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TRANSNITROSATION OF ALICYCLIC *N*-NITROSAMINES CONTAINING SULFUR ATOMS IN FIVE- OR SIX-MEMBERED RINGS

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Abstract – Nitric oxide (NO) is a cell transmitter produced *in vivo*. Alicyclic *N*-nitroso compounds serve as potential NO donors *in vivo* due to the formation of *S*-nitrosoglutathione by transnitrosation. In this study, *N*-nitroso compounds with a thioamide group and endocyclic sulfur atoms in 5- or 6-membered rings were synthesized and the transfer of nitroso group was analyzed. All compounds synthesized exhibited transnitrosation activity under acidic condtions. *N*-Nitroso-1,3-thiazinane-4-carbothioamide exhibited the highest transnitrosation activity.

INTRODUCTION

Nitric oxide (NO), cellular transmitters produced *in vivo*, is considered as a gaseous mediator together with carbon monoxide and hydrogen sulfide.[1,2] NO plays important roles in mammalian physiology and is synthesized *in vivo* via the activity of NO synthases.[3-5] NO plays particularly important roles in biological processes such as blood pressure regulation by modulating vascular relaxation[2,5-7] as well as in immune responses[8,9] and neural signal transduction.[10,11]

S-Nitrosothiols (RSNOs) function as NO transporters *in vivo* and routinely serve as donors of NO bioactivity. RSNOs have been studied as NO donors for therapeutic agents,[6,7,12] and have exhibited potential in the treatment of cardiovascular disorders and infectious diseases as well as the regulation of blood pressure.[6,9,13,14] Notably, *S*-nitrosoglutathione (GSNO), which is the product of *S*-nitrosation of the abundant intracellular antioxidant glutathione, has been detected *in vivo*. Despite a half-life measured in hours, the stability of RSNO is reduced by enzymatic and non-enzymatic degradation, rendering it too low for clinical application.[15,16]

N-Nitrosamines transfer nitrosonium ion (NO^+) to other amines to form corresponding *N*-nitroso derivatives.[17] Furthermore *N*-nitrosamines can transnitrosate the sulfur atoms of proteins to form

RSNOs.[18-20]

Compounds with two sulfur atoms showed the highest GSNO-forming activity, indicating that intramolecular sulfur atoms play an important role in transnitrosation with alicyclic *N*-nitroso compounds.[19] The transnitrosation appeared to proceed via a bridged intermediate pathway.[20] Compound **1** significantly increased the reaction rate of GSNO formation.[20] In this study, we designed 6-membered sulfur-containing ring structure having a thioamide group to enhance the transnitrosation activity because 6-membered compounds were stabilized in the transition state. Additionally, thiomorpholines is an important structural motif that is incorporated into a variety of active pharmaceutical ingredients because of its interesting pharmacological profile.[21] The reaction rate of GSNO formation was investigated by using newly synthesized compound with a 6-membered sulfur-containing 1,3-thiazinane ring (**2**) and 6-membered thiomorpholine ring (**3**) in place of a 5-membered sulfur-containing thiazolidine ring (Figure 1).

