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Syntheses, Structures, and Reactivities of {Fe–NO}^{6} Nitrosyls Derived from Polypyridine-Carboxamide Ligands: Photoactive NO-Donors and Reagents for S-Nitrosylation of Alkyl Thiols

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Two new iron nitrosyls derived from two designed pentadentate ligands *N*,*N*-bis(2-pyridylmethyl)-amine-*N*′-(2-pyridylmethyl)acetamide and *N*,*N*-bis(2-pyridylmethyl)-amine-*N*′-[1-(2-pyridinyl)ethyl]acetamide ($PcPy_3H$ and $MePcPy_3H$, respectively, where H is the dissociable amide proton) have been structurally characterized. These complexes are similar to a previously reported {Fe–NO}^{6} complex, $[(PaPy_3)Fe(NO)](ClO_4)_2$ (**1**) that releases NO under mild conditions. The present nitrosyls, namely $[(PcPy_3)Fe(NO)](ClO_4)_2$ (**2**) and $[(MePcPy_3)Fe(NO)](ClO_4)_2$ (**3**), belong to the same {Fe–NO}^{6} family and exhibit (a) clean ^{1}H NMR spectra in CD_3CN indicating $S = 0$ ground state, (b) almost linear Fe–N–O angles (177.3(5)° and 177.6(4)° for **2** and **3**, respectively), and (c) N–O stretching frequencies (ν_{NO}) in the range 1900–1925 cm^{-1}. The binding of NO at the non-heme iron centers of **1**–**3** is completely reversible and all three nitrosyls rapidly release NO when exposed to light (50 W tungsten bulb). In addition to acting as photoactive NO-donors, these complexes also nitrosylate thiols such as *N*-acetylpenicillamine, 3-mercaptopropionic acid, and *N*-acetyl-cysteine-methyl-ester in yields that range from 30 to 90% in the absence of light. The addition of alkyl or aryl thiolate (RS^-) to the {Fe–NO}^{6} complexes in the absence of dioxygen results in the reduction of the iron metal center to afford the corresponding {Fe–NO}^{7} species.

Introduction

Nitric oxide (NO) has been shown to participate in a variety of physiological functions that include regulation of blood pressure, modulation of neurotransmission, inhibition of platelet aggregation, cell-mediated immune response, and antimicrobial activity.[1−5] NO, a secondary messenger in many biochemical processes, targets metal-heme centers and thiol (−SH) residues in proteins. Since a sudden increase in free NO concentration in cells triggers apoptosis, selective delivery of NO to cancerous cells could result in tumor suppression. Indeed attempts have been made in recent years to deliver NO to hypoxic cancerous cells via the use of organic nitrites (such as GTN), S-nitrosothiols (such as SNAP), or metal complexes (such as SNP, Figure 1).[6,7] The majority of these NO-drugs require heat, light, or metabolic activation to release NO. Among the NO-donors, compounds that release NO upon illumination deserve more attention since they could be utilized in photodynamic therapy against cancer.[6] A close scrutiny of the literature, however, reveals that very few non-heme iron NO complexes have been used to deliver NO. To date, only $Na_2[Fe(CN)_5NO]$ (SNP),[7b] $Na_2[Fe_2S_2(NO)_4]$ (Roussin's Red Salt, RRS),[8] Na[Fe(EDTA)-

