Cleavage of S–S Bond by Nitric Oxide (NO) in the Presence of Oxygen: A Disproportionation Reaction of Two Disulfides

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Disulfide bond was cleaved by a catalytic amount of nitric oxide in the presence of oxygen, which was confirmed by experiments employing two symmetrical disulfides. The reaction resulted in the formation of unsymmetrical disulfides in nearly 50% yields. The steric hindrance of alkyl disulfide slowed the reaction rate, and an electron-donating group on the aryl disulfide promoted the reaction. The substituent and *S*-nitrosothiol effects suggested that the reaction was initialized with an oxidative process by NO⁺.

Key words disulfide; nitric oxide; disproportionation; nitrogen oxide; oxidation; *S*-nitrosothiol

Nitric oxide (NO)¹⁾ is an important biological messenger that plays a role in physiological and pathological conditions such as endothelium-dependent vasorelaxation and septic shock.^{2,3)} In the reaction with organic molecules, NO has been recognized to react with various amines⁴⁾ and thiols⁵⁾ in the presence of oxygen, and the interaction of NO with sulfhydryl group in biomolecules and enzymes has recently gained considerable importance.⁶⁾ In addition, one of the NO donors employed frequently is *S*-nitrosothiol, and there are a lot of papers that treat the decomposition of this compound.⁷⁾ In the course of the cleavage of *S*-nitrosothiol to release NO, the remaining thiol group was converted to disulfide,⁸⁾ and a recent paper showed that *S*-nitrosothiols reacted with thiol groups in protein molecules to afford mixed disulfide compounds.⁹⁾ Thus, disulfide and NO have a close relationship in the biological system, but their interaction has never been reported thus far. We have recently been investigating the chemical reactivity of NO,¹⁰⁾ and found that NO and O₂ reacted with disulfide to cleave the disulfide bond to form an unsymmetrical disulfide when two symmetric disulfides were mixed. The results suggest that the reaction has potential to participate in the biological systems. This paper describes detailed results of the reaction.¹¹⁾

Results and Discussion

Two symmetrical disulfides brought about the disproportionation reaction to form an unsymmetrical disulfide in yields up to 50% in the presence of a catalytic amount of NO and the results are summarized in Chart 1 and Table 1. It was found that the reaction must be performed in the dark; without a shield, the reaction rate became faster, and unreproducible. These facts indicated participation of photosensitive intermediate(s) in the reaction.

In most cases, the reaction converged on the product ratio of 1 : 2 : 1 as shown in Chart 1. When di(*tert*-butyl) disulfide was used, the exchange reaction was not observed (entries 4, 9), and diphenyl disulfide reacted fairly slowly. These results suggested that the reaction was considerably influenced by

steric hindrance. The presence of a hydroxyl group in the substrate slowed the reaction (entry 13), but a carboxyl group did not affect the rate (entries 14, 15). Nitrogen oxides are known to react with hydroxyl groups to give stable esters, thus the active species was assumed to be trapped in the case of entry 13.

Next, the reaction was carried out in the presence of various amounts of NO and O₂ (or NO₂) using diethyl and dibenzyl disulfides (Chart 2, Table 2).

The reaction proceeded by catalytic amounts of NO in air (Table 2, entries 2–4), but not in the absence of NO (entry 1) and/or O₂ (entry 5). Thus, both gases were found to be necessary for the reaction. Since it is well-known that NO and O₂ react rapidly to form active nitrogen oxides such as N₂O₃ and NO₂ (N₂O₄),¹²⁾ the above results indicated the participation of these species in the process. Among nitrogen oxides, dinitrogen trioxide N₂O₃ is formed by the reaction of NO and NO₂. Thus a catalytic amount of NO₂ (0.0005 eq to

Table 1. Formation of Unsymmetrical Disulfide by the Reaction of Two Symmetrical Disulfides in the Presence of a Catalytic Amount of Nitric Oxide in Air

