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# Mild Chemotriggered Generation of a Fluorophore-Tethered Diazoalkane Species via Smiles Rearrangement

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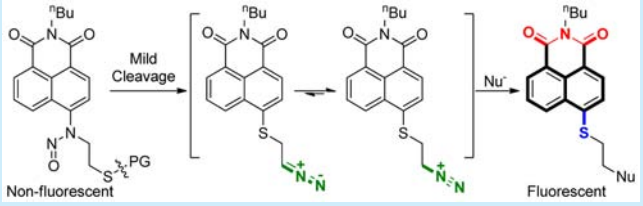
Supporting Information

**ABSTRACT:** In situ generation of diazoalkanes under mild conditions is desired. A mechanism based on a Smiles rearrangement has been devised that releases a fluorophore-labeled unstabilized diazoalkane in the presence of various chemical triggers. Notably, the release of this diazoalkane is accompanied by an intense fluorescence turn-on, which calibrates the release of the diazoalkane. Carboxylic acids can trap this short-lived diazoalkane intermediate and yield the corresponding esters. This transformation has potential for broad applications.




Diazoalkanes exhibit stunningly rich reactivity and are cherished in all branches of chemistry, toxicology, and chemical biology.[1] They are nucleophilic and attack aldehydes, ketones, acyl halides, sulfur, etc.[2] They are 1,3-dipolar and undergo cycloadditions with alkenes and alkynes.[3] They are protonated to generate alkyldiazoniums or lose $N_2$ upon photolysis to give singlet carbenes.[4] They also coordinate to transition metals to form metal−carbene species, whose chemistry has also been extensively explored.[5] Diazoalkanes with an adjacent electron-withdrawing group possess higher stability and are easily handled. They may be routinely prepared via diazotization[6] with a $NO^+$ donor, e.g., $NaNO_2$/HOAc or an organic nitrite, and Regitz diazotransfer[7] from tosyl azide to a cabanion. A recent preparation of diazoalkanes by Myers and Raines[8] involves partial reduction of an azido species by a rationally designed phosphine. In comparison, unstabilized diazoalkanes are not conveniently exploited in practical applications because of their tendency to explode during preparation/handling, their ready decomposition during storage, and their acute/chronic pathological hazards upon inhalation.

The difficulties and hazards associated with the unstabilized diazoalkanes may be circumvented by in situ generation and immediate consumption, as has been showcased in cyclopropanation of olefins by Morandi and Carreira[9] and in esterification of carboxylic acids by Maggini and co-workers.[10] However, the scope of this approach is limited since diazoalkanes are typically prepared by decompositions of acylated/alkylated nitrosoamines or tosylhydrozone under strongly basic conditions (e.g., 6 M KOH), with which not many functional groups are compatible (Figure 1A).[11] Therefore, the development of alternative preparations of unstabilized diazoalkanes under mild conditions is an ongoing research interest. Recent progresses are Swern oxidation of a hydrazine in THF[12] and (difluoroiodo)benzene-mediated oxidation of TBDMS-substituted hydrazones in $CH_2Cl_2$ (Figure 1B).[13] In light of the potential implications of diazoalkanes in mutagenesis[1b] and carcinogenesis and recent interest in their use in bioconjugation,[14] mild preparations of diazoalkanes in neutral aqueous systems[15] are desired. We report herein a novel mechanism that allows triggered generation of a fluorophore-labeled unstabilized diazoalkane in neutral phosphate buffer. Notably, this process is accompanied by a fluorescence turn-on for convenient spectroscopic investigations.

