Firstly, the recombinant proteins, including Siglec-2 protein, Siglec-3 protein and Siglec-5 protein, were purchased as lyophilized powder from Sino Biological Inc.. Then, these proteins were covalently labelled by Monolith NT™ protein labeling kit RED-NHS according to the kit instruction respectively. After further purification of the labelled protein by size-exclusion column chromatography, microscale thermophoresis measurements (MST) were performed using Monolith NT.115 instrument purchased from NanoTemper.

For performing a molecular interaction experiment and measuring the dissociation constant (K_d), a titration series of 16 dilutions is prepared, where the concentration of the labeled protein is kept constant and the concentration of the ligand is varied. After 30 min, 16 binding reactions with different concentrations of ligand were aspirated into Monolith NT™ standard treated capillaries respectively and each time hold the capillary vertically and shake it to position the liquid in center of the Monolith NT™ capillary. Then put the capillaries in the slots on the sample tray, place the sample tray in the instrument and close the instrument door. The MST measurement was then started with the following settings: LED power: 20%; MST power: high; capillary scanning: 16; Fluo. Before: 5s; MST on 5s; Fluo. After: 5 s. After measurement, curve fitting was performed using NT analysis software and the dissociation constant (K_d) could be obtained.

Imaging of Siglecs on living cell membranes.

PC-12 cells and HeLa cells were seeded on glass-bottom culture dishes and cultured in DMEM medium supplemented with 10% (v/v) fetal bovine serum (FBS) at 37°C under 5% CO_2 atmosphere. Before experiment, the cells were pre-cultured until confluence was reached. In fluorescence imaging experiments, PC-12 cells or HeLa cells were incubated with medium containing 50 μM TPE3S or TPE4S for a certain time. Confocal images were obtained using a confocal laser scanning microscopy. For colocalization experiments, after the PC-12 cells were first incubated

with 50 μM TPE3S or TPE4S, the cells were rinsed and incubated in medium containing Lyso-tracker Red or DiD for 30 min. Then, the cells were washed thrice with phosphate buffer solution to remove excess commercial probe and added new culture medium to observe the fluorescence using a confocal laser scanning microscopy.

Supplementary figures

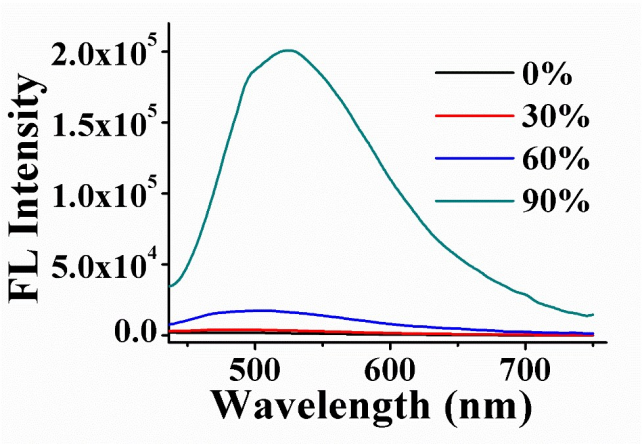


Fig. S1 Fluorescence spectra of 20 μM TPE3S in PBS in the presence of different glycerin contents up to 90%. λ_{ex} = 380 nm.

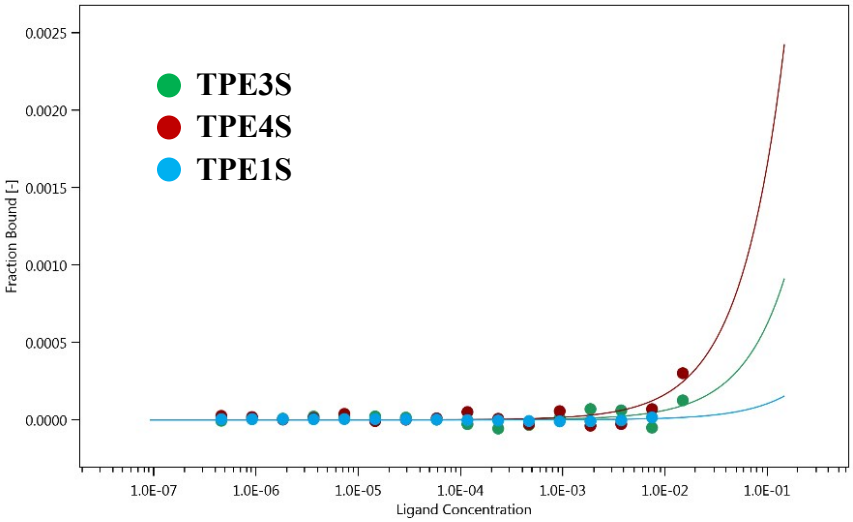


Fig. S2 Thermophoretic analysis of interactions between BSA and ligands in PBS.

Table S1 Comparison between dissociation constants (K_d (mM)) of different complexes formed by different proteins and various ligands.

Ligands	Siglec-2	Siglec-3	Siglec-5	Con A	BSA
TPE3S	0.0142 ± 0.00287	0.0962 ± 0.0137	0.0106 ± 0.00337	--[a]	--
TPE1S	>10	>10	--	--	--
TPE4S	3.17 ± 0.812	1.29 ± 0.221	--	--	--

[a] "—"means no binding

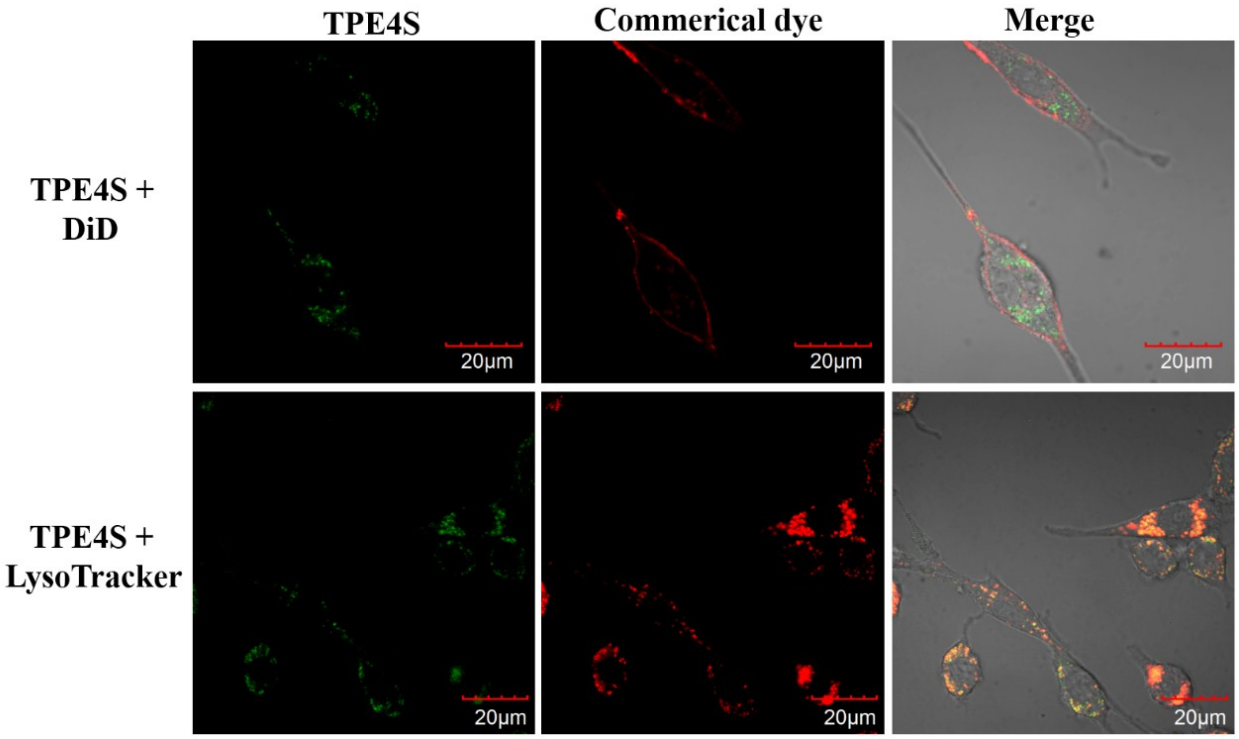


Fig. S3 Fluorescence confocal images of PC-12 cells incubated with TPE4S for 5 h and commercial dyes DiD or LysoTracker (DiD is a commercial available membrane probe, LysoTracker is a commercial available lysosomal probe). Left panel is the confocal image from TPE4S on channel 1 (λ_{ex} = 405 nm, λ_{em} = 440-540 nm); Middle panel is the confocal image from DiD (λ_{ex} = 635 nm, λ_{em} = 650-750 nm) or LysoTracker Red (λ_{ex} = 559 nm, λ_{em} = 570-620 nm) on channel 2. Scale bars = 20 μm.

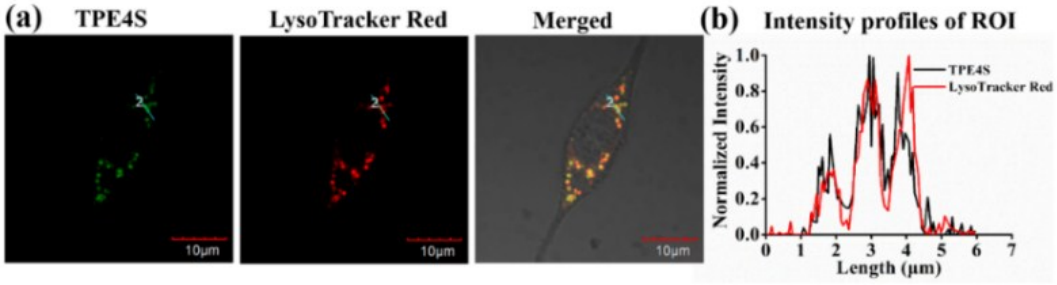


Fig. S4 Colocalization studies with LysoTracker Red and TPE4S for staining PC-12 cells. (a) Confocal images of PC-12 cells stained with TPE4S and LysoTracker Red. The cells were incubated with TPE4S (50 μM) for 5 h, and then the medium was replaced with fresh medium containing LysoTracker Red and incubated for another 30 min. Left panel is the confocal image from TPE4S on channel 1 (λ_{ex} = 405 nm, λ_{em} = 440-540 nm); Middle panel is the confocal image from LysoTracker Red on channel 2 (λ_{ex} = 559 nm, λ_{em} = 570-620 nm). Scale bars = 10 μm. (b) Intensity profiles of region of interest (ROI) cross PC-12 cell in (a).

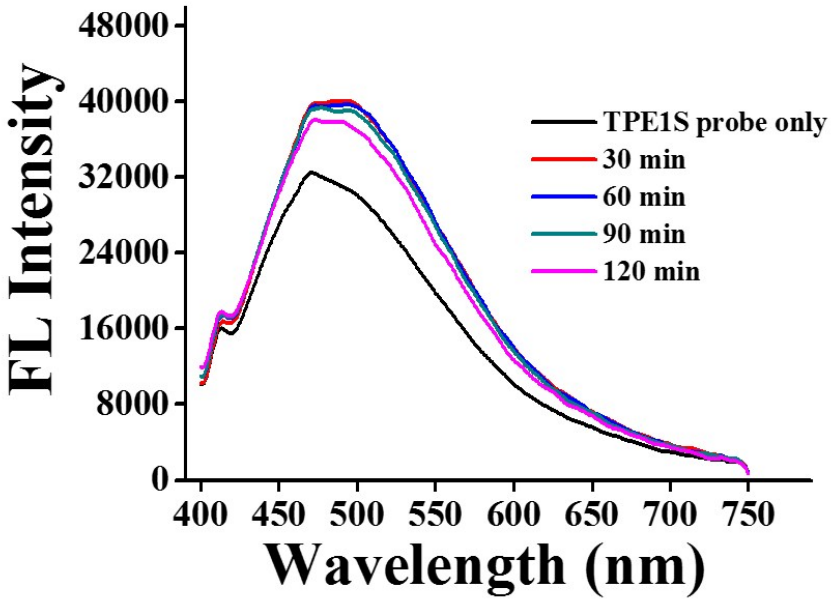


Fig. S5 Fluorescence response of 20 μM TPE1S to 50 mU/mL sialidase from *Clostridium perfringens*. Spectra were acquired before and 30, 60, 90, and 120 min after sialidase was added. λ_{ex} = 360 nm.

