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Surporting information

Visible-light Activatable Organic CO-releasing Molecules (PhotoCORMs) that Simultaneously Generate Fluorophores

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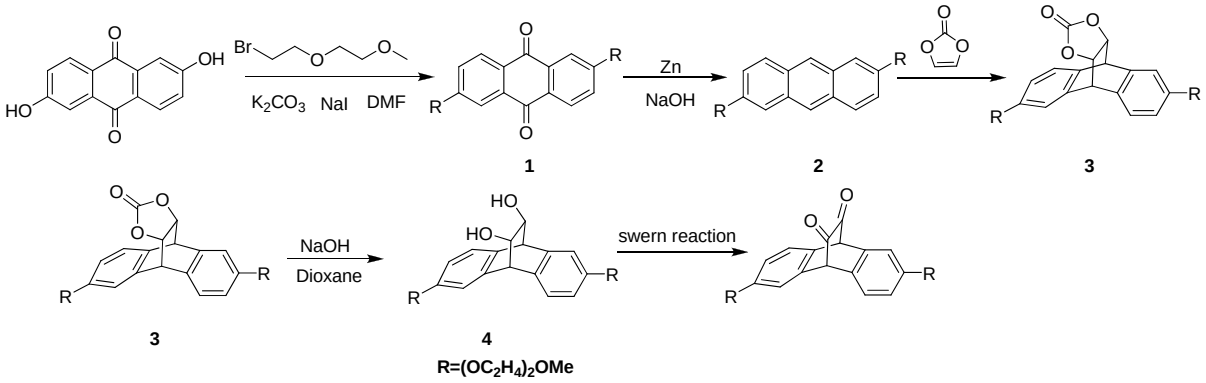
Synthetic Methods:

All chemicals are obtained from commercial sources and used without further purification. The 1H spectroscopic measurements are performed using Varian 500 NMR and Varian 300 NMR spectrometer with tetramethysilane (TMS) as internal reference; chemical shifts are reported in units downfield from TMS. The UV/Vis absorption spectra are obtained with an Varian Cary 50Scan spectrophotometer 1 cm quartz Suprasil cells. High resolution mass spectrometer (HRMS) are measured in Agilent 6210 TOF-MS.

9,10-dihydro-9,10-ethanoanthracene-11,12-dione (DK1)

DK1 was prepared as yellow solid following a literature procedure. [1] ^{1}H NMR (ppm, 500 MHz, $CDCl_3$) : δ = 7.48 (m, 4H), 7.38 (m, 4H), 5.01 (s, 4H). IR (KBr, cm^{-1}): 1752, 1732 (C=O stretch). UV/Vis (DMSO): $\lambda_{max}(\varepsilon)$ = 465 nm. HRMS calcd. $C_{16}H_{10}O_2$, $[M+Na]^+$ = 257.0573; found 257.0583.

Scheme S1. Synthesis of DK2



2,6-bis(2-(2-methoxyethoxy)ethoxy)anthracene-9,10-dione (1).

2,6-dihydroxyanthracene-9,10-dione (5 g, 20.8 mmol) was dissolved with sonication in 100 mL of dry DMF. Then, 14.4 g (104.1 mmol) of dry K_2CO_3, 8.0 g (43.7 mmol) of 1-bromo-2-(2-methoxyethoxy)ethane, and a catalytic amount of NaI were added and the mixture heated to reflux for 2 h. The crude product was poured into ice-cold 1 M aqueous HCl, and filtrated. The solid was redissolved in CH_2Cl_2 and washed with water, the organic fraction was dried over $MgSO_4$, the solvent evaporated, and the resulting product subjected to silica gel column chromatography (CH_2Cl_2 to CH_2Cl_2:CH_3OH 1%) to obtain a light yellow solid (6.1 g, 65.9% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.22 (d, 2H, J = 8.4 Hz), 7.72 (d, 2H, J = 2.7 Hz), 7.26 (dd, 2H, J_{12} = 2.7 Hz, J_{13} = 8.7 Hz), 4.33 (t, 4H, J = 4.8 Hz), 3.92 (t, 4H,

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J = 4.8 Hz), 3.74 (m, 4H), 3.59 (m, 4H), 3.39 (s, 6H).

2,6-bis(2-(2-methoxyethoxy)ethoxy)anthracene (2).

To a 10% NaOH solution (50 mL) of compound 1 (3.0 g, 6.7 mmol) was added zinc powder (9.3 g, 141.1 mmol). The mixture was stirred for two days at 100 °C, cooled to room temperature and then filtered. The filtered cake was washed with CH_2Cl_2. The organic solution was removed in vacuo, and the residue was purified by silica gel column chromatography (Hexane: CH_2Cl_2 = 10:1) to get the yellow solid (1.5 g, 53.6% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.17 (s, 2H), 7.82 (d, 2H, J = 10.2 Hz), 7.17 (m, 4H), 4.29 (t, 4H, J = 4.5 Hz), 3.95 (t, 4H, J = 4.8 Hz), 3.76 (m, 4H), 3.60 (m, 4H), 3.41 (s, 6H).

