Supporting Information

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

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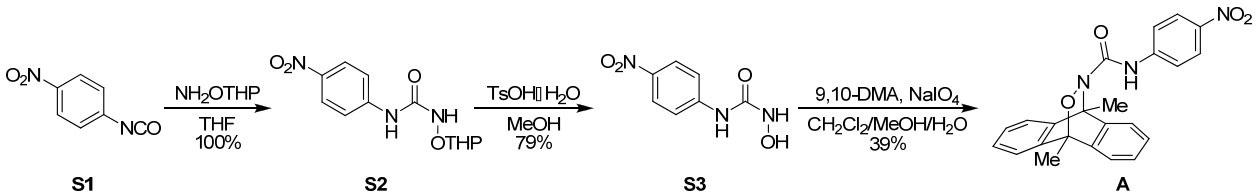
Details of ref. 6.

C. G. Tocchetti, W. Wang, J. P. Froehlich, S. Huke, M. A. Aon, G. M. Wilson, G. D. Benedetto,; B. O'Rourke, W. D. Gao, D. A. Wink, J. P. Toscano, M. Zaccolo, D. M. Ber, H. H. Valdivia, H. Cheng, D. A. Kass, and N. Paolocci, *Circ. Res.* 2007, **100**, 96-104.

<Synthesis>

General Methods. Melting points were determined using a Yanaco micro melting point apparatus or a Büchi B-545 melting point apparatus and were left uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra and carbon nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on a JEOL JNM-LA500 or JEOL JNM-A500 spectrometer in solvent as indicated. Chemical shifts (δ) are reported in parts per million (ppm) relative to the internal standard tetramethylsilane (TMS). Elemental analysis was performed with a Yanaco CHN CORDER NT-5 analyzer, and all values were within ±0.4% of the calculated values. UV/vis spectra were measured using an Agilent 8453 spectrometer. IR spectra were recorded on an Avatar360. GC-MS analyses were performed on a Shimadzu GCMS-QP2010. FAB-MS were recorded on a JEOL JMS-SX102A mass spectrometer. Reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, and Kanto Kagaku and used without further purification. Flash chromatography was performed using Silica Gel 60 (particle size 0.046–0.063 mm) supplied by Merck.

Scheme S1. Preparation of **A**.

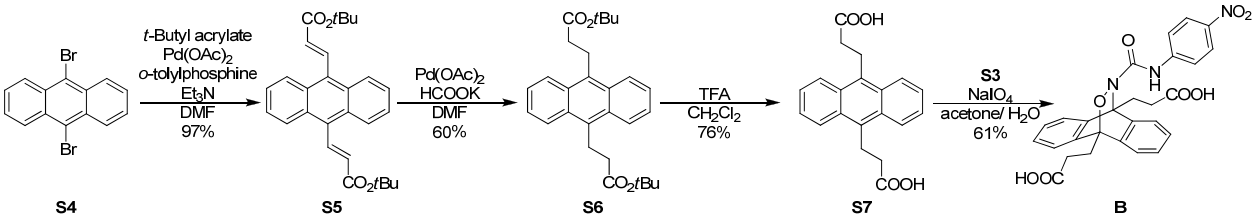


***N*-(Tetrahydropyran-2-yl)oxy-*N'*-(4-nitrophenyl)urea (S2).** To a solution of NH_2OTHP (936 mg, 8 mmol) in dehydrated THF (16 mL) was added **S1** (1313 mg, 8 mmol) dropwise at 0 °C. The reaction mixture was stirred for 40 min at 0 °C. The solvent was removed, and purification of the residue by silica gel flash chromatography (AcOEt/*n*-hexane = 2/3) gave 2256 mg (100%) of **S2** as a yellow solid: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ; ppm) 8.63 (1H, s), 8.21 (2H, d, J = 9.0 Hz), 7.63 (2H, d, J = 9.0 Hz), 7.33 (1H, s), 4.86 (1H, m), 4.09 (1H, m), 3.66 (1H, m), 1.90 (2H, m), 1.64 (4H, m).

***N*-Hydroxy-*N'*-(4-nitrophenyl)urea (S3).** To a solution of **S2** (1950 mg, 6.9 mmol) in MeOH (150 mL) was added $TsOH \cdot H_2O$ (119 mg, 0.69 mmol). The reaction mixture was stirred for 23 h at room temperature. The solution was concentrated under reduced pressure, and purification of the residue by silica gel flash chromatography (AcOEt/*n*-hexane = 3/1) gave 1068 mg (79%) of **S3** as a yellow solid: ^{1}H-NMR (DMSO-d_6, 500 MHz, δ; ppm) 9.51 (1H, s), 9.29 (1H, s), 9.16 (1H, s), 8.16 (2H, d, J = 9.5 Hz), 7.92 (2H, d, J = 9.5 Hz).

9,10-Dihydro-9,10-dimethyl-*N*-(4-nitrophenyl)-9,10-(epoxyimino)anthracene-11-carboxamide (A). To a suspension of 9,10-DMA (103 mg, 0.5 mmol) in CH_2Cl_2 (8.0 mL) and MeOH (1.0 mL) containing $NaIO_4$ (213 mg, 1 mmol) was added a solution of **S3** (197 mg, 1 mmol) in MeOH (8.0 mL) and H_2O (0.8 mL) dropwise at 0 °C. The reaction mixture was stirred for 3 h at 0 °C, and diluted with CH_2Cl_2 and H_2O. Aqueous $Na_2S_2O_3$ was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2. The organic layers were combined with the separated CH_2Cl_2 layer, and then dried over Na_2SO_4. The solvent was removed, and purification of the residue by silica gel flash chromatography (AcOEt/*n*-hexane = 1/20 to 1/3) gave 79 mg (39%) of **A** as a yellow solid. A part (28.0 mg) of the solid was recrystallized from AcOEt/*n*-hexane to give 24.6 mg of pale yellow crystals: decomp. point 138.4–139.2 °C; R_f = 0.40 (AcOEt/*n*-hexane = 1/3); UV/vis (CH_3CN): $\varepsilon_{323\ nm} = 1.73 \times 10^4\ M^{-1}cm^{-1}$; IR (KBr): ν = 3368 cm^{-1} (N-H), 1687 cm^{-1} (C=O), 1596 cm^{-1} (N-H), 1506 cm^{-1} (NO_2), 1340 cm^{-1} (NO_2); ^{1}H-NMR (DMSO-d_6, 500 MHz, δ; ppm) 9.30 (1H, s), 8.08 (2H, d, J = 9.0 Hz), 7.65 (2H, d, J = 9.5 Hz) 7.54 (2H, m), 7.51 (2H, m), 7.31 (4H, m), 2.60 (3H, s), 2.33 (3H, s); ^{13}C-NMR (DMSO-d_6, 125 MHz, δ; ppm) 159.05, 144.85, 141.97, 141.16, 140.23, 127.28, 127.14, 124.37, 121.72, 120.92, 112.34, 79.81, 63.92, 16.86, 14.91; MS (FAB) *m/z*: 402 ($[M+H]^+$); Anal. Calcd. for $C_{23}H_{19}N_3O_4$: C, 68.82; H, 4.77; N, 10.47. Found: C, 68.75; H, 4.86; N, 10.45.

