Electronic Supporting Information

A Self-Assembled Pt^{II}_8 Metallosupramolecular Tubular Cage as Dual Warhead Antibacterial Agent in Water

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Contents:

1. Experimental Section

Materials and Methods: All chemicals and solvents were purchased from commercial sources and used directly without further purification. Bruker 400 and 500 MHz instrument were used to record NMR spectra and TMS (Me_4Si) was used as an internal standard. An electrospray ionization (ESI) technique equipped Q-TOF instrument was used to record high resolution mass spectra in standard spectroscopic grade solvents. Electronic absorption spectra were recorded on Shimadzu UV-2600 UV−visible spectrophotometer. A Carl-Zeiss Ultra 55 instrument with an operating voltage of 3-20 kV was used to perform Scanning Electron Microscopy (SEM). Atomic force micrograph (AFM) was performed using JPK Instruments, USA. Philips 60W LED lamp (400-700 nm) with white light was used for visible light source.

Synthesis Procedure for Ligand L: 4,7-Dibromobenzo[c]-1,2,5-thiadiazole (0.6 g, 2.04 mmol) and bis(4-pyridyl)amine (1g, 6 mmol), anhydrous $CuSO_4$ (0.16g, 1.0 mmol), K_2CO_3 (1.12g, 8.16 mmol) and 18-Crown-6 (0.01g, 0.04 mmol) were taken in a two-neck round-bottom flask containing diphenyl ether (40 mL). The reaction mixture was degassed with N_2 and stirred at 50°C for 30 minutes followed by addition of 18-Crown-6 (0.01g, 0.04 mmol). Finally, it was stirred vigorously at 170ºC for 4 days under N_2 atmosphere. After completion of the reaction, the solvent was removed under reduced pressure. The crude product was purified by column chromatography using neutral alumina as the stationary phase and THF/$CHCl_3$ (1:1) as eluent to afford reddish brown solid (65%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) = 8.50 (d, 8H), 7.50 (s, 2H), 6.99 (d, 8H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 152.5, 151.9, 151.2, 134.7, 127.7, 116.8. HRMS (ESI): $C_{26}H_{19}N_8S$, $[M+H]^+$ = 475.1447 (calculated) found: 475.0831 (100%).

Synthesis Procedure for PMB1: L (0.020g, 0.0426 mmol) and *cis*-[$Pt(NO_3)_2$(eda)] (0.033g, 0.0852 mmol) were taken in a 4 mL glass vial and dissolved in 2 mL Millipore water. The resulting orange solution was stirred at 55 °C for 12 hours. The solution was centrifuged, and the clear supernatant was treated with acetone to afford an orange precipitate which was recrystallized by slow vapour diffusion of acetone to afford pure crystalline product. Yield of isolated pure **PMB1**:0.047 g (90%). ^{1}H NMR (D_2O, 500 MHz): δ (ppm) = 8.58 (d, 2H), 8.29 (d, 2H), 8.12 (s, 2H), 8.08 (br, 4H), 7.62 (d, 2H), 7.07 (d, 2H), 6.98 (br, 2H), 2.92(s, 8H). ESI-MS (m/z) = 1419.144 $[M-4PF_6]^{4+}$, 898.4466 $[M-6PF_6]^{6+}$, 749.1027 $[M-7PF_6]^{7+}$, 637.6000 $[M-8PF_6]^{8+}$.

Synthesis of [L_{Me4}][4NO_3]: A 100 mL round bottom flask was charged with **L** (0.047g, 0.1 mmol) and iodomethane (0.142g, 1 mmol) in acetonitrile. This mixture was heated to 60°C and kept for overnight. An orange precipitate was observed upon completion of the reaction. This precipitate was washed several times with cold ether, and this was suspended in 8 mL methanol. To this solution $AgNO_3$ (0.035g, 0.207 mmol) was added and the resulting mixture was stirred in dark for 12 h. The resulting precipitate was filtered, and the residual solvent was concentrated, and cold diethyl ether was added to afford greenish yellow precipitate. It was washed three times with diethyl ether (10 mL) and finally dried to afford greenish yellow solid (0.030g, 76%). ^{1}H NMR (D_2O, 400 MHz): δ (ppm) = 8.70 (d, 8H), 8.15 (s, 2H). 7.84 (d, 8H), 4.34 (s, 12H) ppm. ^{13}C NMR (100 MHz, D_2O): δ (ppm) =156.20, 152.23, 146.95, 133.50, 131.55, 119.7, 47.4 ppm. ESI-MS (m/z) = 720.2089 $[M\text{-}NO_3]^+$ (calcd = 720.1826), 329.1059 (100%) $[M\text{-}2NO_3]^{2+}$ (calcd = 329.1035).

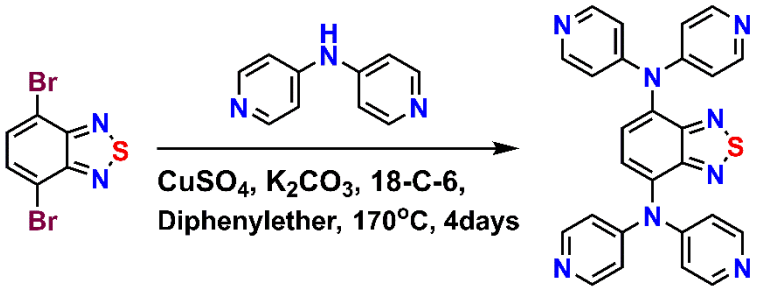
2. X-ray Crystallographic Study:

Single-crystal X-ray diffraction data of **PMB1** were collected at the X-ray diffraction beamline (XRD1) of the Elettra Synchrotron of Trieste (Italy), with a Pilatus 2M image plate detector and a monochromatic wavelength of 0.700 Å. The full data set was collected at 100 K with a nitrogen stream, and the structure was solved by direct methods using the SHELX-2007 software package incorporated in WinGX.[1] The structure was solved by direct methods and Fourier analyses. Then it was refined by the full-matrix least-squares method based on F^2 with all observed reflections using the SHELX-2013[2] program incorporated in WinGX. The non-hydrogen atoms in the main fragment were refined with anisotropic displacement parameters, and hydrogen atoms were fixed at geometrical positions. The SQUEEZE option of PLATON was used in the final cycles of refinement to account for the contribution of disordered solvent molecules to the calculated structure factors.

Table S1: Crystallographic Data and Refinement Parameters of **PMB1.**

Identification Code	**PMB1**
Empirical fromula	$C_{120}H_{104}N_{62}O_{42}Pt_8S_4$
Formula weight	4775.61
Temperature (K)	100 (2)
Wavelength	0.700
Crystal system	Triclinic
Space group	*P*-1
Unit cell dimentions	a = 17.301 (4) b = 18.321 (4) c = 19.320 (4) α = 92.09 (3) β = 104.30 (3) γ = 96.67 (3)
Volume ($Å^3$)	5881 (2)
Z	1
δ (g/cm^{-3})	1.349
Mu (mm^{-1})	4.839
F(000)	2282.0
GooF	1.507
R	0.1053
wR2	0.3321
CCDC No.	1960583

3. Synthetic Schemes and Characterization of Ligand and Cage:



Scheme S1: Synthetic route for the preparation of the ligand **L**.

