# Selective detection of the reduced form of glutathione (GSH) over the oxidized (GSSG) form using a combination of glutathione reductase and a Tb(III)-cyclen maleimide based lanthanide luminescent 'switch on' assay

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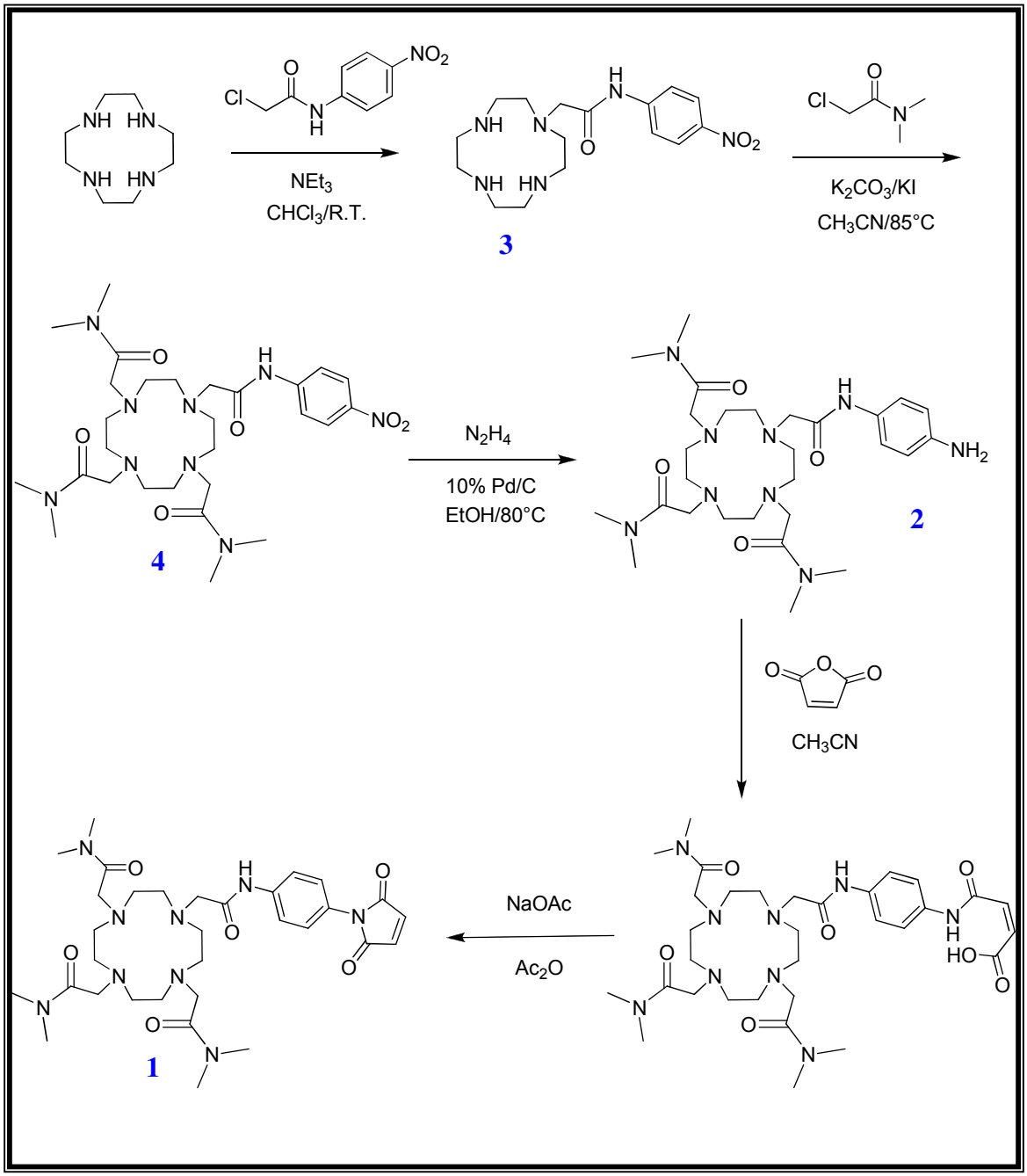
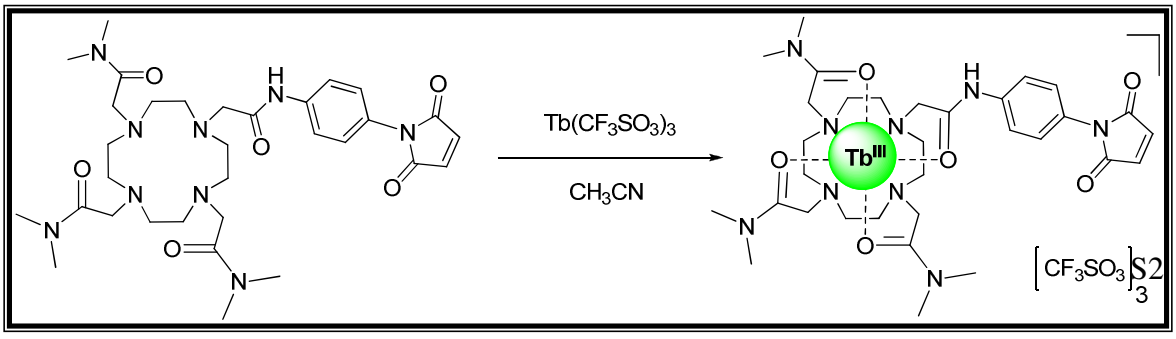
## 1. Experimental Section: Synthesis of 1 and 1.Tb

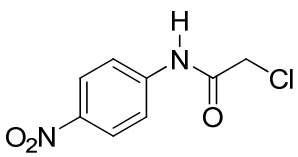


**1** **1.Tb**

**General Procedures.** 2-Chloro-*N*-(4-nitro-phenyl)-acetamide[1] and 1-{4-(nitro-phenylcarbamoyl-methyl)}-1,4,7,10-tetraazacyclododecane[2] **3** were synthesised according to reported procedures. All starting materials were obtained from Sigma-Aldrich, Strem chemicals and Fluka. High grade solvents (Methanol and Acetonitrile) were used in the synthesis of the ligand and the complexes. Infrared spectra were recorded on a Mattson Genesis II FTIR spectrometer equipped with a Gateway 2000 4DX2-66 workstation. Nuclear magnetic spectra were recorded at 400.13 MHz and 600.13 MHz using Bruker Spectrospin DPX-400 instrument and Bruker AV600 instrument respectively. Chemical shifts are expressed in parts per million (ppm or δ) downfield from the standard, followed by the number of protons, splitting pattern, coupling constant (if applicable) and assignment of proton. Tetramethylsilane (TMS) was used as the internal reference standard.$^{13}$C NMR spectra were recorded at 100.61 MHz and 150.9 MHz using a Bruker Spectrospin DPX-400 instrument and Bruker AV600 instrument respectively. Electro mass spectra was recorded on a Mass Lynx NT V 3.4 on a Waters 600 controller connected to a 996 photodiode array detector with HPLC-grade methanol as carrier solvents. Accurate molecular weights were determined by a peak-matching method, using leucine enkephaline (H-Tyr-Gly-Gly-Phe-Leu-OH) as the standard reference (m/z = 556.2771); all accurate mass were reported within ±5 ppm of the expected mass. UV-visible absorption spectra were recorded by means of a Varian CARY 50 spectrophotometer. The solvents used were of HPLC grade. The wavelength range was 220 nm to 450 nm with a scan rate of 600 nm min$^{-1}$. Both luminescence and lifetime measurements were carried out on a Varian Carey Eclipse Fluorimeter equipped with a 1.0 cm path length quartz cell.

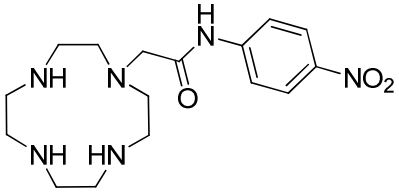
**2-Chloro-*N*-(4-nitro-phenyl)-acetamide[1]**




A solution of 4-nitro-phenylamine (0.900 g, 6.51 mmol) and $Et_3N$ (1.98 g, 19.53 mmol) in freshly distilled $CH_2Cl_2$ (50 mL) was cooled below 0°C in an acetone/ice bath. A solution of chloroacetyl chloride (0.750 g, 6.51 mmol) in $CH_2Cl_2$ (20 mL) was added dropwise over a 30 min period. After complete addition, the reaction mixture was allowed to reach room temp. and

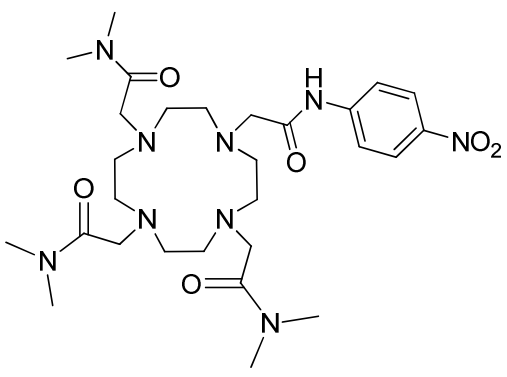
stirred under argon for 48 h. The resulting brown solution was washed with 0.1 M HCl (4 x 50 mL) and $H_2O$ (2 x 25 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to yield a brown solid (0. 85 g, 61% yield). Recrystallisation from EtOH yielded brown crystals (0.54g, 39%). m.p. 184-185°C; HRMS (m/z, $ES^-$) Calculated for $C_8H_6N_2O_3Cl$ m/z = 213.0067 [M - H]. Found m/z = 213.0063; $^1H$ NMR (400 MHz, $d_6$-$(CD_3)_2CO$, $\delta_H$) 8.53 (bs, 1H, N-H), 8.28 (d, 2H, $J$ = 9 Hz, Ar-H), 7.80 (d, 2H, $J$ = 9 Hz, Ar-H), 4.27 (s, 1H, $CH_2$); $^{13}C$ NMR (100 MHz, $d_6$-$(CD_3)_2CO$, $\delta_c$): 165. 61, 144.59, 142.59, 125.09, 119.08, 43.60; IR $\upsilon_{max}$ ($cm^{-1}$): 3228, 3163, 3105, 2941, 1685, 1622, 1597, 1567, 1503, 1405, 1334, 1255, 1198, 1172, 1112, 968, 923, 871, 850, 772, 748, 718.