Scheme S2. Preparation of **B**.



Di *tert*-butyl 3, 3'-(9,10-anthracenediyl) bis 2-propenoate (S5) A solution of **S4** (3360 mg, 10 mmol), *t*-butyl acrylate (29 mL, 200 mmol), Et_3N (28 mL, 200 mmol), $Pd(OAc)_2$ (246.96 mg, 1.10 mmol), and tri-*o*-tolylphosphine (669.6 mg, 2.20 mmol) in DMF (100 mL) was heated at 120 °C for 4 hours. After cooling, water and AcOEt were added, and separated. The organic solution was evaporated under reduced pressure to give a residue, which was purified by silica gel flash chromatography (AcOEt/*n*–hexane = 1/10) to give **S5** (4163 mg, 97 %) as a yellow solid: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ; ppm) 8.51 (2H, d, J = 16.1 Hz), 8.26 (4H, dd, J = 3.2, 6.8 Hz), 7.53 (4H, dd, J = 3.2, 6.8 Hz), 6.32 (2H, d, J = 16.1 Hz), 1.63 (18H, s).

Di *tert*-butyl 9,10-anthracenedipropanoate (S6)
$Pd(OAc)_2$ (89.8 mg, 0.40 mmol) was added to a stirred solution of **S5** (1722.2 mg, 4.0 mmol) in DMF (300 mL) at 80°C, and HCOOK (1776.6 mg, 21.1 mmol) was added. The reaction mixture stirred for 29 hours. Then, AcOEt and water were added to the cooled mixture. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by silica gel flash column chromatography (toluene/*n*–hexane = 1/4) to give 1046.4 mg (60 %) of **S6** as a yellow solid: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ; ppm) 8.33 (4H, dd J = 3.2, 6.8 Hz), 7.54 (4H, dd, J = 3.2, 6.8 Hz), 3.92 (4H, t, J = 8.51 Hz), 2.69 (4H, t, J = 8.51 Hz), 1.49 (18H, s).

9,10-Anthracenedipropanoic acid (S7) 11 mL of TFA was added to a solution of **S6** (1046 mg, 2.41 mmol) in 20 mL of CH_2Cl_2. The reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was concentrated by evaporation *in vacuo* and the residue was suspended in *n*–hexane. Filtration and recrystallization from THF/ *n*–hexane gave 591 mg (76%) of **S7** as a yellow solid: ^{1}H-NMR (DMSO, 500 MHz, δ; ppm) 12.33 (2H, br), 8.36 (4H, dd J = 3.0, 7.0 Hz), 7.59 (4H, dd, J = 3.2, 6.9 Hz), 3.86 (4H, t, J = 8.2 Hz), 2.62 (4H, t, J = 8.1 Hz).

9,10-Dihydro-*N*-(4-nitrophenyl)-9,10-(epoxyimino)-11-carbamylanthracene-9,10-dipropanoic acid (B).
To a suspension of **S7** (180.1 mg, 0.56 mmol) and $NaIO_4$ (239.6 mg, 1.12 mmol) in 25 mL of acetone was added a solution of **S3** (220.5 mg, 1.12 mmol) in 7 mL of acetone and 15 mL of H_2O over a period of 10 minutes at 0 °C. The reaction mixture was stirred for 8 hours at 0 °C, then

poured into water, and the whole was extracted with AcOEt. The AcOEt layer was separated, washed with 4 N HCl and brine, and dried over Na_2SO_4. Filtration and concentration in *vacuo*, and precipitation with *n*–hexane gave **B** (175.4 mg, 61 %) as a light yellow solid: decomp. point 116.5–118.2 °C; R_f = 0.49 (AcOEt only); UV/vis (DMSO/milli Q = 1/9): $\varepsilon_{326\,nm} = 1.54 \times 10^4$ $M^{-1}cm^{-1}$; ^{1}H-NMR (DMSO, 500 MHz, δ; ppm) 9.20 (1H, s), 8.12 (2H, d, J = 9.4 Hz), 7.65 (2H, d, J = 9.1 Hz), 7.51 (4H, m), 7.33-7.28 (4H, m), 3.4 (2H, overlap), 3.15 (2H, t, J = 6.3 Hz), 2.86 (2H, t, J = 5.7 Hz), 2.79 (2H, t, J = 8.2 Hz); ^{13}C-NMR (CD_3OD, 125 MHz, δ; ppm); 178.23, 177.37, 160.84, 146.06, 145.96, 144.07, 128.56, 128.30, 125.51, 123.29, 122.57, 119.91, 119.83, 82.88, 67.85, 31.17, 29.28, 26.21, 23.89; MS (FAB) *m/z*: 518 ($[M+H]^+$); HRMS (FAB) calcd for $C_{27}H_{23}N_3O_8$, 518.15632; found, 518.15437; Anal. Calcd. for $C_{23}H_{19}N_3O_4 \cdot H_2O$: C, 60.56; H, 4.71; N, 7.85. Found: C, 60.20; H, 4.85; N, 7.63.

Angeli's salt (trioxodinitrate). Angeli's salt was prepared at >98% purity from butyl nitrate and hydroxylamine hydrochloride by the method of Smith and Hein:[1] $\varepsilon_{248\,nm}$ obsd, 8200 $M^{-1}cm^{-1}$ in 1 N NaOH; [lit.[2] 8300 $M^{-1}cm^{-1}$].

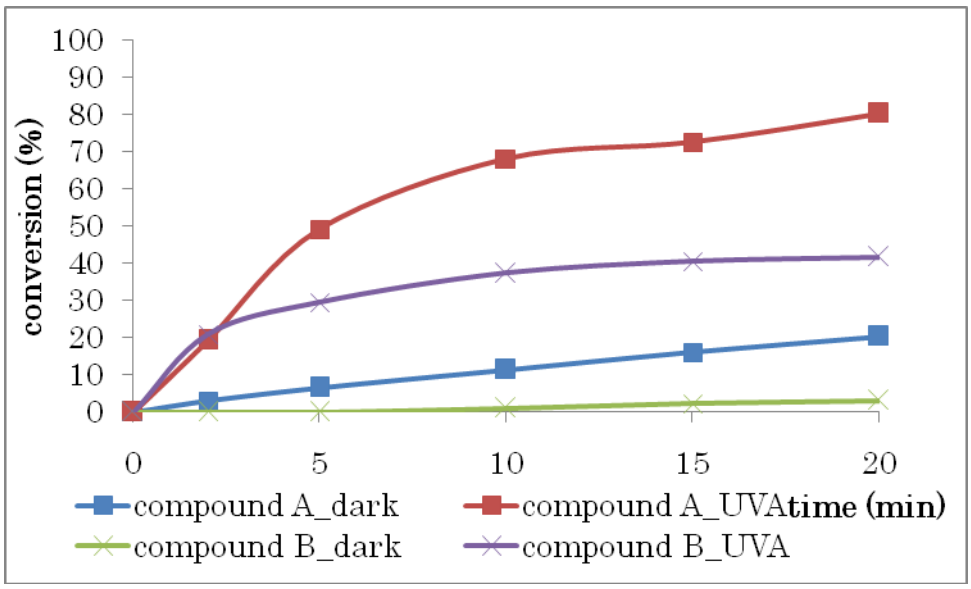
(1) Smith, P. A.; Hein, G. E. *J. Am. Chem. Soc.* **1960**, *82* (21), 5731–5740.
(2) Boghosian, R. A.; McGuinness, E. T. *Biochim. Biophys. Acta* **1979**, *567*, 278–286.

