Supplementary Data

An AIE active fluorescent sensor for measuring Fe^{3+} in aqueous media and iron deficiency anemia drug

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Experimental

General methods

The UV-Vis absorption and fluorescence spectra of the samples were recorded using Shimadzu UV-3600 Plus UV-VIS-NIR Spectrophotometer and Agilent Technologies Cary Eclipse Fluorescence Spectrophotometer, respectively. For the fluorescence measurements, sample solutions were excited at 300 nm and fluorescence intensities were recorded between 310-600 nm. Fourier Transform Infrared (FTIR) measurements of the samples were recorded with Bruker VERTEX 70v FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on 400 (100) MHz Bruker spectrometer and were reported in δ units with SiMe$_4$ as the internal standard. The data for ^{1}H NMR are recorded as follows: chemical shift (d, ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quarted, p = pentet, m = multiplet, bs = broad singlet, bd = broad doublet) and coupling constant(s) in Hz, integration. High-resolution mass spectrometry measurements were recorded on a Q-TOF mass spectrometer.

Materials

The metal ions including salts of NaCl, LiCl, KCl, CaCl$_2$·2H$_2$O, AgCH$_3$COO, MgCl$_2$·6H$_2$O, ZnCl$_2$·H$_2$O, CuCl$_2$·2H$_2$O, BaCl$_2$·2H$_2$O, HgCl$_2$, CrCl$_2$, CdCl$_2$, Pb(CH$_3$COOH)$_2$·3H$_2$O, MnCl$_2$·4H$_2$O, CoCl$_2$·6H$_2$O, FeSO$_4$·7H$_2$O, CrCl$_3$, AlCl$_3$ and FeCl$_3$, tetrahydrofuran (THF) and ethanol (EtOH) were sourced from Sigma-Aldrich. The stock solution of **TPE-TAP** was prepared at a concentration of 1.0x10^{-3} M in ethanol, and samples were freshly prepared at desired concentrations after evaporating ethanol. The experiments were conducted at room temperature (25 ^{o}C) with a final probe concentration of 50 μM.

Synthesis of TPE-TAP
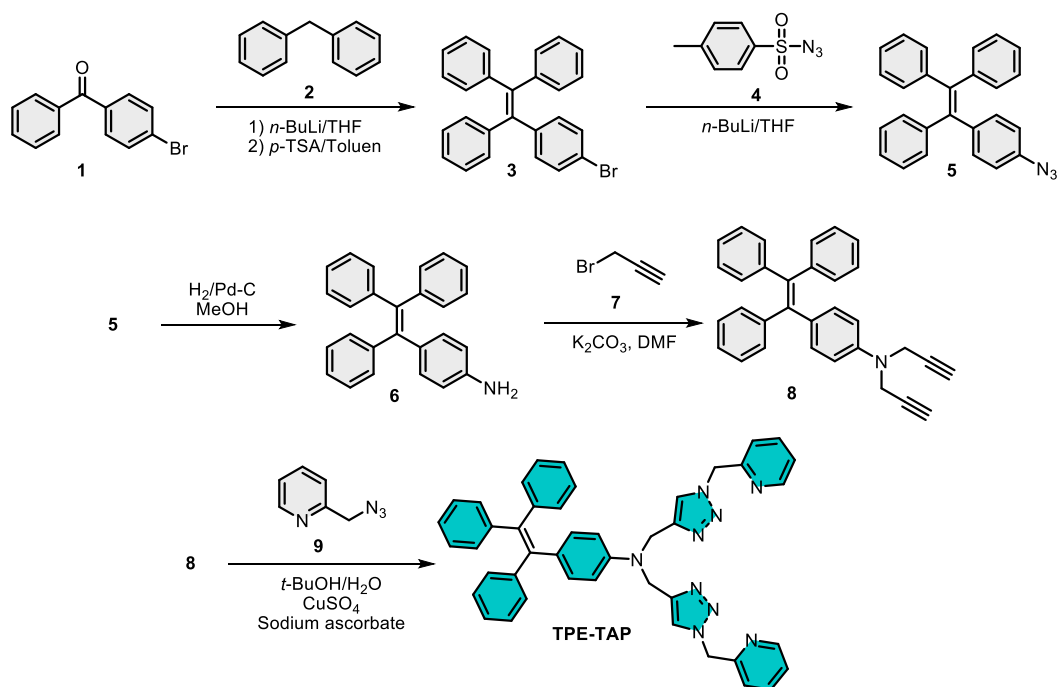
2-(4-Bromophenyl)ethene-1,1,2-triyl)tribenzene (3)[1]

To a stirred solution of diphenylmethane (2.00 g, 11.9 mmol) in dry THF (70 mL) *n*-butyllithium (2.5 M in hexane, 4.76 mL, 11.9 mmol) was added dropwise at 0 °C under nitrogen atmosphere. The resulting mixture was stirred for 2 h at 0 °C. To this mixture was added a solution of 4-bromobenzophenone (2.59 g, 9.91 mmol) in THF (30 mL), and the reaction was allowed to stir at room temperature for 12 h. After completion, the reaction mixture was quenched by adding an aqueous solution of ammonium chloride and then the mixture was extracted with dichloromethane (3 × 50 mL). The organic layers were combined and dried over anhydrous Na_2SO_4, and the solvent was evaporated to give a crude alcohol intermediate. The alcohol intermediate was dissolved in toluene (60 mL), and *p*-toluenesulfonic acid (PTSA, 500 mg) was added to it and refluxed for 16 h. After the mixture was cooled to rt was evaporated on a rotary evaporator to give a crude residue which was purified by silica gel chromatography with hexane to give compound **3** (3.3 g, 80%) as white solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.24 – 7.18 (m, AA' part of AA'BB' system, 2H), 7.16 – 7.06 (m, =CH, 9H), 7.05 – 6.94 (m, =CH, 6H), 6.93 – 6.85 (m, BB' part of AA'BB' system, =CH, 2H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 143.54, 143.5, 143.4, 142.8, 141.7, 139.8, 133.1, 131.4, 131.4, 131.4, 131.0, 128.1, 127.1, 127.2, 126.8, 126.78, 126.7, 120.6.

