Electronic Supplementary Material (ESI) for ChemComm.

A phototoxic thulium complex exhibiting intracellular ROS production upon 630 nm excitation in cancer cells

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Supplementary Information

General experimental conditions

All solvents were purchased in analytical, HPLC or spectroscopy grade and used as received. Photofrin was purchased from Movianto Germany. All substances which were purchased from commercial were used as received.

In vitro Singlet Oxygen Detection

The detection of singlet oxygen production in methanol was carried out using an indirect method *via* the decomposition of 1,3-diphenylisobenzofuran (DPBF) (410 nm absorption maximum decrease) For the measurements, 1% (V) of a saturated DPBF solution was dissolved in methanol. Afterwards the respective compound was added from a 25 mM stock solution in DMSO to reach the indicated final concentrations. If needed, DMSO was added to reach a final DMSO concentration of 0.1%. The UV-Vis spectra were recorded on a setup that included a Shimadzu UV-1900 spectrophotometer equipped with Hellma optical fibres and two Thorlabs KSC101 shutters (one in the instrument and one in the Arc lamp beam line). The irradiation was conducted using a 150 W Xe-Arc discharge lamp equipped with a 590 nm longpass filter, leading to an irradiance of 100 μW/cm^2. An absorption spectrum was recorded prior to the irradiation and repeated in the stated time periods. The setup was tested for 60 minutes with 1% saturated DPBF solution in methanol containing 0.1% DMSO without any compound added. No degradation of the DPBF was observed during this period (see Figure S.16). During the irradiation, a spectrum was recorded every 5 minutes showing a constant degradation of DPBF as was indicated by the continuous decrease of the maximum at 410 nm.

Stability Study

An ESI-MS study was carried out, which is considered a non-quantitate method that may allow for the observation of potential decomposition products. The compound **TmL** was first dissolved in DMSO and 10 µL of this stock solution were dissolved in 500 µL of PBS (Dulbecco's Phosphate-Buffered Saline, pH = 7.4). Spectra were recorded after a few minutes, 24 hours and 48 hours. During this study, no new thulium containing signals could be detected.

Cytotoxicity and Phototoxicity assay

HeLa cells were obtained from Merck Ireland, HeLa cell line, human epithelioid cervix carcinoma. Dulbecco's Modified Eagle's Medium (DMEM), containing 10% fetal calf serum, 1% penicillin and streptomycin. HeLa cells were detached from the seeding dishes or flasks with trypsin and EDTA, harvested by centrifugation, and then resuspended again in cell culture medium to yield the desired concentration of cells. The assays were carried out on 96 well plates with 6000 cells per well. After 24 h of incubation at 37 °C with 5% (or 10%) CO_2, the cells were treated with appropriate concentrations of the investigated dissolved in medium and added to each well up to a final volume of 200 µl per well (with a total DMSO concentrations of 1%). As negative control, one series of cells was only treated with 1% DMSO.

For the photocytotoxicity studies specifically, cells were preincubated for 1 h with investigated the compound and then irradiated with an Amuza inc. LED Array System, equipped with a 96 well 590 nm LED array. For this, the plates were irradiated in an incubator for 1 h with a total radiant exposure level of 1.8 J/cm^2. Afterwards, the plates were incubated for another 22 h and then treated continued in the same way as for the non-irradiated plates.

The cells were then incubated for 24 h followed by addition of 50 µl MTT dye dissolved in DPBS buffer (2.5 mg/ml) for 80% of the wells leaving a minimum of two wells as absorption correction for the cell staining compounds. After an incubation time of 2 h, the medium was removed and 200 µl DMSO were added. The formazan crystals were dissolved, and the absorption was measured on a BMG Labtech CLARIOstar plus v. 5.70 or a Tecan Infinite 200 pro plate reader, respectively, at a wavelength of 550 nm and a reference wavelength of 620 nm.[1,2]

Cell Imaging

Uptake and colocalization studies

HeLa cells were seeded on ibidi 8-well microplates at a concentration of 25,000 cells per well in 250 µL cell culture medium. The cells were then incubated for 24 h at 37 °C and 5% CO_2, before the indicated concentration of the investigated compound was added to reach a final volume of 350 µL per well (with a DMSO concentrations of 1%). After the specified incubation time, the supernatant was aspired off, the cells were washed with PBS (Dulbecco's Phosphate-Buffered Saline), and then covered with phenol-red-free medium for the duration of the microscopy examinations. The imaging was carried out using a Leica TCS DMi8 laser scanning confocal microscope (LCSM) at 37 °C equipped with a white light laser light source using Leica HyD hybrid detectors. A 630 nm white light laser was used to excite **TmL** and emission was collected between 640 and 795 nm. DRAQ 7, a nuclear staining dye was added (3 µM) to distinguish intact live cells from damaged cells. DRAQ 7 was excited at 633 nm and emission was collected between 650–750 nm. In colocalization studies, the cells were co-stained with commercial dye SYTO 11 Green (0.5 µM/ 15 min) to stain the nucleus or BioTracker 488 Green (100 nM; 25 min) to stain mitochondria. The SYTO 11 dye was excited at 508 nm and emission was collected between 517 – 530 and BioTracker 488 was excited at 488 nm and emission was collected between 510 – 520 nm. All experiments were repeated twice to prove reproducibility.

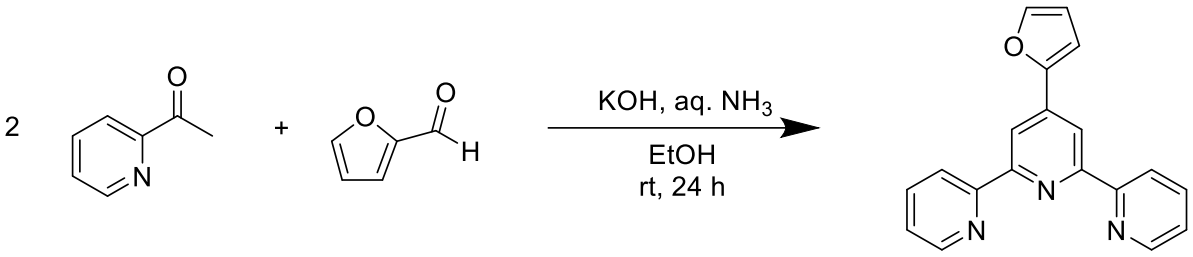
Intracellular ROS detection studies

HeLa cells were seeded as described above and then treated with **TmL** at a concentration of 1 µM, 5 µM and 10 µM and incubated for 1 h at 37 °C in the absence of light. Following incubation, the supernatant was removed, and cells were washed with PBS prior to adding H_2DCFDA (5 µM) in PBS for 30 min at 37 °C. The cells were imaged by collecting the emission from the ROS dye between 517 – 527 nm following excitation at 494 nm. Emission from the ROS dye was collected again post continuous irradiation at 630 nm to excite the **TmL** complex. An image of the **TmL** complex in cells was also collected with excitation at 630 and emission set at 640 – 795 nm. Control cells were prepared as described above and were treated only with the ROS dye (5 µM/ 30 min). Emission from the ROS dye was collected prior and post irradiation at 630 nm. Positive control cells were prepared by treating cells with Photofrin 10 µM for 1 h

at 37 °C in the absence of light. Following incubation, the Photofrin/media solution was replaced with the ROS dye in PBS. Emission from the ROS dye was collected again between 517 and 527 nm prior and post continuous irradiation. Cells were imaged directly using a Leica TCS DMi8 confocal microscope (63 X oil immersion objective lens) with a heated stage at 37 °C. All experiments were repeated twice to proof reproducibility.

Synthetic Procedure

Synthesis of 4'-(furan-2-yl)-2,2':6',2''-terpyridine [1]



Scheme S 1: Synthesis of 4'-(furan-2-yl)-2,2':6',2''-terpyridine [1].

A volume of 46.3 ml (50 g, 412 mmol) 2-acetylpyridine was dissolved in 1000 ml ethanol in a 3 l round bottom flask. Afterwards, 17.1 ml (19.8 g, 206 mmol) furfuraldehyde were added through a syringe. Then under vigorous stirring, 31.8 g (567 mmol) potassium hydroxide and 600 ml aqueous ammonia (25% NH_3) were added to the reaction mixture, which was then stirred for 24 h at room temperature. Over the course of the reaction, the solution turned from pale yellow to dark brown and a colourless solid precipitated. The reaction mixture was passed through a glass sintered funnel and the remaining solid was washed with an ice-cold mixture of ethanol and water (1:1 V/V) until the washings were colourless. The solid was dried under vacuum to obtain 4'-(furan-2-yl)-2,2':6',2''-terpyridine **1** as an off-white powder in 44% yield, 27.3 g, 91.21 mmol.

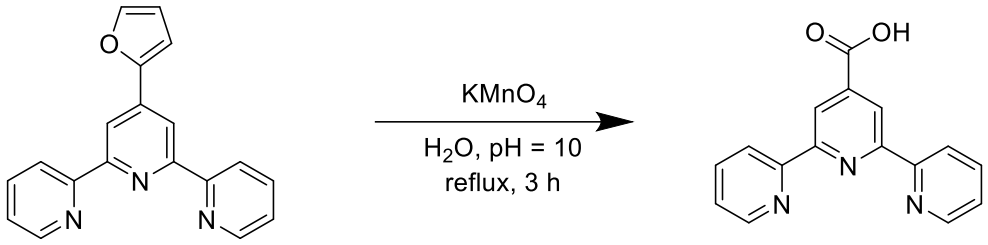
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 – 8.68 (m, 4H), 8.65 (dt, *J* = 8.0, 1.1 Hz, 2H), 7.88 (ddd, *J* = 8.0, 7.5, 1.8 Hz, 2H), 7.59 (dd, *J* = 1.8, 0.7 Hz, 1H), 7.36 (ddd, *J* = 7.5, 4.8, 1.2 Hz, 2H), 7.14 (dd, *J* = 3.5, 0.8 Hz, 1H), 6.56 (dd, *J* = 3.5, 1.8 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ: 156.1, 155.9, 152.0, 149.1, 143.9, 139.7, 137.2, 124.1, 121.5, 115.4, 112.3, 109.4.

MS (ESI+): *m / z* 299.87 $[M+H]^+$.

EA: Anal. calc. for $C_{19}H_{13}N_3O$: C, 75.17; H, 4.34; N, 14.03. found: C, 74.81; H, 4.28; N, 14.32.

Synthesis of [2,2':6',2''-terpyridine]-4'-carboxylic acid [2]



Scheme S 2: Synthesis of [2,2':6',2''-terpyridine]-4'-carboxylic acid [2].

To a 2 l round bottom flask, 4'-(furan-2-yl)-2,2':6',2''-terpyridine **1** 23.75 g (79.3 mmol) was added followed by 1280 ml water. Potassium hydroxide was added to the resulting suspension until the pH of the solution was ≈ 10 as determined by pH indicator paper. Afterwards, 3.47 g (21.94 mmol) of potassium permanganate was added and the reaction mixture was refluxed for 3 h. After cooling down to room temperature, the mixture was filtered through a pad of Celite (approx. 3 cm). The filtrate was recovered and acidified to pH ≈ 5 by dropwise addition of concentrated HCl, resulting in a white gel. The gel was passed through a glass sintered funnel and the resulting filter cake was washed with 900 ml water to obtain a colourless sticky solid. The solid was coevaporated with chloroform (2 x 200 ml) and dried under reduced pressure. The desired product **2** was obtained as a colourless powder in 28% yield, 6.57 g, 23.69 mmol.