<Analysis>

General Methods. Photoirradiation with ultraviolet A was performed by using the light source (100 W mercury lamp) of a fluorescence microscope (Olympus BX60/BX-FLA) with a WU filter (330–380 nm band-pass filter) under Ar- or He-purged anaerobic conditions. The light intensity was attenuated to 1.5% with a combination of a 6% ND filter and a 25% ND filter.

Determination of Photoinduced Conversion by Measurement of Absorption Spectral Change. (a) A solution of compound **A** or **B** (1.5 mM, 20 μL) in DMSO was diluted with DMSO (1330 μL) and Milli Q water (150 μL) and the solution was transferred into a cuvette. (b) A solution of compound **B** (0.20 mM, 150 μL) in DMSO was diluted with Milli Q water (1350 μL) and the solution was transferred into a cuvette. UV/vis spectra were recorded by using an Agilent 8453 spectrometer after 0, 2, 5, 10, 15, and 20 min of photoirradiation at room temperature.

(a) compound **A** or **B** in DMSO/water (9/1)



(b) compound **B** in DMSO/water (1/9)

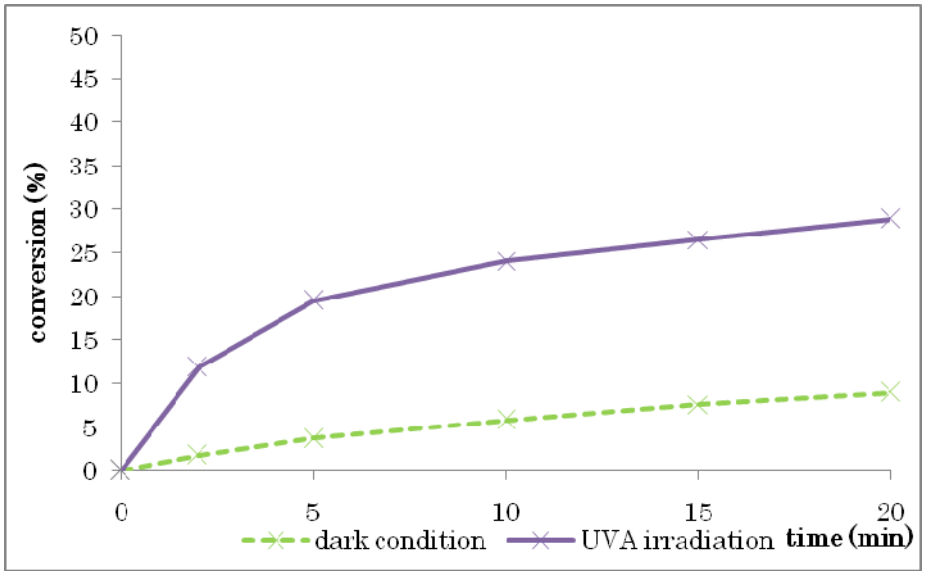
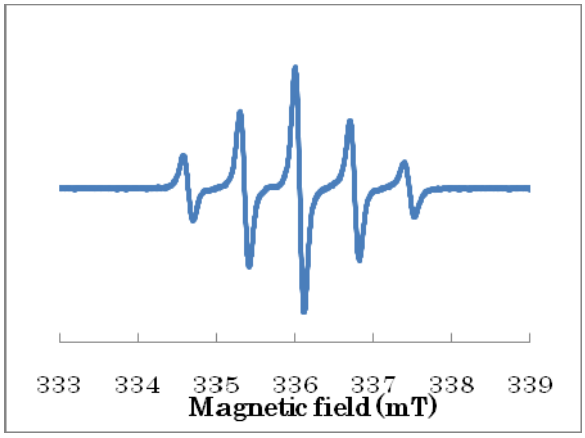


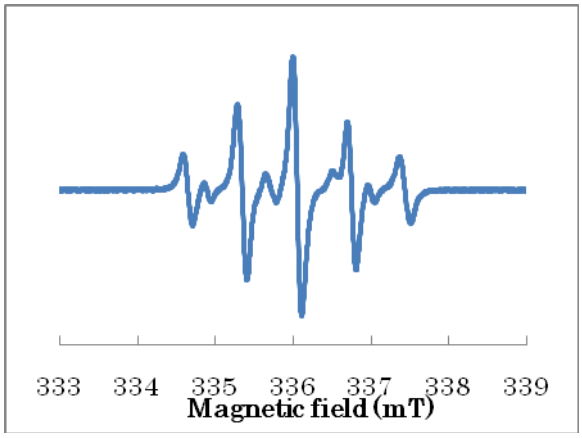
Figure S1. Conversion of (a) compound **A** or **B** in DMSO/water (9/1) and (b) that of compound **B** in DMSO/water (1/9), calculated from the increase in the absorption of 9,10-DMA at 402 nm or compound **S7** at 399 nm.

NO Production from Compound B on Photoirradiation in Low-polarity Solvent. A solution of compound **A** (100 μM) or compound **B** (100 μM) and carboxy-PTIO (100 μM) was dissolved in DMSO/ 50 mM Tris buffer - pH 7.5 (9/1 or 1/9). This mixture was subjected to photoirradiation or without photoirradiation for 10 min at room temperature in a flat EPR tube. EPR spectra were taken on a JES-RE2X spectrometer (JEOL Co. Ltd., Tokyo, Japan). The measurement conditions were follows; microwave power, 10 mW; frequency, 9.4200 GHz; field, 336.6 mT; sweep width, 7.5 mT; sweep time, 1 min; modulation width, 0.050 mT; gain, 125; and time constant; 0.03 s.