2,6-bis(2-(2-methoxyethoxy)ethoxy)-9,10,11,15-tetrahydro-9,10-[4,5]epidioxoloanthracen-13-one (3). The compound 2 (1.5 g, 3.6 mmol) was added into 5 mL of vinylene carbonate. The mixture was allowed to stir at 180^{o}C for two days. The solution was removed in vacuo. The residue was purified by silica gel column chromatography (Hexane: ethyl acetate 20%) to get light yellow oil (1.5 g, 82.8% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ =7.25 (d, 1H, J=8.5 Hz), 7.14 (d, 1H, J = 8.0 Hz), 6.95 (t, 2H, J = 2.5 Hz), 6.76 (dd, 1H, J_{12} = 2.5 Hz, J_{13} = 8.5 Hz), 6.74 (dd, 1H, J_{12} = 2.5 Hz, J_{13} = 8.5 Hz), 4.83 (s, 2H), 4.55 (d, 2H, J = 6.5 Hz), 4.11 (t, 4H, J = 5.0 Hz), 3.83 (t, 4H, J = 5.0 Hz), 3.70 (t, 4H, J = 4.5 Hz), 3.57 (m, 4H), 3.39 (s, 6H).

2,6-bis(2-(2-methoxyethoxy)ethoxy)-9,10-dihydro-9,10-ethanoanthracene-11,12-diol (4). A solution of compound 3 (0.6 g, 0.10 mmol) in 1,4-dioxane (5 mL) was added 4 M NaOH aq. (5 mL) under N_2 atmosphere. After refluxed for 2 h, the reaction mixture was cooled to room temperature, and neutralized with 1 M HCl. After the addition of water, the mixture was extracted with $CHCl_3$, and the combined organic layers were washed with water and brine, dried over Na_2SO_4. The solvent was removed under reduced pressure and the mixture was purified by silica gel column chromatography to get yellow oil (550 mg, 96% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.20 (d, 1H, J = 8.1 Hz), 7.14 (d, 1H, J = 8.1 Hz), 6.92 (d, 1H, J = 2.1 Hz), 6.87 (d, 1H, J = 2.1 Hz), 6.68 (dd, 1H, J_{12} = 2.1 Hz, J_{13} = 8.1 Hz), 6.64 (dd, 1H, J_{12} = 2.4 Hz, J_{13} = 8.4 Hz), 4.22 (d, 2H, J = 3.6 Hz), 4.08 (m, 4H), 3.96 (s, 2H), 3.80 (t, 4H, J = 4.8 Hz), 3.68 (t, 4H, J = 4.5 Hz), 3.53 (m, 4H), 3.36 (s, 6H), 2.62 (br, 2H).

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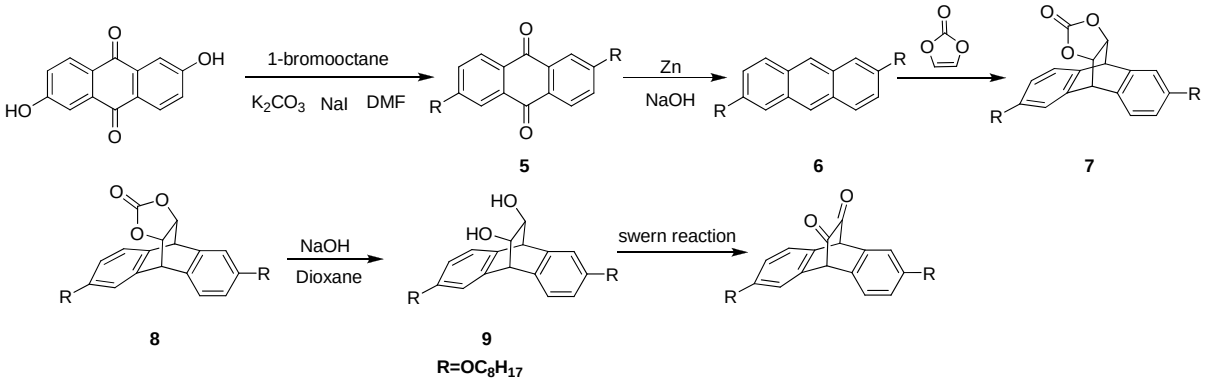
2,6-bis(2-(2-methoxyethoxy)ethoxy)-9,10-dihydro-9,10-ethanoanthracene-11,12-dione (DK2)

To a mixture of dry-DMSO (0.52 mL, 7.4 mmol) and dry-CH_2Cl_2 (5 mL) was added trifluoroacetic anhydride (0.95 mL, 6.7 mmol) dropwise at -78^{0}C under an N_2 atmosphere. After 10 min, the compound 4 (100 mg, 0.21 mmol) dissolved in CH_2Cl_2 was added dropwise. After stirring for 1.5 h, Et_3N (2.2 mL, 15.4 mmol) was added dropwise, and stirring was continued for an additional 1.5 h. After the temperature of the reaction mixture was left to rise up to room temperature, the mixture was poured into 1 M HCl and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and removed in vacuo. The crude product was purified by silica gel column chromatography (CH_2Cl_2) to get red oil (70 mg, 70.6% yield). ^{1}H NMR (300 MHz, $CDCl_3$) : δ = 7.32 (d, 2H, J = 8.1 Hz), 6.98 (s, 2H), 6.88 (d, 2H, J = 7.8 Hz), 4.81 (s, 2H), 4.11(s, 4H), 3.83 (s, 4H),3.68 (s, 4H),3.55 (s, 4H), 3.37 (s, 6H). IR (KBr, cm^{-1}): 1731, 1695 (C=O stretch). UV/Vis (DMSO): $\lambda_{max}(\varepsilon)$ = 465 nm. HRMS calcd. $C_{26}H_{30}O_8$, $[M+Na]^+$ = 493.1833; found 493.1835.

