Electronic supplementary information

An aggregation-induced emission based "turn-on" fluorescent chemodosimeter for the selective detection of ascorbate ions

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Experimental

Chemicals

Propargyl bromide and 4-hydroxybenzophenone were purchased from Sigma Aldrich (India). $TiCl_4$ was purchased from Spectrochem Pvt. Ltd. Mumbai (India). Sodium azide, Zn powder, copper sulphate and sodium ascorbate were purchased from SD Fine Chemicals Ltd. Mumbai (India). All other chemicals and solvents were obtained from different commercial suppliers and were used without further purification.

Instrumentation

NMR spectra were recorded on Bruker AV300 NMR spectrometer. Mass spectra were obtained from Waters Q-TOF micro mass spectrometer (ESI^+) and Agilent 6400B LC-MS (ESI^+). Fluorescence spectra were taken on a JASCO FP-6300 spectrofuorometer, the slit width was 2.5 nm for both excitation and emission. Absorption spectra were recorded on a JASCO V570 UV/Vis/NIR spectrophotometer at room temperature. IR spectra were recorded on Shimadzu DR-8031 FTIR Spectrophotometer.

General Procedure

THF was dried over sodium (whenever required) and freshly distilled before use. The reactions were monitored by thin layer chromatography (TLC) carried out on 0.25-mm silica gel plates (60F-254) using UV light (254 or 365 nm). Stock solutions of compounds **1** and **2** (10 mM) were prepared in THF and that of ascorbate (10 mM) and Cu^{2+} (10 mM) were prepared in deionized water (MilliQ, 18Ω). For study of the effect of different cations and anions stock solutions were prepared by dissolving corresponding metal sulphates or chlorides for cations and sodium salts for anions in deionized water (MilliQ, 18Ω). The solutions of real sample were prepared from commercially available tablet 'CELIN' manufactured by Glaxo Smith Kline (one tablet contains 500 mg of ascorbic acid as per the declaration by the company). All solutions were subjected to filtration through 0.22 μm syringe filter in order to avoid any interference by any particulate matter in fluorescence measurement.

Synthesis of TPE derivatives:

(*E/Z*)-1,2-bis-(4-hydroxyphenyl)-1,2-diphenylethylene (TPE-OH):[1]
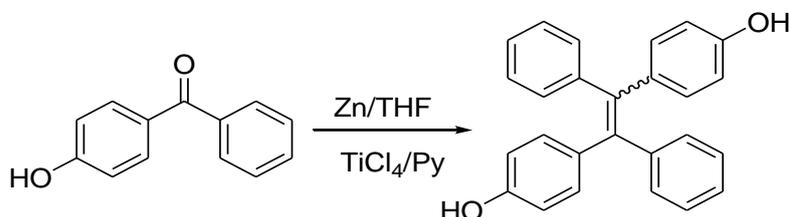
A three-necked flask equipped with a magnetic stirrer was charged with zinc powder (0.8 g, 12 mmol) and 20 mL anhydrous THF under nitrogen atmosphere. The mixture was cooled to 0 to -5 °C and $TiCl_4$ (0.65 mL, 6 mmol) was slowly added by a syringe. The suspension was warmed to room temperature and stirred for 30 min, then heated at reflux for 2.5 h. The mixture was again cooled to 0 to -5 °C, charged with pyridine (0.5 mL, 6 mmol) and stirred for 10 min. The solution of 4-hydroxybezophenone (475 mg, 2.4 mmol) in 10 mL of THF was added slowly. After addition, the reaction mixture was heated at reflux until the 4-hydroxybenzophenone was consumed as revealed by TLC (~8 h). The reaction was quenched by addition of 10% aqueous K_2CO_3 solution and worked up with CH_2Cl_2. The organic layer was collected and concentrated. The crude product was purified by column chromatography to give the desired product, TPE-OH (320 mg, yield: 72%).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.73 (2H, d, *J* = 16.6 Hz, exchangeable); 6.56 (4H, dd, J_1 = 8.8, J_2 = 12.4 Hz); 6.88 (4H, dd, 8.8, J_2 = 12.4 Hz); 6.99-7.12 (10H, m). Mass spectrum (ESI-MS): *m/z* 365.21 [M + H]$^+$.

(*E/Z*)-1,2-bis-(4-(prop-2-ynyloxy)phenyl)-1,2-diphenylethylene (1):
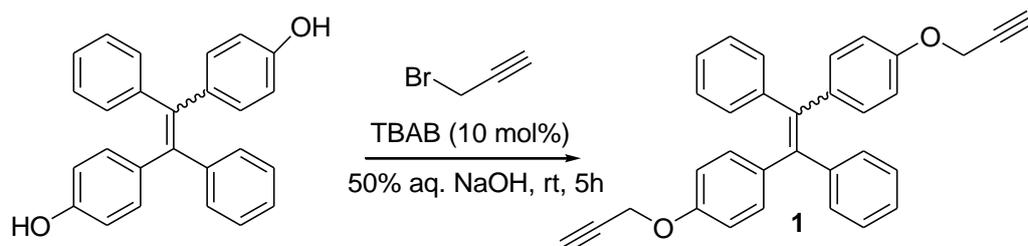
Propargyl bromide (80 wt% in toluene, 0.24 mL, 1.64 mmol) was added to the solution of TPE-OH (100 mg, 0.27 mmol) and tetrabutyl ammonium bromide (19 mg, 10 mol%) in dichloromethane (2 mL) at room temperature. Then, an aqueous 50% NaOH solution (2 mL) was added to the reaction mixture and it was stirred for 5 h. After completion of the reaction, the reaction mixture was diluted with dichloromethane (10 mL). The organic layer was separated out, aqueous layer was extracted with dichloromethane (5 mL × 2), and combined organic layer was washed repeatedly with brine (5 mL × 3), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel 60-120, hexane:EtOAc = 95:5) to afford compound **1** (72 mg, yield: 60%).

^{1}H NMR (400 MHz, CDCl$_3$): δ (ppm) 2.45-2.51 (2H, m), 4.55-4.63 (4H, m), 6.71-6.75 (4H, m), 6.96-7.16 (14H, m). ^{13}C NMR (100 MHz, CDCl$_3$), for major isomer: δ (ppm) 54.6, 54.7, 74.4, 113.5, 124.8, 126.6, 127.4, 128.4, 131.5, 135.9, 142.9, 154.6; for minor isomer: δ (ppm) 75.2, 112.9, 125.2, 126.7, 127.1, 127.3, 130.5, 136.1, 143.0, 155.1, other peaks are merged with the major isomer. Mass spectrum (ESI-MS): *m/z* 441.23 [M + H]$^+$, 463.11 [M + Na]$^+$. IR (cm^{-1}): 3291, 2920, 1596, 1503, 1225, 1031.

