Electronic Supplementary Information

A Yellowish-Green-Light-Controllable Nitric Oxide Donor Based on N-Nitrosoaminophenol
Applicable for Photocontrolled Vasodilation

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1. Experimental Section
A. $^1$H NMR chart of NO-Rosa

B. Experimental procedure
NO$_2^-$/NO$_3^-$ detection with a NO fluorescence probe DAN
Sample solutions of NO-Rosa, (10 µM, 1 mL) were photoirradiated at room temperature for 60 min. Photoirradiation was performed with an MAX-302 (Asahi spectra) equipped with a 530–590 nm band-pass filter (190 mW/cm$^2$ at 570 nm) for NO-Rosa. The calibration curve was prepared by using NaNO$_3$ standard solution and DAN. After reducing NO$_2^-$ to NO$_3^-$ by NO$_3$ reductase, concentration of NO$_3^-$ in the photoirradiated NO-Rosa solution was determined by comparing the fluorescence intensity of naphthalenetriazole with the calibration curve. The fluorescence intensity was recorded on an ARVO-X5 (Perkinelmer) at $\lambda_{ex}$ = 355 nm/$\lambda_{em}$ = 460 nm.

HPLC analysis of photodecomposition of NO-Rosa
NO-Rosa was dissolved in MilliQ water containing 1% DMSO as a cosolvent to prepare 100 µM NO-Rosa solution, and this solution was photoirradiated (530–590 nm, 60 mW/cm$^2$, 15 min).
Detection was done at 565 nm. Gradient conditions: 0 min MeCN (0.1% FA) 30% → 15 min MeCN (0.1% FA) 50% → 20 min MeCN (0.1% FA) 50%; the other solvent was H₂O (0.1% FA).

**LC-MS analysis of photodecomposition products of NO-Rosa**

**NO-Rosa** (100 µM in MilliQ water containing 10% DMSO, 1 mL) was irradiated with yellowish green light (530–590 nm, 140 mW/cm², 15 min), and the solution was analyzed by LC-MS. Detection was done at 565 nm. Gradient conditions: 0 min MeCN (0.1% FA) 30% → 15 min MeCN (0.1% FA) 50% → 20 min MeCN (0.1% FA) 50%; the other solvent was H₂O (0.1% FA).

**Light-toxicity assay for HEK293 cells**

Cell viability was verified by Cell Counting Kit-8 (Dojindo, Kumamoto). HEK293 cells were plated on 96-well plates (100 µL, 1.0×10⁴ cells /well). After incubation under 5% CO₂ atmosphere at 37 °C for 24 hr, the cells were irradiated by yellowish green light (530–590 nm, 40 mW/cm², 15 min) or blue light (470–500 nm, 40 mW/cm², 15 min) with MAX-302 (Asahi Spectra). After irradiation, the cells were incubated another 48 hr under 5% CO₂ atmosphere at 37 °C. CCK solution (10 µL) was added to each well and the cells were incubated for 2 hr under 5% CO₂ atmosphere at 37 °C. The absorption intensity at 450 nm was recorded on an ARVO-X5 (Perkinelmer). Cell viability (%) was determined by dividing the absorption intensity of irradiated group by the absorption intensity of the group without photoirradiation.

2. Figures for supporting data

**Figure S1** Proposed mechanism of NO release from NOBL-1 (1) / NO-Rosa (2)

![Diagram of NO release mechanism](Image)
**Figure S2** ESR spectrum of a solution of Fe-MGD and NO-Rosa without photoirradiation. NO-Rosa (100 μM), MGD (6 mM), and FeSO₄ (1.5 mM) were dissolved in MilliQ water containing 15% DMSO, and the ESR spectrum of the solution was measured without photoirradiation. ESR conditions: microwave power, 10 mW; frequency, 9.4 GHz; field, 330 mT; sweep width, 7.5 mT; sweep time, 4 min; modulation width, 0.125 mT; time constant, 0.10 s.

**Figure S3** Quantitative analysis of NO release from NO-Rosa by DAN. A solution of NO-Rosa (10 μM, 1 mL) was photoirradiated at room temperature for 60 min. Visible light photoirradiation was performed with an Asahi Spectra MAX-302 equipped with a 530–590 nm band-pass filter (190 mW/cm² at 570 nm). NO₂/NO₃ were detected by using a 2,3-diaminonaphthalene (DAN) fluorometric assay kit.

<table>
<thead>
<tr>
<th>NO-Rosa</th>
<th>Fluorescence Intensity (A. U.) Mean±SD</th>
<th>NO Concentration (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO-Rosa</td>
<td>216068±3522</td>
<td>9.8±0.2</td>
</tr>
</tbody>
</table>
**Figure S4** HPLC analysis of photodecomposition. Chromatograms of solution containing NO-Rosa (a) before photoirradiation, and (b) after photoirradiation. NO-Rosa was dissolved in MilliQ water containing 1% DMSO as a cosolvent to prepare 100 μM NO-Rosa solution, and this solution was photoirradiated (530–590 nm, 60 mW/cm², 15 min). Detection was done at 565 nm. Gradient conditions: 0 min MeCN (0.1% FA) 30% → 15 min MeCN (0.1% FA) 50% → 20 min MeCN (0.1% FA) 50%; the other solvent was H₂O (0.1% FA).
Figure S5 LC-MS analysis of photodecomposition products of NO-Rosa

NO-Rosa (100 µM in MilliQ water containing 10% DMSO, 1 mL) was irradiated with yellowish green light (530–590 nm, 140 mW/cm², 15 min), and the solution was analyzed by LC-MS. (a) Detection was done at 565 nm. Gradient conditions: 0 min MeCN (0.1% FA) 30% → 15 min MeCN (0.1% FA) 50% → 20 min MeCN (0.1% FA) 50%; the other solvent was H₂O (0.1% FA). Mass spectra of photodecomposition products at (b) 2 min (S1), (c) 11 min (intermediate 9), (d) 12 min
(intermediate 11) are shown.

**Figure S6** Proposed decomposition mechanism of NO-Rosa

**Figure S7** Red fluorescence images of each sample corresponding to (a)–(d) of Figure 4 (photocontrolled NO release in HEK293 cells).
**Figure S8** HEK293 cell viability determined by Cell Counting Kit-8 after irradiation by different wavelength light. HEK293 cells were irradiated by yellowish green light (530–590 nm, 40 mW/cm\(^2\), 15 min) or by blue light (470–500 nm, 40 mW/cm\(^2\), 15 min). Cell viability was determined by Cell Counting Kit-8. Cell viability after each wavelength range was; 530–590 nm: 82 ± 7%, 470–500 nm: 66±17%, n = 9, *p = 0.026 (Student’s t-test).

**Figure S9** Vasodilation test with photodecomposed sample. NO-Rosa (1 mM in MilliQ water containing 10% DMSO) was decomposed by pre-irradiation with yellowish green light (530–590 nm, 170 mW/cm\(^2\), 30 min). A rat aortic strip was placed in a Magnus tube filled with Krebs buffer at 37 °C. The strip was then pretreated with L-NAME (10 µM) and noradrenaline (10 µM). After equilibration, the photodecomposed sample (originally 10 µM NO-Rosa) was added to the tube. The strip was irradiated with a light source (MAX-303, Asahi Spectra) equipped with a 530–590 nm band-pass filter for 1 min periods as shown. The light intensity at the each irradiation of was (a) 65 mW/cm\(^2\), (b) 130 mW/cm\(^2\).