Theory, Spectroscopy, and Crystallographic Analysis of *S*-Nitrosothiols: Conformational Distribution Dictates Spectroscopic Behavior

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The biological importance of *S*-nitrosothiols (RSNO) and their role in nitric oxide (NO) transport and regulation are now well established.[1−3] However, a concrete mechanistic picture for *S*-nitrosothiol formation, decomposition, and transport, particularly in vivo, is still lacking despite extensive research in this area.[4−6] In part, this is due to only a rudimentary knowledge of the structures and conformations of *S*-nitrosothiols, and how the observed spectroscopic properties are related to the structural features of these substances. Here we present detailed experimental and theoretical results which establish these characteristics.

The stabilities of *S*-nitrosothiols appear to depend on the structure of the organic substituent.[7] Primary species, such as *S*-nitroso-*N*-acetylcysteine, are typically unstable and characterized only spectroscopically,[8] whereas tertiary RSNOs, including that derived from *N*-acetylpenicillamine (SNAP), have been isolated and are indefinitely stable.[9,10] Alkyl substitution also has an effect on both the NMR and UV−vis spectroscopic properties of *S*-nitrosothiols. Primary species are orange-red, whereas tertiary compounds are green. Both ^{1}H and ^{15}N NMR spectra of RSNO compounds show considerable variability in chemical shift, and several demonstrate broad resonances. The relationship between physicochemical properties and bioactivity is not well understood, although primary *S*-nitrosothiols typically produce shorter-lived biological effects than do tertiary.[1]

S-Nitrosothiols exhibit considerable S−N double bond character: as a result, two geometrical isomers are possible. Three crystal structures of *S*-nitrosothiols have been reported. The tertiary nitrosothiols *S*-nitroso-*N*-acetylpenicillamine (SNAP) and *S*-nitrosotriphenylmethanethiol both exist with the SNO moiety exclusively in the anti orientation.[9−11] In contrast, a recent structure of *S*-nitrosohemoglobin demonstrates an out-of-plane CSNO dihedral of nearly 90°.[12] However, in this instance, the electron density was fit to a model derived from the tertiary *S*-nitrosothiol, SNAP. The theory and experiments reported here provide a new and consistent picture of the conformational behavior of S-nitrosothiols, and how it influences spectroscopic properties.

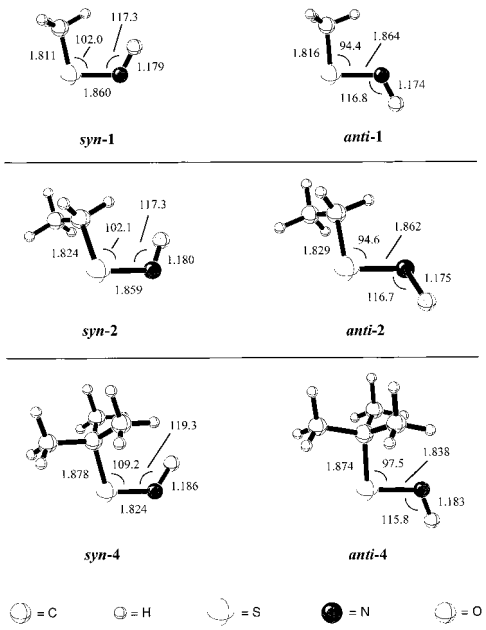

Figure 1. B3LYP/6-311+G* optimized parameters (Å, deg) for syn and anti conformers of *S*-nitrosomethanethiol **1**, *S*-nitrosoethanethiol **2**, and *S*-nitroso-*tert*-butanethiol **4**.

^{15}N NMR spectra were recorded for *S*-nitrosoethanethiol **2**, *S*-nitroso-2-propanethiol **3**, and *S*-nitroso-2-methyl-2-propanethiol **4** (Table 1).[13] In all spectra a single resonance is visible at and above room temperature, showing considerable evidence of exchange broadening. Lowering the temperature to −50 °C resulted in resolution of two distinct sharp signals, assigned to the syn and anti orientations of the RSNO structure. Significantly, both primary and secondary *S*-nitrosothiols show a preponderance of the species responsible for the upfield signal, but the tertiary compound **4** shows a roughly 4:1 ratio of downfield to upfield peaks.

