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Multiple bond-conjugated photoinduced nitric oxide releaser working with two-photon excitation

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ABSTRACT

Four novel nitric oxide (NO) releasers working via two-photon excitation (TPE), based on an acceptor–donor–acceptor (A–D–A) molecular design, were synthesized. Their decomposition and NO release in response to one-photon excitation, and their decomposition in response to two-photon excitation were examined. Their photoinduced decomposition characteristics are discussed.

Nitric oxide (NO) is an endogenous endothelium-derived relaxing factor (EDRF), and plays many important biological roles in mammals, including regulation of blood pressure, neuromodulation, and biodefense.[1] However, research into the functions of NO is difficult, because NO is an unstable free radical. To overcome this difficulty, many NO releasers have been developed,[2] but most of them release NO by spontaneous decomposition, so that it is difficult to control NO release precisely. Controllable NO releasers, which would permit temporal and spatial control of NO release, would be candidates for superior physiologically active reagents and therapeutic agents. To synthesize such compounds, we adopted photo-irradiation as the trigger for NO release.

We previously reported photoinduced NO release from 6-nitrobenzo[*a*]pyrene (6-nitroBaP)[3] via isomerization of the nitro group to nitrite ester, followed by homolytic cleavage. In the case of 6-nitroBaP, the isomerization reaction is easy, since the nitro group is in a non-planar relationship to the pyrene group due to the hydrogen atoms at the two *peri*-positions. We also found photoinduced NO release from 4-substituted-2,6-dimethylnitrobenzenes.[4] In these nitrobenzenes, the nitro group is also non-planar with respect to the benzene ring due to the steric effect of the two methyl groups at the *ortho*-positions, and isomerization of the nitro group can occur easily by photoactivation.

However, the maximum absorption band for NO release from 2,6-dimethylnitrobenzenes is in the UV–A range, which could be harmful to living cells. To overcome this potential problem, we adopted the two-photon excitation (TPE) technique, which offers the advantages not only of excitation at longer wavelength, but also high spatial resolution. Though several NO releasers utilizing TPE have been reported,[5] those molecules contain a nitrosyl-chelated transition metal ion, which is potentially harmful to living cells and is usually metabolically unstable. We recently reported the first TPE-active NO releaser without a nitroxyl moiety, which was designed to contain the fluorescein group as the two-photon absorbing moiety.[6] Here, we report new TPE-active NO releasers based on a different molecular design.

We designed four NO releasers based on acceptor–donor–acceptor (A–D–A) structure (Fig. 1), which is well known to be suitable for TPE.[7] In these molecules, 2,6-dimethylnitrobenzene moieties work as acceptors and NO releasing moieties, and 1,4-dimethoxybenzene moieties as receptors. The two acceptor–donor pairs were connected with a conjugated multiple bond linker. **TB1-DNB** and **TB2-DNB** contain one and two triple bonds, respectively. **DB1-DNB** and **DB2-DNB** contain one and two double bonds, respectively.

The preparation of phosphate ester **5** is shown in Scheme 1. To obtain carboxylic acid **2**, 2-nitromesitylene **1** was oxidized with chromium(VI) oxide. Reduction of **2** with sodium tetrahydroborate gave alcohol **3**. The hydroxyl group of **3** was brominated to give **4**,

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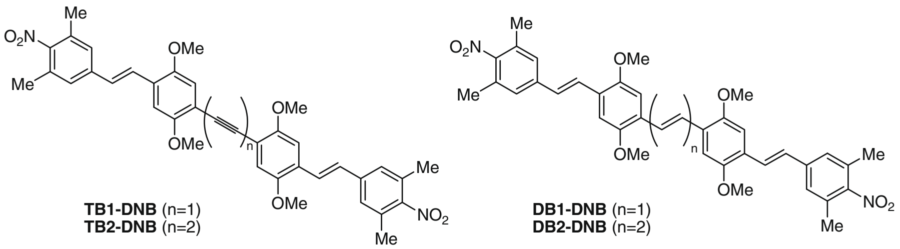
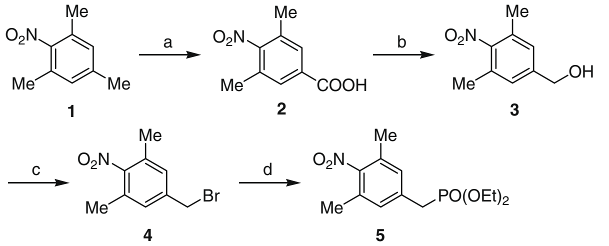