**1-{4-(nitro-phenylcarbamoyl-methyl)}-1,4,7,10-tetraazacyclododecane (3)[2]**



**3** was synthesised according to the reported literature.[2] The product was isolated as an orange viscous oil (0.34 g, 69% yield). HRMS (m/z, $ES^+$) Calculated for $C_{16}H_{26}N_6O_3Na$ m/z = 373.1964 [M + Na]. Found m/z = 373.1962; $^1H$ NMR (400 MHz, $CDCl_3$, $\delta_H$): 10.80 (bs, 1H, N-H) 8.21 (d, 2H, $J$ = 7.5 Hz, Ar-H), 7.97 (d, 2H, $J$ = 7 Hz, Ar-H), 3.35 (s, 1H, 1-$CH_2$CONH), 2.87 (bs, 4H, cyclen-$CH_2$), 2.77 (bs, 12H, cyclen-$CH_2$), 2.21 (bs, 3H, N-H); $^{13}C$ NMR (100 MHz, $CDCl_3$, $\delta_c$): 170.73, 143.92, 142.69, 124.55, 118.54, 59.10, 53.39, 46.92, 46.85, 45.38; IR $\upsilon_{max}$ ($cm^{-1}$) 3183, 2892, 2830, 1691, 1597, 1532, 1504, 1452, 1408, 1329, 1300, 1257, 1215, 1172, 1109, 1074, 941, 869, 851, 799, 750, 707.

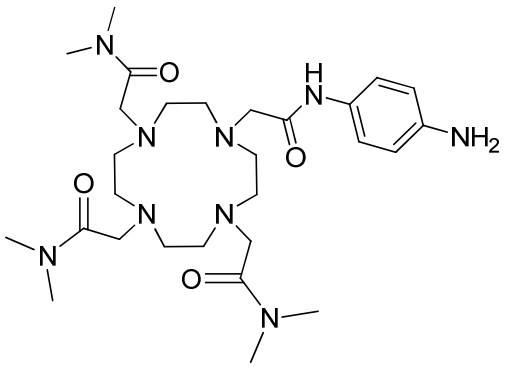
**1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-nitro-phenylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl}-acetamide. (4)**



**3** (0.21 g 0.61 mmol) along with 40 mL of $CH_3CN$ was placed in a 100 mL RBF. KI (0.36g 2.14 mmol) and $K_2CO_3$ (0.30 g 2.14 mmol) were added to the solution, which was then placed in an ice/acetone bath and allowed to cool. A solution of 2 chloro-N,N-dimethyl-acetamide (0.23 g 1.88 mmol) was added quickly and

the solution was stirred at room temp. for 15 mins. The reaction mixture was left stir at 85°C under argon for 5 days. The resulting orange solution was filtered through celite and the solvent removed under reduced pressure. The orange residue was dissolved in $CH_2Cl_2$ and purified by alumina column chromatography using a gradient elution 100 to 80:20 $CH_2Cl_2$:$CH_3OH$. The desired product, after drying under vacuum, was obtained as an orange crystalline solid (0.34 g, 92% yield). m.p. 89-91°C; HRMS (m/z, $ES^+$) Calculated for $C_{28}H_{47}N_9O_6Na$ m/z = 628.3547 [M + Na]. Found m/z = 628.3521; $^1$H NMR (400 MHz, $CDCl_3$, $\delta_H$): 10.56 (bs, 1H, N-H), 8.22 (d, 2H, *J* = 9 Hz, Ar-H), 8.14 (d, 2H, *J* = 9 Hz, Ar-H), 4.0 - 1.8 (bm, 42H, cyclen-$CH_2$ + 4,7,10-$CH_2CON(CH_3)_2$ + 1-$CH_2CONH$); $^{13}$C NMR (100 MHz, $CDCl_3$, $\delta_c$): 171.36, 169.81, 169.73, 144.60, 142.33, 123.94, 119.08, 58.11, 55.22, 55.06, 53.13, 51.77, 36.08, 36.04, 35.23, 35.15; IR $\upsilon_{max}$ ($cm^{-1}$): 3430, 2813, 1695, 1639, 1594, 1551, 1504, 1449, 1401, 1329, 1300, 1257, 1173, 1099, 1063, 1006, 918, 856, 826, 771, 753, 724.

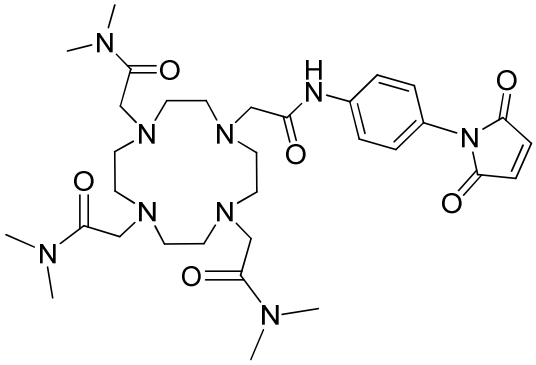
**1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-amino-phenylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl}-acetamide.(2)**



To a stirring solution of **3** (0.33g, 0.55 mmol) and 10% Pd/C catalyst in EtOH (5 mL), a solution of hydrazine monohydrate (0.22 g, 4.40 mmol) was added dropwise. The resulting reaction mixture was microwave irradiated at 80°C for 2 h. Upon reaction completion, the mixture was filtered through celite and the solvent removed under vacuum to yield the product as a brown oil. The brown residue was purified by alumina column chromatography using a gradient elution 100 to 80:20 $CH_2Cl_2$:$CH_3OH$, resulting in the desired product being obtained as an orange viscous oil (0.21 g, 66%), which solidified upon drying under vacuum. HRMS (m/z, $ES^+$) Calculated for $C_{28}H_{50}N_9O_4$ m/z = 576.3986 [M + H]. Found m/z = 576.3965; $^1$H NMR (400 MHz, $CDCl_3$, $\delta_H$): 9.60 (bs, 1H, N-H), 7.56 (d, 2H, *J* = 8.5 Hz, Ar-H), 6.56 (d, 2H, *J* = 8.5 Hz, Ar-H), 3.7 – 2.4 (bm, 42H, cyclen-$CH_2$ + 4,7,10-$CH_2CON(CH_3)_2$ + 1-$CH_2CONH$); $^{13}$C NMR (100 MHz, $CDCl_3$, $\delta_c$): 170.57, 170.40, 169.16, 142.47, 129.71, 120.6854, 114.49, 57.99, 54.60,

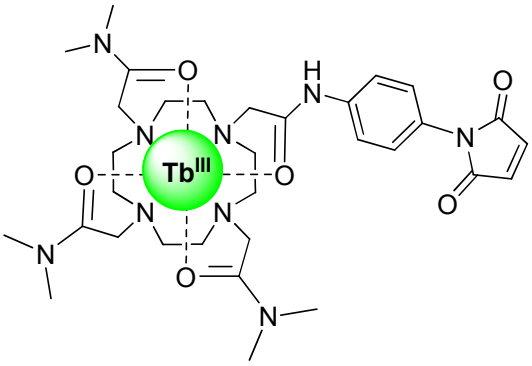
54.40, 50.43, 35.97, 35.81, 35.27, 35.16; IR $\upsilon_{max}$ ($cm^{-1}$): 3236, 2820, 1637, 1514, 1451, 1401, 1345,1296, 1261, 1158, 1101, 1062, 1005, 951, 902, 828, 725.

**2,2',2''-(10-(2-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenylamino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)tris(N,N-dimethylacetamide) (1)**



Maleic anhydride (0.012 g, 0.12 mmol) was added and stirred in 10 mL freshly distilled $CH_3CN$. Once all the maleic anhydride had dissolved, **2** (0.067 g, 0.12 mmol) was added slowly along with 5 mL $CH_3CN$ to the solution using a pressure equalised dropping funnel. The resulting yellow solution was left reflux at 85°C for 24 h. Upon reaction completion, the solvent was reduced to 80 % of its volume and any excess maleic anhydride removed by precipitation out of dry diethyl ether (50 mL) to give an orange viscous oil. The crude product (0.060 g, 0.09 mmol) was re-dissolved in 5 mL acetic anhydride and NaOAc (0.016 g, 0.19 mmol) was added. The reaction mixture was left stir at 100°C for 2 h, which upon cooling to room temp., was poured over a large volume of ice to yield a pale orange oil. The resulting aqueous solution was extracted with $CH_2Cl_2$ (2 x 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to yield **1**, as an orange oil (0.021 g, 27%). HRMS (m/z, $ES^+$): Calculated for $C_{32}H_{50}N_9O_6$ m/z = 656.3884 [M + H]. Found m/z = 656.3891; $^1$H NMR (600 MHz, $CDCl_3$, $\delta_H$): 10.33 (bs, 1H, N-H), 7.97 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.16 (d, 2H, *J* = 8.6 Hz, Ar-H), 6.82 (s, 2H, CH), 3.5 – 2.2 (bm, 42H, cyclen-$CH_2$ + 4,7,10-$CH_2CON(CH_3)_2$ + 1-$CH_2CONH$) $^{13}$C NMR (150 MHz, $CDCl_3$, $\delta_c$): 170.48, 170.34, 169.23, 138.76, 133.76, 125.83, 125.64, 119.80, 57.93, 54.55, 54.46, 35.89, 35.70, 35.30, 35.13; IR $\upsilon_{max}$ ($cm^{-1}$): 3010, 2818, 1713, 1639, 1514, 1456, 1397, 1346, 1315, 1256, 1148, 1102, 1005, 951, 901, 827, 800, 689.

