Supplementary Material (ESI) for Chemical Communications

Supporting Information

Highly Stable Dendritic Trityl Radicals as Oxygen and pH Probe

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I. EPR Spectroscopy

EPR spectra were recorded at room temperature using X-band spectrometer with high sensitivity resonator. The following acquisition parameters were used in the data acquisition: modulation frequency, 100 kHz; microwave frequency, 9.79 GHz; modulation amplitude, 0.03-0.25 G; time constant, 41-164 ms; scan time, 42-84 s; and microwave power, 2 mW.

II. Cyclic Voltammetry

Cyclic voltammetry was performed on a potentiostat and computer-controlled electroanalytical system. Electrochemical measurements were carried out in a 10 mL cell equipped with a glassy carbon working electrode (7.07 mm^2), a platinum-wire auxiliary electrode and a Ag/AgCl reference electrode. Solutions of dentritic trityl radicals (1 mM) were degassed by bubbling with the nitrogen gas before the detection. The redox potentials were calculated according to the relation $E = (E^a_p+E^c_p)/2$.

III. Stability Studies towards Biological Oxidoreductants

Solutions of glutathione (1 mM), ascorbate (1 mM) and hydrogen peroxide (1 mM) in PBS buffer (pH 7.4, 50 mM) were used. Superoxide anion radical ($O_2^{\bullet-}$) was generated by adding xanthine oxidase (0.02 U mL^{-1}) to a solution containing hypoxanthine (0.4 mM), DTPA (1 mM) and TAM radical in PBS buffer (pH 7.4, 50 mM). Hydroxyl radical ($HO^{\bullet}$) was generated by Fenton system using ferrous ammonium sulfate (0.1 mM) and hydrogen peroxide (1 mM). Methyl radical ($^{\bullet}CH_3$) was generated by Fenton system plus DMSO (5%, v/v). Alkylperoxyl radical ($ROO^{\bullet}$) was produced by thermal decomposition of

2,2'-azobis-(2-amindinopropane) dihydrochloride (AAPH, 1 mM) at 37 ºC. Appropriate spin trapping EPR experiments with the spin trap 5-tert-butoxycarbonyl 5-methyl-1-pyrroline N-oxide (BMPO) were carried out to verify the production of the radicals. The spectra were recorded 30 min after mixing the TAM radicals solution (10 μM) with various oxidoreductants. The control experiments containing the TAM radical only were also performed. Each experiment was conducted at least three times.

IV. Determination of the Product Purity

The TAM radical purity was determined using TEMPO as standard. In a typical experiment, purified trityl was weighed and dissolved in water. EPR spectrum was obtained and peak area was determined using double integration. The concentration of the trityl radical was then determined using a standard curve of known concentration of TEMPO versus peak area (Figure S1). Each experiment was done in triplicate. The paramagnetic purity for DTR1 and DTR2 was determined to be 98.7 and 97.6%, respectively.

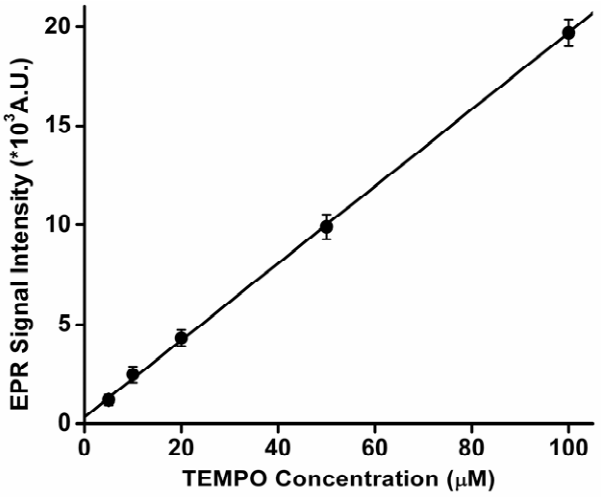


Figure S1. The EPR signal integration of TEMPO as a function of the concentration in aqueous solution.

V. Acid Titration of the TAM Radicals as Detected by UV-vis and EPR Spectroscopies

The EPR and UV-vis spectra were recorded after incremental addition of HCl solution (1 M) to the TAM radical solution (20 μM) in PBS buffer (20 mM).

