Supporting Information for

Bioorthogonally-Activated Tetraphenylene-Tetrazine Aggregation-Induced Emission Fluorogenic Probes

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Supplemental Figures and Tables

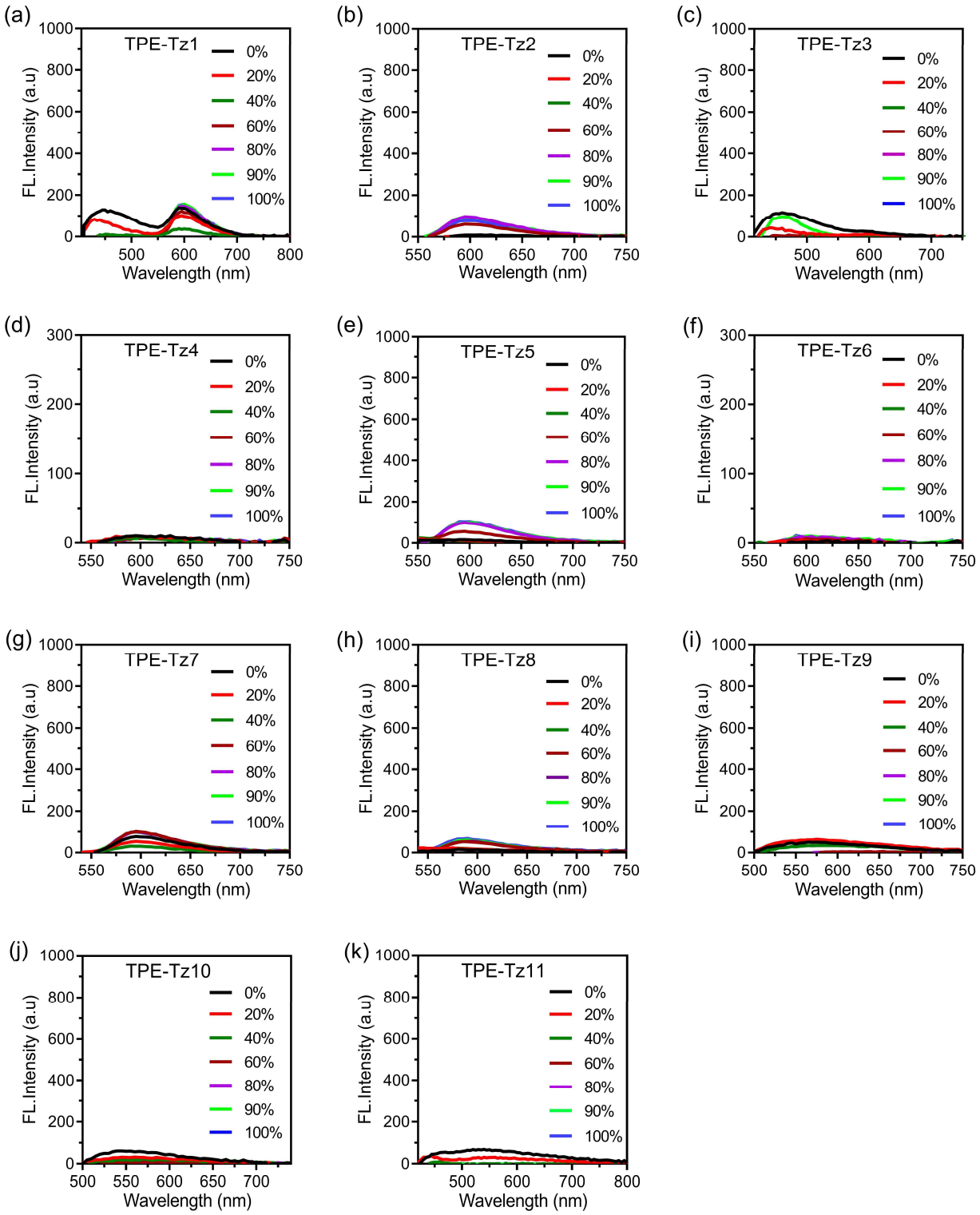


Fig. S1 Fluorescence emission spectra of TPE-Tz1 ~ TPE-Tz11 in DMSO/H_2O mixed solution (10 μM) with different water fractions (λ_{exc}=365 nm).

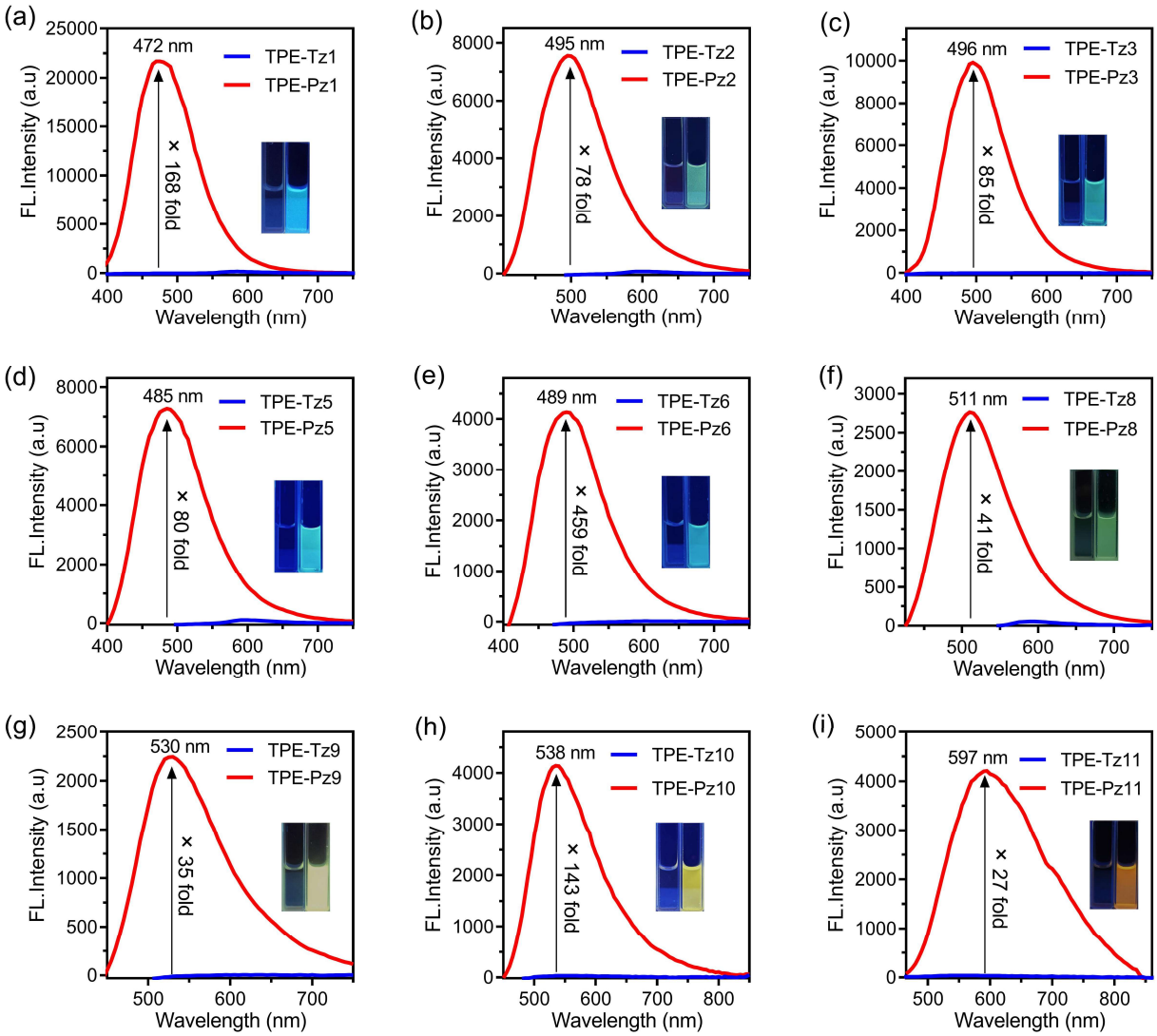


Fig. S2 Fluorescence emission spectra and fluorescent images of TPE-Tz1/Pz1 (a), TPE-Tz2/Pz2 (b), TPE-Tz3/Pz3 (c), TPE-Tz5/Pz5 (d), TPE-Tz6/Pz6 (e), TPE-Tz8/Pz8 (f), TPE-Tz9/Pz9 (g) in H_2O (10 μM), and TPE-Tz10/Pz10 (h), TPE-Tz11/Pz11 (i) in DMSO (10 μM) (λ_{exc}=365 nm).

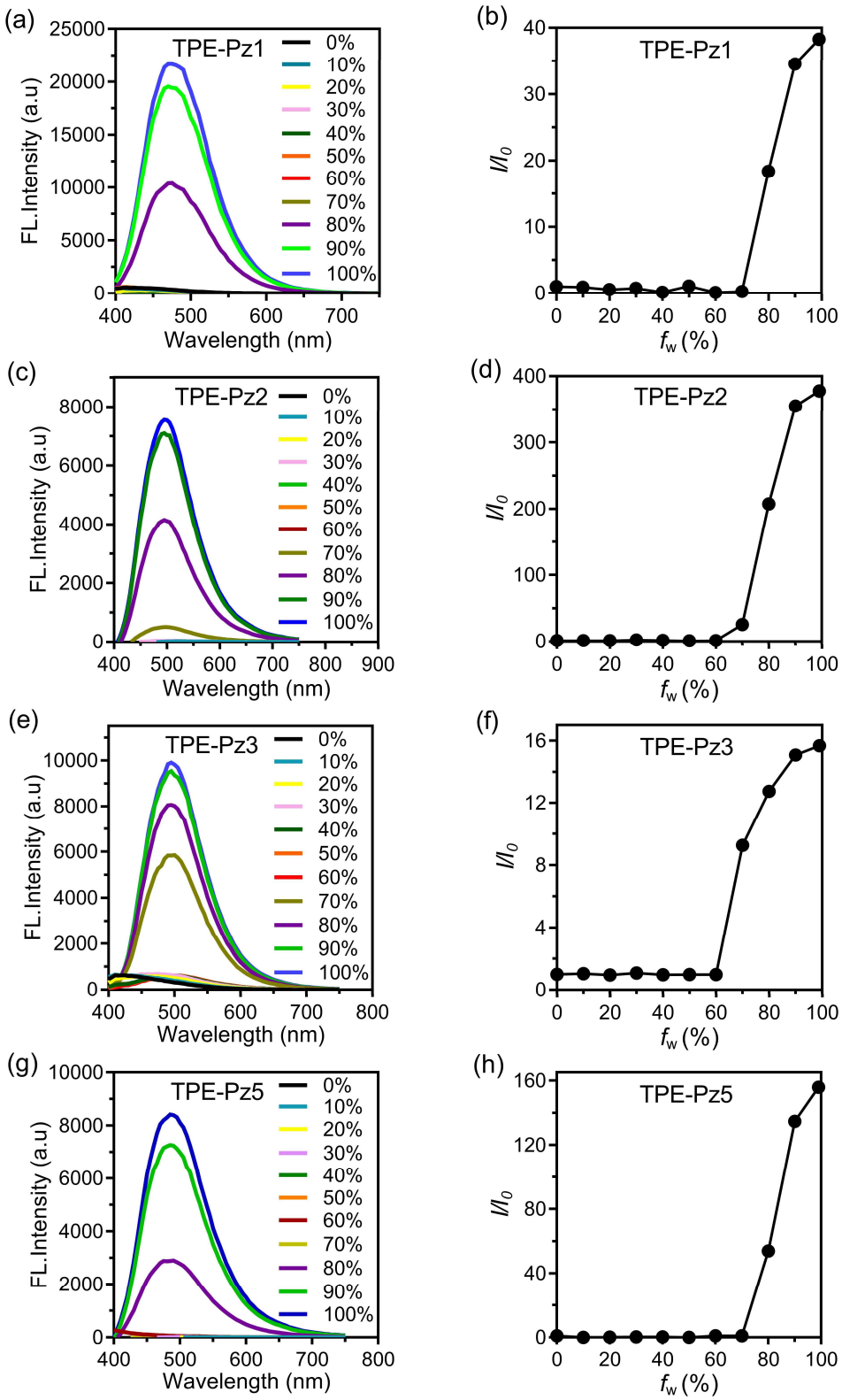


Fig. S3 Fluorescence emission spectra of TPE-Pz1 (a), TPE-Pz2 (c), TPE-Pz3 (e), TPE-Pz5 (g) in DMSO/H_2O mixed solution (10 μM) with different water fractions (λ_{exc}=365 nm). Plot of relative emission intensity (I/I_0) vs water fractions for TPE-Pz1 (b), TPE-Pz2 (d), TPE-Pz3 (f), TPE-Pz5 (h).

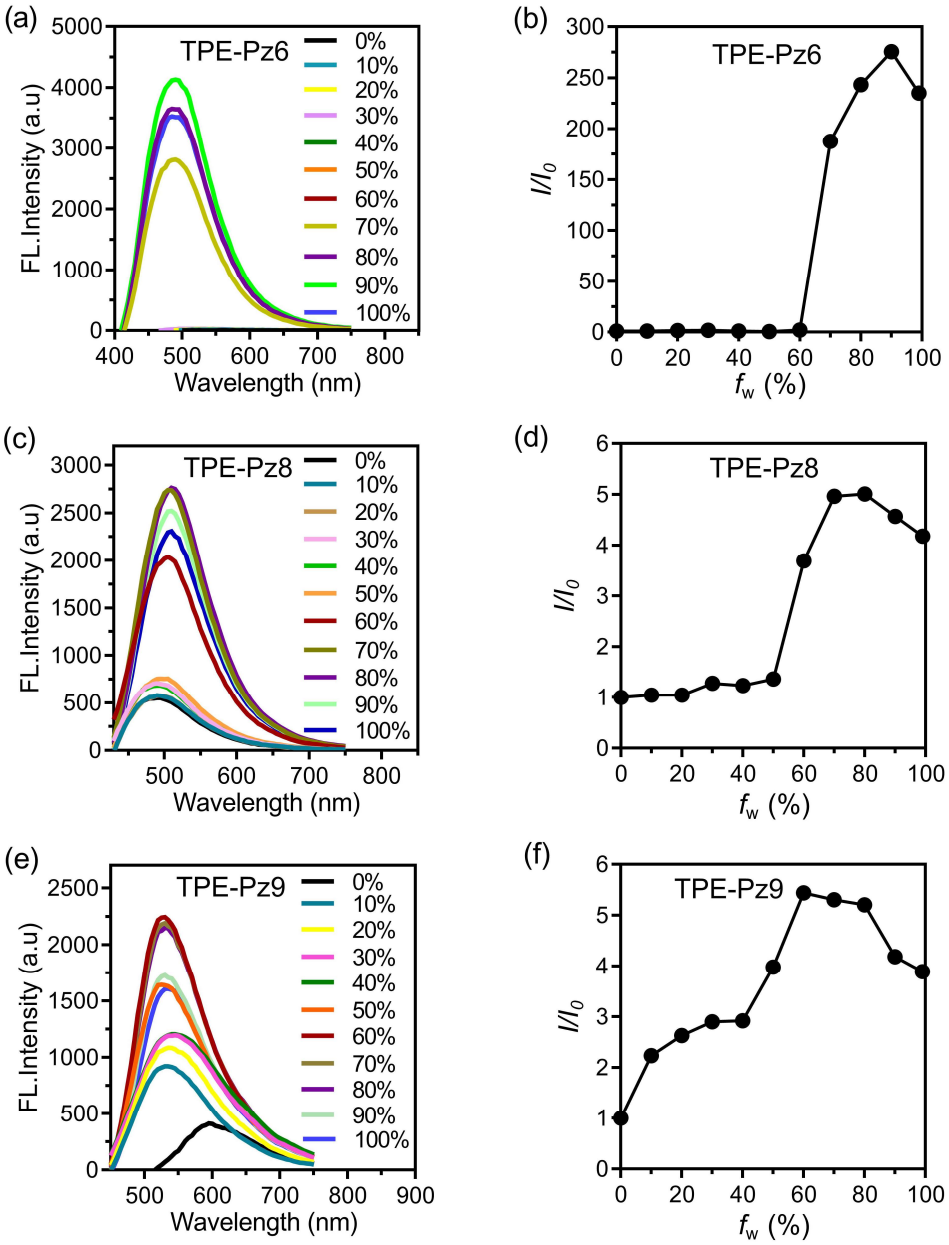


Fig. S4 Fluorescence emission spectra of TPE-Pz6 (a), TPE-Pz8 (c), TPE-Pz9 (e) in DMSO/H_2O mixed solution (10 μM) with different water fractions (λ_{exc}=365 nm). Plot of relative emission intensity (I/I_0) vs water fractions for TPE-Pz6 (b), TPE-Pz8 (d), TPE-Pz9 (f).

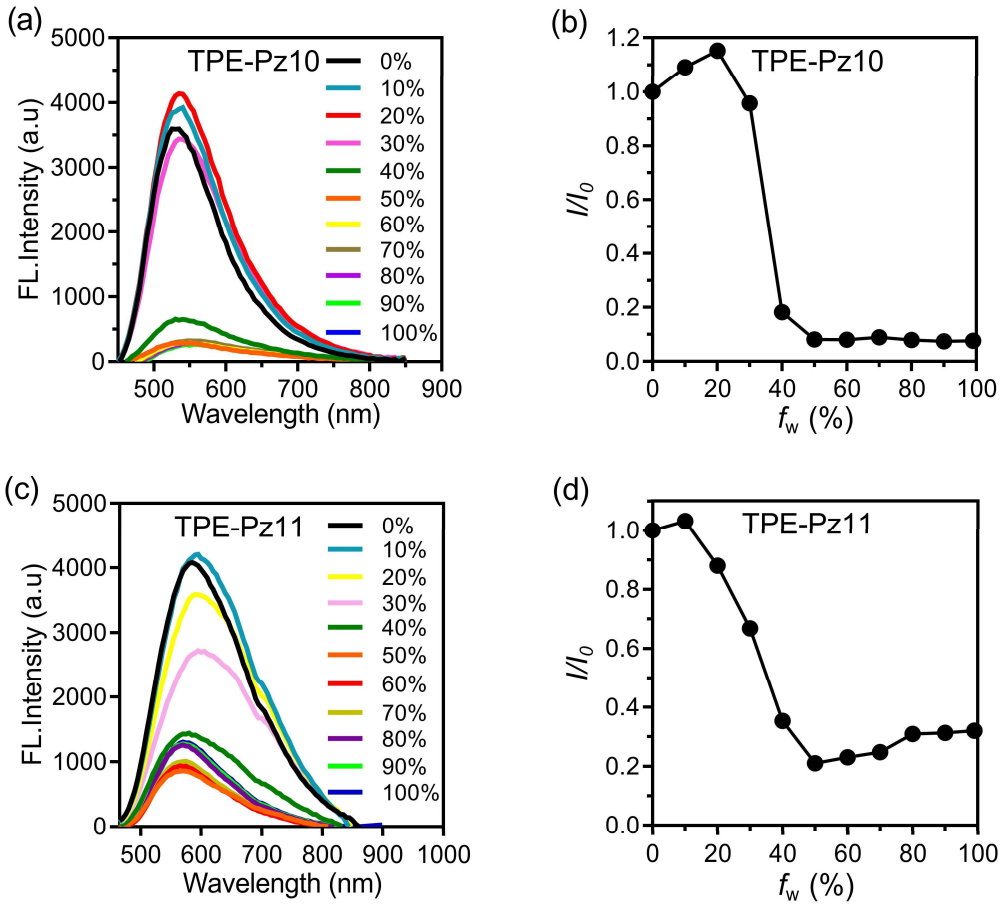


Fig. S5 Fluorescence emission spectra of TPE-Pz10 (a), TPE-Pz11 (c) in DMSO/H_2O mixed solution (10 μM) with different water fractions (λ_{exc}=365 nm). Plot of relative emission intensity (I/I_0) vs water fractions for TPE-Pz10 (b), TPE-Pz11 (d).

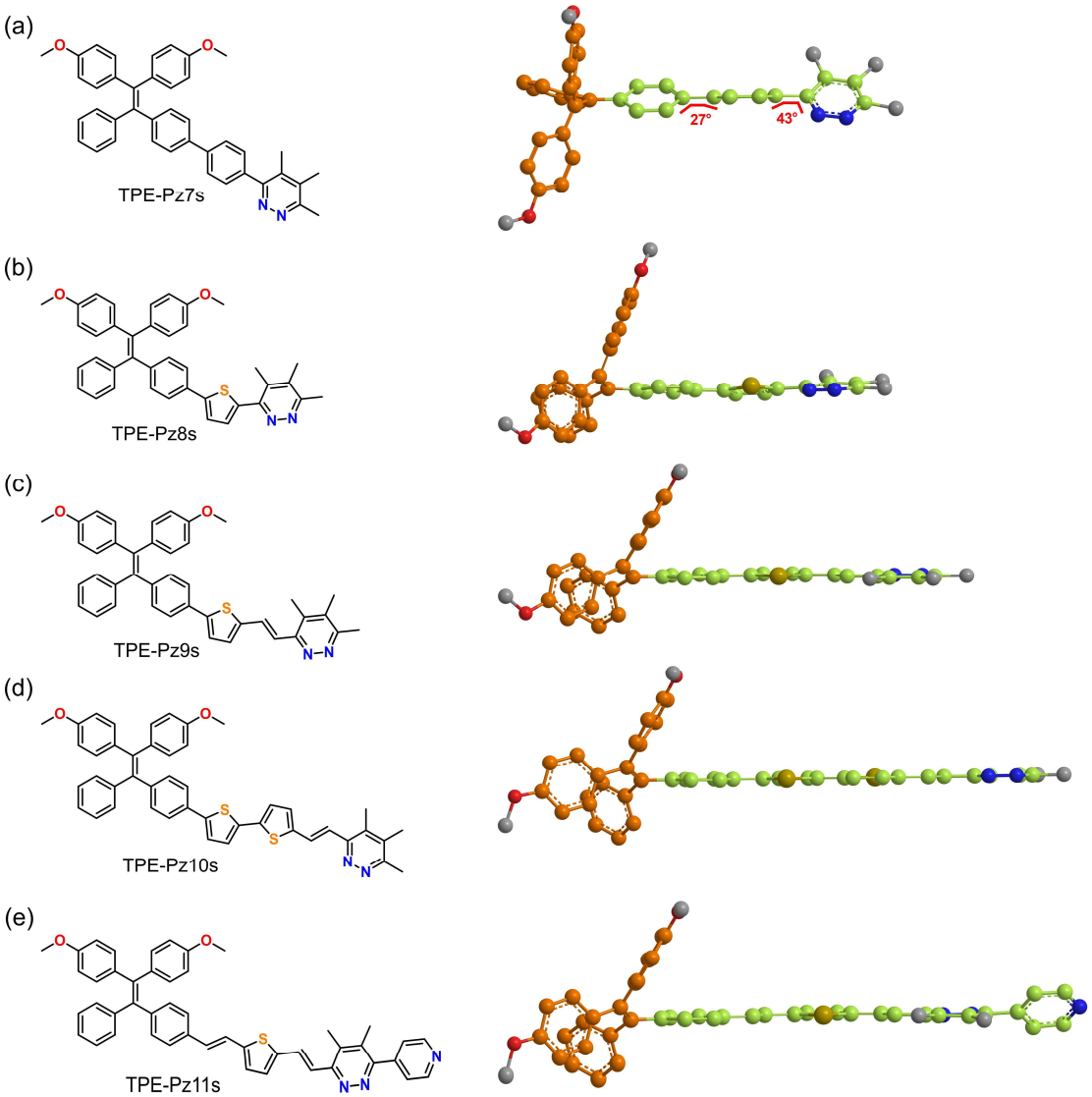


Fig. S6 Optimized 3D structure of TPE-Pz7s (a), TPE-Pz8s (b), TPE-Pz9s (c), TPE-Pz10s (d), TPE-Pz11s (e).

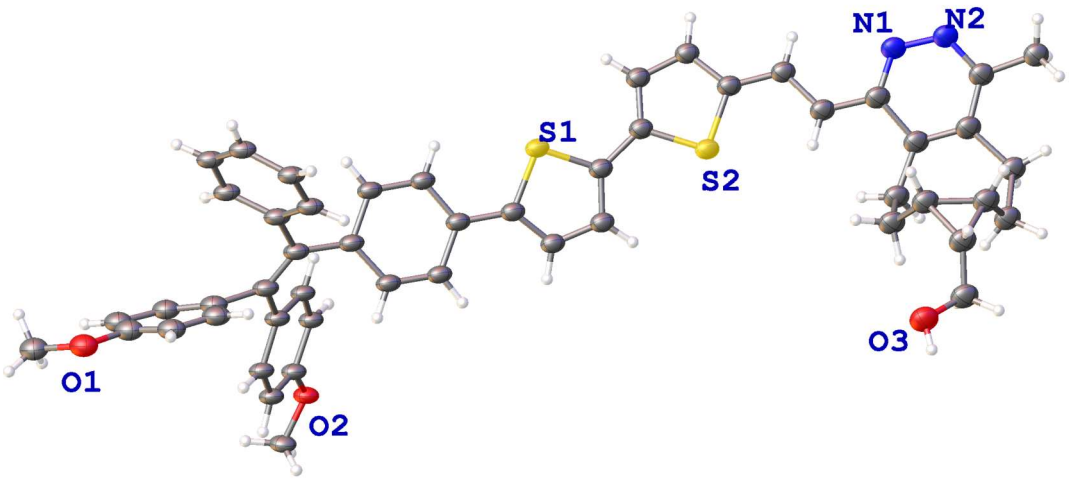


Fig. S7 Thermal ellipsoid plot of the crystal structure of TPE-Pz10 (50% ellipsoid probability).

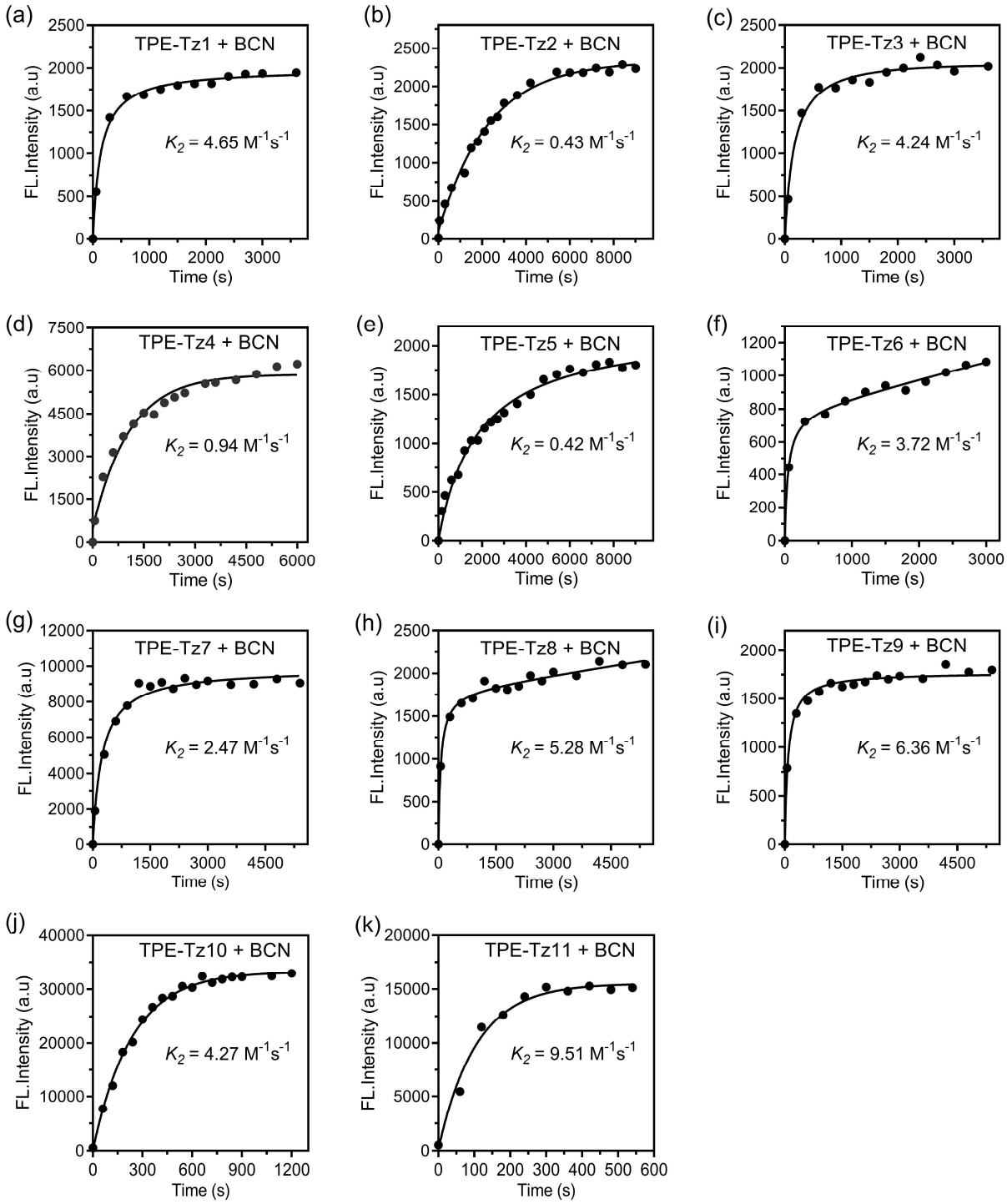


Fig. S8 Reaction kinetic analysis for the reaction between TPE-Tz1 ~ TPE-Tz11 (0.1 mM) and BCN (1 mM) in PBS buffer (pH=7.4) at 37℃. The fluorescence intensity of the mixture solution at different time interval was recorded at λ_{em} of each probe using TECAN fluorescence plate reader (λ_{exc}=365 nm). The reaction kinetics was hypothesized as pseudo-first order reaction, and the observed rate constants (k_{obs}) were calculated using the one phase exponential association equation. The second order rate constants (k_2) were calculated using the equation: $k_2 = k_{obs}$/[BCN reagent].

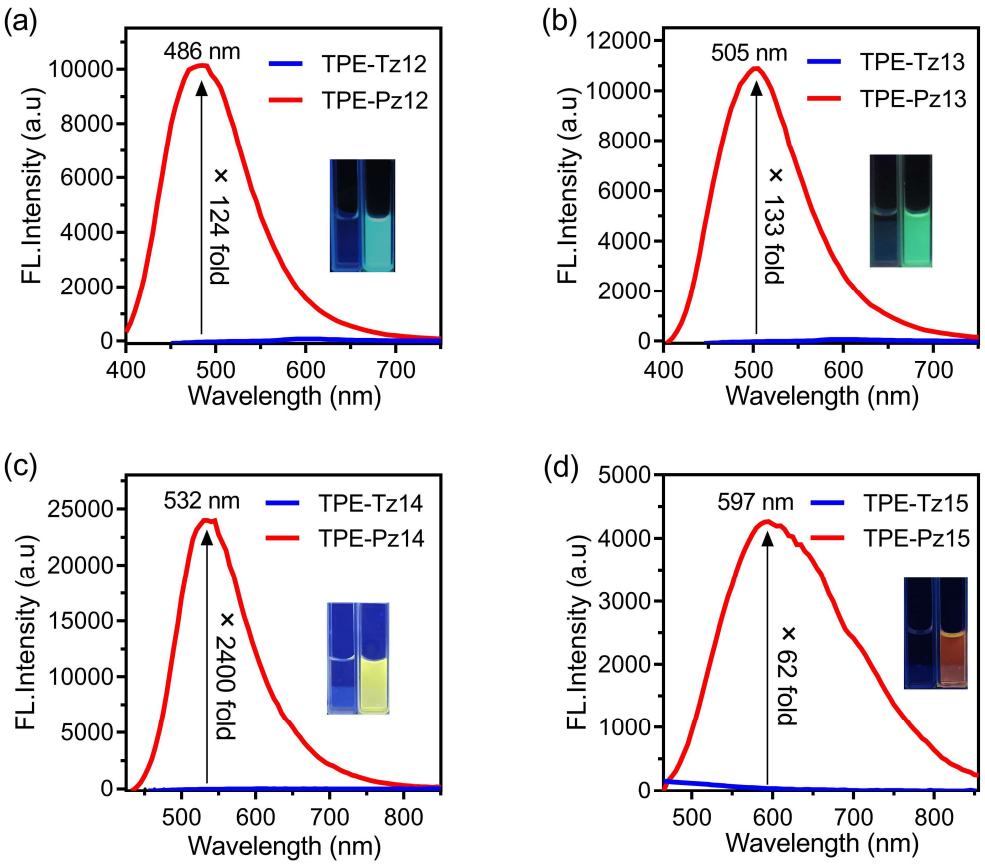


Fig. S9 Fluorescence emission spectra and fluorescent images of TPE-Tz12/Pz12 (a), TPE-Tz13/Pz13 (b), TPE-Tz14/Pz14 (c) in H_2O (10 μM), and TPE-Tz15/Pz15 in DMSO (10 μM) (d) (λ_{exc}=365 nm).

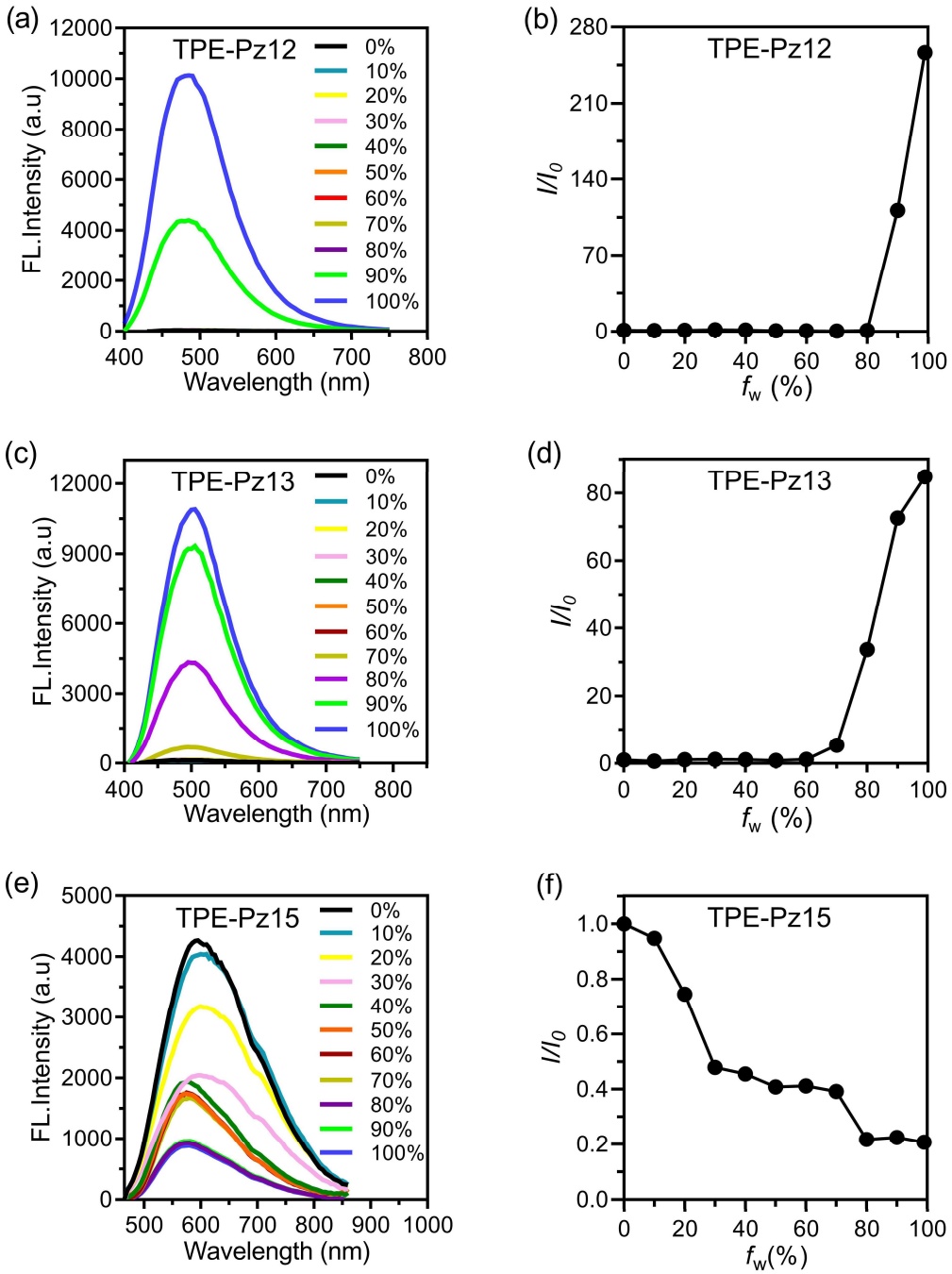


Fig. S10 Fluorescence emission spectra of TPE-Pz12 (a), TPE-Pz13 (c), TPE-Pz15 (e) in DMSO/H_2O mixed solution (10 μM) with different water fractions (λ_{exc}=365 nm). Plot of relative emission intensity (I/I_0) vs water fractions for TPE-Pz12 (b), TPE-Pz13 (d), TPE-Pz15 (f).

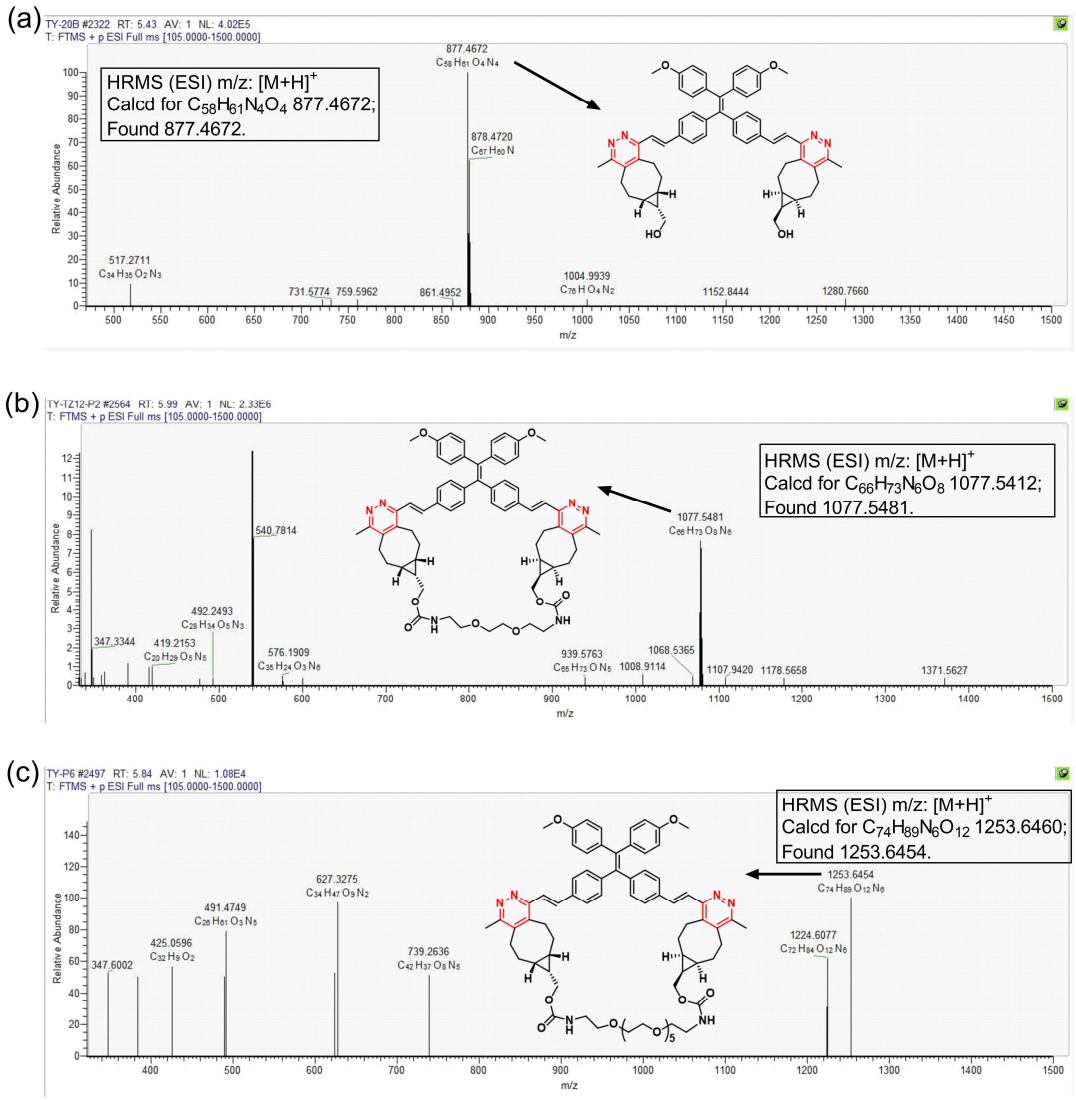


Fig. S11 Mass spectrum of the reaction mixture of TPE-Tz14 (100 μM) with BCN (200 μM) (a), **L1** (100 μM) (b), or **L2** (100 μM) (c) in DMSO.

