Alternative photoinduced release of HNO or NO from an acyl nitroso compound, depending on environmental polarity†

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A hydrophilic hetero-Diels–Alder cycloadduct was synthesized as a novel photocontrollable donor of reactive nitrogen species. Production of either nitric oxide (NO) or nitroxyl (HNO) was photoinduced from this compound, depending on the environmental polarity.

Nitric oxide (NO) is one of the most familiar reactive nitrogen species (RNS). In the 1980s, NO was found to be biologically synthesized in mammalian systems,[1] and since then both the chemical and biological properties of NO have been extensively investigated. Recent studies have shown that nitroxyl (HNO),[2] a one-electron-reduced form of NO, has many pharmacological properties, including positive inotropy,[3] vasodilation,[4] and cardioprotection.[5] HNO exerts these effects *via* mechanisms different from those of NO, and is a promising candidate for treatment of heart failure.[6] However, HNO has varied chemical properties, including dimerization (N_2O formation),[7] high pK_a value,[8] high thiophilicity,[9] reductive nitrosylation,[10] and unique reactivity with phosphines.[11] So, to elucidate the biological potential of HNO in detail, novel HNO donors which can release HNO under precise temporal and spatial control are needed.

King *et al.* reported that cycloadducts of *N*-hydroxyurea derivatives and 9,10-dimethylanthracene (DMA) can act as spontaneous HNO donors, though DMA itself is toxic.[12] We applied this basic reactivity to design photoinducible HNO donors such as compound **A**, which can release HNO in response to UV-A irradiation, although our donors have poor hydrophilicity.[13] To improve the hydrophilic character, we designed and synthesized compound **B**, which consists of a hydrophilic anthracene derivative[14] and *N*-hydroxyurea derivative (Fig. 1, Scheme 1). Compound **B** was found to be well soluble (>100 μM) in buffer solution containing 0.1% DMSO. It was expected that not only would the cycloadduct be hydrophilic, but also the anthracene derivative would be more easily excreted due to the increased water solubility so that toxicity *via* metabolic bioactivation might be decreased.

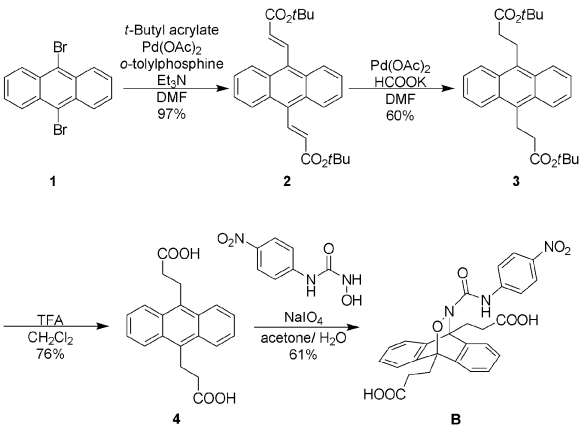
During the characterization of compound **B**, we found that the amounts of N_2O formed were almost the same level between the dark condition and photoirradiated condition (Fig. 2B), although conversion of compound **B** to the anthracene derivative and *p*-nitroaniline was very largely increased upon photoirradiation in photometric analysis (Fig. 2A). In GC-MS analysis, compound **B** yielded little N_2O compared to compound **A** in the same reaction solvent, whereas compounds **A** and **B** showed the accelerated effect on conversion by photoirradiation in photometric analysis.

Therefore, we hypothesized that compound **B** might release an RNS other than HNO in response to photoirradiation, and we thus focused on NO.

When compounds **A** and **B** were irradiated with UVA for 10 min in 90% DMSO solvent, it was found that compound **B** released NO, while compound **A** hardly did, as determined by electron paramagnetic resonance (EPR) analysis using a typical NO-selective trapping reagent, carboxy-2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO) (see ESI†)

We next examined the role of water. The NO release from compound **B** in response to photoirradiation was measured by the EPR spin trapping method in DMSO/50 mM Tris buffer (pH 7.5) with various mixing ratios. Release of NO decreased as the ratio of buffer was increased, that is, as the polarity increased, and in 1/9 DMSO/50 mM Tris buffer (pH 7.5), NO was not observed (Fig. 3) while in 9/1 DMSO/50 mM Tris buffer (pH 7.5), the amount of NO (36.4 μM) released by photoirradiation corresponded to about 90% of the conversion (see ESI†)

These observations suggested the involvement of HNO and NO releasing pathways in the photoinduced conversion of compound **B** (Scheme 2). In the HNO release pathway, it is considered that an acyl nitroso compound would be released



Scheme 1 Synthesis of compound **B**.

