Electronic Supplementary Material (ESI) for Chemical Communications

Fluorescent Imaging and Quantificational Detection of Free Tryptophan in Serum within Confined Metal–Organic Tetrahedron

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Contents

1. Materials and Methods.

All chemicals were of reagent grade quality obtained from commercial sources and used without further purification.

^{1}H NMR spectra was measured on a Varian INOVA 400 M spectrometer.

ESI mass spectra were carried out on a HPLC–Q–Tof MS spectrometer using methanol as mobile phase.

Cells were imaged by Nikon eclipase TE2000–5 inverted fluorescence microscopy. HeLa cells were cultured in 1640 supplemented with 10% FCS (Invitrogen) or DMEM culture mediumt10% FBSt1% PSt1% Glutmax. Cells were seeded on 18 mm glass coverslips.

Uv-*vis* spectra were measured on a HP 8453 spectrometer.

The CD spectra were measured on JASCO J–810. Stock solutions of MTrp was made up in HPLC grade DMF and allowed to equilibrate overnight prior to use. A solution of Ce–**TTS** 30 μM in pure DMF was placed in a CD cell (300 μL). After mixing by shaking, and the CD spectrum was recorded on a JASCO J–810 spectropolarimeter at 298 K.

Isothermal Titration Microcalorimetry (ITC) experiments were performed on a MicroCal iTC_{200}. ITC experiments were performed at 298 K. Solutions of MTrp in DMF were prepared, allowed to equilibrate overnight, then injected stepwise (3 μL aliquots) into the sample cell containing a solution of the receptor Ce-**TTS** in DMF (0.1 mM).

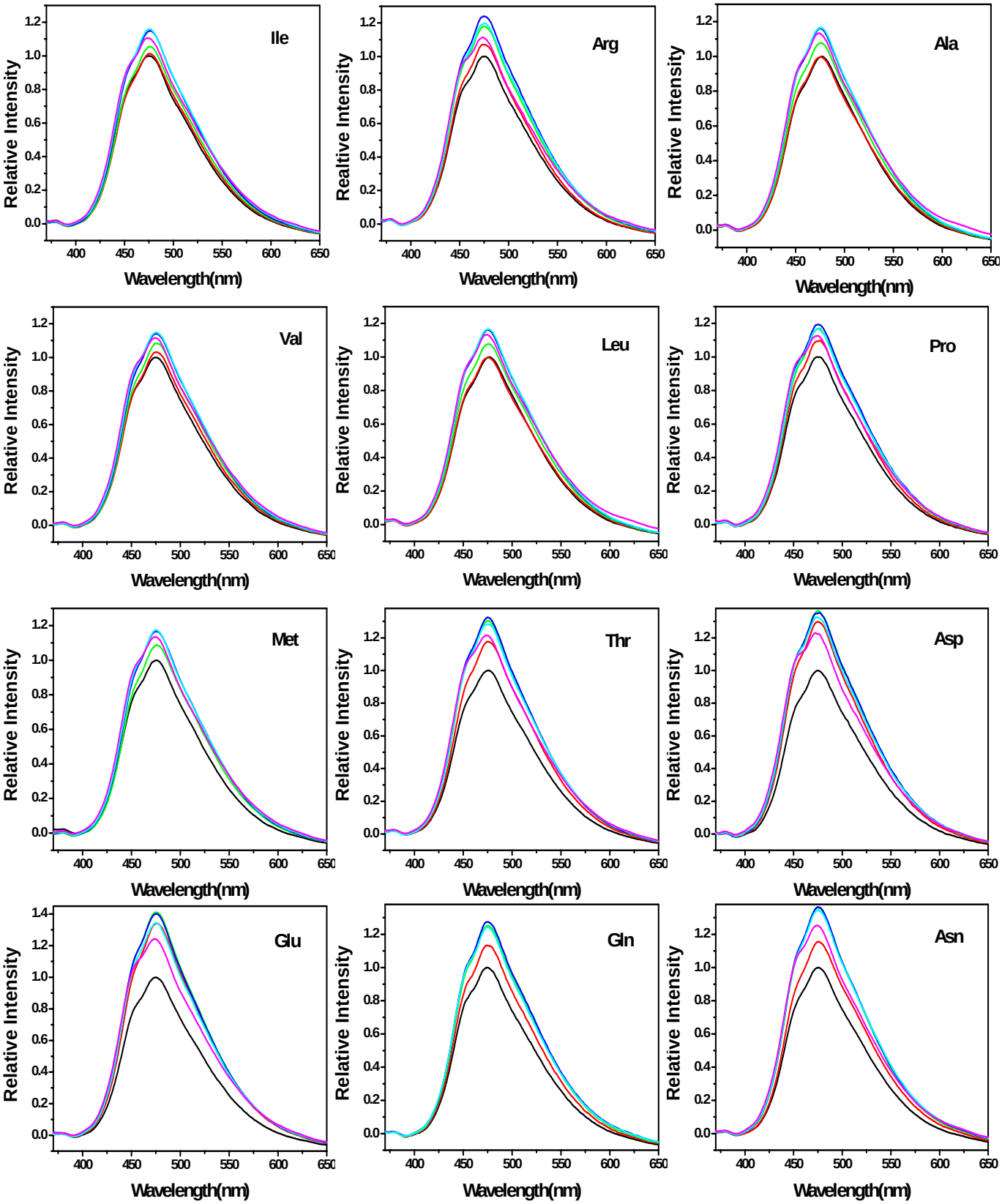
The solution fluorescent spectra were measured on JASCO FP–6500. Both excitation and emission slit widths were 2 nm. The solution of Ce–**TTS**, was prepared in DMF. And the high concentration stock solutions of amino acids (2.0×10^{-2} M) were prepared directly in DMF/H_2O=1/1 (v/v) solvents, the intensity was recorded at 470 nm, excitation at 350 nm.