Scheme S2. Synthesis of **DK3**



2,6-bis(octyloxy)anthracene-9,10-dione (5)

2,6-dihydroxyanthracene-9,10-dione (4 g, 16.7 mmol) was dissolved with sonication in 80 mL of dry DMF. Then, 11.5 g (82.3 mmol) of dry K_2CO_3, 6.75 g (43.7 mmol) of 1-bromooctane, and a catalytic amount of NaI were added and the mixture heated to reflux for 2 h. The crude product was poured into ice-cold 1 M aqueous HCl, and filtrated. The solid was redissolved in CH_2Cl_2 and washed with water, the organic fraction was dried over $MgSO_4$, the solvent evaporated, and the resulting product subjected to silica gel column

chromatography (Hexane: ethyl acetate 20%) to obtain a light yellow solid (4.3 g, 59.5% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.21 (d, 2H, J = 9.0 Hz), 7.71 (d, 2H, J = 3.0 Hz), 7.25 (dd, 2H, J_{12} = 2.0 Hz, J_{13} = 8.5 Hz), 4.15 (t, 4H, J = 6.5 Hz), 1.85 (m, 4H), 1.49 (m, 4H), 1.32 (m, 16H), 0.90 (t, 6H, J = 7.0 Hz).

2,6-bis(octyloxy)anthracene (6).

To a 10% NaOH solution (40 mL) of compound 5 (2.3 g, 5.3 mmol) was added zinc powder (7.3 g, 111.6 mmol). The mixture was stirred for two days at 100 °C, cooled to room temperature and then filtered. The filtered cake was washed with CH_2Cl_2. The organic solution was removed in vacuo, and the residue was purified by silica gel column chromatography (Hexane: ethyl acetate 10%) to get the yellow solid (1.3 g, 60.4 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.18 (s, 2H), 7.84 (d, 2H, J = 9.0 Hz), 7.16 (m, 4H), 4.11 (t, 4H, J = 6.5 Hz), 1.88 (m, 4H), 1.53 (m, 4H), 1.36 (m, 16H), 0.91 (t, 6H, J = 6.5 Hz).

2,6-bis(octyloxy)-9,10,11,15-tetrahydro-9,10-[4,5]epidioxoloanthracen-13-one (7).

The compound 6 (1.0 g, 2.3 mmol) was added into 5 mL of vinylene carbonate. The mixture was allowed to stir at 180^{0}C for two days. The solution was removed in vacuo. The residue was purified by silica gel column chromatography (Hexane: ethyl acetate 20 %) to get light yellow oil (0.9 g, 80.5% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.25 (dd, 2H, J_{12} = 2.0 Hz, J_{13} = 8.0 Hz), 6.92 (t, 2H, J = 2.0 Hz), 6.75 (m, 2H), 4.85 (s, 2H), 4.54 (d, 2H, J = 6.5 Hz), 3.92 (t, 4H, J = 6.5 Hz), 1.75 (m, 4H), 1.43 (m, 4H), 1.30 (m, 16H), 0.89 (t, 6H, J = 7.0 Hz).

2,6-bis(octyloxy)-9,10-dihydro-9,10-ethanoanthracene-11,12-diol (8).

A solution of compound 7 (0.4 g, 0.77 mmol) in 1,4-dioxane (4 mL) was added 4 M NaOH aq. (4 mL) under N_2 atmosphere. After refluxed for 2 h, the reaction mixture was cooled to room temperature, and neutralized with 1 M HCl. After the addition of water, the mixture was extracted with $CHCl_3$, and the combined organic layers were washed with water and brine, dried over Na_2SO_4. The solvent was removed under reduced pressure and the mixture was purified by silica gel column chromatography to get yellow oil (340 mg, 89% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.23 (d, 1H, J = 8.1 Hz), 7.16 (d, 1H, J = 8.4 Hz), 6.94 (d, 1H, J = 2.7 Hz), 6.70 (dd, 1H, J_{12} = 2.4 Hz, J_{13} = 8.1 Hz), 6.64 (dd, 1H, J_{12} = 2.4 Hz, J_{13} = 8.1 Hz), 4.25 (m, 2H), 4.01 (s, 2H), 3.90 (m, 4H), 2.09 (m, 2H), 1.74 (m, 4H), 1.32 (m, 20H), 0.87 (t,