Entry	R	R'	Reaction time (h)	Yield of 2 (%) ^{a)}
1	Me	Et	1	49
2	Me	Bu	1	40
3	Me	<i>sec</i> -Bu	1	47
4	Me	<i>tert</i> -Bu	5	0
5	Me	Bn	1	49
6	Me	Ph	1 (3)	2 (50)
7	Me	<i>p</i> -MePh	1 (3)	18 (50)
8	Me	<i>p</i> -MeOPh	1	48
9	Et	<i>tert</i> -Bu	5	0
10	Et	Bn	1 (5)	12 (47)
11	Et	Ph	1 (5)	0 (8)
12	Bu	Bn	1 (5)	2 (17)
13	Me	–CH ₂ CH ₂ OH	5	35
14	Me	–CH ₂ CO ₂ H	1	50
15	Me	–CH ₂ CH(NHCbz)CO ₂ H	1	49

a) The yield was estimated based on the stoichiometry shown in Chart 1.

Chart 1

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Dedicated to the memory of Dr. Kyosuke Tsuda.

Chart 2

Table 2. The Effect of NO and O₂ (or NO₂) on the Reaction of Diethyl and Dibenzyl Disulfides

Entry	Amount of NO (eq)	Amount of O ₂ (or NO ₂) (eq)	Yield of 3 (%) ^{a)}			
			Reaction time (h)			
			0.5	1	3	5
1	0	In air (ca.0.18)	0	0	0	0
2	0.0025	In air (ca.0.18)	3	5	10	18
3	0.005	In air (ca.0.18)	2	13	44	47
4	0.01	In air (ca.0.18)	37	47	47	47
5	0.005	0	0	0	0	0
6	0.005	0.005	1	2	21	36
7	0.005	0.05	2	12	40	50
8	0.005	NO ₂ 0.0005	0	2	28	43
9	0.05	NO ₂ 0.0005	0	3	27	41
10	0	NO ₂ 0.0005	0	1	4	5
11	0	NO ₂ 0.005	17	30	47	49

a) The yield was estimated based on the stoichiometry shown in Chart 2.

Table 3. The Solvent Effect on the Reaction of Diethyl and Dibenzyl Disulfides

Entry	Solvent	NO (eq)	Yield of 3 (%) ^{a)}			
			Reaction time (h)			
			1	3	5	24
1	CH ₃ CN	0.005	12	42	48	48
2	CH ₃ CH ₂ CN	0.005	0	0	0	0
3	CH ₃ CH ₂ CH ₂ CN	0.005	0	0	0	0
4	(CH ₃) ₃ CCN	0.005	2	4	8	30
5	ClCH ₂ CH ₂ Cl	0.005	50	50	49	—
6	BrCH ₂ CH ₂ Br	0.005	5	15	20	—
7	CH ₂ Cl ₂	0.05	2	13	38	—
8	CH ₂ Br ₂	0.05	50	50	48	—
9	CH ₃ CN+H ₂ O (0.05 eq)	0.005	8	41	49	48
10	CH ₃ CN+H ₂ O (0.5eq)	0.005	2	16	31	46
11	CH ₃ CN+H ₂ O (5 eq)	0.005	0	0	0	39 ^{b)}

a) The yield was estimated based on the stoichiometry shown in Chart 2. b) 72 h.

the total amount of disulfides) was added to the reaction mixture of two disulfides (1.0 eq of each) and NO (0.005 eq), and the disproportionation reaction proceeded in spite of the absence of O₂ (entry 8). In the presence of the same amount of NO₂ (0.0005 eq), the increase of NO by 10 times (from 0.005 to 0.05) did not affect the reaction yields (entries 8, 9). These facts suggest that N₂O₃ is the active species, and NO₂ is completely transformed to N₂O₃ under these conditions. As another possible active species, NO₂ was applied to the reaction (entries 10, 11), and it was found that 0.005 eq of NO₂ afforded a result between those of entries 3 and 4. Thus, NO₂ (probably in the form of N₂O₄) was also reactive toward disulfides.

