Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Electronic Supplementary Information

Dicyclen-TPE zinc complex as a novel fluorescent ensemble for nanomolar pyrophosphate sensing in 100% aqueous solution

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Experimental Section

General

Mass spectrometer (ESI-MS) and High Resolution Mass Spectrometer (HRMS) data were recorded on a Finnigan LCQDECA and a Bruker Daltonics Bio TOF mass spectrometer, respectively. The ^{1}H NMR and ^{13}C NMR spectra measured on a Bruker AM400 NMR spectrometer and the δ scale in ppm referenced to residual solvent peaks or internal tetramethylsilane (TMS). Absorption spectra recorded on Hitachi U1900 spectrophotometer at 298 K. Fluorescence emission spectra were obtained using FluoroMax-4 Spectrofluoro-photometer (HORIBA Jobin Yvon) at 298 K. Unless otherwise indicated, all syntheses and manipulations were carried out under N2 atmosphere. All the solvents were dried according to the standard methods prior to use. All of the solvents were either HPLC or spectroscopic grade in the optical spectroscopic studies.

Details for fluorescence measurements and UV−Vis.

Stock solutions of, **dCT·Zn** (10 M) was prepared in distilled water as stock solutions for each measurement. Chloride (Hg^{2+}, Ba^{2+}, Mn^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}), nitrate (Ca^{2+}, Ni^{2+}, Cr^{3+}, Cu^{2+}, Al^{3+}, Cd^{2+}, Zn^{2+}, Pb^{2+}, Ag^{+}) and perchlorate (K^{+}, Na^{+}, Li^{+}, Mg^{2+}) (50mM) were prepared in distilled water. Stock solutions of anions as sodium salts ($P_2O_7^{4-}$, $H_2PO_4^{-}$, HPO_4^{2-}, PO_4^{3-}, Cl^{-}, Br^{-}, F^{-}, I^{-}, NO_3^{-}, Ci_3^{-}, AcO^{-}, N_3^{-}, CN^{-}, SO_4^{2-}, SO_3^{2}-, S^{2-}, HCO_3^{-}, CO_3^{2-}) (10mM) and ATP, ADP, AMP, Glu, Asp, oxalic acid and malonic acid (10mM) were prepared in distilled water. The fluorescence studies performed in HEPES buffer (10 mM, pH = 7.4). Each time a 3 mL of receptor solution (10 μM) was filled in a quartz cell of 1 cm of optical path length and the stock solution of metal ion or anion was dropped into a quartz cell using a microsyringe. The excitation and emission slits of fluorescence spectra were set at 5.0 nm if not specified. For absorption studies, the final concentrations of receptors were kept constant at 10μM, and the procedure used for the titrations was the same as that used for fluorescence titrations.

Determination of the fluorescence quantum yield

The fluorescence quantum yield (Q) is defined as the ratio of the number of photons emitted and the number of photons of the excitation light absorbed while the fluorescent substance absorbed photons. People usually use ratio method for the determination of the fluorescence quantum yield.

$$Q_x = Q_r\left(\frac{A_r(\lambda_r)}{A_x(\lambda_x)}\right)\left(\frac{I(\lambda_r)}{I(\lambda_x)}\right)\left(\frac{n_x{}^2}{n_r{}^2}\right)\left(\frac{D_x}{D_r}\right)$$

In this equations ***I (a)*** is the relative intensity of the exciting light at wavelength A, ***n*** is the average refractive index of the solution to the luminescence, ***D*** is the integrated area under the corrected emission spectrum, and ***A(a)*** is the absorbance of the solution at the exciting wavelength ***a***. Subscripts x and r refer to the sample and reference solutions, respectively.[1]

In this paper, we use the same wavelength to excite the sample and the reference solution. They have same solvent (H_2O), the middle two formulas of the equations are 1. That means we only need to determine the absorbance and the emission spectra of peak area integration. We use quinine sulphate solution as reference, Qr = 0.560. The result of the calculation is the fluorescence quantum yield increase from 0.426% to 1.757% after **dCT·Zn** complex with the PPi.

Determination of binding constant

The interaction between zinc complex and PPi was analyzed according to the Benesi-Hildebrand equation[2] for spectrofluorometric titration:

$$\frac{1}{F - F_{min}} = \frac{1}{K(F_{max} - F_{min})[P_2O_7{}^{4-}]^{0.5}} + \frac{1}{F_{max} - F_{min}}$$

Here, F_{min} is the fluorescence intensity of free zinc complex, F is the intensity measured with $P_2O_7{}^{4-}$, F_{max} is the intensity measured with an excess of $P_2O_7{}^{4-}$, and K is the binding constant. The value of K was obtained from a plot of $(F_{max} - F_{min}) / (F_{max} - F)$ against $1/[P_2O_7{}^{4-}]^{0.5}$ where K is equal to the 1/slope.

Calculations for detection limit

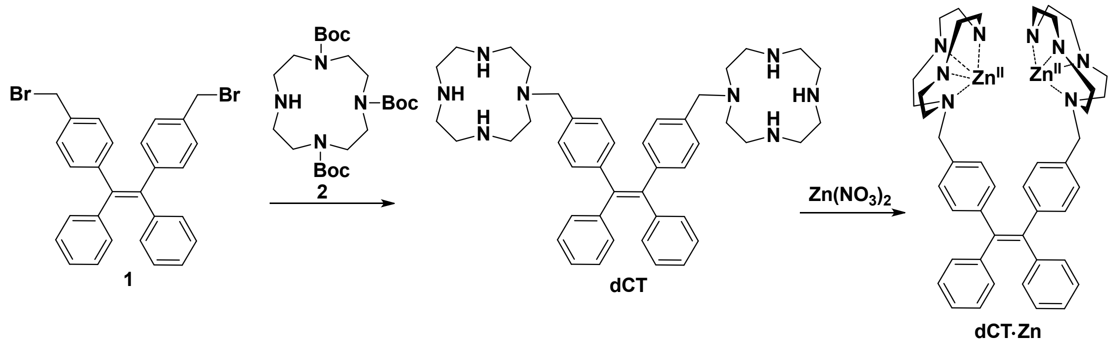
The detection limit was calculated on the basis of the fluorescence titration using the following equation:

$$DL = \frac{3\sigma}{k}$$

Here, σ is the standard deviation of blank measurement, k is the slop between the ratio of emission intensity versus [PPi]. The fluorescence emission of **dCT** was measured 10 times to obtain the standard deviation of blank measurement. [2]

Synthesis

Compounds **1**, **2** were prepared according to the literature procedures.[3]



Synthesis and characterization of dCT.

A mixture of **1** (900 mg, 1.74 mmol), **2** (2.06 g, 4.36 mmol) and K_2CO_3 (1.20 g, 8.70 mmol) in acetonitrile was refluxed over night. After the solution was concentrated, the residue was added dichloromethane (150 mL), and the organic phase was washed with water and brine followed by drying over Na_2SO_4. The solvent was removed under reduced pressure; the crude product was purified by silica gel column eluting with EtOAc/PE (2:1, v/v) to give the product as a pale yellow solid (1.50g), yield 66.1%.
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.11-7.07 (m, 6H), 7.00-6.68 (br, 12H), 3.64 (s, 4H), 3.56 (br, 8H), 3.33-3.15 (br, 16H), 2.64 (br, 8H), 1.50-1.45 (br, 54H) ppm;
^{13}C NMR(100 MHz, $CDCl_3$): δ 155.7, 143.5, 142.9, 142.8, 140.7, 131.2, 129.7, 127.7, 126.6, 126.5, 79.4, 79.3, 60.4, 49.9, 48.4, 47.5, 47.1, 28.5;

The yellow solid achieved above (1.30g, 1.00mmol) was dissolved in a saturated of HCl-methanol solution (150mL) and stirred overnight at room temperature. The solvent and excess HCl were removed under reduced pressure, giving a yellow solid. The solid was dissolved in water and added pretreated anion-exchange resin until the solution is alkaline. Freeze-dried the solution to remove water and obtained the product as a pale yellow flocculent solid (650mg), yield 92.9%.
^{1}H NMR (400 MHz, D_2O): δ 7.14-6.91 (br, 18H), 3.72 (s, 4H), 3.08-3.07 (m, 16H), 2.92 (m, 8H), 2.76 (m, 8H)ppm;
^{13}C NMR(100 MHz, D_2O): δ 143.4, 141.0, 140.9, 132.8, 132.7, 131.3, 131.1, 131.0, 129.5, 128.0, 126.9, 56.0, 47.6, 47.5, 44.4, 44.3, 42.0, 41.9;
HRMS: (ESI) *m/z* calcd for $C_{44}H_{61}N_8$ $[M + H]^+$: 701.5014, found: 701.5017.