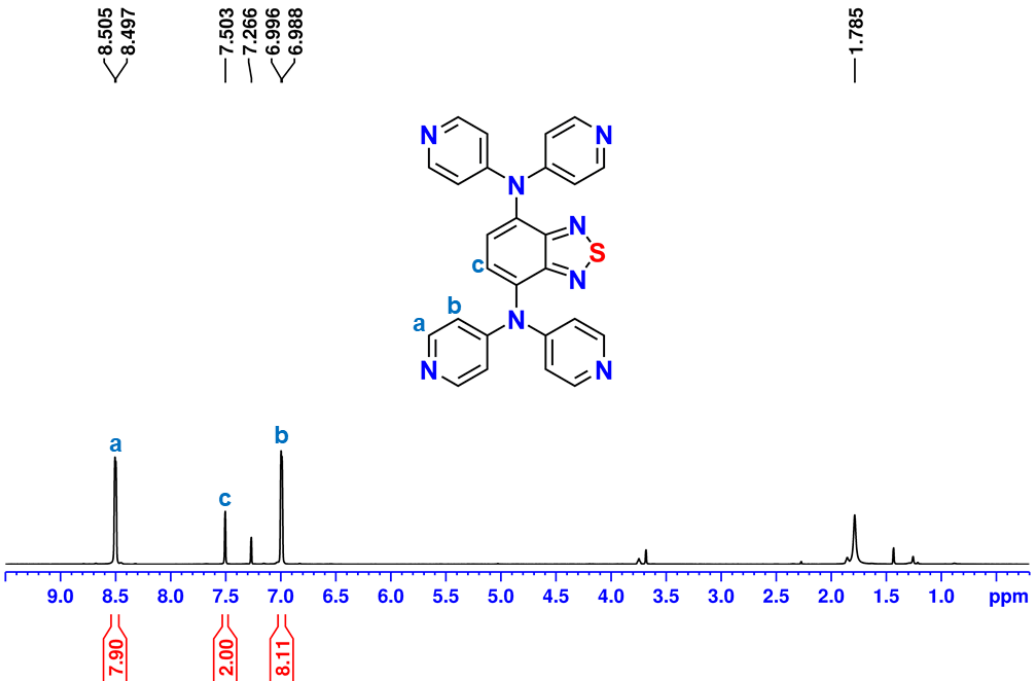


Figure S1. [1] H-NMR spectrum of **L** in $CDCl_3$.

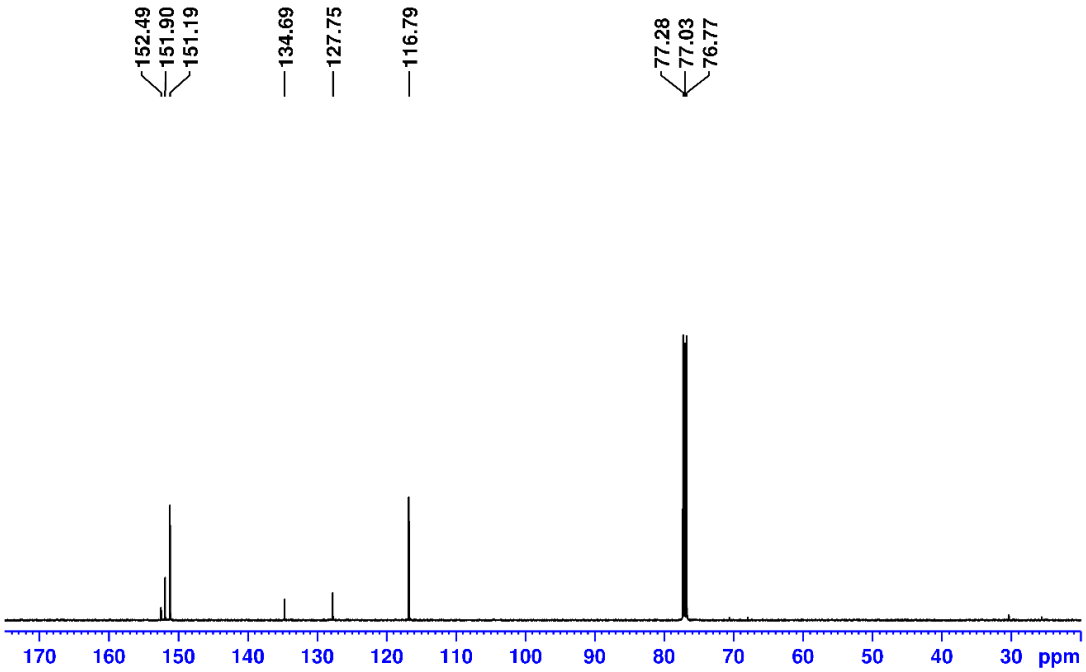


Figure S2. ^{13}C-NMR spectrum of **L** in $CDCl_3$.

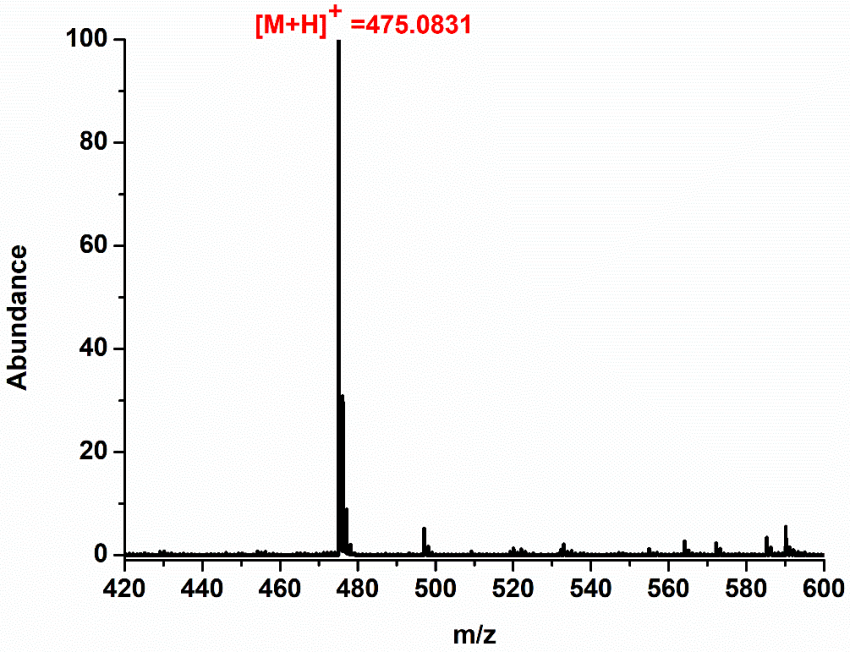


Figure S3: Mass spectrum of the ligand **L** (M = L).

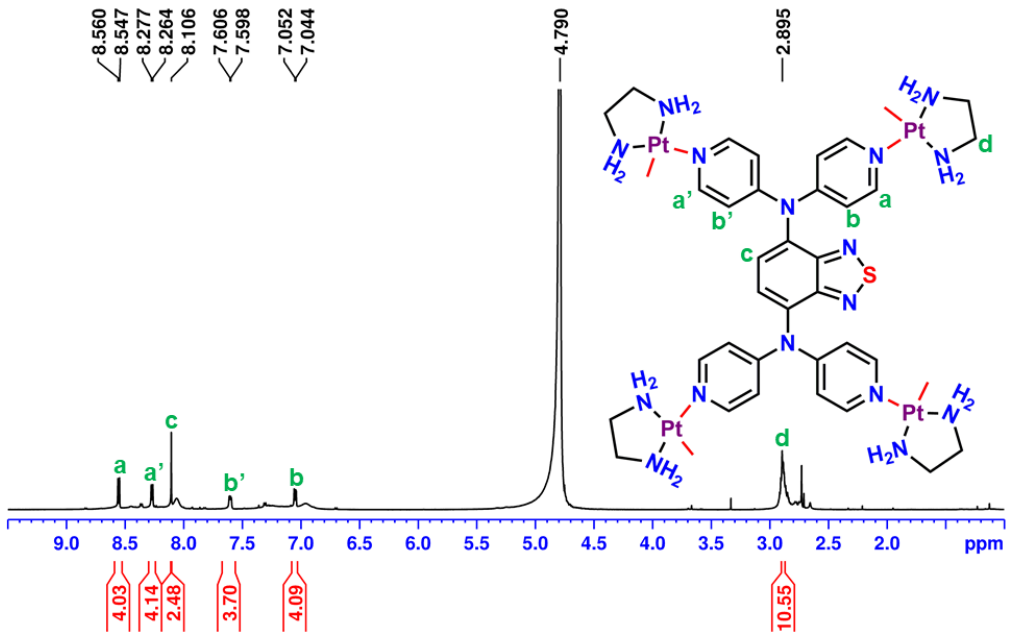


Figure S4. [1] H-NMR spectrum of **PMB1** in D_2O.