(*E/Z*)-1,2-bis-(4-(4-azidobutoxy)phenyl)-1,2-diphenylethylene (2):
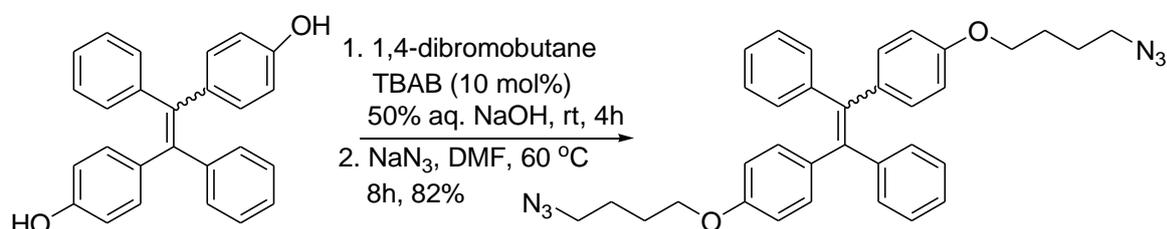
1,4-dibromobutane (0.17 mL, 1.4 mmol) was added to the solution of TPE-OH (100 mg, 0.27 mmol) and tetrabutyl ammonium bromide (19 mg, 10 mol%) in dichloromethane (2 mL) at room temperature. Then, an aqueous 50% NaOH solution (2 mL) was added to the reaction mixture and it was stirred for 4 h. After completion of the reaction, the reaction mixture was diluted with dichloromethane (10 mL). The organic layer was separated out, aqueous layer was extracted with dichloromethane (5 mL × 2), combined organic layer was washed repeatedly with brine (5 mL × 3), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford crude dibromo-TPE derivative (152 mg) in sufficiently pure form.

To the crude dibromo-TPE derivative in DMF (2 mL) was added sodium azide (35 mg, 0.54 mmol) and the suspension was heated at 60 °C for 8 h. After completion of the reaction, the

mixture was diluted with water (25 mL) and extracted with EtOAc (3 x 15 mL). The combined organic layer was washed with brine (2 x 10 mL), dried over anhydrous Na_2SO_4 and filtered. Evaporation of the solvent afforded the crude azide, which was purified by column chromatography (silica gel 60-120, hexane:EtOAc = 95:5) to afford compound **2** (122 mg, 82% over two-step).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.72-1.88 (8H, m); 3.32-3.37 (4H, m); 3.88-3.94 (4H, m); 6.62 (4H, dd, J_1 = 8.8, J_2 = 12.5 Hz); 6.93 (4H, dd, J_1= 8.8, J_2 = 12.5 Hz); 6.98-7.13 (10H, m). ^{13}C NMR (100 MHz): δ (ppm) 25.4, 25.8, 49.3, 65.9, 112.8, 125.4, 126.8, 130.6, 131.8, 135.7, 138.9, 143.5, 156.5. Mass spectrum (ESI-MS): m/z 559.12 $[M + H]^+$. IR (cm^{-1}): 2931, 2090, 1498, 1241, 1030.