Figure 1. Chemical structures of 2,6-dimethylnitrobenzene derivatives designed and synthesized as TPE-mediated NO releasers.



Scheme 1. Reagents and conditions: (a) CrO_3, AcOH; (b) $NaBH_4$, BF_3 etherate; (c) PBr_3; (d) $P(OEt)_3$, $(n\text{-}Bu)_4NI$, 120 °C.

which was converted into phosphate ester **5** by means of the Arbuzov reaction.

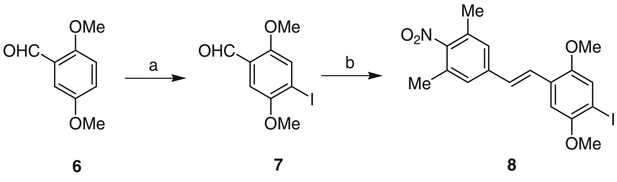
Synthesis of the intermediate **8** is shown in Scheme 2. Iodination of 2,5-dimethoxybenzaldehyde **6** gave iodide **7**. Conversion of aldehyde **7** into alkene **8** was achieved via Horner–Wadsworth–Emmons reaction with **5**.

Preparation of the intermediates **11** and **12** is shown in Scheme 3. Iodide **7** was converted into alkyne **9** via Sonogashira coupling. The TMS group of **9** was removed by hydrolysis with aqueous NaOH solution to give **10**, which was converted into **11** by means of Horner–Wadsworth–Emmons reaction with **5**. Treatment of alkyne **11** with Schwartz reagent and pinacolborane gave borate **12**.

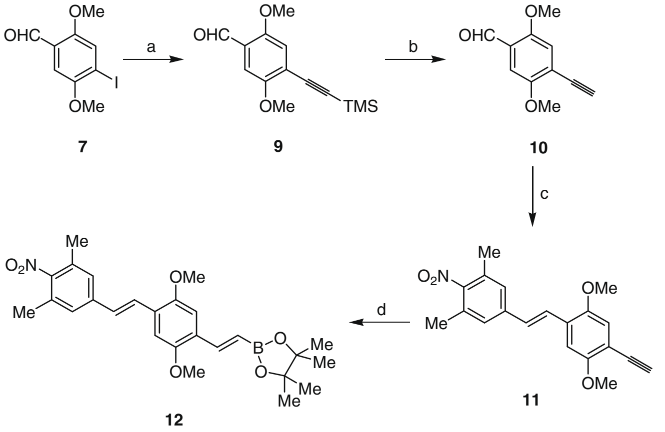
Intermediate **15** was prepared as shown in Scheme 4. After conversion of iodide **7** into alkene **13** via the Heck reaction, **13** was treated with *N*-iodosuccinimide to give iodide **14**. Aldehyde **14** was converted into **15** by means of Horner–Wadsworth–Emmons reaction with **5**.

Preparation of **TB1-DNB**, **TB2-DNB**, **DB1-DNB**, and **DB2-DNB** is shown in Scheme 5. **TB1-DNB** was prepared from iodide **8** and alkyne **11** via Sonogashira coupling. **TB2-DNB** was synthesized from two molecules of **11** by the use of Glaser coupling. **DB1-DNB** was prepared from **8** and **12**, and **DB2-DNB** was prepared from **12** and **15**, both via Suzuki–Miyaura coupling.