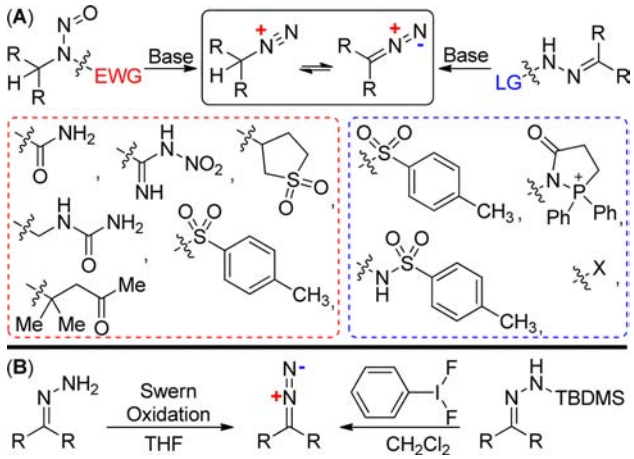



**Figure 1.** Approaches for generation of unstabilized diazoalkanes and the corresponding alkyldiazonium species.

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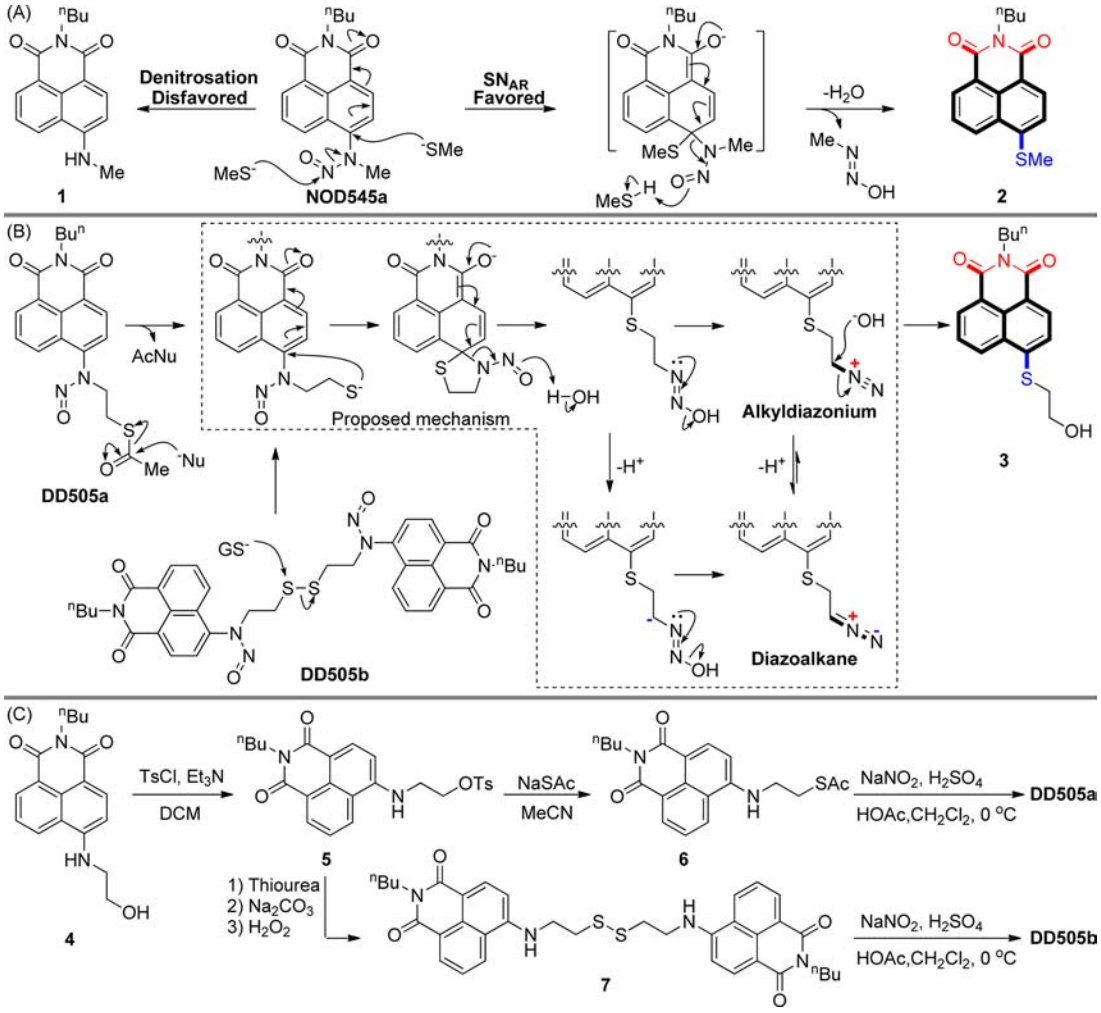