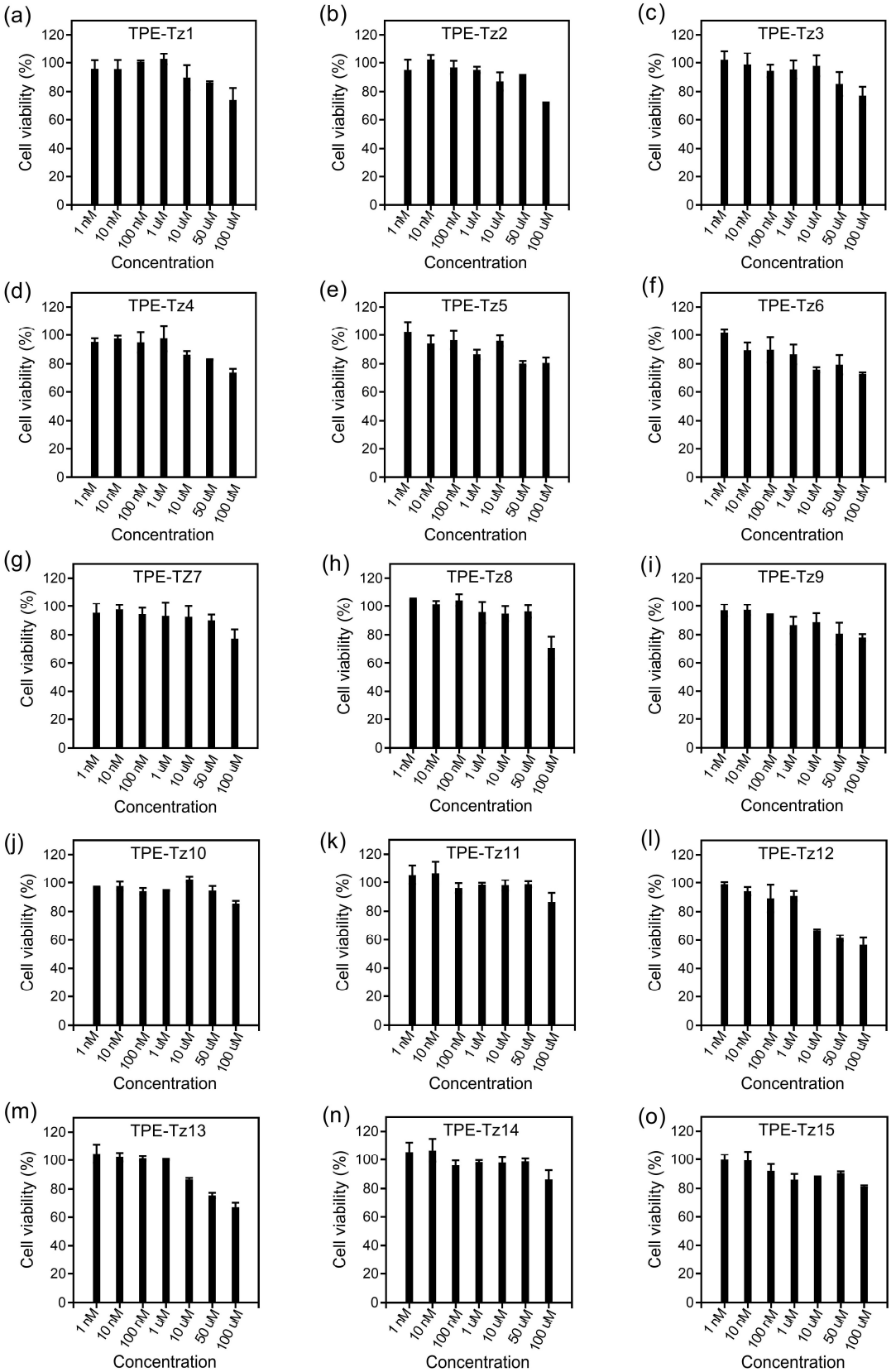


Fig. S12 Cytotoxicity of TPE-Tz1 (a), TPE-Tz2 (b), TPE-Tz3 (c), TPE-Tz4 (d), TPE-Tz5 (e), TPE-Tz6 (f), TPE-Tz7 (g), TPE-Tz8 (h), TPE-Tz9 (i), TPE-Tz10 (j), TPE-Tz11 (k), TPE-Tz12 (l), TPE-Tz13 (m), TPE-Tz14 (n), and TPE-Tz15 (o) against HeLa cells based on MTT assay. The cells were incubated with each TPE-tetrazine probe for 48 h.

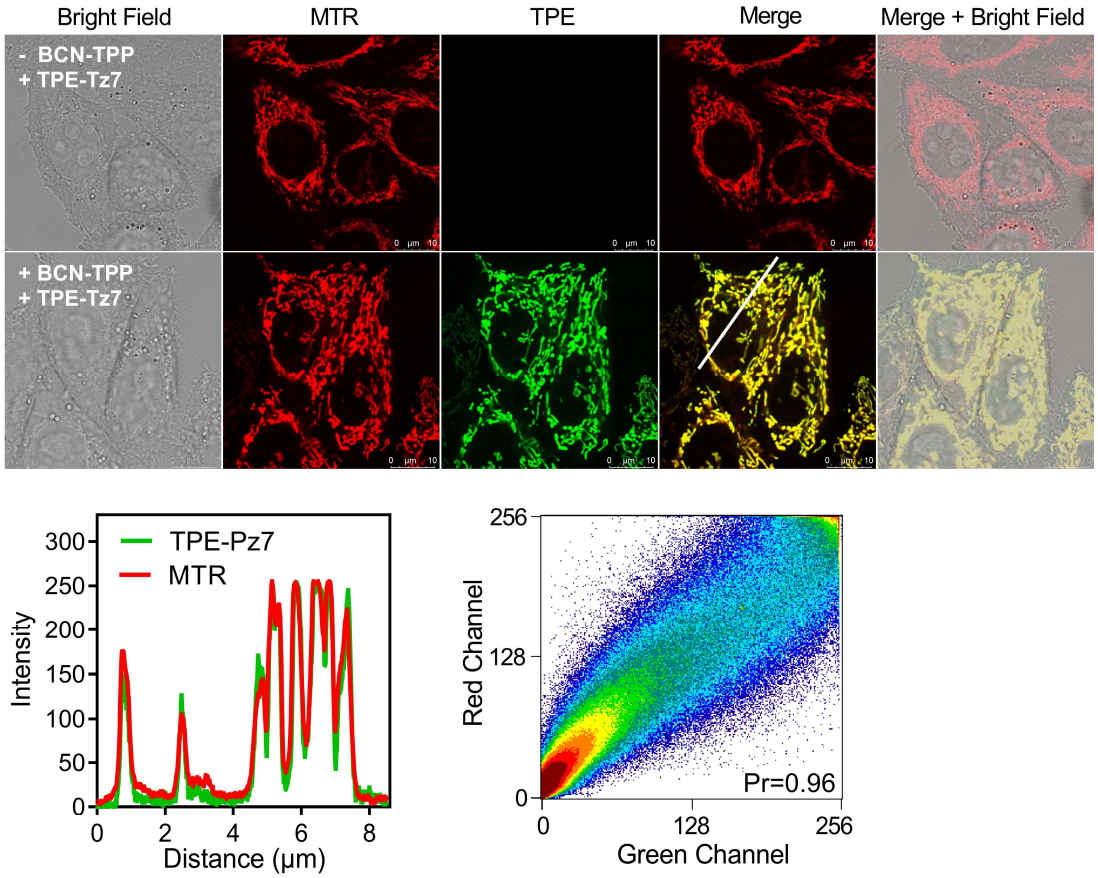


Fig. S13 Confocal images of HeLa cells labeled by BCN-TPP/TPE-Tz7. The cells were treated with BCN-TPP (1 μM) and Mito Tracker Red (MTR, 200 nM) for 1 h, and then with TPE-Tz7 (1 μM) for 1 h. Scale bar: 10 μm. MTR: 200 nM; BCN-TPP: 1 μM; TPE-Tz7: 1 μM.

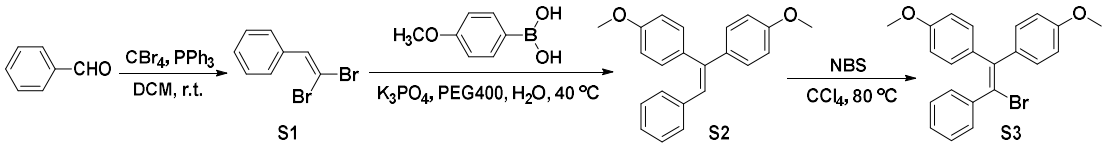
Table S1 Crystal data and structure refinement for TPE-Pz10.

	Single crystal of TPE-Pz10
Empirical formula	$C_{51}H_{46}N_2O_3S_2$
Formula weight	799.02
Temperature/K	150.00(10)
Crystal system	monoclinic
Space group	P2/c
a/Å	17.146(7)
b/Å	10.393(2)
c/Å	24.936(13)
α/°	90
β/°	97.90(4)
γ/°	90
Volume/Å^3	4401(3)
Z	4
ρ_{calc}g/cm^3	1.206
μ/mm^{-1}	1.437
F(000)	1688.0
Crystal size/mm^3	0.14 × 0.12 × 0.1
Radiation	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	5.204 to 133.186
Index ranges	-20 ≤ h ≤ 20, -12 ≤ k ≤ 11, -29 ≤ l ≤ 29
Reflections collected	16034
Independent reflections	7774 [R_{int} = 0.0131, R_{sigma} = 0.2744]
Data/restraints/parameters	7774/903/537
Goodness-of-fit on F^2	1.058
Final R indexes [I>=2σ (I)]	R_1 = 0.0752, wR_2 = 0.2164
Final R indexes [all data]	R_1 = 0.0824, wR_2 = 0.2393
Largest diff. peak/hole / e Å^{-3}	0.91/-1.01

General Information

Compound **BCN-TPP** was prepared by the same procedure according to the reported literature[1]. Compound **L1**, **L2** were purchased from Confluore Biotechnology. Mito Tracker Red (MTR) was purchased from Beyotime. All the other solvents and chemicals were purchased from commercial sources and used directly without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz, 500 MHz, or 600 MHz spectrometer. Chemical shifts are referenced to the residual solvent peak and reported as δ units in ppm (in NMR description, s = singlet, d = doublet, t = triplet, q = quartet and m = multiple), and all coupling constant (J) values are given in hertz. ESI-HRMS data were measured on Thermo LCQ Deca XP Max mass spectrometer equipped with an ion trap mass analyzer. Silica gel flash column chromatography was performed on Biotage Isolera one. Fluorescence emission spectra were recorded on Tecan Spark™ 10M Multimode Microplate Reader. Confocal laser scanning microscope imaging was conducted using a Leica TCS SP8 X Confocal Microscope.

Experimental Procedures and Characterization Data



Synthesis of (2,2-dibromovinyl)benzene (S1)

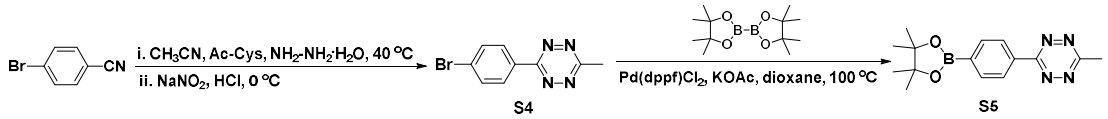
To a solution of carbon tetrabromide (1.25 g, 3.8 mmol) in DCM (30 mL) was added triphenylphosphine (2.0 g, 7.6 mmol) under argon protection. The reaction solution was cooled to 0 °C using an ice bath and then benzaldehyde (200 mg, 1.9 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 15:1) to afford the titled compound as yellow oil (310 mg, 62.8% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (dd, J = 1.8, 0.7 Hz, 1H), 7.52 (dd, J = 1.4, 0.7 Hz, 1H), 7.48 (s, 1H), 7.37 (d, J = 0.7 Hz, 1H), 7.35 – 7.34 (m, 2H).

Synthesis of 4,4'-(2-phenylethene-1,1-diyl)bis(methoxybenzene) (S2)

To a solution of **S1** (400 mg, 1.5 mmol) in a mixture of PEG400 and water (V_{PEG400} : V_{H_2O} = 1:3) was added 4- methoxyphenylboronic acid (580 mg, 3.8 mmol), $Pd(OAc)_2$ (8.5 mg, 0.04 mmol) and K_2CO_3 (650 mg, 3.0 mmol). The reaction mixture was stirred at 40 °C using an oil bath for 24 h under argon protection. Then the reaction was quenched with H_2O (10 mL) and extracted with EtOAc (3×20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was isolated by silica gel flash column chromatography to afford the titled compound (228.2 mg, 47.2% yield) as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (s, 1H), 7.24 (s, 1H), 7.11 (m, J = 7.6, 5H), 7.05 – 7.01 (m, 2H), 6.89 – 6.79 (m, 5H), 3.82 (s, 3H), 3.81 (s, 3H).

Synthesis of 4,4'-(2-bromo-2-phenylethene-1,1-diyl)bis(methoxybenzene) (S3)

To a solution of **S2** (500 mg, 1.5 mmol) in CCl_4 (20 mL) was added NBS (337.5 mg, 1.9 mmol). The reaction mixture was stirred at 80 °C using an oil bath for 3 h and then concentrated. The residue was isolated by silica gel flash column chromatography to afford the titled compound as yellow solid (510 mg, 81.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.27 (m, 4H), 7.19 – 7.13 (m, 3H), 6.89 (d, J = 8.8 Hz, 2H), 6.84 (d, J = 8.9 Hz, 2H), 6.58 (d, J = 8.9 Hz, 2H), 3.83 (s, 3H), 3.69 (s, 3H).

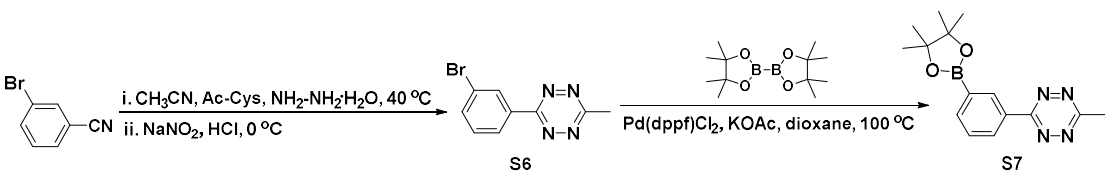


Synthesis of 3-(4-bromophenyl)-6-methyl-1,2,4,5-tetrazine (S4)

To a solution of 4-bromobenzonitrile (400 mg, 2.2 mmol) in CH_3CN (4 mL) was added *N*-acetyl-L-cysteine (360 mg, 2.2 mmol) under argon protection. The reaction solution was cooled to 0 °C using an ice bath and the hydrazine hydrate (1.7 mL, 35.2 mmol) was added dropwise, and then the reaction mixture was stirred at 40 °C using an oil bath for 16 h. Upon completion, the reaction solution was cooled to 0 °C, then a water solution of sodium nitrite (1.87 g, 22.0 mmol) was slowly added, followed by slow addition of 2 N HCl during which the solution was stirred intensely and turned bright red, and gas evolved. Addition of 2 N HCl continued until gas evolution ceased and the pH value was 3-4. Then the reaction mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 10:1) to afford the titled compound as purple solid (334 mg, 60.6% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 8.8 Hz, 2H), 3.10 (s, 3H).

Synthesis of 3-methyl-6-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1,2,4,5-tetrazine (S5)

To a solution of **S4** (400 mg, 1.6 mmol) in 1,4-dioxane (20 mL) was added bis(pinacolato)diboron (608 mg, 2.4 mmol), $Pd(dppf)Cl_2$ (2.9 mg, 0.04 mmol) and KOAc (468.8 mg, 4.8 mmol) under argon protection. The reaction mixture was stirred at 100°C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as purple solid (368 mg, 77.6% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (d, J = 8.8 Hz, 2H), 8.05 (d, J = 8.8 Hz, 2H), 3.05 (s, 3H), 1.43 (s, 12H).



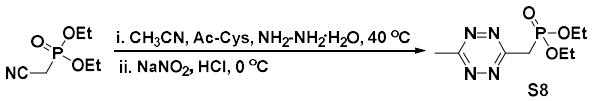
Synthesis of 3-(3-bromophenyl)-6-methyl-1,2,4,5-tetrazine (S6)

To a solution of 3-bromobenzonitrile (400 mg, 2.2 mmol) in CH_3CN (4 mL) was added *N*-acetyl-L-cysteine (360 mg, 2.2 mmol) under argon protection. The reaction solution was cooled to 0 °C using an ice bath and the hydrazine hydrate (1.71 mL, 35.2 mmol) was added dropwise, and then the reaction mixture was stirred at 40 °C using an oil bath for 16 h. Upon completion, the reaction solution was cooled to 0 °C, then a water solution of sodium nitrite (1.87 g, 22.0 mmol) was slowly added, followed by slow addition of 2 N HCl during which the solution was stirred intensely and turned bright red, and gas evolved. Addition of 2 N HCl continued until gas evolution ceased and the pH value was 3-4. Then the reaction mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 10:1) to afford the titled compound as purple solid (396.8 mg, 71.9% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (t, *J* = 1.9 Hz, 1H), 8.57 (d, *J* = 7.9 Hz, 1H), 7.82 – 7.75 (m, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 3.15 (s, 3H).

Synthesis of 3-methyl-6-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1,2,4,5-tetrazine (S7)

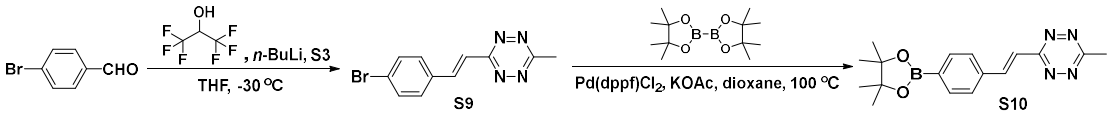
To a solution of **S6** (400 mg, 1.6 mmol) in 1,4-dioxane (20 mL) was added bis(pinacolato)diboron (608 mg, 2.4 mmol), $Pd(dppf)Cl_2$ (2.9 mg, 0.04 mmol) and KOAc (468.8 mg, 4.8 mmol) under argon protection. The reaction mixture was stirred at 100°C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as purple solid (343 mg, 72.3% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.08 (s, 1H), 8.70 (d, *J* = 7.4 Hz, 1H), 8.14 – 8.04 (m, 1H), 7.64 (m, 1H), 3.14 (s, 3H), 1.42 (s, 12H).

Synthesis of diethyl ((6-methyl-1,2,4,5-tetrazin-3-yl)methyl)phosphonate (S8)[2]



S8

To a solution of 2-(diethylphosphonyl)acetonitrile (177 mg, 1.0 mmol) in CH_3CN (4 mL) was added *N*-acetyl-L-cysteine (163 mg, 1.0 mmol) under argon protection. The reaction solution was cooled to 0 °C using an ice bath and the hydrazine hydrate (777 μL, 16.0 mmol) was added dropwise, and then the reaction mixture was stirred at 40 °C using an oil bath for 16 h. Upon completion, the reaction solution was cooled to 0 °C, then a water solution of sodium nitrite (904 mg, 10.0 mmol) was slowly added, followed by slow addition of 2 N HCl during which the solution was stirred intensely and turned bright red, and gas evolved. Addition of 2 N HCl continued until gas evolution ceased and the pH value was 3-4. Then the reaction mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (DCM/MeOH = 20:1) to afford the titled compound as purple oil (118 mg, 48.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.27 – 4.14 (m, 4H), 3.93 (d, *J* = 22.4 Hz, 2H), 3.06 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 6H).

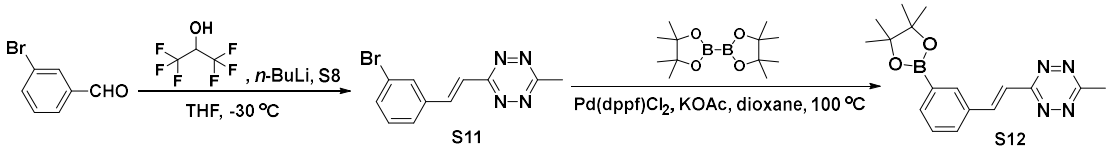


Synthesis of (*E*)-3-(4-bromostyryl)-6-methyl-1,2,4,5-tetrazine (S9)

To a solution of 1,1,1,3,3,3-hexafluoro-2-propanol (167.3 μL, 1.6 mmol) in dry THF (10 mL) was added 1.6 M n-BuLi (1.0 mL, 1.6 mmol) at -20 °C under argon protection. The reaction was stirred for 30 min at -20 °C and further cooled to -30 °C. The solution of THF containing 4-bromobenzaldehyde (130.7 mg, 0.71 mmol) and **S3** (260 mg, 1.06 mmol) was added to the mixture dropwise and stirred for 8 h at -30 °C. The reaction mixture was quenched by H_2O (20 mL) and extracted with EtOAc (3×20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as purple solid (165.4 mg, 84.5% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (d, *J* = 16.3 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.58 (d, *J* = 8.6 Hz, 2H), 7.49 (d, *J* = 16.3 Hz, 1H), 3.10 (s, 3H).

Synthesis of (*E*)-3-methyl-6-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)styryl)-1,2,4,5-tetrazine (S10)

To a solution of **S9** (200 mg, 0.72 mmol) in 1,4-dioxane (20 mL) was added bis(pinacolato)diboron (274.9 mg, 1.08 mmol), $Pd(dppf)Cl_2$ (1.5 mg, 0.02 mmol) and KOAc (212.4 mg, 2.16 mmol) under argon protection. The reaction mixture was stirred at 100°C using an oil bath for 6 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as purple solid (212 mg, 90.9% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.39 – 8.37 (m, 1H), 7.95 – 7.93 (m, 2H), 7.76 – 7.73 (m, 2H), 7.57 (m, 1H), 7.30 – 7.32 (m, 2H), 3.11 (s, 3H), 1.42 (s, 12H).

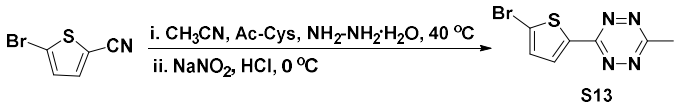


Synthesis of (*E*)-3-(3-bromostyryl)-6-methyl-1,2,4,5-tetrazine (S11)

To a solution of 1,1,1,3,3,3-hexafluoro-2-propanol (167.3 μL, 1.6 mmol) in dry THF (10 mL) was added 1.6 M n-BuLi (1.0 mL, 1.6 mmol) at -20 °C under argon protection. The reaction was stirred for 30 min at -20 °C and further cooled to -30 °C. The solution of THF containing 3-bromobenzaldehyde (130.7 mg, 0.71 mmol) and **S8** (260 mg, 1.06 mmol) was added to the mixture dropwise and stirred for 8 h at -30 °C. The reaction mixture was quenched by H_2O (20 mL) and extracted with EtOAc (3×20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as purple solid (172.5 mg, 87.7% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (dd, *J* = 16.2, 2.2 Hz, 1H), 7.90 – 7.81 (m, 1H), 7.64 (d, *J* = 7.7 Hz, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.50 (m, 1H), 7.37 (m, 1H), 3.11 (s, 1H).

Synthesis of (*E*)-3-methyl-6-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)styryl) -1,2,4,5-tetrazine (S12)

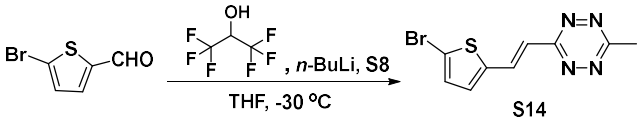
To a solution of **S11** (124 mg, 0.44 mmol) in 1,4-dioxane (20 mL) was added bis(pinacolato)diboron (170.4 mg, 0.66 mmol), $Pd(dppf)Cl_2$ (1.5 mg, 0.02 mmol) and KOAc (129.4 mg, 1.32 mmol) under argon protection. The reaction mixture was stirred at 100°C using an oil bath for 6 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as purple solid (135 mg, 94.7% yield).



S13

Synthesis of 3-(5-bromothiophen-2-yl)-6-methyl-1,2,4,5-tetrazine (S13)

To a solution of 5-brono-thiophene-2-carbonitrile (200 mg, 1.06 mmol) in CH_3CN (4 mL) was added *N*-acetyl-L-cysteine (174 mg, 1.06 mmol) under argon protection. The reaction solution was cooled to 0 °C using an ice bath and the hydrazine hydrate (852 μL, 16.96 mmol) was added dropwise, and then the reaction mixture was stirred at 40 °C using an oil bath for 16 h. Upon completion, the reaction solution was cooled to 0 °C, then a water solution of sodium nitrite (904 mg, 10.6 mmol) was slowly added, followed by slow addition of 2 N HCl during which the solution was stirred intensely and turned bright red, and gas evolved. Addition of 2 N HCl continued until gas evolution ceased and the pH value was 3-4. Then the reaction mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography to afford the titled compound as purple solid (75 mg, 27.5% yield). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.00 (d, J = 4.0 Hz, 1H), 7.21 (d, J = 4.0 Hz, 1H), 3.04 (s, 3H).

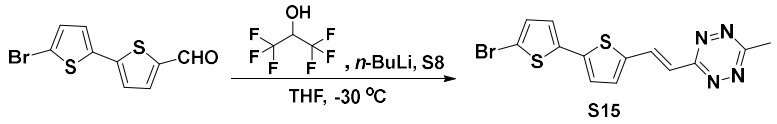


S14

Synthesis of (*E*)-3-(2-(5-bromothiophen-2-yl)vinyl)-6-methyl-1,2,4,5-tetrazine (S14)

To a solution of 1,1,1,3,3,3-hexafluoro-2-propanol (201.5 μL, 1.92 mmol) in dry THF (10 mL) was added 1.6 M n-BuLi (1.2 mL, 1.92 mmol) at -20 °C under argon protection. The reaction was stirred for 30 min at -20 °C and further cooled to -30 °C. The solution of THF containing 5-bromothiophene-2-carbaldehyde (100 mg, 0.52 mmol) and **S8** (200 mg, 0.82 mmol) was added to the mixture dropwise and stirred for 8 h at -30 °C. The reaction mixture was quenched by H_2O (20 mL) and extracted with EtOAc (3×20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel flash column chromatography to afford the titled compound as purple solid (82.5 mg,

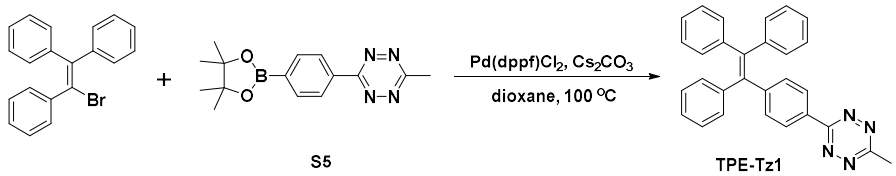
48.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, *J* = 15.9 Hz, 1H), 7.14 (s, 1H), 7.11 – 7.07 (m, 1H), 7.06 (d, *J* = 3.9 Hz, 1H), 3.03 (s, 3H).



Synthesis of (*E*)-3-(2-(5'-bromo-[2,2'-bithiophen]-5-yl)vinyl)-6-methyl-1,2,4,5-tetrazine (S15)

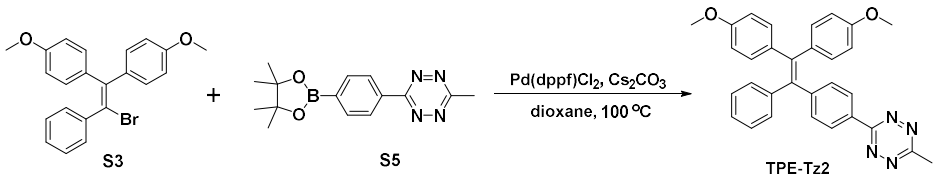
To a solution of 1,1,1,3,3,3-hexafluoro-2-propanol (201.5 μL, 1.92 mmol) in dry THF (10 mL) was added 1.6 M n-BuLi (1.2 mL, 1.92 mmol) at -20 °C under argon protection. The reaction was stirred for 30 min at -20 °C and further cooled to -30 °C. The solution of THF containing 5'-bromo-[2,2'-bithiophene]-5-carbaldehyde (200 mg, 0.73 mmol) and **S8** (180 mg, 0.73 mmol) was added to the mixture dropwise and stirred for 8 h at -30 °C. The reaction mixture was quenched by H_2O (20 mL) and extracted with EtOAc (3×20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel flash column chromatography to afford the titled compound as purple solid (180.0 mg, 67.4% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 – 8.29 (m, 1H), 7.72 (dt, *J* = 5.1, 1.0 Hz, 1H), 7.62 (dt, *J* = 3.7, 0.8 Hz, 1H), 7.24 – 7.11 (m, 2H), 2.90 (s, 3H).

Synthesis of 3-methyl-6-(4-(1,2,2-triphenylvinyl)phenyl)-1,2,4,5-tetrazine (TPE-Tz1)



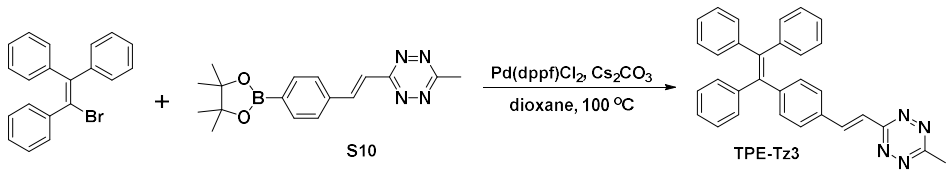
To a solution of (2-bromoethene-1,1,2-triyl)tribenzene (335.2 mg, 1.0 mmol) in dioxane (10 mL) was added **S5** (328 mg, 1.1 mmol), $Pd(dppf)Cl_2$ (2.9 mg, 0.04 mmol) and Cs_2CO_3 (971.4 mg, 3.0 mmol) under argon protection. The reaction mixture was stirred at 100°C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as pink solid (245 mg, 57.4% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, *J* = 8.5 Hz, 2H), 7.22 (s, 1H), 7.13 – 7.09 (m, 10H), 7.08 – 7.01 (m, 6H), 3.05 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 164.0, 148.6, 143.3, 143.2, 143.1, 142.6, 139.9, 132.3, 131.4, 131.3, 131.3, 129.6, 127.9, 127.9, 127.7, 127.3, 126.9, 126.8, 21.2; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{29}H_{23}N_4$ 427.1917; Found 427.1916.

Synthesis of 3-(4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)phenyl)-6-methyl-1,2,4,5-tetrazine (TPE-Tz2)



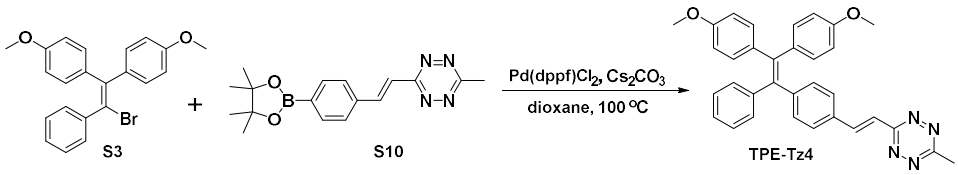
To a solution of **S3** (266 mg, 0.7 mmol) in 1,4-dioxane (20 mL) was added **S5** (200 mg, 0.7 mmol), Pd(dppf)Cl_2 (2.9 mg, 0.04 mmol) and Cs_2CO_3 (440 mg, 1.4 mmol) under argon protection. The reaction mixture was stirred at 100°C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as red solid (180 mg, 52.9% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.38 – 8.33 (m, 2H), 7.34 – 7.25 (m, 3H), 7.18 (m, 2H), 7.10 (m, 2H), 7.01 (m, 4H), 6.71 – 6.67 (m, 4H), 3.79 (s, 3H), 3.78 (s, 3H), 3.10 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 164.1, 158.5, 158.4, 149.3, 143.7, 141.9, 138.2, 135. 9, 135.9, 132.7, 132.7, 132.3, 131.4, 129.2, 127.9, 127.3, 126.5, 113.3, 113.1, 55.1, 21.2; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{31}H_{27}N_4O_2$ 487.2129; Found 487.2114.

Synthesis of (*E*)-3-methyl-6-(4-(1,2,2-triphenylvinyl)styryl)-1,2,4,5-tetrazine (TPE-Tz3)



To a solution of (2-bromoethene-1,1,2-triyl)tribenzene (204 mg, 0.61 mmol) in 1,4-dioxane (10 mL) was added **S10** (197.6 mg, 0.61 mmol), Pd(dppf)Cl_2 (1.5 mg, 0.02 mmol) and Cs_2CO_3 (592.5 mg, 1.83 mmol) under argon protection. The reaction mixture was stirred at 100°C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 3:1) to afford the titled compound as red solid (176.6 mg, 64.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 16.2 Hz, 2H), 7.46 – 7.29 (m, 3H), 7.15-7.09 (m, 11H), 7.07 – 7.00 (m, 6H), 3.02 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 164.8, 146.2, 143.45, 143.34, 142.09, 140.73, 140.19, 133.11, 132.03, 131.40, 131.37, 131.34, 127.90, 127.9, 127.7, 127.5, 126.8, 126.7, 126.7, 120.1, 21.2; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{31}H_{25}N_4$ 453.2001; Found 453.2035.

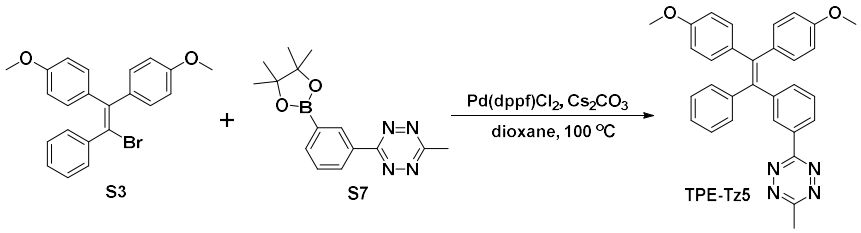
Synthesis of (*E*)-3-(4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)styryl)-6-methyl-1,2,4,5-tetrazine (TPE-Tz4)



To a solution of **S3** (90 mg, 0.23 mmol) in 1,4-dioxane (10 mL) was added **S10**

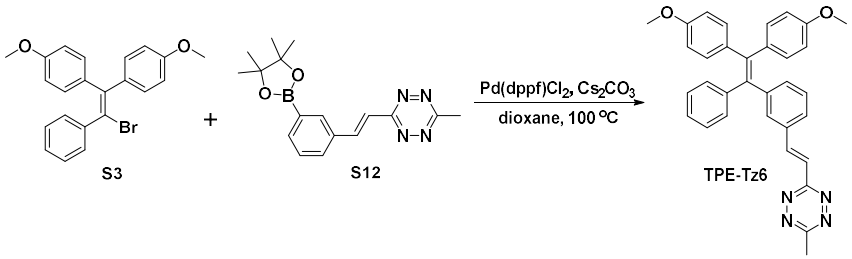
(81 mg, 0.25 mmol), Pd(dppf)Cl_2 (1.5 mg, 0.02 mmol) and Cs_2CO_3 (147.60 mg, 0.45 mmol) under argon protection. The reaction mixture was stirred at 100°C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as red solid (48.6 mg, 41.69% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (d, *J* = 16.2 Hz, 1H), 7.49 – 7.44 (m, 2H), 7.39 (dd, *J* = 16.3, 1.7 Hz, 1H), 7.19 – 7.11 (m, 5H), 7.08 (d, *J* = 6.4 Hz, 2H), 7.04 – 6.95 (m, 4H), 6.75 – 6.64 (m, 4H), 3.79 (s, 3H), 3.77 (s, 3H), 3.07 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.2, 164.9, 158.45, 158.3, 146.88, 143.9, 141.4, 140.8, 138.5, 136.1, 132.8, 132.8, 132.7, 132.1, 131.5, 127.9, 127.6, 126.4, 119.9, 113.3, 113.1, 55.2, 55.1, 21.2; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{33}H_{29}N_4O_2$ 513.2285; Found 513.2286.

Synthesis of 3-(3-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)phenyl)-6-methyl-1,2,4,5-tetrazine (TPE-Tz5)



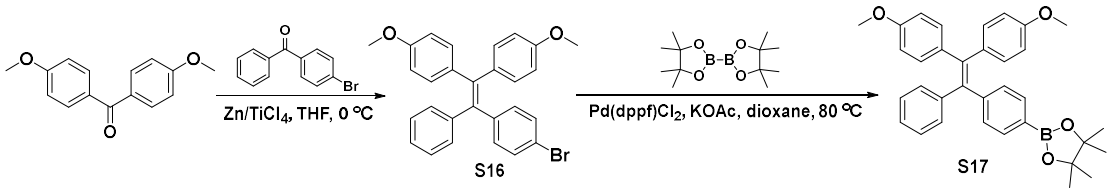
To a solution of **S3** (266 mg, 0.7 mmol) in 1,4-dioxane (20 mL) was added **S7** (200 mg, 0.7 mmol), Pd(dppf)Cl_2 (2.9 mg, 0.04 mmol) and Cs_2CO_3 (440 mg, 1.4 mmol) under argon protection. The reaction mixture was stirred at 100°C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as red solid (201 mg, 59.1% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.39 – 8.26 (m, 2H), 7.35 (m, 2H), 7.19 – 7.08 (m, 5H), 7.06 – 6.97 (m, 4H), 6.74 – 6.61 (m, 4H), 3.78 (d, *J* = 1.8 Hz, 3H), 3.73 (d, *J* = 2.0 Hz, 3H), 3.08 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1, 164.1, 158.3, 158.2, 145.8, 143.6, 141.3, 138.3, 135.9, 135.9, 135.7, 132.7, 132.6, 131.4, 131.4, 130.8, 128.7, 127.9, 126.4, 125.7, 113.2, 113.1, 55.1, 55.1, 21.1; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{31}H_{27}N_4O_2$ 487.2129; Found 487.2122.

Synthesis of (E)-3-(3-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)styryl)-6-methyl-1,2,4,5-tetrazine (TPE-Tz6)



To a solution of **S3** (90 mg, 0.23 mmol) in 1,4-dioxane (10 mL) was added **S12** (81 mg, 0.25 mmol), Pd(dppf)Cl_2 (1.5 mg, 0.02 mmol) and Cs_2CO_3 (147.60 mg, 0.45 mmol) under argon protection. The reaction mixture was stirred at 100°C using an oil bath for

8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as red solid (54 mg, 46.2% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 – 8.12 (m, 1H), 7.44 (d, *J* = 7.5 Hz, 2H), 7.27 – 7.20 (m, 2H), 7.19 – 7.07 (m, 6H), 7.01 (td, *J* = 9.0, 4.3 Hz, 4H), 6.70 (m, 4H), 3.79 (s, 3H), 3.77 (s, 3H), 3.07 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 164.8, 158.3, 158.2, 145.2, 143.8, 141.2, 141.0, 138.4, 136.1, 134.6, 133.4, 132.6, 132.6, 131.4, 131.2, 128.4, 127.9, 127.9, 126.4, 125.8, 120.2, 113.3, 113.1, 55.2, 55.1, 21.2; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{33}H_{29}N_4O_2$ 513.2285; Found 513.2280.