In order to obtain information about the reaction mechanism, the solvent effect was investigated, and the results are summarized in Table 3.

Acetonitrile and 1,2-dichloroethane were found to be the best solvents for the reaction (Table 3, entries 1, 5).¹³⁾

Chart 3

Table 4. Substituent Effects on the Reaction of Aryl Methyl Disulfides with a Catalytic Amount of Nitric Oxide in Air.

Entry	X	Yield of 5 (%) ^{a)}		
		Reaction time (min)		
		10	30	60
1	H	0	39	47
2	Me	26	50	—
3	Cl	0	8	35

a) The yield was estimated based on the stoichiometry shown in Chart 3.

Among the nitriles, only acetonitrile and pivalonitrile yielded **3** (entries 1—4). The α-hydrogens of the nitrile group in propionitrile or butyronitrile were assumed to be abstracted more readily than those of acetonitrile, thus we speculate that the active species in the reaction medium might be quenched by the reaction with the α-hydrogens in propionitrile or butyronitrile. Addition of water to the reaction fairly slowed the reaction (entries 9—11), because the amount of the active species was catalytic, and N₂O₃ and NO₂ are rapidly hydrolyzed by water. In other solvents such as methanol, dimethylformamide (DMF) ether, and tetrahydrofuran (THF) the reaction did not proceed within 5 h.

Next, unsymmetrical aryl methyl disulfides were employed for the reaction (Chart 3, Table 4).

The data shown in Table 4 suggest that electron rich substrates reacted faster than those having an electron-withdrawing group. Thus, the reaction was supposed to proceed *via* an oxidative step. It was shown that superoxide,¹⁴⁾ which is also a ubiquitous biological radical, reacted with disulfide in a reductive manner,¹⁵⁾ thus nitrogen oxides are suggested to have the opposite reactivity toward disulfides.

There have been a few reports concerning the reaction of disulfides with nitrogen oxides, and Oae *et al.* reported the stoichiometrical reactions of N₂O₄ with disulfides.¹⁶⁾ It was suggested that the reaction was initialized by S-oxygenation to form thiosulfinate **6**, which was nitrosated by N₂O₄ to give S-nitrosothiol **7**. And S-nitrosothiol thus obtained was further decomposed by N₂O₄ to disulfides, thiosulfonates, or sulfonates depending upon the reaction conditions (Chart 4).¹⁷⁾

In our reaction system, it should be possible that an intermediary radical species formed in the above scheme is responsible for the radical chain reaction to obtain the mixture of three disulfides.

When diethyl disulfide was allowed to react with NO (0.1 eq) in air, the ¹H-NMR of the mixture showed two major

products (product **A**, **B**) other than the starting material. After 3 d, the measurement of the same sample showed that the peaks of the product **A** disappeared and those of **B** and diethyl disulfide increased instead. There have been several reports that thiosulfonates were unstable and readily decomposed to thiosulfonate and disulfide,¹⁸⁾ thus we assumed that the spectral change was derived from the disproportionation of two thiosulfonates into a thiosulfonate and a disulfide (Chart 5). During the disproportionation, there might be radi-

Chart 4

Chart 5

Chart 6

Table 5. Formation of Unsymmetrical Disulfide by the Reaction of Two Symmetrical Disulfides in the Presence of a Catalytic Amount of *S*-Nitrosothiol

Entry	<i>S</i> -Nitrosothiol (eq to the substrate)	Reaction time (h)	Conditions	Yield of Et-S-S-R (%)
1	8 (0.005)	24	In air	47 ^{a)}
2	8 (0.0005)	24	In air	47 ^{a)}
3	8 (0.0005)	24	Under Ar	36 ^{a)}
4	9 (0.0005)	24	In air	10 ^{a)}
5	9 (0.0005)	24	Under Ar	10 ^{a)}
6	9 (0.005)	5	In air	48 ^{a)}
		24		42 ^{a)}
7	9 (0.005)	5	Under Ar	5 ^{a)}
		24		47 ^{a)}
8	9 (0.005)	16	Under Ar	46 ^{a)}
9	9 (0.005)	16	Under Ar	16 ^{b)}

a) The yield of benzyl ethyl disulfide. b) The yield of ethyl phenyl disulfide.