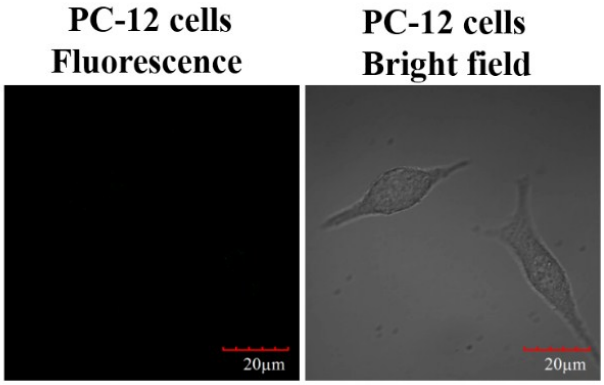


Fig. S6 Fluorescence confocal images of PC-12 cells incubated with TPE1S for 5 h. Left panel is the confocal image from TPE1S on channel 1 (λ_{ex} = 405 nm, λ_{em} = 440-540 nm); Right panel is the bright field. Scale bars = 20 μm.

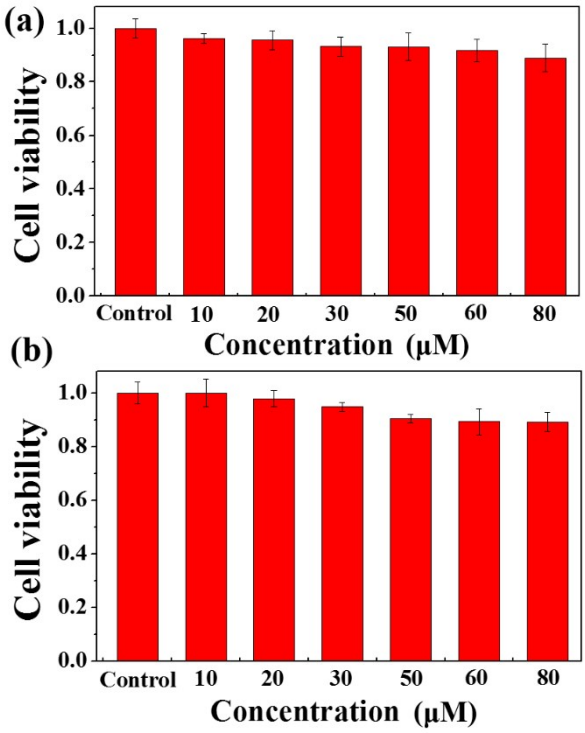


Fig. S7 (a) In vitro cytotoxicity of TPE3S against PC-12 cells at different concentrations after 24 h. (b) In vitro cytotoxicity of TPE4S against PC-12 cells at different concentrations after 24 h.

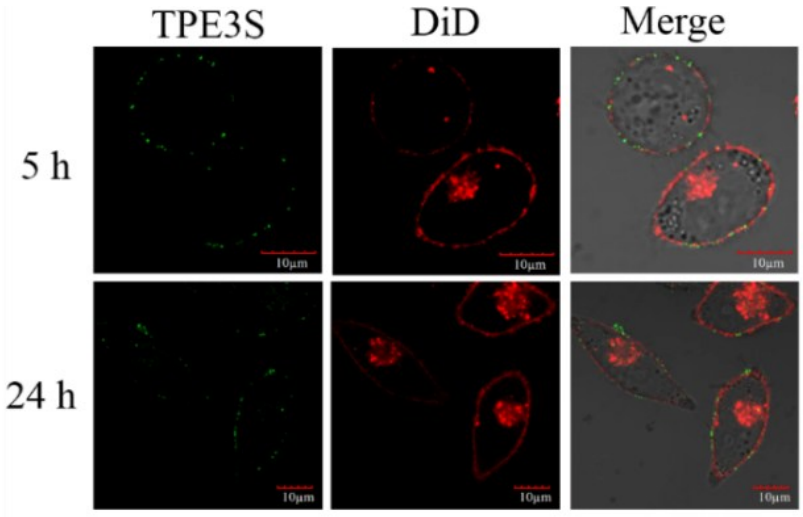


Fig. S8 Fluorescence confocal images of HeLa cells incubated with TPE3S (50 μM) and commercial dye DiD (DiD is a membrane probe). Left panel is the confocal image from TPE3S on channel 1 (λ_{ex} = 405 nm, λ_{em} = 440-540 nm); Middle panel is the confocal image from DiD on channel 2 (λ_{ex} = 635 nm, λ_{em} = 650-750 nm). Scale bars = 10 μm.

