

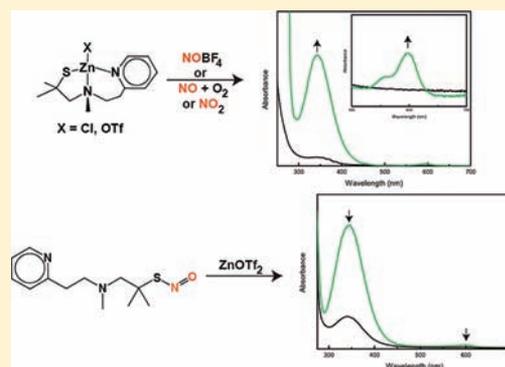
Zinc Thiolate Reactivity toward Nitrogen Oxides: Insights into the Interaction of Zn^{2+} with *S*-Nitrosothiols and Implications for Nitric Oxide Synthase

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Supporting Information

ABSTRACT: Zinc thiolate complexes containing N_2S tridentate ligands were prepared to investigate their reactivity toward reactive nitrogen species, chemistry proposed to occur at the zinc tetracysteine thiolate site of nitric oxide synthase (NOS). The complexes are unreactive toward nitric oxide (NO) in the absence of dioxygen, strongly indicating that NO cannot be the species directly responsible for *S*-nitrosothiol formation and loss of Zn^{2+} at the NOS dimer interface in vivo. *S*-Nitrosothiol formation does occur upon exposure of zinc thiolate solutions to NO in the presence of air, however, or to NO_2 or NOBF_4 , indicating that these reactive nitrogen/oxygen species are capable of liberating zinc from the enzyme, possibly through generation of the *S*-nitrosothiol. Interaction between simple Zn^{2+} salts and preformed *S*-nitrosothiols leads to decomposition of the $-\text{SNO}$ moiety, resulting in release of gaseous NO and N_2O . The potential biological relevance of this chemistry is discussed.



INTRODUCTION

Nitric oxide (NO) functions in the immune, nervous, and circulatory systems to regulate physiological processes, including macrophage cytotoxicity, neuronal signaling, and vasodilation. Biological NO is synthesized in a five-electron oxidation of *L*-arginine at the active site of nitric oxide synthases (NOSs), of which there are neuronal, endothelial, and inducible NOS isoforms.^{1–4} These enzymes are head-to-head homodimers,⁵ containing a tetrahydrobiopterin binding pocket and a tetracysteine thiolate coordination site for zinc at the dimer interface between oxygenase domains. NOS dimer formation and activity require binding of tetrahydrobiopterin.^{6,7} The absence of zinc destabilizes the NOS dimer and strongly favors dissociation of the enzyme into its composite monomers.^{8–11} NOS activity consequently relies on zinc binding to the tetracysteine thiolate site, and the enzyme may utilize this structural feature to enable a feedback mechanism for regulating NO production.^{8–11}

Exposure of NOS to NO in aerobic aqueous solutions leads to *S*-nitrosylation of zinc-binding cysteine residues with concomitant dissociation of zinc from the tetrathiolate site. This chemistry destabilizes the NOS homodimer, which dissociates into monomers, halting NO production. Metallothioneins exposed to either aerobic NO or biologically relevant *S*-nitrosothiols also lose Zn^{2+} from tetracysteine thiolate sites with concomitant formation of *S*-nitrosothiol.^{12,13} Comparison of studies conducted aerobically and anaerobically reveals that it is not NO per se, but an oxidation product thereof, that may be responsible for thiolate nitrosation in these systems.^{14–16} The generation, translocation, and action of NO

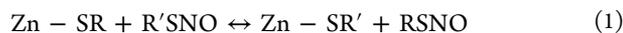
and other reactive nitrogen and oxygen species (RNOS) in biological systems are described in a recent review.¹⁷

The reactions of cobalt, nickel, and copper complexes with nitrogen oxides, as well as reactions of zinc thiolates with RNOS, have been the subject of much recent investigation.^{18–24} Anaerobic NO is unreactive toward the zinc thiolates, but NO_2 , or NO in the presence of dioxygen, causes *S*-nitrosothiol formation. Transnitrosation reactions occur upon exposure of zinc thiolates to external *S*-nitrosothiols.

In the present investigation, we have explored the reactivity of zinc thiolates toward reactive nitrogen species. This work was inspired by the aforementioned studies of NOS and metallothioneins with NO, which expose a need to uncover principles that underlie the chemistry of NO-containing compounds with zinc tetracysteine thiolates. We are also interested in the interaction between zinc thiolates and *S*-nitrosothiols. Although many of the relevant biological targets of NO contain polythiolate zinc centers, we restrict our studies to zinc complexes containing a single thiolate ligand in order to interrogate a unique site. The interaction of transition metals with *S*-nitrosothiols has been described for copper,^{19,25–27} iron,^{28–30} and iridium,^{31,32} inter alia,^{33,34} but much less is known about the reactivity of *S*-nitrosothiols with zinc. Previous studies of *S*-nitrosothiol interactions with zinc centers coordinated by monodentate thiolates describe reversible transnitrosation events (eq 1), whereby transfer of “NO⁺” from

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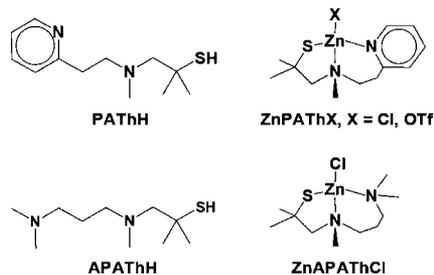
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an exogenous *S*-nitrosothiol to a coordinated thiolate leads to dissociation of the newly formed *S*-nitrosothiol and coordination of the thiolate that is generated by transnitrosation.^{22,24} This work provides insight into the transnitrosation chemistry of coordinated zinc thiolates, and it inspires further investigation into the nature of the interaction between Zn^{2+} and *S*-nitrosothiols.

We selected for study the previously described ligand PAtH^{35-37} and an alkyl analogue, APAtH (Chart 1), to

Chart 1. N_2S Tridentate Chelating Ligands and the Related Zn^{2+} Coordination Compounds



generate mononuclear zinc thiolates. *gem*-Dimethyl groups at the *ipso*-carbon provide steric bulk at the sulfur atom, blocking formation of thiolate-bridged metal dimers or oligomers. In addition, *gem*-dimethyl groups may stabilize tertiary *S*-nitrosothiol species that can form upon exposure of the zinc thiolates to RNOS .³⁴ The tridentate coordination mode of the PAtH and APAtH ligands assures tight binding of zinc,^{37,38} a feature that favors its retention following exposure to RNOS and facilitates analysis of the products of zinc/*S*-nitrosothiol reactions. We herein describe the preparation and characterization of the desired zinc thiolates and their reactivity toward biologically relevant nitrogen oxides, specifically NO , NO^+ , and NO_2 . Reactions of zinc salts with preformed *S*-nitrosothiols are also presented.