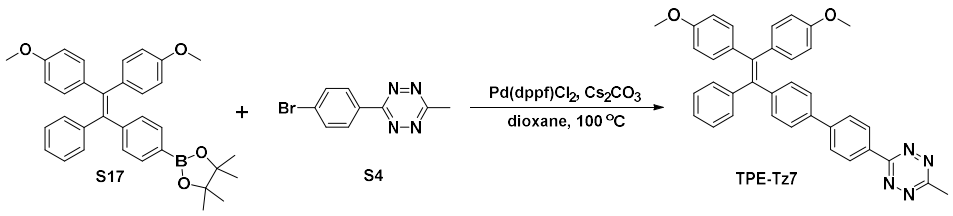
Synthesis of 4,4'-(2-(4-bromophenyl)-2-phenylethene-1,1-diyl)bismethoxybenzene (S16)

To a solution of 4,4'-dimethoxybenzophenone (2.0 mg, 8.26 mmol) in dry THF (20 mL) was added 4-bromobenzophenon (2.34 g, 9.0 mmol) and zinc powder (3.1 g, 33.0 mmol) under argon protection. The reaction solution was cooled to 0 °C using ice bath and 1 M $TiCl_4$ (33.0 mL, 33.0 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 4 h, then quenched by water. The reaction mixture was extracted with DCM. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 10:1) to afford the titled compound as white solid (1.6 g, 41.1% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 – 7.17 (m, 2H), 7.12 – 7.06 (m, 4H), 6.99 (dt, *J* = 7.2, 1.6 Hz, 3H), 6.95 – 6.85 (m, 4H), 6.68 – 6.57 (m, 4H), 3.75 (s, 3H), 3.72 (s, 3H).

Synthesis of 2-(4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (S17)

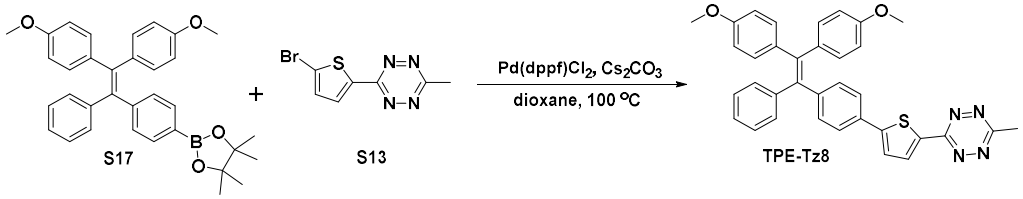
To a solution of **S16** (600 mg, 1.26 mmol) in dry 1,4-dioxane (15 mL) was added bis(pinacolato)diboron (186 mg, 1.92 mmol), $Pd(dppf)Cl_2$ (9.2 mg, 0.012 mmol) and KOAc (378 mg, 3.78 mmol) under argon protection. The reaction mixture was stirred at 80°C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as yellow solid (414 mg, 63.3% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 – 7.48 (m, 2H), 7.09 – 7.05 (m, 3H), 7.04 – 6.97 (m, 4H), 6.92 (dd, *J* = 8.7, 1.5 Hz, 4H), 6.65 – 6.57 (m, 4H), 3.73 (s, 3H), 3.72 (s, 3H), 1.31 (s, 12H).

Synthesis of 3-(4'-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)-[1,1'-biphenyl]-4-yl)-6-methyl-1,2,4,5-tetrazine (TPE-Tz7)



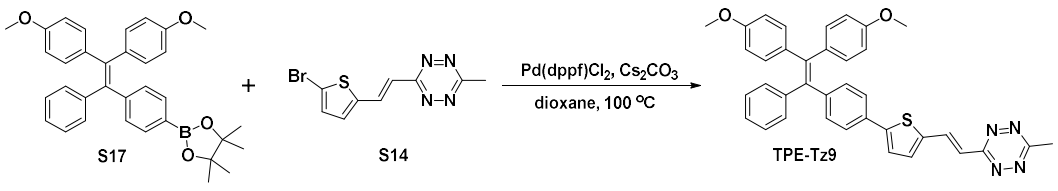
To a solution of **S17** (50 mg, 0.1 mmol) in 1,4-dioxane (5 mL) was added **S4** (24.2 mg, 0.1 mmol), $Pd(dppf)Cl_2$ (1.5 mg, 0.02 mmol) and Cs_2CO_3 (63 mg, 0.2 mmol) under argon protection. The reaction mixture was stirred at 100°C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as red solid (22 mg, 40.6% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 8.6 Hz, 2H), 7.77 (d, *J* = 8.6 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.12 (d, *J* = 8.2 Hz, 5H), 7.06 (dd, *J* = 7.8, 1.9 Hz, 2H), 6.99 (d, *J* = 8.9 Hz, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 6.65 (dd, *J* = 10.9, 8.8 Hz, 4H), 3.74 (s, 3H), 3.73 (s, 3H), 3.09 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.3, 132.8, 132.7, 132.1, 131.6, 128.4, 127.9, 127.6, 126.4, 126.3, 113.3, 113.1, 55.2, 21.2; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{37}H_{31}N_4O_2$ 563.2442; Found 563.2433.

Synthesis of 3-(5-(4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)phenyl)thiophen-2-yl)-6-methyl-1,2,4,5-tetrazine (TPE-Tz8)



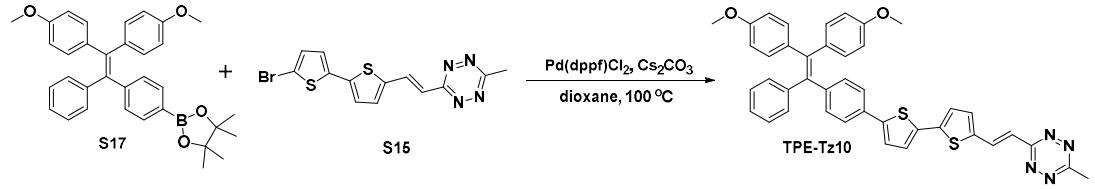
To a solution of **S17** (73 mg, 0.14 mmol) in 1,4-dioxane (5 mL) was added **S13** (36.1 mg, 0.14 mmol), $Pd(dppf)Cl_2$ (1.5 mg, 0.02 mmol) and Cs_2CO_3 (91.2 mg, 0.28 mmol) under argon protection. The reaction mixture was stirred at 100°C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as red solid (24.2 mg, 30.4% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 4.0 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 2H), 7.37 (d, *J* = 4.0 Hz, 1H), 7.16 – 7.01 (m, 7H), 6.96 (dd, *J* = 18.7, 8.8 Hz, 3H), 6.65 (dd, *J* = 15.2, 8.8 Hz, 4H), 3.74 (d, *J* = 4.3 Hz, 7H), 3.02 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 162.1, 158.4, 158.2, 151.5, 145.4, 144.0, 141.1, 138.4, 136.1, 136.1, 133.8, 132.7, 132.7, 132.6, 132.6, 132.6, 132.2, 132.2, 131.5, 131.4, 130.9, 127.9, 127.7, 126.4, 125.4, 124.5, 114.7, 113.3, 113.1, 113.0, 55.2, 55.1, 21.2; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{35}H_{29}N_4O_2S$ 569.1933; Found 569.1946.

Synthesis of (*E*)-3-(2-(5-(4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)phenyl)thiophen-2-yl)vinyl)-6-methyl-1,2,4,5-tetrazine (TPE-Tz9)



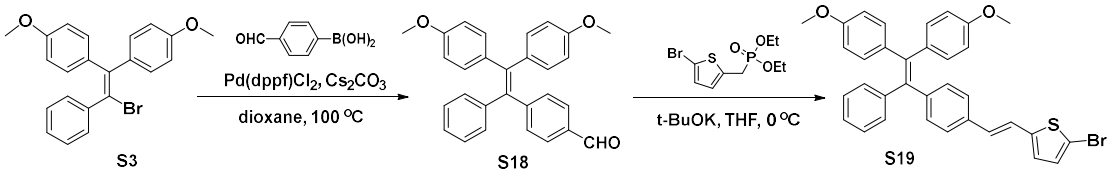
To a solution of **S17** (42 mg, 0.08 mmol) in 1,4-dioxane (5 mL) was added **S14** (23.0 mg, 0.08 mmol), $Pd(dppf)Cl_2$ (1.5 mg, 0.02 mmol) and Cs_2CO_3 (52.5 mg, 0.16 mmol) under argon protection. The reaction mixture was stirred at 100 °C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 3:1) to afford the titled compound as red solid (12.1 mg, 25.5% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (dd, *J* = 15.9, 0.6 Hz, 1H), 7.38 (d, *J* = 8.5 Hz, 2H), 7.28 (d, *J* = 3.9 Hz, 1H), 7.13 – 7.09 (m, 3H), 7.04 (m, 4H), 6.95 (dd, *J* = 18.1, 8.8 Hz, 5H), 6.65 (dd, *J* = 14.3, 8.8 Hz, 5H), 3.74 (s, 3H), 3.73 (s, 3H), 3.02 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 164.9, 158.3, 158.2, 147.7, 144.9, 144.0, 140.9, 139.5, 138.4, 136.2, 133.6, 132.7, 132.6, 132.3, 132.1, 131.5, 131.1, 127.9, 126.3, 125.2, 123.9, 118.8, 113.2, 113.1, 55.2, 55.1, 21.2; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{37}H_{31}N_4O_2S$ 595.2162; Found 595.2203.

Synthesis of (*E*)-3-(2-(5'-(4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)phenyl)-[2,2'-bithiophen]-5-yl)vinyl)-6-methyl-1,2,4,5-tetrazine (TPE-Tz10)



To a solution of **S17** (129 mg, 0.25 mmol) in 1,4-dioxane (15 mL) was added **S15** (100 mg, 0.27 mmol), $Pd(dppf)Cl_2$ (1.5 mg, 0.02 mmol) and Cs_2CO_3 (242 mg, 0.75 mmol) under argon protection. The reaction mixture was stirred at 100 °C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 2:1) to afford the titled compound as red solid (42.4mg, 26.1% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, *J* = 15.8 Hz, 1H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.22 – 7.16 (m, 3H), 7.15 – 7.09 (m, 5H), 7.06 – 7.00 (m, 4H), 6.97 (d, *J* = 8.7 Hz, 2H), 6.93 (d, *J* = 8.6 Hz, 2H), 6.74 – 6.55 (m, 4H), 3.74 (s, 3H), 3.73 (s, 3H), 3.02 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 164.8, 158.3, 144.7, 140.7, 136.2, 133.2, 132.7, 132.3, 132.1, 131.5, 127.8, 126.3, 125.8, 124.9, 124.3, 123.8, 118.9, 113.2, 113.0, 55.1, 55.1, 21.2; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{41}H_{33}N_4O_2S_2$ 651.1844; Found 651.1868.

Synthesis of 4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)benzaldehyde (S18)

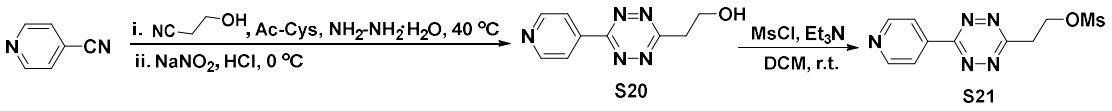


To a solution of **S3** (200 mg, 0.5 mmol) in 1,4-dioxane (15 mL) was added 4-formylphenylboronic acid (91.0 mg, 0.6 mmol), $Pd(dppf)Cl_2$ (1.5 mg, 0.02 mmol) and Cs_2CO_3 (328.0 mg, 1.0 mmol) under argon protection. The reaction mixture was stirred at 100 °C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as

yellow solid (197.9 mg, 93.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 7.60 (d, *J* = 8.3 Hz, 2H), 7.19 – 7.07 (m, 5H), 7.04 – 6.97 (m, 2H), 6.94 – 6.89 (m, 4H), 6.65 – 6.60 (m, 4H), 3.73 (s, 3H), 3.73 (s, 3H).

Synthesis of (*E*)-2-(4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)styryl)-5-bromothiophene (S19)

To a solution of **S18** (198 mg, 0.47 mmol) in dry THF (15 mL) was added diethyl ((5-bromothiophen-2-yl)methyl)phosphonate (176.9 mg, 0.56 mmol). The reaction solution was cooled to 0 °C using an ice bath and then t-BuOK (74.0 mg, 0.66 mmol) was added. The reaction mixture was stirred at room temperature for 3 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 10:1) to afford the titled compound as yellow solid (211.2 mg, 77.4% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 (d, *J* = 8.1 Hz, 2H), 7.15 (d, *J* = 7.5 Hz, 3H), 7.09 – 6.95 (m, 10H), 6.80 – 6.76 (m, 2H), 6.72 – 6.64 (m, 4H), 3.79 (s, 3H), 3.78 (s, 3H).



Synthesis of 2-(6-(pyridin-4-yl)-1,2,4,5-tetrazin-3-yl)ethan-1-ol (S20)

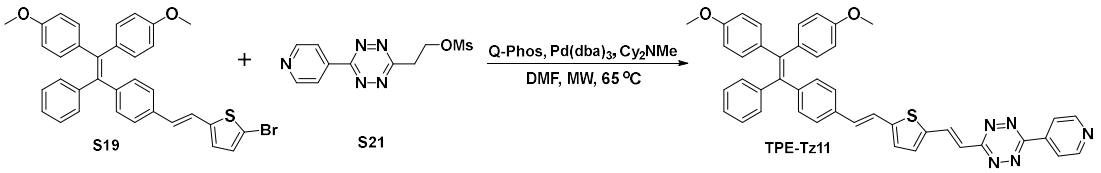
To a solution of 4-cyanopyridine (200 mg, 1.92 mmol) in 3-hydroxypropanenitrile (675 μL, 9.6 mmol) was added *N*-acetyl-L-cysteine (313.5 mg, 1.92 mmol) under argon protection. The reaction solution was cooled to 0 °C using an ice bath and the hydrazine hydrate (1.49 mL, 30.7 mmol) was added dropwise, and then the reaction mixture was stirred at 40 °C using an oil bath for 16 h. Upon completion, the reaction solution was cooled to 0 °C, then a water solution of sodium nitrite (1.63 g, 19.2 mmol) was slowly added, followed by slow addition of 2 N HCl during which the solution was stirred intensely and turned bright red, and gas evolved. Addition of 2 N HCl continued until gas evolution ceased and the pH value was 3-4. Then the reaction mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography to afford the titled compound as purple solid (136 mg, 34.9% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (d, *J* = 6.1 Hz, 2H), 8.34 (d, *J* = 6.2 Hz, 2H), 4.80 (t, *J* = 5.9 Hz, 1H), 3.99 (q, *J* = 5.5, 5.0 Hz, 2H), 3.45 (t, *J* = 6.3 Hz, 2H).

Synthesis of 2-(6-(pyridin-4-yl)-1,2,4,5-tetrazin-3-yl)ethyl methanesulfonate (S21)

To a solution of **S20** (320 mg, 1.57 mmol) in dry DCM (30 mL) was added TEA (175.3 mg, 1.73 mmol) under argon protection. The reaction solution was cooled to 0 °C using an ice bath and the methanesulfonyl chloride (282 μL, 1.73 mmol) was added dropwise, and then the reaction mixture was stirred at room temperature for 2 h, then concentrated. The residue was purified by silica gel flash column chromatography (DCM/MeOH = 20:1) to afford the titled compound as purple solid (208 mg, 47.2% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (d, *J* = 5.3 Hz, 2H), 8.50 (d, *J* = 5.1 Hz, 2H),

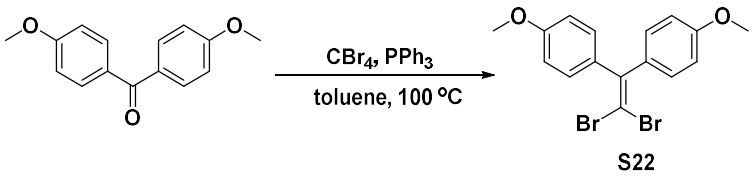
4.98 (t, *J* = 6.0 Hz, 2H), 3.93 (t, *J* = 6.0 Hz, 2H), 3.08 (s, 3H).

Synthesis of 3-((*E*)-2-(5-((*E*)-4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)styryl) thiophen-2-yl)vinyl)-6-(pyridin-4-yl)-1,2,4,5-tetrazine (TPE-Tz11)



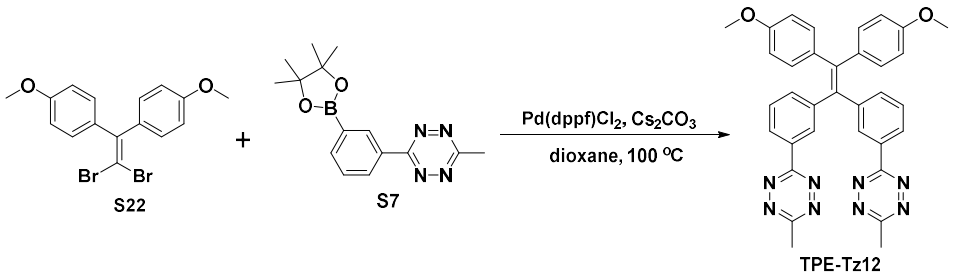
To a 10 mL microwave reaction tube equipped with a stir bar, **S19** (56 mg, 0.1 mmol), **S21** (24 mg, 0.08 mmol), Q-phos (9.0 mg, 0.01 mmol), $Pd_2(dba)_3$ (8.0 mg, 0.008 mmol) and *N*,*N*-dicyclohexylmethylamine (51.0 mg, 0.26 mmol) were dissolved in anhydrous DMF (4 mL). The reaction was protected with argon gas and then heated by microwave irradiation (65 °C, 60 min). The reaction solution was cooled to room temperature and EtOAc (30 mL) was added before washing with water (3×20 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by a preparative TLC plate to afford the titled compound as orange solid (20.4 mg, 35.5% yield). ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.92 (d, *J* = 5.2 Hz, 2H), 8.52 (d, *J* = 15.8 Hz, 1H), 8.44 (d, *J* = 5.1 Hz, 2H), 7.59 (d, *J* = 3.8 Hz, 1H), 7.48 – 7.38 (m, 3H), 7.33 – 7.26 (m, 2H), 7.15 (dd, *J* = 13.3, 7.6 Hz, 4H), 7.11 – 6.92 (m, 8H), 6.72 (dd, *J* = 19.6, 8.2 Hz, 4H), 3.76 (s, 3H), 3.74 (s, 3H); ^{13}C NMR (101 MHz, Acetone-d_6) δ 165.6, 161.6, 158.6, 158.4, 151.0, 147.1, 144.7, 144.217, 140.9, 139.9, 139.2, 138.8, 136.2, 136.1, 134.5, 134.3, 133.4, 132.4, 132.4, 131.7, 131.2, 130.4, 123.0, 127.8, 126.3, 126.1, 121.2, 120.8, 119.0, 113.1, 113.0, 54.5, 54.5; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{43}H_{34}N_5O_2S$ 684.2428; Found 684.2408.

Synthesis of 4,4'-(2,2-dibromoethene-1,1-diyl)bis(methoxybenzene) (S22)



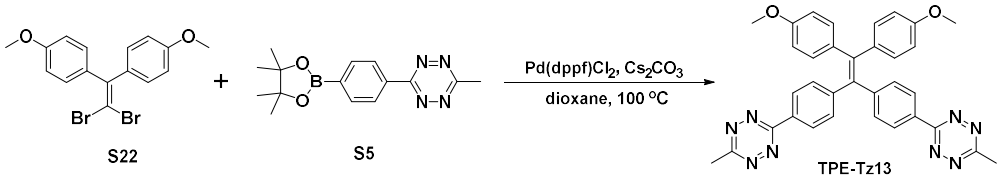
To a solution of carbon tetrabromide (1.1 g, 3.3 mmol) in toluene (30 mL) was added triphenylphosphine (1.7 mg, 6.6 mmol) under argon protection. The reaction solution was cooled to 0 °C using an ice bath and then bis(4-methoxyphenyl)methanone (400 mg, 1.6 mmol) was added. The reaction mixture was stirred at 100 °C for 24 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 10:1) to afford the titled compound as yellow solid (112 mg, 17.04% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 – 7.23 (m, 4H), 6.90 – 6.87 (m, 4H), 3.84 (s, 6H).

Synthesis of 6,6'-((2,2-bis(4-methoxyphenyl)ethene-1,1-diyl)bis(3,1-phenylene)) bis(3-methyl-1,2,4,5-tetrazine) (TPE-Tz12)

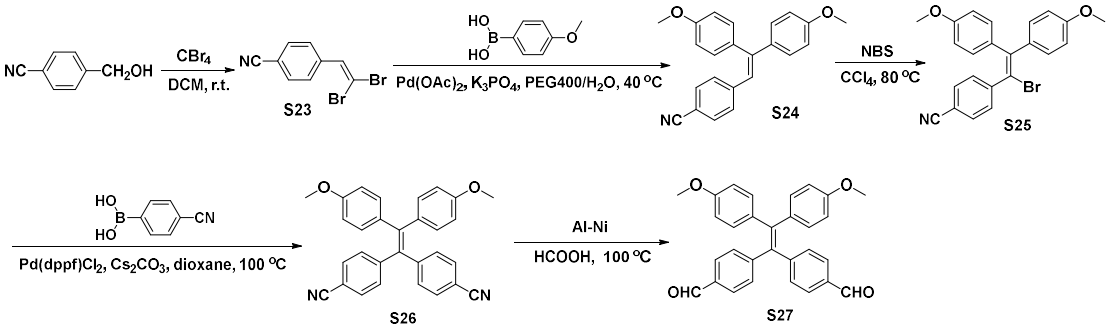


To a solution of **S22** (40 mg, 0.1 mmol) in 1,4-dioxane (3 mL) was added **S7** (60 mg, 0.2 mmol), $Pd(dppf)Cl_2$ (1.5 mg, 0.02 mmol) and Cs_2CO_3 (52 mg, 0.4 mmol) under argon protection. The reaction mixture was stirred at 100 °C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 3:1) to afford the titled compound as red solid (21.2mg, 18.3% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 – 8.29 (m, 4H), 7.33 (dd, *J* = 5.2, 0.8 Hz, 4H), 7.00 (d, *J* = 8.8 Hz, 4H), 6.63 (d, *J* = 8.8 Hz, 4H), 3.70 (s, 6H), 3.02 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1, 164.0, 158.5, 145.1, 142.6, 137.3, 135.7, 135.5, 132.7, 131.6, 130.7, 128.9, 126.0, 113.3, 55.1, 21.1; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{34}H_{29}N_8O_2$ 581.2408; Found 581.2408.

Synthesis of 6,6'-((2,2-bis(4-methoxyphenyl)ethene-1,1-diyl)bis(4,1-phenylene)) bis(3-methyl-1,2,4,5-tetrazine) (TPE-Tz13)



To a solution of **S22** (40 mg, 0.1 mmol) in 1,4-dioxane (3 mL) was added **S5** (60 mg, 0.2 mmol), $Pd(dppf)Cl_2$ (1.5 mg, 0.02 mmol) and Cs_2CO_3 (52 mg, 0.4 mmol) under argon protection. The reaction mixture was stirred at 100 °C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 3:1) to afford the titled compound as red solid (24.4 mg, 21.1% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, *J* = 8.5 Hz, 4H), 7.27 (d, *J* = 2.0 Hz, 2H), 7.25 (d, *J* = 0.9 Hz, 2H), 7.00 (d, *J* = 8.8 Hz, 4H), 6.66 (d, *J* = 8.8 Hz, 4H), 3.74 (s, 6H), 3.06 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 164.0, 158.8, 148.61, 143.6, 137.2, 135.4, 132.8, 132.4, 130.1, 129.6, 127.6, 116.3, 113.4, 55.2, 21.2; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{34}H_{29}N_8O_2$ 581.2335; Found 581.2369.



Synthesis of 4-(2,2-dibromovinyl)benzonitrile (S23)

To a solution of carbon tetrabromide (1.0 g, 3.0 mmol) in DCM (30 mL) was added triphenylphosphine (1.6 g, 6.1 mmol) under argon protection. The reaction solution was cooled to 0 °C using an ice bath and then benzaldehyde (200 mg, 1.5 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 20:1) to afford the titled compound as yellow oil (395 mg, 90.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 – 7.59 (m, 4H), 7.49 (s, 1H).

Synthesis of 4-(2,2-bis(4-methoxyphenyl)vinyl)benzonitrile (S24)

To a solution of **S23** (200 mg, 0.7 mmol) in a mixture of PEG400 and water (V_{PEG400} : V_{H_2O} = 1:3) was added 4-methoxyphenylboronic acid (265 mg, 1.7 mmol), $Pd(OAc)_2$ (6.3 mg, 0.04 mmol) and K_3PO_4 (296 mg, 1.4 mmol). The reaction mixture was stirred at 40 °C using an oil bath for 24 h under argon protection. Then the reaction was quenched with H_2O (10 mL) and extracted with EtOAc (3×20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was isolated by silica gel flash column chromatography to afford the titled compound (157.6 mg, 66.2% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.5 Hz, 2H), 7.26 (s, 1H), 7.24 (s, 1H), 7.10 – 7.01 (m, 4H), 6.86 (d, *J* = 2.7 Hz, 2H), 6.84 (d, *J* = 2.7 Hz, 2H), 6.78 (s, 1H), 3.83 (s, 3H), 3.81 (s, 3H).

Synthesis of 4-(1-bromo-2,2-bis(4-methoxyphenyl)vinyl)benzonitrile (S25)

To a solution of **S24** (500 mg, 1.5 mmol) in CCl_4 (20 mL) was added NBS (391.0 mg, 2.2 mmol). The reaction mixture was stirred at 80 °C using an oil bath for 3 h and then concentrated. The residue was isolated by silica gel flash column chromatography to afford the titled compound (582.4 mg, 94.6% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 8.5 Hz, 2H), 7.26 (s, 2H), 7.18 – 7.14 (m, 4H), 7.01 (d, *J* = 2.7 Hz, 2H), 6.98 (d, *J* = 2.7 Hz, 2H), 3.86 (s, 3H), 3.84 (s, 3H).

Synthesis of 4,4'-(2,2-bis(4-methoxyphenyl)ethene-1,1-diyl)dibenzonitrile (S26)

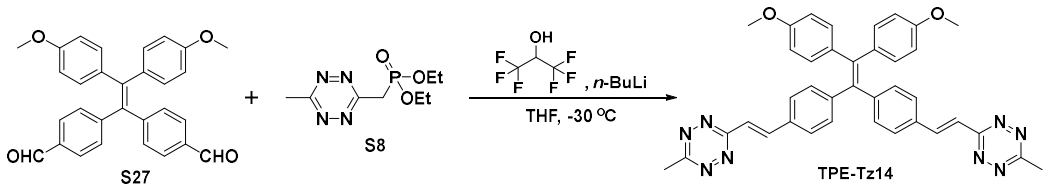
To a solution of **S25** (620 mg, 1.47 mmol) in 1,4-dioxane (20 mL) was added 4-cyanophenylboronic acid (217 mg, 1.47 mmol), $Pd(dppf)Cl_2$ (11 mg, 0.01 mmol) and Cs_2CO_3 (1.43 g, 4.4 mmol) under argon protection. The reaction mixture was stirred at 100 °C using an oil bath for 8 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as yellow solid (550 mg, 84.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.8 (d, *J* = 8.4 Hz, 4H), 7.28 (d, *J* = 8.2 Hz, 4H), 7.12 (d, *J* = 8.9 Hz, 4H), 6.82 (d, *J* = 8.8 Hz, 4H), 3.84 (s, 6H).

Synthesis of 4,4'-(2,2-bis(4-methoxyphenyl)ethene-1,1-diyl)dibenzaldehyde (S27)

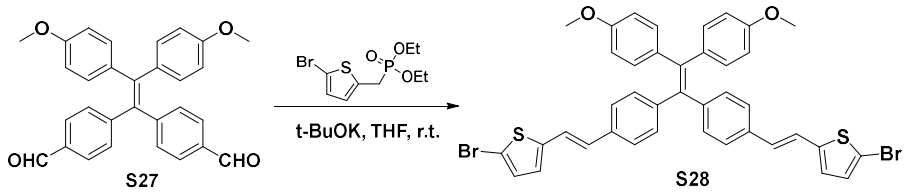
To a solution of **S26** (300 mg, 0.67 mmol) in 75% HCOOH (12 mL) was added Al-Ni (3.23 g, 4.47 mmol) under argon protection. The reaction mixture was stirred at 100 °C using an oil bath for 5 h. The mixture was extracted with EtOAc (3×20 mL).

The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 5:1) to afford the titled compound as luminous yellow solid (200 mg, 65.8% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.90 (s, 2H), 7.63 (d, *J* = 8.4 Hz, 4H), 7.15 (d, *J* = 8.2 Hz, 4H), 6.92 (d, *J* = 8.9 Hz, 4H), 6.64 (d, *J* = 8.8 Hz, 4H), 3.74 (s, 6H).

Synthesis of 6,6'-((1*E*,1'*E*)-((2,2-bis(4-methoxyphenyl)ethene-1,1-diyl)bis(4,1-phenylene))bis(ethene-2,1-diyl))bis(3-methyl-1,2,4,5-tetrazine) (TPE-Tz14)



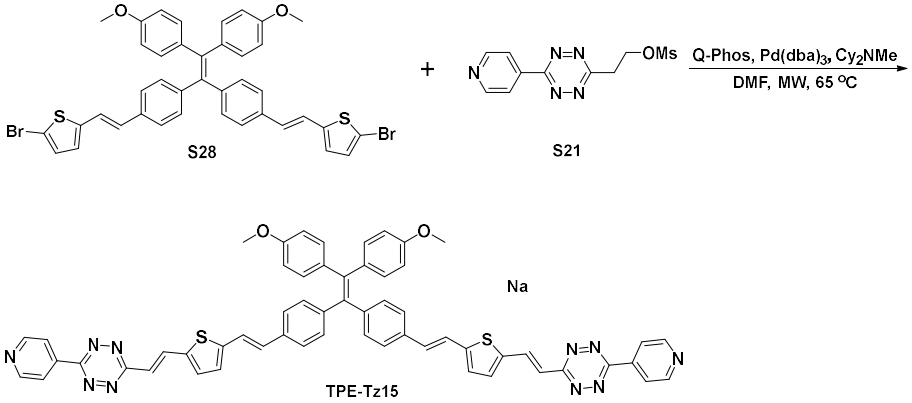
To a solution of 1,1,1,3,3,3-hexafluoro-2-propanol (201.5 μL, 1.92 mmol) in dry THF (10 mL) was added 1.6 M n-BuLi (1.2 mL, 1.92 mmol) at -20 °C under argon protection. The reaction was stirred for 30 min at -20 °C and further cooled to -30 °C. The solution of THF containing **S27** (200 mg, 0.44 mmol) and **S8** (182 mg, 0.98 mmol) was added to the mixture dropwise and stirred for 8 h at -30 °C. The reaction mixture was quenched by H_2O (20 mL) and extracted with EtOAc (3×20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel flash column chromatograph (PE/EtOAc = 2:1) to afford the title compound as red solid (68.4 mg, 24.3% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 16.3 Hz, 2H), 7.44 (d, *J* = 8.1 Hz, 4H), 7.36 (d, *J* = 16.2 Hz, 2H), 7.10 (d, *J* = 8.0 Hz, 4H), 6.97 (d, *J* = 8.7 Hz, 4H), 6.66 (d, *J* = 8.8 Hz, 4H), 3.74 (s, 6H), 3.03 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 164.9, 158.6, 146.4, 142.5, 140.7, 137.6, 135.7, 133.1, 132.8, 132.1, 127.7, 120.1, 113.3, 55.2, 21.2; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{38}H_{33}N_8O_2$ 633.2682; Found 633.2648.



Synthesis of 5,5'-((1*E*,1'*E*)-((2,2-bis(4-methoxyphenyl)ethene-1,1-diyl)bis(4,1-phenylene))bis(ethene-2,1-diyl))bis(2-bromothiophene) (S28)

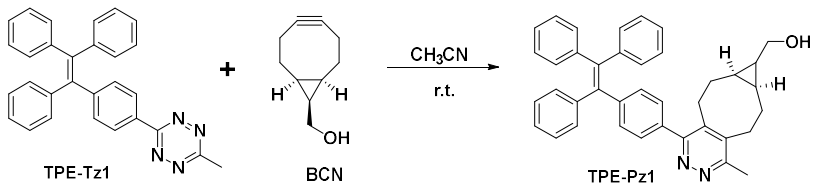
To a solution of **S27** (90 mg, 0.2 mmol) in dry THF (15 mL) was added diethyl ((5-bromothiophen-2-yl)methyl)phosphonate (156.6 mg, 0.5 mmol). The reaction solution was cooled to 0 °C using an ice bath and then t-BuOK (56.3 mg, 0.5 mmol) was added. The reaction mixture was stirred at room temperature for 3 h, then concentrated. The residue was purified by silica gel flash column chromatography (PE/EtOAc = 10:1) to afford the titled compound as yellow solid (48.6 mg, 31.7% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (d, *J* = 8.0 Hz, 4H), 7.04 – 6.88 (m, 12H), 6.75-6.72 (m, 3H), 6.70 – 6.61 (m, 5H), 3.74 (s, 6H).

Synthesis of 6,6'-((1*E*,1'*E*)-(((1*E*,1'*E*)-((2,2-bis(4-methoxyphenyl)ethene-1,1-diyl) bis(4,1-phenylene))bis(ethene-2,1-diyl))bis(thiophene-5,2-diyl))bis(ethene-2,1-diyl))bis(3-(pyridin-4-yl)-1,2,4,5-tetrazine) (TPE-Tz15)



To a 10 mL microwave reaction tube equipped with a stir bar, **S28** (54 mg, 0.09 mmol), **S21** (66 mg, 0.24 mmol), Q-phos (16.6 mg, 0.024 mmol), $Pd_2(dba)_3$ (8.8 mg, 0.01 mmol) and *N*,*N*-dicyclohexylmethylamine (72.8 mg, 0.36 mmol) were dissolved in anhydrous DMF (4 mL). The reaction was protected with argon gas and then heated by microwave irradiation (65 °C, 60 min). The reaction solution was cooled to room temperature and EtOAc (30 mL) was added before washing with water (3×20 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by a preparative TLC plate to afford the titled compound as red solid (32.0 mg, 35.2% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 – 8.84 (m, 2H), 8.44 (d, J = 15.9 Hz, 1H), 8.35 – 8.29 (m, 2H), 7.62 (d, J = 3.8 Hz, 1H), 7.46 – 7.19 (m, 10 H), 7.13 (t, J = 3.6 Hz, 2H), 7.05 – 6.82 (m, 12H), 6.77 (d, J = 15.4 Hz, 1H), 6.72 – 6.65 (m, 5H), 3.65 (s, 6H); ^{13}C NMR (151 MHz, Acetone) δ 166.4, 162.5, 159.5, 151.9, 147.9, 145.9, 145.4, 144.9, 142.1, 140.7, 140.2, 139.3, 137.0, 135.6, 135.5, 135.2, 134.3, 133.4, 133.4, 133.2, 132.7, 132.6, 131.9, 131.3, 129.4, 128.9, 128.6, 127.8, 127.3, 127.1, 126.8, 126.6, 125.5, 122.1, 121.9, 121.7, 119.9, 114.0, 114.0, 111.1, 55.4; HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{58}H_{42}N_{10}O_2S_2Na$ 997.2826; Found 997.2893.

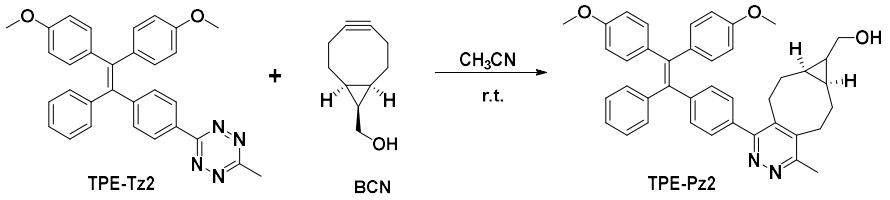
Synthesis of ((6aR,7aS)-1-methyl-4-(4-(1,2,2-triphenylvinyl)phenyl)-6,6a,7,7a,8,9-hexahydro-5*H*-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz1)



To a solution of **TPE-Tz1** (20 mg, 0.046 mmol) in CH_3CN (5 mL) was added **BCN** (14.0 mg, 0.092). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as white solid (22.4 mg, 88.9% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (d, J = 8.3 Hz, 2H), 7.12 – 7.01 (m, 17H), 3.70 (d, J = 7.8 Hz, 1H), 3.02 – 2.98 (m, 1H), 2.86 – 2.80 (m, 2H), 2.73 (s, 4H), 2.45 –

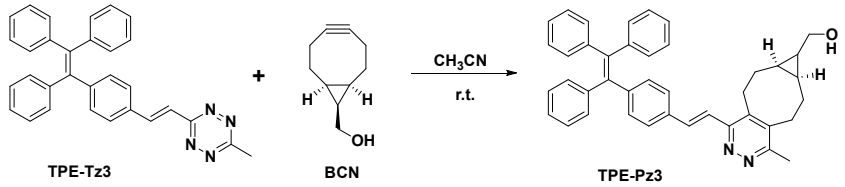
2.32 (m, 1H), 2.14 – 2.03 (m, 1H), 1.33 – 1.27 (m, 2H), 1.16 – 1.17 (m, 1H), 0.96 – 0.87 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.8, 157.5, 144.0, 143.7, 143.6, 143.4, 141.6, 140.8, 140.5, 139.6, 135.9, 131.5, 131.4, 131.4, 131.3, 128.6, 127.7, 127.7, 127.7, 126.6, 126.6, 126.6, 59.4, 29.7, 29.4, 27.9, 27.3, 20.6; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{39}H_{37}N_2O$ 549.2900; Found 549.2907.

Synthesis of ((6aS,7aR)-1-(4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)phenyl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5H-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz2)



To a solution of **TPE-Tz2** (20 mg, 0.041 mmol) in CH_3CN (5 mL) was added **BCN** (12.4 mg, 0.082). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as white solid (22.3 mg, 89.2% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 (d, *J* = 7.9 Hz, 2H), 7.19 – 7.08 (m, 7H), 7.01 (dd, *J* = 8.6, 4.1 Hz, 4H), 6.71 – 6.66 (m, 4H), 3.77- 3.75 (m, 9H), 3.05 (m, 1H), 2.87 (m, 2H), 2.78 (s, 4H), 2.48 – 2.38 (m, 1H), 2.14 – 2.12 (m, 1H), 1.33 – 1.31 (m, 1H), 1.18 – 1.15 (m, 1H), 0.96 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.9, 158.2, 158.2, 157.5, 144.6, 143.9, 140.8, 140.7, 138.8, 136.3, 136.2, 135.7, 132.7, 132.6, 131.5, 131.30, 128.6, 127.8, 126.3, 113.1, 113.1, 59.4, 55.2, 55.1, 27.9, 27.2, 20.7; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{41}H_{41}N_2O_3$ 609.3112; Found 609.3116.