**Figure 2.** (A) Formation of fluorescent **2** from the reaction of **NOD545a** with $MeS^-$. (B) Proposed mechanism of chemotriggered release of an alkyldiazonium/diazoalkane species from **DD505a,b** in aqueous media. (C) Synthesis of **DD505a,b**.

This work has evolved from the chemostability study of **NOD545**s,[16] a class of novel phototriggered and photocalibrated NO donors from our group, against thiol-mediated transnitrosation.[17] Unexpectedly, a blue-emitting species, which was identified to be **2**, rather than the green-emitting compound **1**, was gradually generated upon treatment with $MeS^-$ (~5 M) (Figure 2A). Mechanistically, the formation of **2** suggests that a nucleophilic aromatic substitution ($S_NAr$) of methyl nitrosamine (Me−N═N−OH) on an electron-deficient naphthalimide scaffold by $MeS^-$ occurred. Since methyl nitrosamine is a precursor of diazomethane,[11] this transformation constitutes a novel thiol-triggered release of diazoalkane in aqueous media with a concomitant fluorescence turn-on

The implications of this transformation may be greatly promoted if it could occur at a much reduced level of thiol. We resorted to the principle of *proximity-based catalysis*,[18] i.e. rendering an intermolecular reaction intramolecular, to achieve this goal, and designed **DD505a,b**, the thiols of which are differently protected and hang in close proximity to the electrophilic position of the naphthalimide core (Figure 2B). Upon facile removal of the protective group in **DD505a,b**, a sulfide is freed up and can then initiate a nucleophilic attack to release the 1° nitrosamine. As the nucleophile (sulfide) and the leaving group (1° nitrosamine) are intramolecular, this transformation is reminiscent of a classic Smiles rearrangement.[19] Compounds **DD505a,b** were readily prepared by a three-step cascade in good overall yield (Figure 2C).

The thioacetate in **DD505a** is prone to hydrolysis under basic conditions. We estimate that the $pK_a$ of this thiol is ca. 10 on the basis of the $pK_a$'s of *N*-acetylcysteine (9.52) and cystamine (10.75). Therefore, a carbonate buffer (50 mM) at pH 10.0 was chosen to hydrolyze the thioacetate of **DD505a**. **DD505a** absorbs maximally at 350 nm with a molar absorptivity of 15 200 $cm^{-1}$ $M^{-1}$ and is nonfluorescent. Upon addition of **DD505a** (10 $\mu$M) to the aforementioned carbonate buffer at pH 10, the UV−vis absorption and fluorescence spectra of the resulting solution were immediately collected every 10 min. The absorption band of **DD505a** at 350 nm decreased and a new absorption band at 400 nm increased with an isosbestic point at 370 nm, suggesting a clean one-to-one-type conversion (Figure 3A). The concomitant fluorescence enhancement with a maximum emission intensity at 505 nm was also followed with excitation at 400 nm (Figure 3A). The identity of this fluorescent product was unambiguously identified to be **3** using UV−vis, fluorescence, and $^1H$ NMR spectroscopies and mass spectrometry (Figures S7−S9). This transformation was complete in ca. 6 h (Figure 3B), and by the Beer−Lambert law **3** was estimated to be generated in 92% yield upon hydrolysis of **DD505a** at pH 10 in carbonate buffer.