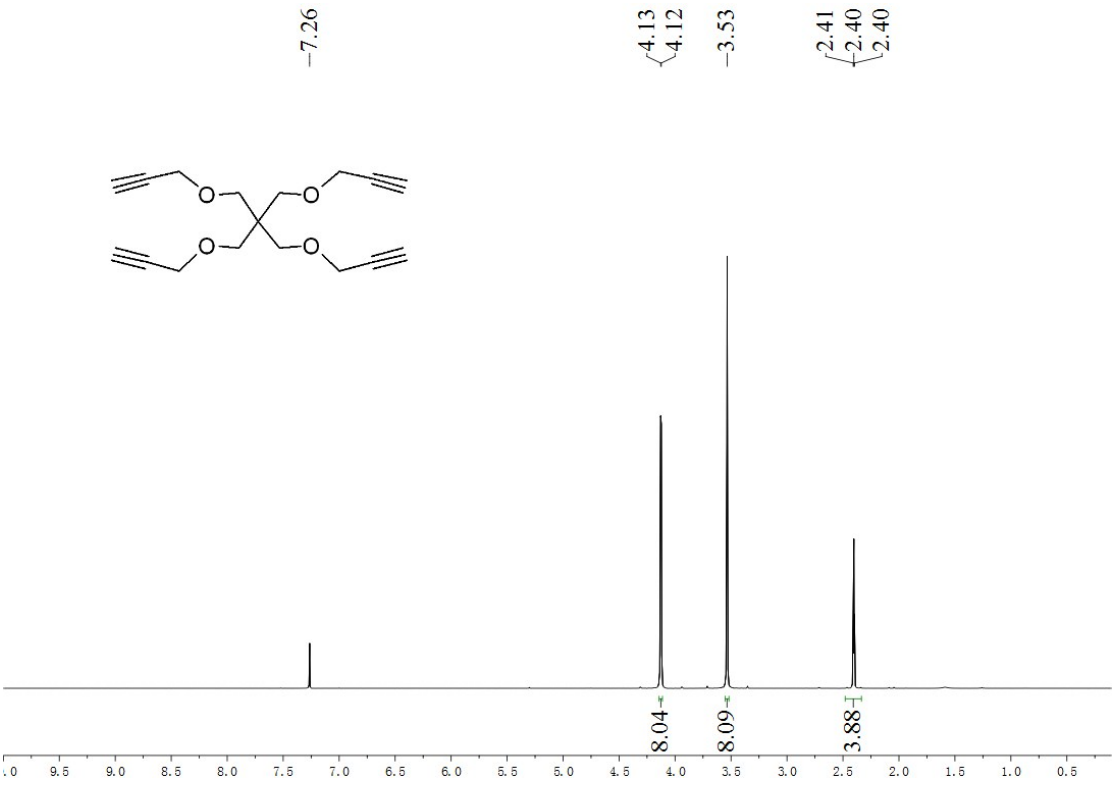


Fig. S9 ^{1}H NMR spectrum of Tetrakis(2-propynyloxymethyl)methane

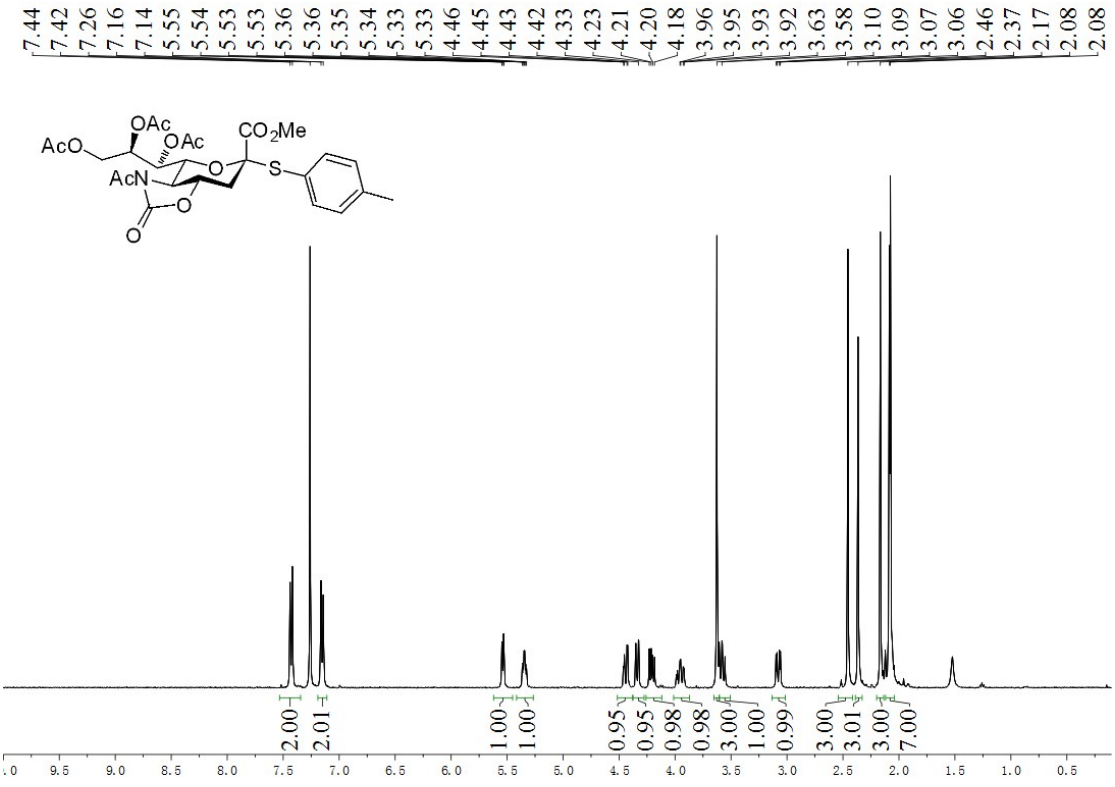


Fig. S10 ^{1}H NMR spectrum of Compound **Sia-donor**

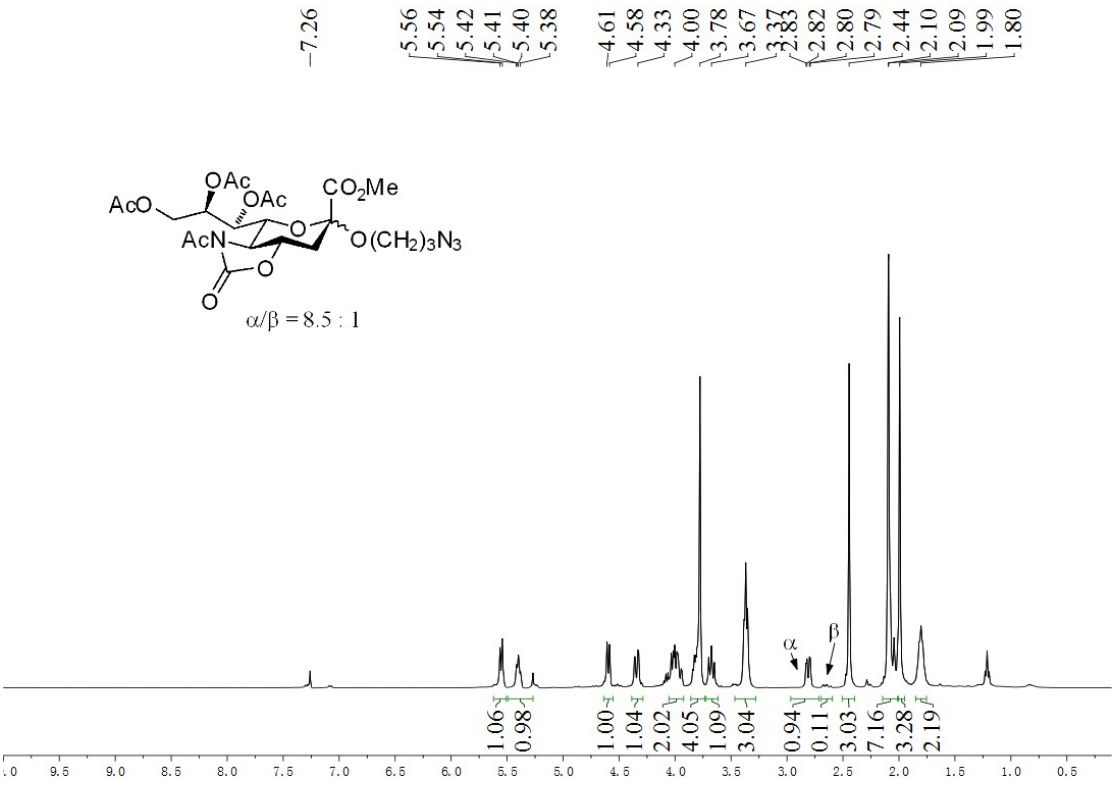


Fig. S11 ^{1}H NMR spectrum of Compound **Sia-N_3**.