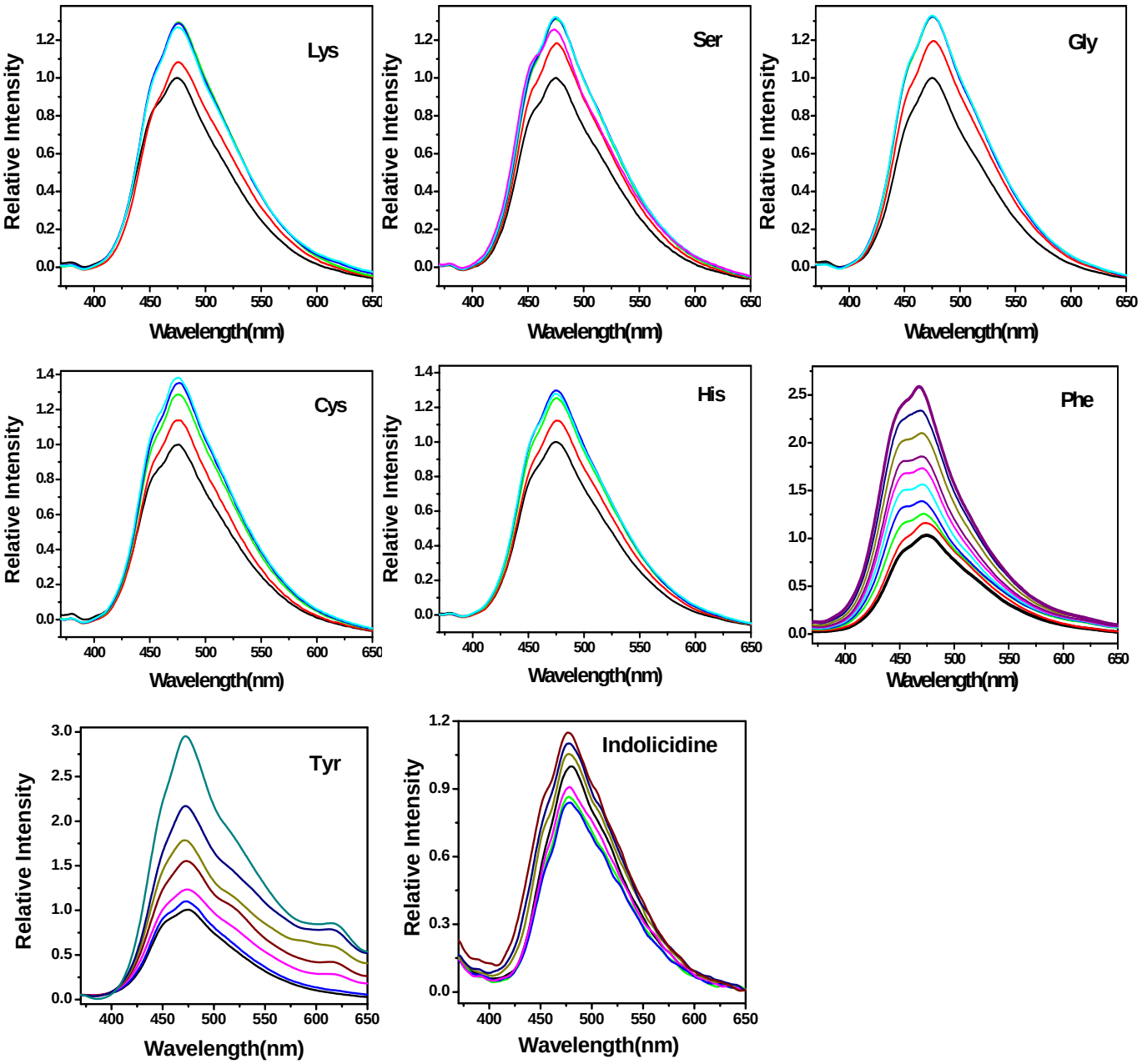
Cells in 48-well plates (Costar, Corning, USA) were exposed at 37 °C to increase concentrations of complex in complete culture medium for 48 h (5 mM stock solution in DMSO). After washing with phosphate-buffered saline (PBS), the supernatants were replaced with fresh medium and cell survival was measured using the 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazolium bromide (MTT) test. MTT (Merck) was added at 250 μg/mL and incubation was continued for 2 h. Then the cell culture supernatants were removed, the cell layer was dissolved in *iso*-propanol/0.04 N HCl, and the absorbance at 540 nm was measured in a 96-well multiwell-plate reader (iEMS Reader MF, Labsystems, Bioconcept, Switzerland) and compared with the values of control cells incubated without complexes. Experiments were conducted in triplicate wells and repeated for three times.[S1,S2]

General HPLC detection for Trp in serums[S3]: Taking one part of serum in a plastic tube, adding an equal volume of 5.0 % perchloric acid solution, the suspension was then vigorously shaken and then centrifuged at 10000 rpm for 5 min. The dissociative Trp was extracted through the above classical sample treatment to remove the potential peptides containing Trp resides, and taking the supernatant liquid for test.