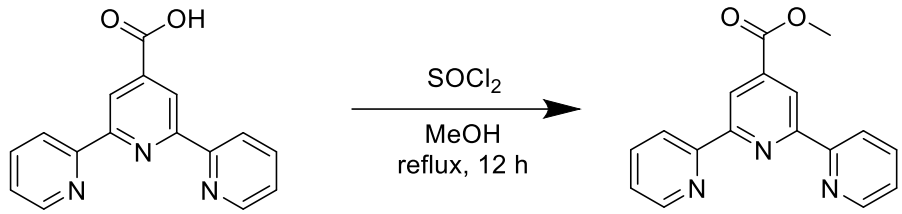
^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.84 (s, 1H), 8.94 – 8.55 (m, 6H), 8.03 (td, J = 7.7, 1.9 Hz, 2H), 7.54 (ddd, J = 7.7, 4.7, 1.2 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ: 166.0, 156.0, 154.2, 149.5, 140.6, 137.6, 124.8, 120.9, 119.6.

MS (ESI+): *m / z* 277.96 $[M+H]^+$.

EA: Anal. calc. for $C_{16}H_{11}N_3O_2$: C, 69.24; H, 3.97; N, 15.15. found: C, 68.96; H, 3.84; N, 15.40.

Synthesis of methyl [2,2':6',2''-terpyridine]-4'-carboxylate [3]



Scheme S 3: Synthesis of methyl [2,2':6',2''-terpyridine]-4'-carboxylate [3].

In a 100 ml twin-neck round-bottom flask, 0.57 g (2.06 mmol) [2,2':6',2''-terpyridine]-4'-carboxylic acid **2** were dissolved in 25 ml methanol and the solution was cooled down to 0 °C. Afterwards, 0.6 ml (0.98 g, 8.22 mmol) thionyl chloride was added at 0 °C under vigorous stirring. After complete addition, the reaction mixture was heated to reflux for 12 h and then cooled down to room temperature. The solution was dumped on 100 ml of ice-water and neutralized with potassium carbonate. The aqueous solution was then extracted with chloroform (3 x 75 ml) and the combined organic phase was then washed with brine (1 x 75 ml) afterwards and dried with Mg_2SO_4. The organic solution was then filtered and the filtrate was concentrated under reduced pressure and then dried in vacuum to obtain the product **3** as an off-white solid in 92% yield 0.55 g, 1.88 mmol.

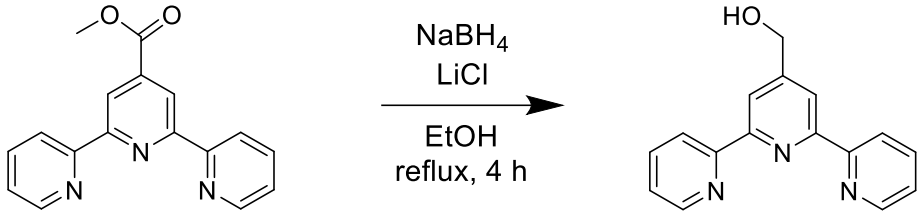
^{1}H NMR (300 MHz, Chloroform-*d*) δ: 9.00 (s, 2H), 8.76 (dt, *J* = 4.7, 1.2 Hz, 2H), 8.69 – 8.58 (m, 2H), 7.90 (td, *J* = 7.8, 1.8 Hz, 2H), 7.38 (ddd, *J* = 7.6, 4.8, 1.2 Hz, 2H), 4.01 (s, 3H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ: 165.9, 156.5, 155.4, 149.4, 139.9, 137.2, 124.4, 121.5, 120.6, 52.8.

MS (ESI+): *m / z* 314.26 $[M+Na]^+$.

EA: Anal. calc. for $C_{17}H_{13}N_3O_2$: C, 70.03; H, 4.46; N, 14.42. found: C, 69.95; H, 4.41; N, 14.65.

Synthesis of [2,2':6',2''-terpyridin]-4'-ylmethanol [4]



Scheme S 4: Synthesis of [2,2':6',2''-terpyridin]-4'-ylmethanol [4].

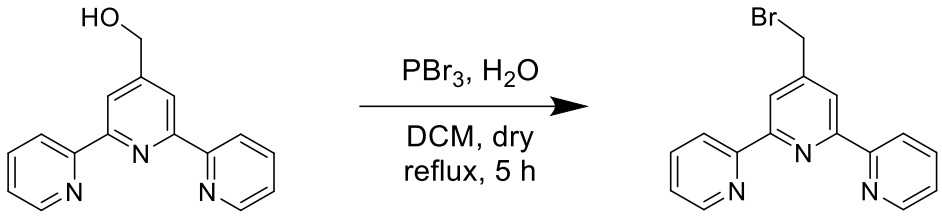
In a 500 ml twin-neck round-bottom flask, 5.00 g (17.16 mmol) [2,2':6',2''-terpyridine]-4'-carboxylate **3** were dissolved in 190 ml ethanol. Afterwards, 1.95 g (51.49 mmol) sodium borohydride and 2.18 g (51.49 mmol) lithium chloride were added to the solution. The mixture was heated to reflux for 4 h and then cooled down to room temperature. All volatiles were removed under reduced pressure and 50 ml water was added to the remaining slurry. The aqueous phase was extracted with dichloromethane (3 x 150 ml) and the combined organic solution was dried with Na_2SO_4 and filtered afterwards. The organic solution was then filtered and the filtrate was concentrated under reduced pressure. The product **4** was obtained as a colourless powder in 91% yield, 4.13 g, 15.67 mmol.

^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.75 – 8.68 (m, 2H), 8.62 (d, J = 1.1 Hz, 2H), 8.44 (s, 2H), 8.00 (td, J = 7.7, 1.8 Hz, 2H), 7.52 – 7.44 (m, 2H), 5.61 (t, J = 5.3 Hz, 1H), 4.74 (d, J = 4.5 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d_6) δ: 155.2, 154.7, 154.2, 149.2, 137.4, 124.3, 120.8, 118.0, 61.9.

MS (ESI+): m / z 285.87 $[M+Na]^+$.

Synthesis of 4'-(bromomethyl)-2,2':6',2''-terpyridine [5]



Scheme S 5: Synthesis of 4'-(bromomethyl)-2,2':6',2''-terpyridine [5].

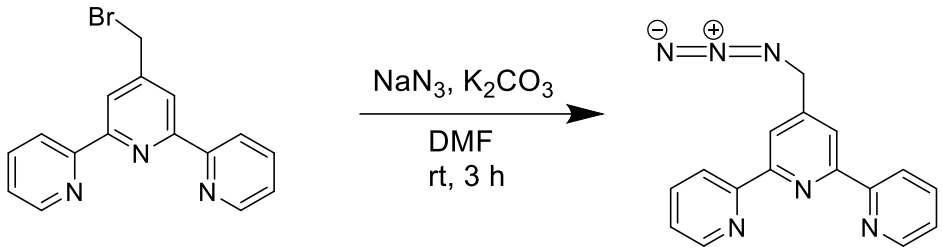
In a 250 ml twin-neck Schlenk flask, 4.00 g (15.19 mmol) [2,2':6',2''-terpyridin]-4'-ylmethanol **4** was dissolved in 90 ml dry dichloromethane and the solution was cooled to 0 °C. Then, 4.33 ml (12.34 g, 45.57 mmol) phosphorus tribromide and 0.55 ml (45.57 mmol) water were added slowly at 0 °C. The mixture was stirred for another 15 min at room temperature and then heated to reflux for 5 h. Afterwards the solution was allowed to cool to room temperature and then neutralized with saturated $NaHCO_3$ until pH ≈ 7. Another 50 ml water were added and the mixture was extracted with dichloromethane (3 x 150 ml). The combined organic phases were dried with Na_2SO_4 and filtered afterwards. The solvent was removed under reduced pressure to obtain the product **5** as a pale brown powder in 84% yield, 4.14 g, 12.69 mmol.

^{1}H NMR (400 MHz, DCM-d_2) δ: 8.75 – 8.67 (m, 2H), 8.63 (d, J = 1.1 Hz, 2H), 8.51 (s, 2H), 7.89 (td, J = 7.7, 1.8 Hz, 2H) ,7.37 (m, 2H), 4.62.

^{13}C NMR (101 MHz, DCM-d_2) δ: 156.5, 155.9, 149.6, 148.7, 137.3, 124.5, 121.5, 121.1, 31.8.

MS (ESI+): *m / z* 326.3 $[M+H]^+$,248.3 $[M-Br]^+$.

Synthesis of 4'-(azidomethyl)-2,2':6',2''-terpyridine [6]



Scheme S 6: Synthesis of 4'-(azidomethyl)-2,2':6',2''-terpyridine [6].

In a 100 ml round bottom flask, 2.00 g (6.13 mmol) 4'-(bromomethyl)-2,2':6',2''-terpyridine **5** was dissolved in 50 ml *N*,*N*-dimethylformamide. Then, 0.79 g (6.13 mmol) potassium carbonate was added and the mixture was stirred at room temperature in the dark for 3 h. Afterwards, 100 ml water was added and the reaction mixture was extracted with dichloromethane (3 x 100 ml). The combined organic layers were extracted with brine (2 x 100 ml), dried with Na_2SO_4 and filtered afterwards. All volatiles were removed under reduced pressure and a brown oil was obtained. The remaining oil was dissolved in 20 ml of EtOH and the product was crystallized in the freezer overnight. The product **6** was obtained as pale-yellow crystalline powder in 84% yield, 4.14 g, 12.69 mmol.

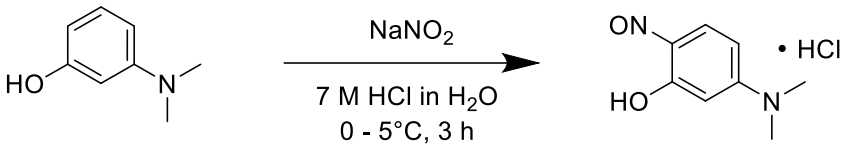
^{1}H NMR (400 MHz, DCM-d_2) δ: 8.76 – 8.60 (m, 4H), 8.45 (s, 2H), 7.89 (td, J = 7.7, 1.8 Hz, 2H), 7.43 – 7.31 (m, 2H), 4.61 (s, 2H).

^{13}C NMR (101 MHz, DCM-d_2) δ: 156.4, 156.0, 149.6, 147.0, 137.3, 124.5, 121.5, 119.9, 54.2.

MS (ESI+): m / z 289.4 $[M+H]^+$.

IR (ATR-IR): $\tilde{\nu}$ = 2099 cm^{-1}.

Synthesis of 5-(dimethylamino)-2-nitrosophenol hydrochloride [7]



Scheme S 7: Synthesis of 5-(dimethylamino)-2-nitrosophenol hydrochloride [7].

In a 100 ml round bottom flask, 8.60 g (62.7 mmol) 3-(dimethylamino)phenol was dissolved in 40 ml of a 7 M aqueous HCl. The mixture turned red immediately and was cooled down to 0 °C. A solution of 4.37 g (63.3 mmol) sodium nitrite in 5 ml water was slowly added over the course of 30 minutes and the mixture was stirred at 0 °C for another 3 h. The resulting suspension was filtered through a glass sintered funnel and the remaining yellow solid was washed with 150 ml saturated aqueous sodium acetate solution until the colour changed to red. The solid was redissolved in acetone and filtered through a pad of silica (approx. 3 cm). Afterwards, all volatiles were removed under reduced pressure to obtain the product **7** as red crystals in 53% yield, 6.69 g, 33.12 mmol.

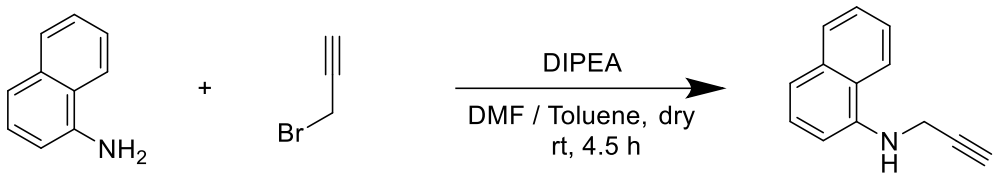
^{1}H NMR (400 MHz, acetone-d_6) δ: 7.36 (dd, J = 9.9 Hz, 1H), 6.64 (dd, J = 9.9, 2.6 Hz, 1H), 5.69 (d, J = 2.6 Hz, 1H), 3.21 (s, 6H).