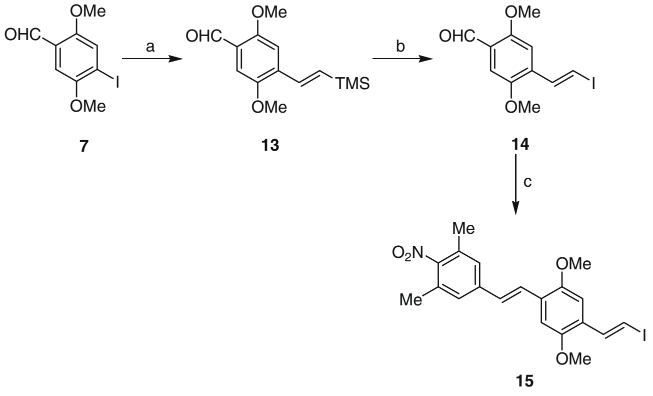
First, we measured the absorption spectra of the four compounds in DMSO solution (Fig. 2). The absorption maximum wavelengths of **TB1-DNB** and **TB2-DNB** were shorter than those of **DB1-DNB** and **DB2-DNB**, probably because *sp*^{2} carbons in the linker double bond of the latter compounds are more efficiently conjugated with *sp*^{2} carbons in benzene rings, as compared with the *sp* carbons in the former compounds (Table 1).



Scheme 2. Reagents: (a) I_2, $AgNO_3$, MeOH; (b) **5**, NaH, THF.

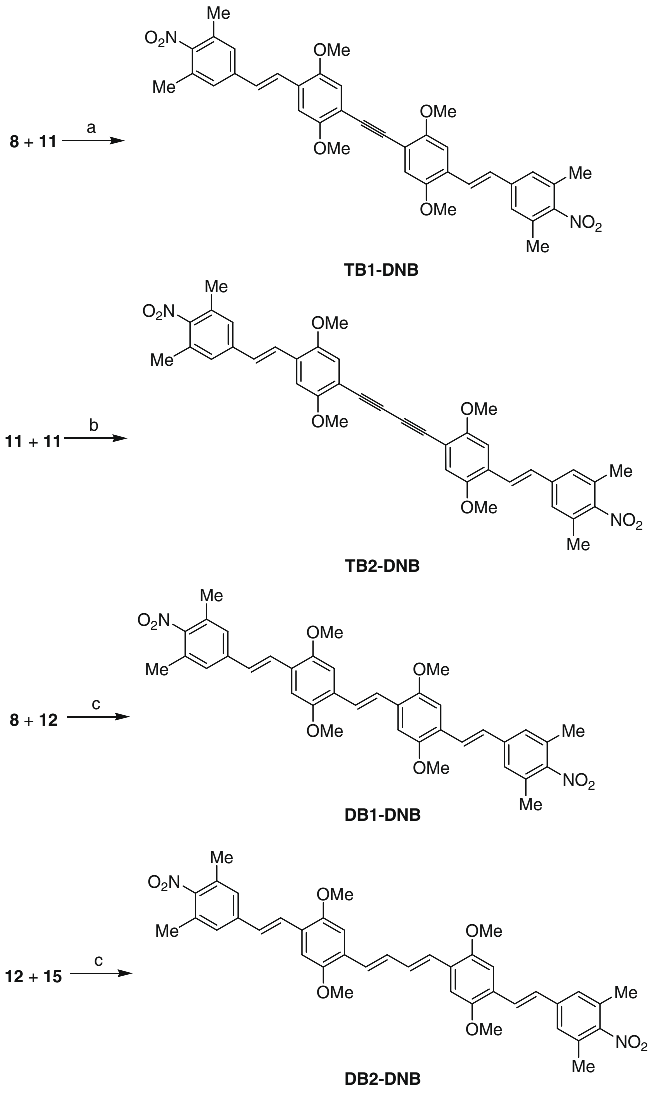


Scheme 3. Reagents: (a) trimethylsilylacetylene, $PdCl_2$, PPh_3, CuI, piperidine; (b) 2 N-NaOH aq, MeOH, $CHCl_3$; (c) **5**, NaH, THF; (d) pinacolborane, Cp_2ZrHCl, CH_2Cl_2.