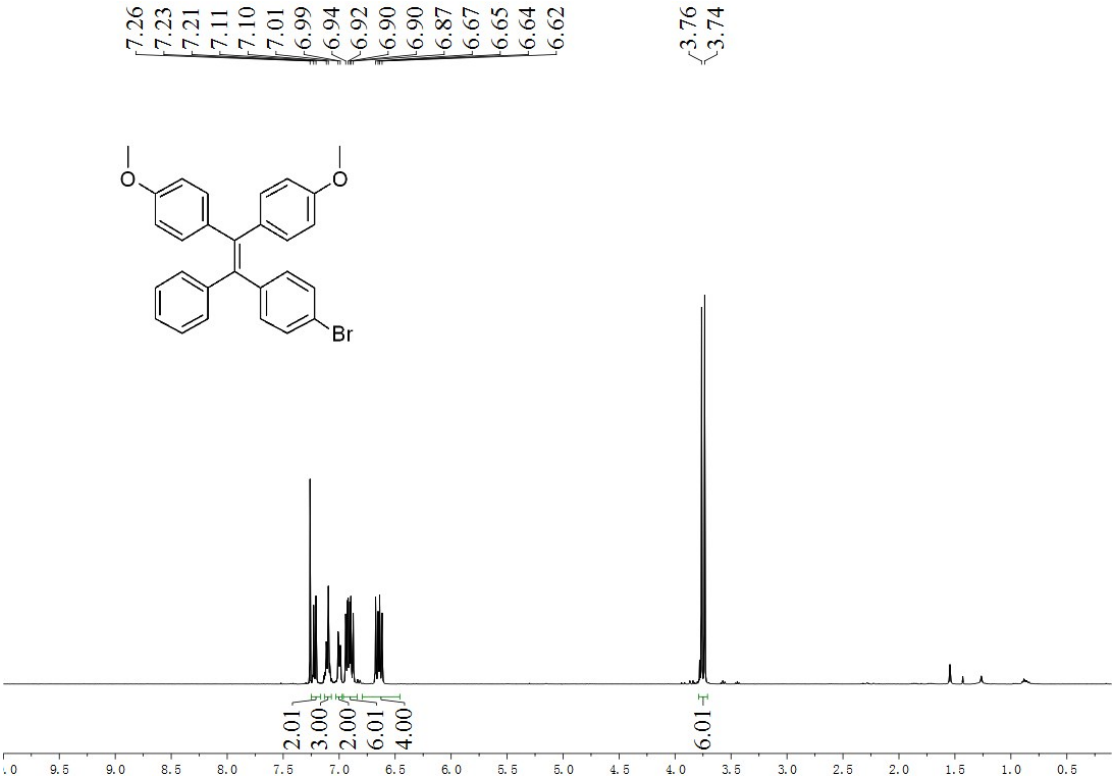


Fig. S12 ^{1}H NMR spectrum of Compound **TPE1.**

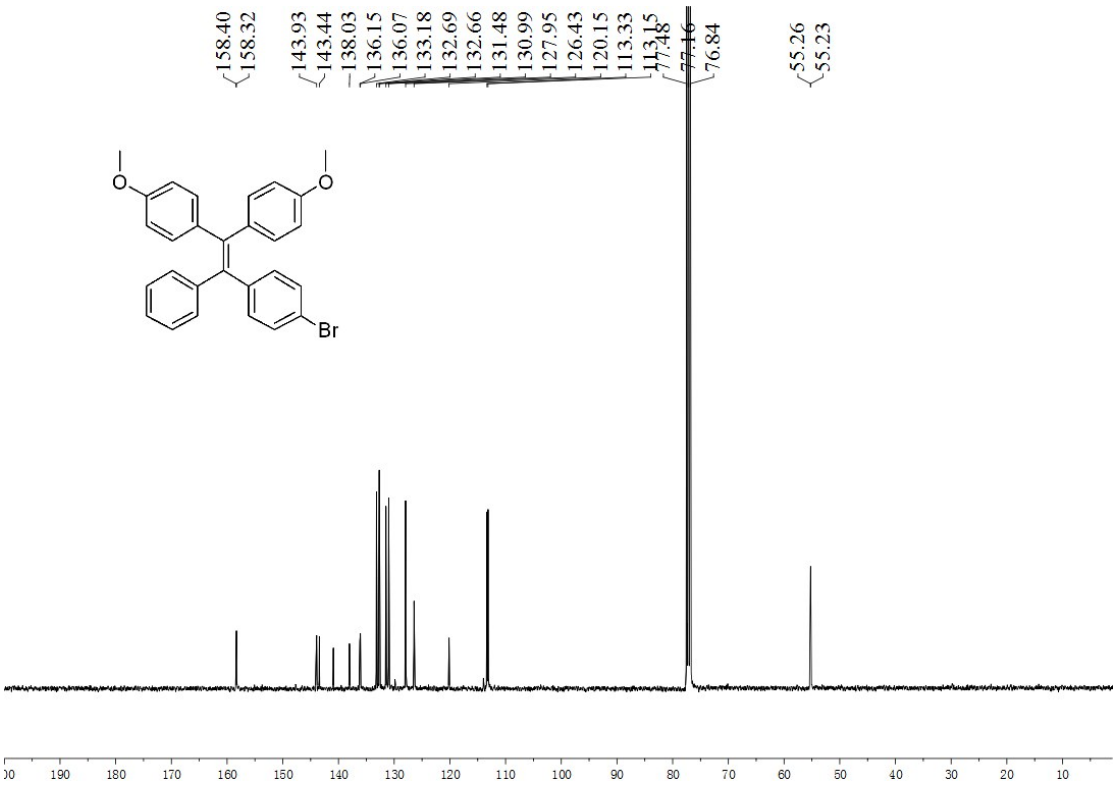


Fig. S13 ^{13}C NMR spectrum of Compound **TPE1.**

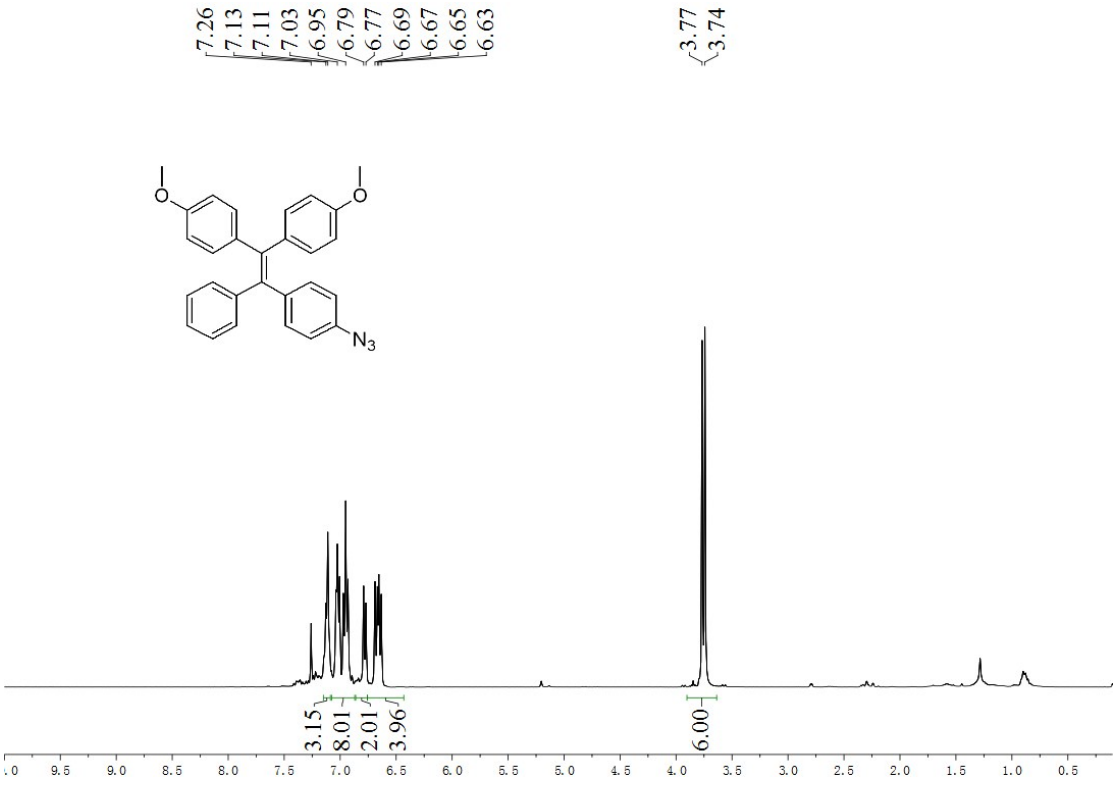


Fig. S14 ^{1}H NMR spectrum of Compound **TPE2.**

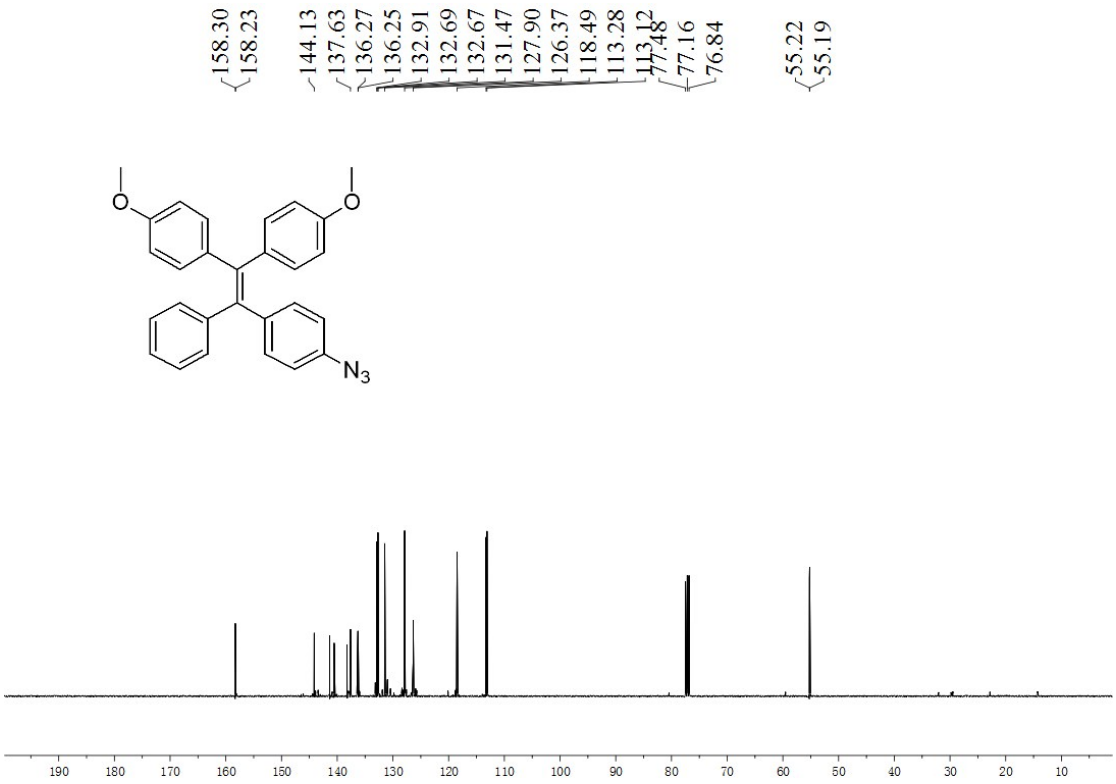


Fig. S15 ^{13}C NMR spectrum of Compound **TPE2.**

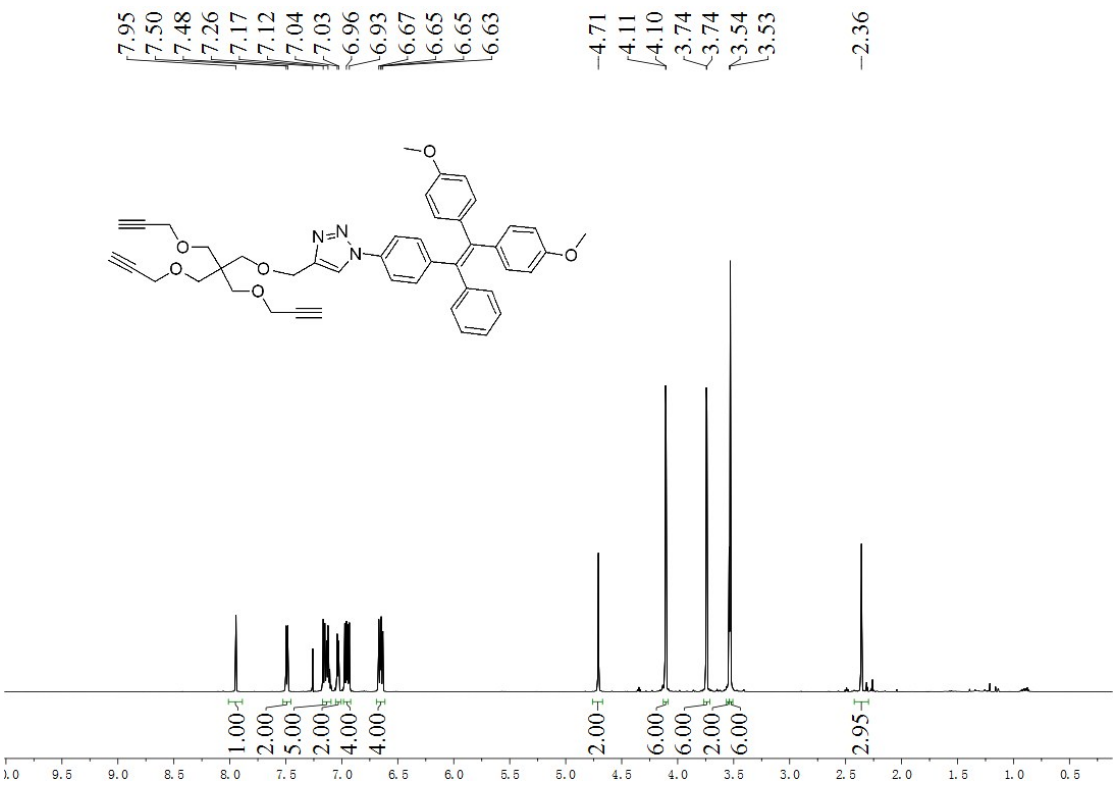