(a) compound **A** in DMSO/water = 9/1



(b) compound **B** in DMSO/Tris buffer = 9/1



(c) compound **B** in DMSO/Tris buffer = 1/9

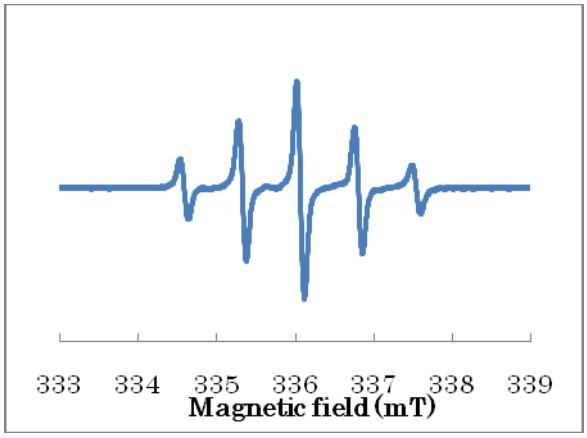
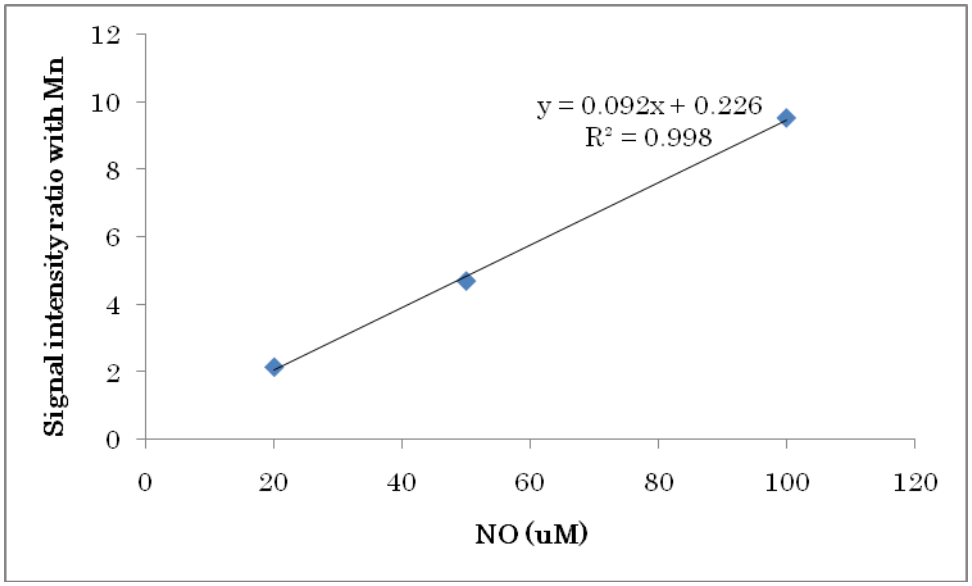


Figure S2-1. EPR spectra of (a) compound **A** and (b) **B** in DMSO/Tris buffer (9/1) and (c) compound **B** in DMSO/Tris buffer (1/9) with carboxy-PTIO in the presence of manganese as an external standard.

The amount of releasing NO from compound B was estimated by using NOC-7 in EPR study in DMSO/Tris buffer (9/1). A solution of compound B (100 μM) and carboxy-PTIO (50 μM) was dissolved in DMSO/ 50 mM Tris buffer pH 7.5 (9/1). This mixture was photoirradiated for 10 min at room temperature in a flat EPR tube. EPR spectra were taken on a JES-RE2X spectrometer (JEOL Co. Ltd., Tokyo, Japan). Calibration curve was made based on NOC-7, which was completely decomposed in 37°C for 45 min. The measurement conditions were follows; microwave power, 10 mW; frequency, 9.4200 GHz; field, 336.6 mT; sweep width, 7.5 mT; sweep time, 1 min; modulation width, 0.050 mT; gain, 630; and time constant; 0.03 s.

(a)



(b)

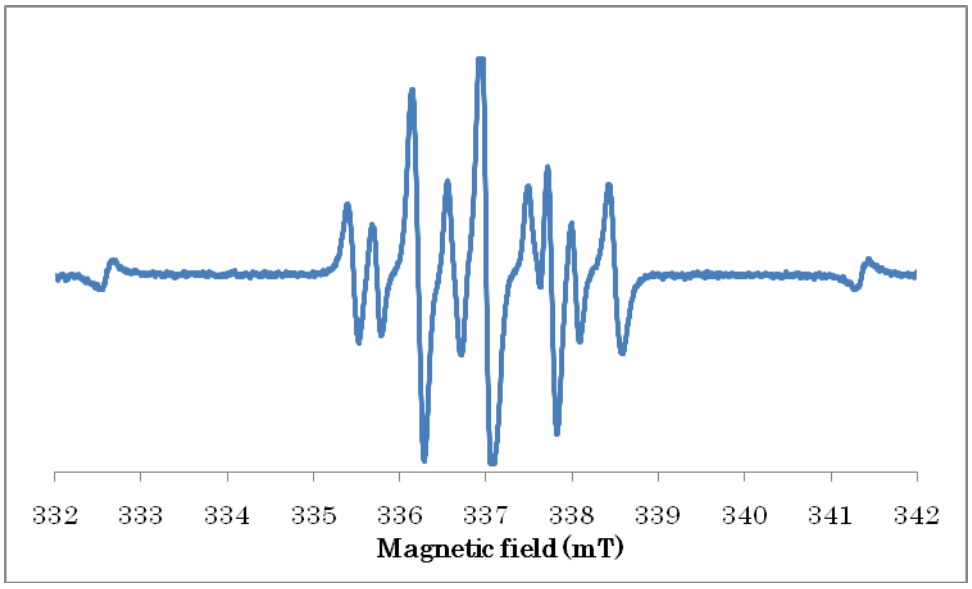


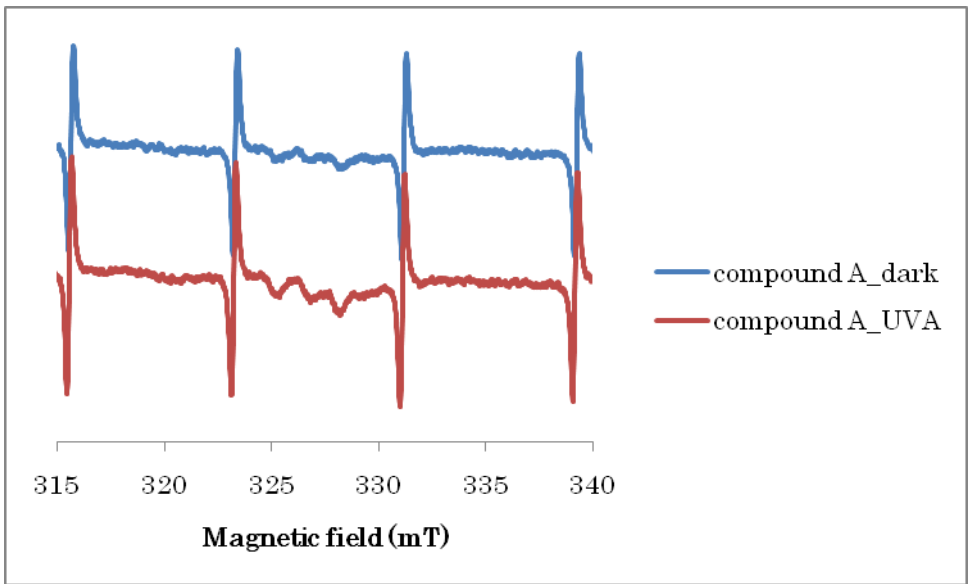
Figure S2-2. (a) calibration curve. (b) EPR spectra of compound **B** (100 μM) in DMSO/Tris buffer pH 7.5 (= 9/1) with carboxy-PTIO after photoirradiation for 10 min in the presence of manganese as an external standard. NO concentration was calculated as 36.4 μM.