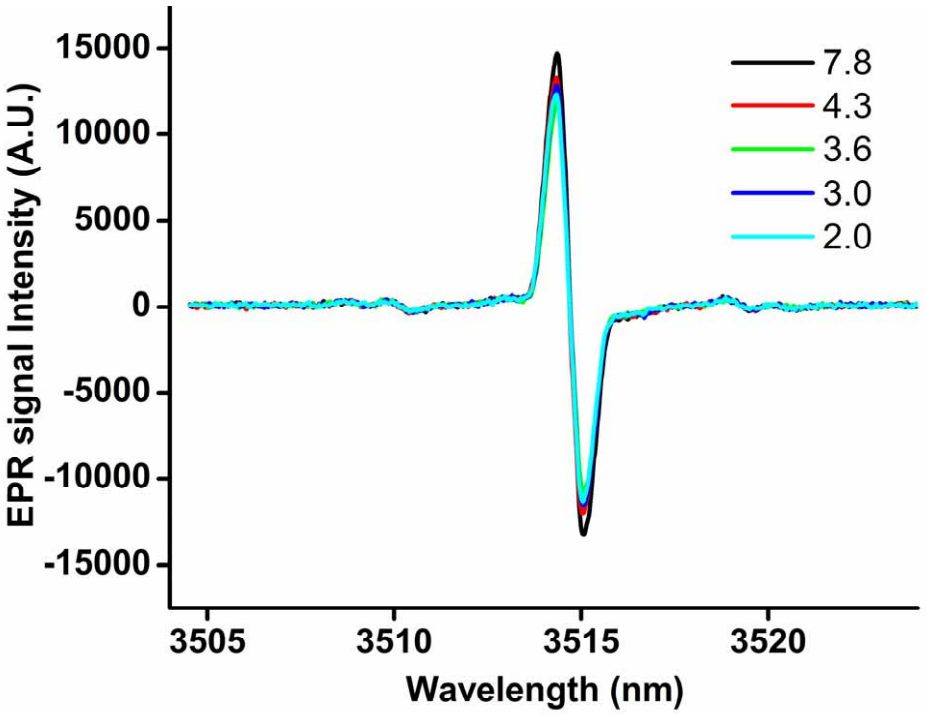


Figure S2. EPR spectra of DTR1 as a function of pH

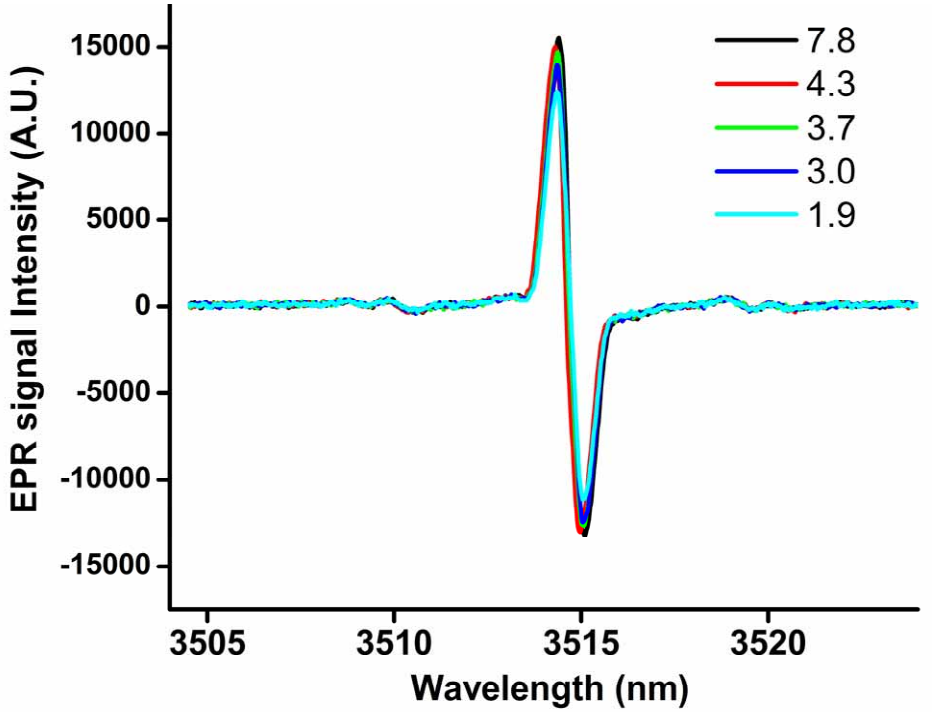


Figure S3. EPR spectra of DTR2 as a function of pH

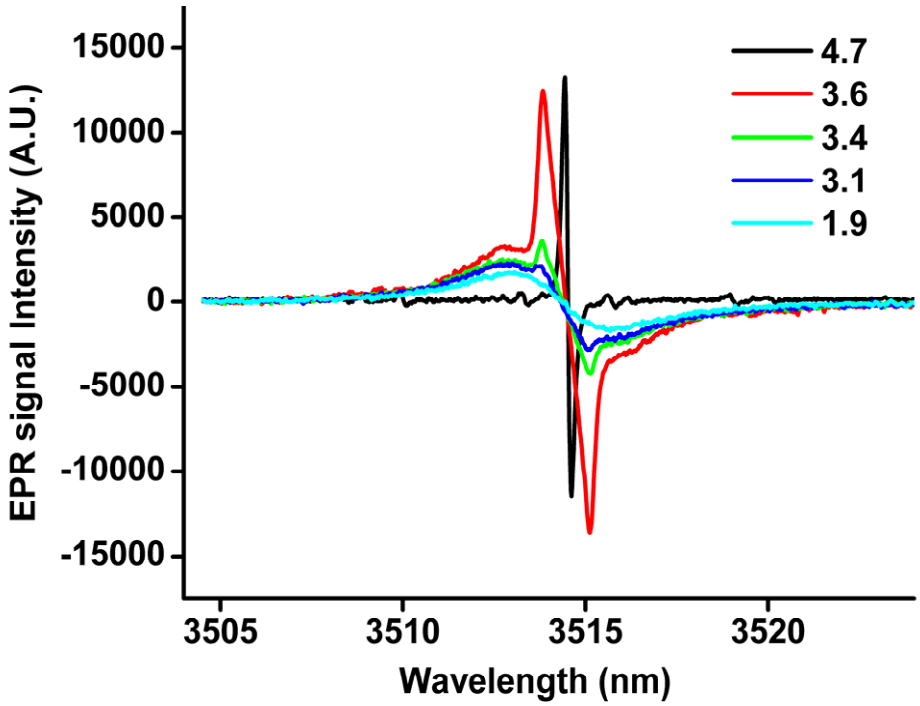


Figure S4. EPR spectra of CT-03 as a function of pH

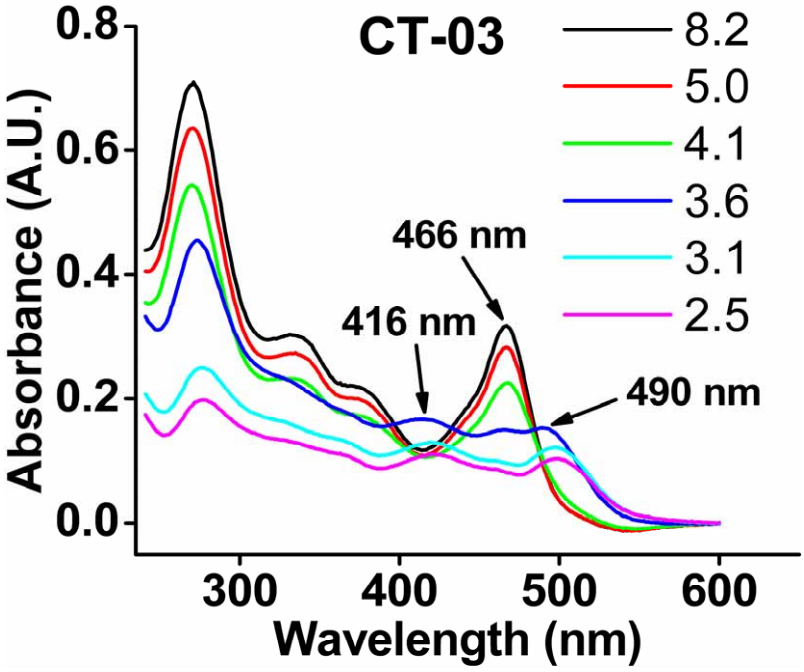


Figure S5. UV-Vis spectra of CT-03 as a function of pH. While the peak around 466 nm is due to the carboxylate form of CT-03, two peaks around 416 nm and 490 nm are attributed to the carboxylic acid form.

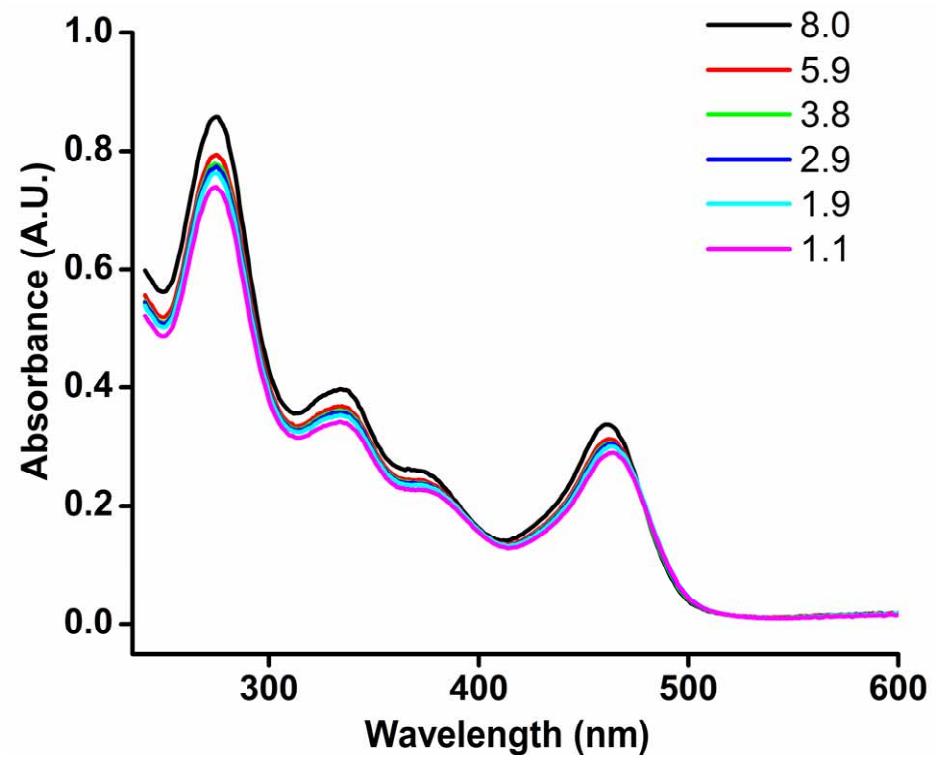


Figure S6. UV-Vis spectra of DTR1 as a function of pH

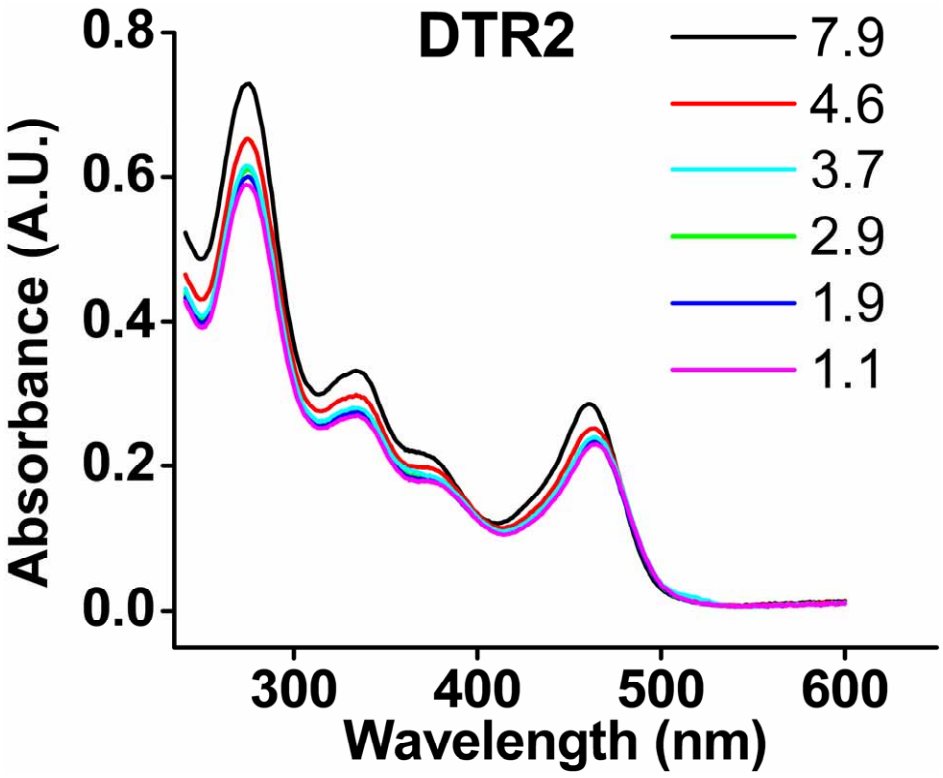


Figure S7. UV-Vis spectra of DTR2 as a function of pH

VI. Oxygen Sensitivity

A solution of trityl radical (10 μM) in PBS buffer was transferred into a gas-permeable Teflon tube (i.d. = 0.8 mm) and was sealed at both ends. The sealed sample was placed inside a quartz EPR tube with open ends. Nitrogen or N_2/O_2 gas mixture with varying concentrations of O_2 was allowed to bleed into the EPR tube. After 20 min of equilibration, the EPR spectrum was recorded and the peak-peak linewidth was calculated.