(2-(4-Azidophenyl)ethene-1,1,2-triyl)tribenzene (5)[2]

*n-B*utyllithium (2.5 M in hexane, 1.17 mL, 2.92 mmol) was added dropwise to a solution of 2-(4-bromophenyl)ethene-1,1,2-triyl)tribenzene (3) (1.0 g, 2.43 mmol) in 40 mL anhydrous THF at -78 °C under nitrogen. After the mixture was stirred for 2 h at this temperature a solution of 4-methylbenzenesulfonyl

azide (575 mg, 2.92 mmol) in THF (10 mL) was added dropwise and stirred at −78 °C for 1 h, the mixture was warmed slowly to room temperature and stirred overnight. Afterward, saturated NH_4Cl solution was added to quench the reaction, and THF was evaporated.Then, dichloromethane (DCM) was added to extract the product three times. The organic layer was combined and washed with water and brine and dried over Na_2SO_4. After filtration and solvent evaporation, the crude product was purified by a silica gel column chromatography using petroleum ether to give compound **5** as a Pale yellow solid (845 mg, 93%).

4-(1,2,2-triphenylvinyl)aniline (6)[2]

(2-(4-Azidophenyl)ethene-1,1,2-triyl)tribenzene (800 mg, 2.14 mmol) was dissolved in MeOH (15 mL), and 10% Pd/C (15 mg) was added. The mixture was stirred in H2 atmosphere for 12 h. The Pd/C was removed by filtration and the solvent was evaporated to obtained pure compound 6 yellow solid (710 mg, 95%).

N,N-di(prop-2-yn-1-yl)-4-(1,2,2-triphenylvinyl)aniline (8)

To a solution of 4-(1,2,2-triphenylvinyl)aniline (500 mg, 1.44 mmol) in DMF (15 mL) potassium carbonate (1.2 g, 8.6 mmol) was added and stirred for 5 min at room temperature. A solution of propargyl bromide (1.1 mL, 10.1 mmol) in DMF (5 mL) was added to the mixture dropwise. The reaction mixture is stirred at room temperature for 24h. Then the reaction mixture was quenched by adding water and then the mixture was extracted with diethyl ether (3 × 50 mL). The organic layers were combined and dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (v/v = 99:1) to give compound **8** as a orange oil (366 mg, 60%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.56 – 6.97 (m, 15H), 6.90 (d, J = 8.8 Hz, 2H), 6.73 (d, J = 8.8 Hz, 2H), 4.13 (d, J = 2.3 Hz, 4H), 2.72 (t, J = 2.3 Hz, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 146.5, 144.5, 144.4, 141.1, 139.9, 134.2, 132.1, 131.43, 131.4, 131.3, 128.0, 127.8, 126.52, 126.5, 126.4, 114.2, 79.6, 73.3, 39.7 (2C signal overlaps). HRMS (APCI-TOF) m/z: $[M + H]^+$ calcd for $C_{32}H_{26}N$, 424.2060; found 424.2061.

TPE-TAP

N,N-di(prop-2-yn-1-yl)-4-(1,2,2-triphenylvinyl)aniline (**3**) (100 mg, 0.24 mmol), $CuSO_4·$(16.8 mg, 0.08 mmol) and sodium ascorbate (16.4 mg, 0.08 mmol) were added to a solution of 2-(azidomethyl)pyridine (95.0 mg, 0.71 mmol) in t-BuOH/H_2O (1:1, v/v; 20 mL). The solution was refluxed for 5 h at 100 °C. The reaction mixture was cold to room temperature and extracted with CH_2Cl_2 (2×20 mL). The organic layers

were combined and dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (v/v = 4:6) to give **TPE-TAP** as a yellow solid (106 mg, 65%, mp: 207.5 - 208.5°C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, J = 4.7 Hz, 2H), 7.65 (t, J = 7.7 Hz, 2H), 7.52 – 7.38 (m, 2H), 7.24 (dd, J = 7.2, 4.6 Hz, 2H), 7.16 – 6.94 (m, 17H), 6.81 (d, J = 8.5 Hz, 2H), 6.56 (d, J = 8.5 Hz, 2H), 5.59 (s, 4H), 4.58 (s, 4H).^{13}C NMR (100 MHz, $CDCl_3$) δ 154.8, 149.9, 146.7, 145.9, 144.6, 144.4, 144.3, 141.0, 139.5, 137.6, 133.2, 132.6, 131.7, 131.6, 127.9, 127.8, 126.5, 126.3, 123.6 (2C), 123.0, 122.95, 122.4, 122.3, 113.0, 55.8, 46.8. HRMS (ESI-TOF) m/z: [M + H]$^+$ calcd for $C_{44}H_{38}N_9$ 692.3245; found 692.3243.

2.4. The sensing of metal ions

Milli-Q water was used to create 1.0×10^{-3} M stock solutions of all metal ions, including Na^+, K^+, Li^+, Ag^+, Ca^{2+}, Mg^{2+}, Cd^{2+}, Ba^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, Cr^{2+}, Hg^{2+}, Fe^{2+}, Co^{2+}, Mn^{2+}, Cr^{3+}, Al^{3+} and Fe^{3+}. Next, 200 μl of each metal ion stock solution was added to 5 mL bottles containing 50 μM probe at room temperature. Fluorescence and absorption measurements were conducted to record the spectroscopic changes of **TPE-TAP**. The Parker-Rees technique was used to determine the fluorescence quantum yield values of **TPE-TAP** in the absence and presence of metal ions, which is given by the following equation.

$$\Phi_S = \Phi_r \left(\frac{D_S}{D_r}\right) \left(\frac{\eta_S^2}{\eta_r^2}\right) \left(\frac{1-10^{-ODr}}{1-10^{-ODs}}\right) \qquad \text{(S1)}$$