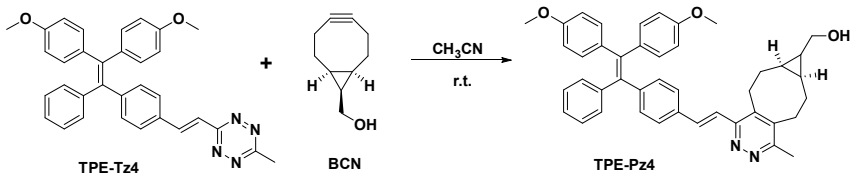
Synthesis of ((6aR,7aS)-1-methyl-4-((*E*)-4-(1,2,2-triphenylvinyl)styryl)-6,6a,7,7a,8,9-hexahydro-5*H*-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz3)



To a solution of **TPE-Tz3** (20 mg, 0.044 mmol) in CH_3CN (5 mL) was added **BCN** (13.3 mg, 0.088). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as white solid (20.3 mg, 79.9% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 15.7 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.17 – 6.90 (m, 18H), 3.71 (d, *J* = 7.8 Hz, 2H), 3.00 – 2.82 (m, 3H), 2.70 (s, 3H), 2.33 (p, *J* = 6.4 Hz, 2H), 1.29 (d, *J* = 19.7 Hz, 1H), 1.09 (q, *J* = 8.3 Hz, 1H), 0.92 – 0.74 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.9, 155.2, 144.3, 143.7, 143.7, 143.6, 141.4, 140.5, 135.6, 134.8, 131.8, 131.4, 131.4, 127.9, 127.8, 127.7, 126.7, 126.6, 126.6, 126.5, 120.9, 59.5, 29.7, 27.1, 26.2, 20.9; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{41}H_{39}N_2O$

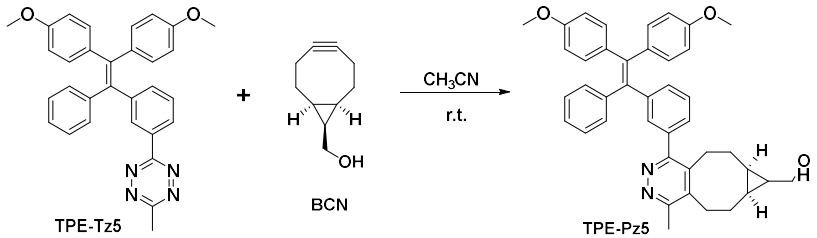
575.2984; Found 575.3018.

Synthesis of ((6aS,7aR)-1-((*E*)-4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)styryl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5*H*-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz4)



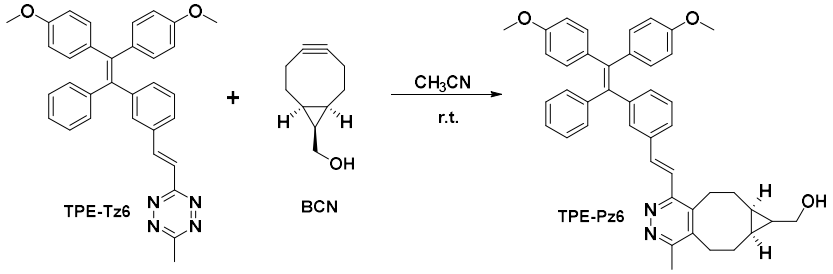
To a solution of **TPE-Tz4** (20 mg, 0.04 mmol) in CH_3CN (5 mL) was added **BCN** (12.0 mg, 0.08). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as yellow solid (18.0 mg, 70.7% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 15.6 Hz, 1H), 7.34 – 7.29 (m, 2H), 7.25 – 7.13 (m, 1H), 7.13 – 7.07 (m, 3H), 7.05 – 6.99 (m, 4H), 6.98 – 6.89 (m, 4H), 6.69 – 6.58 (m, 4H), 3.74 (s, 3H), 3.72 (s, 4H), 3.70 (s, 1H), 3.00 – 2.81 (m, 4H), 2.69 (s, 3H), 2.35 – 2.33 (m, 2H), 1.60 (d, *J* = 6.9 Hz, 2H), 1.10 (p, *J* = 8.3 Hz, 1H), 0.82 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.3, 158.2, 157.1, 155.3, 145.0, 144.2, 140.7, 138.8, 136.4, 135.6, 134.5, 132.7, 131.9, 131.5, 127.9, 126.8, 126.3, 120.9, 113.2, 113.1, 59.5, 55.2, 55.2, 27.2, 26.2, 21.0; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{43}H_{43}N_2O_3$ 635.3268; Found 635.3267.

Synthesis of ((6aS,7aR)-1-(3-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)phenyl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5*H*-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz5)



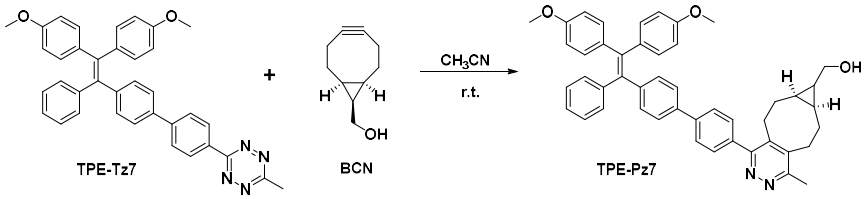
To a solution of **TPE-Tz5** (20 mg, 0.041 mmol) in CH_3CN (5 mL) was added **BCN** (12.4 mg, 0.082). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as white solid (19.8 mg, 79.27% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 (dd, *J* = 8.8, 2.7 Hz, 2H), 7.20 – 7.06 (m, 7H), 7.06 – 7.01 (m, 2H), 6.98 – 6.93 (m, 2H), 6.72 – 6.63 (m, 4H), 3.77 (d, *J* = 5.3 Hz, 6H), 3.69 – 3.67(m, 2H), 3.04 – 2.91 (m, 1H), 2.81 (d, *J* = 14.2 Hz, 1H), 2.76 (s, 3H), 2.51 (m, 1H), 2.44 – 2.33 (m, 2H), 1.62 (m, 1H), 1.30 (q, *J* = 6.8 Hz, 3H), 1.09 (t, *J* = 8.4 Hz, 1H), 0.97 – 0.54 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.0, 158.2, 158.0, 157.5, 144.3, 144.2, 140.6, 138.9, 137.5, 136.4, 136.1, 132.9, 132.6, 132.2, 131.5, 128.1, 127.8, 127.2, 126.2, 113.2, 113.1, 59.2, 55.2, 55.1, 27.6, 27.0, 22.4, 20.4; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{41}H_{41}N_2O_3$ 609.3112; Found 609.3107.

Synthesis of ((6aS,7aR)-1-((*E*)-3-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)styryl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5*H*-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz6)



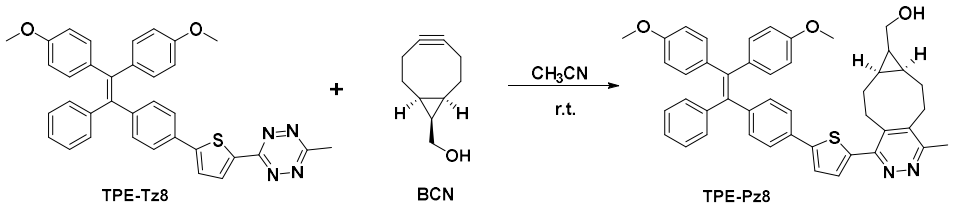
To a solution of **TPE-Tz6** (20 mg, 0.039 mmol) in CH_3CN (5 mL) was added **BCN** (11.7 mg, 0.078). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as white solid (20.5 mg, 82.8% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 15.7 Hz, 1H), 7.34 (s, 2H), 7.15 (q, *J* = 9.5, 9.0 Hz, 4H), 7.11 – 7.05 (m, 3H), 7.04 – 6.96 (m, 5H), 6.68 (t, *J* = 8.1 Hz, 4H), 3.76 (d, *J* = 14.0 Hz, 8H), 2.98 (q, *J* = 7.7, 6.7 Hz, 2H), 2.94 – 2.86 (m, 2H), 2.75 (s, 3H), 1.65 (s, 2H),2.45 – 2.25 (m, 2H), 1.15 (t, *J* = 8.6 Hz, 1H), 0.85 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.1, 156.9, 155.2, 144.6, 144.0, 140.5, 140.2, 138.9, 136.3, 136.3, 136.2, 136.1, 132.6, 131.8, 131.4, 130.2, 128.2, 127.8, 126.2, 125.6, 120.8, 113.2, 113.1, 59.4, 55.2, 55.1, 27.1, 26.1, 20.8; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{43}H_{43}N_2O_3$ 635.3268; Found 635.3276.

Synthesis of ((6aS,7aR)-1-(4'-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)-[1,1'-biphenyl]-4-yl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5*H*-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz7)



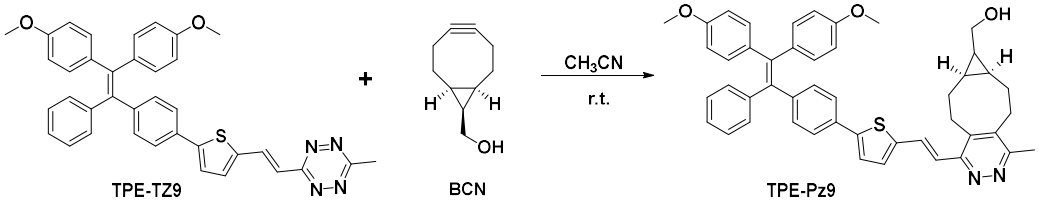
To a solution of **TPE-Tz7** (20 mg, 0.035 mmol) in CH_3CN (5 mL) was added **BCN** (10.6 mg, 0.07). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as yellow solid (21.8 mg, 89.8% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, *J* = 7.8 Hz, 2H), 7.54 (d, *J* = 7.8 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.14 (dt, *J* = 16.2, 8.3 Hz, 7H), 7.04 (d, *J* = 8.2 Hz, 2H), 7.00 (d, *J* = 8.2 Hz, 2H), 6.70 (dd, *J* = 11.8, 8.3 Hz, 4H), 3.81-3.79 (m, 8H), 3.08 (d, *J* = 8.2 Hz, 1H), 2.97 (m, 1H), 2.92 (s, 2H), 2.83 (s, 3H), 2.56 – 2.40 (m, 1H), 2.25 (s, 1H), 1.30 (s, 1H), 1.20 (t, *J* = 8.4 Hz, 1H), 1.01 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.8, 158.2, 158.1, 157.5, 144.3, 143.8, 140.9, 140.5, 138.7, 137.8, 136.4, 136.4, 132.6, 131.9, 131.5, 129.7, 127.8, 126.7, 126.3, 126.2, 113.2, 113.0, 59.5, 55.2, 55.1, 28.1, 27.2, 20.6; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{47}H_{45}N_2O_3$ 685.3425; Found 685.3463.

Synthesis of ((6aS,7aR)-1-(5-(4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)phenyl) thiophen-2-yl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5*H*-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz8)



To a solution of **TPE-Tz8** (20 mg, 0.035 mmol) in CH_3CN (5 mL) was added **BCN** (10.6 mg, 0.07). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as yellow solid (22.9 mg, 94.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.36 (m, 2H), 7.09 (q, *J* = 7.5, 5.9 Hz, 4H), 7.06 – 7.01 (m, 3H), 7.00 – 6.95 (m, 4H), 6.95 – 6.90 (m, 4H), 6.64 (m, 4H), 3.73 (d, *J* = 4.6 Hz, 9H), 3.07 (d, *J* = 38.6 Hz, 3H), 2.89 – 2.81 (m, 1H), 2.74 (d, *J* = 4.4 Hz, 3H), 2.40 (s, 2H), 1.16 (s, 2H), 1.02 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.3, 158.2, 146.8, 144.2, 144.1, 140.6, 139.0, 138.6, 136.3, 136.3, 132.7, 132.0, 131.5, 131.5, 129.1, 127.8, 126.3, 125.1, 123.0, 113.2, 113.0, 59.5, 55.2, 55.1, 28.0, 27.2, 20.7; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{45}H_{43}N_2O_3S$ 691.2989; Found 691.2999.

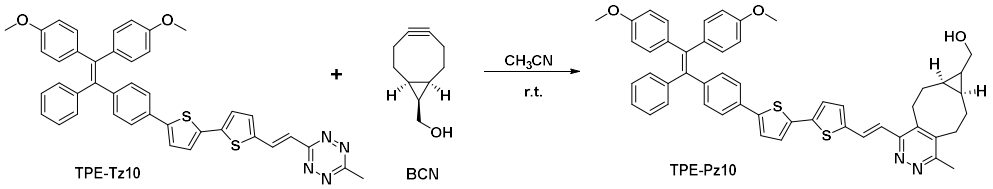
Synthesis of ((6aS,7aR)-1-((*E*)-2-(5-(4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl) phenyl)thiophen-2-yl)vinyl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5*H*-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz9)



To a solution of **TPE-Tz9** (20 mg, 0.034 mmol) in CH_3CN (5 mL) was added **BCN** (10.1 mg, 0.068). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as yellow solid (21.9 mg, 91.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, *J* = 15.2 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 3.7 Hz, 1H), 7.20 – 7.13 (m, 5H), 7.11 – 7.07 (m, 3H), 7.06 (d, *J* = 1.8 Hz, 1H), 7.03 (d, *J* = 8.7 Hz, 2H), 6.98 (d, *J* = 8.7 Hz, 2H), 6.75 – 6.63 (m, 4H), 3.80 (s, 3H), 3.79 (s, 3H), 3.77 (s, 1H), 3.16 – 2.89 (m, 4H), 2.76 (s, 3 H), 2.51 – 2.37 (s, 2H), 1.35 – 1.25 (m, 3H), 1.2 – 1.18 (m, 1H), 0.96 – 0.86 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.3, 158.2, 144.2, 144.1, 141.1, 140.7, 138.5, 136.3, 132.7, 132.0, 131.5, 131.5, 130.3, 127.8, 126.3, 124.9, 123.6, 113.2, 113.0, 59.5, 55.2, 55.1, 29.7, 27.2, 26.3, 20.6; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{47}H_{45}N_2O_3S$ 717.3145; Found 717.3184.

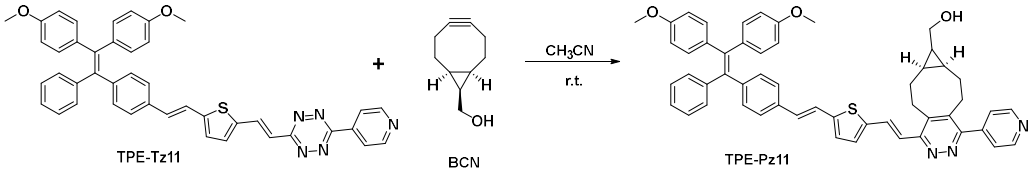
Synthesis of ((6aS,7aR)-1-((*E*)-2-(5'-(4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl) phenyl)-[2,2'-bithiophen]-5-yl)vinyl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5*H*-

cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz10)



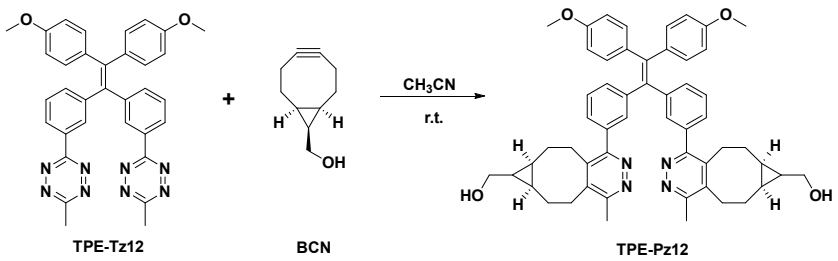
To a solution of **TPE-Tz10** (20 mg, 0.03 mmol) in CH_3CN (5 mL) was added **BCN** (9.0 mg, 0.13 mmol). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as orange solid (18.8 mg, 76.5% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.0 Hz, 2H), 7.17 (s, 2H), 7.11 (q, *J* = 5.6, 5.0 Hz, 5H), 7.06 – 7.00 (m, 4H), 6.97 (d, *J* = 8.7 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 6.85 (s, 1H), 6.64 (dd, *J* = 12.9, 8.7 Hz, 4H), 3.74 (s, 3H), 3.73 (s, 3H), 3.03 (s, 5H), 2.46 (s, 2H), 1.87 – 1.51 (m, 6H), 0.87 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.3, 158.2, 144.1, 140.8, 138.9, 138.5, 136.2, 135.4, 132.7, 132.6, 132.1, 131.5, 127.8, 126.3, 125.9, 124.9, 123.8, 113.2, 113.0, 55.16, 55.13, 31.96, 29.73, 22.73; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{51}H_{47}N_2O_3S_2$ 799.2950; Found 799.2985.

Synthesis of ((6aS,7aR)-1-((E)-2-(5-((E)-4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)styryl)thiophen-2-yl)vinyl)-4-(pyridin-4-yl)-6,6a,7,7a,8,9-hexahydro-5*H*-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz11)



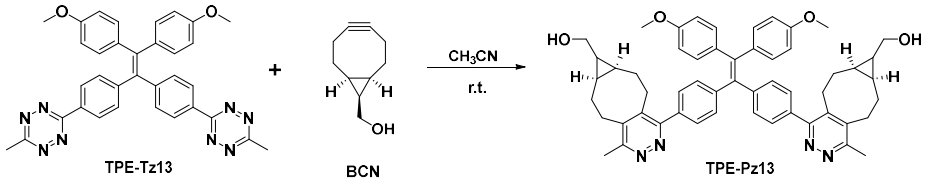
To a solution of **TPE-Tz11** (20 mg, 0.03 mmol) in CH_3CN (5 mL) was added **BCN** (8.8 mg, 0.06 mmol). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as orange solid (19.5 mg, 82.7% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.70 (d, *J* = 6.0 Hz, 2H), 8.05 (d, *J* = 15.2 Hz, 1H), 7.49 (d, *J* = 6.0 Hz, 2H), 7.41 – 7.22 (m, 5H), 7.16 – 7.05 (m, 4H), 6.96 – 6.79 (m, 9H), 6.67 (dd, *J* = 17.3, 8.8 Hz, 4H), 3.64 (d, *J* = 6.2 Hz, 6H), 3.46 (s, 2H), 3.07 (d, *J* = 9.3 Hz, 1H), 2.91 (s, 2H), 2.67 – 2.65 (m, 1H), 2.24 (s, 1H), 1.95 (s, 1H), 1.63 (s, 2H), 0.95 – 0.80 (m, 1H), 0.66 (s, 2H); ^{13}C NMR (101 MHz, DMSO) δ 158.3, 158.2, 155.2, 150.1, 146.2, 144.2, 144.1, 144.0, 140.8, 140.6, 138.8, 136.2, 136.1, 134.8, 132.5, 131.7, 131.3, 131.2, 129.1, 128.8, 128.6, 128.4, 126.8, 126.5, 124.6, 122.2, 121.1, 113.8, 113.7, 57.5, 55.4, 55.4; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{53}H_{48}N_3O_3S$ 806.3411; Found 806.3409.

Synthesis of ((6aR,7aS)-1-(3-(1-(3-((6aS,7aR)-7-(hydroxymethyl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5*H*-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-1-yl)phenyl)-2,2-bis(4-methoxyphenyl)vinyl)phenyl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5H-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz12)



To a solution of **TPE-Tz12** (20 mg, 0.034 mmol) in CH_3CN (5 mL) was added **BCN** (20.7 mg, 0.14 mmol). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as white solid (25.4 mg, 89.4% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (d, *J* = 14.4 Hz, 4H), 7.23 (d, *J* = 7.2 Hz, 2H), 7.10 (s, 2H), 7.06 – 6.98 (m, 4H), 6.74 – 6.66 (m, 4H), 3.78 (d, *J* = 1.7 Hz, 8H), 3.61 (t, *J* = 10.1 Hz, 2H), 2.94 (dd, *J* = 14.9, 7.5 Hz, 2H), 2.80 (d, *J* = 8.2 Hz, 2H), 2.74 (d, *J* = 2.6 Hz, 6H), 2.47 (d, *J* = 17.7 Hz, 3H), 2.33 (q, *J* = 11.8, 7.1 Hz, 6H), 1.29 (s, 4H), 1.15 – 1.01 (m, 2H), 0.88 – 0.86 (m, 2H), 0.68 – 0.48 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.7, 158.3, 158.2, 157.6, 144.0, 141.2, 141.2, 138.2, 138.2, 137.6, 137.6, 136.0, 135.9, 132.9, 132.9, 132.0, 132.0, 131.4, 128.5, 128.4, 127.5, 127.4, 113.3, 59.1, 59.0, 55.2, 29.7, 27.7, 27.5, 20.5, 20.4; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{54}H_{57}N_4O_4$ 825.4374; Found 825.4369.

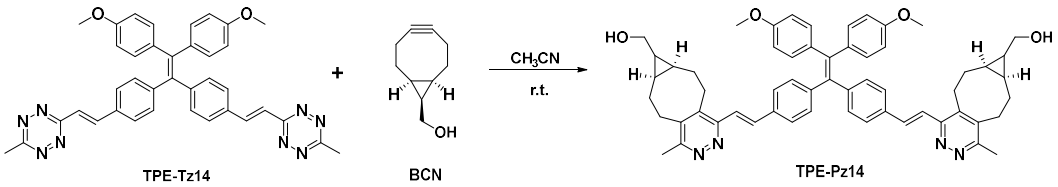
Synthesis of ((6aR,7aS)-1-(4-(1-(4-((6aS,7aR)-7-(hydroxymethyl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5*H*-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-1-yl)phenyl)-2,2-bis(4-methoxyphenyl)vinyl)phenyl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5H-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz13)



To a solution of **TPE-Tz13** (20 mg, 0.034 mmol) in CH_3CN (5 mL) was added **BCN** (20.7 mg, 0.14 mmol). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as white solid (23.3 mg, 82.0% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15 (d, *J* = 7.9 Hz, 4H), 7.11 (d, *J* = 7.9 Hz, 4H), 6.95 (d, *J* = 8.3 Hz, 4H), 6.60 (d, *J* = 8.3 Hz, 4H), 3.69 (s, 6H), 3.66 (d, *J* = 7.9 Hz, 4H), 3.00 – 2.90 (m, 2H), 2.84 – 2.75 (m, 4H), 2.69 (s, 8H), 2.33 (d, *J* = 7.0 Hz, 2H), 2.06 (s, 2H), 1.46 (s, 5H), 1.08 (s, 2H), 0.87 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.86, 158.35, 157.49, 144.26, 141.43, 140.79, 139.58, 138.23, 136.05, 135.78, 132.73, 131.38, 128.74, 113.16, 59.41, 55.18, 27.92, 27.23, 20.68; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{54}H_{57}N_4O_4$ 825.4374; Found 825.4362.

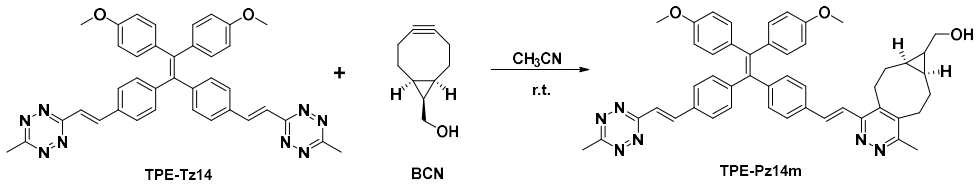
Synthesis of ((6aR,7aS)-1-((*E*)-4-(1-(4-((*E*)-2-((6aS,7aR)-7-(hydroxymethyl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5*H*-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-1-

yl)vinyl)phenyl)-2,2-bis(4-methoxyphenyl)vinyl)styryl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5H-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz14)



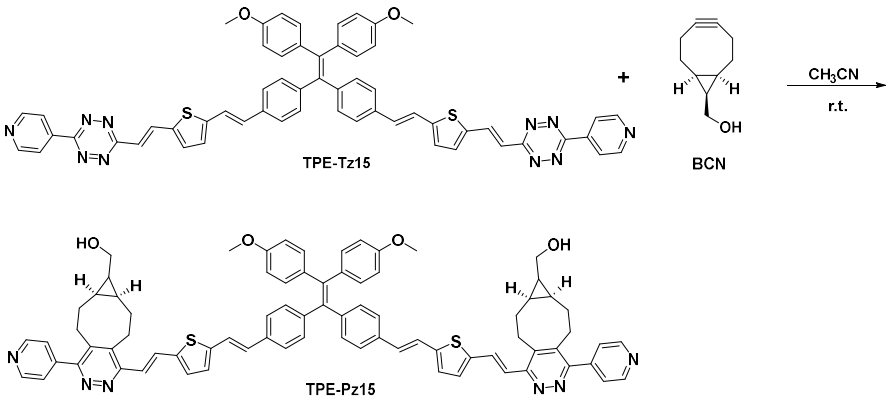
To a solution of **TPE-Tz14** (20 mg, 0.032 mmol) in CH_3CN (5 mL) was added **BCN** (19.0 mg, 0.13 mmol). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as yellow solid (26.2 mg, 94.6% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ7.82 (d, *J* = 15.6 Hz, 2H), 7.33 (d, *J* = 8.1 Hz, 4H), 7.26 (d, *J* = 8.2 Hz, 2H), 7.03 (d, *J* = 8.2 Hz, 4H), 6.96 (d, *J* = 8.8 Hz, 4H), 6.65 (d, *J* = 8.7 Hz, 4H)., 3.74 (s, 6H), 3.71 (d, *J* = 7.9 Hz, 2H), 3.01 – 2.83 (m, 8H), 2.70 (s, 6H), 2.39 – 2.31 (m, 4H), 1.24 (s, 4H), 1.13 – 1.07 (m, 2H), 0.87 – 0.78 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.3, 156.9, 155.3, 144.8, 141.2, 140.2, 138.9, 138.2, 136.2, 135.7, 134.5, 132.7, 131.9, 126.9, 120.8, 113.2, 59.4, 55.2, 29.7, 27.1, 26.2, 20.8; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{58}H_{61}N_4O_4$ 877.4672; Found 877.4687.

Synthesis of ((6aS,7aR)-1-((*E*)-4-(2,2-bis(4-methoxyphenyl)-1-(4-((*E*)-2-(6-methyl-1,2,4,5-tetrazin-3-yl)vinyl)phenyl)vinyl)styryl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5*H*-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz14m)



To a solution of **TPE-Tz14** (10 mg, 0.016 mmol) in CH_3CN (2 mL) at 0℃ was drop-added a solution of **BCN** (2.4 mg, 0.016 mmol) in CH_3CN. The reaction mixture was stirred at 0℃ for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as yellow solid (7.6 mg, 63.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 16.5 Hz, 1H), 7.84 (d, *J* = 15.5 Hz, 1H), 7.42 (dd, *J* = 8.6, 2.0 Hz, 2H), 7.35 (dt, *J* = 12.5, 3.2 Hz, 3H), 7.28 (d, *J* = 2.1 Hz, 1H), 7.09 (dd, *J* = 8.3, 1.9 Hz, 2H), 7.03 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.96 (dd, *J* = 8.8, 2.2 Hz, 4H), 6.65 (dd, *J* = 9.0, 2.2 Hz, 4H), 3.79 – 3.66 (m, 8H), 3.08 – 2.83 (m, 8H), 2.72 (d, *J* = 1.9 Hz, 3H), 2.35 (dd, *J* = 11.5, 5.2 Hz, 2H), 1.31 – 1.23 (m, 1H), 1.16 – 1.05 (m, 1H), 0.84 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166. 2, 164.9, 158.5, 158.5, 155.3, 146.7, 144.6, 141.9, 140.8, 137.9, 136.0, 135.9, 134.6, 132.9, 132.7, 132.7, 132.2, 131.9, 127.7, 127.0, 120.7, 119.9, 113.3, 113.233, 59.5, 55.2, 55.2, 27.2, 26.3, 21.2, 20.7. HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{48}H_{47}N_6O_3$ 755.3665; Found 755.3681.

Synthesis of ((6aR,7aS)-1-((*E*)-2-(5-((*E*)-4-(1-(4-((*E*)-2-(5-((*E*)-2-((6aS,7aR)-7-(hydroxymethyl)-4-(pyridin-4-yl)-6,6a,7,7a,8,9-hexahydro-5*H*-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-1-yl)vinyl)thiophen-2-yl)vinyl)phenyl)-2,2-bis(4-methoxyphenyl)vinyl)styryl)thiophen-2-yl)vinyl)-4-(pyridin-4-yl)-6,6a,7,7a,8,9-hexahydro-5H-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methanol (TPE-Pz15)



To a solution of **TPE-Tz15** (20 mg, 0.02 mmol) in CH_3CN (5 mL) was added **BCN** (6.2 mg, 0.8 mmol). The reaction mixture was stirred at room temperature for 30 min, then concentrated. The residue was purified by a preparative TLC plate (DCM/MeOH = 20:1) to afford the titled compound as orange solid (16.2 mg, 68.2% yield). ^{1}H NMR (600 MHz, DMSO) δ 8.70 (d, *J* = 4.8 Hz, 3H), 8.06 (d, *J* = 15.3 Hz, 1H), 7.53 – 7.22 (m, 12H), 7.13 (dd, *J* = 9.3, 3.6 Hz, 3H), 7.07 – 6.78 (m, 13H), 6.71 – 6.63 (m, 4H), 3.64 (d, *J* = 2.9 Hz, 6H), 3.46 (t, *J* = 7.7 Hz, 4H), 3.07 (s, 2H), 2.89 (d, *J* = 23.6 Hz, 2H), 2.65 (dt, *J* = 14.8, 7.1 Hz, 1H), 2.31 – 2.18 (m, 1H), 2.02 – 1.91 (m, 2H), 1.71 – 1.53 (m, 3H), 1.47 – 1.37 (m, 1H), 1.27 – 1.14 (m, 4H), 0.85 (dt, *J* = 44.2, 7.8 Hz, 6H).^{13}C NMR (151 MHz, DMSO) δ 158.3, 155.1, 150.1, 146.2, 144.8, 143.9, 143.9, 143.5, 142.8, 140.8, 138.4, 136.1, 135.0, 134.8, 134.7, 132.6, 132.3, 131.8, 131.1, 130.1, 129.0, 128.7, 128.5, 128.4, 127.7, 127.6, 127.5, 127.1, 126.7, 126.5, 126.3, 125.7, 124.6, 122.2, 121.5, 121.0, 113.7, 110.7, 88.1, 57.4, 55.4, 40.2, 29.5, 29.1, 29.0, 28.0, 27.0, 22.5; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{78}H_{71}N_6O_4S_2$ 1219.4890; Found 1219.4902.

Reaction kinetics measurement

To a solution of 0.1 mM TPE probe in PBS buffer (pH=7.4) was added 10-fold excess of BCN reagent (1 mM) at 37 °C. The fluorescence intensity of the mixture solution at different time interval was recorded at λ_{em} of each probe using TECAN fluorescence plate reader (λ_{exc}=365 nm). The reaction kinetics was hypothesized as pseudo-first order reaction, and the observed rate constants (k_{obs}) were calculated using the one phase exponential association equation. The second order rate constants (k_2) were calculated using the equation: $k_2 = k_{obs}$/[BCN reagent].

X-ray single crystal diffraction

Suitable crystals of TPE-Pz10 were obtained by slowly evaporating a mixture of

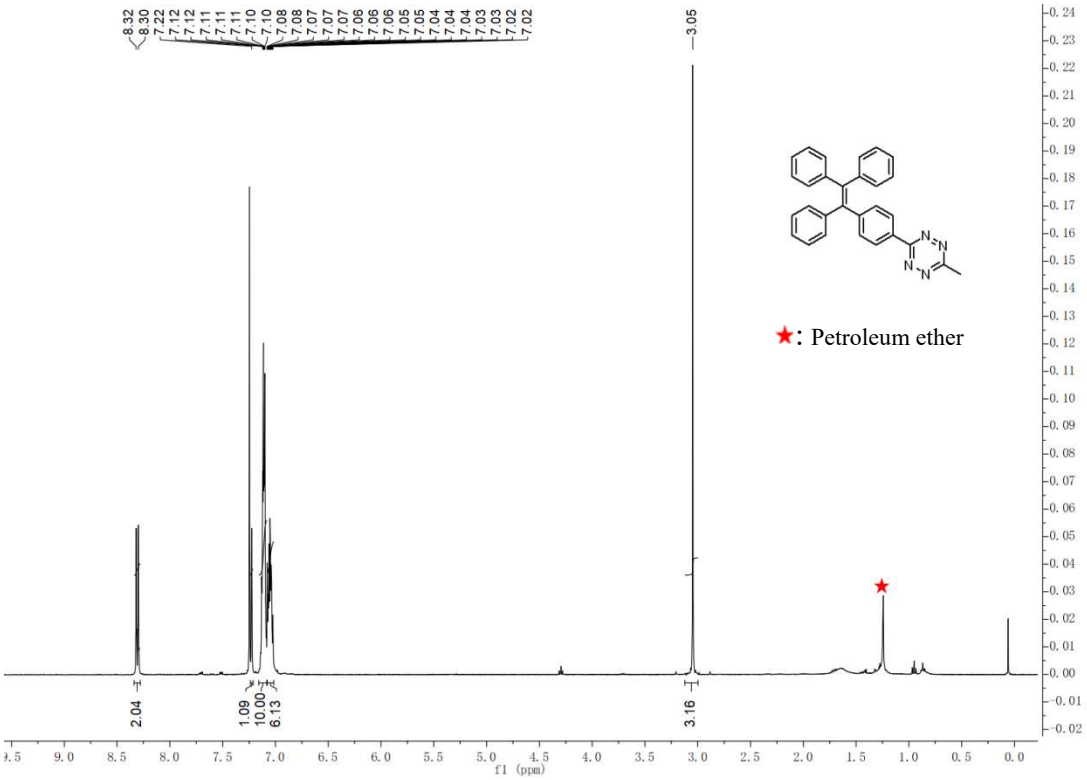
chloroform and methanol solution (1:1, v/v) at ambient temperature. A colorless crystal was mounted on a glass fiber at a random orientation. The data were collected at 100 K by a diffractometer Rigaku Oxford Diffraction Supernova Dual Source, Cu at Zero equipped with an AtlasS2 CCD using Cu Kα radiation (1.54178 Å), and processed using CrysAlisPro. The structures were solved by direct methods using Olex2 software, and the nonhydrogen atoms were located from the trial structure and then refined anisotropically with SHELXL-2018 using a full-matrix least squares procedure based on F^2. The weighted *R* factor, *wR* and goodness-of-fit *S* values were obtained based on F^2. The hydrogen atom positions were fixed geometrically at the calculated distances and allowed to ride on the parent atoms. Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Center and allocated with the deposition numbers: CCDC 2191457.

Reference

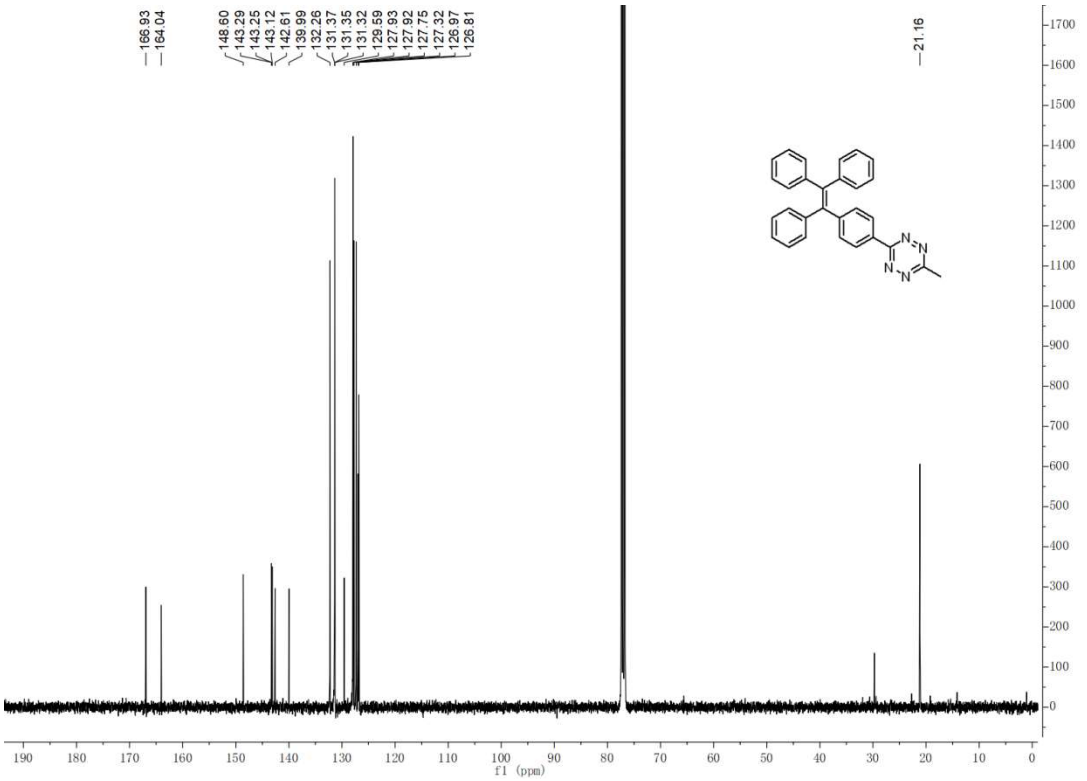
1. Teng, Y.; Yang, H.; Li, X.; Wang, Y. C.; Yin, D. L.; Tian, Y. L., A Bioorthogonal-Activated Fluorescence Turn-On Probe Based on Nitrone-Modified 1,8-Naphthalimide for Live-Cell Imaging. *Chin. J. Chem.* **2022**, 40, 209-214.

2. Mao, W.; Shi, W.; Li, J.; Su, D.; Wang, X.; Zhang, L.; Pan, L.; Wu, X.; Wu, H., Organocatalytic and Scalable Syntheses of Unsymmetrical 1,2,4,5-Tetrazines by Thiol-Containing Promotors. *Angew. Chem. Int. Ed.* **2019**, 58, 1106-1109.