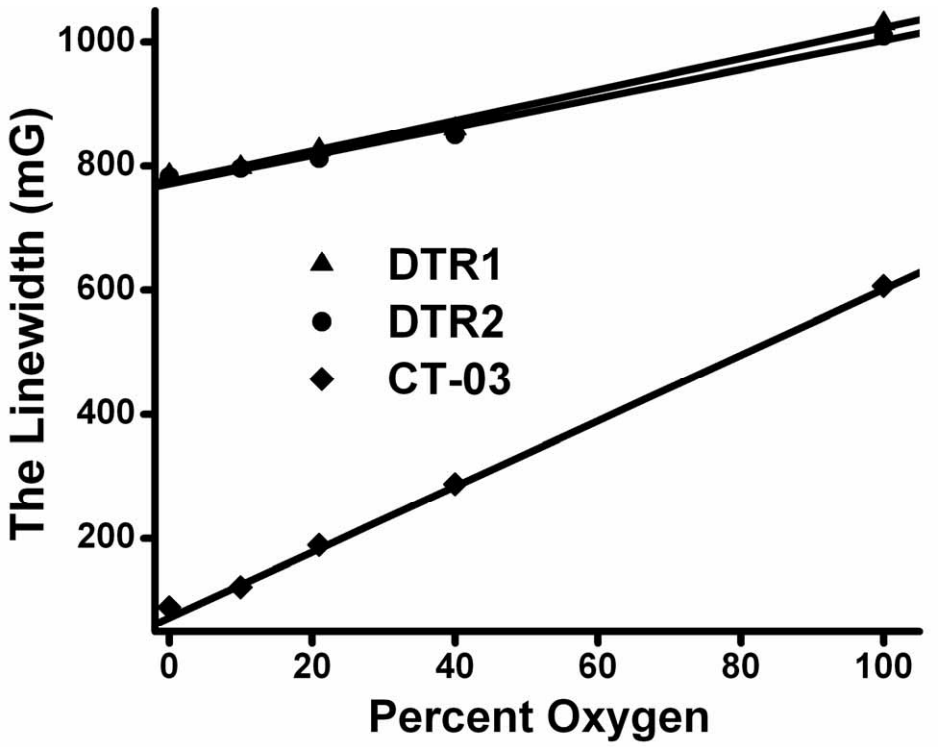


Figure S8. Plots of linewidths of (▲) DTR1; (●) DTR2; (♦) CT-03 as a function of O_2 concentration in PBS buffer (50 mM, pH 7.4).

VII. Binding of Cu^{2+} into DTR2

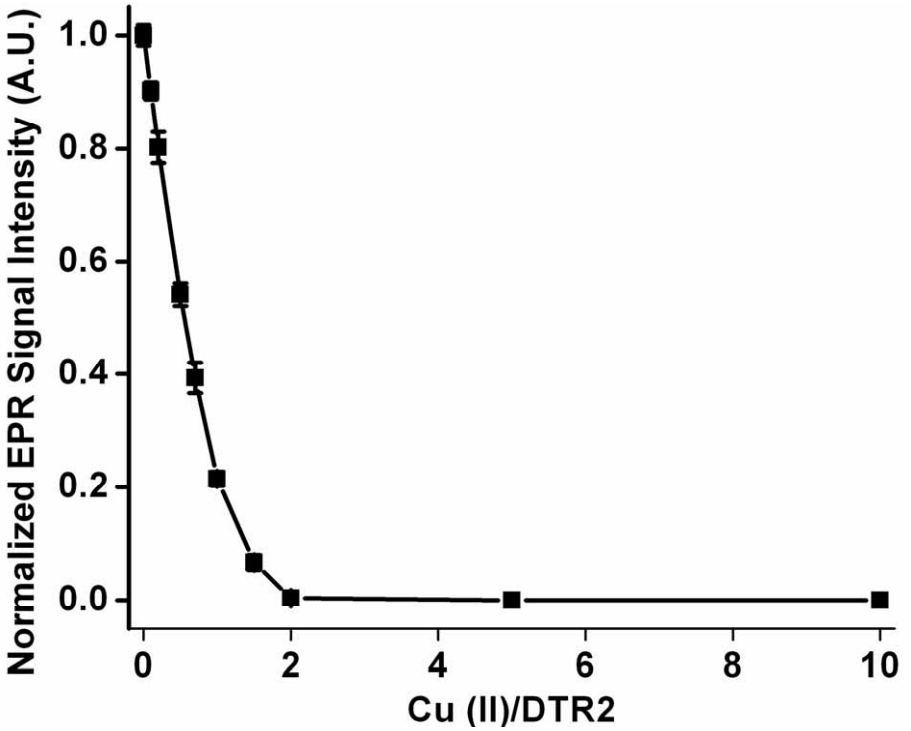


Figure S9. The EPR signal intensity as a function of the number of Cu^{2+} ions per DTR2. Various concentrations of $CuSO_4$ solution in water was added to the unbuffered aqueous DTR2 solution (10 μM). After 10 min, the EPR spectra were recorded at room temperature.

VIII. pH dependence of the EPR intensity of the DTR2-Cu^{2+} complex

The DTR2- Cu^{2+} complex was formed by adding Cu^{2+} (25 μM) to the aqueous solution of DTR2 (10 μM), which were stayed for 10 min at room temperature. Then, acidic titration with HCl solution (1M) was carried out. After acidic titration, the resulting solution was also exposed to alkaline titration with NaOH (1M).

