Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Ascorbate-assisted nitric oxide release from photocontrollable nitrosonium ion releasers for potent *ex vivo* photovasodilation

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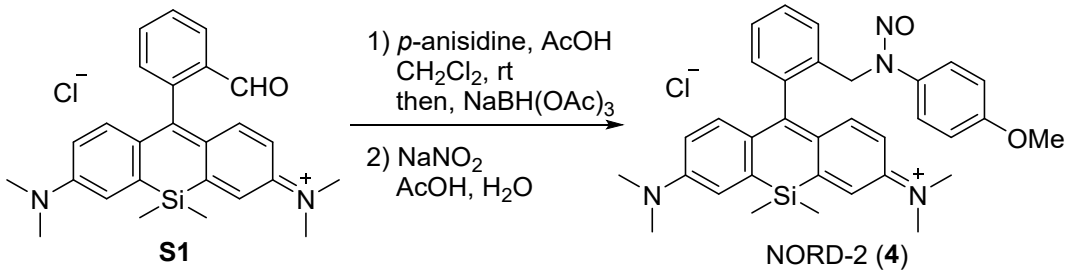
1. General information

Proton nuclear magnetic resonance spectra (^{1}H NMR) and carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded on a JEOL JNM-ECZ500R spectrometer. Chemical shifts (δ) are reported in parts per million relative to the internal standard, tetramethylsilane. Elemental analysis was performed with MICRO CORDER JM11, and all values were within ±0.4 % of the calculated values. Ultraviolet-visible-light absorption spectra were recorded on an Agilent 8453 spectrometer. Fluorescence spectra were recorded on an RF-5300 PC (Shimadzu). Irradiation was conducted with LEDs (CL-1501, Asahi Spectra). All other reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, FUJIFILM Wako Pure Chemical Corp., Nacalai Tesque, Kanto Kagaku, Kishida Kagaku, Junsei Kagaku or Dojindo, and used without purification. MPLC purification was performed using YFLC-Wprep2XY-S (Yamazen).

2. Experimental section

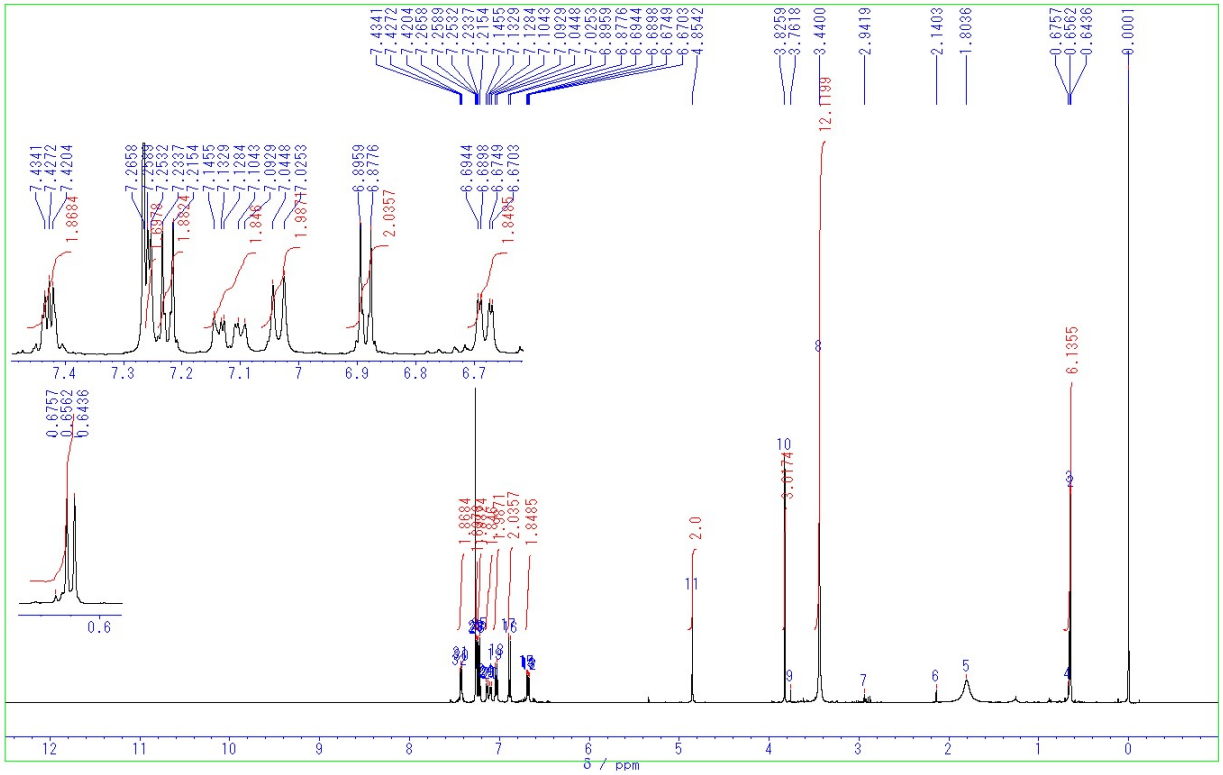
Synthesis of **4**



S1 NORD-2 (**4**)

To a solution of **S1** (90 mg, 0.200 mmol) and *p*-anisidine (26 mg, 0.211 mmol, 1.1 equiv.) in CH_2Cl_2 (2 mL) was added AcOH (0.2 mL). The mixture was stirred at room temperature for 30 min, and then $NaBH(OAc)_3$ (126 mg, 0.595 mmol, 3.0 equiv.) was added. Stirring was continued for 10 min, then the reaction mixture was diluted with CH_2Cl_2 and washed with 2 N NaOH (10 mL×3). The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with brine and dried over Na_2SO_4. Filtration, and evaporation *in vacuo* gave a crude white solid. To a solution of the residue in AcOH (2 mL) was added a solution of $NaNO_2$ (15 mg, 0.217 mmol, 1.1 equiv.) in water (2 mL) on an ice-water bath. The reaction mixture was stirred on the ice-water bath for 20 min, and then brine (40 mL) and 2 N HCl (1 mL) were added. The mixture was extracted with CH_2Cl_2 three times. The organic layer was dried over Na_2SO_4. Filtration, evaporation *in vacuo*, and purification by MPLC (CH_2Cl_2/MeOH = 94/6 → 90/10 → 70/30) gave 80 mg (68%) as a navy blue solid: ^{1}H NMR ($CDCl_3$ 500 MHz, δ; ppm) 7.43 (2H, dd, J = 3.6 Hz, 3.6 Hz), 7.26 (2H, d, J = 2.8 Hz), 7.22 (2H, d, J = 9.0 Hz), 7.15–7.09 (2H, m), 7.04 (2H, d, J = 9.6 Hz), 6.89 (2H, d, J = 9.0 Hz), 6.68 (2H, dd, J = 2.5 Hz, 9.6 Hz), 4.85 (2H, s), 3.83 (3H, s), 3.44 (12H, brs), 0.66 (3H, s), 0.64 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz, δ; ppm)166.60, 159.15, 154.19, 148.38, 141.17, 137.42, 134.53, 132.30, 129.63, 129.39, 127.56, 127.37, 127.03, 121.35, 121.26, 114.72, 114.25, 55.72, 45.97, 41.26, –0.67, –0.69; MS (ESI) m/z: 519 ([M–NO]$^+$); Anal. Calcd. for $C_{33}H_{37}ClN_4O_2Si·7/2H_2O$: C, 61.14; H, 6.84; N, 8.64. Found: C, 61.31; H, 6.82; N, 8.63.

H NMR Spectrum of **4**



NO detection using NO electrode

A solution (total volume 10 mL) of each PeT-driven compound (10 μM) in HEPES buffer (100 mM, pH 7.3, DMSO 0.1%) was irradiated at 37 ºC using a LED (CL-1501, Asahi Spectra) with a 568 nm LED head-unit (CL-H1-568-9-1) equipped with a 530–590 nm band pass filter, or a 660 nm LED head-unit (CL-H1-660-9-1). The light intensity at 568 nm was 33 mW cm^{-2} and that at 660 nm was 112 mW cm^{-2}. The NO release was measured with an NO electrode, ISO-NOP (World Precision Instruments), and recorded on a LabChart7 (ADInstruments). For detection of NO in the presence of sodium ascorbate, the indicated amount of sodium ascorbate was added before light irradiation.