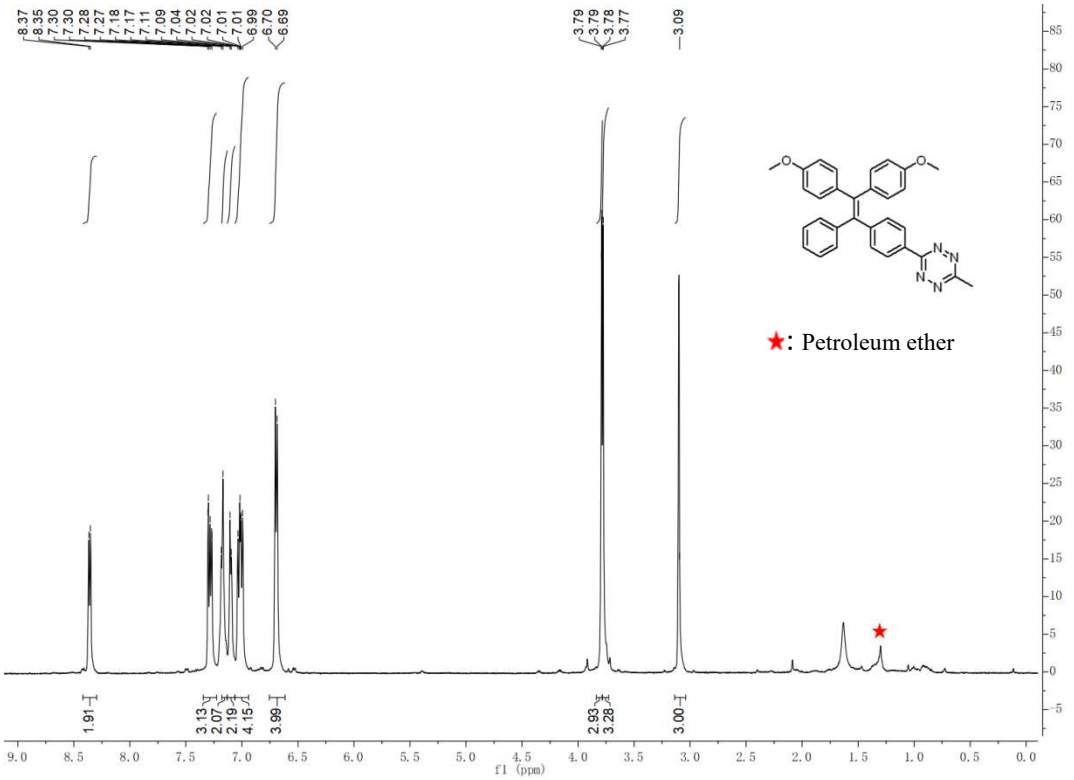
^{1}H NMR of compound **TPE-Tz1** (400 MHz in $CDCl_3$)



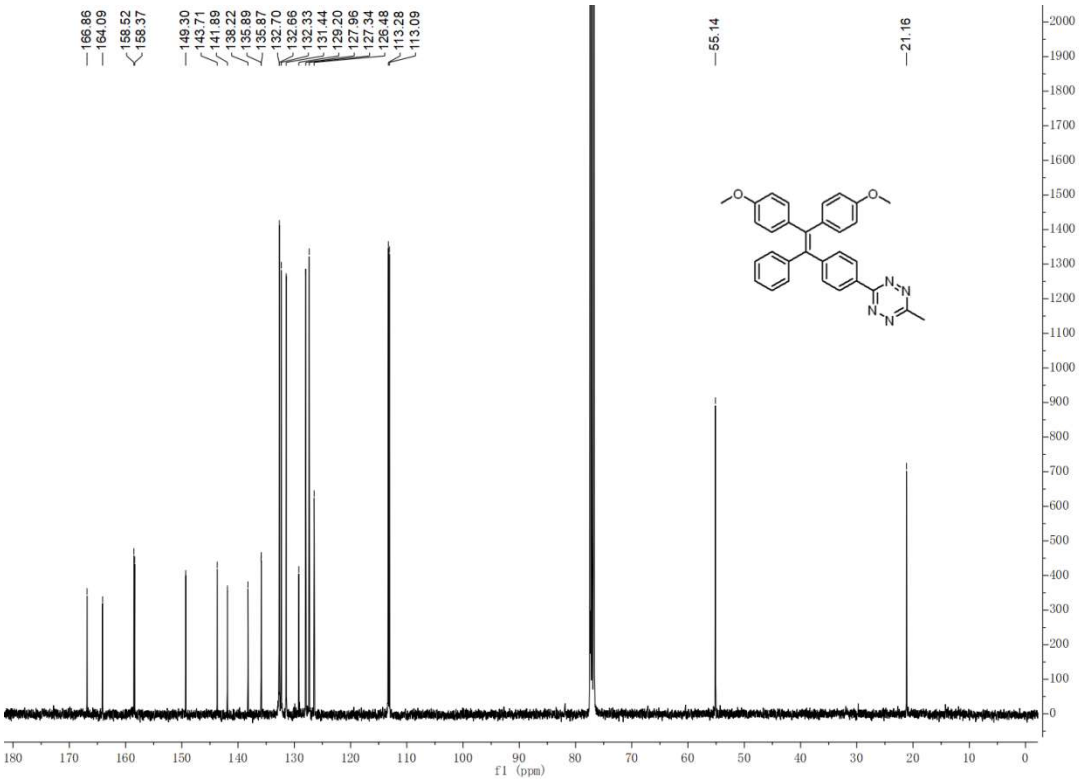
^{13}C NMR of compound **TPE-Tz1** (101 MHz in $CDCl_3$)



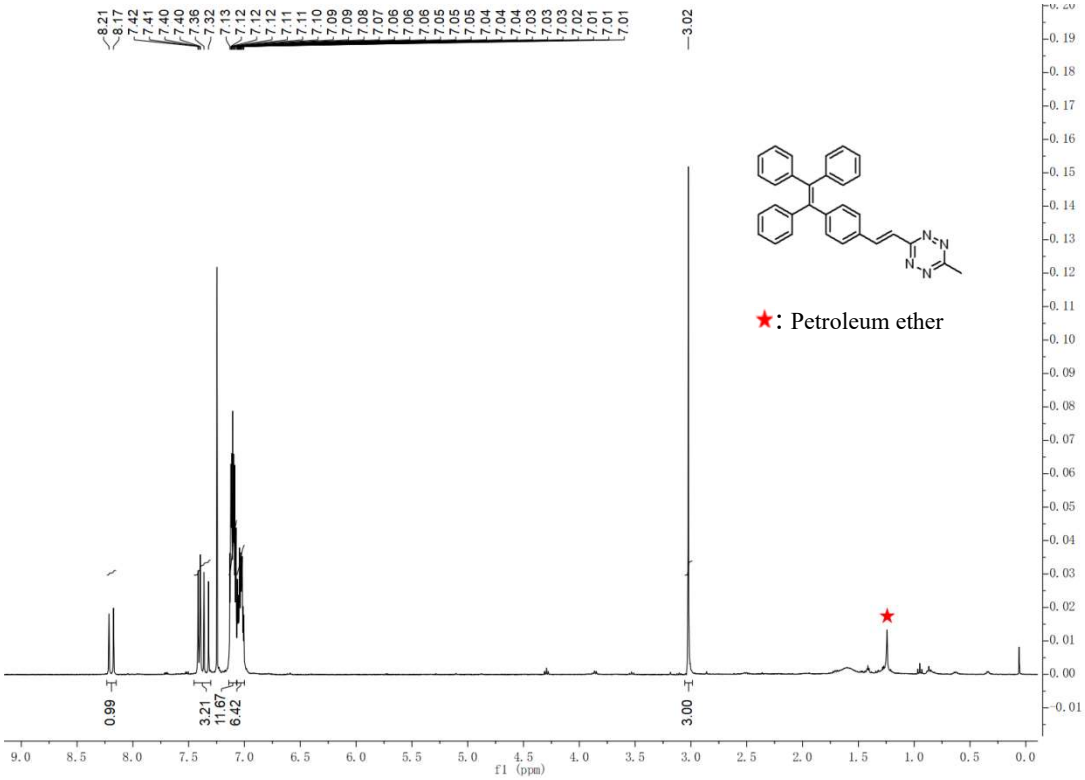
^{1}H NMR of compound **TPE-Tz2** (500 MHz in $CDCl_3$)



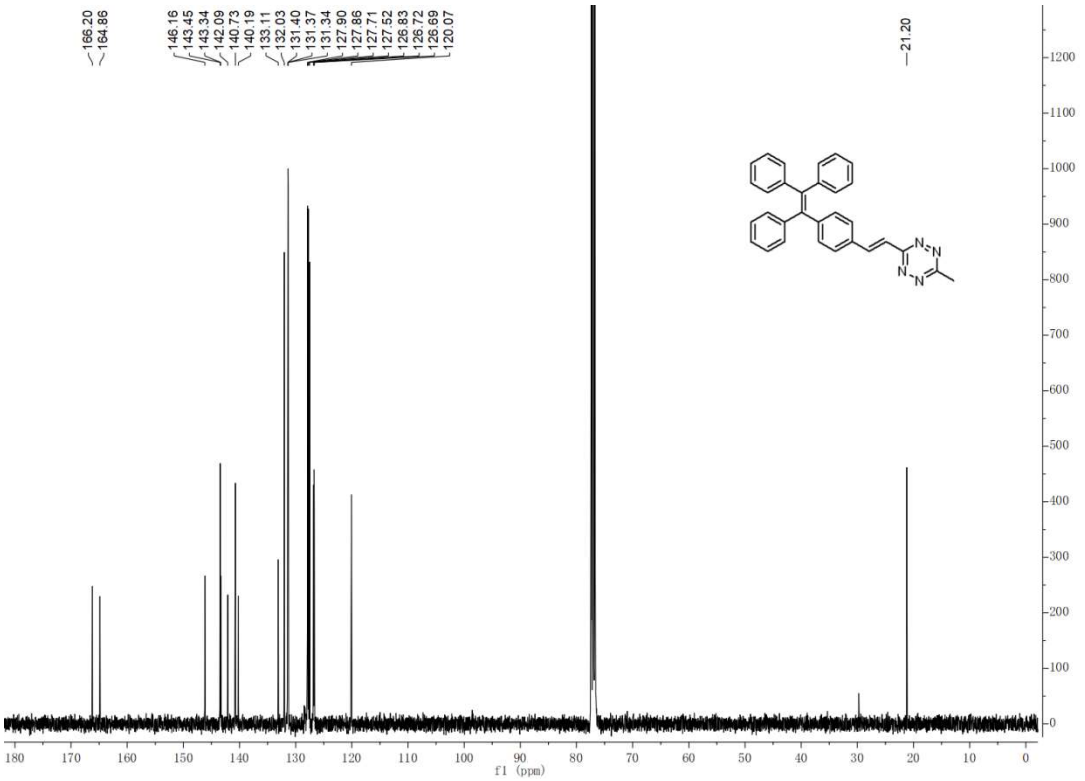
^{13}C NMR of compound **TPE-Tz2** (101 MHz in $CDCl_3$)



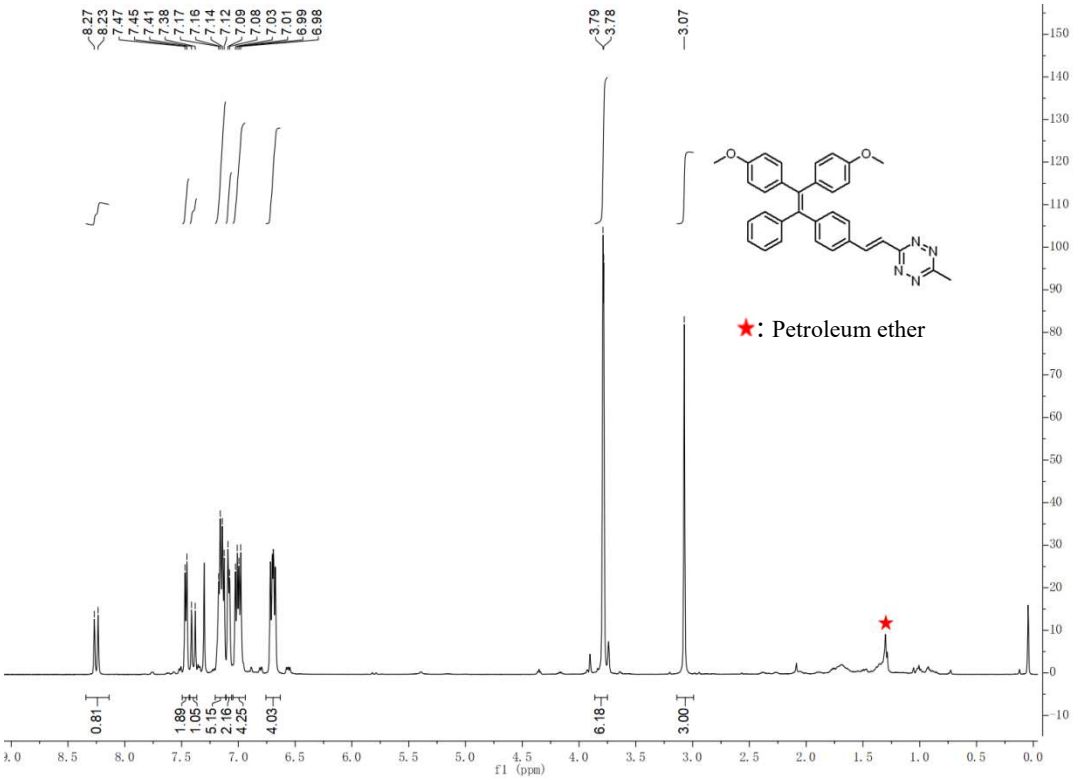
^{1}H NMR of compound **TPE-Tz3** (400 MHz in $CDCl_3$)



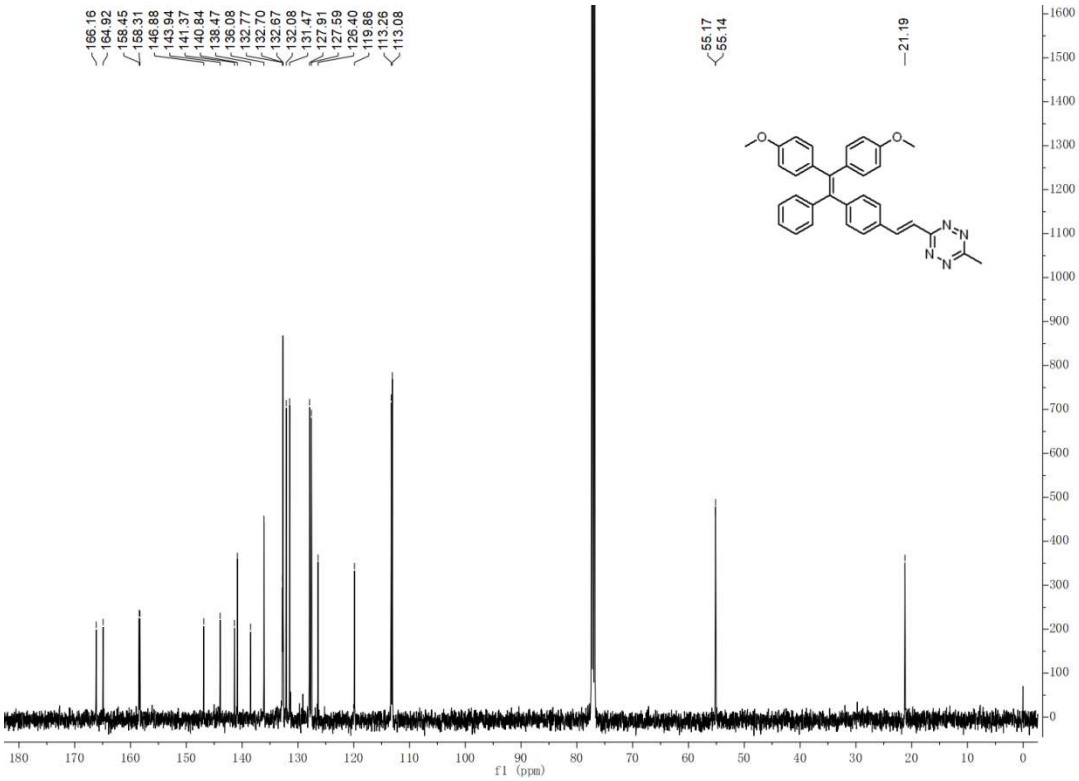
^{13}C NMR of compound **TPE-Tz3** (101 MHz in $CDCl_3$)



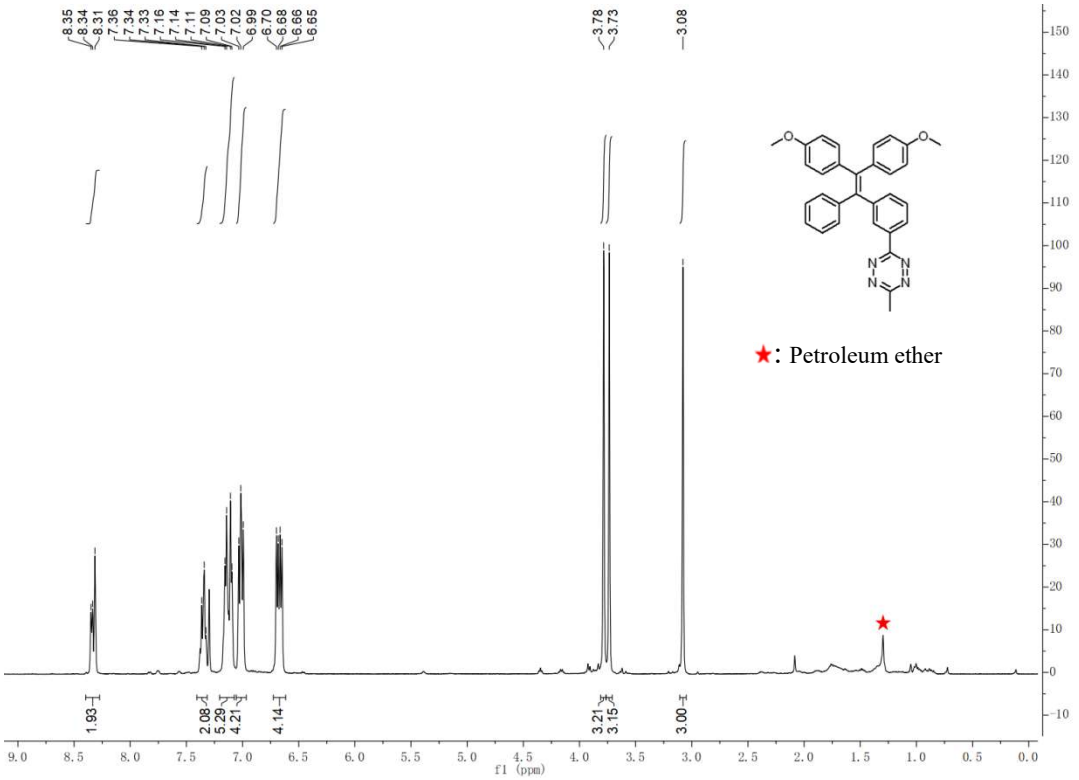
^{1}H NMR of compound **TPE-Tz4** (500 MHz in $CDCl_3$)



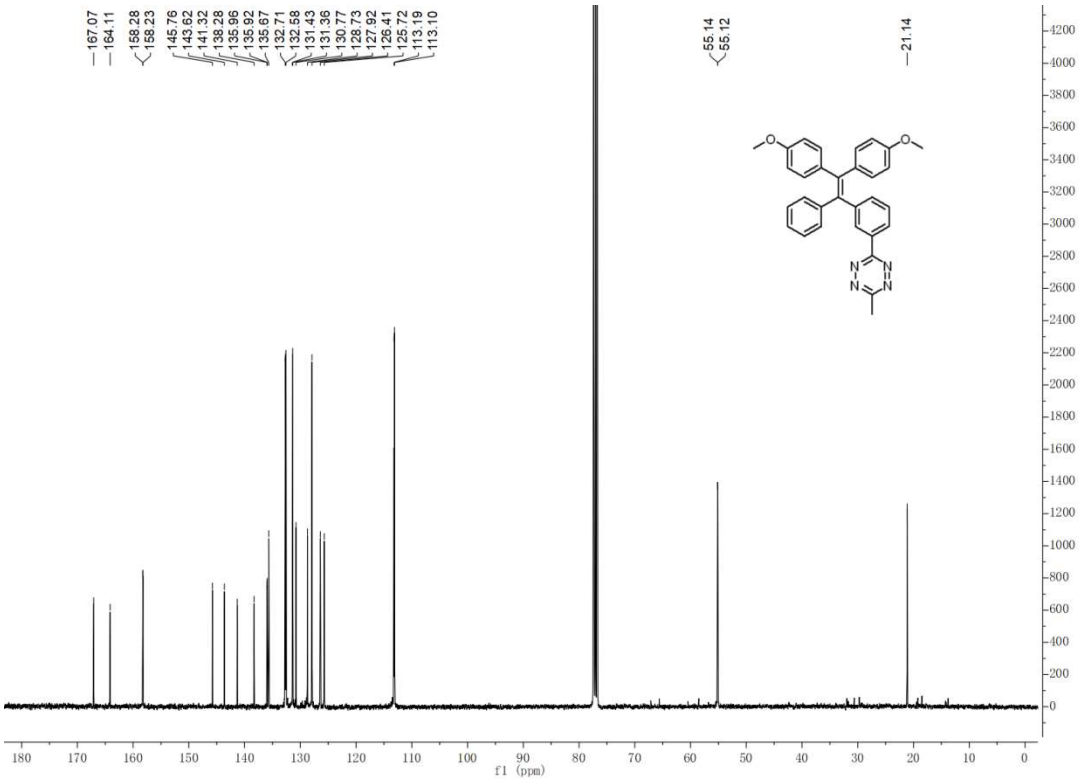
^{13}C NMR of compound **TPE-Tz4** (126 MHz in $CDCl_3$)



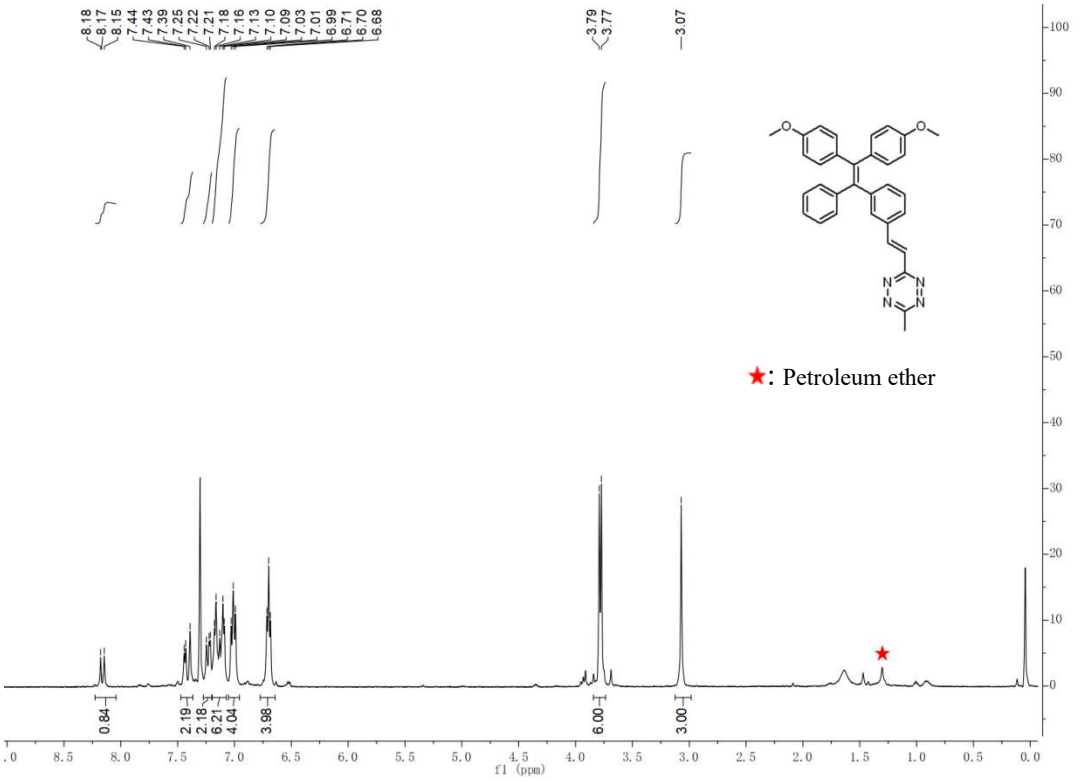
^{1}H NMR of compound **TPE-Tz5** (500 MHz in $CDCl_3$)



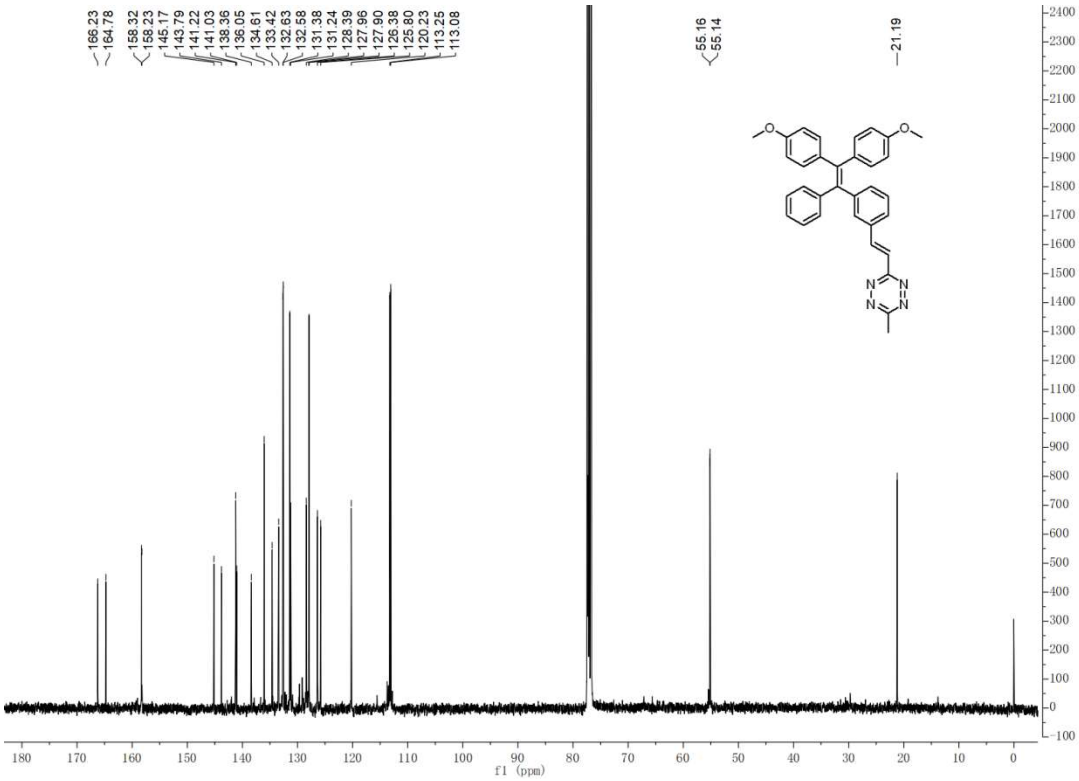
^{13}C NMR of compound **TPE-Tz5** (101 MHz in $CDCl_3$)



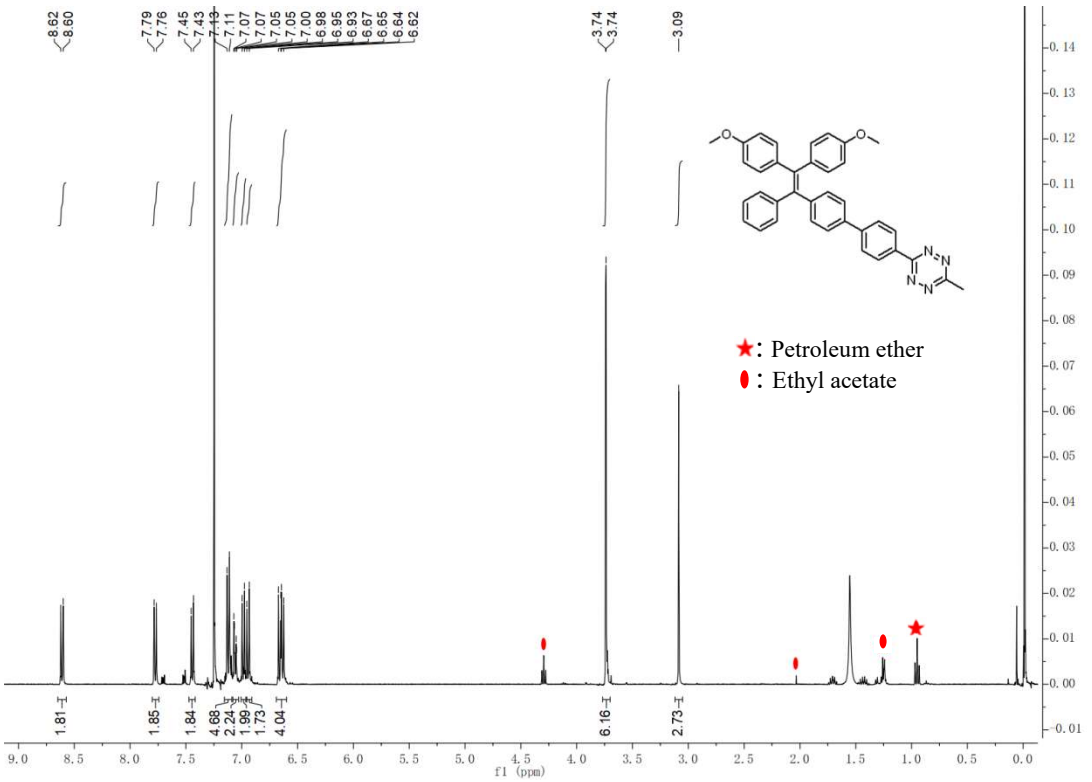
^{1}H NMR of compound **TPE-Tz6** (500 MHz in $CDCl_3$)



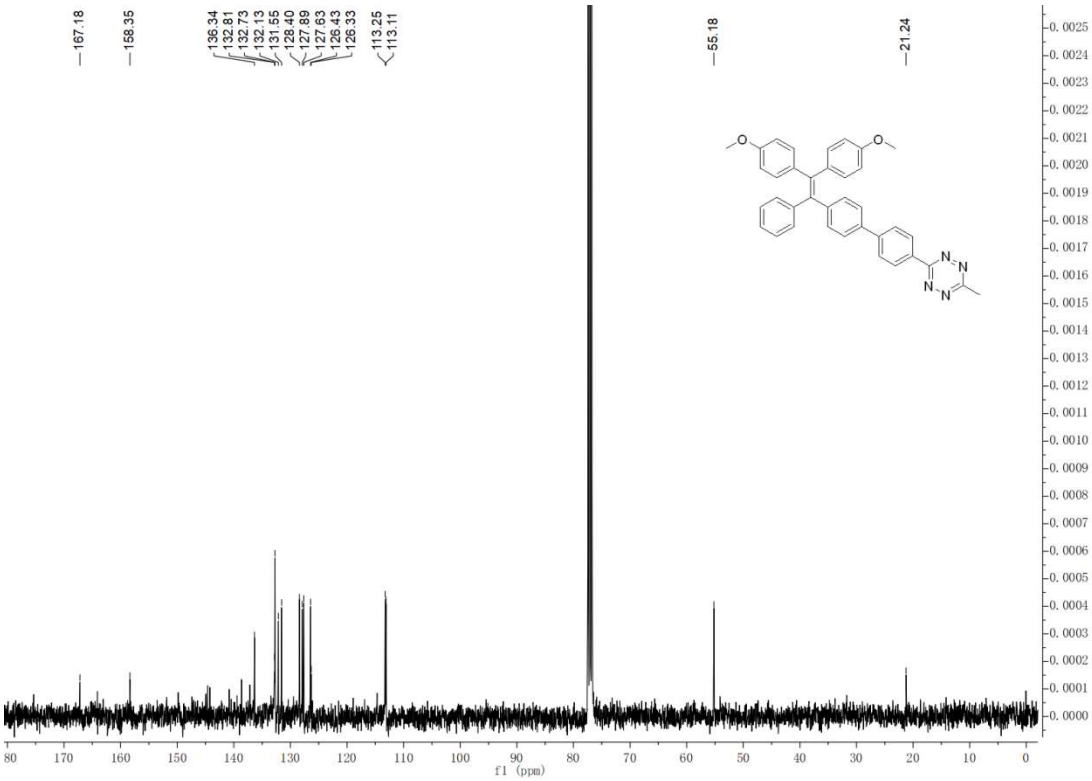
^{13}C NMR of compound **TPE-Tz6** (101 MHz in $CDCl_3$)



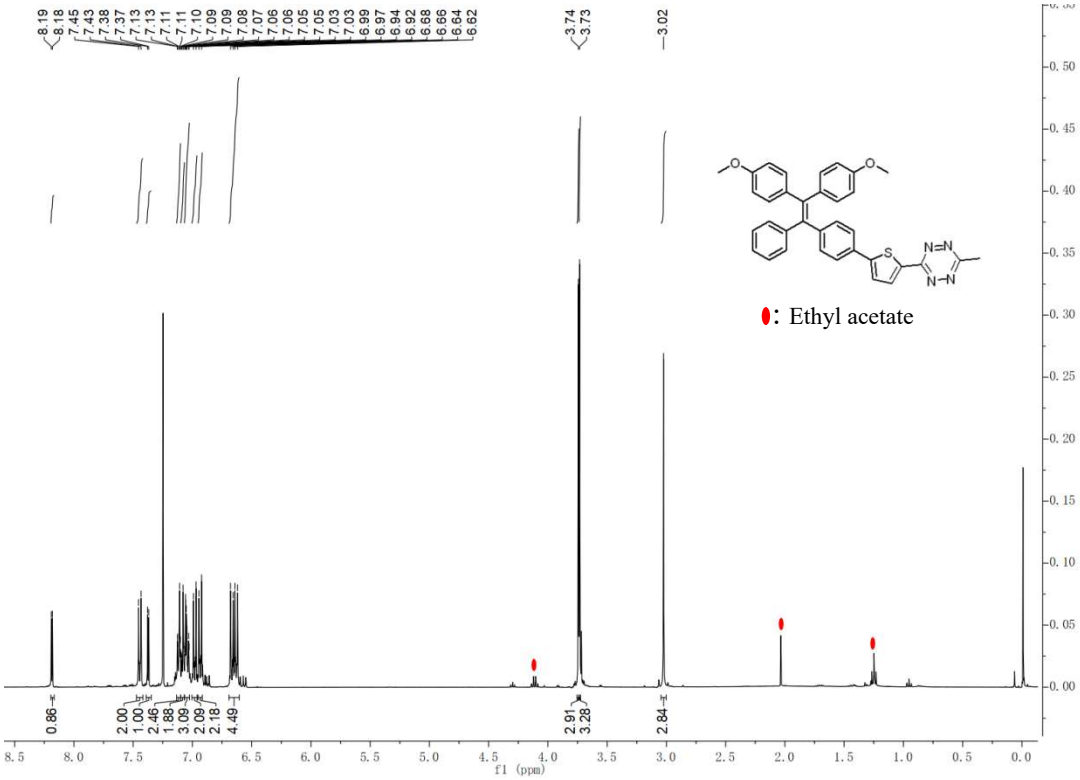
^{1}H NMR of compound **TPE-Tz7** (400 MHz in $CDCl_3$)



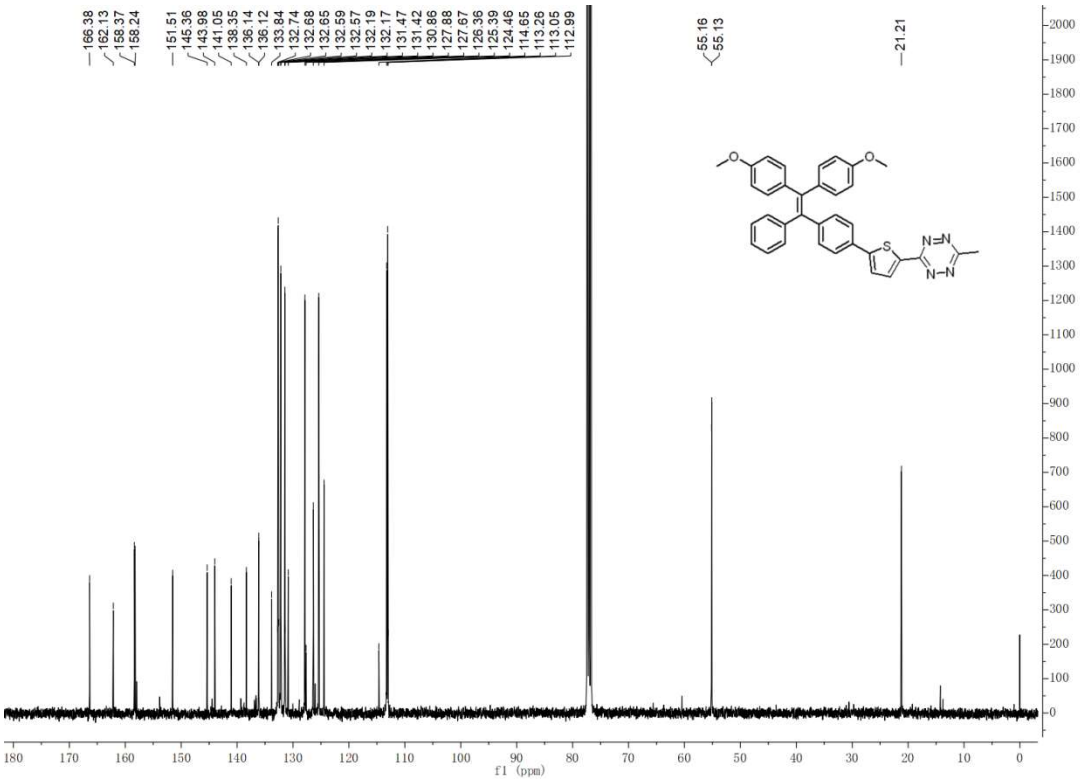
^{13}C NMR of compound **TPE-Tz7** (101 MHz in $CDCl_3$)



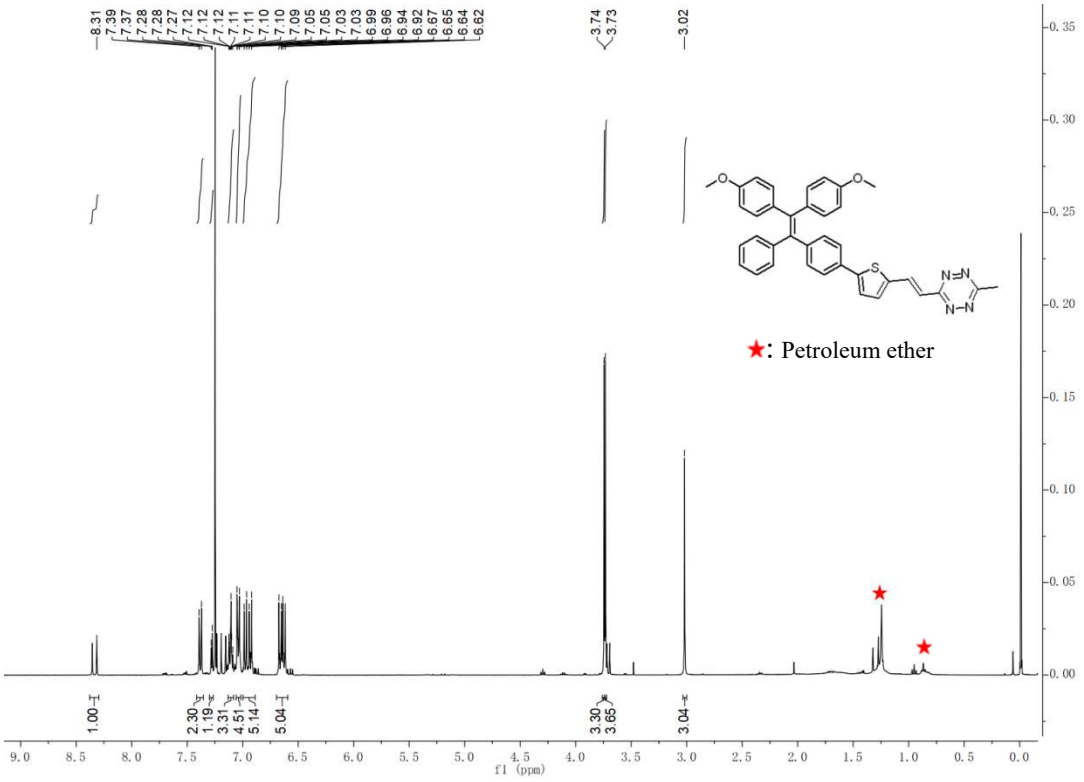
^{1}H NMR of compound **TPE-Tz8** (400 MHz in $CDCl_3$)



