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EFFECT OF SULFUR-CONTAINING SIDE CHAINS ON TRANSNITROSATION OF *N*-NITROSO COMPOUNDS OF THIAZOLIDINES

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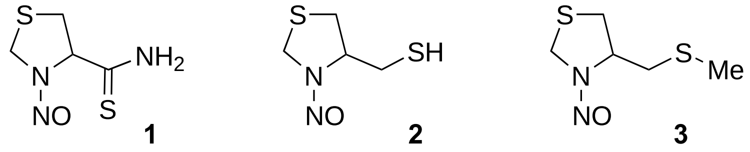
Abstract – *S*-Nitrosoglutathione (GSNO) acts as a potential nitric oxide donor *in vivo*. 3-Nitroso-1,3-thiazolidine-4-thiocarboxamide (**1**) exhibited a high GSNO-formation activity. In this study, we synthesized novel compounds in which methylthiol (**2**) or methylthiomethyl (**3**) group was incorporated into the thiazolidine skeleton instead of a thioamide group. Pseudo-first-order rate constants for GSNO formation for the reaction between the compounds and glutathione followed the order **1**>>**2**≒**3**. Introduction of another substituent containing a sulfur atom into the thioamide group this time resulted in a decrease in transnitrosation activity. These differences in activity suggested that compounds **2** and **3** transnitrosate via a different reaction pathway than **1**.

INTRODUCTION

Nitric oxide (NO) is a cell transmitter synthesized *in vivo* from arginine via NO synthase.[1-3] NO is involved in many biological processes, including vasodilation,[4-6] inhibition of platelet aggregation,[3,7] immune responses,[8,9] and nerve signal transduction.[10,11] Due to its chemical reactivity, NO has a short half-life (5-10 seconds).[12] *S*-Nitrosothiols (RSNOs) which are thought to act as NO transporters *in vivo*, are routinely used as donors for NO bioactivity, and they are also being investigated as NO donors for therapeutic agents.[13-15] As NO donor, RSNOs are expected to be useful therapeutic agents for treating cardiovascular, infectious, and other diseases.[16,17] In particular, *S*-nitrosoglutathione (GSNO), the product of *S*-nitrosation of glutathione, is an abundant intracellular antioxidant that has been detected *in vivo*. However, therapeutic applications of RSNOs may be limited by their short half-lives.[18,19]

N-Nitrosamines reportedly transfer nitrosonium ion (NO^+) to other amines to form the corresponding *N*-nitroso derivatives.[20] In the absence of a nitrosating donor, *N*-nitrosamines nitrosate protein sulfur atoms to form RSNOs.[21,22] Although many *N*-nitrosodialkylamines are potent carcinogens, some *N*-nitrosamines, such as *N*-nitrosoproline and *N*-nitrosothioproline, are nonmutagenic and noncarcinogenic.[23] Compound **1** (Figure 1), a derivative of *N*-nitrosothioproline in which the carboxyl group was converted to a thioamide group, was shown to exhibit greater GSNO-formation activity than *N*-nitrosothioproline.[21] We demostrated that the intramolecular sulfur atom plays an important role in transnitrosation by alicyclic *N*-nitroso compounds.[22] In this study, we synthesized three compounds in which the thiazolidine ring substituents were changed from a thioamide group to a methylthiol group and further methylated thiols and then evaluated the nitroso-transfer ability of these compounds.