ESR Measurements

A quartz ESR tube (internal diameter: 4.0 mm) containing a deaerated MeCN solution of sample was irradiated in the cavity of the ESR spectrometer with the focused light of a 60 W LED lamp (λ = 405 nm) (Pi Photonics Inc., Japan) at –130 ˚C. ESR spectra in frozen MeCN were measured under non-saturating microwave power conditions using a JEOL X-band spectrometer (JES-X320) with an attached variable temperature apparatus. The magnitude of modulation was chosen to optimize the resolution and the signal-to-noise (S/N) ratio of the observed spectra when the maximum slope linewidth (ΔHmsl) of the ESR signals was unchanged with a larger modulation magnitude. The g values and the hyperfine coupling (*hfc*) constants were calibrated with a Mn^{2+} marker.

Theoretical Calculations

Density functional theory (DFT) calculations were performed with Gaussian09 (Revision C.02, Gaussian, Inc.). The calculations were performed on a 16-processor high-performance computer (ForScientist XD1, HPC Systems Inc., Japan).

Changes in absorption spectra of irradiated solutions of PeT-driven compounds

Light irradiation of a solution of each compound was conducted under the same conditions as described above. The absorption spectrum of an aliquot of each solution (250 μL) was measured using an Agilent 8453.

Monitoring the formation of NAT using HPLC

A solution (total volume 10 mL) of each PeT-driven compound (10 μM), and 2,3-diaminonaphthalene (10 μM) in HEPES buffer (100 mM, pH 7.3, DMSO 0.1%) was irradiated by an LED (CL-1501, Asahi Spectra) with a 568 nm LED head-unit (CL-H1-568-9-1) equipped with a 530–590 nm band pass filter, or a 660 nm LED head-unit (CL-H1-660-9-1). The light intensity at 568 nm was 34 mW cm^{-2} and that at 660 nm was 108 mW cm^{-2}. An aliquot of each solution (20 μL) was loaded onto an Inertsil ODS column (5 μm; 150 × 4.6 mm) fitted on a Shimadzu HPLC system, and the eluates were monitored with a fluorescence detector (ex. 360 nm, em. 460 nm). MilliQ water containing 0.1% FA (A) and MeCN containing 0.1% FA (B) were used as developing solvents; 0 min, B 5% → 2 min, B 5% → 3 min, B 20% → 15 min, B 80% → 17 min, B 80% → 18 min, B 100% → 23 min, 100% → 24 min, B 5% → 30 min, B 5%.

NO release quantum yield

Measurement of the amount of NO released during irradiation: A solution of each compound (10 μM) in 100 mM HEPES buffer (pH 7.3, total volume: 3 mL) containing 0.1% DMSO was placed in a plastic cuvette and irradiated at 520 nm for **1**, 504 nm for **2**, and 600 nm for **3** or **4** (band width: 10 nm) for 1 min with the Xe lamp of a fluorescence spectrometer, RF5300 (Shimadzu). The amount of NO released was measured with an ISO-NOP (World Precision Instruments) and recorded on a LabChart7 (ADInstruments). The Φ_{NO} values of **1**, **3**, and **4** were calculated with reference to the Φ_{NO} of **1** and **3** reported previously (1.01×10^{-3}, and 3.85×10^{-3}, respectively). For **2**, the number of irradiated photons was calculated as follows.

Measurement of the number of photons: An aqueous solution (3 mL) of Reinecke's salt (10 mM) was irradiated under the same conditions as described above. After irradiation, an aliquot (100 μL) was mixed with 400 μL of an aqueous solution containing 0.5 M $HClO_4$ and 0.1 M $Fe(NO_3)_3$ and 400 μL of MilliQ water. The absorption spectrum of the mixture was recorded on an Agilent 8453 spectrometer. The absorption spectrum of the non-irradiated mixture was also recorded. The photon number was calculated from the absorption difference. The calculations were performed with reference to the quantum yield of Reinecke's salt for thiocyanate anion release (Φ = 0.30).

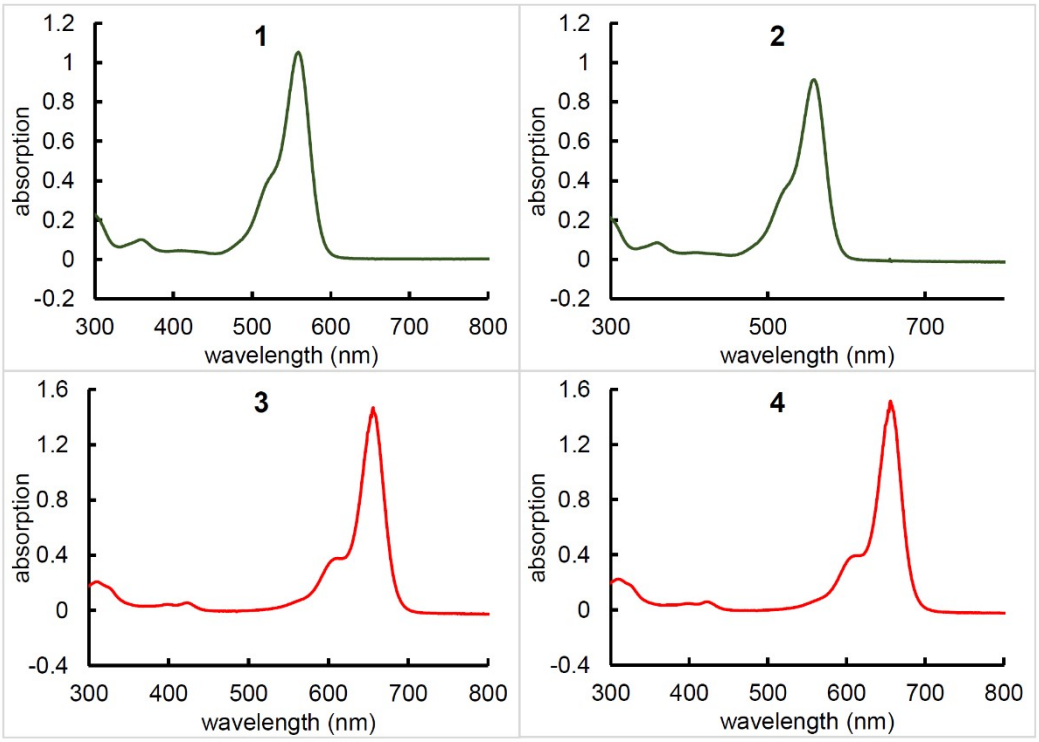
Tracking the concentration of **1** or **3** using HPLC for determination of reaction rate

A solution (total volume 10 mL) of each PeT-driven compound (2, 5, or 10 μM) in HEPES buffer (100 mM, pH 7.3, DMSO 0.1%) was irradiated by an LED (CL-1501, Asahi Spectra) with a 568 nm LED head-unit (CL-H1-568-9-1) equipped with a 530–590 nm band pass filter, or a 660 nm LED head-unit (CL-H1-660-9-1). The light intensity at 568 nm was 3.3 mW cm^{-2} and that at 660 nm was 10 mW cm^{-2}. An aliquot of each solution (20 μL) was loaded onto a Shim-pack Velox C18 (5 μm; 150 × 4.6 mm) fitted on a Shimadzu HPLC system, and the eluates were monitored with a fluorescence detector ($\lambda_{ex}/\lambda_{flu}$ was 558/587 nm for **1** or **2**, 656/676 nm for **3** or **4**). MilliQ water containing 0.1% FA (A) and MeCN containing 0.1% FA (B) were used as developing solvents; 0 min, B 5% → 1.33 min, B 5% → 2 min, B 20% → 10 min, B 80% → 11.33 min, B 80% → 12 min, B 100% → 15.33 min, 100% → 16 min, B 5% → 20 min, B 5%.