Fig. S16 ^{1}H NMR spectrum of Compound **TPE3.**

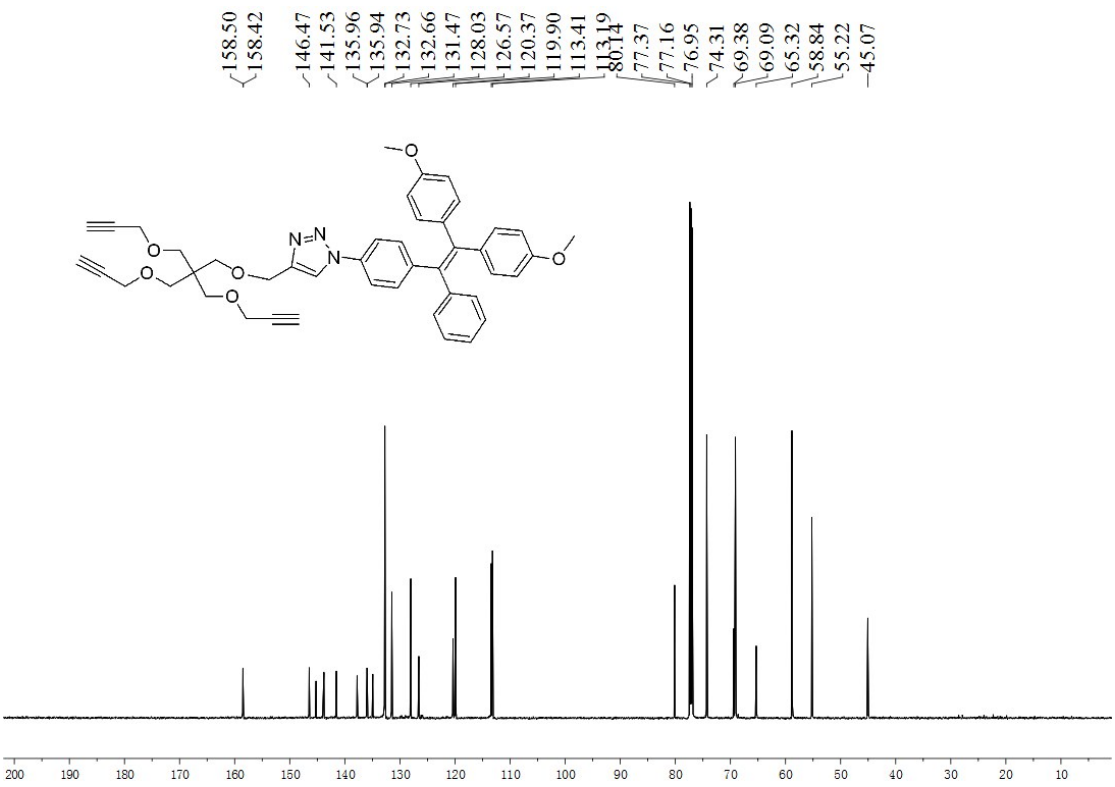


Fig. S17 ^{13}C NMR spectrum of Compound **TPE3.**

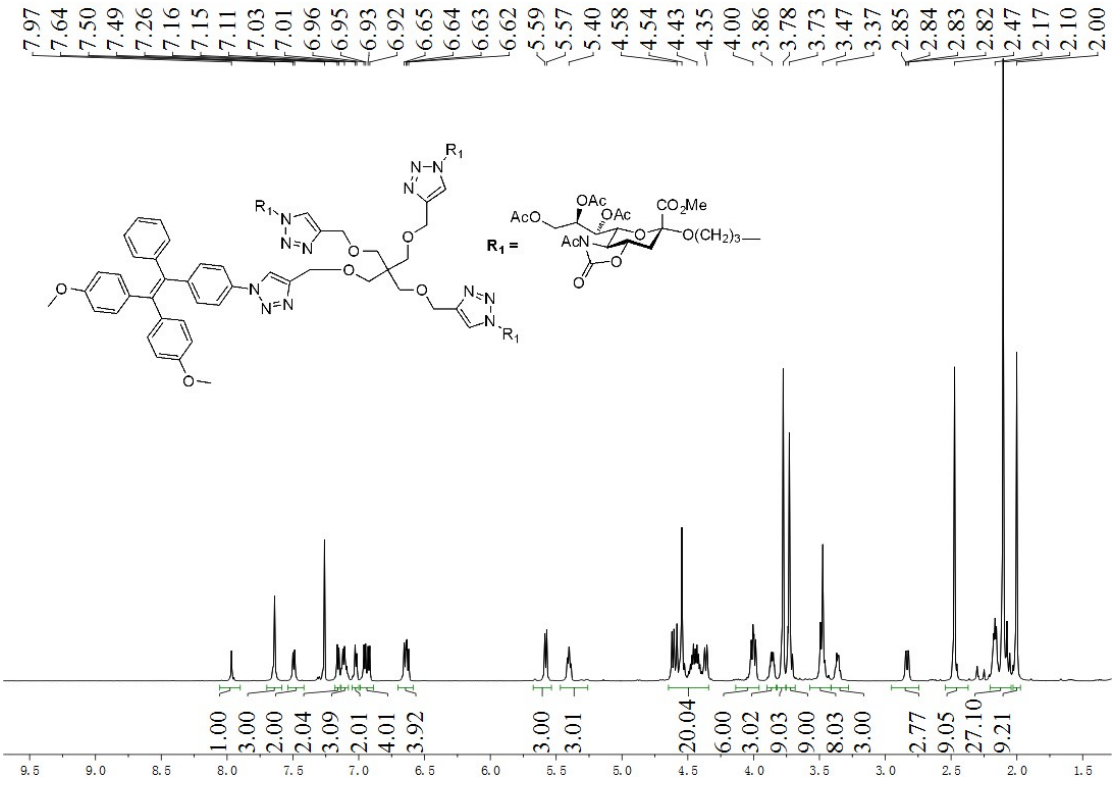


Fig. S18 ^{1}H NMR spectrum of Compound **TPE3S-Ac.**

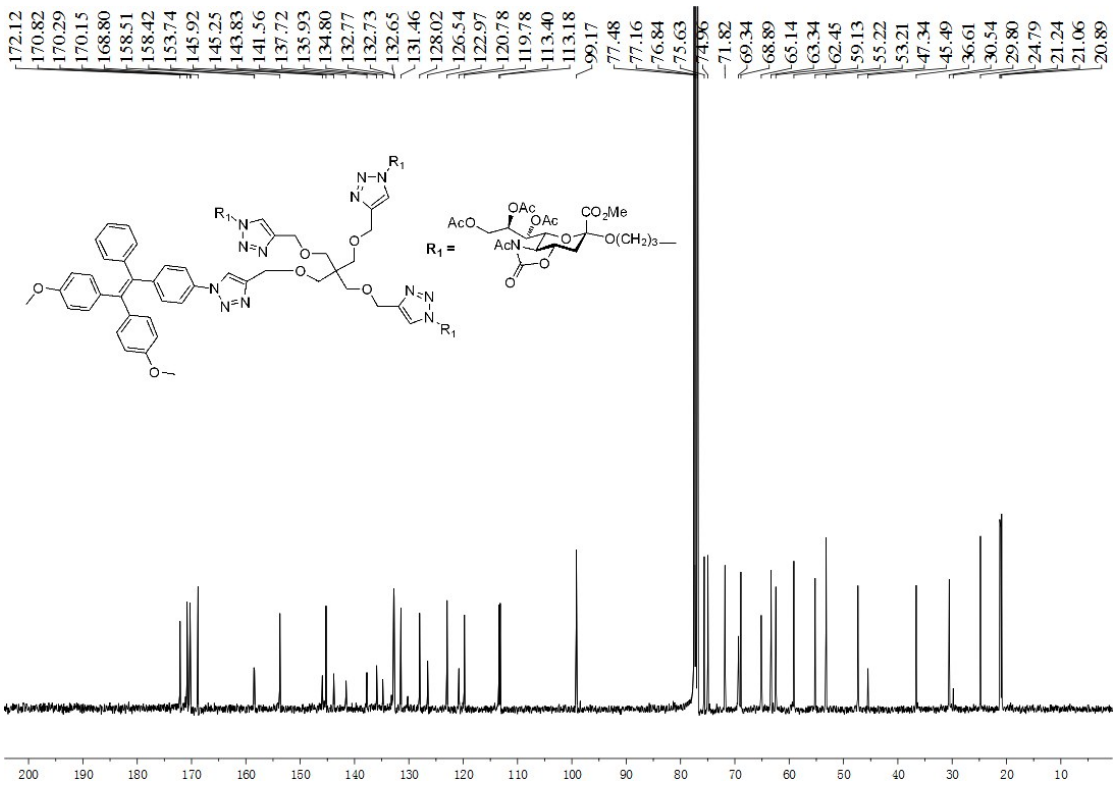


Fig. S19 ^{13}C NMR spectrum of Compound **TPE3S-Ac.**

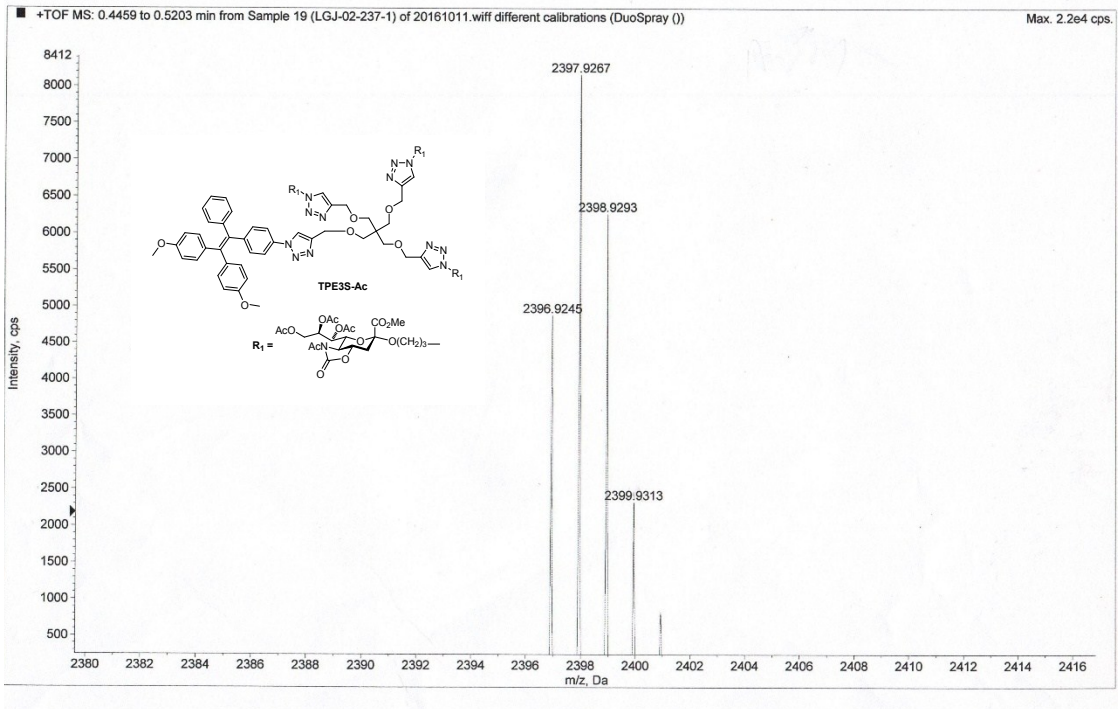
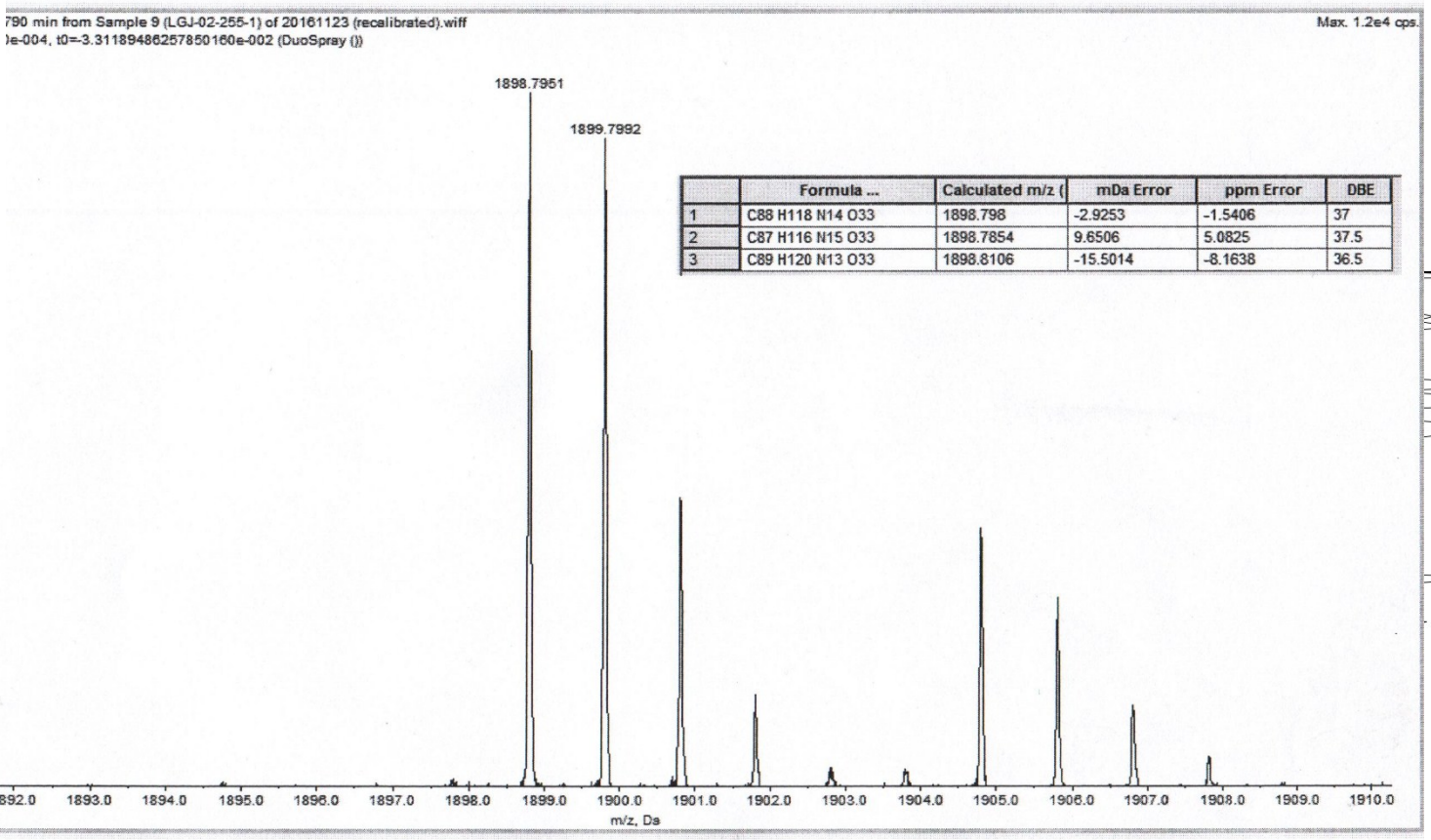


Fig. S20 HRMS spectrum of Compound **TPE3S-Ac.**