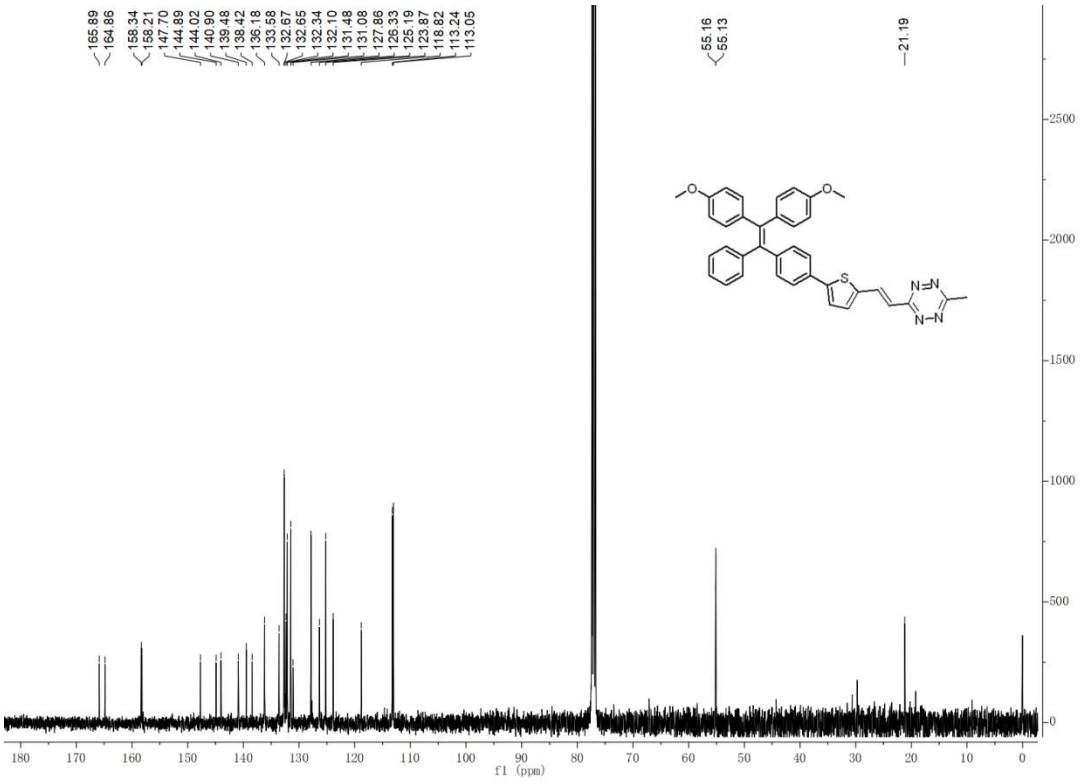
^{13}C NMR of compound **TPE-Tz8** (101 MHz in $CDCl_3$)



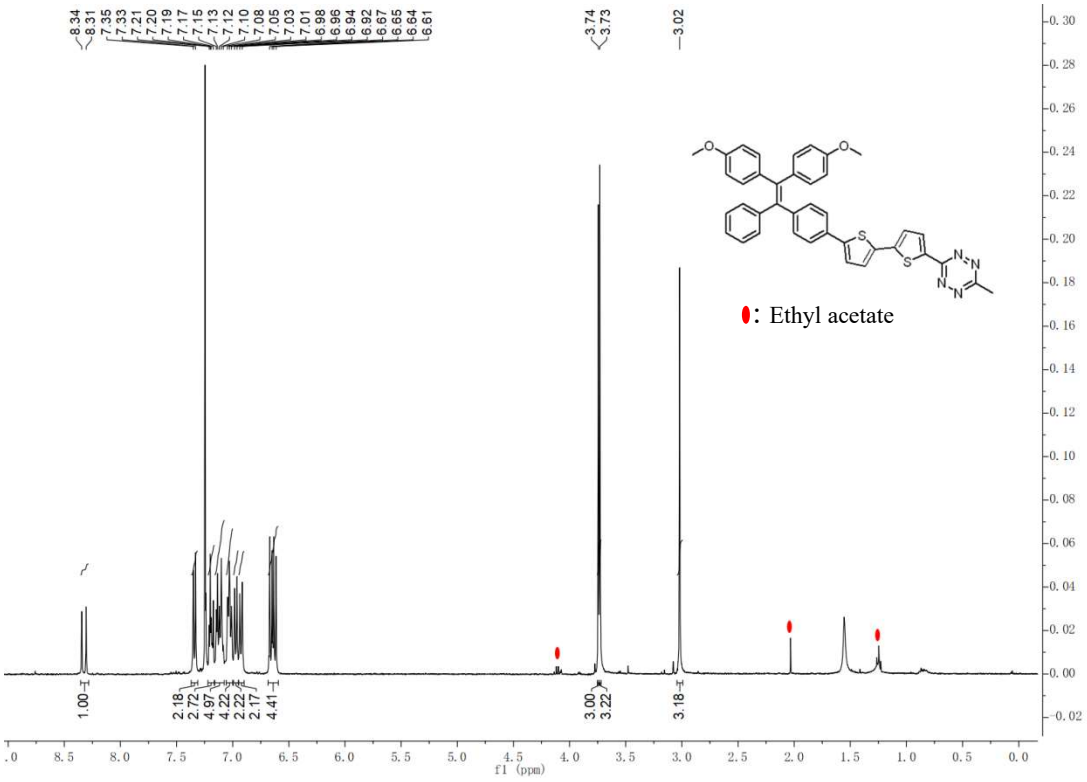
^{1}H NMR of compound **TPE-Tz9** (400 MHz in $CDCl_3$)



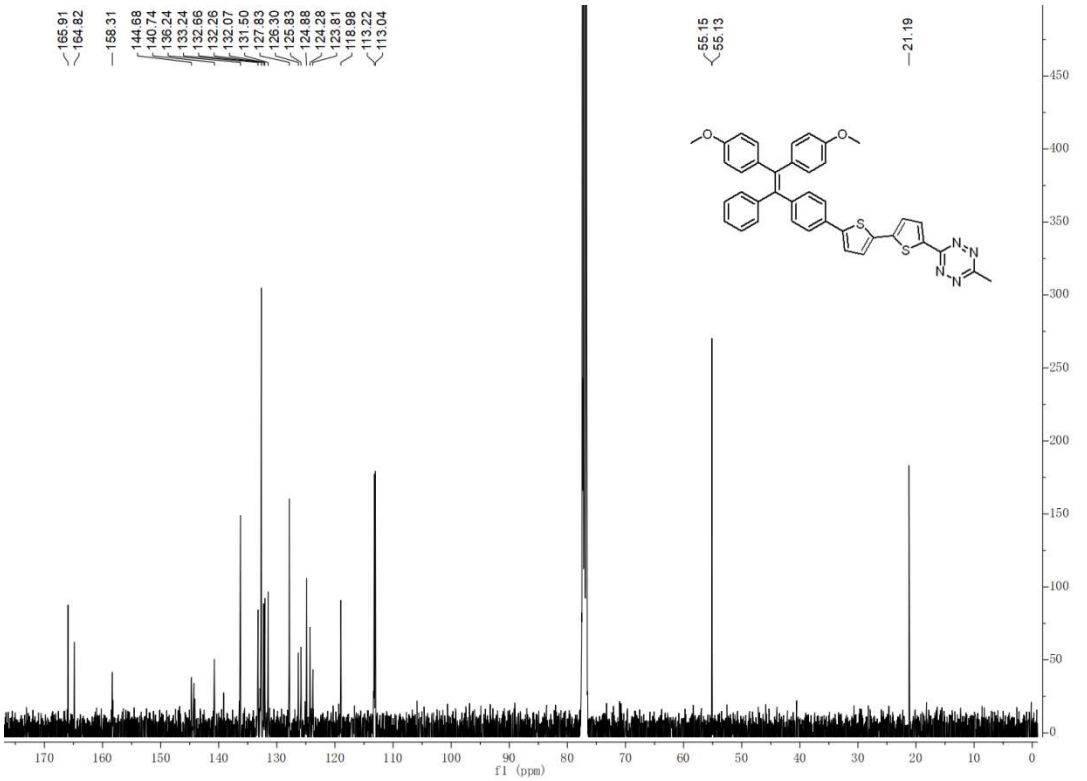
^{13}C NMR of compound **TPE-Tz9** (101 MHz in $CDCl_3$)



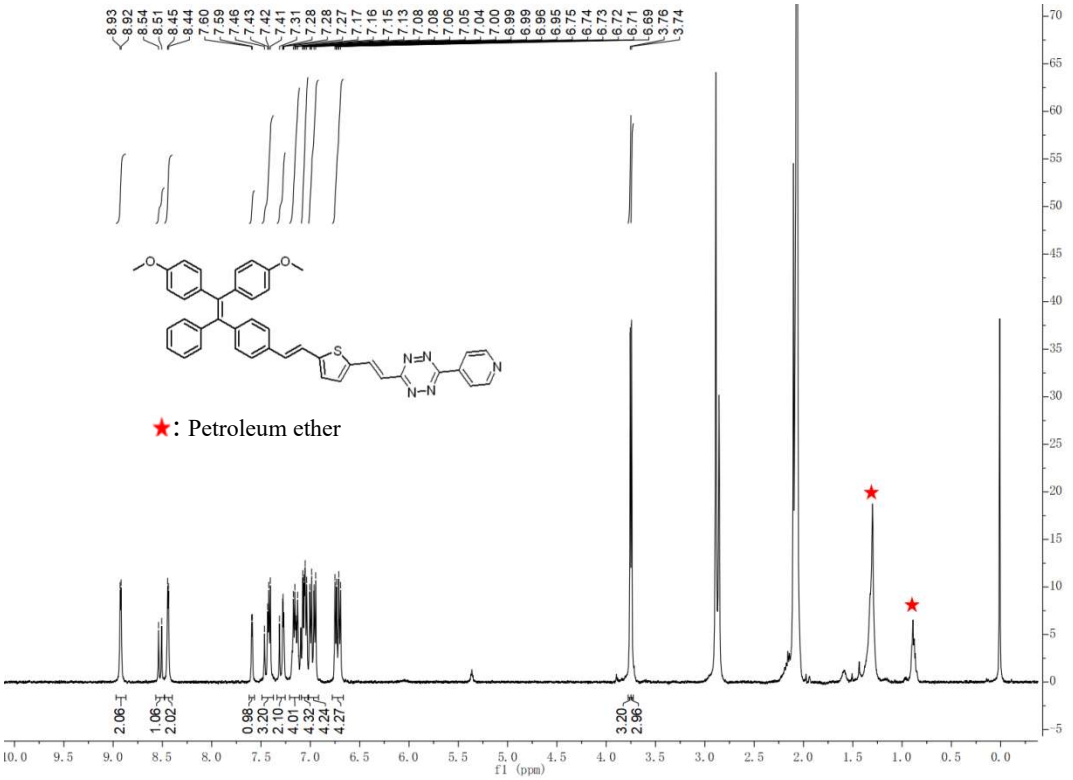
^{1}H NMR of compound **TPE-Tz10** (400 MHz in $CDCl_3$)



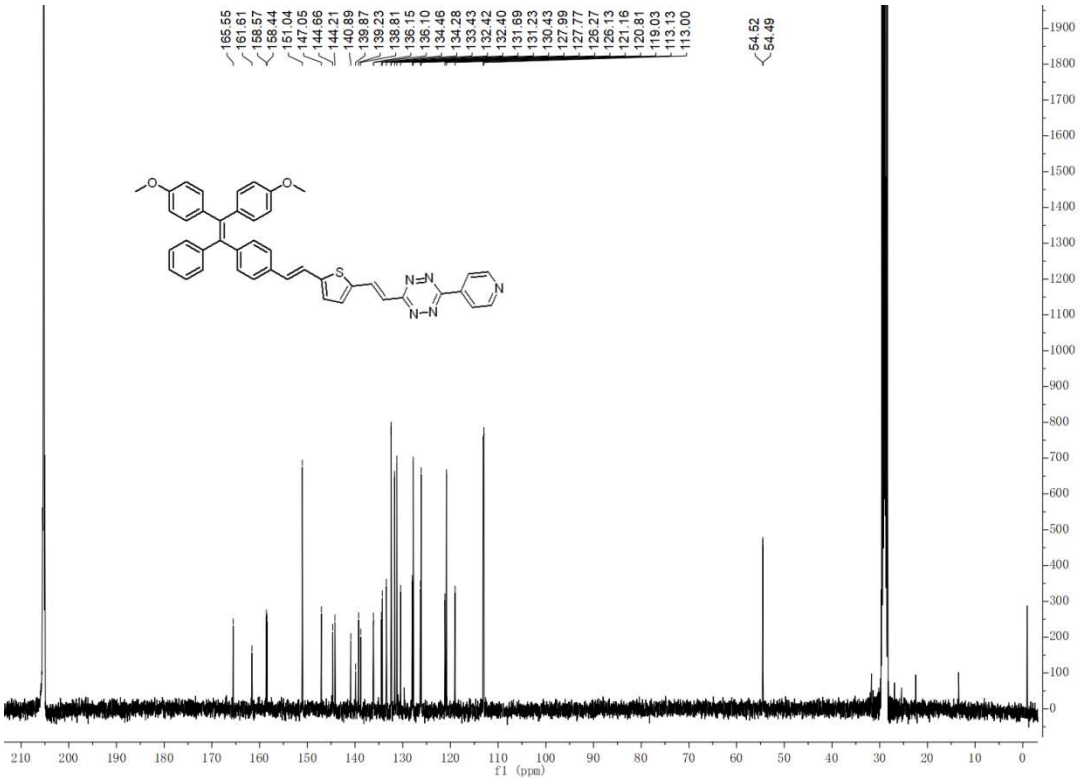
^{13}C NMR of compound **TPE-Tz10** (101 MHz in $CDCl_3$)



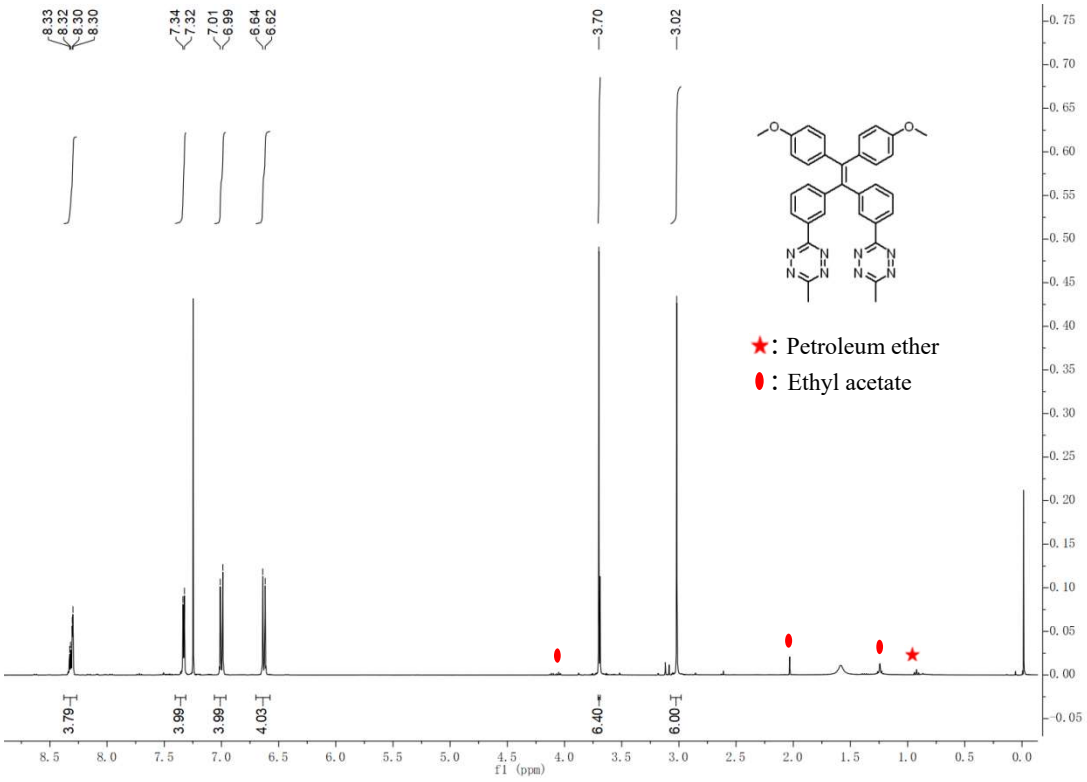
^{1}H NMR of compound **TPE-Tz11** (500 MHz in Acetone-d_6)



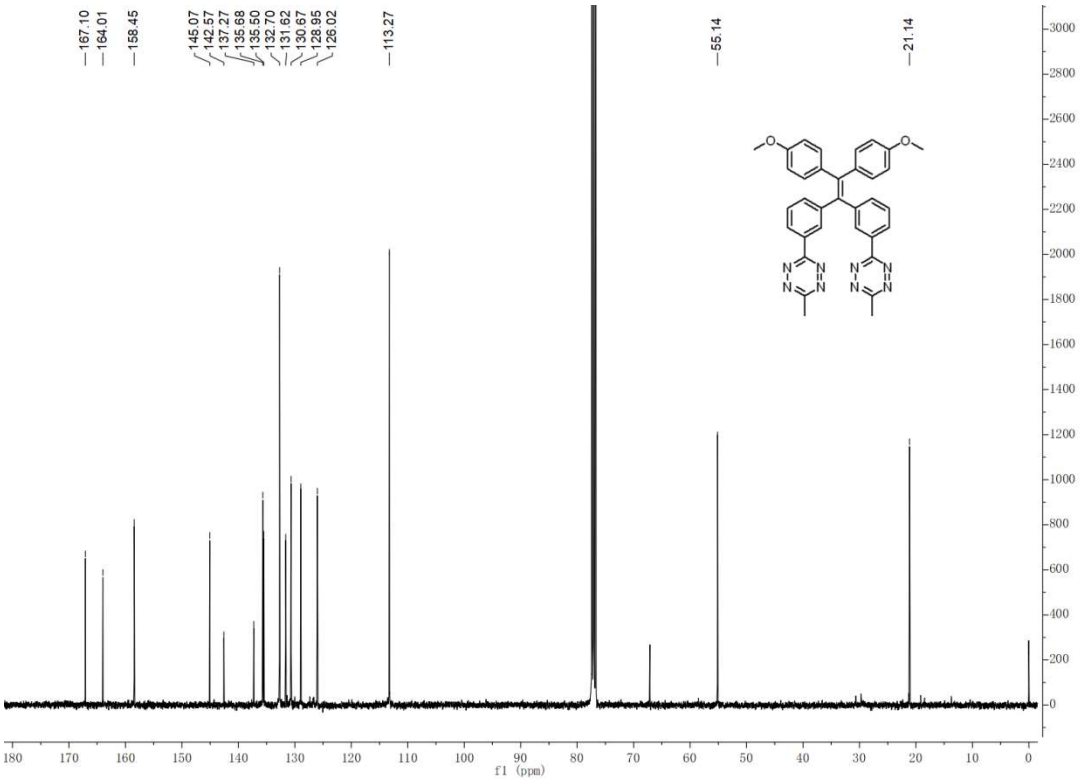
^{13}C NMR of compound **TPE-Tz11** (101 MHz in Acetone-d_6)



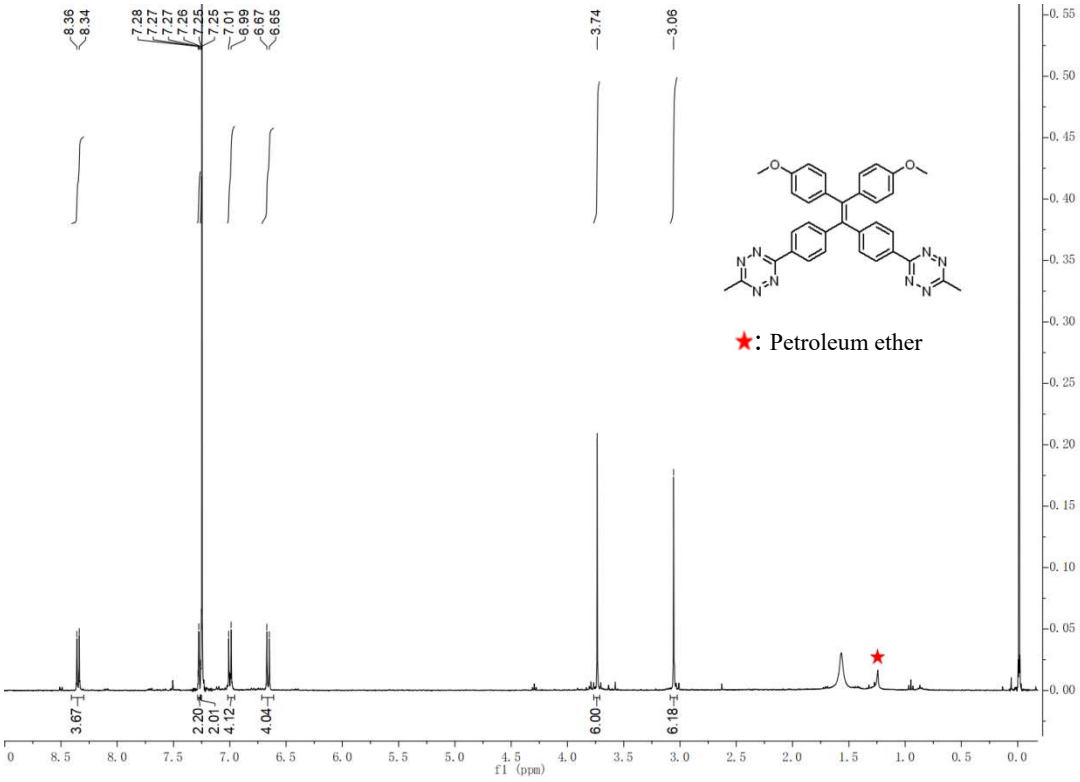
^{1}H NMR of compound **TPE-Tz12** (400 MHz in $CDCl_3$)



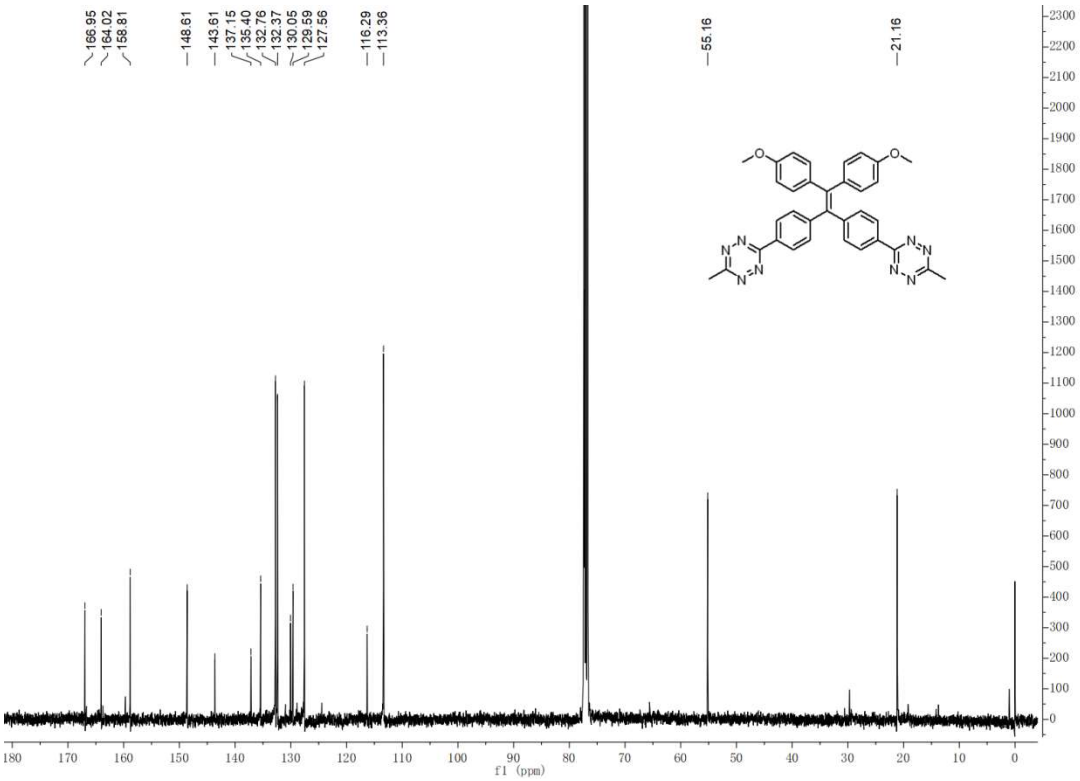
^{13}C NMR of compound **TPE-Tz12** (101 MHz in $CDCl_3$)



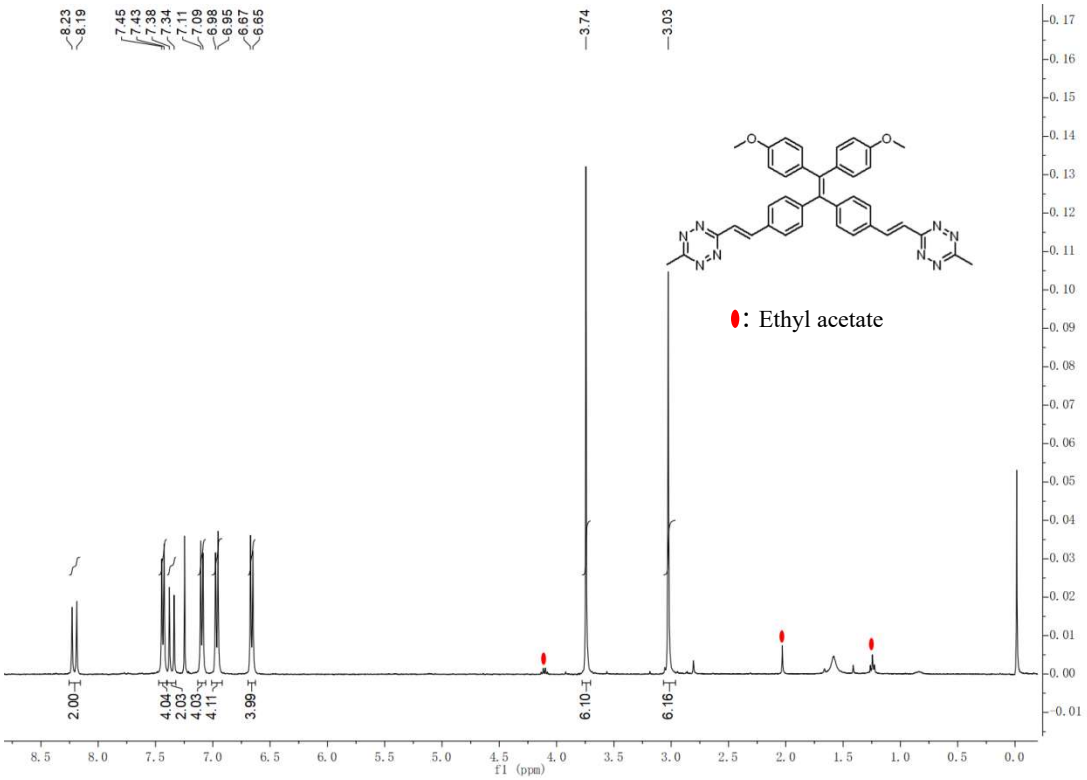
^{1}H NMR of compound **TPE-Tz13** (400 MHz in $CDCl_3$)



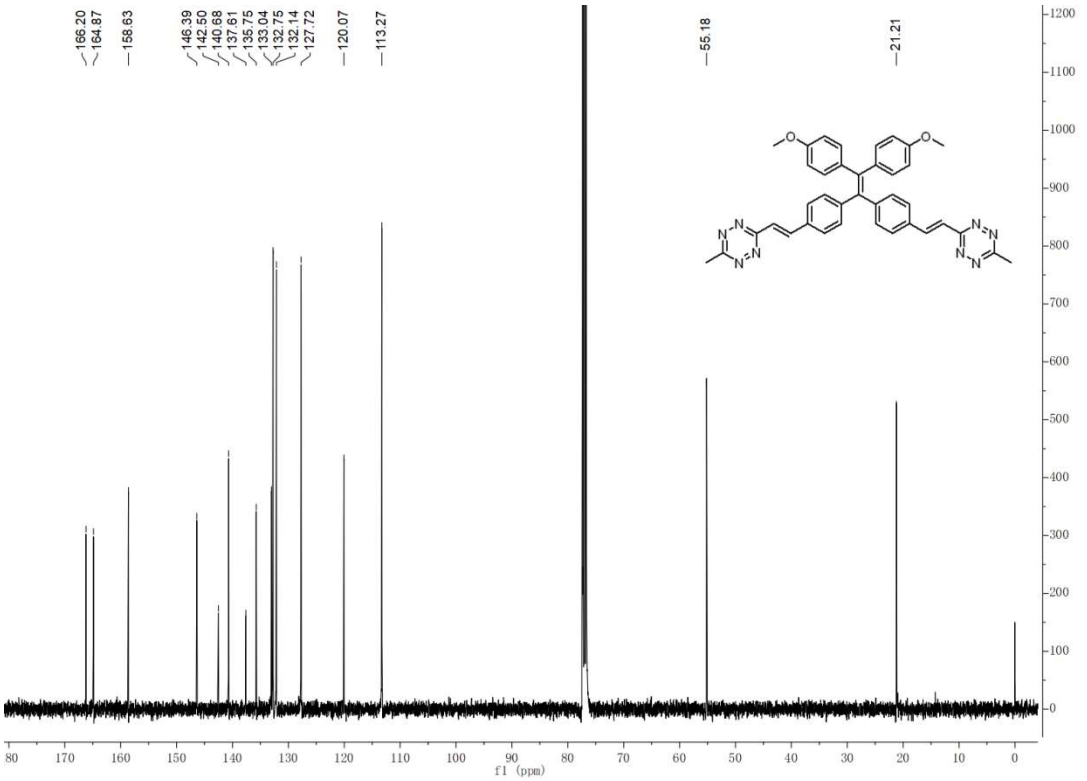
^{13}C NMR of compound **TPE-Tz13** (101 MHz in $CDCl_3$)



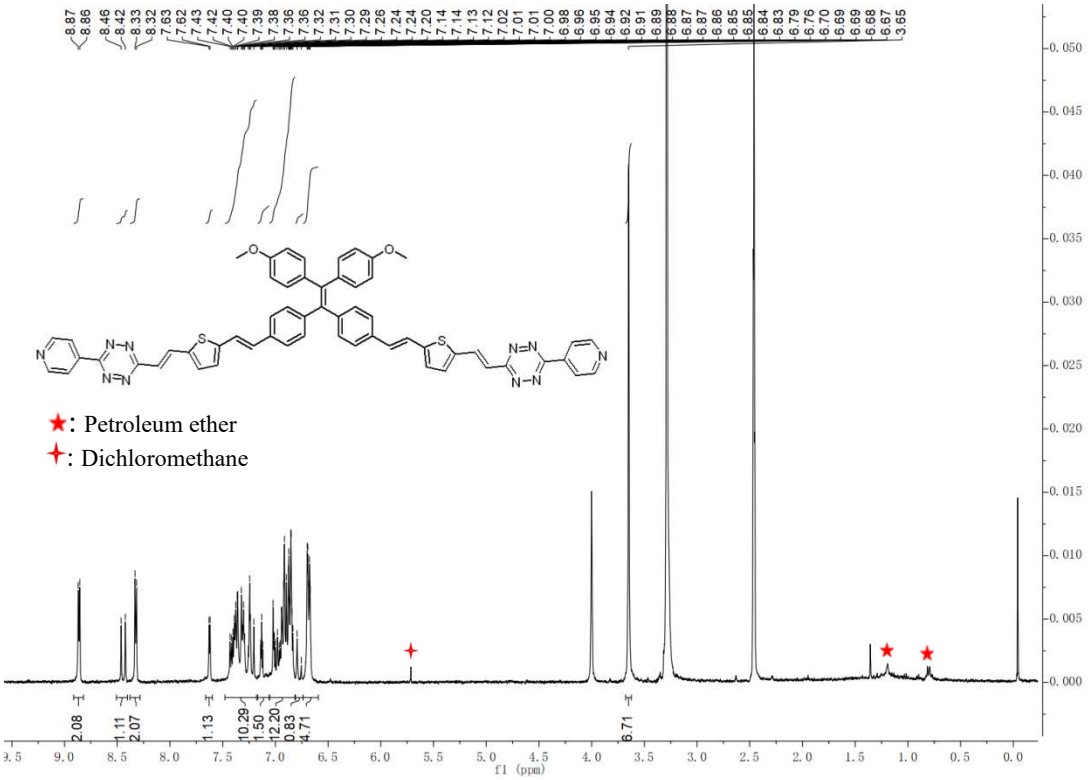
^{1}H NMR of compound **TPE-Tz14** (400 MHz in $CDCl_3$)



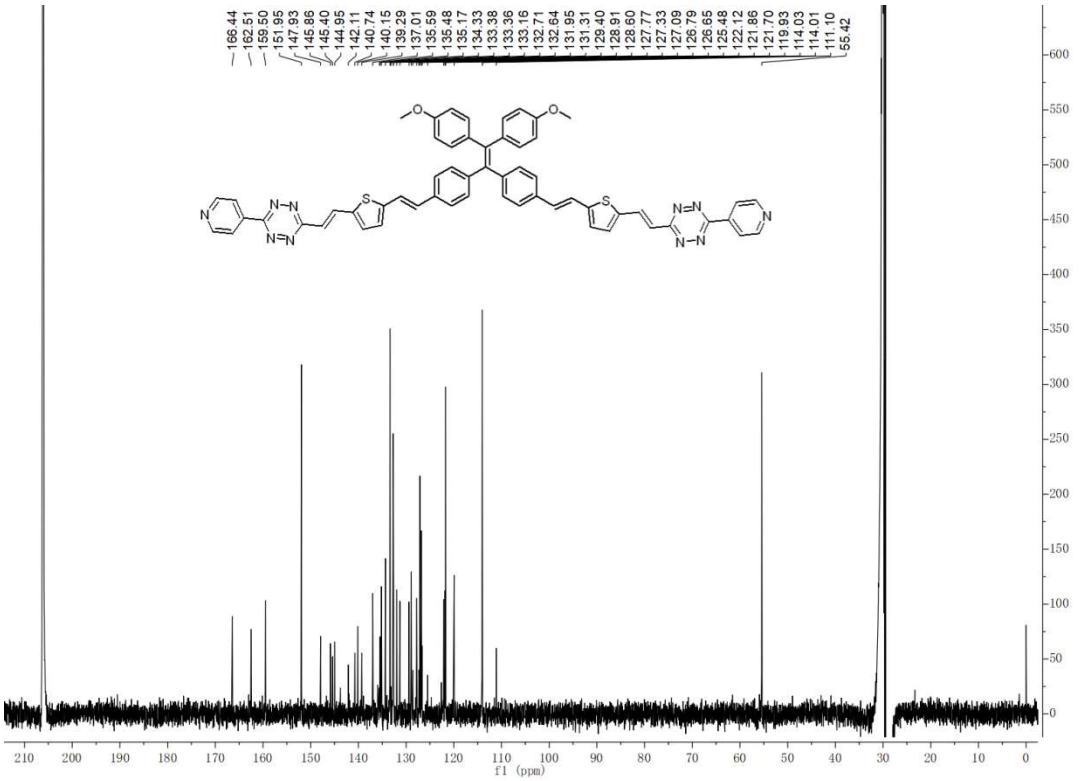
^{13}C NMR of compound **TPE-Tz14** (101 MHz in $CDCl_3$)



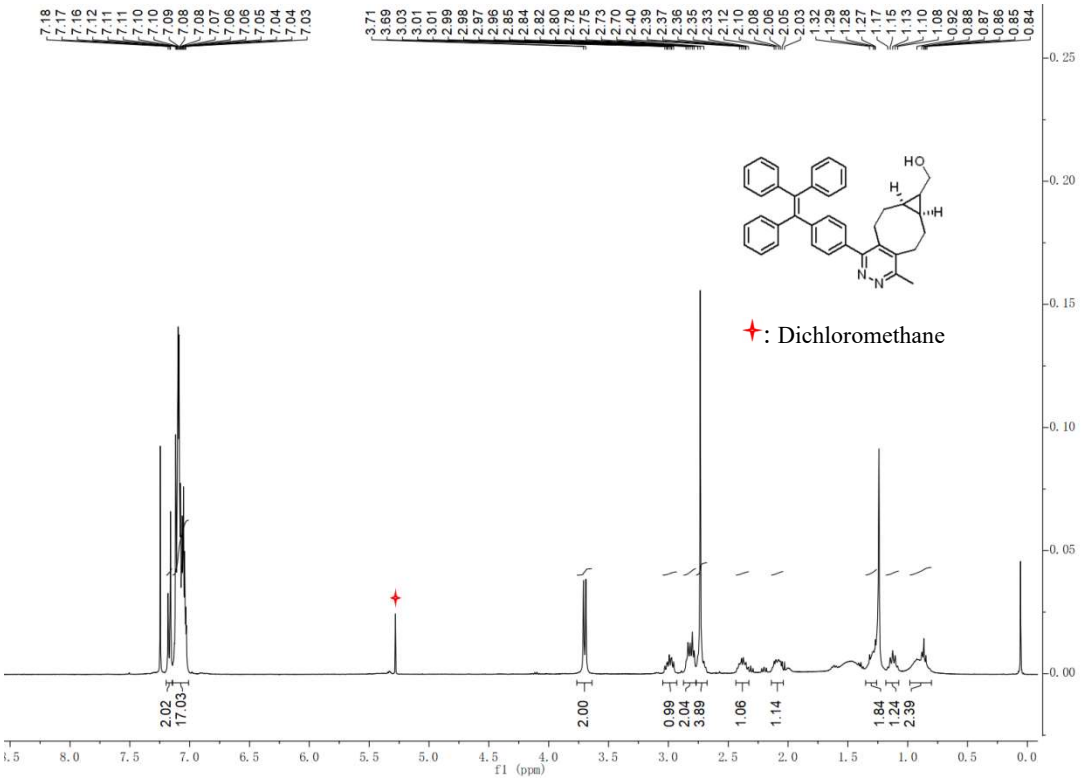
^{1}H NMR of compound **TPE-Tz15** (400 MHz in Acetone-d_6)



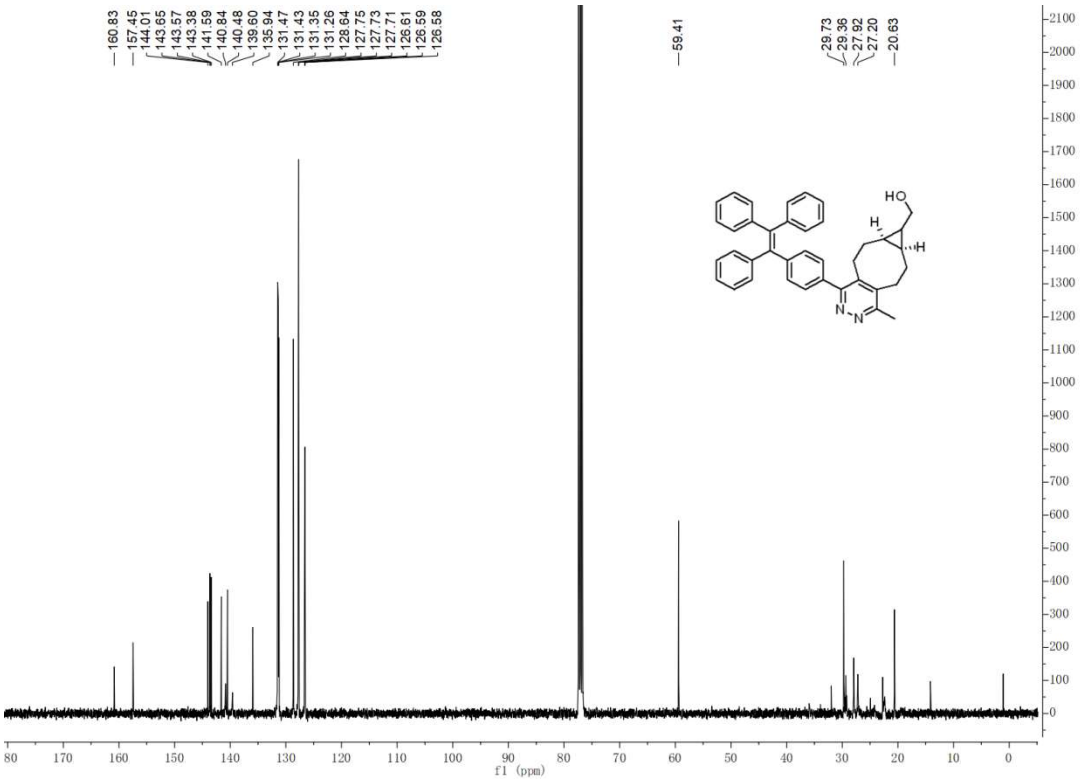
^{13}C NMR of compound **TPE-Tz15** (151 MHz in Acetone-d_6)