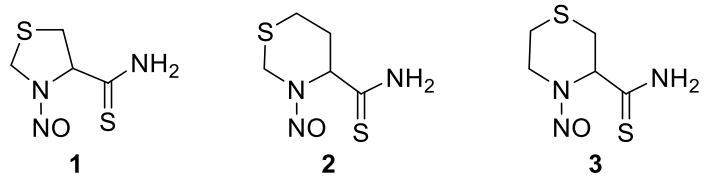
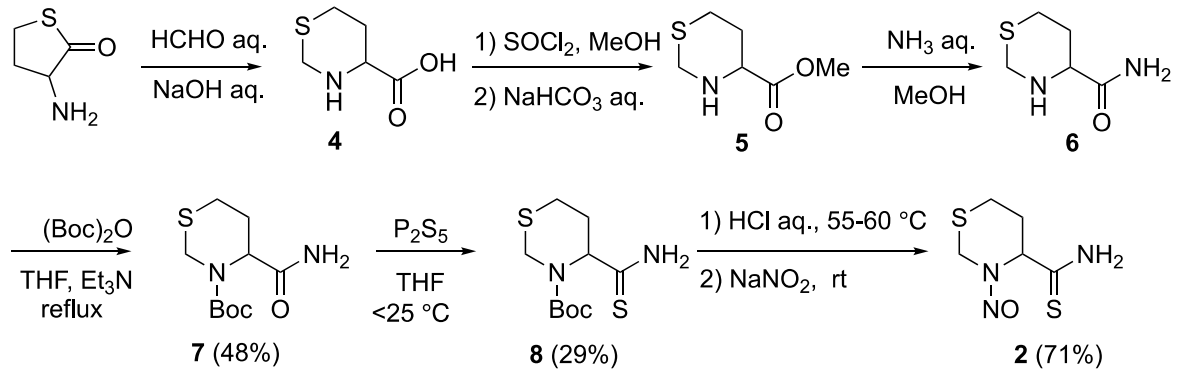


Figure 1. Structures of the alicyclic *N*-nitrosamines

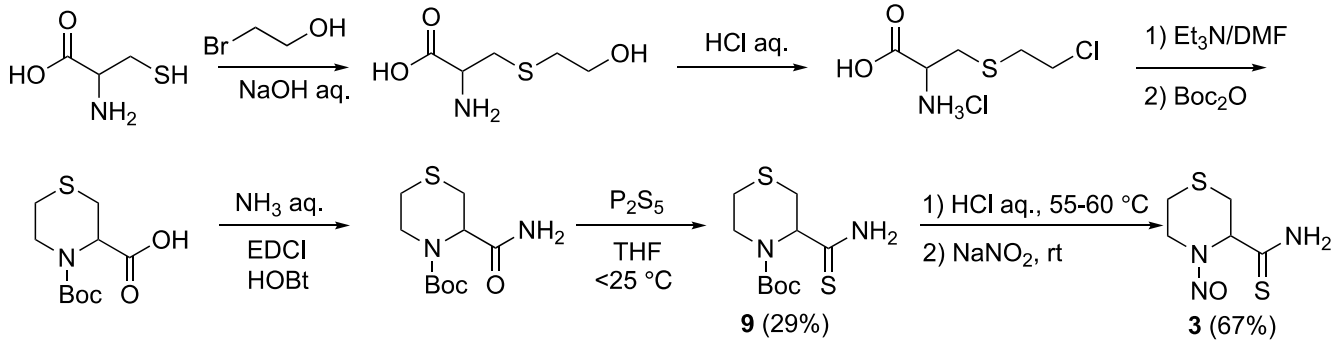
RESULTS AND DISCUSSION

We designed *N*-nitroso compounds incorporating 6-membered thiazinane ring (*N*-nitroso-1,3-thiazinane-4-carbothioamide, **2**) and thiomorpholine ring (*N*-nitrosothiomorpholine-3-carbothioamide**, 3**) containing a sulfur-atom. The compounds were thought to pass through a bridged intermediate state which would be a more stable transition state than compound **1**.

1,3-Thiazinane-4-carboxylic acid (**4**) was prepared as described by Musser et al.[22] Methyl 1,3-thiazinane-4-carboxylate (**5**) was prepared via a reaction between **4** and $SOCl_2$ in MeOH. Aqueous ammonia solution was added to **5** to obtain 1,3-thiazinane-4-carboxamide (**6**).[19] Compound **6** reacted with $(Boc)_2O$ to give *t*-butyl 4-carbamoyl-1,3-thiazinane-3-carboxylate (**7**). *t*-Butyl 4-carbamothioyl-1,3-thiazinane-3-carboxylate (**8**) was prepared from a reaction with **7** and P_2S_5 under ultrasonication. The Boc group was then removed under acidic conditions and was subsequently nitrosated with $NaNO_2$ to afford **2**. Compounds **2**, **7** and **8** were synthesized as novel compounds.



