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Visible-Light-Triggered Release of Nitric Oxide from N-Pyramidal Nitrosamines

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Abstract: Although many organic/inorganic compounds that release nitric oxide (NO) upon photoirradiation (phototriggered caged-NOs) have been reported, their photoabsorption wavelengths mostly lie in the UV region, because X–NO bonds (X = heteroatom and metal) generally have rather strong π-bond character. Thus, it is intrinsically difficult to generate organic compounds that release NO under visible light irradiation. Herein, the structures and properties of N-pyramidal nitrosamine derivatives of 7-azabicyclo-[2.2.1]heptanes that release NO under visible light irradiation are described. Bathochromic shifts of the absorptions of these nitrosamines, attributed to HOMO (n)–LUMO (π*) transitions associated with the nonplanar structure of the N–NO moiety, enable the molecules to absorb visible light, which results in N–NO bond cleavage. Thus, these compounds are innate organic caged-NOs that are uncaged by visible light.

Keywords: cage compounds · cleavage reactions · nitrogen oxides · nitrosamines · photolysis

Introduction

Phototriggered release of nitric oxide (NO)[1] from organic/inorganic compounds (that is, phototriggered caged-NOs) has been studied for decades, and reported compounds include O^2-*ortho*-nitrobenzyl-substituted diazeniumdiolate (NONOate) derivatives,[2,3] aliphatic[4] and aromatic nitrosamines,[5] aromatic nitro compounds,[6] and metal nitrosyls (M-NOs),[7] such as iron, manganese, and ruthenium complexes of NO. However, these compounds mostly require irradiation at UV wavelengths for cleavage of the relevant bond to release NO. The reason for this is that X–NO bonds (X = heteroatom and metal) generally have rather strong π-bond character and their photoabsorption wavelengths lie in the UV range, so it is intrinsically difficult to generate compounds that release NO under visible light irradiation. On the other hand, a two-photon excitation technique to achieve NO release with near-infrared light is under development.[7] Furthermore, some metal nitrosyls can undergo M–NO bond cleavage to release NO under visible light irradiation.[8] However, few organic caged-NOs that release NO on exposure to visible light have been reported so far. Herein, we study new aspects of the structures and properties of N-pyramidalized bicyclic nitrosamine derivatives of 7-azabicyclo[2.2.1]heptanes,[9] and find that these nitrosamines can release NO under visible light irradiation. NO is an important biological signaling molecule, and therefore molecules that can provide spatially and temporally controlled release of NO are of interest as potential tools for biological research. Although there may be concern about potential mutagenicity in the use of *N*-nitrosamines as an NO source, the timescale of the biological action of NO released from caged-NOs is as short as seconds to minutes, whereas the timescale of the genotoxic effect of nitrosamines is as long as days to months, so that mutagenicity need not be a major concern in the application of *N*-nitrosamines as tools for experimental NO release. In any case, the present bicyclic nitrosamines have been reported to be nonmutagenic in the typical Ames assay, apparently due to their characteristic strained structures.[10]

Results and Discussion

Spectroscopic features of bicyclic and monocyclic nitrosamines: We have previously shown that the amino-nitrogen atom of nitrosamine derivatives of 7-azabicyclo-[2.2.1]heptanes is pyramidal and the N–NO bond tends to be weak.[9] Electronic absorption spectra of various synthesized nitrosamines (Scheme 1) were measured in DMSO (e.g., **3** and **16**, Figure 1 and Table 1). A comparison of the

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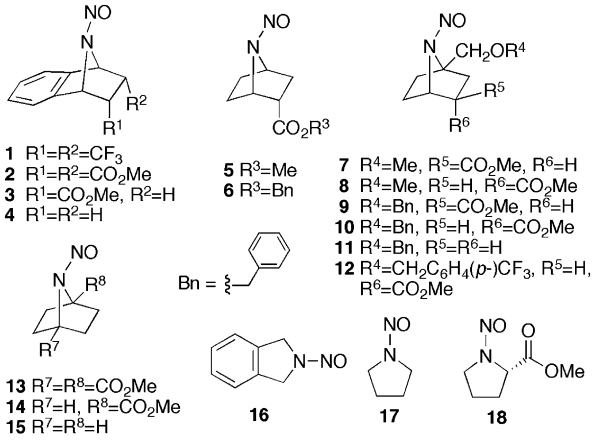
Supporting information (including details of the synthesis of *N*-nitrosamines **3** and **4**) for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201101427.



Scheme 1. Bicyclic and monocyclic nitrosamines. Bn = benzyl.

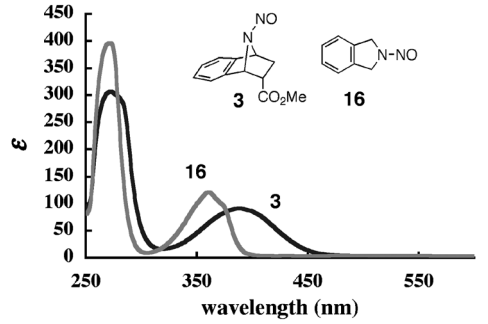


Figure 1. UV spectra of bicyclic (**3**) and monocyclic (**16**) nitrosamines in DMSO.

UV/Vis spectra of these bicyclic nitrosamines (**1**–**15**, e.g., **3**) with those of the monocyclic derivatives (**16**–**18**, e.g., **16**) showed significant bathochromic shifts, and the absorption tails into the visible light region (up to 500 nm) in the case of the bicyclic nitrosamines. The absorption peaks at around 300–450 nm can be attributed to HOMO (n)-to-LUMO (π*) transitions (Figure 2).[4a] The magnitudes of the HOMO–LUMO excitation energies in the $S_0 \rightarrow S_1$ transition, obtained by means of time-dependent (TD) DFT calculations of the most stable conformers (Table 1), were also consistent with this observation. Conformations involving *cis*/*trans* isomers of the N–NO group and bending of the NO group have little effect on the calculated λ_{max} values (for other conformers, see Table S1 in the Supporting Information). These bathochromic shifts can be attributed to the pyramidal character of the N–NO bond in the bicyclic nitrosamines, that is, a reduction of the double-bond character of the N–NO bond (Figure S1 in the Supporting Information). Lowering of the LUMO level due to the attenuated p-orbital overlap in the N-pyramidalized nitrosoamino functionality is assumed to contribute mainly to the reduction of the magnitudes of the HOMO–LUMO excitation energies in the $S_0 \rightarrow S_1$ transition (Figure 3). As the pyramidalization is enhanced by the introduction of a bridgehead substituent(s), the bathochromic shift of λ_{max} will be magnified (see θ values of **5**/**8**, **6**/**10**, and **15**/**14**/**13** in Table 1 and Figure S1 in the Supporting Information). Thus, the nitrosamine derivatives of 7-azabicyclo[2.2.1]heptanes can absorb visible light, and so we expected that these nitrosamines would work as organic caged-NOs, the N–NO bonds of which would be cleaved upon visible light absorption because of the antibonding

Table 1. Absorption spectral data and calculated structural parameters.

Nitrosamine	λ_{max} [nm] (exptl)[a]	Conformer[b]	λ_{max} [nm] (calcd)[c,d]	θ [°][e] (X-ray)[f]
1	394	*endo*	408.9	343.7
2	390	*endo*	404.5	345.0 (349.8)
3	389	*cis–endo*	403.9	344.1
4	385	*endo*	400.9	344.3
5	382	*cis–endo*	403.1	345.7
6	380	*cis–endo*	403.2	345.5
7	375	*cis–exo*	404.4	345.5
8	383	*cis–endo*	403.1	346.7
9	379	*cis–endo*	404.8	345.2
10	383	*cis–endo*	403.2	346.7
11	377	*trans–endo*	399.0	346.8
12	383	*cis–endo*	404.9	346.1
13	405	–	401.5	342.4
14	392	*cis*	397.5	343.8
15	375	–	398.6	345.9
16	372	–	374.4	360.0
17	360	–	371.8	359.2
18	361	*cis*	368.9	359.7

[a] In DMSO at RT. [b] For asymmetrical compounds, the term *cis* (*trans*) refers to the NO group of the nitrosamine on the same side (opposite side) with respect to the dangling ester or bridgehead substituent. Symmetrical compounds have no stereoisomers (–). The tilting of the N–NO group on the same side as (opposite to) the dangling substituent is defined as the *endo* (*exo*) conformation here. [c] TDDFT: B3LYP/6-31+G(d) was used. [d] Calculated first singlet excitation, attributed to n–π* transition within the N–NO group (except for **6** and **12**, n(NO)–π*(aromatic ring) transition). [e] Calculated at the B3LYP/6-31G(d) geometry optimization level. θ is the summation of the three calculated bond angles around the nitrogen atom of N–(NO). [f] Ref. [9a].

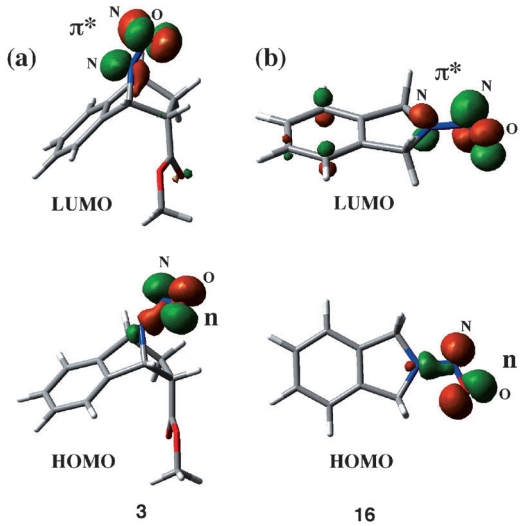


Figure 2. HOMOs and LUMOs of a) bicyclic nitrosamine **3** and b) monocyclic nitrosamine **16** associated with the n–π* transition.

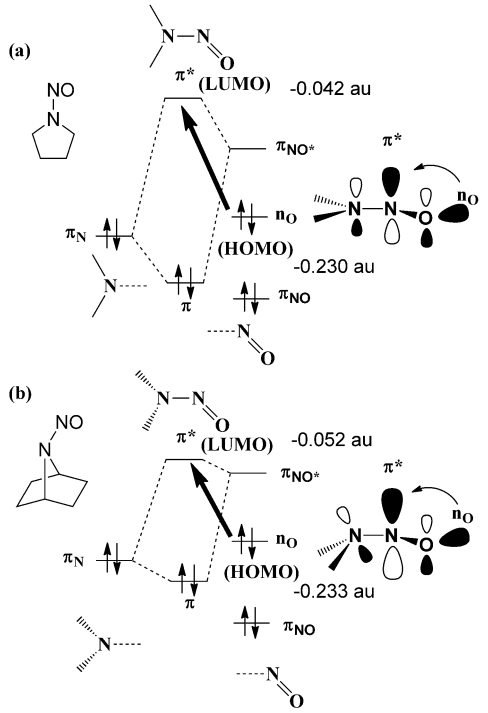


Figure 3. Orbital interactions within the nitrosoamino functionality and HOMO–LUMO transition; a) monocyclic and b) bicyclic nitrosamines.

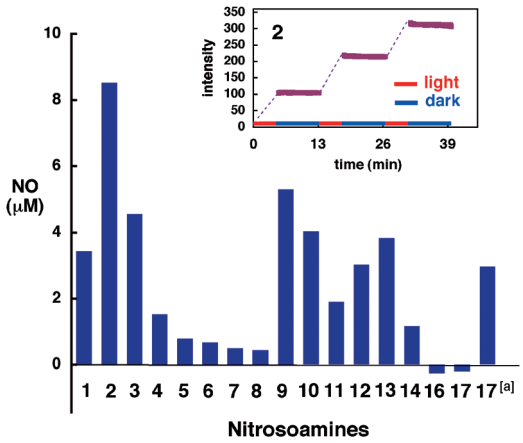
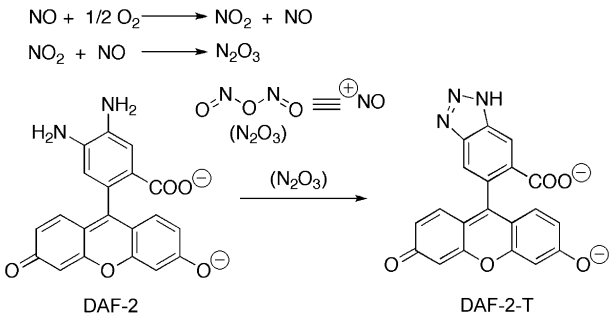


Figure 4. Concentration [μM] of NO released under visible light irradiation of nitrosamines **1**–**17** (96.2 μM) at 420 nm for 10 min in the presence of DAF-2 (9.62 μM) in PBS buffer containing 1.7% DMSO. [a] Irradiated at 360 nm. Inset: Fluorescence increase of **2** in response to successive light–dark–light exposures. A solution of **2** was irradiated at 420 nm for 5 min and the fluorescence intensity (excitation at 492 nm and emission at 520 nm) was measured for 8 min (i.e., in the dark). This sequence was repeated three times. No increase of the fluorescence could be seen in the dark periods. In terms of yields for NO release, the scale unit of the vertical axis (NO(μM)) should read 1.03% yield in the range between 0 and 10.3%.

character between the amino N atom and the NO moiety in the LUMO (Figure 2a).

Detection of N–NO bond cleavage under visible light irradiation: The synthesized nitrosamine derivatives were dissolved in phosphate-buffered saline (PBS, pH 7.4) containing 1.7% DMSO and irradiated with visible light (420 nm) at 20°C. Release of NO in aqueous aerobic solution can be detected and measured by using diaminofluorescein (DAF-2),[11] a fluorescent probe for NO, based on formation of the fluorescent triazole (DAF-2-T) through the nitrosation reaction of the DAF-2 diamine with nitrous anhydride (N_2O_3) or other NO^+ equivalents formed by the reaction of NO with dioxygen (Scheme 2).[11,12] As shown in Figure 4, under irradiation at 420 nm, the bicyclic nitrosamines (**1**–**14**) released NO, whereas the monocyclic nitrosamines (**16**–**18**) did not.



Scheme 2. Reaction of DAF-2 to generate fluorescent DAF-2-T.

The amount of NO released was proportional to the duration of light exposure (Figure 5a). When a solution of the monocyclic nitrosamine **17** was irradiated at 360 nm, which corresponds to the absorption maximum of the n–π* transition of **17** (Table 1), the fluorescence increased owing to the formation of DAF-2-T, apparently through the generation of NO by cleavage of the N–NO bond (Figure 4), in accordance with the previous observation[4] and the antibonding character of the N–NO bond in the LUMO of the monocyclic *N*-nitrosamine (see Figure 2b). Furthermore, the fluorescence increase was significantly suppressed in the cases of **2**, **3**, **9**, **10**, and **17** (irradiation at 420 nm, except **17** (360 nm)) when dissolved dioxygen was expelled from the solution by bubbling of Ar gas before light irradiation (Figure 5b). These observations are consistent with the postulate that the N–NO bond cleavage upon photoexcitation proceeded at least partially in a homolytic manner to generate NO. To demonstrate that release occurs only during irradiation, light–dark–light sequences were examined (Figure 4, inset). The amount of NO released correlated well with the time period of irradiation, and no release was observed during the dark phase. The control experiments, shown in Figure 5a, also confirmed no significant formation of NO in the absence of light exposure. These results exclude the possibility that the nitrosamines themselves nitrosate DAF-2 to generate fluorescent DAF-2-T.

