Electronic Supplementary Material (ESI) for Chemical Communications.

Lysosome targeted visible light induced Photo-CORM for simultaneous CO-release and singlet oxygen generation

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Experimental section:

Materials: $Mn(CO)_5Br$, silver triflate, di-(2-picolyl)amine, 4-Bromo-1,8-naphthalic anhydride and 4-(2-Aminoethyl)morpholine were purchased from Sigma-Aldrich. Solvents were purified and/or dried by standard techniques prior to use for all syntheses.

Analytical Methods:

^1H NMR and ^{13}CNMR spectra were recorded AV 300 MHz Bruker NMR spectrometer using $CDCl_3$ and DMSO-d_6 as the solvents. Tetra methyl silane (TMS) as an internal standard for ^1H NMR, whereas trichlorofluoromethane (CCl_3F) as an internal standard for ^{19}F NMR. UV-Vis spectra were recorded on an Analytic Jena Specord S 600 UV-Vis spectrometer. Mass spectra (EI, ESI) were obtained by the use of a Finnigan MAT SSQ 710 or MAZ95XL device.

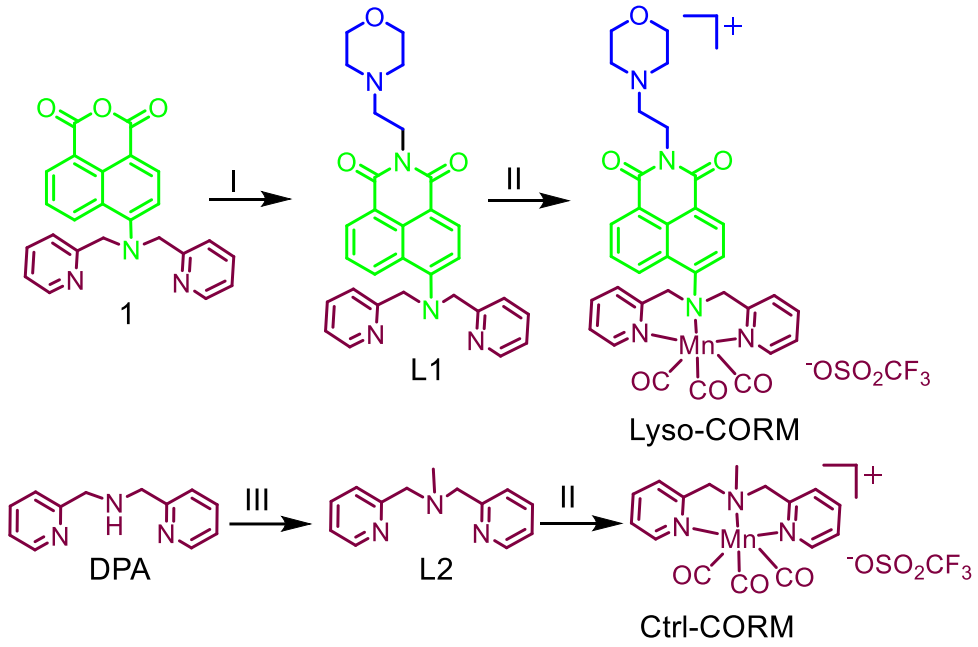
General experimental methods for UV-Vis and fluorescence studies:

1 mM stock solution of **Lyso-CORM** and **Ctrl-CORM** were prepared in DMSO and the same solution was used for all the studies after appropriate dilution. Unless and otherwise mentioned, 10 mM and pH 7.3 solution of aq. PBS buffer was used for all spectroscopic studies. For spectroscopic measurements, stock solution of the probe was further diluted by using PBS buffer: DMSO (99:1, v/v) mixture and the effective final concentration were made as 15 μM. All luminescence measurements were done using λ_{Ext} = 405 nm with an emission slit width of 5 nm. Each spectrum was recorded at room temperature with a quartz cuvette at regular time intervals of 20 seconds of 480 nm light exposure (UVP Benchtop 2 UV Transilluminator, ≤10 mW cm^{-2}) at different wavelengths.

Photolysis Experiment: Solution of **Lyso-CORM** (15 μM) in a quartz cuvette were exposed to UV light at 480 nm at every 20 sec intervals to investigate the light-induced CO release studies. Light-induced CO release was confirmed by monitoring changes in the emission intensity at ~ 500 nm in systematic fluorescence studies as well as in absorption spectrum. The power of the light sources was determined with a PM100USB power.

Synthesis and Characterization:

Scheme 1. Methodologies that were adopted for synthesis of **CORMS.**



Reaction conditions: (I) dipicolylamine, 2-methoxyethanol, reflux 48h; (II) 2-morpholinoethan-1-amine,ethanol, reflux 12h; (III) $Mn(CO)_5Br$ followed by $AgOSO_2CF_3$, dry acetone; (III) HCHO, catalytic AcOH, MeOH, $NaBH_4$.

Synthesis of **1**, **L2** and **Ctrl-CORM**: Compound **1**, **L2** and **Ctrl-CORM** were synthesized by according to our previous reports.[1]

Synthesis of **L1**[1]. Compound (**1**) (400 mg, 1.012 mmol) and 2-morpholinoethan-1-amine (250 mg, 2.024 mmol) were dissolved in 30 mL of fresh ethanol. The reaction mixture was allowed to stir for 12h under reflux condition. By monitoring the TLC reaction was stopped. The crude mixture was concentrated and subjected to silica column chromatographic (eluent ethyl acetate and hexane in 1: 1(*v*/*v*)) purification to result pure sticky compound **L1** (yield: ~ 78%) after evaporation of the solvent. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.96 (1H, dd, *J* = 8.5, 0.8 Hz), 8.52 (3H, t, *J* = 5.8 Hz), 8.26 (1H, d, *J* = 8.2 Hz), 7.79 – 7.70 (1H, m), 7.70 – 7.61 (2H, m), 7.46 (2H, d, *J* = 7.8 Hz), 7.26 – 7.15 (3H, m), 4.72 (4H, s), 4.20 (2H, t, *J* = 6.9 Hz), 3.60 – 3.54

(4H,m), 2.59 (2H, t, *J* = 6.9 Hz), 2.52 – 2.46 (4H, m).^{13}C NMR (75 MHz, $CDCl_3$-d_3): *δ* (ppm) 164.50, 163.93, 157.28, 153.75, 149.59, 136.70, 132.01, 131.15, 130.44, 130.18, 126.64, 125.89, 123.28, 122.50, 122.27, 117.54, 116.43, 67.03, 59.85, 56.22, 53.79 and 37.03. ESI-MS (m/z) calculated for $C_{30}H_{29}N_5O_3$: 507.23, observed: 507.129 [**L1**].