The reference material used in the calculation was quinine sulfate (Φ_f=0.55 in 0.5 M H_2SO_4), where D represents the integrated area under the corrected fluorescence spectrum, n represents the refractive index of the solution, and OD represents the optical density at the excitation wavelength (λ_{ex}= 330 nm). The subscripts s and r indicate the sample and reference solutions, respectively.[3]

Using data obtained from fluorescence titration experiments, the detection limit values for the ion detected were determined using the 3s/k equation. In this equation, s refers to the standard deviation of the blank and k represents the slope of the fit line in the fluorescence titration experiment.[4]

Moreover, the Benesi-Hildebrand equation was used to obtain the binding constant (K_a).:

$$\frac{1}{F-F_0} = \frac{1}{K_a(F_{max}-F_0)[M^+]^n} + \frac{1}{F_{max}-F_0} \tag{S2}$$

The equation includes fluorescent intensity values F_0, F, and F_{max}, where F_0 is the intensity in the absence of a metal ion, F is the intensity at a specific metal ion concentration, and F_{max} is the intensity at the saturation concentration of the metal ion. The variables $[M^+]$ and n represent the concentration of the metal ion and the binding stoichiometry between the probe and metal ion, respectively.[4,5] The determination of binding stoichiometry was achieved through Job's plot analysis, which involved using the following equation:

$$F_{job} = (1-X)(F_0 - F) \tag{S3}$$

In this equation, X is the mole fraction of the observed ion, while F_0 and F are the fluorescence intensities of **TPE-TAP** in the absence and presence of ions, respectively.[6]

2.5. Real sample tests

A commercial drug used to treat iron deficiency was selected to determine the usability of **TPE-TAP** in real samples. A stock solution was prepared by making appropriate amounts of iron drug containing 100 mg Fe^{3+} in a granule chassis. The samples 25, 50 and 100 µL volumes were taken from this stock and added to a 5 ml solution containing 50 µM **TPE-TAP**. Then, fluorescence measurements were taken for each sample at room temperature. To verify the accuracy of this new sensor for Fe^{3+} detection, drug samples prepared in water at the same concentrations were also measured by ICP-MS.

References

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4) A. Kushwaha, S. K. Patil and D. Das, *New J. Chem.*, 2018, **42**, 9200–9208.

5) H. Kilic and E. Bozkurt, *J. Photochem. Photobiol. A Chem.*, 2018, **363**, 23–30.