^{13}C NMR (101 MHz, acetone-d_6) δ: 169.2, 159.2, 150.3, 135.5, 114.1, 96.7, 41.2.

MS (ESI+): 167.87 $[M+H]^+$.

IR (ATR-IR): $\tilde{\nu}$ = 1620, 1496, 1474, 1431, 1412, 1389, 1236, 1163, 1088, 903, 806, 700, 617 cm^{-1}.

Synthesis of *N*-(prop-2-yn-1-yl)naphthalen-1-amine [8]



Scheme S 8: Synthesis of *N*-(prop-2-yn-1-yl)naphthalen-1-amine [8].

In a 500 ml twin-neck Schlenk flask, 20.00 g (140 mmol) naphthalen-1-amine was dissolved in 100 ml dry DMF and 30.9 ml DIPEA was added to the solution. Afterwards, 100 ml of a 16.13% propargyl bromide solution in dry toluene were added dropwise to the reaction mixture over the course of 1 h. The reaction mixture was stirred for another 4.5 h at room temperature and then quenched by addition of 100 ml water. The mixture was extracted with ethyl acetate (3 x 200 ml) and the unified organic layers were extracted with water (1 x 200 ml) and brine (1 x 200 ml). The organic phase was then concentrated under reduced pressure and the crude product was purified by flash chromatography (R_f = 0.37 *n*-hexane/EtOAC, 9:1) using a gradient of *n*-hexane and cyclohexane/ethyl acetate (1:1 V/V) from 10% to 70%. The product **8** was obtained as brown oil in 46% yield, 11.7 g, 64.6 mmol.

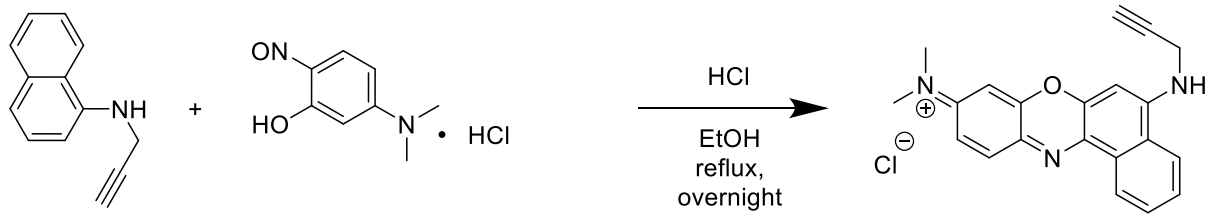
^{1}H NMR (400 MHz, DCM-d_2) δ: 7.89 – 7.79 (m, 2H), 7.55 – 7.46 (m, 2H), 7.45 – 7.39 (m, 1H), 7.38 – 7.32 (m, 1H), 6.76 (d, J = 1.2 Hz, 1H), 4.64 (s, 1H), 4.20 – 4.10 (m, 2H), 2.36 (t, J = 2.5 Hz, 1H).

^{13}C NMR (101 MHz, DCM-d_2) δ: 142.6, 134.7, 129.0, 126.7, 126.3, 125.4, 124.2, 120.3, 118.9, 105.8, 81.3, 71.6.

MS (ESI+): 182.08 $[M+H]^+$.

IR (ATR-IR): $\tilde{\nu}$ = 3289, 1582, 1524, 1477, 1408, 1279, 1117, 787, 768, 667, 640 cm^{-1}.

Synthesis of *N*-methyl-*N*-(5-(prop-2-yn-1-ylamino)-9*H*-benzo[*a*]phenoxazin-9-ylidene)methanaminium chloride [9]



Scheme S 9: Synthesis of *N*-methyl-*N*-(5-(prop-2-yn-1-ylamino)-9*H*-benzo[*a*]phenoxazin-9-ylidene)methanaminium chloride [9].

In a 100 ml round bottom flask, 1.00 g (5 mmol) of *N*-(prop-2-yn-1-yl)naphthalen-1-amine **8** and 1.00 g (5 mmol) of 5-(dimethylamino)-2-nitrosophenol hydrochloride **7** was dissolved in 20 ml ethanol. The solution was cooled down to 0 °C and 500 µl concentrated aqueous HCl was added. The solution was heated to reflux overnight and a colour change from yellow to deep blue was observed. After cooling down room temperature, all volatiles were removed under reduced pressure to obtain a black slurry. The crude was purified by flash chromatography (R_f = 0.15 DCM/MeOH, 95:5) using a gradient of dichloromethane and dichloromethane/methanol (4:1 V/V) from 0% to 90%. The product **9** was obtained as dark green powder in 62% yield, 1.11 g, 3.06 mmol.

^{1}H NMR (400 MHz, MeOD) δ: 8.97 (d, *J* = 8.1 Hz, 1H), 8.29 (d, *J* = 8.3 Hz, 1H), 8.00 - 7.90 (m, 2H), 7.89 – 7.80 (m, 1H) 7.37 (dd, *J* = 9.5 Hz, 2.4 Hz, 1H), 7.09 (s, 1H), 6.99 (d, *J* = 2.6 Hz 1H), 4.55 (d, *J* = 2.6 Hz, 2H), 3.37 (s, 6H), 2.98 (d, J = 2.2 Hz, 1H).

^{13}C-DEPT 45 NMR (101 MHz, MeOD) δ: 134.4, 133.2, 131.2, 125.9, 124.0, 117.9, 97.4, 95.3, 75.2, 41.5, 34.6.

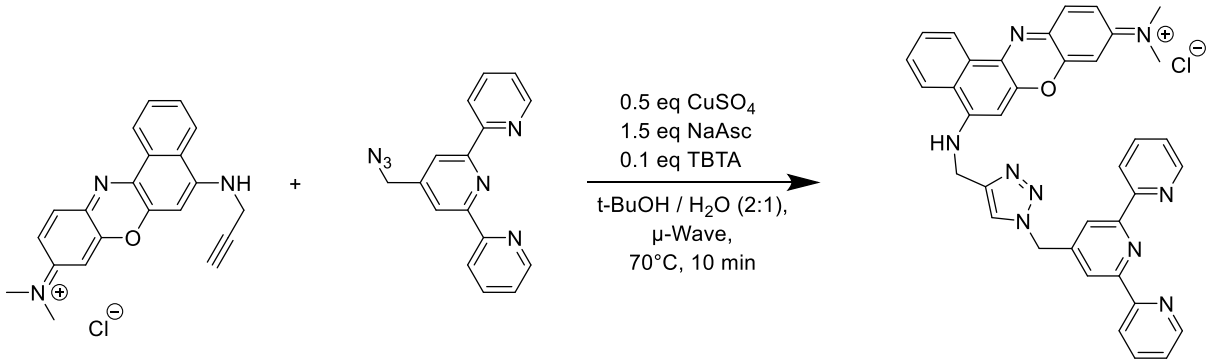
MS (ESI+): 327.99 $[M+H]^+$.

IR (ATR-IR): $\tilde{\nu}$ = 3235, 3034, 2916, 1641, 1584, 1535, 1427, 1354, 1319, 1294, 1204, 1175, 1132, 1051, 1005, 907, 860, 797, 756, 718, 644 cm^{-1}.

UV-Vis: $\lambda_{max, abs.}$ (MeOH) = 639 nm.

Fluorescence: $\lambda_{max, em.}$ (MeOH) = 681 nm.

Synthesis of *N*-(5-(((1-([2,2':6',2''-terpyridin]-4'-ylmethyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)-9*H*-benzo[*a*]phenoxazin-9-ylidene)-*N*-methylmethanaminium chloride [L]



Scheme S 10: Synthesis of *N*-(5-(((1-([2,2':6',2''-terpyridin]-4'-ylmethyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)-9*H*-benzo[*a*]phenoxazin-9-ylidene)-*N*-methylmethanaminium chloride [L].

In a 10 ml microwave tube, 50 mg (0.17 mmol) 4'-(azidomethyl)-2,2':6',2''-terpyridine **6** and 63.1 mg (0.17 mmol) compound **9** were dissolved in 5 ml *t*-BuOH and water (2:1 V/V). The solution was degassed with argon for 10 min and 14.58 mg (0.03 mmol) TBTA was added. Then, 21.65 mg (0.09 mmol) $CuSO_4$ and 51.54 mg (0.26 mmol) sodium ascorbate were added. The mixture was then transferred into a microwave tube, sealed, and brought to react in lab microwave at 70 °C for 10 min. After cooling down to room temperature, all volatiles were removed under reduced pressure. The remaining solid was redissolved in DCM/MeOH/$NH_{3(aq)}$ (10:10:4 V/V/V) and the resulting solution was filtered. The filtrate was then purified by flash chromatography (R_f = 0.6 DCM/MeOH/$NH_{3(aq)}$, 7:2:1 V/V/V) using a gradient of dichloromethane and dichloromethane/methanol/aqueous ammonia (25%) (10:10:4 V/V/V) from 0% to 75%. The obtained, deprotonated product was then treated with aqueous HCl and all volatiles were removed under reduced pressure afterwards to obtain product **L** as dark blue powder in 82% yield, 93 mg, 0.14 mmol.

^{1}H NMR (400 MHz, MeOD) δ: 8.94 (s, 1H) , 8.89 (s, 2H), 8.70 – 8.54 (m, 6H), 7.99 (ddd, *J* = 7.3, 5.7, 1.2 Hz, 2H), 7.85 (d, *J* = 8.2 Hz, 1H), 7.45 (t, *J* = 7.8 Hz, 1H,), 7.39 – 7.31 (m, 2H), 6.91 (s, 1H), 6.82 – 6.76 (m, 2H), 6.11 (s, 2H), 6.02 (d, *J* = 2.1 Hz, 1H), 5.12 (s, 2H), 3.14 (s, 6H).

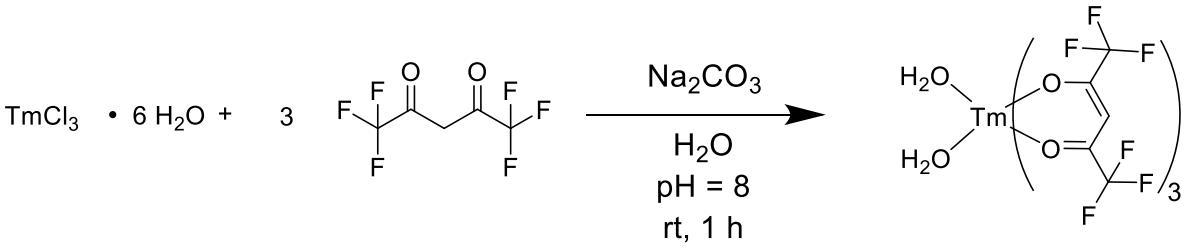
MS (ESI+): 616.20 $[M+H]^+$.

IR (ATR-IR): $\tilde{\nu}$ = 1581, 1323, 1290, 1201, 1174, 1139, 1005, 785, 717 cm^{-1}.

UV-Vis: $\lambda_{max,\ abs.}$ (MeOH) = 636 nm.

Fluorescence: $\lambda_{max,\ emm.}$ (MeOH) = 669 nm.

Synthesis of diaquatris(hfac)thulium(III) [18Tm]



Scheme S 11: Synthesis of diaquatris(hfac)thulium(III) [18Tm].

In a 100 ml round bottom flask, 2.18 ml (3.25 g ,15.7 mmol) 1,1,1,5,5,5-hexafluoro-2,4-pentanedione was dissolved in 50 ml water and the pH was adjusted with sodium carbonate to pH = 8. Afterwards, a solution of 2.00 g (5.2 mmol) thulium chloride hexahydrate in 20 ml water was added slowly under vigorous stirring. The solution was stirred at room temperature for 3 h and then extracted with diethyl ether (3 x 100 ml). The combined organic layers were washed with brine (1 x 100 ml), then dried with Na_2SO_4 and filtered afterwards. The solvent was removed under reduced pressure and the remaining waxy solid was recrystalised from *n*-hexane. The product **18Tm** was obtained as pale-pink crystals in 64% yield, 2.75 g, 3.33 mmol.