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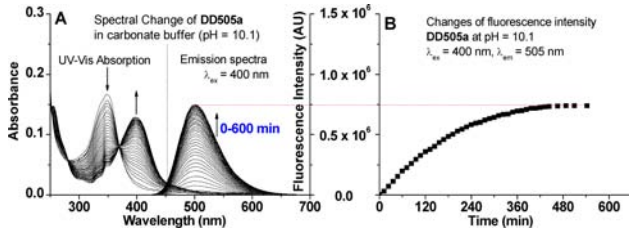


**Figure 3.** (A) UV−vis absorption and fluorescence emission spectral changes of **DD505a** in carbonate buffer (160 mM, pH 10.1). (B) Corresponding fluorescence enhancement at 505 nm, with excitation at 400 nm, as a function of reaction time.

Higher pH promotes the hydrolysis of the thioester and release of the diazoalkane species (Figure S1A−D), and vice versa (Figure S1E,F). Alternatively, sulfide (e.g., $CH_3S^-$) can also trigger this transformation (Figure S1G,H). The thiol in **DD505b** is protected in the form of a disulfide, which is labile toward disulfide exchange with a variety of biological substrates, including glutathione (GSH).[20] **DD500b** (5 $\mu$M) was dissolved in neutral phosphate buffer (50 mM, pH 7.4) containing 10% DMSO, and GSH (5 mM) was added in one portion. The characteristic absorption and emission from **3** was observed (Figure S2).

The thioacetate of **DD505a** can also be deprotected with dimethylamine ($HNMe_2$) in organic solvents. Stirring $HNMe_2$ (18 equiv in dry THF) with **DD505a** solution (500 mg) in $CH_3CN$ at room temperature for 30 min yielded compound **8** in a ca. 67% yield. A longer reaction time is needed if a lower dose of $HNMe_2$ is used. When **DD505a** (150 mg) was dissolved in $CH_3CN$ with various carboxylic acids (3 equiv) before $HNMe_2$ (18 equiv) was added, the reactive diazomethane intermediate could also be trapped by various aliphatic and aromatic carboxylic acids (Figure 4). The desired esters **9** were isolated in yields of 12−45% along with **8** in a decreased yield of ∼40%. Generally, esters from aliphatic carboxylic acids (**9a**−**f**) were obtained in comparatively high yields of 30−40%, with the exception of 1-adamantanecarboxylic acid, whose ester **9f** was obtained in 18% yield, presumably because of steric shielding. The yields of aromatic esters (**9g**−**p**) showed a strong dependence on the pattern of substitution on the aryl ring. It seems that the less acidic aromatic carboxylic acids are more reactive toward esterification. Ones with strong electron-withdrawing groups or ortho-substituted ones are not as reactive with the diazoalkane intermediate. The reaction is also feasible in organic solvents including THF, diethyl ether, dioxane, $CH_2Cl_2$, EtOAc, etc.

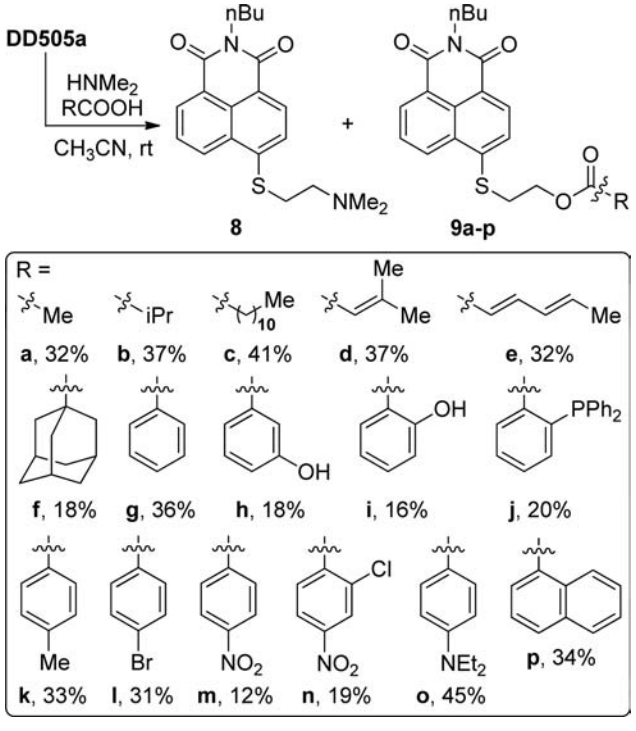


**Figure 4.** Diazoalkane species derived upon reaction of dimethylamine with **DD505a** can be trapped by various carboxylic acids in $CH_3CN$.

