Electronic Supplementary Material (ESI) for Dalton Transactions.

Supplementary Information

Lipophilic Ruthenium Salen Complexes: Incorporation into Liposome Bilayers and Photoinduced Release of Nitric Oxide

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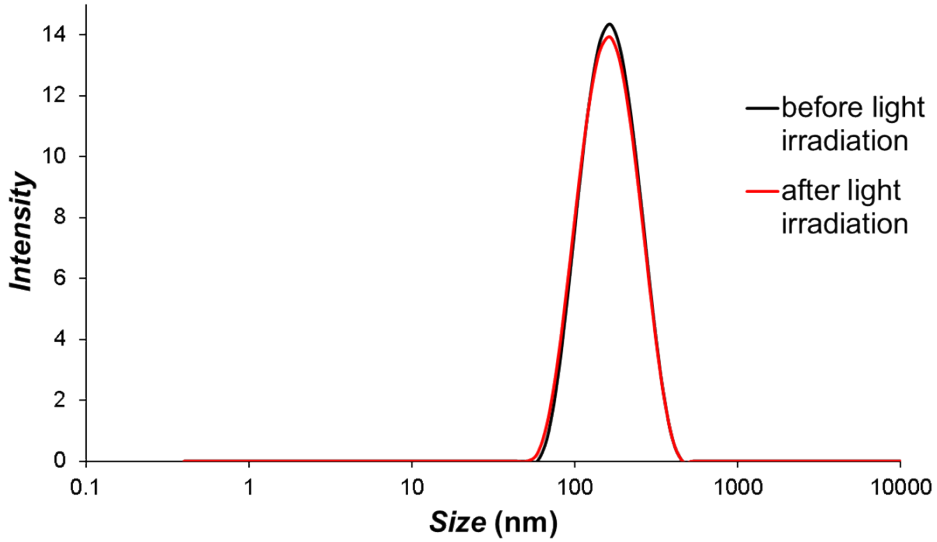


Fig. S1 Particle size distribution of **1**_Lipo measured with DLS. $Z_{average}$ diameters before and after light irradiation are 141 nm and 136 nm, respectively.

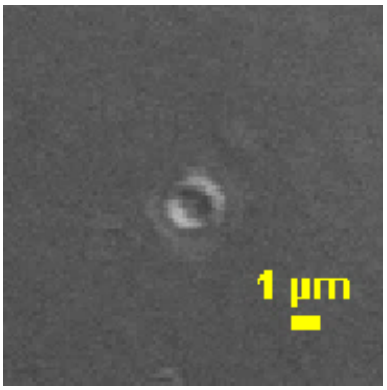


Fig. S2 Confocal laser scanning microscopy image of **1**_Lipo.

