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Seymour, Christopher Peter
Tohda, Rei
Tsubaki, Motonari
Hayashi, Masahiko
Matsubara, Ryosuke

(Citation)
Journal of Organic Chemistry,82(18):9647-9654

(Issue Date)
2017-09-15

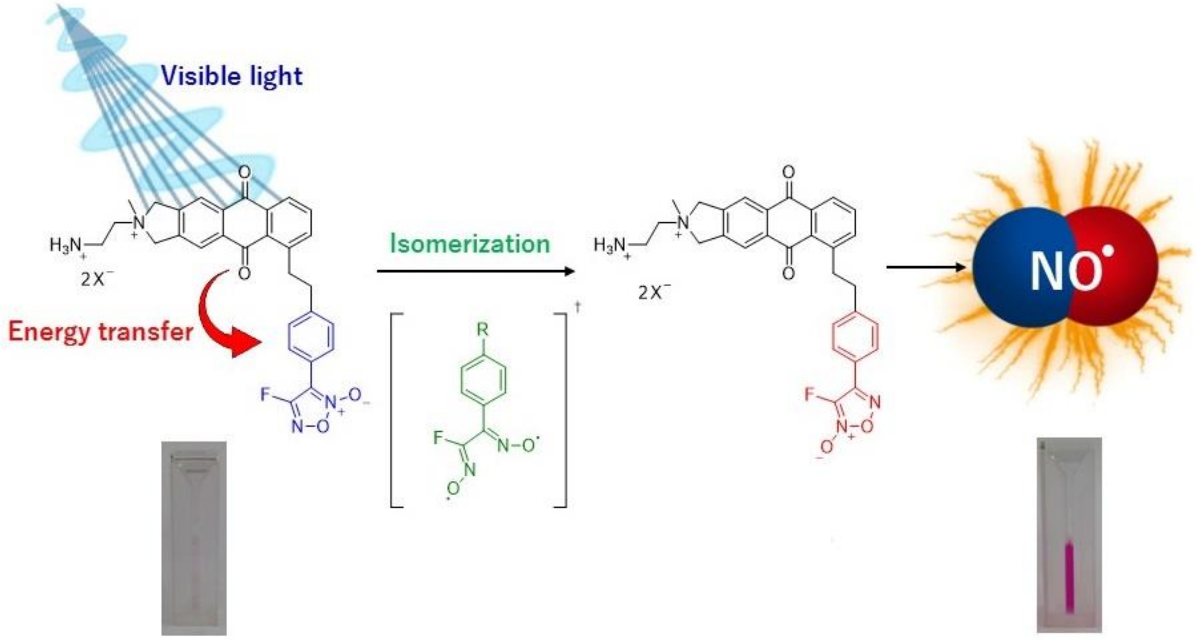
(Resource Type)
journal article

(Version)
Accepted Manuscript

Photosensitization of fluorofuroxans and its application to the development of visible light-triggered nitric oxide donor

Christopher Peter Seymour, Rei Tohda, Motonari Tsubaki, Masahiko Hayashi and Ryosuke Matsubara*

Department of Chemistry, Graduate School of Science, Kobe University, Nada, Kobe 657-8501, Japan



Abstract: Nitric oxide (NO) is an endogenous signaling molecule used in multiple biochemical processes. The development of switchable NO donors that deliver an NO payload under spatiotemporal control harbors many medicinal benefits. Previously, 4-fluorofuroxans were found to function as a UV light-induced NO donor under physiological conditions based on the photo-induced isomeriazation mechanism; however, the isomerization of fluorofuroxans with longer wavelength light is desired in light of its further application into living systems. Herein, we report the use of photosensitizers in the photochemical isomerization of fluorofuroxan, enabling the use of visible light to induce isomerization. Among the tried photosensitizers, anthraquinone derivatives showed a good sensitizing ability to isomerize 4-fluorofuroxan to 3-fluorofuroxan using visible light. This new phenomenon was applied to the synthesis of a water soluble anthraquinone-fluorofuroxan all-in-one molecule which demonstrated promising NO-releasing ability using 400–500 nm irradiation. A high level of control is displayed with 'on' and 'off' NO-release functionality suggesting that photosensitizer-furoxan hybrids would make valuable donors. Furthermore, unlike most furoxans, NO is released in the absence of thiol cofactor.

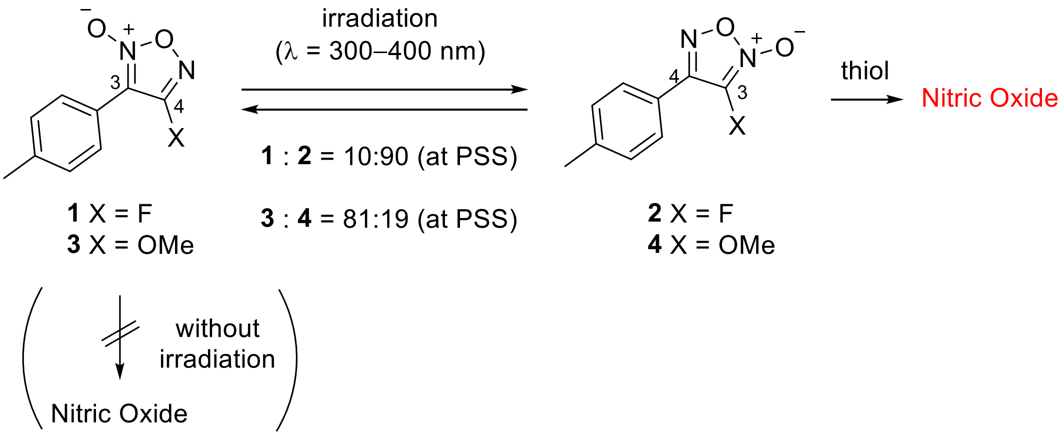
1. Introduction

Nitric oxide (NO) is a reactive radical gas with a short lifetime and area of effect.[1] NO mediates a variety of biological effects such as vasodilation, inhibition of platelet aggregation, cell apoptosis and neurotransmission.[2,3,4] Research has been conducted into NO therapeutics given the many medicinal benefits. However, gaseous NO cannot be reliably administered, therefore stable NO donor compounds have been developed to deliver NO exogenously.[5] Many NO donor families exist, the most common being the NONOates[6] and S-nitrosothiols.[7] The shortfall of current donors is the lack of quantitative and spatiotemporal control over the NO-release which limits application.

Photoinduced nitric oxide donors (PINODs) are an attractive solution to the spatiotemporal control issues of exogenous NO delivery. Light can be used to induce otherwise impossible reactivity without remaining in the system after completion, furthermore reactivity can be tuned by altering the parameters of irradiation such as wavelength, amplitude and time. Various types of PINODs are known such as caged NONOates,[8] metal nitrosyls,[9] nitroarenes[10] and *N*-nitrosoamines.[11]

Despite promising advances, there are drawbacks for many PINODs such as toxicity of the byproducts following uncaging and low stability of the parent donors prior to release.[12] Therefore the development of PINODs utilizing new photo-switching mechanisms is necessary.

Recently our laboratory reported a PINOD based on the furoxan structural motif (Scheme 1).[13] Furoxan is a known NO donor but prior to our laboratories work has rarely exhibited PINOD character.[14,15] We discovered that fluorofuroxan exhibits PINOD character when the thermodynamically stable 4-fluoro isomer **1** is isomerized to the 3-isomer **2** under UV irradiation. In the presence of a thiol cofactor **2** releases NO readily whereas **1** discharges only marginal quantities of NO. Under continuous irradiation moderate NO-release was achieved. Following the success of the fluorofuroxan we developed alkoxy furoxan derivatives that released NO in moderate to high yields, despite lower total isomerization at the photo stationary state.[16]



Scheme 1. Isomerization of furoxans under UV irradiation and following NO-release (our previous results). PSS = photostationary state.

However, there is a significant drawback in the previous works preventing further application, which is the short UV wavelength required to induce isomerization because fluorofuroxan **1** does not absorb above 320 nm. UV irradiation is problematic in a practical sense because high energy radiation is damaging to organic tissue and penetrates poorly. Furoxan PINODs that are photoactive under longer wavelengths of irradiation would present an attractive approach to spatiotemporal in vivo NO-release.

