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A double bond-conjugated dimethylnitrobenzene-type photolabile nitric oxide donor with improved two-photon cross section

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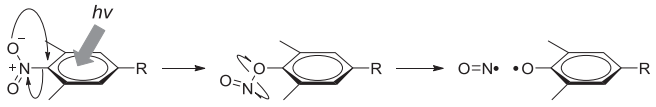
ABSTRACT

Photocontrollable NO donors enable precise spatiotemporal release of NO under physiological conditions. We designed and synthesized a novel dimethylnitrobenzene-type NO donor, Flu-DNB-DB, which contains a carbon–carbon double bond in place of the amide bond of previously reported Flu-DNB. Flu-DNB-DB releases NO in response to one-photon activation in the blue wavelength region, and shows a greatly increased two-photon cross-section (δ_u) at 720 nm (Flu-DNB: 0.12 GM, Flu-DNB-DB: 0.98 GM). We show that Flu-DNB-DB enables precisely controlled intracellular release of NO in response to 950 nm pulse laser irradiation for as little as 1 s. This near-infrared-light-controllable NO source should be a valuable tool for studies on the biological roles of NO.

Nitric oxide (NO) is endogenously synthesized by nitric oxide synthase (NOS) and is a key regulator of various physiological signaling events, serving as an endothelial derived relaxing factor (EDRF),[1] a neurotransmitter,[2] and an immune regulator.[3] Because NO is a gaseous molecule and is unstable under physiological conditions, many NO donors that can store and release NO have been developed for biological NO research.[4] Among them, photocontrollable NO donors can be used to release NO with high spatiotemporal precision, so they are particularly suitable for studies of the physiological actions of NO. For photocontrolled release, light in the visible to near-infrared range is preferable due to its low cytotoxicity and high tissue penetration, whereas ultraviolet light (UV) has higher cytotoxicity and lower penetration. For this reason, photocontrollable NO donors activatable by two-photon excitation (TPE) have been developed.[5] One-photon excitation involves excitation of a dye from the ground state to an excited state by absorption of a single photon. However, the same excitation process can be mediated by absorption of two photons (typically in the near-infrared range) at a sufficiently high photon density, typically generated with a femtosecond (fs)-pulse laser. We have shown that the 2,6-dimethylnitrobenzene (DNB) scaffold is available for photocontrollable NO donors triggered by UV–visible light (Scheme 1),[6] and we developed DNB tethered to fluorescein (Flu-DNB (**1**, Fig. 1)) as a NO donor for TPE. Although Flu-DNB worked as a TPE-activatable NO donor in vivo, the value of two-photon uncaging cross section (δ_u), a measure of the photodecomposition efficiency in response to TPE, was smaller than that of Bhc-Glu, a photocontrollable glutamate activated by TPE (for Flu-DNB: 0.12 GM, for Bhc-OAc: 0.95 GM).[7] A large value of δ_u is preferable for biological research to avoid the need for high donor concentrations and high irradiation doses.



Scheme 1. Proposed mechanism of photoinduced NO release from 2,6-dimethylnitrobenzene.

To improve the performance of Flu-DNB, we focused on the amide bond. We expected that replacement of this bond with a carbon–carbon double bond would increase the absorption coefficient due to more efficient conjugation, and provide a larger two-photon cross-section. The designed compound, named Flu-DNB-DB (**2**, Fig. 1), was synthesized as shown in Scheme 2. In brief, 4-bromobenzaldehyde (**3**) was coupled with trimethylsilylacetylene to form benzaldehyde derivative **4** which was converted

