Inorganic Chemistry

A Monohydrosulfidodinitrosyldiiron Complex That Generates N₂O as a Model for Flavodiiron Nitric Oxide Reductases: Reaction Mechanism and Electronic Structure

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ABSTRACT: Flavodiiron nitric oxide reductases (FNORs) protect microbes from nitrosative stress under anaerobic conditions by mediating the reduction of nitric oxide (NO) to nitrous oxide (N₂O). The proposed mechanism for the catalytic reduction of NO by FNORs involves a dinitrosyldiiron intermediate with a $[hs-{FeNO}^7]_2$ formulation, which produces N₂O and a diferric species. Moreover, both NO and hydrogen sulfide (H₂S) have been implicated in several similar physiological functions in biology and are also known to cross paths in cell signaling. Here we report the synthesis, spectroscopic and theoretical characterization, and N₂O production activity of an unprecedented monohydrosulfidodinitrosyldiiron compound, with a [(HS)hs-{FeNO}⁷/hs-{FeNO}⁷] formulation, that models the key dinitrosyl intermediate of FNORs. The generation of N₂O from this unique compound follows a semireduced pathway, where one-electron reduction generates a reactive hs-{FeNO}⁸ center via the occupation of an Fe–NO antibonding orbital. In contrast to the well-known reactivity of H₂S and NO, the coordinated hydrosulfide remains unreactive toward NO and acts only as a spectator ligand during the NO reduction process.

■ INTRODUCTION

Nitric oxide (NO) plays important roles in cardiovascular maintenance,¹ nerve signal transduction,²⁻⁴ and the immune defense mechanism of mammals against invading pathogens.³⁻⁵ While inducible NO synthase can produce up to micromolar concentrations of NO as a response to bacterial infection,⁶ few microbes such as Thermotoga maritima, Moorella thermoacetica, and Desulfovibrio gigas express the enzyme flavodiiron nitric oxide reductase (FNOR), which reduces the toxic molecule NO to less toxic nitrous oxide (N_2O) .⁷⁻¹⁰ The FNOR activity thus protects the microbes from nitrosative stress under anaerobic conditions.¹¹ The active site of FNORs comprises two nonheme Fe centers, each one usually coordinated by two histidines and a glutamate.^{12,13} The coordination spheres are completed by two bridging ligands: a hydroxide and an aspartate. While four different mechanisms have been proposed for the reduction of NO to N_2O by FNORs,^{7-9,12-14} a recent study has revealed that the reaction proceeds through the successive formation of monoand dinitrosyldiiron species, of which the latter produces

N₂O.¹⁵ Quite similar to NO, hydrogen sulfide (H₂S) has also been recognized as a possible endogenous gasotransmitter¹⁶ and has been implicated in several physiological processes¹⁷ such as cardiovascular effects,¹⁸ vasodilation,^{19,20} and neuromodulation.^{21,22} H₂S (pK_a = 6.9) remains as an equilibrium mixture of hydrosulfide (HS⁻) and H₂S with a ratio of 3:1 at a biological pH of 7.4. Therefore, any or both of these two species may act as ligands for the metal ions available in biological systems. NO and H₂S have also been implicated to cross paths in cell signaling, via the formation of HNO, (H)SNO, (H)SSNO, and polysulfides.^{23,24}

Special Issue: Renaissance in NO Chemistry

Received: February 10, 2021



Functional model complexes of FNORs include cisdinitrosyldiiron complexes that can produce N_2O in quantitative yield either directly (by N.L.)²⁵ or upon reduction at room temperature (by N.L.)²⁶ via a semireduced mechanism (by N.L. and F.M.)²⁷ or upon photolysis at low temperature.²⁴ However, a trans-dinitrosyldiiron complex was found (by F.M.) to be ineffective toward the production of N_2O^2 Recently, we have reported the only example of a model mononitrosyldiiron compound³⁰ and its dinitrosyl analogue,³¹ both of which could produce N₂O upon reduction by following the superreduced and semireduced pathways, respectively.³ However, the effect of coligands on the N2O yield and NO reduction activity of model complexes has not been explored yet. Furthermore, despite the intriguing reactions of $H_2S/HS^$ with NO in biological systems, studies on the reactivity of H₂S/HS⁻ toward NO in nonheme diiron complexes are unavailable in the literature. Elucidating the mechanism of NO reduction by FNORs as well as understanding the interaction of NO with H₂S/HS⁻ in functional nonheme diiron complexes is therefore of significant interest.

The reactivity of H₂S/HS⁻ toward NO in nonheme iron complexes has been explored so far only in the context of sodium nitroprusside, 3^{2-35} dinitrosyl- and mononitrosyliron complexes, 3^{6-40} and iron–sulfur clusters, 4^{1-46} which, however, are distinctly different classes of compounds compared with nonheme diiron complexes. Considering the importance of the reactivity of NO with H2S/HS in biology, we planned to investigate the reactivity of NO toward nonheme diiron(II) hydrosulfide complexes in the context of FNOR activity. Recently, one of us (A.M.) reported a series of nonheme diiron(II) hydrosulfide complexes^{47,48} that provide an excellent opportunity for investigating the reactivity of HS⁻ and NO in this class of diiron complexes. Here we report the synthesis, extensive characterization, and NO reduction activity of an unprecedented monohydrosulfidodinitrosyldiiron compound, $[Fe_2(N-Et-HPTB)(SH)(NO)_2(DMF)](BF_4)_2 [1(BF_4)_2, where$ N-Et-HPTB⁴⁹ is the anion of N,N,N',N'-tetrakis[2-(l-ethylbenzimidazolyI)]-2-hydroxy-1,3-diaminopropane], which features a $[(HS)hs-{FeNO}^7/hs-{FeNO}^7]$ formulation. To the best of our knowledge, there is no analogue with any dinucleating ligand system in the literature for the unique structural features of complex 1.