Fig. S21 ^{1}H NMR spectrum of Compound



	Formula ...	Calculated m/z (	mDa Error	ppm Error	DBE
1	C88 H118 N14 O33	1898.798	-2.9253	-1.5406	37
2	C87 H116 N15 O33	1898.7854	9.6506	5.0825	37.5
3	C89 H120 N13 O33	1898.8106	-15.5014	-8.1638	36.5

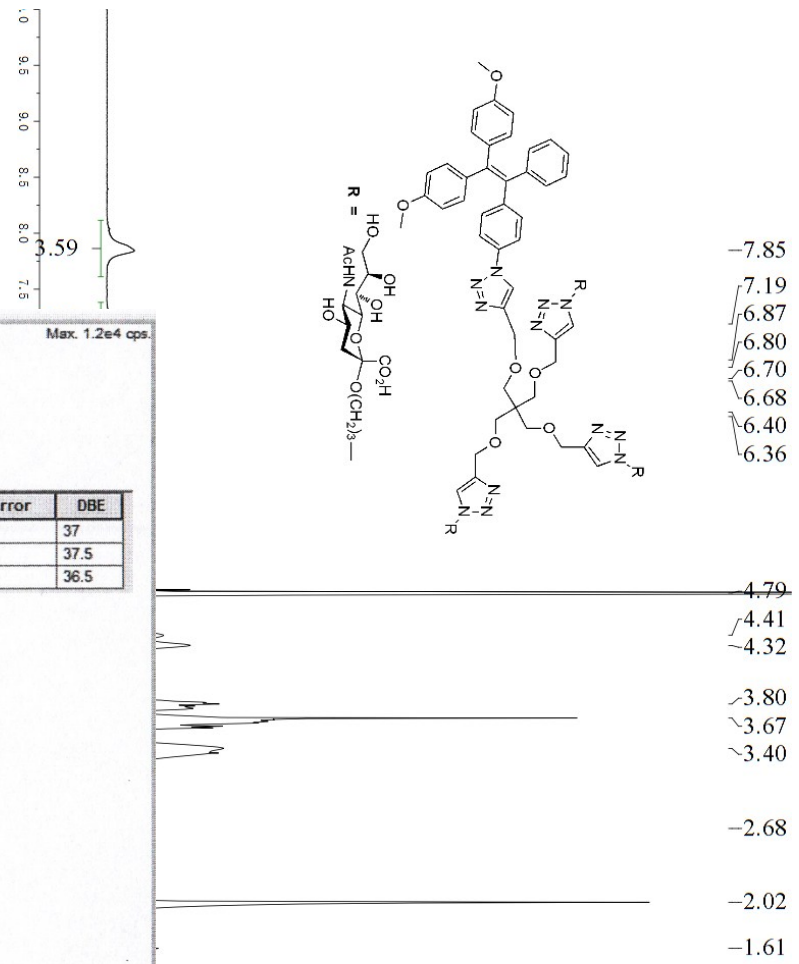


Fig. S22 HRMS spectrum of Compound **TPE3S.**

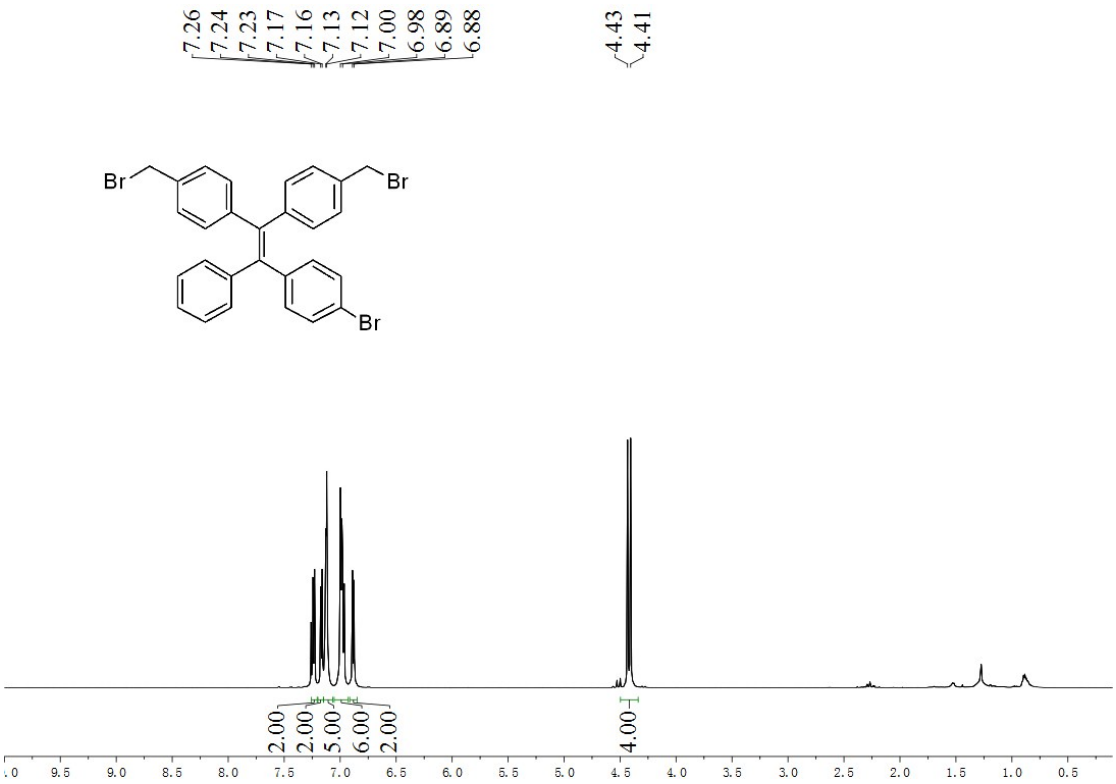


Fig. S23 ^{1}H NMR spectrum of Compound **C1**.