**Complex 1.Tb**




Ligand **1** (0.018 g, 0.03 mmol) and $Tb(CF_3SO_3)_3$ (0.017 g, 0.03 mmol). were dissolved in freshly distilled $CH_3CN$ (5 mL). The resulting solution was refluxed for 15 hrs. After removal of the solvent under vacuum, the complexes were isolated by dissolving in a minimal amount of MeOH and precipitated from swirling dry diethyl ether (200 mL). The desired product was obtained as an orange solid (0.033 g, 93%). HRMS (m/z, MALDI) Calculated for $C_{34}H_{49}N_9O_{12}F_6S_2Tb$ m/z = 1112.2100 [M +2($CF_3SO_3$)]. Found m/z = 1112.2052; $^1$H NMR (400 MHz, $CDCl_3$, $\delta_H$): 250.67, 227.27, 203.42, 104.35, 101.37, 72.01, 71.45, 70.46, 49.92, 48.16, 41.84, 41.18, 39.41, 15.23, 14.02, 12.02, 7.89, 7.56, 7.32, 6.93, 5.98, 4.71, 3.26, 2.93, 2.13, 1.97, 1.36, -138.37, -110.92, -108.43, -107.07, -92.78, -90.06, -89.60, -85.75, -83.70, -68.51, -65.56, -63.51, -376.63, -366.43, -364.16, -357.81, -353.73; IR $\upsilon_{max}$ ($cm^{-1}$): 3453, 1715, 1619, 1564, 1515, 1460, 1403, 1245, 1225, 1156, 1080, 1028, 957, 910, 824, 690.

## 2. Spectrophotometric Titrations

Unless otherwise stated, all titrations were carried out in buffered water solutions (20 mM HEPES, 135 mM KCl, pH 7.4) or (0.1M TRIS, 135mM, pH 7.4). UV-visible absorption spectra were recorded by means of a Varian CARY 50 spectrophotometer. Both luminescence and lifetime measurements were carried out on a Varian Carey Eclipse Fluorescence spectrophotometer equipped with a 1.0 cm path length quartz cell. All solvents used were of HPLC grade. The settings used for each type of measurement are as stated.

**UV-Vis. Spectrophotometer settings**

| |
|---|
| **Scan:** 200-800 nm |
| **Ex. Slit:** 1 nm |
| **Em. Slit:** 1 nm |
| **Scan Rate:** 600 nm $min^{-1}$ |

**Luminescence settings: Lanthanide Luminescence**

| | |
|---|---|
| **Mode**: Phosphorescence | **Excitation:** 256 nm |
| **Total Decay:** 0.02 s | **Scan:** 450-650 nm |
| **Flash:** 1 | **Delay:** 0.1 ms |
| **Gate:** 2 ms | **PMT Voltage:** 580V |
| **Excitation slit width:** 20 nm | **Emission slit width:** 10 nm |
| **Cycle:** 100 | **Average time:** 0.1 s |
| **Data interval:** 1 nm | |

**Luminescence settings: Lifetime studies**

The technique employed to determine the hydration number (*q*) was to measure the excited state lifetimes ($^5D_0$) of the $Tb^{III}$ complex in $H_2O$ ($\tau_{H2O}$) and $D_2O$ ($\tau_{D2O}$), by indirect excitation of the $Eu^{III}$ at 282 nm. The Horrocks modified equation developed by Parker *et al.*[3] was then used to calculate the number of metal bound water molecules. Lifetimes were measured as an average of 5 measurements all agreeing to within 5% of each other. The general settings used are as stated below.

| | |
|---|---|
| **Indirect excitation:** 256 nm | **Total Decay:** 20 ms |
| **Emission: $Tb^{III}$** – 545 nm | **Delay** 0.2 ms |
| **No Cycles:** 100 | **PMT Voltage:** High |
| **Flash:** 1 | **Emission slit width:** 10 nm |
| **Gate:** 0.1 ms | |
| **Excitation slit width:** 20 nm | |

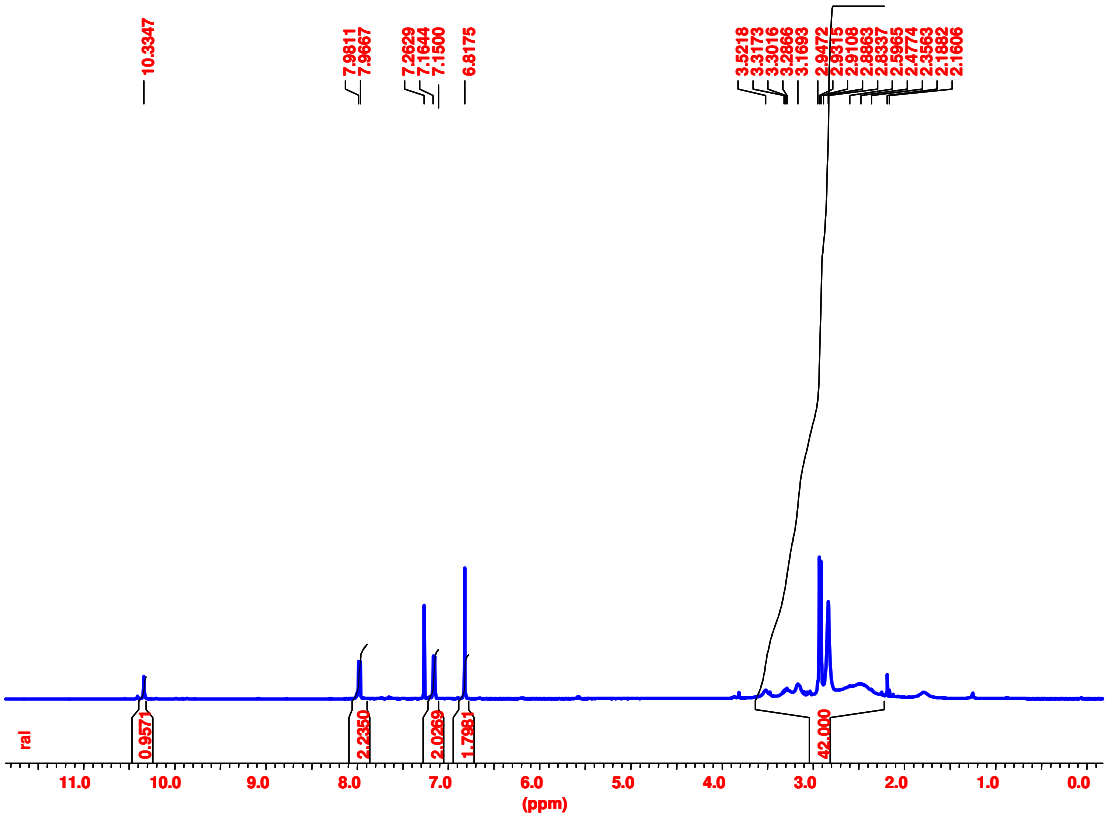
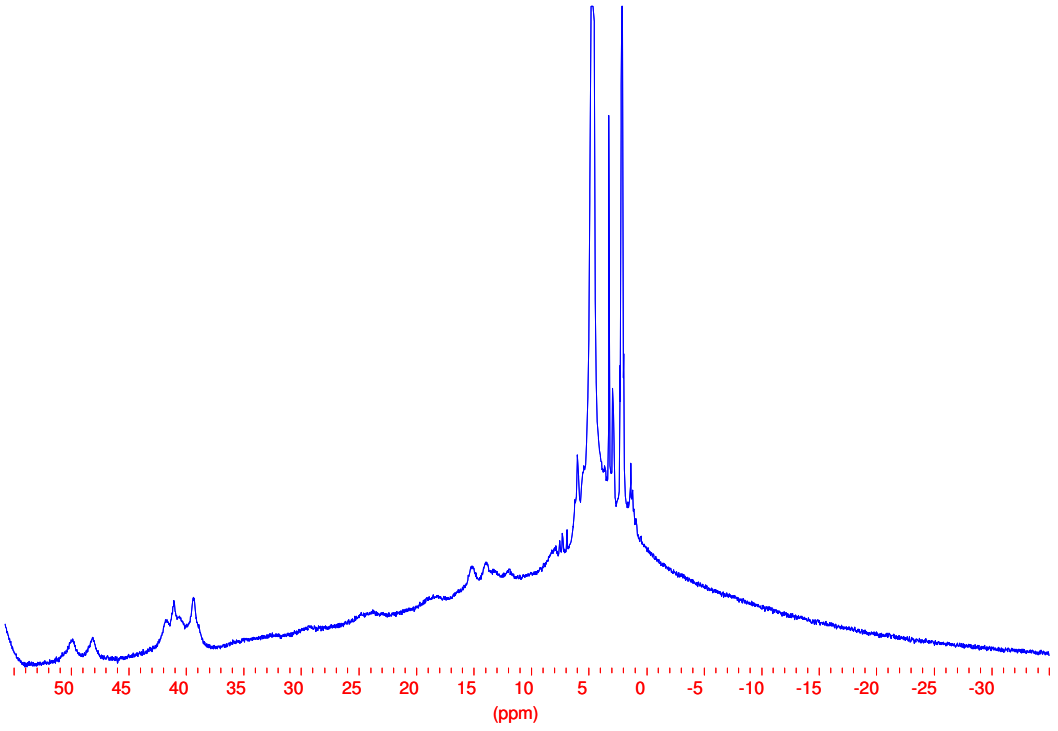