EXPERIMENTAL SECTION

Preparation of Compounds. All reactions and manipulations were performed under a pure argon atmosphere using either standard Schlenk techniques or an inert atmosphere box. Solvents were dried following standard procedures.^{50,51} Fe(BF₄)₂·6H₂O, Fe(ClO₄)₂. xH_2O , Fe(OTf)₂, Cp₂Fe, Cp₂Co, Et₃N, and NaS^tBu were obtained from commercial sources and used without further purification. HN-Et-HPTB^{49,52} and tritylnitrosothiol (Ph₃CSNO)⁴³ were prepared following the procedures reported in the literature. In the preparations that follow, all of the filtrations were performed through Celite, and solvent removal steps were carried out in vacuo inside an inert (argon gas) atmosphere box. Yields are for recrystallized compounds and are the average of individual yields obtained from multiple batches of reactions, calculated using the corresponding molecular weights of the compound shown in Table S1.

 $[Fe_2(N-Et-HPTB)(SH)(NO)_2(DMF)](BF_4)_2$ [1(BF_4)_2]. To a solution of $[Fe_2(N-Et-HPTB)(SH)(H_2O)](BF_4)_2$ ·DMF [2(BF_4)_2·DMF; DMF = *N*,*N*-dimethylformamide] (0.04 mmol, 53.28 mg) in 1 mL of DMF was added Ph₃CSNO (0.32 mmol, 96.0 mg) in 1 mL of DMF, and the resulting solution was stirred for 4 h to obtain a dark-green solution. The solution was filtered, and diethyl ether (Et₂O) was allowed to diffuse into the filtrate overnight at -35 °C, with an additional 1 day

of standing at room temperature, to afford greenish-brown blockshaped crystals. The crystals were washed several times with tetrahydrofuran (THF) and Et₂O followed by drying under vacuum to yield 38.0 mg (81%) of 1(BF₄)₂. The identity of the compound obtained was confirmed by a single-crystal X-ray structure determination. Anal. Calcd for C₄₆H₅₇B₂F₈Fe₂N₁₃O₄S₁ [1(BF₄)₂]: C, 47.09; H, 4.90; N, 15.52. Found: C, 46.85; H, 5.15; N, 15.39. ESI-MS in acetonitrile (MeCN; saturated with NO). Found (calcd) for [Fe₂(*N*-Et-HPTB)(SH)(NO)₂]³⁺: *m*/*z* 464.1357 (464.1347). Absorption spectrum [DMF; λ_{max} nm (ε_{M} , M⁻¹ cm⁻¹)]: 342 (3450 ± 40), 525(245 ± 35), 600 (210 ± 5). IR: ν_{SH} = 2516 cm⁻¹ (KBr pellet), ν_{NO} = 1785 cm⁻¹ (KBr pellet), 1796 cm⁻¹ (in MeCN). Mössbauer: δ = 0.65 mm s⁻¹ (ΔE_Q = 1.39 mm s⁻¹) at 80 K. XPS: S 2p at 162.0 eV. EPR: EPR-silent at 4 K.

Alternative Synthesis of $1(BF_4)_2$ Using NO Gas. To a solution of $2(BF_4)_2$ ·DMF (0.04 mmol, 53.28 mg) in 2 mL of DMF was added NO gas (purged) for 2 min. The solution was filtered, and Et₂O was allowed to diffuse into the filtrate overnight at -35 °C with an additional 1 day of standing at room temperature to afford greenishbrown block-shaped crystals. The crystals were washed several times with THF and Et₂O followed by drying under vacuum to yield 40.2 mg (85%) of $1(BF_4)_2$. The identity of the compound obtained was confirmed by single-crystal X-ray structure determination and IR spectroscopy.

 $[Fe_2(N-\tilde{Et}-HPTB)(SH)(NO)_2(DMF)](CIO_4)_2$ [1(CIO_4)_2]. To a mixture of HN-Et-HPTB (0.08 mmol, 57.8 mg), Et₃N (0.12 mmol, 12.1 mg), and NaStBu (0.12 mmol, 15 mg) in 2 mL of DMF was added $Fe(ClO_4)_2 \cdot 6H_2O$ (0.16 mmol, 58.0 mg) with stirring, and the resultant slurry was stirred for 6 h. The reaction mixture was filtered. Et₂O was added to the filtrate to afford [Fe₂(N-Et-HPTB)(SH)- $(H_2O)](ClO_4)_2$ ·DMF [2(ClO₄)₂·DMF] as a pale-yellow solid, which was used directly in the next step. To a solution of $2(ClO_4)_2$ ·DMF (0.04 mmol, 54.36 mg) in 1 mL of DMF was added Ph₃CSNO (0.32 mmol, 96.0 mg) in 1 mL of DMF, and the resulting solution was stirred for 4 h to obtain a dark-green solution. The solution was filtered, and Et2O was allowed to diffuse into the filtrate overnight at -35 °C with an additional 1 day of standing at room temperature to afford greenish-brown block-shaped crystals. The crystals were washed several times with THF and Et₂O followed by drying under vacuum to yield 37.0 mg (77%) of $1(ClO_4)_2$. The identity of 1(ClO₄)₂ was confirmed by single-crystal X-ray structure determination. Anal. Calcd for $C_{46}H_{57}Cl_2Fe_2N_{13}O_{12}S_1\cdot DMF\cdot H_2O$ [1(ClO₄)₂. DMF·H₂O]: C, 45.63; H, 5.16; N, 15.20. Found: C, 45.38; H, 5.59; N, 14.88. ESI-MS in MeCN. Found (calcd) for [Fe2(N-Et- $(HPTB)(SH)(NO)_2]^{2+}$: m/z 464.1347 (464.1347). IR (KBr pellet): $\nu_{\rm SH} = 2509 \text{ cm}^{-1}, \ \nu_{\rm NO} = 1782 \text{ cm}^{-1}.$