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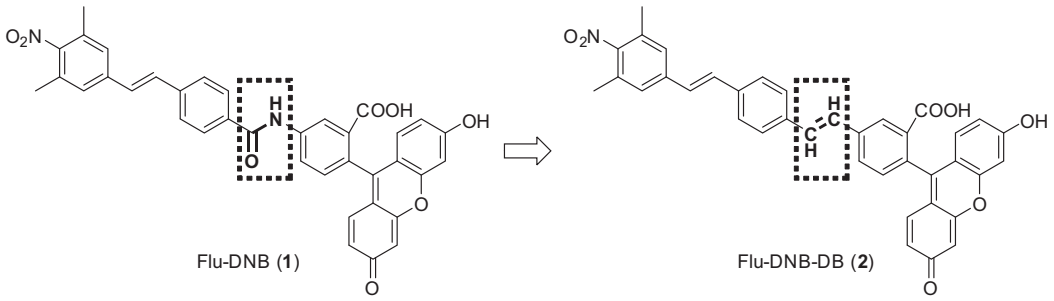
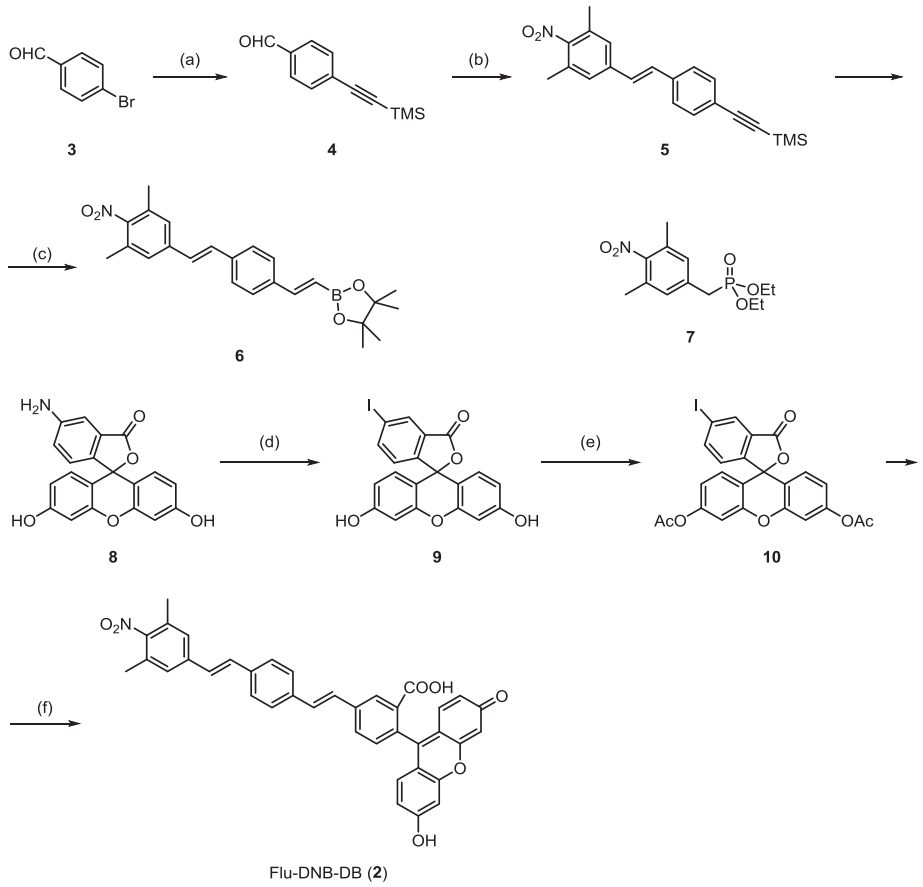


Figure 1. Structures of Flu-DNB (**1**) and the newly designed NO donor, Flu-DNB-DB (**2**).



Scheme 2. Synthesis of Flu-DNB-DB (**2**). Reagents and conditions: (a) CuI, $PdCl_2$, PPh_3, NEt_3, trimethylsilylacetylene, THF, rt, 65%; (b) **7**, NaH, THF, 0 °C, then, **4**, THF, 0 °C → rt, quant.; (c) 4,4,5,5-tetramethyl-1,3,2-dioxaborolane, Cp_2ZrHCl, CH_2Cl_2, 44%; (d) $NaNO_2$, HCl, 0 °C, then KI, 0 °C; 75%; (e) Ac_2O, DMAP, pyridine, rt, 84%; (f) **6**, $Pd(PPh_3)_4$, CsF, DME, 53%.

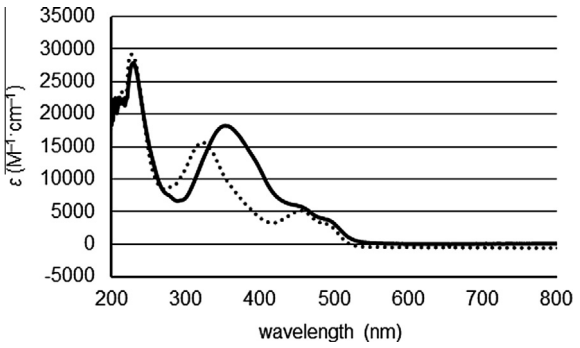


Figure 2. Absorption spectra of Flu-DNB (dashed line) and Flu-DNB-DB (solid line). The concentration was 10 μM in water containing 0.1% DMSO.

to nitrostilbene derivative **5** by Horner–Wadsworth–Emmons reaction with phosphonate **7**. The alkyne moiety was changed to borate **6** by using Cp_2ZrHCl. Commercially available aminofluorescein **8** was converted to iodofluorescein **9**, which was acetylated to afford **10**. Finally, Flu-DNB-DB (**2**) was synthesized by Suzuki–Miyaura cross coupling of **6** and **10**. Its structure and purity were confirmed by ^1H NMR, ^{13}C NMR, mass spectrometry, and elemental analysis.

In the absorption spectrum of Flu-DNB-DB (Fig. 2), the absorption maximum around 300–400 nm (λ_{max} = 359 nm, $\varepsilon = 1.8 \times 10^4\ M^{-1}\ cm^{-1}$) was red-shifted in comparison with that of Flu-DNB (λ_{max} = 322 nm, $\varepsilon = 1.6 \times 10^4\ M^{-1}\ cm^{-1}$). This shift is probably a result of the extended conjugation due to conversion of the amide bond of Flu-DNB to the carbon–carbon double bond.