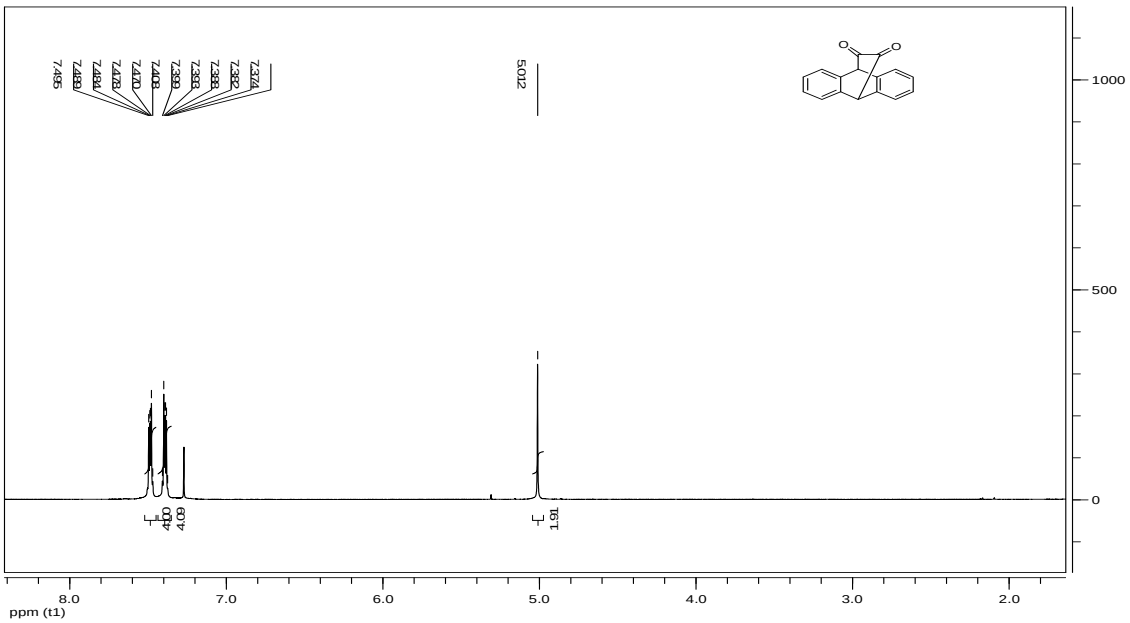
6H, J = 6.6 Hz).

2,6-bis(octyloxy)-9,10-dihydro-9,10-ethanoanthracene-11,12-dione (DK3)

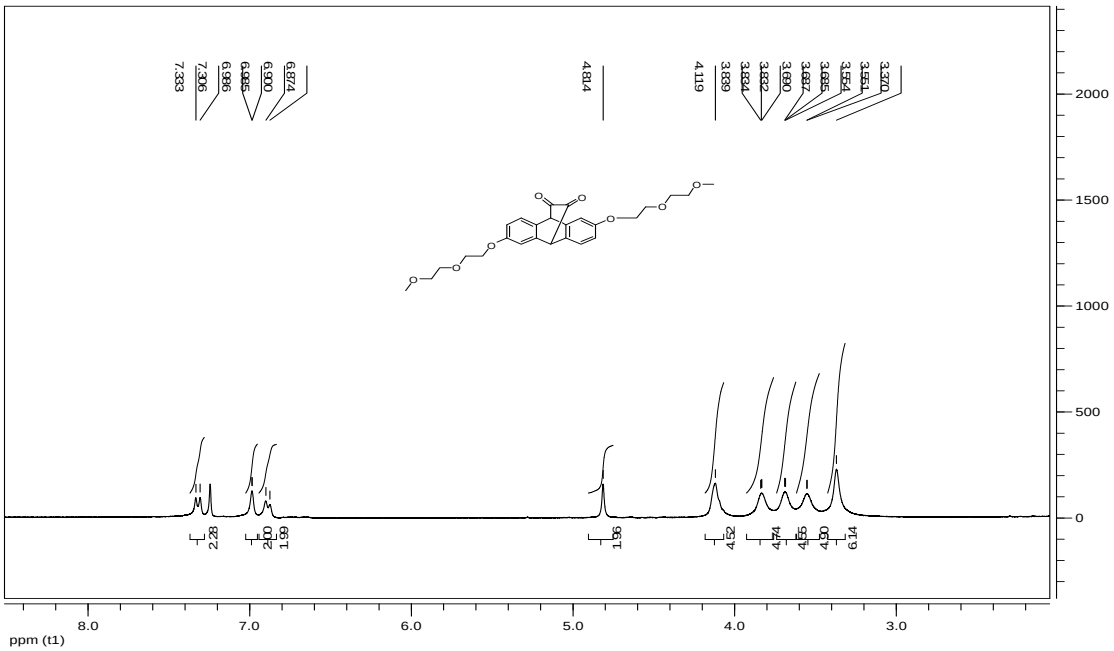
To a mixture of dry-DMSO (0.50 mL, 7.1 mmol) and dry-CH_2Cl_2 (5 mL) was added trifluoroacetic anhydride (0.90 mL, 6.5 mmol) dropwise at -78^{0}C under an N_2 atmosphere. After 10 min, the compound 8 (100 mg, 0.20 mmol) dissolved in CH_2Cl_2 was added dropwise. After stirring for 1.5 h, Et_3N (2.0 mL, 14.7 mmol) was added dropwise, and stirring was continued for an additional 1.5 h. After the temperature of the reaction mixture was left to rise up to room temperature, the mixture was poured into 1 M HCl and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and removed in vacuo. The crude product was purified by silica gel column chromatography (Hexane: ethyl acetate 25 %) to get red solid (71 mg, 71.6 % yield). δ = 7.34 (d, 2H, J = 8.5 Hz), 6.97 (d, 2H, J = 2.5 Hz), 6.89 (d, 1H, J = 2.5 Hz), 6.87 (d, 1H, J = 2.5 Hz), 4.83 (s, 2H), 3.95 (t, 4H, 6.5 Hz), 1.77 (m, 4H), 1.43 (m, 4H), 1.30 (m, 4H), 0.89 (t, 6H, J = 6.5 Hz). IR (KBr, cm^{-1}) 1752, 1734 (C=O stretch). UV/Vis (DMSO): $\lambda_{max}(\varepsilon)$ = 467 nm. HRMS calcd. $C_{32}H_{42}O_4$, $[M+Na]^+$ = 513.2975; found 513.2999.