[Fe₂(N-Et-HPTB)(SH)(NO)₂(DMF)](OTf)₂ [1(OTf)₂]. To a mixture of HN-Et-HPTB (0.08 mmol, 57.8 mg), Et₃N (0.12 mmol, 12.1 mg), and NaS'Bu (0.12 mmol, 15 mg) in 2 mL of DMF was added Fe(OTf)₂ (0.16 mmol, 56.6 mg) with stirring, and the resultant slurry was stirred for 6 h. The reaction mixture was filtered. Et₂O was added to the filtrate to afford [Fe2(N-Et-HPTB)(SH)(H2O)](OTf)2·DMF $[2(OTf)_2 \cdot DMF]$ as a pale-yellow solid, which was directly used in the next step. To a solution of $2(OTf)_2$ ·DMF (0.04 mmol, 58.50 mg) in 1 mL of DMF was added Ph₃CSNO (0.32 mmol, 96.0 mg) in 1 mL of DMF, and the resulting solution was stirred for 4 h to obtain a darkgreen solution. The solution was filtered, and Et₂O was allowed to diffuse into the filtrate overnight at -35 °C with an additional 1 day of standing at room temperature to afford greenish-brown blockshaped crystals. The crystals were washed several times with THF and Et₂O followed by drying under vacuum to yield 40.0 mg (74%) of 1(OTf)₂. The identity of 1(OTf)₂ was confirmed by single-crystal Xray structure determination. Anal. Calcd for C48H57F6Fe2N13O10S3. 3DMF·Et₂O [1(OTf)₂·3DMF·Et₂O]: C, 46.04; H, 5.57; N, 14.08. Found: C, 45.76; H, 5.44; N, 14.37. ESI-MS in MeCN. Found (calcd) for $[Fe_2(N-Et-HPTB)(SH)(NO)_2]^{2+}$: m/z 464.1347 (464.1347). IR (KBr pellet): $\nu_{SH} = 2532 \text{ cm}^{-1}$, $\nu_{NO} = 1782 \text{ cm}^{-1}$. Reaction of $1(BF_4)_2$ with 1 equiv of Cp_2Co . To a solution of

Reaction of $1(BF_4)_2$ with 1 equiv of Cp_2Co . To a solution of $1(BF_4)_2$ (0.085 mmol, 100 mg) in 5 mL of CH_2Cl_2 was added a solution of Cp_2Co (0.085 mmol, 16.1 mg) in 2 mL of CH_2Cl_2 . The

color of the solution immediately changed from greenish to dark brown. The resulting solution was stirred for 1 h and evaporated to dryness. The residue was washed twice with MeOH, dissolved in 2 mL of DMF, and filtered. Et_2O was allowed to diffuse into the filtrate overnight at room temperature to yield a light-brown crystalline solid (61 mg).

Reaction of $1(BF_4)_2$ with 2 equiv of Cp_2CO . To a solution of $1(BF_4)_2$ (0.085 mmol, 100 mg) in 5 mL of CH_2Cl_2 was added a solution of Cp_2Co (0.17 mmol, 32.2 mg) in 2 mL of CH_2Cl_2 . The color of the solution immediately changed from greenish to dark brown. The resulting solution was stirred for 1 h and evaporated to dryness. The residue was washed twice with MeOH, dissolved in 2 mL of DMF, and filtered. Et_2O was allowed to diffuse into the filtrate overnight at room temperature to yield a light-brown crystalline solid (55 mg).

General Physical Methods. Elemental analysis was performed using a PerkinElmer 2400 series II CHNS analyzer. A cyclic voltammetry study of $1(BF_4)_2$ (10⁻³ M) in DMF was performed using a CHI620E electrochemical analyzer (CH Instruments, USA). A three-electrode setup comprised of a glassy carbon working electrode, a platinum wire auxiliary electrode, and a silver wire pseudoreference electrode was employed. Tetra-n-butylammonium hexafluorophosphate (0.1 M) was used as the supporting electrolyte. Electrochemical potentials were referenced internally to the ferrocenium/ferrocene couple at 0.0 V. Electronic absorption spectra were recorded using a Cary 60 UV-vis spectrophotometer. IR spectra of the solid samples as KBr pellets were recorded using a PerkinElmer Spectrum BX Fourier transform infrared (FT-IR) spectrometer. The solution FT-IR data were measured on a PerkinElmer FT-IR spectrometer (Frontier) instrument. The Mössbauer spectrum was recorded using an alternating constant WissEl Mössbauer spectrometer, consisting of an MR 360 Drive Unit, an MV-1000 velocity transducer, and an LND 45431 proportional counter mounted on an LINOS precision bench. The system was operated in a horizontal transmission geometry with the source, absorber, and detector in a linear arrangement. The temperature was controlled and maintained using a Janis SHI closed-cycle helium cryostat. Measurements were performed at 80 K. Data acquisition was performed using a 512channel analyzer. Isomer shifts were referenced versus α -iron metal foil at ambient temperatures. Simulation of the experimental data was performed using the Mfit program. Magnetic susceptibility measurement of $1(BF_4)_2$ was conducted on a Quantum-Design MPMS XL-5 SQUID magnetometer, equipped with a 5 T magnet. The polycrystalline sample was contained in a gel bucket, covered with a drop of low-viscosity perfluoropolyether-based inert oil Fomblin Y45 to fix the crystals, and fixed in a nonmagnetic sample holder. The maximum measuring temperature of 210 K was chosen because of the pour point of the oil, in order to keep the oil in the frozen state and to avoid an orientation of the crystals parallel to the magnetic field. Each raw data file for the measured magnetic moment was corrected for the diamagnetic contribution of the gel bucket and of the inert oil. The raw data were corrected for the diamagnetic contribution of the compound using $\chi_{\rm M}^{\rm dia}({\rm sample}) = -0.5M \times 10^{-6} \,{\rm cm}^3 \cdot {\rm mol}^{-1}$. The data were fit with the *julX* program⁵³ using the appropriate Heisenberg-Dirac-van Vleck (HDvV) spin Hamiltonian for isotropic exchange coupling and Zeeman splitting (eq 1).