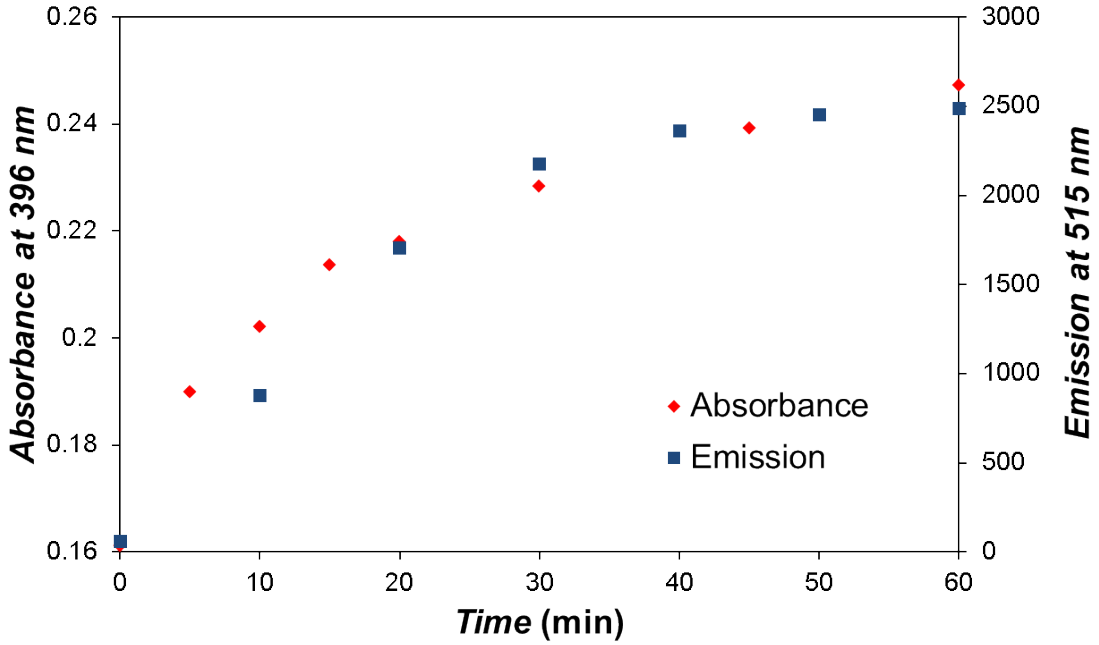


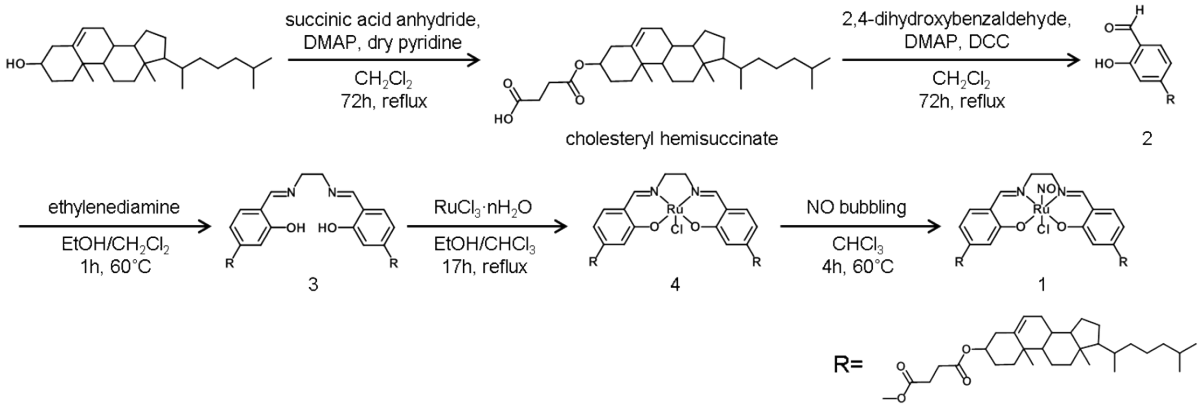
Fig. S3 Time profiles of the absorbance at 396 nm of **1**_Lipo (from Fig. 3), and the emission at 515 nm of the mixture of **1**_Lipo and DAF-2 (from Fig. 4)

Materials

DMPA, DMPG, and cholesterol were purchased from Avanti Polar Lipids. All chemicals were purchased from commercial sources and used without further purification.

Synthesis of [Ru(L)Cl(NO)] (1)

(L = *N,N'*-ethylene-bis(4-cholesteryl-hemisuccinate-salicylideneamine))



Scheme S1 Synthetic route of [Ru(L)Cl(NO)] (**1**)

4-cholesteryl hemisuccinate-salicylaldehyde (2)

Cholesteryl hemisuccinate was prepared by literature method.[1] 2,4-dihydroxybenzaldehyde (0.83 g, 4.0 mmol), *N,N'*-dicyclohexylcarbodiimide (DCC) (0.82 g, 4.0 mmol) and DMAP (20 mg, 0.16 mmol) were suspended in dry dichloromethane (10 ml) under nitrogen. A solution of cholesteryl hemisuccinate (1.94 g, 4.0 mmol) in dry dichloromethane (10 ml) was added slowly. The reaction mixture was stirred at room temperature for 1 h and heated at 40˚C for 16 h. The white solid was separated by filtration and washed with dichloromethane. The filtrate was concentrated to about 5 ml by evaporation, and the crude product was purified by column chromatography on silica gel (1/30 = methanol/dichloromethane). **2** was obtained as a white powder (1.43 g, 2.4 mmol, yield: 59%).