NMR Spectra of the PhotoCORMs (CDCl3, 500 MHz)

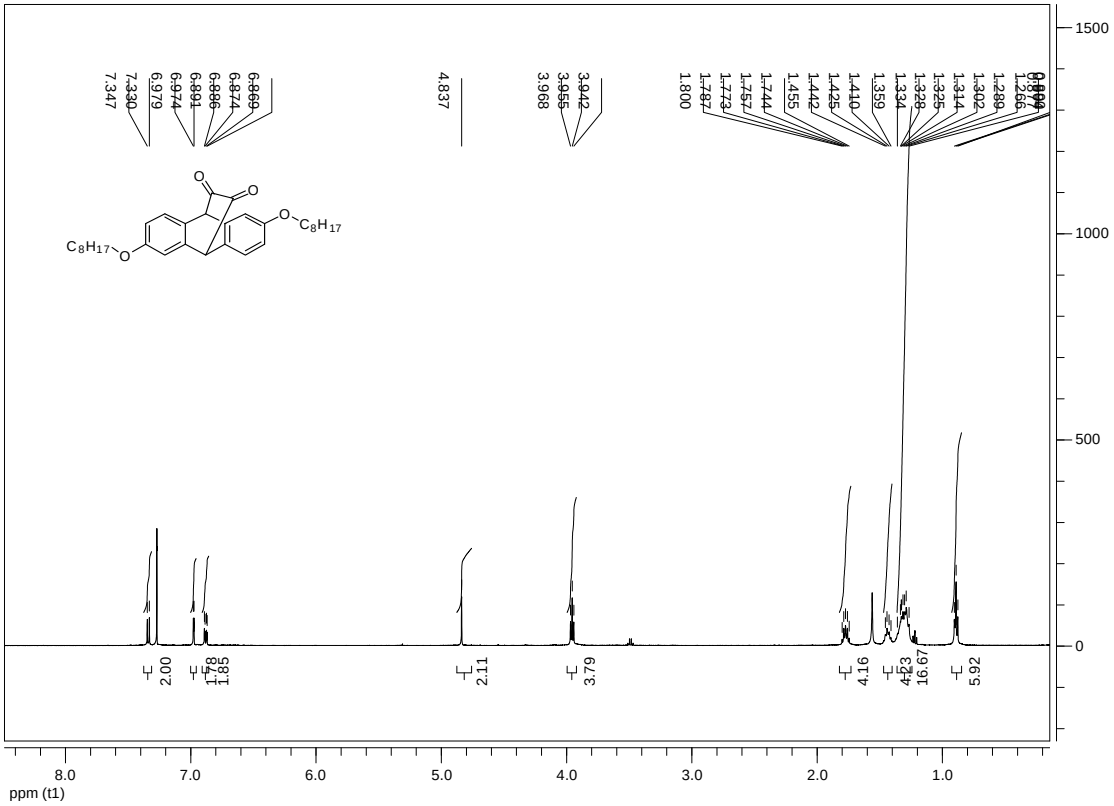
NMR of DK1



NMR of DK2



NMR of DK3



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Micelle preparation

The diketone compound is dissolved in DCM (5 mL). The solution is mixed with Pluronic F 128NF (100 mg) in water (5 mL). The resulting mixture is stirred at room temperature for 48 h to slowly evaporate the DCM.