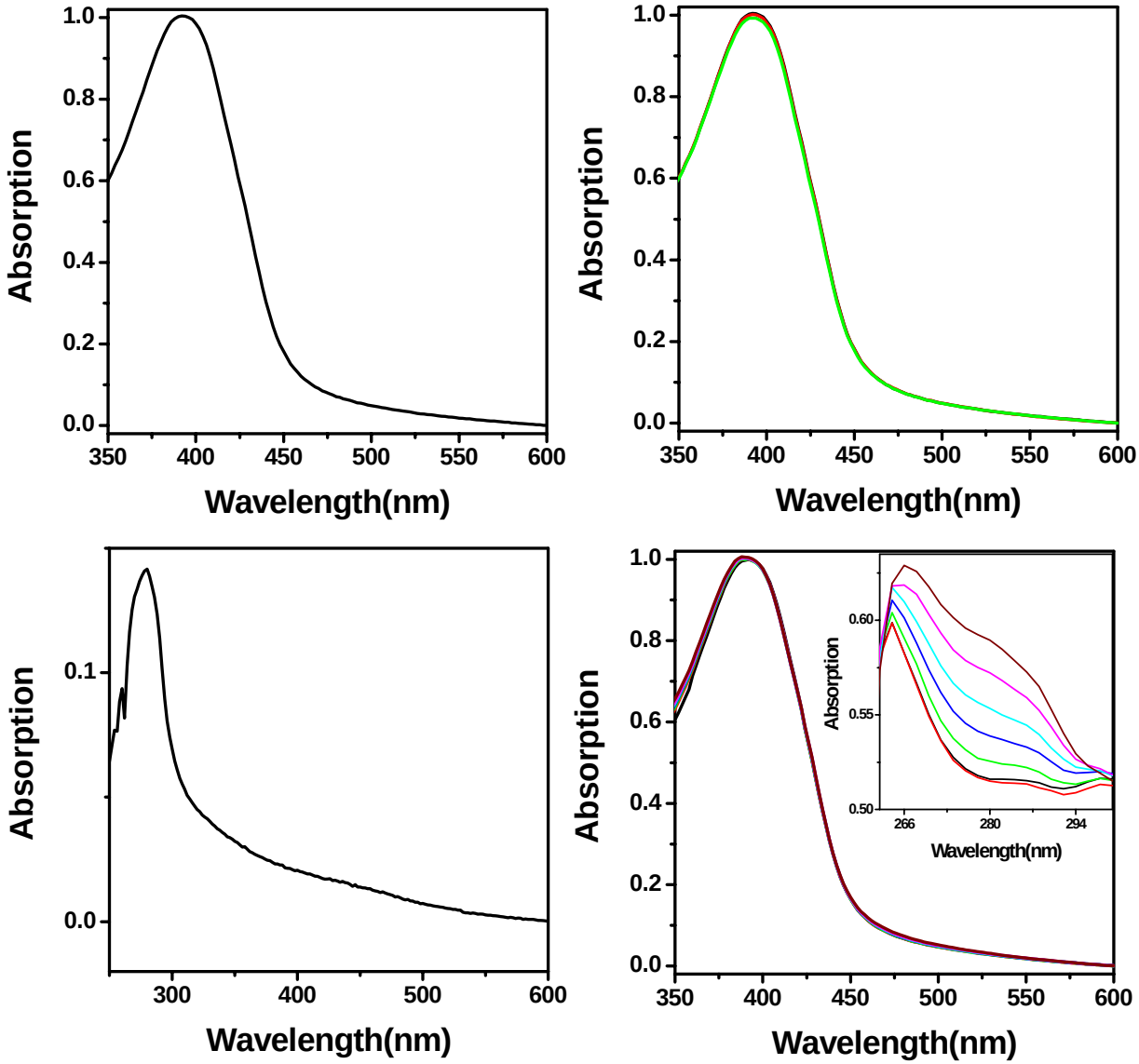
Fluorescent detection for Trp in serums by Ce-TTS: In our developed emission method, the measurements were simple displayed upon the addition of 20 μL of serum sample directly to the solution of Ce-**TTS** (15 μM) in DMF/H_2O, excitation at 350 nm.

2. Figure S1 Families of various fluorescence spectra of Ce–**TTS** (15 μM) upon the addition of 15.0 mM of different amino acids and 80.0 mM of Indolicidin (IL).