We quantified the amount of NO by spin trapping method using carboxy-PTIO as an NO spin trap and one of the NONOates, NOC-7 (1-Hydroxy-2-oxo-3-(N-methyl-3-aminopropyl)-3-methyl-1-triazene) as an authentic NO releaser. One mol of NOC-7 was reported to produce 2 mol NO, so we made a calibration curve of NO adduct of carboxy-PTIO by using NOC-7. By this calibration curve, we measured and calculated the amount of NO released from the compound upon photoirradiation. As a result, 36.4 μM of NO was detected from 100 μM of compound **B** for 10 min photoirradiation.

Under this experimental condition, the conversion of compound **B** was calculated as about 40%, so that it was found that most of the converted compound was related to the NO formation in the low polarity solvent.

EPR Measurement of Ferrous Nitrosyl Complex Formed from HNO and Heme. (a) A solution of hemin (10 mM, 50 μL) in DMSO was diluted with DMSO (845 μL) and Milli Q water (100 μL). (b) A solution (500 μL) of hemoglobin (500 μM) in DMSO/50 mM Tris buffer (pH 7.5) = 1/9 was prepared. A solution of compound **A** or **B** (500 μM, 500 μL) in DMSO/Tris buffer (1/9) was added. The resulting mixture (1000 μL) was photoirradiated or not for 10 min at room temperature, then transferred to an EPR tube and frozen in liquid nitrogen (77 K). EPR spectra were taken on a JES-RE2X spectrometer (JEOL Co. Ltd., Tokyo, Japan). The measurement conditions were follows; microwave power, 10 mW; frequency, 9.145 GHz; field, 336.5 mT; sweep width, 40 mT; sweep time, 1 min; modulation width, 0.63 mT; gain, (a) 500, (b) 1000; and time constant; 0.03 s.

(a)



(b)

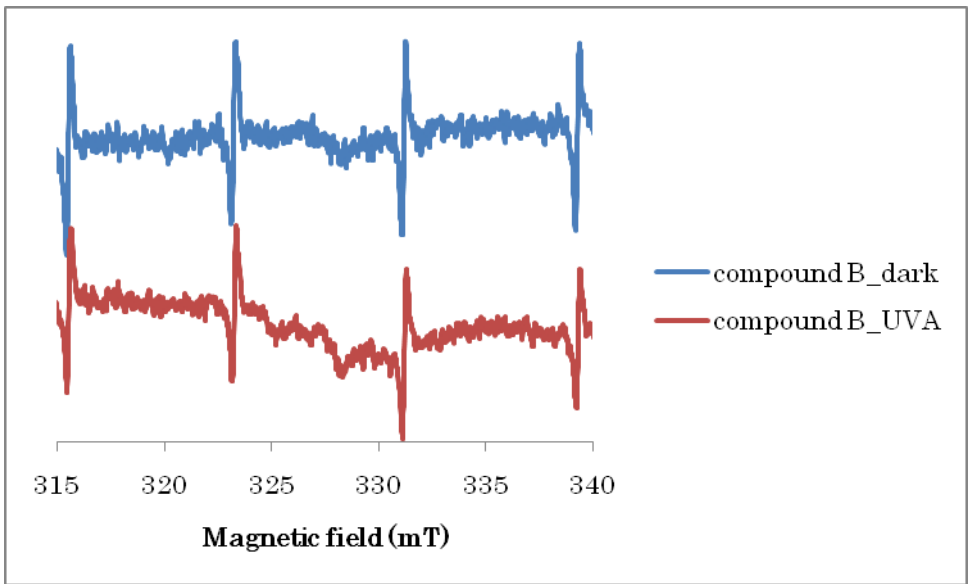
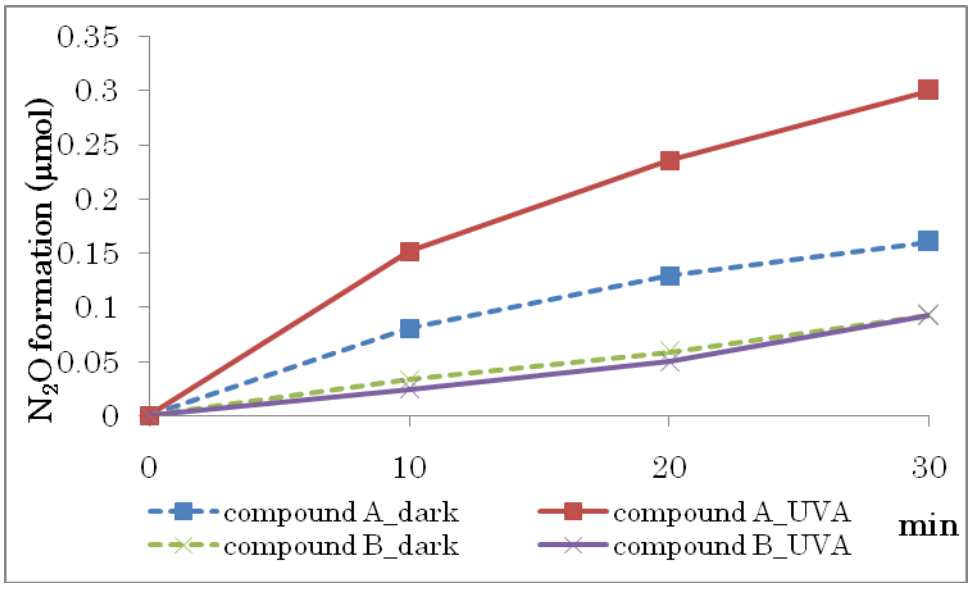


Figure S4. EPR spectra of (a) compound **A** in DMSO/water (9/1) with hemin and (b) compound **B** in DMSO/Tris buffer (1/9) with hemoglobin. Spectra were measured either in the dark (top), or under photoirradiation (bottom) in the presence of manganese as an external standard.