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† Electronic supplementary information (ESI) available: Complete ref. 6*a*. Details of the synthesis of compounds **A** and **B**, and details of their photometric, EPR, GC-MS and NMR analysis. See DOI: 10.1039/c001502d

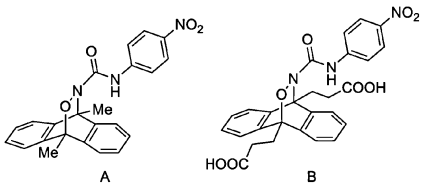


Fig. 1 Our photo-inducible HNO donors.

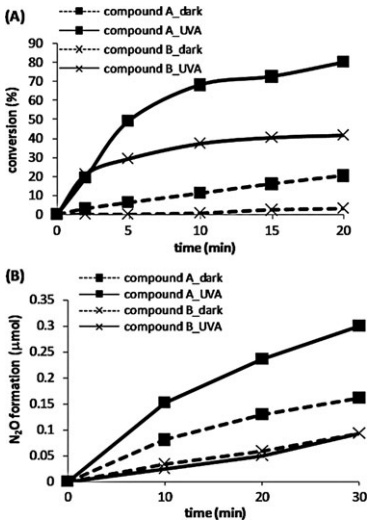


Fig. 2 (A) Conversion in photometric analysis and (B) N_2O formation in GC-MS analysis of compounds **A** and **B** in 90% DMSO solvent.

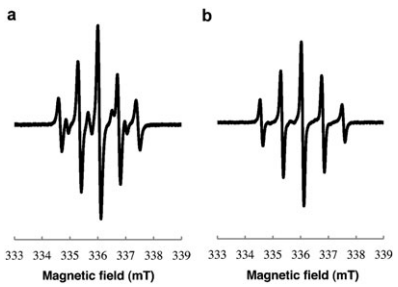
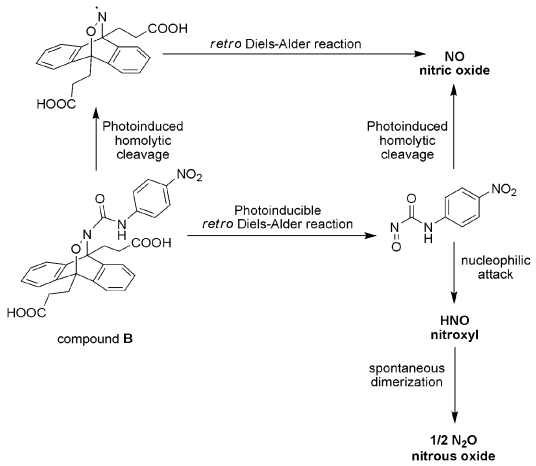


Fig. 3 Detection of NO release from compound **B** by EPR with carboxy-PTIO in (a) 9/1 DMSO/50 mM Tris buffer (pH 7.5), and (b) 1/9 DMSO/50 mM Tris buffer (pH 7.5).

by photo-triggered retro-Diels–Alder reaction, and attacked by water with release of HNO, in the same manner as in thermal decomposition. This pathway was found to occur in high-polarity solvent in our experiment.

The other pathways are concerned with NO release. One possible pathway is that an acyl nitroso compound would be released by photo-inducible retro-Diels–Alder reaction, and homolytic cleavage of the C–N bond adjacent to the carbonyl group would occur by photoirradiation before the attack of nucleophile, resulting in NO release. Another possible route is that photoirradiation induces N–C(O) bond homolysis to produce the acyl radical plus the Diels–Alder adduct of NO with the anthracene derivative. These pathways were observed in low-polarity solvent.

Next, we measured N_2O release in 1/9 DMSO/50 mM Tris buffer by means of GC-MS analysis. While the amounts of



Scheme 2 Proposed mechanisms of HNO and NO release.

N_2O in low-polarity solvent did not increase both under dark condition and under photoirradiated condition (Fig. 2B), time-dependent release of N_2O was observed in this high polarity solvent. When the amount of N_2O under photoirradiated condition was compared to that under dark condition, apparent facilitation was observed upon photoirradiation (Fig. 4 and ESI†). Therefore, compound **B** in 1/9 DMSO/Tris buffer (pH 7.5) released HNO upon UVA irradiation.

Moreover, in both cases of compounds **A** and **B**, N_2O was not detected in the presence of 2-mercaptoethanol in the reaction solvent (see ESI†) This indicates that HNO was trapped by 2-mercaptoethanol, thus precluding N_2O formation.