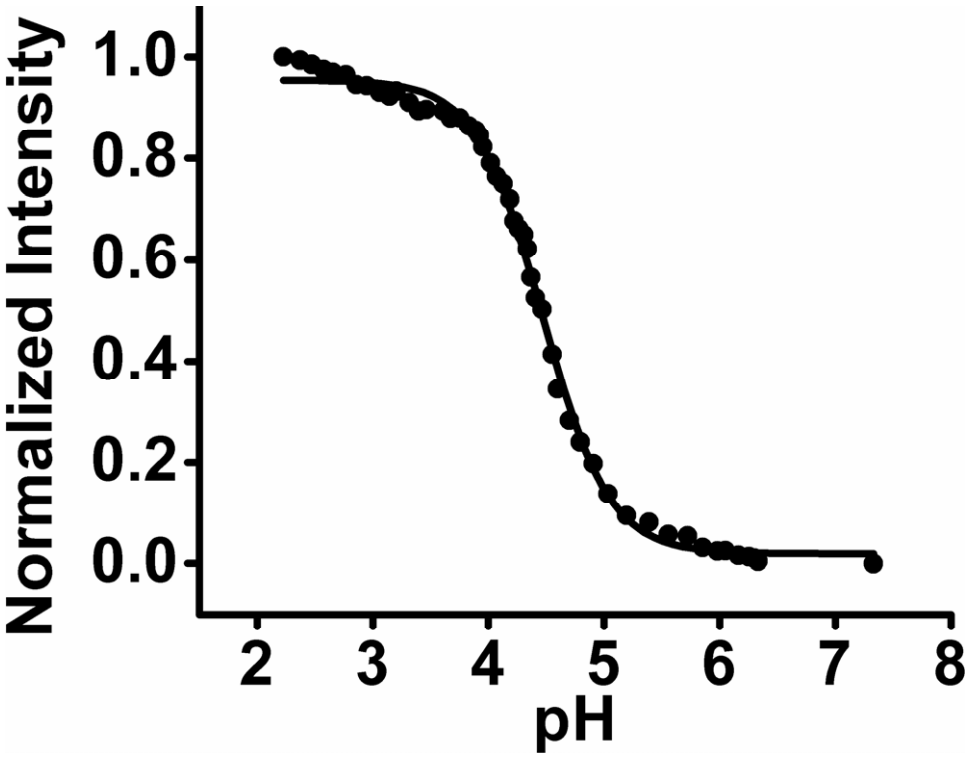
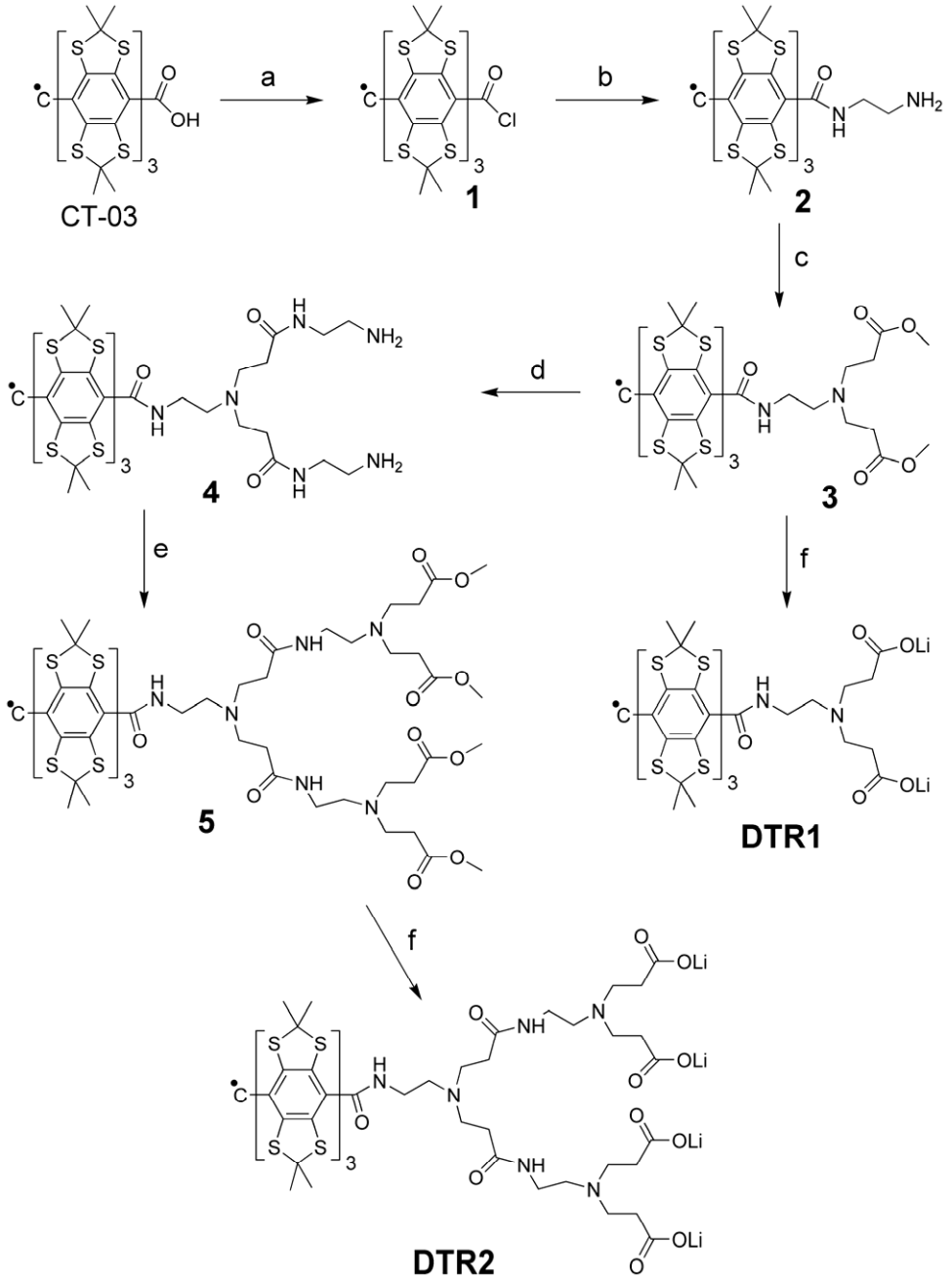


Figure S10. Another trial on pH dependence of the EPR intensity of the DTR2-Cu^{2+} complex

IX. Synthesis of DTR1 and DTR2:



Scheme S1. Reagents and conditions. (a) $(COCl)_2$, CH_2Cl_2, 2h; (b) ethylenediamine, CH_2Cl_2, overnight, 47% (two steps); (c) methyl acrylate, MeOH, 3 days, 86%; (d) ethylenediamine, MeOH, 7 days, 78%; (e) methyl acrylate, MeOH, 5 days, 84%; (f) LiOH, MeOH, 3h, quantitative.

a. General. All other reagents were obtained commercially and used without further purification and reactions were carried out under dry nitrogen atmosphere using standard Schlenk techniques unless otherwise indicated. The TAM radical CT-03 was synthesized using tetra-*tert*-butylthiobenzene as a starting material according to the previous procedure (Dhimitruka, et al, *Bioorg. Med. Chem. Lett.* **2007**, 6801).

b. Synthesis of Compound 2

To the solution of CT-03 (0.215 g, 0.215 mmol) in 20 mL of dichloromethane was added dropwise a large excess of oxalyl chloride (250 μL, 2.87 mmol). The solution was stirred for 5 min at room temperature and then catalytic amounts of DMF were added to initiate the reaction. The reaction mixture was stirred for 3h at room temperature. The solvent and the residual oxalyl chloride were removed under vacuum affording a red solid **1** which was directly used in the next step without further purification.

The resulting red solid was redissolved in 20 mL of dichloromethane and slowly added to the solution of ethylenediamine (1.8 mL, 26.9 mmol) in 30 mL of dichloromethane. The reaction mixture was stirred overnight at room temperature. The solvent and excess ethylenediamine were removed under vacuum. The residue was dissolved in 20 mL of dichloromethane and the insoluble solids were filtered. The resulting solution was acidified by HCl solution (1M) to pH 3 and extracted with dichloromethane (3 x 20 mL). The aqueous layer was neutralized with NaOH solution (1M) and extracted with dichloromethane (3 x 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. A green solid **2** (0.114 g, 47%) was obtained. IR (cm^{-1}, neat): 3357, 3279, 2965, 2935, 2856, 1663, 1456, 1437, 1366, 1329,

1296, 1236, 1217, 1170, 1150, 1111, 1057, 1008, 988, 943; MS (MALDI-TOF, M^+, m/z): 1125.321 (measured), 1125.114 (calculated).

c. Synthesis of Compound 3

To the solution of compound **2** (105 mg, 0.093 mmol) in MeOH (10 mL) were added methyl acrylate (0.56 mL, 6.22 mmol). The reaction mixture was stirred for 3 days at room temperature. The progress of reaction was monitored by observing the variation of the IR peak intensity ratio between 1733 and 1650 cm^{-1} of the reaction mixture. The solvent and excess methyl acrylate were then removed under vacuum. The crude product was separated by chromatography using the eluent (20:1 CH_2Cl_2:MeOH) to afford a green solid **3** (132 mg, 86%). IR (cm^{-1}, neat): 3367, 2953, 2915, 2843, 1733, 1651, 1509, 1452, 1435, 1366, 1237, 1196, 1170, 1150, 1114, 1041, 1012, 925, 842, 732, 700; MS (MALDI-TOF, M^+, m/z): 1641.518 (measured), 1641.335 (calculated).

d. Synthesis of Compound 4

To the solution of compound **3** (65 mg, 0.04 mmol) in MeOH (5 mL) were added ethylenediamine (2 mL, 30 mmol). The reaction mixture was stirred for 7 days at room temperature. The progress of reaction was monitored by observing the decrease of the IR peak intensity at 1733 cm^{-1} of the reaction mixture. The separation and purification procedures were similar to that of the compound **2**. A green solid **4** (56 mg, 78%) was obtained. IR (cm^{-1}, neat): 3352, 3282, 2930, 2922, 2851, 1639, 1563, 1477, 1433, 1383, 1367, 1314, 1242, 1166, 1150, 1025, 942, 821; MS (MALDI-TOF, $[M+H]^+$, m/z): 1810.671 (measured), 1810.560 (calculated).

e. Synthesis of Compound 5

To the solution of compound **4** (27 mg, 0.015 mmol) in MeOH (2 mL) were added methyl acrylate (0.5 mL, 55 mmol). The reaction mixture was stirred for 6 days at room temperature. The progress of reaction was monitored by observing the variation of the IR peak intensity ratio between 1733 and 1647 cm^{-1} of the reaction mixture. The solvent and excess of methyl acrylate were removed under vacuum. The crude product was separated by chromatography using the eluent (30:1 CH_2Cl_2:MeOH) to afford the green solid **5** (35.6 mg, 84%). IR (cm^{-1}, neat): 3367, 2953, 2915, 2843, 1736, 1651, 1509, 1452, 1435, 1384, 1366, 1237, 1196, 1170, 1150, 1114, 1041, 1012, 925, 842, 790, 773, 732, 700; MS (MALDI-TOF, $[M+H]^+$, m/z): 2842.931 (measured), 2843.031 (calculated).

f. Synthesis of DTR1 and DTR2

To the solution of compound **4** or **5** in MeOH was added solid LiOH (50 eq). The reaction mixture was stirred for 3 h at room temperature. The solid was then filtered and solvent was removed under vacuo. The resulting residue was redissolved in water and purified by column chromatography on Sephadex LH-20 using water as the eluent to afford the green solid DTR1 or DTR2. The paramagnetic purity of DTR1 and DTR2 was determined using TEMPO as standard, as described Section IV. DTR1: MS (MALDI-TOF, $C_{64}H_{81}N_6O_{15}S_{12}^{\bullet}$, m/z): 1557.351 (measured), 1557.241 (calculated); UV (λ_{max}, nm, water): 275 (ε = 42.9 mM^{-1} cm^{-1},), 336 (ε = 20.1 mM^{-1} cm^{-1}), 460 (ε = 17.0 mM^{-1} cm^{-1}); DTR2: MS (MALDI-TOF, $C_{112}H_{166}N_{18}O_{33}S_{12}^{\bullet}$, m/z): : 2674.949 (measured), 2674.844 (calculated); UV (λ_{max}, nm, water): 275 (ε = 42.7 mM^{-1} cm^{-1},), 334 (ε = 19.6 mM^{-1} cm^{-1}),

460 (ε = 16.8 mM^{-1} cm^{-1}).