***Figure S1a***. $^{1}$H NMR ($CDCl_3$, 400 MHz) of ligand **1**.



***Fi···re S1b.*** $^{1}$H NMR ($D_2O$, 400 MHz) of complex **1.Tb**.

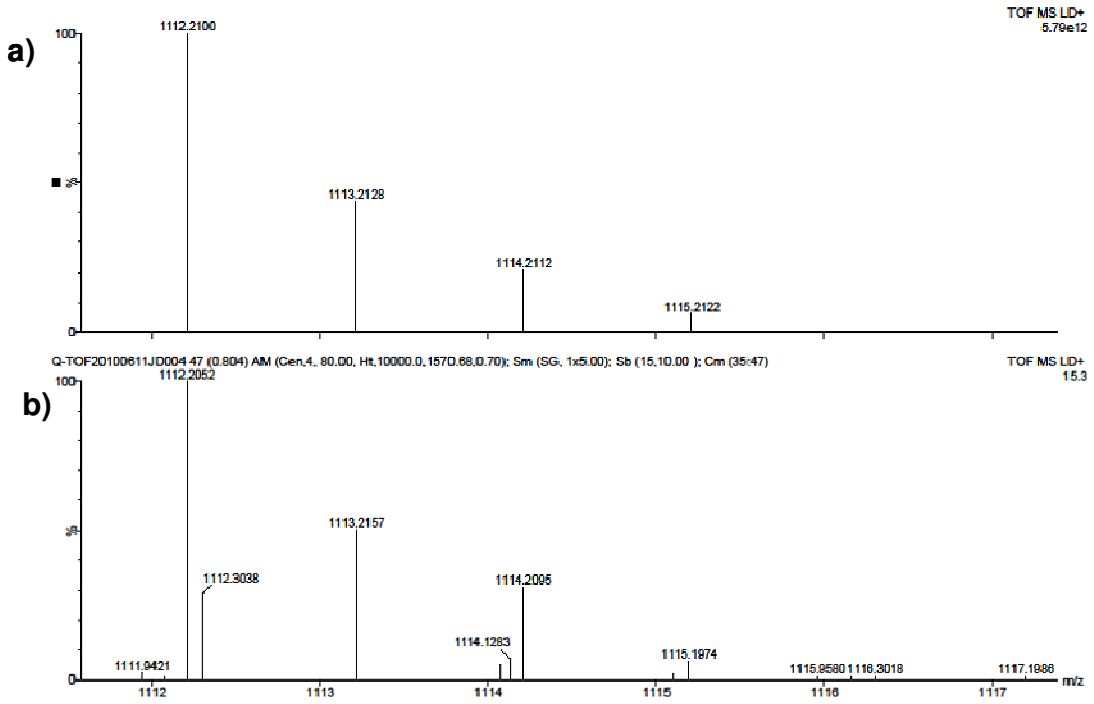



***Figure S1c:*** (b) HRMS spectrum of **1.Tb** obtained by MALDI-TOF analysis showing the $Tb^{III}$ characteristic isotopic pattern, compared with its theoretical isotopic distribution model. (a)

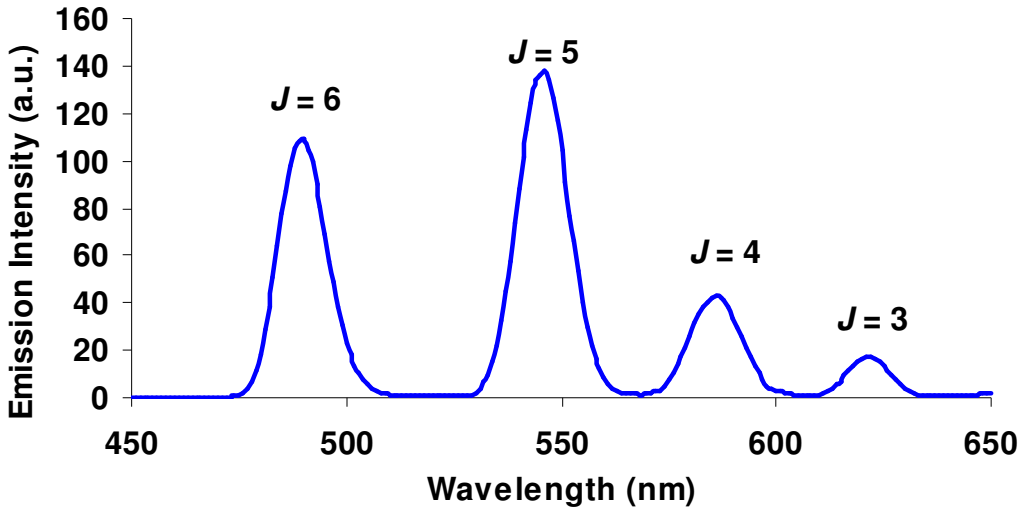


***Figure S2.*** Lanthanide luminescence spectra of **1.Tb** in $H_2O$, using an excitation wavelength of 256 nm.

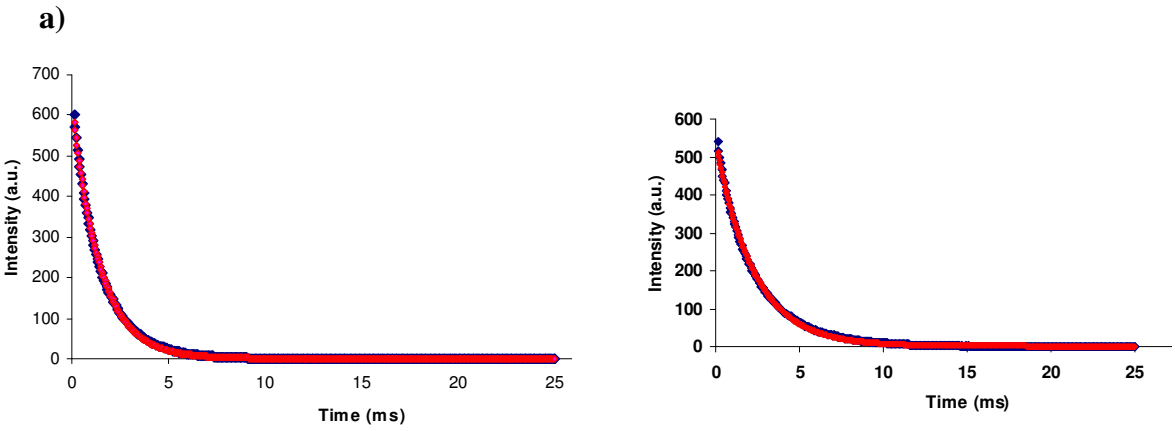



***Figure S3.*** Luminescence decay of **1.Tb** (fit to a mono exponential function) in a) $H_2O$ and b) $D_2O$

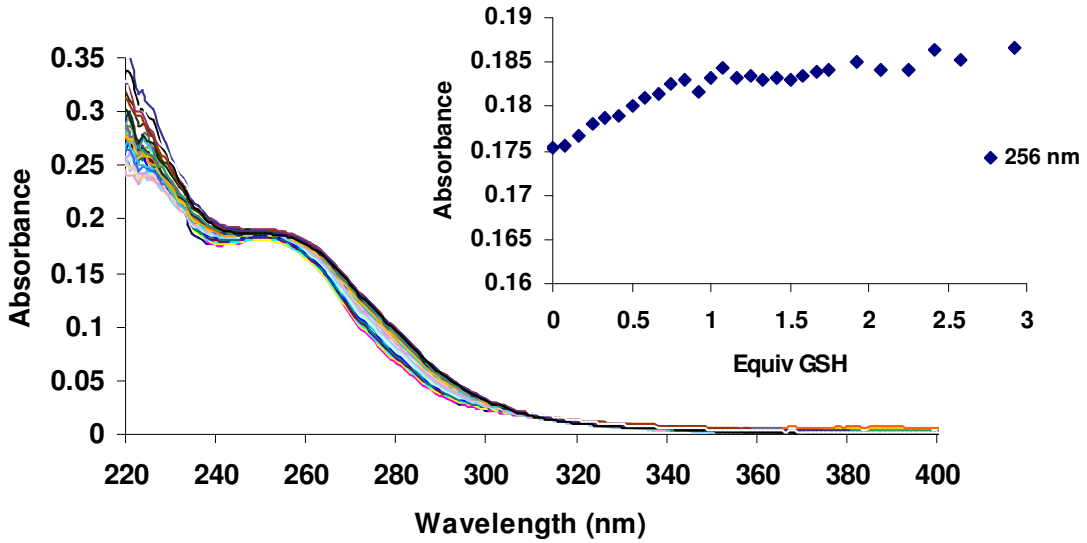


***Figure S4.*** Overall absorption spectrum of **1.Tb** (10 μM) as a function of GSH concentration [pH 7.4 aqueous solution (20mM HEPES, 135mM KCl)]. ***Insert:*** Absorbance at 256 nm as a function of GSH equiv added.