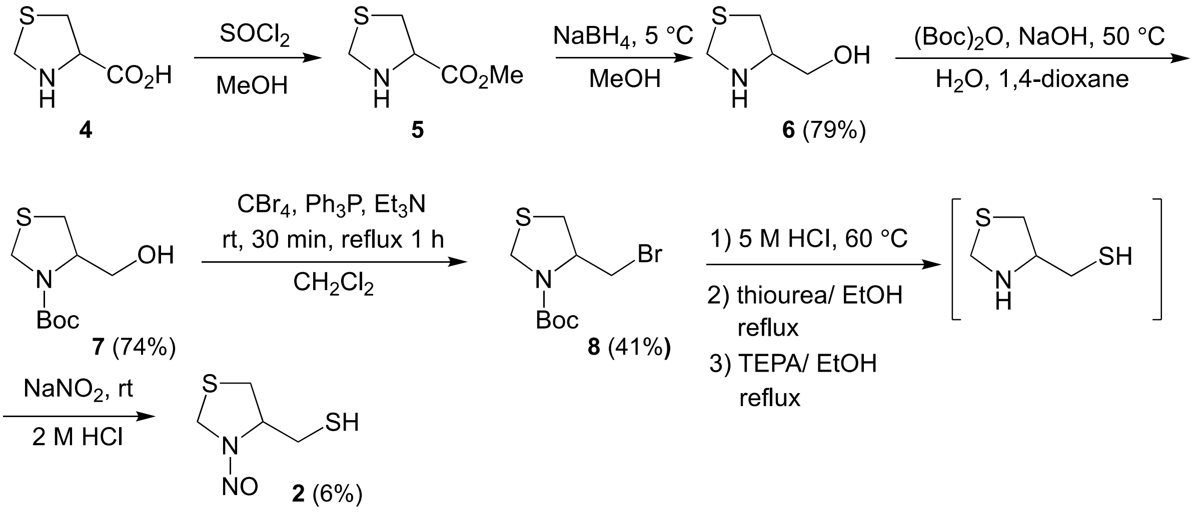


1 **2** **3**

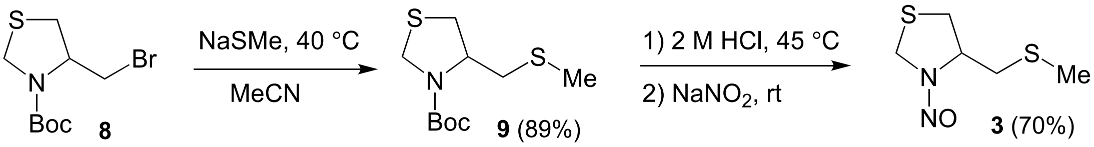
Figure 1. Structures of alicyclic *N*-nitrosamines

RESULTS AND DISCUSSION

In order to create compounds with greater nitroso group-transfer activity than compound **1**, we designed **2** with a thiol group instead of a thioamide group and **3** with an electron-donating methyl group introduced into **2** (Figure 1). The procedures for synthesizing compounds **2** and **3** are shown in Schemes 1 and 2. Methyl 1,3-thiazolidine-4-carboxylate (**5**) was prepared from L-thioproline (**4**) as previously reported.[21] 1,3-Thiazolidin-4-ylmethanol (**6**) was prepared via reduction of **5** in MeOH (Yield 79%). The nitrogen atom of **6** was protected with a *t*-butoxycarbonyl (Boc) group to prepare *t*-butyl 4-(hydroxymethyl)-1,3-thiazolidine-3-carboxylate (**7**, 74%). Compound **7** was brominated via Appel reaction to prepare *t*-butyl 4-(bromomethyl)-1,3-thiazolidine-3-carboxylate (**8**, 41%). After deprotection of **8** by acidification with HCl, thiourea and tetraethylenepentamine (TEPA) in EtOH were added for thiolation. The resulting product was acidified with HCl and nitrosated with $NaNO_2$ to prepare 3-nitroso-(1,3-thiazolidin-4-yl)methanethiol (**2**, 6%). *t*-Butyl 4-[(methylthio)methyl]thiazolidine-3-carboxylate (**9**) was obtained by thiomethylation of **8** using NaSMe (Yield 89%). 4-[(Methylthio)methyl]-3-nitrosothiazolidine (**3**) was prepared by deprotection of **9** under acidic conditions (HCl) and nitrosation using $NaNO_2$ (Yield 70%). Compounds **2**, **3**, **8**, and **9** were newly synthesized.



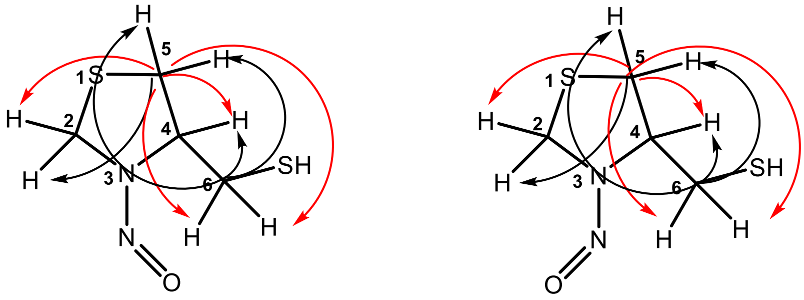
Scheme 1. Synthesis of **2**



Scheme 2. Synthesis of **3**

For the novel compounds **2**, **3**, **8** and **9**, ^{1}H and ^{13}C NMR and high resolution mass spectrometer (HRMS) spectra were measured. In terms of mass, the expected molecular ion peak was confirmed. Compounds **8** and **9** were assigned via ^{1}H and ^{13}C NMR, and confirmed as the intended compounds. However, for compounds **2** and **3**, *E/Z* isomer peaks were detected, overlapping peaks were detected in ^{1}H NMR, and multiple peaks were detected in ^{13}C NMR, making it difficult to assign these compounds via one-dimensional NMR. The structures were therefore deduced from a combination of ^{1}H-^{1}H COSY, HMQC and HMBC experiments.