To assign the structures of the two species, we carried out hybrid density functional theory calculations[14] of the structures of all three *S*-nitrosothiols, plus *S*-nitrosomethanethiol **1**, for which calculations have recently been reported.[11] B3LYP/6-311+G* minima for the syn and anti forms of **1** (the former possessing an eclipsed C−H bond),[15] **2**, and **4** are provided in Figure 1. MP2/6-311+G* geometry optimizations and QCISD(T)/6-311+G* single-point calculations on **1** further confirm the syn preference. Also of note is the preferred eclipsing of an α-CH and anti-clinal orientation of the alkyl chain with respect to the SNO moiety in *syn*-**2**, in direct accord with that found in the crystal structure of **5** (Figure 2). Relative syn/anti energies and equilibria are provided in Table 1. The calculations demonstrate an electronic preference for the syn form, similar to the factors governing the conforma-

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(1) Stamler, J. S. *Curr. Top. Microbiol. Immunol.* **1995**, 196, 19−36.
(2) Butler, A. R.; Williams, D. L. H. *Chem. Soc. Rev.* **1993**, 233.
(3) Stamler, J. S. *Cell* **1994**, *78*, 931−936.
(4) Singh, S. P.; Wishnok, J. S.; Keshive, M.; Deen, W. M.; Tannenbaum, S. R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14428−14433.
(5) Munro, A. P.; Williams, D. L. H. *Can. J. Chem.* **1999**, *77*, 550−556.
(6) Singh, R. J.; Hogg, N.; Joseph, J.; Kalyanaraman, B. *J. Biol. Chem.* **1996**, *271*, 18596−18603.
(7) Roy, B.; d'Hardemare, A.; Fontcave, M. *J. Org. Chem.* **1994**, *59*, 7019−7026.
(8) Askew, S. C.; Barnett, D. J.; McAninly, J.; Williams, D. L. H. *J. Chem. Soc., Perkin Trans. 2* **1995**, 741−745.
(9) Field, L.; Dilts, R. V.; Ravichandran, R.; Lenhert, P. G.; Carnahan, G. E. *J. Chem. Soc., Chem. Commun.* **1978**, 249.
(10) Carnahan, G. E.; Lenhert, P. G.; Ravichandran, R. *Acta Crystallogr.* **1978**, *B34*, 2645−2648.
(11) Arulsamy, N.; Bohle, D. S.; Butt, J. A.; Irvine, J. A.; Jordan, P. A.; Sagan, E. *J. Am. Chem. Soc.* **1999**, *121*, 7115−7123.
(12) Chan, N.-L.; Rogers, P. H.; Arnone, A. *Biochemistry* **1998**, *37*, 16459−16464.

(13) For NMR experiments, *S*-nitrosothiols were prepared as follows: to 600 μL of toluene-d_8 was added 0.06 mmol of thiol, followed by 0.03 mmol of ^{15}N-labeled *tert*-butyl nitrite (prepared as described by Field et al.),[9] to give final concentrations of approximately 100 mM *S*-nitrosothiol and 100 mM thiol. Nitrosation with equimolar *tert*-butyl nitrite (to give *S*-nitrosothiol in the absence of thiol) gave identical NMR shifts and syn/anti populations for trial compounds.

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Table 1. Relative Free Energies, Conformational Equilibria, and Spectral Properties of *S*-Nitrosothiols **1**−**4**

S-nitrosothiol	ΔG_{rel} (kcal/mol)[a]	[anti]/[syn] (calc)[b]	[anti]/[syn] (exp)[c]	^{15}N shift (calc)[d]	^{15}N shift (exp)[e]	average ^{15}N shift (calc)[b]	average ^{15}N shift (exp)[c]	λmax,vis (calc)[f]	λmax,vis (exp)
CH_3SNO (**1**)		0.27				381.2			
syn	0			365.9				520.1	
anti	0.78			438.1				556.9	
CH_3CH_2SNO (**2**)		0.47	0.37			398.7	397.0		(orange-red)
syn	0			378.4	378.4			531.0	520
anti	0.45			441.8	447.2			561.2	552
$(CH_3)_2CHSNO$ (**3**)		0.84	0.43			405.1	401.5		(orange-red)
syn	0			378.4	381.9			532.6	524
anti	0.11			436.8	446.7			560.4	556
$(CH_3)_3CSNO$ (**4**)		7.69	4.78			470.5	452.4		(green)
syn	1.19			419.5	403.3			571.2	550
anti	0			477.1	462.7			591.6	596

[a] From scaled B3LYP/6-311+G* vibrational frequencies and entropic correction at 298 K. [b] From Boltzmann distribution at 298 K. [c] 298 K. [d] GIAO-B3LYP/6-311++G(3df,2df,2p)//B3LYP/6-311+G*. *syn*-**2** set equal to 378.4 ppm. [e] 183 K. [f] TD-B3LYP/6-311++G(3df,2df,2p)//B3LYP/6-311+G*.