Detection of Ascorbate

The fluoroscence study was completed by adding 15 μL (10 mM) of each of probe **1** and probe **2** in deionized water (2.7 mL). To this solution required volume of sodium ascorbate (10 mM) and 15 μL (10 mM) of Cu^{2+} solution were added and required volume of THF and water were added to make it 3 mL of 93% H_2O-THF solution. The solution was stirred at room temperature for 2 h. Then, the solution was taken in 3 mL cuvette and fluorescence response was measured. For equivalent study the concentration of sodium ascorbate was changed from 0.1-3.0 equivalent. For each reaction mixture the fluorescence spectrum was recorded. The same procedure was followed for the other metal ions and anions.
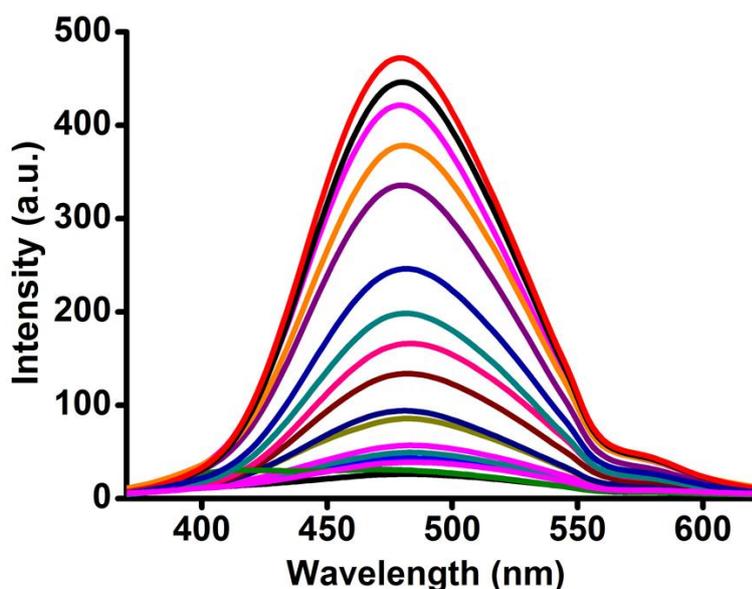
Figure S1. Fluorimetric response of a mixture of **1** and **2** (50 μM each) in the presence of Cu^{2+} (50 μM) upon addition of sodium ascorbate (0 –150 μM) [excitation wavelength is 350 nm].
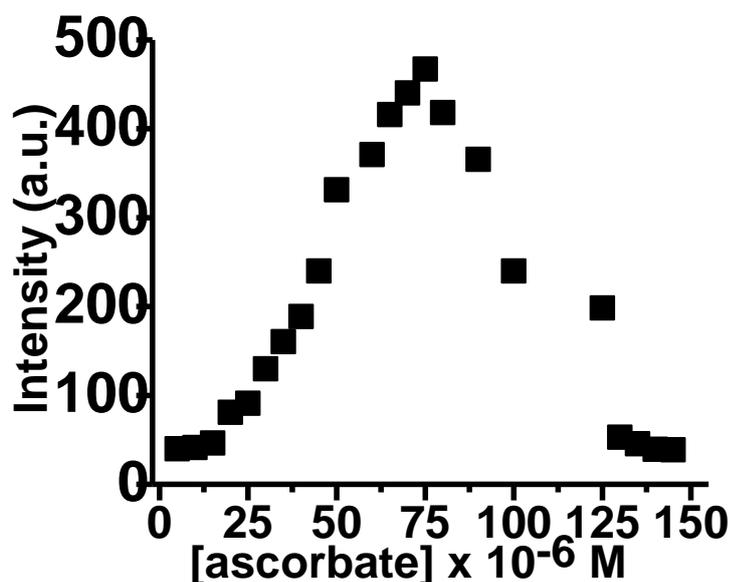
Figure S2. Plot of the increment in fluorescence emission vs. concentration of ascorbate ions. Fluorimetric response was measured from a mixture of **1** and **2** (50 μM each) in the presence of Cu^{2+} (50 μM) upon incremental addition of sodium ascorbate [λ_{ex} = 350 nm, λ_{em} = 480 nm]. The plot is showing a two-phase increment (slow increase from 5 to 15 μM and sharp increase from 20 to 75 μM) and then gradual drop in florescence intensity (after 75 μM).
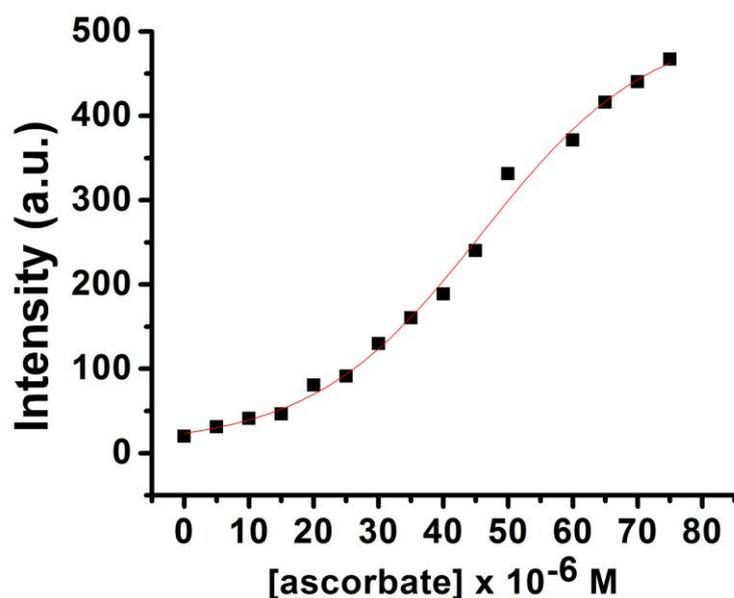
Figure S3. The expanded part of Fig. S2 (concentration range 0—75 μM) clearly showing the change in fluorescence intensity was initially less and the sharp increase in fluorescence was observed after 15 μM.
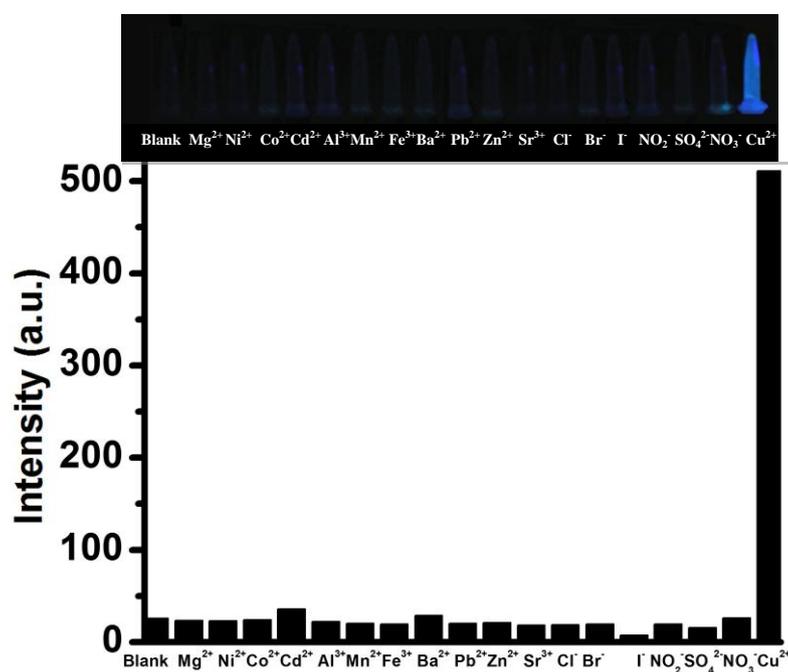
Figure S4. Maximum fluorescence response of a mixture of **1** and **2** in 93% H_2O-THF upon addition of different metal ions (Mg^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Al^{3+}, Mn^{2+}, Fe^{3+}, Ba^{2+}, Pb^{2+}, Sr^{2+}) in presence of a fixed concentration of ascorbate (50 μM) and anions (Cl^-, Br^-, I^-, NO_3^-, NO_2^-) in presence of a fixed concentration of Cu^{2+} (50 μM).