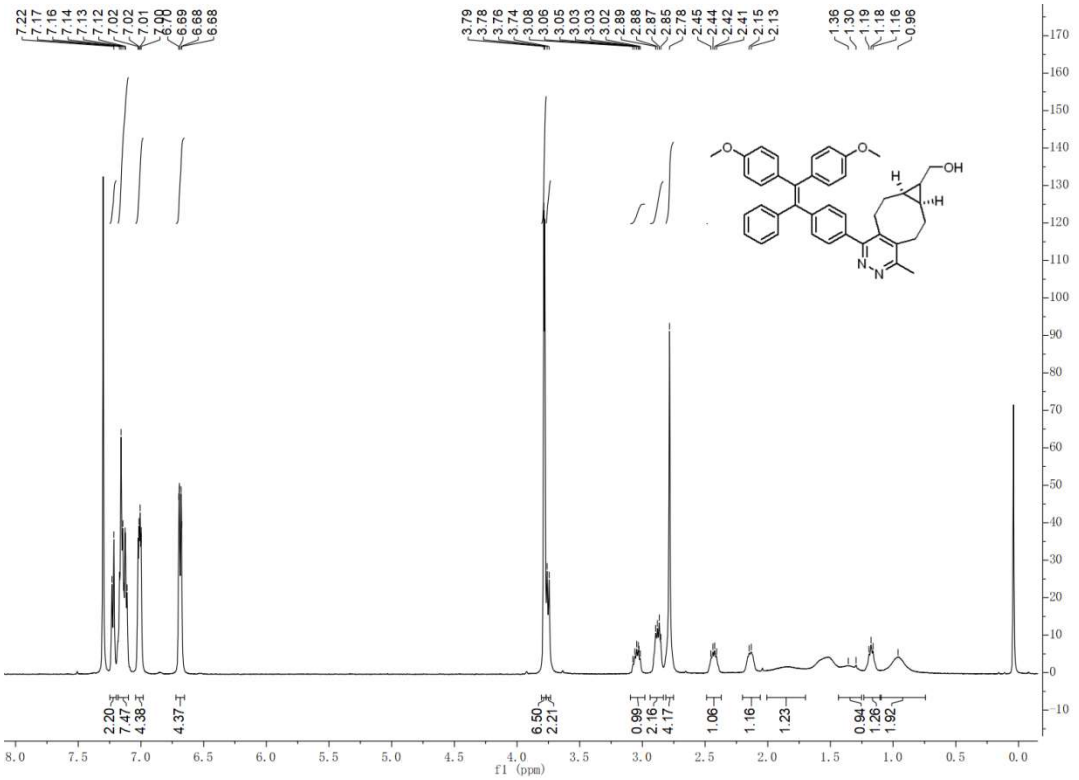
^{1}H NMR of compound **TPE-Pz1** (400 MHz in $CDCl_3$)



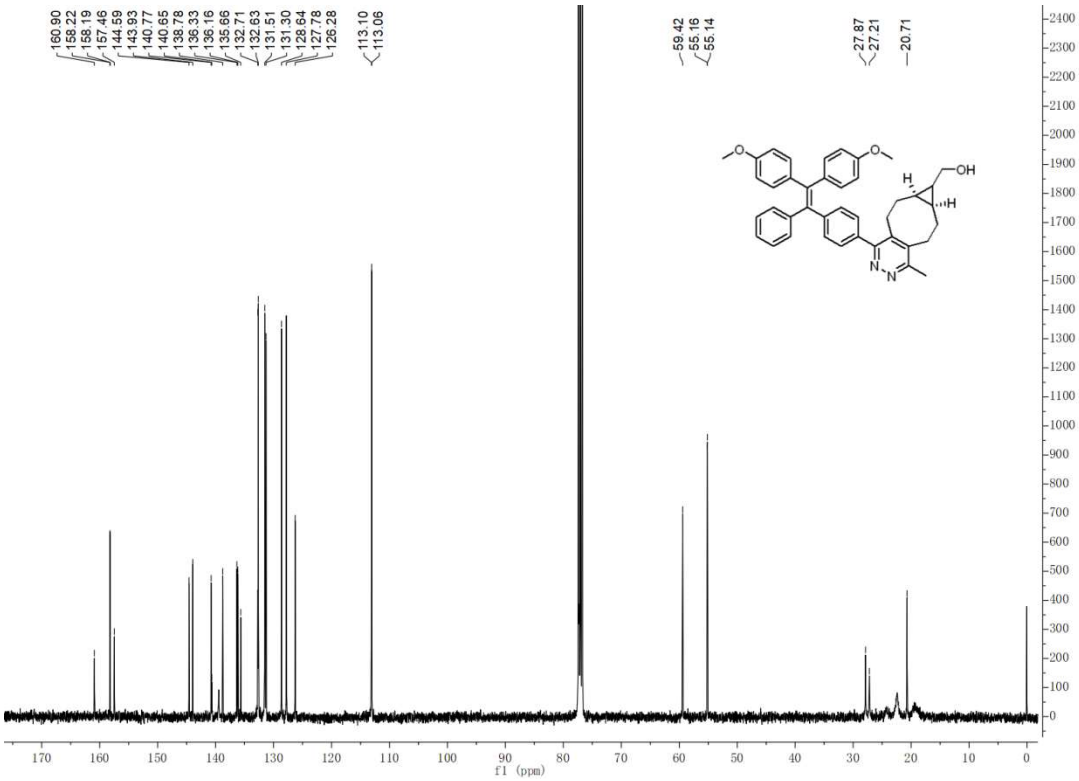
^{13}C NMR of compound **TPE-Pz1** (101 MHz in $CDCl_3$)



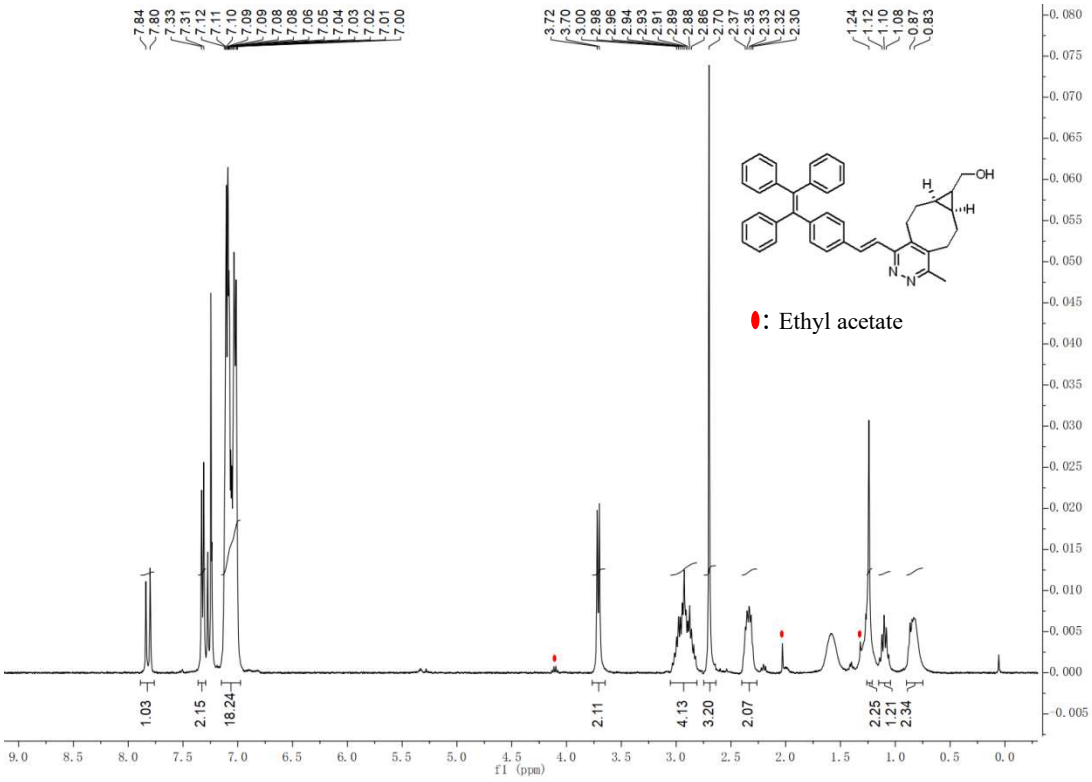
^{1}H NMR of compound **TPE-Pz2** (500 MHz in $CDCl_3$)



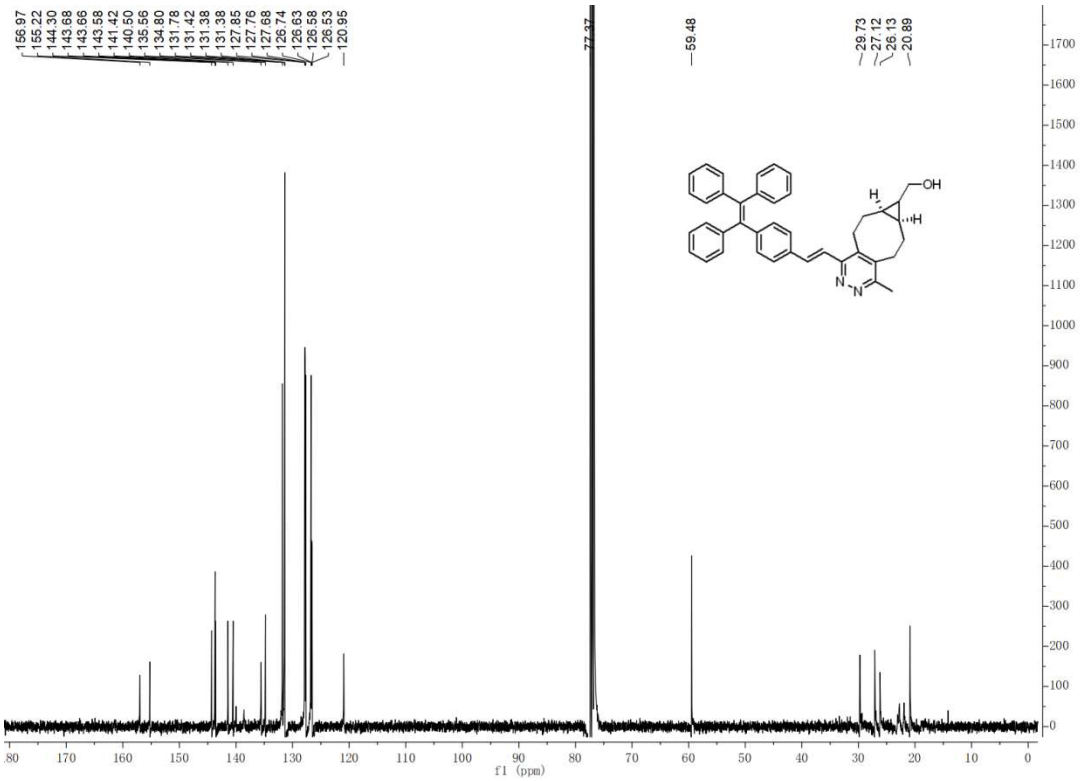
^{13}C NMR of compound **TPE-Pz2** (101 MHz in $CDCl_3$)



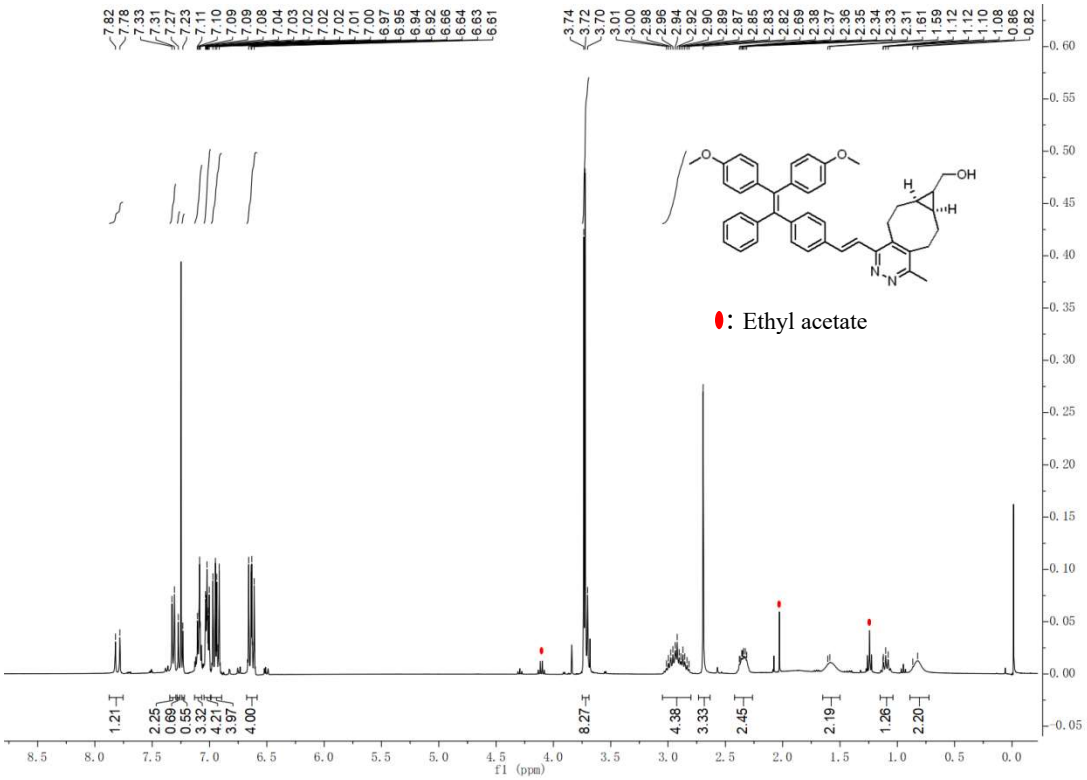
^{1}H NMR of compound **TPE-Pz3** (400 MHz in $CDCl_3$)



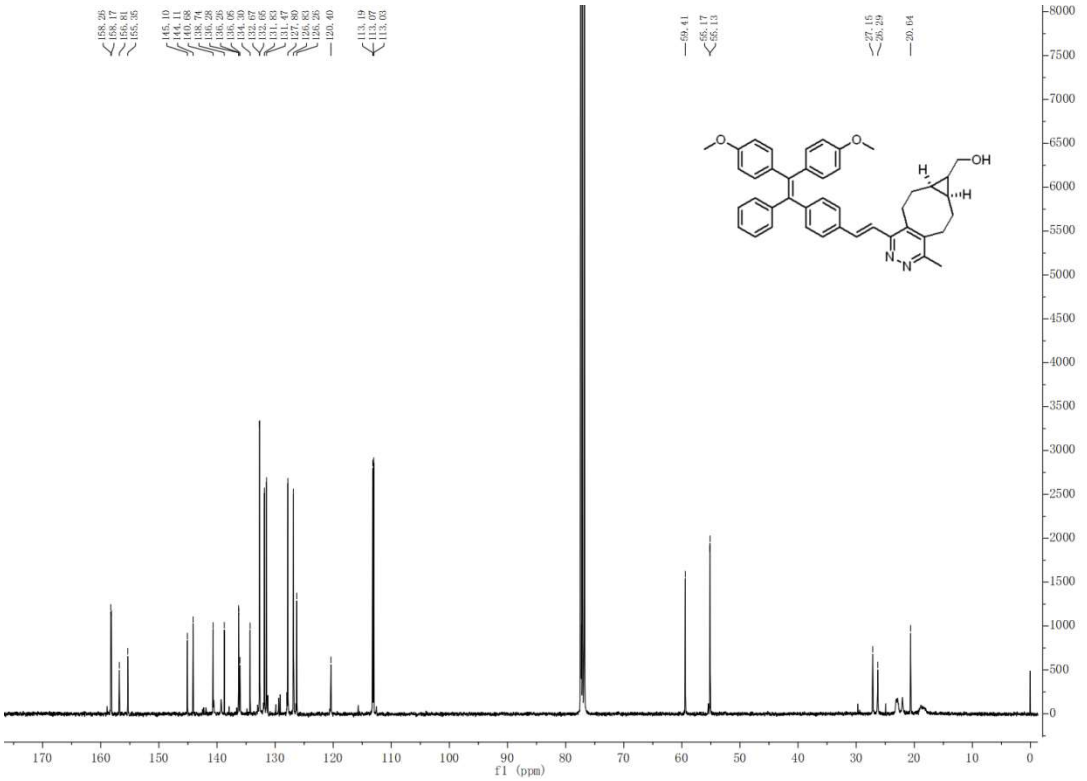
^{13}C NMR of compound **TPE-Pz3** (101 MHz in $CDCl_3$)



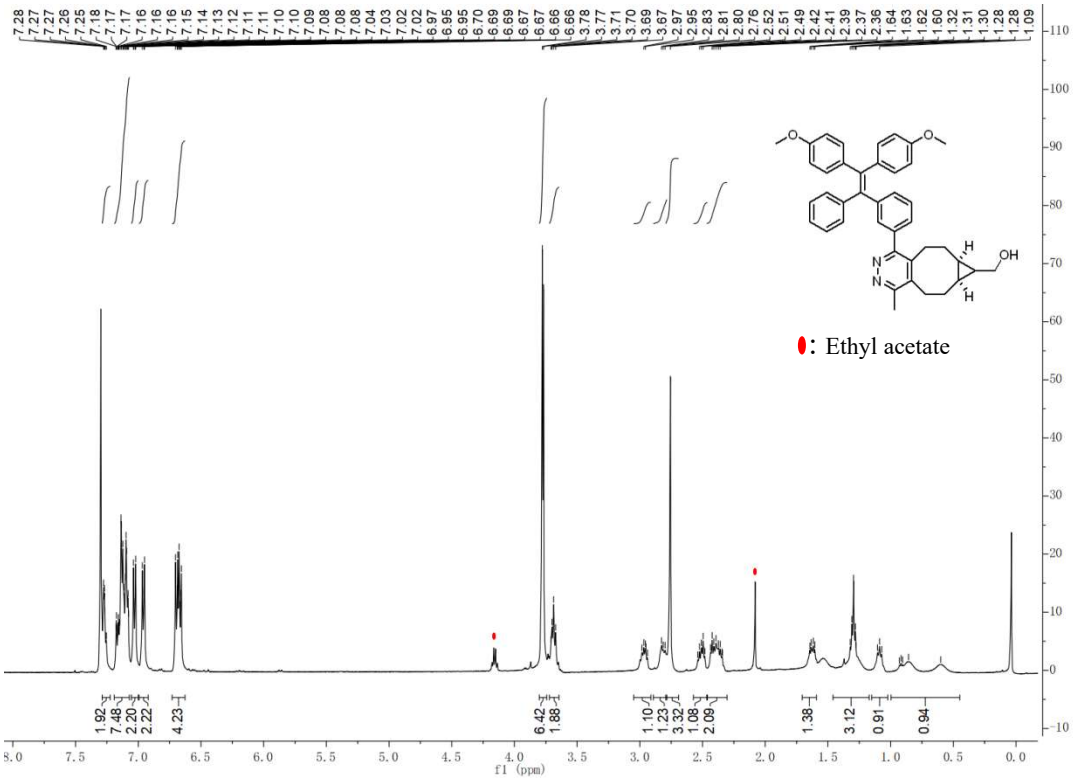
^{1}H NMR of compound **TPE-Pz4** (400 MHz in $CDCl_3$)



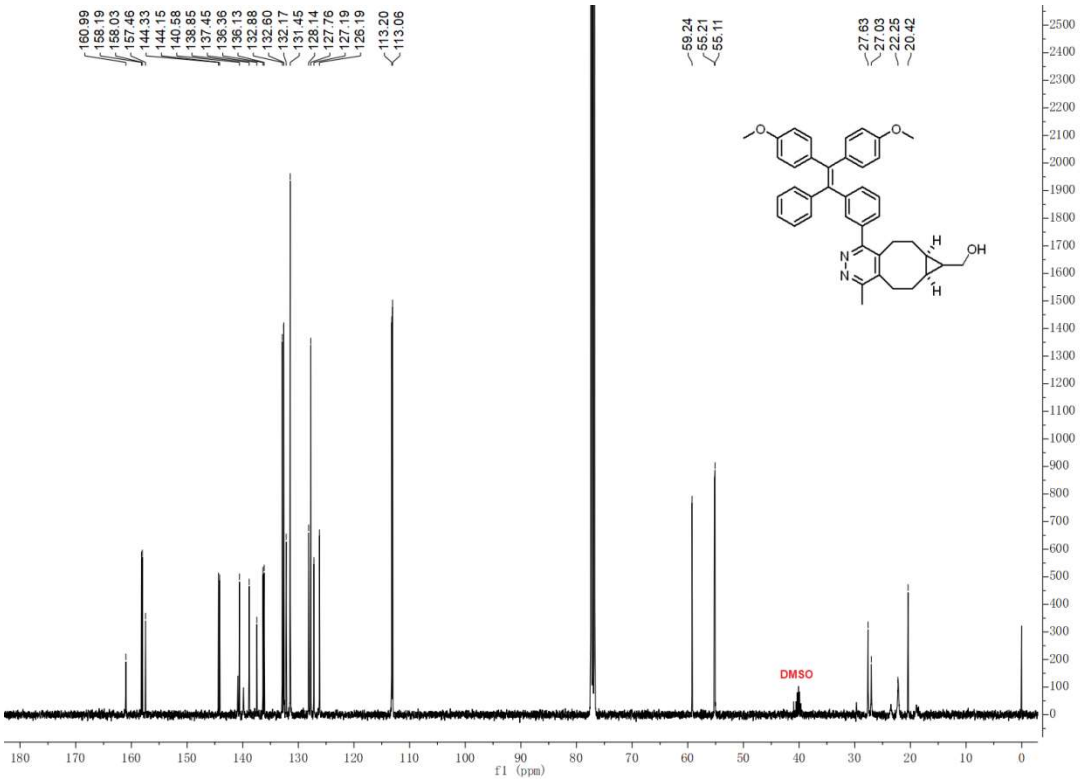
^{13}C NMR of compound **TPE-Pz4** (101 MHz in $CDCl_3$)



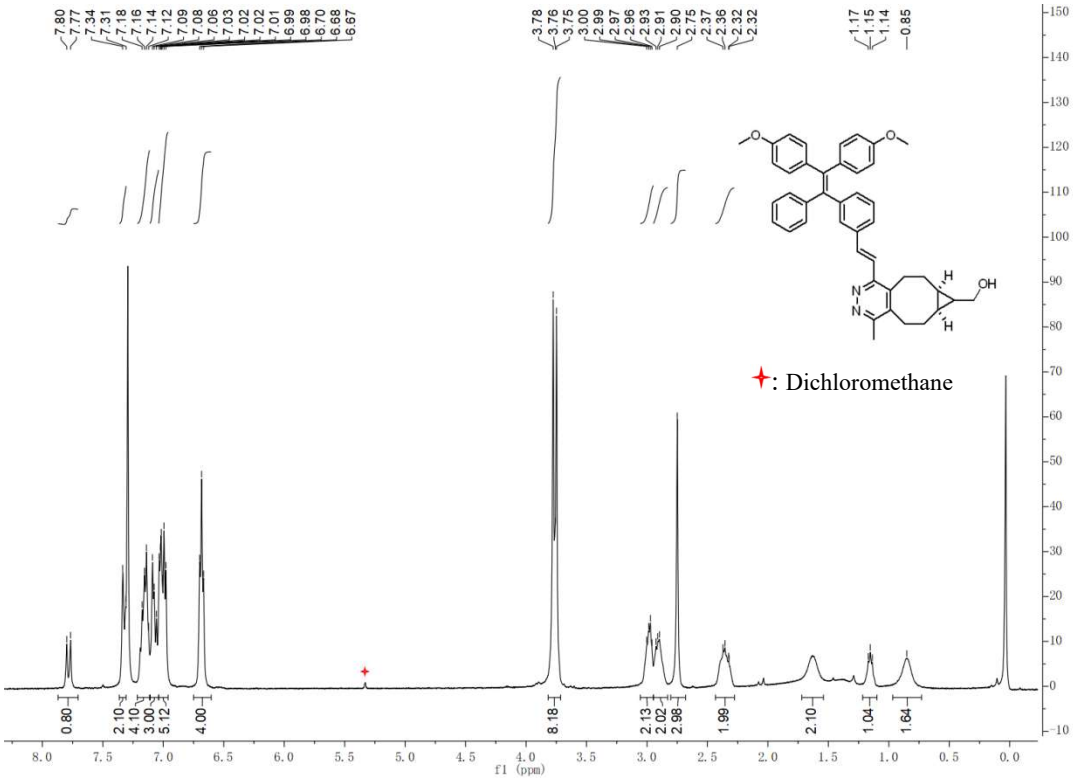
^{1}H NMR of compound **TPE-Pz5** (500 MHz in $CDCl_3$)



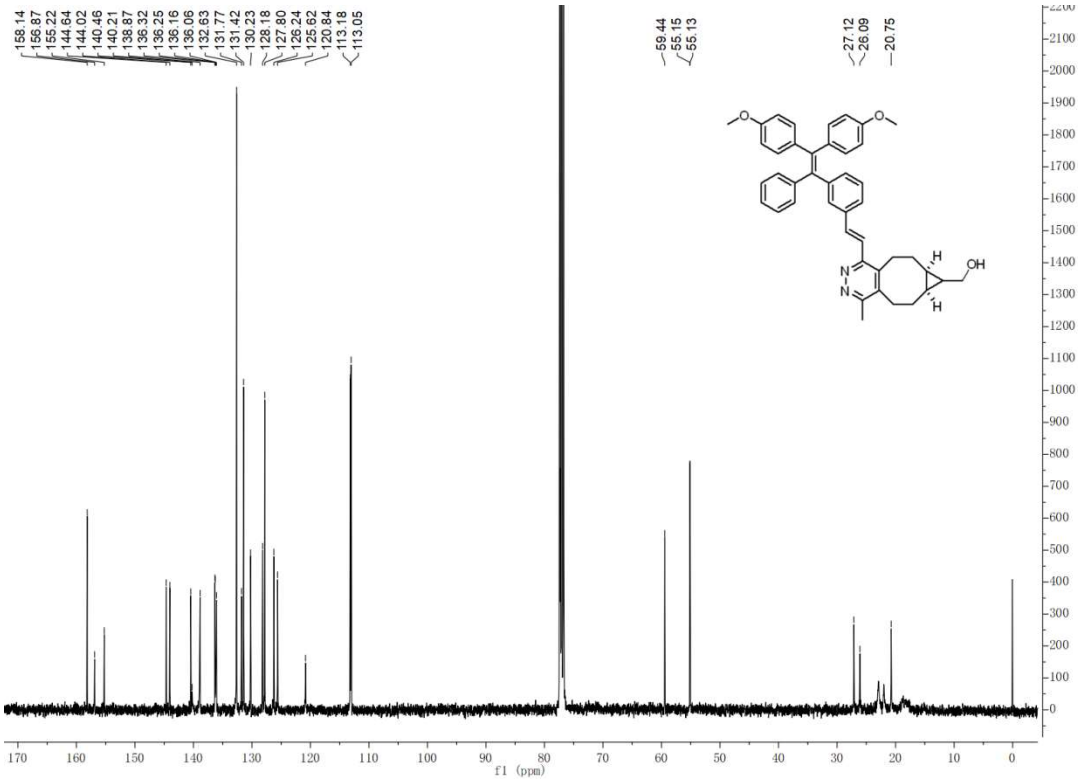
^{13}C NMR of compound **TPE-Pz5** (101 MHz in $CDCl_3$)



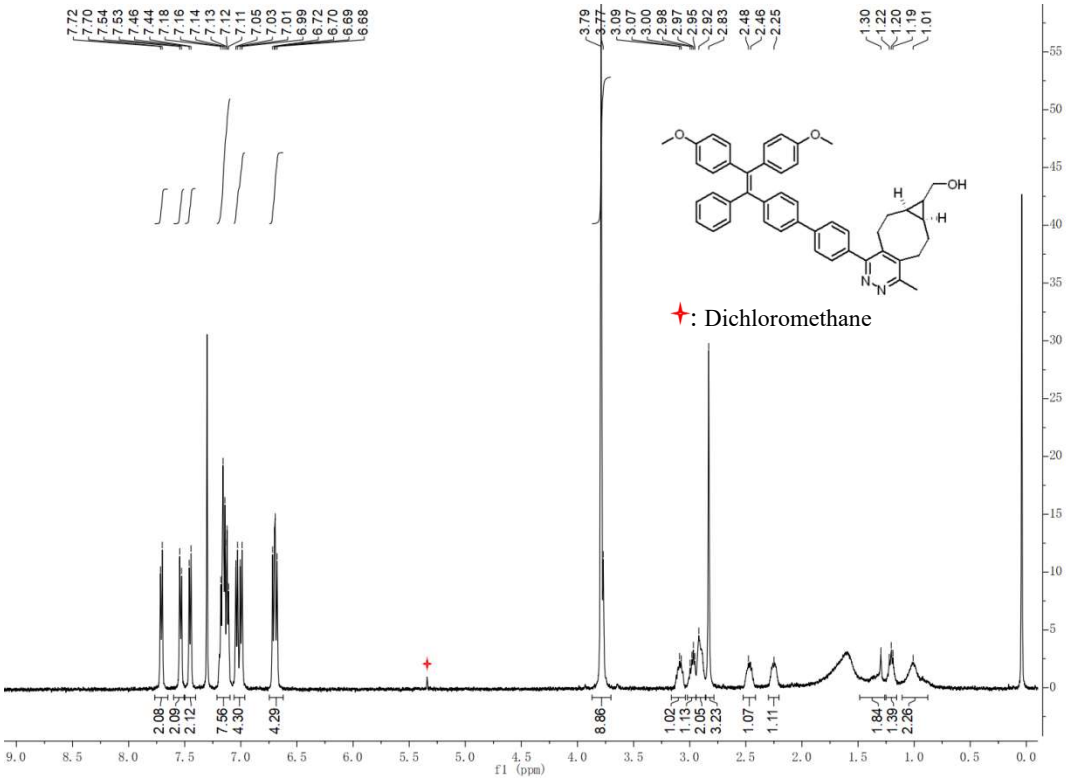
^{1}H NMR of compound **TPE-Pz6** (500 MHz in $CDCl_3$)



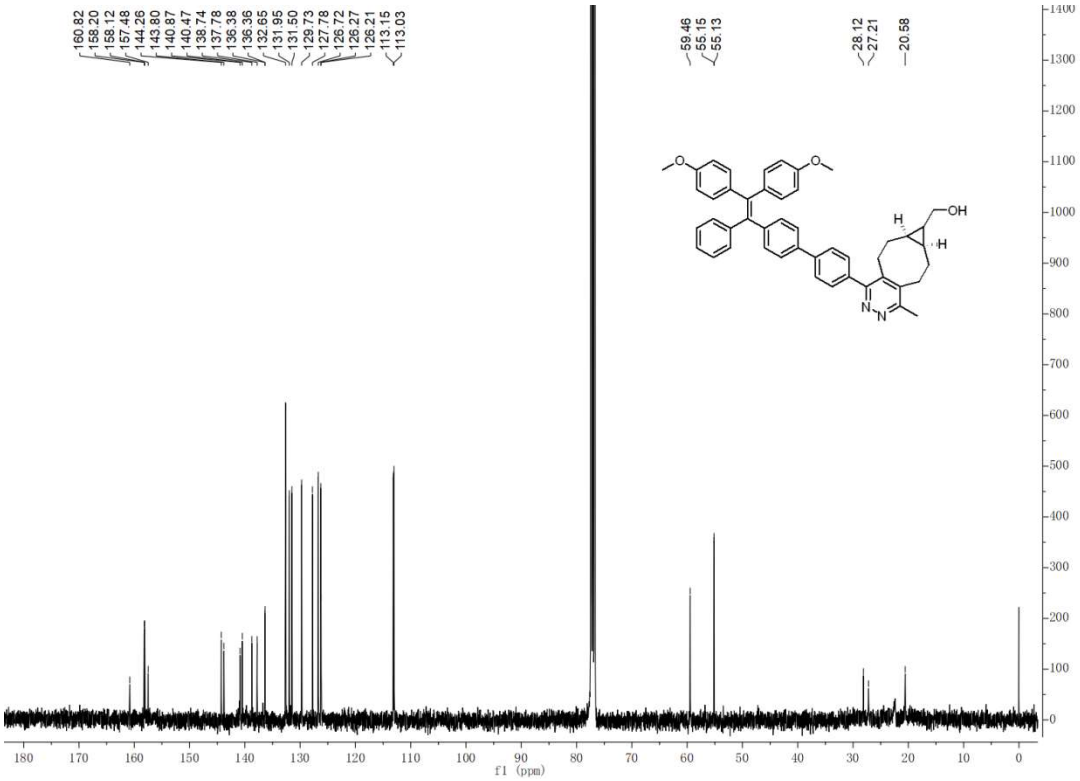
^{13}C NMR of compound **TPE-Pz6** (101 MHz in $CDCl_3$)



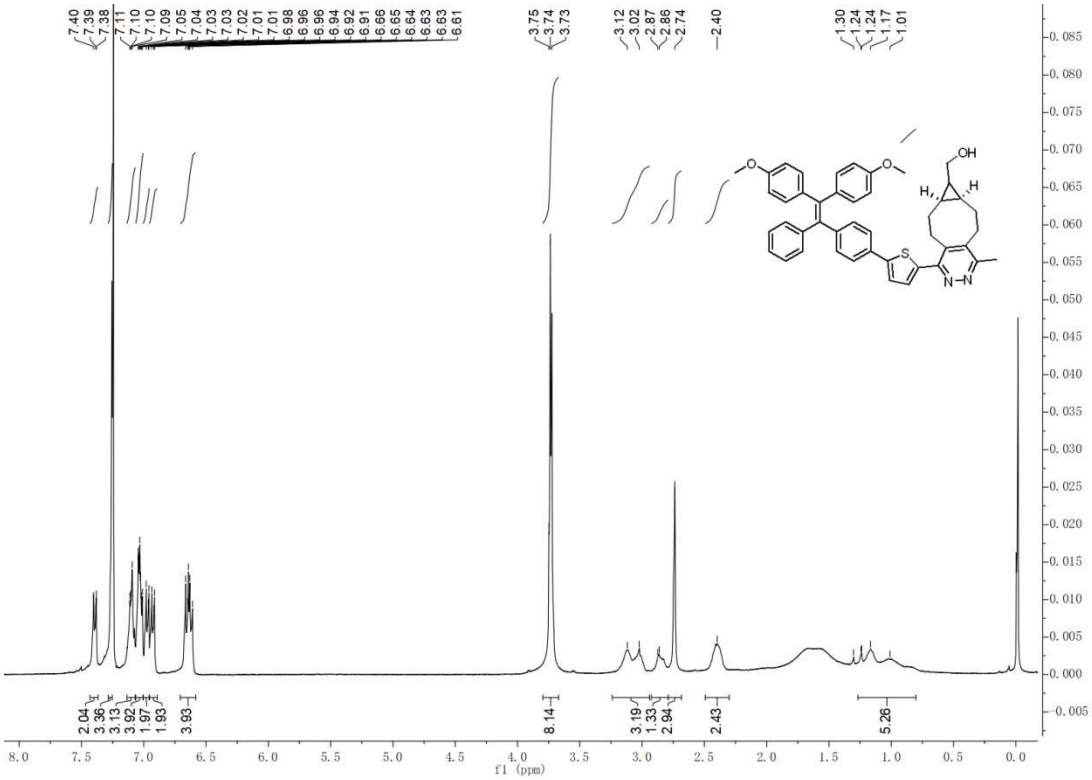
^{1}H NMR of compound **TPE-Pz7** (500 MHz in $CDCl_3$)



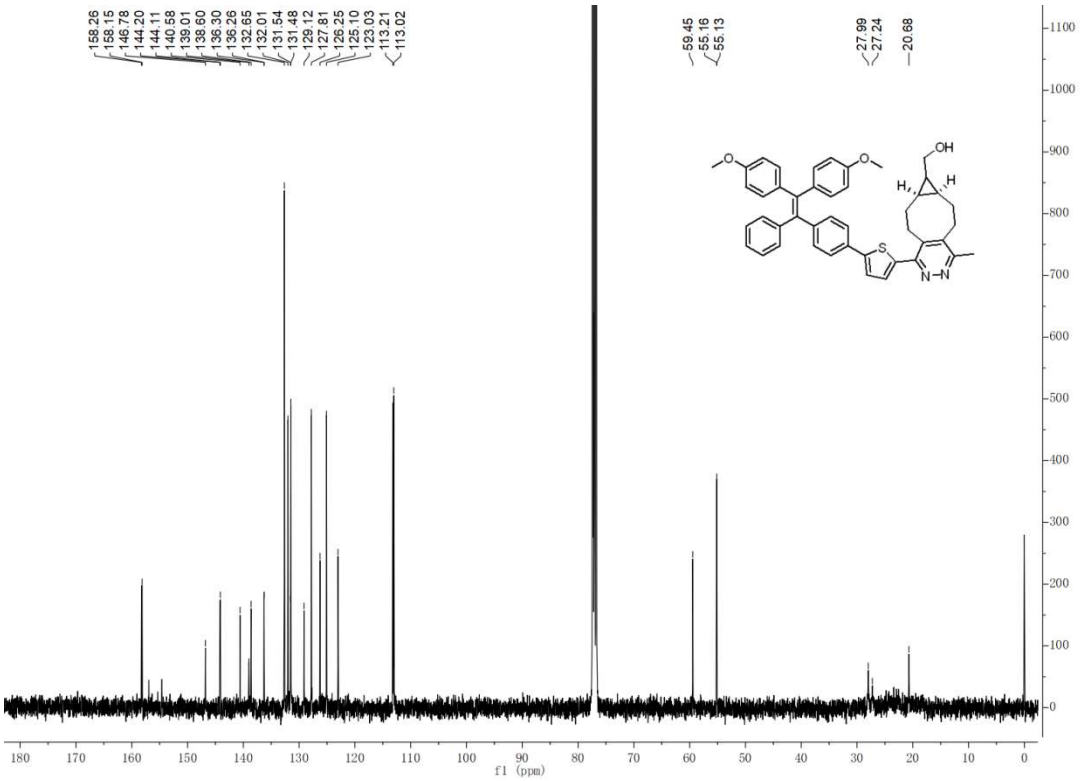
^{13}C NMR of compound **TPE-Pz7** (101 MHz in $CDCl_3$)



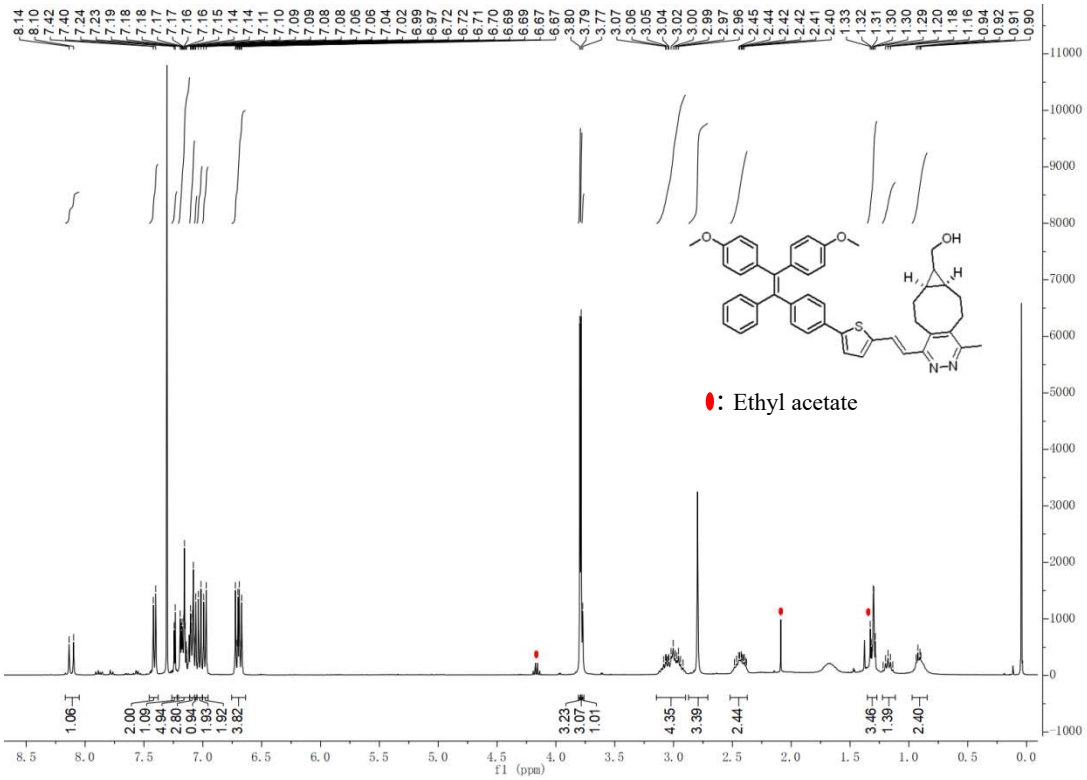
^{1}H NMR of compound **TPE-Pz8** (400 MHz in $CDCl_3$)