In ^{1}H NMR analysis, compound **2** exhibited overlapping peaks at 3.2 to 3.4 ppm, and multiple peaks were observed in ^{13}C NMR analysis. The ^{1}H-^{1}H COSY correlations between 5.57 ppm (dd, J = 5.5, 10.3 Hz) and 5.18 ppm (d, J = 10.3 Hz), and between 4.76 pm (dd, J = 3.4, 12.4 Hz) and 4.51 pm (dd, J = 3.1, 12.1 Hz) allowed assignment of the signals of the H2. From the correlation between 5.35 ppm (m), 3.39 ppm (m) and 3.21 ppm (m), the correlation between 5.03 ppm (m), 3.39 ppm (m), 3.21 ppm (m) and 2.80 ppm (m) and the integral ratio of ^{1}H NMR spectra, 5.35 ppm (m) was confirmed to be the H4, *Z* form, and 5.03 ppm (m) was confirmed to be the H4, *E* form. From this result, we identified the respective peaks indicative of H bound to C2 and C4 of the *E/Z* isomers. HMQC identified C2 and C4 binding to H2 and H4. According to HMBC analysis (Figure 2), the carbon atoms at 33.5, 33.0, and 32.9 ppm are C5 because they are coupled to H2 and H4, and the carbon atoms at 41.8, 41.5, and 41.0 ppm are C6 because they are not coupled to H2. From the results of HMBC and HMQC analysis, H5 and H6 were confirmed, and it was also confirmed that the 1H of H5 and H6 in the *Z* form were present at 3.21 and 3.39 ppm, respectively (Table 1).



2(*E*) **2**(*Z*)

Figure 2. HMBC correlations of **2**

Table 1. ^{1}H- and ^{13}C-NMR spectral data for compounds **2** and **3** (600 and 150 MHz in $CDCl_3$)

C	2 (*E*)		2(*Z*)		3(*E*)		3(*Z*)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	5.57 (1H, dd, 5.5, 10.3) 5.18 (1H, d, 10.3)	52.8 52.7	4.76 (1H, dd, 3.4, 12.4) 4.51 (1H, dd, 3.1, 12.1)	47.0 46.9	5.58 (1H, d, 10.3) 5.16 (1H, d, 10.3)	52.9	4.77 (1H, d, 12.4) 4.49 (1H, d, 12.4)	47.0
4	5.03 (1H, m)	57.3 57.2	5.35 (1H, m)	62.6 62.6 62.5	4.82(1H, m)	57.3	5.13 (1H, *m*)	62.7
5	3.50 (1H, m) 3.21 (1H, m)	33.5	3.39 (1H, m) 3.21 (1H, m)	33.0 32.9	3.27 (1H, dd, 6.9, 12.4) 3.19 (1H, dd, 4.1, 7.6)	33.8	3.38 (1H, dd, 5.9, 12.1) 3.21 (1H, dd, 3.4, 11.7)	33.0
6	3.21 (1H, m) 2.80 (1H, m)	38.0 37.7	3.39 (1H, m) 3.21 (1H, m)	41.8 41.5 41.0	2.81(1H, dd, 3.5, 13.8) 2.65 (1H, dd, 8.3, 13.8)	34.6	3.10 (1H, dd, 4.8, 13.8) 3.04 (1H, dd, 9.0, 13.8)	37.7
SMe	-	-	-	-	2.17 (3H, s)	16.0	2.22 (3H, s)	16.1

Assignments were based on COSY, HMQC, and HMBC experiments.
Values in parentheses indicate coupling constants in Hz.

Compound **3** exhibited less complex ^{1}H and ^{13}C NMR spectra compared with compound **2**. However, in ^{1}H NMR analysis, peaks at 3.0 to 3.2 ppm overlapped, making assignment difficult. ^{13}C NMR analysis confirmed a carbon-atom number peak corresponding to the *E*/*Z* isomer. ^{1}H NMR analysis confirmed the *E*/*Z* forms H2, H4, and SMe (Table 1). In HMQC analysis, binding carbon atoms confirmed the identified hydrogen atom. HMBC analysis confirmed H6 coupling with the carbon of SMe, *E*-form 3.04 ppm (dd, J = 9.0, 13.8 Hz) and 2.65 ppm (dd, J = 8.3, 13.8 Hz), *Z*-form 3.09 ppm (dd, J = 5.2, 13.4 Hz) and 3.07

ppm (m). As C5 couples with H2, the *E*-form C5 was assigned to 33.8 ppm and the *Z*-form C-5 to 33.0 ppm. The hydrogen atom attached to the C5 carbon atom was assigned based on HMQC and ^{1}H-^{1}H COSY data.

For all compounds, the atoms could not be assigned based on ^{1}H NMR spectra obtained by overlapping; instead, the compounds were identified based on the integral ratio and two-dimensional NMR data.