Synthesis and characterization of dCT·Zn.

To the methanol solution (15mL) of **dCT** (600 mg, 0.857 mmol) was added $Zn(NO_3)_2 \cdot 6H_2O$ (750 mg, 2.52 mmol) and stirred the solution overnight at room temperature. The solvent was evaporated under reduced pressure to give the yellow solid. Washed the solid with ethanol (5×10 mL) and centrifuged the mixture to afford the pure product as pale yellow solid. Yield (460 mg, 49.9%).
^{1}H NMR (400 MHz, DMSO-*d*6): δ 7.17-6.98 (br, 18H), 4.51 (s, 4H), 4.33 (s, 2H), 3.77-3.75 (d, 4H, $J = 8$Hz), 2.93 (s, 4H), 2.72-2.67 (br, 22H) ppm;

^{13}C NMR(100 MHz, DMSO-*d*6): δ 143.2, 131.0, 128.4, 128.3, 49.1, 44.8, 44.0, 42.4; HRMS: (ESI) *m/z* calcd for $C_{44}H_{63}N_9O_6NaZn_2$ [M + 3OH + 2Zn + NO_3 + Na]$^+$: 964.3376, found: 964.3015.

References

[1] BENESI, H. A.; HILDEBRAND, J. H., *J. Am. Chem. Soc.* **1949**, *71*, 2703-2707.

[2] Datta, B. K.; Mukherjee, S.; Kar, C.; Ramesh, A.; Das, G. *Anal. Chem.* **2013**, *85*, 8369-8375.

[3] a) Li, D.; Yu, J.; Xu, R. *Chem. Commun.* **2011**, *47*, 11077-11079; b) Vögtle, F.; Wester, N. *Justus Liebigs Annalen der Chemie* **2006**, *1978*, 545-551; c) Yu, Y.; Li, J.; Chen, S.; Hong, Y.; Ng, K. M.; Luo, K. Q.; Tang, B. Z. *ACS Appl. Mater. Interfaces* **2013**, *5*, 4613-4616; d) Wang, J.-H.; Wang, C.-C.; Zhang, D.; Liu, C.-J.; Ye, Y.; Zhao, Y. *Phosphorus, Sulfur Silicon Relat. Elem.* **2013**, *188*, 54-58

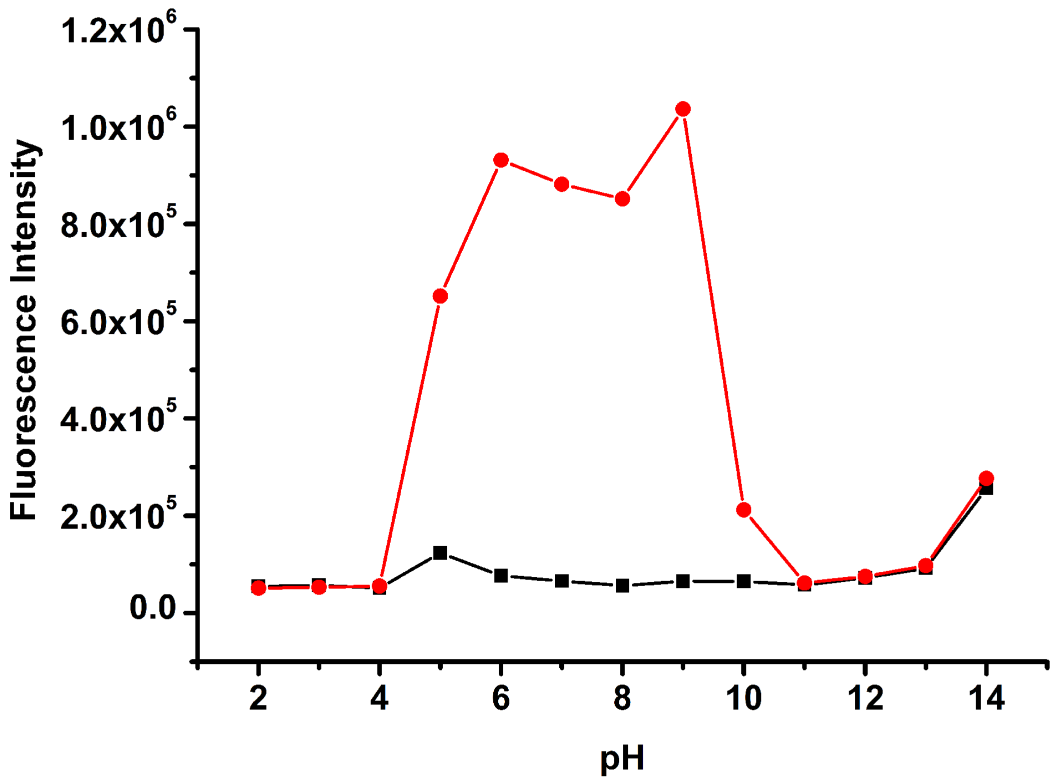


Figure S1. Effect of pH on the fluorescence intensity at 468nm of **dCT·Zn** (black, ■) and **dCT·Zn** + 0.5eq. PPi (red, ●) in aqueous solution. The pH of solution was adjusted by NaOH (1M) and HCl (1M).