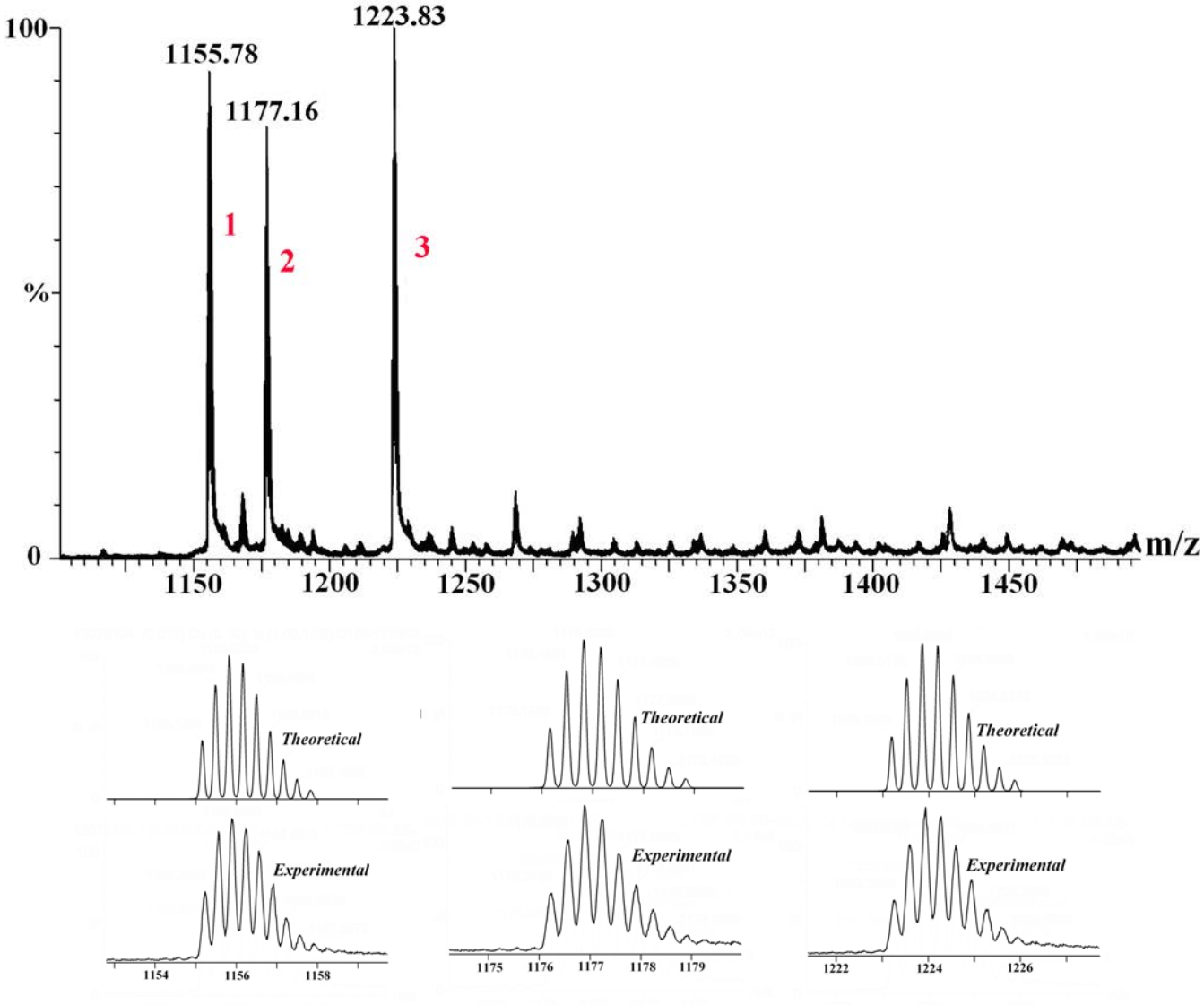


3. Figure S2 Top: Families of UV-vis spectra of Ce–**TTS** (5 μM, left) upon the addition of 7.5 mM of Trp (right). Bottom: Families of UV-vis spectra of serum (20 μL in 2 ml DMF right) and the UV-vis spectra of Ce–**TTS** (5 μM) upon the addition of 20 μL of serum in 2 ml DMF(left) .



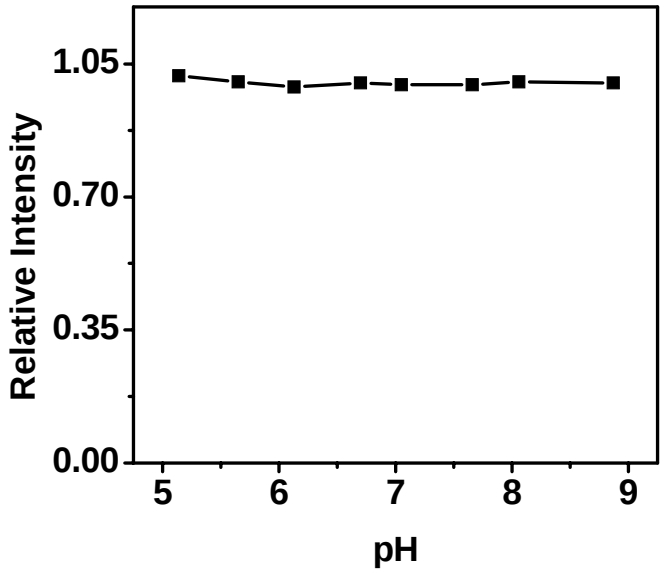
4. Host-guest properties between compound Ce–**TTS and Trp**.

