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S-Nitrosothiol and Nitric Oxide Reactivity at Zinc Thiolates

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S-Nitrosothiols undergo reversible transnitrosation reactions at tris(pyrazolyl)boratozinc thiolates ^{iPr2}TpZn-SR. These zinc thiolates are unreactive toward anaerobic NO but rapidly react with NO in the presence of O_2 or anaerobically with NO_2 to release the *S*-nitrosothiol RSNO with formation of the corresponding zinc nitrate.

Nitric oxide (NO) is implicated in numerous biological roles ranging from vasodilation in the cardiovascular system[1] to signaling in the respiratory system[2] to host defense against microbial pathogens.[3] While the various NO synthases generate NO in vivo, NO itself is unstable in the plasma with an estimated half-life of 3−5 s.[4] Considerably more oxygen-stable *S*-nitrosothiols (RSNOs) such as *S*-nitrosocysteine and *S*-nitrosoglutathione circulate at near micromolar levels in the blood.[5] Capable of serving as NO and NO^+ donors,[6,7] RSNOs have been implicated in a wide variety of physiological functions that often mirror those observed for NO itself.[1,8] The nature of the molecular species involved in the biological reactivity of RSNOs, however, is clouded by the facile decomposition of RSNOs into free NO and disulfides by a copper-catalyzed process.[6,9]

The cleavage or formation of zinc thiolate linkages in biology is often connected to physiological function.[10] In this context, both NO and RSNOs have been reported to modify Zn−SR linkages in biological environments. For instance, Maret and co-workers have shown that NO and RSNOs release Zn^{2+} ions from the sulfur-rich binding domains in metallothioneins (MTs) with concomitant formation of RSNOs and/or disulfides.[11] The release of zinc from MTs by RSNOs and NO has spurred investigation into the significance of both zinc and RSNOs in cellular signal transduction[11,12] as well as their roles in respiratory function.[13] Similarly, RSNOs and NO reversibly inhibit DNA transcription of some zinc fingers,[14] metalloproteins in which Zn−thiolate bonds play especially important structural roles.

Matrix metalloproteinases (MMPs) are a class of zinc enzymes involved in tissue remodeling connected to both normal and pathological processes such as inflammation, wound healing, and cancer.[15] The His_3Zn^{2+} site responsible for MMP activity requires prior disruption of a Zn−SCys linkage in the enzyme's latent form to allow for substrate binding and its subsequent cleavage. Both NO and RSNOs have been proposed to activate this "cysteine switch", suggesting a molecular basis for NO and RSNO regulation of MMP activity.[16]

While transnitrosation between RSNOs and free thiols as well as the corresponding thiolate anions has been observed in a variety of media,[17,18] NO does not react readily under anaerobic conditions with free thiols HSR or thiolate anions $^-$SR in the absence of an oxidant.[6] In contrast, few molecular level details are known for transnitrosation at transition-metal thiolates,[19] though *reductive nitrosylation* of M−SR

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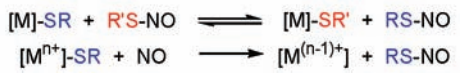
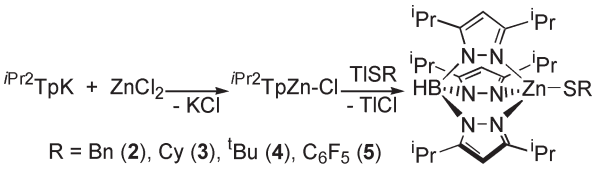
Scheme 1. Transnitrosation (Top) and Reductive Nitrosylation (Bottom) at Metal Thiolates [M]−SR