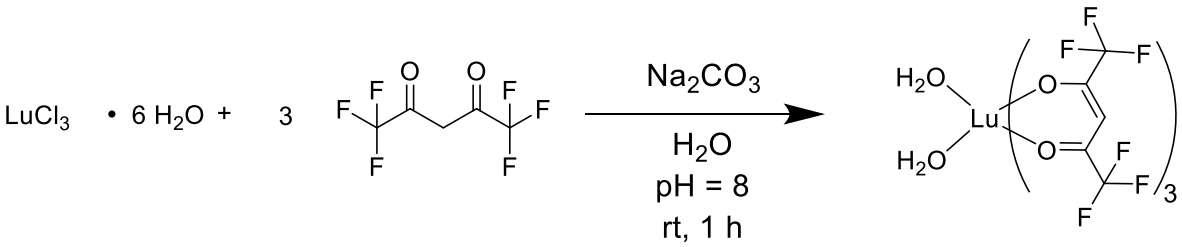
^{19}F NMR (235 MHz, DCM-d_2) δ: -105.4.

EA: Anal. calc. for $C_{15}H_7TmF_{18}O_8$: C, 21.81; H, 0.85; N, 0.00. found: C, 22.07; H, 0.79; N, 0.05.

MS (ESI+): *m / z* 698.67 [M- 2 H_2O-hfac+2 acetone]$^+$.

IR (ATR-IR): $\tilde{\nu}$ = 3493, 1740, 1647, 1618, 1568, 1539, 1477, 1353, 1254, 1221, 1204, 1146, 1103, 808, 743, 664 cm^{-1}.

Synthesis of diaquatris(hfac)lutetium(III) [18Lu]



Scheme S 12: Synthesis of diaquatris(hfac)lutetium(III) [18Lu].

In a 50 ml round bottom flask, 0.61 ml (0.91 g, 4.37 mmol) 1,1,1,5,5,5-hexafluoro-2,4-pentanedione was dissolved in 15 ml water and the pH was adjusted with sodium carbonate to pH = 8. Afterwards, a solution of 0.57 g (1.46 mmol) lutetium chloride hexahydrate in 20 ml water was added slowly under vigorous stirring. The solution was stirred at room temperature for 3 h and then extracted with diethyl ether (3 x 50 ml). The combined organic layers were washed with brine (1 x 50 ml), then dried with Na_2SO_4 and filtered afterwards. The solvent was removed under reduced pressure and the remaining waxy solid was recrystalised from *n*-hexane. The product **18Lu** was obtained as colourless crystals in 49% yield, 0.60 g, 0.72 mmol.

^{1}H NMR (400 MHz, DCM-d_2) δ: 6.16 (s, 3H).

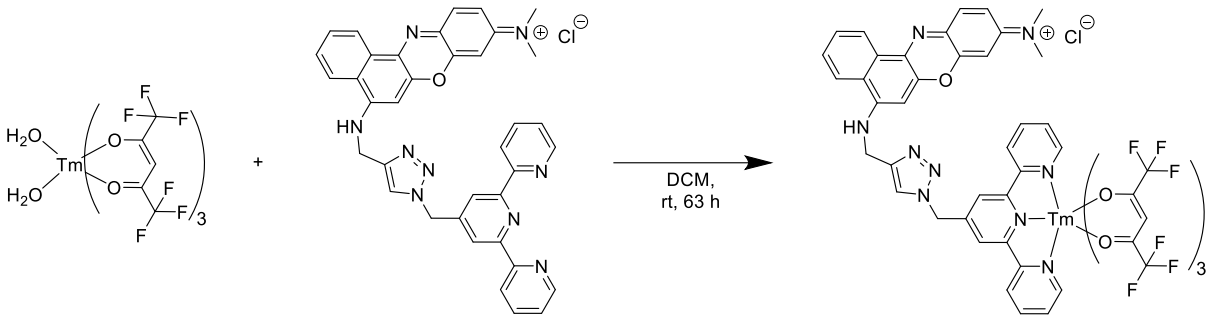
^{19}F NMR (235 MHz, DCM-d_2) δ: -77.4.

EA: Anal. calc. for $C_{15}H_7LuF_{18}O_8$: C, 21.65; H, 0.85; N, 0.00. found: C, 21.80; H, 0.79; N, 0.04.

MS (ESI+): *m / z* 706.59 [M- 2 H_2O-hfac+2 acetone]$^+$.

IR (ATR-IR): $\tilde{\nu}$ = 1740, 1651, 1562, 1539, 1506, 1473, 1351, 1254, 1217, 1202, 1146, 1103, 804, 665 cm^{-1}.

Synthesis of tris(hfac)(L)thulium [TmL]



Scheme S 13: Synthesis of tris(hfac)(L)thulium [TmL].

In a 100 ml round bottom flask, 30 mg (46 µmol) of ligand **15** and 38 mg (46 µmol) diaquatris(hfac)thulium(III) **18Tm** were dissolved in 50 ml dichloromethane. Due to the poor solubility of the ligand and the high solubility of the target compound, the solution turned from a pale blue to an intense blue over time. After stirring for 63 h, the solution was filtered and all volatiles of the filtrate were removed under reduced pressure. The product **19Tm** was obtained as blue solid in 55% yield, 36.5 mg, 25 µmol.

^{19}F NMR (235 MHz, DCM-d_2) δ: -99.9.

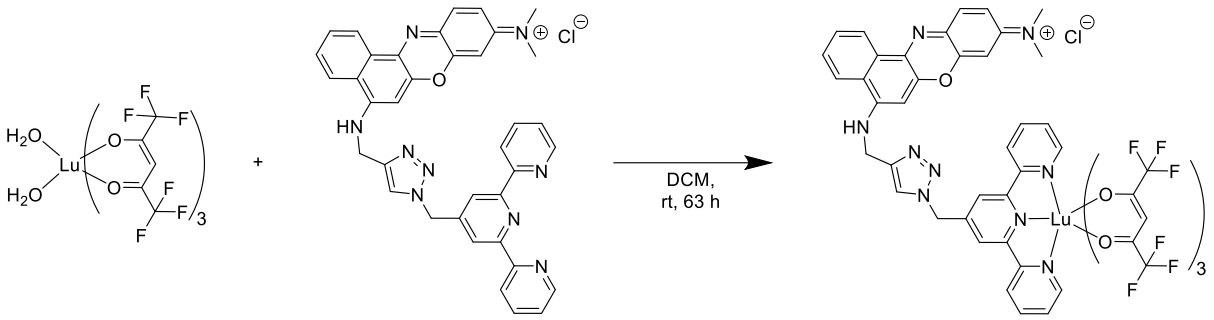
MS (ESI+): *m / z* 1197.61 $[M\text{-hfac}]^+$.

IR (ATR-IR): $\tilde{\nu}$ = 1653, 1253, 1203, 1139, 796 cm^{-1}.

UV-Vis: $\lambda_{max,\ abs.}$ (MeOH) = 635 nm.

Fluorescence: $\lambda_{max,\ em.}$ (MeOH) = 676 nm.

Synthesis of tris(hfac)(L)lutetium [LuL]



Scheme S 14: Synthesis of tris(hfac)(L)lutetium [LuL].

In a 100 ml round bottom flask, 30 mg (46 µmol) of ligand **15** and 38 mg (46 µmol) diaquatris(hfac)lutetium(III) **18Lu** were dissolved in 50 ml dichloromethane. Due to the poor solubility of the ligand and the high solubility of the target compound, the solution turned from a pale blue to an intense blue over time. After stirring for 63 h, the solution was filtered and all volatiles of the filtrate were removed under reduced pressure. The product **19Lu** was obtained as blue solid in 63% yield, 41.9 mg, 29 µmol.

^{19}F NMR (235 MHz, DCM-d_2) δ: -77.3.

MS (ESI+): *m / z* 1203.52 $[M\text{-hfac}]^+$.

IR (ATR-IR): $\tilde{\nu}$ = 1656, 1504, 1254, 1200, 1136, 1099, 796 cm^{-1}.

UV-Vis: $\lambda_{max,\ abs.}$ (MeOH) = 636 nm.

Fluorescence: $\lambda_{max,\ em.}$ (MeOH) = 672 nm.

Compound Characterisation

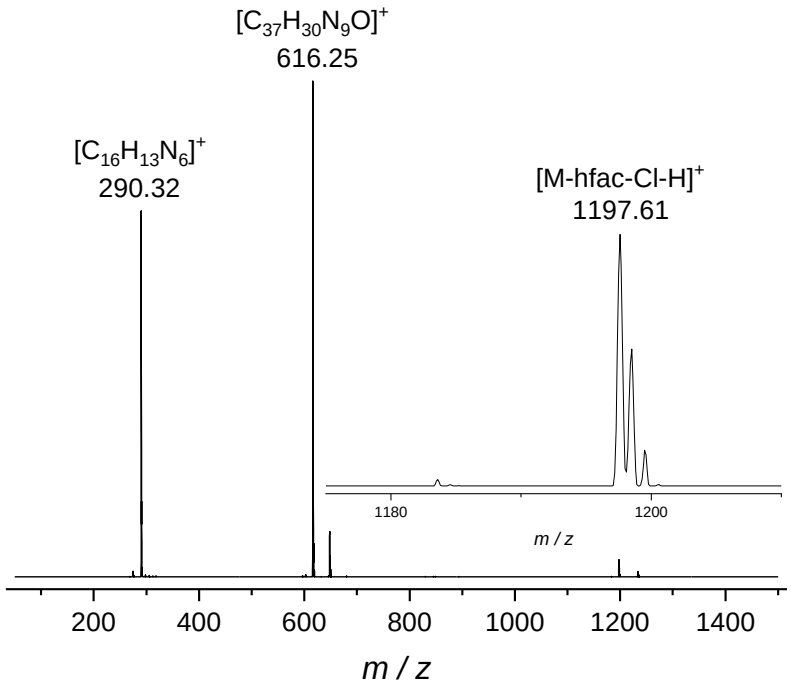


Figure S. 1: ESI-MS (+) of complex **TmL.**

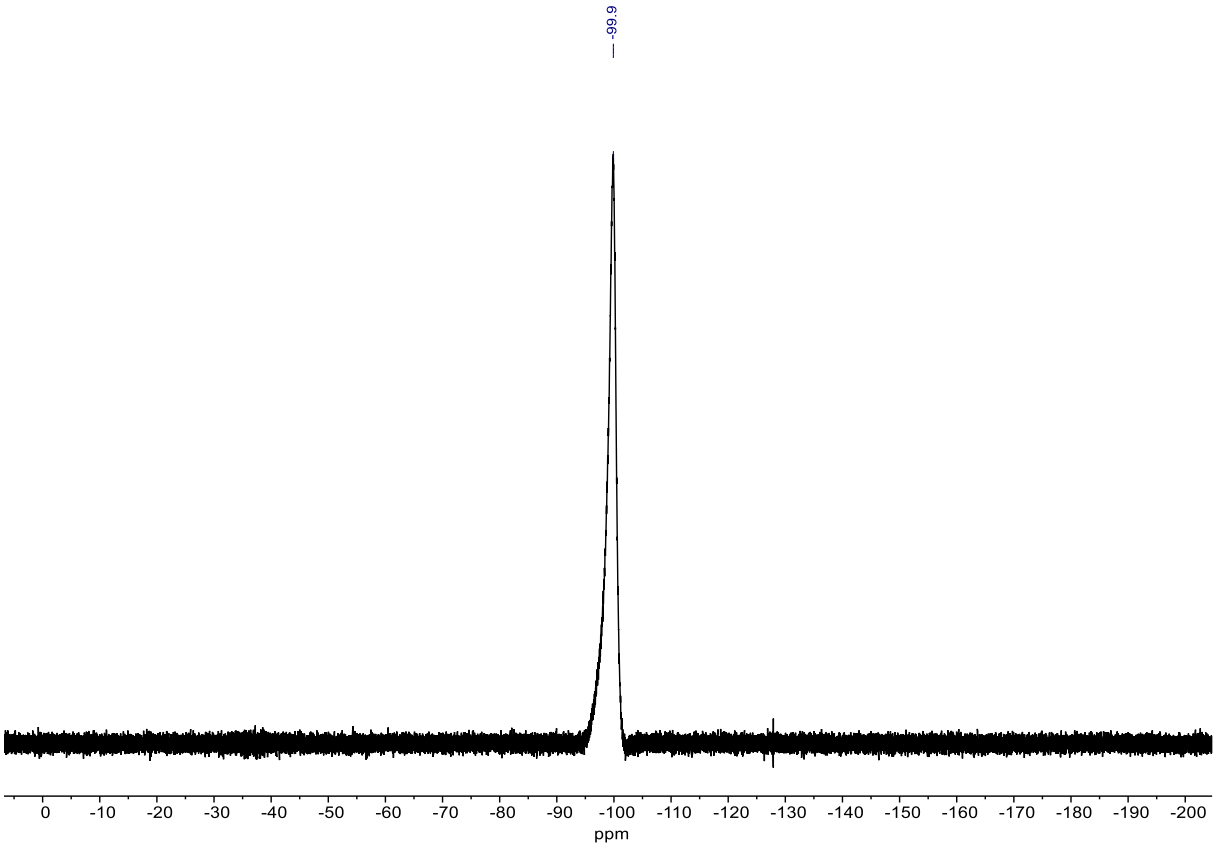


Figure S. 2: ^{19}F NMR of of complex **TmL** in DCM-d_2.