Irradiation of a solution of the bicyclic nitrosamine **3** and cobalt(II) *meso*-tetraphenylporphyrin (TPPCo) in THF at 480 nm caused the intermolecular transfer of NO from **3** to TPPCo. Herein, light at 420 nm was not used to avoid the absorption peaks of TPPCo. Electronic absorption spectros-

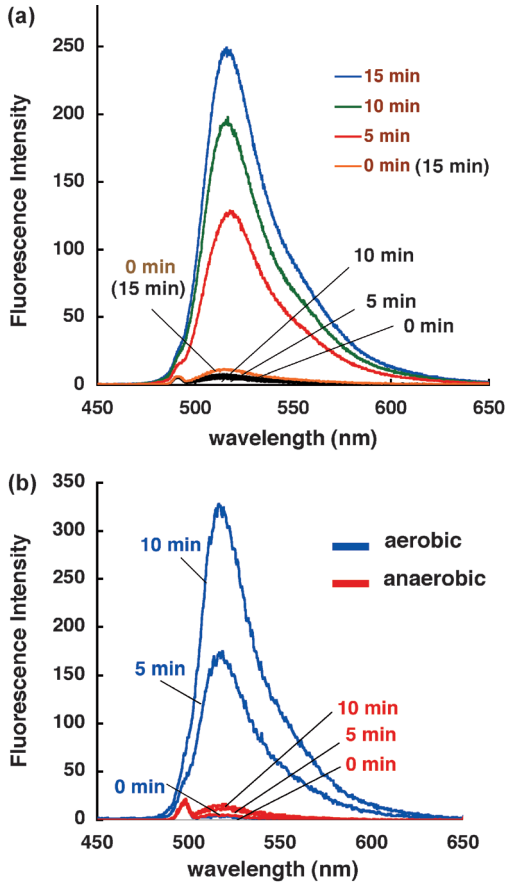


Figure 5. Effects of a) light exposure and b) the presence of dioxygen on the fluorescence increase of DAF-2 during irradiation of nitrosamine **3** at 420 nm. a) The amount of fluorescent DAF-2-T was proportional to the duration of light exposure. No significant formation of DAF-2-T was observed in the dark (black lines) for 15 min (orange line). b) The formation of fluorescent DAF-2-T was observed under aerobic conditions (blue lines), but was significantly suppressed (also in the cases of **2**, **9**, and **10** irradiated at 420 nm and **17** irradiated at 360 nm; not shown) after removal of dissolved dioxygen by bubbling of Ar gas (anaerobic conditions, red lines).

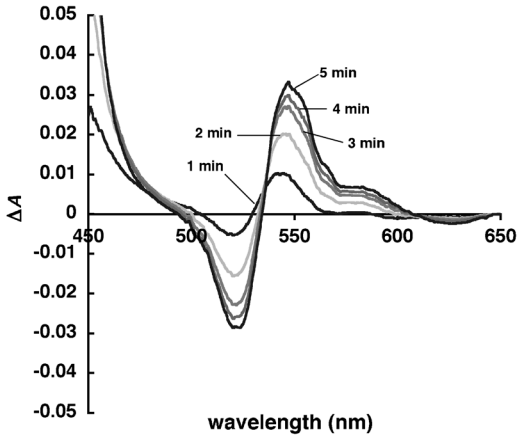


Figure 6. Difference spectra obtained during photolysis of **3** in the presence of TPPCo in THF. $[\mathbf{3}]_0 = 1$ mM, $[\mathrm{TPPCo}]_0 = 100$ μM in THF. The solution was irradiated with visible light at 480 nm and the spectra were measured every minute for 5 min. The change in absorbance (ΔA) during photolysis was obtained by subtracting the initial spectrum from the spectra recorded at different photolytic uncaging times. A decrease of absorption at 520 nm due to consumption of TPPCo with a concomitant increase of the absorption at 545 nm due to formation of TPPCo(NO) were observed.

copy showed clean conversion of TPPCo into TPPCo(NO) (Figure 6). The spectral change during photolysis was represented by difference spectra (Figure 6), obtained by subtracting the initial spectrum from the spectra recorded at different photolytic uncaging times: a decrease of absorption at 520 nm due to consumption of TPPCo, with a concomitant increase of the absorption at 545 nm due to formation of TPPCo(NO), was observed. These spectral changes are consistent with those previously reported for authentic TPPCo(NO) and conversion of TPPCo to TPPCo(NO) by other NO donors,[5a,7c] thereby confirming the photodependent formation of NO from nitrosamine **3**.

Structure dependence of N–NO bond cleavage and uncaging efficiency: Among the bicyclic derivatives, the compounds bearing a fused aromatic ring (**1**–**3**) or an aromatic bridgehead substituent (**9**–**11**) tended to release NO more efficiently than the compounds without such features (e.g., **5** and **7**/**8**; see Figure 4). An aromatic electron-withdrawing substituent (*p*-CF_3 in **12**) retarded the NO release relative to the unsubstituted case (**10**). The distal aromatic ring of **6** had no significant effect on the NO release as compared with the methyl ester **5**. These phenomena may be explained in terms of interactions of NO or NO^+ with the aromatic ring[13] to stabilize the released NO species or acceleration of the reaction of NO with O_2 within the hydrophobic interior;[14] such interactions have been investigated both experimentally and theoretically. This result indicates that NO release can be tuned by changing the substituents, which is one of the advantages of organic caged compounds.

To evaluate the efficiency of uncaging of these bicyclic nitrosamines, we determined the quantum yield of uncaging (Φ_{uncage}) of **3** by comparison with that of *N*,*N*′-bis(carboxymethyl)-*N*,*N*′-dinitroso-*para*-phenylenediamine sodium salt (**BNN5Na**) under irradiation at 350 nm.[5a] Net uncaging efficiency can be represented in terms of $\varepsilon\Phi_{\mathrm{uncage}}$, wherein Φ_{uncage} is the quantum yield of uncaging and ε is the molar absorption coefficient at a specified wavelength. A solution of **BNN5Na** (9.62 μM) or **3** (96.2 μM) in 1.7% DMSO–PBS at pH 7.4 was irradiated for 5 min at 350 nm in the presence of DAF-2 (9.62 μM). From the relative magnitude of the fluorescence intensity of the formed DAF-2-T (emitted at 520 nm), the value of $\varepsilon\Phi_{\mathrm{uncage}}$ of **3** was extrapolated to be $1.8\times10^2\,\mathrm{M^{-1}cm^{-1}}$ ($\Phi_{\mathrm{uncage}} = 1.17$) with 350 nm light. The corresponding value $\varepsilon\Phi_{\mathrm{uncage}}$ of **BNN5Na** was reported to be $2.4\times10^4\,\mathrm{M^{-1}cm^{-1}}$ with 350 nm light ($\Phi_{\mathrm{uncage}} = 1.87$ for uncaging two N–NO bonds).[5a] Therefore, the quantum yield of uncaging (Φ_{uncage}) of **3** is as high as that of **BNN5Na** (0.94 after a simple correction with respect to a single N–O bond), and both show a higher net uncaging efficiency than those for reported *ortho*-nitrobenzyl ester caged-NOs

($\varepsilon\Phi_{uncage} = 15–70\,M^{-1}cm^{-1}$).[2] A relationship between DAF-2-T formation and irradiation wavelength (350–500 nm) was also examined in the case of **3** (Figure S2 in the Supporting Information). The irradiation-wavelength-dependent formation of DAF-2-T is approximately proportional to the intensity of the absorption of **3** (see Figure 1).

To analyze the photoirradiation products of these bicyclic nitrosamines, a solution of nitrosamine **1** (10 mM) in $CDCl_3$ was irradiated with visible light (500 nm) at 20 °C in the presence of triphenylmethanethiol (10 mM) to trap the dissociated NO and prevent regeneration of the nitrosamine. In this experiment, light at 500 nm was used to avoid absorption peaks of *S*-nitrosotriphenylmethanethiol at 603 nm and around 400 nm. After 24 h of irradiation, the ^{1}H NMR spectra showed that 20% of the nitrosamine had been converted to the corresponding amine under these conditions (Figure S3 in the Supporting Information). The formation of the resultant amine and *S*-nitrosotriphenylmethanethiol was confirmed by the detection of these species by means of ESI-TOF mass spectrometry (data not shown). After 24 h without light irradiation, less than 3% of the nitrosamine had been converted to the corresponding amine (see Figure S3 in the Supporting Information). Therefore, it seems reasonable to conclude that at least one of the photolysis products of nitrosamine **1** is the corresponding amine.

Conclusion

We have found that nitrosamine derivatives of 7-azabicyclo-[2.2.1]heptanes act as innate organic caged-NOs, which undergo N–NO bond cleavage upon exposure to visible light at wavelengths longer than 420 nm (e.g., up to 480 nm), thereby releasing NO. Furthermore, the *N*-nitrosoamino functionality embedded in the bicyclic 7-azabicylo-[2.2.1]heptane structure lacks mutagenicity in the typical Ames assay, that is, it is inert to metabolic α-hydroxylation, which is the trigger of mutagenic events.[10] All these properties arise from the nature of the bicyclic structure and the resultant pyramidal structure of the nitrosamino moiety. Bathochromic shifts of the absorptions of these nitrosamines, attributed to HOMO (n)–LUMO (π*) transitions associated with the nonplanar structure of the N–NO moiety, enable the molecules to absorb visible light, which results in N–NO bond cleavage. These bicyclic *N*-nitrosamines are stable at room temperature and in sunlight under the usual conditions. The applicability of the present bicyclic nitrosamines as tools for spatially and temporally controlled release of NO in biological research is currently being investigated in our laboratories.

Experimental Section

General methods of photochemical experiments: Fluorescence spectroscopic studies were performed on a Perkin–Elmer LS55 instrument. UV/Vis absorption spectra were recorded on Shimadzu UV-1650PC and JASCO V-550 UV/Vis spectrometers. Photoirradiation experiments in cuvettes were carried out in a monochromator unit (Bunko-Keiki Co., Ltd., Japan) equipped with a 500 W xenon lamp (Usio Inc., Japan) as a light source. The light intensity was measured with a Nova Display (OPHIR Japan Ltd., Japan).

DAF-2 assay: An aliquot of a stock solution of DAF-2 in DMSO (5 μL, 5 mM) and an aliquot of a stock solution of the nitrosamine in DMSO (40 μL, 6.25 mM) were diluted by adding PBS buffer (2555 μL, pH 7.4) in a $1.0\times1.0\,cm^2$ quartz cuvette; the final concentrations were 9.615 μM DAF-2 and 96.15 μM nitrosamine in PBS containing 1.7% DMSO. The cuvette containing the prepared solution was held on a cell holder placed in the light path of a monochromator, and illuminated at (420±20) nm ($2.3\,mW\,cm^{-2}$ at 420 nm) for a specified period. After each irradiation, the fluorescence spectrum was recorded in the range from 450 to 650 nm, with 492 nm excitation. The setup parameters were as follows: excitation slit: 5.0 nm, emission slit: 2.5 nm, scan speed: $200\,nm\,min^{-1}$, photomultiplier voltage: auto. When dissolved dioxygen was expelled from the solution, the solution was bubbled with Ar gas for 20 min before the photoirradiation. The fluorescence intensity of DAF-2-T was converted to concentration of NO on the basis of the calibration curve method: an aliquot of a stock solution of DAF-2 in DMSO (40 μL, 5 mM) and DMSO (320 μL) were diluted by adding PBS buffer (20.44 mL, pH 7.4; PBS–DMSO (containing 1.7% DMSO)). An aliquot of saturated PBS stock solution (2.5, 5.0, 10, or 20 μL, 1.9 mM) of NO (gas)[15] was added to an aliquot of the above DAF-2 solution (2600 μL, 9.62 μM) in a glass vial equipped with a magnetic stirrer. The final concentrations of NO were 1.9, 3.8, 7.6, and 15.2 μM, respectively. Each solution was stirred at RT for 4 h, and the fluorescence was measured in a quartz cuvette in a similar manner to that described above. The results were used to prepare the analytical curve ($r > 0.99$).

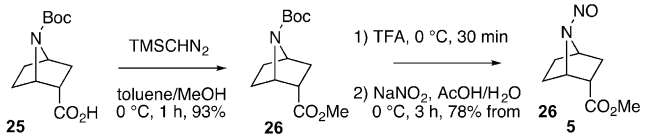
TPPCo assay: For this assay, PBS buffer and THF were purchased from Dojindo, Japan and cobalt(II) *meso*-tetraphenylporphyrin (TPPCo) was purchased from Aldrich, Milwaukee. An aliquot of a stock solution TPPCo in THF (1500 μL, 200 μM) and an aliquot of a stock solution of the nitrosamine in THF (1500 μL, 2 mM) were added to a $1.0\times1.0\,cm^2$ quartz cuvette; the final concentrations were 100 μM TPPCo and 1 mM nitrosamine in THF. The cuvette containing the prepared solution was held on a cell holder placed in the light path of a monochromator, and illuminated at (480±20) nm ($1.8\,mW\,cm^{-2}$ at 480 nm) for a specified period. After each irradiation, the electronic absorption spectrum was recorded in the range from 450 to 650 nm. The spectral change during photolysis was represented by difference spectra (Figure 4), obtained by subtracting the initial spectrum from the spectra recorded at different photolytic uncaging times.

General methods for synthesis: All the reagents were commercially available and used without further purification, unless otherwise noted. All the NMR data were recorded on a Bruker Avance 400 NMR spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR spectroscopy). $CDCl_3$ was used as the solvent, unless otherwise noted. Chemical shifts (δ) are reported in ppm with respect to internal standard tetramethylsilane (TMS, $\delta = 0$ ppm) or undeuterated residual solvent (i.e., $CHCl_3$, $\delta = 7.27$ ppm). Coupling constants are given in hertz. Coupling patterns are indicated as follows: m = multiplet, d = doublet, s = singlet, br = broad. High-resolution mass spectrometry (HRMS) was carried out in electron spray ionization (ESI) time-of-flight (TOF) detection mode and the mass spectra were recorded on a Bruker micrOTOF-05 instrument. Column chromatography was carried out on silica gel (silica gel 60N, 100–210 μm; Kanto Chemicals, Japan). All the melting points were measured with a Yanaco Micro Melting Point Apparatus and are uncorrected. Microwave heating was carried out on a Biotage Initiator system (400 W). Combustion analyses were carried out in the microanalysis laboratory of our faculty.

Synthesis of *N*-nitrosamines: The bicyclic nitrosamines **1** and **2** and the monocyclic *N*-nitrosamines **16** and **17** had been synthesized previously[9,10,16] and were used in this work. The experimental details of the synthesis of some of the other nitrosamines were described in refs. [9,10]

and the details of the synthesis of **3** and **4** are shown in the Supporting Information.