^{1}H-NMR (600 MHz, $CDCl_3$): δ = 0.67 (s, 3H, -CH_3), 0.80 - 1.62 (m, 40H, -CH-, -CH_2-,-CH_3), 1.74 - 2.02 (m, 5H, -CH-, -CH_2-), 2.33 (d, 2H, -C=C-CH_2-), 2.75 - 2.95 (m, 4H, -(C=O)-CH_2-CH_2-(C=O)-), 4.54 - 4.71 (m,1H, -(C=O)-O-CH-), 5.37 (d, 1H, -C=C-H), 7.01 (t, 1H, phenyl H), 7.34 (d, 1H, phenyl H), 7.48 (d, 1H, phenyl H), 9.93 (s, 1H, -CHO), 11.1 (s, 1H, -OH) ppm.

***N,N'*-ethylene-bis (4-cholesteryl-hemisuccinate-salicylideneamine) (H_2 L) (3)**

2 (0.82 g, 1.4 mmol) was dissolved in a mixture of ethanol (100 ml) and dichloromethane (30 ml). After the solution was heated at 60˚C, ethylenediamine (48 μl, 0.70 mmol) was added, resulting in the yellow precipitation. The reaction mixture was stirred at 60˚C for 4 h. Dichloromethane was removed from the reaction mixture by heating at 70˚C without a condenser. After cooling reaction mixture in the ice bath for 4 h, yellow solid was collected by filtration. **3** was dried under vacuum (0.63 g, 0.51 mmol, yield: 75%).

Elemental analysis: Calcd. for [H_2 L]: H, 9.12; N, 2.26; C, 75.69, Found: H, 9.04; N, 2.26; C, 75.89.

^{1}H-NMR (600 MHz, $CDCl_3$): δ = 0.67 (s, 6H, -CH_3), 0.80 - 1.62 (m, 71H, -CH-, -CH_2-,-CH_3), 1.70 - 2.08 (m, 11H, -CH-, -CH_2-), 2.33 (d, 2H, -C=C-CH_2-), 2.70 - 2.85 (m, 8H, -(C=O)-CH_2-CH_2-(C=O)-), 3.92 (s, 4H, =N-CH_2-CH_2-N=) , 4.64 - 4.66 (m,2H, -(C=O)-O-CH-), 5.37 (d, 2H, -C=C-H), 6.63 (d, 2H, phenyl H), 6.67 (s, 2H, phenyl H), 7.21-7.23 (d, 2H, phenyl H), 8.320 (s, 2H, -CH=N-) ppm.

[Ru(L)Cl] (4)

3 (0.50 g, 0.40 mmol) and $RuCl_3$· nH_2O (0.15 g, 0.60 mmol) were suspended in a mixture of dry ethanol (15 ml) and dry chloroform (15 ml) under nitrogen. The reaction mixture was stirred at 100°C for 17 h, resulting in the color change of the solution from red brown to deep green. The mixture was cooled at 0°C for 24 h. Then, ethanol (10 ml) was added to the reaction mixture, and deep green precipitation was collected by filtration. The precipitation was dissolved in chloroform, and insoluble products were removed by filtration. **4** was obtained as a deep green solid by evaporation of the filtrate (0.11 g, 0.082 mmol, yield: 20%).

MALDI-TOF MS: Found: m,*z*=1395.8, Calcd. for [Ru(L)Cl]+Na^+: m/z=1395.2.

[Ru(L)Cl(NO)] (1)

4 (0.095 g, 0.068 mmol) was dissolved in dry chloroform (45 ml) under nitrogen, and dissolved oxygen was removed by freeze-pump-thaw cycling (3 sets). Then, nitric oxide was bubbled through the solution for 4 h at 60°C resulting in a reddish brown solution. The mixture was purified by a silica gel column chromatography (1/100 = methanol/dichloromethane). The red brown fraction was collected, and dried under vacuum to obtain **1** as a brown solid (0.034 g, 0.024 mmol, yield: 24%).

Elemental analysis: Calcd. for [Ru(L)Cl(NO)]· H_2O: H, 7.95; N, 2.96; C, 65.96. Found: H, 7.89; N, 2.90; C, 66.24.

^{1}H-NMR (600 MHz, $CDCl_3$): δ = 0.67 (s, 6H, -CH_3), 0.80 - 1.62 (m, 81H, -CH-, -CH_2-,-CH_3), 1.85 - 2.01 (m, 11H, -CH-, -CH_2-), 2.33 (d, 2H, -C=C-CH_2-), 2.70 - 2.87 (m, 8H, -(C=O)-CH_2-CH_2-(C=O)-), 3.88-4.23 (m, 4H, =N-CH_2-CH_2-N=) , 4.64 (d,2H, -(C=O)-O-CH-), 5.37 (d, 2H, -C=C-H), 6.51 (d, 2H, phenyl H), 6.98 (s,2H, phenyl H), 7.22-7.24 (d, 2H, phenyl H), 8.20 (s, 2H, -CH=N-) ppm.