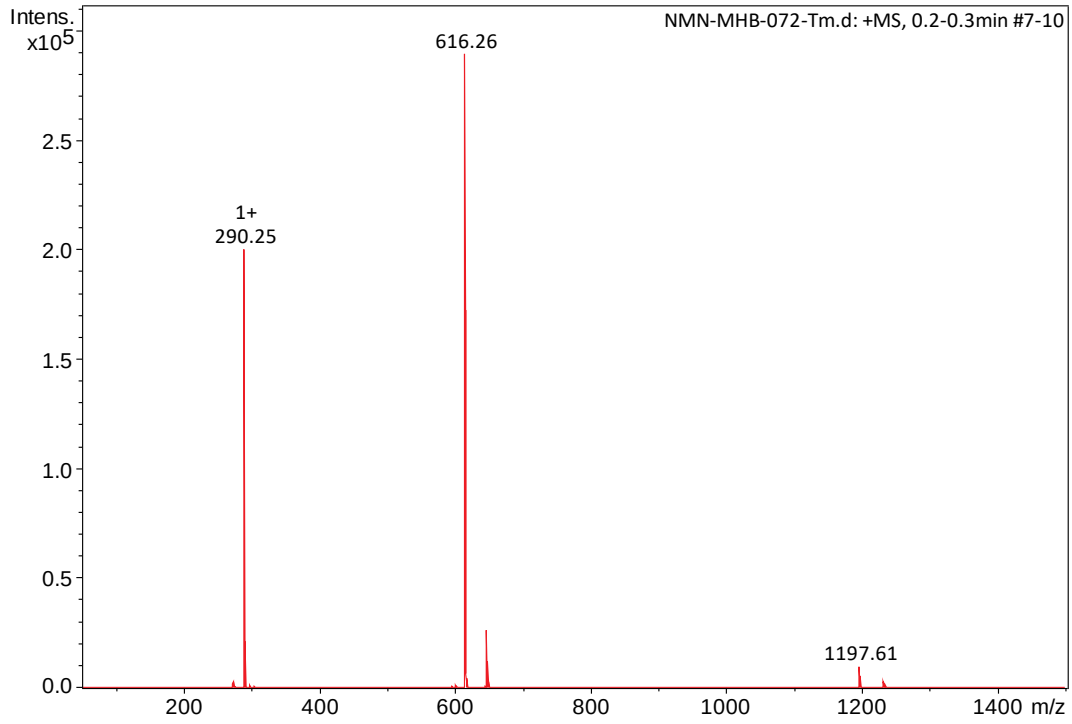


Figure S. 3: ESI-MS (+) of complex **TmL** – Stability study in PBS after 0 hours

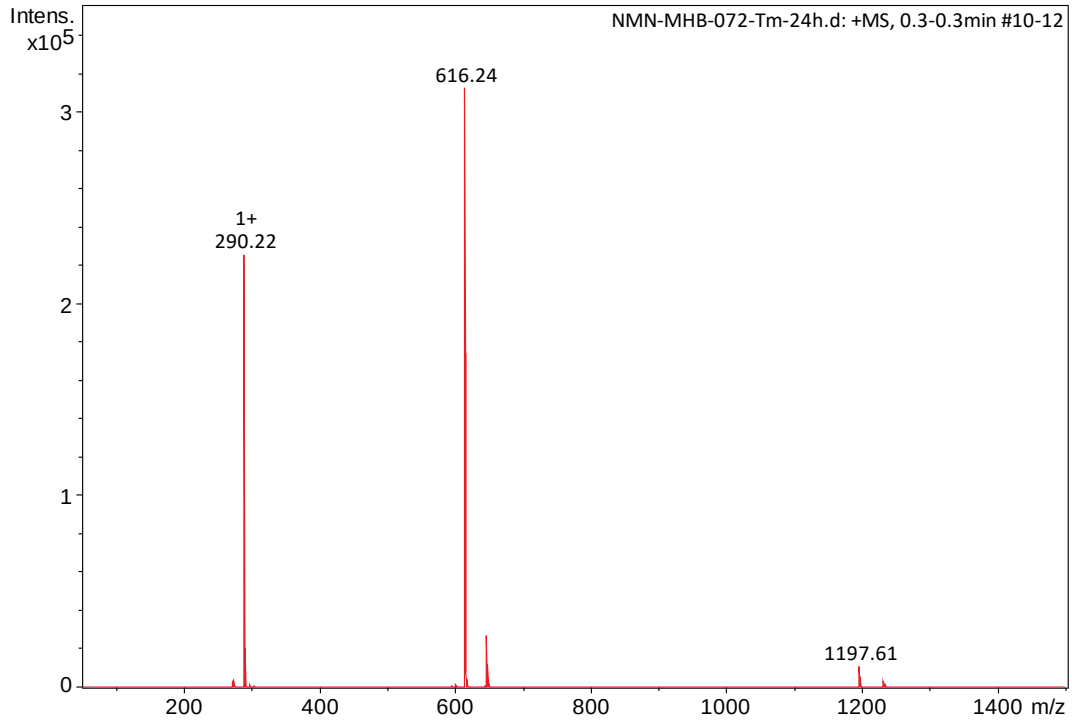


Figure S. 4: ESI-MS (+) of complex TmL – Stability study in PBS after 24 hours

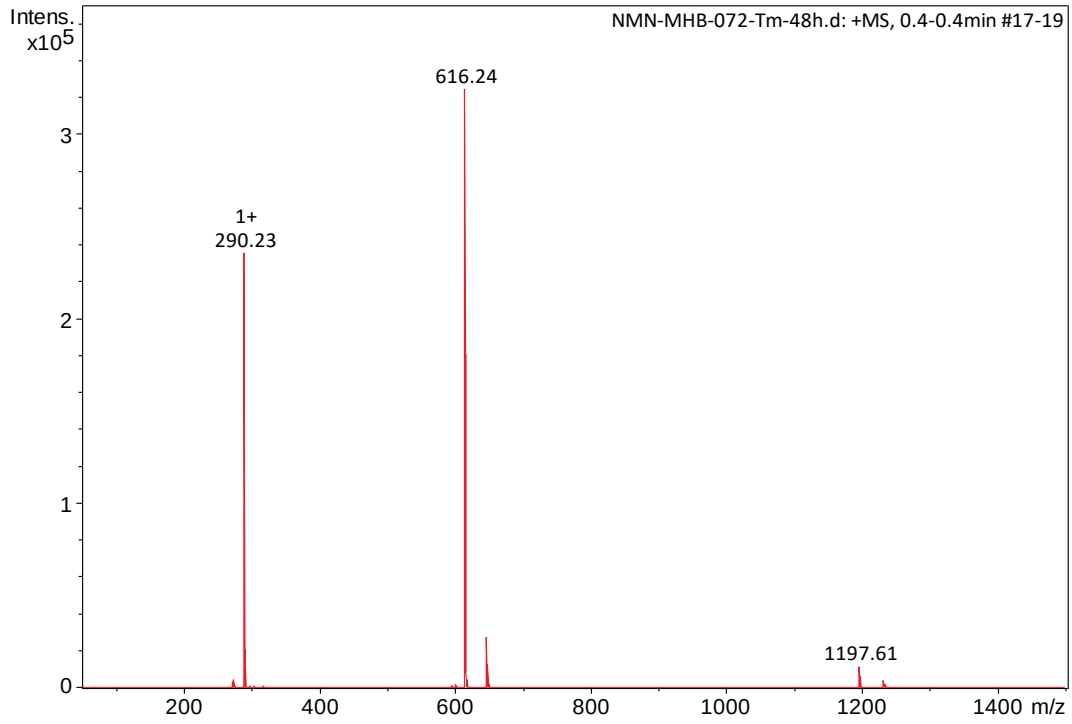


Figure S. 5: ESI-MS (+) of complex **TmL** – Stability study in PBS after 48 hours

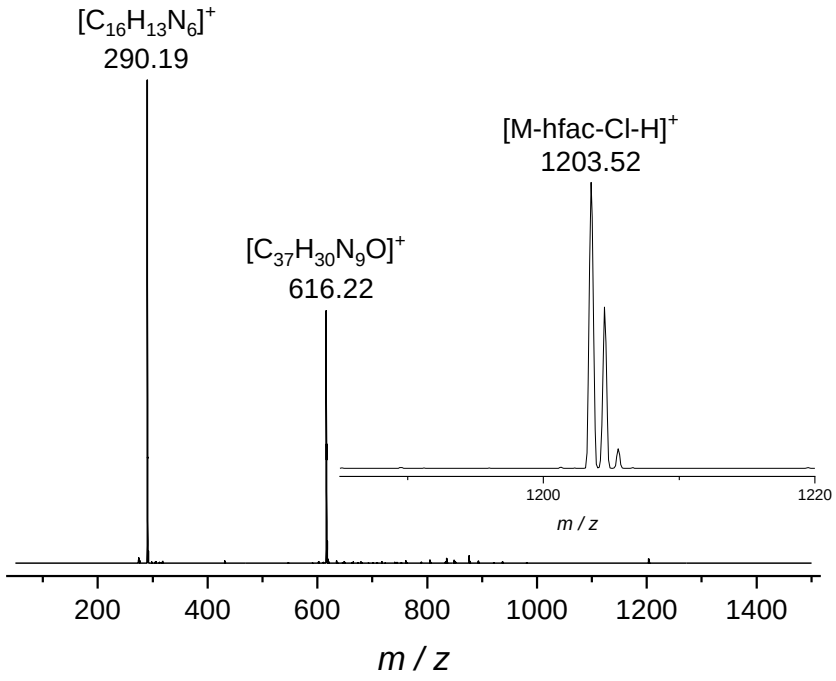


Figure S. 6: ESI-MS (+) of complex **LuL**.