4.1 Figure S3 ESI–MS spectra of Ce–**TTS** upon the addition of 2 equiv of Trp in DMF/CH_3OH in present of KOH (0.1 *m*M).

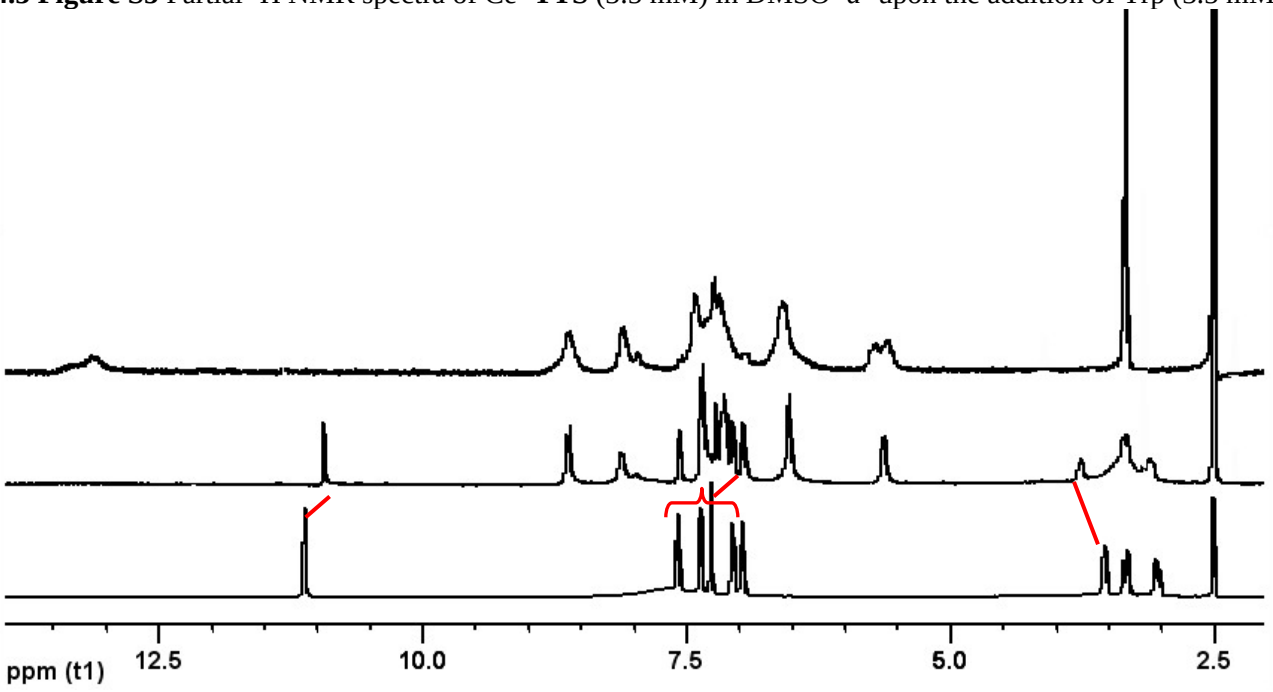


Peak number	value	Specie Assigned
1	1155.78	$[Ce_4(HTTS)_3(H_2TTS)]^{3-}$
2	1177.16	$[Ce_4(HTTS)_3(H_2TTS)\cdot 2CH_3OH]^{3-}$
3	1223.83	$[Ce_4(HTTS)_3(H_2TTS)\supset Trp]^{3-}$

4.2 Figure S4 The fluorescence intensity on 470 nm of Ce–**TTS** (1.5 μM) in DMF/H_2O=7/3 upon the addition of hydrochloric acid and NaOH.