Scheme 2. Synthesis of Zinc Thiolates **2**−**5**



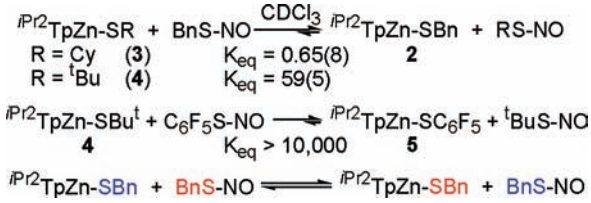
linkages with NO is possible at redox-active metal centers (Scheme 1).[20] Such transnitrosation reactions are thought to proceed via nitroxyl disulfide intermediates $[RSN(O)NSR']^-$ theoretically considered by Houk et al.[21] and observed via ^{15}N NMR spectroscopy by Estrin et al. in the exchange between *S*-nitrosocysteine ethyl ester and its thiolate anion in methanol.[18]

We recently demonstrated transnitrosation between the β-diketiminatozinc thiolate $\{[Me_2NN]Zn\}_2(\mu\text{-}SBu^t)_2$ and CySNO to give equilibrium quantities of $\{[Me_2NN]Zn\}_2(\mu\text{-}SCy)_2$ and ^{t}BuSNO.[19] Unfortunately, the dinuclear nature of these zinc complexes clouded the thermodynamic preferences for transnitrosation equilibria. Herein we utilize well-defined tris(pyrazolyl)boratozinc complexes[22] ^{iPr2}TpM-SR to examine RSNO and NO reactivity at strictly mononuclear zinc thiolate sites.

Tris(pyrazolyl)boratozinc thiolates[23−25] ^{iPr2}TpZn-SR [R = Bn (**2**) Cy (**3**), ^{t}Bu (**4**), C_6F_5 (**5**)[25]] are prepared by salt metathesis reactions between ^{iPr2}TpZn-Cl (**1**) and the corresponding thallium thiolate Tl-SR (Scheme 2). Reaction of the secondary and tertiary zinc thiolates **3** and **4** with BnSNO (generated by the addition of Tl-SBn in $CDCl_3$ to dry NO $[BF_4]$) allows for transnitrosation equilibria to be observed at room temperature in $CDCl_3$, as outlined in Scheme 3. While there is no significant bias in the equilibrium between the secondary zinc thiolate **3** and the primary *S*-nitrosothiol BnSNO [K_{eq} = 0.65(8)], transnitrosation favors the formation of the primary zinc thiolate **2** and tertiary *S*-nitrosothiol ^{t}BuSNO [K_{eq} = 59(5)]. These equilibria data qualitatively mirror the trend observed in reactions between zinc thiolates **3** and **4** and the thiol HSBn [K_{eq} = 3.2(4) and 750(50), respectively]. Moreover, the reaction between tertiary zinc thiolate **4** and the electron-poor *S*-nitrosothiol C_6F_5SNO results in complete conversion to the zinc arylthiolate **5** and ^{t}BuSNO.

While the above transnitrosation equilibria were generally complete within 5 min at approximate 0.1 mM concentrations

Scheme 3. Transnitrosation Equilibria at Zinc Tholates in $CDCl_3$



of each reactant, transnitrosation between BnSNO with the bulkier ^{iPr2}TpZn-SBu^t complex is qualitatively slower than with the smaller cyclohexyl derivative ^{iPr2}TpZn-SCy. To more quantitatively define the time scale for transnitrosation, we monitored degenerate transnitrosation between ^{iPr2}TpZn-SBn and BnSNO by ^{1}H NMR spectroscopy in $CDCl_3$ (Scheme 3).

Irradiation of the *S*-nitrosothiol PhCH_2SNO resonance decreased the intensity of the ^{iPr2}TpZn-SCH_2Ph signal, demonstrating saturation transfer between these two exchanging species. The pseudo-first-order rate constant via saturation transfer (Figure S5 in the Supporting Information) increases linearly with an increase in the RSNO concentration, leading to a second-order rate law k[Zn-SR][RSNO] with a rate constant 2.0(2) M^{-1} s^{-1} at 60 °C. No significant decomposition (less than 5%) of the RSNOs was observed under these conditions. Transnitrosation with RSNOs at zinc thiolates is significantly faster than alkylation[26] or disulfide exchange[23] in related model complexes. For instance, transnitrosation of ^{iPr2}TpZnSCH_2Ph (**2**) with PhCH_2SNO is 500 times faster than alkylation by MeI in $CDCl_3$ at 60 °C [k = 3.8(3) × 10^{-3} M^{-1} s^{-1}; Scheme 4].