In order to overcome this problem, we focused on photosensitization. Photosensitization enables reactivity that would not necessarily be available through typical reaction pathways.[17] We expected that the use of a photosensitizer that absorbs lower energy light and then transfers the energy to furoxan could lead to sensitized isomerization and NO-release under biologically inert wavelengths. Installing both the photosensitizer and fluorofuroxan in one molecule would make the self-contained PINOD that is activated by longer wavelengths of irradiation (Figure 1). To the best of our knowledge there have been no reports of furoxan photosensitization so far. In this report we demonstrate for the first time that 4-fluorofuroxans can be excited under visible light irradiation (λ = 400–500 nm) in the presence of triplet sensitizers and isomerize to the 3-regioisomer. Based on the finding that anthraquinone is a suitable photosensitizer for 4-fluorofuroxans, we designed and synthesized a novel PINOD **5** bearing both 4-fluorofuroxan and anthraquinone units. PINOD **5** showed visible light-induced NO-releasing capability in the buffered solution. Unexpectedly, in contrast to most known furoxans that require a thiol cofactor for NO-release, **5** released NO under irradiation in the absence of a thiol cofactor.

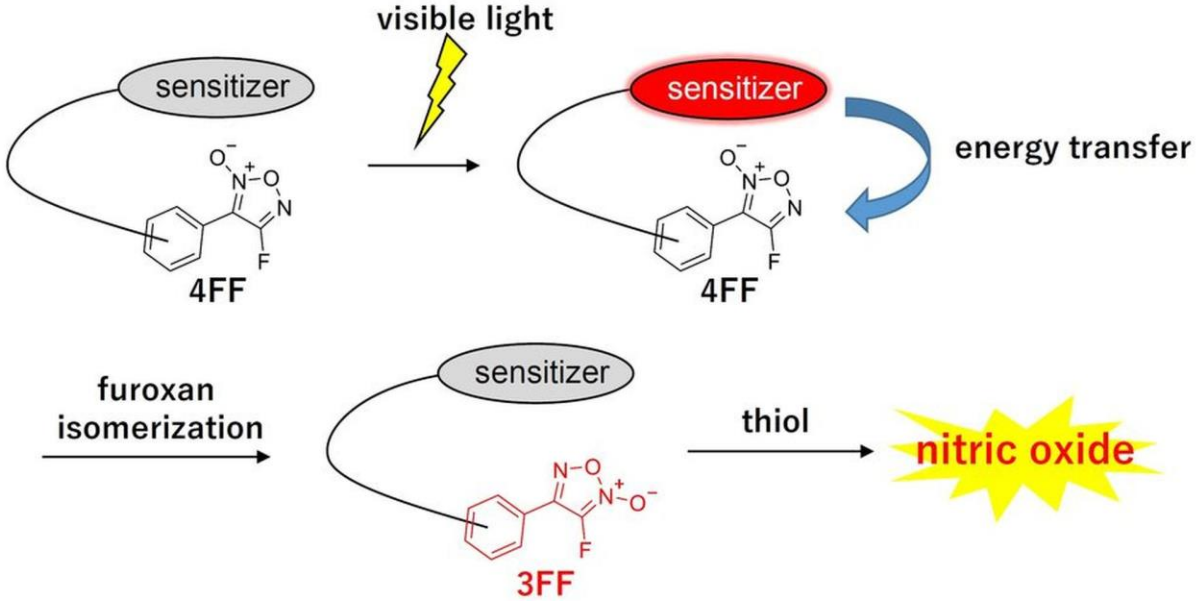


Figure 1. Illustration diagram of concept for visible light-triggered NO-release via sensitization of 4-fluorofuroxan. **4FF** = 4-fluorofuroxan. **3FF** = 3-fluorofuroxan.

2. Results and Discussion

2.1 Sensitization of fluorofuroxan

To know the triplet energy of 4-fluorofuroxan, phosphorescence of 4-fluorofuroxan **1** was measured in glassy frozen 2-methyltetrahydrofuroran (2-MeTHF) at 77K (Figure 2). The phosphorescent spectrum was well-resolved, and maximal phosphorescence wavelength was above 400 nm. Judging from this spectrum, the photosensitization of 4-fluorofuroxan by light longer than 400 nm was expected to be possible using appropriate triplet photosensitizers

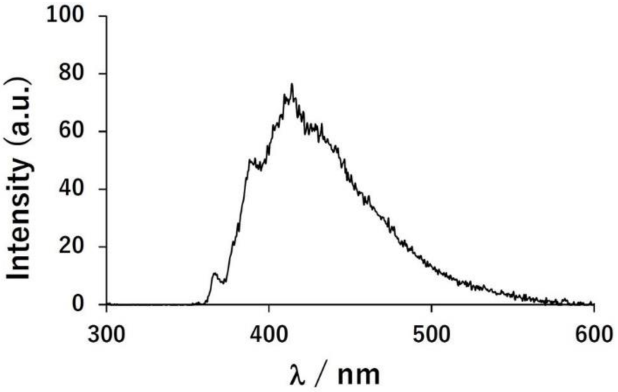
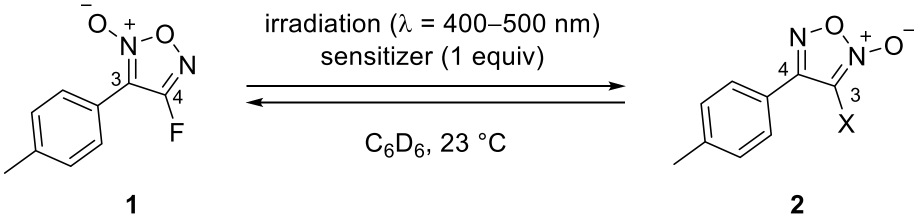


Figure 2. Phosphorescence of **1** (1 mM in 2-MeTHF at 77K). Ex = 280 nm.

Potential photosensitizers were screened by irradiating an NMR tube containing a 1:1 molar solution of sensitizer and furoxan in 5 mM deaerated C_6D_6 (Table 1). Samples were exposed to visible light in the range of 400–500 nm and relative isomerization was measured at 15 minute intervals up to 90 minutes by ^{1}H NMR spectroscopy. In most cases the PSS was reached between 15 and 30 minutes of irradiation.

Table 1. Photosensitized furoxan isomerization under visible irradiation[a]



Entry	Sensitizer	Yield of **2** /%[b]		
		at 15 min	at 30 min	at PSS
1	anthraquinone	59	75	76
2	1,2-benzanthraquinone	96	96	96
3	benzil	79	92	94
4	phenothiazine	8	14	–[c]
5	DDQ	0[d]	0[d]	–[d]
6	benzophenone	1	2	>6[e]
7	none	0	0	0

[a] Sensitizer (1 equiv), **1** (1 equiv) in deaerated C_6D_6 (5 mM). [b] Determined by ^{1}H NMR analysis using dodecane as an internal standard. [c] Phenothiazine decomposed and byproduct started to form after 1 h of irradiation. [d] DDQ reacted with furoxan. [e] PSS was not reached after 1.5 h of irradiation.