Synthesis of **Lyso-CORM**: In the dark and under a nitrogen atmosphere, $Mn(CO)_5Br$ (81 mg, 0.295 mmol) and silver triflate (76 mg, 0.295 mmol) were dissolved in 10 mL of dry acetone. The reaction mixture was stirred at 60°C for 1.5 h. The reaction mixture was filtered under an inert atmosphere to separate the precipitate of silver bromide. To filtrate, ligand **L1** (100 mg, 0.197 mmol) in 8 mL of dry acetone was added and the reaction mixture was heated under reflux for 60 °C for 2h. The reaction mixture was filtered off and washed with dry diethyl ether for several times to get pure **Lyso-CORM** as solid (yield: ~ 69%). ESI-MS (m/z) calculated for $C_{34}H_{29}F_3MnN_5O_9S$: 795.10, observed: 646.10 [**Lyso-CORM** - OSO_2CF_3]. ^{1}H NMR (DMSO-d_6: 300 MHz) *δ* (ppm) 9.01 (2H, d, *J* = 5.0 Hz), 8.73 (1H, d, *J* = 8.2 Hz), 8.64 (1H, d, *J* = 7.1 Hz), 8.28 (1H, d, *J* = 8.2 Hz), 8.02 (3H, dd, *J* = 14.2, 6.6 Hz), 7.96 (1H, s), 7.73 – 7.64 (4H, m), 7.64 – 7.51 (m), 5.61 (2H, d, *J* = 16.8 Hz), 4.87 (2H, d, *J* = 17.0 Hz), 4.44 (2H, d), 3.83 (4H, m), 3.21 (6H, m, J = 33.1 Hz). ^{13}C-NMR (125 MHz, DMSO-d_6): *δ* (ppm) 217, 158, 152, 151, 139, 135, 135, 132, 130, 130, 125, 124, 123, 123, 117, 115, 61, 45.

Cell Studies:

Cytotoxicity study: Cytotoxicity of **Lyso-CORM** in HeLa and NIH-3T3 cells were performed using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The cells were seeded in a 96-well plate at a density of $5X10^3$ cells per well overnight followed by the treatment of different concentration of **Lyso-CORM** in DMEM medium containing 10% FBS for 4 hour. Then the cells are replaced with fresh DMEM followed by the light treatment. The cells were kept for another 20 hour and then incubated with 3(4,5-dimethyl-thyzoyl-2-yl)2,5 diphenyltetrazolium bromide (MTT) for 4 hour. The crystallized formazan was solubilized in (1:1) DMSO: MeOH and quantified by measuring the absorbance at 595 nm with ELISA plate

reader. Results were expressed as percent viability = [(A595 (treated cells)-background)/(A595 (untreated cells)-background)] x 100. The dark toxicity was measured without the light irradiation.

Laser specification used in two-photon excitation: Laser source at 800 nm was obtained by Ti:Sa mode-locked oscillator and chirped pulse amplifier delivering 80 fs pulses with energies up to 1 mJ at the repetition rate of 1 kHz, having an average power of 1 W (Spitfire, SpectraPhysics).

Confocal laser scanning microscopy (CLSM) studies: HeLa cells were cultivated with VLE DMEM (Biochrom GmbH, Berlin, Germany) containing fetal bovine serum (10%, v/v, FBS Superior, Biochrom GmbH, Berlin, Germany), penicillin (100 U/mL, Biochrom GmbH, Berlin, Germany) and streptomycin (100 µg/mL, Biochrom GmbH, Berlin, Germany) at standard cell culture conditions. Twenty-four hours prior to use, the cells were seeded with a density of 105 cells/cm^2 into imaging dishes with polymer coverslip bottom (ibidi GmbH, Planegg/Martinsried, Germany). Seeding medium was replaced by each 2.5 mL freshly prepared 50 µM **Lyso-CORM** dilution containing 1% DMSO (v/v) and incubated each for 50 minutes at standard cell culture conditions. Seeding medium was replaced by FluoroBrite™ DMEM (GibcoTM, Paisley, Scotland, United Kingdom) supplemented with GlutaMAX™ (2 mmol, GibcoTM, Paisley, Scotland, United Kingdom) respectively the corresponding 50 µM **Lyso-CORM** dilution containing 1% DMSO (v/v) and incubated each for 50 minutes at standard cell culture conditions. One set of **Lyso-CORM** treated cells was irradiated with a 405 nm high-power LED (10 mW/cm^2, H2A1 series from Roithner Lasertechnik GmbH, Vienna, Austria) for 10 minutes, while control experiments were accomplished by avoiding exposure to ambient light. For CLSM measurements, the medium was removed, cells washed twice with 3 mL DBPS and 2.5 mL FluoroBrite™ DMEM (GibcoTM, Paisley, Scotland, United Kingdom) supplemented with GlutaMAX™ (2 mmol, GibcoTM, Paisley, Scotland, United Kingdom) added. Subsequently, one- and two-photon imaging was performed on an inverted confocal

microscope system (LSM 780, Carl Zeiss, Jena, Germany) equipped with a water-immersed objective (LD C-Apochromat 63×/1.15 W Corr M27, Carl Zeiss, Jena, Germany) offering very high transmission in the UV-vis as well as in the IR region plus high resolution. For two-photon excitation at 790 nm pulsed titanium: sapphire (Ti:Sa) laser emitting 140 fs pulses at 80 MHz repetition rate, tunable in the range from 690 to 1080 nm (Chameleon Ultra II, Coherent GmbH, Dieburg, Germany) was applied. One-photon excitation at 405 nm was conducted with a diode laser (Carl Zeiss, Jena, Germany). Fluorescence emission was recorded at 545 (±55) nm using a gallium arsenide phosphide plus photomultiplier tube array detector (32 GaAsP + 2 PMT, Carl Zeiss, Jena, Germany). For better visibility, brightness and contrast of the recorded images were slightly enhanced with the image processing program ImageJ by treating all images in the same manner.