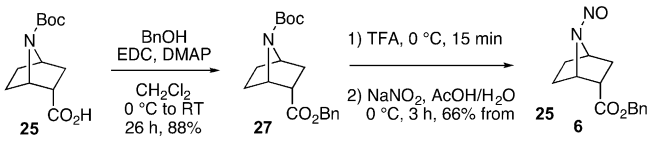
Synthesis of nitrosamine 5:



Synthesis of ***26***: The synthesis of **25** was carried out as described in ref. [17]. Trimethylsilyldiazomethane ($TMSCHN_2$, 2 M solution in Et_2O, 1800 mL, 3.6 mmol) was added dropwise at 0 °C to a solution of the acid **25** (Boc = *tert*-butoxycarbonyl; 358.6 mg, 1.486 mmol) in toluene (16.5 mL) and MeOH (4.0 mL), and the whole was stirred for 1 h at 0 °C. The reaction mixture was evaporated and the residue was purified by column chromatography (*n*-hexane/AcOEt = 7:1 to 5:1) to give **26** (353.1 mg, 1.383 mmol, 93 % yield) as a colorless solid. ^{1}H NMR ($CDCl_3$): δ = 4.37 (br s, 1H; bridgehead), 4.21 (br s, 1H; bridgehead), 3.70 (s, 3H; -CO_2*Me*), 3.05–3.03 (m, 1H), 2.00–1.46 (m, 6H), 1.45 ppm (s, 9H; -Boc).

Synthesis of ***5***: Compound **26** (353.1 mg, 1.383 mmol) was added to trifluoroacetic acid (TFA, 3 mL) at 0 °C and the reaction mixture was stirred for 30 min at this temperature. Then, TFA was evaporated to give the crude amine. An aqueous solution of $NaNO_2$ (288.8 mg, 4.186 mmol) in H_2O (13 mL) was added to a solution of the resultant amine derivative in acetic acid (3 mL) over 1 min at 0 °C, and the whole was stirred for 3 h at 0 °C. The reaction mixture was poured into water (30 mL) and extracted with (Et_2O, 3 × 50 mL). The organic layer was dried over sodium sulfate and evaporated. The residue was purified by column chromatography (*n*-hexane/AcOEt = 4:1 to 2:1) to give **5** (199.0 mg, 1.080 mmol, 78 % yield) as a yellow oil. Two isomers with respect to the N–NO bond were present. ^{1}H NMR ($CDCl_3$): δ = 5.18–5.15 (m, 1H; bridgehead), 5.01–4.96 (m, 1H; bridgehead), 3.77 (s, 1.5H; -CO_2*Me*), 3.73 (s, 1.5H; -CO_2*Me*), 3.19–3.16 (m, 0.5H), 2.99–2.97 (m, 0.5H), 2.18–1.61 ppm (m, 6H); ^{13}C NMR ($CDCl_3$): δ = 171.80, 59.39, 58.57, 52.98, 52.49, 52.32, 45.97, 43.53, 32.75, 30.33, 29.20, 27.06, 25.43, 23.23 ppm; HRMS (ESI-TOF,): *m*/*z* calcd for $C_8H_{12}N_2NaO_3^+$: 207.0746 [*M*+Na]$^+$; found: 207.0746; elemental analysis calcd (%) for $C_8H_{12}N_2O_3 + 1/6H_2O$: C 51.33, H 6.64, N 14.96; found: C 51.57, H 6.45, N 15.01.

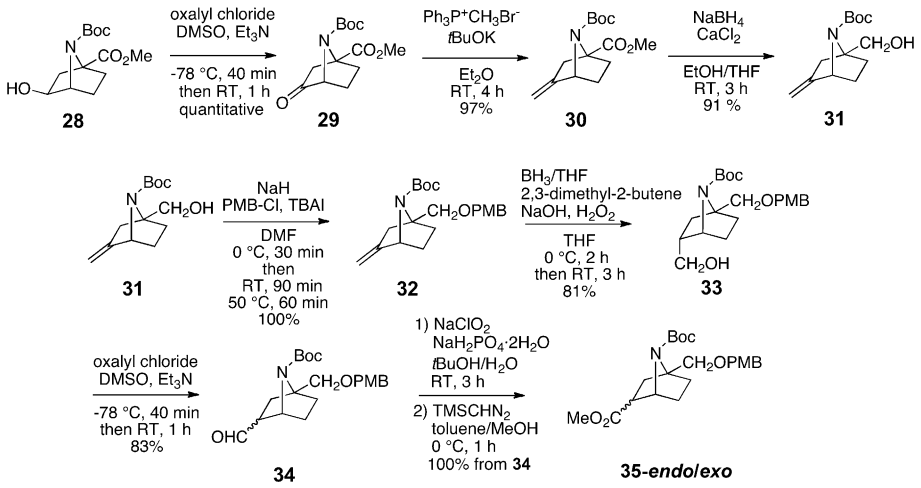
Synthesis of nitrosamine 6:



Synthesis of ***27***: A solution of **25** (121.6 mg, 0.5040 mmol), 4-dimethylaminopyridine (DMAP; 11.2 mg, 0.0917 mmol), and benzyl alcohol (BnOH; 60 μL, 62.7 mg, 0.5798 mmol) in dry CH_2Cl_2 (2 mL) was cooled with stirring in an ice-water bath under Ar. *N*-Ethyl-*N*′-(3-dimethyl(aminopropyl)carbodiimide (EDC; 124.8 mg, 0.6510 mmol) was added, and the reaction mixture was stirred at RT for 26 h. The solution was concentrated to dryness in vacuum, the residue was diluted with water (40 mL), and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was washed with brine (5 mL) and dried over Na_2SO_4. The solvent was removed in vacuum, and the residue was purified by column chromatography (*n*-hexane/AcOEt = 5:1) to give **27** (147.1 mg, 0.4439 mmol, 88 % yield, recrystallized from *n*-hexane/Et_2O) as colorless needles. M.p. 46.2–48.3 °C; ^{1}H NMR ($CDCl_3$): δ = 7.37–7.32 (m, 5H), 5.14 (s, 2H), 4.41 (br s, 1H), 4.21 (br s, 1H), 3.10–3.08 (m, 1H), 1.98–1.64 (m, 6H), 1.44 ppm (s, 9H); HRMS (ESI-TOF): *m*/*z* calcd for $C_{19}H_{25}NNaO_4^+$: 354.1676 [*M*+Na]$^+$; found: 354.1665; elemental analysis calcd (%) for $C_{19}H_{25}NO_4$: C 68.86, H 7.60, N 4.23; found: C 68.89, H 7.56, N 4.25.

Synthesis of ***6***: Compound **27** (134.6 mg, 0.4061 mmol) was dissolved in TFA (3 mL) at 0 °C and the reaction mixture was stirred for 15 min at this temperature. Then, TFA was evaporated to give the crude amine. An aqueous solution of $NaNO_2$ (85.4 mg, 1.238 mmol) in H_2O (3.0 mL) was added to a solution of the resultant amine derivative in acetic acid (1.5 mL) over 1 min at 0 °C, and the mixture was stirred for 3 h at this temperature. The reaction mixture was poured into water (40 mL) and the whole was extracted with Et_2O (3 × 30 mL). The organic layer was washed with water (40 mL) and brine (5 mL), dried over Na_2SO_4, and evaporated to give **6** (70.0 mg, 0.2689 mmol, 66 % yield) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ = 7.40–7.33 (m, 5H), 5.20–5.12 (m, 3H), 5.01–4.96 (m, 1H), 3.24–3.20 (m, 0.5H), 3.04–3.01 (m, 0.5H), 1.77–1.50 ppm (m, 6H); ^{13}C NMR ($CDCl_3$): δ = 171.06, 171.04, 135.32, 128.61, 128.58, 128.49, 128.44, 128.29, 128.21, 66.99, 66.94, 59.34, 58.54, 52.95, 52.46, 46.01, 43.58, 32.61, 30.20, 29.12, 26.97, 25.25, 23.00 ppm; HRMS (ESI-TOF): *m*/*z* calcd for $C_{14}H_{16}N_2NaO_3^+$: 283.1053 [*M*+Na]$^+$; found: 283.1084; elemental analysis calcd (%) for $C_{14}H_{16}N_2O_3$: C 64.60, H 6.20, N 10.76; found: C 64.75, H 6.31, N 10.71.

Synthesis of bridgehead-substituted derivatives:



Synthesis of ***29***: The synthesis of **28** was described in ref. [18]. DMSO (4.5 mL, 63.36 mmol) at −78 °C was added to a solution of oxalyl chloride (3.6 mL, 42.54 mmol) in CH_2Cl_2 (220 mL). The reaction mixture was stirred for 20 min and a solution of **28** (6.738 g, 24.84 mmol) in CH_2Cl_2 (330 mL) was added to this solution at −78 °C. The reaction mixture was stirred for 40 min at −78 °C, and then Et_3N (20.0 mL, 144.28 mmol) was added. The solution was allowed to warm to RT. After stirring for 1 h, the reaction mixture was quenched by the addition of water. The whole was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. Column chromatography of the residue (*n*-hexane/AcOEt = 3:1) gave compound **29** (7.229 g, 100 %) as a yellow oil. ^{1}H NMR ($CDCl_3$): 4.35 (d, *J* = 5.6 Hz, 1H; bridgehead), 3.84 (s, 3H; $COOCH_3$), 2.85 (m, 1H), 2.40–2.29

(m, 2H), 2.19–2.13 (m, 1H), 1.91–1.85 (m, 1H), 1.70–1.63 (m, 1H; CH_2×3), 1.41 ppm (s, 9H; -C(CH_3)$_3$).

Synthesis of ***30***: Methyltriphenylphosphonium bromide (17.993 g, 50.37 mmol) at 0°C was added to a solution of *t*BuOK (5.139 g, 45.80 mmol) in Et_2O (130 mL). The reaction mixture was heated at reflux with stirring for 1 h, and then cooled to RT. A solution of **29** (6.608 g, 24.84 mmol) in Et_2O (130 mL) was added to the above mixture at RT, and the reaction mixture was stirred for an additional 4 h at RT. The reaction mixture was quenched by the addition of water, and the whole was extracted with Et_2O (3×50 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt=5:1) gave compound **30** (6.421 g, 97%) as a yellow oil. ^{1}H NMR ($CDCl_3$): 4.97–4.96 (m, 1H), 4.80 (s, 1H; C=CH_2), 4.54 (d, *J*=4.64 Hz, 1H; bridgehead), 3.81 (s, 3H; COOCH_3), 2.84 (m, 1H), 2.42–2.38 (m, 1H), 2.23–2.20 (m, 1H), 2.11–2.03 (m, 1H), 1.82–1.75 (m, 1H), 1.60–1.54 (m, 1H; CH_2×3), 1.40 ppm (s, 9H; -C(CH_3)$_3$).

Synthesis of ***31***: $CaCl_2$ (5542.5 mg, 49.941 mmol) and $NaBH_4$ (3879.3 mg, 102.55 mmol) at 0°C were added to a solution of **30** (6.421 g, 24.02 mmol) in EtOH (100 mL)/THF (70 mL). The reaction mixture was stirred for 3 h at RT and quenched by the addition of a 10% aqueous solution of citric acid. Then, the inorganic salts were isolated by filtration through a Celite pad and the residue was washed with AcOEt (70 mL). The filtrate was extracted with AcOEt (3×100 mL). The organic phases were combined, washed with brine, dried over Na_2SO_4, and evaporated. Column chromatography (*n*-hexane/AcOEt=3:1) of the residue gave compound **31** (5249.1 mg, 91%) as a colorless oil. ^{1}H NMR ($CDCl_3$): 4.94–4.92 (m, 1H; C=CH_2), 4.88 (br s, 1H; -OH), 4.75 (s, 1H; C=CH_2), 4.48 (d, *J*=4.08 Hz, 1H; bridgehead), 3.96–3.87 (m, 2H; -CH_2OH), 2.62–2.57 (m, 1H), 2.07–2.02 (m, 1H), 1.94–1.85 (m, 2H), 1.65–1.53 (m, 1H), 1.50–1.42 (m, 1H; CH_2×3), 1.44 ppm (s, 9H; -C(CH_3)$_3$).

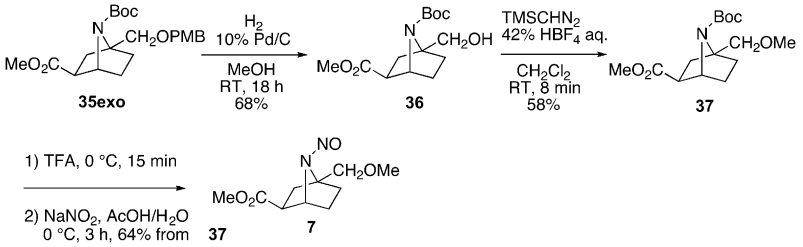
Synthesis of ***32***: NaH (1898.9 mg, 47.47 mmol, about 60%) at 0°C was added to a solution of **31** (5249.1 mg, 21.93 mmol) in DMF (170 mL). The reaction mixture was stirred for 30 min at 0°C. Then *para*-methoxybenzyl chloride (PMB-Cl; 4.4 mL, 32.45 mmol) and tetrabutylammonium iodide (TBAI; 791.2 mg, 2.14 mmol) at 0°C were added. The reaction mixture was stirred for 1.5 h at RT and then heated at 50°C for 1 h. The mixture was poured into water and extracted with Et_2O (3×150 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt=16:1 to 14:1) gave compound **32** (8000.9 mg, 101%) as a colorless oil. ^{1}H NMR ($CDCl_3$): 7.27 (d, *J*=8.72 Hz, 2H), 6.87 (d, *J*=8.68 Hz, 2H; -*Ph*-OMe), 4.91–4.90 (m, 1H), 4.72–4.4.71 (m, 1H; C=CH_2), 4.54 (s, 2H; -O-CH_2-Ph-OMe), 4.50 (d, *J*=4.80 Hz, 1H; bridgehead), 4.08 (d, *J*=9.60 Hz, 1H; -CH_2-O-), 4.04 (d, *J*=9.60 Hz, 1H; -CH_2-O-), 3.81 (s, 3H; -OMe), 2.42–2.30 (m, 2H), 1.91–1.72 (m, 3H), 1.52–1.1.44 (m, 1H; CH_2×3), 1.42 ppm (s, 9H; -C(CH_3)$_3$).

Synthesis of ***33***: BH_3/THF (45 mL, 1.08 M in THF) at 0°C was added to 2,3-dimethyl-2-butene (50 mL, 1.0 M in THF), and the reaction mixture was stirred at 0°C for 1 h. Then, a solution of **32** (7905.2 mg, 21.99 mmol) in THF (80 mL) was added at 0°C, and the reaction mixture was stirred for 2 h at 0°C. Next, 3 N aqueous NaOH (16 mL, 53.7 mmol) and 30% H_2O_2 (16 mL) were added at 0°C. The reaction mixture was stirred for 3 h at RT, then poured into water, and the whole was extracted with CH_2Cl_2 (3×100 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. Column chromatography (*n*-hexane/AcOEt=1:1) of the residue gave compound **33** (6690.0 mg, 81%, *endo*/*exo*=95:5) as a colorless oil. ^{1}H NMR ($CDCl_3$): 7.25 (d, *J*=8.60 Hz, 2H), 6.87 (d, *J*=8.68 Hz, 2H; -Ph), 4.52 (s, 2H; -O-CH_2-Ph), 4.31–4.27 (m, 1H; bridgehead), 4.01–3.94 (m, 2H; -CH_2-OCH$_2$Ph), 3.80 (s, 3H; -Ph-OCH_3), 3.75–3.71 (m, 1H), 3.55–3.50 (m, 1H), 2.43–2.33 (m, 1H; *H*-C(2)), 2.0–1.54 (m, 6H; CH_2×3), 1.43 (s, 9H; -C(CH_3)$_3$), 1.35 ppm (br s, 1H).