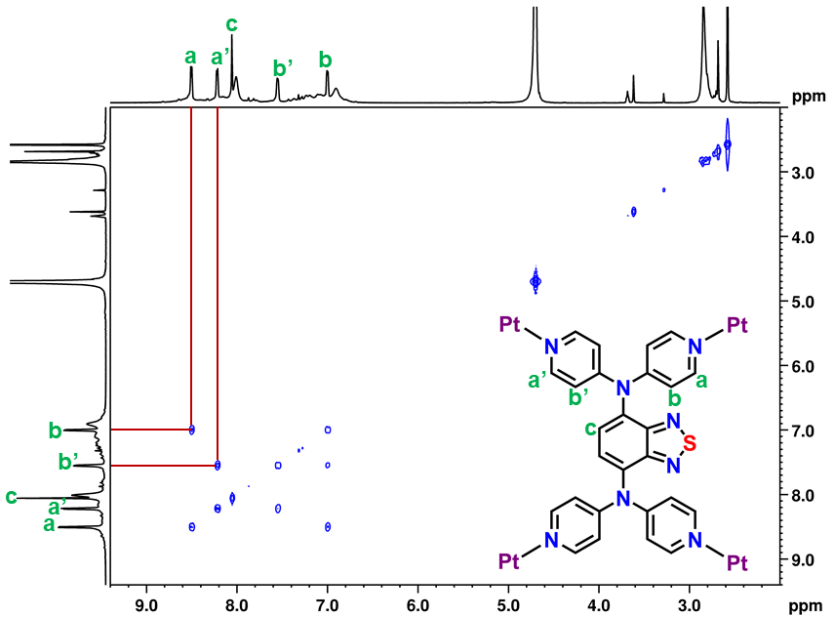


Figure S5. [1] H-[1]H -COSY spectrum of **PMB1** in D_2O.

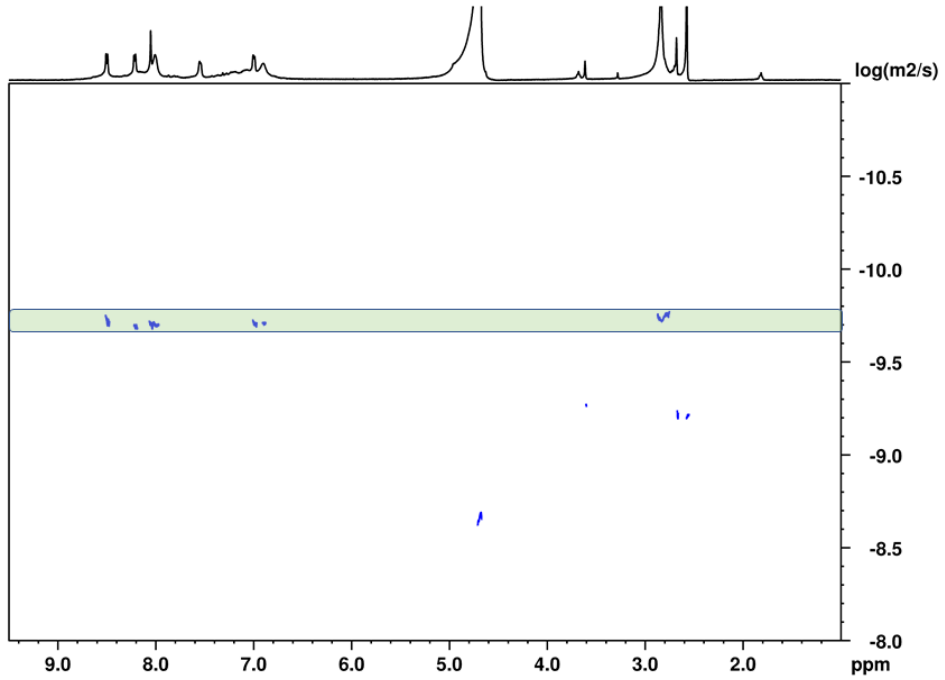


Figure S6. ^{1}H-DOSY of **PMB1** in D_2O.

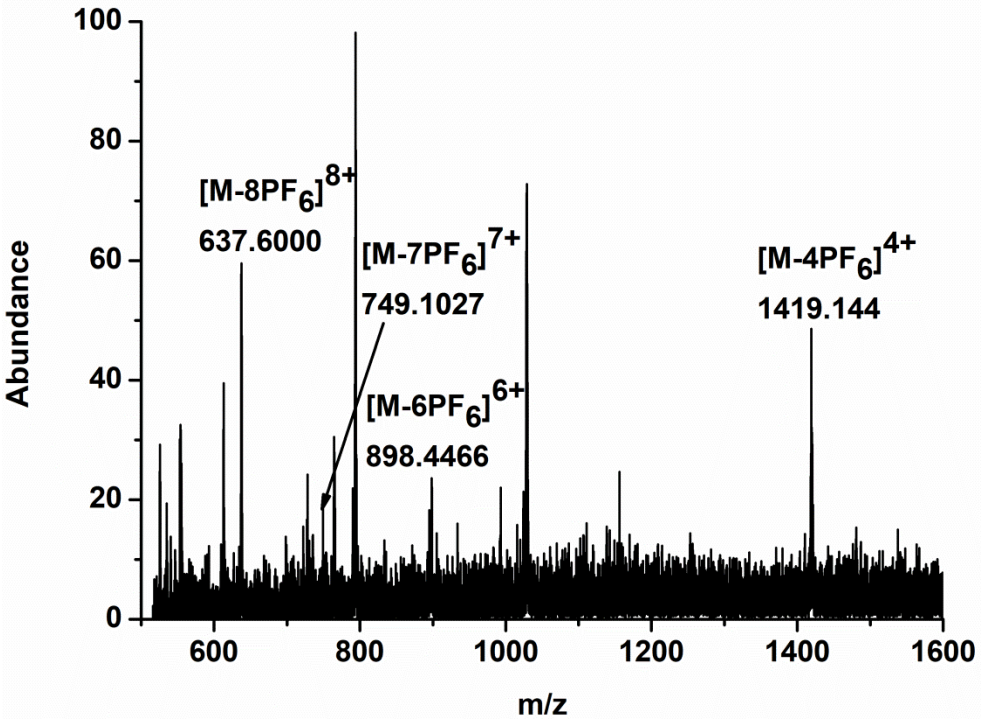


Figure S7. ESI-MS spectrum of PF_6^- analogue of **PMB1** in CH_3CN (M= PMB1).

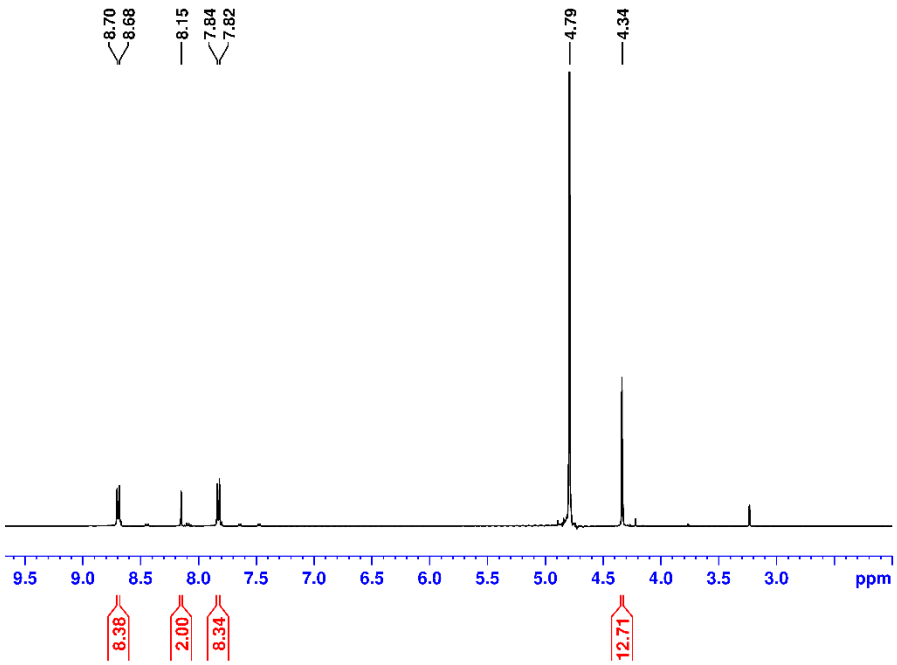


Figure S8. [1] H-NMR spectrum of tetra-methylated ligand **[L_{Me4}][4NO_3]** in D_2O.