Scheme 4. Reagents: (a) vinyltrimethylsilane, $Pd(OAc)_2$, PPh_3, $AgNO_3$, Et_3N, MeCN; (b) *N*-iodosuccimide, MeCN; (c) **5**, NaH, THF.

To investigate NO release from the four compounds through the single photon excitation pathway, DMSO solutions of **TB1-DNB**, **TB2-DNB**, **DB1-DNB,** and **DB2-DNB** were irradiated with 400–440 nm or 450–480 nm light (from a 100 W Hg lamp with suitable band pass filters) in the presence of Fe–DETC complex, which traps NO to yield an Fe–DETC–NO complex that shows triplet signals at around 330 mT in 4 GHz ESR spectrometry. After irradiation, the ESR spectra of the solutions showed typical triplet signals due to the Fe–DETC–NO complex, suggesting that all of the compounds released NO upon photo-irradiation (data are shown in Supplementary data). The amounts of NO released from the compounds were evaluated from the ESR spectra (Table 2). In the case of 15 min irradiation, **TB1-DNB** and **TB2-DNB** released NO in 9–10% molar ratio, but **DB1-DNB** and **DB2-DNB** released only a little



Scheme 5. Reagents: (a) $Pd(PPh_3)_4$, CuI, $AgNO_3$, Et_3N, THF; (b) $Cu(OAc)_2$, THF–pyridine; (c) $PdCl_2(PPh_3)_2$, NaOEt, toluene.

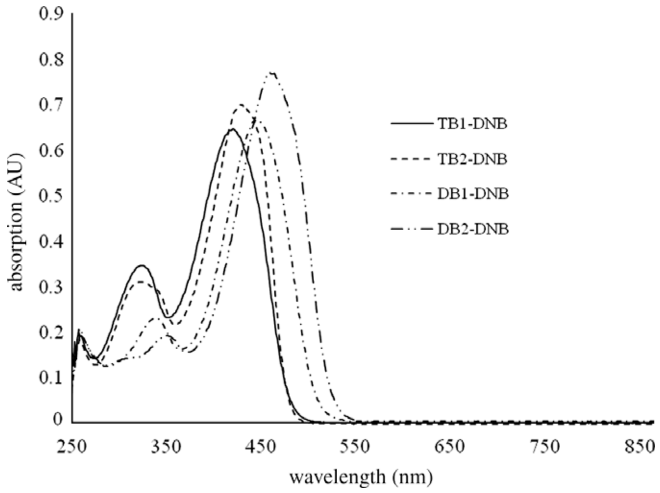


Figure 2. UV–vis spectra of **TB1-DNB**, **TB2-DNB**, **DB1-DNB**, and **DB2-DNB**. Concentrations of all compounds are 10 μM in DMSO.

Table 1
Absorption maximum wavelength of the four compounds and molar absorbance coefficient at this wavelength

Compounds	Absorption maximum wavelength (nm)	Molar absorbance efficient ($L\,mol^{-1}\,cm^{-1}$)
TB1-DNB	324, 421	3.5×10^4, 6.5×10^4
TB2-DNB	323, 430	3.1×10^4, 7.0×10^4
DB1-DNB	339, 445	2.3×10^4, 6.7×10^4
DB2-DNB	352, 459	1.9×10^4, 7.7×10^4

Table 2
Comparison of released NO

Compounds	Amount of released NO (μM)	Rate of released NO to concentration of the compound[c] (%)
TB1-DNB	23.9[a]	9.2[a]
TB2-DNB	24.1[a]	9.7[a]
DB1-DNB	2.61[a], 2.13[b]	1.0[a], 0.86[b]
DB2-DNB	2.40[a], 1.36[b]	0.96[a], 0.54[b]

[a] Photoirradiation wavelength is 400–440 nm.
[b] Photoirradiation wavelength is 450–480 nm.
[c] Rate at 250 μM donor compound.