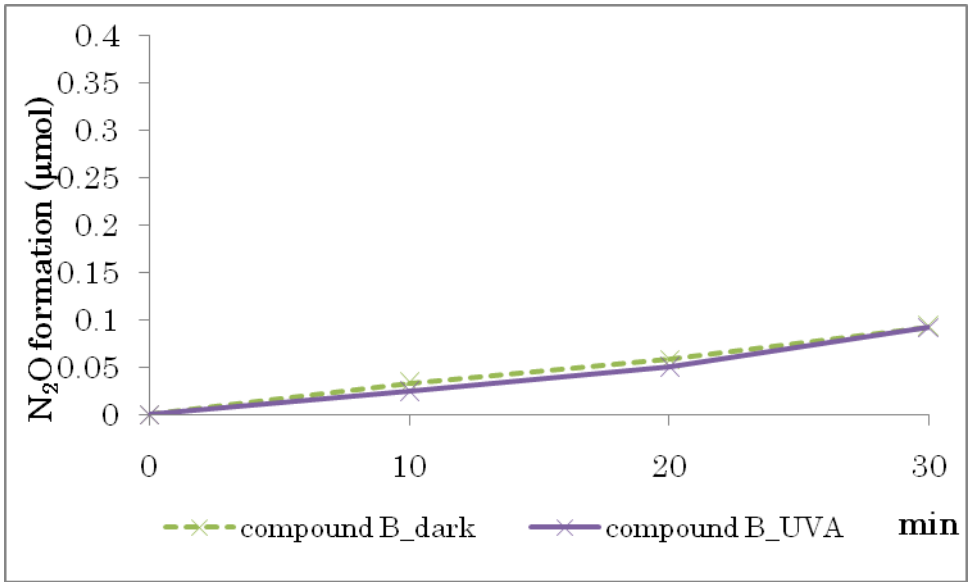
Detection of N_2O by Gas Chromatography. A solution of compound **A** or **B** (1.5 μmol) in DMSO (2700 μL) and 50 mM Tris buffer pH 7.5 (300 μL) or a solution of compound **B** (1.5 μmol) in DMSO (300 μL) and 50 mM Tris buffer pH 7.5 (2700 μL) was placed in a 4-mL cuvette sealed with a rubber septum. For HNO scavenging experiments, 2-mercaptoethanol (1.05 μL) was added to the solution. The sample solution was photoirradiated by UVA, or stood without photoirradiation for designated time at room temperature. The cuvette was then shaken and put onto ice bath for 30 min for equilibration of N_2O in the head space of the cuvette without further decomposition of the compound. An aliquot of the reaction headspace (50 μL) was injected onto a Shimadzu GC-2010 gas chromatograph equipped with a mass spectrometer (QP2010) and a Rt-QPLOT column (0.32 mm × 15 m) expanded with a 15-m inactivated fused silica tube (total 30 m). The GC injector was operated with a split ratio of 0.1 at 200 °C. The carrier gas (He) was set at a flow rate of 2.2 mL/min. The GC oven was held at 35 °C. The MS interface was set to 280 °C. N_2O formation was calculated based on the decomposition of Angeli's salt (1.5 μmol) according to Hughes and Wimbledon.[3]

On the chromatograph in GC-MS analysis, the peak of N_2O was partly overlapped with the large CO_2 peak in many cases. To calculate peak area of N_2O, the area of the peak tail of CO_2 peak overlapped with N_2O peak was processed on the computer.

(a)



(b)



(c)

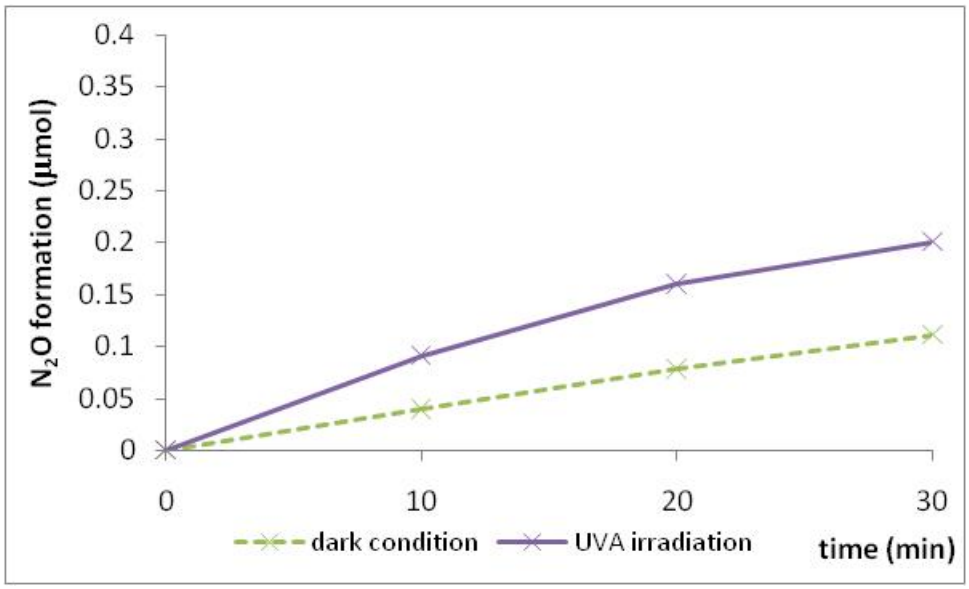


Figure S5. N_2O formation of (a) compound **A** or **B** in DMSO/Tris buffer = 9/1, (b) compound **B** in DMSO/Tris buffer = 9/1 and (c) compound **B** in DMSO/Tris buffer = 1/9

Although 2 mol of NO and 1 mol of HNO can react to produce 1 mol of N_2O, the third step of this reaction (the reaction of HN_3O_2 to N_2O) was reported to have very small kinetic constant compared with HNO dimerization. When NO was the main product of the photodecomposition of compound **B** as shown in the main text, there are little amounts of HNO produced, and it would be very little chance to form N_2O via the reaction of NO with HNO. When HNO was produced

from the compound in the high polarity solvent as we propose, NO was not detected and there were no chance to proceed HNO and NO reaction. So, we considered that almost all the detected N_2O was the results of the dimerization of N_2O.

When 2-mercaptoethanol (1.05 μL, 10 equiv.) was added to the solution in all cases (a)-(c), N_2O was not found at all.

As for the quantitative analysis, the formation of N_2O from HNO was the second order reaction, so that the calibration curve by using Angeli's salt was not expressed as linear regression but fitted with the second order curve. In this calibration curve, the errors would be different between at the small amount of N_2O formation and the large amount of that. Furthermore, Angeli's salt is known to form a certain amount of NO other than HNO, so that it is also make it difficult to determine precise values of N_2O formation.

(3) Hughes, M. N.; Wimbledon, P. E. *J. Chem. Soc. Dalton Trans.* **1976**, 703–707.

pH dependency of the HNO formation (measured as N_2O) from compound B in the low polarity solvent.