Evaluation of transnitrosation activity based on GSNO formation

The transnitrosation activity of the compounds was evaluated based on their capacity to form GSNO. Pseudo–first–order rate constants (k_{obs}) were calculated using the slope of the linear relationship between GSNO formation and time (Figure 3). The transnitrosation results are summarized in Table 2 with k_{obs} values ordered as follows: **1** >> **2** = **3**. The GSNO yields after 24 h of reaction time was calculated by dividing [GSNO formation] by [*N*-nitrosamine consumption] and ranged from 92 to 93%. *N*-Nitrosamine consumption was calculated from the concentrations of compounds after 0 and 24 h. These results indicate that transnitrosation to GSH proceeded selectively without decomposition of the compound.

A chiral center at 4-position in thiazolidine ring results in D and L enantiomers, and the partial double bond character of the N–N=O group also results in *E* and *Z* isomers. 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 ($NH^+—N=O$).[24,25]

Farrelly et al. reported that *E* isomer of asymmetrical nitrosamine was preferentially metabolized.[26] There

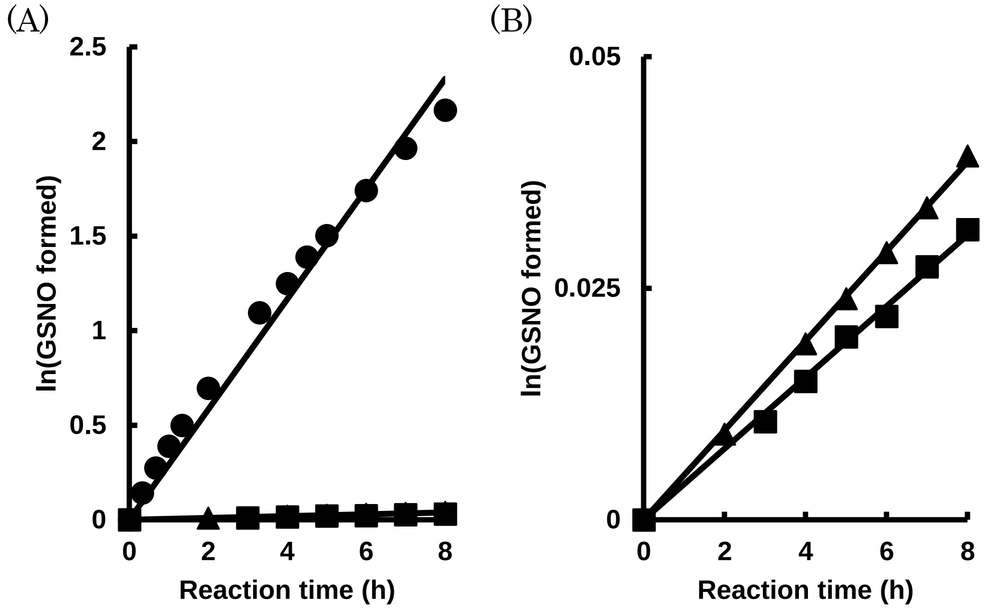


Figure 3. Formation of GSNO by the reaction of *N*-nitrosamines (**1**-**3**) with GSH.
The reaction conditions were as follows: compound **1** (●), **2** (▲), and **3** (■) 0.45 mM; GSH, 5.0 mM, pH 1.5, temperature 37 °C. (B) is an extension of (A).

Table 2. Reaction rate constant (k_{obs}) values and GSNO yield

N-nitrosamine[a]	k_{obs} [b] ($\times 10^{-7}$/s)	GSNO[c] (mM)	*N*-nitrosamine consumption[c] (mM)	GSNO yield[c] (%)
1	1014	0.42	0.45	93
2	31	0.12	0.13	92
3	24	0.098	0.095	93

[a] The reaction conditions are as follows: *N*-nitrosamine, 0.45 mM; GSH, 5.0 mM; pH 1.5; temperature 37 °C; (in the dark).

[b] The k_{obs} values for GSNO formation were calculated from the slope of $\ln\{[GSNO_\infty]/([GSNO_\infty] - [GSNO])\}$ versus time, where GSNO and $GSNO_\infty$ refer to the GSNO concentration at a given time and the final concentration (0.45 mM), respectively. The k_{obs} values were determined using the least-squares method.

[c] Data were acquired after 24 h.

is a possibility that transnitrosation activity of the synthesized compounds were different among *E* or *Z* isomer. The synthesized nitrosamines in NMR spectra have two peaks for one nitrosamines, however, we observed one peak on HPLC analyses and couldn't follow the reactivity of *E* or *Z* isomers.

Buffer catalysis studies of GSNO formation

To further explore the effects of pH on the compounds and GSH in the acidic range, buffer catalysis was investigated at pH 1.5 by measuring k_{obs} values at varying buffer concentrations while maintaining constant pH and ionic strength. The slope of the relationship between k_{obs} and pH was defined as the catalytic rate constant, k_{cat}. Values of k_{obs} unaffected by buffer concentration and the k_{cat} value (0.003) was near zero (Figure 4). Because the pH-rate profile showed a dependence of k_{obs} on pH 1.5, 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.