Photoinduced vasodilation with **1–4**

All animal experiments were performed following the Guiding Principles for the Care and Use of Laboratory Animals of the Science and International Affairs Bureau of the Japanese Ministry of Education, Culture, Sports, Science, and Technology. The study design was reviewed and approved by the Animal Experimentation Ethics Committee of Nagoya City University (No. H29-P-05). Eight-week-old Wistar-ST rat aortic strips were placed in glass tubes filled with Krebs buffer (5 mL) at 37 °C. Each strip was pretreated with N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME, 10 μM) and noradrenaline (NA, 10 μM). After equilibration, sodium ascorbate (10 μM) was added and the system was irradiated with a 568 nm LED equipped with a 530–590 nm band pass filter (40 mW cm^{-2}) or a 660 nm LED (40 mW cm^{-2}). When 3 min had passed after the first irradiation, a test compound (1 nM to 10 μM) was added to the tube. After a further 3 min, the strip was irradiated for 3 min. After a further 3 min, each compound was applied at the next concentration. Several cycles of photoirradiation were conducted, and the vasodilating time and vasodilation% were calculated from smoothed data.

3. Supporting Figures, Schemes, and Table



	1	2	3	4
λ_{max} (nm)	559	559	656	656
ε (M^{-1} cm^{-1})	1.05×10^5	9.14×10^4	1.47×10^5	1.52×10^5

Fig. S1 Absorption spectra, maximum absorption wavelength, and extinction coefficients of **1**-**4** in HEPES buffer (100 mM, pH 7.3, DMSO 0.1%).

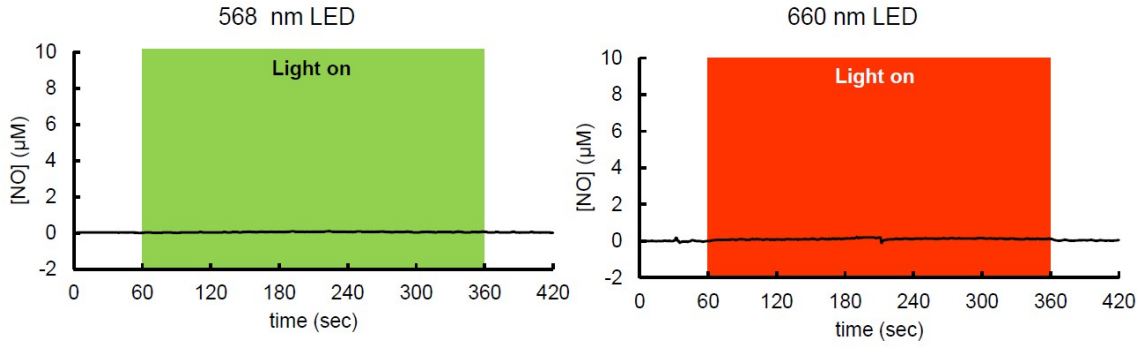


Fig. S2 NO detection in the absence of PeT-driven NO releasers. A solution of HEPES buffer (100 mM, pH 7.3) was irradiated with a 568 nm or 660 nm LED, and the NO concentration was monitored by an NO electrode.

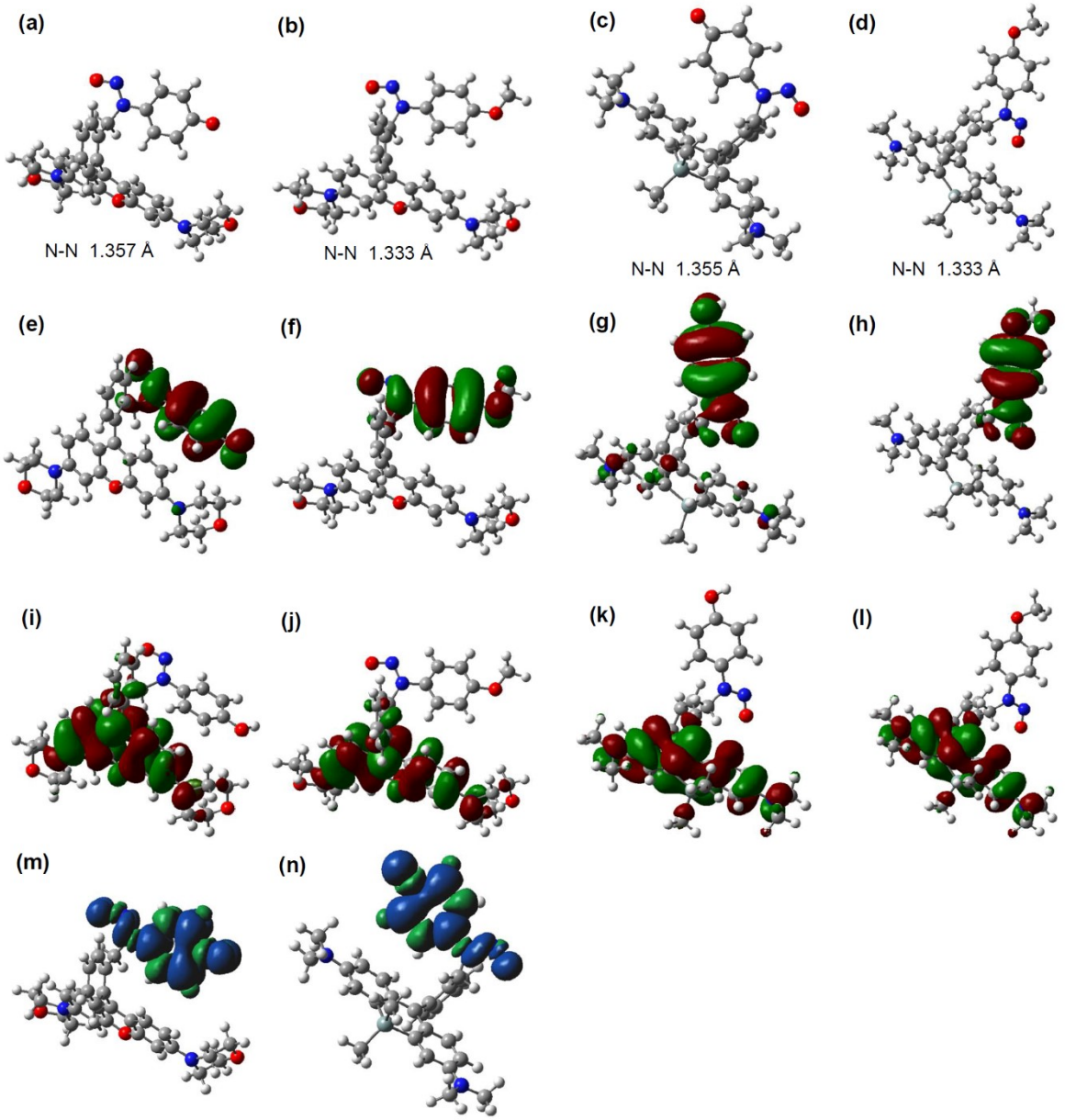


Fig. S3 Optimized structures of **1**(–H•) (a), **2** (b), **3**(–H•) (c), **4** (d); N-N bond length is indicated below the structure; HOMOs of **1**-**3** (e–g), HOMO–1 of **4** (h); LUMOs of **1**–**4** (i–l) and SOMOs of **1**(–H•) (m) and **3**(–H•) (n) calculated at the M06-2X/6-31+G(d) level of theory.