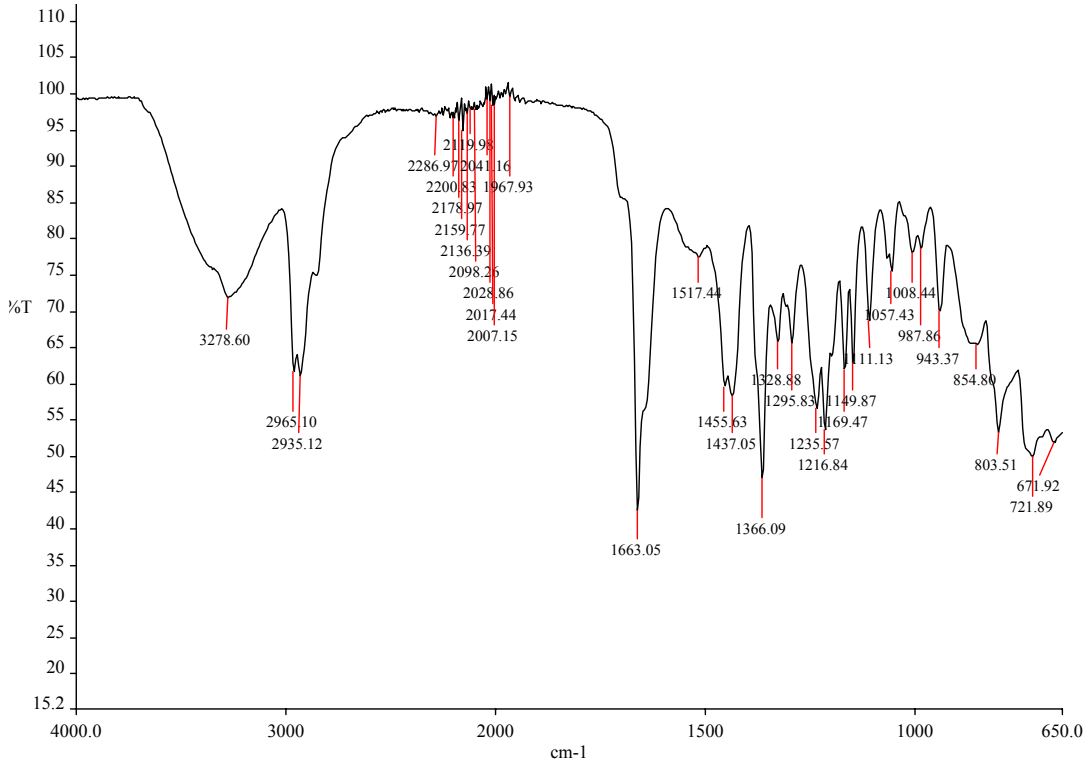


Figure S11. The IR spectrum of compound **2**

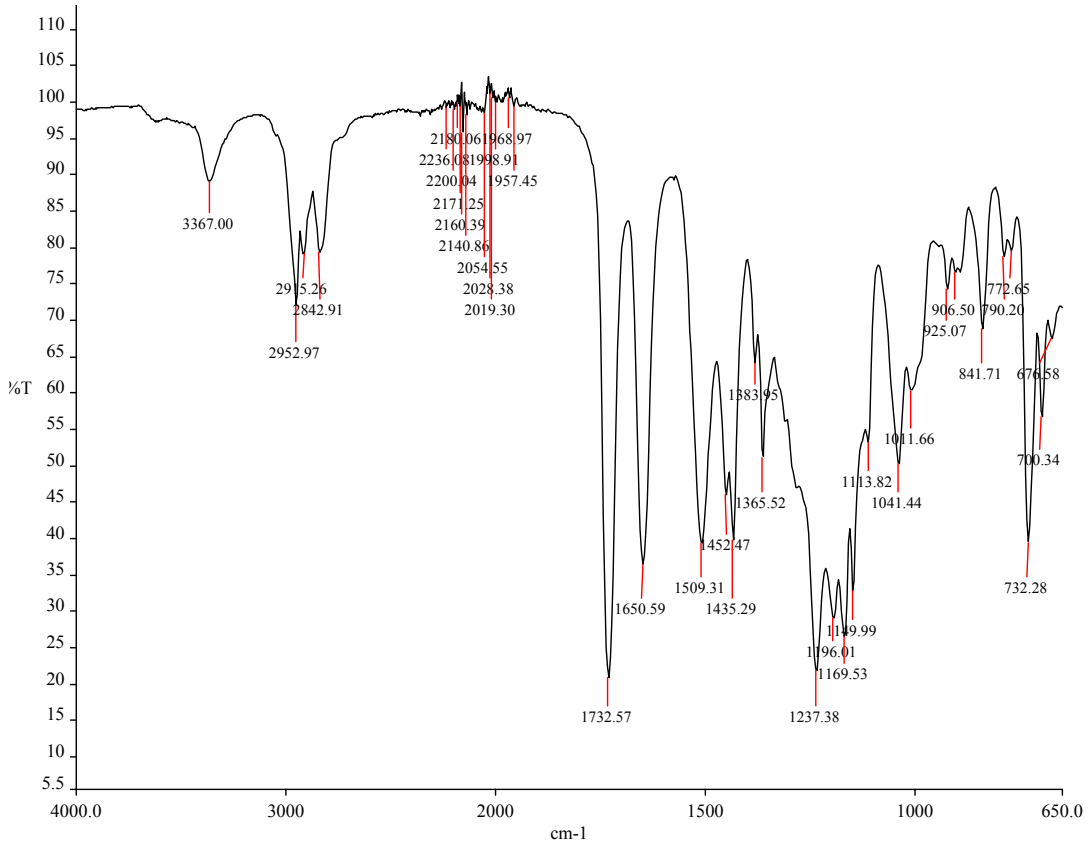


Figure S12. The IR spectrum of compound **3**

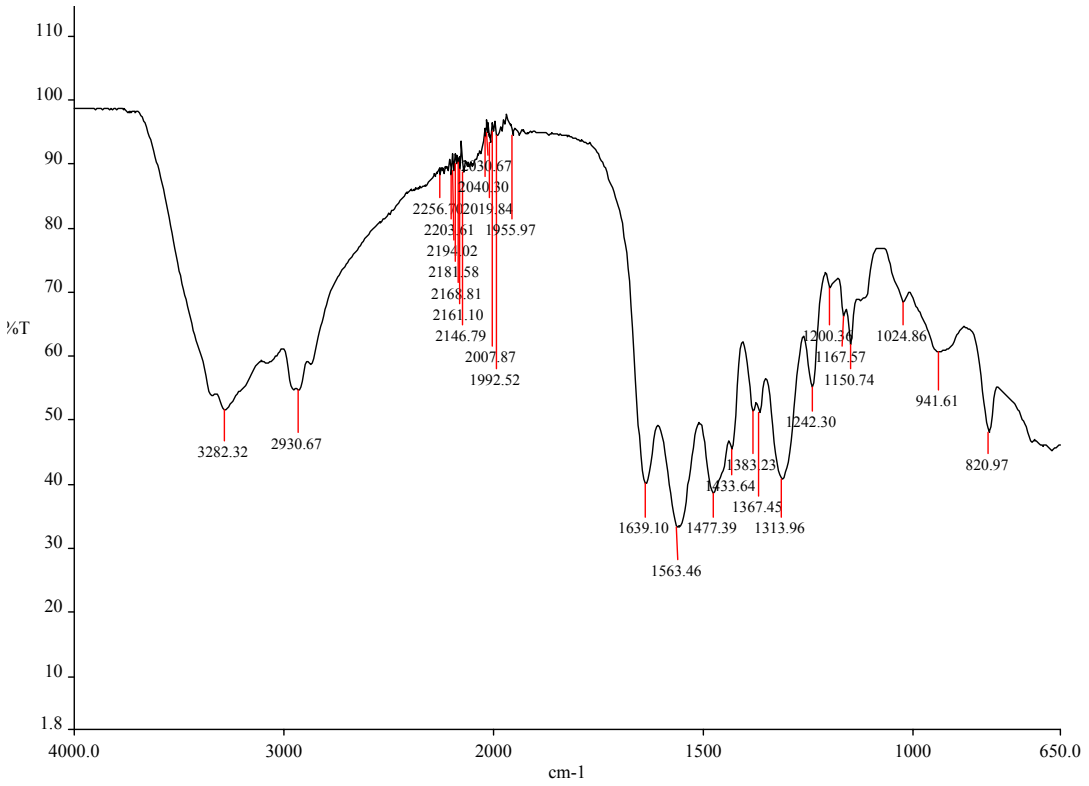


Figure S13. The IR spectrum of compound **4**