Scheme 1 Synthesis of **2**



Scheme 2. Synthesis of **3**

Thiomorpholine-3-carboxylic acid was prepared from cysteine as described by Shiraiwa et al.[22] Thiomorpholine-3-carboxylic acid was protected by treatment with $(Boc)_2O$, amidated by aqueous ammonia solution with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBt), followed by thioamidation with P_2S_5 under ultrasonication to obtain *t*-butyl 3-carbamothioylthiomorpholine-4-carboxylate (**9**). Compound **9** was deprotected and nitrosated according to the same procedure described above to obtain **3** (Scheme 2). Compounds **3** and **9** were newly synthesized.

Evaluation of transnitrosation activity by monitoring GSNO formation

The transnitrosation activity of the compounds was evaluated in terms of their capacity to form GSNO. Pseudo–first–order rate constant (k_{obs}) values were calculated using the slope of the linear relationship between GSNO formation and time (Figure 2). The transnitrosation results are summarized in Table 1, with k_{obs} values ordered as follows: **3** < **1** < **2**. The GSNO yield after 24 h of reaction time was calculated by diving [GSNO formation] by [*N*-nitrosamine consumption]. The GSNO yield was 93% for **1** and 96% for **3**. As the reaction of **2** was completed in 2 h, the GSNO yield was determined as 98% at 2 h. These results indicate that transnitrosation to GSH proceeded selectively without decomposition of the

compound.

Compounds **1**-**3** have a chiral center in ring results in D and L enantiomers, and the partial double bond character of the N ― N = O group also results from *E* and *Z* isomers.[25,26] D- or L-forms of *N*-nitrosothioproline had similar activity for GSNO formation (unpublished data), because transnitrosation of nitrosamines is thought to proceed via *N*-protonation ($^{+}$NH ― N=O).[27]

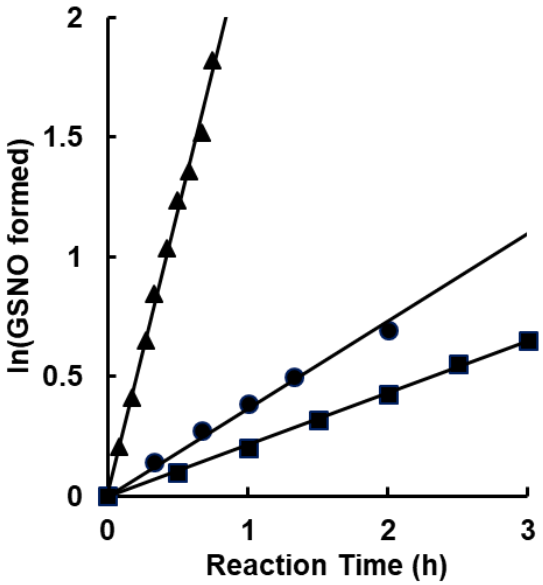


Figure 2. Formation of GSNO by the reaction of *N*-nitrosamines (**1**-**3**) with GSH
The reaction conditions were as follows: compound **1** (●), **2** (▲), and **3** (■) 0.45 mM; GSH, 5.0 mM, pH 1.5, 37 ℃.

Table 1. Reaction rate constant (k_{obs}) values and GSNO yield

N-nitrosamine[a]	k_{obs} [b] ($\times 10^{-7}$/s)	GSNO (mM)	*N*-nitrosamine consumption (mM)	GSNO yield (%)
1	1014	0.42[c]	0.45[c]	93[c]
	6667	0.44[d]	0.45[d]	98[d]
3	599	0.43[c]	0.45[c]	96[c]

a The reaction conditions are as follows: *N*-nitrosamine, 0.45 mM; GSH, 5.0 mM; pH 1.5; 37 ℃; in the dark.
b The k_{obs} for GSNO formation was calculated from the slope in ln{[GSNO$_\infty$]/([GSNO$_\infty$] – [GSNO])} versus time, where GSNO and GSNO$_\infty$ refer to the GSNO concentration at the time and the final concentration (0.45 mM), respectively. The k_{obs} were determined by the method of the least-squares.
c Data were acquired after 24 h.
d Data were acquired after 2 h.