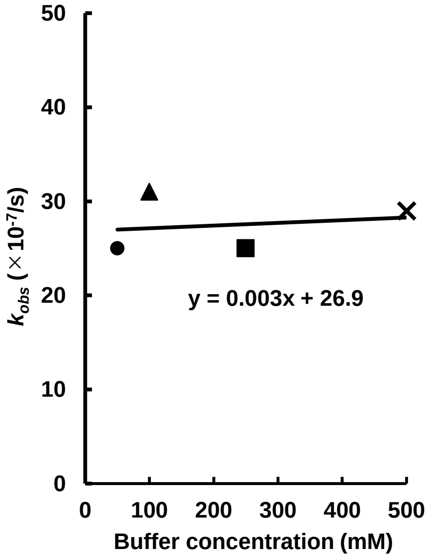


Figure 4. Effect of buffer concentration on the formation of GSNO (k_{obs}) with compound **2** and GSH. The reaction condition were as follows: *N*-nitrosamine, 0.45 mM; GSH, 5.0 mM; pH 1.5; buffer concentration, 50 mM (●), 100 mM(▲), 250 mM(■), 500 mM(×); temperature 37 °C.

However, contrary to expectations, the k_{obs} values of **2** and **3** for GSNO formation were lower than that of **1**. When a methyl group; (which is an electron-donating group) was substituted into the thioamide group, the k_{obs} values for **2** and **3** were lower than that of **1**. The introduction of alkyl groups into the thioamide group is thought to lower the k_{obs} due to steric hindrance.[22] However, the k_{obs} values of **2** and **3** were even lower than would be expected. A thiol group is considered to have less steric hindrance than a thioamide group. Compound **1** is protonated at the nitrogen atom to which the nitroso group is bonded, forming a bridging-type structure between the nitrogen atom of the nitroso group and two sulfur atoms (one in the ring and the other in the substituent).[22]

The sulfur atom of the thioamide group of **1** is bonded to the sp^2 carbon, whereas the exocyclic sulfur atom bonded to the sp^3 carbon in compounds **2** and **3**. The experimentally determined C-S bonds of the side chains of compounds **1**-**3** were 1.678, 1.848, and 1.838 Å (B3LYP/6-311G+[d]), respectively. Compounds **2** and **3** have a longer bond distance than **1**, and the activation energy via the bridging-type structure is increased; therefore it was thought that this structure could not be formed. It is also possible that the structure with S (in substituent) - NO is stabilized; and that the nitroso group does not transfer to GSH. All GSNO yields were the same (Table 2), indicating that any intermediates did not remain in reaction mixture. Therefore, we hypothesized that the nitroso group attached to the nitrogen atom is directly transferred to GSH. Indeed, the k_{obs} values of compounds **2** and **3** corresponded closely to those of 1-nitrosopyrrolidine-2-thiocarboxamide, *N*-nitrosothioproline, and 3-nitroso-1,3-thiazolidine-4-carboxamide containing one sulfur atom.[21] This result suggested that the distance between the sulfur atoms in the thioamide group was more appropriate for the intramolecular transfer of the nitroso group.

Intramolecular transfer via the bridging structure was thought to reduce the energy required for transfer to the nitroso group from these compounds to GSH, leading to the efficient formation of GSNO.

In this study, we synthesized three aliphatic compounds (**1-3**) with a sulfur atom in the side chain of a thiazolidine skeleton with an intracyclic sulfur atom and evaluated GSNO formation via these compounds. All three compounds exhibited transnitrosation activity under acidic conditions. Compound **1** exhibited the strongest activity, whereas compounds **2** and **3** exhibited weak activity. The activity of **2** and **3** was almost the same as that of the compound containing one sulfur atom. Compounds **1-3** contain two sulfur atoms (one in the ring and one in the substituent). Compound **1** is believed to undergo intramolecular transfer of the nitroso group to the sulfur atom of the thioamide group, followed by transnitrosation to GSH. However, the transnitrosation of **2** and **3** to GSH appears to involve transfer via different mechanisms.

EXPERIMENTAL

Materials and Methods

L-Thioproline was obtained from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan). Diphosphorous 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 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]). 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 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[22] (observed mp 121.5-122.0 °C).