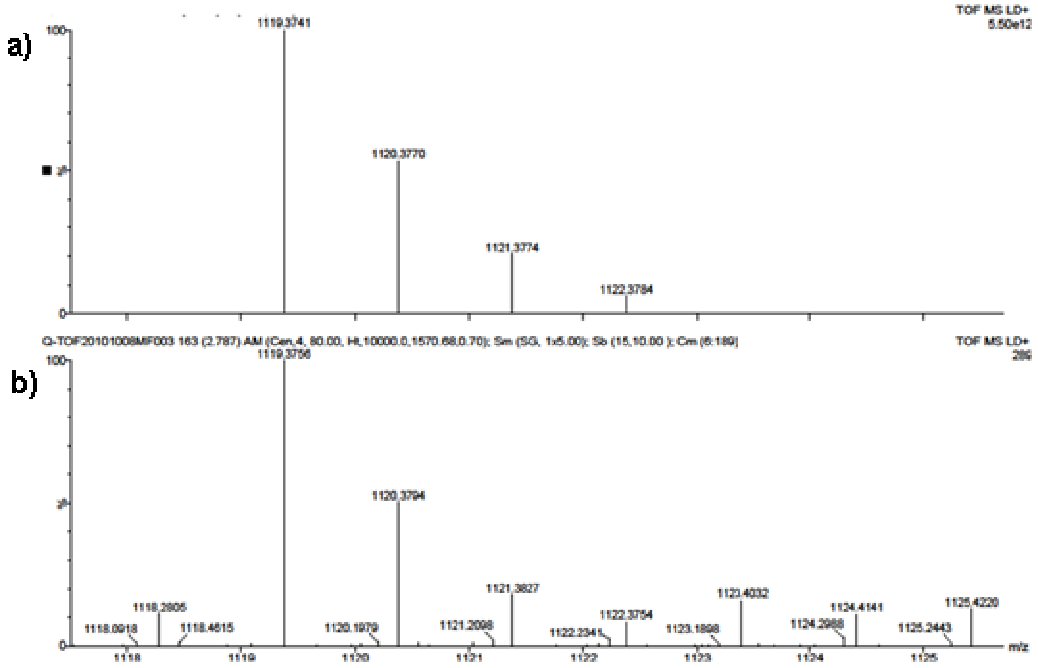



***Figure S5:*** (b) HRMS spectrum of **1.Tb.GSH** obtained by MALDI-TOF analysis showing the $Tb^{III}$ characteristic isotopic pattern, compared with its theoretical isotopic distribution model (a).

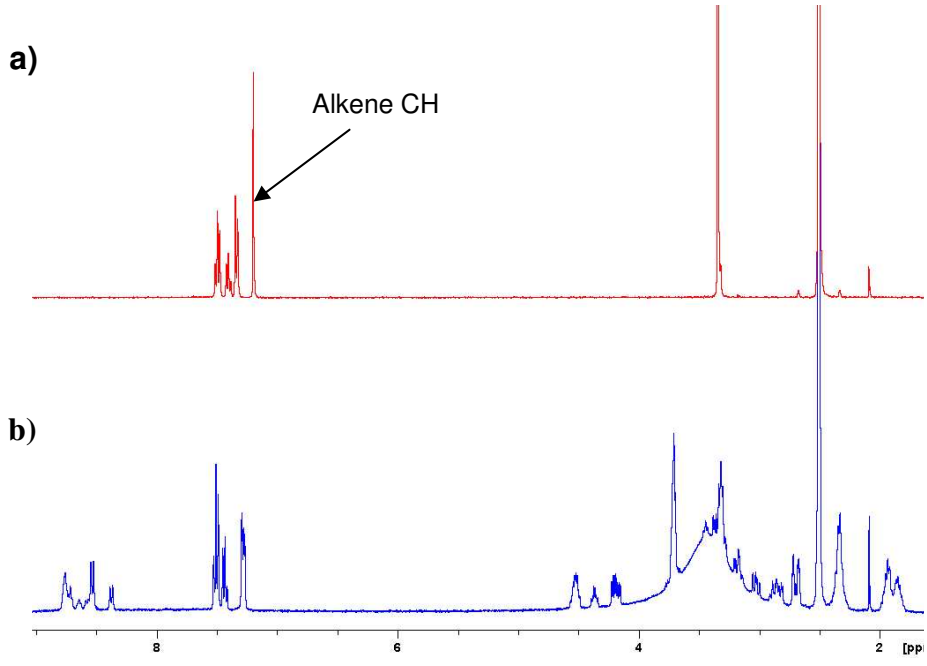


***Figure S6***.a) $^{1}$H NMR spectrum (DMSO, 400 MHz) of N-phenylmaleimide b) $^{1}$H NMR spectrum (DMSO, 400 MHz) of N-phenylmaleimide upon the addition of 1 equiv of GSH.

***Table S1:*** Summary of the lifetimes and *q* values obtained for complex **1.Tb** and **1.Tb.GSH**

| *Compound* | $\tau_{O\text{-}H}$ *(± 0.01 ms)* | $\tau_{O\text{-}D}$ *(± 0.01 ms)* | *q (±0.5)* |
|---|---|---|---|
| **Tb.110** | **1.307** | **1.992** | **1.0** |
| **Tb.110-GSH** | **1.418** | **2.186** | **0.9** |

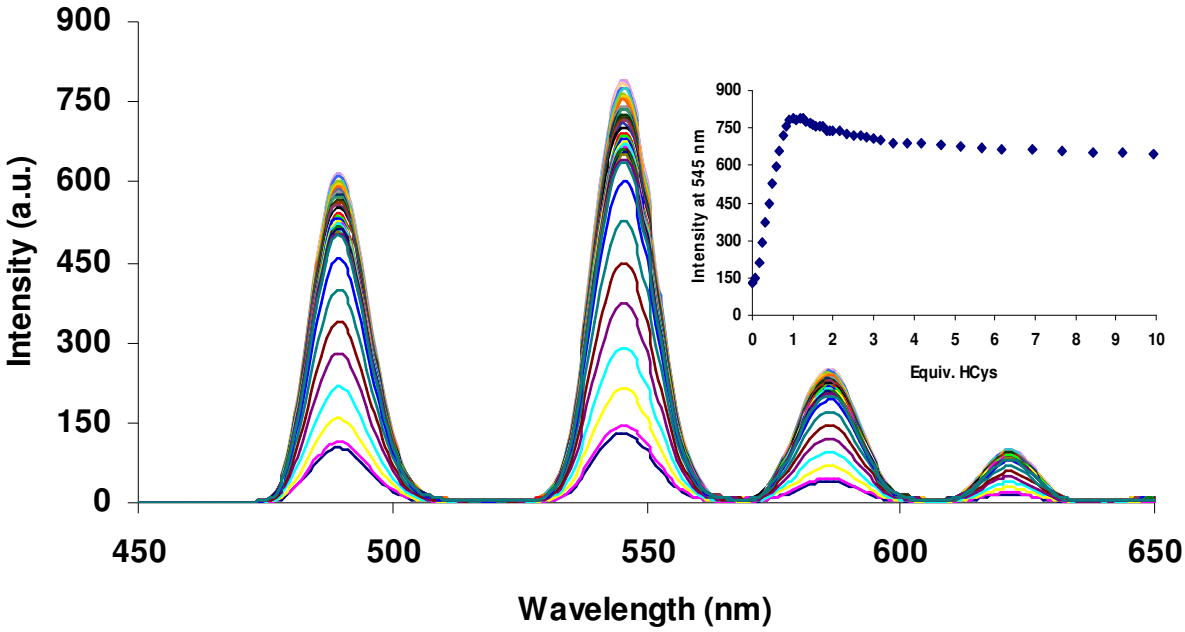



***Figure S7.*** Changes in the $Tb^{III}$ emission of **1.Tb** (10 μM) in the presence of HCys at pH = 7.4 (20 mM HEPES, 135mM KCl). ***Insert:*** Plot of intensity at 545 nm with equiv of HCys added.

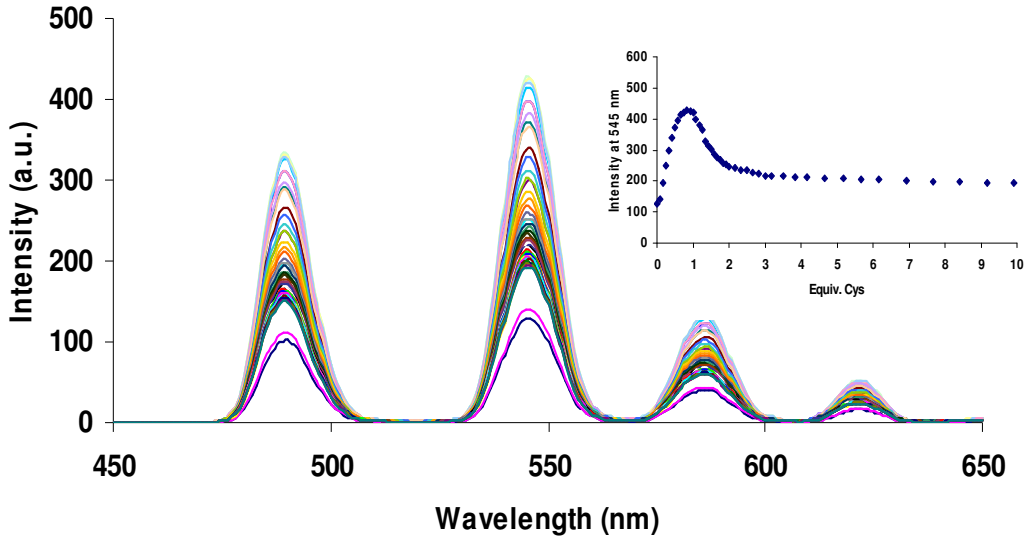



***Figure S8.*** Changes in the $Tb^{III}$ emission of **1.Tb** (10 μM) in the presence of Cys at pH = 7.4 (20 mM HEPES, 135mM KCl). ***Insert:*** Plot of intensity at 545 nm with equiv of Cys added.