Buffer catalysis studies on GSNO formation

The further explore the effects of pH on compound and GSH in the acidic range, buffer catalysis was

investigated in the pH 2.0 by measuring k_{obs} at varying buffer concentrations while maintaining constant pH and ionic strength. The slope of the relationship between k_{obs} and pH is defined as the catalytic rate constant, k_{cat}. The k_{obs} value is unaffected by buffer concentration and the k_{cat} value 0.002 is near zero (Figure 3). Because the buffer catalysis showed a dependence of k_{obs} on pH 2.0, this result suggests that the solution-state reaction is subject to specific acid catalysis as described by Song et al.[27] The transnitrosation reaction proceeded via specific acid catalysis and the protonated **2** was involved in the rate-determining step.

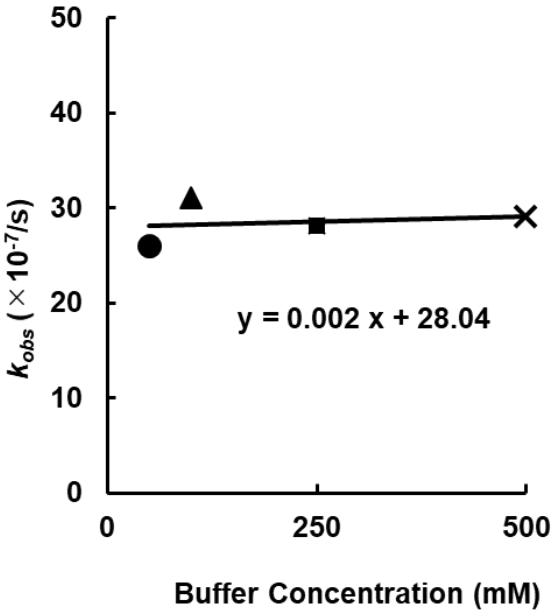


Figure 3. Effect of buffer concentration on the formation of GSNO (k_{obs}) in compound **2** and GSH
The reaction conditions were as follows: *N*-nitrosamine, 0.45 mM; GSH, 5.0 mM; pH 1.5; buffer concentration, 50 mM (●), 100 mM (▲), 200 mM (■), 500 mM (×); 37 ℃.

We focused on GSNO formation from *N*-nitrosamines via transnitrosation, as GSNO is a potential therapeutic drug. *N*-Nitrosothioproline with a thioamide group efficiently produced GSNO via transnitrosation. Based on this result, two types of novel 6-membered alicyclic ring compounds containing a sulfur atom with a thioamide group were synthesized. As compound **1** was determined computationally to form a bridged intermediate between the nitrogen atom of the nitroso group and the two sulfur atoms (one in the ring and one in the thioamide group),[20] we designed compounds **2** and **3** to enhance the transnitrosation activity due to formation of more stable intermediates.

Comparing the transnitrosation of these compounds to GSH, the k_{obs} of **2** was more than 6-fold greater than that of **1**. In contrast the k_{obs} of **3** was half that of **1**. The buffer catalysis studies of compound **2**, which exhibited the highest k_{obs} value, indicated that protonation of the *N*-nitrosamine was the rate-determining step, similar to compound **1**.[20] These data suggested that GSNO is formed through a mechanism similar to that of compound **1**. As compound **1** have a thioamide group, GSH is nitrosated

after the nitroso group is transferred to the sulfur atom of the thioamide group via the bridged structure.[20,29,30] Compounds **2** and **3** were also thought to nitrosate GSH through a similar pathway. To predict difference in k_{obs} value, the activation energy was calculated from the free-energy difference between the protonated compounds and their transition state using density functional theoretical (DFT) calculations (B3LYP/6-31G+[d, p], SMD Water).[31] The calculated activation energies of compounds **1**-**3** were 7.2, 2.7 and 13.5 kcal/mol, respectively. The lower activation energy increase k_{obs}. Our results showed that the order of their activation energies was similar tendency with that of k_{obs}. Compound **2** has the lowest activation energy, indicating that more stable intermediate structure brought high activity of the GSNO formation. In general, the chair conformation in cyclohexane is more favored than the boat conformation. The protonated **2** or **3** had a chair conformation and the protonated **3** was more stable than the protonated **2** as shown in DFT calculations (see Supporting Information Table S2). Meanwhile, the structure of **3** was unstable in transition state because **3** had to generate a boat conformation (see Supporting Information Figure S2).