EXPERIMENTAL SECTION

General Procedures and Starting Materials. Air- and moisture-sensitive materials were handled in an MBraun glovebox under a nitrogen atmosphere. 2-[2-(Methylamino)ethyl]pyridine and *N,N,N'*-trimethyl-1,3-propanediamine were purchased from Sigma-Aldrich and used without further purification. Nitrosonium tetrafluoroborate (97%) was obtained from Strem and used as received. Isobutylene sulfide³⁹ and trityl *S*-nitrosothiol²⁸ were prepared according to published methods. PAtH was synthesized as described^{35,36} and purified by Al_2O_3 column chromatography using 2:1 ethyl acetate/pentane to give the desired product as a colorless oil. ZnPAtHCl was prepared by a method analogous to the published procedure and characterized by ^1H and ^{13}C NMR spectroscopy, as well as X-ray crystallography.⁴⁰ Nitric oxide (NO) was obtained from Airgas and purified according to published methods.^{28,41} Briefly, NO was passed through an Ascarite column and a 6 ft coil containing silica gel at -78°C to remove impurities, and NO was then collected and stored under nitrogen in a gas storage bulb. N_2O_4 was obtained from Sigma-Aldrich and collected in a gas bulb under nitrogen. Purification by successive freeze-pump-thaw steps at -78°C facilitated the removal of NO , N_2O , and N_2O_3 impurities. $\text{Na}^{15}\text{NO}_2$ was purchased from Cambridge Isotope Laboratories and used without purification. *n*-Butanol was obtained from Alfa Aesar and also used as received. *n*-Butyl nitrite was prepared by modification of a published procedure,⁴² in which the reaction scale was $1/40$ th the size of that reported. $\text{APAtH}^{15}\text{NO}$ was prepared in a manner analogous to that reported for APAtHNO

below, using ^{15}N *n*-butyl nitrite to nitrosate the thiol. All gas transfers were performed using gastight syringes under a atmosphere, unless otherwise noted. Acetonitrile (MeCN), tetrahydrofuran (THF), methylene chloride (DCM), pentane, and diethyl ether were saturated with argon, purified by passage through activated alumina, and stored over 4 Å molecular sieves under a nitrogen atmosphere prior to use. Deuterated NMR solvents were obtained from Cambridge Isotope Laboratories and used without further purification. $\text{MeCN-}d_3$ and methylene chloride- d_2 were brought into the glovebox and stored over 4 Å molecular sieves under a nitrogen atmosphere.

Synthesis of ZnPAtHCl . To a solution of ZnPAtHCl (1.0 g, 3.1 mmol, 1 equiv) in ~ 10 mL of MeCN was added thallium triflate (1.10 g, 3.11 mmol, 1 equiv). The resultant suspension was allowed to stir overnight, after which the reaction mixture was filtered. The solvent was removed from the filtrate, and the solid was washed with diethyl ether. The material was dried under vacuum, yielding 0.50 g (37%) of the desired product as an off-white powder. ^1H NMR ($\text{MeCN-}d_3$): δ 1.32 (s, 3H, CH_3), 1.52 (s, 3H, CH_3), 2.64 (d, $J = 13.4$ Hz, 1H, CH_2), 2.73 (s, 3H, CH_3), 2.89 (d, $J = 13.4$ Hz, 1H, CH_2), 2.95–3.26 (m, 4H, CH_2), 7.52–7.56 (m, 2H, $\text{py-}H_\beta + \text{py-}H_\delta$), 8.05 (td, $J = 8$ and 1.2 Hz, 1H, $\text{py-}H_\gamma$), 8.50 (d, $J = 4.8$ Hz, 1H, $\text{py-}H_\epsilon$). ^{13}C NMR ($\text{MeCN-}d_3$): δ 33.39, 33.89, 34.99, 49.14, 49.92, 60.67, 73.89, 125.31, 127.45, 142.97, 150.06, 161.65. ^{19}F NMR ($\text{MeCN-}d_3$): δ -79.41. IR (MeCN , cm^{-1}) 1612, 1571, 1271. Anal. Calcd for $\text{C}_{13}\text{H}_{19}\text{F}_3\text{N}_2\text{O}_3\text{Zn}$: C, 35.66; H, 4.37; N, 6.40. Found: C, 35.33; H, 4.23; N, 6.42.

Synthesis of PAtHNO . To a solution of PAtH (1.0 g, 4.5 mmol, 1 equiv) in DCM (~ 10 mL) was added *i*-amyl nitrite (0.6 mL, 4.5 mmol, 1 equiv). The reaction mixture was allowed to stir overnight in the dark, and the solvent was removed. Silica gel chromatography (1:1 ethyl acetate/pentane) allowed isolation of the pure product as a dark-green oil (0.52 g, 46%). Care was taken to minimize exposure of the reaction mixture and product to light. ^1H NMR ($\text{MeCN-}d_3$): δ 1.80 (s, 6H, CH_3), 2.40 (s, 3H, CH_3), 2.87 (s, 4H, CH_2), 3.06 (s, 2H, CH_2), 7.10–7.14 (m, 1H, $\text{py-}H_\beta$), 7.18 (d, $J = 8.8$ Hz, 1H, $\text{py-}H_\delta$), 7.60 (t, $J = 5.6$ Hz, 1H, $\text{py-}H_\gamma$), 8.44 (d, $J = 4.8$ Hz, 1H, $\text{py-}H_\epsilon$). ^{13}C NMR ($\text{MeCN-}d_3$): δ 27.32, 36.83, 44.83, 60.41, 60.98, 69.01, 122.08, 124.27, 137.07, 150.05, 161.43. IR ($\text{MeCN-}d_3$, cm^{-1}): 1591, 1569, 1484 (ν_{NO}), 1476. Anal. Calcd for $\text{C}_{12}\text{H}_{19}\text{N}_3\text{OS}$: C, 56.89; H, 7.56; N, 16.58. Found: C, 57.01; H, 7.39; N, 16.48.