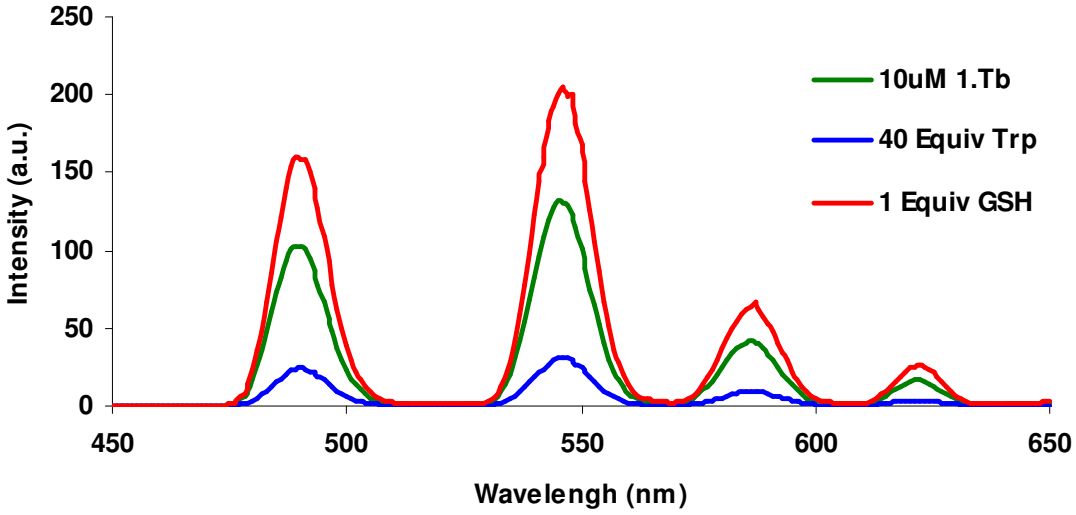



***Figure S9a.*** Changes in the $Tb^{III}$ emission of **1.Tb** (10 μM) in the presence of 40 equiv Trp and upon addition of 1 equiv GSH at pH = 7.4 (20 mM HEPES, 135mM KCl).

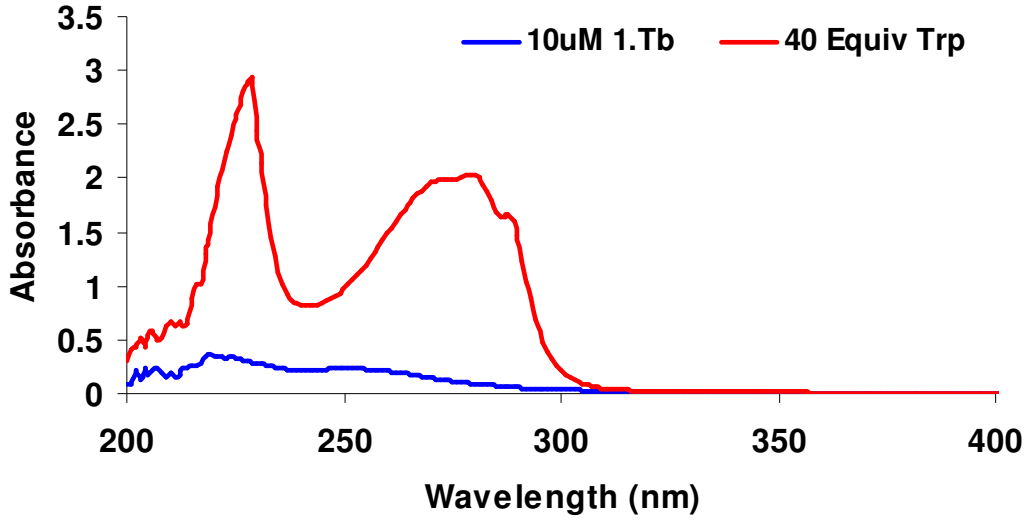



***Figure S9b.*** Changes in the absorption spectrum of **1.Tb** (10 μM) upon the addition of 40 equiv of Trp at pH = 7.4 (20 mM HEPES, 135mM KCl.

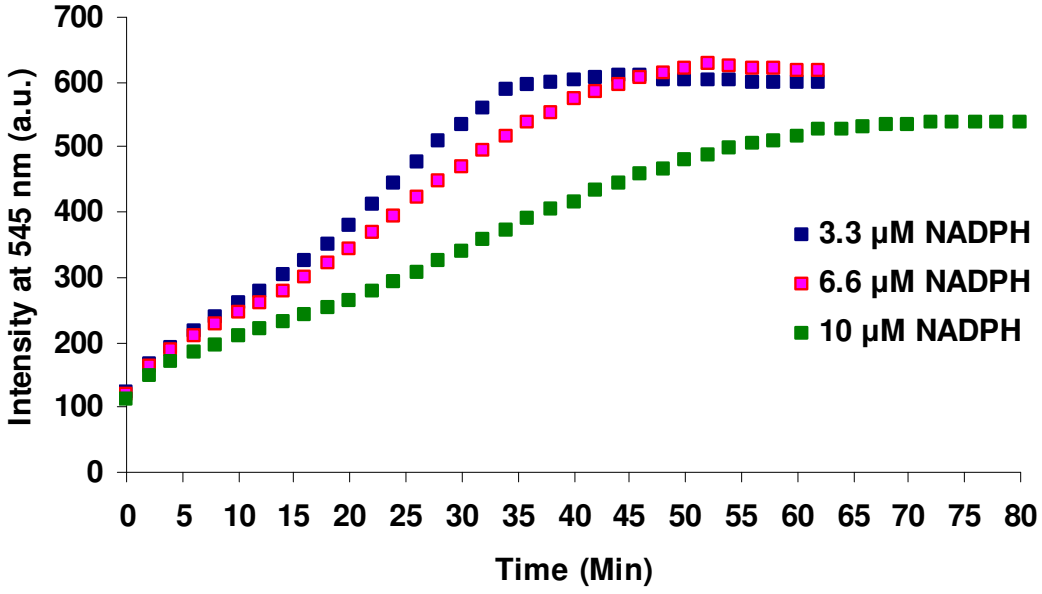



***Figure S10.*** Luminescence response of **1.Tb** (10 μM) at 545 nm as a result of the enzymatic conversion of GSSG to its reduced GSH form using the enzyme glutathione

reductase (4.93 nM) and various concentrations of NADPH. All enzymatic titrations were carried out in aqueous pH 7.4 buffered solutions (0.1 M TRIS, 0135 mM KCl) at 37 °C.

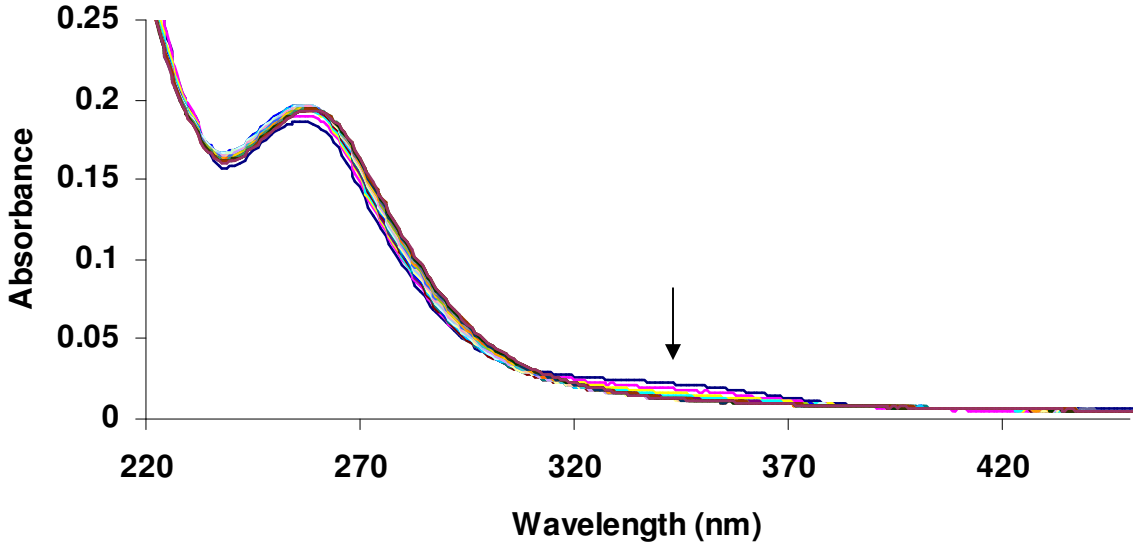



***Figure S11a.*** Overall absorbance response of **1.Tb** (10 μM) as a result of the enzymatic conversion of GSSG to its reduced GSH form using NADPH (3.3 μM) and the enzyme glutathione reductase (19.72 nM). All enzymatic titrations were carried out in aqueous pH 7.4 buffered solutions (0.1 M TRIS, 135 mM KCl) at 37 °C.