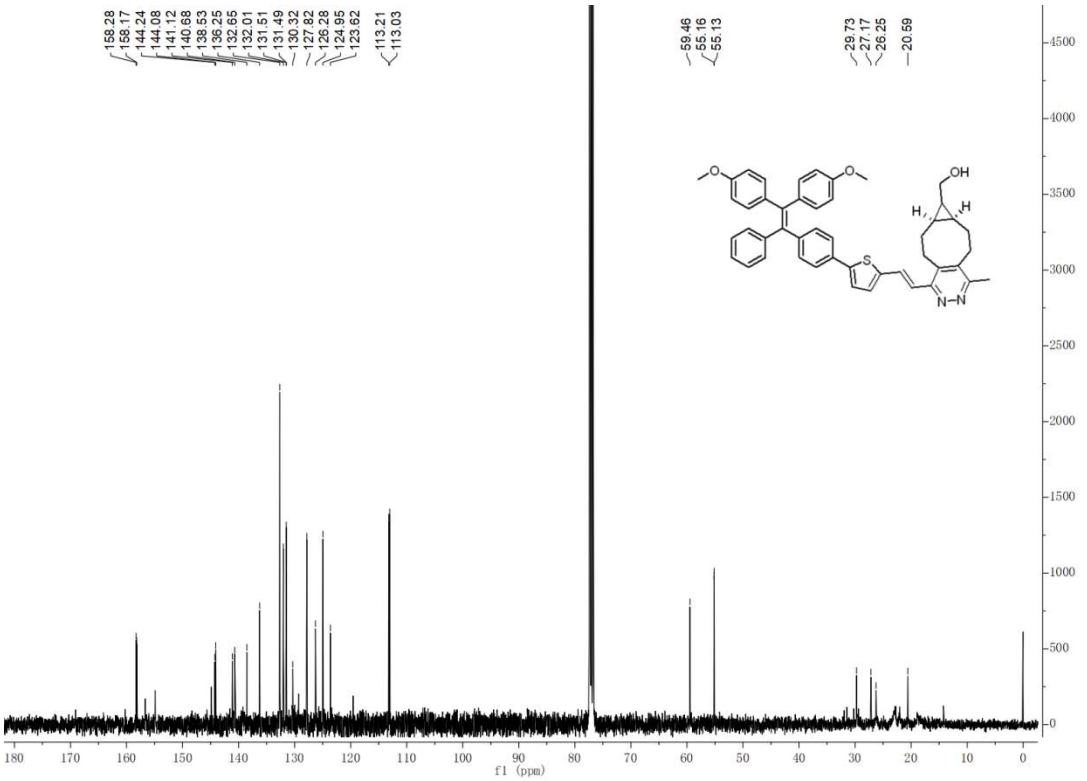
^{13}C NMR of compound **TPE-Pz8** (101 MHz in $CDCl_3$)



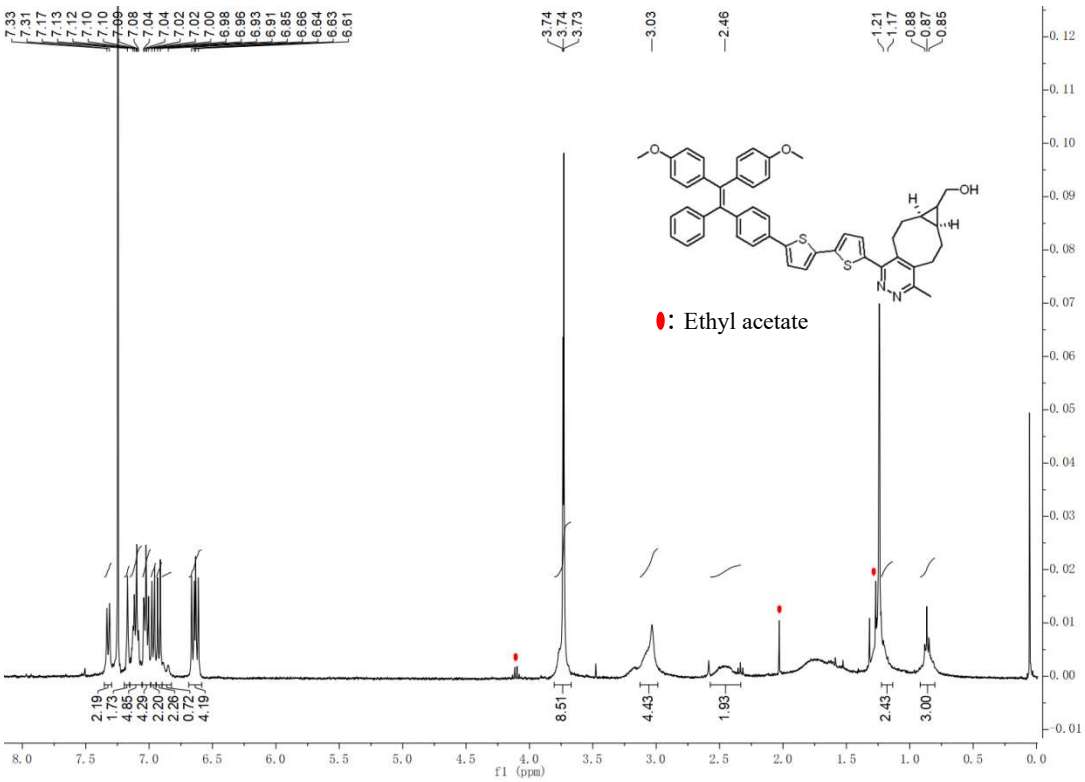
^{1}H NMR of compound **TPE-Pz9** (400 MHz in $CDCl_3$)



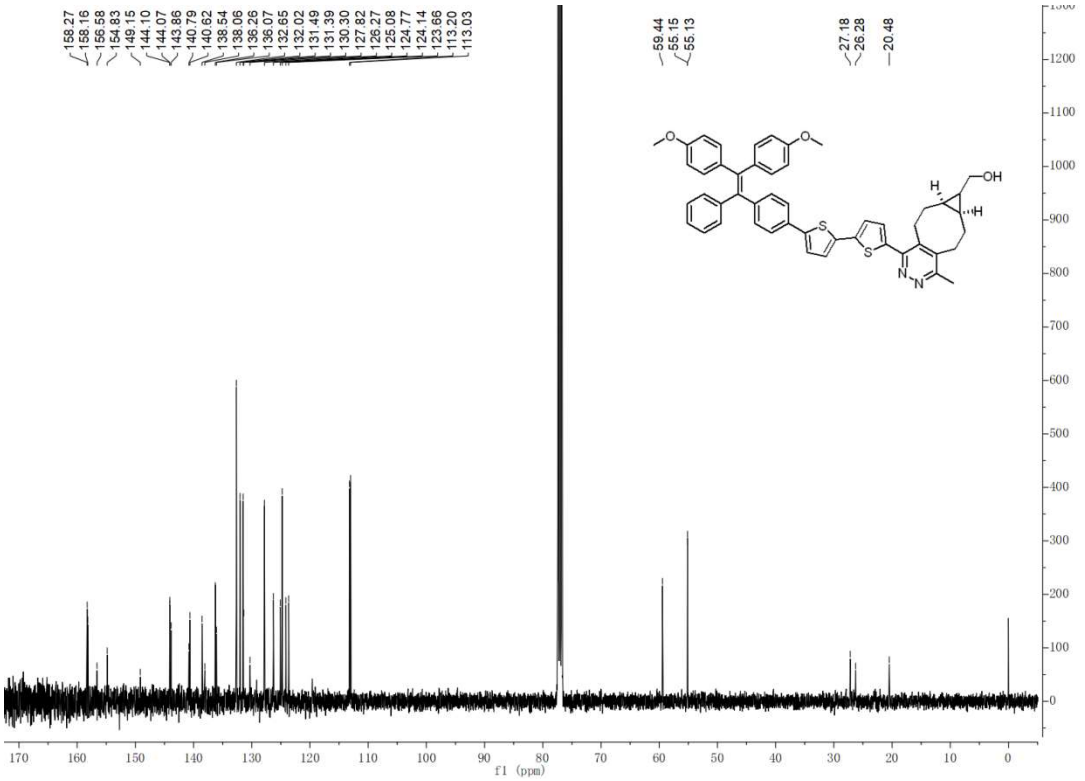
^{13}C NMR of compound **TPE-Pz9** (101 MHz in $CDCl_3$)



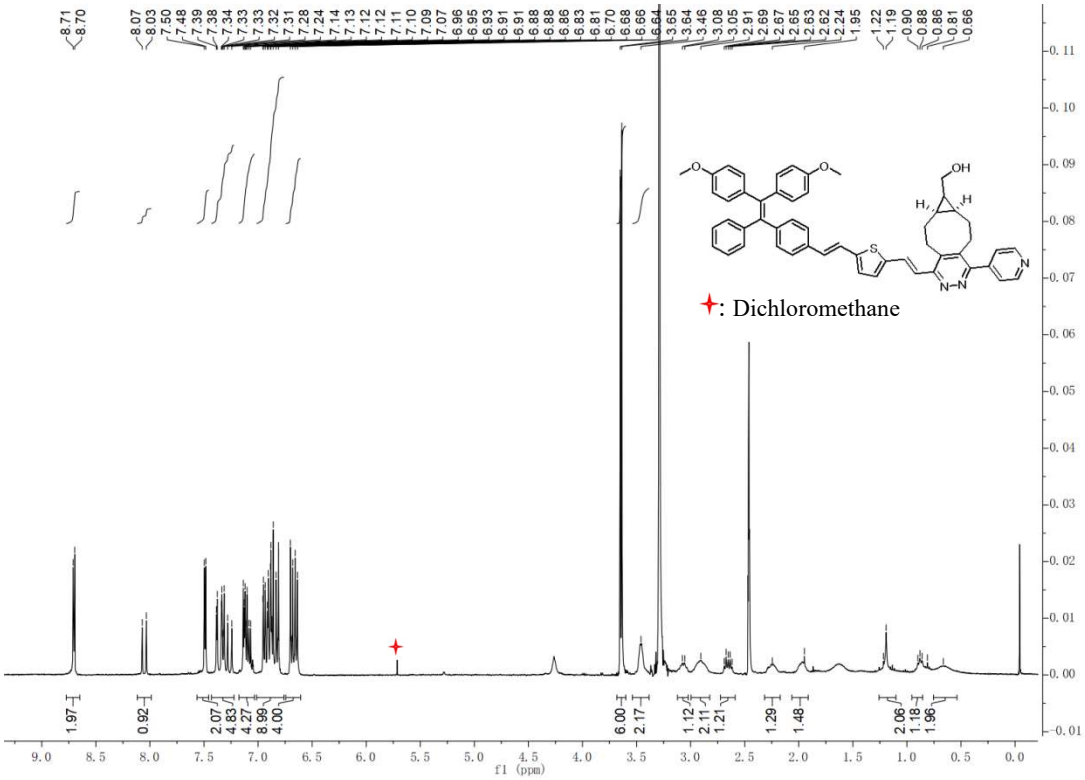
^{1}H NMR of compound **TPE-Pz10** (400 MHz in $CDCl_3$)



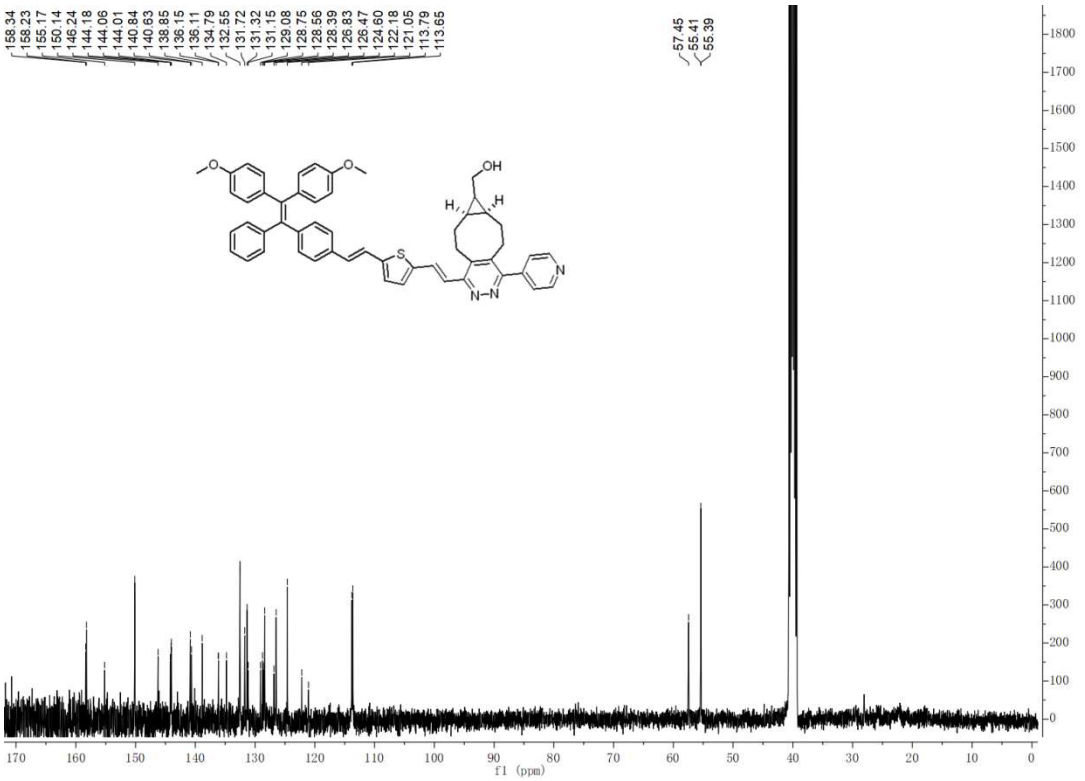
^{13}C NMR of compound **TPE-Pz10** (101 MHz in $CDCl_3$)



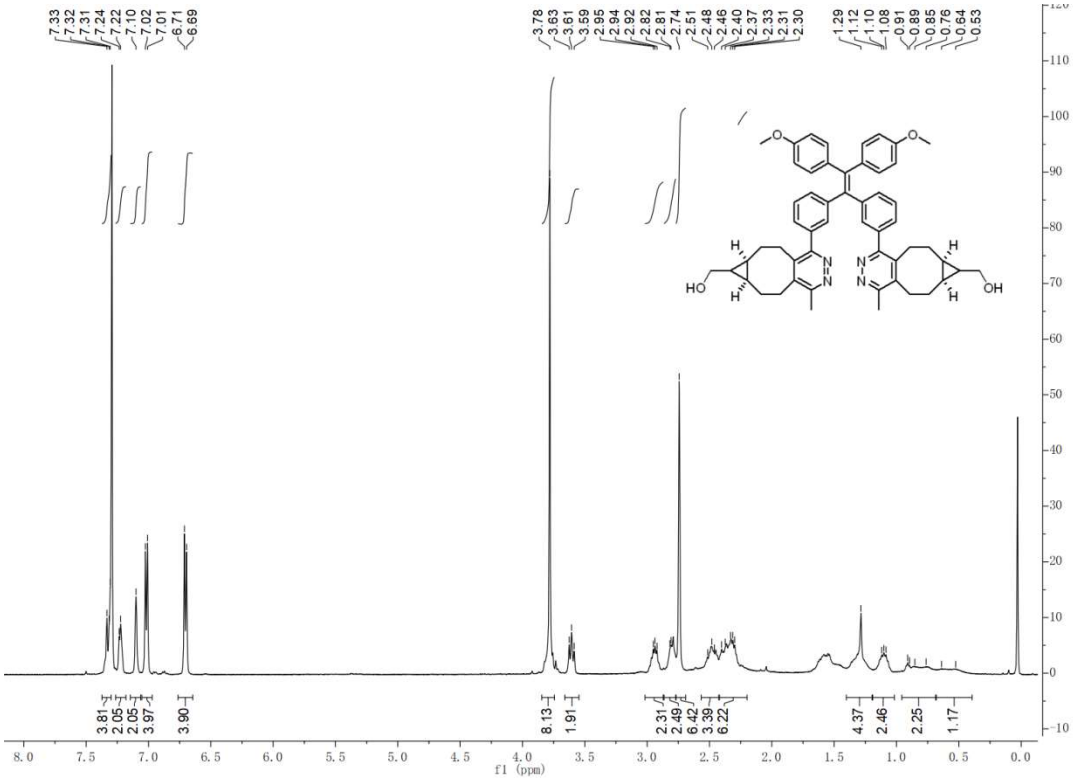
^{1}H NMR of compound **TPE-Pz11** (400 MHz in Acetone-d_6)



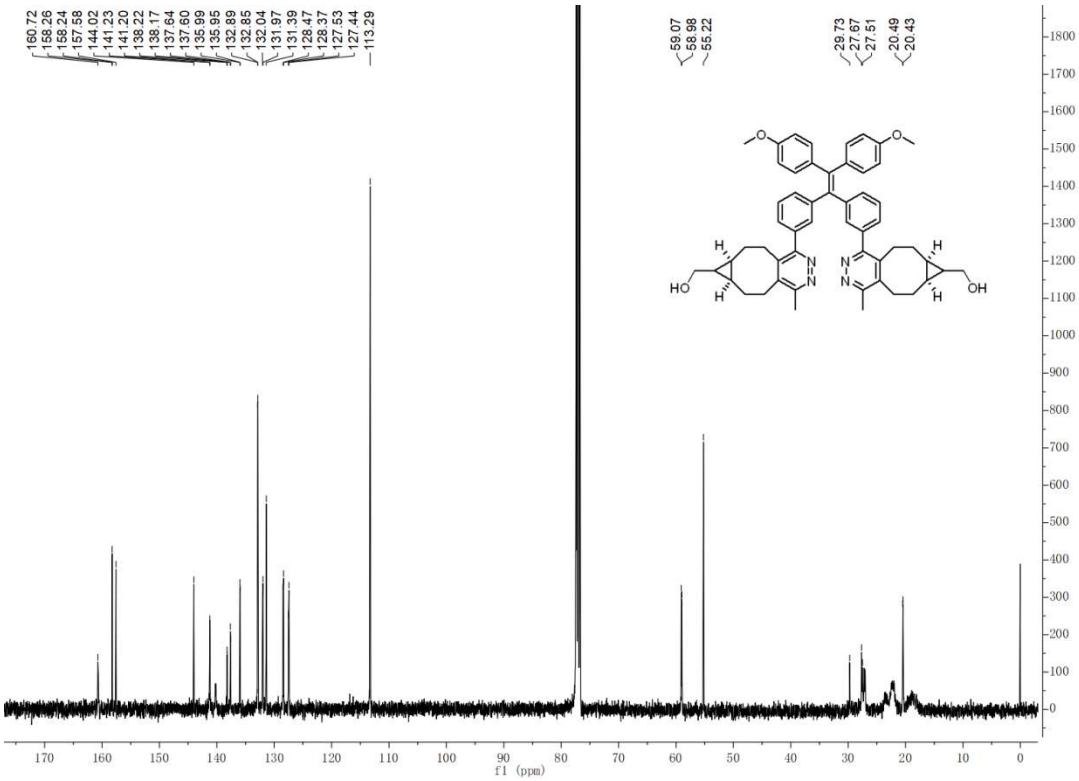
^{13}C NMR of compound **TPE-Pz11** (101 MHz in Acetone-d_6)



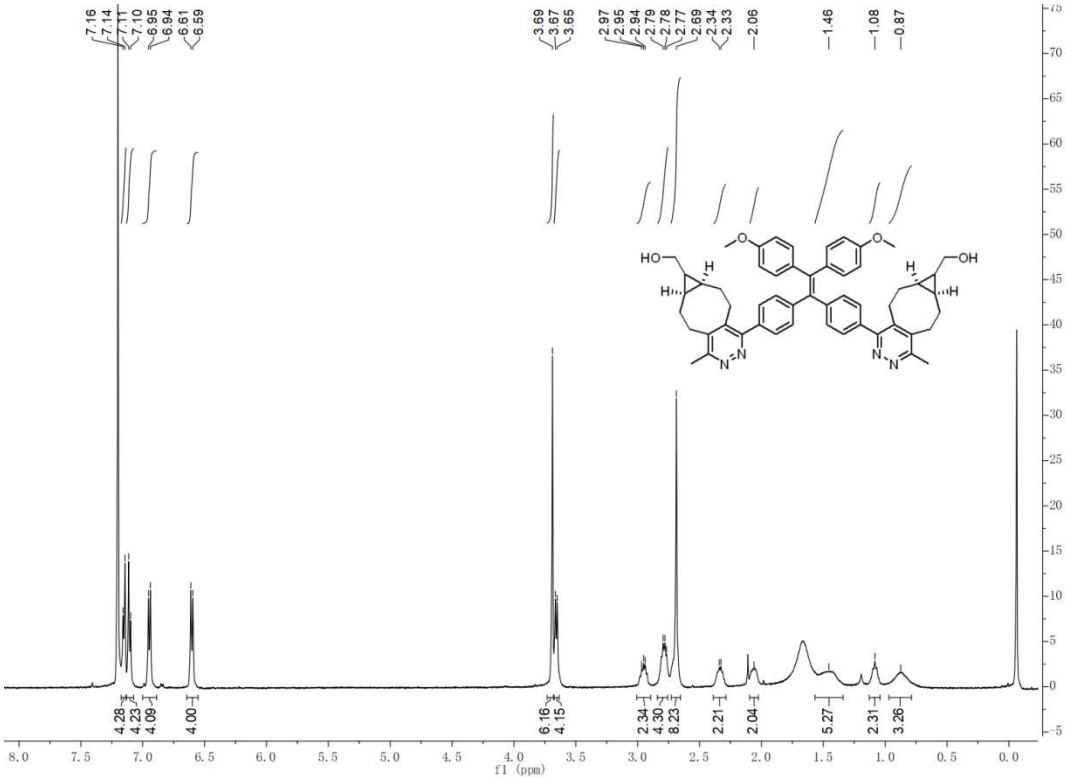
^{1}H NMR of compound **TPE-Pz12** (500 MHz in $CDCl_3$)



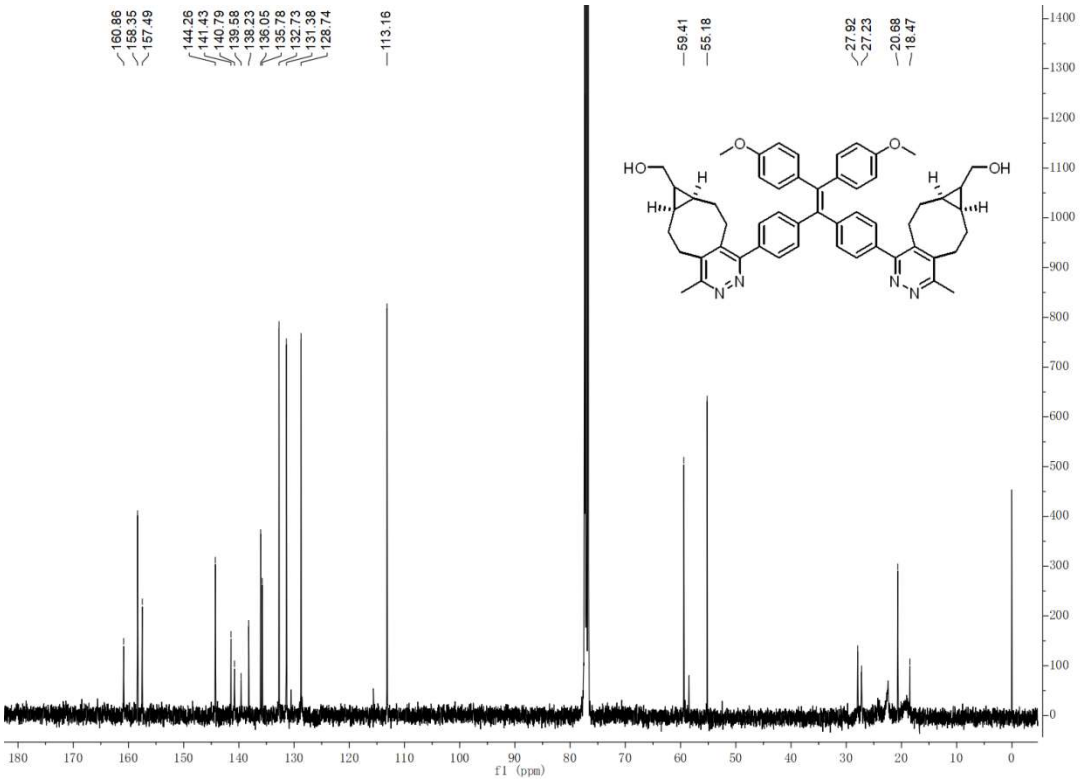
^{13}C NMR of compound **TPE-Pz12** (101 MHz in $CDCl_3$)



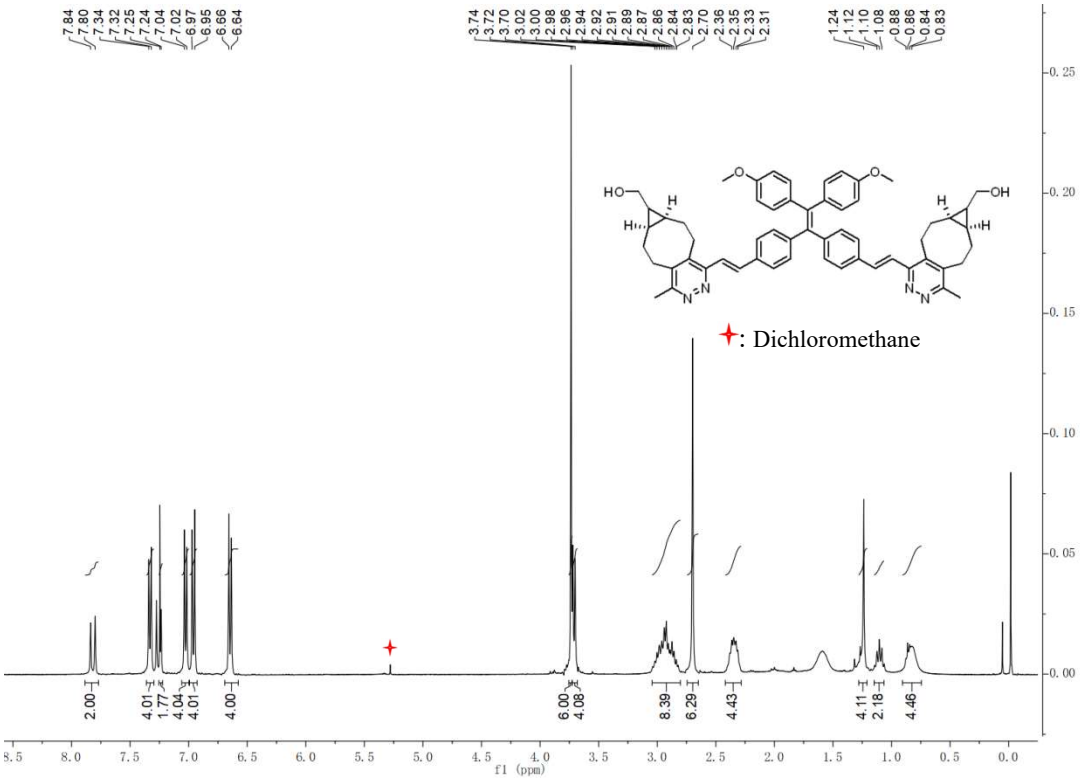
^{1}H NMR of compound **TPE-Pz13** (500 MHz in $CDCl_3$)



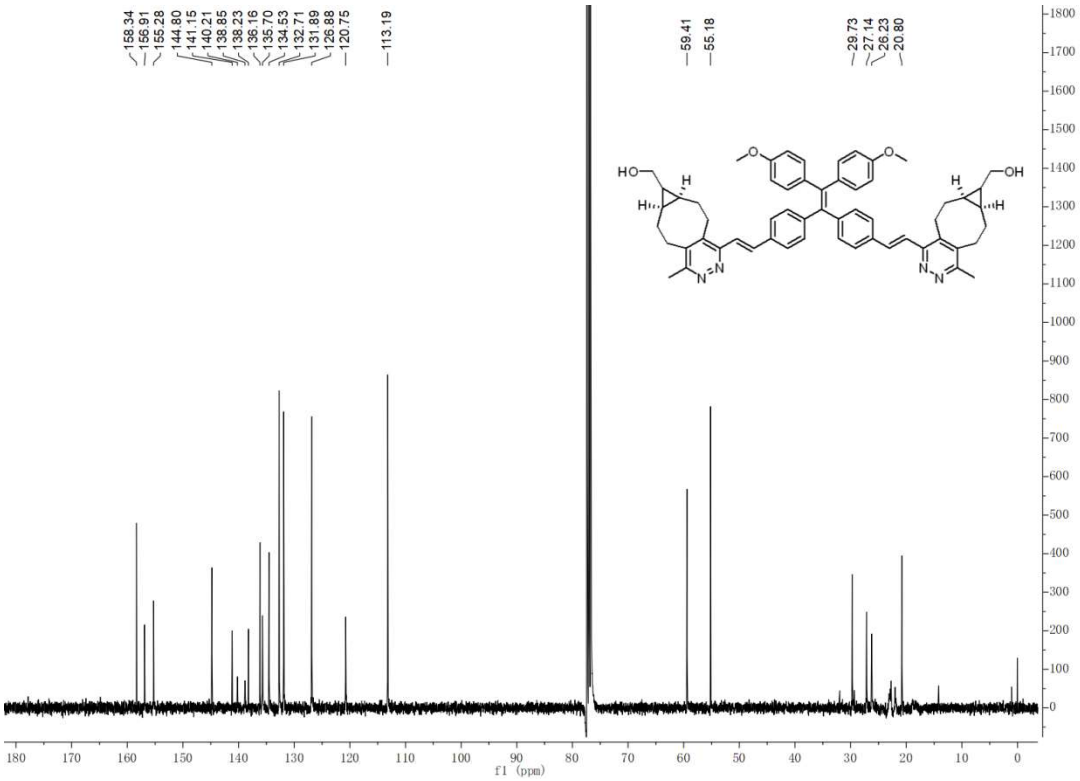
^{13}C NMR of compound **TPE-Pz13** (101 MHz in $CDCl_3$)



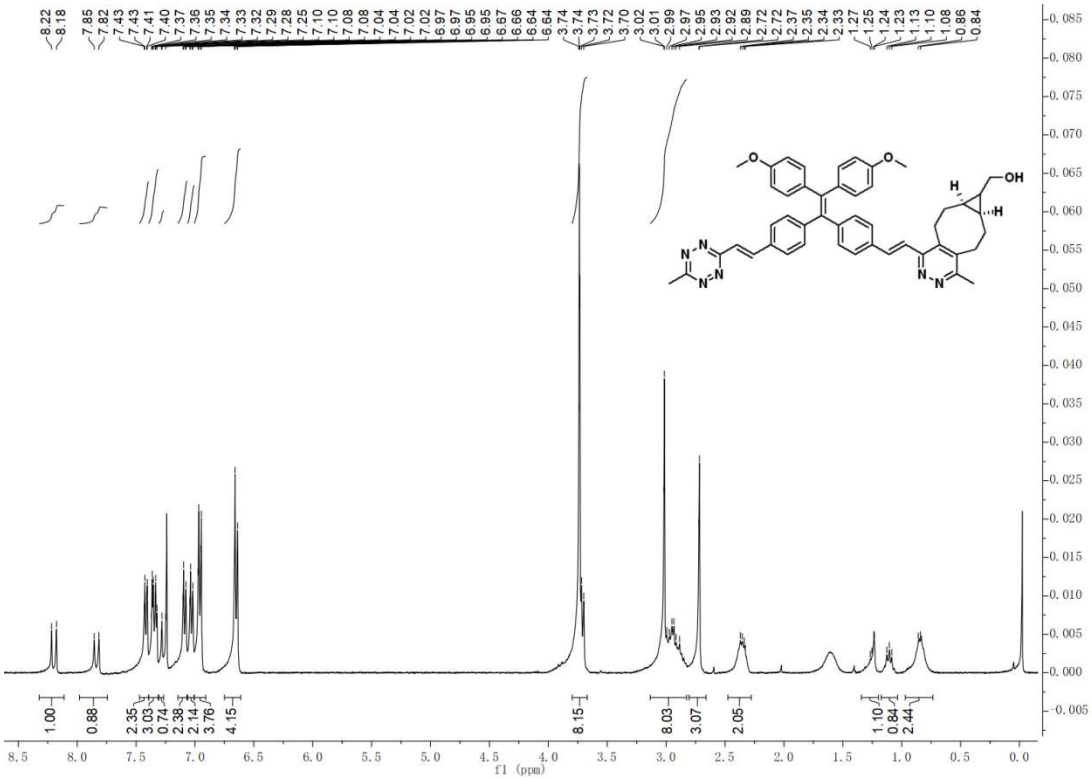
^{1}H NMR of compound **TPE-Pz14** (400 MHz in $CDCl_3$)



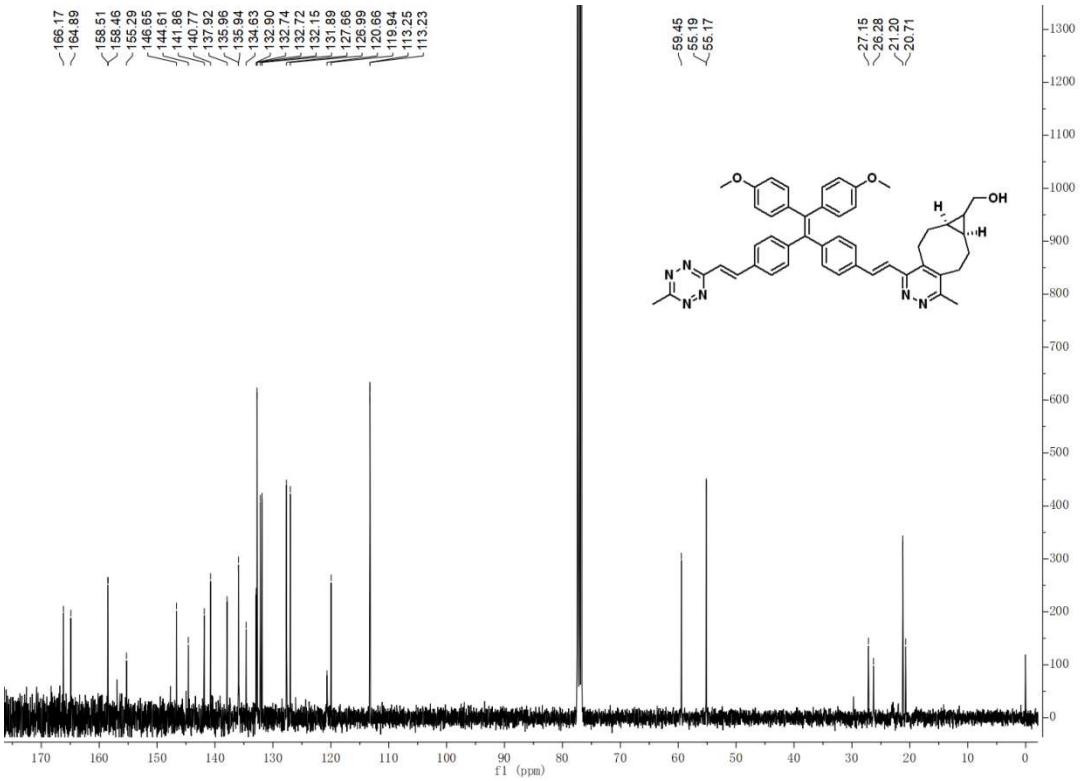
^{13}C NMR of compound **TPE-Pz14** (101 MHz in $CDCl_3$)



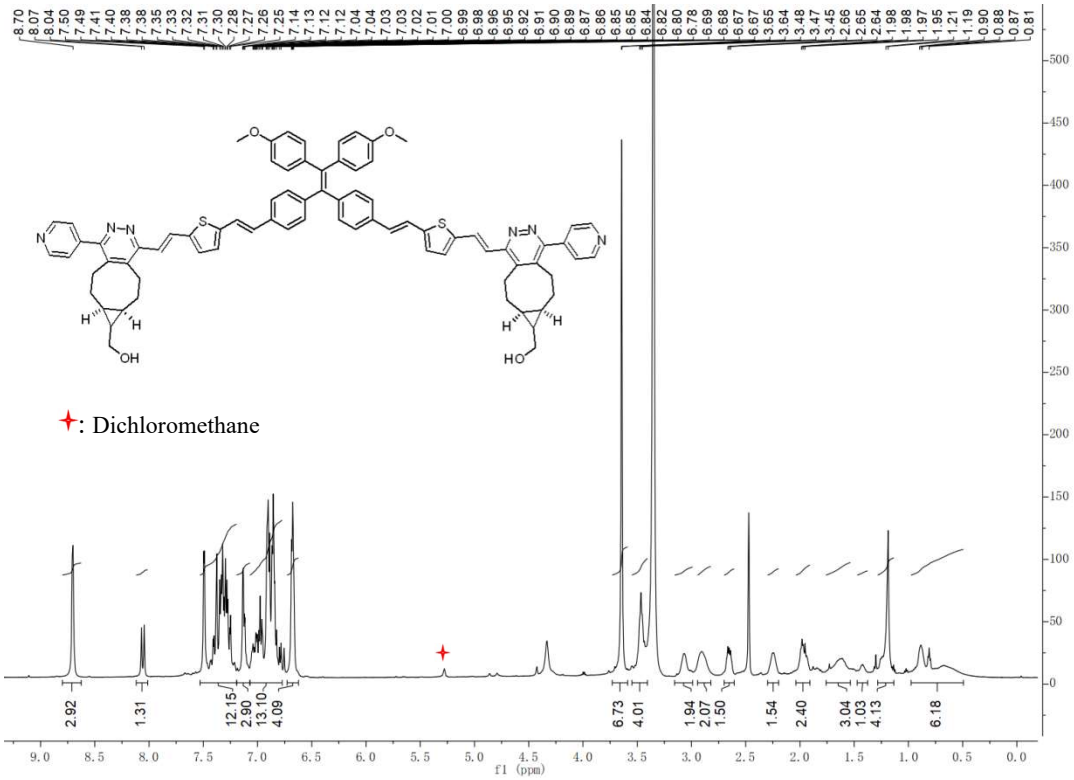
^{1}H NMR of compound **TPE-Pz14m** (400 MHz in $CDCl_3$)



^{13}C NMR of compound **TPE-Pz14m** (101 MHz in $CDCl_3$)



^{1}H NMR of compound **TPE-Pz15** (600 MHz in Acetone-d_6)



^{13}C NMR of compound **TPE-Pz15** (151 MHz in Acetone-d_6)