A solution of **B** (1.5 μmol) in DMSO (2700 μL) and 50 mM Tris buffer (various pH, 300 μL) was placed in a 4-mL cuvette sealed with a rubber septum. After 10 min photoirradiation, the cuvette was then shaken and put onto ice bath for 30 min for equilibration of N_2O in the head space of the cuvette without further decomposition of the compound. An aliquot of the reaction headspace (50 μL) was injected onto a Shimadzu GC-2010 gas chromatograph equipped with a mass spectrometer (QP2010) and a Rt-QPLOT column (0.32 mm × 15 m) expanded with a 15-m inactivated fused silica tube (total 30 m). The GC injector was operated with a split ratio of 0.1 at 200 °C. The carrier gas (He) was set at a flow rate of 2.2 mL/min. The GC oven was held at 35 °C. The MS interface was set to 280 °C. N_2O formation was calculated based on the decomposition of Angeli's salt.

Table **S1**. N_2O formation under photoirradiated condition (compound **B** in DMSO/50 mM Tris buffer (various pH) = 9/1)

pH	N_2O formation (μmol)
5.0	0.043
7.5	0.012
8.5	0.010

The pH dependency of HNO formation (measured as N_2O) from compound **B** in the low polarity solvent was examined. As shown in table **S1**, N_2O formation was depending on the solvent pH. Although the difference of N_2O formation at various pH values may look somewhat small, considering that the nucleophilic attack of water to the acyl nitoroso compound would be less facilitated at the acidic pH, there would be a significant pH dependency in the HNO formation.

The yields of NO and HNO under thermal and photochemical conditions.

1. Measurement of N_2O formation

A solution of **B** (1.5 μmol) in DMSO (2700 μL) and 50 mM Tris buffer (pH 7.5, 300 μL) or in DMSO (300 μL) and 50 mM Tris buffer (pH 7.5, 2700 μL) was placed in a 4-mL cuvette sealed with a rubber septum. After 10 min photoirradiation, the cuvette was then shaken and put onto ice bath for 30 min for equilibration of N_2O in the head space of the cuvette without further decomposition of the compound. An aliquot of the reaction headspace (50 μL) was injected onto a Shimadzu GC-2010 gas chromatograph equipped with a mass spectrometer (QP2010) and a Rt-QPLOT column (0.32 mm × 15 m) expanded with a 15-m inactivated fused silica tube (total 30 m). The GC injector was operated with a split ratio of 0.1 at 200 °C. The carrier gas (He) was set at a flow rate of 2.2 mL/min. The GC oven was held at 35 °C. The MS interface was set to 280 °C. N_2O formation was calculated based on the decomposition of Angeli's salt.

The ratio of observed HNO was calculated based on the results of GC-MS analysis. 1.5 μmol of compound **B** under this assay was photoirradiated for 10 min in each condition's solvents and under both conditions. N_2O formation was calculated based on Angeli's salt decomposition. The calculated N_2O was divided by the theoretical maximum of N_2O (0.75 μmol) released from 1.5 μmol of compound **B**.

2. Measurement of the amount of releasing NO

A solution of compound **B** (100 μM) and carboxy-PTIO (50 μM) was dissolved in DMSO/ 50 mM Tris buffer pH 7.5 (9/1) or (1/9). This mixture was photoirradiated for 10 min at room temperature in a flat EPR tube. EPR spectra were recorded on a JES-RE2X spectrometer (JEOL Co. Ltd., Tokyo, Japan). A caliblarion curve was made based on NOC-7, which was completely decomposed in 37^{o}C for 45 min. The measurement conditions were follows; microwave power, 10 mW; frequency, 9.4200 GHz; field, 336.6 mT; sweep width, 7.5 mT; sweep time, 1 min; modulation width, 0.050 mT; gain, 630; and time constant; 0.03 s.

In this assay, 100 μM of compound **B** was photoirradiated for 10 min under each solvent condition. The ratio of NO formation was calculated based on the signal change of carboxy-PTIO.

Table **S2**. The yields of NO and HNO under dark and photoirradiated conditions (10 min irradiation)

	DMSO/ 50 mM Tris buffer (pH 7.5) = 9/ 1		DMSO/ 50 mM Tris buffer (pH 7.5) = 1/ 9	
condition	dark	photoirradiated	dark	photoirradiated
ratio of observed HNO (%)	4.4	3.2	5.3	12
ratio of observed NO (%)	0	36	0	0
conversion (%)	10*	37	5.8	24**

* The sum of HNO and NO is over the conversion, and the reason was not clear, but this might be included within an error.

**The sum of HNO and NO is about half of the conversion. This might indicate the other decomposition route than releasing HNO, but we did not determine it in this work, but at least it is not a NO-releasing pathway.

Investigation of Intramolecular Hydrogen Bond by NMR Analysis. The NMR spectrum of a solution of compound **B** in DMSO-d_6/50 mM Tris buffer (9/1) was measured with a JEOL JNM-LA500 spectrometer. The pH of the Tris buffer was adjusted from 2 to 8.5.

