Supporting Information

Linkage isomerization in nitrosothiols (RSNOs): The X-ray crystal structure of an S-nitrosocysteine and DFT analysis of its metastable MS\textsubscript{1} and MS\textsubscript{2} 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).
| Compound                                    | N-O (Å) | S-N (Å) | C-S (Å) | S-N-O (°) | C-S-N (°) | \(\phi^a\) | references     |
|---------------------------------------------|---------|---------|---------|-----------|-----------|------------|----------------|
| S-nitroso-L-cysteine methyl ester hydrochloride (major 53%) | cis     | 1.172(4)| 1.819(3)| 1.796(3)  | 117.0(3)  | 102.11(17) | 1.7(4) this work |
| S-nitroso-L-cysteine ethyl ester hydrochloride (major 29%) | cis     | 1.215(11)| 1.762(6)| 1.706(3)  | 115.7(5)  | 106.7(4)   | -2.6(11) [1]   |
| S-nitroso-L-cysteine ethyl ester hydrochloride (minor 18%)  | trans   | 1.215(12)| 1.762(7)| 1.686(3)  | 114.5(6)  | 107.8(5)   | 17(2)         |
| ONSC(Me)\(_2\)CH\(_2\)NHC(O)Me             | 3°      | trans   | 1.206(2) | 1.754(2)  | 1.829(2)  | 114.9(2)   | 99.44(8) 178.5 |
| S-nitroso-N-acetyl-penicillamine\(^b\)      | 3°      | trans   | 1.199(2) | 1.763(2)  | 1.833(1)  | 113.99(11)| 100.80(7) 176.3 |
| S-nitroso-N-acetyl-penicillamine\(^c\)      | 3°      | trans   | 1.206(3) | 1.762(3)  | 1.842(2)  | 114.9(2)   | 96.56(8) 179.7 |
| Ph\(_3\)CSNO                                | 3°      | trans   | 1.177(6) | 1.792(5)  | 1.867(3)  | 114.0(4)   | 102.1(2) 175.7 |
| S-nitrosocaptopril                          | 1°      | cis     | 1.206   | 1.766     | 1.800     | 117.7      | 103.7 0.68 [5] |
| tert-decalin1-SNO                           | 3°      | trans   | 1.307   | 1.744     | 1.875     | 179.3      | [6]           |
| tert-decalin2-SNO                           | 3°      | trans   | 1.231   | 1.703     | 1.815     | 175.0      | [6]           |
| Ar\(_3\)CSNOD\(^d\)                         | 3°      | trans   | 1.205(6) | 1.781(5)  | 1.841(4)  | 111.4(6)   | 104.2(3) 179.6 |
| BpqSNO\(^e\) (major 55%)                    | aryl    | cis     | 1.23(3)  | 1.85(3)   | 1.803(10)| 103.0(25)  | 109.4(10) -21.3(28) [8] |
| BmtSNO\(^f\)                                | aryl    | cis     | 1.204(4) | 1.804(3)  | 1.770(3)  | 117.8(2)   | 101.1(1) 1.1(3) [9] |

\(\phi^a\) 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-tert-butyl-2,6[(2,2”,6,6”-tetramethyl-m-terphenyl-2’-yl)methyl]-benzenethiol.

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| 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 Mb¹             |        |            |     |     |     |        |        |        |          |     |
| 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 Np²            |        |            |     |     |     |        |        |        |          |     |
| 1. wt                | 1Y21   | 1.75       | 1.23| 1.71| 1.77| 121    | 104    | 112    | -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 Trx³           |        |            |     |     |     |        |        |        |          |     |
| 1 Cys62              | 211Y   | 1.70       | 1.19| 1.76| 1.77| 121    | 102    | 113    | 1        |     |
| 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        |     |
| Cys69                |        | 1.19       | 1.80| 1.80| 112  | 101    | 114    | 152      |          |     |
| 3. A Cys69 (60%)     | 2HXK   | 1.65       | 1.21| 1.80| 1.80| 118    | 101    | 115    | 2        |     |
| A Cys69 (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              | 4OO5   | 1.54       | 1.18| 1.75| 1.78| 113    | 103    | 114    | 1        | PDB |
| DDAH-I²              |        |            |     |     |     |        |        |        |          |     |
| Cys83                | 2CI1   | 1.08       | 1.47| 1.65| 1.79| 117    | 102    | 116    | -70      |     |
| PTP1B¹               |        |            |     |     |     |        |        |        |          |     |
| Cys215               | 3E0U   | 2.6        | 1.31| 1.66| 1.80| 146    | 153    | 114    | 41       |     |
| RuBiCO¹              |        |            |     |     |     |        |        |        |          |     |
| 1 Cys460             | 4F0H   | 1.96       | 1.23| 1.65| 1.75| 121    | 111    | 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       |     |

¹ tuna Mb = blackfin tuna myoglobin; two conformations (A and B; 70%) of -CSNO, and (30%) of the cysteine is not S-nitrosated. ² Cimex NP = Cimex lectularius nitrophorin, NP A21V mutant (4L20) and NP F64V mutant (4L21). ³ 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 2HXK at pH 9.0 (home source). Trx C69S/C73S mutant (4OO5). ⁴ DDAH = dimethylarginine dimethylaminohydrolase. ⁵ PTP1B = protein-tyrosine phosphatase 1B. ⁶ RuBiCO = ribulose 1,5 bisphosphate carboxylase/oxygenase, RuBiCO-O₂, derivative (4F0H), RuBiCO-O₂, derivative (4F0K), RuBiCO-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–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 ($\angle$C–S–N=O = $-0.4(9)^\circ$; $\angle$N–C–C–S torsion angle $\chi_1$ = 83.6(3)$^\circ$). (B) The minor cis CSNO component refined with 29% occupancy ($\angle$C–S–N=O = 3.3(15)$^\circ$; $\angle$N–C–C–S torsion angle $\chi_1$ = 177.2(2)$^\circ$). (C) The trans CSNO component refined with 18% occupancy ($\angle$C–S–N=O = 178(2)$^\circ$; $\angle$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 N···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 cysteine sulfoximine (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.