Chart 7

cal species that catalyze the cleavage of disulfides.

The participation of *S*-nitrosothiol in the reaction was examined by the use of triphenylmethylthionitrite (**8**) and *S*-nitroso-*N*-acetylpenicillamine (SNAP) (**9**) as reagents, whose results are summarized in Chart 6 and Table 5. The reaction proceeded in the presence of a catalytic amount of *S*-nitrosothiol, and the presence of air accelerated the reaction progress (entries 2, 3, entries 6, 7). The results suggested that *S*-nitrosothiol enhanced the reaction progress by both pathways which did involve N_2O_3 (or N_2O_4) and did not. Accordingly, it was supposed that *S*-nitrosothiol acted as both NO donor and thiyl radical donor to catalyze the reaction.

In addition, when a catalytic amount of nitrosonium tetrafluoroborate (0.00025 eq to the total amount of disulfides) was used under Ar, the reaction proceeded in 42% yield after 5 h, and in the case of 0.005 eq, the reaction was complete within 1 h (Chart 7). These results suggest that the step of *S*-oxygenation is not absolutely necessary for the disproportionation reaction.

The UV-vis spectrum of *S*-butyl thionitrite was observed when dibutyl disulfide, NO (2 eq), and O_2 (0.2 eq) were allowed to react in acetonitrile.¹⁹⁾ Consequently, there is a possible pathway that the S-S bond was cleaved by the direct attack of NO^+ without *S*-oxidation to afford *S*-nitrosothiol **7** and sulfenium cation **10** (Chart 8). *S*-Nitrosothiol thus formed might be decomposed to NO and a thiyl radical, which would attack another disulfide molecule to give unsymmetrical disulfide and a new thiyl radical, which reiterated the reaction to the statistically most probable 1:2:1 mixture of three disulfides. Moreover, there is a recent paper that claimed a disulfide bond was cleaved by the attack of sulfenium cation to bring about the disproportionation reaction.²⁰⁾ Consequently, if the reaction proceeds *via* the pathway shown in Chart 8, both of the two intermediates will take part in the disproportionation reaction.

In this paper, we described a new disproportionation reaction of disulfide by nitric oxide in the presence of oxygen. The reaction proceeded in a catalytic fashion, and this process might be of importance from the physiological point of view, since disulfide and NO are closely related to each other in biological system. In addition, it was suggested that the S-S bond was cleaved by the direct attack of nitrosation reagents to form *S*-nitrosothiol. Although the reaction be-

Chart 8

NOBF ₄	Reaction Time	Yield (%)
0.00025 equiv	1 h	31
0.00025 equiv	5 h	42
0.005 equiv	1 h	48

came fairly slow in the presence of water, it might progress in lipophilic sites or under the situation of continuous generation of NO.

Experimental

Melting points were measured with Büchi535 micromelting point apparatus and are uncorrected. The NMR spectra were measured with JEOL GX400 and LA500 spectrometers using tetramethylsilane as an internal standard. The abbreviations used are as follows: s, singlet; d, doublet; dd, double doublet; ddd, double double doublet; t, triplet; q, quartet; sext, sextet; m, multiplet. High performance liquid chromatography (HPLC) analysis was carried out using JASCO UVIDEC-100-V TRIOTAR-V system.

Materials Nitric oxide gas (99.9%) was purchased from Takachiho Chemical Company Ltd. and was passed through 10 M NaOH aqueous solution and a column of 4–8 mesh soda lime to remove NO_x impurities.