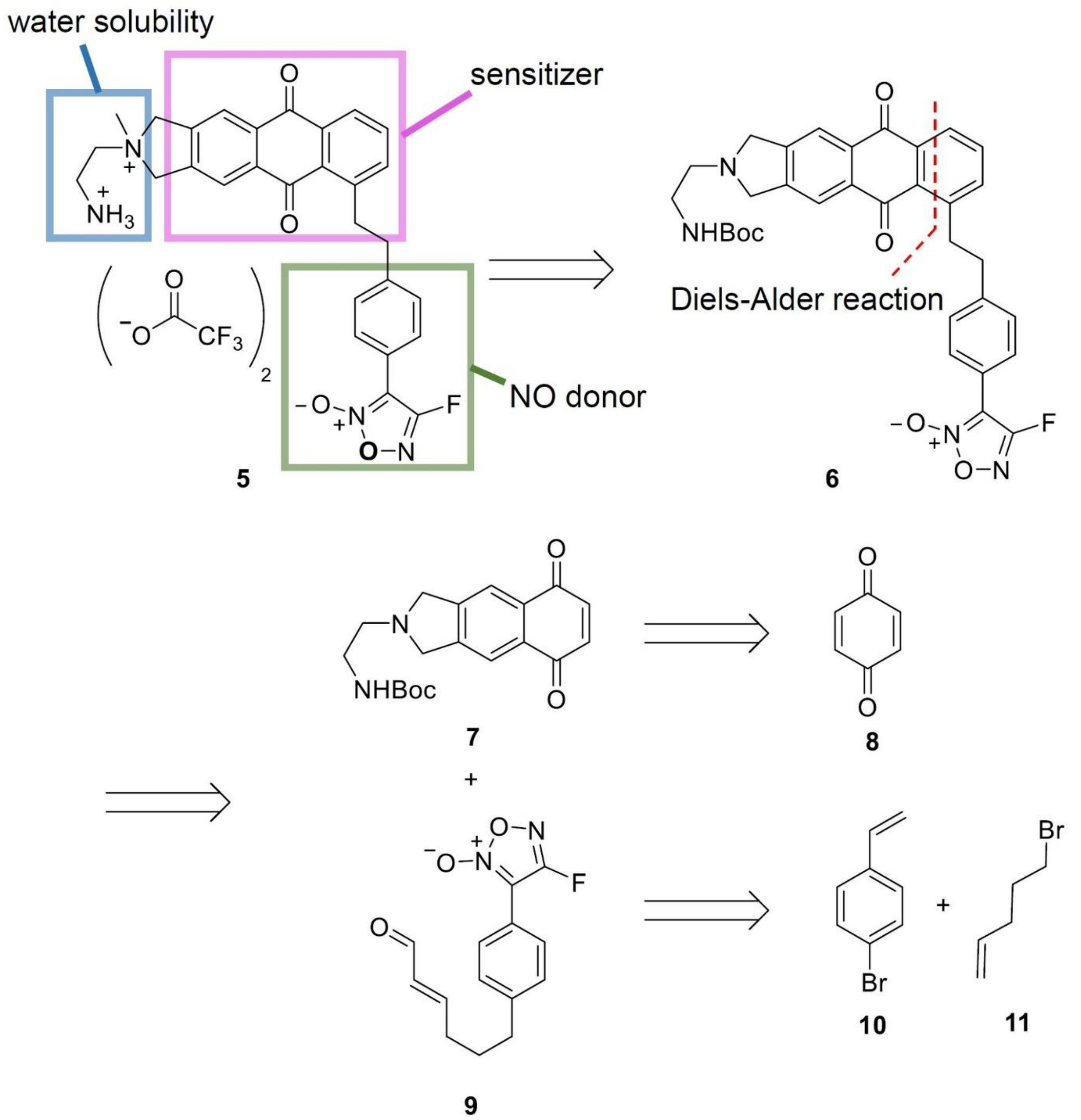
Quinone type sensitizers appeared to be quite effective at inducing isomerization in the furoxan. Anthraquinone produced a good yield of 76% at the PSS which was reached just after 30 minutes of irradiation (entry 1, Table 1). 1,2-Benzanthraquinone was an exceptional sensitizer reaching the PSS within the first 15 minutes of irradiation, furthermore it displayed a high yield at the PSS of 96% (entry 2, Table 1). Benzil was also a good photosensitizer inducing isomerization at a faster rate than anthraquinone and displaying an excellent yield at the PSS (entry 3, Table 1). Phenothiazine appeared to induce minor isomerization in the furoxan, however reaction occurred leading to the build-up of by products and rapid decomposition of the sensitizer (entry 4, Table 1). 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) did not cause isomerization but instead reacted with the furoxan under irradiation. Even if DDQ was a successful sensitizer it is unlikely that it could be used to synthesize an NO donor hybrid given its toxic nature (entry 5, Table 1). Benzophenone, a well-known triplet sensitizer, was not effective inducing only minor isomerization and not reaching the PSS after 90 minutes of irradiation (entry 6, Table 1). Without a suitable photosensitizer there is no isomerization induced under visible light irradiation because the furoxan simply does not absorb such low wavelength light (entry 7, Table 1).[13]

We measured the electrochemical properties of anthraquinone and fluorofuroxan **1**, and examined whether or not the electron transfer sensitization is plausible instead of energy transfer. Cyclic voltammetry experiments on anthraquinone and **1** in CH_3CN using 0.1 M Bu_4NClO_4 as supporting electrolyte revealed the reduction potentials of anthraquinone/anthraquinone$^{\bullet-}$ and $\mathbf{1}^{\bullet+}/\mathbf{1}$ couples to be −0.95 V and +2.11 V versus SCE, respectively.[18] The triplet energy of anthraquinone was reported to be 2.73 eV.[19] Following the Rehm–Weller formalism[20,21] with the assumption that the work function ω is a small contribution,[22] the excited-state reduction potential of anthraquinone was estimated to be +1.78 V versus SCE, which is not comparable to the reduction potential of $\mathbf{1}^{\bullet+}/\mathbf{1}$ couple (+2.11 V versus SCE). Thus the ΔG^0 for the electron transfer from **1** to the excited anthraquinone is a large positive value, suggesting that this pathway is unlikely. We currently believe that the furoxan isomerization using the photosensitizers should proceed via triplet energy transfer, although further investigation is required to unambiguously determine the mechanism.

2.2 Synthesis of furoxan-based visible light-induced NO donor

Anthraquinone, 1,2-benzanthraquinone and benzil were considered for a suitable sensitizer-tethered fluorofuroxan molecule. Previously we found that fast isomerization and subsequent thiol attack to the furoxan are important factors for successful photo-induced NO-release. With this in mind, the isomerization ratio at PSS is not so significant because isomerized furoxan is rapidly replenished upon consumption to maintain said PSS.[16] Indeed, when monitored by ^1H NMR analysis, successful alkoxy furoxan PINODs have shown excellent NO-release without appearing to isomerize to a great extent under irradiation, (e.g. alkoxy furoxan **3**, Scheme 1). Taking this into account we chose not to focus solely on the isomerization properties but also considered the synthetic utility of potential sensitizers. Although the data suggested 1,2-benzanthraquinone and benzil were the better sensitizers with respect to isomerization ratio, after careful consideration they were not selected. We were concerned about the stability of the benzil during synthesis and biological testing, whereas benzanthraquinone presented a challenging synthesis which would add unnecessary regioisomeric complications due to the lack of symmetry. A symmetrical design was preferred to avoid synthetic complexities in purification and analysis, therefore we decided to use an anthraquinone as the core of the molecule, the admirable induced furoxan isomerization rate coupled with molecular symmetry and ease of synthetic modification made the sensitizer attractive.

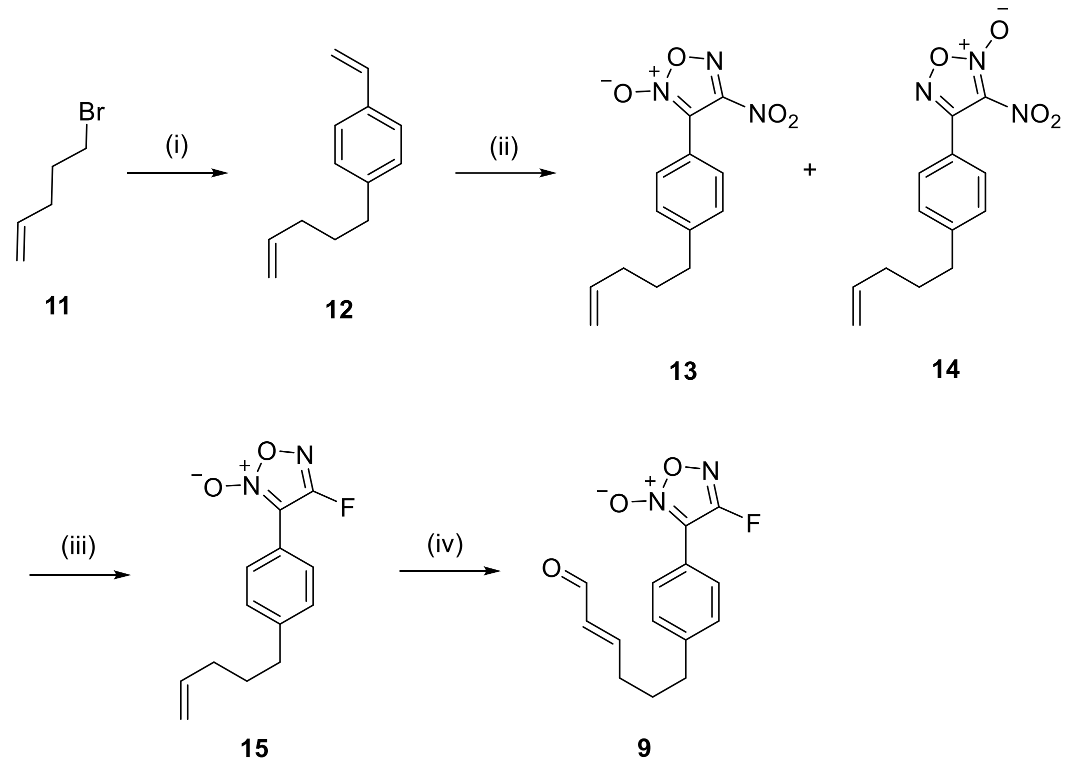
We anticipated that the synthesis of a molecule featuring an anthraquinone core would exhibit poor solubility, so to counter this we employed a quaternary ammonium salt side chain. Another expected issue was the poor stability of the fluorofuroxan, which is prone to reduction (the furoxan ring is electron deficient) and nucleophilic attack to the C4 position even by weak nucleophiles.[23] This meant that mild reaction conditions were necessary to avoid furoxan ring decomposition. Based on these considerations, we designed compound **5** as a furoxan-based visible light-induced NO donor (Scheme 2).