mean diameter 63nm   mean diameter 847nm
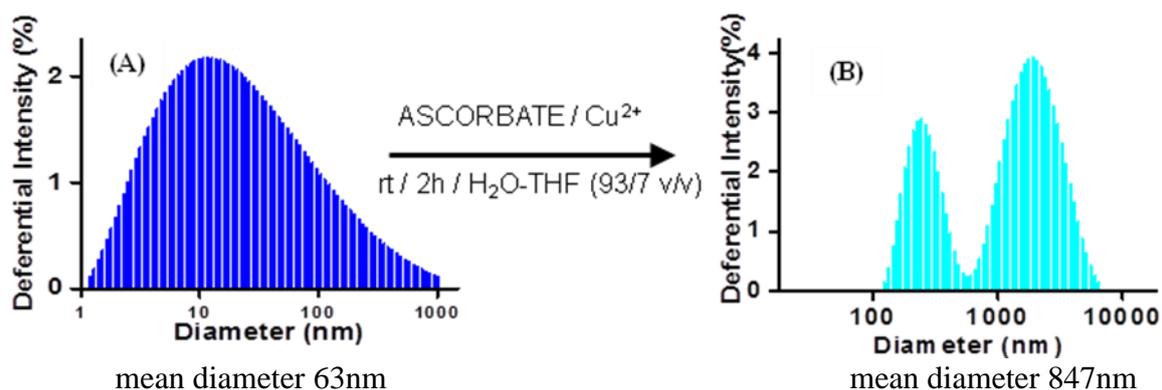
Figure S5. Particle size analysis of a mixture of propargyl-TPE (**1**) and TPE-azide (**2**) [50 μM each] in 93% H_2O-THF before (**A**) and after (**B**) addition of ascorbate and Cu^{2+} (50 μM each).
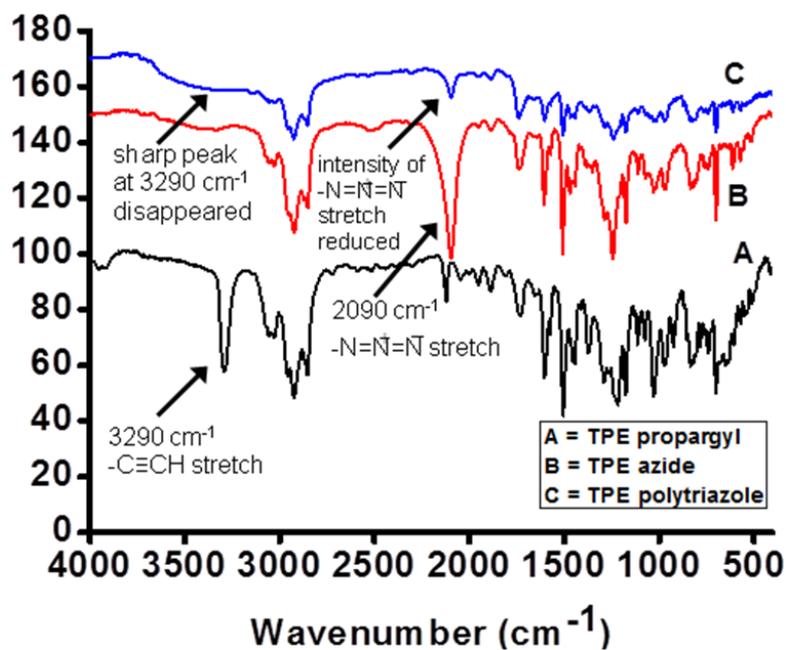
Figure S6. Overlaid IR spectra of propargyl-TPE (A), TPE-azide (B) and TPE-polytriazole (C).
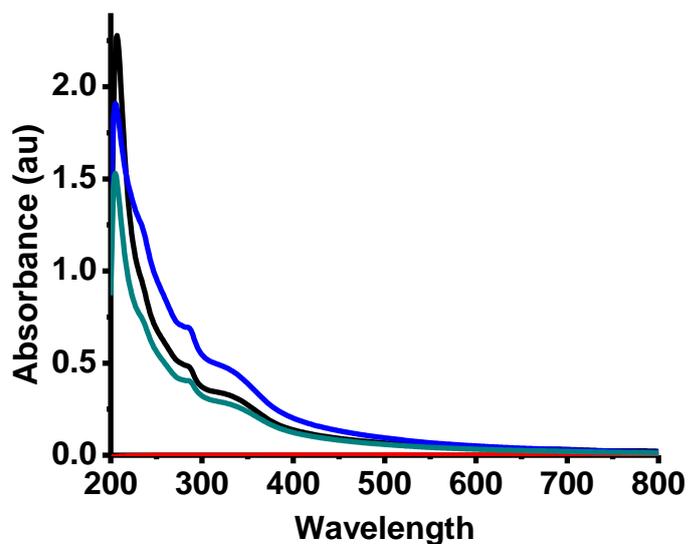
Figure S7. Absorption spectra of TPE-azide (green), propargyl-TPE (black) and mixture of both after 2 h of addition of Cu^{2+}/ascorbate (blue). All three spectra were recorded in 93:7 H_2O-THF as solvent system.

Propergyl-TPE (1)   **TPE-azide (2)**
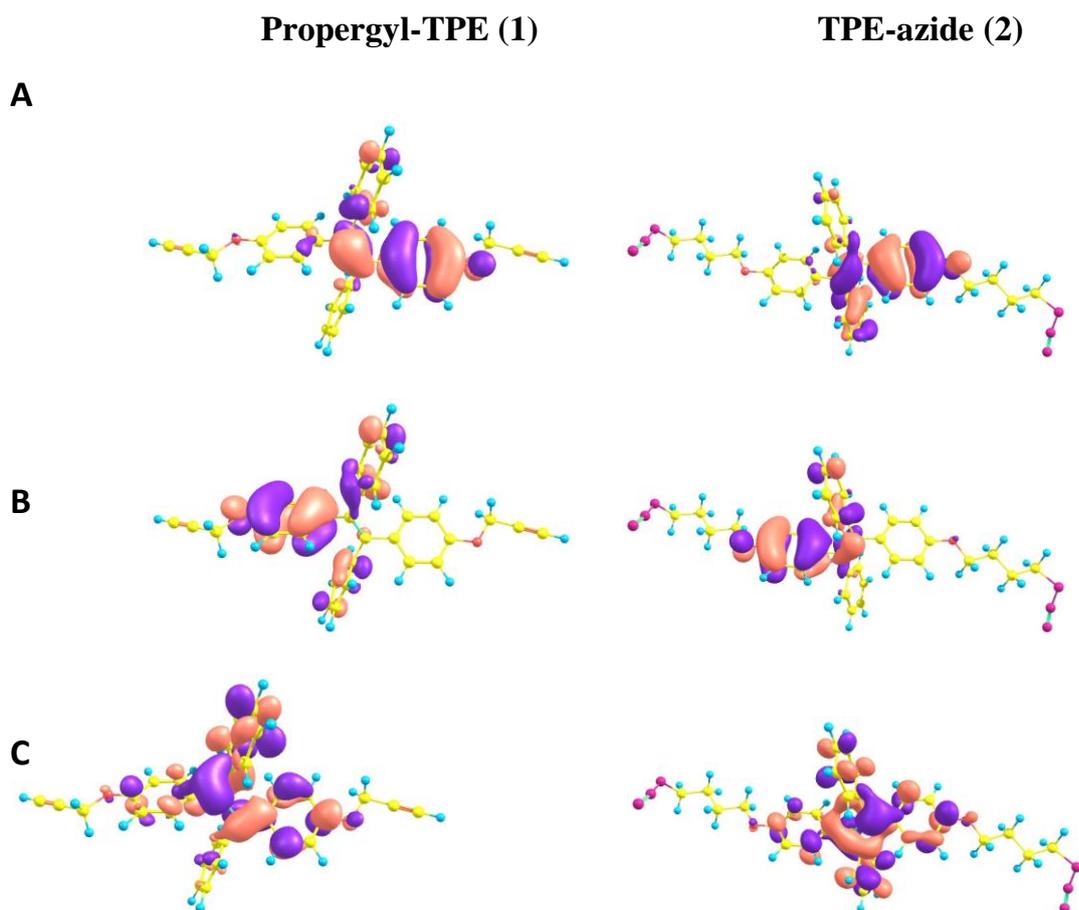
Figure S8. Molecular orbital diagrams of TPE derivatives: (A) HOMO, (B) HOMO-1 and (C) LUMO of **1** and **2**.
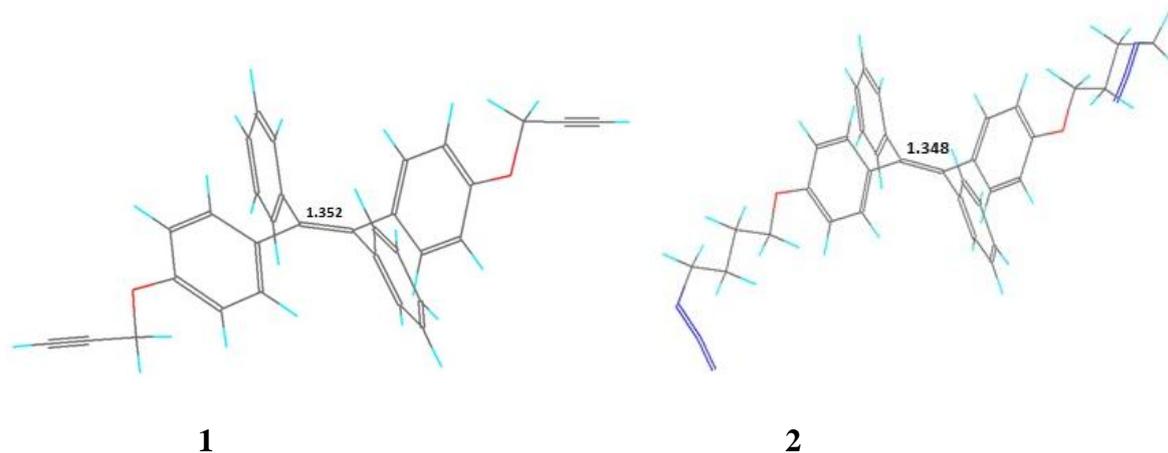
Figure S9. Energy minimized structures of **1** and **2**.