Synthesis of ***34***: DMSO (7.6 mL, 107.00 mmol) at −78°C was added to a solution of oxalyl chloride (7.0 mL, 82.72 mmol) in CH_2Cl_2 (370 mL). The reaction mixture was stirred for 20 min and a solution of **33** (6774.0 mg, 17.95 mmol, obtained in the above reaction) in CH_2Cl_2 (180 mL) was added at −78°C. The reaction mixture was stirred for 40 min at −78°C, and then Et_3N (30 mL, 216.42 mmol) was added. The solution was allowed to warm to RT. After 1 h of stirring, the mixture was quenched by the addition of water and extracted with CH_2Cl_2 (3×100 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt=6:1 to 3:1) gave compound **34** (5563.6 mg, 83%, *endo*/*exo*=2:1) as a yellow oil. ^{1}H NMR ($CDCl_3$): 9.73 (d, *J*=1.40 Hz, 0.67H; -CHO of *endo* isomer), 9.65 (d, *J*=1.80 Hz, 0.33H; -CHO of *exo* isomer), 7.26 (d, *J*=8.64 Hz, 2H), 6.87 (d, *J*=8.72 Hz, 2H; -Ph), 4.62–4.59 (m, 1H; bridgehead), 4.54–4.53 (s, 2H; -O-CH_2-Ph), 4.05–3.96 (m, 2H; -CH_2-OCH$_2$Ph), 3.81 (s, 3H; -Ph-OCH_3), 3.09–3.04 (m, 0.67H; *H*-C(2) of *endo* isomer), 2.53–2.49 (m, 0.33H; *H*-C(2) of *exo* isomer), 2.14–2.10 (m, 1H), 1.92–1.64 (m, 4H), 1.53–1.45 (m, 1H; CH_2×3), 1.44 (s, 6.03H; -C(CH_3)$_3$ of *endo* isomer), 1.36 ppm (s, 2.97H; -C-(CH_3)$_3$ of *exo* isomer).

Synthesis of ***35endo/exo***: 2-Methyl-2-butene (30 mL) and a solution of $NaClO_2$ (12.05 g, 133.26 mmol) and NaH_2PO_4·2H_2O (16.18 g, 103.72 mmol) in H_2O (125 mL) at RT were added to a solution of **34** (5563.6 mg, 14.82 mmol) in *t*BuOH (125 mL), and the reaction mixture was stirred for 3 h at RT. *t*BuOH was evaporated, then the aqueous residue was poured into a 0.5 N aqueous solution of HCl and the whole was extracted with $CHCl_3$ (3×100 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated to give the crude carboxylic acid as a colorless oil. $TMSCHN_2$ (15 mL, 2.0 M Et_2O solution, 30.0 mmol) at 0°C was added to a solution of the resultant carboxylic acid derivative in toluene/MeOH (200 mL/50 mL). The reaction mixture was stirred for 1 h at RT and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt=12:1 to 3:1) gave compound **35-*exo*** (1799.3 mg, 36% yield) as a colorless oil and **35-*endo*** (2982.9 mg, 60% yield) as a colorless oil (5207.0 mg, quantitative yield from **34**, combined yield). **35-*exo***: ^{1}H NMR ($CDCl_3$): 7.27 (d, *J*=8.48 Hz, 2H), 6.87 (d, *J*=8.60 Hz, 2H; -Ph), 4.57 (d, *J*=4.80 Hz, 1H; bridgehead), 4.54 (s, 2H; -O-CH_2-Ph), 4.07 (s, 2H; -CH_2-OCH$_2$Ph), 3.80 (s, 3H; -Ph-OCH_3), 3.70 (s, 3H; -COOCH_3), 2.58–2.54 (m, 1H; *H*-C(2)), 2.24–2.19 (m, 1H), 1.93–1.66 (m, 4H), 1.49–1.43 (m, 1H; CH_2×3), 1.40 ppm (s, 9H; -C(CH_3)$_3$). **35-*endo***: ^{1}H NMR ($CDCl_3$): 7.26 (d, *J*=8.48 Hz, 2H), 6.87 (d, *J*=8.56 Hz, 2H; -Ph), 4.53 (s, 2H; -O-CH_2-Ph), 4.46–4.44 (m, 1H; bridgehead), 4.01 (d, *J*=9.60 Hz, 1H), 3.96 (d, *J*=9.60 Hz, 1H; -CH_2-OCH$_2$Ph), 3.80 (s, 3H; -Ph-OCH_3), 3.70 (s, 3H; -COOCH_3), 3.08–3.02 (m, 1H; *H*-C(2)), 2.11–2.06 (m, 1H), 2.00–1.93 (m, 1H), 1.78–1.69 (m, 3H), 1.51–1.40 (m, 1H; CH_2×3), 1.43 ppm (s, 9H; -C(CH_3)$_3$).

Synthesis of nitrosamine 7:



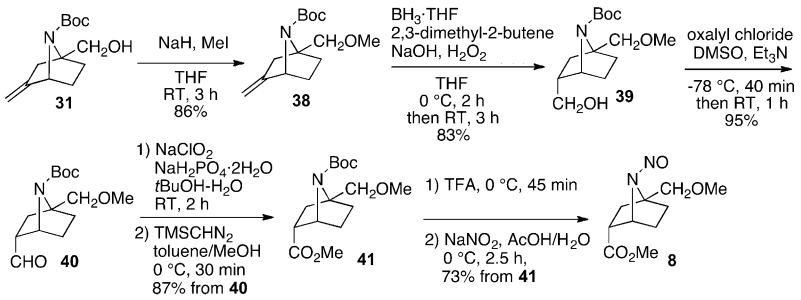
Synthesis of ***36***: Compound **35-*exo*** (750.6 mg, 2.245 mmol) was hydrogenated over 10% Pd/C (170.1 mg) in methanol (75 mL) at RT for 18 h. Pd/C was removed by filtration. The residue obtained after evaporation of the solvent was purified by column chromatography (*n*-hexane/AcOEt=3:1) to afford **36** (436.9 mg, 1.531 mmol, 68% yield) as a colorless oil.

^{1}H NMR ($CDCl_3$): δ = 4.81 (br s, 1H), 4.52–4.50 (m, 1H), 3.92 (d, J = 6.4 Hz, 1H × 2), 3.69 (s, 3H), 2.63–2.59 (m, 1H), 2.38–2.33 (m, 1H), 1.87–1.77 (m, 2H), 1.61–1.41 (m, 2H), 1.38 (s, 9H), 1.38–1.36 ppm (m, 1H); ^{13}C NMR ($CDCl_3$): δ = 173.55, 80.42, 69.15, 61.47, 61.00, 51.96, 46.83, 35.09, 31.38, 28.91, 28.20 ppm (the carbamate carbon atom was not detected); HRMS (ESI-TOF): m/z calcd for $C_{14}H_{23}NNaO_5^+$: 308.1468 $[M+Na]^+$; found: 308.1432.

Synthesis of ***37***: A 42% aqueous solution of HBF_4 (120 μL) at RT was added to a solution of **36** (102.6 mg, 0.3596 mmol) in CH_2Cl_2 (3.5 mL). $TMSCHN_2$ in 2.0 M Et_2O was added in portions over 8 min at RT [175 μL, 525 μL after 1 min, 525 μL after 1 min, 525 μL after 1 min (1750 μL in total, 3.5 mmol in total)]. The reaction mixture was poured into water (30 mL) and the whole was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phase was washed with brine (2 mL), dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt = 4:1) gave compound **37** (62.5 mg, 58%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ = 4.56–4.55 (m, 1H), 4.16 (d, J = 9.6 Hz, 1H), 3.97 (d, J = 9.6 Hz, 1H), 3.68 (s, 3H), 3.40 (s, 3H), 2.56–2.53 (m, 1H), 2.23–2.17 (m, 1H), 1.85–1.65 (m, 3H), 1.64–1.55 (m, 1H), 1.48–1.40 (m, 1H), 1.39 ppm (s, 9H); ^{13}C NMR ($CDCl_3$): δ = 173.70, 154.29, 79.77, 74.44, 67.03, 61.42, 59.38, 51.96, 46.61, 36.98, 32.85, 28.62, 28.20 ppm; HRMS (ESI-TOF):
m/z calcd for $C_{15}H_{25}NNaO_5^+$: 322.1625 $[M+Na]^+$; found: 322.1609.

Synthesis of ***7***: Compound **37** (62.5 mg, 0.2088 mmol) was dissolved in ice-cooled TFA (1 mL) and the reaction mixture was stirred for 15 min at 0 °C. Then, TFA was evaporated to give the crude amine. An aqueous solution of $NaNO_2$ (58.0 mg, 0.8406 mmol) in H_2O (1.0 mL) was added over 1 min at 0 °C to a solution of the amine derivative in acetic acid (0.5 mL), and the whole was stirred for 3 h at this temperature. The reaction mixture was poured into water (30 mL) and extracted with Et_2O (3 × 30 mL). The organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (2 mL) and brine (2 mL), dried over sodium sulfate, and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt = 3:1) gave compound **7** (30.6 mg, 0.1341 mmol, 64% from **37**) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ = 5.29–5.28 (m, 1H), 4.15 (s, 2H), 3.67 (s, 3H), 3.47 (s, 3H), 2.75–2.71 (m, 1H), 2.45–2.18 (m, 1H), 1.92–1.59 ppm (m, 5H); ^{13}C NMR ($CDCl_3$): δ = 172.32, 70.70, 68.14, 59.70, 55.51, 52.41, 44.99, 36.08, 31.93, 26.77 ppm; HRMS (ESI-TOF): m/z calcd for $C_{10}H_{16}N_2NaO_4^+$: 251.1002 $[M+Na]^+$; found: 251.1012.

Synthesis of nitrosamine 8:



Synthesis of ***38***: A solution of **31** (117.7 mg, 0.4918 mmol) in THF (1.2 mL) at 0 °C was added to a solution of NaH (86.3 mg, 2.158 mmol, about 60%) in THF (1.2 mL) and the reaction mixture was stirred for 30 min at 0 °C. Then, MeI (50 μL, 0.8032 mmol) was added at 0 °C. The reaction mixture was stirred for 3 h at RT, poured into 0.5 N aqueous HCl solution, and extracted with Et_2O (3 × 30 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane to *n*-hexane/AcOEt = 3:1) gave compound **38** (106.7 mg, 86%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ = 4.92–4.91 (m, 1H), 4.73–4.72 (m, 1H), 4.50 (d, J = 4.4 Hz, 1H), 4.00 (s, 2H), 3.42 (s, 3H), 2.47–2.41 (m, 1H), 2.30–2.25 (m, 1H), 1.96–1.79 (m, 2H), 1.70–1.63 (m, 1H), 1.55–1.46 (m, 1H), 1.42 ppm (s, 9H); HRMS (ESI-TOF): m/z calcd for $C_{14}H_{23}NNaO_3^+$: 276.1570 $[M+Na]^+$; found: 276.1578.

Synthesis of ***39***: BH_3·THF (1000 μL, 1.0 M in THF) at 0 °C was added to 2,3-dimethyl-2-butene (1000 μL, 1.0 M in THF), and the reaction mixture was stirred for 1 h at 0 °C. Then, a solution of **38** (106.7 mg, 0.4212 mmol) in THF (2 mL) was added at 0 °C, and the reaction mixture was stirred for 2 h at 0 °C. Next, 3 N aqueous NaOH (340 μL, 1.02 mmol) and 30% H_2O_2 (340 μL) were added at 0 °C, and the whole was stirred for 3 h at RT. The reaction mixture was poured into water and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. Column chromatography (*n*-hexane/AcOEt = 1:1) of the residue gave compound **39** (95.2 mg, 83%, *endo*/*exo* = 87:13) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ = 4.31–4.29 (m, 0.87H), 4.25–4.24 (m, 0.13H), 3.97 (d, J = 9.6 Hz, 1H), 3.92 (d, J = 9.6 Hz, 1H), 3.77–3.71 (m, 1H), 3.56–3.50 (m, 1H), 2.40–2.37 (m, 1H), 2.01–1.94 (m, 1H), 1.77–1.70 (m, 3H), 1.44 (s, 9H), 1.29–1.15 ppm (m, 2H); HRMS (ESI-TOF): m/z calcd for $C_{14}H_{25}NNaO_4^+$: 294.1676 $[M+Na]^+$; found: 294.1646.

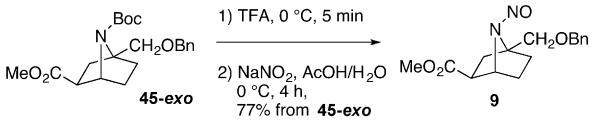
Synthesis of ***40***: DMSO (50 μL, 0.7040 mmol) at −78 °C was added to a solution of oxalyl chloride (50 μL, 0.5909 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred for 20 min and a solution of **39** (95.2 mg, 0.3508 mmol) in CH_2Cl_2 (7 mL) was added at −78 °C. The reaction mixture was stirred for 40 min at −78 °C, and then Et_3N (200 μL, 1.443 mmol) was added. The solution was allowed to warm to RT. After 1 h of stirring, the reaction mixture was quenched by addition of water. The mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. Column chromatography (*n*-hexane/AcOEt = 2:1) of the residue gave compound **40** (90.0 mg, 95%, *endo*/*exo* = 89:11) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ = 9.74 (s, 0.89H), 9.67 (s, 0.11H), 4.62–4.60 (m, 1H), 4.02 (d, J = 9.6 Hz, 0.11H), 3.99 (d, J = 9.6 Hz, 0.11H), 3.96 (d, J = 9.6 Hz, 0.89H), 3.93 (d, J = 10.0 Hz, 0.89H), 3.43 (s, 3H), 3.11–3.06 (m, 0.89H), 2.54–2.50 (m, 0.11H), 2.10–2.05 (m, 1H), 1.93–1.46 (m, 5H), 1.45 ppm (s, 9H); HRMS (ESI-TOF): m/z calcd for $C_{14}H_{23}NNaO_4^+$: 292.1519 $[M+Na]^+$; found: 292.1510.