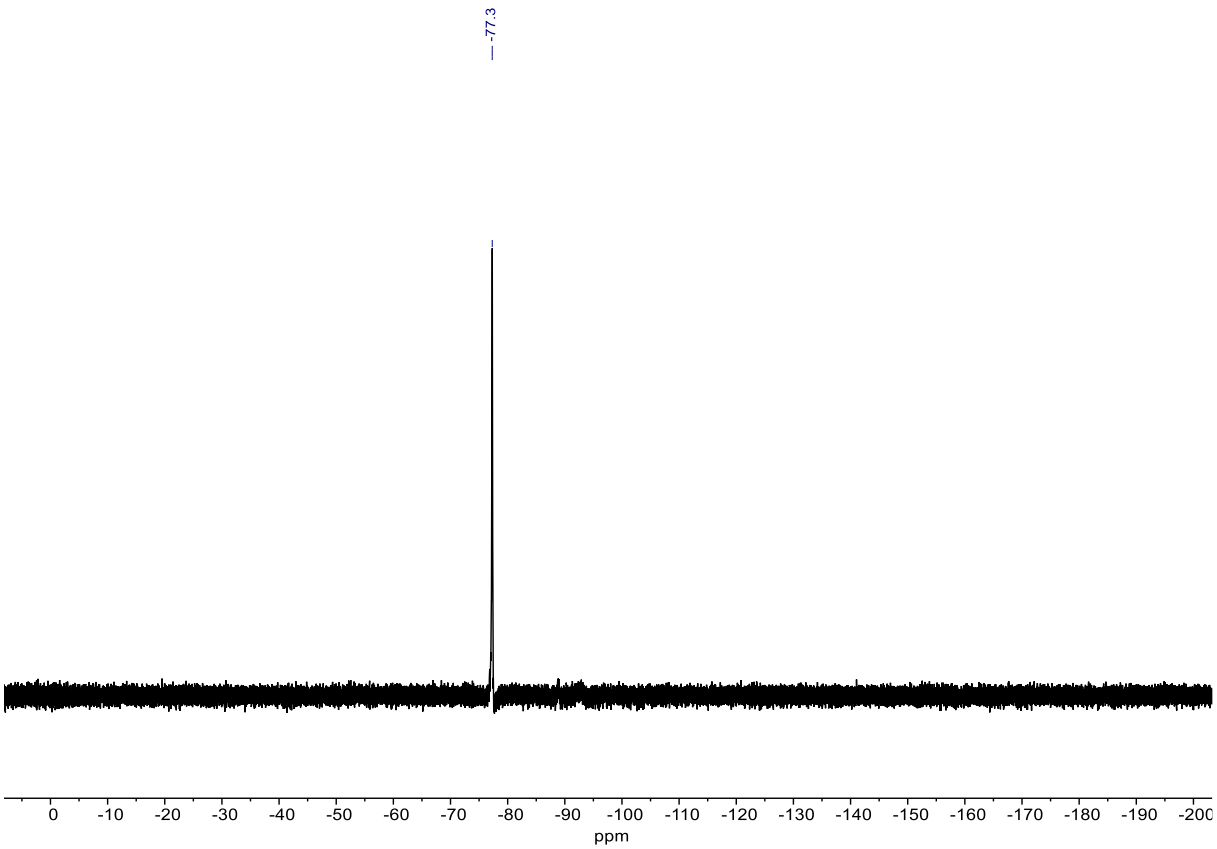


Figure S. 7: ^{19}F NMR of of complex **LuL** in DCM-d_2.

Absorption and Emission

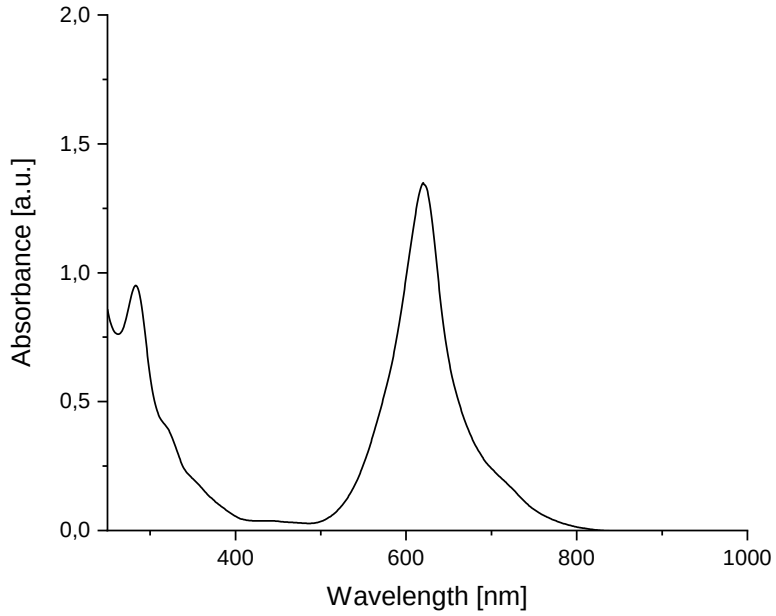


Figure S. 8: UV-Vis spectrum of ligand L at 25 µM in PBS with 0.1% DMSO.

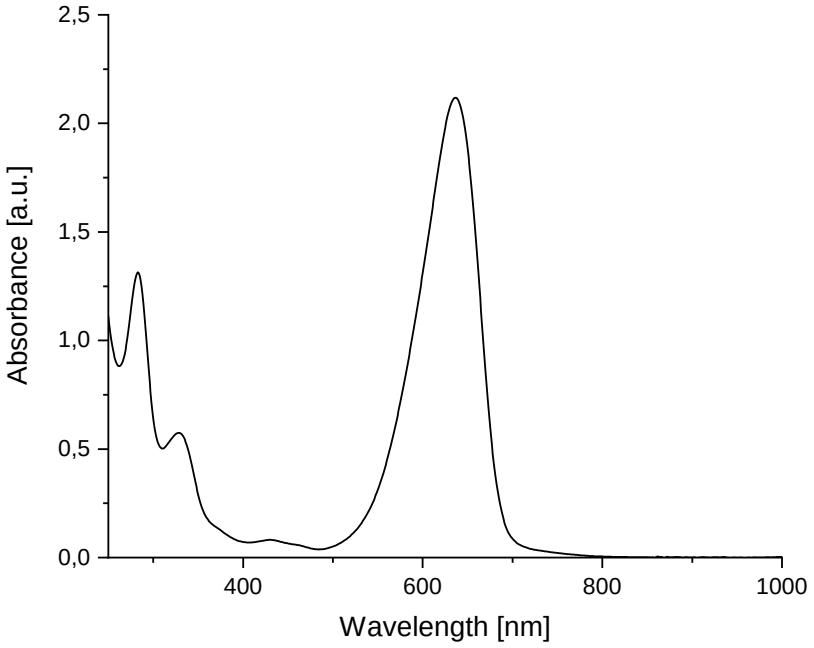


Figure S. 9: UV-Vis spectrum of ligand **L** at 25 µM in MeOH with 0.1% DMSO.

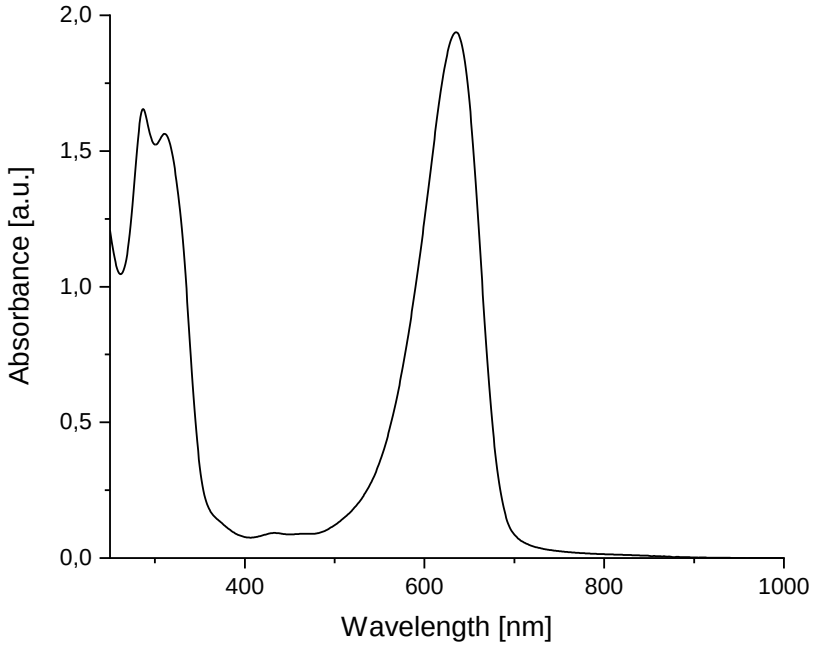


Figure S. 10: UV-Vis spectrum of ligand **TmL** at 25 µM in PBS with 0.1% DMSO.

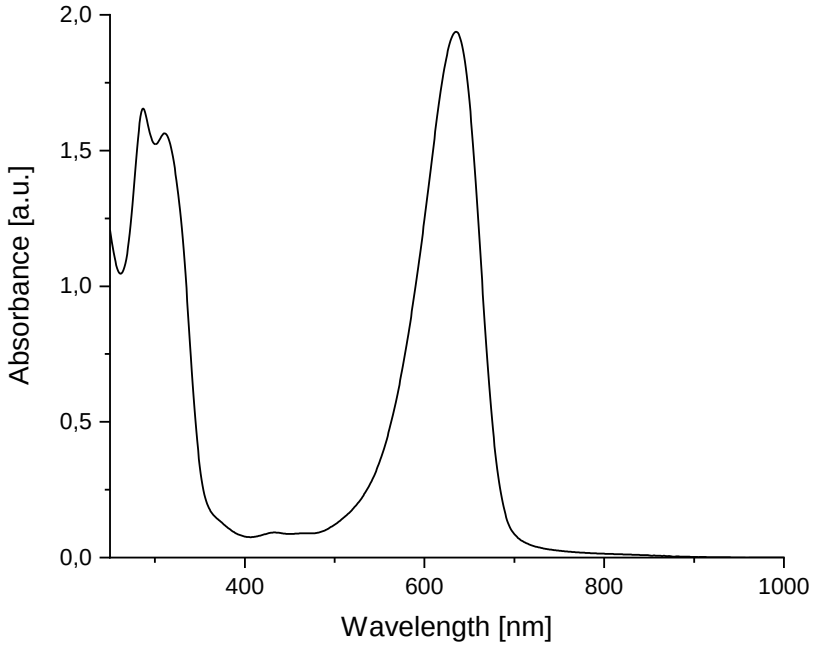


Figure S. 11: UV-Vis spectrum of complex **TmL** at 25 µM in MeOH with 0.1% DMSO.

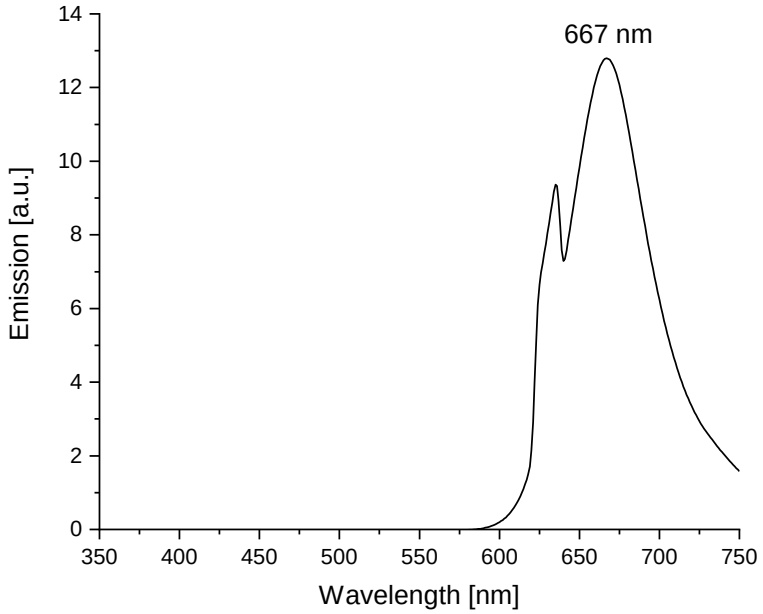


Figure S. 12: Fluorescence emission spectra of ligand **L** at 25 µM in PBS with 0.1% DMSO, 630 nm excitation (exc. slit = 5 nm, em. slit = 5 nm).