Synthesis of (3-nitroso-1,3-thiazolidin-4-yl)methanethiol (2)

*1,3-Thiazolidin-4-ylmethanol (**6**)*

Methyl 1,3-thiazolidine-4-carboxylate hydrochloride was prepared according to a previously reported procedure.[22] Methyl 1,3-thiazolidine-4-carboxylate hydrochloride (3.76 g, 0.02 mol) was dissolved in sat. $NaHCO_3$ (15 mL), and the mixture was extracted with CH_2Cl_2 (15 mL × 4). The organic phase was dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure to afford a tan-yellow oil (2.96 g, 98%). Sodium borohydride (1.52 g, 2.0 eq.) was added slowly to a solution of the crude methyl 1,3-thiazolidine-4-carboxylate in a distilled MeOH (20 mL) below 5 °C under a nitrogen gas flow and stirred overnight. Water (10 mL) was added to the reaction mixture, which was then extracted with CH_2Cl_2 (15 mL × 5). The combined organic phase was dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure to afford a tan-yellow oil (1.89 g, 79%). ^{1}H NMR (600 MHz, δ ppm in CD_3OD); 4.16 (d, J = 9.3 Hz, 1H, H2), 4.04 (d, J = 9.0 Hz, 1H, H2), 3.68 (d, J = 5.1 Hz, 2H, H6), 3.30 (m, 1H, H4), 2.95 (dd, J = 6.3, 10.2 Hz, 1H, H5), 2.69 (dd, J = 8.1, 10.2 Hz, 1H, H5).

*t-Butyl 4-(hydroxymethyl)-1,3-thiazolidine-3-carboxylate (**7**)*

A solution of $(Boc)_2O$ (2.85 g, 1.2 eq.) in 1,4-dioxane (10 mL) was added dropwise to a solution of crude 1,3-thiazolidin-4-ylmethanol (1.28 g, 10.7 mmol) in 0.5 M NaOH (20 mL) and stirred at 50 °C for 30 min. Water (10 mL) was added to the reaction mixture, which was then extracted with CH_2Cl_2 (15 mL × 4), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure to afford a colorless oil. The crude product was purified several times on a silica gel column (*n*-hexane:EtOAc [3:1], UV 254 nm & I_2 vapor) to afford a colorless oil (1.73 g, 74%). ^{1}H NMR (600 MHz, δ ppm in $CDCl_3$); 4.61 (d, J = 9.0 Hz, 1H, H2), 4.35 (br, 1H, H4), 4.28 (d, J = 9.6 Hz, 1H, H2), 3.71 (t, J = 5.7 Hz, 2H, H6), 3.15 (dd, J = 6.6, 11.7 Hz, 1H, H5), 2.90 (br, 1H, H5), 1.48 (s, 9H, *t*-butyl).

*t-Butyl 4-(bromomethyl)-1,3-thiazolidine-3-carboxylate (**8**)*

t-Butyl 4-(hydroxymethyl)-1,3-thiazolidine-3-carboxylate (0.98 g, 4.47 mmol) and carbon tetrabromide (1.92 g, 1.3 eq.) were dissolved in CH_2Cl_2 (15 mL), added dropwise to a solution of triphenylphosphine (1.52 g, 1.3 eq.) in CH_2Cl_2 (10 mL), and then triethylamine (0.80 mL, 1.3 eq.) was added dropwise under a nitrogen gas flow. The mixture was stirred for 30 min at room temperature and then refluxed for 1 h. After the reaction mixture was cooled to room temperature, *n*-hexane (60 mL) was added, the CH_2Cl_2 was evaporated, and then a brown solid appeared. The solid was filtered off, and the filtrate was evaporated under reduced pressure to give a brown solid. The crude product was purified twice on a silica gel column ($CHCl_3$, UV 254 nm & I_2 vapor and *n*-hexane:CH_2Cl_2 [1:1], UV 254 nm & I_2 vapor) to afford a colorless oil (514 mg, 41%). ^{1}H NMR (600 MHz, δ ppm in CD_3CN); 4.54 (br, 1H, H2), 4.40 (br, 1H, H2), 4.23 (br, 1H, H4), 3.53 (m, 2H, H6), 3.21 (br, 1H, H5), 3.07 (br, 1H, H5), 1.45 (s, 9H, *t*-butyl). ^{13}C NMR (150 MHz, δ ppm in CD_3OD); 155.1 (C=O), 82.8 ($-\underline{C}(CH_3)_3$), 82.7 ($-\underline{C}(CH_3)_3$), 62.5 (C5), 50.1 (C2), 35.7 (C4), 34.7 (C4), 33.7 (C6), 33.2 (C6), 28.9 ($-CH_3$). HRMS (ESI-positive) $[M+H]^+$ 282.01531, (calcd. for

$C_9H_{16}NO_2SBr$ 282.01579).