$$\hat{H} = -2J\hat{S}_{1}\hat{S}_{2} + g\mu_{\rm B}\vec{B}(\vec{S}_{1} + \vec{S}_{2}) \tag{1}$$

A Curie-behaved paramagnetic impurity (PI) with spin $S = \frac{5}{2}$ was included in the fit, according to χ (calc) = $(1 - \text{PI})\chi + \text{PI}\chi$ (mono).

Electron paramagnetic resonance (EPR) spectra were recorded using a Bruker X-band EMX spectrometer equipped with an Oxford liquid-helium cryostat. Spectra were recorded on ~2 mM frozen solutions using a 20 mW microwave power and a 100 kHz field modulation at a 1 G amplitude. IR spectroelectrochemistry experiments were performed using a LabOmak UF-SEC thin-layer cell, with platinum mesh working and counter electrodes and a silver wire pseudoreference electrode. X-ray photoelectron spectroscopy (XPS) measurements were performed using a focused monochromatized Al $K\alpha$ X-ray source (1486.8 eV) in the XPS instrument (Omicron Nano Technology 0571), after the sample surface was etched by argon-ion sputtering. The peak fitting was done using *Peak Fit*, version 4.12, with the Gaussian deconvolution method.

 N_2O Yield Calculation. Compound $1(\mathrm{BF}_4)_2$ (about 5 $\mu M)$ was dissolved in 2 mL of $\mathrm{CH}_2\mathrm{Cl}_2$ in a septum-sealed 25 mL round-bottomed flask with a 14/20 joint. A total of 0.5, 1, or 2 equiv of CoCp_2 (with respect to the concentration of 2) was dissolved in 0.5 mL of $\mathrm{CH}_2\mathrm{Cl}_2$ and syringed into the flask. The solution was stirred for exactly 5 min prior to evacuation of the headspace of the flask via cannula transfer to an evacuated Pike HT gas-IR cell (190 mTorr) for exactly 20 s. After subtraction of a $\mathrm{CH}_2\mathrm{Cl}_2$ blank taken under the exact same conditions, the IR spectrum was converted to absorbance and the N–N stretch of N_2O was integrated using a straight-line baseline correction from 2150 to 2275 cm $^{-1}$. The integration was then compared to a standard curve. 54

X-ray Structure Determination. The molecular structures of $1(BF_4)_2$, $1(ClO_4)_2$, and $1(OTf)_2$ were determined by single-crystal Xray structure determinations. Diffraction-quality crystals were obtained as described in the synthesis of the respective compound. Single crystals were coated with Parabar oil and mounted under a 100 K nitrogen cold stream. Data collections were performed on a Bruker D8VENTURE Microfocus diffractometer equipped with a PHOTON II detector, with Mo K α radiation ($\lambda = 0.71073$ Å), controlled by the APEX3 (version 2017.3-0) software package. The raw data were integrated and corrected for Lorentz and polarization effects with the aid of the Bruker APEX II program suite.55 Absorption corrections were performed by using SADABS. Space groups were assigned by systematic absences (determined by XPREP) and analysis of the metric symmetry and further checked by PLATON^{56,57} for additional symmetry. Structures were solved by direct methods and refined against all data in the reported 2θ ranges by full-matrix least squares on F^2 using the SHELXL program suite³⁸ in the OLEX2⁵⁹ interface. H atoms at idealized positions were included in the final refinements. The OLEX2 interface was used for structure visualization as well as for drawing ORTEP^{60,61} plots. The crystallographic data and final agreement factors are provided in Table S1. The individual CIFs include the refinement details and explanations (wherever applicable). CCDC 2035675-2035677 contain the supplementary crystallographic data for this paper.

Computational Methods. Optimization and frequency calculations on complex 1 were performed with Gaussian 09^{62} using the B3LYP functional^{63,64} and 6-311G(d) basis set.⁶⁵⁻⁶⁷ Complex 1 contains two antiferromagnetically coupled Fe centers that were treated by generating four fragments: one fragment containing the N-Et-HPTB⁻ ligand scaffold, one containing the hydrosulfide (SH⁻) ligand, and the other two fragments containing one each of the FeNO units. An initial guess calculation was performed first in Gaussian 09 by making one of the Fe-containing fragments antiferromagnetically coupled to the other one. This guess was then utilized to begin singlepoint and geometry-optimization calculations. Once optimized, a frequency calculation was performed, and the final optimized structure was used in a subsequent Orca 4.0.1.268 single-point calculation to analyze the electronic structure and calculate the Mössbauer parameters. This single-point calculation was performed on the Gaussian 09 B3LYP-optimized structure (without fragments) utilizing the B3LYP functional and 6-311G(d) basis set with the def2/ J auxiliary basis set.⁶⁹ The ferromagnetic single point was calculated first, and the spins at one of the FeNO units were then flipped using the Spin Flip operation in Orca.