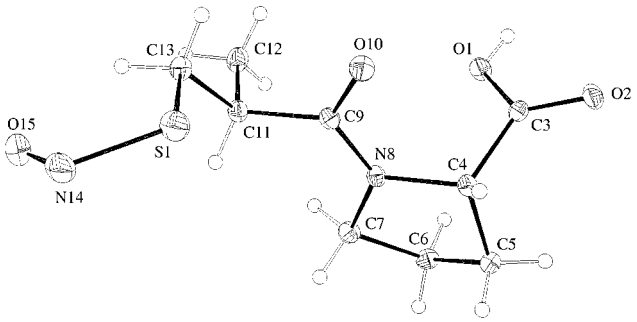

Figure 2. X-ray crystal structure of *S*-nitrosocaptopril **5**. Relevant geometric parameters are found in ref 17.

tional preferences of alkyl esters.[16] A competing steric effect causes the anti orientation to be favored when the R group is large. Thus, primary, and to a lesser extent, secondary *S*-nitrosothiols preferentially adopt a syn conformation, whereas the anti conformer of **4** is favored by ∼1 kcal/mol, in accord with measured values.

Analysis of the data demonstrates 4:1 and 3:1 preferences for the syn orientations of **1** and **2** and a 6:1 preference for the anti orientation of **4** at 25 °C. A van't Hoff analysis of **4** yields an enthalpic difference between the two states of roughly 1.4 kcal/mol. Finally, to establish unambiguously the conformation of a primary *S*-nitrosothiol, *S*-nitrosocaptopril **5** was crystallized and subjected to X-ray analysis. This material gives rise to a ^{15}N NMR shift of 378.9 ppm at −90 °C (a downfield peak was not visible in the baseline noise.) *The X-ray crystal structure of this species clearly shows that the S-nitroso moiety exists excusively in the syn conformation, with a CSNO dihedral of 0.7* ° (Figure 2).[17] This is in striking contrast to the reported dihedral angles of nearly 180° for the two tertiary *S*-nitrosothiols SNAP (N = 176.3°)[10] and *S*-nitroso-triphenylmethanethiol (N = 175.7°).[11]

An appreciable barrier (11−12 kcal/mol) to rotation about the S−N bond is predicted for all of the model *S*-nitrosothiols, in good agreement with the experimental $\Delta G^{\ddagger}$ value of 11.1 kcal/mol for the interconversion of primary species **2** at 273 K, and recent measurements for the interconversion of **4**.[11]

To further validate our spectral assignments, ^{15}N chemical shifts of each species were computed utilizing the B3LYP-GIAO approach.[18] The individual calculated ^{15}N shifts of syn and anti forms of these *S*-nitrosothiols show a substantial effect of conformation, with anti resonances shifted roughly 60 ppm downfield relative to the syn. This surprisingly large prediction is in excellent accord with the experimental values.

TD-DFT calculations[19] of the electronic transitions of **1**−**4** are summarized in Table 1. From spectra and calculated assignments, the visible absorption of *S*-nitrosothiols corresponds to a weak n→π* transition in the 520−590 nm region,[20] superimposed upon the tail of a strong absorption centered near 340 nm resulting from an π→π* transition. This explains the color difference between primary and tertiary *S*-nitrosothiols. The absorption maxima of anti conformers are red-shifted by about 30 nm relative to the syn. Experimental spectra show two n→π* bands in the 500−600 nm region, separated by about 30 nm.

These results suggest that a number of recent interpretations of SNO structure/activity relationships should be reevaluated. For example, the crystal structure of *S*-nitrosohemoglobin[12] should be refit to a *syn*-CSNO model. Additionally, attempts to correlate *S*-nitrosothiol activity with ^{15}N chemical shifts[21] should be reexamined, to determine if these resonances reflect alterations in the syn and anti ^{15}N shifts, or the distribution of the isomeric forms. Further investigation of *S*-nitrosothiol reactivity under various conditions is currently underway.

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(14) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, rev. A.7; Gaussian, Inc.: Pittsburgh, PA, 1998.

(15) A recent study suggested that the energy difference between the syn and anti forms of *S*-nitrosomethanethiol **1** was as small as 0.1 kcal/mol, predicting nearly equal populations of each.[11] The geometry of the syn isomer in that study in fact corresponds to a saddle point, bearing a methyl C−H bond in a staggered arrangement with the SNO function.

(16) Blom, C. E.; Günthard, H. H. *Chem. Phys. Lett.* **1981**, *84*, 267.

(17) Geometric parameters for **5**: *r*(N−O): 1.206 Å; *r*(S−N): 1.766 Å; *r*(C−S): 1.800 Å; θ(S−N−O): 117.7°; θ(C−S−N): 103.7°; ϕ(C−S−N−O): 0.68°.

(18) For a recent review, see: Helgaker, T.; Jaszuski, M.; Ruud, K. *Chem. Rev.* **1999**, *99*, 293−352.

(19) Stratmann, R. E.; Scuseria, G. E.; Frisch, M. J. *J. Chem. Phys.* **1998**, *109*, 8218−8224.

(20) Barrett, J.; Debenham, D. F.; Glauser, J. *J. Chem. Soc., Chem. Commun.* **1965**, 248−249.

(21) Wang, K.; Hou, Y.; Zhang, W.; Ksebati, M. B.; Xian, M.; Cheng, J.-P.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2897−2902.