References:

1. U.R. G, J. Axthelm, P. Hoffmann, N. Taye, S. Glaser, H. Gorls, S.L. Hopkins, W. Plass, U. Neugebauer, S. Bonnet, A. Schiller, J Am Chem Soc, 139 (2017) 4991-4994.
2. V. Ramu, G. Upendar Reddy, J. Liu, P. Hoffmann, R. Sollapur, R. Wyrwa, S. Kupfer, C. Spielmann, S. Bonnet, U. Neugebauer, A. Schiller, Chemistry, 25 (2019) 8453-8458.

^{1}H NMR spectrum of L1

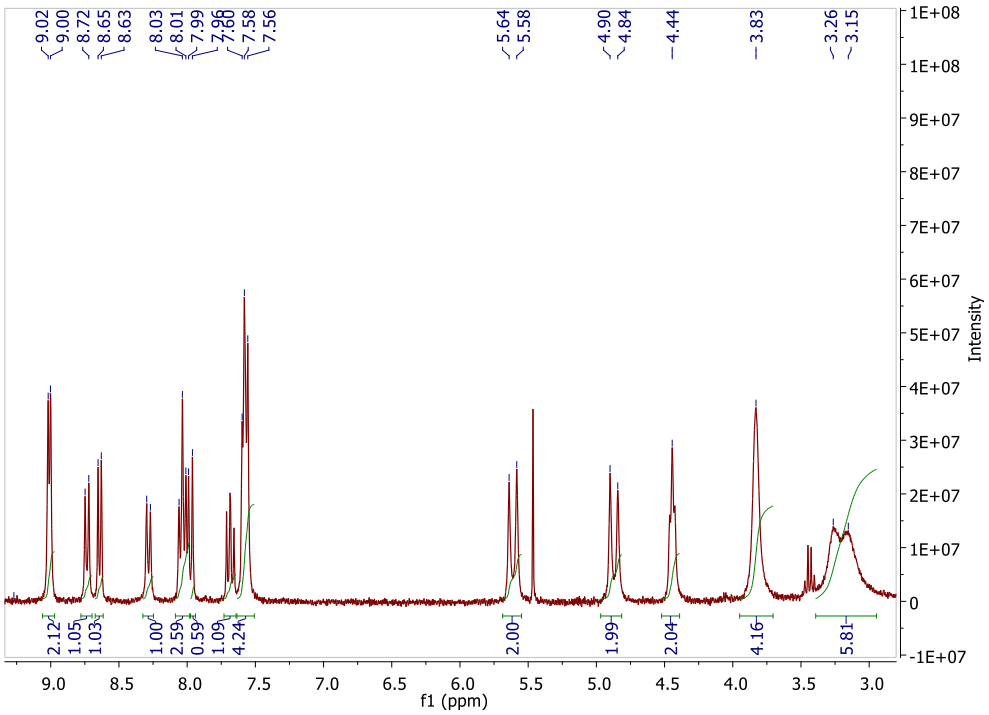


Figure S 1. ^{1}H NMR spectrum of **L1** in $CdCl_3$.

^{13}C NMR spectrum of L1

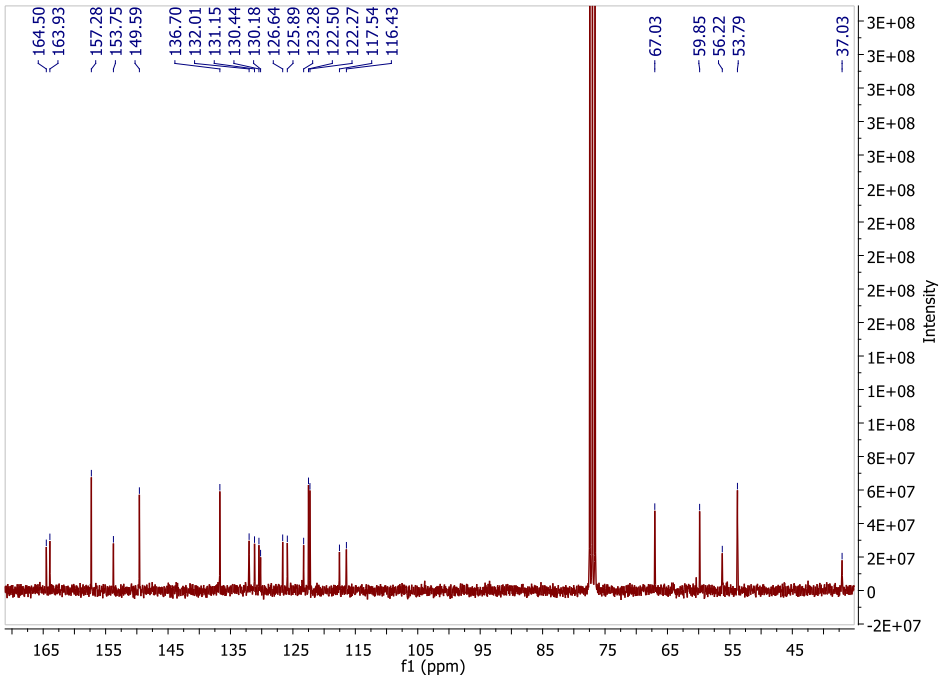


Figure S 2. ^{13}C NMR spectrum of **L1** in $CDCl_3$.