RESULTS AND DISCUSSION

Synthesis and Characterization. Treatment of the diiron(II) hydrosulfide compound, $2(BF_4)_2$,⁴⁷ with either excess Ph₃CSNO or excess NO gas yielded $1(BF_4)_2$ in 81% and 85% yields, respectively. The molecular structure of complex 1 revealed an unsymmetrical diiron(II) unit (Figure 1) where one Fe center coordinates the hydrosulfide and one NO, while the other Fe center is bound to one NO and one





DMF molecule. The reactivity of coordinated thiolates and NO is quite well-known in the literature, ^{31,70,71} although there are few reports for the coexistence of thiolates and NO in the same complex as well.^{72–75} On the other hand, NO is known to be highly reactive toward H_2S ,^{23,24} and to the best of our knowledge, there are no reports in the literature for the coexistence of NO and hydrosulfide in any nonheme dinuclear complex. It was anticipated that the incoming NO may react with the coordinated hydrosulfide to generate functionalities like HSNO on a diiron(II) platform. However, in contrast to the expectation based on the high reactivity of NO and H_2S , the coordinated hydrosulfide ligand in 2 did not react with the incoming NO gas, and, moreover, NO and hydrosulfide coexist in 1 (Figure 1).

These results prompted us to check the possible reactivity of the coordinated hydrosulfide in 2 and 1 with NO⁺ (Scheme 1). Treatment of 2 with 2.5 equiv of $(NO)(BF_4)$ (reduction potential = 0.87 and 1 V vs Cp₂Fe⁺/Cp₂Fe in MeCN and CH₂Cl₂, respectively) led to formation of the mixed-valent diiron(II,III)-SH complex [Fe₂(N-Et-HPTB)(SH)(H₂O)- $(DMF)_2$ ³⁺ (5) in 77% yield. Synthesis [via treatment of 2] with $(Cp_2Fe)(BF_4)$] and characterization of 5 was previously reported by us.⁴⁷ Moreover, treatment of 1 (diNOSH complex) with 1.5 equiv of $(NO)(BF_4)$ again yielded 5 in 81% yield. Reduction of 5 using 1.5 equiv of Cp₂Co yielded 2 in 68% yield. The formation of 5 in the above-mentioned reactions has been confirmed by single-crystal X-ray structure determination, IR spectroscopy, mass spectrometry, and cyclic voltammetry. These results clearly indicate that the coordinated –SH does not react even with NO⁺. Furthermore, 1 was subjected to a base titration study, which was monitored by IR spectroscopy (Figure S1) in order to check whether deprotonation of the coordinated -SH may trigger the reaction with NO. No such reaction, however, could be observed.

The Fe2–SH distance of 2.456(2) Å in 1 is longer than those reported for 2 [2.344(4) Å]⁴⁷ and another diiron(II) bis(hydrosulfide) compound [2.383(1) and 2.377(1) Å].⁴⁸ The Fe–NO distances of 1.761(5) Å (Fe1–N11) and 1.751(5) Å (Fe2–N13), N–O distances of 1.106(6) Å (N11–O2) and 1.150(6) Å (N13–O3), and Fe–N–O angles of 166.9(5)° (Fe1–N11–O2) and 160.3(5)° (Fe2–N13–O3) in 1 are comparable with those reported for the FNOR model compound, $[Fe_2(N-Et-HPTB)(NO)_2(DMF)_2](BF_4)_3$ [3(BF₄)₃],³¹ and its mononitrosyl analogue, $[Fe_2(N-Et-HPTB)(NO)(DMF)_3](BF_4)_3$ [4(BF₄)₃],³⁰ and other nitro-

Scheme 1. Reaction of 1 and 2 with NO^+



Table 1. Selected	Bond Distances,	Angles and	IR Stretching	Frequencies	for $1(BF_4)_2$,	$1(ClO_4)_2, 1$	(OTf) ₂ , and	l Related
Compounds from	the Literature							

compound	Fe-N _{NO} (Å)	N-0 (Å)	Fe-SH (Å)	Fe–O _{DMF} (Å)	FeFe separation (Å)	∠Fe−N−O (deg)	∠Fe1−O1−Fe2 (deg)	$ u_{ m NO} \ (m cm^{-1}) $ (as KBr pellet)	$ u_{ m SH}~(m cm^{-1}) $ (as KBr pellet)
$1(BF_4)_2$	1.761(5), 1.751(5)	1.106(6), 1.150(6)	2.456(2)	2.146(4)	3.749	166.9(5), 160.3(5)	132.4(2)	1785	2516
$1(ClO_4)_2$	1.759(3), 1.761(4)	1.159(5), 1.152(5)	2.406(1)	2.153(3)	3.625	167.4(4), 157.0(4)	127.9(2)	1782	2509
$1(OTf)_2$	1.758(5), 1.853(6)	1.127(6), 0.973(6)	2.435(2)	2.095(4)	3.679	165.2(5), 157.6(7)	129.9(2)	1782	2532
$2(BF_4)_2)^{47}$			2.344(4)		3.677		132.1(3)		2515
$3(BF_4)_3^{31}$	1.729(7), 1.748(7)	1.123(8), 1.147(8)		2.073(6), 2.153(6)	3.669	174.9(8), 164.9(7)	131.1(2)	1782	
$4(BF_4)_3^{30}$	1.775(5)	1.066(7)		2.159(4), 2.339(5), 2.082(4)	3.605	160.5(7)	129.6(2)	1768	