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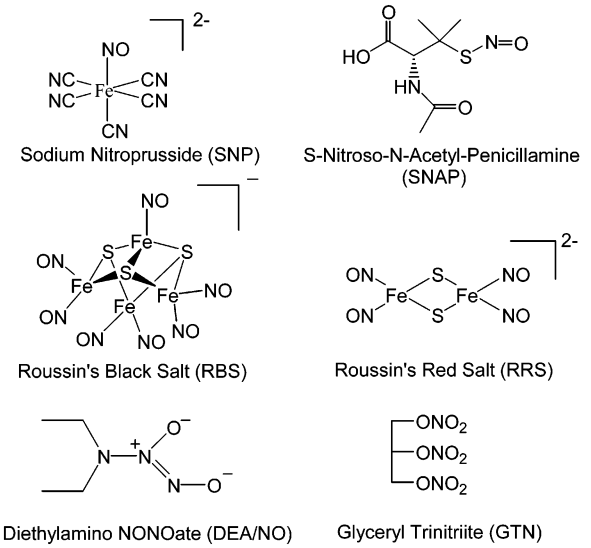
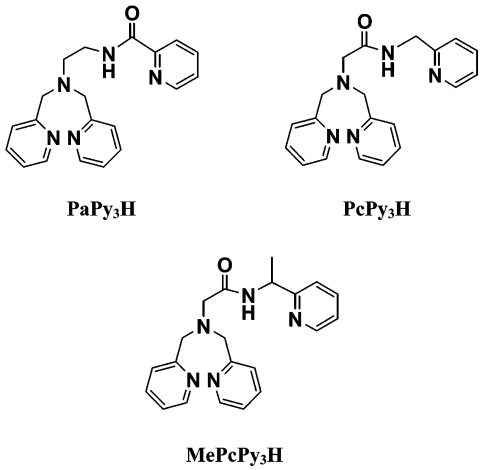


Figure 1. Examples of organic and inorganic NO donors.

(NO)],[9] ($Na_2[Fe_2(SR)_2(NO)_4]$ (Roussin's Red Ester, RRE),[10] and $NH_4[Fe_4S_3(NO)_7]$ (Roussin's Black Salt, RBS)[8b,11] have found some use as NO donor. Use of these inorganic NO donors is often limited due to serious side reactions. For example, release of CN^- from SNP in biological matrix severely limits its use as a NO donor in the treatment of hypertensive emergencies. The photoinduced release of NO from the inorganic NO donors such as SNP and RBS has been employed in photodynamic therapy. Release of NO from these complexes, however, requires high-intensity light in the near UV region (340−440 nm) and the mechanism of NO release is still unknown.[8b]

In our previous work, we have synthesized an Fe(III) nitrosyl complex, $[(PaPy_3)Fe(NO)](ClO_4)_2$ (**1**, where $PaPy_3H$ is *N*,*N*-bis(2-pyridylmethyl)amine-*N*-ethyl-2-pyridine-2-carboxamide) that contains a deprotonated carboxamido nitrogen trans to the bound NO.[12] This {Fe−NO}^{6} nitrosyl (according to the notation of Enemark and Feltham[13]) is photolabile and rapidly releases NO under mild illumination (50 W tungsten lamp). In MeCN, loss of NO from $[(PaPy_3)Fe(NO)](ClO_4)_2$ (**1**) affords the solvent-bound $[(PaPy_3)Fe(MeCN)](ClO_4)_2$ species. The photoreaction is clean (no other secondary byproduct) and quantitative. Since passage of NO through a solution of $[(PaPy_3)Fe(MeCN)](ClO_4)_2$ in MeCN generates **1**, the reaction is also reversible.

To probe the effect(s) of the pyridine-2-carboxamido moiety on the release of NO from **1** upon illumination, we have now synthesized two additional derivatives of the designed ligand $PaPy_3H$, namely *N*-(2-pyridylmethyl)-2-(bis(2-pyridylmethyl)-amine)-ethanamide ($PcPy_3H$) and *N*-(1-(2-pyridylethyl)-2-(bis(2-pyridylmethyl)-amine)-ethanamide ($MePcPy_3H$). In both these ligands, the carboxamide group is *not* conjugated to the pyridine ring although the overall binding characteristics are identical to that of $PaPy_3H$. In this paper we report the syntheses and structures of the two nitrosyls $[(PcPy_3)Fe(NO)](ClO_4)_2$ (**2**) and $[(MePcPy_3)Fe(NO)](ClO_4)_2$ (**3**). The spectroscopic properties of all three nitrosyls **1**−**3** and rates of the NO release from these three nitrosyls are also reported. In biological systems, nitrosothiols derived from NO donors and compounds with SH groups such as glutathione often act as potential NO storage.[7a,14] We were therefore curious to find out whether the metal nitrosyls **1**−**3** could transfer NO to compounds with free thiol groups to generate nitrosothiols. Herein we report that all three nitrosyls transfer NO to alkylthiols such as *N*-acetylpenicillamine, *N*-acetylcysteine, 3-mercaptopropionic acid, and glutathione.