ESI-MS spectrum of L1

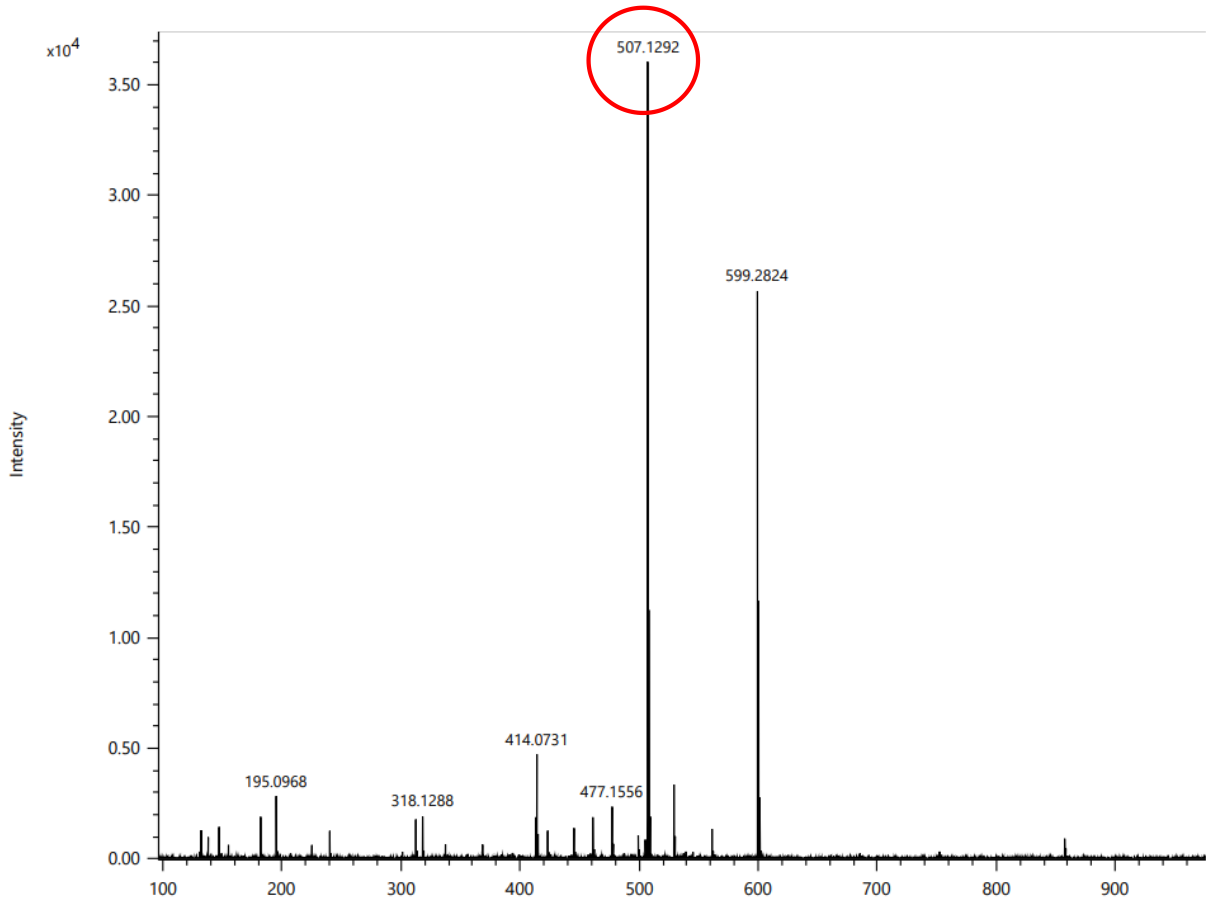


Figure S 3. ESI- MS spectrum of **L1** in CH_3OH.

^{1}H NMR spectrum of Lyso-CORM

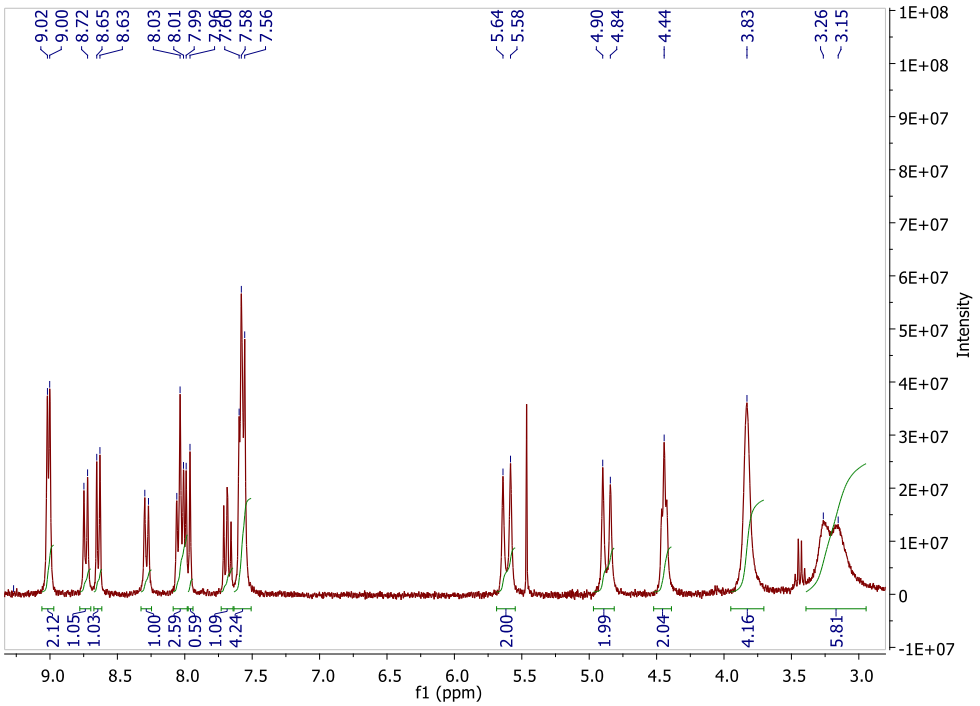


Figure S 4. ^{1}H NMR spectrum of **Lyso-CORM** in DMSO-d6.