Oily disulfides (dimethyl disulfide, diethyl disulfide, dibutyl disulfide, di-*sec*-butyl disulfide, di-*tert*-butyl disulfide) were purchased from Wako Chemicals Co. and used as received. 2,2'-Dithiodiethanol and dithioacetic acid were purchased from Tokyo Kasei Kogyo Co. and used as received. Diphenyl disulfide, di(*p*-tolyl) disulfide and dibenzyl disulfide were recrystallized until analytically pure.

Synthesis of Bis(*p*-methoxyphenyl) Disulfide and Bis(*p*-chlorophenyl) Disulfide The corresponding thiol was oxidized by I₂ and a catalytic amount of KI in acetonitrile at room temperature until the thiol was entirely consumed. Bis(*p*-methoxyphenyl) disulfide was purified by recrystallization from hexane after the evaporation of the reaction solvent (yellow plates; mp 39–39.5°C (lit.²¹) mp 42–44°C)). Bis(*p*-chlorophenyl) disulfide was purified by silica gel column chromatography (hexane) and recrystallization from hexane (pale yellow plates; mp 70.5°C (lit.²²) mp 70.7–71.9°C)).

Synthesis of Unsymmetrical Disulfides Diaryl disulfide and excess amount of dimethyl disulfide were dissolved in acetonitrile or 1,2-dichloroethane, and the vessel was sealed with a septum. NO gas (up to 0.1 eq to diaryl disulfide) was added and the mixture was allowed to react for several hours at room temperature. In the case of bis(*p*-chlorophenyl) disulfide, KO₂ was used for the reaction according to the reported procedure.¹⁵ Methyl *p*-tolyl disulfide²³ and *p*-chlorophenyl methyl disulfide were purified by distillation *in vacuo*. Methyl phenyl disulfide²³ was purified by silica gel column chromatography (hexane).

Other chemicals were of analytical grade and were used as received.

Reaction of Two Disulfides in the Presence of Catalytic Amounts of NO in Air Though the reaction also proceeded on a preparative scale, NMR was used for precise quantitative analyses of volatile disulfides. In the typical procedure, two symmetrical disulfides (0.1 mmol of each) were dissolved in CD₃CN (1 ml), and the reaction vessel was sealed with a septum cap. Then 22 μ l (0.01 eq to each disulfide) of NO was added to the mixture using a gas tight syringe, and the reaction mixture was allowed to react at room temperature in the dark. The reaction mixture was subjected to NMR measurement to estimate the product ratio. Each NMR showed only the signals of unsymmetrical disulfides other than those of two starting materials, and each signal was separated sufficiently to estimate the reaction yields. The spectra of symmetrical disulfides are as follows: dimethyl disulfide (2.41 (s)); diethyl disulfide (1.28 (6H, t, *J*=7.3 Hz), 2.71 (4H, q, *J*=7.3 Hz)); dibutyl disulfide (0.92 (6H, t, *J*=7.3 Hz), 1.40 (4H, sext, *J*=7.3 Hz), 1.64 (4H, tt, *J*=7.5, 7.3 Hz), 2.71 (4H, t, *J*=7.5 Hz)); di(*sec*-butyl) disulfide (0.96 (6H, t, *J*=7.3 Hz), 1.27 (6H, d, *J*=6.8 Hz), 1.46–1.56 (2H, m), 1.62–1.73 (2H, m), 2.73–2.81 (2H, m)); di(*tert*-butyl) disulfide (1.30 (s)); dibenzyl disulfide (3.69 (4H, s), 7.26–7.36 (10H, m)); diphenyl disulfide (7.26–7.30 (2H, m), 7.33–7.38 (4H, m), 7.51–7.55 (4H, m)); di(*p*-tolyl) disulfide (2.30 (6H, s), 7.16 (4H, d, *J*=8.0 Hz), 7.40 (4H, d, *J*=8.0 Hz)); bis(*p*-methoxyphenyl) disulfide (3.78 (6H, s), 6.90 (4H, d, *J*=8.9 Hz), 7.41 (4H, d, *J*=8.9 Hz)); bis(*p*-chlorophenyl) disulfide (7.36 (4H, d, *J*=8.8 Hz), 7.49 (4H, d, *J*=8.8 Hz); 2,2'-dithiodiethanol (2.82 (4H, t, *J*=6.0 Hz), 2.95 (2H, t, *J*=6.0 Hz), 3.73 (4H, q, *J*=6.0 Hz)); dithiodiacetic acid (3.60 (s)); *N,N'*-bis(benzyloxycarbonyl)-L-cysteine (3.02 (2H, dd, *J*=14.0, 8.5 Hz), 3.22 (2H, dd, *J*=14.0, 4.6 Hz), 4.47 (2H, ddd, *J*=8.5, 7.6, 4.6 Hz), 5.08 (4H, s), 6.15 (2H, br d, *J*=7.6 Hz), 7.32–7.37 (10H, m)). The spectra of unsymmetrical disulfides are as follows: ethyl methyl disulfide (1.30 (3H, t, *J*=7.3 Hz), 2.40 (3H, s), 2.74 (2H, q, *J*=7.3 Hz)); butyl methyl disulfide (0.92 (3H, t, *J*=7.3 Hz), 1.36–1.46 (2H, m), 1.61–1.70 (2H, m), 2.39 (3H, s), 2.74 (2H, t, *J*=7.3 Hz)); *sec*-butyl methyl disulfide (0.97 (3H, t, *J*=7.3 Hz), 1.29 (3H, d, *J*=6.8 Hz), 1.48–1.59 (1H, m), 1.62–1.74 (1H, m), 2.39 (3H, m), 2.83 (1H, sext, *J*=6.8 Hz)); benzyl methyl disulfide (2.18 (3H, s), 3.94 (2H, s), 7.26–7.38 (5H, m)); methyl phenyl disulfide (2.46 (3H, s), 7.26–7.30 (1H, m),