Experimental Section

2-(Aminomethyl)-pyridine, bromoacetate, *p*-chlorobenzene thiol, sodium nitroprusside, and bromoacetylbromide were purchased from Aldrich Chemical Co. and used without further purification. The Fe(III) starting material $[Fe(DMF)_6](ClO_4)_3$,[15] 1-(2-pyridyl)-ethylamine,[16] bis(2-pyridylmethyl)amine (BPA),[17] and $PaPy_3H$[18] were synthesized by following the published procedures. $NH_4[Fe_4S_3(NO)_7]$ and $(Et_4N)[S\text{-}p\text{-}C_6H_4Cl]$ were synthesized by literature methods.[19,20] *S*-Nitroso-mercaptopropanoic acid and *S*-nitroso-*N*-acetyl-cysteine methyl ester were synthesized by the method described by Hart[21] while *S*-nitroso-*N*-acetyl penicillamine was synthesized by the method of Field and co-workers.[22] *S*-nitroso-glutathione was synthesized as previously reported.[23] Acetonitrile (MeCN), ethanol (EtOH), methanol (MeOH), triethylamine (Et_3N), and diethyl ether (Et_2O) were obtained from Fischer Chemical Co. and were distilled from CaH_2, $Mg(OEt)_2$, $Mg(OMe)_2$, sodium, and

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sodium/benzophenone, respectively, prior to use. Nitric oxide gas was purchased from Johnson Mathew Chemical Co. and was purified from higher oxides by passage through a long KOH column before use in reactions.

Synthesis Safety Note. Transition metal perchlorates should be handled with great caution and be prepared in small quantities as metal perchlorates are hazardous and may explode upon heating.

Methyl(bis(2-pyridylmethyl)amino)acetate. This intermediate was synthesized according to a modified literature procedure.[24] A mixture of 8.10 g (58.6 mmol) of potassium carbonate and 2.75 g (15 mmol) of methyl bromoacetate was added to a solution of 3.00 g (15 mmol) of bis(2-pyridylmethyl)amine (BPA) in 20 mL of anhydrous DMF under dinitrogen. The resulting suspension was protected from light and stirred at 35 °C for 72 h. The solvent was then removed under vacuum and the residue was dissolved in 50 mL of chloroform ($CHCl_3$). Following sequential washes with aqueous HCl (pH 5), saturated brine solution, and concentrated NaOH solution, the $CHCl_3$ layer was finally dried with anhydrous $MgSO_4$ and filtered. Removal of the solvent afforded the desired compound as an oil (yield 2.92 g, 72%). ^{1}H NMR (303 K, $CDCl_3$, 500 MHz), δ (ppm from TMS): 3.44 (s, 2H); 3.65 (s, 3H); 3.96 (s, 4H); 7.10 (m, 2H); 7.52 (m, 2H); 7.60 (m, 2H); 8.48 (m, 2H).