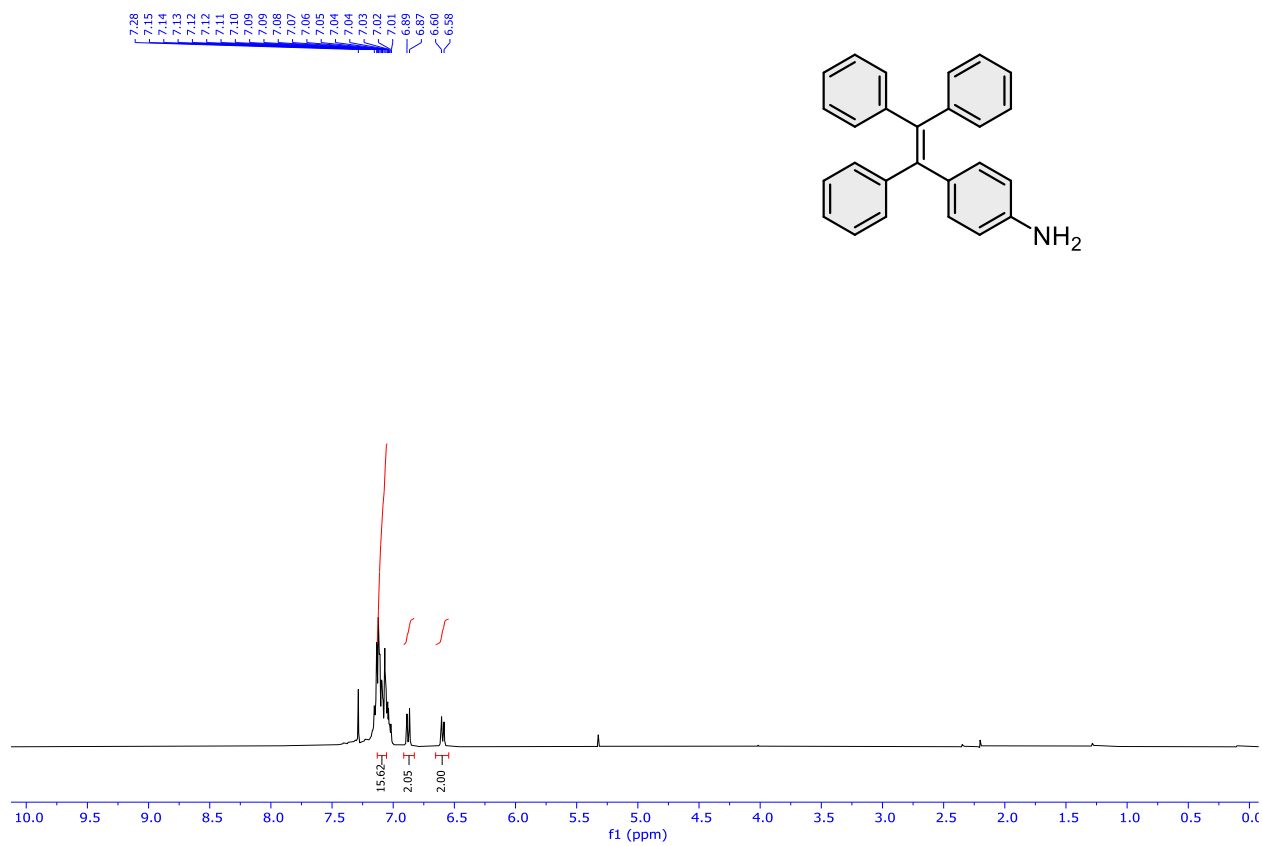6) Y. Zhang, G. Wang and J. Zhang, *Sensors Actuators B Chem.*, 2014, **200**, 259–268.
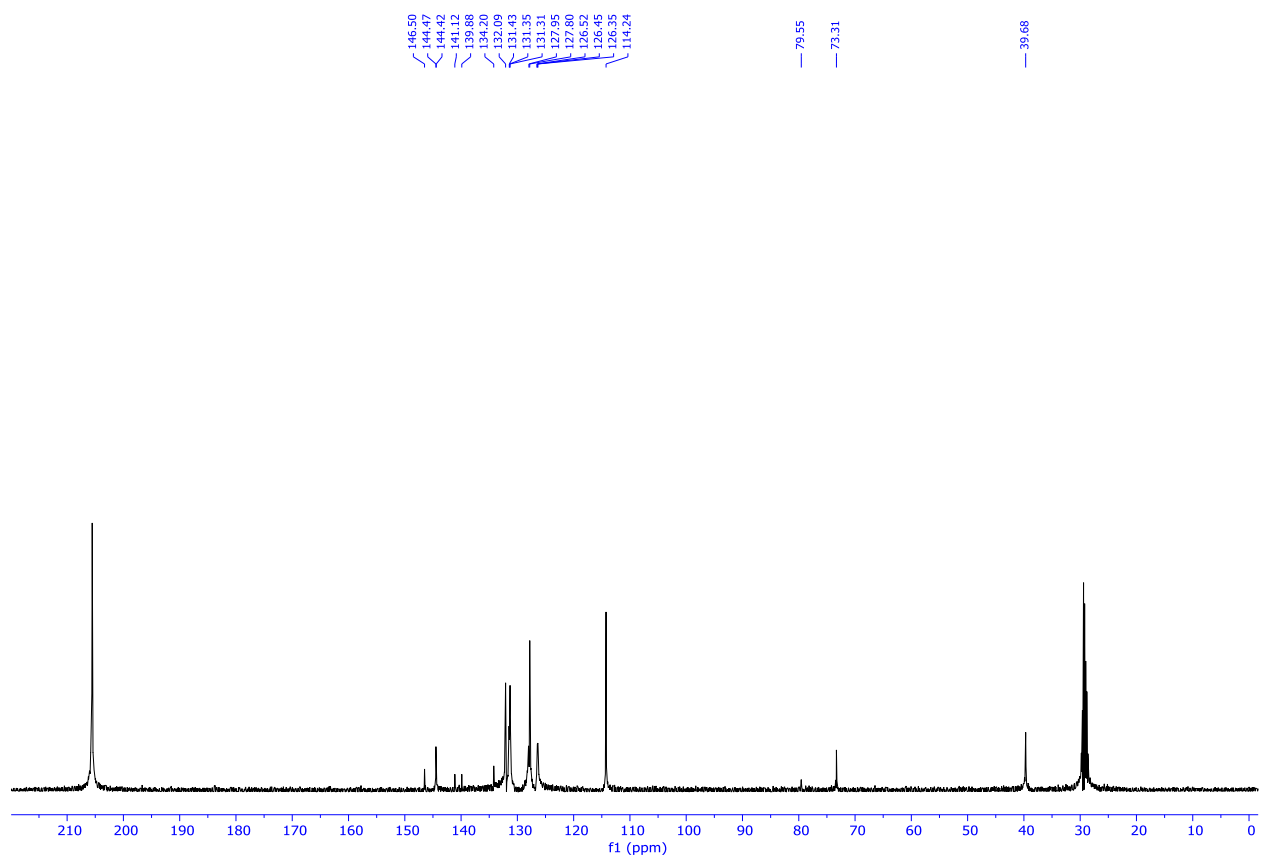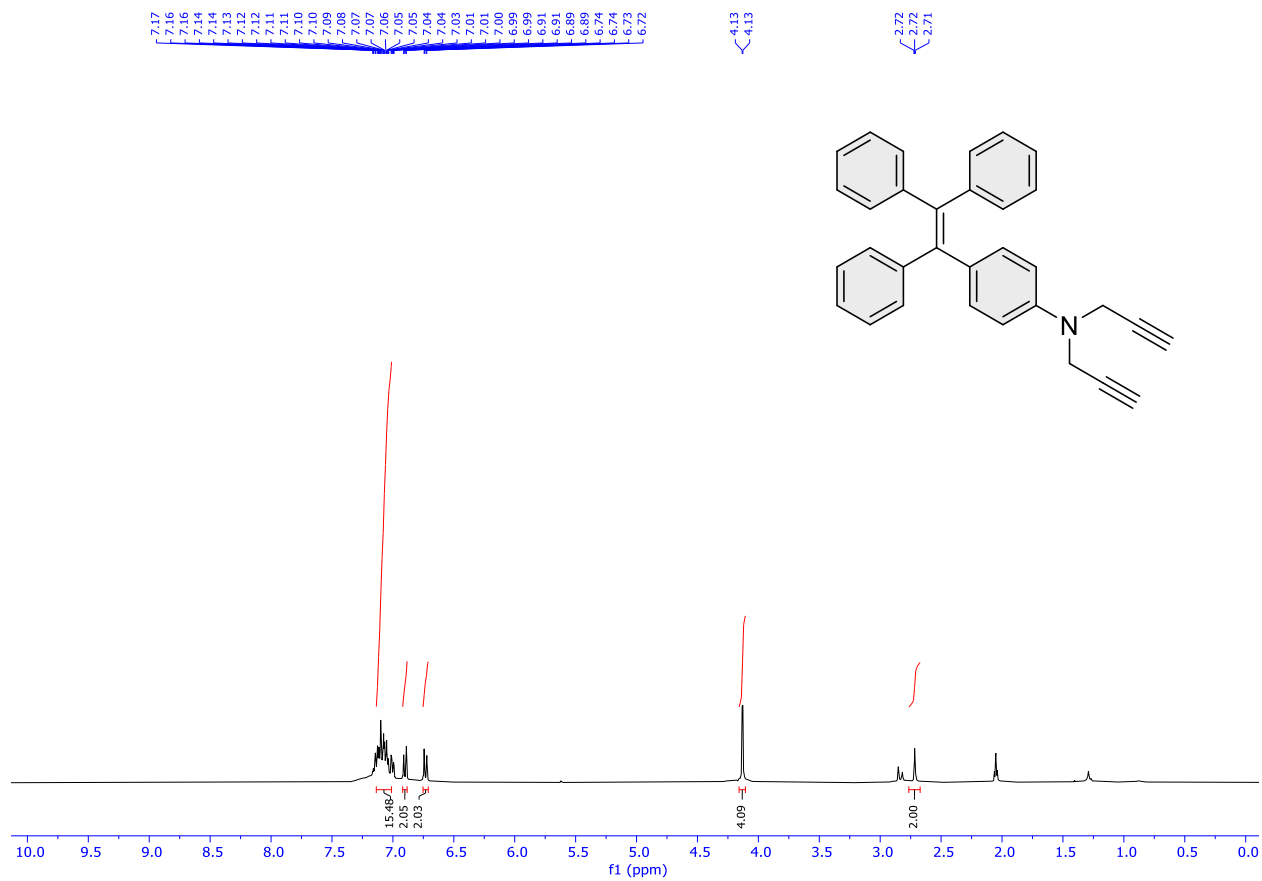