Synthesis of ***41***: 2-Methyl-2-butene (760 μL) and a solution of $NaClO_2$ (283.7 mg, 3.137 mmol) and NaH_2PO_4·$2H_2O$ (419.7 mg, 2.690 mmol) in H_2O (3 mL) at RT were added to a solution of **40** (90.0 mg, 0.3342 mmol) in *t*BuOH (3 mL), and the reaction mixture was stirred for 2 h at RT. *t*BuOH was evaporated and the aqueous residue was poured into a 10% aqueous solution of citric acid, and extracted with $CHCl_3$ (3 × 20 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated to give the crude carboxylic acid as a colorless oil. $TMSCHN_2$ (400 μL, 2.0 M in Et_2O) at 0 °C was added to a solution of the resultant carboxylic acid derivative in CH_2Cl_2 (3 mL)/MeOH (1 mL). The reaction mixture was stirred for 30 min at RT, then evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt = 9:1) gave compound **41** (86.8 mg, 87%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.47–4.44 (m, 1H), 3.96 (d, J = 9.6 Hz, 1H), 3.91 (d, J = 9.6 Hz, 1H), 3.70 (s, 3H), 3.42 (s, 3H), 3.09–3.03 (m, 1H), 2.07–1.45 (m, 6H), 1.44 ppm (s, 9H); HRMS (ESI-TOF): m/z calcd for $C_{15}H_{25}NNaO_5^+$: 322.1615 $[M+Na]^+$; found: 322.1586.

Synthesis of ***8***: Compound **41** (86.8 mg, 0.2900 mmol) was dissolved in TFA (1 mL) at 0 °C and the reaction mixture was stirred for 45 min at this temperature. Then, TFA was evaporated to give the crude amine. An aqueous solution of $NaNO_2$ (74.7 mg, 1.083 mmol) in H_2O (2 mL) was added to a solution of the resultant amine derivative in acetic acid (1 mL) over 1 min at 0 °C, and the whole was stirred for 2.5 h at this temperature. The reaction mixture was poured into water (50 mL) and ex-

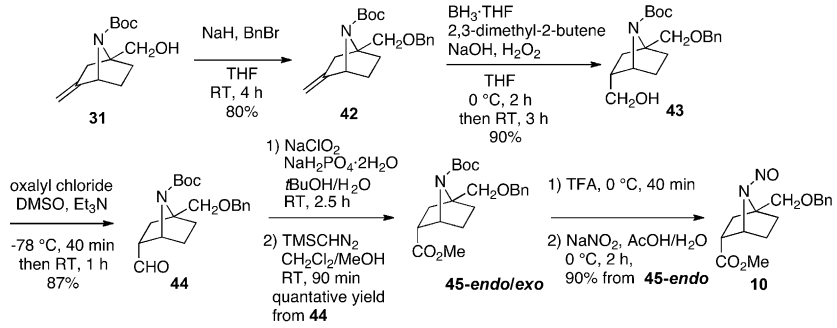
tracted with Et_2O (3×25 mL). The organic layer was washed with water and brine (5 mL), dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt = 3:1) gave compound **8** (48.6 mg, 0.2129 mmol, 73% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ = 5.21–5.18 (m, 1H), 4.12 (d, J = 10.4 Hz, 1H), 4.09 (d, J = 10.8 Hz, 1H), 3.73 (s, 3H), 3.48 (s, 3H), 3.03–2.99 (m, 1H), 2.25–1.63 ppm (m, 6H); ^{13}C NMR ($CDCl_3$): δ = 171.76, 70.26, 69.53, 59.65, 54.40, 52.28, 43.15, 35.09, 31.89, 23.02 ppm; HRMS (ESI-TOF): *m*/*z* calcd for $C_{10}H_{16}N_2NaO_4^+$: 251.1002 [*M*+Na]$^+$; found: 251.1001; elemental analysis calcd (%) for $C_{10}H_{16}N_2O_4$: C 52.62, H 7.07, N 12.27; found: C 52.62, H 6.91, N 12.27.

Synthesis of nitrosamine 9:



Synthesis of ***9***: The synthesis of **45-*exo*** is described in connection with the synthesis of nitrosamine **10**. Compound **45-*exo*** (61.4 mg, 0.1634 mmol) was dissolved in TFA (1 mL) at 0°C (in an ice-water bath) and the reaction mixture was stirred for 5 min at this temperature. Then, the acid was evaporated to give the crude amine. An aqueous solution of $NaNO_2$ (33.8 mg, 0.4902 mmol) in H_2O (2 mL) was added over 1 min at 0°C to a solution of the resultant amine derivative in acetic acid (1.5 mL). The reaction mixture was stirred for 4 h at 0°C, then poured into water (50 mL) and the whole was extracted with Et_2O (3×30 mL). The organic layer was washed with water (20 mL), dried over Na_2SO_4, and evaporated to give a residue, which was purified by column chromatography (*n*-hexane/AcOEt = 2:1) to afford **9** (38.2 mg, 0.1255 mmol, 77% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ = 7.34–7.28 (m, 5H; aromatic), 5.29 (br s, 1H; bridgehead), 4.66 (s, 2H; benzyl), 4.24 (s, 2H; C*H*$_2$OBn), 3.67 (s, 3H; -CO$_2$*Me*), 2.76–2.72 (m, 1H), 2.47–2.44 (m, 1H), 2.10–2.05 (m, 1H), 1.95–1.80 (m, 3H), 1.66–1.61 ppm (m, 1H); ^{13}C NMR ($CDCl_3$): δ = 172.36, 137.92, 128.43, 127.67, 73.77, 68.27, 55.56, 52.45, 45.07, 36.27, 32.07, 26.83 ppm (one quaternary carbon atom cannot be detected); HRMS (ESI-TOF): *m*/*z* calcd for $C_{16}H_{20}N_2NaO_4^+$: 327.1321 [*M*+Na]$^+$; found: 327.1286; elemental analysis calcd (%) for $C_{16}H_{20}N_2O_4 \cdot 2/3H_2O$: C 60.75, H 6.80, N 8.86; found: C 60.36, H 6.43, N 8.90.

Synthesis of nitrosamine 10:



Synthesis of ***42***: A solution of **31** (368.0 mg, 1.538 mmol) in THF (3.5 mL) at 0°C was added to a suspension of NaH (261.5 mg, 6.538 mmol, about 60%) in THF (3.5 mL) and the reaction mixture was stirred for 30 min at 0°C. Then, BnBr (290 μL, 2.44 mmol) was added at 0°C. The reaction mixture was stirred for 4 h at RT, then poured into a 0.5 N aqueous HCl solution (50 mL) and the whole was extracted with Et_2O (3×40 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt = 10:1) gave compound **42** (404.3 mg, 80%) as a colorless oil. ^{1}H NMR ($CDCl_3$): 7.36–7.25 (m, 5H; Ph), 4.92–4.91 (m, 1H), 4.73 (s, 1H; C=C*H*$_2$), 4.6 (s, 2H; -O-*CH*$_2$-Ph), 4.51 (d, J = 4.6 Hz, 1H; bridgehead), 4.13–4.06 (m, 2H; -C*H*$_2$-OPh), 2.48–2.44 (m, 1H), 2.36–2.33 (m, 1H), 1.97–1.71 (m, 3H), 1.53–1.47 (m, 1H; C*H*$_2$×3), 1.42 ppm (s, 9H; -C(C*H*$_3$)$_3$).

Synthesis of ***43***: BH_3·THF (1250 μL, 1.0 M in THF) at 0°C was added to 2,3-dimethyl-2-butene (1250 μL, 1.0 M in THF) and the reaction mixture was stirred for 1 h at 0°C. Then, a solution of **42** (169.1 mg, 0.5133 mmol) in THF (2.5 mL) was added at 0°C, and the whole was stirred for 2 h at 0°C. Next, 3 N aqueous NaOH (400 μL, 1.2 mmol) and 30% H_2O_2 (400 μL) were added at 0°C. The reaction mixture was stirred for 3 h at RT, poured into water, and extracted with CH_2Cl_2 (3×30 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt = 1:1) gave compound **43** (160.0 mg, 90%, *endo*/*exo* = 93:7) as a colorless oil. ^{1}H NMR ($CDCl_3$): 7.34–7.25 (m, 5H; -Ph), 4.59 (s, 2H; -O-*CH*$_2$-Ph), 4.31–4.29 (m, 0.93H; a bridgehead proton of *endo* isomer), 4.26–4.25 (m, 0.07H; a bridgehead proton of *exo* isomer), 4.07–3.99 (m, 2H; -C*H*$_2$-OCH$_2$Ph), 3.75–3.71 (m, 0.93H), 3.55–3.50 (m, 0.93H; -C*H*$_2$OH×2 of *endo* isomer), 3.46–3.36 (m, 0.14H; -C*H*$_2$OH×2 of *exo* isomer), 2.43–2.33 (m, 0.93H; *H*-C(2) of *endo* isomer), 2.02–1.45 (m, 6.07H; *H*-C(2) of *exo* isomer + C*H*$_2$×3), 1.43 (s, 8.37H; -C(C*H*$_3$)$_3$ of *endo* isomer), 1.42 ppm (s, 0.63H; -C(C*H*$_3$)$_3$ of *exo* isomer).

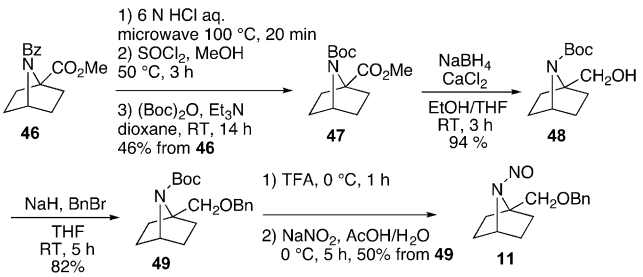
Synthesis of ***44***: DMSO (145 μL, 2.04 mmol) at −78°C was added to a solution of oxalyl chloride (140 μL, 1.65 mmol) in CH_2Cl_2 (15 mL). The reaction mixture was stirred for 20 min and a solution of **43** (350.6 mg, 1.009 mmol) in CH_2Cl_2 (20 mL) was added at −78°C. Stirring was continued for 40 min at −78°C, and then Et_3N (568 μL, 4.09 mmol) was added. The solution was allowed to warm to RT. After 1 h of stirring, the reaction mixture was quenched by addition of water and extracted with CH_2Cl_2 (3×50 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt = 4:1) gave compound **44** (315.3 mg, 87%, *endo*/*exo* = 93:7) as a colorless oil. ^{1}H NMR ($CDCl_3$): 9.74 (d, J = 1.36 Hz, 0.93H; -CHO of *endo* isomer), 9.66 (d, J = 1.72 Hz, 0.07H; -CHO of *exo* isomer), 7.35–7.26 (m, 5H; -Ph), 4.63–4.61 (m, 3H; bridgehead + -O-*CH*$_2$-Ph), 4.09–4.00 (m, 2H; -C*H*$_2$-OCH$_2$Ph), 3.10–3.05 (m, 0.93H; *H*-C(2) of *endo* isomer), 2.54–2.50 (m, 0.07H; *H*-C(2) of *exo* isomer), 2.19–1.46 (m, 6H; C*H*$_2$×3), 1.44 (s, 8.37H; -C(C*H*$_3$)$_3$ of *endo* isomer), 1.39 ppm (s, 0.63H; -C(C*H*$_3$)$_3$ of *exo* isomer).

Synthesis of ***45endo/exo***: 2-Methyl-2-butene (2 mL) and a solution of $NaClO_2$ (721.9 mg, 7.982 mmol) and $NaH_2PO_4 \cdot 2H_2O$ (1059.8 mg, 6.793 mmol) in H_2O (8 mL) at RT were added to a solution of **44** (315.3 mg, 0.8772 mmol) in *t*BuOH (8 mL), and the reaction mixture was stirred for 2.5 h at RT. *t*BuOH was evaporated and the aqueous residue was poured into a 10% aqueous solution of citric acid and extracted with $CHCl_3$ (3×30 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated to give the crude carboxylic acid (439.0 mg) as a colorless oil. $TMSCHN_2$ (1.0 mL, 2.0 M in Et_2O) at 0°C was added to a solution of the resultant carboxylic acid derivative in CH_2Cl_2 (6 mL)/MeOH (2 mL), and the reaction mixture was stirred for 90 min at RT, then evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt = 10:1) gave compound **45**-*endo*/*exo* (335.6 mg, 0.8938 mmol, quantitative yield from **44**) as a colorless oil. **45**-*endo*: ^{1}H NMR ($CDCl_3$): δ = 7.35–7.26 (m, 5H), 4.61 (s, 2H), 4.47–4.45 (m, 1H), 4.06–3.98 (m, 2H), 3.70 (s, 3H), 3.09–3.03 (m, 1H), 2.13–2.10 (m, 1H), 2.09–1.96 (m, 1H), 1.80–1.71 (m, 3H), 1.51–1.45 (m, 1H), 1.44 ppm (s, 9H). **45**-*exo*: ^{1}H NMR ($CDCl_3$): δ = 7.36–7.25 (m, 5H; Ph), 4.61 (s,

2H; -O-CH_2-Ph), 4.59 (d, J=4.68 Hz, 1H; bridgehead), 4.14–4.09 (m, 2H; -CH_2-OPh), 3.70 (s, 3H; -COOCH_3), 2.59–2.55 (m, 1H; H-C(2)), 2.26–2.21 (m, 1H), 1.95–1.68 (m, 4H), 1.55–1.43 (m, 1H; CH_2×3), 1.40 ppm (s, 9H; -C(CH_3)$_3$).

Synthesis of ***10***: Compound **45**-*endo* (74.7 mg, 0.1990 mmol) was dissolved in TFA (1.0 mL) at 0°C and the reaction mixture was stirred for 40 min at this temperature. Then, TFA was evaporated to give the crude amine. An aqueous solution of $NaNO_2$ (60.0 mg, 0.8696 mmol) in H_2O (1.0 mL) was added over 1 min at 0°C to a solution of the resultant amine derivative in acetic acid (0.5 mL), and the whole was stirred for 2 h at this temperature. The reaction mixture was poured into water (40 mL) and extracted with Et_2O (30 mL ×3). The organic layer was washed with brine (5 mL), dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt=3:1) gave compound **10** (54.5 mg, 0.1791 mmol, 90% from **45**-*endo*) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ=7.36–7.30 (m, 5H), 5.21 (m, 1H), 4.66 (s, 2H), 4.20 (d, J=10.8 Hz, 1H), 4.16 (d, J=10.4 Hz, 1H), 3.73 (s, 3H), 3.02–2.99 (m, 1H), 2.28–1.62 ppm (m, 6H); ^{13}C NMR ($CDCl_3$): δ= 171.68, 137.74, 128.32, 127.67, 127.55, 73.58, 69.52, 67.71, 54.35, 52.20, 43.09, 35.15, 31.97, 22.96 ppm; HRMS (ESI-TOF): m/z calcd for $C_{16}H_{20}N_2NaO_4^+$: 327.1321 $[M+Na]^+$; found: 327.1309; elemental analysis calcd (%) for $C_{16}H_{20}N_2O_4$: C 63.14, H 6.62, N 9.20; found: C 63.00, H 6.60, N 9.12.