^{13}C NMR spectrum of Lyso-CORM

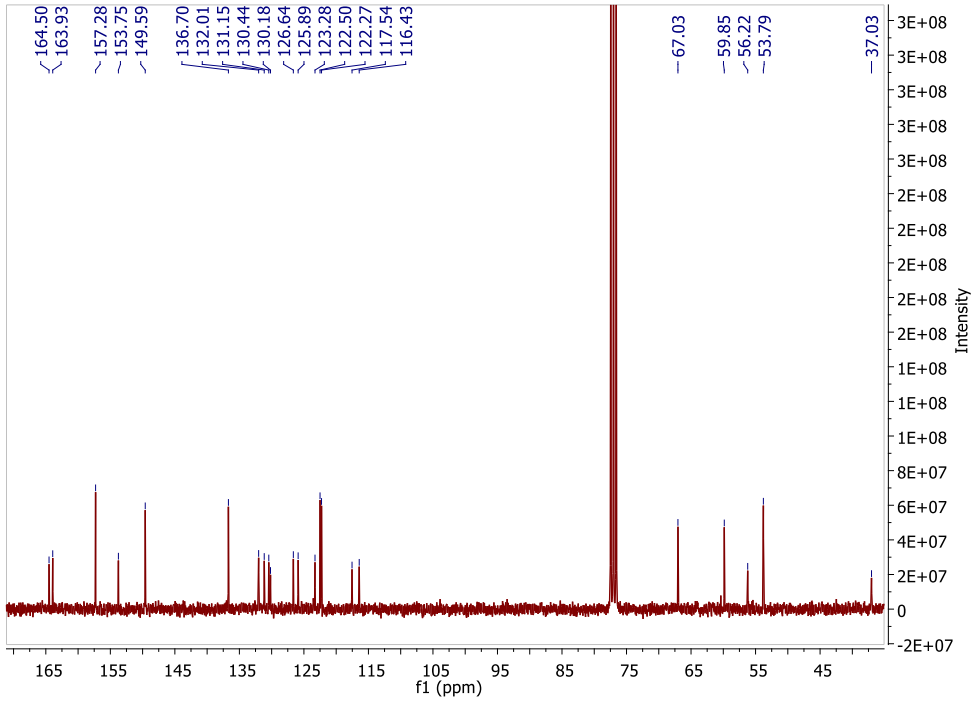


Figure S 5. ^{13}C NMR spectrum of **Lyso-CORM** in DMSO-d6.

IR spectrum of Lyso-CORM.

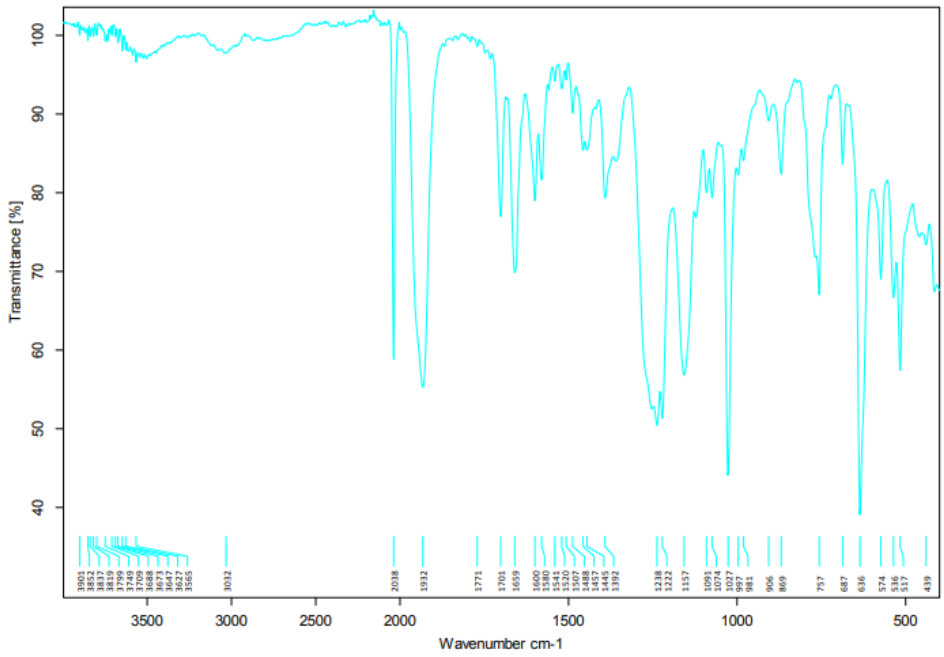


Figure S 6.IR spectrum of **Lyso-CORM**.