In the present work theoretical studies are carried out at semiempirical-CI (PM3/CI and ZINDO/CI) and *ab initio* RHF/6-311G level of calculations. The PM3/CI[2,3] calculations are done in MOPAC, while ArgusLab software has been employed to predict the singlet-singlet transition properties using ZINDO/CI[4-7] calculations. Restricted Hartree-Fock (RHF) calculations have been carried out in GAMESS suite of programs.[8] The predicted absorption peak (284 nm) for compound **1** from the ZINDO/CI level of study almost matches the experimentally observed value. This transition is characterized by substantially large oscillator strength (0.55) and transition moment values. Compound **2** is found to have an intense absorption at 274 nm (36, 530 cm^{-1}) with an oscillator strength value of roughly 0.36. Strong absorptions are also predicted between 265 nm and 270 nm; however, they correspond to slightly lower transition moment and oscillator strength values (0.23 to 0.12).

Sensitivity of probes 1 and 2 at lower concentration range of ascorbate

To determine the sensitivity of probes **1** and **2** at lower concentration range of ascorbate titration was carried out by adding aliquots of ascorbate (AA) solution (from 5 μM to 15μM) to a mixture of propargyl-TPE (**1**) and TPE-azide (**2**) (50 μM each) in 93/7 v/v H$_2$O–THF in the presence of Cu^{2+} (50 μM). The change in fluorescence intensity was plotted as a function of [AA] within the concentration range 0–15 μM and a linear curve was obtained with R^2 = 0.9918 (Fig. S10).
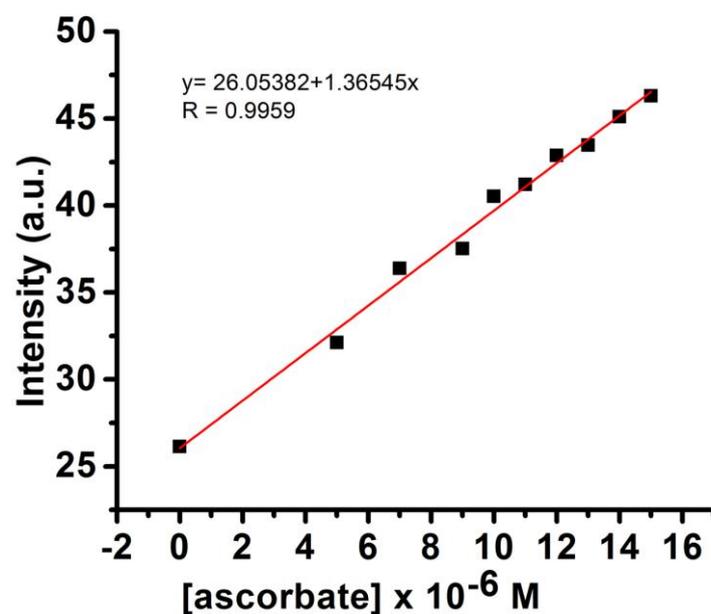
Figure S10. Sensitivity of probes **1** and **2** towards ascorbate. At lower concentration (concentration range 0─15 μM).

Detection limit

For detection limit, PL emission growth ratio [$(I - I_0)/I_0$] was plotted as a function of square of concentration of AA within the range 0─40 μM to get a linear curve with good linearity (Fig. S10).[9] Curve-fit equation: $(I - I_0)/I_0 = 0.13225 + 0.00397 [AA]^2$, $R^2 = 0.99375$. The last point of the linear regression curve is considered as the detection limit. Thus, the detection limit of this sensing system for ascorbic acid arrived at 5.0 μM.