In this study, we synthesized novel 6-membered sulfur-containing aliphatic *N*-nitroso compounds with thioamide group (**2**, **3**). GSNO formation was evaluated, including compound **1**. All three compounds exhibited transnitrosation activity under acidic conditions. The GSNO formation activity of **2** was greater than that of **1** and **3**. Since the GSNO yield was 90% or more for all compounds, it could be presumed that all the nitroso groups were transferred from the compound to GSH. The formation of stable intermediate was efficiently accelerated the transnitrosation. The compounds in this study are expected to be useful in medicine development for GSNO-related diseases.

EXPERIMENTAL

Materials and Methods

Diphosphorus pentasulfide was purchased from Sigma-Aldrich Co., Inc. (St. Louis, MO, USA). Diethylenetriamine-*N,N,N',N'',N'''*-pentaacetic acid (DTPA) was acquired from Dojindo Laboratories (Kumamoto, Japan). Other reagents were purchased from Fuji film Wako Pure Chemical Industries (Osaka, Japan).

Reaction progress was monitored using thin-layer chromatography (TLC) on silica gel 60 F254 (0.25 mm, Merck, Darmstadt, Germany) and aluminum oxide 150 F254 neutral plates (Merck). Column chromatography was performed using silica gel 60 (0.01-0.063 mm, Merck). Melting points were determined using a Yanaco MP-J3 micro-melting-point apparatus (Kyoto, Japan) without correction. HPLC was performed using a Shimadzu LC system (SPD-20A UV spectrometric detector, LC-20AD pumps, Kyoto, Japan) and column (Capcell Pak UG-120 [5 mm, 250 × 4.6 mm, Osaka soda, Osaka, Japan] or Chromolith Performance RP-18e [5 μm, 100 × 4.6 mm, Merck]). NMR spectra were recorded

using a JEOL JMS-600 spectrometer (Tokyo, Japan). Chemical shifts are expressed in ppm and shifted downfield from TMS. High-resolution mass spectra (HRMS) were collected using a JEOL AccuTOF LC-plus 4G mass spectrometer.

Preparation of alicyclic *N*-nitrosamines

Compound **1** was prepared using a previously reported procedure[20] (observed mp 121.5-122.0 °C). Compound **4** was prepared according to the reported by Musser et al.,[21] and **6** was prepared according to the procedure reported by Inami et al.[19] Thiomorpholine-3-carboxylic acid was prepared from cysteine according to the procedure reported by Shiraiwa et al.,[23] and *t*-butyl 4-carbamoyl-thiomorpholine-3-carboxylate was prepared according to the procedure reported by Cai et al.[24]