Synthesis of nitrosamine 11:



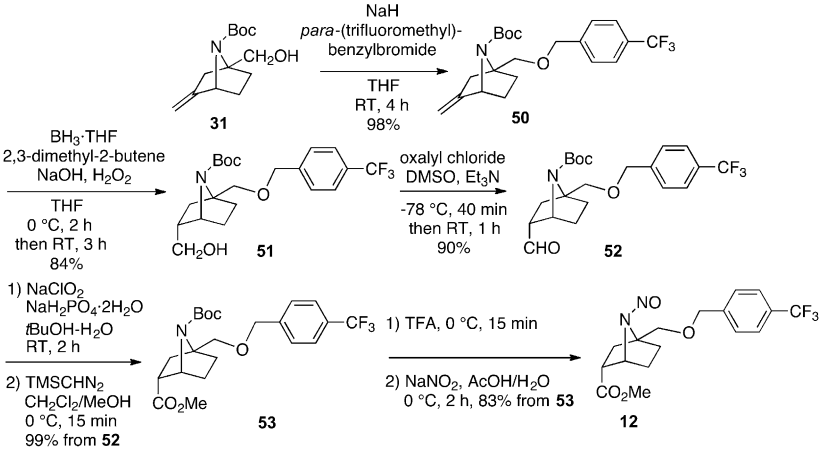
Synthesis of ***47***: The synthesis of compound **46** (Bz=benzoyl) is described below in the synthesis of nitrosamine **14**. Compound **46** (131.5 mg, 0.5071 mmol) was suspended in an aqueous 6N HCl solution (4 mL) and heated at 100°C under microwave irradiation for 20 min. The solvent was evaporated and the resultant crude salt of the amine was washed with Et_2O (3×10 mL). A suspension of the crude salt product in MeOH (20 mL) was cooled to 0°C with stirring. $SOCl_2$ (2.0 mL) was carefully added dropwise. The mixture was then warmed to 50°C, and after 3 h it was cooled to RT. The solvent was removed by rotary evaporation to afford the crude ester amine salt, which was washed with Et_2O (3×10 mL). A solution of $(Boc)_2O$ (189.6 mg, 0.8687 mmol) in dioxane (1 mL) was added at RT to a solution of the resultant amine derivative and Et_3N (1 mL) in dioxane (1 mL), and the whole was stirred for 14 h at RT. The reaction mixture was evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt= 6:1 to 3:1) gave compound **47** (59.5 mg, 0.2331 mmol, 46% from **46**) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ=4.29–4.27 (m, 1H), 3.76 (s, 3H), 2.18–2.11 (m, 2H), 1.92–1.87 (m, 2H), 1.75–1.68 (m, 2H), 1.49–1.43 (m, 2H), 1.38 ppm (s, 9H); ^{13}C NMR ($CDCl_3$): δ=171.66, 156.41, 80.57, 68.64, 59.59, 52.01, 33.32, 29.20, 27.97 ppm; HRMS (ESI-TOF): m/z calcd for $C_{13}H_{21}NNaO_4^+$: 278.1363, $[M+Na]^+$; found: 278.1375.

Synthesis of ***48***: $CaCl_2$ (24.9 mg, 0.2244 mmol) and $NaBH_4$ (18.2 mg, 0.4811 mmol) at 0°C were added to a solution of **47** (28.8 mg, 0.1128 mmol) in EtOH (500 μL)/THF (300 μL). The reaction mixture was stirred for 3 h at RT, and was quenched by the addition of a 10% aqueous solution of citric acid. The whole was diluted with water (30 mL) and extracted with AcOEt (3×20 mL). The organic phases were combined, washed with brine (2 mL), dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt=1:1) gave compound **48** (24.0 mg, 0.1056 mmol, 94%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ=4.86 (br s, 1H), 4.23–4.21 (m, 1H), 3.89 (d, J= 7.2 Hz, 2H), 1.89–1.71 (m, 4H), 1.47–1.34 (m, 4H), 1.43 ppm (s, 9H); ^{13}C NMR ($CDCl_3$): δ=155.11, 80.06, 69.08, 61.97, 58.31, 31.77, 29.22, 28.33 ppm; HRMS (ESI-TOF): m/z calcd for $C_{12}H_{21}NNaO_3^+$: 250.1414 $[M+Na]^+$; found: 250.1385.

Synthesis of ***49***: NaH (40.7 mg, 1.02 mmol, about 60%) at 0°C was added to a solution of **48** (57.2 mg, 0.2070 mmol) in THF (500 μL) and the reaction mixture was stirred for 30 min at 0°C. BnBr (60 μL, 0.50 mmol) at 0°C was added to the reaction mixture, and stirring was continued for 5 h at RT. The reaction mixture was poured into 0.5N aqueous HCl solution (50 mL), and the whole was extracted with Et_2O (3×20 mL). The combined organic phase was washed with brine (2 mL), dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane to *n*-hexane/AcOEt=9:1) gave compound **49** (65.7 mg, 0.2070 mmol, 82%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ= 7.37–7.24 (m, 5H), 4.61 (s, 2H), 4.28–4.26 (m, 1H), 4.07 (s, 2H), 1.81–1.66 (m, 6H), 1.45–1.38 (m, 2H), 1.43 ppm (s, 9H); ^{13}C NMR ($CDCl_3$): δ=155.40, 138.77, 128.21, 127.47, 127.34, 79.35, 73.30, 72.49, 67.15, 58.66, 33.71, 28.80, 28.31 ppm; HRMS (ESI-TOF): m/z calcd for $C_{19}H_{27}NNaO_3^+$: 340.1883; $[M+Na]^+$ found: 340.1874.

Synthesis of ***11***: Compound **49** (65.7 mg, 0.2070 mmol) was dissolved in TFA (1 mL) at 0°C and the reaction mixture was stirred for 1 h at 0°C. Then, the acid was evaporated and the residue was adjusted to pH 11 with 2N aqueous NaOH and extracted with Et_2O (3×40 mL). The organic layer was dried over Na_2SO_4 and evaporated to give the crude amine (42.2 mg). An aqueous solution of $NaNO_2$ (124.3 mg, 1.801 mmol) in H_2O (600 μL) was added over 1 min at 0°C to a solution of the resultant amine derivative in acetic acid (300 μL), and the whole was stirred for 5 h at this temperature. The reaction mixture was poured into water (50 mL), extracted with Et_2O (3×40 mL), and the organic solution was dried over sodium sulfate and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt=5:1) gave compound **11** (25.3 mg, 0.1027 mmol, 50% from **49**) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ=7.36–7.28 (m, 5H), 5.04–5.02 (m, 1H), 4.66 (s, 2H), 4.18 (s, 2H), 1.99–1.95 (m, 2H), 1.84–1.78 (m, 4H), 1.61–1.56 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): δ=138.01, 128.36, 127.67, 127.62, 73.68, 68.38, 68.33, 52.91, 32.38, 27.21 ppm; HRMS (ESI-TOF): m/z calcd for $C_{14}H_{18}N_2NaO_2^+$: 269.1260 $[M+Na]^+$; found: 269.1251; elemental analysis calcd (%) for $C_{14}H_{18}N_2O_2$: C 68.27, H 7.37, N 11.37; found: C 68.46, H 7.58, N 11.06.

Synthesis of nitrosamine 12:



Synthesis of ***50***: A solution of **31** (96.7 mg, 0.4041 mmol) in THF (900 μL) at 0 °C was added to a solution of NaH (31.2 mg, 1.30 mmol, about 60 %) in THF (900 μL) and the reaction mixture was stirred for 30 min at 0 °C. 4-(Trifluoromethyl)benzyl bromide (140 μL, 0.905 mmol) at 0 °C was added to the reaction mixture, and stirring was continued for 4 h at RT. The mixture was poured into a 0.5 N aqueous solution of HCl, and the whole was extracted with Et_2O (3×30 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane to *n*-hexane/AcOEt = 4:1) gave compound **50** (157.0 mg, 98 %) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ = 7.59 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.8 Hz, 2H), 4.93 (m, 1H), 4.74 (m, 1H), 4.66 (s, 2H), 4.52–4.51 (m, 1H), 4.15 (d, *J* = 9.6 Hz, 1H), 4.11 (d, *J* = 9.6 Hz, 1H), 2.50–2.45 (m, 1H), 2.36–2.31 (m, 1H), 1.95–1.71 (m, 3H), 1.55–1.49 (m, 1H), 1.41 ppm (s, 9H); HRMS (ESI-TOF): *m*/*z* calcd for $C_{21}H_{26}F_3NNaO_3^+$: 420.1757 [*M*+Na]$^+$; found: 420.1726.

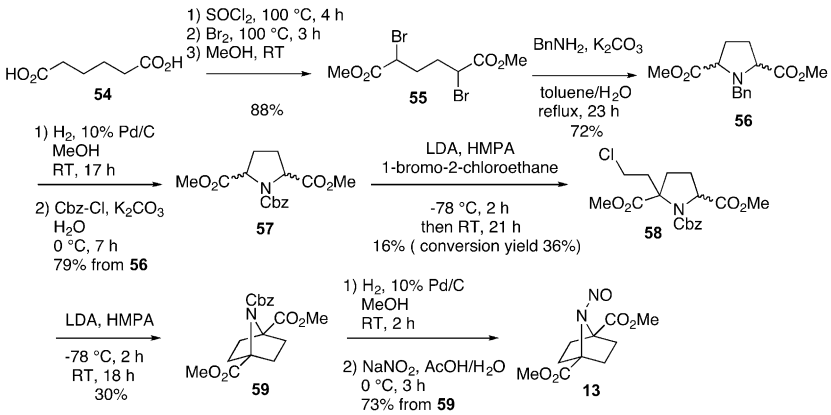
Synthesis of ***51***: BH_3·THF (820 μL, 1.08 M in THF) at 0 °C was added to 2,3-dimethyl-2-butene (920 μL, 1.0 M in THF), and the reaction mixture was stirred for 1 h at 0 °C. Then, a solution of **50** (157.0 mg, 0.3950 mmol) in THF (1.8 mL) was added to the mixture at 0 °C, and stirring was continued for 2 h at 0 °C. Next, 3 N aqueous NaOH (365 μL, 1.10 mmol) and 30 % H_2O_2 (365 μL) at 0 °C were added to the reaction mixture, and stirring was continued for 3 h at RT. The mixture was poured into water and the whole was extracted with CH_2Cl_2 (3×30 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt = 1:1) gave compound **51** (137.8 mg, 84 %, *endo*/*exo* = 89:11) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ = 7.55 (d, *J* = 8.04 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 4.63 (s, 2H), 4.30–4.29 (m, 0.89H), 4.27–4.25 (m, 0.11H), 4.07 (d, *J* = 9.6 Hz, 1H), 4.02 (d, *J* = 9.2 Hz, 1H), 3.74–3.70 (m, 1H), 3.54–3.49 (m, 1H), 2.43–2.37 (m, 1H), 2.02–1.56 (m, 6H), 1.42 (s, 9H), 1.17–1.14 ppm (m, 1H); HRMS (ESI-TOF): *m*/*z* calcd for $C_{21}H_{28}F_3NNaO_4^+$: 438.1863 [*M*+Na]$^+$; found: 438.1853.

Synthesis of ***52***: DMSO (150 μL, 2.112 mmol) at −78 °C was added to a solution of oxalyl chloride (130 μL, 1.536 mmol) in CH_2Cl_2 (3 mL). The reaction mixture was stirred for 20 min and a solution of **51** (137.8 mg, 0.3317 mmol) in CH_2Cl_2 (4.5 mL) was added at −78 °C. Stirring was continued for 40 min at −78 °C, and then Et_3N (560 μL, 4.040 mmol) was added. The solution was allowed to warm to RT. After 1 h of stirring, the reaction mixture was quenched by addition of water and extracted with CH_2Cl_2 (3×30 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt = 3:1) gave compound **52** (122.8 mg, 90 %, *endo*/*exo* = 89:11) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ = 9.74 (d, *J* = 1.2 Hz, 0.89H), 9.67 (d, *J* = 1.6 Hz, 0.11H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 4.65 (s, 2H), 4.63–4.61 (m, 1H), 4.09 (d, *J* = 9.6 Hz, 1H), 4.04 (d, *J* = 9.8 Hz, 1H), 3.10–3.07 (m, 0.89Hm), 2.56–2.49 (m, 0.11H), 2.17–2.03 (m, 1H), 1.94–1.66 (m, 4H), 1.51–1.46 (m, 1H), 1.43 (s, 8.01H), 1.38 ppm (s, 0.99H); HRMS (ESI-TOF): *m*/*z* calcd for $C_{21}H_{26}F_3NNaO_4^+$: 436.1706 [*M*+Na]$^+$; found: 436.1699.

Synthesis of ***53***: 2-Methyl-2-butene (600 μL) and a solution of $NaClO_2$ (247.5 mg, 2.737 mmol) and NaH_2PO_4·$2H_2O$ (327.2 mg, 2.097 mmol) in H_2O (2.2 mL) at RT were added to a solution of **52** (122.8 mg, 0.2970 mmol) in *t*BuOH (2.2 mL), and the reaction mixture was stirred for 2 h at RT. *t*BuOH was evaporated and the aqueous residue was poured into a 0.5 N aqueous solution of HCl, and extracted with $CHCl_3$ (3×30 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated to give the crude carboxylic acid as a colorless oil. $TMSCHN_2$ (300 μL, 2.0 M in Et_2O, 0.6 mmol) at 0 °C was added to a solution of the resultant carboxylic acid derivative in CH_2Cl_2 (4.4 mL)/MeOH (2.2 mL). The reaction mixture was stirred for 15 min at 0 °C. The residue obtained by evaporation was purified by column chromatography (*n*-hexane/AcOEt = 14:1 to 4:1) to give compound ***53-exo*** (15.0 mg) as a colorless oil and ***53-endo*** (103.0 mg) as a colorless oil (total amount of **53**: 130.4 mg, 99 % from **52**, combined yield). ***53-endo***: ^{1}H NMR ($CDCl_3$): δ = 7.59 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 4.66 (s, 2H), 4.48–4.46 (m, 1H), 4.08 (d, *J* = 9.6 Hz, 1H), 4.03 (d, *J* = 9.6 Hz, 1H), 3.71 (s, 3H), 3.09–3.04 (m, 1H), 2.14–2.09 (m, 1H), 2.04–2.03 (m, 1H), 1.81–1.71 (m, 3H), 1.53–1.48 (m, 1H), 1.44 ppm (s, 9H); HRMS (ESI-TOF): *m*/*z* calcd for $C_{22}H_{28}F_3NNaO_5^+$: 466.1812 [*M*+Na]$^+$; found: 466.1814. ***53-exo***: ^{1}H NMR ($CDCl_3$): δ = 7.59 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 4.66 (s, 2H), 4.59–4.58 (m, 1H), 4.17 (d, *J* = 9.6 Hz, 1H), 4.14 (d, *J* = 9.6 Hz, 1H), 3.71 (s, 3H), 2.60–2.57 (m, 1H), 2.28–2.23 (m, 1H), 1.94–1.69 (m, 4H), 1.52–1.43 (m, 1H), 1.40 ppm (s, 9H).