CO detection using two-compartment myoglobin test

The setup of the two-compartment test is illustrated in Figure S1. Specifically, myoglobin solution was degassed and mixed with excess amount of $Na_2S_2O_4$ in a sealed Erlenmeyer flask. Reduction of the MbFe(III) to MbFe(II) was confirmed by UV-Vis absorption spectroscopy. DK3 micelle solution in PBS buffer was degassed and stocked in an air-tight syringe. The long needle tip of the syringe was positioned at the very bottom of the MbFe(II) solution. The syringe was chilled in ice-water bath to increase the solubility of CO in water and irradiated by 470nm LED light. The plunger of the syringe was pulled very slowly in order to draw the Mb solution from the flask into the syringe without making gap between the solution and the plunger. The amount of Mb is ~4 times that of the theoretical yield of CO. The absorbance of the mixture solution was recorded again in a quartz cuvette. The concentration of MbCO was calculated by using the following equations.[2]

$$\frac{A_{542}}{A_{iso}} = \frac{\varepsilon_{dB42}[Mb] + \varepsilon_{COB42}[MbCO]}{\varepsilon_{iso}([Mb] + [MbCO])} = \frac{\varepsilon_{dB42}}{\varepsilon_{iso}} - \frac{\varepsilon_{dB42} - \varepsilon_{COB42}}{\varepsilon_{iso}} \cdot \frac{[MbCO]}{[Mb] + [MbCO]}$$

$$\therefore \frac{[MbCO]}{[Mb] + [MbCO]} = \left(\frac{\varepsilon_{dB42}}{\varepsilon_{iso}} - \frac{A_{542}}{A_{iso}}\right) \cdot \frac{\varepsilon_{iso}}{\varepsilon_{dB42} - \varepsilon_{COB42}}$$

Where A_{542} and A_{iso} are the absorbance at 542 and 552nm, and [Mb] and [MbCO] are the concentration of Mb and MbCO.

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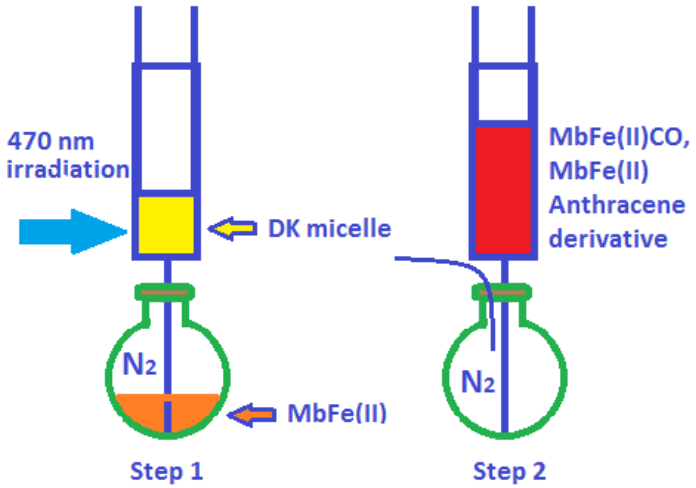

Figure S1. Setup for the two-compartment myoglobin test of CO released from the photoCORM.

CO detection using the Rh complex

To demonstrate the release of CO from the micelles, the compound cis-$[Rh_2(C_6H_4PPh_2)_2(O_2CCH_3)_2](HO_2CCH_3)_2$ was used in a CO-signaling capacity. The complex was mixed with silicon gel with weight ratio of 1:3. Some silica gel powders absorbed with the Rh complex was sealed in a side arm of a round bottom flask filled with an aqueous solution of DK3 micelle. After irradiation of the micelle aqueous solution for ten minutes, the color of the Rh complex changed from violet to orange. (Figure S7)

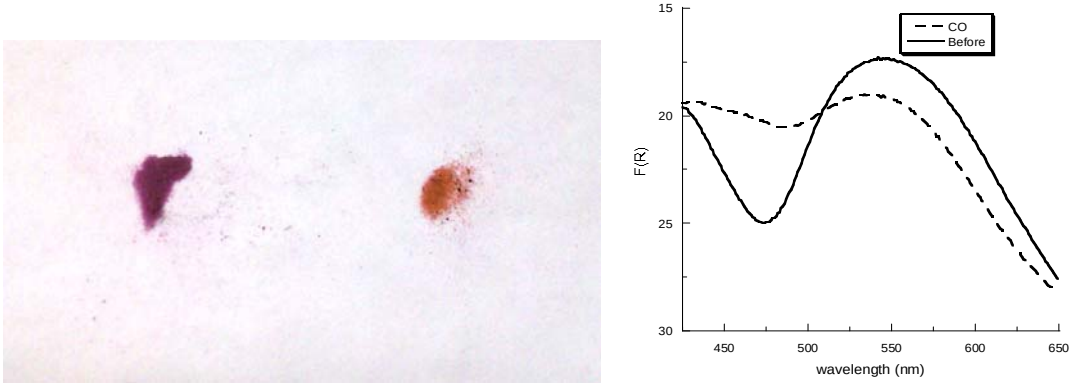

Figure S2: Photograph (left) and reflectance UV-Vis spectra showing silica gel containing adsorbed cis-$[Rh_2(C_6H_4PPh_2)_2(O_2CCH_3)_2](HO_2CCH_3)_2$ before irradiating the micelle solution and after irradiating the micelle solution.