Synthesis of PAtH_2 . Sodium hydride (0.214 g, 8.92 mmol, 1 equiv) was added as a solid to a solution of PAtH (2.0 g, 8.9 mmol, 1 equiv) in ~ 40 mL of THF , and the mixture was allowed to stir overnight. The resulting suspension was left to stand at -30°C to precipitate unreacted NaH . The solution was decanted by pipet into an aluminum-foil-covered flask, and iodine (1.36 g, 5.35 mmol, 0.65 equiv) was added to the stirring solution. After 24 h of stirring, the solvent was removed and the oily residue was partitioned between diethyl ether and a saturated sodium thiosulfate aqueous solution. The desired product was extracted into the ether layer, and the organics were washed with water and dried (MgSO_4). The solvent was removed to yield the desired product as a viscous, bright-yellow oil (1.4 g, 69%). ^1H NMR (CD_2Cl_2): δ 1.18 (s, 12H, CH_3), 2.40 (s, 6H, CH_3), 2.47 (s, 4H, CH_2), 2.81–2.85 (m, 4H, CH_2), 2.88–2.92 (m, 4H, CH_2), 7.08 (dd, $J = 7.2$ and 5.6 Hz, 1H, $\text{py-}H_\beta$), 7.17 (d, $J = 8.0$ Hz, 1H, $\text{py-}H_\delta$), 7.53–7.61 (m, 1H, $\text{py-}H_\gamma$), 8.47 (d, $J = 4.8$ Hz, 1H, $\text{py-}H_\epsilon$). ^{13}C NMR (CD_2Cl_2): δ 26.52, 36.83, 45.02, 51.53, 60.84, 68.70, 121.44, 123.74, 136.47, 149.64, 161.26. IR ($\text{MeCN-}d_3$, cm^{-1}): 1591, 1569, 1475, 1463, 1436. Anal. Calcd for $\text{C}_{24}\text{H}_{38}\text{N}_4\text{S}_2$: C, 64.53; H, 8.57; N, 12.54. Found: C, 64.58; H, 8.70; N, 12.58.

Synthesis of APAtH . To a stirring solution of *N,N,N'*-trimethyl-1,3-propanediamine (10 g, 86 mmol, 1 equiv) in approximately 500 mL of MeCN was added dropwise a solution of isobutylene sulfide (10.2 mL, 103 mmol, 1.2 equiv) in 100 mL of MeCN at room temperature. The reaction mixture was heated to 45°C for 16.5 h. The solvent was removed in vacuo, and the residue was purified by column chromatography (Al_2O_3 , 2:1 pentane/ EtOAc , $R_f \sim 0.55$) to yield the desired product as a colorless oil (6.3 g, 36%). ^1H NMR ($\text{MeCN-}d_3$): δ 1.28 (s, 6H, CH_3), 1.55–1.62 (m, 2H, CH_2), 2.12 (s, 6H, $\text{N-}(\text{CH}_3)_2$), 2.24 (t, $J = 7.2$ Hz, 2H, CH_2), 2.30 (s, 1H, SH), 2.32 (s, 3H, NCH_3), 2.40 (s, 2H, CH_2), 2.50 (t, $J = 7.2$ Hz, 2H, CH_2). ^{13}C NMR

(MeCN- d_3): δ 26.89, 30.75, 45.10, 45.79, 47.03, 58.20, 59.03, 72.82. IR (MeCN- d_3 , cm^{-1}): 1463, 1383, 1363. Anal. Calcd for $\text{C}_{10}\text{H}_{24}\text{N}_2\text{S}\cdot 0.14\text{EtOAc}$: C, 58.53; H, 11.68; N, 12.93. Found: C, 58.22; H, 11.41; N, 13.07. The presence of residual ethyl acetate was confirmed by the ^1H NMR spectrum, and the quantity used for the computed elemental composition was adjusted for the best fit to the results.

Synthesis of ZnAPATHCl. To a solution of APATHH (1.0 g, 4.9 mmol, 1 equiv) in ~ 5 mL of THF was added NaH (0.117 g, 4.89 mmol, 1 equiv) as a solid. The suspension was allowed to stir until all solid material had dissolved and gas evolution ceased. Zinc chloride (0.667 g, 4.89 mmol, 1 equiv) was suspended in 10 mL of THF, and the suspension was added to the deprotonated ligand solution. The reaction was allowed to stir overnight. The solvent was removed in vacuo, the residue was taken up in methylene chloride, and the solution was filtered. Solvent was evaporated from the filtrate, and the resulting residue was washed with diethyl ether to remove unreacted ligand. The remaining solid was dried in vacuo, and the product was isolated as a white powder (0.86 g, 58%). ^1H NMR (MeCN- d_3): δ 1.39 (d, $J = 7.6$ Hz, 6H, CH_3), 1.66–1.74 (m, 1H, CH_2), 1.86–1.95 (m, 1H, CH_2), 2.28 (d, $J = 13.0$ Hz, 1H, CH_2), 2.45 (bs, 6H, $\text{N}(\text{CH}_3)_2$), 2.53 (s, 3H, NCH_3), 2.61 (d, $J = 13.0$ Hz, 1H, CH_2), 2.66–2.80 (m, 3H, CH_2), 2.95–3.00 (m, 1H, CH_2). ^{13}C NMR (MeCN- d_3): δ 22.96, 36.70, 37.23, 45.20, 48.72, 48.98, 49.53, 62.34, 62.83, 73.55. IR (CH_2Cl_2 , cm^{-1}): 1469, 1456. Anal. Calcd for $\text{C}_{10}\text{H}_{23}\text{ClN}_2\text{SZn}\cdot 0.30\text{CH}_2\text{Cl}_2$: C, 37.52; H, 7.21; N, 8.50. Found: C, 37.59; H, 7.22; N, 8.27. The presence of residual methylene chloride was confirmed by ^1H NMR, and the quantity used for the computed elemental composition was adjusted for the best fit to the results.

Synthesis of APATHNO. APATHH (1.0 g, 4.9 mmol, 1 equiv) and *i*-amyl nitrite (0.66 mL, 4.9 mmol, 1 equiv) were combined in 10 mL of methylene chloride, and the solution was stirred overnight in the dark. The solvent was removed, and the green residue was purified by silica gel chromatography (96:4 $\text{CH}_2\text{Cl}_2/\text{MeOH}$). The product was isolated as a dark-green oil (0.67 g, 58%). Care was taken to minimize exposure of the reaction mixture and product to light. ^1H NMR (CD_2Cl_2): δ 1.55–1.62 (m, 2H, CH_2), 1.88 (s, 6H, CH_3), 2.20 (s, 6H, $\text{N}(\text{CH}_3)_2$), 2.27 (t, $J = 7.2$ Hz, 2H, CH_2), 2.32 (s, 3H, NCH_3), 2.50 (t, $J = 7.2$ Hz, 2H, CH_2), 3.01 (s, 2H, CH_2). ^{13}C NMR (CD_2Cl_2): δ 26.06, 27.51, 44.90, 45.46, 57.72, 58.67, 59.81, 69.41. UV–vis [MeCN; λ , nm (ϵ , $\text{M}^{-1}\text{cm}^{-1}$): 346 (554.1), 556 (9.2), 599 (16.8)]. IR (CH_3CN , cm^{-1}): 1477 (ν_{NO}) {1467 (ν_{NO}^{15})}, 1388, 1369. Anal. Calcd for $\text{C}_{10}\text{H}_{23}\text{N}_3\text{OS}$: C, 51.47; H, 9.93; N, 18.01. Found: C, 51.76; H, 9.59; N, 18.23.