Scheme 2. Retrosynthesis of designed PINOD **5**

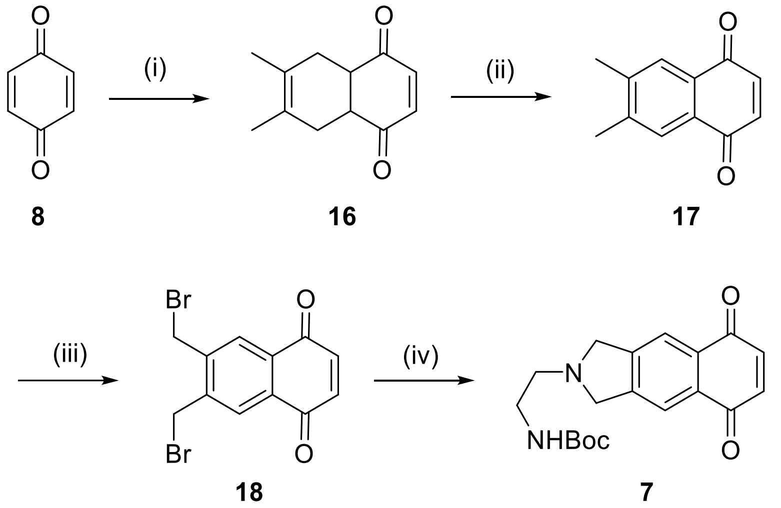
For the key step in the synthesis of **5** we envisioned that an organocatlayzed Diels-Alder reaction based upon work by Lee et al[24] would provide the mild conditions necessary for the union between naphthoquinone **7** and furoxan **9** in one pot. This convergent approach was particularly attractive because both the anthraquinone core and carbon linker to furoxan would be simultaneously furnished. With the conjoined intermediate completed, methylation of tertiary pyrrole amine followed by acid catalyzed Boc deprotection would yield the desired PINOD **5**.

Our convergent synthesis of **5** began with the formation of aldehyde fluorofuroxan **9** in four steps**.** To gain access to the elongated alkyl frame we first reacted 4-bromostyrene (**10**) with 5-bromopentene (**11**) under iron catalyzed Grignard conditions developed by Nakamura et al.[25] The extended styrene **12** was obtained following chromatography in hexanes. Acidic Wieland conditions constructed the furoxan core and afforded compound **13** as an inseparable mixture of 3 and 4 isomers in a 13 : 87 ratio respectively.[26] It is of note that only the conjugated alkene reacted and the nitrofuroxan with a pendant alkene was obtained. During the next fluorination step[13] TBAF reacted exclusively with **13** to form **15** which was easily separated from the unreacted **14** by chromatography. Finally acrolein and **15** were refluxed in dilute toluene with Hoveyda-Grubbs second generation catalyst granting **9**.[27]



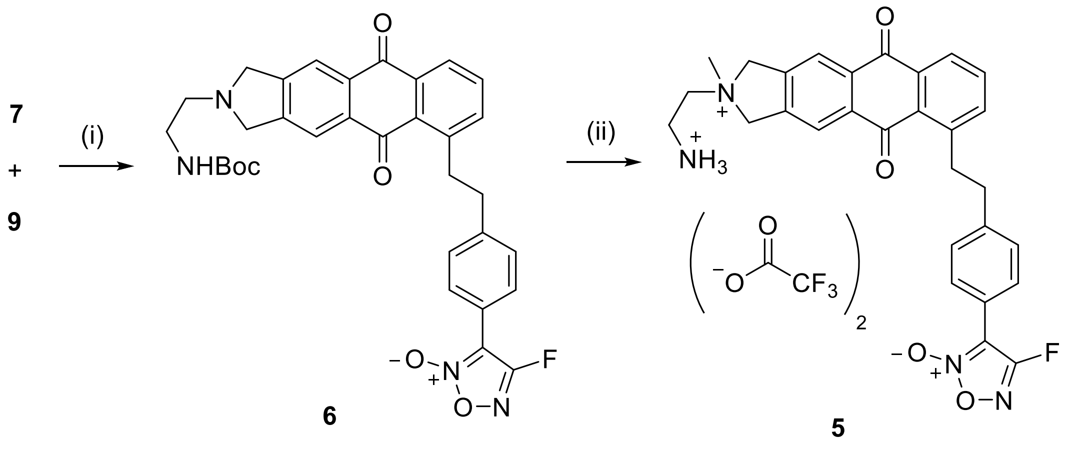
Scheme 3. Synthesis of intermediate **9**. Reagents and conditions: (i) 4-bromostyrene (**10**), Mg turnings, TMEDA, $FeCl_3$, THF, 0 °C, 30 min, 72%; (ii) $NaNO_2$, CH_3CO_2H, DCM, 25 °C, 2 h then 2 M HCl, 25 °C, 14 h, 38% (**13** : **14** = 87:13); (iii) TBAF, THF, 0 °C, 1 h, 72%; (iv) acrolein, Hoveyda-Grubbs second generation catalyst, toluene, 100 °C, 18 h, 76%.

Synthesis of the naphthoquinone **7** began with Lewis acid-catalyzed Diels Alder reaction of 1,4-benzoquinone (**8**) with 2,3-dimethylbutadiene to afford **16** following recrystallization in toluene. The oxidation of **16** initially proved difficult, oxidizing agents such as DDQ and IBX led to poor yields. Utilizing an excess of the relatively mild oxidizing agent MnO_2 we obtained 1,4-naphthoquinone **17**.[28] Benzylic bromination of **17** with NBS led to a mixture of inseparable byproducts along with the desired dibromo product **18**. Recrystallization attempts failed so we decided to perform a two-step synthesis to **7**. Reaction of semi-pure **18** with *N*-Boc-protected ethylenediamine under dilute conditions and iodide catalysis granted **7** following overnight stirring. Initial attempts at accessing **7** were low yielding and tenuous optimization was required. One of the problems was oxidation of the product, probably leading to the full unsaturation of nitrogen-containing five-membered ring. The highly activated alkene was also likely prone to attack from the amine leading to unwanted side reactions. Degassing the solvent helped offset the low yield, furthermore lower reaction temperature, iodide and high dilution were necessary to access acceptable yields. Compound **7** is unstable and will readily oxidize or decompose so should be used immediately in the convergent step.