*3-Nitroso-(1,3-thiazolidin-4-yl)methanethiol (**2**)*

t-Butyl 4-(bromomethyl)-1,3-thiazolidine-3-carboxylate (1.19 g, 4.22 mmol) was dissolved in 2 M HCl (10 mL) and stirred at 60 °C overnight. The mixture was evaporated under reduced pressure to afford a tan-yellow oil. Thiourea (934 mg, 1.5 eq.) was added to a solution of the crude product in EtOH (20 mL) and the mixture was refluxed overnight.[28] Tetraethylenepentamine (1.85 mL, 1.2 eq.) was added dropwise to the mixture and refluxed for 3 h. After the mixture was cooled to room temperature, H_2O (5 mL) was added, and the mixture was saturated with NaCl, and extracted with CH_2Cl_2 (10 mL × 4). The combined organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to yield a yellow oil. The crude product was purified on a silica gel column ($CHCl_3$: MeOH [20:1], UV 254 nm & I_2 vapor) to afford a tan-yellow oil (390 mg, 52%). 1,3-Thiazolidin-4-ylmethanethiol (388 mg, 2.87 mmol) was dissolved in 1 M HCl (10 mL), $NaNO_2$ (219 mg, 1.1 eq.) was added, and the mixture was stirred for 10 min at room temperature. The mixture was then extracted with CH_2Cl_2 (10 mL × 4). The combined organic phase was washed with H_2O (10 mL), sat. $NaHCO_3$ (10 mL), and H_2O (10 mL). The organic phase was dried over Na_2SO_4 and filtered, after which 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 twice on a silica gel column (*n*-hexane:EtOAc [2:1], UV 254 nm & I_2 vapor) to afford a tan-yellow oil (56 mg, 12%). The oil was recrystallized from EtOAc and *n*-hexane to give a white needle like product (30 mg, 6%). Mp 99.0-102.0 °C; ^{1}H NMR (600 MHz, δ ppm in $CDCl_3$); *E*/*Z* = 22 /78; 5.57 (dd, *J* = 5.5, 10.3 Hz, 1H, *E*-H2), 5.35 (m, 1H, *Z*-H4), 5.18 (d, *J* = 10.3 Hz, 1H, *E*-H2), 5.03 (m, 1H, *E*-H4), 4.76 (dd, *J* = 3.4, 12.4 Hz, 1H, *Z*-H2), 4.51 (dd, *J* = 3.1, 12.1 Hz, 1H, *Z*-H2), 3.50 (m, 1H, E-H5), 3.39 (m, 2H, *Z*-H5, *Z*-H6), 3.21 (m, 4H, *Z*-H5, *Z*-H6, *E*-H5, *E*-H6), 2.80 (m, 1H, *E*-H6). ^{13}C NMR (150 MHz, δ ppm in $CDCl_3$); 62.6 (*Z*-C4), 62.6 (*Z*-C4), 62.5 (Z-C4), 57.3 (*E*-C4), 57.3 (*E*-C4), 52.8 (*E*-C2), 52.7 (*E*-C2), 47.0 (*Z*-C2), 46.9 (*Z*-C2), 41.8 (*Z*-C6), 41.5 (*Z*-C6), 41.0 (*Z*-C6), 38.0 (*E*-C6), 37.7 (*E*-C6), 33.5 (*E*-C5), 33.0 (*Z*-C5), 32.9 (*Z*-C5). HRMS (ESI-positive) $[M+H]^+$ 165.01549 (calcd. for $C_4H_9N_2OS_2$ 165.01508).

Synthesis of 4-[(methylthio)methyl]-3-nitrosothiazolidine (3)

*t-Butyl 4-[(methylthio)methyl]thiazolidine-3-carboxylate (**9**)*

NaSMe (950 µL, 2.0 eq.) was added dropwise to a solution of *t*-butyl 4-(hydroxymethyl)-1,3-thiazolidine-3-carboxylate (286 mg, 1.01 mmol) in MeCN (5 mL), and the mixture was stirred at 40 °C for 1.5 h under argon gas flow. Additional NaSMe (140 µL, 0.3 eq.) was added, and the mixture was stirred at 40 °C for 1 h. Water (10 mL) was added the reaction mixture, which was then extracted with CH_2Cl_2 (15 mL × 4). The combined organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give a colorless oil. The crude product was purified on silica gel column ($CHCl_3$, UV 254 nm & I_2 vapor) to afford a colorless oil (226 mg, 89%). ^{1}H NMR (600 MHz, δ ppm in acetone-*d*$_6$); 4.57 (d, *J* = 9.0 Hz, 1H, H2), 4.40 (br, 1H, H4), 4.25 (d, *J* = 7.6 Hz, 1H, H2), 3.20 (br, 1H, H5), 3.05 (br, *J* = 8.9 Hz, 1H, H5), 2.78 (br, 1H, H6), 2.67 (dd, *J* = 9.7 Hz, 1H, H6), 2.16 (s, 3H, $SC\underline{H}_3$), 1.47 (s, 9H, *tert*-butyl). ^{13}C NMR (150 MHz, δ ppm in acetone-*d*$_6$); 153.2 (C=O), 80.2 ($-\underline{C}(CH_3)_3$), 59.4 (C4), 59.2 (C4), 48.4 (C2), 36.7 (C5), 36.3 (C5), 34.9 (C6), 33.9 (C6), 28.0 ($C(\underline{C}H_3)_3$), 15.0 ($S\underline{C}H_3$). HRMS (ESI-positive) $[M+H]^+$ 250.09395 (calcd. for $C_{10}H_{19}NO_2S_2$ 250.09300).