Phosphine-Mediated APATHNO Ligation. *S*-[2-(Diphenylphosphino)phenyl]butanethioate was prepared according to published procedures.^{43,44} To ZnAPATHCl (50 mg, 0.16 mmol, 1 equiv) in ~ 15 mL of MeCN under a nitrogen atmosphere was added solid NOBF_4 (17 mg, 0.15 mmol, 0.9 equiv). The mixture was allowed to stir for 2 h, at which point the phosphine reagent (120 mg, 0.329 mmol, 2 equiv) was added as a solid. An aliquot of the reaction mixture was immediately analyzed by ESI-MS with anaerobic MeCN as the carrier solvent. An $[\text{M} + \text{H}]^+$ peak at m/z 582.1 (calcd m/z 582.27) was observed for the phosphine-ligated APATHNO.

Physical Measurements. ^1H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker Avance spectrometer. Optical spectra were measured on a Varian Cary 50 Bio UV–vis spectrophotometer in 6SQ Starna cells. The Cary WinUV Scanning Kinetics software was used to record optical time-dependent spectra. FT-IR spectra were recorded on a Thermo Nicolet Avatar 360 spectrometer running the OMNIC software package. A CaF_2 cell was used for solution IR spectra. ESI-MS analyses were performed on an Agilent 1100 Series LC/MSD trap spectrometer. An Agilent Technologies 5975C mass selective detector running in electron impact ionization mode was used for EI-MS studies of the reaction headspace.

UV–Vis Studies. All solutions were prepared under a nitrogen atmosphere. Optical spectra were recorded at 25 °C.

Zinc Thiolate Reactions with NO(g). Stock solutions of zinc thiolates were prepared at 10 times the desired reaction concentration. A 2.7 mL volume of MeCN was transferred to a cuvette, and 300 μL of

the zinc thiolate solution was injected into the cell. The cell was sealed with an airtight Teflon cap containing a septum, and 1 mL of headspace was removed from the sealed cuvette by a gastight syringe. NO (1 mL) was withdrawn from the storage bulb by a gastight syringe, and the syringe needle was covered with a septum. The syringe and cuvette were removed from the glovebox, and the cuvette was transferred to the UV–vis spectrophotometer. After one optical scan of the zinc thiolate solution, NO was quickly injected into the cuvette. The cuvette was shaken to mix the reagents and returned to the spectrophotometer. Optical spectra were recorded at regular intervals. For studies with NO + air, a 1 mL volume of air was injected into the reaction cuvette following completion of the NO study, the cuvette was shaken, and the cell was returned to the spectrophotometer. Optical spectra were again recorded at regular intervals.

Zinc Thiolate Reactions with NOBF_4 . Stock solutions of NOBF_4 in MeCN were prepared at 1.1 times the desired reaction concentration. Solutions of zinc thiolates were prepared at 10 times the desired reaction concentration. A 2.7 mL portion of the NOBF_4 solution was transferred to a cuvette, and the cell was sealed with an airtight Teflon cap containing a septum. A 300 μL portion of the zinc thiolate solution was withdrawn by a gastight syringe, and the needle was covered by a septum. The cuvette was transferred to the UV–vis spectrophotometer, and after one optical scan of the nitrosonium tetrafluoroborate solution, the zinc thiolate solution was quickly injected. The cuvette was shaken to mix the reagents and returned to the spectrophotometer. Optical spectra were recorded at regular intervals.

Zinc Thiolate Reactions with $\text{NO}_2(\text{g})$. The procedure and quantities used were identical to those described for the reactions with NO, except that $\text{NO}_2(\text{g})$ was added instead of NO (g).

***S*-Nitrosothiol Reactions with $\text{Zn}(\text{OTf})_2$.** All solutions were prepared under a nitrogen atmosphere.

PATHNO. A 2.52 mM PATHNO solution in MeCN was prepared, and 2.5 mL of the stock solution was sealed in a cuvette. A 1 mL solution of $\text{Zn}(\text{OTf})_2$ (2.3 mg, 0.0063 mmol) dissolved in MeCN was withdrawn by a gastight syringe, and the needle was covered by a septum. The cuvette and syringe were removed from the glovebox, and the cuvette was placed in the spectrophotometer. After one optical scan of the PATHNO solution, the zinc solution was injected into the cuvette. The cell was shaken to mix the reagents and returned to the spectrophotometer. The final solution was 1.8 mM in both reagents. Optical spectra were recorded at regular intervals.

APATHNO. The procedure was identical to that described for PATHNO, with the following exceptions. The prepared solution of *S*-nitrosothiol was 2.1 mM in APATHNO, and the MeCN solution of zinc(II) salt was 5.25 mM in $\text{Zn}(\text{OTf})_2$. The final solution was 1.5 mM in both reagents.

FT-IR Time-Dependent Studies of *S*-Nitrosothiol Reactions with Zinc Salts. All reactions were performed under a nitrogen atmosphere. To a stirring solution of 100 mg (0.429 mmol) of APATHNO in ~ 3 mL of MeCN- d_3 was added 1 equiv of solid ZnX_2 ($\text{X} = \text{Cl}, \text{OTf}$). Aliquots of the reaction mixtures were obtained over the duration of the study and injected into a CaF_2 solution IR cell under a nitrogen atmosphere. The cell was sealed, removed from the glovebox, and placed in the FT-IR spectrometer for analysis. For the study with APATH ^{15}NO , the conditions were analogous except that 50 mg (0.21 mmol) of the *S*-nitrosothiol was used.

^1H NMR Studies. All samples were prepared under a nitrogen atmosphere.

Comparison of the Reactivity of ZnPATHOTf with NO^+ with That of PATHNO with $\text{Zn}(\text{OTf})_2$. Reactivity of ZnPATHOTf with NO^+ . A 1 mL portion of a 7.5 mM stock solution of ZnPATHOTf (0.008 mmol) in MeCN- d_3 was added to 0.9 mg (0.008 mmol) of NOBF_4 . The solution was allowed to stir for 1 h, at which point an aliquot was analyzed by ^1H NMR spectroscopy.

Reactivity of PATHNO with $\text{Zn}(\text{OTf})_2$. PATHNO (0.100 g, 0.395 mmol) in ~ 2 mL of MeCN- d_3 was added to $\text{Zn}(\text{OTf})_2$ (0.144 g, 0.395 mmol). The solution was allowed to stir for 1 h, at which point an aliquot was analyzed by ^1H NMR spectroscopy.

Disulfide Titrations with $\text{Zn}(\text{OTf})_2$. Stock solutions of the disulfide PATH_2 (15 mM) and $\text{Zn}(\text{OTf})_2$ (75 mM) were prepared in MeCN- d_3 .

Samples for ^1H NMR spectroscopic analysis were prepared by adding 250 μL of the PATH_2 stock solution and the appropriate volume of $\text{MeCN-}d_3$ to NMR tubes containing 0.5, 1, 2, or 5 equiv of $\text{Zn}(\text{OTf})_2$ in a total reaction volume of 500 μL . The final concentration of PATH_2 in each NMR tube was 7.5 mM. Samples were analyzed after 1 h of mixing.