y= 0.00397x+0.13225

R^2= 0.99375
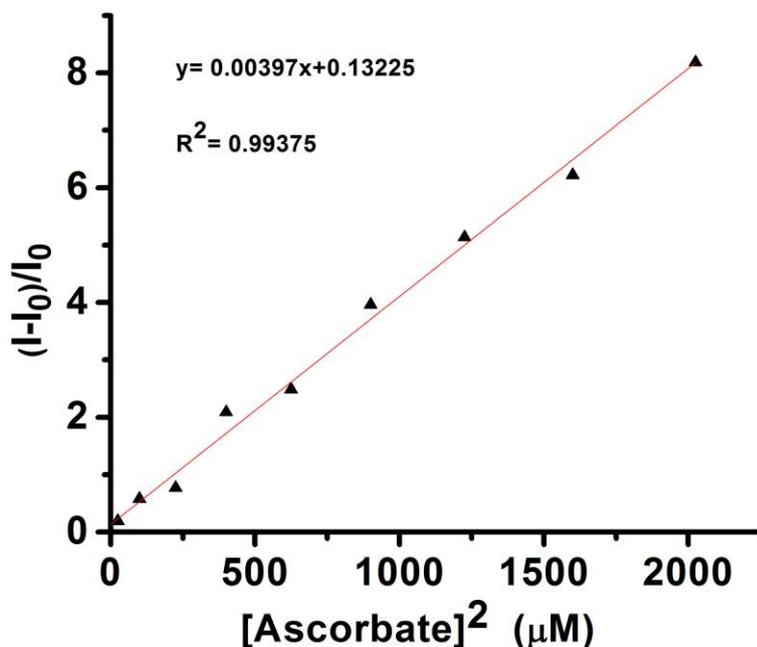
Fig. S11. The plot of fluorescence emission growth ratio $[(I - I_0)/I_0]$ as a function of $[AA]^2$. The values were obtained from the fluorimetric titration experiment of propargyl-TPE (**1**) and TPE-azide (**2**) with ascorbate in the presence of Cu^{2+} (50 μM) at 480 nm. Detection limit was estimated as 5 μM.

Detection of ascorbate in real sample (Vitamin C tablet).

Ten 'CELIN' tablets (wt. 15 g) were finely ground and two solutions of different concentrations were prepared by taking (A) 28.2 mg and (B) 60 mg, and dissolving them in 10 mL of deionized (DI) water. For fluorescence study 15 μL (10 mM) of each of probe **1** and probe **2** were taken in deionized water (2.7 mL), and 15 μL of each of the stock solutions of 'CELIN' tablet and 15 μL (10 mM) of Cu^{2+} were added, respectively. Then, required volume of THF and water were added to make it 3 mL of 93% H_2O-THF solution. The solution was stirred at room temperature for 2 h and fluorescence response was measured thereafter. The concentration of ascorbate found as 25.6 μM for solution A and 56.2 μM for solution B which closely matched with the amount of ascorbic acid present in each tablet.
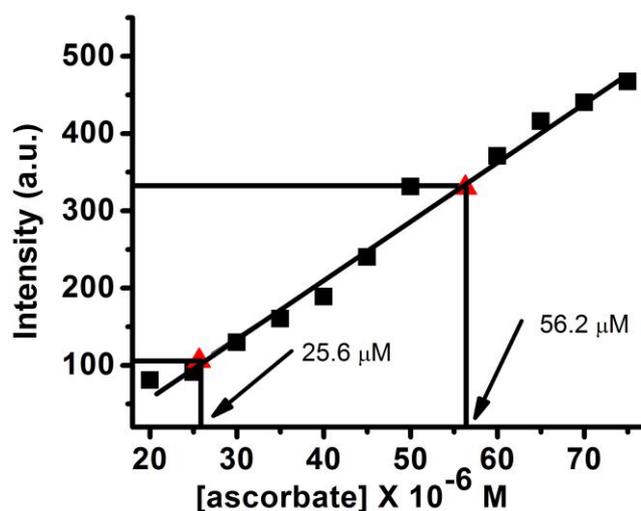
Figure S12. A plot of the intensities of different real samples onto the calibration curve to quantify the level of ascorbate ions in those samples.

Table S1. Real sample analysis

Sample no.	Concentration found from graph (µM)	Actual concentration* (µM)	Error %
1.	25.6	26.70	(-) 4.1
2.	56.2	56.81	(-) 1.0

*each CELIN tablet (wt 1.5 g) contains 200 mg of ascorbic acid and 338 mg of sodium ascorbate (is equiv. to 500 mg of ascorbic acid per tablet).