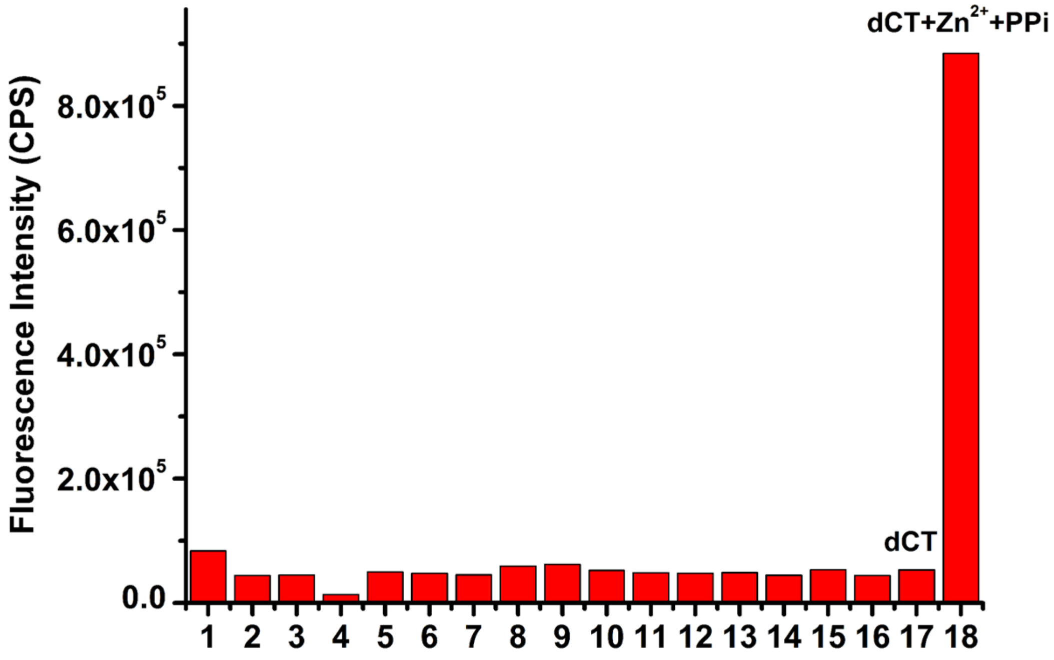


Figure 1. Fluorescence spectra of sensor **dCT** (10 µM) in HEPES buffer (10mM, pH=7.4) in the presence of various metal ions (50 µM): (1) Pb^{2+}; (2) Ni^{+}; (3) Mn^{2+}; (4) Cu^{2+}; (5) Fe^{3+}; (6) Cr^{3+}; (7) Co^{2+}; (8) Ag^{+}; (9) Hg^{2+}; (10) Ca^{2+}; (11) Na^{+}; (12) K^{+}; (13)

Mg^{2+}; (14) Li^{+}; (15) Ba^{2+}; (16) Zn^{2+}; (17) none; (18) Zn^{2+} + PPi.

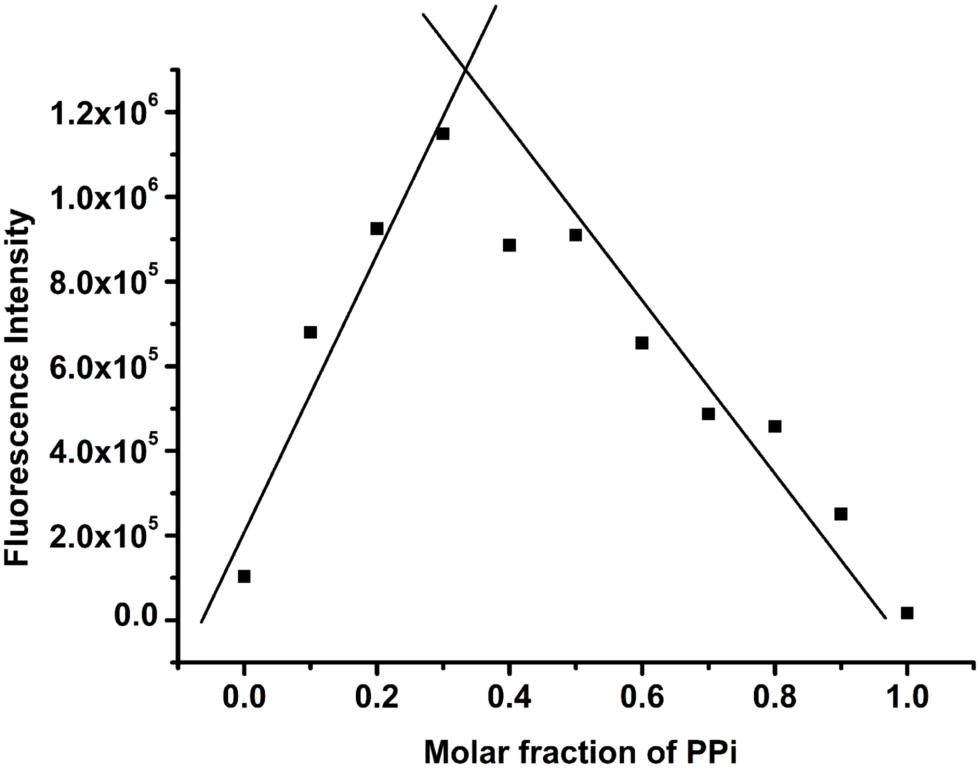
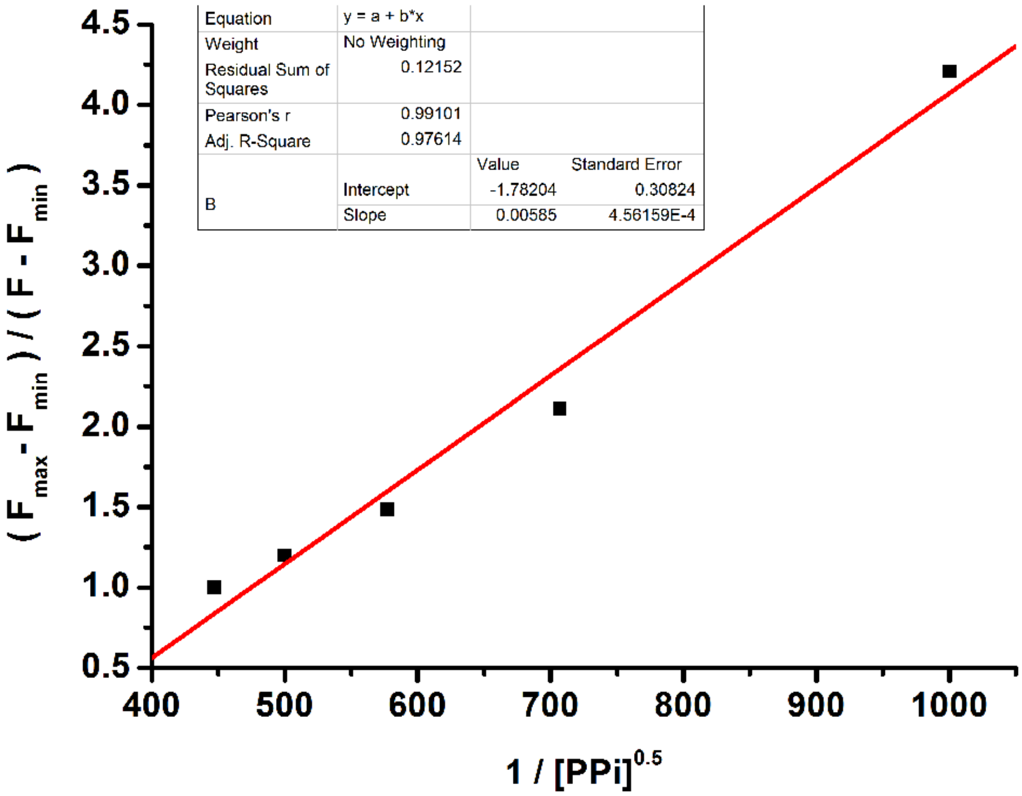


Figure S3. Job's plot of **dCT·Zn** and PPi. The total concentration of **dCT·Zn** and PPi were kept at 10 μM in HEPES (10mM, pH = 7.4).



Equation	y = a + b*x		
Weight	No Weighting		
Residual Sum of Squares	0.12152		
Pearson's r	0.99101		
Adj. R-Square	0.97614		
		Value	Standard Error
B	Intercept	-1.78204	0.30824
	Slope	0.00585	4.56159E-4

Figure S4. Benesi-Hildebrand plots (λem = 468nm) of **dCT·Zn**, assuming 2:1 stoichiometry for association between **dCT·Zn** and PPi, giving $K_{dCT} = 170.94\ M^{-2}$.