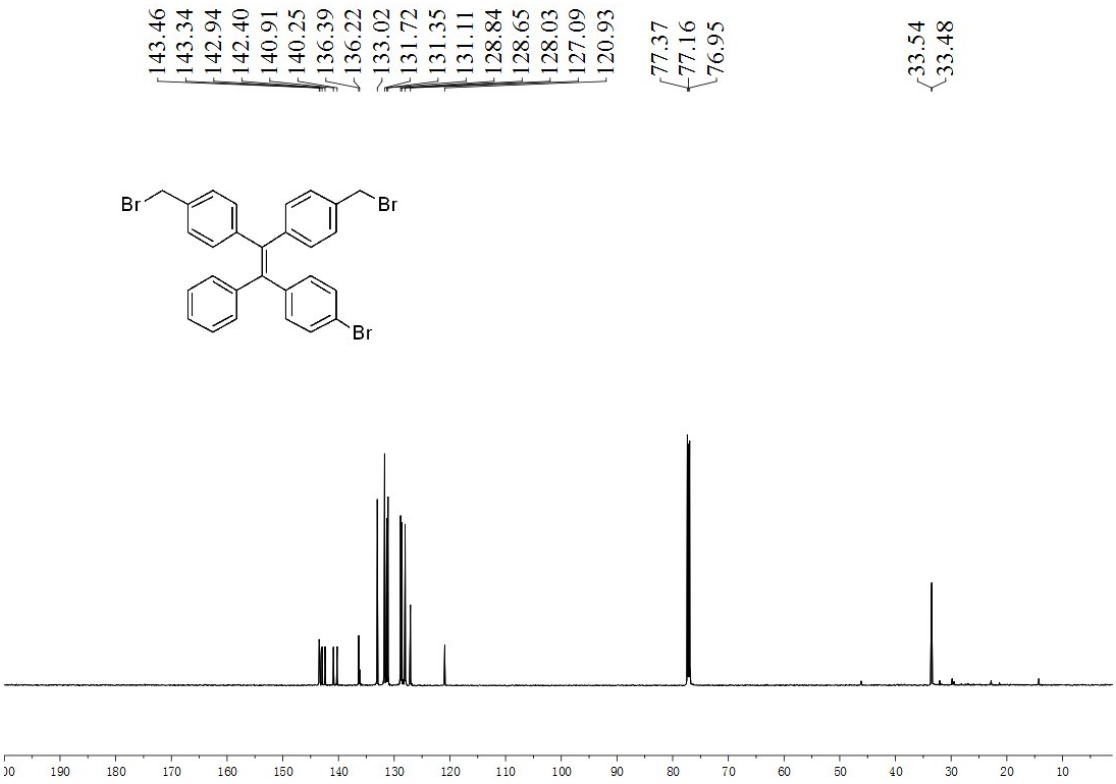


Fig. S24 ^{13}C NMR spectrum of Compound **C1.**

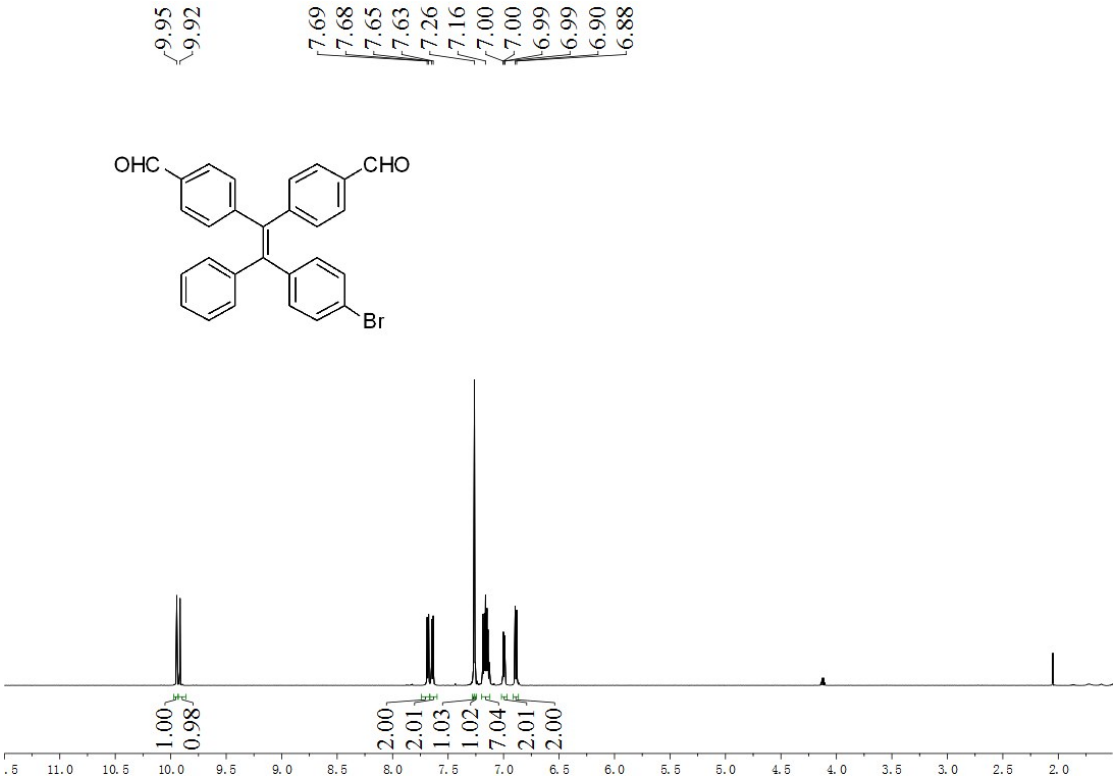


Fig. S25 ^{1}H NMR spectrum of Compound **C2.**

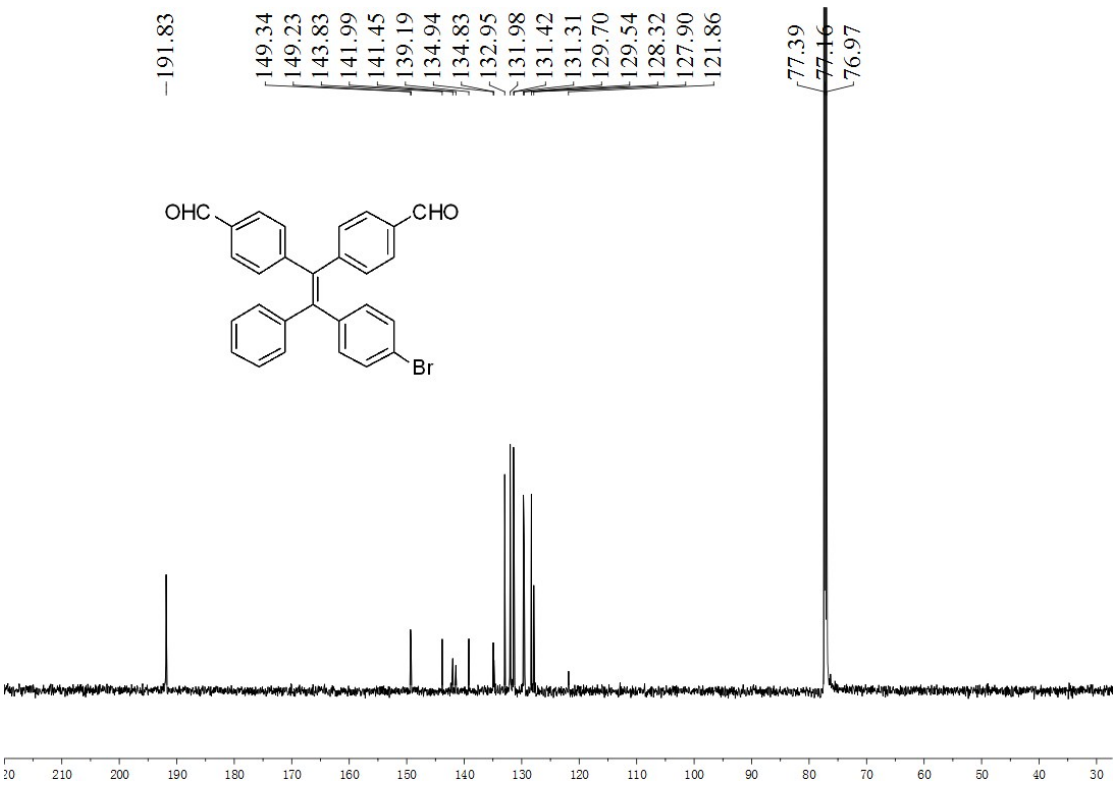


Fig. S26 ^{13}C NMR spectrum of Compound **C2.**

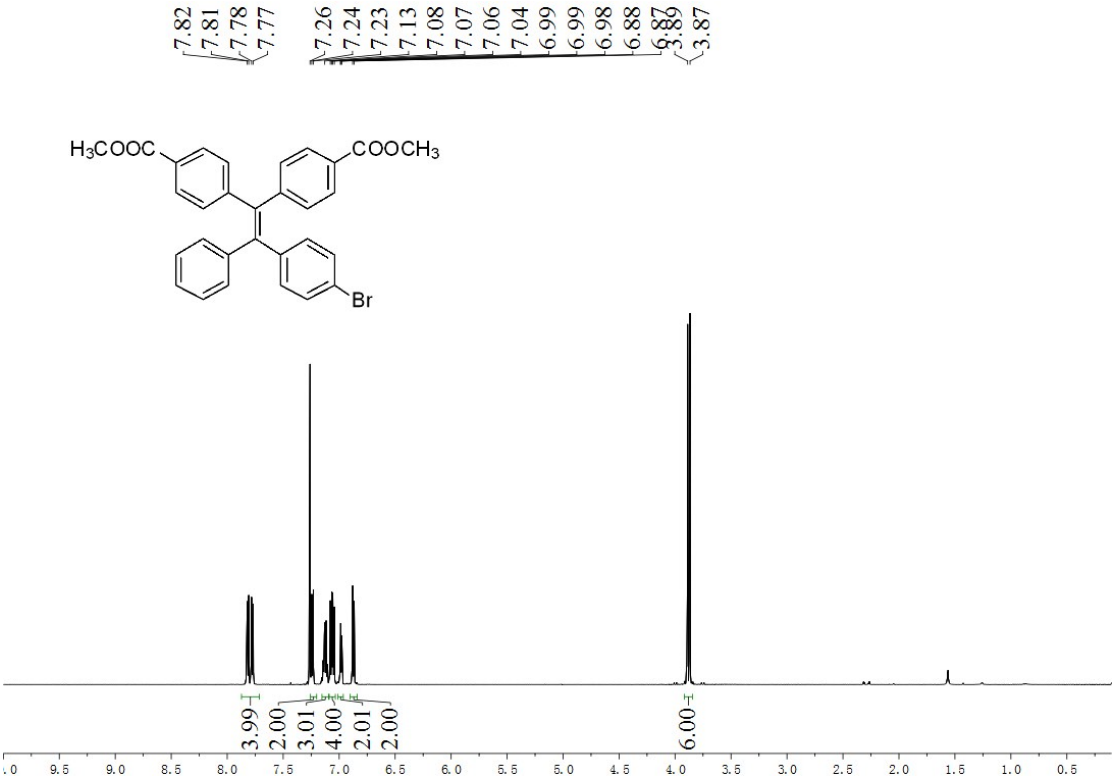


Fig. S27 ^{1}H NMR spectrum of Compound **C4.**

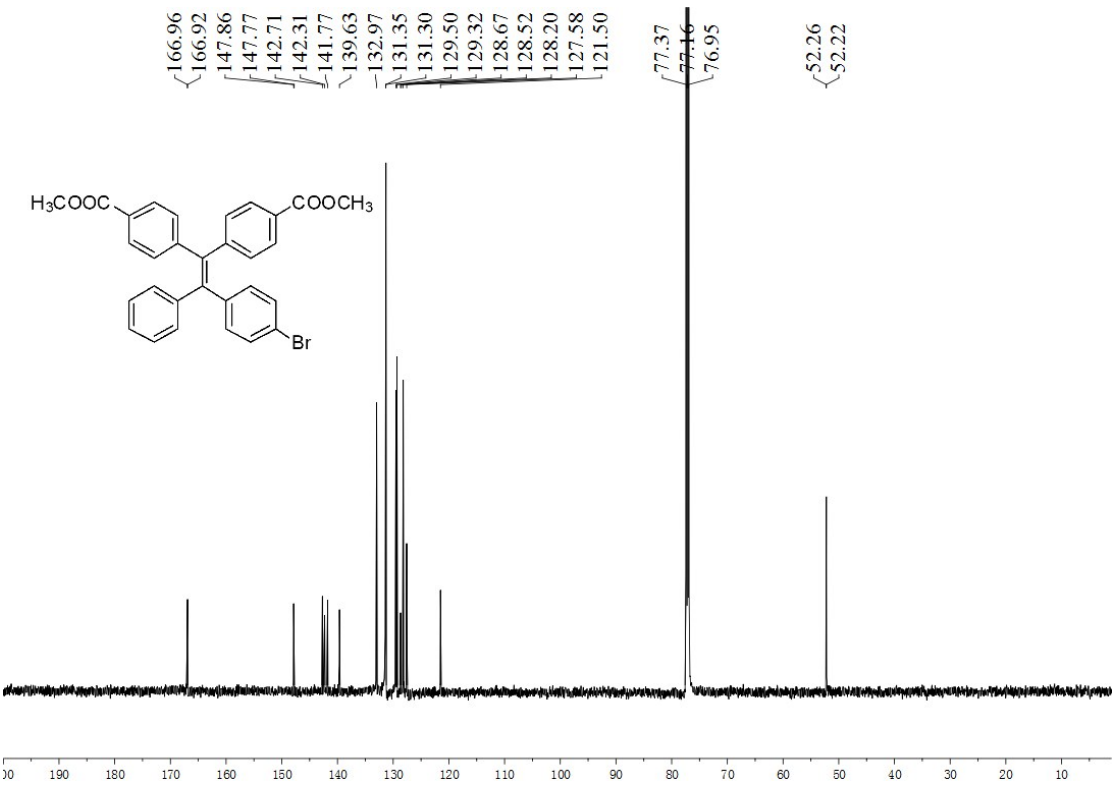


Fig. S28 ^{13}C NMR spectrum of Compound **C4.**

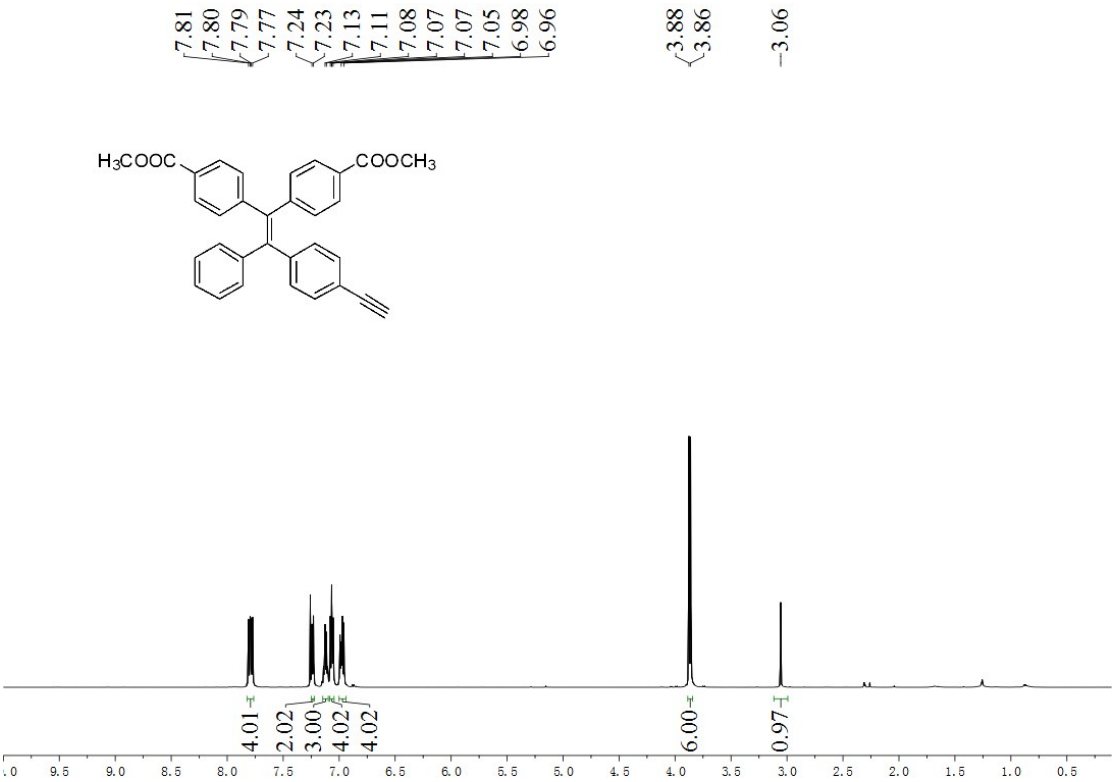


Fig. S29 ^{1}H NMR spectrum of Compound **C6.**

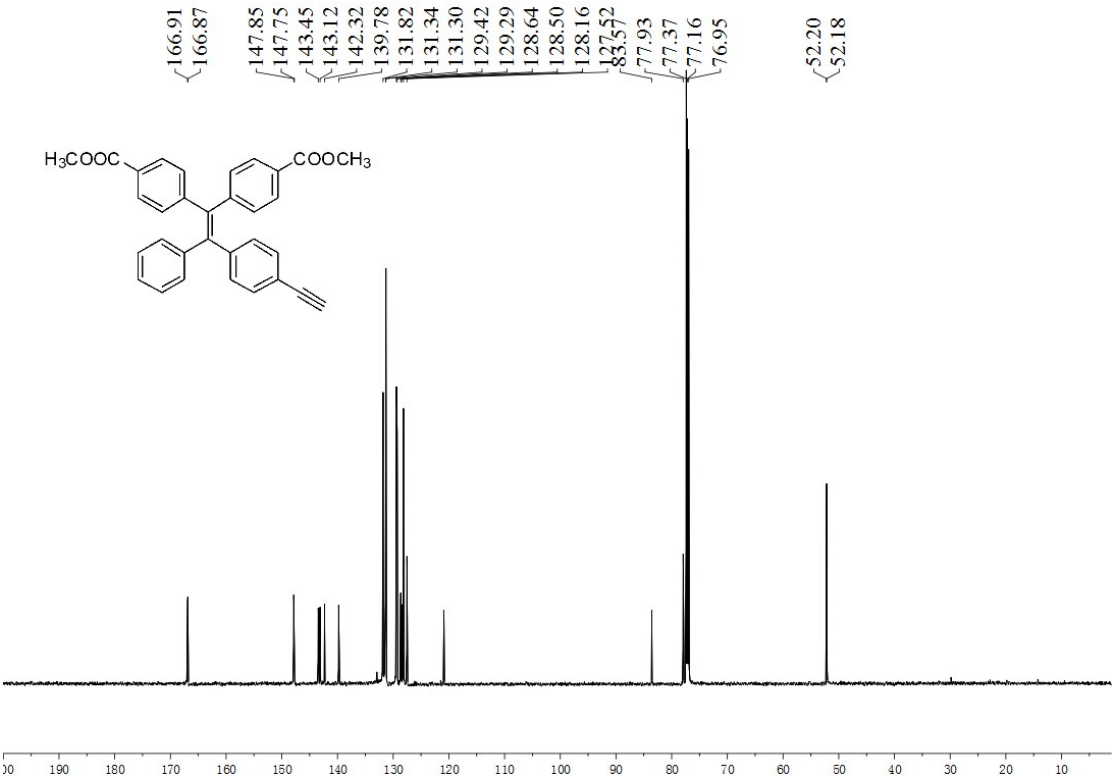


Fig. S30 ^{13}C NMR spectrum of Compound **C6.**