References

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^{1}H NMR of **propergyl-TPE (1)**
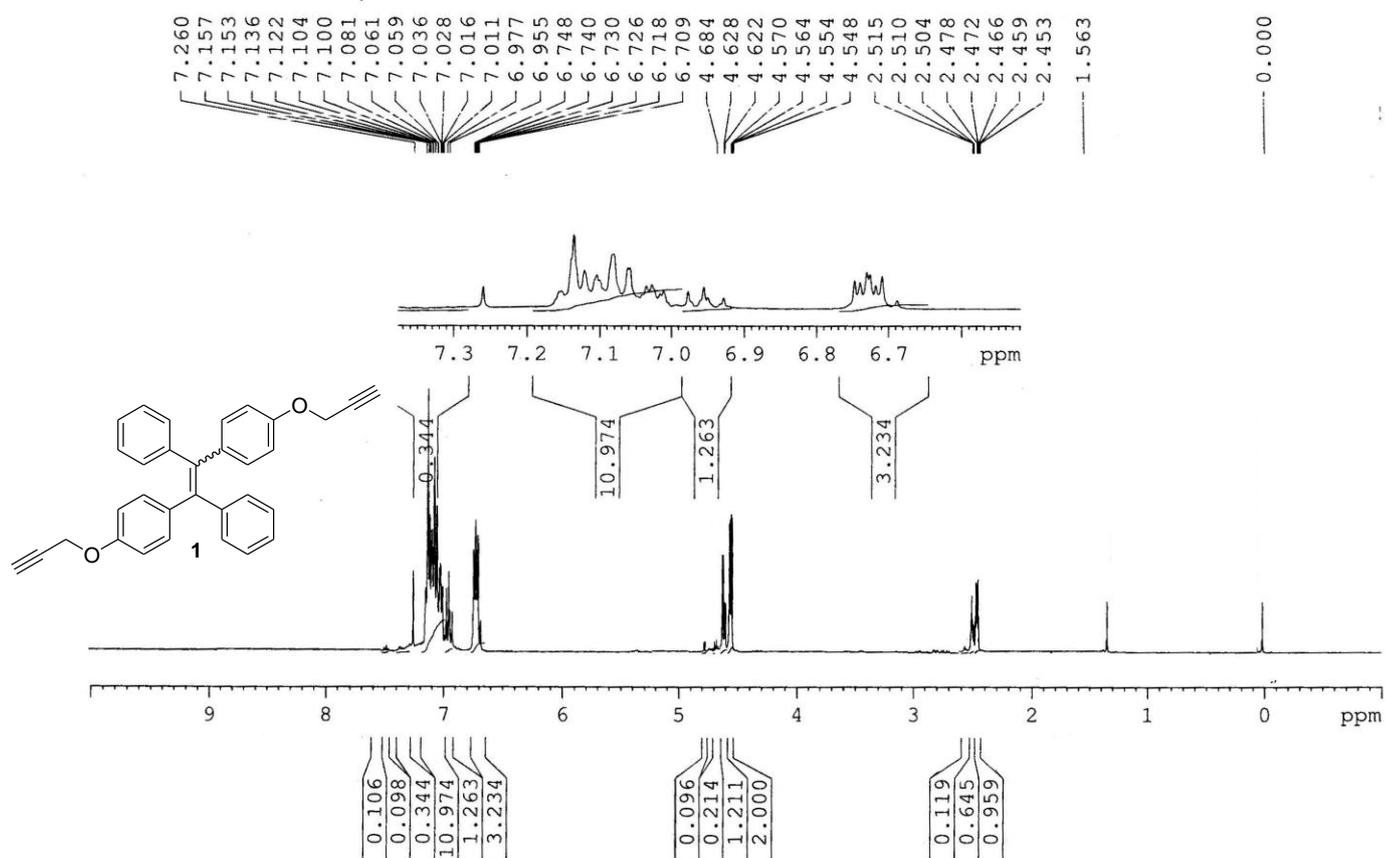
^{13}C NMR of **propergyl-TPE (1)**
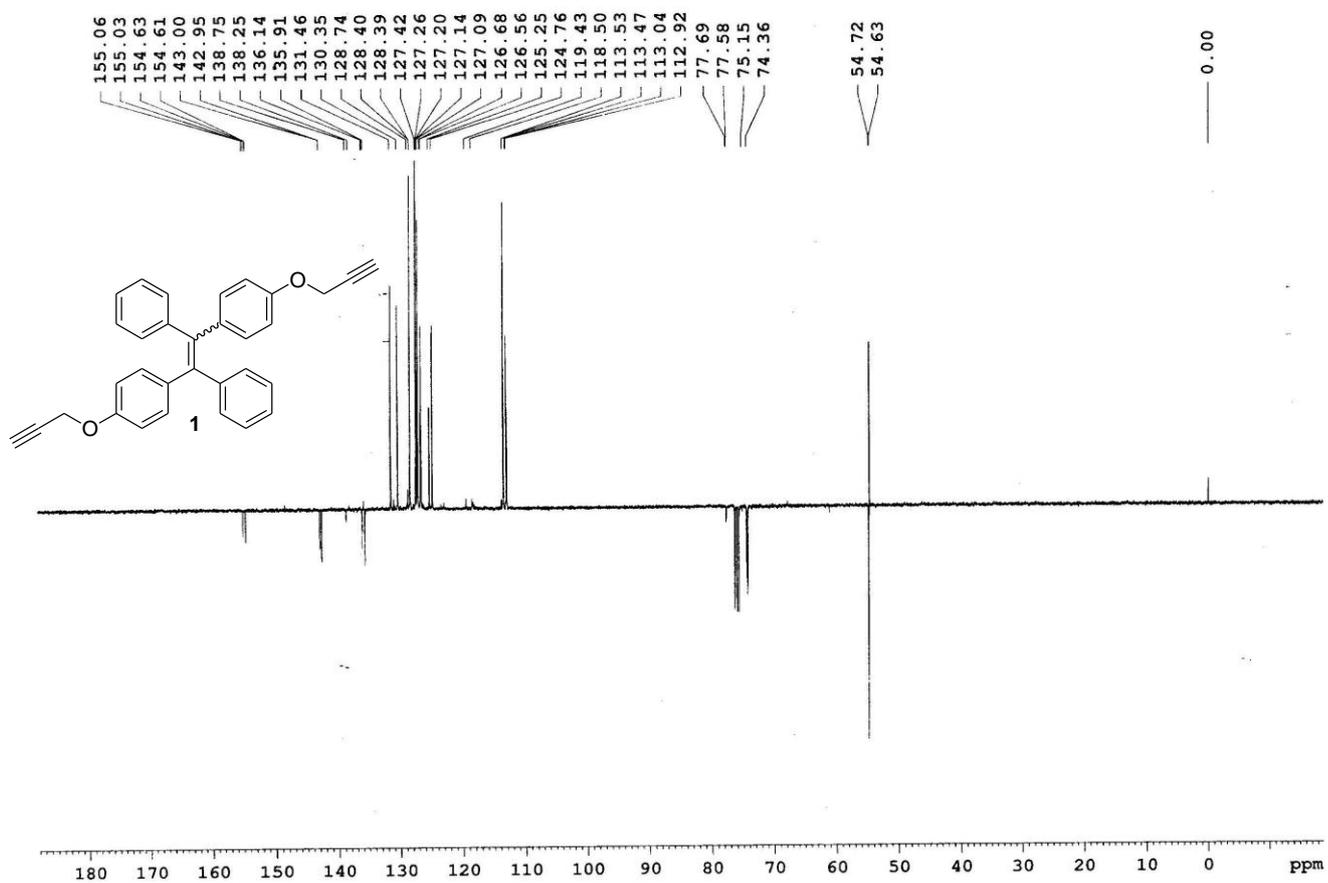
^{1}H NMR of **TPE-azide (2)**