EI-MS Studies of Zinc Thiolate with NO^+ and of *S*-Nitrosothiol with Zinc Salts. All solutions were prepared under a nitrogen atmosphere. NOBF_4 (0.154 g, 1.32 mmol) was placed in a custom-made gastight cell in ~ 3 mL of methylene chloride in a glovebox under a nitrogen atmosphere. The cell was removed from the glovebox and exposed to an argon purge. This procedure ensured that high concentrations of nitrogen would not mask peaks in the m/z 30 region. ZnAPATHCl (0.40 g, 1.3 mmol) was dissolved in ~ 1 mL of methylene chloride under a nitrogen atmosphere and was transferred by a gastight syringe to the reaction cell under an argon flow. The cell was sealed, and the reaction mixture was allowed to stir for 1.5 h prior to analysis. The cell was then connected to a helium gas flow and to the mass spectrometer, and the connecting cables were purged thoroughly prior to analysis of the reaction headspace. Headspace analysis was undertaken with the mass spectrometer operating in selective ion mode. Analogous reaction conditions were employed for the reactions of APATHNO with ZnCl_2 and with $\text{Zn}(\text{OTf})_2$. ZnCl_2 (58.4 mg, 0.428 mmol, 1 equiv) and MeCN were added to the reaction cell in the glovebox, and a solution of APATHNO (100 mg, 0.428 mmol, 1 equiv) was injected into the cell after an argon purge. A sample of $\text{Zn}(\text{OTf})_2$ (77.9 mg, 0.214 mmol, 1 equiv) was exposed to APATHNO (50 mg, 0.21 mmol, 1 equiv) in MeCN in a parallel experiment. For the control experiment with APATHNO , a solution containing 50 mg of the *S*-nitrosothiol was added to the reaction cell in the glovebox. The cell was then sealed, removed from the glovebox, and purged with argon. The headspace of the stirring solution was analyzed by EI-MS after 2 h. All reactions were carried out in the dark to prevent photodecomposition of *S*-nitrosothiol.

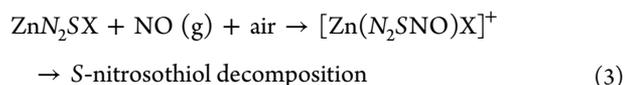
RESULTS AND DISCUSSION

Preparation of Compounds. To prepare for studies of the reactivity of the zinc thiolate moiety, we first synthesized the PATHH ligand from isobutylene sulfide and 2-[2-(methylamino)ethyl]pyridine according to previously published procedures.^{35,36} Deprotonation of the ligand followed by ZnCl_2 addition afforded the chloride complex ZnPAThCl in decent yield (Scheme 1a). Structural parameters for ZnPAThCl match previously reported values.⁴⁰ Reaction of thallium triflate with an MeCN solution of ZnPAThCl gave ZnPAThOTf , which was obtained in 37% yield (Scheme 1b). The *S*-nitrosothiol PAThNO was prepared as a green oil for spectral comparison

with zinc thiolate/nitrogen oxide reaction products by treating PATHH with *i*-amyl nitrite (Scheme 1c).

We also synthesized the new ligand APATHH , an alkyl propylaminothioliolate, to eliminate $\text{C}=\text{C}$ and $\text{C}=\text{N}$ stretches that appear in the characteristic IR $\text{N}-\text{O}$ spectral stretching region for *S*-nitrosothiols and thereby facilitate the interpretation of IR spectroscopic studies (Figure S1 in the Supporting Information). APATHH retains the coordinating atoms afforded by PATH but replaces the spectrally interfering pyridyl moiety of the latter with a tertiary amine. Condensation of *N,N,N'*-trimethyl-1,3-diaminopropane with isobutylene sulfide readily yielded APATHH (Scheme 2a). The zinc complex ZnAPATHCl was prepared by analogy to the synthesis of ZnPAThCl (Scheme 2b). We were unable to obtain X-ray-quality crystals of ZnAPATHCl . Given the similarity between the coordination environments of PATHH and APATHH , however, we expect the structural characteristics of ZnAPATHCl to be similar to those of ZnPAThCl .⁴⁰ The *S*-nitrosothiol APATHNO was obtained by transnitrosation of APATHH with *i*-amyl nitrite (Scheme 2c), and the disulfide PATH_2 (Scheme 1d) was prepared by iodine oxidation of $[\text{PATH}]^-$ thiolate. These compounds were synthesized for spectral comparison with the products of reactions between the zinc complexes and RNOS .

Exposure of Zinc PATH and APATH Complexes to NO under Various Conditions. No reaction was observed by UV-vis spectroscopy upon addition of excess NO to anaerobic MeCN solutions of ZnPAThCl , ZnPAThOTf , and ZnAPATHCl . Injection of air into the cuvette led to the immediate appearance of peaks in the UV-vis spectrum that are characteristic of tertiary *S*-nitrosothiols (eqs 2 and 3, Figure 1, and Figures S2 and S3 in the Supporting Information).³⁴ The "0 min" trace reflects immediate appearance of a spectral band arising from the *S*-nitrosothiol, which then decays over time.



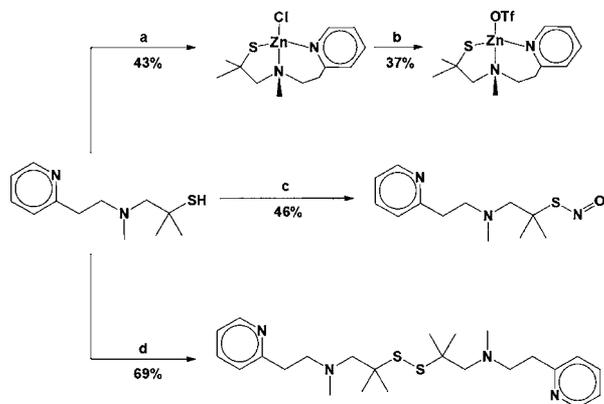
The lack of reactivity under anaerobic conditions is consistent with previous findings,⁴⁵ which reveal Zn^{2+} -based model systems that utilize β -diketiminato²⁴ and tris(pyrazolyl)borate²² scaffolds to be inert to anaerobic $\text{NO} (\text{g})$. This finding has important implications for NO in biology. Specifically, this result requires that it is not NO itself, but an oxidation product thereof, that is responsible for *S*-nitrosothiol formation at biological zinc thiolate sites, such as those in NOS and metallothioneins. NO has been previously described as a poor nitrosating agent for thiols.⁴⁶ The NO -mediated thiolate nitros(yl)ation reaction is an electron-releasing process (eq 4), and in the absence of a viable electron acceptor, no reaction is observed. In biological milieu, however, dioxygen is present



to oxidize NO to NO_2 , for example, thereby enabling *S*-nitrosothiol formation (eqs 5 and 6). In accordance with the redox nature of NO , the reported reaction between the model complex ${}^{i\text{Pr}}_2\text{TpZnS}^t\text{Bu}$ and NO/O_2 mixtures, or $\text{NO}_2 (\text{g})$, led to *S*-nitrosothiol formation and isolation of ${}^{i\text{Pr}}_2\text{TpZn}(\text{O}_2\text{NO})$.²² Our results are consistent with this *S*-nitros(yl)ation chemistry, although we did not explore the fate of the Zn^{2+} ion in the NO/O_2 chemistry.