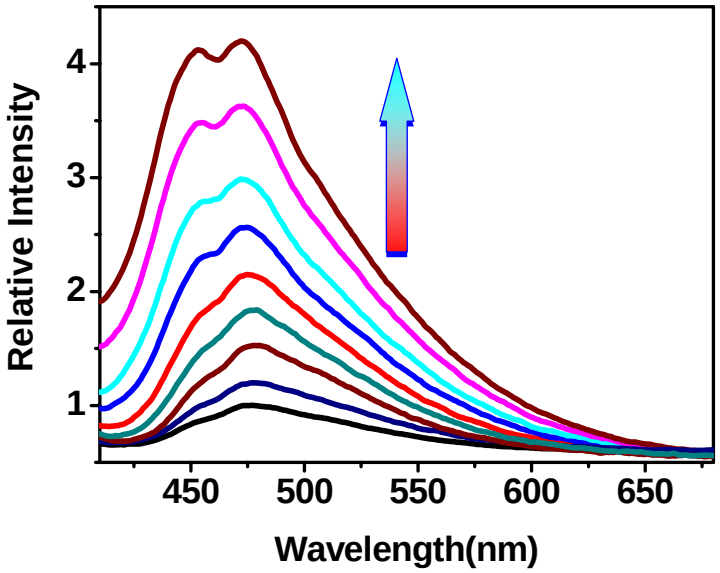


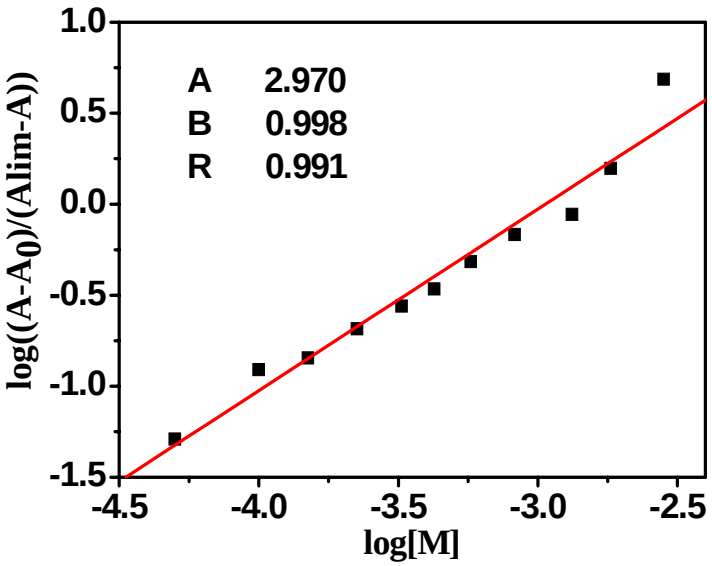
4.3 Figure S5 Partial ^{1}H NMR spectra of Ce–**TTS** (3.5 mM) in DMSO–d^6 upon the addition of Trp (3.5 mM).



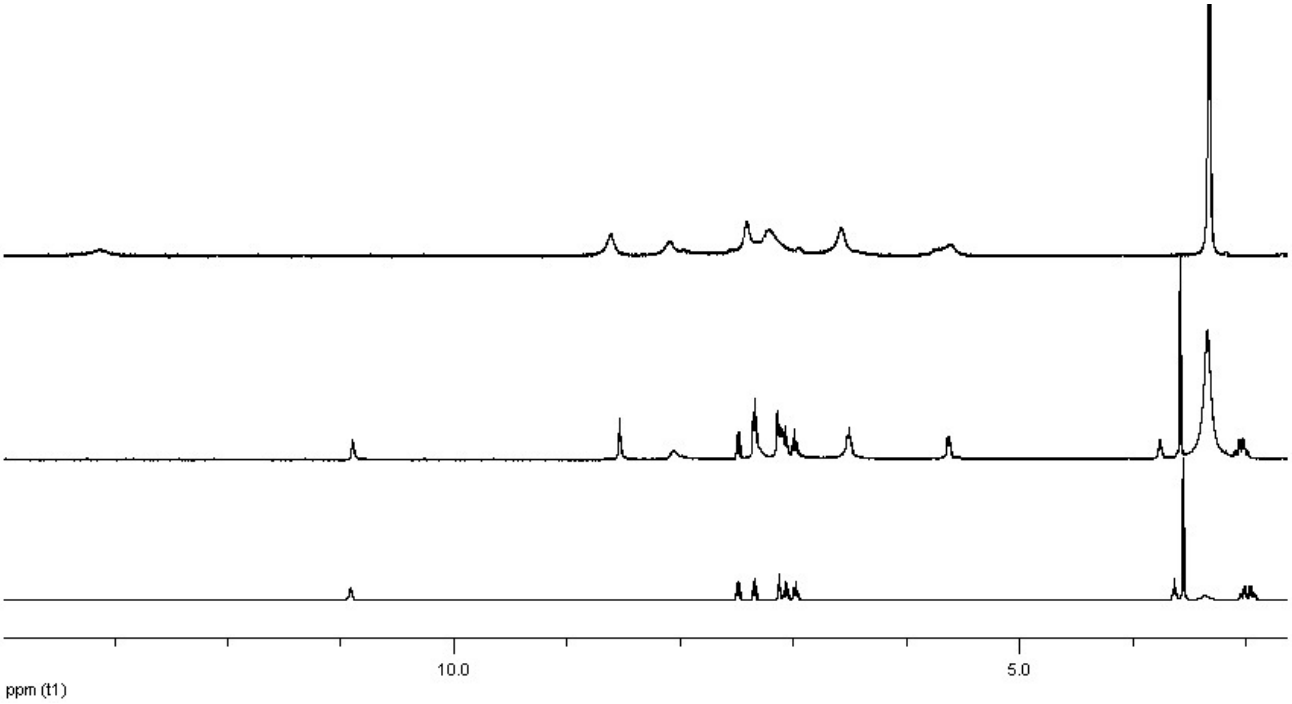
5. Host–guest properties between compound Ce–**TTS and MTrp**.

5.1 Figure S6 Top: Family of luminescence spectra of Ce–**TTS** (15 μM in DMF solution) upon the addition of MTrp up to 7.8 mM. Bottom: Luminescence intensity of the above solution recorded in 470 nm. Excitation at 350 nm.