Mechanistically, the thiolate from cleavage of either the thioester of **DD505a** or the disulfide of **DD505b** can potentially denitrosate the nitrosamine. However, this transformation was not observed during our studies with **DD505a,b**. This can be rationalized. First of all, it is recognized that among two resonance structures of nitrosamine, diazenium oxide is the major contributing one (Figure 5A). The coexistence of cis and trans isomers in the NMR spectrum of this type of compound, including **DD505a,b**, supports this notion. With this resonance structure, it is immediately obvious that the denitrosation pathway is a disfavored 5-endo-trig-type attack, according to Baldwin's rule, while the Smiles rearrangement pathway is a favored 5-exo-trig-type attack (Figure 5B).[21] We also obtained the crystal structure of **DD505a,** which provides additional insights (Figures 5C and S3). The coplanarity of atoms of C10, N2, C13, N3, and O3 supports the presence of double-bond character between the two nitrogen atoms. Also, a dihedral angle of as large as 75.57° is measured between the naphthalimide plane and the nitrosamine plane. Such a near-orthogonal conformation brings the nucleophilic sulfur atom and the electrophilic site of the naphthalimide core in close proximity and renders the 5-exo-trig pathway entropically convenient.

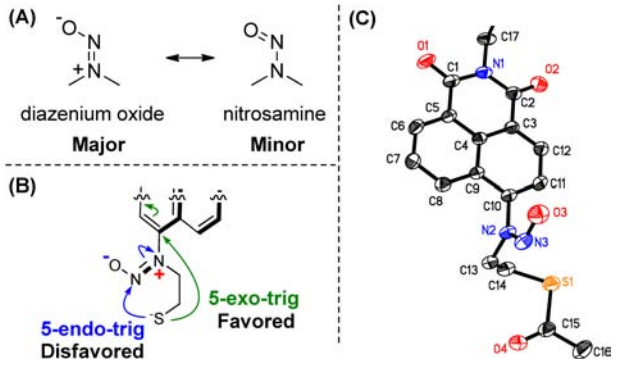


**Figure 5.** (A) The two resonance structures of a nitrosamine. (B) The two potential nucleophilic attack pathways upon cleavage of the thiol protection in **DD505a,b**. (C) Crystal structure of **DD505a** with the *n*-butyl chain partially omitted.

In conclusion, we have devised a novel mechanism to allow chemotriggered release of an unstabilized diazoalkane from **DD505a,b**, which process is accompanied by a strong fluorescence turn-on. Depending on the nature of the protective groups, the tested chemical triggers include hydroxide, methyl sulfide, glutathione, and dimethylamine. We emphasize that the scope of the trigger is not limited to these few nucleophiles. Since both thioacetate and disulfide are


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biological, **DD505a,b** may be enzymatically triggered. Additionally, in conjunction with a "releasable linker", the diverse protective chemistry of hydroxyl and amino groups, which has been routinely employed in the construction of prodrugs, may also be exploited.[22] Moreover, this process is feasible not only in organic solvents but also in different aqueous buffers of various pH. The high specificity of the decomposition pathways of **DD505a,b** is explained well by Baldwin's rules. The chemistry reported herein has broad implications in an array of applications.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02303.

General experimental methods, syntheses and characterizations, and additional spectral studies (PDF)
Crystallographic data for **DD505a** (CIF)

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### Notes

The authors declare no competing financial interest.

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