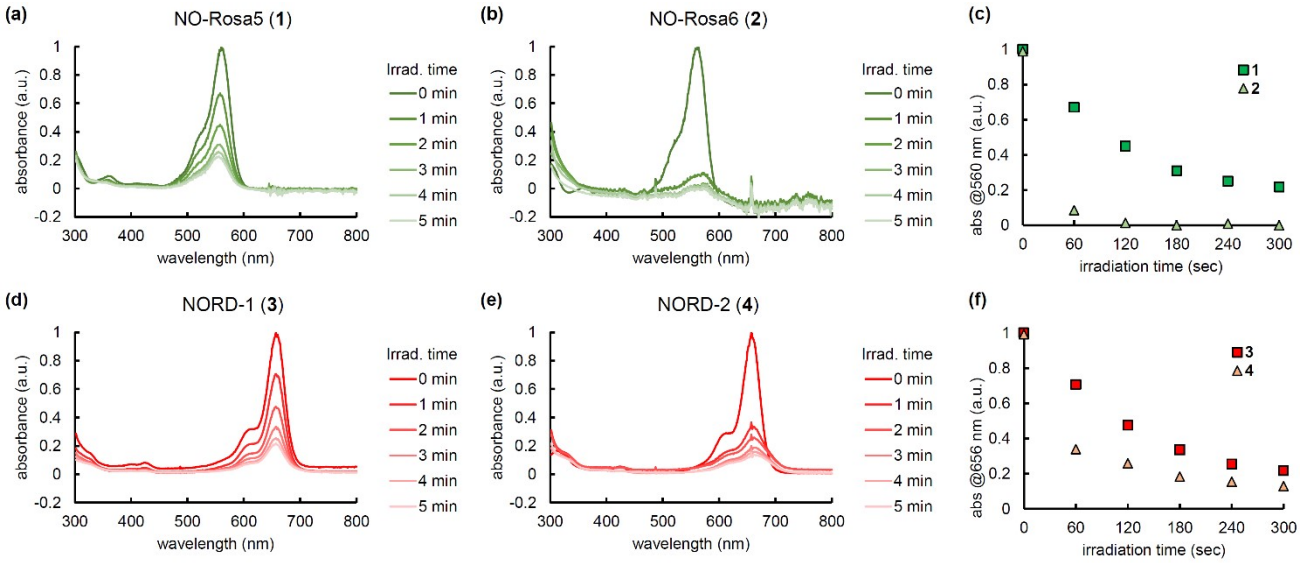


Fig. S4 Changes in absorption spectra of irradiated solutions of each compound (a, b, d, and e) and time-dependent change of each absorption peak (c and f). A solution of the indicated compound (10 μM) in HEPES buffer (100 mM, pH 7.3, DMSO 0.1 %) was irradiated with a 568 nm LED equipped with a 530-590 nm band pass filter (34 mW cm^{-2}) or a 660 nm LED (108 mW cm^{-2}).

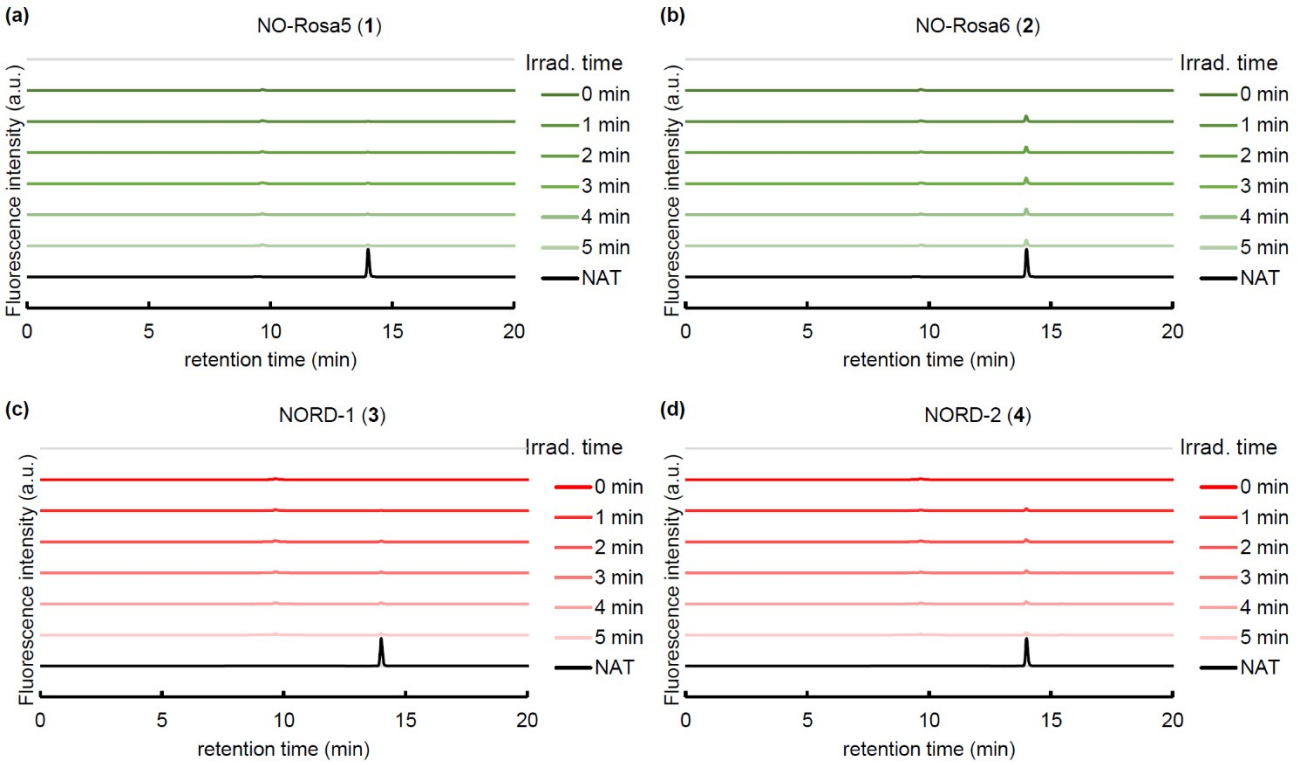


Fig. S5 Fluorescence detection of 2,3-naphthotriazole (NAT) after photoreaction of 2,3-diaminonapthalene (DAN) and each PeT-driven compound. An aliquot of each solution (20 μL) was loaded onto an Inertsil ODS column (5 μm; 150 × 4.6 mm) fitted on a Shimadzu HPLC system, and the eluates were monitored with a fluorescence detector (ex. 360 nm, em. 460 nm).

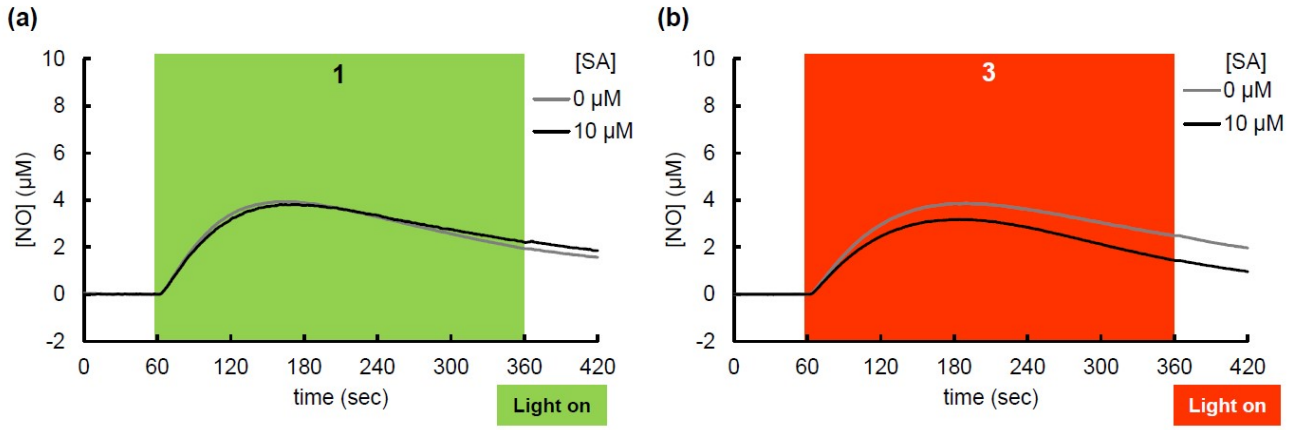


Fig. S6 Detection of ascorbate-assisted NO release from a solution of **1** (a) or **3** (b) in the presence of the indicated concentrations of sodium ascorbate (SA) using an NO electrode. A solution (total volume 10 mL) of each compound in HEPES buffer (100 mM, pH 7.3) was irradiated using a 568 nm or 660 nm LED, and NO was measured by an NO electrode (ISO-NOP).