Scheme 1. Synthesis of PATH Species



a 1. NaH , THF 2. ZnCl_2 ; b TIOTf , MeCN ; c *iso*-amyl nitrite, CH_2Cl_2 ; d 1. NaH , THF 2. I_2

Scheme 2. Synthesis of APATH Species

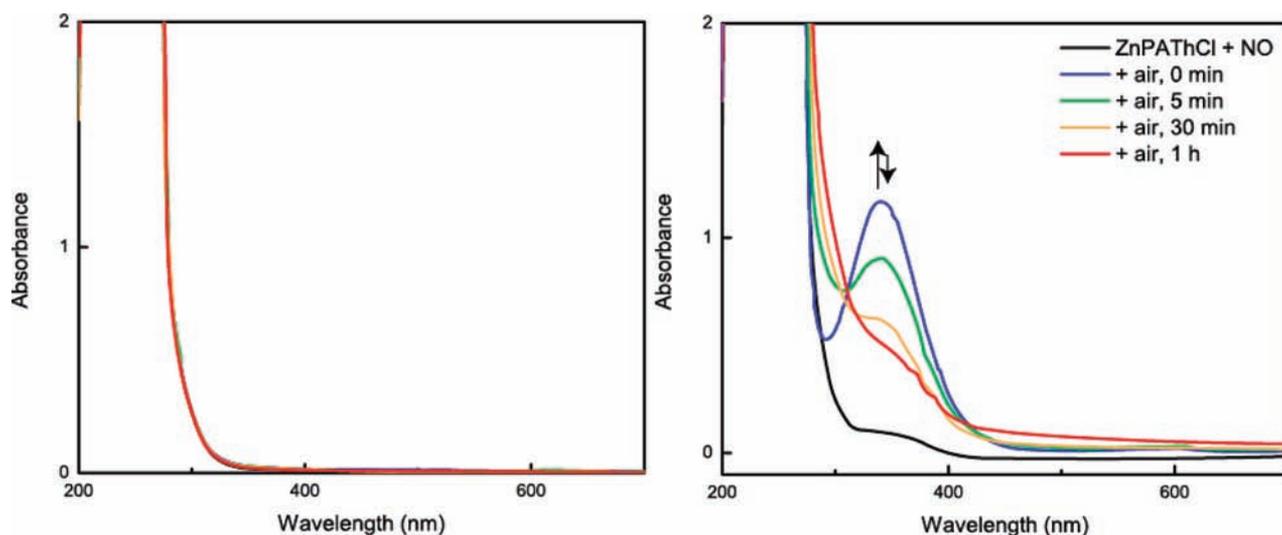
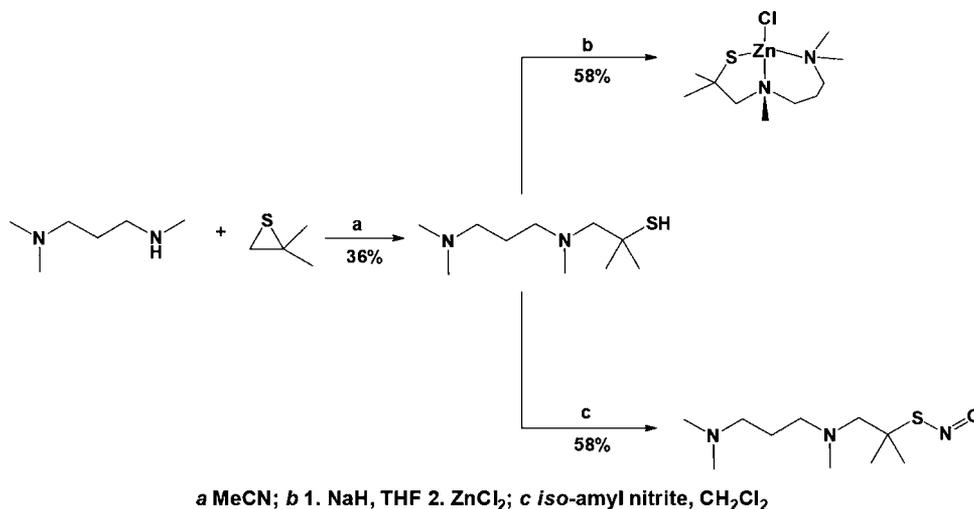
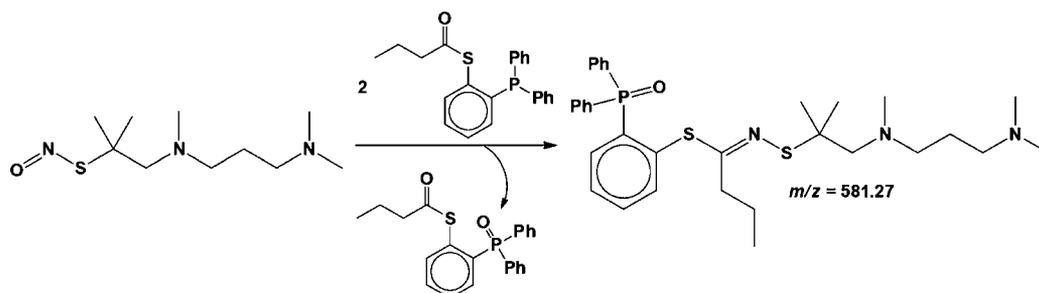


Figure 1. UV-vis reactivity profiles for anaerobic 1.5 mM ZnPATHCl + excess NO (g) (left) and 1.5 mM ZnPATHCl + excess NO (g) + air (right). Recorded in MeCN.

Scheme 3. Traceless Ligation of APATHNO by Phosphine-Mediated N=O Bond Scission.^{43,44}



Reactions of Zinc PATH and APATH Complexes with NOBF₄. We next examined the reactivity of nitrosium tetrafluoroborate with zinc thiolates. Exposure of ZnPATHCl, ZnPATHOTf, and ZnAPATHCl to 1 equiv of NOBF₄ in MeCN led to the appearance of peaks in the optical spectra corresponding to tertiary S-nitrosothiols (Figure S4 in the Supporting Information). S-Nitrosothiol formation was dem-

onstrated for the reaction of ZnAPATHCl with NO⁺ by trapping APATHNO and studying the products by mass spectrometry (Scheme 3).^{43,44} Utilization of phosphine-mediated N=O bond scission and ESI-MS analysis of products confirmed that APATHNO formed upon exposure of ZnAPATHCl to NO⁺ (Figure S5 in the Supporting Information). We therefore conclude that optical peaks attributed to tertiary S-nitrosothiol formation in reactions of zinc thiolates and RNOS are correctly assigned.