***t*-Butyl 4-carbamoyl-1,3-thiazinane-3-carboxylate (7)**: To a solution of 1,3-thiazinane-4-carboxamide (1.78 g, 12.2 mmol) in THF (5 mL) was added a solution of $(Boc)_2O$ (4.0 g, 1.5 eq.) in THF (10 mL) and Et_3N (5 mL). The mixture was refluxed overnight and extracted with CH_2Cl_2 (15 mL × 4). The combined organic layer was washed with saturated NaCl aq. (50 mL), dried over anhydrous Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure to give an orange oil (2.74 g, 91%). The crude product was purified on a silica gel column ($CHCl_3$-EtOAc=1:1) to obtain a tan-yellow solid (1.87 g, yield 48%) and white prism crystal (recrystallized from $CHCl_3$ and *n*-hexane); mp 137.0-138.0 °C; ^{1}H NMR (600 MHz, 50 °C, DMSO-d_6); δ 7.33 (br, 1H, NH_2), 7.08 (br, 1H, NH_2), 4.67 (br, 2H, H2), 4.35 (br, 1H, H4), 2.81 (t, *J* = 11.7 Hz, 1H, H6), 2.57 (t, *J* = 13.8 Hz, 1H, H6), 2.30 (t, *J* = 12.4 Hz, 1H, H5), 1.82 (br, 1H, H5), 1.42 (s, 9H, *t*-butyl); ^{13}C NMR (150 MHz, DMSO-d_6); δ 171.8 (CONH), 153.8 (CO in Boc), 153.6 (CO in Boc), 80.2 ($\underline{C}(CH_3)_3$), 55.2 (C4), 53.6 (C4), 43.7 (C2), 42.3 (C2), 28.1 (CH_3), 27.3 (C5), 27.0 (C5), 24.1 (C6), 23.8 (C6); HRMS (ESI-positive) $[M+H]^+$:247.11239 (calcd. for $C_{10}H_{18}N_2O_3S$ 247.11109).

General procedure for thiolation of carboxamides. Diphosphorus pentasulfide (1.1 eq.) was added to a solution of the corresponding carboxamides in THF (20 mL). The reaction mixture was ultrasonicated at <25 °C until the starting material disappeared in the TLC chromatograms. After addition of acetone, the reaction mixture was filtered and concentrated under reduced pressure. The crude product was purified on a silica gel column to afford the single desired product.

***t*-Butyl 4-carbamothioyl-1,3-thiazinane-3-carboxylate (8)**: white prism crystals (recrystallized from $CHCl_3$ and *n*-hexane); yield 29%; mp 167.0-169.0 °C (decomp.); ^{1}H NMR (600 MHz, 50 °C, $CDCl_3$); δ 7.58 (br, 1H, NH_2), 7.49 (br, 1H, NH_2), 4.97 (br, 1H, H2), 4.73 (d, *J* = 11.7 Hz, 1H, H2), 4.23 (d, *J* = 13.8 Hz, 1H, H4), 3.19 (br, 1H, H6), 2.97 (m, 1H, H6), 2.59 (d, *J* = 13.1 Hz, 1H, H5), 2.14 (m, 1H, H5), 1.51 (s, 9H, *t*-butyl). ^{13}C NMR (150 MHz, $CDCl_3$); δ 206.0 (C=S), 154.7 (C=O), 82.3 ($\underline{C}(CH_3)_3$), 62.1 (C4),

59.4 (C4), 43.5 (C2), 42.2 (C2), 28.2 (CH_3), 27.6 (C5), 23.3 (C6); HRMS (ESI-positive) $[M+H]^+$: 263.08900 (calcd. for $C_{10}H_{18}N_2O_2S_2$ 263.08825).

***t*-Butyl 3-carbamothioylthiomorpholine-4-carboxylate (9)**: white prism crystals (recrystallized from $CHCl_3$ and *n*-hexane); yield 29%; mp 136.0-138.2 °C; ^{1}H NMR (600 MHz, 50 °C, $CDCl_3$); δ 7.48 (br, 2H, NH_2), 5.12 (t, *J* = 4.8 Hz, 1H, H3), 4.29 (d, *J* = 13.1 Hz, 1H, H5), 3.62 (dd, *J* = 4.8, 13.8 Hz, 1H, H5), 3.14 (m, 1H, H2), 2.95 (dd, *J* = 4.8, 13.8 Hz, 1H, H2), 2.75 (dt, *J* = 3.9, 12.2 Hz, 1H, H6), 2.50 (d, *J* = 13.1 Hz, 1H, H6), 1.49 (s, 9H, *t*-butyl); ^{13}C NMR (150 MHz, $CDCl_3$); δ 205.3 (C=S), 155.2 (C=O), 82.0 ($\underline{C}(CH_3)_3$), 60.9 (C3), 41.7 (C5), 29.0 (C2), 28.3 (CH_3), 26.0 (C6); HRMS (ESI-positive) $[M+H]^+$: 263.09117 (calcd. for $C_{10}H_{18}N_2O_2S_2$ 263.08825).