Figure 2. XPS spectra for $1(BF_4)_2$: (a) full range; (b) S 2p.

sylated diiron compounds (Table 1).^{26,29,76} The metric parameters of $1(ClO_4)_2$ and $1(OTf)_2$ (Figure S2) are similar to those obtained for $1(BF_4)_2$ (Table 1). The presence of a coordinated hydrosulfide in 1 was confirmed by the appearance of ν_{SH} at 2516 cm⁻¹ (Figure S3), which is nearly identical with that reported for 2 $(2515 \text{ cm}^{-1})^{47}$ and for another diiron(II) hydrosulfide compound.⁴⁸ The presence of sulfur in 1 was further confirmed by XPS of $1(BF_4)_2$, which showed the characteristic peak for sulfur (S 2p)^{47,48} at 162.0 eV (Figure 2b). The $\nu_{\rm NO}$ value of 1785 cm⁻¹ (as KBR pellet, Figure S3; 1796 cm⁻¹ in MeCN, Figure S4) for $1(BF_4)_2$ falls within the range of 1720-1840 cm⁻¹ typically observed for high-spin (hs) nonheme {FeNO}⁷ complexes.^{26,27,29-31,76-78} The amide vibration from the metal-coordinated DMF is observed at 1654 cm⁻¹ (as KBR pellet), while the solution IR spectrum shows the vibrations from metal-coordinated DMF and free DMF at 1654 and 1670 cm^{-1} , respectively, which is consistent with literature reports.^{28,30,31} The electronic absorption spectrum of $1(BF_4)_2$ (Figure S5) shows three distinct transitions and is in agreement with the data for other nonheme hs-{FeNO}⁷ complexes.^{9,30,31,76} The broad features observed at 600 nm ($\varepsilon = 210 \pm 5 \text{ M}^{-1} \text{ cm}^{-1}$) and 525 nm ($\varepsilon =$ $245 \pm 35 \text{ M}^{-1} \text{ cm}^{-1}$) are attributed to mixed d-d and $NO^{-}(\pi^{*})$ -to-Fe^{III} charge-transfer transition.^{9,79} Compound $1(BF_4)_2$ exhibits an additional shoulder at 425 nm ($\varepsilon = 518$ \pm 75 \tilde{M}^{-1} cm⁻¹), which sits on the tail of the strong UV band at 342 nm (3450 \pm 40 M⁻¹ cm⁻¹). Mass spectrometry of 1 (in MeCN in the presence of NO; Figure S6) showed the molecular ion peak at m/z 464.1357 for [Fe₂(N-Et-HPTB)- $(SH)(NO)_2$ ²⁺ (calcd m/z 464.1347). Despite the different



Figure 3. 57 Fe Mössbauer spectrum of a polycrystalline sample of $1(BF_4)_2$ at 80 K.

ligation of the two Fe centers, the ⁵⁷Fe Mössbauer spectrum of $1(BF_4)_2$ (Figure 3) features only one quadrupole doublet with an isomer shift, $\delta = 0.65$ mm s⁻¹. Very similar isomer shifts have been reported for 3 (0.64 mm s⁻¹)³¹ and other complexes that feature one or more hs-{FeNO}⁷ units.^{27,29–31,76,77} Similar to 3,³¹ complex 1 was also found to be EPR-silent. A superconducting quantum interference device (SQUID) magnetometry measurement of solid $1(BF_4)_2$ revealed significant antiferromagnetic coupling with J = -30.8 cm⁻¹ between the two hs-{FeNO}⁷ units (Figure 4), which is very similar to that observed previously (-28.3 cm⁻¹) for 3.³¹ This result reflects the Fe^{III}–NO⁻ electronic structure of the two hs-{FeNO}⁷ units.³¹ Together, the molecular structure determi-



Figure 4. SQUID magnetometry measurements of $1(BF_4)_2$. The solid red line represents the best fit using $S_1 = S_2 = \frac{3}{2}$ for 1 and the indicated parameters.

nation, elemental analysis, mass spectrometry, SQUID magnetometry, electronic absorption, IR, Mössbauer, EPR, and XPS measurements thus confirm the formulation of 1 as a $[(HS)hs-\{FeNO\}^7/hs-\{FeNO\}^7]$ species. Complexes 1–3 and the dinucleating ligand HN-ET-HPTB are shown in Scheme 2.

A cyclic voltammetry study of 1 showed three irreversible reduction processes at -1.0, -1.18, and -1.26 V (Figure 5). In comparison, 3 showed two irreversible reduction processes at -1.18 and -1.30 V,³¹ while its mononitrosyl analogue, $4(BF_4)_3$,³⁰ showed only one irreversible reduction at -1.02 V under identical conditions.³⁰ We therefore propose that the three reduction events for 1 arise because of the partial dissociation of one NO to generate some amount of a mononitrosylated species under electrochemical conditions in solution. This result suggests that the NO ligands in 1 are labile. In contrast, complex 3 (without the SH⁻ ligand) does not show this behavior, which indicates that it is likely the NO ligand coordinated to the Fe–SH unit that is labile. The new redox couple generated in the return scan at $E_{pa} = -0.570$ ($E_{pc} = -0.630$) for 1 may be attributed³¹ to the presence of an



Figure 5. Cyclic voltammetric trace (multiple scans) for $1(BF_4)_2$ in DMF.