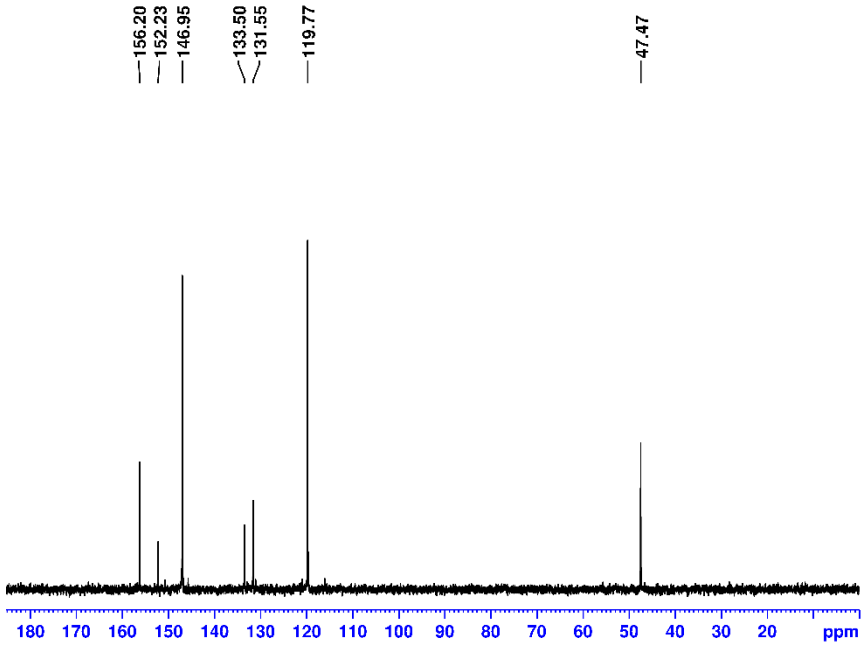


Figure S9. [13] C-NMR spectrum of tetra-methylated ligand **[L_{Me_4}][4NO_3]** in D_2O.

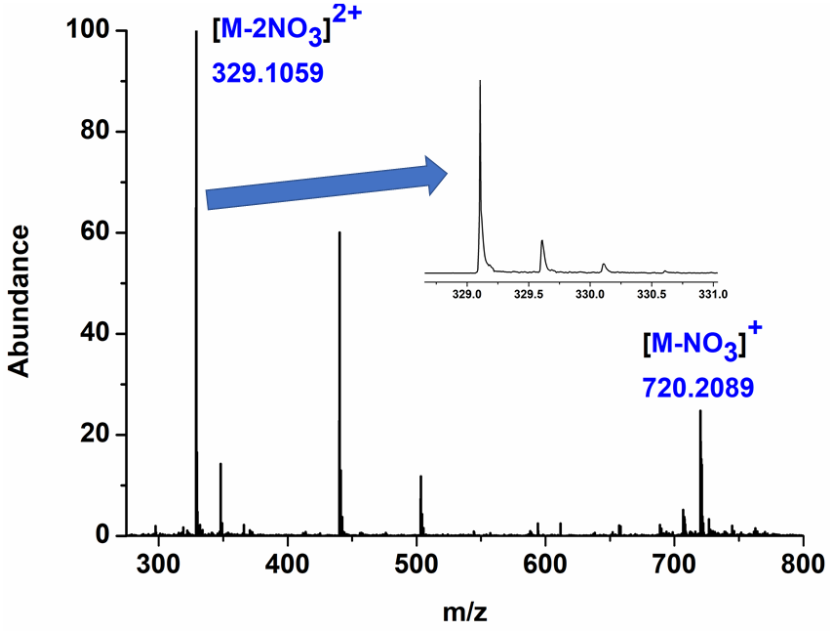


Figure S10: Mass spectrum of tetra-methylated ligand **[L_{Me_4}][4NO_3]** (M = **[L_{Me_4}][4NO_3]**).

4. Photophysical studies:

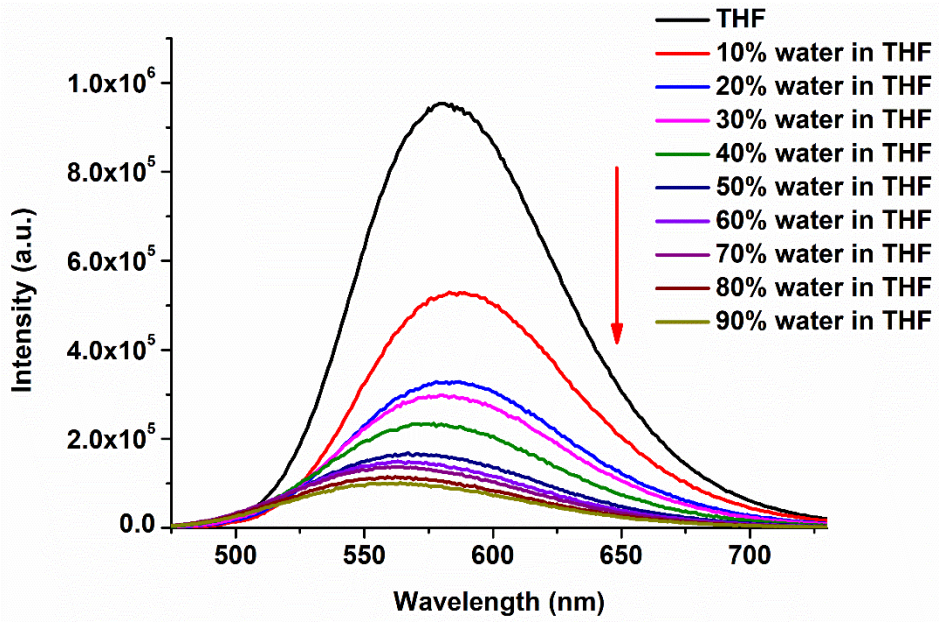


Figure S11: Emission spectra of ligand (**L**) in different solvent fractions of water in THF (10μM solution), λ_{max} = 435 nm.