***N*,*N*-Bis(2-pyridylmethyl)-amine-*N*′-(2-pyridylmethyl)acetamide ($PcPy_3H$).** A mixture of 1.0 g (3.7 mmol) of methyl(bis(2-pyridylmethyl)amino)acetate, 1.60 g (14.8 mmol) of 2-(aminomethyl)-pyridine, and 0.4 mL of pyridine was refluxed for 4 h. The thick brown solution was then taken up in 100 mL of $CHCl_3$ and washed successively with an aqueous HCl solution (pH 5, 5 × 100 mL), saturated brine solution (5 × 100 mL), and concentrated NaOH solution (5 × 100 mL). Next, the $CHCl_3$ solution was dried with anhydrous $MgSO_4$ and filtered. Removal of the solvent afforded the ligand as brown oil (yield 3.77 g, 98%). ^{1}H NMR (303 K, $CDCl_3$, 500 MHz), δ (ppm from TMS): 3.38 (s, 2H); 3.86 (s, 4H); 4.59 (d, 2H); 7.10 (m, 2H); 7.14 (t, 1H); 7.20 (d, 1H); 7.37 (d, 2H); 7.58 (m, 3H); 8.44 (m, 2H), 8.52 (d, 1H); 9.19 (t, 1H). Selected IR frequency (NaCl plate) $\nu_{CO} = 1660$ cm^{-1}.

***N*,*N*-Bis(2-pyridylmethyl)-amine-*N*′-[1-(2-pyridinyl)ethyl]acetamide ($MePcPy_3H$).** This ligand was synthesized according to a modified literature procedure.[25] A solution of 2.00 g (16.4 mmol) of 1-(2-pyridyl)ethylamine and 1.66 g (16.4 mmol) of Et_3N in 25 mL THF was added dropwise to a solution of 3.30 g (16.4 mmol) of bromoacetylbromide in 20 mL of THF at 0 °C over a period of 20 min. The white precipitate of Et_3NHBr was filtered off and the filtrate was added to a solution of 3.27 g (16.4 mmol) of bis(2-pyridylmethyl)amine and 1.66 g (16.4 mmol) of Et_3N in 20 mL of THF. The solution was then refluxed for 20 h. Next, it was filtered to remove the Et_3NHBr (white precipitate) and the solvent was removed. The resulting red-brown oil was then taken up in 100 mL of $CHCl_3$ and washed successively with an aqueous HCl solution (pH 5, 5 × 100 mL), saturated brine solution (5 × 100 mL), and concentrated NaOH solution (5 × 100 mL). The $CHCl_3$ solution was dried with anhydrous $MgSO_4$ and filtered. Removal of the solvent afforded the ligand as brown oil (yield 3.67 g, 62%). ^{1}H NMR (303 K, $CDCl_3$, 500 MHz), δ (ppm from TMS): 1.50 (d, 3H); 3.32 (q, 2H); 3.88 (s, 4H); 5.16 (p, 1H); 7.14 (m, 3H); 7.25 (d, 1H); 7.38 (d, 1H); 7.47 (d, 2H); 7.60 (m, 3H), 8.51 (d, 2H); 8.57 (d, 1H), 9.16 (d, 1H). Selected IR frequency (NaCl plate) $\nu_{CO} = 1666$ cm^{-1}.

$[(PaPy_3)Fe(MeOH)](ClO_4)_2$. A slurry of 0.50 g (0.63 mmol) of $[Fe(DMF)_6](ClO_4)_3$ in 10 mL of MeOH was added to a solution of 0.22 g (0.63 mmol) of $PaPy_3H$ and 0.018 g (0.76 mmol) of NaH in 10 mL of MeOH. The mixture became homogeneous and turned red within 20 min of stirring. The desired complex was obtained as a dark red microcrystalline precipitate from this solution upon further stirring (3 h). It was filtered and dried under vacuum (yield 0.27 g, 67%). Anal. Calcd for $C_{21}H_{24}N_5O_{10}Cl_2Fe$: C, 39.83, H, 3.82, N, 11.06. Found: C, 39.80, H, 3.97, N, 11.11. Selected IR bands (KBr pellet, cm^{-1}): 3554 (w, ν_{OH}); 3075 (w); 3029 (w); 2947 (w); 1645 (s, ν_{CO}); 1634 (w); 1567 (m); 1488 (w); 1471 (m); 1434 (s); 1385 (m); 1367 (m); 1286 (m); 1089 (s, ν_{ClO4}); 1026 (m); 788 (m); 761 (s); 626 (s, ν_{ClO4}).