7.36–7.40 (2H, m), 7.54–7.56 (2H, m)); methyl (*p*-tolyl) disulfide (2.33 (3H, s), 2.44 (3H, s), 7.20 (2H, d, *J*=8.1 Hz), 7.44 (2H, d, *J*=8.1 Hz)); (*p*-methoxyphenyl) methyl disulfide (2.44 (3H, s), 3.80 (3H, s), 6.94 (2H, d, *J*=8.9 Hz), 7.50 (2H, d, *J*=8.9 Hz)); (*p*-chlorophenyl) methyl disulfide (2.45 (3H, s), 7.38 (2H, d, *J*=8.8 Hz), 7.53 (2H, d, *J*=8.8 Hz)); benzyl ethyl disulfide (1.21 (3H, t, *J*=7.3 Hz), 2.51 (2H, q, *J*=7.3 Hz), 3.92 (2H, s), 7.26–7.37 (5H, m)); ethyl phenyl disulfide (1.28 (3H, t, *J*=7.3 Hz), 2.78 (2H, q, *J*=7.3 Hz), 7.24–7.30 (1H, m), 7.33–7.39 (2H, m), 7.52–7.59 (2H, m)); benzyl butyl disulfide (0.87 (3H, t, *J*=7.3 Hz), 1.27–1.35 (2H, m), 1.51–1.58 (2H, m), 2.48 (2H, t, *J*=7.3 Hz), 3.91 (2H, s), 7.26–7.36 (5H, m)); 2-hydroxyethyl methyl disulfide (2.41 (3H, s), 2.83 (2H, t, *J*=6.1 Hz), 2.93 (1H, t, *J*=6.1 Hz), 3.74 (2H, q, *J*=6.1 Hz)); 2-carboxymethyl methyl disulfide (2.46 (3H, s), 3.50 (3H, s)); *N*-benzyloxycarbonyl-L-cysteinyl methyl disulfide (2.39 (3H, s), 3.00 (1H, dd, *J*=14.0, 8.8 Hz), 3.23 (1H, dd, *J*=14.0, 4.3 Hz), 4.49 (1H, ddd, *J*=8.8, 8.2, 4.3 Hz), 5.09 (2H, s), 6.17 (1H, d, *J*=8.2 Hz), 7.30–7.39 (5H, m)).