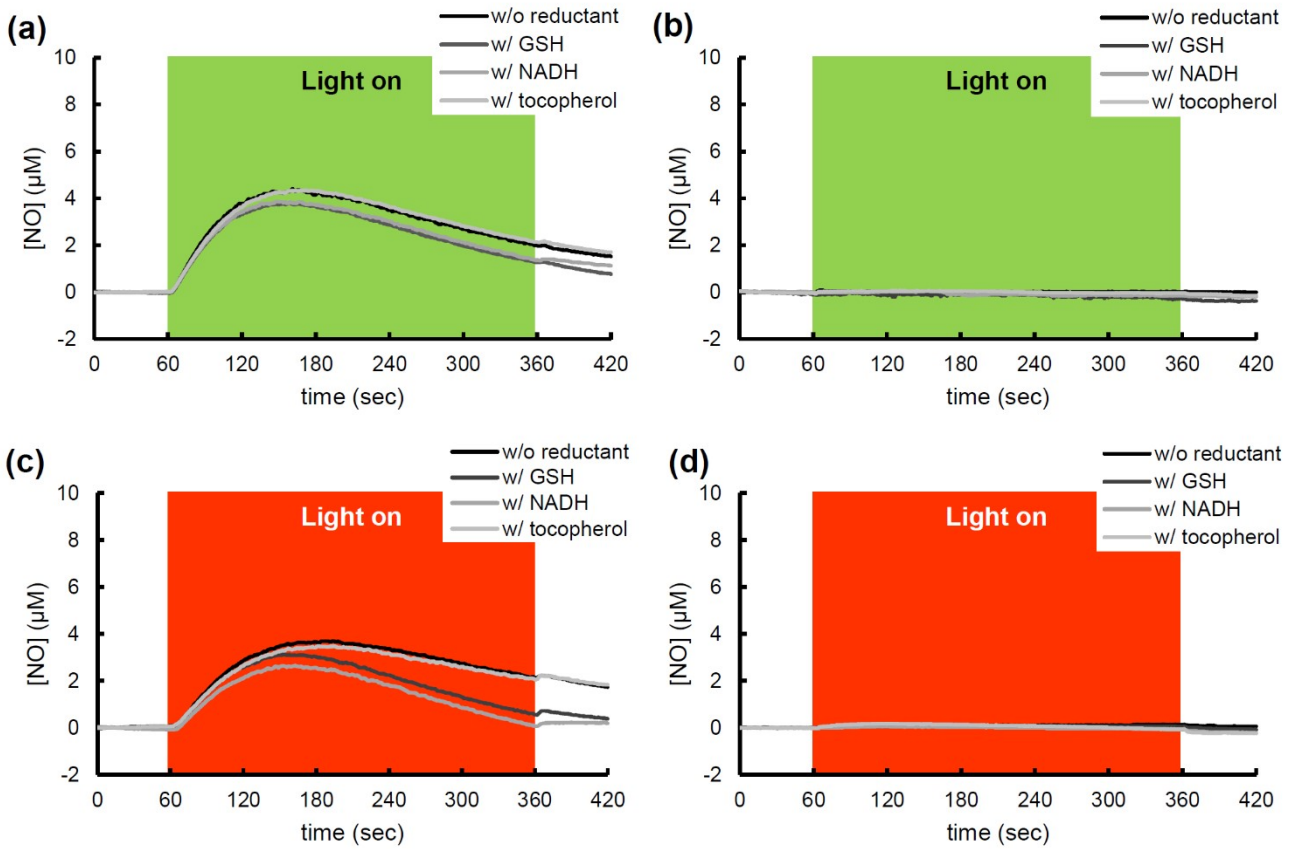


Fig. S7 Detection of reductant-assisted change of NO release from a solution of **1** (a), **2** (b), **3** (c). or **4** (d) in the presence of the indicated reductant (GSH: glutathione 1 mM, NADH: nicotine adenine dinucleotide 100 μM, or tocopherol 100 μM). A solution (total volume 10 mL) of each compound in HEPES buffer (100 mM, pH 7.3) was irradiated using a 568 nm or 660 nm LED, and NO was measured by an NO electrode (ISO-NOP).

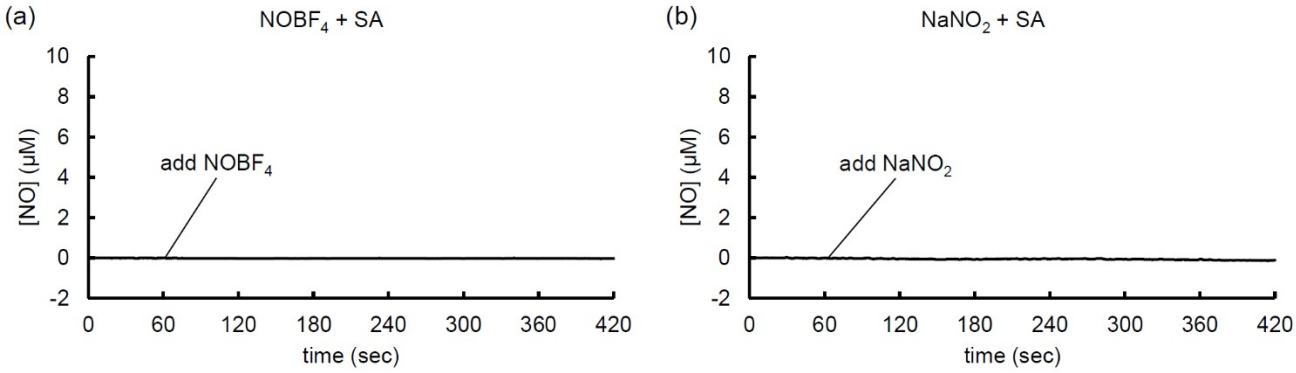
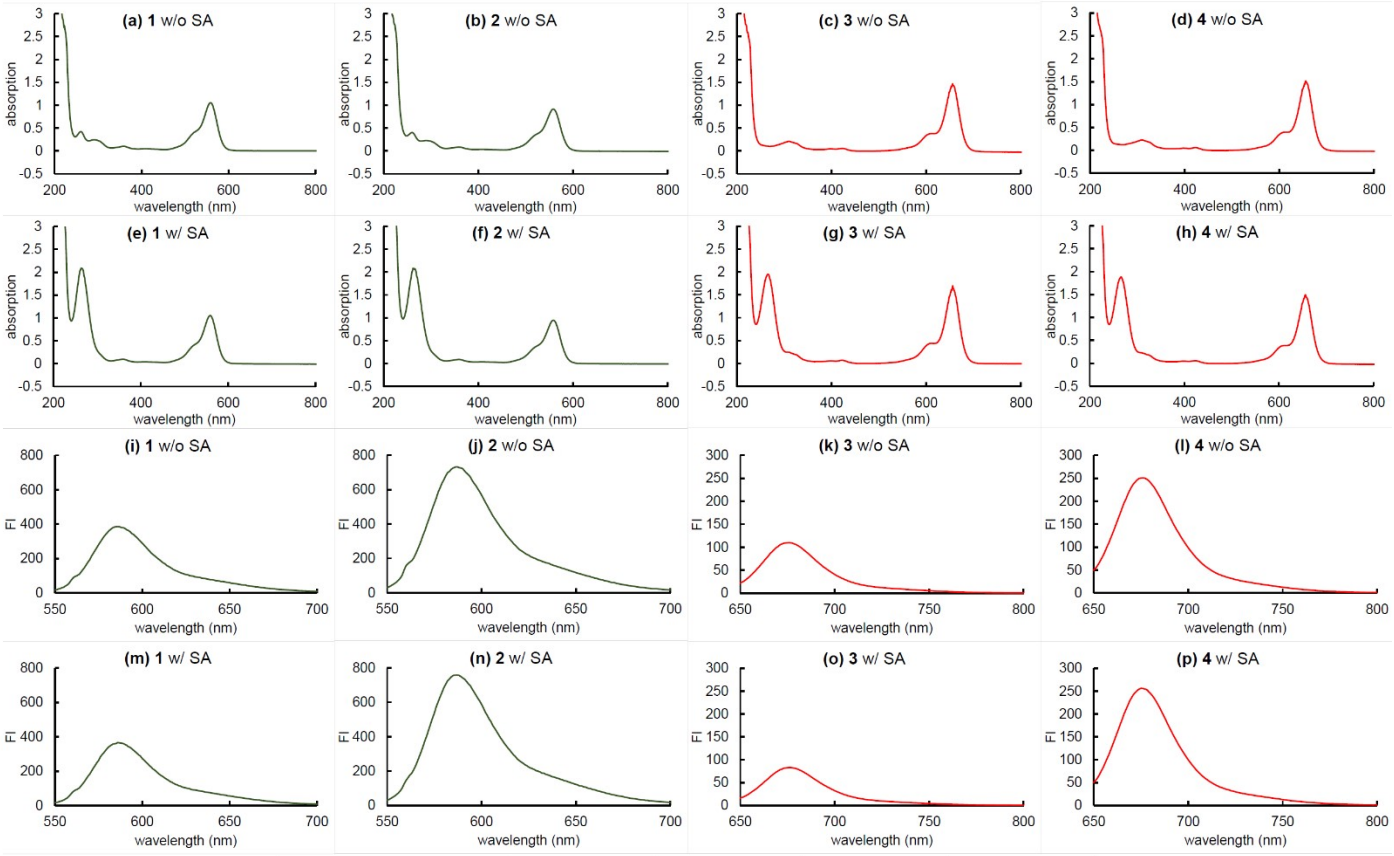
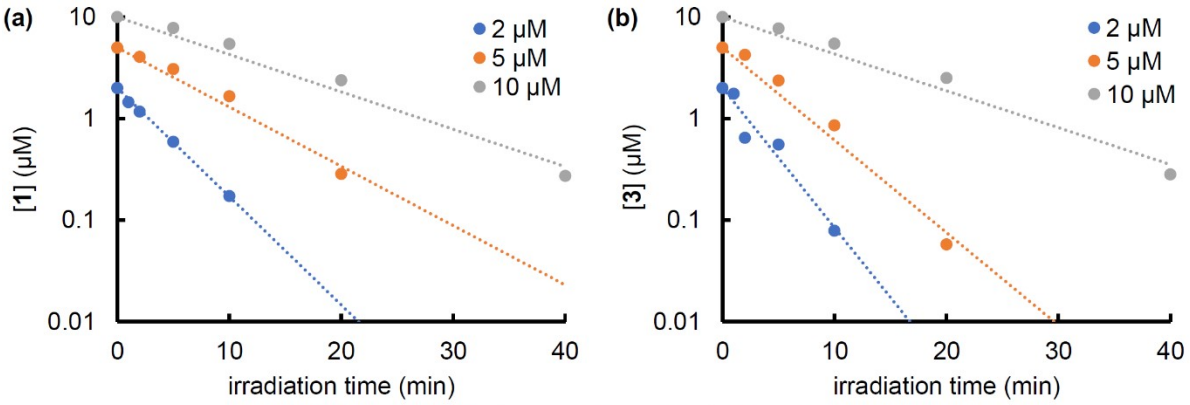