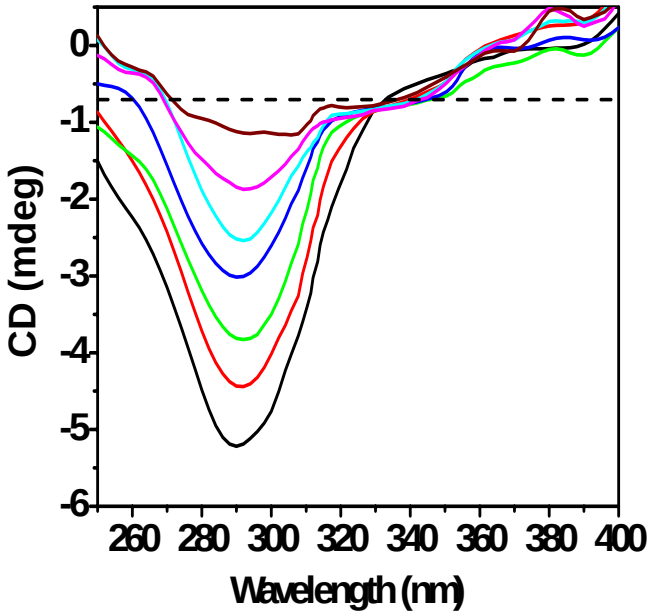




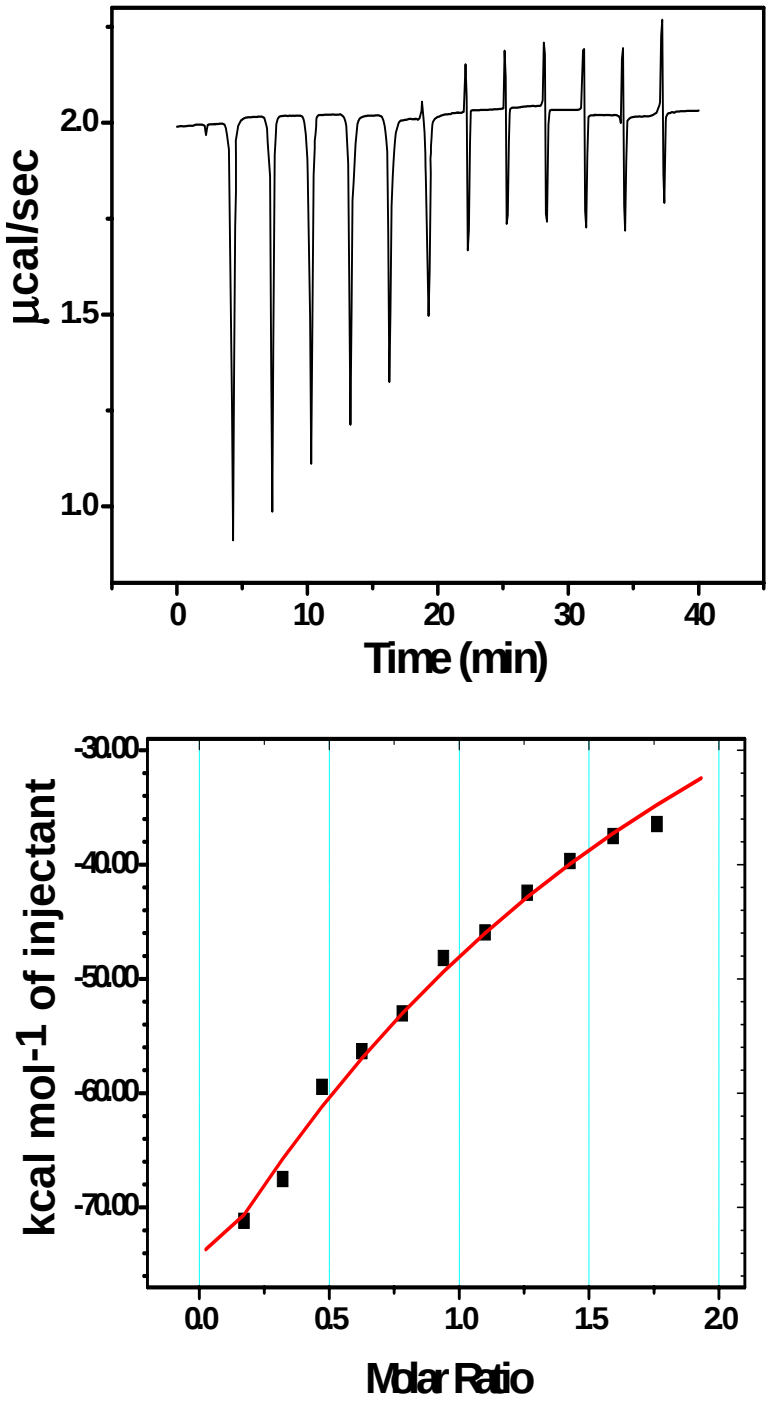
5.2 Figure S7 Partial ^{1}H NMR spectra of Ce–**TTS** (3.5 mM) in DMSO-d^{6} upon the addition of 1 eq. of MTrp.