Scheme 4. Synthesis of napthoquinone intermediate **7**. Reagents and conditions: (i) benzoquinone, $BF_3 \bullet OEt_2$, 2,3-dimethylbutadiene, toluene, −10 °C, 80 min, 76%; (ii) MnO_2, toluene, 100 °C, 2 h, 70%; (iii) NBS, $(PhCO_2)_2$, benzene, 100 °C, 18 h; (iv) *N*-Boc ethylenediamine, K_2CO_3, KI, MeCN, -10 °C, 18 h, 17% (2 steps)

The reaction between **7** and **9**[24] was initially difficult due to decomposition of both partners during reaction. The use of toluene as the solvent resulted in low yields which were improved upon by switching to chloroform, this enhanced the solubility of reactants and increased the yield to 38% (Scheme 5). With the key intermediate **6** in hand, the pyrrole amine was qauternerized by overnight stirring with excess MeI. Upon completion excess MeI was evacuated and subsequent Boc deprotection by treatment with trifluoroacetic acid (TFA) formed a terminal quaternary ammonium ion. The TFA was removed in vacuo and the oily product sonicated in Et_2O whereupon it precipitated out and was collected by filtration, thus completing the synthesis of **5**.



Scheme 5. Organocatalyzed Diels-Alder reaction and subsequent modification to **5**. Reagents and conditions: (i) proline, benzoic acid, $CHCl_3$, 55 °C, 15 h, 38%; (ii) MeI, DCM, 40 °C, 20 h then TFA, DCM, 25 °C, 1 h, 80%.

2.3 NO-releasing ability under visible light

The in vitro NO-releasing ability of compound **5** was tested using the Griess method.[29] Compound **5** (100 μM) was dissolved in a 50 mM phosphate buffer containing 5mM of ʟcysteine[30] and was subjected to seven hours of continuous irradiation (λ= 400–500 nm). Aliquots were taken at regular intervals and the NO% determined spectroscopically after treatment with Griess reagent (Figure 3). To our delight NO was released from **5** under visible light irradiation. The total release was up to a similar percentage release as **2** under UV irradiation, although the rate was slightly slower (Figure 3a, blue line).[13] Initial release of NO was rapid reaching up to 20% after one hour of irradiation, under prolonged irradiation the rate of NO-release slowed. This is a similar trend shared by other NO donors which seem to have intrinsic NO-release limits. Only background NO-release (up to 2%) was detected without irradiation in the presence of cysteine after 1 hour (data not shown). To our surprise irradiation in the absence of cysteine led to significant NO-release with initial rates comparable to that with thiol present (Figure 3a, orange line). Reports of furoxans that release significant quantities of NO in the absence of thiol are rare and the exact reason for NO-release is unknown.[30] Compound **5** is a particularly unusual example since the furoxan does not release NO unless irradiated. These results suggest that in addition to the thiol-mediated irradiation-independent NO-release from the 3-fluorofuroxan, there might be another NO-release pathway where the photochemically excited fluorofuroxan or secondary transient intermediate releases NO without thiol cofactor assistance. In any case, the exact mechanism of the sensitized release is currently unknown and is the topic of further research in our laboratory.

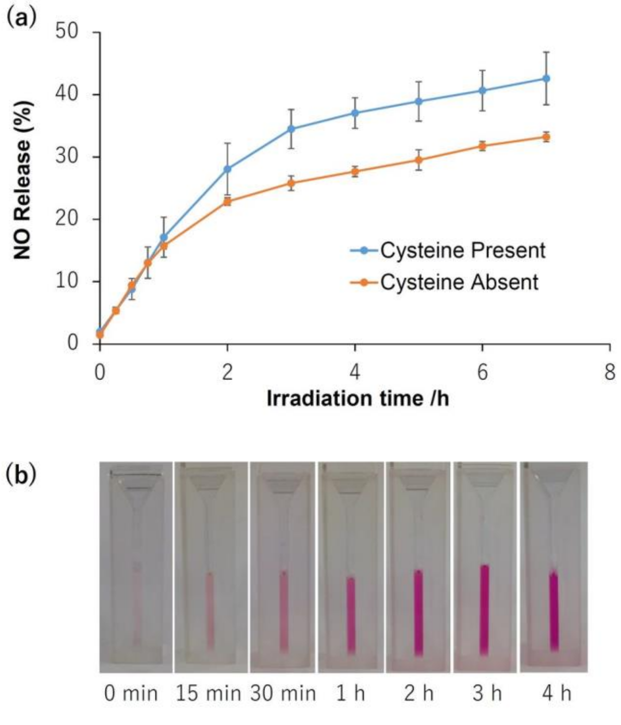


Figure 3. (a) NO-release curve of **5** (100 μM) in 1% DMSO 50 mM phosphate buffer containing or omitting 5 mM of L-cysteine under 400–500 nm irradiation at 37 °C. (b) Pictures of cuvettes containing a solution of **5** (in the presence of L-cysteine) after irradiation at the indicated period and Griess reagent.

With the NO-release confirmed to be dependent on irradiation we decided to investigate the level of control over release (Figure 4). Compound **5** was subjected to 1-hour rotations of irradiation under 400–500 nm and ambient light, aliquots were taken at intervals. Figure 4 shows that as soon as irradiation is ceased, the NO-release almost completely stops with any increments attributed to background release. This demonstrates a high level of control over

NO-release with compound **5**, therefore it may be possible to release a specific quantity of NO with appropriate sample concentration and irradiation time.

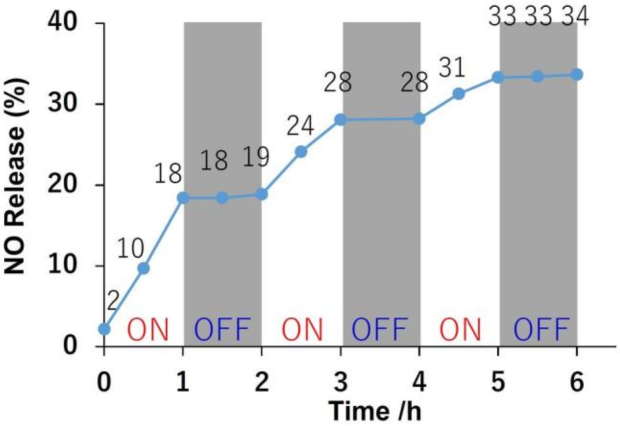


Figure 4. NO-release curve of **5** (100 uM) in 1% DMSO 50 mM phosphate buffer containing 5 mM of L-cysteine under 1-hour rotations of 400–500 nm irradiation and ambient light at 37 °C

Following successful in vitro NO-release we attempted to release NO under spatiotemporal control in vivo. HeLa cancer cells were initially incubated with NO_{550}, a fluorescent probe for NO detection,[31] and then treated with **5** for 30 min. After washed with PBS buffer, the cells were irradiated with 400–500 nm light for 1 hour. To our regret the cells exhibited no significant fluorescence derived from the reaction of NO_{550} and NO (See Figure S3 for the detail). Due to the non-fluorescent nature of **5**, it could not be determined whether or not **5** permeated and entered the cells. Despite these disappointing preliminary in vivo results, since there are unlimited combinations of photosensitizers and peripheral substituents to facilitate the cellular uptake, in the future we would be likely able to develop a visible light-induced NO donor suitable for in vivo use based on the developed concept.

3. Conclusion

Triplet photosensitization of furoxans was demonstrated for the first time. The sensitization led to the visible light-induced isomerization of 4-fluorofuroxan to 3-fluorofuroxan. We have designed and synthesized a prototype water-soluble furoxan-based visible light-induced NO donor. The key steps in its synthesis are the selective furoxan ring formation from the diene and organocatalyzed anthraquinone formation. Water solubility is endowed by quarternalization of the nitrogen atoms. The synthesized molecule showed visible light-responsive NO-releasing ability in physiological conditions. The present work provides not only a concept to utilize biologically inert visible light for furoxan photochemistry, but also a new mode of NO-release switching of furoxans, which is anticipated to be applicable to the development of other switching systems for NO-release, e.g. by turning on/off the light sensitivity of photosensitizer with external stimuli.