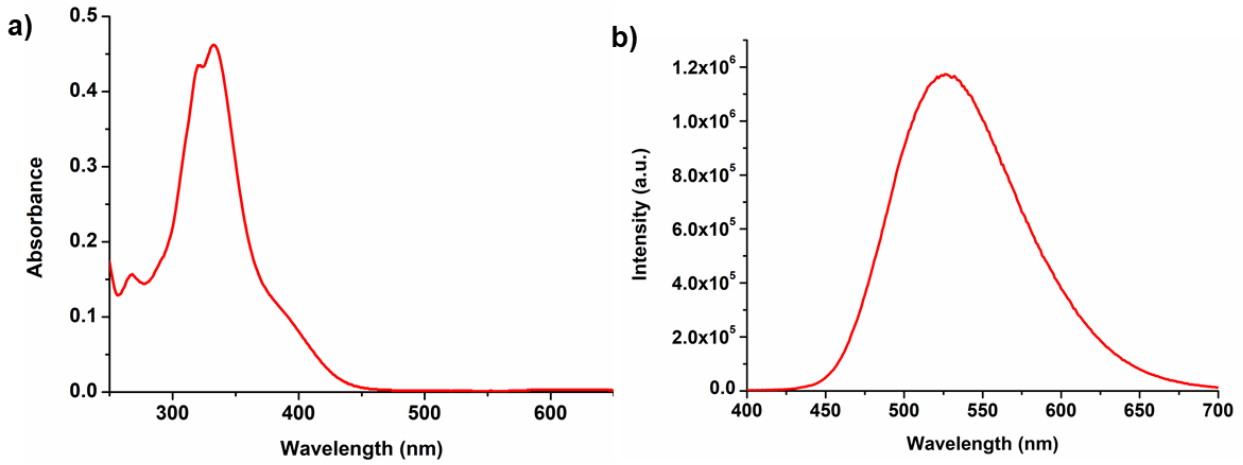


Figure S12: Absorption (a) and emission (b) spectra of tetra-methylated ligand **[L_{Me_4}][4NO_3]** in 10 µM aqueous solution.

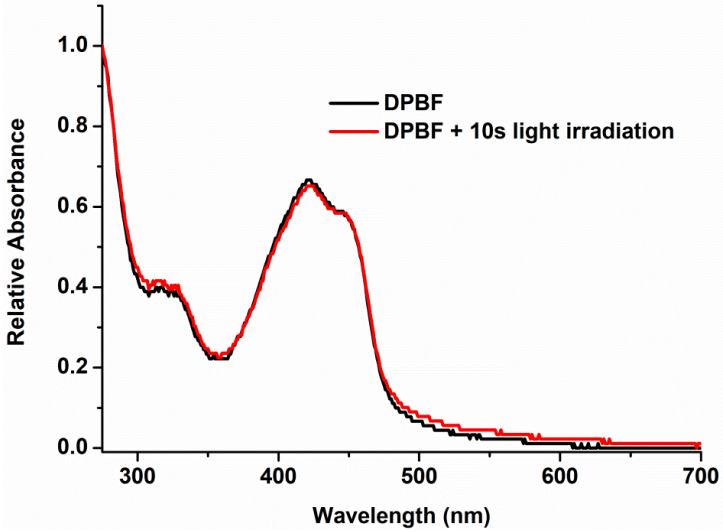


Figure S13: Absorption spectra of DPBF (20 µM) without **PMB1** as a function of irradiation time.

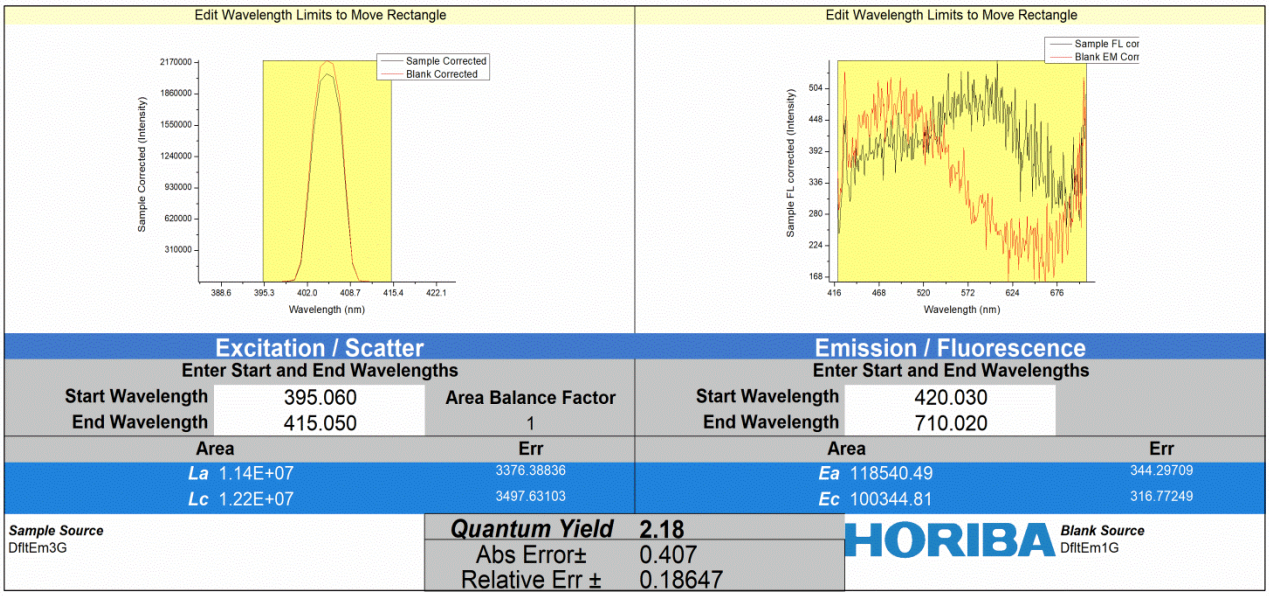


Figure S14. Absolute fluorescence quantum yield of **L** in water.

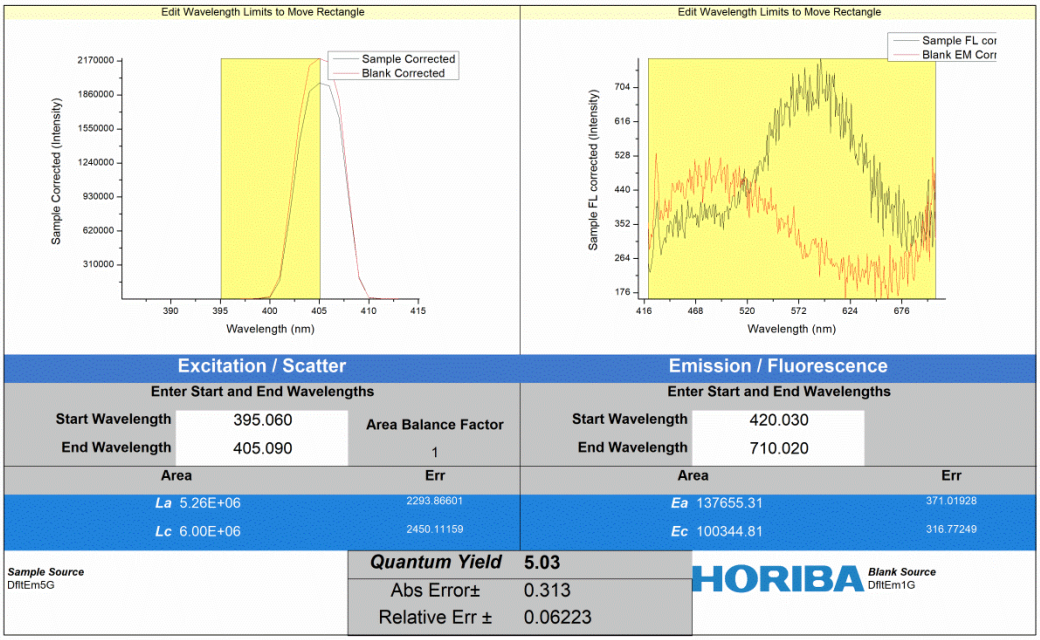


Figure S15. Absolute fluorescence quantum yield of **PMB1** in water.

5.1 Minimum inhibitory concentration (MIC) for PMB1, Pt acceptor, Ligand (L) and water soluble charged analogue of L ([LMe$_4$][4NO$_3$]) and minimum bactericidal concentration (MBC) for PMB1, Pt acceptor and Ligand (L):

The antibacterial activity of cage and its building components was investigated with methicillin resistant *Staphylococcus aureus* (MRSA, USA300) as gram positive bacteria and *Pseudomonas aeruginosa* (PA,) as gram negative bacteria. The freeze-dried bacterial species were revived by transferring into nutrient augur plates. The primary culture was obtained by taking few colonies of bacteria from augur plate and cultured in Luria broth media (LB, HiMedia 20 g/L) overnight for 10-12 h. The secondary culture was prepared by sub-cultureing 50 µl of primary culture in 5 mL of fresh LB media until it reaches the mid-log phase (A_{600nm} =0.3). For our experiments, the optical density of seeding bacteria was adjusted to A_{600nm}= 0.01 (10^6 to 10^7 bacteria per mL).