*4-[(Methylthio)methyl]-3-nitrosothiazolidine (**3**)*

A solution of *t*-butyl 4-[(methylthio)methyl]thiazolidine-3-carboxylate (224 mg, 0.9 mmol) in 2 M HCl (9 mL) was stirred at 45 °C for 2 h under argon gas flow. After the mixture was cooled to room temperature, a solution of $NaNO_2$ (76 mg, 1.2 eq. in H_2O [1 mL]) was added, and the mixture was stirred for 30 min under argon gas flow. The mixture was then extracted with CH_2Cl_2 (15 mL × 4). The combined organic phase was dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure to give a red-colored oil. The crude product was purified twice on a silica gel column ($CHCl_3$, UV 254 nm & I_2 vapor and *n*-hexane:CH_2Cl_2 [1:3], UV 254 nm & I_2 vapor) to afford a tan-yellow oil (112 mg, 70%). ^{1}H NMR (600 MHz, δ ppm in $CDCl_3$); *E*/*Z* =24/76; 5.58 (d, *J* = 10.3 Hz, 1H, *E*-2), 5.16 (d, *J* = 10.3 Hz, 1H, *E*-2), 5.13 (m, 1H, *Z*-4), 4.82, (m, 1H, *E*-4), 4.77 (d, *J* = 12.4 Hz, 1H, *Z*-2), 4.49 (d, *J* = 12.4 Hz, 1H, *Z*-2), 3.38 (dd, *J* = 5.9, 12.1 Hz, 1H, *Z*-5), 3.20 (m, 2H, *E*-5, *Z*-5), 3.09 (dd, *J* = 5.2, 13.4 Hz, 1H, *Z*-6), 3.07 (m, 1H, *Z*-6), 3.04 (dd, *J* = 9.0, 13.8 Hz, 1H, *E*-6), 2.81 (dd, *J* = 3.5, 13.8 Hz, 1H, *E*-5), 2.65 (dd, *J* = 8.3, 13.8 Hz, 1H, *E*-6), 2.22 (s, 3H, *Z*-CH_3), 2.17 (s, 3H, *E*-CH_3). ^{13}C NMR (150 MHz, δ ppm in $CDCl_3$); 62.6 (*Z*-4), 57.2 (*E*-4), 52.8 (*E*-2), 47.0 (*Z*-2), 37.6 (*Z*-6), 34.6 (*E*-6), 33.8 (*E*-5), 33.0 (*Z*-5), 16.1 (*Z*-CH_3), 15.9 (*E*-CH_3). HRMS (ESI-positive) $[M+H]^+$ 179.03049 (calcd. for $C_5H_{10}N_2OS_2$ 179.03073).

Reaction of compounds 1–3 with GSH

GSH (50 mM), DTPA (24 μM) and L-phenylalanine (internal standard [IS], 20 mM) 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. Chromatographic separation was achieved using a Capcel-Pak UG120 (5 μm, 250 × 4.6 mm) column at room temperature. The mobile phase for **1** consisted of MeCN:0.05% trifluoroacetic acid (TFA) (5:95) at 1.0 mL/min. The mobile phase for **2** consisted of solvent A (0.05% TFA) and solvent B (MeOH) for gradient elution, and was delivered at a flow rate of 1.0 mL/min under the following conditions: 5% solvent B at 0 min, 5% solvent B at 1 min, 60% solvent B at 15 min, 60% solvent B at 25 min, 5% solvent B at 25.01 min. The mobile phase for **3** consisted of solvent A (0.05% TFA) and solvent B (MeOH) for gradient elution, and was delivered at a flow rate of 1.0 mL/min under the following conditions: 4% solvent B at 0 min, 4% solvent B at 1 min, 60% solvent B at 18 min, 60% solvent B at 25 min, 4% solvent B at 25.01 min. GSNO was detected at 335 nm, and the IS and compounds were detected at 258 nm.

GSNO was synthesized according to the procedure reported by Akhter et al. (λ_{max} [H_2O]: 335 nm [ε=914] [lit. λ_{max}: 335 nm (ε=912)[29]]). 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.[30,31] All experiments were conducted in the dark due to the light instability of GSNO.[30,31]

Kinetic analysis of transnitrosation reactions

Reaction rate constants were determined by monitoring GSNO formation at 37 °C. The k_{obs} values were determined by graphical analysis of the initial linear portion of curves obtained from plots of ln $\{[GSNO_\infty]/([GSNO_\infty] - [GSNO])\}$ versus t. 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 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 1.5 using HCl. The reaction mixtures were prepared and analyzed as previously described.

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