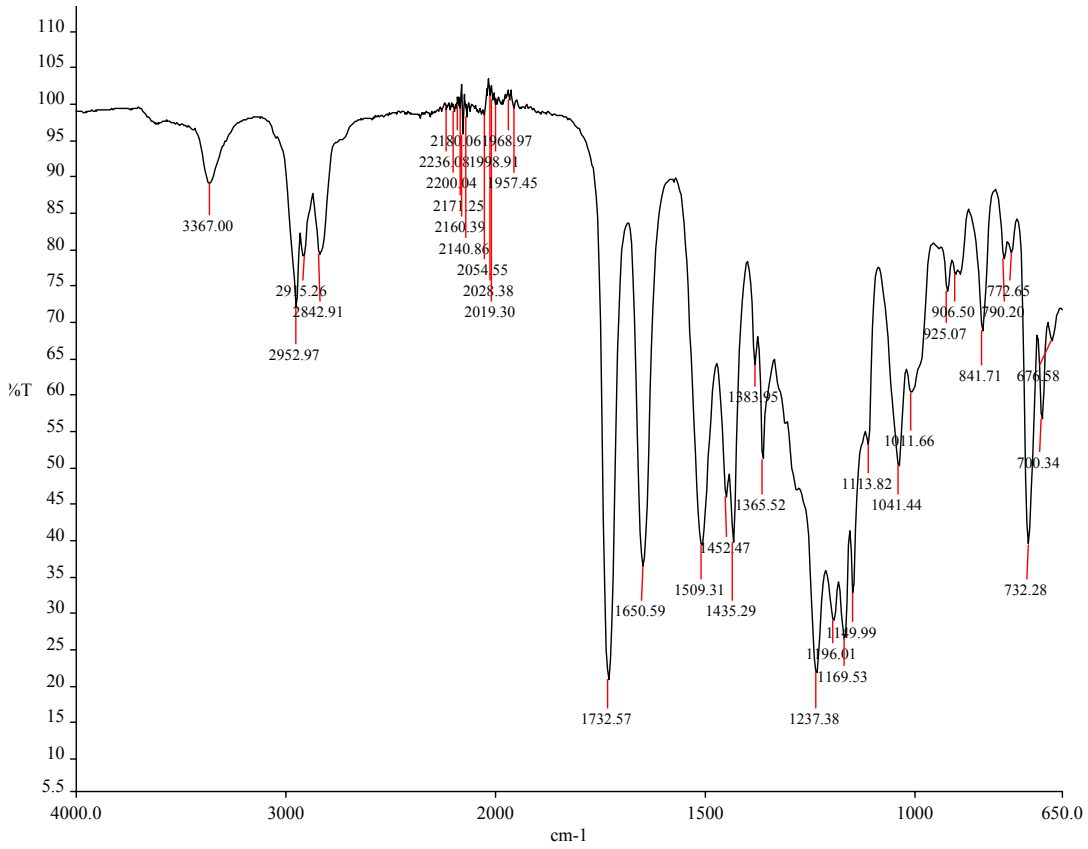


Figure S14. The IR spectrum of compound **5**

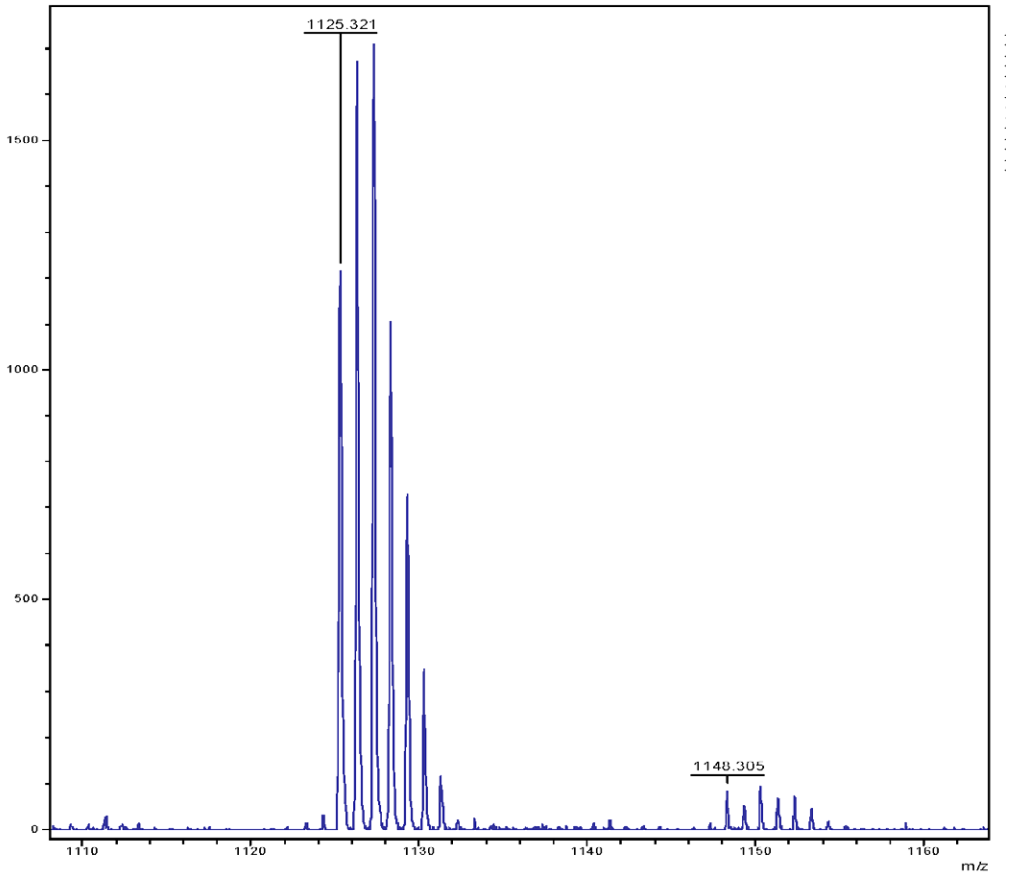


Figure S15. The High Resolution Mass Spectrum of compound **2**

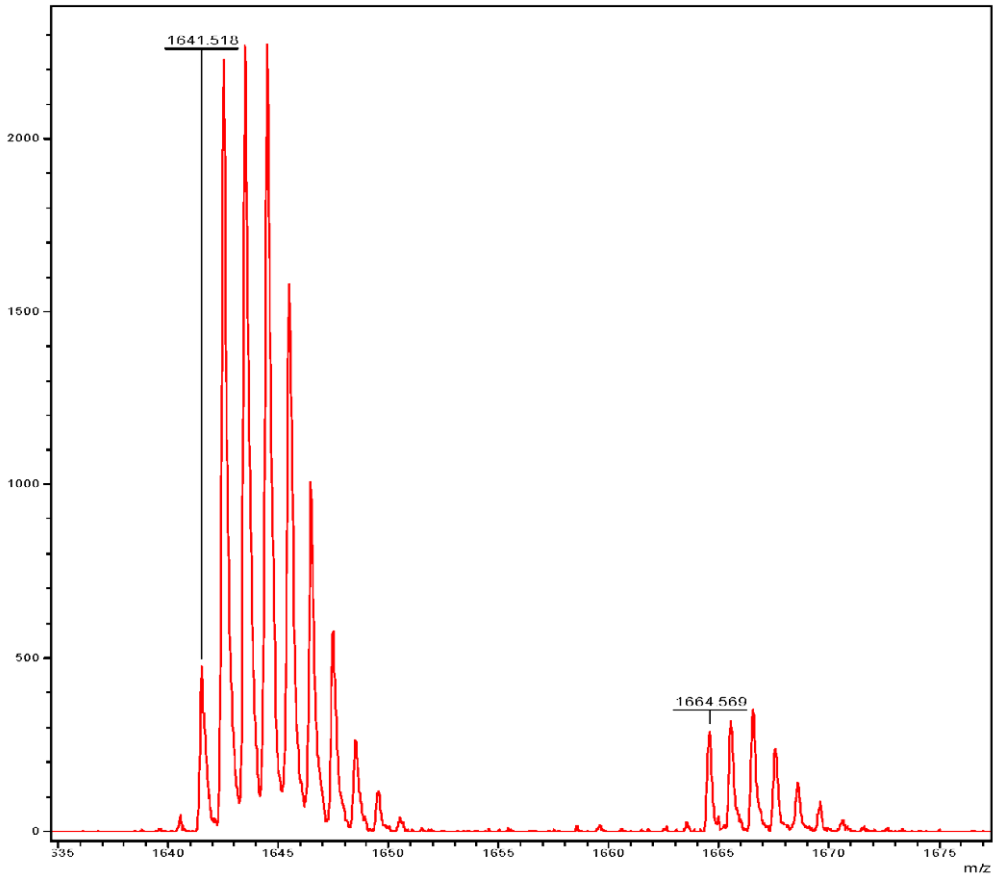


Figure S16. The High Resolution Mass Spectrum of compound **3**