Fig. S8 NO detection using an NO electrode during reaction between sodium ascorbate (SA) and nitrosonium tetrafluoroborate ($NOBF_4$) (a) or sodium nitrite ($NaNO_2$) (b). To a solution (total volume 10 mL) of SA (10 μM) in HEPES buffer (100 mM, pH 7.3) was added a solution of $NOBF_4$ in MeCN (final 10 μM) or an aqueous solution of $NaNO_2$ (final 10 μM). NO was measured by an NO electrode (ISO-NOP).



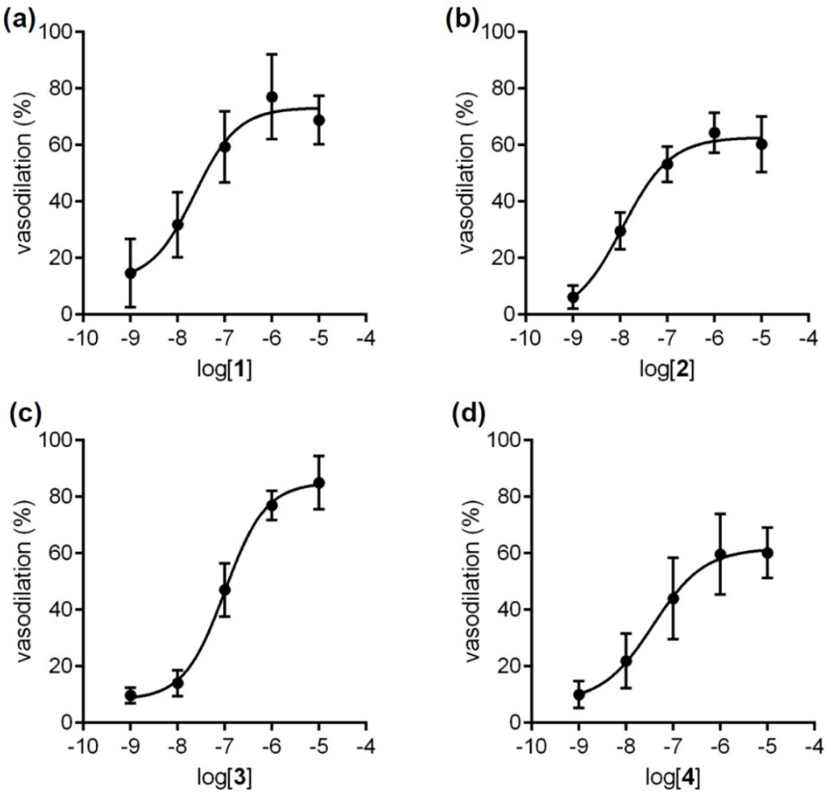
		1	2	3	4
λ_{max} (nm)	w/o SA	559	559	656	656
	w/ SA	559	559	656	656
ε (M^{-1} cm^{-1})	w/o SA	1.05×10^5	9.14×10^4	1.47×10^5	1.52×10^5
	w/ SA	1.05×10^5	9.47×10^4	1.70×10^5	1.50×10^5
λ_{flu} (nm)	w/o SA	587	587	676	676
	w/ SA	586	587	676	675
Φ_{flu}	w/o SA	0.033	0.067	0.12	0.27
	w/ SA	0.031	0.074	0.080	0.28

Fig. S9 Effects of SA on absorption and fluorescence spectra: (upper) absorption spectra in the absence of SA (a–d) and in the presence of SA (e–h), and fluorescence spectra in the absence of SA (i–l) and in the presence of SA (m–p); (bottom) values of absorption maximum wavelength (λ_{max}), extinction coefficient of λ_{max} (ε), absorption maximum wavelength (λ_{flu}), and fluorescence quantum yields (Φ_{flu}) in each solution of a PeT-driven compound.



Initial conc.(μM)	k_{PD} of **1** (sec^{-1})	k_{PD} of **3** (sec^{-1})
2	14.8	19.0
5	8.10	12.6
10	5.10	5.04

Fig. S10 Changes in photodecomposition rate depending on initial concentration of PeT-driven compounds: The concentration of **1** (a) or **3** (b) after photoirradiation was monitored by HPLC with fluorescence detection. The light intensity for **1** was 3.3 mW cm^{-2} and that for **3** was 11 mW cm^{-2}. k_{PD}: photodecomposition rate.



compound	**1**	**2**	**3**	**4**
EC_{50} (nM)	22.9	11.3	101	37.4
E_{max} (%)	68.8	60.3	84.9	60.1

Fig. S11 Effective concentration curves of **1**-**4** and EC_{50} values. The results are means ± SE (n = 4).

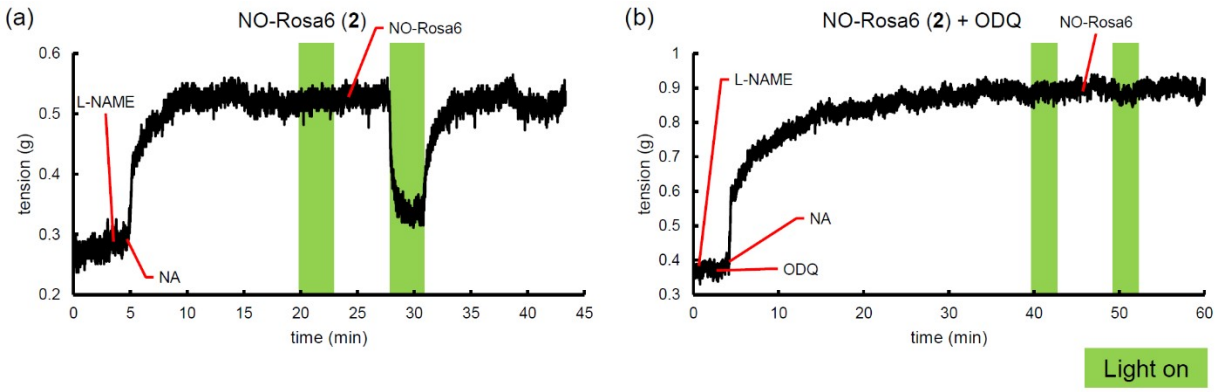


Figure S12 Change in the tension of rat aorta *ex vivo* induced by green-light-mediated NO release from **2** in the presence of a nitric oxide synthetase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 10 μM) (a). Rat aorta in a glass tube was treated with noradrenaline (NA, 10 μM) followed by **2** (10 μM). An sGC inhibitor, ODQ (10 μM), was added before the addition of NA (b). The tube was irradiated with a MAX303 (Asahi Spectra) equipped with a 530–590 nm band pass filter (20 mW cm^{-2}) for 3 min before and 3 min after the addition of **2**.