Synthesis of ***12***: Compound **53** (103.0 mg, 0.2324 mmol) was dissolved in TFA (1 mL) at 0 °C and the reaction mixture was stirred for 15 min at this temperature. Then, TFA was evaporated to give the crude amine. An aqueous solution of $NaNO_2$ (70.8 mg, 1.026 mmol) in H_2O (1 mL) was added to a solution of the resultant amine derivative in acetic acid (0.5 mL) over 1 min at 0 °C, and the whole was stirred for 2 h at this temperature. The reaction mixture was poured into water (30 mL) and extracted with Et_2O (3×20 mL). The organic layer was washed with water and brine, and dried over Na_2SO_4. The residue obtained by evaporation was purified by column chromatography (*n*-hexane/AcOEt = 3:1) to give compound **12** (71.7 mg, 0.1926 mmol, 83 % yield) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ = 7.61 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H), 5.23–5.20 (m, 1H), 4.72 (s, 2H), 4.24–4.18 (m, 2H), 3.74 (s, 3H), 3.03–3.01 (m, 1H), 2.29–2.16 (m, 2H), 2.01–1.97 (m, 1H), 1.89–1.60 ppm (m, 3H); ^{13}C NMR ($CDCl_3$): δ = 171.71, 141.93, 129.9 (q, J_{CF} = 32 Hz; *C*-CF_3), 127.51, 125.42 (q, J_{CF} = 3.6 Hz; *o*-*C*H-C-CF_3), 125.35 (q, J_{CF} = 3.7 Hz; *o*-*C*H-C-CF_3), 124.06 (q, J_{CF} = 263.1 Hz; *o*-*C*H-C-CF_3), 72.86, 69.52, 68.15, 54.45, 52.31, 43.17, 35.17, 31.96, 23.04 ppm; HRMS (ESI-TOF): *m*/*z* calcd for $C_{17}H_{19}F_3N_2NaO_4^+$: 395.1189 [*M*+Na]$^+$; found: 395.1184; elemental analysis calcd (%) for $C_{17}H_{19}F_3N_2O_3$: C 54.84, H 5.14, N 7.52; found: C 54.68, H 5.18, N 7.46.

Synthesis of nitrosamine 13:[19]



Synthesis of ***55***: Thionyl chloride (3262 mg, 27.42 mmol) was added over 30 min at 0 °C to adipic acid (**54**, 2100.0 mg, 14.370 mmol) and the whole was heated to 100 °C with stirring for 4 h. Then, the reaction mixture was cooled to RT and bromine (5.0 g, 62.58 mmol) was added dropwise over 10 min at RT. Stirring was continued for 19 h at RT and then the mixture was heated at 100 °C for 3 h and further cooled to RT. Argon was passed through the reaction mixture to remove excess bromine, and MeOH was added dropwise to the resultant brown mixture. The reaction mixture was evaporated and the residue was purified by column chromatography

(*n*-hexane/AcOEt = 3:1) to give **55** (4217.8 mg, 12.70 mmol, 88%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ = 4.27–4.23 (m, 2H), 3.79 (s, 6H), 2.34–2.07 ppm (m, 4H).

Synthesis of ***56***: A mixture of **55** (24.315 g, 73.24 mmol), benzylamine ($BnNH_2$, 8 mL, 7800 mg, 73.2 mmol), K_2CO_3 (13.32 g, 96.38 mmol), toluene (50 mL), and water (25 mL) was heated at reflux for 23 h. After cooling to RT, the organic layer was separated and the aqueous layer was extracted with AcOEt (3 × 100 mL). The combined organic layer was washed with brine (5 mL), dried over Na_2SO_4, and evaporated, and the residue was purified by column chromatography (*n*-hexane/AcOEt = 5:1 to 3:1) to give **56** (4.6833 g (16.89 mmol) + 10.011 g (36.10 mmol), 72% in total as a mixture of *cis*/*trans* isomers) as a yellow oil. ***56***-*cis*/*trans*: ^{1}H NMR ($CDCl_3$): δ = 7.33–7.21 (m, 5H), 3.96 (d, *J* = 13.2 Hz, 1H), 3.79 (d, *J* = 13.2 Hz, 1H), 3.85–3.82 (m, 2H), 3.64 (s, 6H), 2.36–2.25 (m, 2H), 1.98–1.89 ppm (m, 2H); HRMS (ESI-TOF): *m*/*z* calcd for $C_{15}H_{19}NNaO_4^+$: 300.1206 [*M*+Na]$^+$; found: 300.1199. ***56***-*trans*/*cis*: ^{1}H NMR ($CDCl_3$): δ = 7.33–7.21 (m, 5H), 3.92 (s, 2H), 3.58 (s, 6H), 3.46–3.43 (m, 2H), 2.09–2.04 ppm (m, 4H); HRMS (ESI-TOF): *m*/*z* calcd for $C_{15}H_{19}NNaO_4^+$: 300.1206 [*M*+Na]$^+$; found: 300.1248.

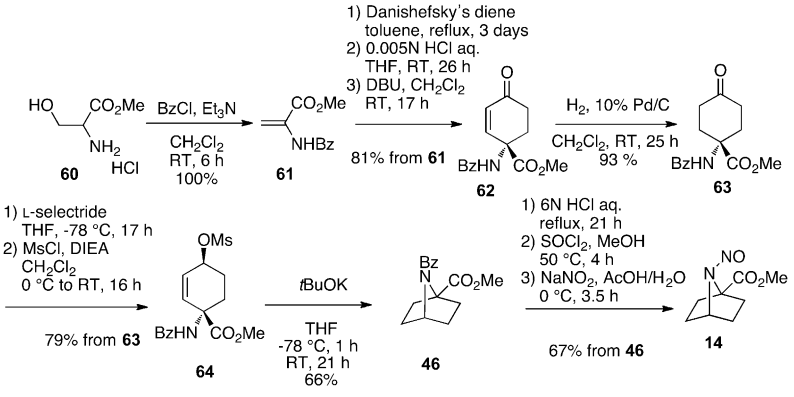
Synthesis of ***57***: A solution of **56** (4.9697 g, 17.92 mmol) in MeOH (600 mL) was hydrogenated catalytically (10% Pd/C 500 mg) at RT for 17 h. The mixture was filtered, the filtrate was evaporated, and the residue was subjected to column chromatography (*n*-hexane/AcOEt/MeOH = 10:10:1). The crude amine (3.3648 g) was obtained as a pale yellow oil. Cbz-Cl (Cbz = carbobenzyloxy; 3100 μL, 3751 mg, 21.99 mmol) was added at 0 °C to a solution of the obtained amine and K_2CO_3 (3.2490 g, 23.51 mmol) in water (40 mL), and the whole was stirred for 7 h at this temperature. The solution was extracted with AcOEt (70 mL × 3). The organic layer was washed with water (2 × 25 mL) and brine (2 mL), dried over Na_2SO_4, and evaporated, and the residue was subjected to column chromatography (*n*-hexane/AcOEt = 3:1 to 1:1). Compound **57** (4577.6 mg, 14.24 mmol, 79% yield) was obtained as a colorless oil. ***57***-*cis*/*trans*: ^{1}H NMR ($CDCl_3$): δ = 7.36–7.28 (m, 5H), 5.23 (d, *J* = 12.4 Hz, 1H), 5.03 (d, *J* = 12.4 Hz, 1H), 4.61–4.53 (m, 2H), 3.75 (s, 3H), 3.57 (s, 3H), 2.39–2.25 (m, 2H), 2.06–1.99 ppm (m, 2H). ***57***-*trans*/*cis*: ^{1}H NMR ($CDCl_3$): δ = 7.37–7.27 (m, 5H), 5.18–5.10 (m, 2H), 4.51–4.48 (m, 1H), 4.43–4.40 (m, 1H), 3.77 (s, 3H), 3.64 (s, 3H), 2.26–2.13 ppm (m, 4H).

Synthesis of ***58***: At −30 °C (acetone–dry ice bath), *n*BuLi (2200 μL, 1.65 M in *n*-hexane, 3.63 mmol) was added to a solution of diisopropylamine (500 μL, 361 mg, 3.568 mmol) in dry THF (4 mL). The whole was stirred for 30 min at −30 °C, then cooled to −78 °C. Next, a solution of **57** (968.4 mg, 3.014 mmol) and hexamethylphosphoramide (HMPA; 2610 μL, 2688 mg, 15.00 mmol) in dry THF (8 mL) was added to the above mixture (LDA = lithium diisopropylamide). The whole was stirred for 2 h at this temperature and 1-bromo-2-chloroethane (750 μL, 1292 mg, 9.011 mmol) was added at −78 °C. Stirring was continued for 21 h at RT. The reaction mixture was poured into a 0.5 N aqueous solution of HCl (30 mL), and the whole was extracted with $CHCl_3$ (3 × 50 mL). The combined organic phase was washed with brine (2 mL), dried over Na_2SO_4, and evaporated. Open column chromatography (*n*-hexane/AcOEt = 3:1 to 1:1) of the residue gave **58** (187.0 mg, 0.4872 mmol; 16% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ = 7.35–7.27 (m, 5H), 5.23 and 5.02 (d of AB system, *J* = 12 Hz, 0.8H), 5.14 and 5.08 (d of AB system, *J* = 12.8 Hz, 1.2H), 4.65 (dd, *J* = 3.2, 8.4 Hz, 0.4H), 4.58 (dd, *J* = 3.2, 7.8 Hz, 0.6H), 3.76 (s, 1.2H), 3.72 (s, 1.8H), 3.63 (s, 1.8H), 3.44 (s, 1.2H), 3.79–3.71 (m, 2H), 2.86–2.79 (m, 0.6H), 2.67–2.61 (m, 0.4H), 2.58–2.51 (m, 1H), 2.29–2.27 (m, 2H), 2.15–2.12 (m, 1H), 2.20–1.83 ppm (m, 1H); HRMS (ESI-TOF): *m*/*z* calcd for $C_{18}H_{22}ClNNaO_6^+$: 406.1028 [*M*+Na]$^+$; found: 406.1015.

Synthesis of ***59***: At −40 °C (an acetone–dry ice bath), *n*BuLi (650 mL, 1.65 M in *n*-hexane, 1.073 mmol) was added to a solution of diisopropylamine (150 μL, 108.3 mg, 1.070 mmol) in dry THF (0.5 mL). The whole was stirred at −40 °C for 30 min and cooled to −78 °C, then a solution of **58** (316.5 mg, 0.8246 mmol) and HMPA (720 μL, 741.6 mg, 4.138 mmol) in dry THF (1.5 mL) was added. The reaction mixture was stirred for 2 h at −78 °C and for 18 h at RT, then poured into a 0.5 N aqueous solution of HCl (30 mL) and extracted with $CHCl_3$ (3 × 30 mL). The combined organic phase was washed with brine (5 mL), dried over Na_2SO_4, and evaporated. Purification of the residue by open column chromatography (*n*-hexane/AcOEt = 3:1 to 1:1) gave **59** (86.6 mg, 0.2493 mmol, 30% yield) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ = 7.38–7.28 (m, 5H), 5.03 (s, 2H), 3.59 (s, 6H), 2.31 (d, *J* = 6.8 Hz, 4H), 1.84 ppm (d, *J* = 6.8 Hz, 4H).

Synthesis of ***13***: A solution of **59** (86.6 mg, 0.2493 mmol) in MeOH (9 mL) was hydrogenated catalytically (10% Pd/C 8.4 mg) at RT for 2 h. The mixture was filtered, the filtrate was evaporated, and the residue was subjected to column chromatography (*n*-hexane/AcOEt/MeOH = 10:10:1) to give the corresponding amine (47.3 mg) as a white solid. An aqueous solution of $NaNO_2$ (53.7 mg, 0.7782 mmol) in H_2O (2.5 mL) was added over 1 min at 0 °C to a solution of the resultant amine derivative in acetic acid (0.6 mL). The whole was stirred for 3 h at this temperature, diluted with water (30 mL), and extracted with Et_2O (3 × 30 mL). The organic layer was washed with H_2O (20 mL) and brine (2 mL), dried over sodium sulfate, and evaporated. The residue was subjected to column chromatography (*n*-hexane/AcOEt = 2:1) to give **13** (43.9 mg, 0.1812 mmol, 73% yield, recrystallized from CH_2Cl_2/*n*-hexane) as yellow cubes. M.p. 177.1–178.3 °C; ^{1}H NMR ($CDCl_3$): δ = 3.86 (s, 6H), 2.33–2.31 (m, 4H), 1.96–1.95 ppm (m, 4H); ^{13}C NMR($CDCl_3$): δ = 168.85, 69.94, 52.79, 31.66 ppm; HRMS (ESI-TOF): *m*/*z* calcd for $C_{10}H_{14}N_2NaO_5^+$: 265.0800 [*M*+Na]$^+$; found: 265.0806; elemental analysis calcd (%) for $C_{10}H_{14}N_2O_5$: C 49.58, H 5.83, N 11.56; found: C 49.46, H 5.76, N 11.46.

Synthesis of nitrosamine 14:[20]



Synthesis of ***61***: Et_3N (2.18 g, 21.52 mmol) and BzCl (2.06 g, 14.65 mmol) were added in portions to a solution of L-serine methyl ester hydrochloride (**60**; 1.0086 g, 6.483 mmol) in CH_2Cl_2 (20 mL) under an Ar atmosphere. The mixture was stirred for 6 h at RT and then washed with a saturated aqueous solution of $NaHCO_3$ (2 × 50 mL), dried over Na_2SO_4, and evaporated. The resultant white powder was dissolved in CH_2Cl_2 (15 mL), then 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 1.16 g, 7.62 mmol) was added at +5 °C (a water–ice bath) under an Ar atmosphere. After 2.5 h of stirring at +5 °C, the reaction mixture was washed with water (50 mL) and a saturated aqueous solution of $NaHCO_3$ (2 × 50 mL). The organic layer was dried over Na_2SO_4 and evaporated to give compound **61** (1525.7 mg, 100%) as a brown oil. ^{1}H NMR ($CDCl_3$): δ =

8.53 (brs, 1H), 7.85–7.83 (m, 2H), 7.57–7.53 (m, 1H), 7.51–7.46 (m, 2H), 6.80 (s, 1H), 6.00 (s, 1H), 3.90 ppm (s, 3H).

Synthesis of ***62***: *trans*-1-Methoxy-3-trimethylsilyloxy-1,3-butadiene (Danishefsky's diene; 2.5 mL, 12.84 mmol) was added to a solution of methyl 2-benzamidoacrylate (**61**; 1330 mg, 6.483 mmol) in dry toluene (65 mL) under an Ar atmosphere. The whole was heated at reflux with stirring for 3 days, then evaporated, and a 0.005 N aqueous solution of HCl/THF (1:4, 25 mL) was added to the residue. The reaction mixture was stirred for 26 h at RT, then evaporated, and the residue was purified by column chromatography (hexane/ethyl acetate = 1:1 to 1:2) to give the crude Diels–Alder adduct. The crude mixture was dissolved in CH_2Cl_2 (50 mL), and DBU (2110 μL, 14.11 mmol) was added at 0 °C. The reaction mixture was stirred for 17 h at RT and washed with a 0.5 N aqueous solution of HCl (50 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 40 mL) and the combined organic phase was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography (*n*-hexane/ethyl acetate = 1:1), to yield enone **62** (1434.4 mg, 81 % yield) as a white amorphous solid. ^{1}H NMR ($CDCl_3$): δ = 7.81–7.78 (m, 2H), 7.57–7.53 (m, 1H), 7.49–7.44 (m, 2H), 7.14–7.11 (d, J = 11.6 Hz, 1H), 6.82 (brs, 1H), 6.19–6.17 (d, J = 10.0 Hz, 1H), 3.85 (s, 3H), 2.67–2.53 ppm (m, 4H).