The Effect of NO and O₂ (or NO₂) on the Reaction of Diethyl and Dibenzyl Disulfides Dibenzyl disulfide (1 mmol) was dissolved in acetonitrile (10 ml), and argon was bubbled into the solvent for 15 min except for the reactions in air. Then the reaction vessel was sealed with a septum rubber, and diethyl disulfide was introduced by a syringe. NO and O₂ gas were added by the use of a Hamilton gas tight syringe. The mixture was allowed to react in the dark at room temperature. The reaction was monitored using HPLC analysis (column: Merck Lichrospher Si60 (5 μ m), eluent; hexane: dichloromethane=2:1).

Reaction of Unsymmetrical Disulfides with NO Aryl methyl disulfide (0.2 mmol) was dissolved in CD₃CN (1 ml), and the reaction vessel was sealed with a septum cap. Then 22 μ l of NO was added to the mixture using a gas tight syringe, and the reaction mixture was allowed to react at room temperature in the dark. The reaction mixture was subjected to NMR measurement to estimate the product ratio.

Reaction of Diethyl Disulfide with NO in Air Diethyl disulfide (1 mmol) was dissolved in CH₃CN (10 ml), and the reaction vessel was sealed with a septum cap. Then 2.2 ml of NO was added to the mixture using a gas tight syringe, and the reaction mixture was allowed to react at room temperature in the dark for 3 h. Then the solvent was evaporated off, and the residue was subjected to NMR measurement. The sample was stored in the dark for 3 d at ambient temperature, then the NMR was re-measured. The reaction mixture after 3 h showed the spectra (in CDCl₃) mentioned below: diethyl disulfide; 1.32 (t), 2.71 (q). Product **A** (diethyl thiosulfinate); 1.42 (t), 1.48 (t), 3.07–3.25 (m). After 3 d, the spectra derived from product **A** had entirely disappeared, and product **B** was the only compound formed other than diethyl disulfide. Product **B** (diethyl thiosulfonate); 1.44 (t), 1.48 (t), 3.16 (q), 3.33 (q).

Reaction of Two Disulfides with *S*-Nitrosothiol Triphenylmethylthionitrite (**8**) was synthesized according to the reported method.²⁴ A certain amount of *S*-nitrosothiol was dissolved in CD₃CN (1 ml), and argon was bubbled into the solvent for 15 min except for the reactions in air. Then the reaction vessel was sealed with a septum rubber, and the CD₃CN solution of two disulfides was introduced by a syringe. The mixture was allowed to react at room temperature in the dark. The reaction progress was monitored by NMR analysis.

Reaction with Nitrosonium Tetrafluoroborate Dibenzyl disulfide (1 mmol) was dissolved in acetonitrile (10 ml), and argon was bubbled into the solvent for 15 min except for the reactions in air. Then the reaction vessel was sealed with a septum rubber, and diethyl disulfide (1 mmol) was introduced by a syringe. Then the acetonitrile solution (100 μ l) of nitrosonium tetrafluoroborate (0.5 or 10 μ mol) was added to the mixture, which was allowed to stir at room temperature. Product analysis was carried out using HPLC.

Reaction of Dibutyl Disulfide with NO and O₂: Detection of *S*-Nitrosothiol NO (45 ml) and O₂ (4.5 ml) was added to a reaction vessel degassed with Ar bubbling containing acetonitrile (10 ml). Then dibutyl disulfide (1 mmol) was added and the mixture was allowed to stand for 10 min, and the solution was subjected to UV–vis spectrum measurement (551.5 nm). A similar spectrum was observed by the reaction with diethyl disulfide. By comparison to the reported data, the yield of *S*-nitrosothiol was estimated at about 2%.

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