Comparison of partial ^{1}H NMR spectra of L1 and Lyso-CORM

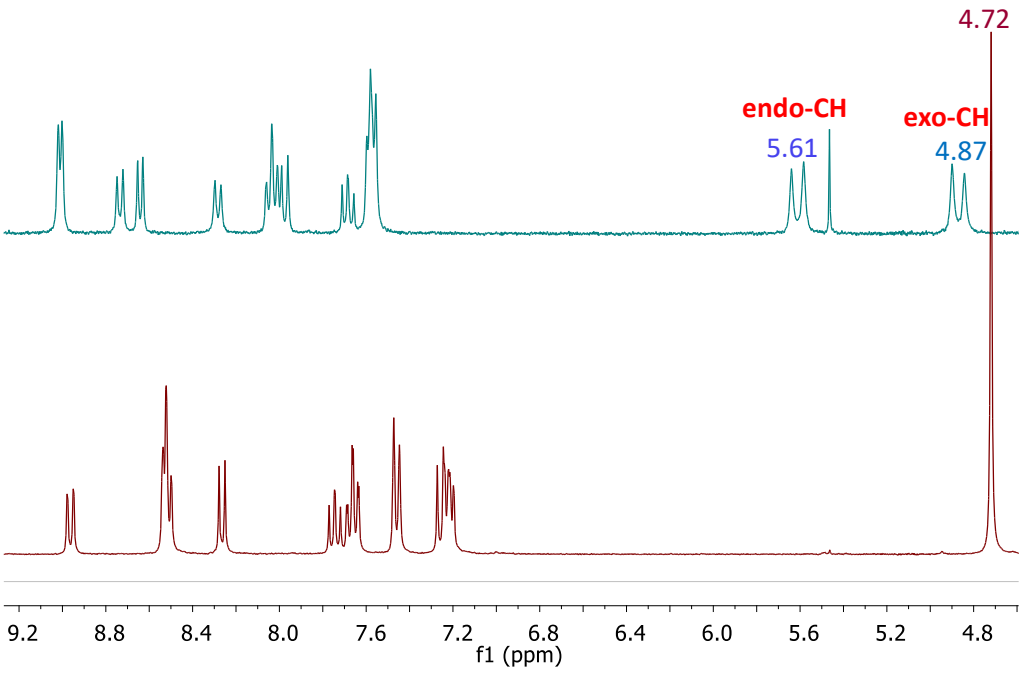


Figure S 7. Partial ^{1}H NMR spectra of **L1** (below) and **Lyso-CORM** (top). The two doublets of **Lyso-CORM** at δ = 5.61 and 4.87 ppm correspond to endo-CH_2 and exo-CH_2 protons.

UV and fluorescence spectra of of L1, Lyso-CORM & Ctrl-CORM

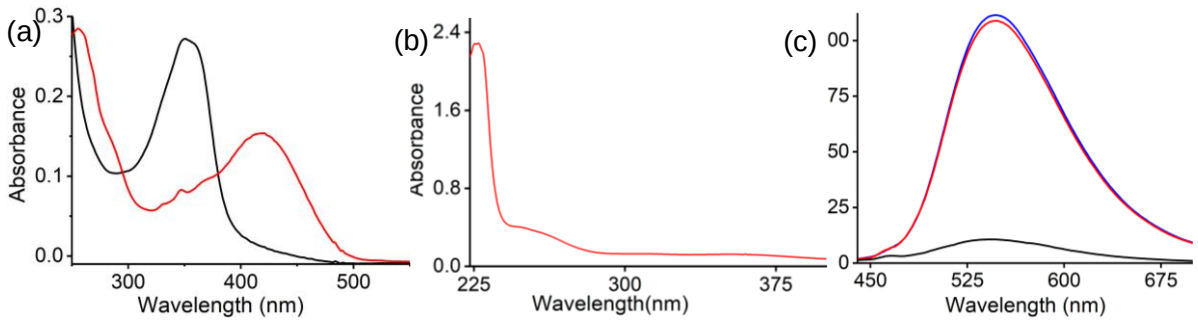


Figure S 8. (a) UV−vis absorption spectrum of **L1** (red line), **Lyso-CORM** (black line); (b) UV−vis absorption spectrum of **Ctrl-CORM** (red line); (c) luminescence spectrum of **L1** (blue line) and Lyso-CORM before (black) and after(red) photo-induced CO-release, (λ_{Ext} = 405 nm, slit width 5/5).

UV-vis spectral changes of Lyso-CORM under dark conditions

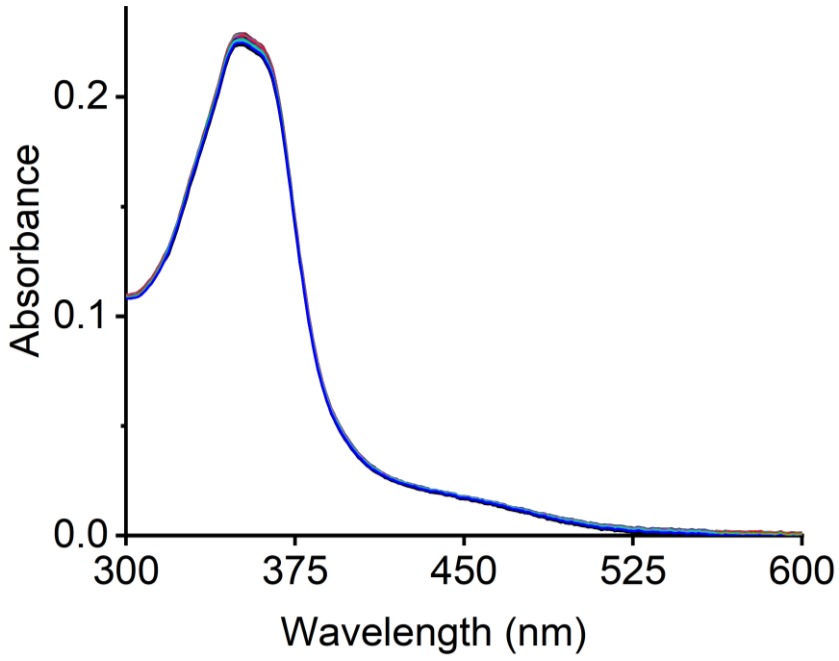


Figure S 9. Eelectronic absorption spectral traces of Lyso-CORM (20 μM) in aq.-PB: DMSO (98:1, v/v) recorded at dark conditions for every 30 min min intervals up to 8 h.

Myoglobin (Mb) assay & Influence of sodium dithionite for Lyso-CORM on CO-release