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Cell biology tests

Cell culture Acute myeloid leukemia cell line KG1 was purchased from American Tissue Culture Collection (ATCC). The KG1 cells were derived from AML patient and are suspension cells. For all conditions, KG1 cells were seeded in a 24-well plate in IMDM media (ATCC) + 20% fetal bovine serum (FBS; Hyclone) at 0.5 x 106 cells per mL of culture. All culture conditions were incubated overnight in a 37^{o}C, 5% CO_2 incubator prior to irradiating and imaging. After irradiation and imaging, cell cultures were maintained at 37^{o}C, 5% CO_2 for 72 hours to observe possible effects on viability, cell growth, and morphology.

Loading and activation of the photoCORM in KG1. To test the effects of the photoCORM on cell viability and growth, KG1 cells were seeded at 0.5 x 106/mL in 500 µL of growth media in a 24-well plate and incubated overnight as is, with vehicle control (pluronic solution), inactive control (the anthracene derivative), or photoCORM at 10, 25 and 40 µM concentration. To prevent premature light activation, the cell culture plates were protected from light during the duration of the experiment. The following day KG1 cells were pelleted at 250 X g for 5 minutes and placed in 1 mL of DPBS, 2 mM Glutamax (Invitrogen), 25 mM α-D glucose (Acros Organics) at a cell density of 0.5 x 106/mL. The cell suspensions were then split into two different plates of an experimental plate to be irradiated and a control non-irradiated plate. The control plate was completely covered in aluminum foil to avoid light activation. Both plates were placed in a 37^{o}C incubator for the irradiation process. A light source (wavelength of 470 nm) was placed at a 10 cm distance from the experimental plate. KG1 cells were then irradiated with six 30 second pulses, with gentle mixing in between irradiation. After irradiation, both irradiated and non-irradiated cells were washed and resuspended in 500 µL growth media and cultured in humidified atmosphere with 5% CO_2 at 37^{o}C.

Fluorescence microscopy Fluorescence was observed using a Nikon Eclipse 80i microscope with an attached X-Cite series 120 lamp and a DAPI emission filter set. Images (Figure S3-S5) were captured at 10 × and 20 × magnification with a high-resolution digital camera (Nikon Digital Sight).

Growth and viability of KG1 Cells (Figure S6) were counted daily using hemacytometer

and viability was assessed by flow cytometry (Canto II, Beckman Coulter) using ApoScreen with Annexin V-FITC and propidium Iodide (Beckman Coulter) according to manufacturers protocol. A gate was placed to remove non-cell events. Viable cells were defined as negative for Annexin V-FITC and propidium iodide.

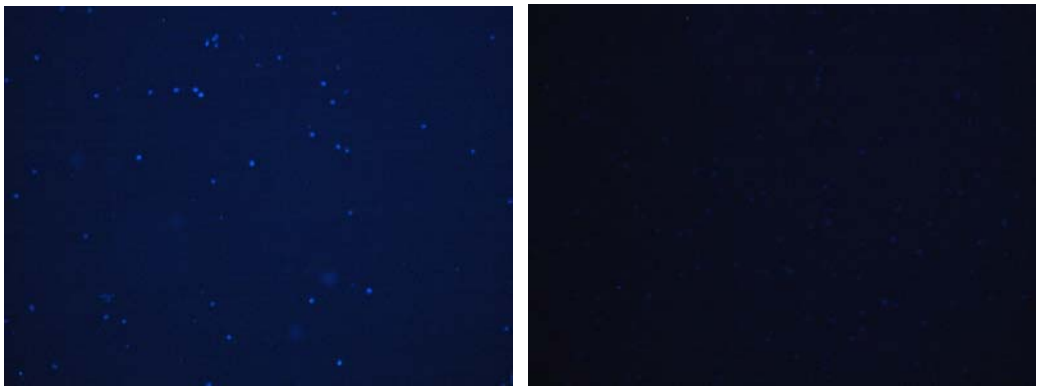

Figure S3. KG1 cells incubated with 25 μM DK3 micelle after three 30-second pulse irradiation of 470 nm light (left), and before irradiation (right).

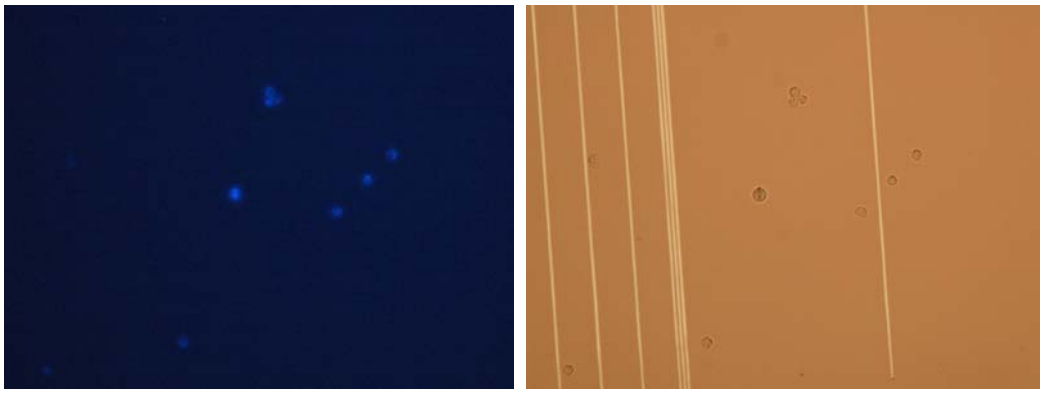

Figure S4. Dark field (left) and bright field imaging of KG1 cells incubated with 25 μM DK3 micelle after 470 nm irradiation.