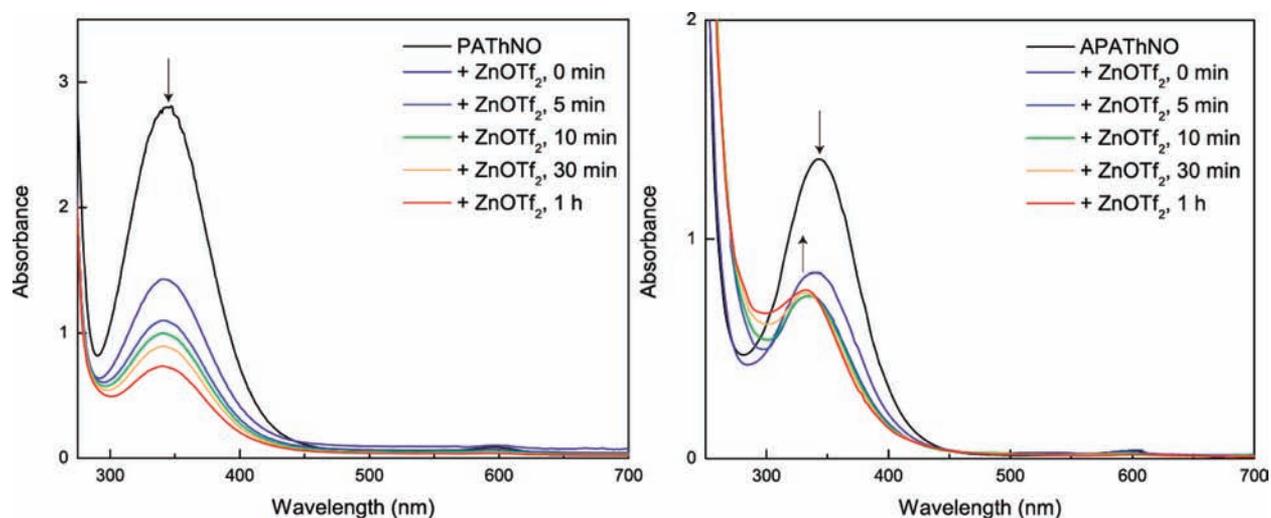
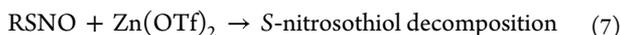


Figure 2. Disappearance of characteristic *S*-nitrosothiol peaks occurring upon exposure of 1.8 mM PATHNO (left) and 1.5 mM APATHNO (right) to 1 equiv of Zn(OTf)₂ in MeCN. Reactions were monitored for 1 h.

Reactions of *S*-Nitrosothiols with Zinc(II) Salts.

PATHNO and APATHNO were prepared to examine the interaction between these preformed tertiary *S*-nitrosothiols and Zn²⁺. Optical spectroscopic studies of reaction mixtures containing 1 equiv of Zn(OTf)₂ and PATHNO or APATHNO revealed a decrease in the intensity of the peaks at ~340 nm compared to optical spectra of *S*-nitrosothiol solutions lacking Zn²⁺ (Figure 2 and eq 7). This spectroscopic change corresponds to decay of the $n_{\text{O}} \rightarrow \pi^*$ transitions for each *S*-nitrosothiol³⁴ in the presence of Zn(OTf)₂. Peaks at 602 nm in the spectra of PATHNO and APATHNO disappear completely upon addition of Zn(OTf)₂. A new peak, corresponding to an



as-of-yet unidentified species, grows in at 330 nm after 1 h in the APATHNO/Zn(OTf)₂ reaction spectrum (Figure 2). IR spectroscopic analysis of the APATHNO reaction with ZnCl₂ revealed the appearance of three bands in the 1600 cm⁻¹ region of the spectrum, and two bands were similarly observed for the reaction of APATHNO with Zn(OTf)₂ (Figure S6 in the Supporting Information). Concomitant disappearance of the ν_{NO} feature of APATHNO at 1477 cm⁻¹ was observed for reactions with both zinc(II) salts (Figure S6 in the Supporting Information). Additionally, inner-sphere coordination of a single triflate anion was deduced for the reaction with Zn(OTf)₂, judging by the appearance of a band at 1311 cm⁻¹, a signature for covalent binding of the sulfonate moiety.⁴⁷ There is a corresponding decrease in the intensity of the asymmetric sulfonate ion stretching frequency at 1287 cm⁻¹ from the outer-sphere triflate anion.⁴⁷ Use of ¹⁵N-isotopically labeled APATHNO revealed that the bands in the 1600 cm⁻¹ IR spectral region for reactions with ZnCl₂ and Zn(OTf)₂ showed negligible changes compared to the unlabeled *S*-nitrosothiol, indicating that these peaks are not caused by stretches of N-containing moieties in the product(s) (Figure S7 in the Supporting Information).

There is a dependence on the anion in the reactions of zinc salts with the *S*-nitrosothiol, as inferred from the presence of an additional band in the IR spectra of the ZnAPATHCl/NOBF₄ and APATHNO/ZnCl₂ reaction mixtures that is absent in solutions containing a triflate counterion (Figures S6 and S7 in the Supporting Information). The better coordinating ability of

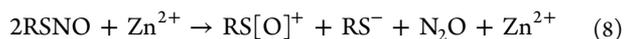
chloride compared to triflate may control the availability of binding sites for the -SNO moiety on zinc, or Cl⁻ may participate directly in the reaction.

To further explore the chemistry between Zn²⁺ and the -SNO moiety of *S*-nitrosothiols, we recorded ¹H NMR spectra for reactions of ZnPATHOTf with NOBF₄ and of PATHNO with Zn(OTf)₂ (Figure S8 in the Supporting Information). All of the peaks observed in the PATHNO/Zn(OTf)₂ reaction mixture are present in the ZnPATHOTf/NOBF₄ ¹H NMR spectrum. Spectroscopic comparison of mixtures suggests that PATHNO binds zinc(II) in a process which, on the basis of infrared spectroscopic evidence, is accompanied by coordination of a triflate counteranion. In the related ZnPATHOTf reaction with NOBF₄, NO⁺ attacks the thiolate, such that the reactive species in both instances is [Zn(PATHNO)(OTf)]⁺. In addition, the ¹H NMR spectrum contains a new singlet at 2.05 ppm. This peak may arise from the *gem*-dimethyl groups of a metastable intermediate, possibly coordinated *S*-nitrosothiol. We were unable to trap and identify this species.