NO. According to the UV–vis spectra, the absorption coefficients of **DB1-DNB** and **DB2-DNB** are larger than those of **TB1-DNB** or **TB2-DNB** in this irradiation range. Low efficiency of NO release from **DB1-DNB** and **DB2-DNB** might be due to insufficient excitation energy to isomerize the nitro group to nitrite ester in the irradiation range of 450–480 nm. It is considered that photo isomerization of the dimethylnitrobenzene moiety would require 440 nm or shorter wavelength photoirradiation.

We next examined TPE-based decomposition of the four compounds. DMSO solutions of **TB1-DNB** and **TB2-DNB** were irradiated at 800 nm to 860 nm and DMSO solutions of **DB1-DNB** and **DB2-DNB** were irradiated at 800 nm to 880 nm with a pulse laser (Tsunami; Spectra-Physics), and the products were analyzed by HPLC. In this wavelength range, none of the four compounds showed conventional single photon absorption. A parameter of two-photon decomposition efficiency, the two-photon uncaging cross section (δ_u)[8], was calculated for each compound, and the results are shown in Figure 3. It was found that **TB2-DNB** was decomposed to the greatest extent among the four compounds. Although there is little difference between **TB1-DNB** and **TB2-DNB** in one-photon excita-

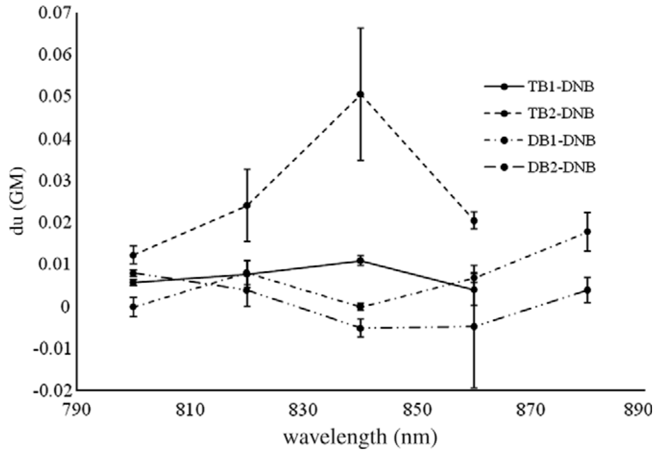


Figure 3. Two-photon uncaging cross section (δ_u) spectra of the four compounds.

Figure 4. ESR spectra of [$(DETC)_2$–Fe–NO] complex after 840 nm pulse laser irradiation in the presence of **TB2-DNB**. Samples contained 250 μM **TB2-DNB**, 8 mM DETC, and 2 mM $FeSO_4$ in DMSO; ESR spectra were recorded after photoirradiation for 8 min with a modulation width of 0.5 G and a microwave power of 10 mW.

tion, there is a marked difference in TPE. It seems that **TB2-DNB** collects photons more efficiently than **TB1-DNB** because of its larger conjugation area.

We also demonstrated TPE-based NO release from **TB2-DNB** which is the most decomposed compound by TPE. A DMSO solution of **TB2-DNB** with Fe–DETC complex was irradiated 840 nm pulse laser and the solution was measured by ESR. The ESR spectra of the solution showed typical triplet signals assigned as the Fe–DETC–NO complex, suggesting that **TB2-DNB** released NO by TPE (Fig. 4).

In conclusion, we demonstrated photoinduced NO generation from novel 2,6-dimethylnitrobenzene derivatives and photoinduced decomposition via TPE. Among these compounds, **TB1-DNB** and **TB2-DNB**, containing triple bonds, were more extensively decomposed than double bond-containing **DB1-DNB** and **DB2-DNB**. **TB2-DNB** is particularly well decomposed via the TPE process, which is more efficient than in the case of **TB1-DNB**.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.120.

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