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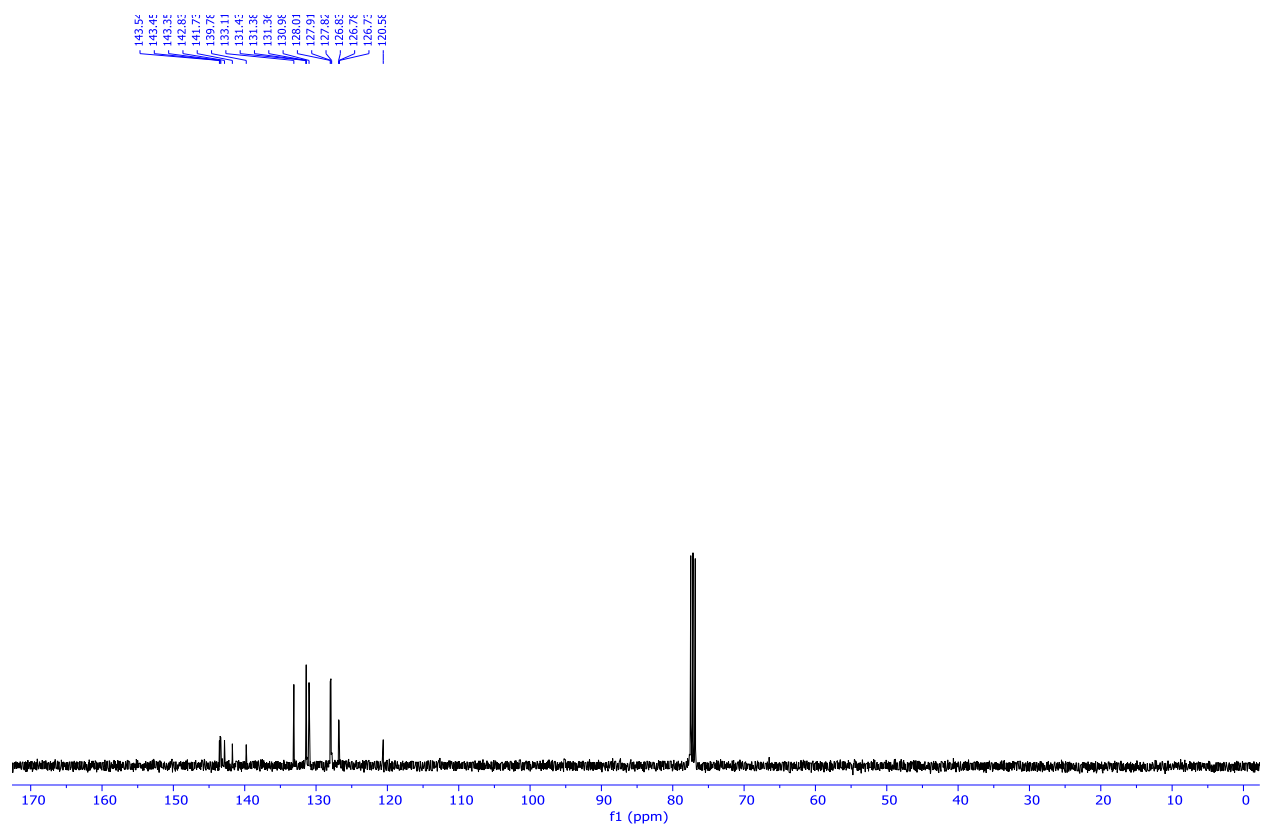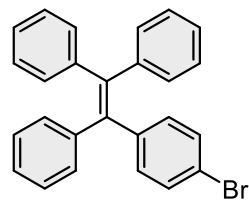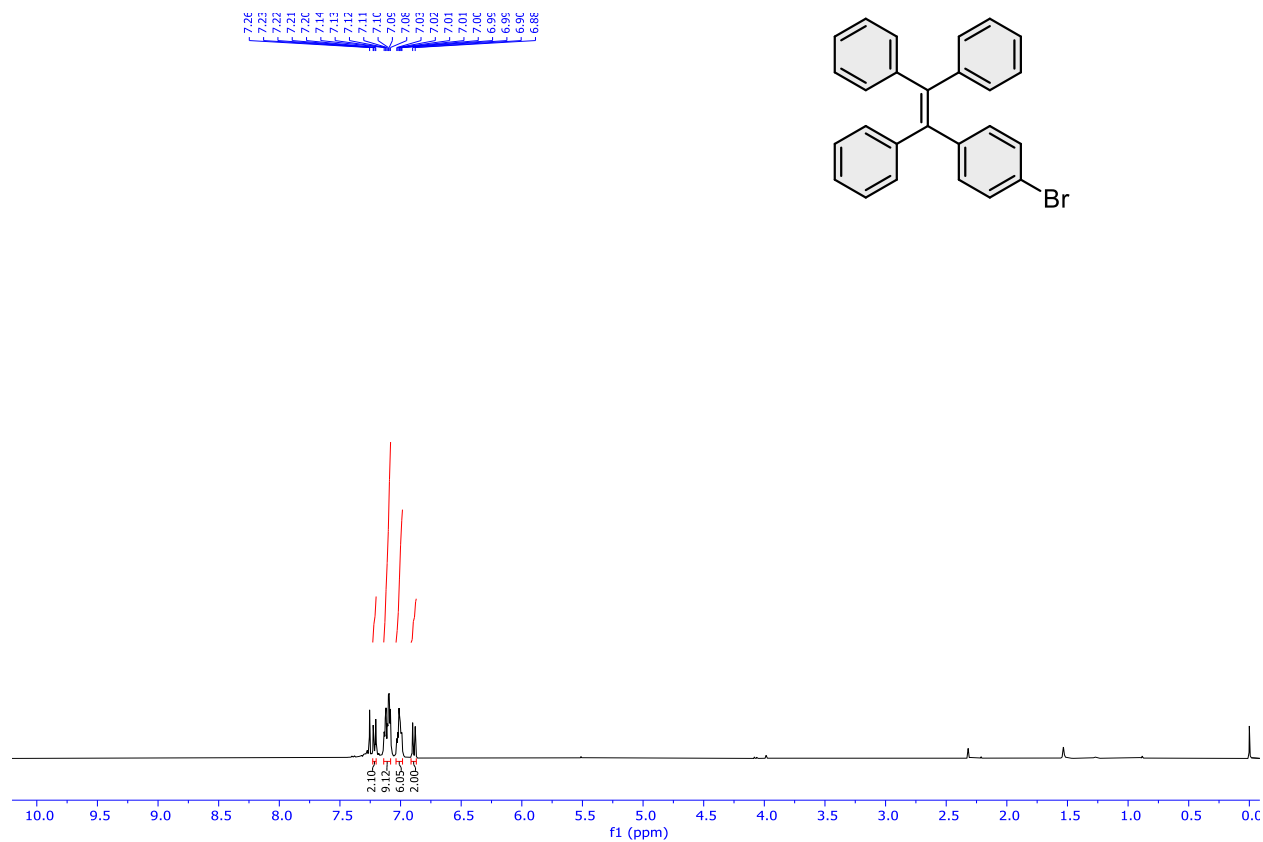
¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz) spectra