 $Fe^{II}-O-Fe^{II}/Fe^{II}-OH-Fe^{II}$ species, formed from reduction of the initial reaction product, a mixed-valent $Fe^{II}-O-Fe^{III}$ species, which is generated upon release of N_2O from reduced 1.

Generation of N₂O by Complex 1. Considering the similarity in the structural parameters and spectroscopic properties of 1 with the FNOR functional model complex 3^{31} , the N₂O production activity of 1 was examined. Complex 1 does not produce N₂O in solution in the absence of a reductant (Figure S4), similar to 3 and other previously reported dinitrosyldiiron complexes^{26,28,76} and the dinitrosyl adducts of ribonucleotide reductase and methane monoxygenase.⁹ However, 1 generates N₂O upon chemical (by cobaltocene) and electrochemical reduction at room temperature. The latter was examined by IR spectroelectrochemistry (Figure 6), which confirmed the formation of N₂O from 1 without any detectable intermediates. Rapid production of N₂O upon reduction of 1 by 2 equiv of cobaltocene was also confirmed by IR spectroscopic analysis of the reaction headspace. Integration of the N–N stretching band of N₂O

Scheme 2. Complexes 1-3 and the Dinucleating Ligand HN-ET-HPTB



https://doi.org/10.1021/acs.inorgchem.1c00429 Inorg. Chem. XXXX, XXX, XXX–XXX



Figure 6. Generation of N₂O upon the electrochemical reduction of $1(BF_4)_2$. Experimental conditions: $[1(BF_4)_2] = 11.6$ mM in CH₂Cl₂; hold potential = -1.5 V versus silver wire; 0.1 mM (Et₄N)(BF₄).

against a calibration curve revealed \sim 91% yield of N₂O [based on the concentration of $1(BF_4)_2$] within 5 min of the cobaltocene addition (Figure S10). Furthermore, it was observed that even 1 equiv of cobaltocene was sufficient for the production of N_2O from 1 with 84% yield. Thus, 1 is following a semireduced mechanism for the production of N_2O . A semireduced mechanism was previously reported²⁷ by two of us (N.L. and F.M.) for a dinitrosyldiiron compound, where it was shown that this mechanism constitutes an efficient pathway for the reduction of NO to N₂O by model systems and potentially by FNORs as well. Recently, we have shown that 3 also follows a semireduced pathway for the reduction of NO to N₂O and that a broad $S = \frac{1}{2}$ EPR signal $(\langle g \rangle = 1.55, 1.79, 2.03)$ is generated because of the formation of a mixed-valent diiron(II,III) species upon one-electron reduction of 3.³¹ In line with these results, a mixture of 1 and 1 equiv of cobaltocene indeed generated an $S = \frac{1}{2}$ EPR signal $(\langle g \rangle = 2.05, 2.05, 2.03;$ Figure 7; see also Figure S15 for EPR simulation of the mixed-valent reduction product) and thus further confirmed the semireduced mechanism for the reduction of NO to N₂O by 1. The semireduced mechanism is also supported by the mass spectrometric study of a reaction mixture involving 1 and 1 equiv of cobaltocene, which indicated the formation of a mixed-valent diiron(II,III) species, $[Fe_2(N-Et-HPTB)(\mu-O)]^{2+}$, along with its hydroxo-bridged dimer, $[Fe_4(N-Et-HPTB)_2(\mu-OH)_4]^{4+}$ (Figures S11 and S12). On the other hand, a reaction mixture involving 1 and 2 equiv of cobaltocene indicated the formation of all-ferrous tetrameric species, $[Fe_4(N-Et-HPTB)_2(\mu-OH)_2(H_2O)_2]^{4+}$ and $[Fe_4(N-Et-HPTB)_2(\mu-OH)_2(H_2O)_2]^{4+}$ Et-HPTB)₂(μ -OH)₂]⁴⁺ (Figures S13 and S14). These results further indicate that the coordinated hydrosulfide is likely lost after N₂O formation.

Theoretical Calculation. Because of the asymmetric nature of 1 (Figure 1), density functional theory (DFT) was employed to evaluate the electronic structure of its one-electron-reduced form and the site of reduction. The B3LYP/ 6-311(G)d-optimized structure of 1 shows Fe–NO bond lengths of 1.787 and 1.793 Å at the hs-{FeNO}⁷(SH) and hs-{FeNO}⁷(DMF) sites, respectively, and Fe–N–O bond angles



Figure 7. Left: EPR spectra of $1(BF_4)_2$ and $2(BF_4)_2$ in MeCN demonstrating that both of the compounds are initially EPR-silent. Upon oneelectron reduction of 1 at room temperature in CH_2Cl_2 , a S = 1/2 signal arises. EPR conditions: $[1(BF_4)_2] = \sim 2$ mM, $[2(BF_4)_2] = 2$ mM, 9.336 GHz microwave frequency, 20 mW microwave power, 1 G modulation amplitude, and 10.24 ms time constant. EPR data were recorded at 4 K. Right: Spin count simulation of the EPR spectrum resulting from the reduction of 1 with 1 equiv of $CoCp_2$ (taken in CH_2Cl_2). Simulation parameters: $g_x = g_y = 2.05$, $g_z = 2.03$; $s_{e(x,y,z)} = 0.017$.