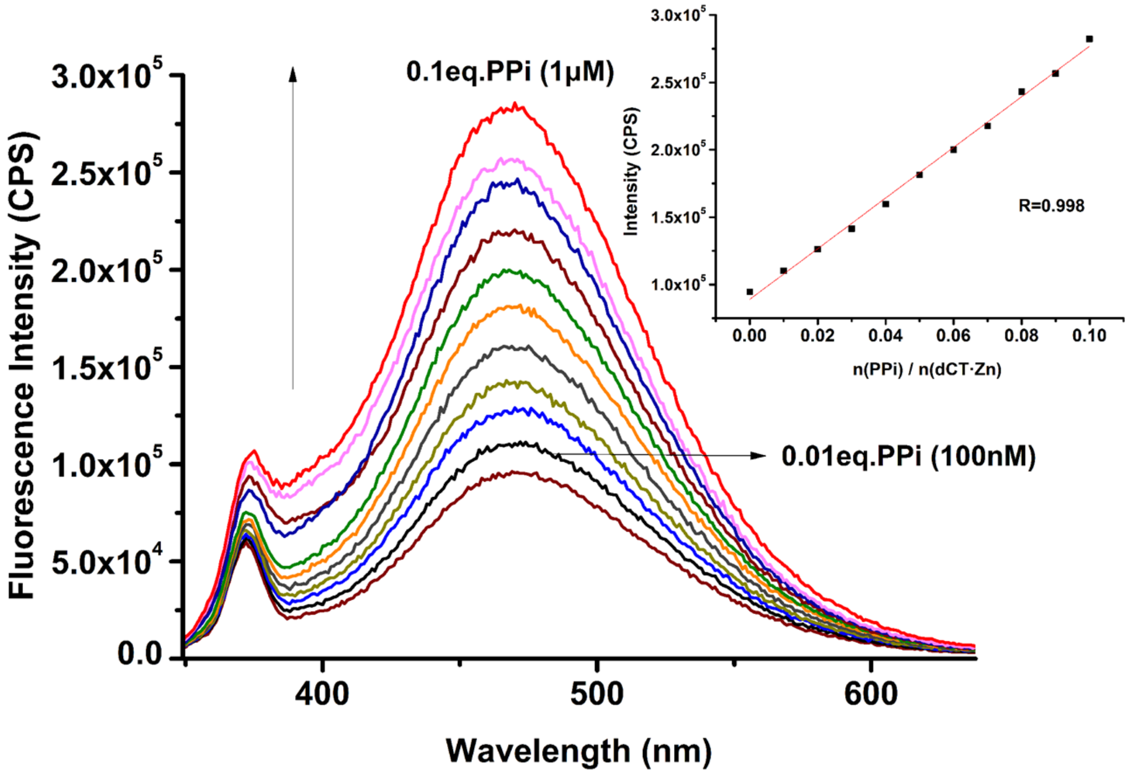


Figure S5. Fluorescence titration of **dCT·Zn** (10 μM) with PPi (0-0.10eq.) in HEPES (pH = 7.4, 10mM). Inset shows the fluorescence change at 468nm as a function of the amount of PPi.

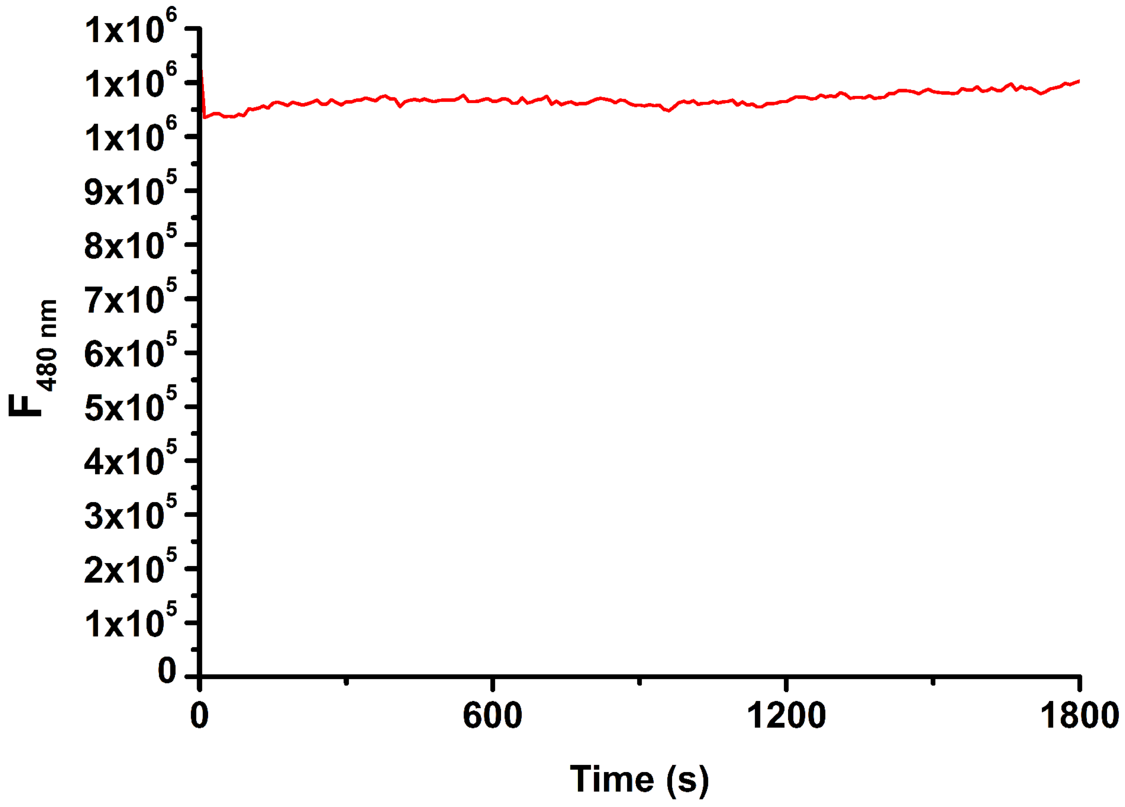


Figure S6. Fluorescence response of **dCT·Zn** (10μM) in the presence of PPi (5μM), λex=329 nm, λem=468 nm.

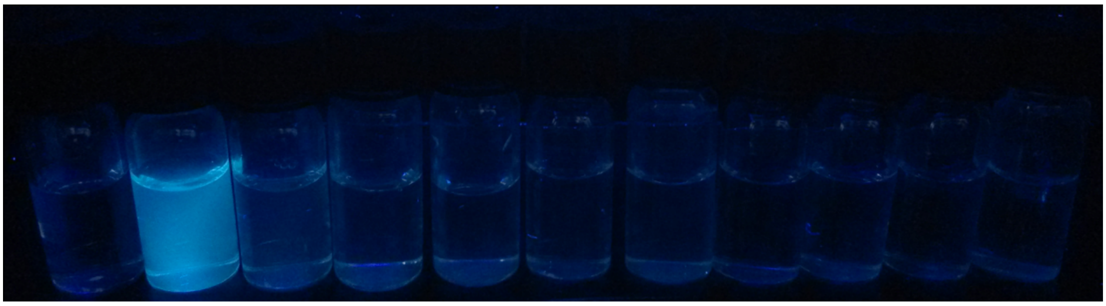


Figure S7. The color change of **dCT·Zn** (20 μM) in HEPES (10 mM, pH = 7.4) under a UV lamp (365 nm) by addition of 0.5 equiv. different anions (from left to right: no anion, PPi, ATP, ADP, AMP, PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, Ci^{3+}, Glu, Asp).