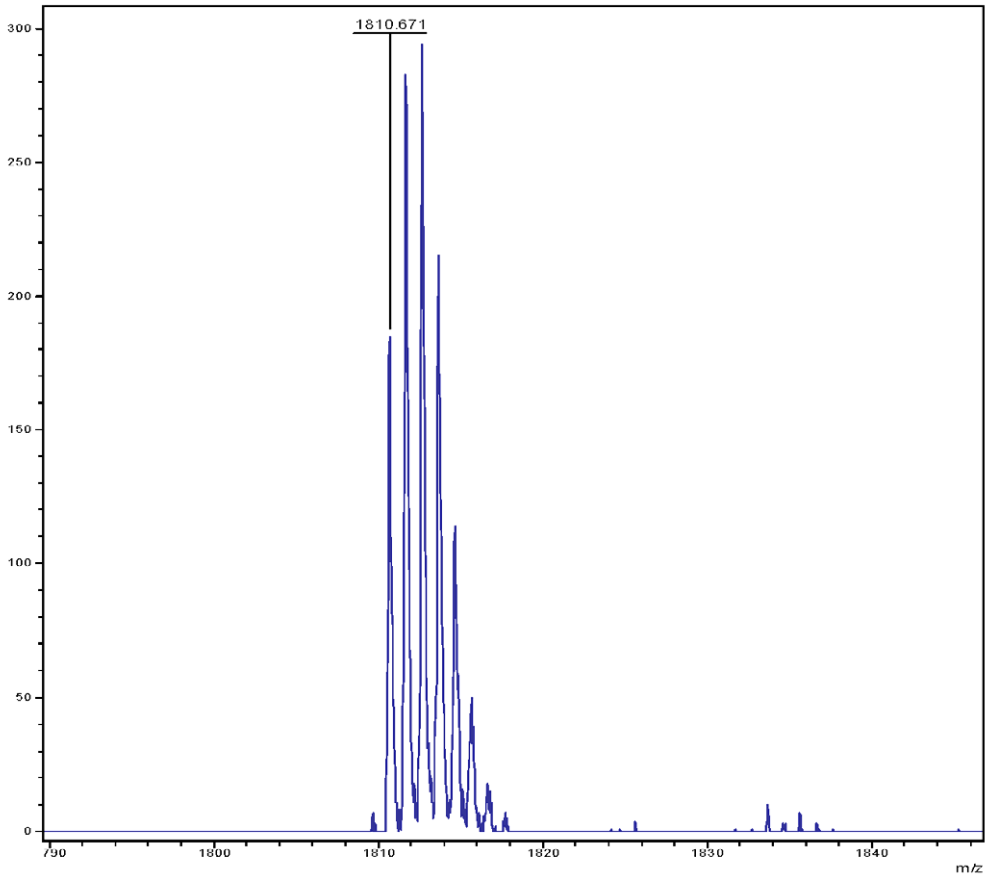


Figure S17. The High Resolution Mass Spectrum of compound **4**

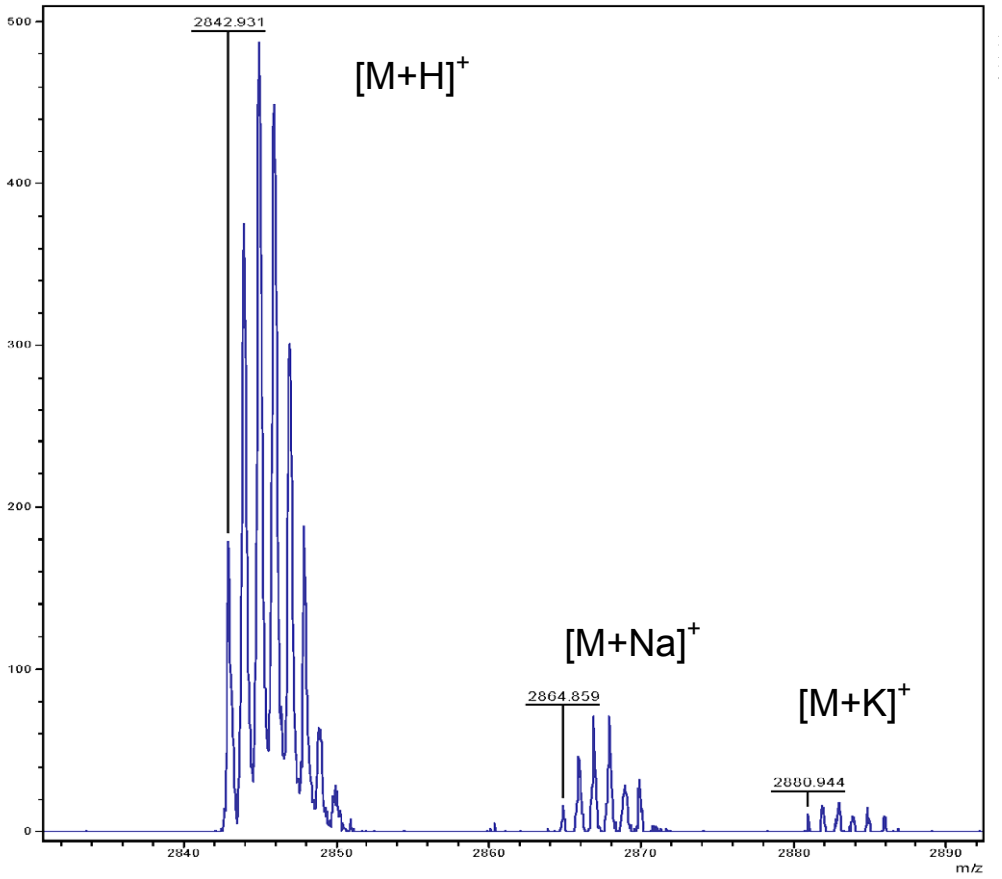


Figure S18. The High Resolution Mass Spectrum of compound **5**

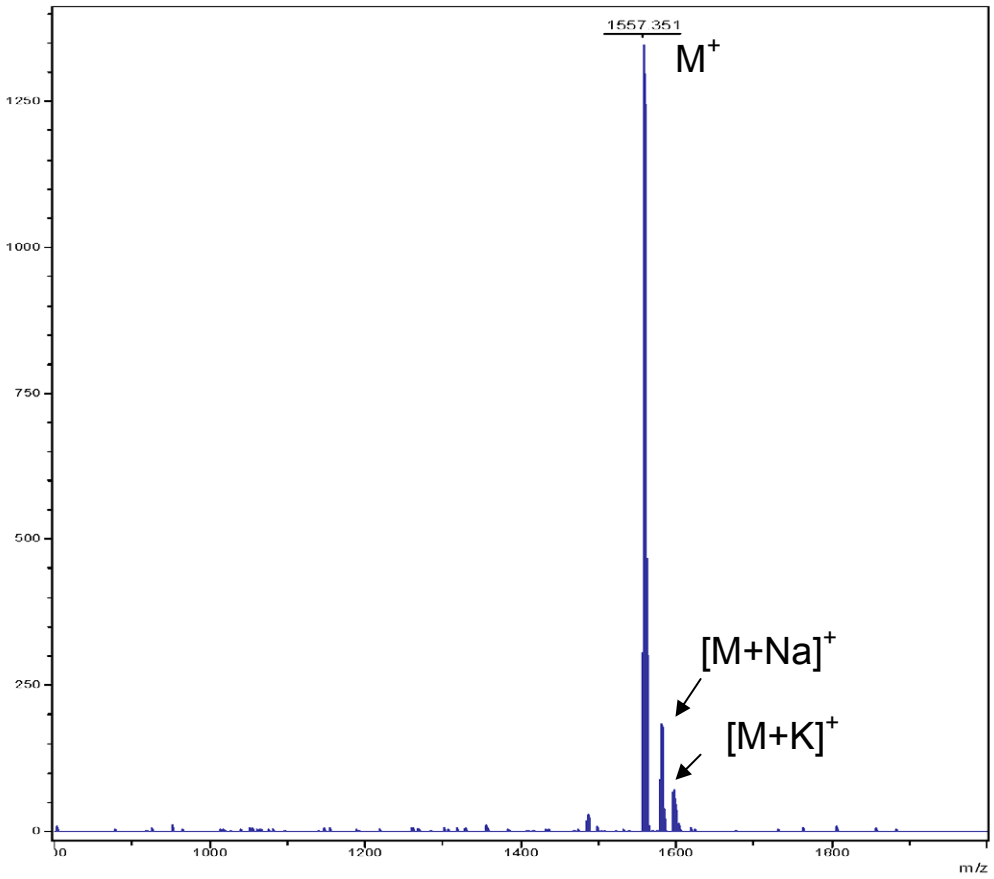


Figure S19. The High Resolution Mass Spectrum of DTR1