of 156.2° and 167.6° (Table S2). These values are all typical for hs-{FeNO}⁷ complexes⁹ and are in good agreement with the structural parameters from the crystal structure of 1 (Tables 1 and S2). The B3LYP calculations for 1 overestimate the frequencies of each N–O stretch, predicting values of 1811 and 1861 cm⁻¹ for the hs-{FeNO}⁷(SH) and hs-{FeNO}⁷(DMF) units, respectively, which is commonly observed when using hybrid functionals like B3LYP.⁸⁰

The one-electron reduction to the hs-{FeNO}⁷(SH)/hs-{FeNO}⁸(DMF) state leads to an elongation of the Fe-NO and N–O bond lengths at the hs-{FeNO}⁸(DMF) center from 1.793 and 1.164 Å to 1.806 and 1.207 Å, respectively (Table S3). The Fe–N–O bond angle becomes more bent at 149.8°, and the N–O stretching frequency significantly decreases to 1671 cm⁻¹. It is important to note that in the gas-phase DFT-optimized structure of the hs-{FeNO}⁷(SH)/hs-{Fe-NO}⁸(DMF) state, the DMF ligand twists such that the DMF does not coordinate to the Fe center but instead provides a moderately strong hydrogen-bonding interaction to NO (H–N distance = 2.90 Å). In solution, the reduced Fe center likely loses the DMF ligand to the bulk solvent. Single-point calculations on the optimized structure show that the additional electron occupies the $d_{yz_{-}}\pi^*_{y}$ orbital (Figure 8),



Figure 8. Contour plot of the Fe–NO antibonding $d_{yz}_{z}\pi^{*}_{y}$ orbital that is occupied upon one-electron reduction of 1 to the hs-{FeNO}⁷(SH)/hs-{FeNO}⁸(DMF) state.

which is antibonding with respect to the Fe–NO bond. In line with this, the spin density at the hs-{FeNO}⁸(DMF) unit increases (Table S4), suggesting a decreased covalency in the Fe–NO bond. This finding is also in agreement with the predicted, dramatic drop in the N–O stretch of the hs-{FeNO}⁸(DMF) center. The occupation of the antibonding $d_{yz}\pi^*_y$ orbital leads to a strong activation of the corresponding hs-{FeNO}⁸ unit, as previously discussed.⁸⁰

Interestingly, the geometry optimizations of the hs- $\{FeNO\}^7(SH)/hs-\{FeNO\}^8(DMF)$ and $hs-\{FeNO\}^8(SH)/hs-\{FeNO\}^7(DMF)$ states ultimately converged to two slightly



Figure 9. Overlay of the two hs-{FeNO}⁷(SH)/hs-{FeNO}⁸(DMF) structures generated from geometry optimizations on the initial hs-{FeNO}⁷(SH)/hs-{FeNO}⁸(DMF) and hs-{FeNO}⁸(SH)/hs-{FeNO}⁷(DMF) states. It is evident from the figure that the main structural difference lies in the Fe–SH distance.

different hs-{FeNO}⁷(SH)/hs-{FeNO}⁸(DMF) structures (overlaid in Figure 9 and structural parameters are compared in Table S3). The structure resulting from the hs-{FeNO}⁷(SH)/hs-{FeNO}⁸(DMF) starting point is 5.3 kcal mol⁻¹ lower in energy than the structure resulting from the hs-{FeNO}⁸(SH)/hs-{FeNO}⁷(DMF) starting point. In both cases, Orca single-point calculations on the optimized structures show that the antibonding $d_{yz}_{x}\pi^*_{y}$ orbital of the hs-{FeNO}⁸(DMF) center is occupied upon one-electron reduction. A PES scan along the hs-{FeNO}⁷(SH)/hs-{FeNO}⁸(DMF) N-N reaction coordinate indicates that N-N coupling has a 10.9 kcal/mol activation barrier (Figure S16), similar to previous calculations indicating a 10 kcal/mol activation barrier for N-N coupling within the hs-{FeNO}⁷/hs-{FeNO}⁸ state of the BPMP⁻ ligand scaffold.⁸⁰

CONCLUSION

In summary, an unprecedented monohydrosulfidodinitrosyldiiron compound $[1(BF_4)_2]$ has been synthesized and extensively characterized. Complex 1 features a [(HS)hs- ${FeNO}^7/hs-{FeNO}^7$ formulation and produces N₂O in high yield following a semireduced mechanism, where oneelectron reduction generates a reactive hs-{FeNO}⁸ center via the occupation of an Fe-NO antibonding orbital. Isolation of 1 confirms that in stark contrast to the known reactivity of H₂S and NO,^{23,24} the Fe-bound hydrosulfide in 1 does not react with NO. Interestingly, the coordinated hydrosulfide in 1 and 2 does not even react with NO⁺ either. In spite of featuring a unique molecular structure, the structural parameters and spectroscopic properties of the FeNO units as well as the N₂O production activity of 1 are quite similar to that observed for the previously reported functional model complex of FNORs, 3.³¹ These results thus strongly indicate that the coordinated hydrosulfide is acting as a spectator ligand during the generation of N₂O by 1.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.inorgchem.1c00429.

Experimental procedure, spectroscopic data and N_2O yield calculation (PDF). (PDF)

Accession Codes

CCDC 2035675–2035677 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by SERB, India (Grant EMR/2017/ 000828 to A.M.) and CSIR India [Grant 01(2972)/19/EMR-II to A.M.] and by the National Science Foundation (Grant CHE-2002885 to NL). N.P. acknowledge CSIR, India, for SRF. F.M. acknowledges support from the Georg-August-University Göttingen.

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