of **3** in CDCl₃.
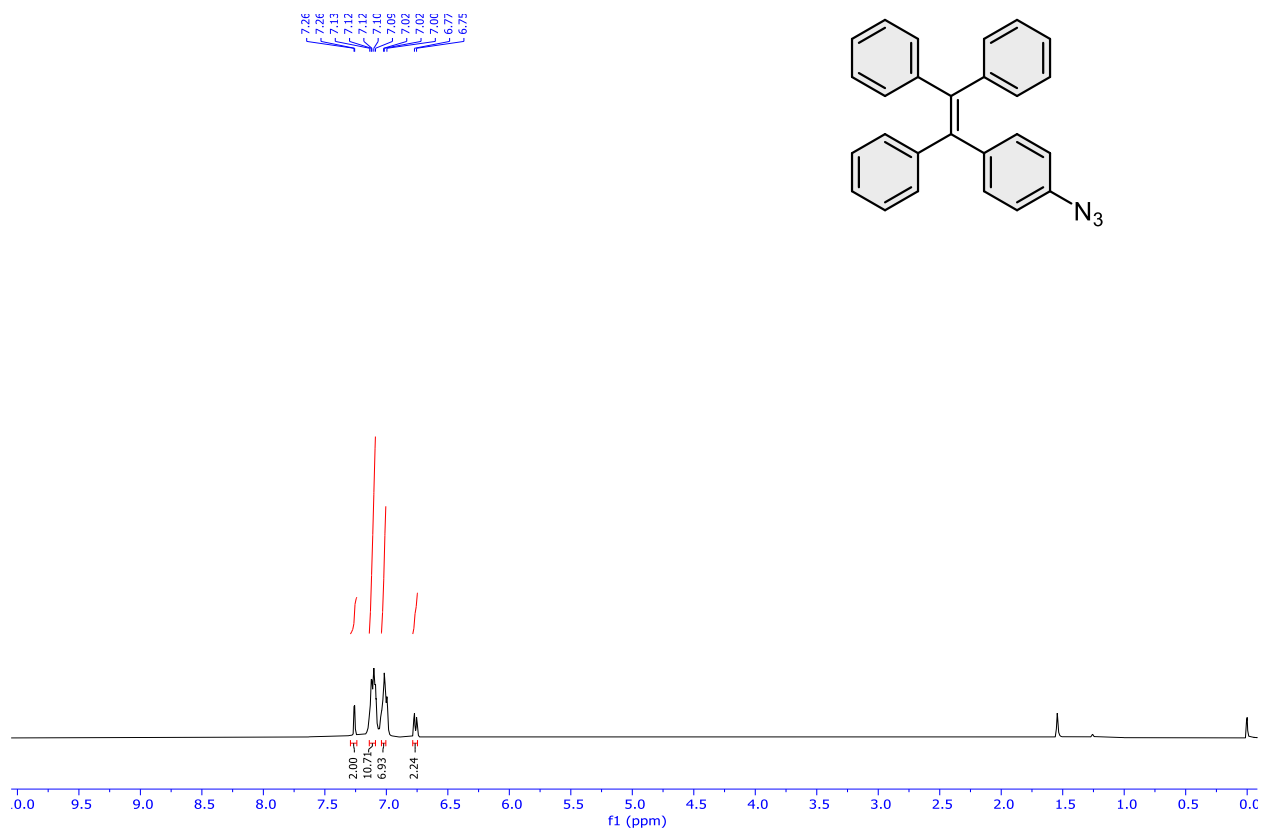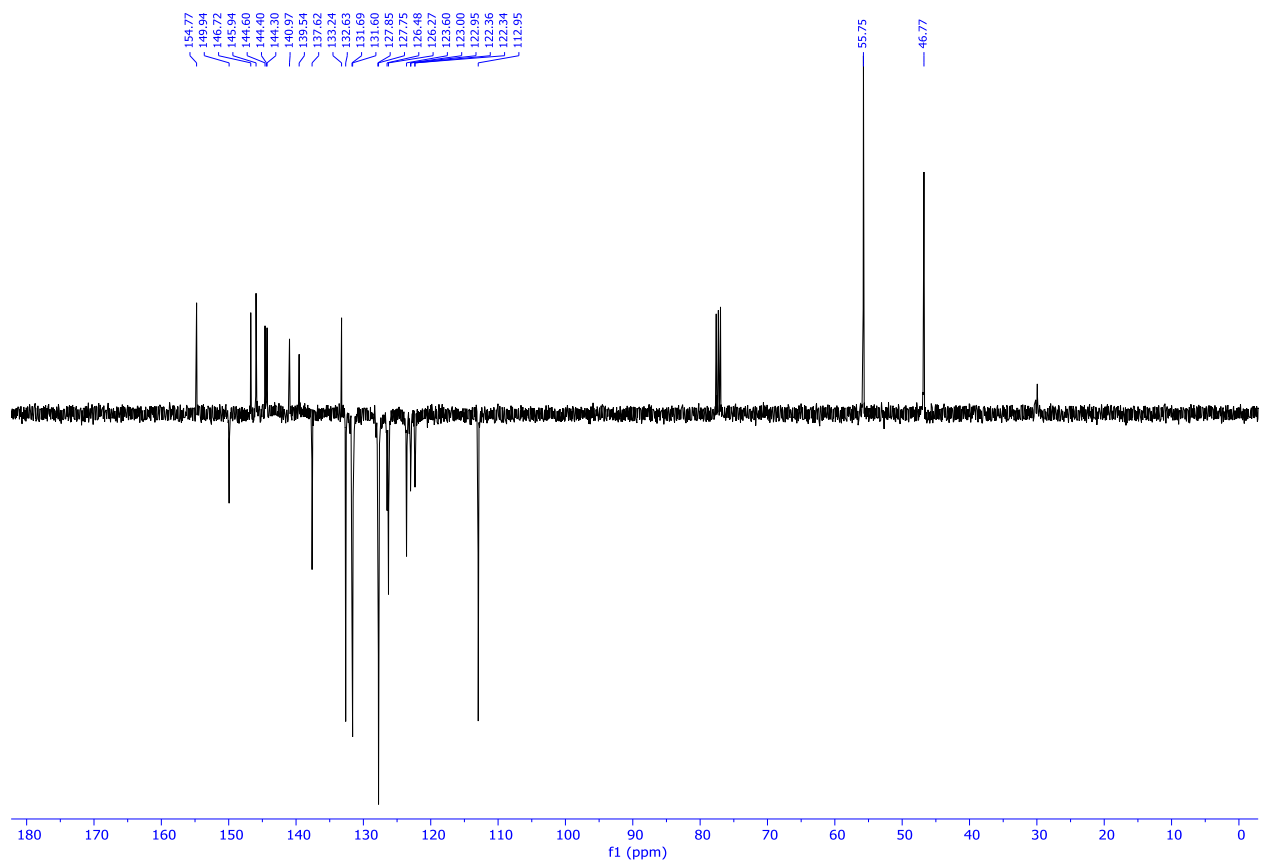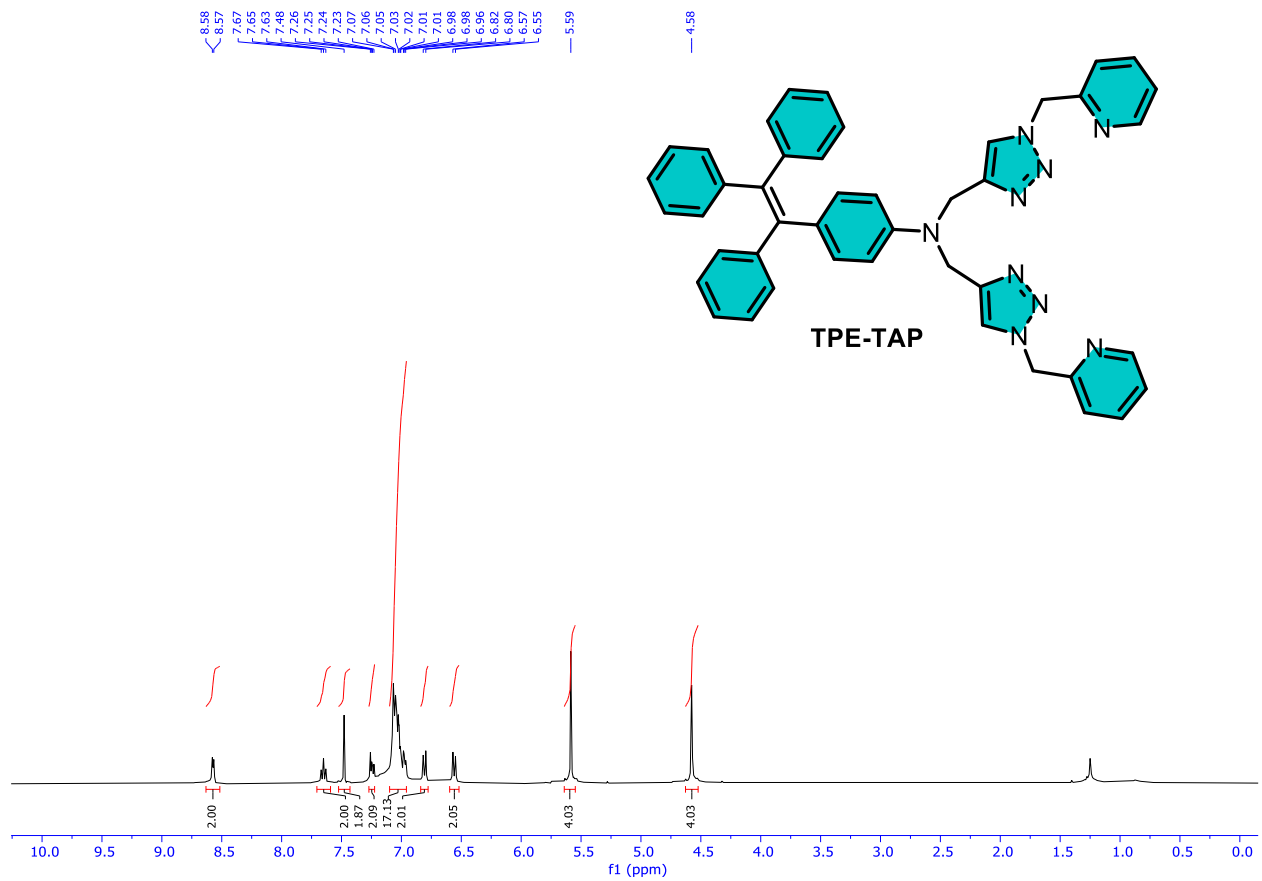
^{1}H-NMR (400 MHz) spectrum of **5** in CDCl$_3$.