None of the zinc thiolates **2**−**5** react with NO_{gas} in $CDCl_3$ under anaerobic conditions; standing for 12 h with ca. 8 equiv of NO results in no change in their ^{1}H NMR spectra. No reaction is observed with excess O_2 under similar conditions. The addition of 8 equiv of NO to a O_2-saturated $CDCl_3$ solution of **4**, however, results in an immediate color change to green owing to essentially the quantitative formation of ^{t}BuSNO confirmed by its ^{1}H NMR signal at δ 1.92 ppm (Scheme 5). The addition of 8 equiv of anaerobic NO_2 gas to a $CDCl_3$ solution of **4** also provides similar results.

In the reaction of **4** with NO/O_2 or NO_2, only one new ^{iPr2}TpZn species **7** is produced. X-ray analysis of the colorless product identifies it as ^{iPr2}TpZn(NO_3), which has unsymmetric κ^2 bonding of the nitrate anion to the Zn center with Zn−O distances of 1.954(2) and 2.488(3) Å. This unsymmetric bonding mode is seen in other crystallographically characterized TpZn(NO_3) complexes.[27] Because N_2O_3 has been suggested as a potential nitrosating reagent in reactions of NO under aerobic conditions, which could generate the nitrite anion after transfer of NO^+,[28] ^{iPr2}TpZn(NO_2) (**8**) was independently prepared

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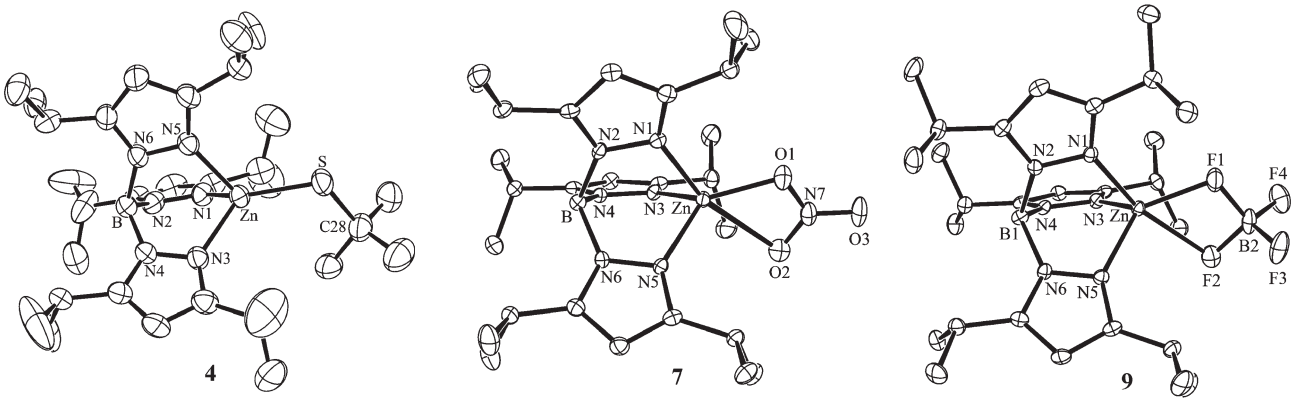
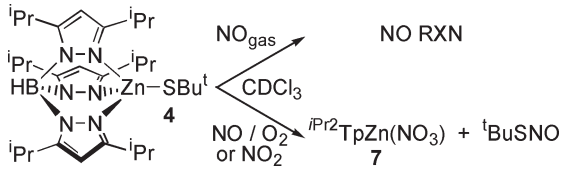


Figure 1. X-ray structures of ^{iPr2}TpZn-SBu^t (**4**), ^{iPr2}TpZn(NO$_3$) (**7**), and ^{iPr2}TpZn(BF$_4$) (**9**) (**4** was collected at 173 K, and **7** and **9** were collected at 100 K).