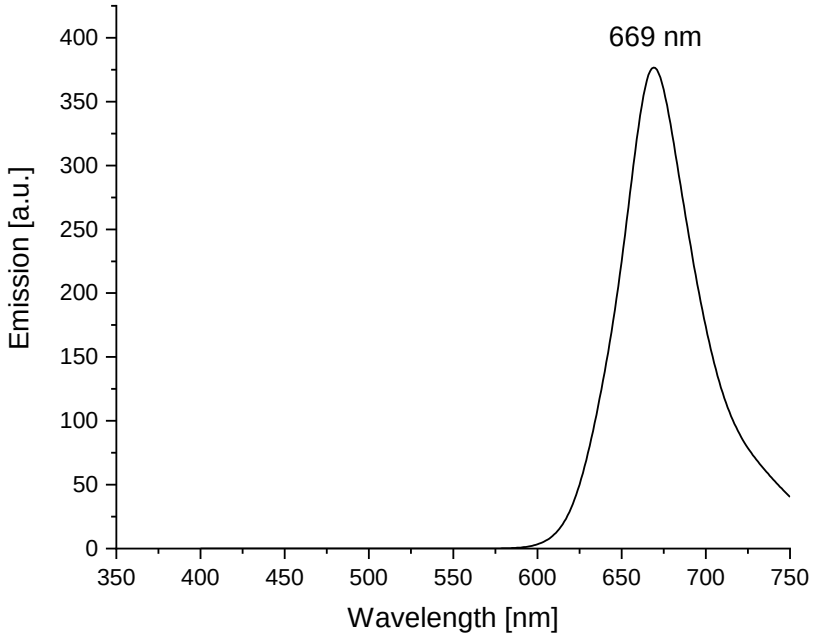


Figure S. 13: Fluorescence emission spectra of ligand **L** at 25 µM in MeOH with 0.1% DMSO, 630 nm excitation (exc. slit = 5 nm, em. slit = 5 nm).

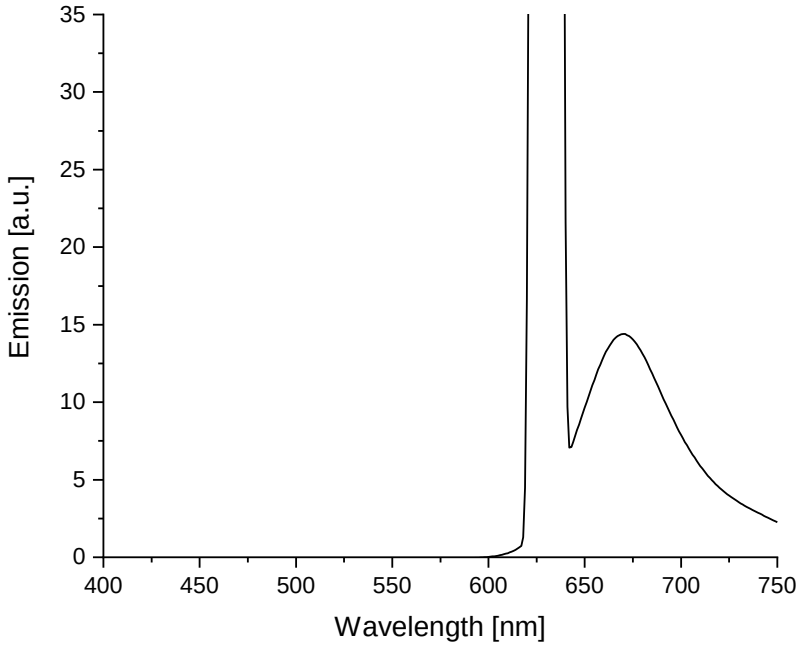


Figure S. 14: Fluorescence emission spectrum of target compound **TmL** at 25 µM in PBS with 0.1% DMSO, 630 nm excitation (exc. slit = 5 nm, em. slit = 5 nm).

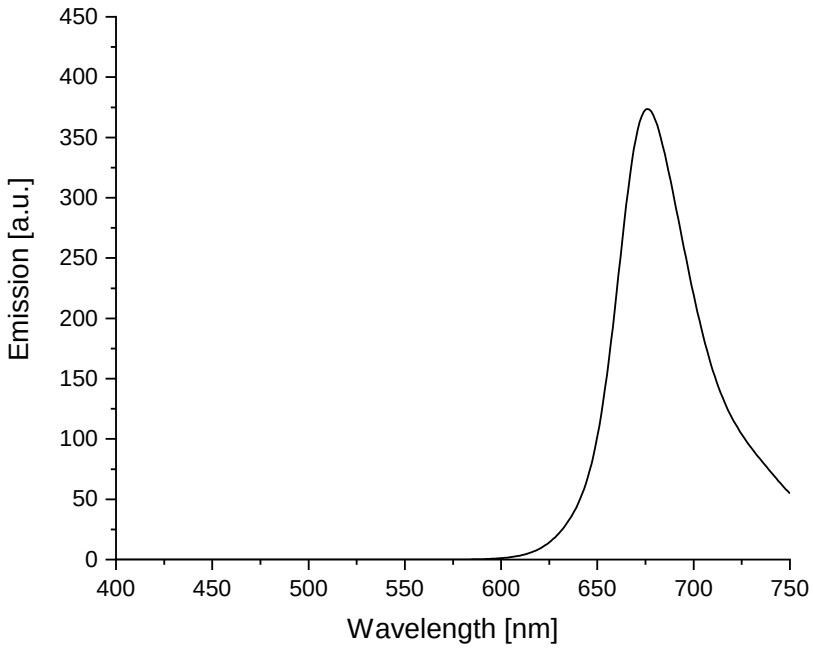


Figure S. 15: Fluorescence emission spectrum of target compound **TmL** at 25 µM in PBS with 0.1% DMSO, 630 nm excitation (exc. slit = 5 nm, em. slit = 5 nm).

In solution Singlet Oxygen Detection

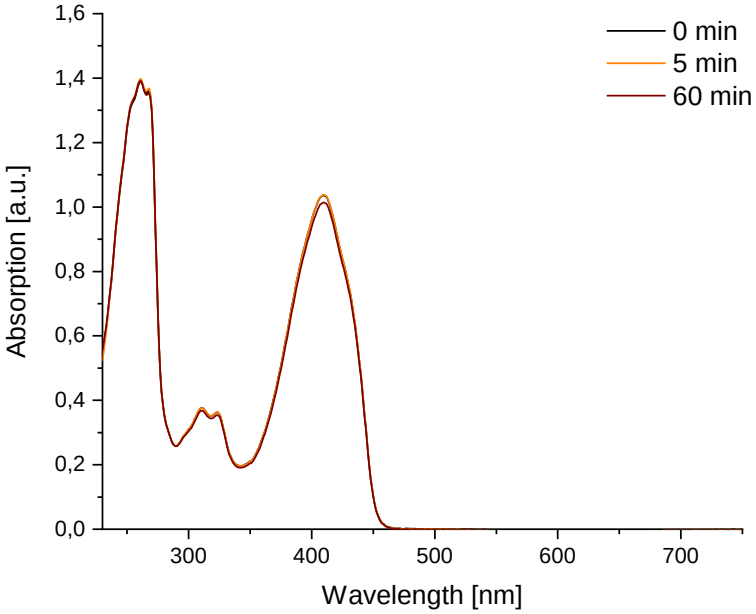


Figure S. 16: UV-Vis spectra of 1% saturated DPBF solution in MeOH with 0.1% DMSO upon irradiation over 60 minutes

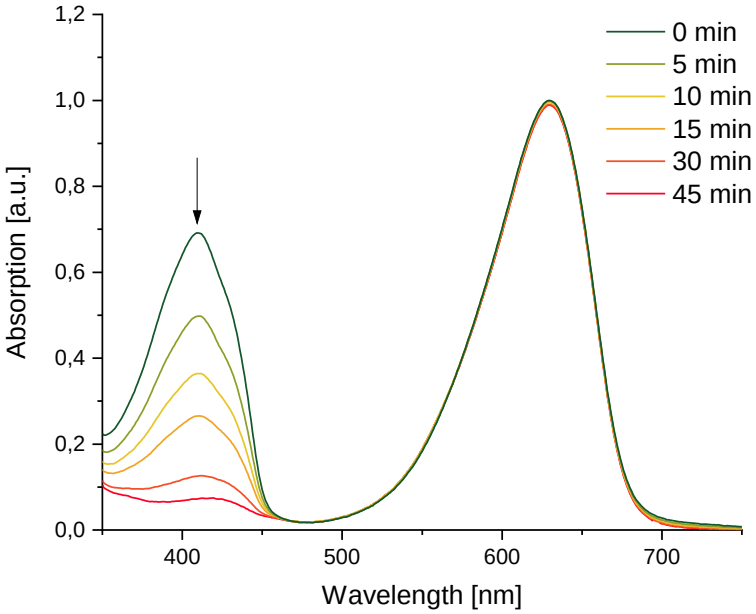


Figure S. 17: UV-Vis spectra of 10 µM **L** and 1% saturated DPBF solution in MeOH with 0.1% DMSO upon irradiation over 45 minutes.

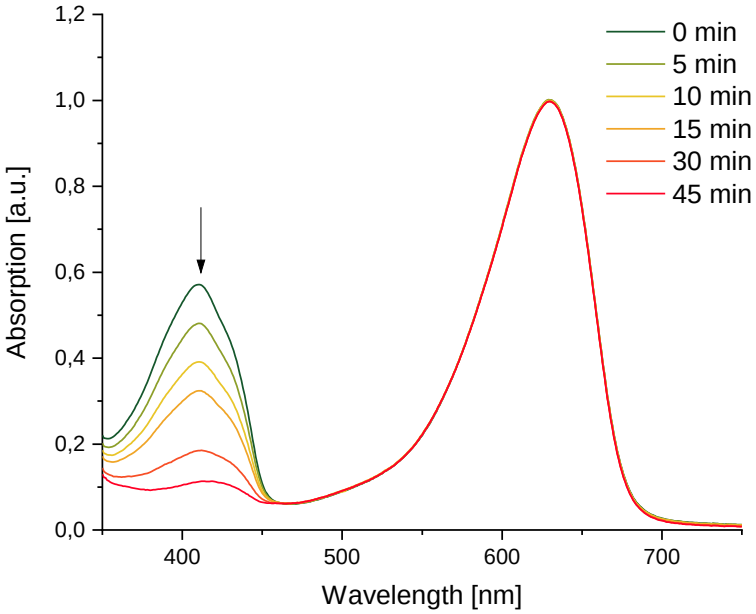


Figure S. 18: UV-Vis spectra of 10 µM **LuL** and 1% saturated DPBF solution in MeOH with 0.1% DMSO upon irradiation over 45 minutes.

Cell studies

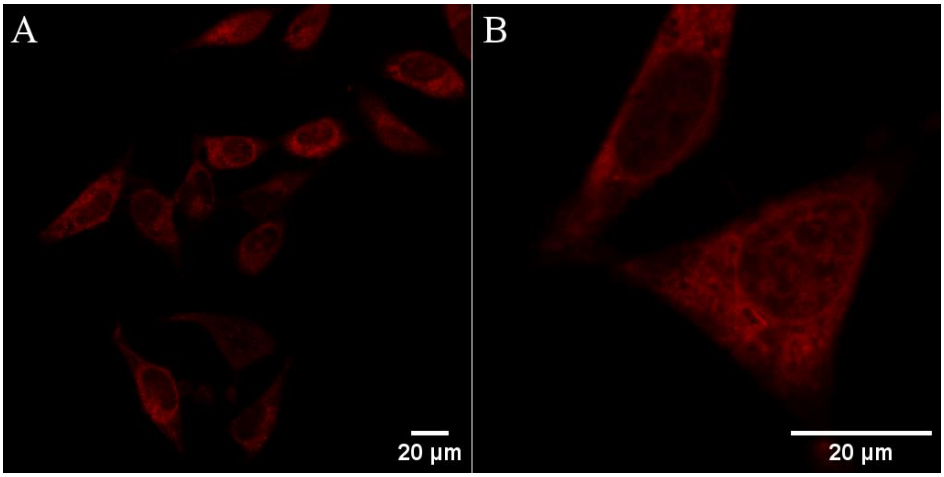


Figure S. 19 Uptake of **TmL** live HeLa cells. Cells were treated with the complex at 10 µM for 1 h at 37 °C at 5% CO_2 in the dark. A 630 nm white light laser was used to excite **TmL** and emission was collected between 640 and 795 nm.