General procedure for nitrosation of thiocarboxamides. The corresponding thiocarboxamides were dissolved in MeOH and acidified to pH 3 using 2 M HCl. The reaction mixture was stirred at 55-60 °C until the starting material disappeared in the TLC chromatograms. After the solution cooled to room temperature, sodium nitrite (1 eq.) was added, and the mixture was stirred for 1 h at room temperature until the deprotected compound disappeared. The reaction mixture was extracted with CH_2Cl_2, and the combined organic layer was washed with water, dried over anhydrous Na_2SO_4 and filtered, a small quantity of silica gel was added to the filtrate, and the mixture was evaporated. The residue adsorbed onto the silica gel was purified on silica-gel column (2.5% MeOH-$CHCl_3$, 254 nm & I_2 vapor) to afford the single desired compound.

***N*-Nitroso-1,3-thiazinane-4-carbothioamide (2)**: tan-yellow prism crystals (recrystallized from $CHCl_3$); yield 71%; mp 149.0-149.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$); *E* / *Z* = 35 / 65; δ 7.63 (br, 1H, *Z*-$CONH_2$), 7.32 (br, 1H, *E*-$CONH_2$), 7.07 (br, 1H, *E*-$CONH_2$), 6.90 (br, 1H, *Z*-$CONH_2$), 5.78 (dd, *J* = 3.8, 5.9 Hz, 1H, *Z*-H4), 5.75 (dd, *J* = 2.4, 5.9 Hz, 1H, *E*-H4), 5.55 (d, *J* = 13.2 Hz, 1H, *Z*-H2), 5.53 (d, *J* = 13.8 Hz, 1H, *E*-H2), 5.20 (d, *J* = 13.8 MHz, 1H, *Z*-H2), 3.91 (d, *J* = 13.2 MHz, 1H, *E*-H2), 3.50 (dt, *J* = 2.8 MHz, 1H, *Z*-H5), 3.41 (m, 2H, *E*-H5), 2.99 (m, 1H, *Z*-H5), 2.65 (m, 1H, *E*- and *Z*-H6), 2.33 (m, 1H, *E*-H6), 2.09 (m, 1H, *Z*-H6); ^{13}C NMR (150 MHz, $CDCl_3$); 202.4 (*E*-C=S), 201.4 (*Z*-C=S), 65.4 (*Z*-C2), 54.7 (*E*-C4), 47.8 (*E*-C2), 36.4 (*Z*-C4), 31.4 (*Z*-C6), 29.3 (*E*-C6), 24.2 (*Z*-C5), 23.6 (*E*-C5); HRMS (ESI-negative) $[M-H]^-$: 190.01360 (calcd. for $C_5H_9N_3OS_2$ 190.01143).

***N*-Nitrosothiomorpholine-3-carbothioamide (3)**: tan-yellow needle crystals (recrystallized from $CHCl_3$; yield 67%; mp 117.0-120.0 °C; ^{1}H NMR (600 MHz, $CDCl_3$); *E* / *Z* = 67 /33; δ 7.70 (br, 1H, *Z*-NH_2), 7.43 (br, 1H, *E*-NH_2), 7.12 (br, 1H, *Z*-NH_2), 6.83 (br, 1H, *E*-NH_2), 5.89 (dd, *J* = 4.8, 9.6 Hz, 1H, *E*-H3), 5.86 (dd, *J* = 3.6, 7.2 Hz, 1H, *Z*-H3), 5.28 (dt, *J* = 13.8, 3.4 Hz, 1H, *Z*-H5), 5.13 (dq, *J* = 13.8, 2.1 Hz, 1H, *E*-H5), 4.13 (m, 1H, *E*-H5), 4.01 (dt, *J* = 14.0, 2.4 Hz, 1H, *Z*-H5), 3.76 (dq, *J* = 14.3, 2.1 Hz, 1H, *Z*-H2), 3.17 (m, 1H, *E*-H2), 3.02 (ddd, *J* = 14.5, 6.0, 2.6 Hz, 1H, E-H6), 2.94 (ddd, *J* = 14.6, 10.8, 3.3 Hz, 1H,