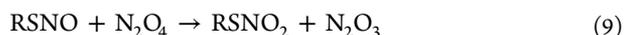
The complexity of the NMR spectra of these reaction mixtures precluded definitive identification of the products. However, being aware of the propensity of *S*-nitrosothiols to decompose to the corresponding disulfides over time,³⁴ we prepared the disulfide PATH₂ as a control to assess whether exposure of zinc thiolates to nitrosonium ion, or of *S*-nitrosothiol to Zn²⁺, could result in disulfide formation. ¹H NMR spectral titration studies of PATH₂ with Zn(OTf)₂ revealed formation of various species, depending on the ratio of zinc to disulfide in solution (Figure S9 in the Supporting Information). The ¹H NMR spectra of neither the free disulfide nor disulfide solutions containing Zn²⁺ matched the ¹H NMR spectra of the zinc thiolate/NOBF₄ or *S*-nitrosothiol/Zn(OTf)₂ reaction mixtures (Figure S10 in the Supporting Information). From these results, we conclude that disulfide is not a product of *S*-nitrosothiol exposure to Zn²⁺. It therefore appears likely that zinc promotes heterolytic, not homolytic, cleavage of the S-N bond of *S*-nitrosothiols. These results complement previous studies of the transnitrosation reactions between monodentate zinc(II)-bound thiolates and exogenous *S*-nitrosothiols (eq 1, *vide supra*).^{22,24}

Finally, we analyzed the reaction headspace of ZnAPATHCl/NOBF₄, APATHNO/ZnCl₂, and APATHNO/Zn(OTf)₂ reac-

tion mixtures by EI-MS to determine whether gaseous species are generated by *S*-nitrosothiols in the presence of Zn^{2+} . NO and N_2O were detected in all instances (Figures S11–S13 in the Supporting Information). NO_2 was not observed. The $ZnCl_2/APATHNO$ reaction was also monitored for the possible formation of Cl_2 or $NOCl$ (Figure S12 in the Supporting Information). Neither was observed, indicating that the chloride anion is unlikely to participate in any redox chemistry. The headspace of a control solution of *APATHNO* was examined by EI-MS, and neither NO nor N_2O was present (Figure S14 in the Supporting Information). This control experiment confirms that NO and N_2O are true reaction products and not the result of *S*-nitrosothiol autodecomposition. A possible reaction pathway that accounts for Zn^{2+} -mediated decomposition of *S*-nitrosothiols with concomitant generation of N_2O , and not disulfide, involves thiolate oxidation to generate transient $RS[O]^+$ species, which may undergo further decomposition. The existence of reactive $RS[O]^+$ species is described in the literature.⁴⁸ A total of 2 equiv of *S*-nitrosothiol may react to form N_2O , $RS[O]^+$, and RS^- . The resultant thiolate may coordinate Zn^{2+} to regenerate the starting complex. Equation 8 describes the proposed reaction. We also suggest that Zn^{2+} , by forming a stable complex with the resulting thiolate, may activate *S*-nitrosothiols to release NO^+ , which may, in turn, lead to catalytic *S*-nitrosothiol decomposition and generation of NO .⁴⁹



Reactions of Zinc PATH and APATH Complexes with NO_2 . Exposure of *ZnPATHCl*, *ZnPATHOTf*, and *ZnAPATHCl* to excess NO_2 led to immediate appearance and subsequent decay of optical features attributed to *S*-nitrosothiols (Figure S15 in the Supporting Information). The decomposition of *S*-nitrosothiols in the presence of excess nitrogen dioxide has been reported.^{50,51} N_2O_4 equilibrates with NO_2 and oxidizes *S*-nitrosothiols to thionitrates (eq 9), which lack the optical features of *S*-nitrosothiols.



Potential Biological Relevance. Consistent with prior results, we find that an isolated zinc thiolate bond is unreactive toward NO in the absence of an oxidizing agent. We propose, on the basis of spectral changes of *S*-nitrosothiols in the presence of Zn^{2+} , that zinc, in its role as a Lewis acid, activates the *RSNO* moiety, possibly stabilizing RS^- with release of NO^+ . Biological consequences for NOS may be the need for zinc to dissociate from nitrosated thiolates in order to maintain physiological reversibility of the nitrosation reaction. Without such dissociation, interaction of Zn^{2+} with *S*-nitrosothiols could result in thiolate oxidation that would block conversion back to the biologically useful form of the enzyme.^{52,53} Irreversible modification of zinc thiolates in transcription factors following oxidative stress provides a precedent for the biological irreversibility that we propose here.^{54,55}

SUMMARY AND CONCLUSIONS

NO under anaerobic conditions is unreactive toward the zinc thiolates examined in this work, which implies that it is not NO itself, but an oxidized form thereof, that is the active species responsible for *S*-nitrosothiol formation and demetalation in NOS and metallothioneins in biology. Zinc thiolate reactivity with NO in the presence of dioxygen supports this hypothesis,

and studies with NO_2 indicate that nitrogen dioxide is a viable species for inducing zinc thiolate nitrosation in vivo. Exposure of *ZnPATHCl*, *ZnPATHOTf*, and *ZnAPATHCl* to NO^+ in the form of nitrosonium tetrafluoroborate also leads to thiolate nitrosation. Addition of $NOBF_4$ to *ZnPATHOTf*, or of $Zn(OTf)_2$ to *PATHNO*, most likely generates a coordinated *S*-nitrosothiol as a transient species, but for both *PATHNO* and *APATHNO*, interaction of the $-SNO$ moiety with Zn^{2+} promotes its decomposition. NO and N_2O were detected in the headspace of solutions containing Zn^{2+} and *S*-nitrosothiol, irrespective of whether the latter was prepared independently or generated in situ. The absence of disulfide products from reaction mixtures is characteristic of heterolytic, not homolytic, cleavage of the $S-N$ bond in the presence of Zn^{2+} , the role of which may be to activate the $-SNO$ moiety toward heterolysis, possibly by stabilizing the thiolate formed in the reaction. Oxidized thiolates may be the ultimate reaction products, as proposed in eq 8.

ASSOCIATED CONTENT

Supporting Information

IR spectra of *PATHNO* and related species; optical spectra of reactions of $NO \pm$ air, NO_2 , and $NOBF_4$ with zinc thiolates; ESI-MS spectrum of the reaction between *ZnAPATHCl*, $NOBF_4$, and phosphine; solution IR comparisons of the reactions of *APATHNO* and *APATH¹⁵NO* with $ZnCl_2$ and $Zn(OTf)_2$; 1H NMR spectra of *ZnPATHOTf/NOBF₄*, *PATHNO/Zn(OTf)₂*, and *PATH₂/Zn(OTf)₂* reactions; EI-MS analyses of the headspaces of reaction mixtures containing *ZnAPATHCl/NOBF₄*, *APATHNO/ZnCl₂*, *APATHNO/Zn(OTf)₂*, and *APATHNO*. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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