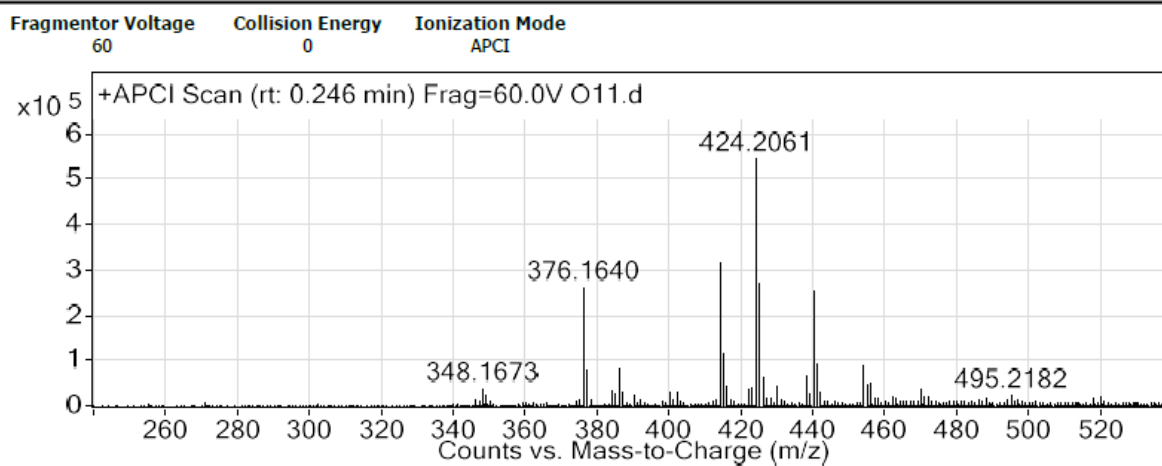^{1}H-NMR (400 MHz) spectrum of **6** in CDCl$_3$.