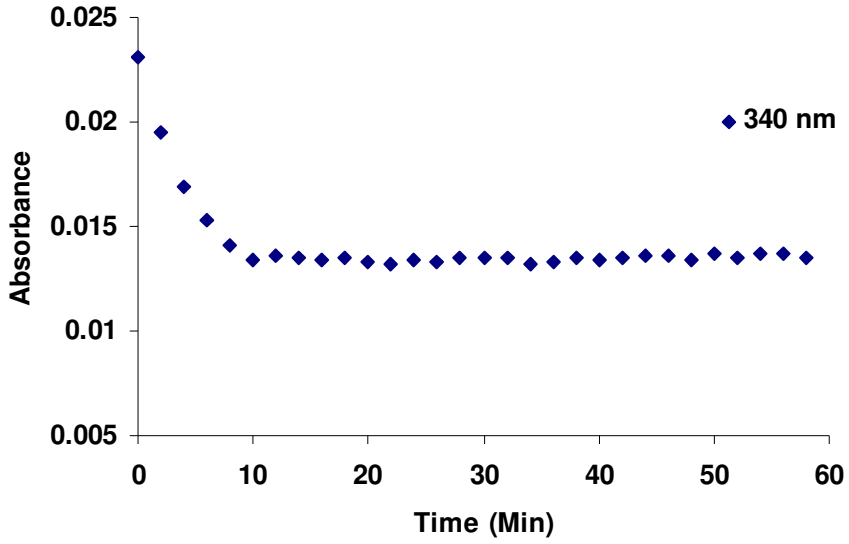


***Figure S11b.*** Absorbance response of **1.Tb** (10 μM) at 340 nm as a result of the enzymatic conversion of GSSG to its reduced GSH form using NADPH (3.3 μM) and the enzyme glutathione reductase (19.72 nM). All enzymatic titrations were carried out in aqueous pH 7.4 buffered solutions (0.1 M TRIS, 135 mM KCl) at 37 °C.

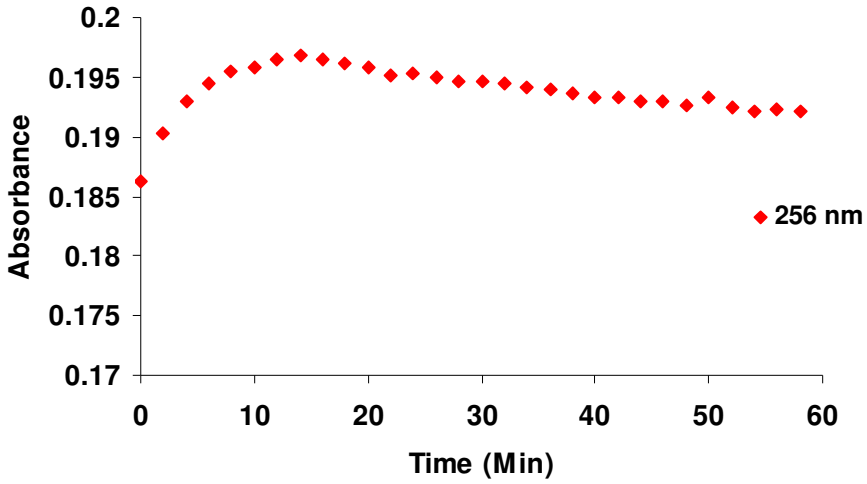



***Figure S11c.*** Absorbance response of **1.Tb** (10 μM) at 256 nm as a result of the enzymatic conversion of GSSG to its reduced GSH form using NADPH (3.3 μM) and the enzyme glutathione reductase (19.72 nM). All enzymatic titrations were carried out in aqueous pH 7.4 buffered solutions (0.1 M TRIS, 0.135 M KCl) at 37 °C.

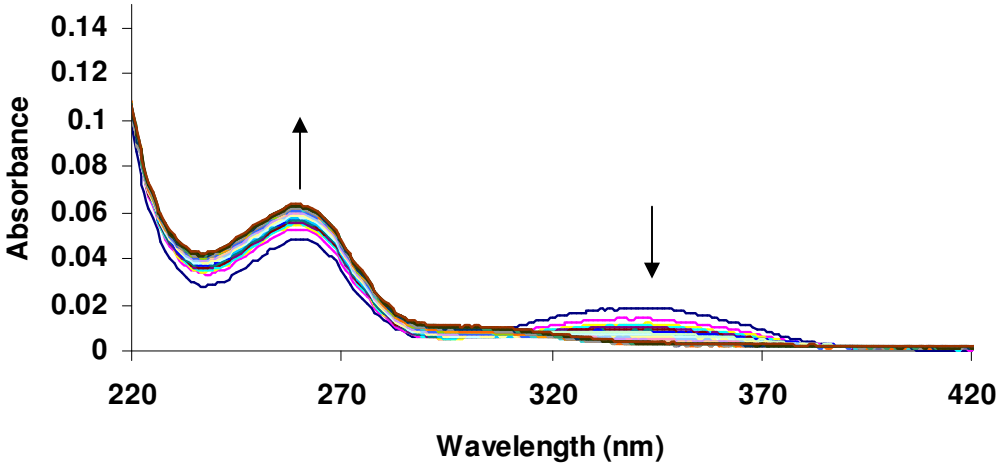


***Figure S12.*** Overall absorbance response of the enzymatic conversion of GSSG to its reduced GSH form using NADPH (3.3 μM) and the enzyme glutathione reductase (19.72 nM). All enzymatic titrations were carried out in aqueous pH 7.4 buffered solutions (0.1 M TRIS, 135 mM KCl) at 37 °C.

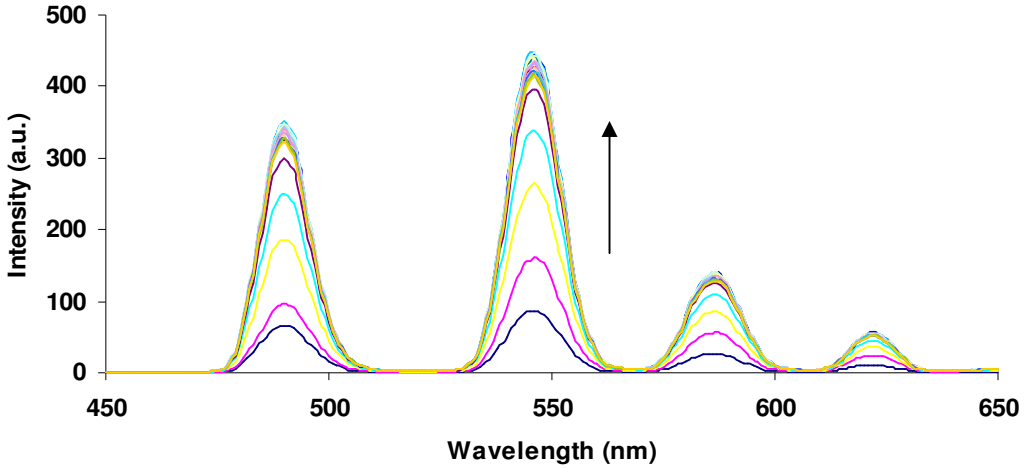



***Figure S13a.*** Overall luminescence response of **1.Tb** (10 μM) as a result of the enzymatic conversion of GSSG to its reduced GSH form using the enzyme glutathione reductase (4.93 nM) and NADPH (3.3 μM) in 40 equiv of Ala, Asp, His, Arg, Phe, Ser, Val, IIe, GSSG, GSH, Pro, Thr, Tyr, Gly, Leu, Lys, Met, Glu, Sar and Asn. All enzymatic titrations were carried out in aqueous pH 7.4 buffered solutions (0.1 M TRIS, 135 mM KCl) at 37 °C.

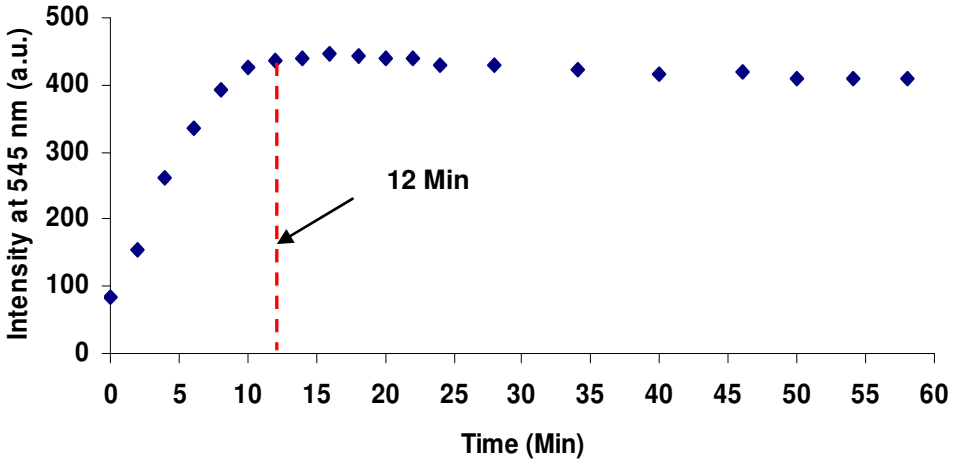


***Figure S13b.*** Luminescence response of **1.Tb** (10 μM) at 545 nm as a result of the enzymatic conversion of GSSG to its reduced GSH form using the enzyme glutathione reductase (4.93 nM) and NADPH (3.3 μM) in 40 equiv of Ala, Asp, His, Arg, Phe, Ser, Val, IIe, GSSG, GSH, Pro, Thr, Tyr, Gly, Leu, Lys, Met, Glu, Sar and Asn. All enzymatic titrations were carried out in aqueous pH 7.4 buffered solutions (0.1 M TRIS,