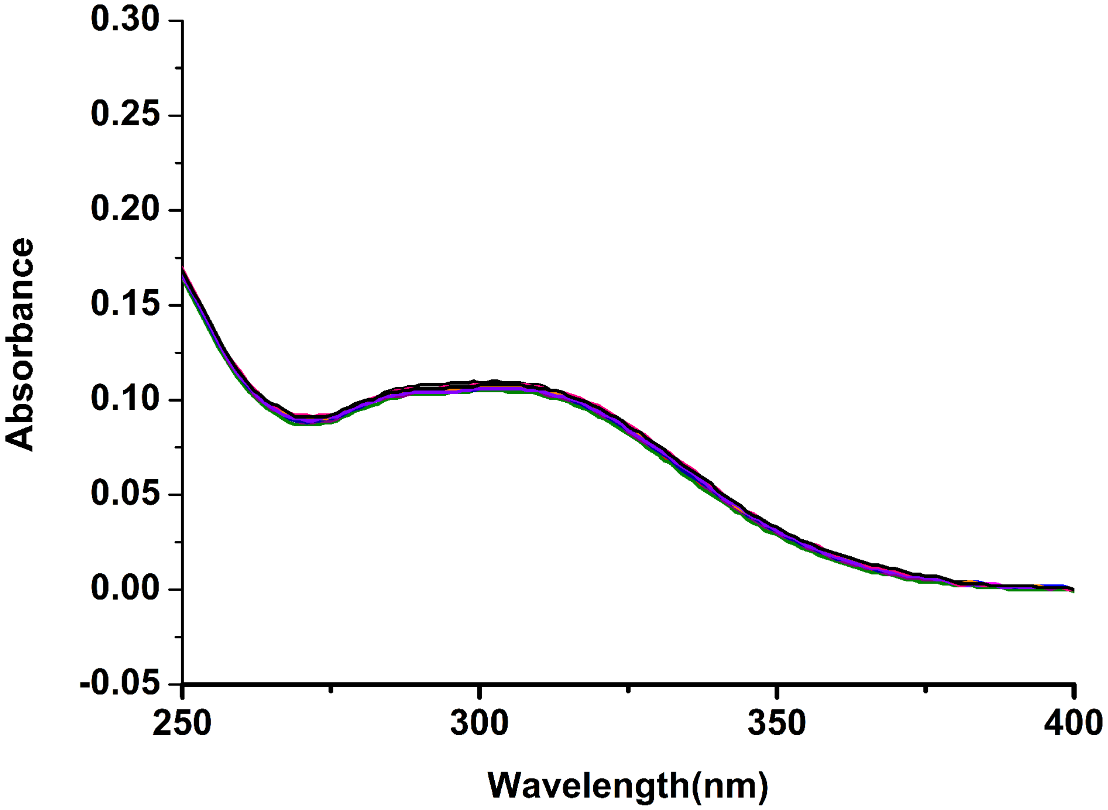


Figure S8. UV-vis titration of **dCT** (10 μM) with Zn^{2+} (0-25.0 equiv.) in HEPES (pH=7.4, 10mM).

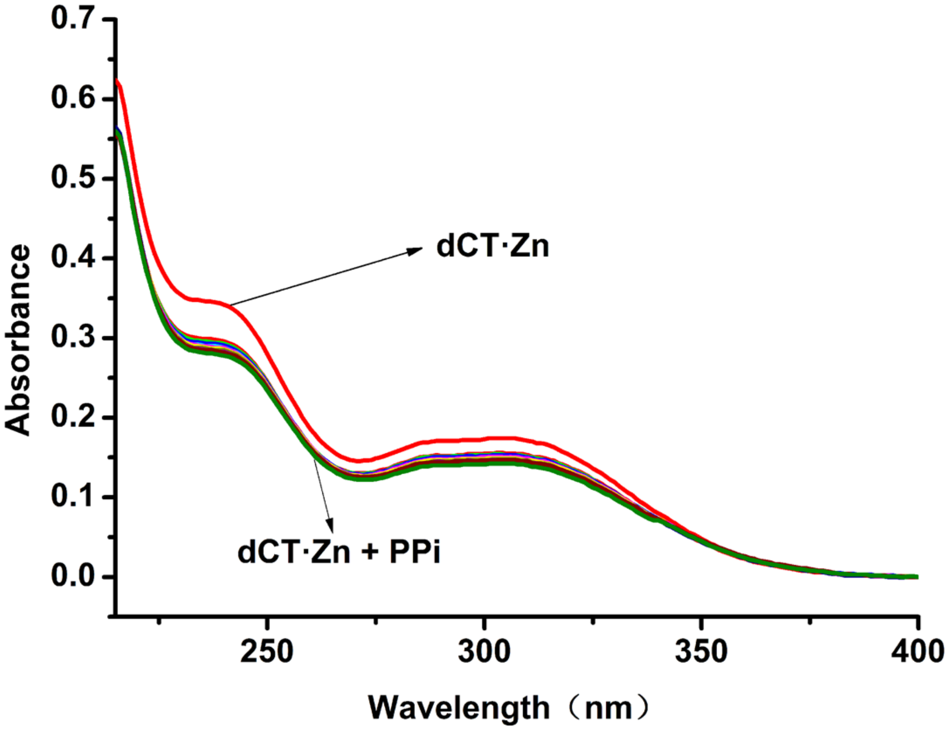


Figure S9. UV-vis titration of **dCT·Zn** (10μM) with PPi (0.1~1.0, 2.0, 4.0 equiv.) in HEPES (pH=7.4, 10mM).

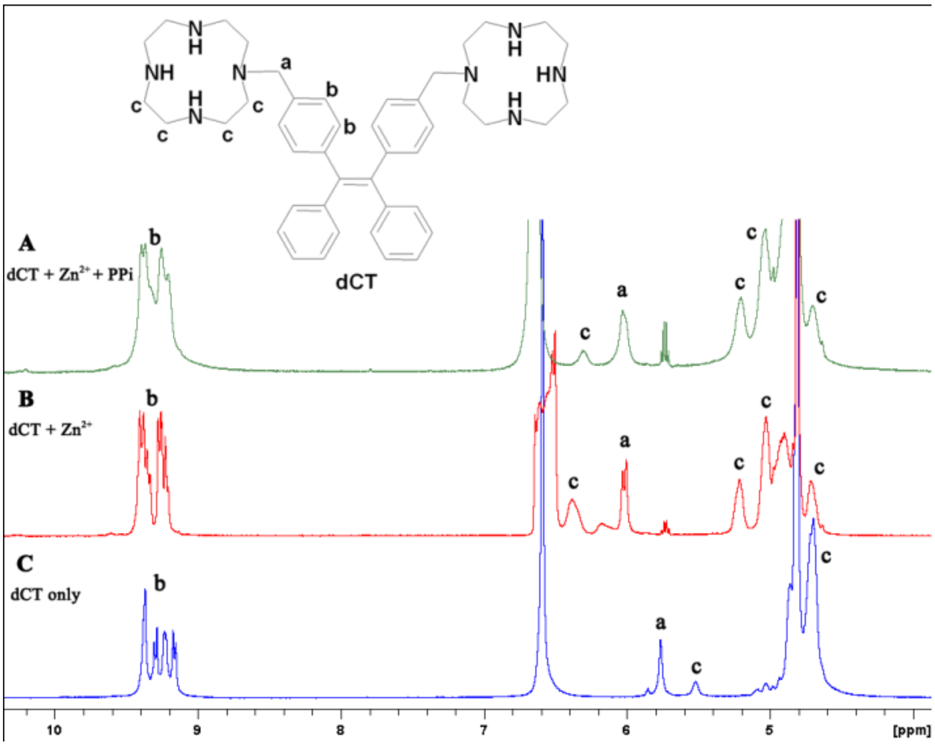
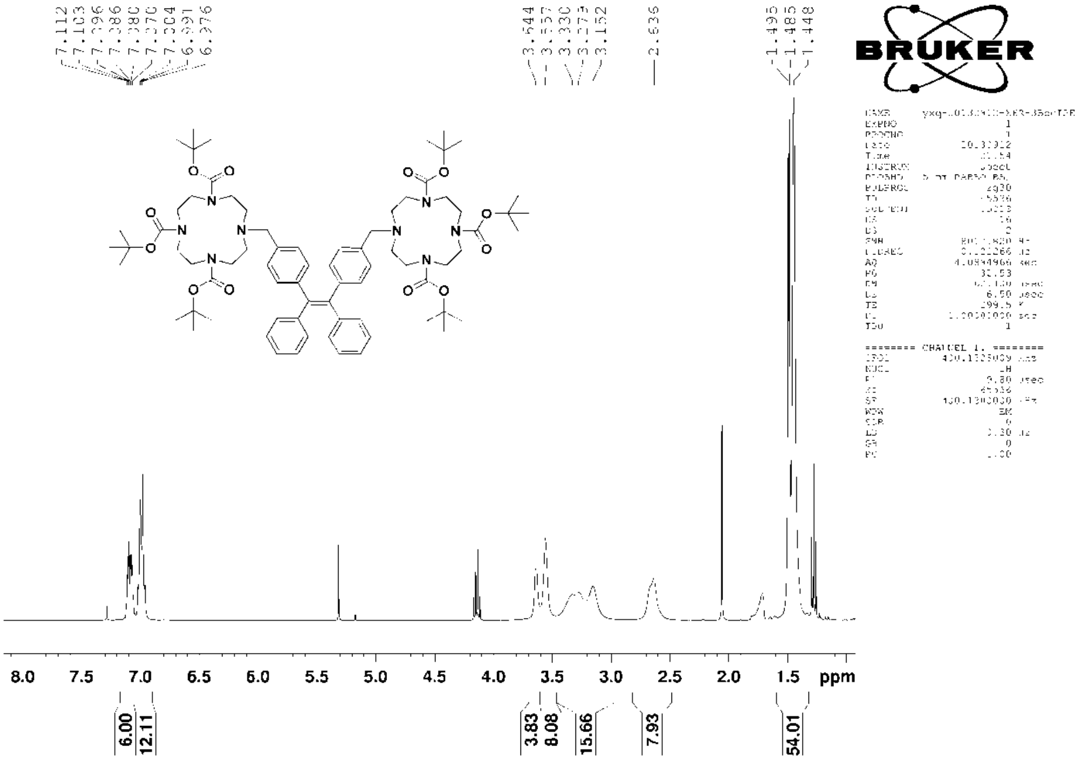