Preparation of [Ru(L)Cl(NO)]_Lipo (1_Lipo)

1_Lipo was prepared by Bangham method. **1** dissolved in chloroform (1.0 mM, 200 μl) and 1,2-ditetradecanoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt)(DMPG) dissolved in chloroform/methanol (1/1) (20 mM, 200 μl) and cholesterol dissolved in chloroform (20 mM, 40 μl) were mixed in chloroform (2.0 ml). The organic solvent was removed by a rotary evaporation yielding a thin lipid film. Then, the lipid film was hydrated with Tris/HCl buffer (pH 7.4, 20 mM, 2.0 ml) at 37°C for 24 h. The resulting suspension was sonicated at 37°C for 10 min, and extruded 21 times through two layers of a polycarbonate membrane with a mean pore diameter of 1.0 μm. The suspension was purified by Sephadex G-25 using Tris/HCl buffer (pH 7.4, 20 mM) to obtain **1**_Lipo. The ruthenium concentration of **1**_Lipo was determined by ICP-MS (Agilent 7500c).

Preparation of DAF-2 encapsulating liposomes (DAF-2@Lipo)

DAF-2@Lipo was prepared by freeze-thaw method. 1,2-ditetradecanoyl-sn-glycero-3-phosphate (sodium salt) (DMPA) dissolved in chloroform/methanol/miliQ (65/35/1) (20 mM, 200 μl) and cholesterol dissolved in chloroform (20 mM, 40 μl) were mixed in chloroform/methanol=2/1 (2.0 ml). The organic solvent was removed by a rotary evaporation yielding a thin lipid film. The lipid film was hydrated with Tris/HCl buffer (pH 7.4, 20 mM, 2.0 ml) containing DAF-2 (20 μM) at 57°C for 24 h. The resulting suspension was sonicated at 57°C for 3 min, and frozen with liquid nitrogen and then thawed at 57°C with water bath to concentrate DAF-2 in the inner aqueous phase of liposomes. The freeze-thaw cycle was repeated five times. The suspension was purified by Sephadex G-25 using Tris/HCl buffer (pH 7.4, 20 mM) to obtain DAF-2@Lipo.

NO photorelease experiments of samples were carried out using a xenon lamp (XFL-300, Yamashita Denso, Corp. Japan), and the wavelength range was 400-750 nm.

Generation of photoproducts of 1 for UV-Visible analysis

The chloroform solution of **1** (5.0 x10^{-2} mM) was degassed by nitrogen bubbling for 20 min. The degassed solution was irradiated with Xe lamp (400-750 nm) under nitrogen at 20°C. UV-vis spectra of the solution were measured (wavelength range: 300-800 nm).

Generation of photoproducts of 1 for IR analysis

The chloroform solution of **1** (2.0 mM) was degassed by nitrogen bubbling for 20 min at 20˚C. The degassed solution was irradiated with Xe lamp (400-750 nm) under nitrogen for 30 min at 20°C. Then, the solid green residue was obtained by removing the organic solvent using a rotary evaporation. The residue was kept inside a desiccator for 12h.

Generation of photoproducts of 1_Lipo for UV-Vis absorption analysis

Suspension of **1_Lipo** ([Ru] = 11.2 μM) was irradiated with Xe lamp (400-750 nm) at 20˚C. UV-vis spectra of the solution were measured (wavelength range: 300-800 nm).

Detection of NO from 1_Lipo using DAF-2

Suspension of **1**_Lipo ([Ru] = 3.3 μM) and DAF-2 (10 μM) in 20 mM Tris/HCl buffer (pH 7.4) was irradiated with Xe lamp (400-750 nm) at 20˚C. Emission spectra of the reaction solution were measured (λ_{ex} = 495 nm, wavelength range: 500-650 nm).