Experimental Section

Unless otherwise noted, all reactions were carried out in well cleaned glasswares with magnetic stirring. Operations were performed under an atmosphere of dry argon using Schlenk and vacuum techniques, unless otherwise noted. All starting materials were obtained from commercial sources or were synthesized using standard procedures. Melting points were measured on a Yanaco MP-500D and are not corrected. ^{1}H, ^{13}C NMR, and ^{19}F spectra (400, 100, and 376 MHz, respectively) were recorded on a Bruker Avance III HD 400 using TMS (0 ppm), $CDCl_3$ (77.0 ppm), and C_6F_6 (−164.9 ppm) as an internal standard, respectively. The following abbreviations are used in connection with NMR; s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sep = septet, and m = multiplet. Mass spectra were measured using a JEOL JMS-T100LP (DART method, ambient ionization). Preparative column chromatography was performed using Kanto Chemical silica gel 60 N (spherical, neutral), Fuji Silysia BW-4:10MH silica gel or YMC_GEL Silica (6 nm I-40-63 μm). Thin layer chromatography (TLC) was carried out on Merck 25 TLC silica gel 60 F254 aluminium sheets. Phosphorescence spectrum was recorded on a spectrofluorometer (Jasco FP-6500) using phosphorescence measurement mode. Cyclic voltammetric measurements were performed using an ALS CHI606S electrochemical analyzer. 4-Fluorofuroxan **1** was synthesized according to a previously reported method.[13]

Phosphorescence measurement of 4-fluorofuroxan 1. Phosphorescence of 4-fluorofuroxan **1** was measured for a 1 mM 2-MeTHF solution of **1** kept in a quartz ESR tube (Φ 5 mm) at 77 K. The delay time, gate time and excitation wavelength were set to be 9 ms, 9 ms and 280 nm, respectively.

Photosensitized isomerization of 4-fluorofuroxan 1 to 3-fluorofuroxan 2 (Table 1). A solution of 4-fluorofuroxan (0.5 mg, 2.4 μM) and sensitizer (2.4 μM) in deaerated C_6D_6 (0.5 mL) was prepared in a Pyrex NMR tube. The solution was irradiated with 400–500 nm light (a 300W Xenon lamp, Asahi Spectra MAX-303 equipped with a 300- to 600-nm ultraviolet-visible module, and a combination of a 400-nm long-pass and 500-nm short-pass filters). The reaction progress was monitored by ^{1}H NMR analysis (Figure S1). The yield was determined from the peak integration relative to dodecane internal standard (1 μL).

Redox property. Cyclic voltammetric measurements were performed at 298 K with solvents being deaerated by Ar bubbling for 30 min before each measurement. The supporting electrolyte was 0.10 M $TBAClO_4$. A conventional three-electrode cell was used with a platinum working electrode and a platinum wire as a counter electrode. The cyclic voltammograms were recorded with respect to the $Ag/AgNO_3$ (10 mM) reference electrode at a sweep rate of 50 mV/s. The oxidation potential (determined as the peak potential) was corrected to the SCE scale by adding 0.31 V, on the basis of the measurement of the redox potential (+0.09 V in the cell system used) of the Fc/Fc^+ couple as the internal standard.

Synthesis of furoxan 9 (Scheme 3). *1-Ethenyl-4-(pent-4-en-1-yl)benzene (**12**).* To a mixture of 5-bromopent-1-ene (11.3 mL, 95.6 mmol), $FeCl_3$ (47.8 mL of a 0.1 M THF solution, 4.78 mmol) was added at 0 °C a mixture of 4-ethenylphenylmagnesium bromide (115 mL of a 1 M THF solution, 114.8 mmol) and TMEDA (17.2 mL, 114.8 mmol) dropwise over 2.5 hours at a rate that keeps the reaction mixture yellow (4-ethenylphenylmagnesium bromide was prepared by dropwise addition of a 1 M solution of 4-ethenylphenyl bromide in THF to Mg turnings at a rate which maintained gentle reflux, following addition the mixture was stirred for a further 30 minutes). Once addition was completed the reaction was stirred for a further 20 minutes. Saturated aqueous NH_4Cl was added followed by 1 M HCl and water, the reaction mixture was extracted thrice with EtOAc and the organics washed with brine, dried over $MgSO_4$ and filtered. Solvent was removed in vacuo and the residue purified by column chromatography in hexanes to yield a colorless oil (11.82 g, 68.6 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 7.33 (2H, d, J = 8.0 Hz), 7.15 (2H, d, J = 8.0 Hz), 6.70 (1H, dd, J = 17.6, 11.2 Hz), 5.84 (1H, ddt, J = 17.0, 10.4, 6.8 Hz), 5.71 (1H, dd, J = 17.6, 0.8 Hz), 5.20 (1H, dd, J = 10.8, 1.2 Hz), 5.03 (1H, dq, J = 17.2, 2.0 Hz), 4.98 (ddt, J = 10.0 Hz, 3.2 Hz, 1.2 Hz), 2.62 (2H, t, J = 7.6 Hz), 2.01 (2H, q, J = 6.8 Hz), 1.72 (2H, quin, 7.6 Hz); δ_C (100 MHz, $CDCl_3$) 142.3, 138.6, 136.7, 135.2, 128.7, 126.2, 114.8, 112.9, 35.1, 33.3, 30.6; IR (neat) 3081, 3000, 2973, 2927, 2853, 1641, 1620, 1511, 1438, 1406, 988, 902, 836, 817 cm^{-1}. HRMS (DART): Calcd for $C_{13}H_{16}$ (M+H)$^+$: 173.1325; found 173.1330.

*4-Nitro-3-[4-(pent-4-en-1-yl)phenyl]furoxan (**13**).* Nitrofuroxans **13** and **14** were synthesized according to a reported method.[26] To a stirred suspension of $NaNO_2$ (961 mg, 13.9 mmol) in a 0.2 M CH_2Cl_2 solution of **12** (300 mg, 1.74 mmol) was added AcOH (796 µL, 13.9 mmol) over 30 minutes at room temperature. Two hours after starting the AcOH addition, a 2 M solution of HCl (5.2 mL, 10.5 mmol) was added. The reaction mixture was stirred for 14 h at rt, and then extracted three times with CH_2Cl_2. The combined organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo. The residue was purified by silica gel to yield **13** and **14** (181 mg, 0.66 mmol, 38%) as an 87:13 (**13**:**14**) mixture. The reaction can be conducted on a larger scale at the cost of diminished yield as byproduct formation increases. Data reported is for the major isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.52 (2H, dt, J = 8.4, 2.0 Hz), 7.37 (2H, d, J = 8.8 Hz), 5.83 (1H, ddt, J = 17.0, 10.4, 6.4 Hz), 5.04 (1H, dq, J = 16.8, 2.0 Hz), 5.01 (1H, ddt, J = 10.4, 2.0, 1.2 Hz), 2.71 (2H, t, J = 8 Hz), 2.11 (2H, q, J = 6.4 Hz), 1.76 (2H, quin, J = 7.2 Hz); δ_C (100 MHz, $CDCl_3$) Major isomer **13;** 158.2, 147.1, 138.1, 129.4, 128.6, 116.5, 115.2, 109.2, 35.3, 33.2, 30.2; distinguishable minor isomer **14**; 147.4, 138.1, 129.2, 128.6; IR (neat) 3075, 2924, 2856, 1612, 1560, 1519, 1492, 1357, 1270, 1115, 1069, 983, 909, 839, 812, 785, 755 cm^{-1}. HRMS (DART): Calcd for $C_{13}H_{13}N_3O_4$ (M+H)$^+$: 276.0979; found 276.0984.

*4-Fluoro-3-[4-(pent-4-en-1-yl)phenyl]furoxan (**15**).* To a solution containing the mixed isomers of **13** and **14** (1 g, 3.63 mmol) in THF (7.3 mL) was added dropwise a 1 M solution of tetrabutylammonium fluoride (TBAF) in THF (3.63 mL, 3.63 mmol) at 0 °C. After 1 hour the reaction was diluted with water and extracted thrice with DCM. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The residue was purified by column chromatography to yield **15** as a colourless oil (652 mg, 2.63 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 7.89 (2H, d, J = 8.4 Hz), 7.37 (2H, d, J = 8.8 Hz), 5.83 (1H, ddt, J = 17.2, 10.4, 6.4 Hz), 5.06–4.98 (2H, m), 2.70 (2H, t, J = 8.0 Hz), 2.10 (2H, q, J = 7.6 Hz), 1.75 (2H, quin, J = 8.0 Hz); δ_C (100 MHz, $CDCl_3$) 161.0 (d, $J_{C\text{–}F}$ = 258.4 Hz), 145.5, 137.1, 128.5, 124.8 (d, $J_{C\text{–}F}$ = 3.8 Hz), 116.9, 114.1, 104.9 (d, $J_{C\text{–}F}$ = 26.4 Hz), 34.2, 32.1, 29.1; IR (neat) 3072, 2975, 2924, 2856, 1612, 1568, 1522, 1476, 1411, 1338, 1321, 1154, 1124, 1093, 977, 909, 825, 800, 733, 634, 608, 590 cm^{-1}. HRMS (DART): Calcd for $C_{13}H_{13}FN_2O_2$ (M+H)$^+$: 249.1034; found 249.1039.