Figure S10. ^{1}H NMR (400MHz) spectra of **dCT** in DMSO-*d6* and D_2O (v/v = 2:1): (A) **dCT** + 2 equiv.Zn^{2+} +0.5 equiv. PPi; (B) **dCT** +2 equiv.Zn^{2+}; (C) **dCT** only.

Table S1. Crustallographic Data for dCT·Zn·2NO$_3$·2Cl$^-$.

Empirical formula	$C_{44}H_{54}Cl_2N_{10}O_6Zn_2$
Formula weight	1020.61
Temperature/K	145.2(3)
Crystal system	triclinic
Space group	P-1
a/Å	13.7485(11)
b/Å	14.2439(12)
c/Å	16.1369(12)
α/°	96.785(7)
β/°	111.858(7)
γ/°	102.580(7)
Volume/Å^3	2792.4(4)
Z	2
ρ_{calc}mg/mm^3	1.214
m/mm^{-1}	1.003
F(000)	1060.0
Crystal size/mm^3	0.35 × 0.18 × 0.12
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection	5.724 to 52.744°
Index ranges	$-15 \leqslant h \leqslant 17$, $-17 \leqslant k \leqslant 16$, $-18 \leqslant l \leqslant 20$
Reflections collected	23678
Independent reflections	11355 [R_{int} = 0.0561, R_{sigma} = 0.1103]
Data/restraints/parameters	11355/1/541
Goodness-of-fit on F^2	1.074
Final R indexes [I>=2σ (I)]	R_1 = 0.1131, wR_2 = 0.3085
Final R indexes [all data]	R_1 = 0.1470, wR_2 = 0.3414
Largest diff. peak/hole / e Å^{-3}	2.93/-1.54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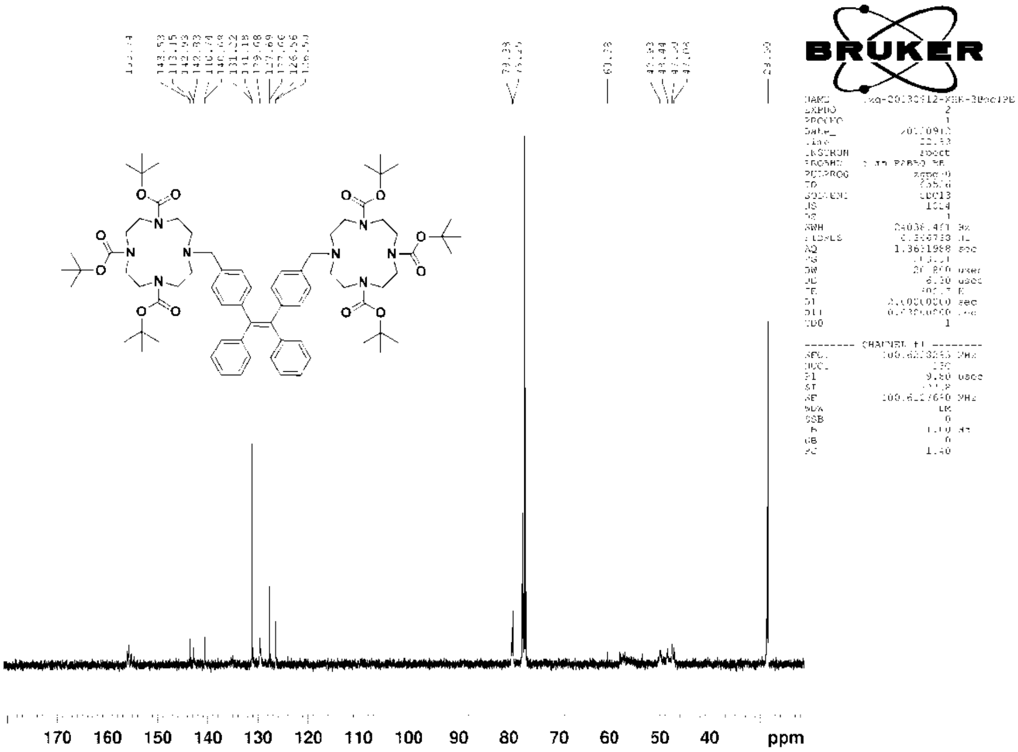
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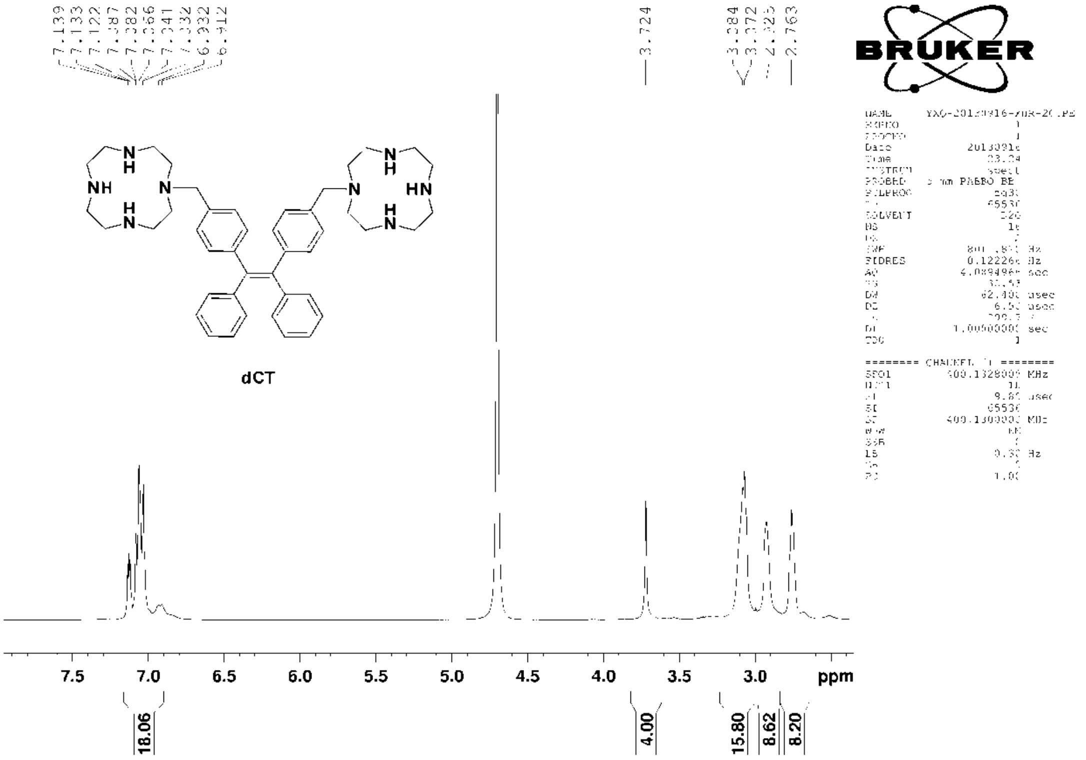
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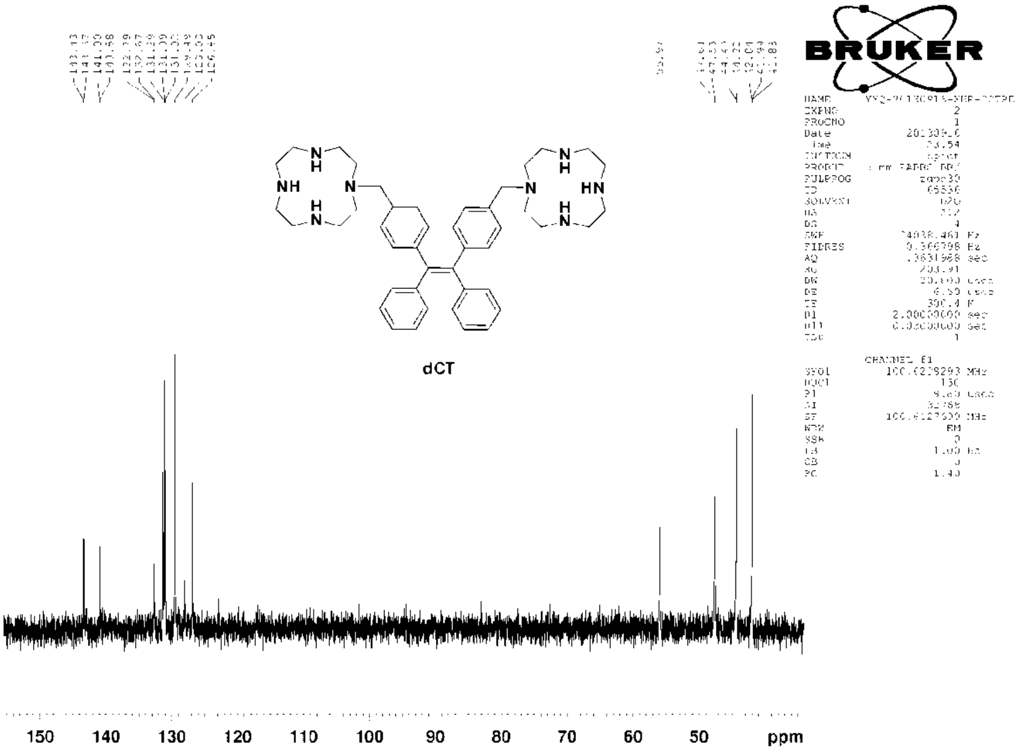
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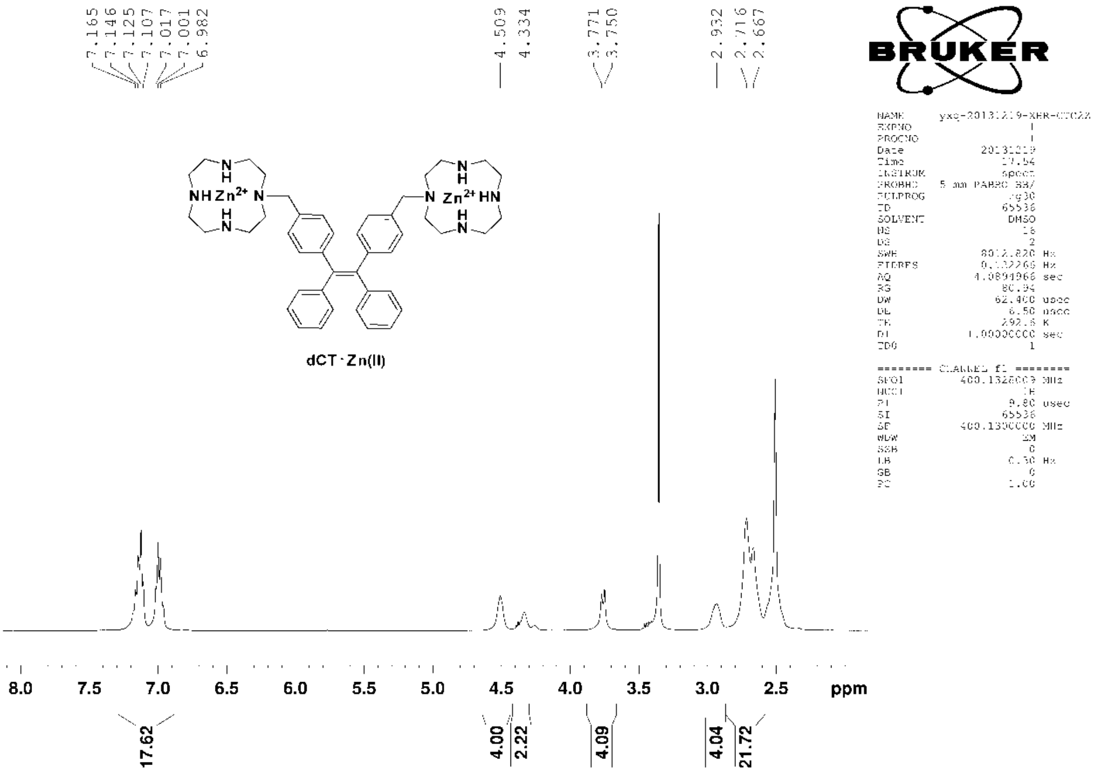
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Date 20131219
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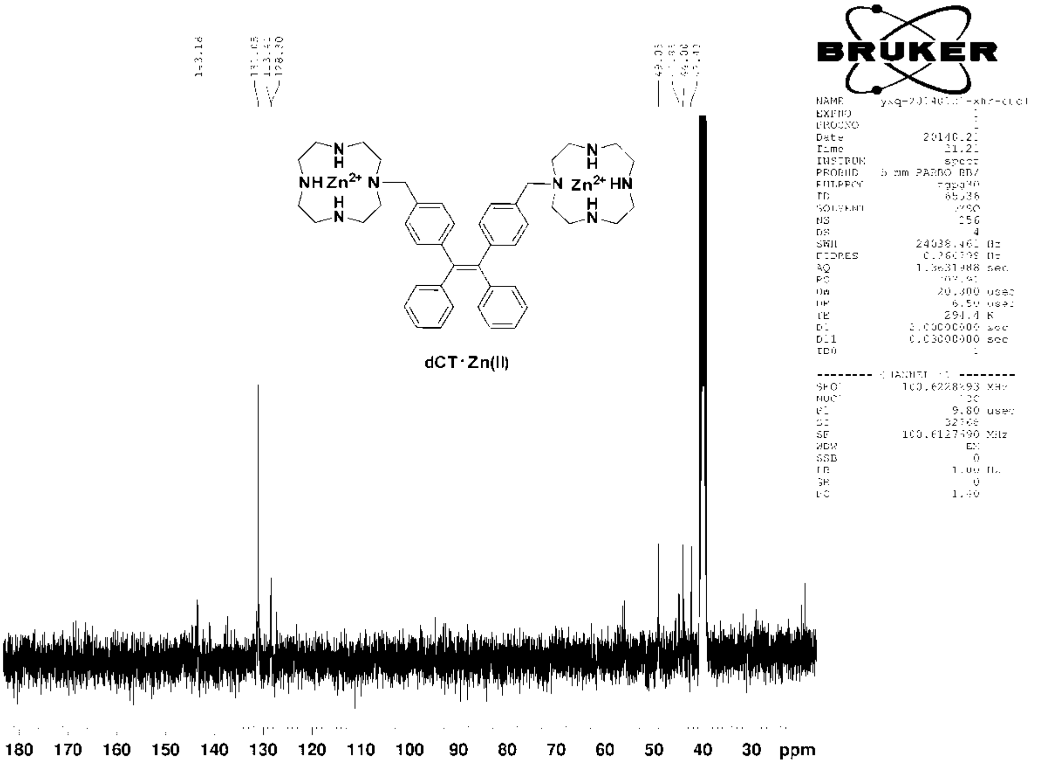