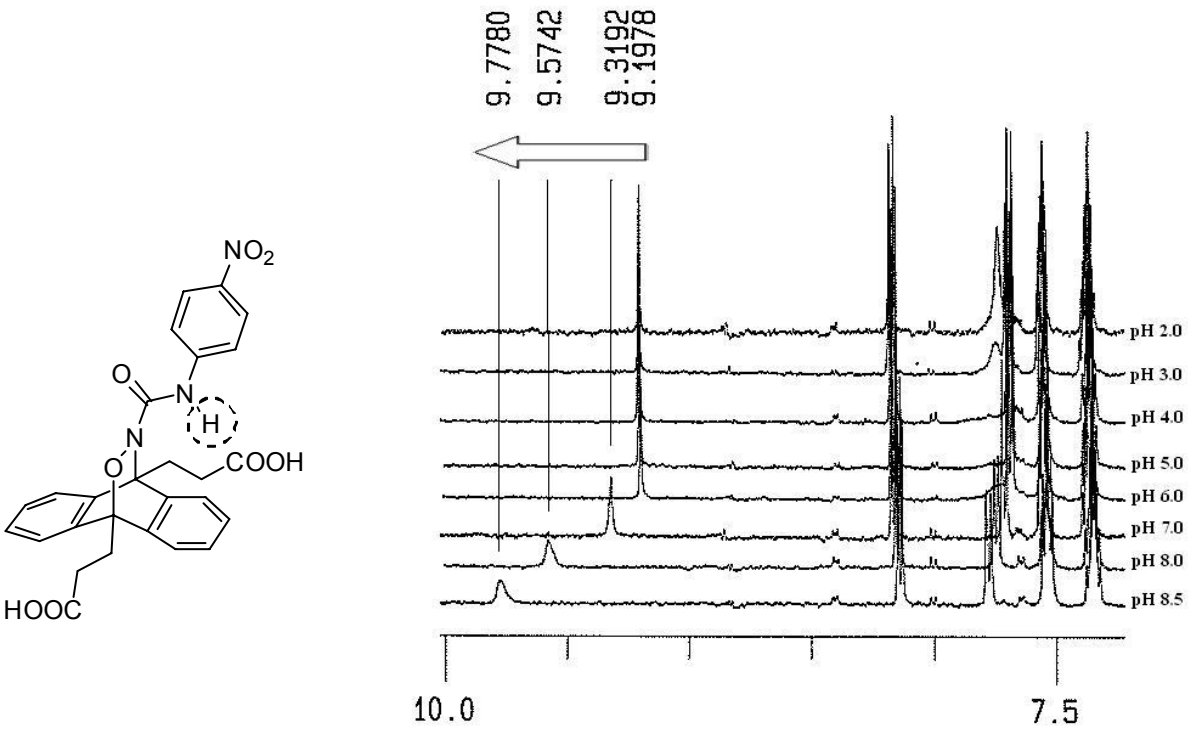


Figure **S6**. The signal due to the N-H bond moved downfield and changed to a broad peak pH-dependently, reflecting the formation of an intramolecular hydrogen bond.

DFT Calculation with Spartan '08 of the Stable Conformation of Compound B in Dianionic Form. The stable structure of compound **B** in dianionic form was computed by means of density functional theory (using the B3LYP method and 6-31G*), using Spartan '08.

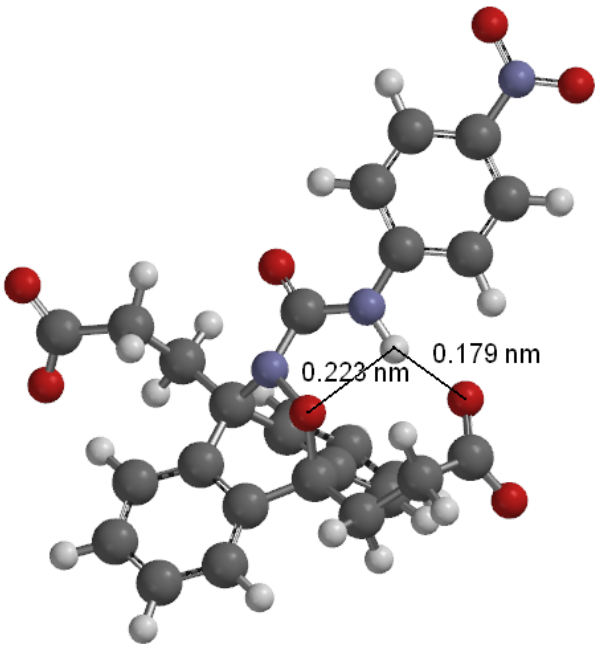


Figure S7. Calculated stable structure of compound **B** obtained with Spartan '08, indicating the presence of two intramolecular hydrogen bonds.

An NMR titration using tetrabutylammonium acetate. The NMR spectrum of a solution of compound **B** (1.9 mM, 1mL) in DMSO-d_6/50 mM Tris buffer pH 7.5 (9/1) was measured with tetrabutylammonium acetate (0, 0.5, 1.0, 5.0, 10 equiv.) using a JEOL JNM-LA500 spectrometer.

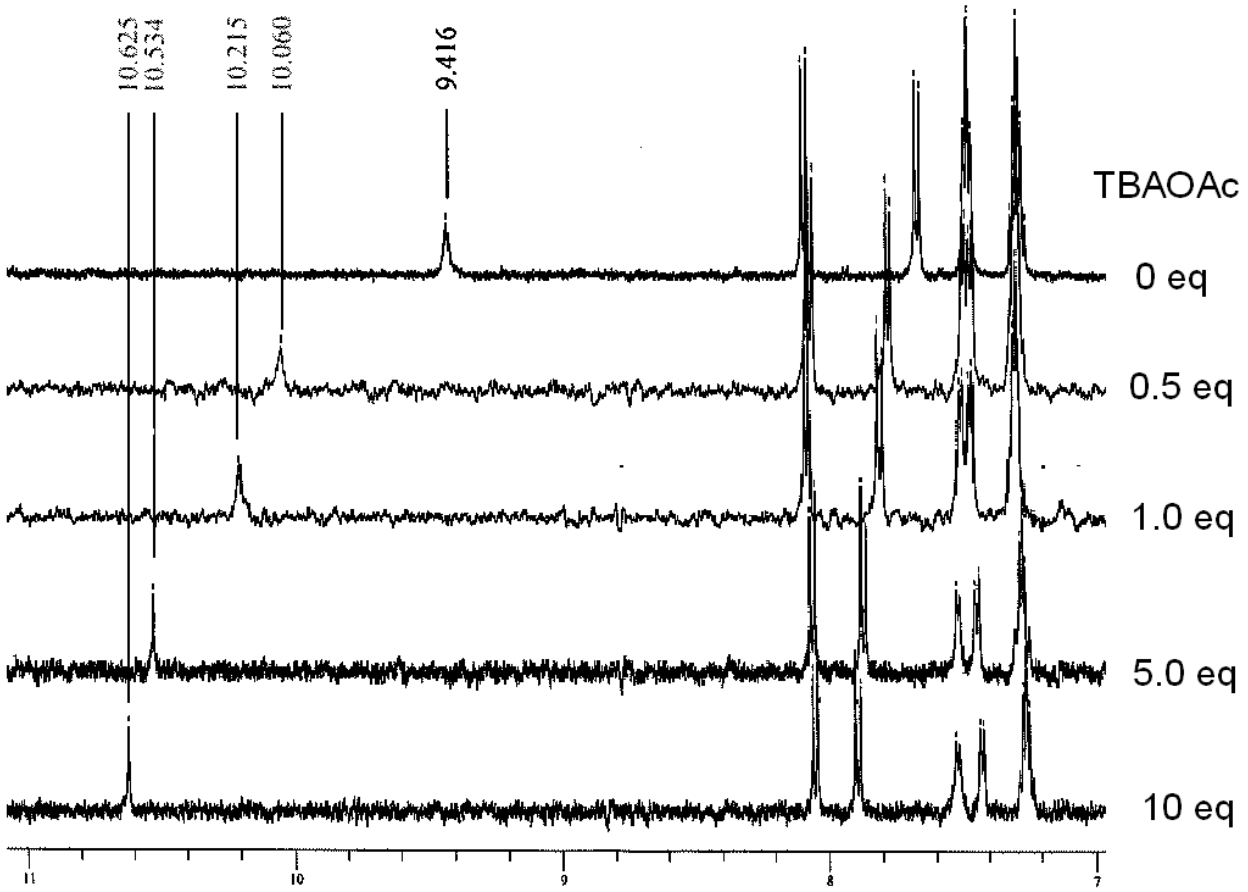


Figure S8. ^{1}H-NMR titration of compound **B** with TBAOAc (tetrabutylammonium acetate) in DMSO-d_6/50 mM Tris buffer pH 7.5 (9/1).