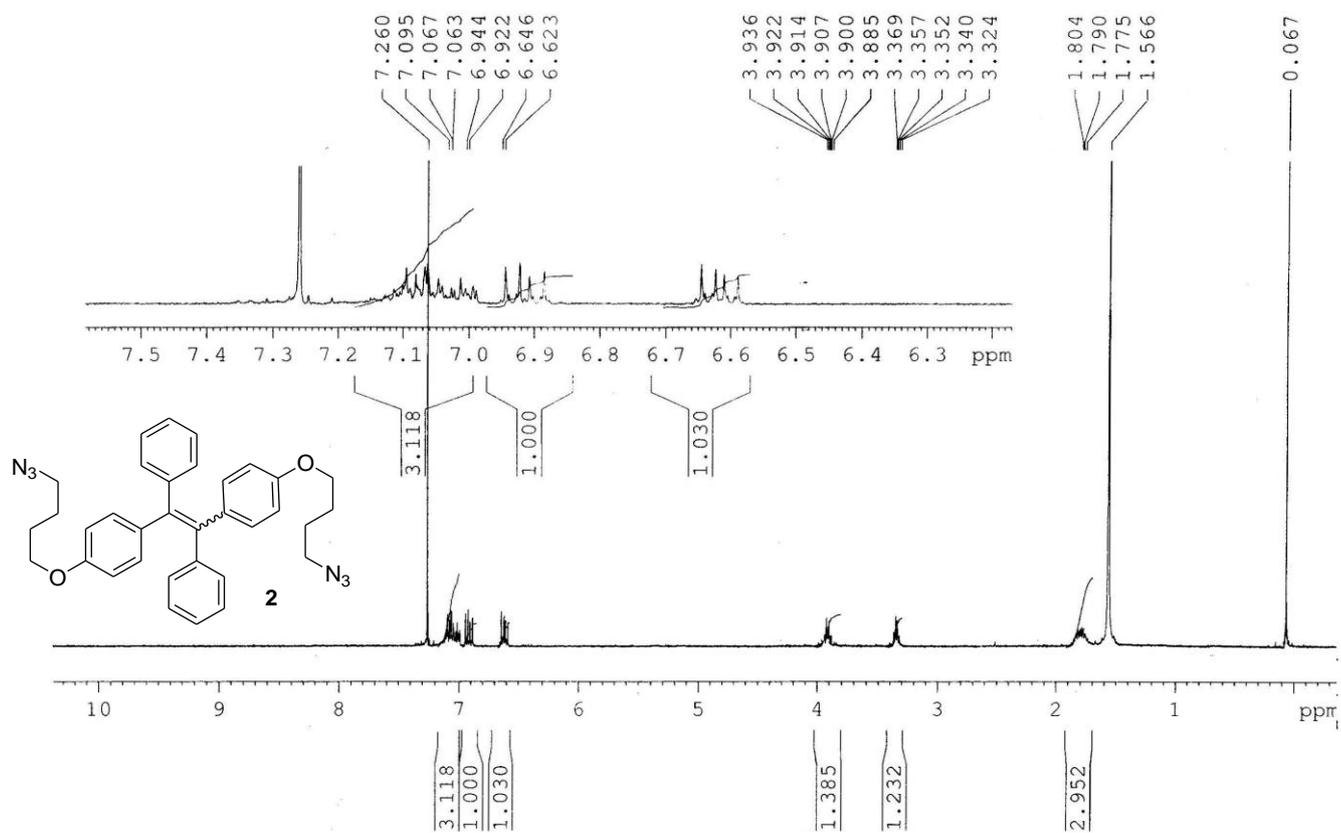
^{13}C NMR of **TPE-azide (2)**
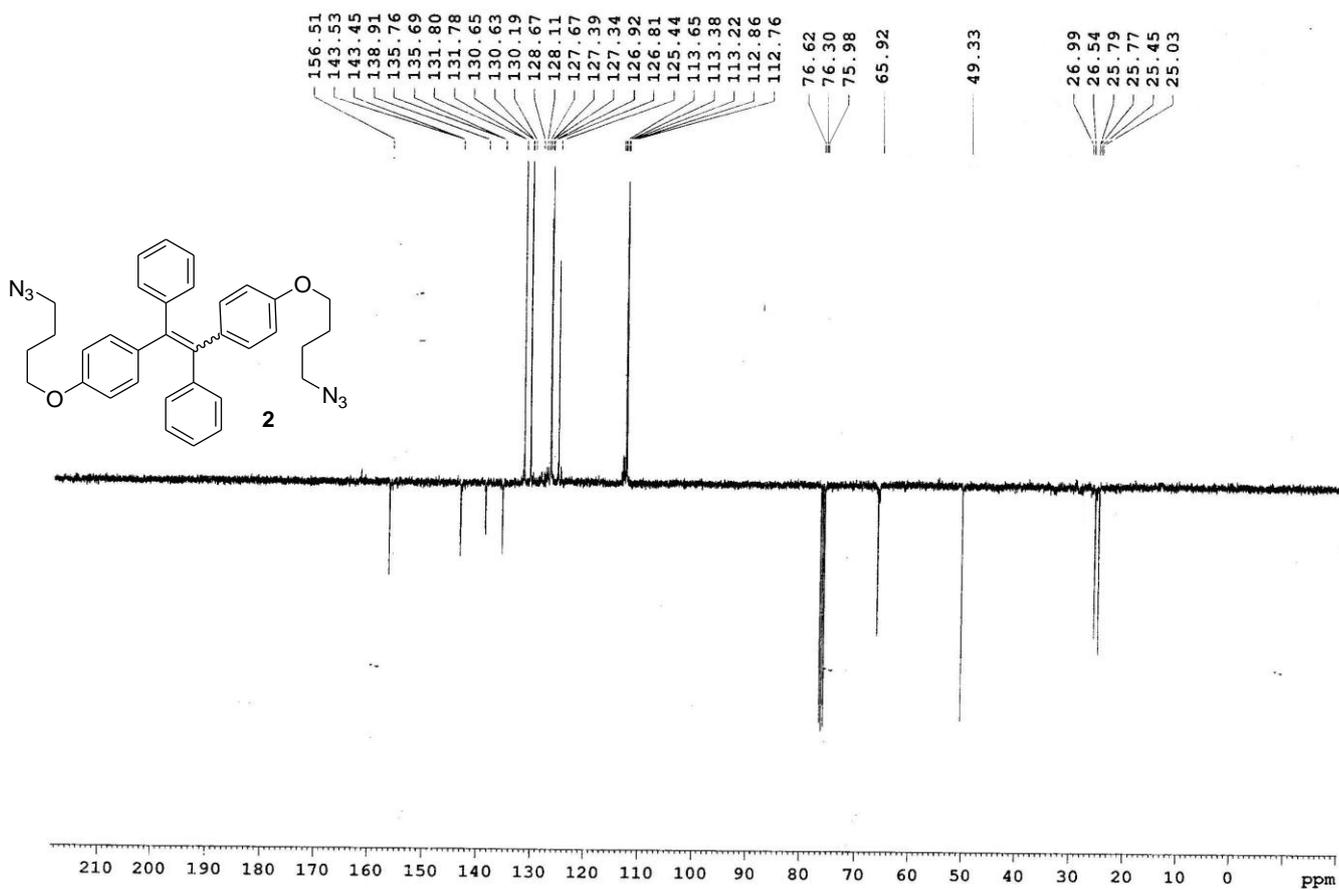
ESI-MS of the reaction mixture containing 50 μM each of alkyne 1, azide 2, Cu^{2+} and ascorbate in 93:7 H_2O-THF (taken after 10 min).