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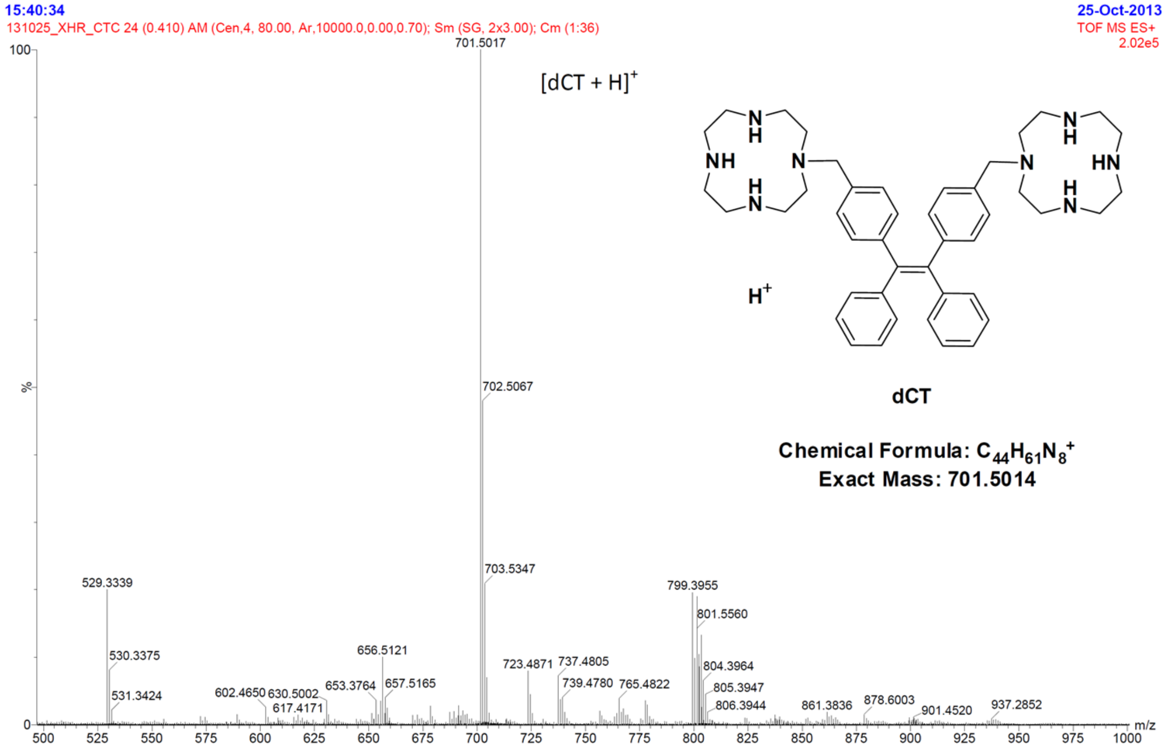
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25-Oct-2013
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800
825
850
875
900
925
950
975
1000
m/z
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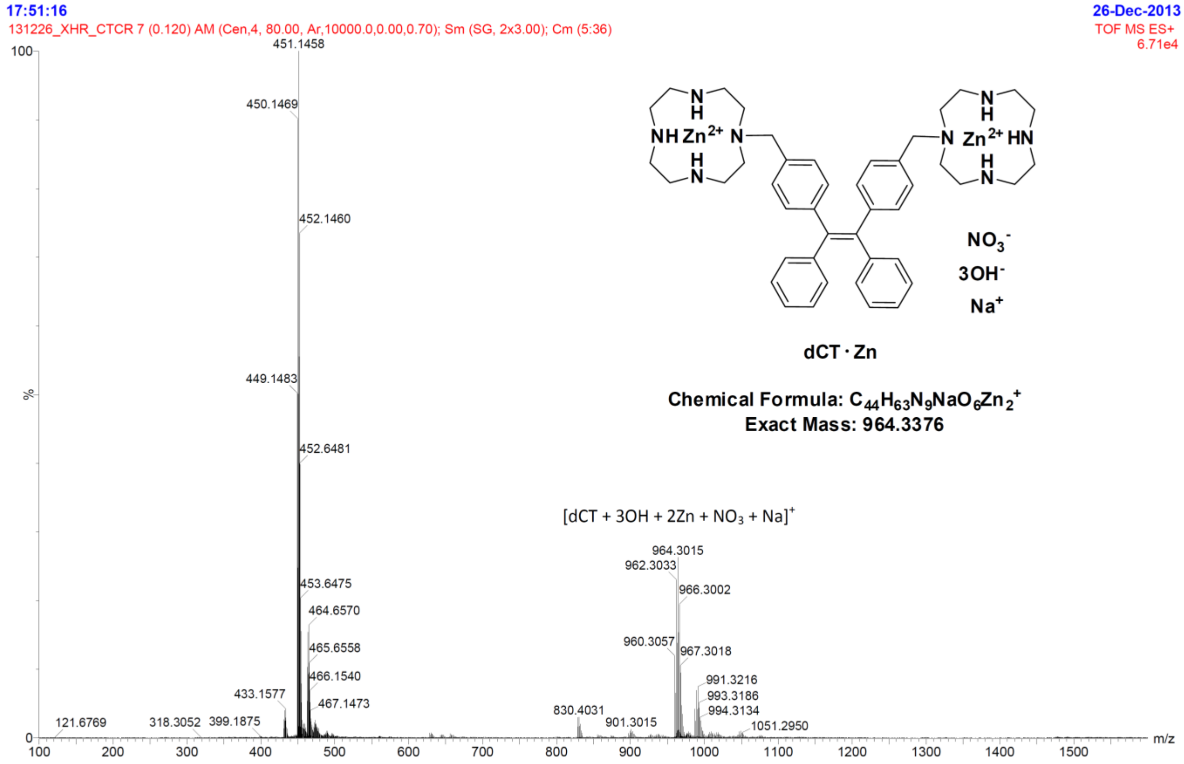
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26-Dec-2013
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450.1469
452.1460
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962.3033
966.3002
960.3057
967.3018
991.3216
993.3186
994.3134
1051.2950
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
m/z
[dCT + 3OH + 2Zn + NO_3 + Na]$^+$
NH
H
N
Zn^{2+}
HN
NO_3^-
$3OH^-$
Na^+
dCT · Zn
Chemical Formula: $C_{44}H_{63}N_9NaO_6Zn_2^+$
Exact Mass: 964.3376