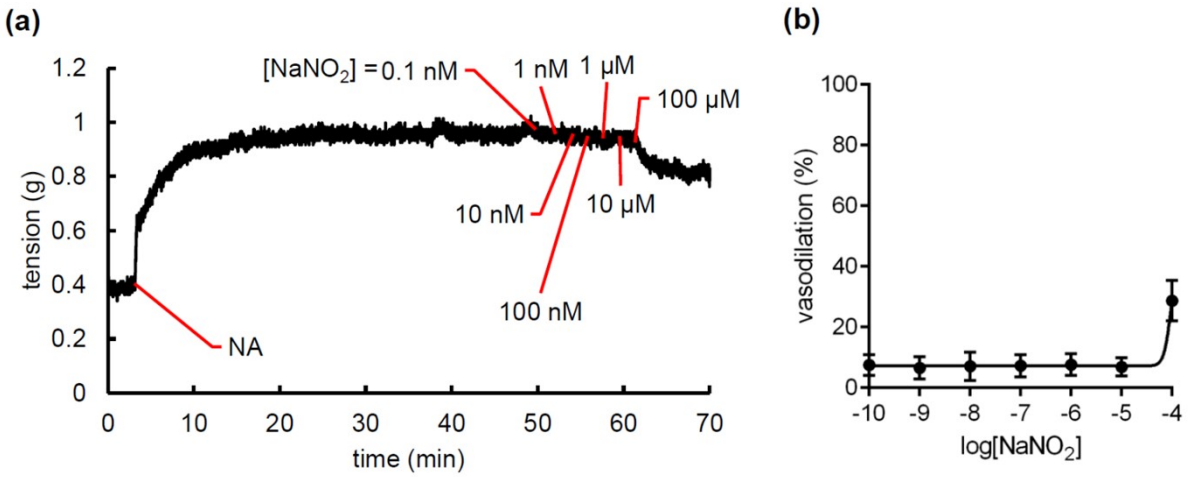


Fig. S13 (a) Change in the tension of *ex vivo* rat aorta induced by sodium nitrite ($NaNO_2$). Rat aorta in a glass tube was treated with noradrenaline (NA, 10 μM) followed by $NaNO_2$ (indicated concentration); (b) vasodilation% in each concentration. Data are expressed as mean ± SE (shown as error bars, n = 6).

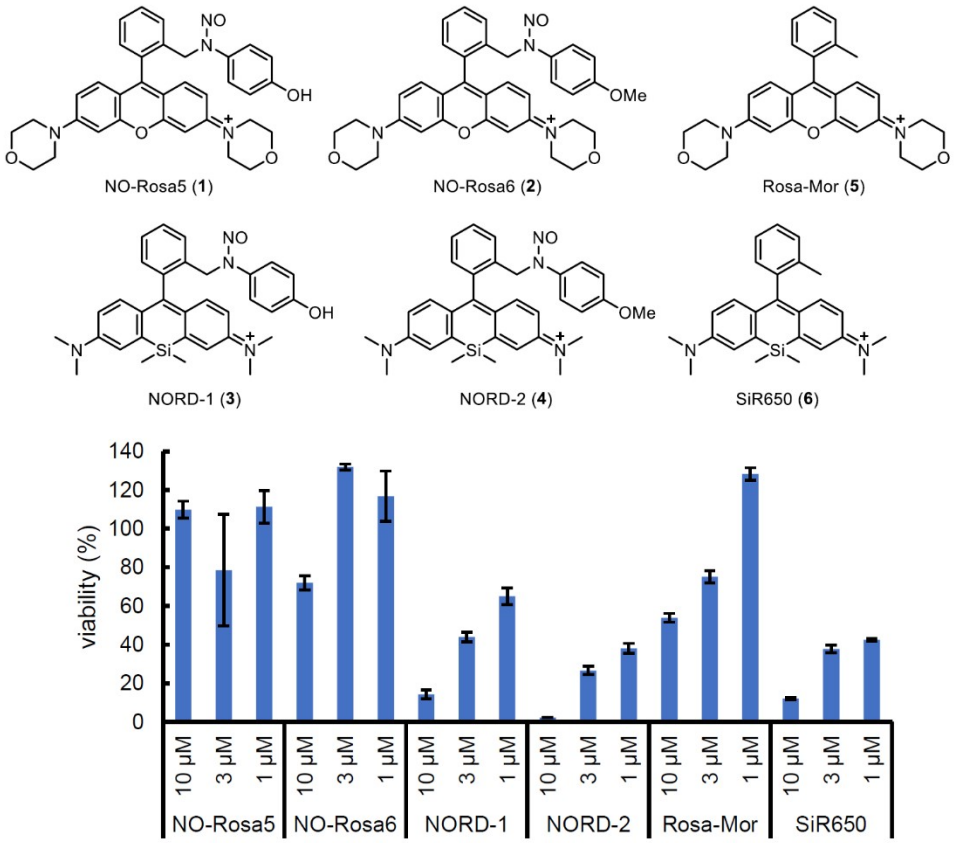
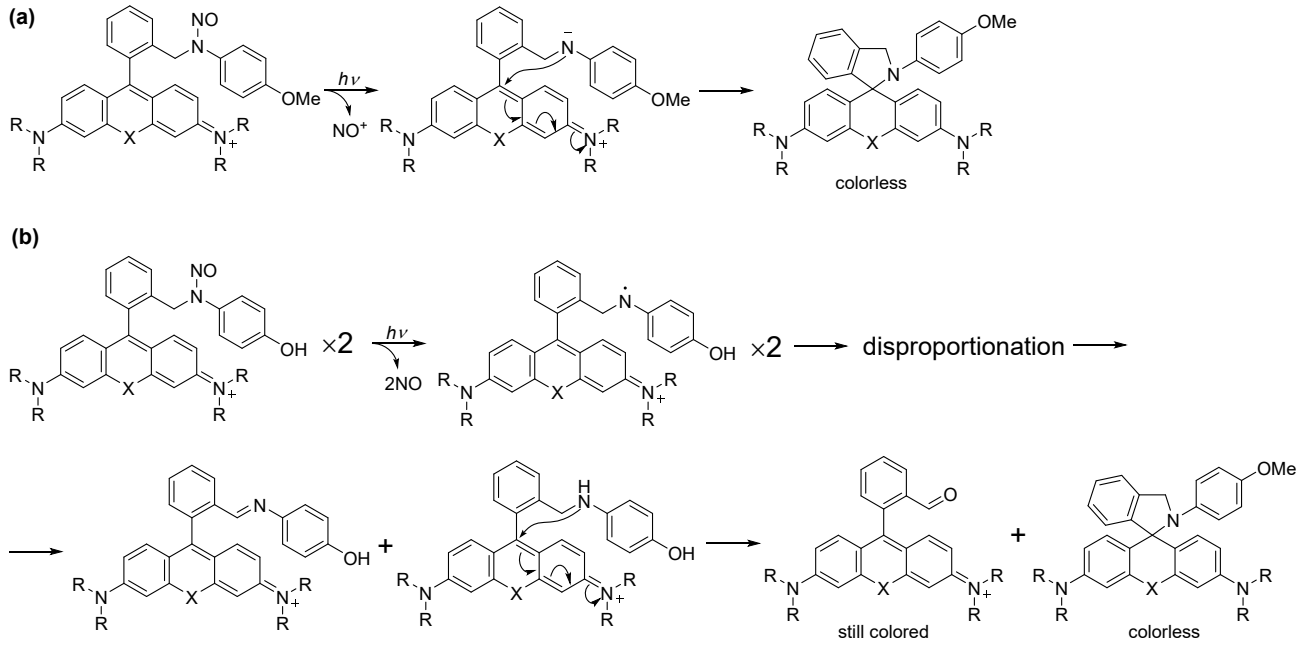


Fig. S14 Cell viability assay using WST-8. Cells were exposed to each compound overnight and viability was examined using WST-8 (Dojindo, Japan).