5 mL test tubes with tight caps were used to estimate minimum inhibitory concentration. Phosphate saline buffer (PBS 5 mM, pH 7.4) was used to prepare all the dilutions for working solution concentrations. Bacterial suspension of 1.5 mL with A_{600nm}= 0.01 was added to 1.5mL of working solution prepared in test tube. Bacteria aren't washed before light irradiation and done the irradiation in LB medium and we have not done any preincubation before light irradiation. The dark experiment was performed by covering the test tube with aluminum foil and light experiment was performed under continuous exposer of 40W LED white bulb for 12 h. The experimental setup is shown in Figure S17. The growth curve was obtained by measuring optical density of 100 µL from treated solution after each 1 h interval over a period of 12 h. The minimum inhibitory concentration (MIC) was the concentration where there was no growth or 95% decline in growth.

For minimum bactericidal concentration (MBC), after MIC measurement the same test tube was incubated for another 12 h at 37°C under CO_2 atmosphere and then the bacterial solution from treated tube was streaked on a nutrient agar plate. The concentration at which there is no colony formation of bacteria has been designated as MBC.

To examine photobleaching of **PMB1**, the absorption spectrum of 1 µM aqueous solution of the barrel was recorded under similar experimental condition. No significant photobleaching was observed.

Figure S16. Experimental setup for photoirradiated antibacterial activity.

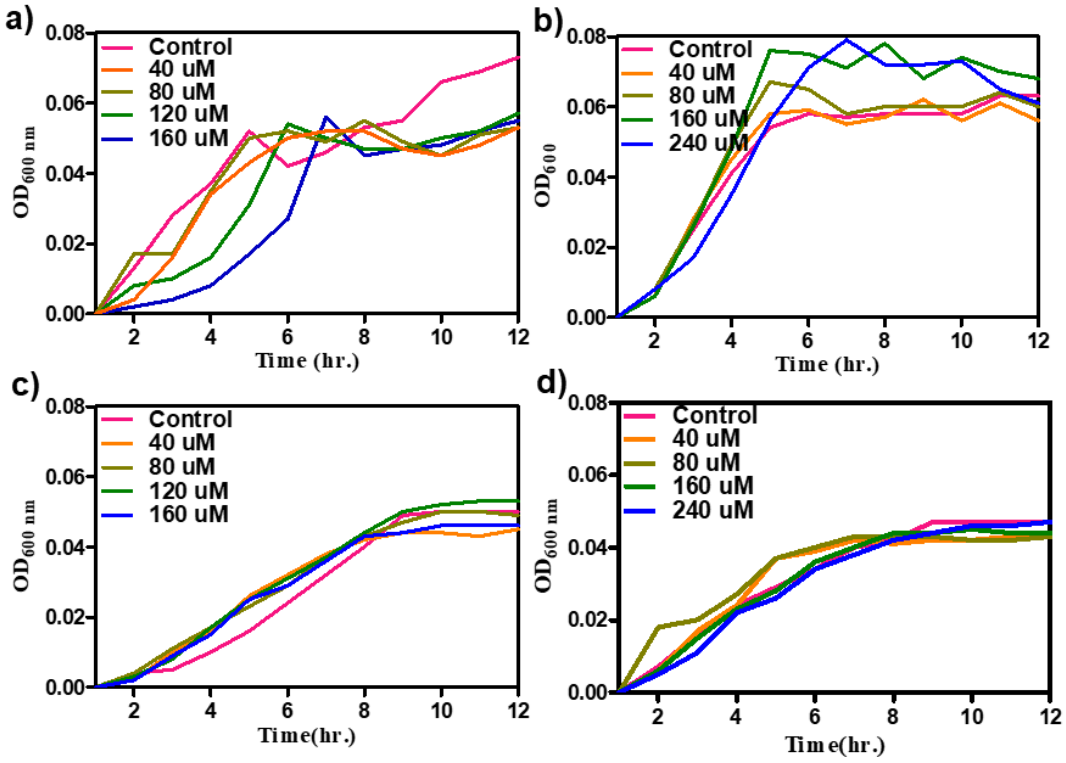


Figure S17. Bacterial growth curve of MRSA and PA in presence of light. (a, c) bacteria treated with ligand (**L**) in presence of light (b, d) treated with Pt acceptor.

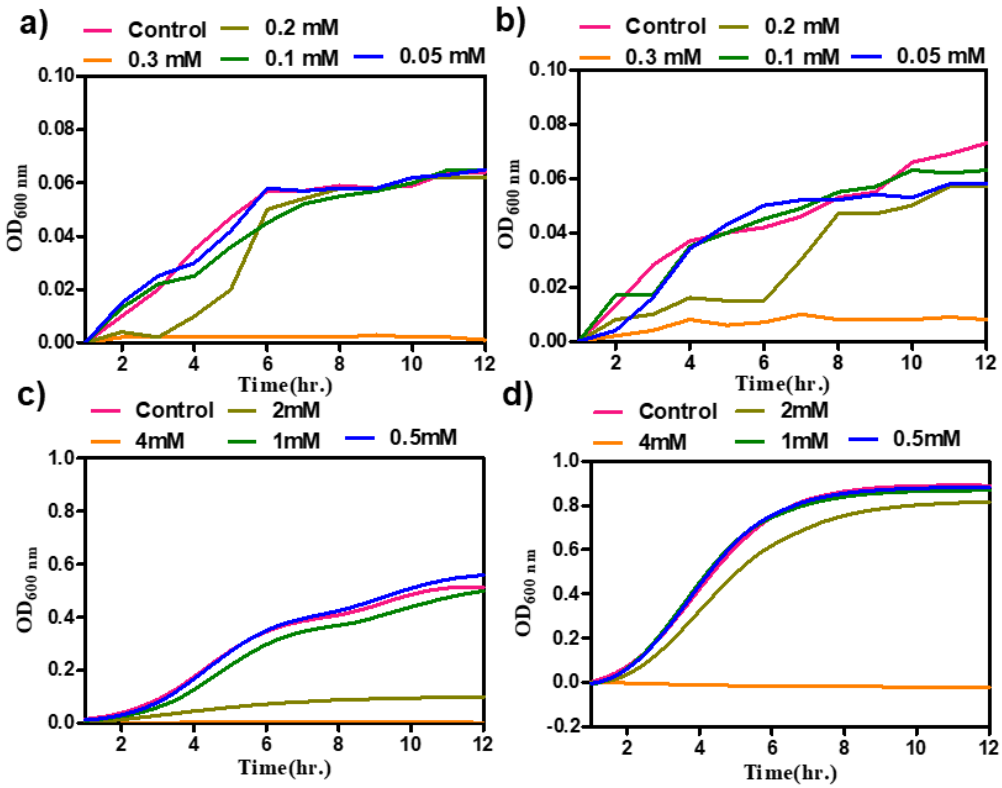


Figure S18. Bacterial growth curve of MRSA and PA in presence of light (a, b) bacteria treated with water soluble tetra-methylated ligand **[L_{Me_4}][4NO_3]** in absence of light (c, d) respectively.