Scheme 4. Alkylation of **2** with MeI to Give Zinc Iodide **6**

$$\underset{\mathbf{2}}{^{iPr2}\text{TpZn-SBn}} + \text{Me-I} \xrightarrow[\text{CDCl}_3]{} \underset{\mathbf{6}}{^{iPr2}\text{TpZn-I}} + \text{BnS-Me}$$

Scheme 5. NO and NO_2 Reactivity of Zinc Thiolate **4**



by the reaction of $Zn(ClO_4)_2$ with ^{iPr2}TpK and $NaNO_2$ in MeOH (Scheme 6). The X-ray structure of the zinc nitrite complex **8** is closely related to that of **7** with Zn−O distances of 1.981(2) and 2.430(2) Å (Figure S14 in the Supporting Information). Importantly, nitrate/nitrite exchange between ^{iPr2}TpZn fragments is slow in a $CDCl_3$ solution at room temperature; an overlapping eight-line pattern is observed in the ^{1}H NMR spectrum of a mixture of **7** and **8**, resulting from four sets of diastereotopic CH*Me*$_2$ groups. Thus, our evidence points to the sole formation of the zinc nitrate **7** when zinc thiolate **4** is exposed to NO with an excess of O_2. Under these conditions, the active nitrosating reagent behaves analogously to NO_2 which can exhibit electrophilic reactivity owing to its equilibrium with N_2O_4 reacting as [NO$^+$][NO$_3^-$].[29]

Similarly, treatment of **4** with $NOBF_4$ results in the formation of ^{iPr2}TpZn(BF$_4$) (**9**) along with ^{t}BuSNO (Scheme 7). The zinc-containing product **9** was confirmed by an independent synthesis through the salt metathesis reaction of **1** with $AgBF_4$. The BF_4 anion exhibits κ^2 coordination in **9** with Zn−F1 and Zn−F2 bond distances of 1.997(2) and 2.379(2) Å, respectively. The X-ray structure of **9** appears to be an unusual example of Zn-BF_4 coordination, though related κ^2

Scheme 6. Independent Synthesis of Zinc Nitrate **7** and Zinc Nitrite **8**

$$\underset{\mathbf{8}}{^{iPr2}\text{TpZn(NO}_2\text{)}} \xleftarrow[\text{MeOH}]{\text{Zn(ClO}_4\text{)}_2,\ \text{NaNO}_2} {^{iPr2}\text{TpK}} \xrightarrow[\text{MeOH}]{\text{Zn(NO}_3\text{)}_2} \underset{\mathbf{7}}{^{iPr2}\text{TpZn(NO}_3\text{)}}$$

Scheme 7. Nitrosation of Zinc Thiolate **4** with $NOBF_4$

$$\underset{\mathbf{4}}{^{iPr2}\text{TpZn-SBu}^t} + \text{NOBF}_4 \xrightarrow[\text{CDCl}_3]{} \underset{\mathbf{9}}{^{iPr2}\text{TpZn(BF}_4\text{)}} + {}^t\text{BuSNO}$$

binding of the BF_4 anion has been observed in Ni[30] and Cu[31] complexes.

These studies with well-defined, mononuclear zinc thiolates demonstrate that thiolate nitrosation with reagents capable of serving as equivalents of NO$^+$ such as RSNOs and aerobic NO is quite facile, particularly so when considering the steric bulk of the ^{iPr2}Tp ancillary ligand. Owing to the redox stability of the Zn^{2+} ion, the Zn−thiolate bond exhibits no anaerobic NO reactivity under similar conditions. Moreover, transnitrosation with the *S*-nitrosothiol $PhCH_2SNO$ is much faster than the conceptually related alkylation reaction with MeI. Future studies will explore transnitrosation at various zinc coordination environments as well as probe the nature of the zinc intermediates in transnitrosation.

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Supporting Information Available: Complete experimental details as well as X-ray crystallographic data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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