Scheme S1 A plausible decomposition mechanism of PeT-driven NO releasers (a) or NO^+ releasers (b)



Scheme S2

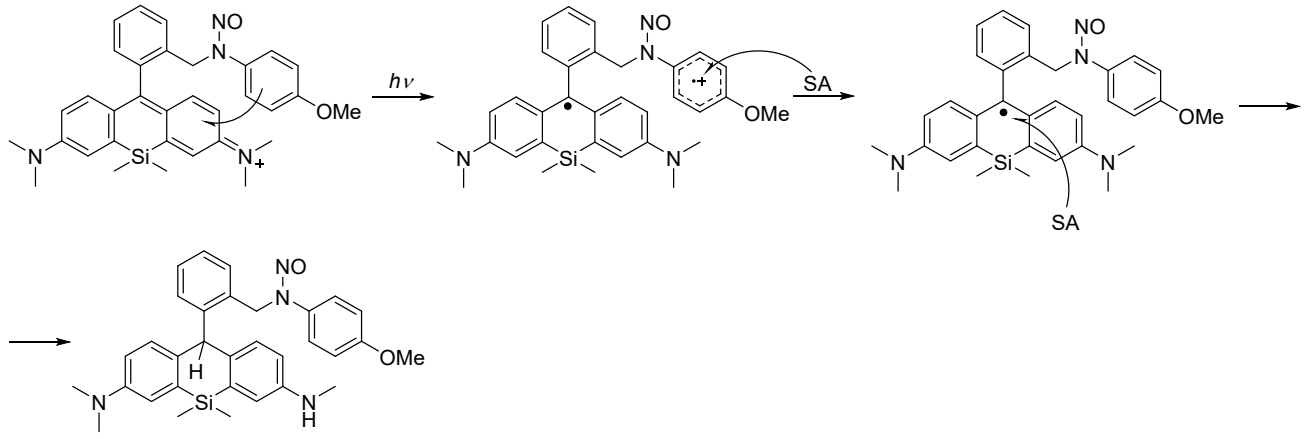


Table S1 Light-controllable NO-releasing compounds working at wavelengths over 500 nm

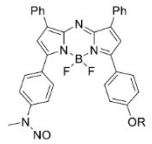
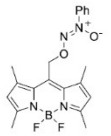
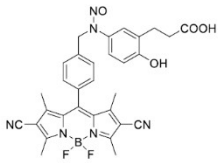
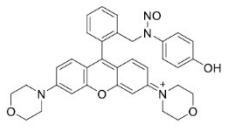
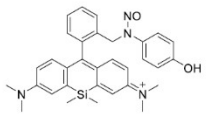
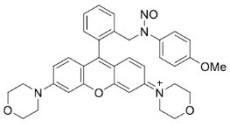
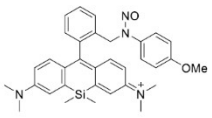
	λ for NO release	Φ	εΦ	Ref
	681/678 nm	n.d.	n.d.	*J. Am. Chem. Soc.* **2018**, *140*, 11686
	532 nm	8.0×10^{-3}	550	*Chem. Eur. J.* **2017**, *23*, 9026
	500 nm	1.90×10^{-3}	205	*J. Am. Chem. Soc.* **2014**, *136*, 7085
	559 nm	1.01×10^{-3}	98.2	*Sci. Rep.* **2019**, *9*, 1430
	656 nm	3.85×10^{-3}	365	*ACS. Chem. Biol.* **2020**, *15*, 2958.
	559 nm	9.84×10^{-3} (w/ SA)	**932**	This paper
	656 nm	4.97×10^{-3} (w/ SA)	**746**	This paper

Table S2 Calculation of Φ_{NO} values

For NO-Rosa5 (**1**)					
NO-Rosa5 (**1**)	1st	2nd	3rd	average	stdev
[NO] (μM)	1.33	1.29	1.21		
amount of NO (mol)	3.99E-09	3.87E-09	3.63E-09		
number of absorbed photons (mol)	3.95E-06	3.83E-06	3.59E-06		
number of irradiated photons (mol)	7.19E-06	6.97E-06	6.54E-06	6.90E-06	
NO-Rosa5 (**1**) +AA	1st	2nd	3rd	average	stdev
[NO] (μM)	0.96	1	1.29		
amount of NO (mol)	2.88E-09	3.00E-09	3.87E-09		
number of absorbed photons (mol)	3.54E-06				
ΦNO	8.14E-04	8.48E-04	1.09E-03	9.19E-04	1.53E-04

For NO-Rosa6 (**2**)					
actinometer	1st	2nd	3rd	average	
A450 (before irrad.)	1.732042	1.712958	1.787256		
[SCN-] (M)	4.03E-04	3.98E-04	4.16E-04		
amount of SCN- (mol)	1.05E-05	1.04E-05	1.08E-05		
A'450 (after irrad.)	1.820659	1.823478	1.932876		
[SCN-] (M)	4.23E-04	4.24E-04	4.50E-04		
amount of SCN- (mol)	1.11E-05	1.11E-05	1.17E-05		
⊿SCN (mol)	5.38E-07	6.71E-07	8.84E-07		
photons absorbed by RS (mol)	1.92E-06	2.40E-06	3.16E-06		
photons irradiated to the cell (mol)	2.14E-06	2.67E-06	3.52E-06	2.77E-06	
[NO] (μM)	1st	2nd	3rd		
NO-Rosa6 (**2**) + AA	2.04	2.19	2.35		
amount of NO (mol)	1st	2nd	3rd		
NO-Rosa6 (**2**) + AA	6.12E-09	6.57E-09	7.05E-09		
number of photons absorbed by NO-Rosa6 (mol)					
NO-Rosa6 (**2**) + AA	6.69E-07				
ΦNO	1st	2nd	3rd	average	stdev
NO-Rosa6 (**2**) + AA	9.15.E-03	9.82.E-03	1.05.E-02	9.84E-03	6.95E-04

Table S2 (continued) Calculation of Φ_{NO} values

NORD-1 (**3**)	1st	2nd	3rd	average	stdev
[NO] (μM)	0.94	0.91	0.96		
amount of NO (mol)	2.82E-09	2.73E-09	2.88E-09		
photons absorbed by NORD-1 (mol)	7.32E-07	7.09E-07	7.48E-07		
photons irradiated to NORD-1 solution (mol)	1.31E-06	1.27E-06	1.34E-06	1.31E-06	

NORD-1 (**3**) + AA	1st	2nd	3rd	average	stdev
[NO] (μM)	0.9	0.72	0.65		
amount of NO (mol)	2.7E-09	2.16E-09	1.95E-09		
photons absorbed by NORD-2 (mol)	6.88E-07				
ΦNO	3.93E-03	3.14E-03	2.84E-03	3.30E-03	5.63E-04

NORD-2 (**4**) +AA	1st	2nd	3rd	average	stdev
[NO] (μM)	0.98	1.25	1.04		
amount of NO (mol)	2.94E-09	3.75E-09	3.12E-09		
photons absorbed by NORD-2 (mol)	6.58E-07				
ΦNO	4.47E-03	5.70E-03	4.74E-03	4.97E-03	6.47E-04

Table S3 Φ_{NO} values of the compounds. ND means not detected

	1		**2**		**3**		**4**	
Φ_{NO} ($\times 10^3$)	SA (–)	SA (+)	SA (–)	SA (+)	SA (–)	SA (+)	SA (–)	SA (+)
	1.01	0.919	ND	9.84	3.85	3.30	ND	4.97