Supporting Information

Linkage isomerization in nitrosothiols (RSNOs): The X-ray crystal structure of an S-nitrosocysteine and DFT analysis of its metastable MS₁ and MS₂ isomers

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Table S1. Selected data for structurally characterized non-protein RSNO compounds.

Table S2. Selected data for structurally characterized protein RSNO moieties.

Figure S1. Packing diagram for cySNO-methyl ester.HCl

Figure S2. Crystal structure of cySNO-ethyl ester.HCl

Figure S3. Packing diagram for cySNO-ethyl ester.HCl.

Figure S4. DFT calculated %-orbital contributions for the HOMOs and LUMOs at the 6-311+G(2df,p) level.

Table S3. Comparison of method and basis sets for the geometry optimizations of *cis* cySNO-methyl ester (**1**).

Table S1. Selected data for structurally characterized non-protein RSNO compounds

Compound			N-O (Å)	S-N (Å)	C-S (Å)	S-N-O (°)	C-S-N (°)	ϕ^a	references
<i>S</i> -nitroso-L-cysteine methyl ester hydrochloride	1°	<i>cis</i>	1.172(4)	1.819(3)	1.796(3)	117.0(3)	102.11(17)	1.7(4)	this work
<i>S</i> -nitroso-L-cysteine ethyl ester hydrochloride	1°								this work
(major 53%)		<i>cis</i>	1.215(11)	1.762(6)	1.706(3)	115.7(5)	106.7(4)	-2.6(11)	
(minor 29%)		<i>cis</i>	1.215(12)	1.762(7)	1.686(3)	114.5(6)	107.8(5)	17(2)	
(minor 18%)		<i>trans</i>	1.215(12)	1.762(7)	1.699(4)	115.1(7)	99.3(9)	165(3)	
ONSC(Me) ₂ CH ₂ NHC(O)Me	3°	<i>trans</i>	1.206(2)	1.754(2)	1.829(2)	114.9(2)	99.44(8)	178.5	[1]
<i>S</i> -nitroso- <i>N</i> -acetyl-penicillamine ^b	3°	<i>trans</i>	1.199(2)	1.763(2)	1.833(1)	113.99(11)	100.80(7)	176.3	[2,3]
<i>S</i> -nitroso- <i>N</i> -acetyl-penicillamine ^c	3°	<i>trans</i>	1.206(3)	1.762(3)	1.842(2)	114.9(2)	96.56(8)	179.7	[4]
Ph ₃ CSNO	3°	<i>trans</i>	1.177(6)	1.792(5)	1.867(3)	114.0(4)	102.1(2)	175.7	[4]
<i>S</i> -nitrosocaptopril	1°	<i>cis</i>	1.206	1.766	1.800	117.7	103.7	0.68	[5]
<i>tert</i> -decalin1-SNO	3°	<i>trans</i>	1.307	1.744	1.875			179.3	[6]
<i>tert</i> -decalin2-SNO	3°	<i>trans</i>	1.231	1.703	1.815			175.0	[6]
Ar ₃ CSNO ^d	(major) 3°	<i>trans</i>	1.205 (6)	1.781(5)	1.841(4)	111.4(6)	104.2(3)	179.6	[7]
	(minor) 3°	<i>cis</i>	1.189(12)			123.6(7)		7.3	
BpqSNO ^e	(major 55%)	aryl <i>cis</i>	1.23(3)	1.85(3)	1.803(10)	103.0(25)	109.4(10)	-21.3(28)	[8]
BmtSNO ^f		aryl <i>cis</i>	1.204(4)	1.804(3)	1.770(3)	117.8(2)	101.1(1)	1.1(3)	[9]

^a Defined as the C-S-N-O torsion angle. ^b Crystallized from methanol/water. ^c Crystallized from acetonitrile. ^d Ar = 3,5-bis(2,6-dimethylphenyl)phenyl; the nitroso O-atom is disordered over two positions to give a mixture of *anti* and *syn* conformers in a ratio of 0.67:0.33. ^e Bpq = 2,6-di(3,5-diAr)phenyl; Ar = 2,6-diisopropylphenyl. Only major conformation data was reported. ^f Bmt = 4-*t*-butyl-2,6[(2,2'',6,6'')-tetramethyl-*m*-terphenyl-2'-yl)methyl]-benzenethiol.

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Table S2. Selected data for structurally characterized protein RSNO moieties (Å, deg).