$[(PcPy_3)Fe(NO)](ClO_4)_2$·MeCN (2·MeCN). A slurry of 0.67 g (0.84 mmol) of $[Fe(DMF)_6](ClO_4)_3$ in 10 mL of MeOH was added to a solution of 0.30 g (0.84 mmol) of $PcPy_3H$ (0.30 g, 0.84) and 0.023 g (0.96 mmol) of NaH in 10 mL of MeOH. The mixture became homogeneous within 20 min of stirring and the color turned to deep red. Upon further stirring, $[(PcPy_3)Fe(MeOH)](ClO_4)_2$ precipitated out of the solution as a light tan precipitate which was collected on a sintered glass funnel (yield 0.28 g, 50%). [Selected IR bands (KBr pellet, cm^{-1}): 3557 (w, ν_{OH}); 3080 (m); 2960 (w); 1634 (s, ν_{CO}); 1609 (w); 1486 (m); 1445 (s); 1360 (s); 1089 (s, ν_{ClO4}); 1026 (m); 861 (s); 761 (s); 626 (s, ν_{ClO4}).] A batch of 0.23 g (0.37 mmol) of this $[(PcPy_3)Fe(MeOH)](ClO_4)_2$ complex was added to 12 mL of MeCN and the mixture was stirred for 20 h. The solution slowly turned purple (a strong band with λ_{max} at 535 nm) during this time. Next, it was degassed and a stream of purified NO gas was slowly passed through it for 10 min. The solution was then stored at −20 °C for 8 h. The dark red microcrystalline precipitate of complex **2** thus obtained was collected on a sintered glass funnel and dried (yield 0.16 g, 67%). Slow diffusion of Et_2O into a solution of **2** in MeCN in the dark afforded crystals of **2**·MeCN, which were suitable for crystallographic analysis. ^{1}H NMR (303 K, CD_3CN, 500 MHz) δ (ppm from TMS): 4.19 (s, 2H); 4.89 (d, 2H); 5.00 (d, 2H); 5.26 (s, 2H); 6.78 (d, 2H); 7.45 (t, 2H); 7.73 (d, 2H); 7.84 (t, 1H); 7.94 (d, 1H); 8.13 (m, 2H), 8.35 (m, 1H); 8.87 (d, 1H). Selected IR bands (KBr pellet, cm^{-1}) 3085 (w), 2947 (w), 2285 (w, ν_{CN}), 1897 (s, ν_{NO}), 1622 (s, ν_{CO}), 1486 (w), 1466 (w), 1447 (w), 1400 (s), 1297 (w), 1227 (w), 1089 (s, ν_{ClO4}), 907 (m), 819 (w), 769 (s), 624 (s). Electronic absorption in MeCN, λ_{max}, nm (ϵ = M^{-1} cm^{-1}): 363 (2040), 500 (925).

$[(MePcPy_3)Fe(NO)](ClO_4)_2$·1.75MeCN (3·1.75MeCN). A slurry of 0.44 g (0.56 mmol) of $[Fe(DMF)_6](ClO_4)_3$ in 15 mL of EtOH was added to a solution of 0.20 g (0.56 mmol) of $MePcPy_3H$ and 0.015 g (0.62 mmol) of NaH in 15 mL of EtOH, and the mixture was stirred for 1 h. The mixture eventually turned light green and the $[(MePcPy_3)Fe(EtOH)](ClO_4)_2$ complex separated out as a tan precipitate which was collected on a sintered glass funnel (yield 0.23 g, 62%). Selected IR bands (KBr pellet, cm^{-1}): 3560 (w, ν_{OH}); 3067 (w); 2933 (w); 1634 (s, ν_{CO}); 1608 (w); 1481 (w); 1445 (m); 1388 (w); 1290 (w); 1090 (s, ν_{ClO4}); 1026 (m); 861 (m); 770 (m); 627 (s, ν_{ClO4}). A batch of 0.15 g (0.23 mmol) of this $[(MePcPy_3)Fe(EtOH)](ClO_4)_2$ complex was added to 16 mL of MeCN and the mixture was stirred for 20 h. The solution slowly turned purple (a strong band with λ_{max} at 535 nm) during this time. Next, the solution was degassed and NO was passed through it for 5 min. It was then stirred in the dark for 2 h. Finally, a batch of 20 mL of Et_2O was layered onto the solution and the reaction flask was stored at −20 °C. The dark red microcrystals of **3** thus obtained