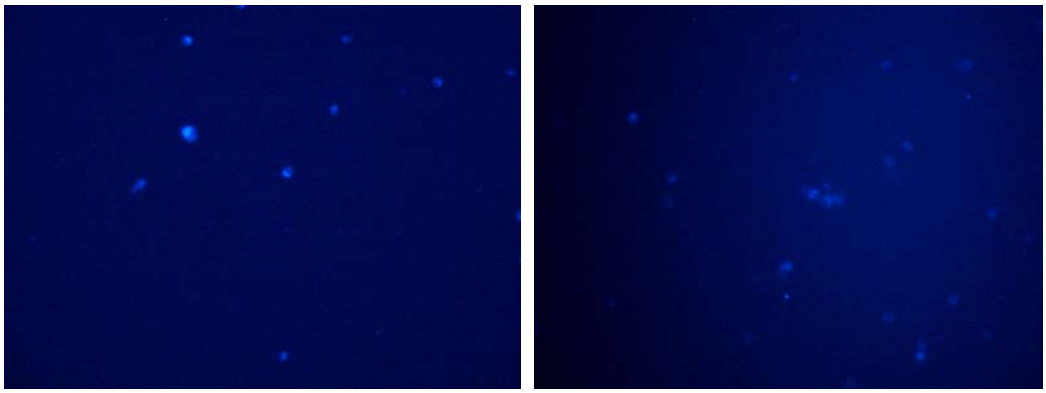

Figure S5. KG1 cells incubated with 40 μM DK3 micelle after three 30-second pulse irradiation of 470 nm light. The left image was taken immediately after irradiation. The right image was taken after 72 hours. The fluorescence was weaker but still observable.

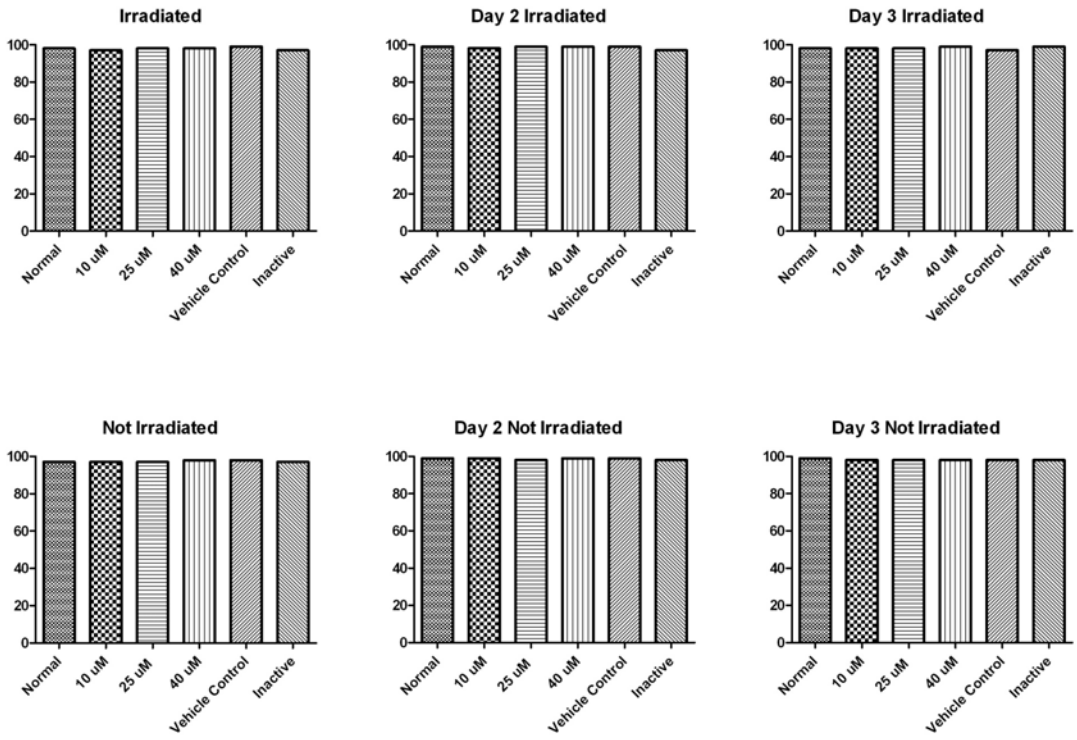

Figure S6. Cell viability tests of the irradiated and not irradiated samples. (Normal: KG1 cell only, vehicle control: KG1 cell with Pluronic, inactive: KG1 cell with the anthracene derivative.)

Reference

(1) Mondal, R.; Shah, B. K.; Neckers, D. C. *J. Am. Chem. Soc.* **2006**, *128*, 9612-9613.

(2) Hasegawa, U.; Van der Vlies, A. J.; Simeoni, E.; Wandrey, C.; Hubbell, J. A. *J. Am. Chem. Soc.* **2010**, *132*, 18273-18280