^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra of **8** in DMSO-*d6*.

^{1}H-NMR (400 MHz) and ^{13}C-APT NMR (100 MHz) spectra of **TPE-TAP** in CDCl$_3$.

Fragmentor Voltage	Collision Energy	Ionization Mode
60	0	APCI

+APCI Scan (rt: 0.246 min) Frag=60.0V O11.d

x10 5

424.2061

376.1640

348.1673

495.2182

Counts vs. Mass-to-Charge (m/z)

The HRMS (APCI-TOF) mass spectrum of **8**

Fragmentor Voltage	Collision Energy	Ionization Mode
90	0	ESI

+ESI Scan (rt: 0.100 min) Frag=90.0V click.d

x10 6

* 692.3243

346.6654

145.0746

520.2473

808.0040 922.0098

Counts vs. Mass-to-Charge (m/z)
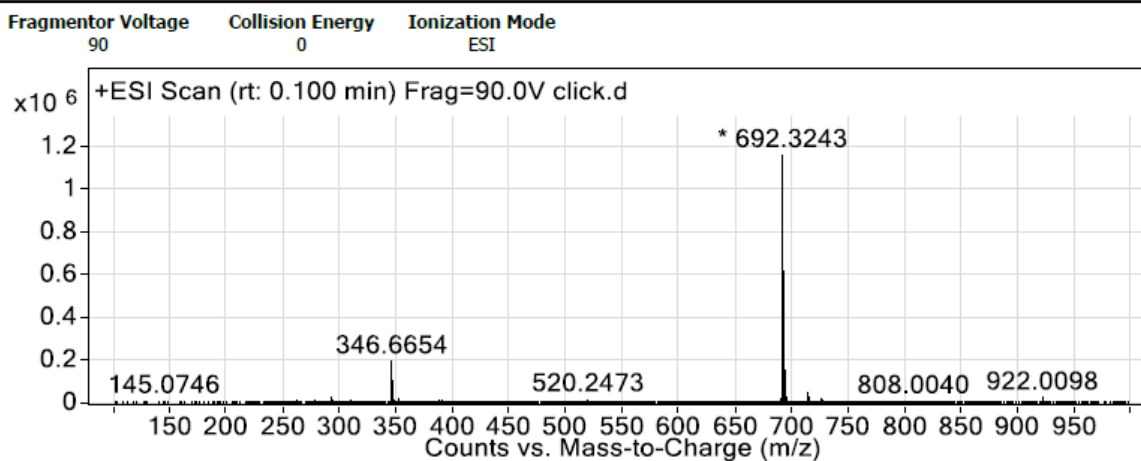
The HRMS (ESI-TOF) mass spectrum of **TPE-TAP**