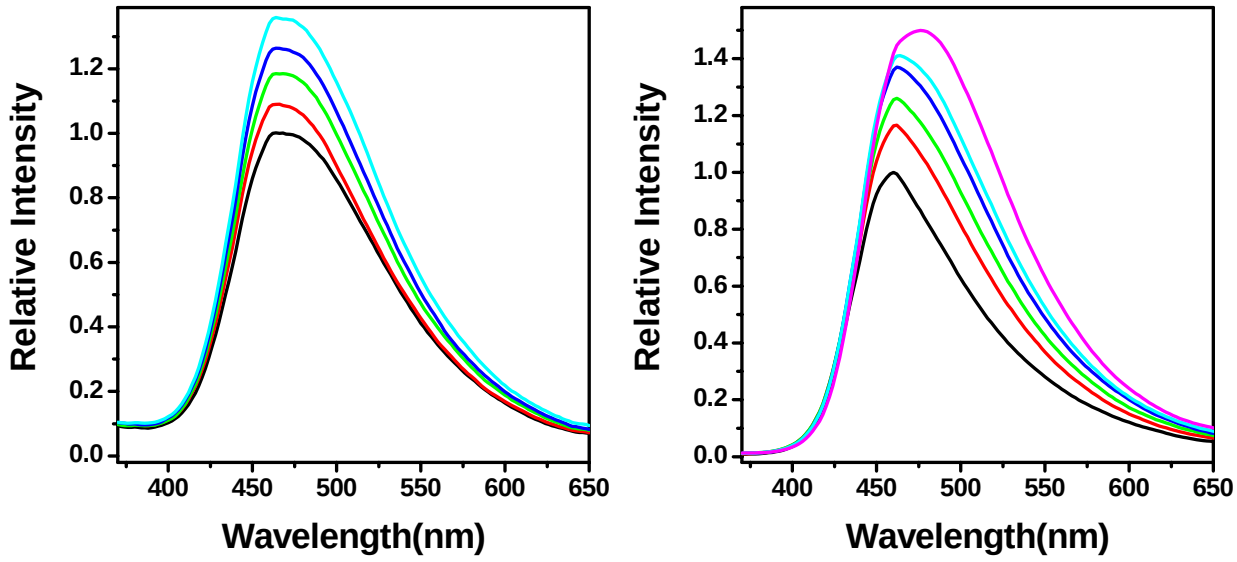
5.3 Figure S8 CD spectra of MTrp in DMF solution.



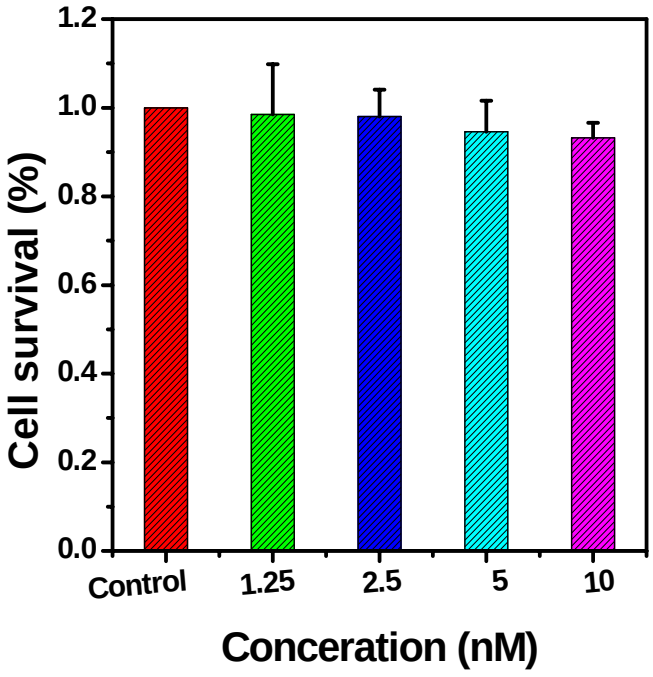
5.4 Figure S9 Isothermal microcalorimetric profile of the titration of Ce–**TTS** (0.1 mM in DMF) with MTrp (1 mM in DMF) at 25℃.



6 Figure S10 Left: Family of luminescence spectra of Ce–**TTS** (15 μM) upon the addition of calf serum; Right: Family of luminescence spectra of Ce–**TTS** (15 μM) upon the addition of man serum. Excitation at 350 nm.



7 Figure S11 Cells were exposed for 24 h to increasing concentrations of Ce–**TTS** and their survival was determined using the MTT cell survival assay.



8. Reference:

S1. F. Schmitt, J. Freudenreich, N. P. E. Barry, L. J. Jeanneret, G. S. Fink and B. Therrien, *J. Am. Chem.Soc.* 2012, **134**, 754.

S2. F. Schmitt, M. Auzias, P, Stepnicka, Y. Sei, K. Yamaguchi, G. S. Fink, B. Therrien, L. J. Jeanneret, *J. Biol. Inorg. Chem.* 2009, **14**, 693.

S3. J. H. Wen, Z. R. Geng, Y. X. Yin, Z. Zhang and Z. L. Wang, *Dalton Trans.* 2011, **40**, 1984; A. Özcan and Y. Sahin, *Biosensors and Bioelectronics* 2012, **31**, 26.