*(2E)-6-[4-(4-Fluoro-furoxan-3-yl)phenyl]hex-2-enal (**9**).* To a stirred solution of **15** (300 mg, 1.21 mmol) and Hoveyda-Grubbs second generation catalyst (22.7 mg, 36.3 µmol) was added acrolein monomer and the mixture heated to 100 °C for 28 hours. Upon reaction completion the solvent was removed in vacuo and crude material purified by column chromatography on silica gel using EtOAc/hexane to give **9** (252.2 mg, 0.913 mmol, 76%) as a pale yellow crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 9.52 (1H, d, J = 7.6 Hz), 7.91 (2H, d, J = 8.0 Hz), 7.37 (2H, d, J = 8.8 Hz), 6.85 (1H, dt, J = 15.6, 6.8 Hz), 6.14 (1H, ddt, J = 15.6, 8.0, 1.2 Hz), 2.75 (2H, t, J = 8.0 Hz), 2.39 (2H, qd, J = 8.0, 1.6 Hz), 1.89 (2H, quin, J = 7.6 Hz); δ_C (100 MHz, $CDCl_3$) 193.8, 162.0 (d, $J_{C\text{–}F}$ = 258.4 Hz), 157.4, 145.4, 133.4, 129.4, 126.0 (d, $J_{C\text{–}F}$ = 3.7 Hz), 118.3, 105.8 (d, $J_{C\text{–}F}$ = 26.5 Hz), 35.2, 32.0, 29.0; IR (neat): 2951, 2935, 2870, 2829, 2739, 1679, 1612, 1568, 1522, 1473, 1408, 1340, 1326, 1305, 1292, 1150, 1120, 1091, 974, 831, 814, 725, 598 cm^{-1}. HRMS (DART): Calcd for $C_{14}H_{13}FN_2O_3$ (M+H)$^+$: 277.0983; found 277.0989; m.p. 53–56 °C.

Synthesis of naphthalene-1,4-dione 7 (Scheme 4). *6,7-Dimethyl-4a,5,8,8a-tetrahydronaphthalene-1,4-dione (**16**).* To a stirred solution of 1,4-benzoquinone (10.0 g, 92.5 mmol) and BF_3•OEt_2 (1.14 mL, 9.25 mmol) in toluene was added 2,3-dimethyl-1,3-butadiene (12.6 mL, 111 mmol) dropwise at −16 °C over 10 minutes. After addition completion the reaction was allowed to warm to rt over 80 minutes. Upon completion the mixture was washed twice with brine and the organics dried over Na_2SO_4, filtered and solvent condensed under vacuum. The product was recrystallized from toluene to yield yellow crystals (13.3 g, 70.0 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 6.65 (2H, s), 3.22–3.17 (2H, m), 2.40 (2H, apparent d, J = 17.2 Hz), 2.08 (2H, apparent d, J = 16.6 Hz), 1.63 (6H, s); δ_C (100 MHz, $CDCl_3$) 200.3, 139.3, 123.3, 47.1, 30.3, 18.9; IR (neat) 2919, 2876, 2828, 1679, 1602, 1441, 1427, 1373, 1354, 1262, 1196, 1095, 887, 864, 848, 805, 749, 682 cm^{-1}; HRMS (DART): Calcd for $C_{12}H_{14}O_2$ $(M+H)^+$: 191.1067; found 191.1072; m.p. 119–121 °C.

*6,7-Dimethylnaphthalene-1,4-dione (**17**).* To a stirred solution of **16** (3.54 g, 18.6 mmol) in toluene (140 mL) was added portion wise activated MnO_2 (17.4 g, 200 mmol) and refluxed for 2 hours. The reaction mixture was cooled and filtered through celite by washing with EtOH. The solvent was condensed and crude material purified by column chromatography in EtOAc/hexanes to yield a yellow solid (2.42 g, 12.7 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 7.83 (2H, s), 6.90 (2H, s), 2.41 (6H, s); δ_C (100 MHz, $CDCl_3$) 185.3, 143.8, 138.5, 129.9, 127.4, 20.2; IR (neat) 2948, 2920, 1664, 1595, 1447, 1392, 1341, 1326, 1307, 1221, 1144, 1057, 1017, 994, 972, 835, 769, 712, 626 cm^{-1}; HRMS (DART): Calcd for $C_{12}H_{10}O_2$ $(M+H)^+$:187.0754; found 187.0759; m.p. 121–123 °C.

*tert-Butyl [2-(5,8-dioxo-1,3,5,8-tetrahydro-2H-benzo[f]isoindol-2-yl)ethyl]carbamate (**7**).* A stirred solution of **17** (340 mg, 1.79 mmol), NBS (732.6 mg, 4.12 mmol) and benzoyl peroxide (21.7 mg, 0.09 mmol) in benzene (18 mL) was heated to reflux for 24 h. Upon reaction completion the mixture was washed twice with distilled water, the organic layer was dried over $MgSO_4$, filtered and solvent condensed in vacuo. The residue was filtered through a plug of silica with EtOAc/hexane to yield 6,7-bis(bromomethyl)naphthalene-1,4-dione (**18**) as a crude green solid. The impure material **18** was stirred in a mixture containing K_2CO_3 (795 mg, 5.76 mmol) and KI (120 mg, 0.72 mmol) in degassed MeCN (720 mL). To the mixture was added *tert*-butyl (2-aminoethyl)carbamate (248.2 mg, 1.44 mmol) at −10 °C and stirred for 15 hours. Upon reaction completion the reaction mixture was concentrated and water was added, the mixture was extracted thrice with EtOAc. The combined organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude material was purified by column chromatography to yield **7** (103 mg, 0.301 mmol, 17% over two steps) as an orange solid. Compound **7** should be used immediately in the next step as it is unstable and prone to spontaneous oxidation. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 7.91 (2H, s), 6.96 (2H, s), 5.01 (1H, broad s), 4.06 (4H, s), 3.34 (2H, q, J = 5.2 Hz), 2.90 (2H, t, J = 6.0 Hz), 1.45 (9H, s); δ_C (100 MHz, $CDCl_3$): 185.0, 156.0, 149.7, 146.6, 138.5, 131.5, 120.4, 58.6, 54.7, 39.0, 28.4; IR (neat) 3368, 3048, 3013, 2980, 2980, 2930, 281, 2761, 1688, 1663, 1608, 1524, 1435, 1385, 1362, 1327, 1305, 1273, 1240, 1153, 1129, 1040, 964, 838 cm^{-1}; HRMS (DART): Calcd for $C_{19}H_{22}N_2O_4$ $(M+H)^+$: 343.1652; found 343.1658; m.p. 110 °C (decomposed).