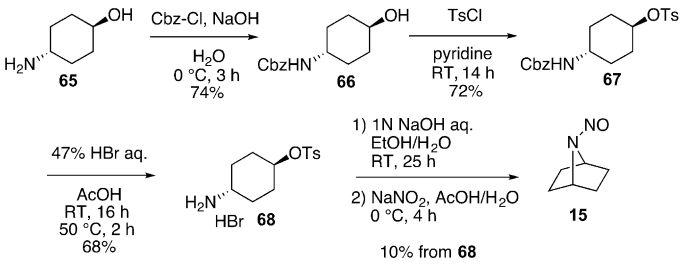
Synthesis of ***63***: A solution of enone **62** (1434.4 mg, 5.249 mmol) in dry CH_2Cl_2 (140 mL) was hydrogenated at atmospheric pressure for 25 h at RT, with 10 % Pd/C(145.4 mg) as the catalyst. After the removal of the catalyst, the solvent was evaporated. The residue was purified by column chromatography (*n*-hexane/ethyl acetate = 1:1) to give compound **63** (1339.9 mg, 93 % yield) as a white amorphous solid. ^{1}H NMR ($CDCl_3$): δ = 7.80–7.78 (m, 2H), 7.57–7.53 (m, 1H), 7.49–7.44 (m, 2H), 6.41 (brs, 1H), 3.79 (s, 3H), 2.55–2.51 ppm (m, 8H).

Synthesis of ***64***: L-Selectride (6.0 mL, 1 M solution in THF, 6.0 mmol) was added dropwise at −78 °C under an Ar atmosphere to a solution of compound **63** (1339.9 mg, 4.867 mmol) in dry THF (25 mL). After 17 h of stirring at −78 °C, the reaction was quenched by the addition of saturated aqueous NH_4Cl (10 mL). The resulting mixture was allowed to warm to RT, evaporated, and the residue was washed with ethyl acetate (1 × 40 mL) and $CHCl_3$/2-propanol (3:1, 2 × 40 mL). Evaporation of the solvent gave a residue that was purified by column chromatography (*n*-hexane/ethyl acetate = 1:9), to give a mixture of *trans*/*cis* alcohols (1228.3 mg). Diisopropylethylamine (DIEA; 1500 μL, 8.70 mmol) and methanesulfonyl chloride (MsCl; 700 μL, 26.2 mmol) were added to a solution of the mixture of crude alcohols in dry CH_2Cl_2 (25 mL), at 0 °C under an Ar atmosphere. The solution was allowed to warm to RT and, after 16 h of stirring at RT, the mixture was washed with a 5 % aqueous solution of $NaHCO_3$ (10 mL). The separated organic phase was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography (*n*-hexane/ethyl acetate = 1:1), to obtain compound **64** (1366.2 mg, 79 % yield from ketone **63**) as a white solid. ^{1}H NMR ($CDCl_3$): δ = 7.79–7.75 (m, 2H), 7.56–7.51 (m, 1H), 7.47–7.43 (m, 2H), 6.18 (brs, 1H), 6.97 (brs, 1H), 3.77 (s, 3H), 3.06 (s, 3H), 2.41–1.89 ppm (m, 8H).

Synthesis of ***46***: A 1 M solution of *t*BuOK (539.7 mg, 4.810 mmol) in THF (5 mL) was added under an Ar atmosphere to a solution of **64** (1366.2 mg, 3.844 mmol) in dry THF (40 mL) at −78 °C. After stirring for 1 h at −78 °C, the whole was allowed to warm to RT and stirring was continued at RT for 21 h. The reaction was quenched by the addition of a 2 N aqueous HCl solution (15 mL) and the resulting mixture was extracted with ethyl acetate (1 × 50 mL) and CH_2Cl_2 (2 × 40 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to give a residue, which was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 7:3), to give compound **46** (653.9 mg, 66 % yield, recrystallized from *n*-hexane/$CHCl_3$) as colorless cubes. M.p. 107.0–109.3 °C; ^{1}H NMR ($CDCl_3$): δ = 7.70–7.68 (m, 2H), 7.50–7.46 (m, 1H), 7.42–7.38 (m, 2H), 4.27 (dd, J = 4.4, 4.4 Hz, 1H), 3.82 (s, 3H), 2.40–2.32 (m, 2H), 1.98–1.89 (m, 2H), 1.85–1.79 (m, 2H), 1.62–1.52 ppm (m, 2H); elemental analysis calcd (%) for $C_{15}H_{17}NO_3$: C 69.48, H 6.61, N 5.40; found: C 69.50, H 6.60, N 5.41.

Synthesis of ***14***: Compound **46** (131.5 mg, 0.5071 mmol) was suspended in 6 N aqueous HCl solution (40 mL) and the whole was heated at reflux for 21 h. The solvent was evaporated, the residue was dissolved in water, and the solution was washed with diethyl ether (2 × 5 mL). The aqueous layer was evaporated to give a crude amine salt as a white solid. A suspension of the amine salt in MeOH (10 mL) was cooled to 0 °C and stirred under Ar, followed by careful dropwise addition of $SOCl_2$ (1.0 mL). The mixture was then warmed to 50 °C. After 4 h, the reaction mixture was cooled to RT. The solvent was removed by rotary evaporation to afford the crude amine ester as a white solid. An aqueous solution of $NaNO_2$ (170.1 mg, 2.465 mmol) in H_2O (3 mL) was added over 1 min at 0 °C to a solution of the resultant amine ester derivative in acetic acid (1.5 mL), and the whole was stirred for 3.5 h at 0 °C. The reaction mixture was extracted with Et_2O (3 × 40 mL), and the organic layer was washed with saturated aqueous $NaHCO_3$ (5 mL) and brine (5 mL), and dried over Na_2SO_4. The organic solvent was evaporated to give **14** (62.3 mg, 0.3382 mmol, 67 % from **46**) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ = 5.12–5.03 (m, 1H), 3.89–3.78 (m, 3H), 2.33–1.61 ppm (m, 8H); ^{13}C NMR ($CDCl_3$): δ = 169.16, 69.53, 66.48, 60.04, 54.04, 33.30, 30.59, 29.61, 27.92 ppm; HRMS (ESI-TOF): m/z calcd for $C_8H_{12}N_2NaO_3^+$: 207.0740 $[M+Na]^+$; found: 207.0721.

Synthesis of nitrosamine 15:[21]



Synthesis of ***66***: An aqueous solution of NaOH (2.1 g, 15 mL) and benzyloxycarbonyl chloride (6.61 mL, 8.00 g, 46.9 mmol) was added slowly at 0 °C to a solution of *trans*-4-aminocyclohexanol (**65**; 5.03 g, 43.67 mmol) in water (40 mL). The mixture was stirred for 3 h at 0 °C, then water (40 mL) was added. The whole was extracted with CH_2Cl_2 (3 × 150 mL), and the organic layer was washed with water (50 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and evaporated to give a residue, which was purified by column chromatography ($CHCl_3$/AcOEt = 1:1) to give **66** (8.07 g, 32.37 mmol, 74 %) as a white solid. ^{1}H NMR ($CDCl_3$): δ = 7.36–7.31 (m, 5H), 5.08 (s, 2H), 4.57 (brs, 1H), 3.64–3.58 (m, 1H), 3.50 (brs, 1H), 2.05–1.96 (m, 4H), 1.25–1.21 (m, 3H), 1.19–1.15 ppm (m, 2H); HRMS (ESI-TOF): m/z calcd for $C_{14}H_{19}NNaO_3^+$: 272.1257 $[M+Na]^+$; found: 272.1263.

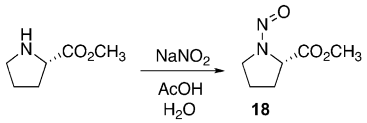
Synthesis of ***67***: *para*-Toluenesulfonyl chloride (TsCl; 2505.8 mg, 13.14 mmol) was added at 0 °C to a solution of **66** (2474.3 mg, 9.925 mol) in dry pyridine (25 mL) and the whole was stirred at RT for 14 h, then evaporated, and water (40 mL) was added to the residue. The whole was extracted with $CHCl_3$ (3 × 40 mL) and the organic layer was washed with 0.5 N aqueous HCl solution (40 mL) and brine (5 mL). The organic layer was dried over Na_2SO_4 and evaporated to give a solid residue, which was recrystallized from EtOH to give **41** (2887.9 mg, 7.157 mmol, 72 %) as colorless cubes. M.p. 114.1–116.0 °C; ^{1}H NMR ($CDCl_3$): δ = 7.78 (d, J = 8.4 H, 2H), 7.36–7.31 (m, 7H), 5.06 (s, 2H), 4.55 (brs, 1H), 4.46–4.39 (m, 1H), 3.49 (brs, 1H), 2.45 (s, 3H), 2.02–1.91 (m, 4H), 1.61–1.58 (m, 2H), 1.24–1.18 ppm (m, 2H); HRMS (ESI-TOF): m/z calcd for $C_{21}H_{25}NNaO_5S^+$: 426.1346 $[M+Na]^+$; found: 426.1353; elemental analysis calcd (%) for $C_{21}H_{25}NO_2S$: C 62.51, H 6.25, N 3.47; found: C 62.41, H 6.22, N 3.43.

Syntesis of ***68***: A solution of 47 % aqueous hydrobromic acid (4 mL) in acetic acid (9 mL) at RT was added to a solution of **67** (1660.1 mg, 4.114 mmol) in acetic acid (40 mL). The whole was stirred for 16 h at RT and heated at 50 °C for 2 h. The solvent was then evaporated and the solid residue was recrystallized from AcOEt to give **68** (985.8 mg, 2.814 mmol, 68 % yield) as colorless cubes. M.p. 145.5–147.4 °C; ^{1}H NMR

($CDCl_3$): $\delta = 8.07$ (brs, 3H), 7.78 (d, $J = 8.4$ Hz, 2H), 7.35 (d, $J = 8.4$ Hz, 2H), 4.41 (brs, 1H), 3.25 (brs, 1H), 2.46 (s, 3H), 2.16–2.13 (m, 2H), 2.04–2.01 (m, 2H), 1.65–1.55 ppm (m, 4H); elemental analysis calcd (%) for $C_{13}H_{20}BrNO_3S$: C 44.58, H 5.76, N 4.00; found: C 44.30, H 5.46, N 4.01.

Synthesis of ***15***: A 1 N aqueous solution of NaOH was added to a solution of **68** (985.8 mg, 2.814 mmol) in EtOH (56 mL)/H_2O (24 mL) at RT and the mixture was stirred for 25 h at RT. EtOH was evaporated and the aqueous residue was adjusted to pH 1 by using 2 N aqueous HCl, and the whole was washed with CH_2Cl_2 (2×10 mL). Then, the aqueous residue was adjusted to pH 12 with a 2 N aqueous solution of NaOH, and extracted with Et_2O (4×60 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated to give the crude amine (229.3 mg) as a white solid. An aqueous solution of $NaNO_2$ (71.4 mg, 1.035 mmol) in H_2O (2 mL) was added over 1 min at 0 °C to a solution of the resultant amine derivative (50.6 mg) in acetic acid (1 mL), and the whole was stirred for 1 h at 0 °C. Then, $NaNO_2$ (484.8 mg, 7.026 mmol) was added and the reaction mixture was stirred for 4 h at 0 °C and allowed to warm to RT. It was then poured into water (30 mL) and extracted with Et_2O (3×15 mL). The organic layer was washed with brine (2 mL), dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (*n*-hexane/AcOEt = 5:1) gave compound **15** (7.9 mg, about 10%) as a yellow solid. ^{1}H NMR ($CDCl_3$): $\delta = 4.99$ (dd, $J = 4.8$, 4.8 Hz, 1H), 4.90 (dd, $J = 4.8$, 4.8 Hz, 1H), 2.09–1.94 (m, 2H), 1.82–1.76 (m, 2H), 1.75–1.67 (m, 2H), 1.61–1.52 ppm (m, 2H); HRMS (ESI-TOF): *m*/*z* calcd for $C_6H_{10}N_2NaO^+$: 149.0685 $[M+Na]^+$; found: 149.0713.

Synthesis of 18:



18

An aqueous solution of $NaNO_2$ (206.1 mg, 2.99 mmol, 1.5 equiv with respect to the amine) in water (3 mL) was added at 0 °C (in an ice-water bath) to a solution of L-proline methyl ester (330.5 mg, 2.00 mmol) in a mixture of acetic acid (2 mL) and water (1 mL). The whole was stirred at 0 °C for 2 h, and was diluted with CH_2Cl_2 (60 mL). The organic layer was separated, washed with water (30 mL), saturated aqueous $NaHCO_3$ (30 mL), and then brine (30 mL), and dried over $MgSO_4$. The organic solvent was evaporated to give a viscous pale yellowish oil (138. 7 mg), which was purified by column chromatography (AcOEt/*n*-hexane = 1:2 to 1:1) to give the *N*-nitroso derivative **18** (132.9 mg, 34% yield) as a pale yellowish oil. ^{1}H NMR ($CDCl_3$; two isomers with respect to the N–NO bond were present in a ratio of 2:1): 5.29 (m, 0.33H), 4.52 (m, 0.67H), 4.44 (m, 0.67H×2), 3.80 (s, 0.33H×3; OCH_3), 3.72 (s, 0.67H×3; OCH_3), 3.69 (m, 0.33H×2), 2.33 (m, 0.67H×2), 2.22 (m, 0.33H×2), 2.08 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): 170.84, 169.09, 61.90, 57.85, 52.85, 52.53, 49.87, 45.55, 28.87, 27.75, 23.21, 21.14 ppm; HRMS (MALDI-TOF): *m*/*z* calcd for $C_6H_{11}N_2O_3^+$: 159.0764 $[M+H]^+$; found: 159.0721; elemental analysis calcd (%) for $C_6H_{10}N_2O_3$; C 45.57, H 6.37, N 17.71; found: C 45.33, H 6.42, N 17.63.

Calculation methods: All calculations were performed by using a suite of the Gaussian 03 programs.[22] Ground-state minimum structures were obtained by geometry optimization at the B3LYP/6-31G(d) level of theory. The ground-state geometries were confirmed by the number of imaginary frequencies. Zero-point energy corrections calculated at the B3LYP/6-31G+(d) level were applied with unscaling. The vertical excitation energies in the gas phase were calculated by using time-dependent density functional theory (TDDFT) at the B3LYP/6-31+G(d) level. The lowest singlet excitation was considered for each molecule.

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[12] In the present study, we found a linear relationship between the concentration (fluorescence intensity) of DAF-2-T and that of the formed NO in the range from 0 to 15.2 μM (NO). The reaction yield of NO with DAF-2 to give DAF-2-T was 2.3%.

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