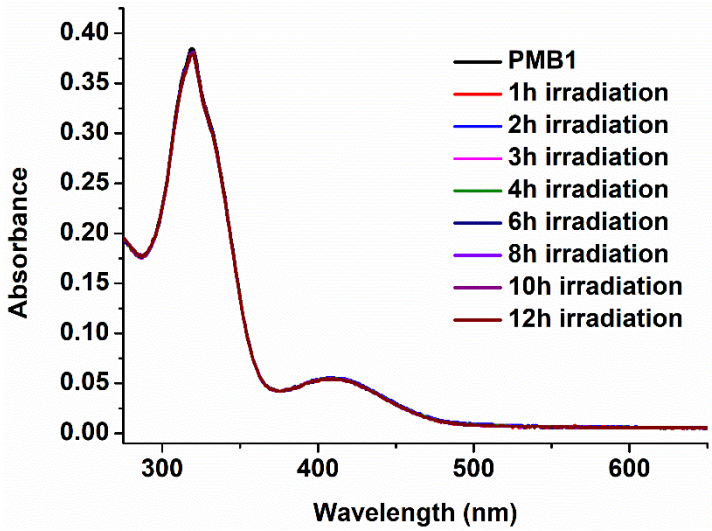


Figure S19. Absorption spectrum of **PMB1** (1 μM) under continuous exposer of 40W LED white bulb at different time intervals over a period of 12 h.

5.2 Estimation of oxidative stress:

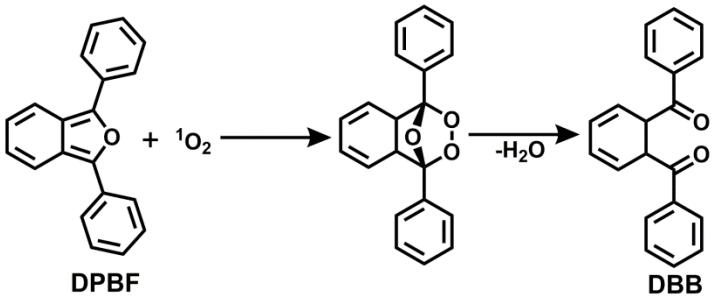
Ellman's assay was performed to determine the oxidative stress induced by cage and its composition. MIC of MRSA for cage and ligand was used for the experiment. 0.4 mM of glutathione (GSH) solution in 50 mM bicarbonate buffer in pH- 8.6 was taken with cage and ligand for oxidative stress estimation through loss of GSH. Untreated GSH solution was taken as negative control while H_2O_2 (10 mM) with GSH solution was considered as positive control. The treated solution along with control were exposed to 40W LED white light while a control experiment was carrying with side by side in absence of light. At different time intervals (15 min, 30 min, 45 min, 60 min), 100 µL from stock solution was added to 100 µl of 2 mM 5,5′- dithiobis (2-nitrobenzoic acid) (DTNB, SRL Chem) in 50 mM TRIS-HCl (pH 8.3, SRL Chem) buffer taken in a 96-well plate. After 5 minutes, the absorbance was measured at 412 nm using plate reader. The percentage loss of glutathione was calculated as,

$$\%loss = (1 - \frac{\text{absorbance at 412 nm of the sample at a particular time}}{\text{absorbance at 412 nm of negative control at 0 min}}) \times 100$$

The concentration of all solutions mentioned here are the final concentrations.

5.3 Determination of singlet oxygen generation:

Singlet oxygen generation was determined in water using 40W white LED light as the light source. The generation of singlet oxygen was monitored by following the decrease in absorption of 1,3-diphenylisobenzofuran (DPBF) in different time intervals (light exposer time) at 417 nm. This experiment was performed by taking 1 µM aqueous solution of **PMB1** and 20 µM (stock in acetonitrile) DPBF solution. The decrease of absorbance happened due to photooxidation of DPBF sensitized by the cages.[3]



Scheme S2: Reaction scheme of singlet oxygen with DPBF.

Singlet oxygen quantum yield (Φ_Δ) was calculated using similar experiment setup according to literature procedure[4]. Chloride complex of $[Ru(bpy)_3]^{2+}$ was used as the reference photosensitizer to compare ($\Phi_\Delta^R = 0.22$)[5] while calculating the singlet oxygen quantum yield in relative method. The singlet oxygen quantum yield (Φ_Δ) for the tetra-methylated ligand **[L_{Me4}][4NO_3]** is found to be 19%.

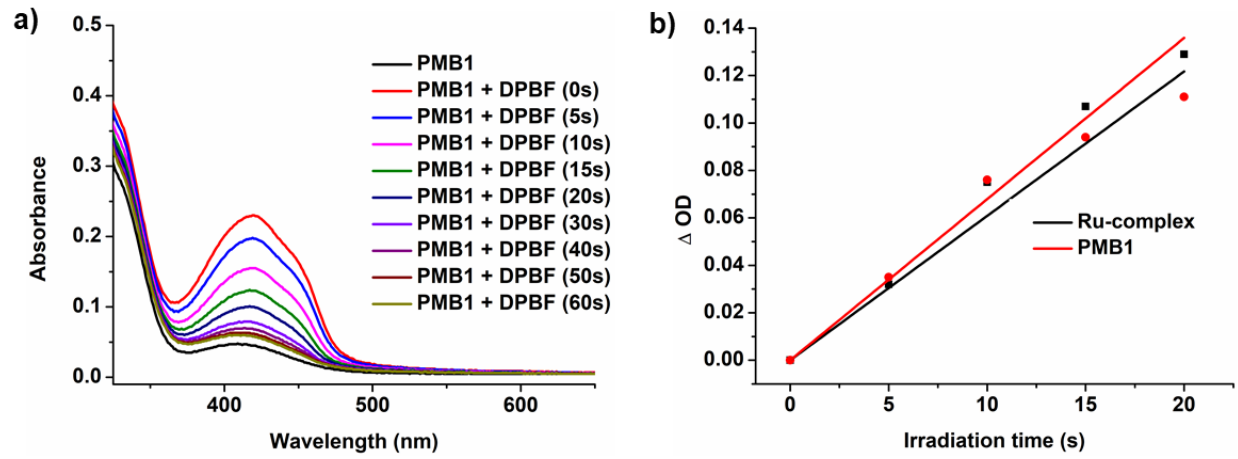


Figure S20: (a) Changes in absorption spectra of DPBF in presence of **PMB1** in aqueous solution, irradiated at different intervals of time. (b) Plot of ΔOD vs irradiation time at 417 nm of DPBF.

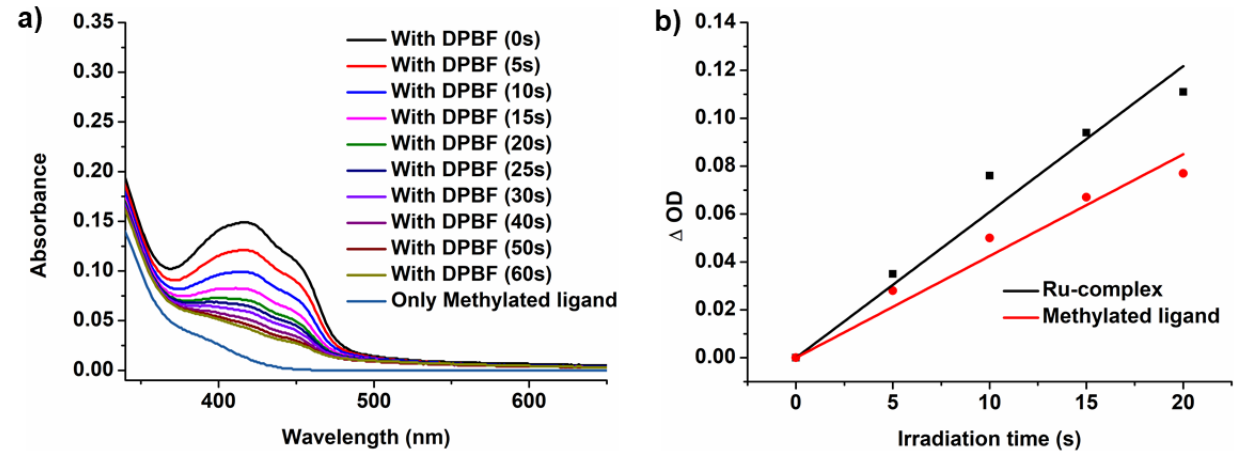


Figure S21: (a) Changes in absorption spectra of DPBF in presence of tetra-methylated ligand **[L_{Me4}][4NO_3]** in aqueous solution, irradiated at different intervals of time. (b) Plot of ΔOD vs irradiation time at 417 nm of DPBF.