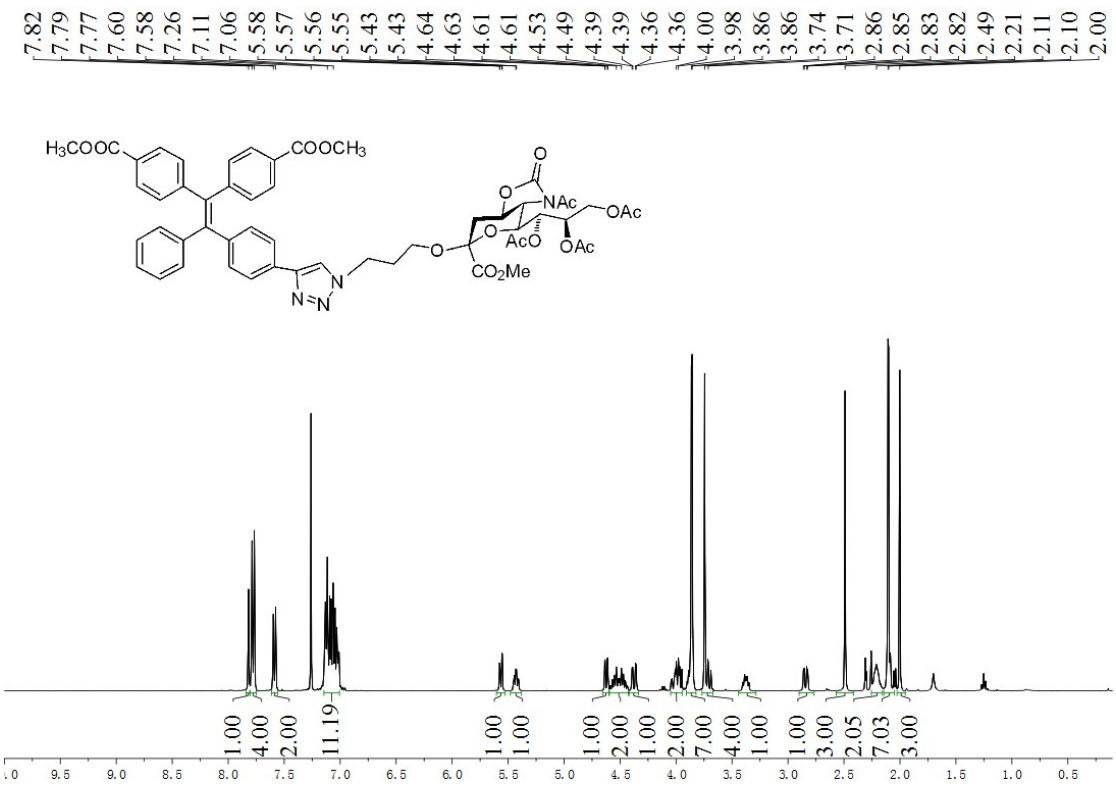


Fig. S31 [1]H NMR spectrum of Compound **TPE1S-Ac.**

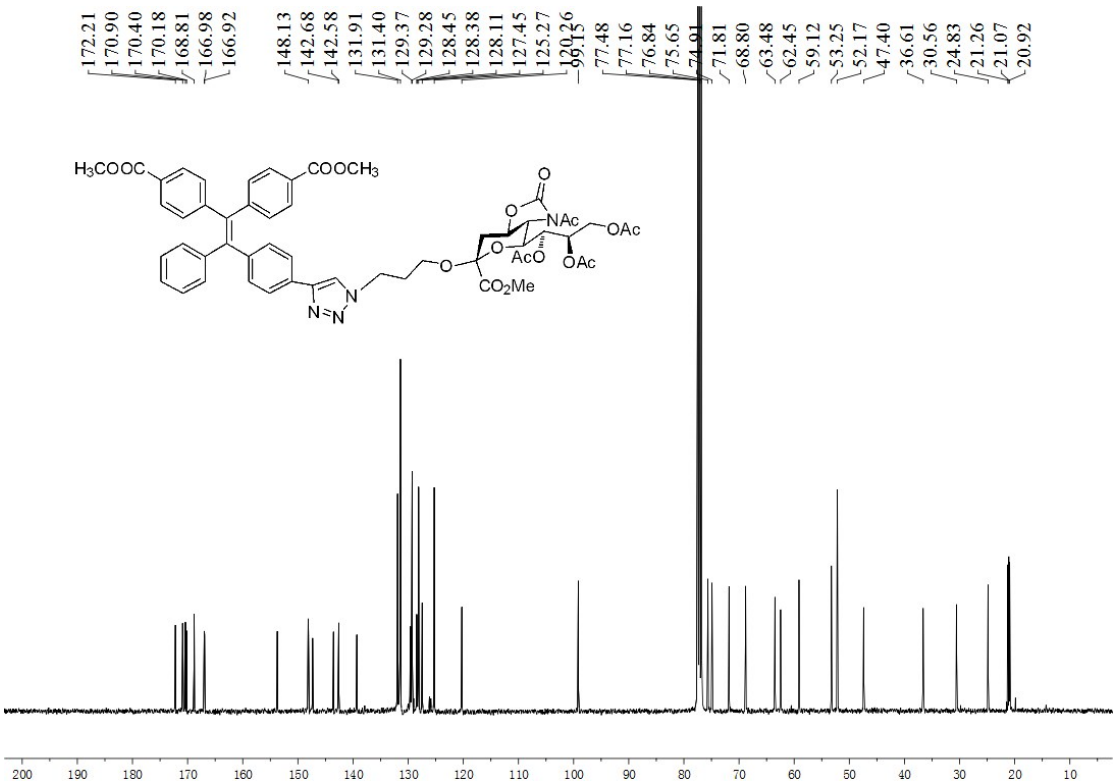


Fig. S32 [13]C NMR spectrum of Compound **TPE1S-Ac.**

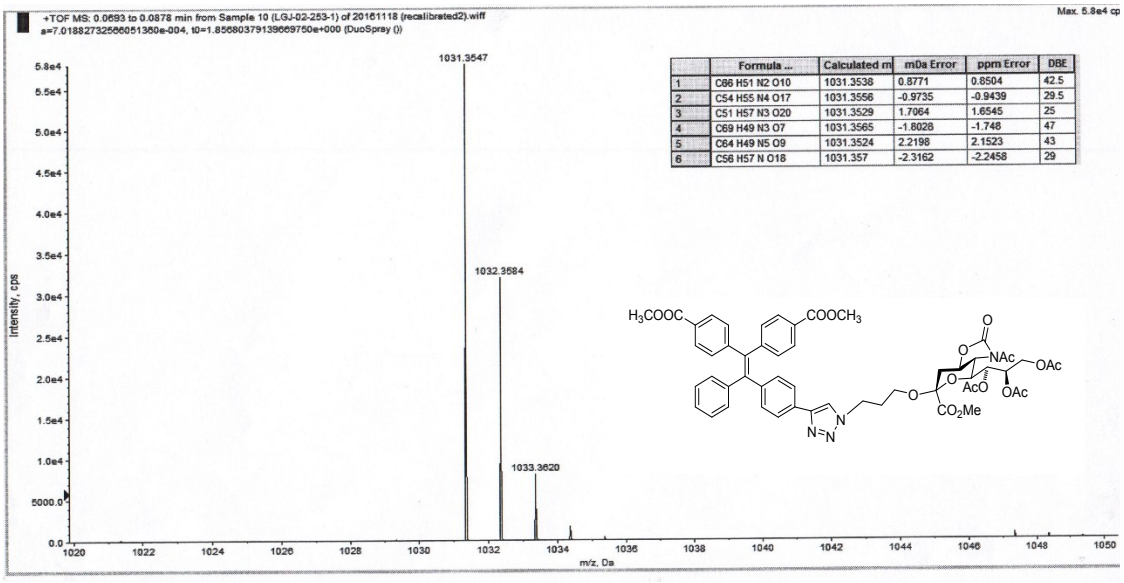


Fig. S33 HRMS spectrum of Compound **TPE1S-Ac.**

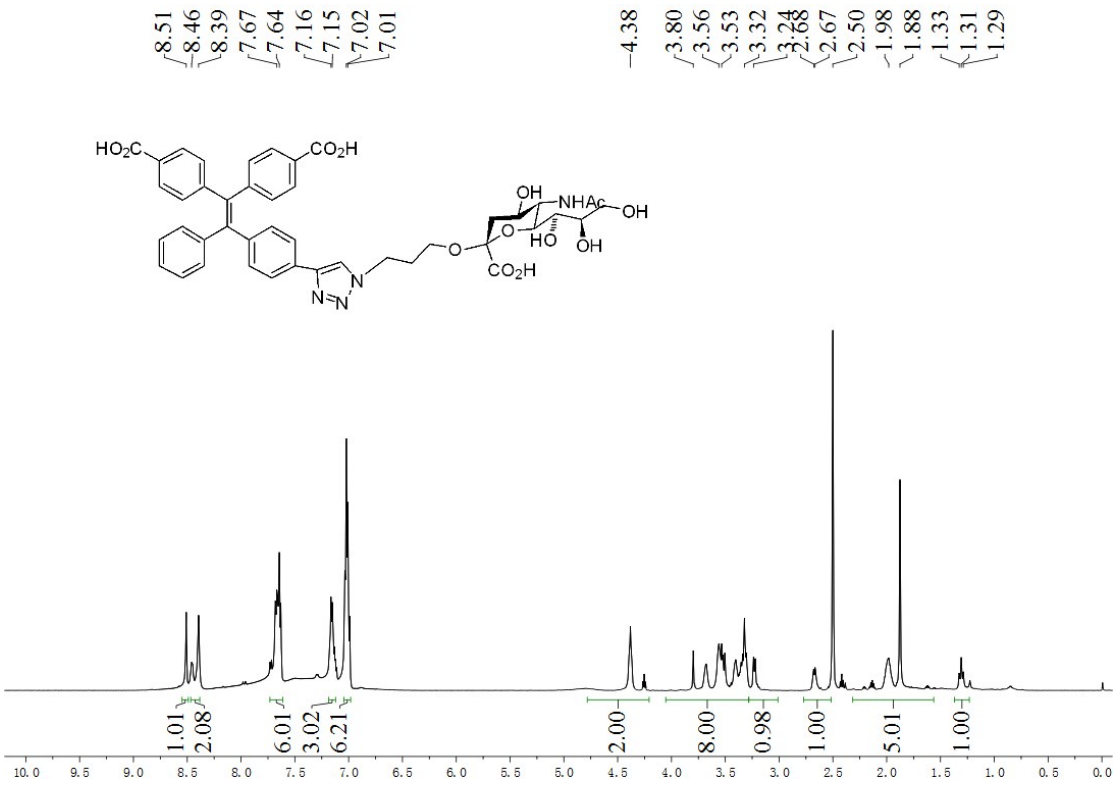


Fig. S34 [1]H NMR spectrum of Compound **TPE1S.**

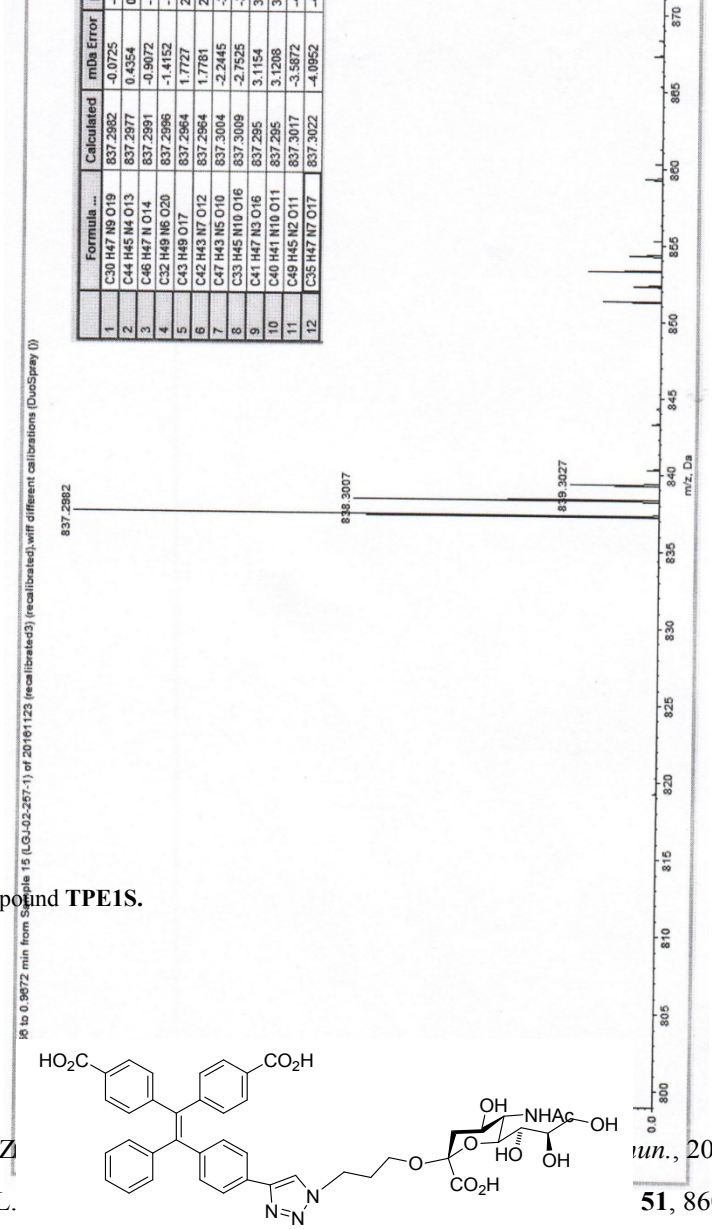


Fig. S35 HRMS spectrum of Compound **TPE1S.**

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（2）X.-t. Zhang, Z.-y. Gu, L. **51**, 8606.

（3）X. Du, J. Qi, Z. Zhang, D. Ma and Z. Y. Wang, *Chem. Mater.*, 2012, **24**, 2178.