Z-H6), 2.86 (m, 1H, *E*-H6), 2.80 (dd, *J* = 14.5, 4.8 Hz, 1H, *E*-H2), 2.58 (m, 1H, *Z*-H2), 2.55 (br, 1H, *Z*-H6); ^{13}C NMR (150 MHz, $CDCl_3$); δ 202.1 (*Z*-C=S), 200.4 (*E*-C=S), 66.5 (*Z*-C3), 56.5 (*E*-C3), 48.9 (*E*-C5), 39.2 (*Z*-C5), 31.7 (*Z*-C3), 29.9 (*E*-C3), 27.8 (*E*-C6), 26.7 (*Z*-C6); HRMS (ESI-negative) $[M-H]^-$: 190.01039 (calcd. for $C_5H_9N_3OS_2$ 190.01143).

Reaction of 1–3 with GSH

GSH (50 mM), L-phenylalanine (internal standard [IS], 20 mM) and DTPA (24 μM) were dissolved in 0.1 M sodium phosphate buffer (pH 7.4). The *N*-nitroso compound (4.5 mM) was dissolved in MeCN. Aliquots of GSH (300 μL, 5.0 mM), IS (300 μL, 2.0 mM), and DTPA (150 μL, 1.2 μM) were mixed, and the pH was adjusted to 1.5 using HCl for a total volume of 2.7 mL. The reaction was initiated by adding **1**–**3** (300 μL, 0.45 mM) at 37 °C. Aliquots were collected at specified intervals, and the GSNO yield was determined via HPLC using a Capcel-Pak UG120 (5 μm, 250 × 4.6 mm) column with MeCN / 0.05% trifluoroacetic acid (5/95) as the eluent at 1.0 mL/min for **1** and a Chromolith Performance RP-18e (5 μm, 100 × 4.6 mm) column with MeOH / 0.05% trifluoroacetic acid (0-3 min; 5/95, 3-17 min; 5-60/95-40, 7-12 min; 60/40, 12-16 min; 5/95) as the eluent at 1.0 mL/min for **2** and **3** at 335 nm (GSNO) and 258 nm (IS and compounds).

GSNO was synthesized according to the procedure reported by Akhter et al. (λ_{max} [H_2O]: 335 nm [ε=908] [lit. λ_{max}: 335 nm (ε=912)[32]]). Calibration curves for GSNO or *N*-nitroso compounds were prepared using L-phenylalanine as an IS. The concentration of GSNO and *N*-nitroso compounds were determined from the respective standard curves. As the reaction mixture was contaminated with transition metals, DTPA was added as a transition metal ion chelator to stabilize the GSNO.[33,34] All experiments were conducted in the dark due to the light instability of GSNO.[33,34]

Kinetic analysis of transnitrosation reactions

Reaction rate constants were determined by monitoring the GSNO formation at 37 °C. The k_{obs} values were determined by graphical analysis of the initial linear portion of the curve obtained from a plot of ln {[$GSNO_\infty$]/([$GSNO_\infty$] − [GSNO])} versus time. The theoretical value for $GSNO_\infty$ was the initial concentration of the *N*-nitroso compound. All rate values were calculated using the least-squares method, and each experiment was conducted in triplicate.

The quantity of *N*-nitroso residue was determined simultaneously with GSNO formation. The yield of *S*-transnitrosation from *N*-nitrosamines at 24 h (**1**, **3**) or 2 h (**2**) was calculated by dividing the percentage of GSNO formed by the percentage of *N*-nitroso compound consumed.

Buffer catalysis studies

GSH, IS, and DTPA were dissolved in 0.05-0.5 M sodium phosphate buffer (pH 7.4), and the pH was adjusted to 2.0 using HCl. The reaction mixtures were prepared and analyzed as previously described.

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