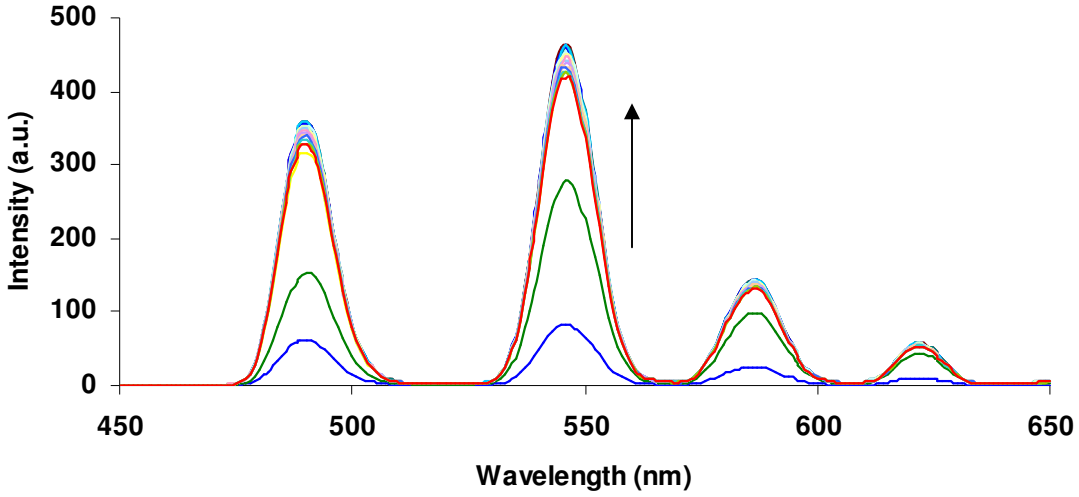



***Figure S13c.*** Overall luminescence response of **1.Tb** (10 μM) as a result of the enzymatic conversion of GSSG to its reduced GSH form using the enzyme glutathione reductase (9.86 nM) and NADPH (3.3 μM) in 40 equiv of Ala, Asp, His, Arg, Phe, Ser, Val, IIe, GSSG, GSH, Pro, Thr, Tyr, Gly, Leu, Lys, Met, Glu, Sar and Asn. All enzymatic titrations were carried out in aqueous pH 7.4 buffered solutions (0.1 M TRIS, 135 mM KCl) at 37 °C.

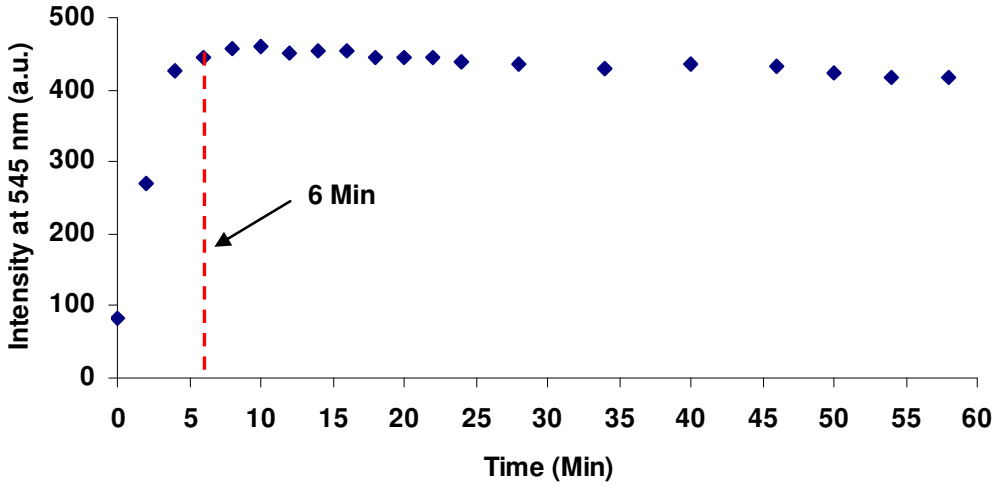


***Figure S13d.*** Luminescence response of **1.Tb** (10 μM) at 545 nm as a result of the enzymatic conversion of GSSG to its reduced GSH form using the enzyme glutathione reductase (9.86 nM) and NADPH (3.3 μM) in 40 equiv of Ala, Asp, His, Arg, Phe, Ser, Val, IIe, GSSG, GSH, Pro, Thr, Tyr, Gly, Leu, Lys, Met, Glu, Sar and Asn. All enzymatic titrations were carried out in aqueous pH 7.4 buffered solutions (0.1 M TRIS, 135 mM KCl) at 37 °C.

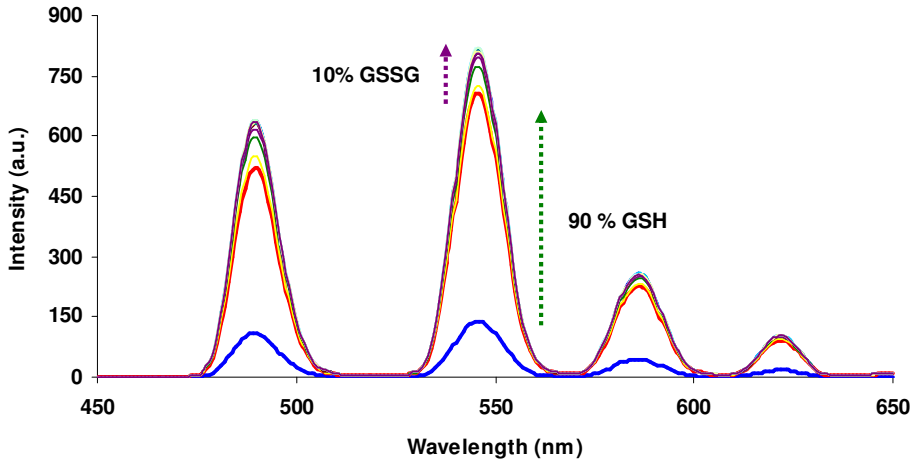



***Figure S14a.*** Overall luminescence response of **1.Tb** (10 μM) in a 90%:10% (GSH:GSSG) solution mixture. The initial GSH response was recorded followed by addition of 3.3 μM NADPH and 4.93 nM glutathione reductase to allow for the enzymatic conversion of the GSSG in solution to its reduced GSH form. All enzymatic titrations were carried out in aqueous pH 7.4 buffered solutions (0.1 M TRIS, 135 mM KCl) at 37 °C.

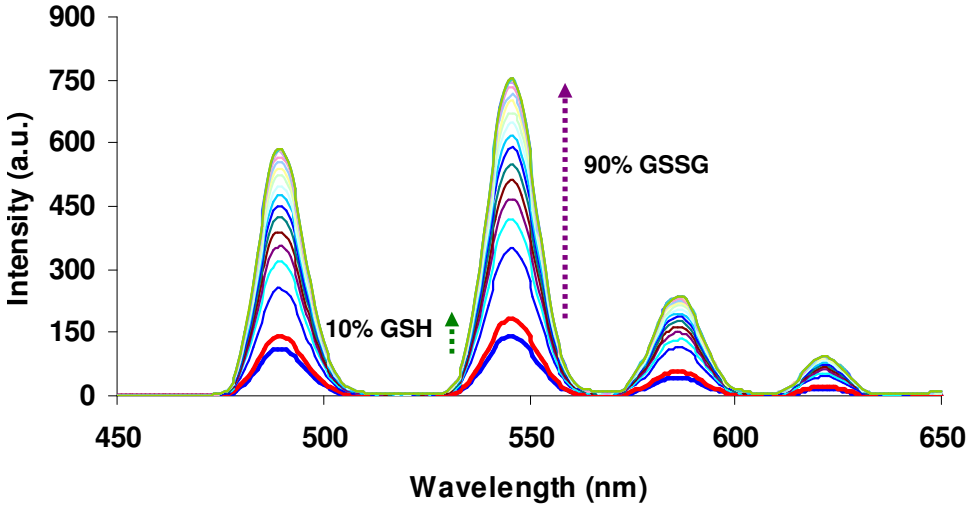


***Figure S14b.*** Overall luminescence response of **1.Tb** (10 μM) in a 10%:90% (GSH:GSSG) solution mixture. The initial GSH response was recorded followed by addition of 3.3 μM NADPH and 4.93 nM glutathione reductase to allow for the enzymatic conversion of the GSSG in solution to its reduced GSH form. All enzymatic titrations were carried out in aqueous pH 7.4 buffered solutions (0.1 M TRIS, 135 mM KCl)

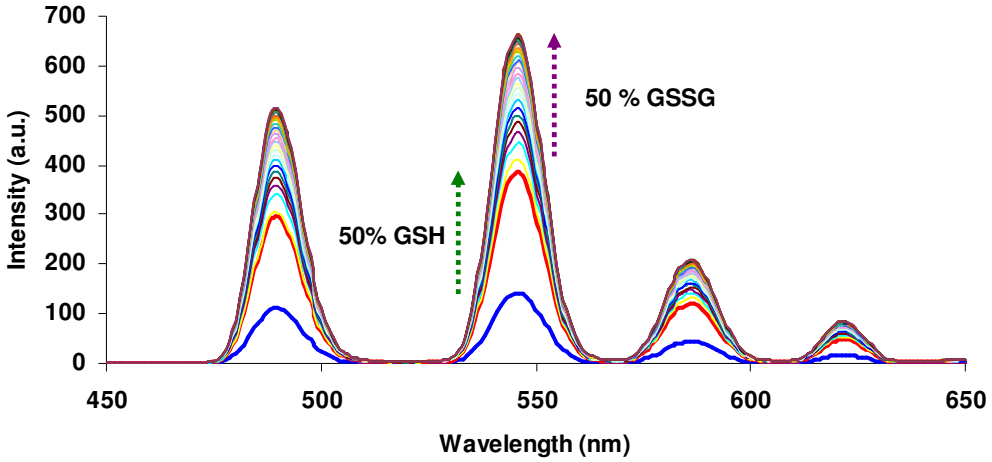



***Figure S14c.*** Overall luminescence response of **1.Tb** (10 μM) in a 50%:50% (GSH:GSSG) solution mixture. The initial GSH response was recorded followed by addition of 3.3 μM NADPH and 4.93 nM glutathione reductase to allow for the enzymatic conversion of the GSSG in solution to its reduced GSH form. All enzymatic titrations were carried out in aqueous pH 7.4 buffered solutions (0.1 M TRIS, 135 mM KCl) at 37 °C.

**References:**

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