S-Nitrosated Protein	PDB ID	Resoln (Å)	N-O	S-N	C-S	∠S-N-O	∠C-S-N	∠C-C-S	∠C-S-N=O	ref.
tuna Mb^c										[1]
wt (40%)	2NRM	1.09	1.21	1.81	1.77	116	105	113	-4	
wt (30%)			1.20	1.80	1.79	115	99	115	1	
Cimex NP^d										
1. wt	1Y21	1.75	1.23	1.71	1.77	121	104	112	-2	[2]
2. A21V	4L20	1.68	1.21	1.83	1.77	117	88	114	178	PDB
3. F64V	4L21	1.65	1.22	1.82	1.78	123	96	114	0	PDB
human Trx^e										
1 Cys62	211Y	1.70	1.19	1.76	1.77	121	102	113	1	[3]
Cys69 (33% NO)			1.21	1.80	1.79	119	103	115	1	
2 A Cys69 (50% NO)	2IFQ	1.20	1.20	1.78	1.76	123	102	113	1	
C Cys69			1.19	1.80	1.80	112	101	114	152	
3 A Cys62 (60%)	2H XK	1.65	1.21	1.80	1.80	118	101	115	2	
A Cys62 (40%)			1.22	1.81	1.78	118	100	110	-0.4	
A Cys69			1.21	1.81	1.77	119	101	114	1	
B Cys62			1.21	1.81	1.79	118	104	117	-0.6	
B Cys69			1.20	1.80	1.78	118	106	117	3	
C Cys62			1.22	1.80	1.77	118	99	109	5	
C Cys69 (70%)			1.20	1.81	1.83	123	103	116	3	
C Cys69 (30%)			1.23	1.81	1.78	114	103	109	179	
4 Cys62	4O O5	1.54	1.18	1.75	1.78	113	103	114	1	PDB
DDAH-I^f										[4]
Cys83	2C H1	1.08	1.47	1.65	1.79	117	102	116	-70	
PTP1B^g										[5]
Cys215	3E U0	2.6	1.31	1.66	1.80	146	153	114	41	
RuBisCO^h										[6]
1 Cys460	4F0H	1.96	1.23	1.65	1.75	121	113	110	73	
2 Cys460	4F0K	2.05	1.26	1.66	1.77	129	102	112	91	
3 Cys460	4F0M	2.25	1.26	1.65	1.76	128	106	108	76	

^a tuna Mb = blackfin tuna myoglobin; two conformations (A and B; 70%) of -CSNO, and (30%) of the cysteine is not S-nitrosated. ^d Cimex NP = *Cimex lectularius* nitrophorin, NP A21V mutant (4L20) and NP F64V mutant (4L21). ^e human Trx = human thioredoxin. There are three molecules (A, B, C) in the asymmetric unit. The 211Y data was collected from crystals at pH 7.0 (home source); the 2IFQ at pH 9.0 (synchrotron data); and 2H XK at pH 9.0 (home source). Trx C69S/C73S mutant (4O O5). ^f DDAH = dimethylarginine dimethylaminohydrolase. ^g PTP1B = protein-tyrosine phosphatase 1B. ^h RuBisCO = ribulose 1,5 biphosphate carboxylase/oxygenase, RuBisCO-O₂ derivative (4F0H), RuBisCO-CO₂ derivative (4F0K), RuBisCO-H₂O derivative (4F0M).

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Figure S1. Packing diagram for the unit cell of cySNO-methyl ester.HCl. The chloride anions were hydrogen bonded with nitrogen atoms from the amine groups with an N $\cdots$ Cl distance of 3.089 Å. The distance between the chloride and sulfur atom is 3.635 Å.

Figure S2. The crystal structure of cySNO-ethyl ester hydrochloride showing the three rotamers with respect to the C–S–N=O moiety. (A) The major *cis* CSNO component refined with 53% occupancy ($\angle\text{C–S–N=O} = -0.4(9)^\circ$; $\angle\text{N–C–C–S}$ torsion angle $\chi_1 = 83.6(3)^\circ$). (B) The minor *cis* CSNO component refined with 29% occupancy ($\angle\text{C–S–N=O} = 3.3(15)^\circ$; $\angle\text{N–C–C–S}$ torsion angle $\chi_1 = 177.2(2)^\circ$). (C) The *trans* CSNO component refined with 18% occupancy ($\angle\text{C–S–N=O} = 178(2)^\circ$; $\angle\text{N–C–C–S}$ torsion angle $\chi_1 = 90.6(6)^\circ$). Thermal ellipsoids are drawn at the 30% level.

Figure S3. The crystal packing of cySNO-ethyl ester hydrochloride in the unit cell. The chloride anions were hydrogen bonded with nitrogen atoms from the amine groups with an $\text{N}\cdots\text{Cl}$ distance of 3.178 Å. The distance between the chloride and sulfur atom is 3.434 Å.

Figure S4. DFT calculated % orbital contributions for the HOMOs and LUMOs at the 6-311+G(2df,p) level.

Table S3. Comparison of Method and Basis Sets for the Geometry Optimizations of *cis* cysSNO-methyl ester (**1**).

Method	Basis set	C–S (Å)	S–N (Å)	N–O (Å)	∠C–S–N (°)	∠S–N–O (°)	∠C–S–N–O (°)	∠N–C–C–S (°)
X-ray ^[a]		1.797(4)	1.819(4)	1.171(6)	102.1(2)	117.1(3)	1.7(5)	75.4(4)
B3P86	6-31+G(d,p)	1.807	1.806	1.192	102.3	117.8	1.0	60.6
B3P86	6-311+G(2df,p)	1.798	1.786	1.184	102.7	118.4	0.9	60.9
B3P86	6-311++G(3df,3pd)	1.793	1.779	1.184	102.8	118.5	0.9	61.1
B3LYP	6-31+G(d,p)	1.824	1.831	1.194	102.5	117.9	0.8	61.3
B3LYP	6-311+G(2df,p)	1.815	1.813	1.185	102.9	118.5	0.8	61.7
B3LYP	6-311++G(3df,3pd)	1.810	1.805	1.185	103.1	118.5	0.7	61.8

^[a] Determined as its hydrochloride salt.