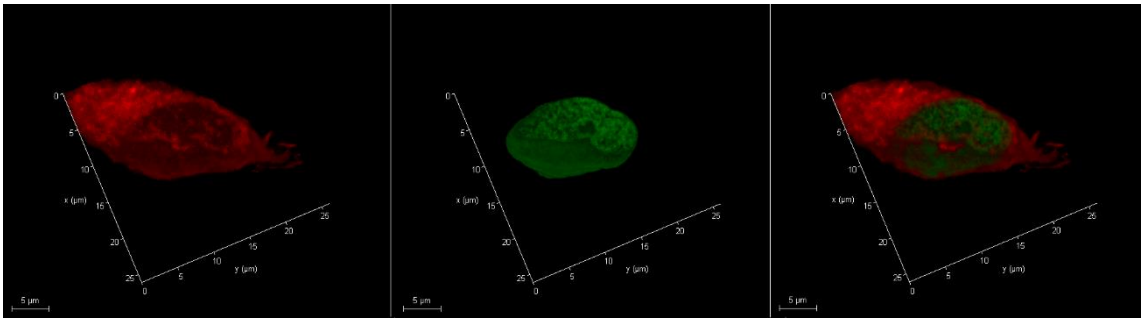


Figure S. 20: Z-stack images of a single live HeLa cell stained with **TmL** at 10 µM/ 1 h and co-stained with SYTO 11 Green. The overlay of the **TmL** (in red) and SYTO 11 Green (in green) emission are shown. A 630 nm white light laser was used to excite **TmL** and emission was collected between 640 and 795 nm and SYTO 11 Green was excited at 508 nm and emission was collected between 517 – 530.

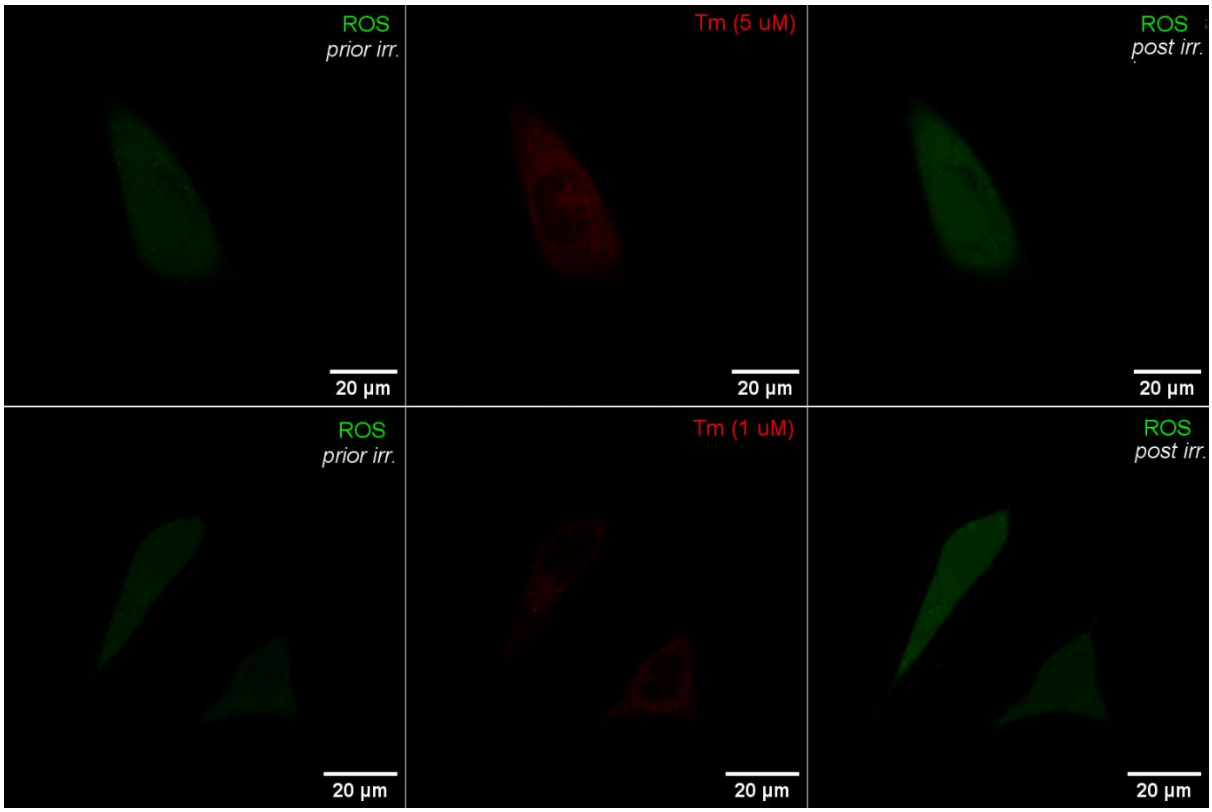


Figure S. 21: Intracellular ROS detection in HeLa cells pre-treated with **TmL** at 5 µM (top) and 1 µM (bottom) for 1 h in the absence of light. Cells were washed with PBS post incubation with the complex followed by addition of H_2DCFDA (5 µM/ 30 min). Emission from the ROS dye was collected (λ_{exc} 494 nm; λ_{em} 517 – 527 nm) before and after continuous irradiation at 630 nm. An image of the **TmL** complex in cells was also collected with excitation at 630 and emission set at 640 – 795 nm.

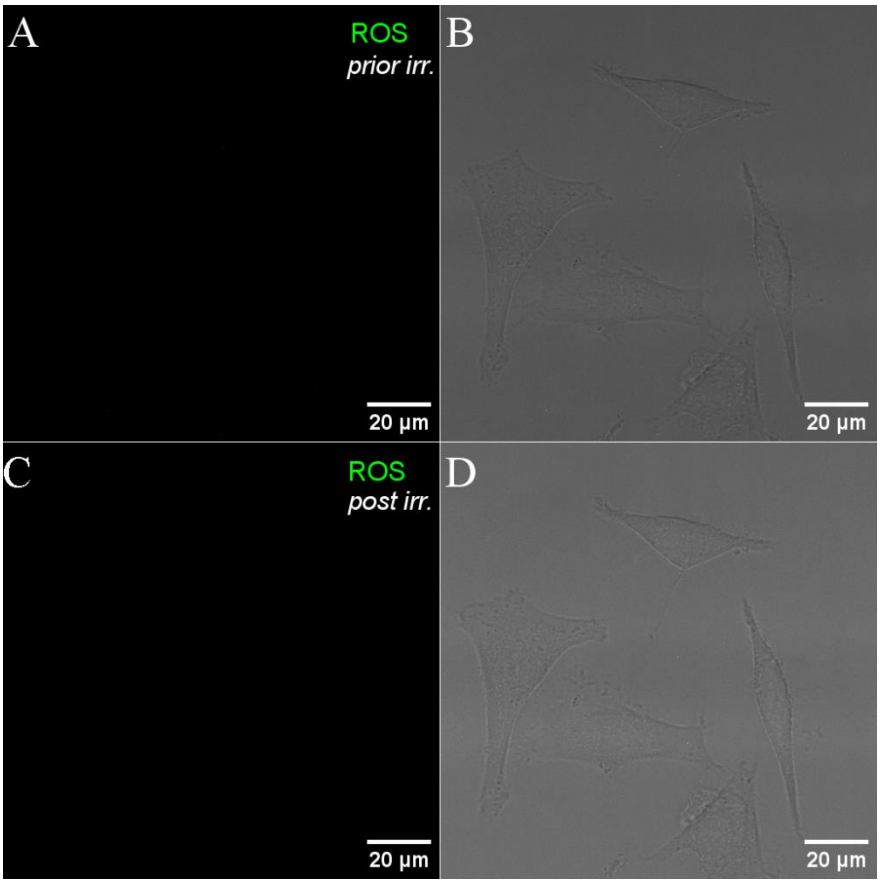


Figure S. 22 Intracellular ROS detection in non-treated (control) HeLa. Cells were seeded for imaging and incubated with the ROS detection dye, H_2DCFDA (5 µM/ 30 min). Emission from the ROS dye was collected (λ_{exc} 494 nm; λ_{em} 517 – 527 nm) before (A, B) and after (C, D) continuous irradiation at the experimental conditions for the TmL (λ_{exc} at 630 nm). In the absence of the **TmL** complex, no emission from the ROS dye was observed post-irradiation.

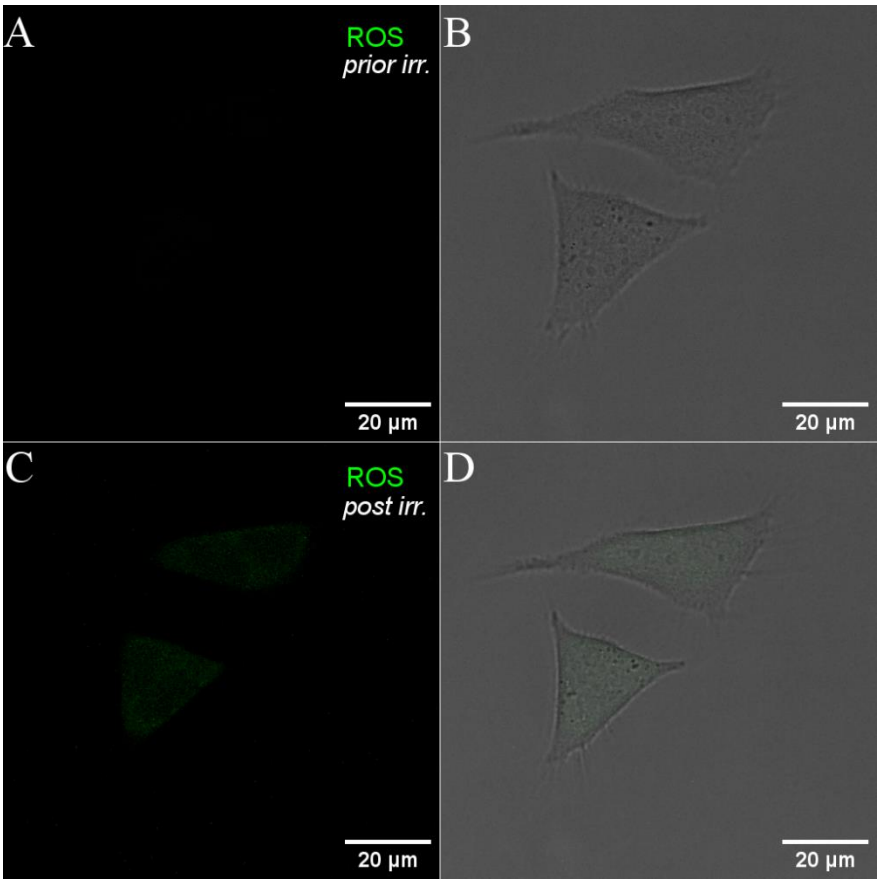


Figure S. 23: Intracellular ROS detection in HeLa cells pre-treated with Photofrin (positive control). Cells were seeded for imaging, pre-treated with Photofrin (10 µM/ 1 h), washed with PBS and incubated with H_2DCFDA (5 µM/ 30 min). Emission from the ROS dye was collected (λ_{exc} 494 nm; λ_{em} 517 – 527 nm) before (A, B) and after (C, D) continuous irradiation. Emission from the ROS dye was observed after continuous irradiation.

Notes and References

1 T. Mosmann, *Journal of Immunological Methods*, 1983, **65**, 55–63.

2 D. Obitz, R. G. Miller and N. Metzler-Nolte, *Dalton Trans.*, 2021, **50**, 13768–13777.