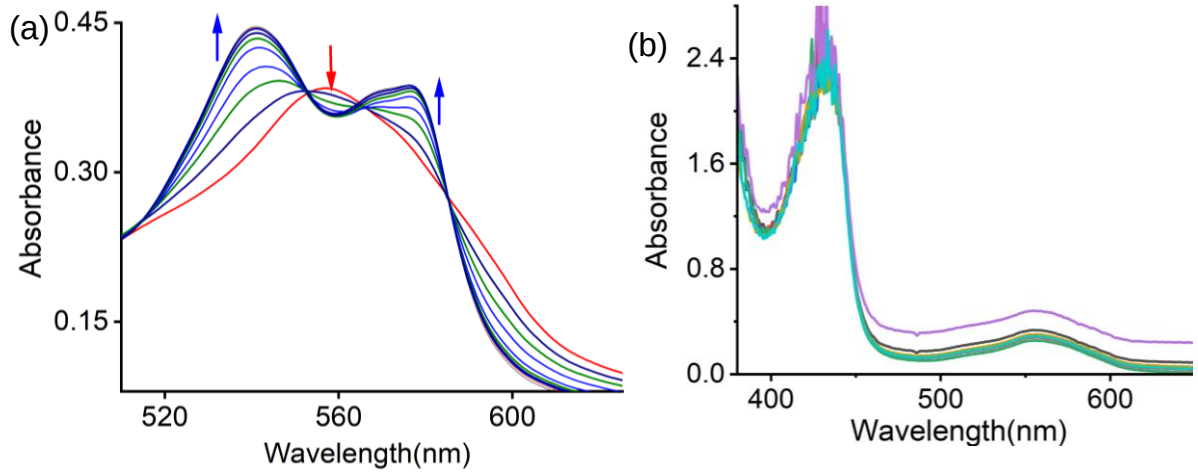


Figure S 10. (a) Conversion of reduced Mb to Mb-CO in a mixture of **Lyso-CORM** (20 μM) and reduced Mb (80 μM) in phosphate buffer (pH 7.4) upon exposure to light (λ_{irr} = 480 nm).(b) Electronic absorption spectral traces of **Lyso-CORM** (20 μM) in presence of sodium dithionite and myoglobin (80 μM) recorded at dark conditions for every 6 min intervals.

Light controlled CO-release

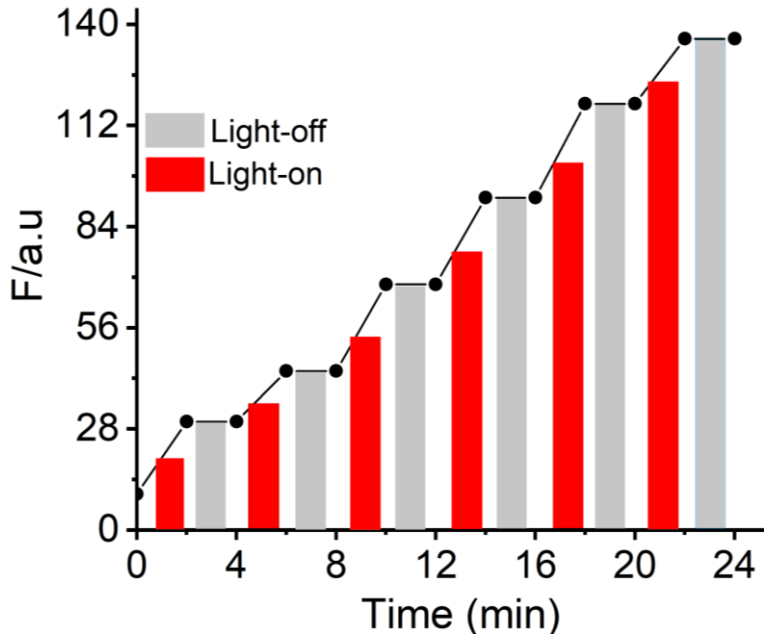


Figure S 11. Controlled CO-release profile of **Lyso-CORM** under light irradiation for different on/off cycles by using fluorescence intensity.

To identify the magnetism and oxidation state of the Mn core in the inactive product, X-band ESR spectroscopy was performed. The X-band ESR spectrum (at 293 K) of the photolyzed solution of Lyso-CORM exhibits a six-line spectrum indicative of a paramagnetic Mn(II) species (Figure S 12b). Further, the paramagnetic nature of the inactive product was shown by ^{1}H NMR. The signals for all protons of Lyso-CORM became broad upon irradiation. This confirms that the Mn (II) species (d^5, high spin) is still bound to the DPA ligand. The photolyzed solution was evaporated to dryness, and the residue was subjected to IR spectroscopy. The solid displayed no CO stretching vibrations in the region of 2100−1900 cm^{-1}, strongly confirming that all the CO molecules were released during the irradiation (Figure S 12a).

IR and EPR spectral changes of Lyso-CORM before and after irradiation

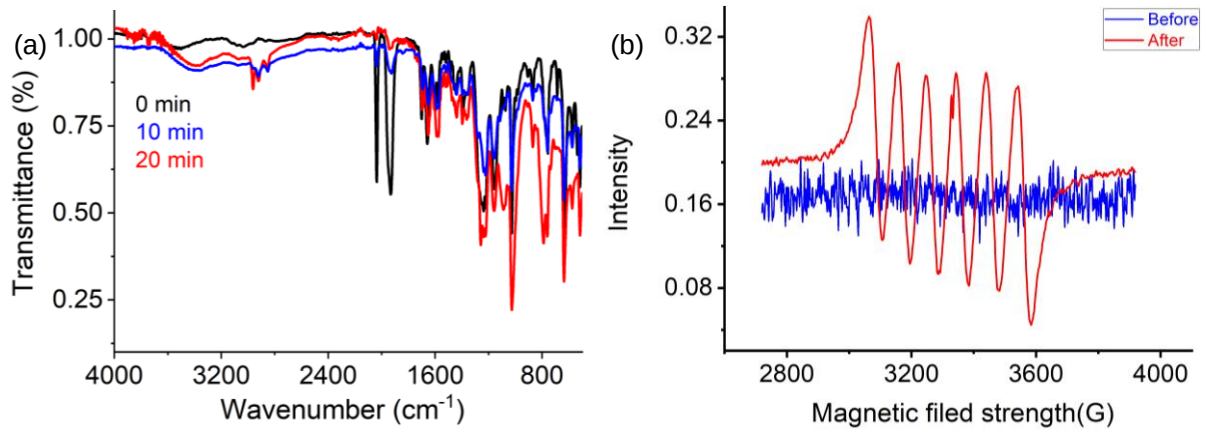


Figure S 12. (a) ATR-IR spectra of **Lyso-CORM** before and after irradiation. The spectrum shows the loss of CO vibration bands (between 1900-2100 cm^{-1}) after the irradiation at 480 nm. (b) X-band ESR spectra (at 293 K) of **Lyso-CORM** (3 mM) before (**Lyso-CORM** exist in Mn^{I}-d^6 , low spin, diamagnetic) after photolysis (**Lyso-CORM** exist in Mn^{II}-d^5 , high spin, paramagnetic with g = 2.08 G) in DMSO−water (1:1, v/v) medium. Microwave frequency, 9.32 GHz; modulation amplitude, 0.6 mT and modulation frequency, 100kHz.