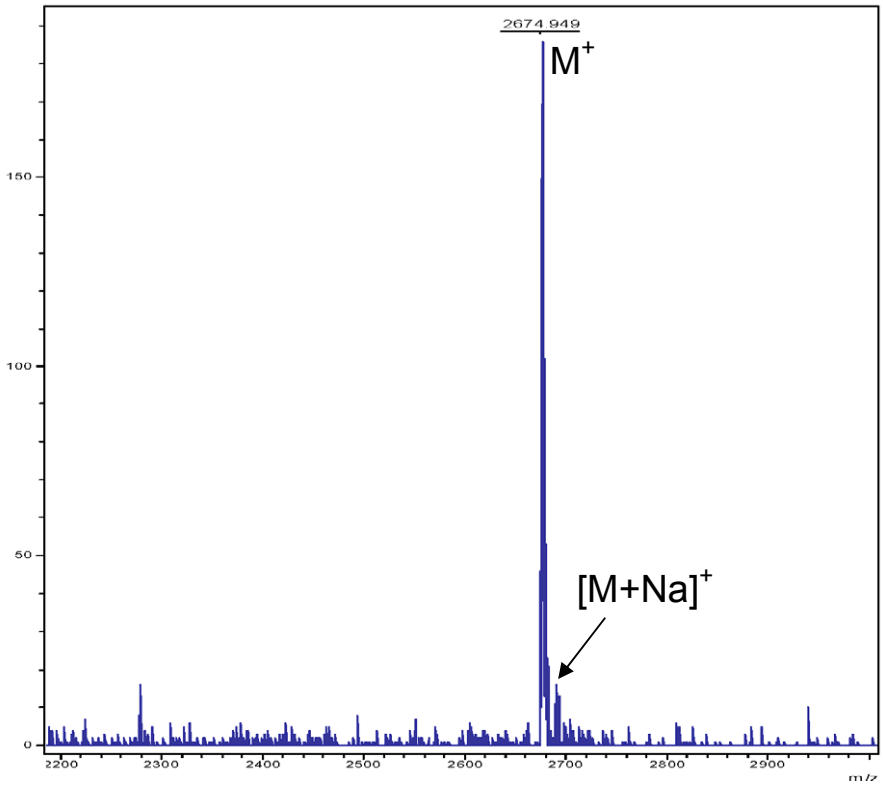


Figure S20. The High Resolution Mass Spectrum of DTR2

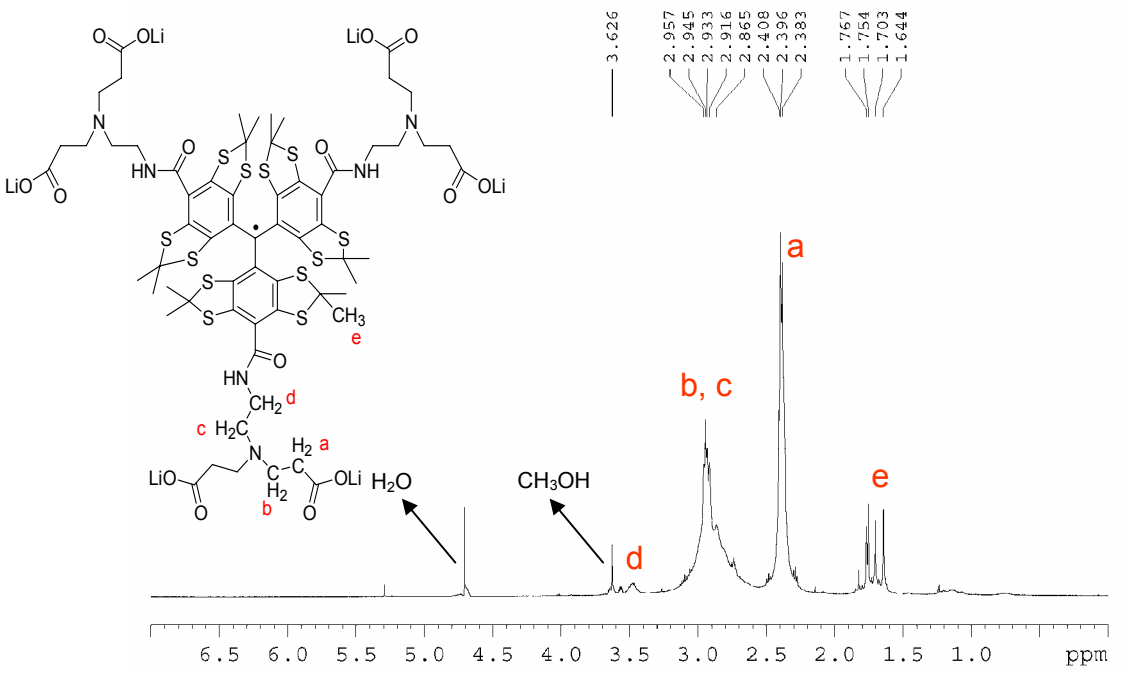


Figure S21. ^{1}H-NMR spectra of DTR1.

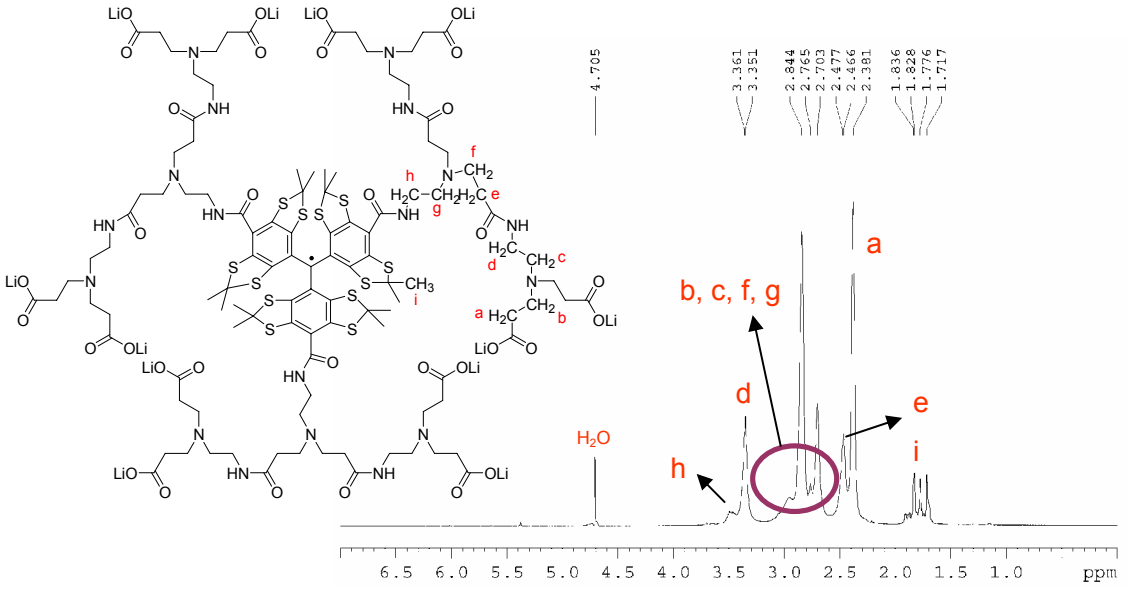


Figure S22. ^{1}H-NMR spectra of DTR2.