5.4 Membrane Depolarization Assay:

The MRSA bacteria was harvested at mid log phase culture ($A_{600nm} \approx 0.3$) and centrifuged at 5000 rpm for 5 min. The pellet was suspended in 5 mM glucose and 5 mM HEPES buffer (pH 7.2) mixed in 1:1 ratio. Again, the bacterial solution in buffer was centrifuged and washed to collect the pellet. The washed pellet was again resuspended in 5 mM HEPES buffer, 5 mM glucose, and 100 mM KCl solution mixed in a 1:1:1 ratio. Then, 2 μL of 5 mM 3,3′- dipropylthiadicarbocyanine iodide dye (DISC3, TCI Chemicals) was added to 100 μL of bacterial suspension in 96 well plate and incubated for 30 min. MIC of **PMB1**, ligand (**L**) and Pt complex were added to the 96 well plate containing bacterial solution and DISC3 dye. The fluorescence of bacterial suspension was monitored with an excitation wavelength of 622 nm and an emission wavelength of 670 nm, for the next 2 h with a lag time of 10 min. The increase in florescence shows the membrane depolarization of the bacterial membrane.

5.5 Cell Viability Assay:

Human cervical cancer cells (HeLa) and human embryonic kidney (HEK293) normal cell lines were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) at 37 °C in 5% CO_2.

Cell cytotoxicity of cage was evaluated by considering human cervical cancer cells (HeLa cells) and human embryonic kidney (HEK293) normal cell lines. Both the cells were cultured in 96 well plates (20,000 cells per well; 80% confluency) and incubated for 24 h in a 37°C humidified incubator (5% CO_2). Then after 24 h incubation, cells were washed with PBS buffer and old medium was replaced with fresh medium containing cage and again incubated for 24 h at different concentrations. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT assay was performed to measure cell viability. The MTT agent was added to each well and co- incubated for another 3h. Then, the cultured medium was replaced with 200 μL DMSO. The relative cell viability was obtained by measuring absorbance for formazan at 570nm.

The phototoxicity of both the cell lines was measured by irradiating treated and untreated cells for 15 min in each 1 h interval for 12 h. Then, the cells were further incubated for another 12 h in incubator without irradiation. The above written MTT assay procedure was followed to measure toxicity. The phototoxicity was determined by using 40W white LED light as the light source.

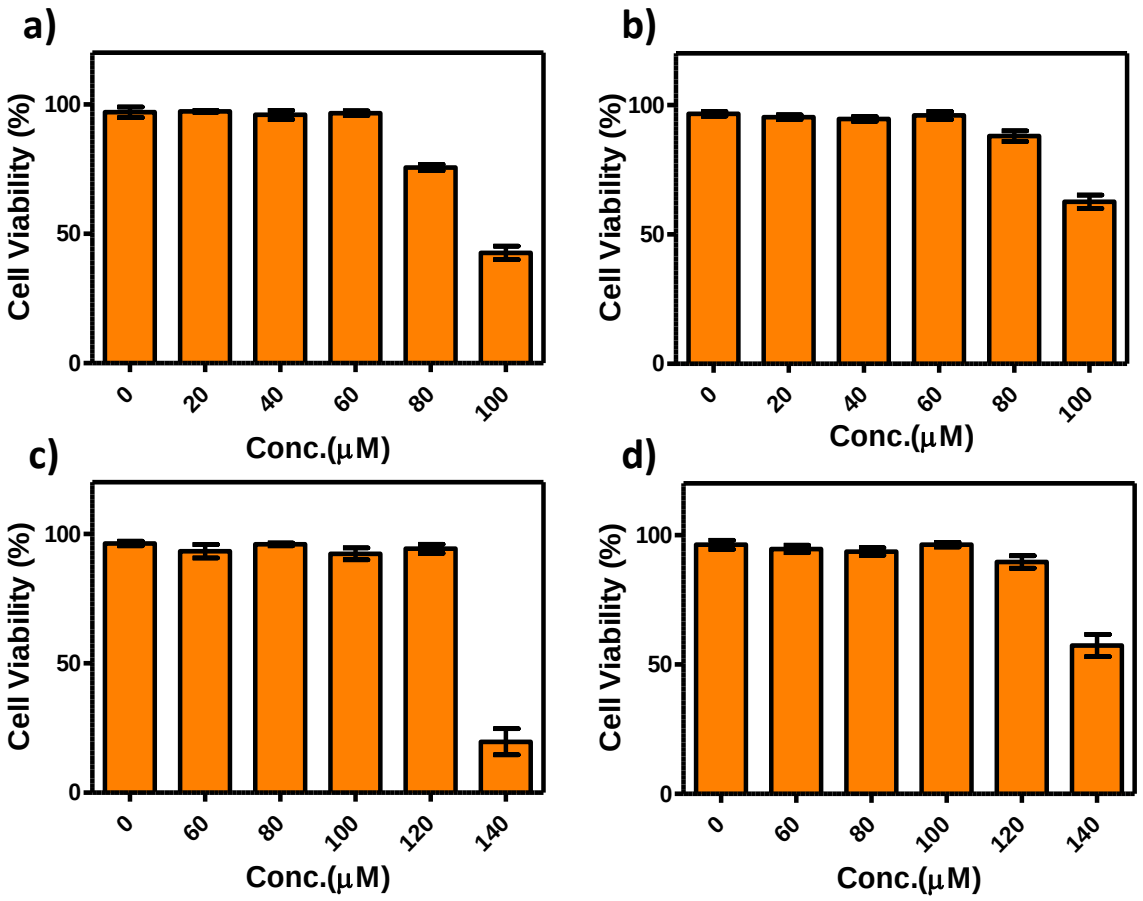


Figure S22: Cell viability of HEK293 (a) with irradiation (b) without irradiation and HeLa cells (c) with irradiation (d) without irradiation at different concentrations of **PMB1**.

5.6 SEM Sample Preparation:

MRSA bacteria was cultured in LB media and harvested in mid- log phase (A_{600nm}= 0.5) by spinning at 5000 rpm for 6 min. Then the bacterial pellet was dispersed in PBS buffer and treated with MIC of **PMB1** for 1 h. After 1h of incubation, it was centrifuged, and the pellet was fixed with 3% glutaraldehyde for 1 h. Then again it was centrifuged at 5000 rpm for 6 min. After fixation it was dehydrated using 30%, 40%, 50%, 60%, 70%, 80%, 90% ethanol gradient and finally it was dispersed in 100% ethanol and drop- cast on silicon wafer, sputtered with gold prior to imaging in SEM.

5.7 AFM sample preparation:

The AFM sample was prepared by following the procedure of SEM sample preparation. But the dehydration after fixing with glutaraldehyde was avoided and both treated and untreated bacterial solution was drop casted on atomically smooth mica foil. Then, the sample was desiccated under high vacuum overnight prior to imaging in AFM instrument (JPK Instruments, USA).

References:

(1) (a) Sheldrick, G. Program for the refinement of crystal structures, *SHELXL97* **1997**;

(b) Farrugia, L. J. WinGX suite for small-molecule single-crystal crystallography, *J. Appl. Crystallogr.* **1999**, *32*, 837.

(2) Sheldrick, G. M. SHELXT–Integrated space-group and crystal-structure determination, *Acta. Crystallogr. Sect. A: Found. Adv.* **2015**, *71*, 3.

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(5) Adarsh, N.; Avirah, R. R.; Ramaiah, D. Tuning Photosensitized Singlet Oxygen Generation Efficiency of Novel Aza-BODIPY Dyes, *Org. Lett.* **2010**, *12*, 5720.