From these results, it was considered that compound **B** is relatively stable, and that HNO release depends at least in part on the concentration of surrounding nucleophiles, such as H_2O. When the concentration of nucleophile is sufficiently high that nucleophilic attack on the acyl nitroso compound is rapid, hydrolysis occurs before photolysis, resulting in HNO release. On the other hand, in low-polarity solvent, the acyl nitroso compound derived from compound **B** is homolytically cleaved by photoirradiation to produce NO before it can be attacked by a nucleophile. Another possible way of releasing NO from compound **B** is by homolytically cleavage followed by retro-Diels–Alder reaction.

Importantly, since this characteristic was not observed in compound **A**, there must be an additional factor, other than

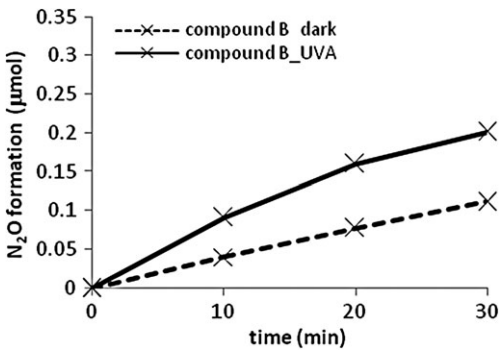


Fig. 4 GC-MS analysis of N_2O formation from compound **B** in 1/9 DMSO/Tris buffer (pH 7.5).

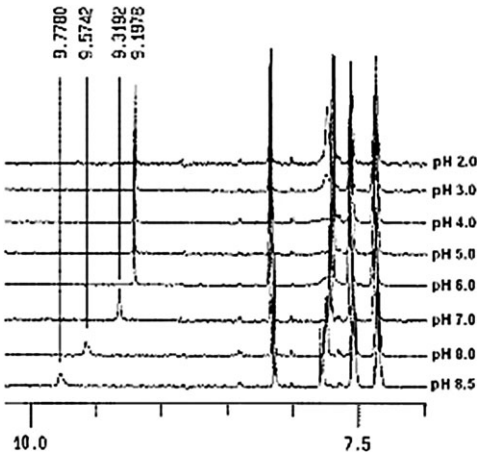


Fig. 5 Evidence of intramolecular hydrogen bonding in NMR spectra of compound **B** in 9/1 DMSO/Tris buffer at various pH values.

nucleophile concentration, which further slows the nucleophilic attack of H_2O in low-polarity solvent.

^{1}H NMR analysis indicated the possibility of intramolecular hydrogen bond formation[15] involving the NH group in compound **B** (Fig. 5). The NH signal in 9/1 DMSO/buffer was broadened and there was a downfield chemical shift at neutral to basic pH. In this pH range, the stable structure of compound **B** should be the dianionic form, and the existence of a hydrogen bond between the carboxylate and NH group was also reasonable as ascertained from the result of DFT calculation of a stable conformation with Spartan '08 (see ESI†). Furthermore, hydrogen bond formation was also strongly suggested from the dramatic chemical shift change of the NH signal in an NMR titration using tetrabutylammonium acetate (TBAOAc)[16] (see ESI†).

These results suggest that the acyl nitroso moiety and an anthracene moiety are associated through a hydrogen bond, which might serve to increase the Diels–Alder cycloadduct, contributing to the unexpected stability of compound **B** in 9/1 DMSO/Tris buffer solvent.

It is assumed that the acyl nitroso moiety would be in close proximity to the anthracene moiety after retro-Diels–Alder reaction, and this would sterically inhibit the nucleophilic attack of water, and facilitate the hetero-Diels–Alder cycloaddition. Such a proximity effect would not occur in compound **A**.

Thus, an intramolecular hydrogen bond is suggested to configure the molecular properties for photoinduced conversion, and this hydrogen bond would contribute to both the stability of compound **B** and NO production in response to UVA irradiation. A high content ratio of Tris buffer would favor solvation of the carboxylate anion, weakening the intramolecular hydrogen bond. For this reason, HNO is dominantly photo-released from compound **B** in 1/9 DMSO/Tris buffer.

Many NO donors[17] and several HNO donors[2] have been developed, but this is the first report of a photoinducible donor that can release either HNO or NO, depending on the polarity of the solvent. Further, this is the first report that an acyl nitroso compound can release NO by photodecomposition, though *N*-hydroxyurea was reported to release NO *via* an oxidation reaction.[18]

The release of HNO/NO from compound **B** is controllable by varying the UVA exposure and solvent polarity. The present findings should also be helpful to improve HNO donor design.

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