Standard curve for nitric oxide determination

To quantify the amount of NO released from **1**_Lipo, a standard curve for the DAF-2T fluorescence intensity was prepared using NO donor, NOC7 (1-Hydroxy-2-oxo-3- (*N*-methyl-3-aminopropyl)-3-methyl-1-triazene (half-time: 5 min under 37˚C, pH 7.4)). NOC7 (1.62 mg, 10 μmol) was dissolved in NaOH aq (1.0 ml, 0.10 M), and the solution was diluted 100 times with NaOH aq (0.10 M) to prepare 100 μM NOC7 solution. Arbitrary amount of the NOC7 solution (2.5, 5.0, 10, 20, 30, 40, 50 μl) was added to DAF-2 solutions (400 μl, 50 μM), and diluted to 2.0 ml (total volume) with Tris/HCl buffer (pH 7.4, 20 mM). After these solutions were incubated at 37˚C for 2 h, they were cooled to 20˚C. Then, emission spectra were measured (λ_{ex} = 495 nm).

Concentration of NO was calculated assuming that NOC7 released 2 equivalents NO (slope: 1102.7; y-intercept: 1.7301; R^2: 0.999).

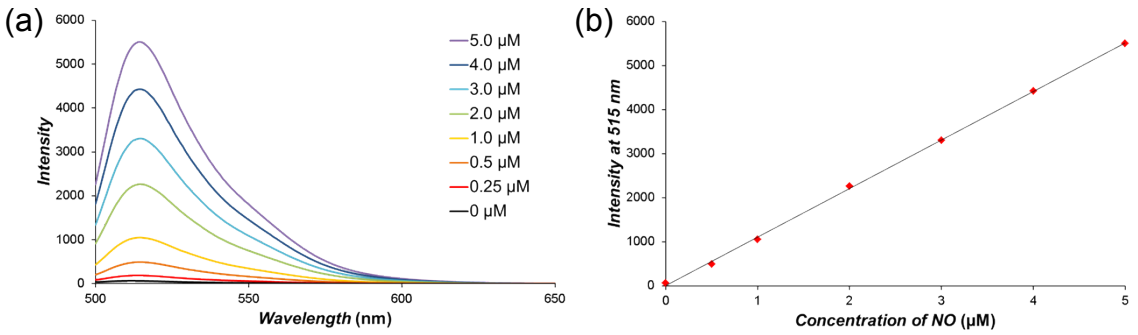


Fig. S4 (a) Emission spectra of DAF-2 solutions at each NO concentration , and (b) standard curve for the DAF-2T fluorescence intensity.

Detection of NO transport between 1_Lipo and DAF-2@Lipo using emission analysis

Suspension of **1**_Lipo ([Ru] = 33.7 μM) and DAF-2@Lipo were mixed at the ratio of 1:20 (v:v). The mixture was irradiated with Xe lamp (400-750 nm) at 20˚C. Emission spectra of the reaction solution were measured (λ_{ex} = 495 nm, wavelength range: 500-650 nm).

Observation of NO transport between 1_Lipo and DAF-2@Lipo using confocal laser scanning microscopy

Suspension of **1**_Lipo ([Ru] = 33.7 μM) and DAF-2@Lipo were mixed at the ratio of 1:20 (v:v). Aliquots of the mixture before and after 40 min irradiation with Xe lamp (400-750 nm) at 20˚C (λ_{ex} = 405, 488, 561 nm) were observed by a confocal laser scanning microscopy.

Physical Measurement

^{1}H-NMR analyses were measured with JEOL 600MHz NMR. Elemental analyses of C, H, and N atoms were measured with Yanaco CHN Corder MT-5. IR spectra were measured with a JASCO FT/IR-4200 spectrophotometer. UV-Vis absorption and emission spectra were measured with JASCO V-630 and FP-8200, respectively. MALDI-TOF MS was measured with Bruker Doltonics auto flex II. 2, 5-dihydroxybenzoic acid was used as a matrix. Confocal laser scanning microscopic images were snapped with Nikon C2si. The size distribution of the liposomes was

measured by Dynamic light scattering (DLS) (Malvern Instruments Ltd). The $Z_{average}$ diameter values are the means of 3 repeat measurements.

Reference

1. P. Pescador, N. Brodersen, H. A. Scheidt, M. Loew, G. Holland, N. Bannert, J. Liebscher, A. Herrmann, D. Huster and A. Arbuzova, *Chem. Commun.*, **2010**, *46*, 5358–5360.