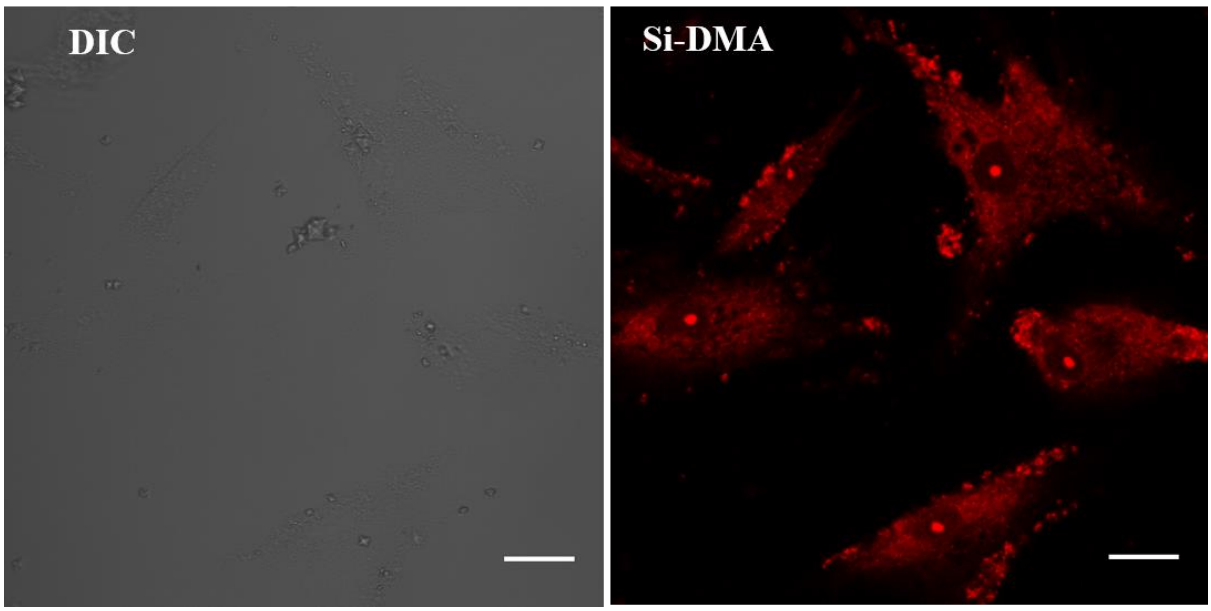


Figure S13. Si-DMA assay in **Lyso-CORM** treated HeLa cells followed by light irradiation. Strong red fluorescence indicates huge singlet oxygen generation. The scale bar corresponds to 20 µm.

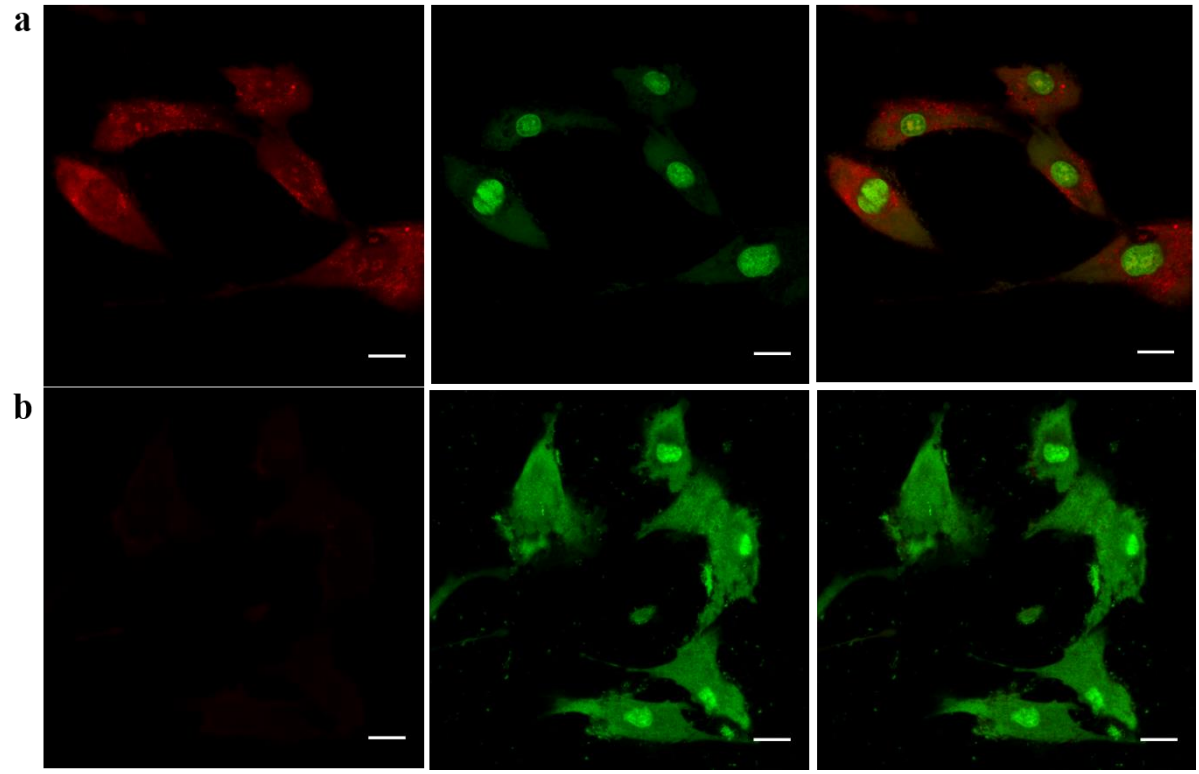


Figure S14. Lysosomal membrane integrity was measured in (a) control (b) **Lyso-CORM** treated HeLa cells using acridine orange (AO). The increase in the green fluorescence intensity indicates the loss of the lysosomal membrane integrity after Lyso-CORM treatment followed by light irradiation. Scale bar corresponds to 20 µm.