Synthesis of fluorofuroxan 5 (Scheme 5). *tert-Butyl [2-(6-{2-[4-(4-fluorofuroxan-3-yl)phenyl]ethyl}-5,10-dioxo-1,3,5,10-tetrahydro-2H-naphtho[2,3-f]isoindol-2-yl)ethyl]carbamate (**6**).* A mixture of **7** (100 mg, 0.292 mmol), **9** (129 mg, 0.468 mmol), L-proline (6.7 mg, 0.058 mmol) and benzoic acid (3.4 mg, 0.03 mmol) in $CHCl_3$ (1.5 mL) was stirred at 50 °C for 15 hours. After reaction completion the solvent was condensed in vacuo and the residue purified by silica chromatography using DCM/EtOAc to afford **6** (67.1 mg, 0.112 mmol, 38%) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm): 8.31 (1H, dd, J = 8.0, 1.2 Hz), 8.12 (1H, s), 8.10 (1H, s), 7.92 (2H, d, J = 7.6 Hz), 7.65 (1H, t, J = 7.6 Hz), 7.52 (2H, d, J = 8.4 Hz), 7.48 (1H, dd, J = 8.4, 1.2 Hz), 5.05 (1H, broad s), 4.10 (4H, s), 3.56 (2H, t, J = 8 Hz), 3.36 (2H, apparent q, J = 5.2 Hz), 3.05 (2H, t, J = 7.6 Hz), 2.93 (2H, apparent t, J = 6.4 Hz), 1.46 (9H, s); δ_C (100 MHz, $CDCl_3$): 184.9, 183.4, 162.1 (d, $J_{C–F}$ = 258.3 Hz), 156.0, 147.0, 146.4, 145.9, 144.6, 137.8, 135.5, 134.5, 133.3, 132.3, 131.0, 129.8, 126.8, 125.9 (d, $J_{C–F}$ = 3.7 Hz), 121.2, 120.5, 118.1 (d, $J_{C–F}$ = 4.2 Hz), 105.9 (d, $J_{C–F}$ = 26.6 Hz), 79.4, 58.7, 58.6, 54.8, 39.1, 37.6, 37.2 28.4; IR (neat) 2958, 2927, 2846, 1712, 1666, 1606, 1584, 1522, 1471, 1321, 1298 1252, 1168, 1153, 1115, 981, 726 cm^{-1}; HRMS (DART): Calcd for $C_{33}H_{21}FN_4O_6$ $(M+H)^+$: 599.2300; found 599.2306; m.p. 209 °C (decomposed).

*2-(2-Aminoethyl)-6-[4[(4-fluorofuroxan-3-yl)phenyl]ethyl]-2-methyl-5,10-dioxo-2,3,5,10-tetrahydro-1H-naphtho[2,3-f]isoindol-2-ium bistrifluoroacetate (**5**).* Compound **6** (60 mg, 0.100 mmol) was stirred in DCM (1 mL) and MeI (32 μL, 0.500 mmol) was added and the reaction warmed to 40 °C for 20 hours. The solvent and excess MeI were removed under vacuum. To the crude material was added DCM (0.5 mL) and cooled to −10 °C, TFA (0.5 mL) was added the mixture stirred for 1 hour. The solvent and excess TFA were removed in vacuo and the brown oil sonicated in Et_2O for 1 hour whereupon it solidified and was filtered to afford **5** (58.9 mg, 0.08 mmol, 80%) as a brown solid. ^{1}H NMR (400 MHz, D_2O) δ_H(ppm): 8.14 (1H, s), 8.09 (1H, dd, J = 7.6, 1.2 Hz), 8.03 (1H, s), 7.68–7.64 (3H, m), 7.56 (1H, t, J = 7.6 Hz), 7.23 (2H, d, J = 8.0 Hz), 5.17–5.06 (2H, m), 4.05–4.01 (2H, m), 3.62–3.58 (2H, m), 3.51–3.46 (2H, m), 3.33 (3H, s), 2.94 (2H, t, J = 7.2 Hz); δ_C (100 MHz, d_6-DMSO): 183.9, 182.4, 162.5 (d, $J_{C–F}$ = 256.3 Hz), 158.2 (q, $J_{C–F}$ = 32.5 Hz), 145.5, 144.6, 139.8, 139.2, 138.2, 135.2, 134.6, 134.0, 133.0, 130.4, 129.4, 126.3 (d, $J_{C–F}$ = 3.1 Hz), 122.6, 121.8, 118.2, 106.9 (d, $J_{C–F}$ = 27.4 Hz), 68.9, 59.6, 49.9, 36.6, 36.3, 33.3; IR (neat) 3006, 2929, 2860, 1667, 1614, 1523, 1473, 1415, 1327, 1298, 1197, 1173, 1125, 976, 836, 796, 719 cm^{-1}. HRMS (DART): Calcd for $C_{27}H_{21}FN_3O_4$ $(M–2CF_3CO_2–C_2H_4NH_2)^+$: 470.1511; found 470.1516.

Measurement of the NO-releasing ability of compound 5 with visible light-irradiation (Figure 3). A 100 μM stock solution of **5** was prepared by adding a 50 mM phosphate buffer (pH 7.4) either containing or omitting 5 mM L-cysteine to a 10 mM solution of **5** in DMSO (100 μL, 1 μmol) to bring the total volume up to 10 mL. 8 mL of the stock solution was added to a Pyrex vial, which was placed in a 37 °C oil bath and then irradiated using 400–500 nm light. At intervals, aliquots (0.5 mL) were taken out, to which the Griess reagent (40 μL) was added. After the samples were stood for 30 min, the absorbance of the samples at 520 nm was measured, and the percent nitrite (NO_2^-) (mol/mol) was determined from a calibration curve prepared in advance by using $NaNO_2$ standard solutions (20–100 μM) treated with the Griess reagent.

The Griess reagent was prepared by diluting a mixture of 1 g of sulfanilamide, 50 mg of *N*-naphthylethylenediamine dihydrochloride, and 2.5 mL of 85% phosphoric acid with distilled water to a final volume of 25 mL.

Measurement of NO-releasing ability of compound 5 without light-irradiation (Figure 3). A 100 μM stock solution of **5** was prepared by adding a 50 mM phosphate buffer (pH 7.4) containing 5 mM L-cysteine to a 10 mM solution of **5** in DMSO (100 μL, 1 μmol) to bring the total volume up to 10 mL. 8 mL of the stock solution was added to a Pyrex vial, which was placed in a 37 °C oil bath. The solution was maintained at 37 °C for 1 h. Then, 3 mL of the solution was added to an empty quartz cuvette, followed by the addition of 250 μL of the Griess reagent. After 10 min of stirring, the absorbance at 520 nm was measured, and the percent nitrite (NO_2^-)(mol/mol) was determined from a calibration curve prepared in advance by using $NaNO_2$ standard solutions (20–100 μM) treated with the Griess reagent.

In vivo experiments on NO imaging. HeLa cells (Riken BioResource Center) were cultured in high glucose DME medium (Sigma-Aldrich) containing 10 %(v/v) FBS, 1 %(v/v) Penicillin-Streptomycin (P/S) (Sigma-Aldrich) at 37 °C in a CO_2 incubator (5% CO_2). Then, the HeLa cells were seeded at a density of 1.5×10^5 cells/mL in DME medium in a 35 mm glass bottom dish. After 24 hours of culture, the cells were incubated with 10 μM NO_{550} for 30 min in a CO_2 incubator (5 % CO_2) at 37 °C. After the incubation, the cells were washed thrice with PBS buffer and incubated with 30 μM compound **5** or 30 μM NOC7 (DOJINDO) in DME medium for 30 min. After 30 min of the incubation, the DME medium was removed and the cells were washed thrice with PBS buffer. The cells incubated with **5** were exposed to the light in the wavelength of 400–500 nm for 1 hour to liberate NO and were then washed thrice with PBS buffer. The fluorescence derived from NO_{550} in the cells was then analyzed using a fluorescence microscope (BZ-X700; KEYENCE) equipped with BZ-X filter GFP and a 40x objective lens with the exposure time of 1 second. The fluorescence images are shown in Figure S3.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publication website at DOI: The detailed photosensitized isomerization data, CV data, in vivo NO imaging and copies of ^1H, ^{13}C, and ^{19}F NMR spectra of all new compounds (PDF).

AUTHOR INFORMATOION

Corresponding Author
*E-mail: matsubara.ryosuke@people.kobe-u.ac.jp
ORCID
Ryosuke Matsubara: 0000-0001-8941-2391

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Prof. Atsunori Mori and Prof. Kentaro Okano for their help on mass analysis. We also thank Prof. Yasuhiro Kobori, Prof. Takashi Tachikawa and Mr. Fumitoshi Ema for their help on phosphorescence analysis. This work was financially supported by JSPS KAKENHI Grant Numbers JP16K18844 and JP17J00025, Futaba Electronics Memorial Foundation, Suzuken Memorial Foundation, Inamori Foundation, and Daiichi Sankyo Foundation of Life Science. CPS wishes to thank the MEXT for the Japanese Government Scholarship Program.

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