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were collected on a sintered glass funnel and dried in the absence of light (yield 0.07 g, 47%). Crystals of **3**·1.75MeCN, suitable for X-ray studies, were grown in the absence of light by diffusing Et_2O into a dilute solution of **3** in MeCN. ^{1}H NMR (303 K, CD_3CN, 500 MHz) δ (ppm from TMS): 8.88 (d, 1H); 8.38 (t, 1H); 8.13 (q, 2H); 7.91 (d, 1H); 7.84 (t, 1H); 7.75 (d, 2H); 7.48 (d, 2H); 7.11 (d, 1H); 6.55 (d, 1H); 5.65 (d, 1H); 5.00 (m, 2H); 4.90 (d, 2H); 4.36 (d, 1H); 3.42 (q, 1H); 1.60 (d, 3H). Selected IR bands (KBr pellet, cm^{-1}): 3079 (w); 2962 (w); 2284 (w, ν_{CN}); 1918 (s); 1628 (s, ν_{CO}); 1613 (s); 1489 (w); 1470 (w); 1452 (w); 1386 (m); 1299 (w); 1090 (s, ν_{ClO4}); 908 (w); 806 (w); 771 (m); 624 (s, ν_{ClO4}); 539(w). Electronic absorption in MeCN λ_{max}, nm ($\epsilon = M^{-1}\ cm^{-1}$): 360 (2012), 505 (735).

Transnitrosylation of RSH by [(L)Fe(NO)](ClO$_4$)$_2$. In a typical experiment, a 1:1 mixture of the solid [(L)Fe(NO)](ClO$_4$)$_2$ and RSH (RSH = *N*-acetyl penicillamine, 3-mercaptopropionic acid, glutathione, or *N*-acetyl cysteine methyl ester)was placed in a 20-mL Schlenk flask in the absence of light. Next, 10 mL of MeCN was added and the solution was stirred for 15 min (only in case of GSH, 10 mL of water was used). A 100- μL portion of the reaction mixture was then removed and analyzed on a Spectra-Physics UV 2000 HPLC (Grace VYDAC reverse-phase C18 column, 5-μm particle size, 250 × 4.6 mm, 10-μL loop volume) using an isocratic elution with a mixture of MeCN and H_2O. All wavelength readings were taken at 220 and 340 nm. The retention times were as follows: *S*-nitroso-*N*-acetyl penicillamine (SNAP), 4.36 min at 65:35 MeCN/H_2O; *S*-nitroso-mercaptopropionic acid (SNMP), 9.30 min at 20:80 MeCN/H_2O; *S*-nitroso-*N*-acetyl cysteine methyl ester (SNACE), 8.00 min at 80:20 MeCN/H_2O; and *S*-nitroso-glutathione (GSNO), 8.38 min at 6:94 MeOH/H_2O. The areas under the peaks were determined by using authentic samples of known concentration run under identical conditions. The eluting samples from each run were collected from the HPLC and their identities were confirmed by electrospray ionization mass spectroscopy.

Physical Measurements. Absorption spectra were recorded on a Varian Cary 50 spectrophotometer. A Perkin-Elmer 1600 FTIR spectrophotometer was employed to monitor the infrared spectra. ^{1}H NMR spectra were recorded at 298 K on a Varian 500 MHz instrument. The HPLC analysis was performed on a Spectra-Physics UV 2000 high-pressure liquid chromatograph system with an UV detector. Electrospray mass spectra were recorded on an ESI-ZMD (Micromass 4000) mass spectrometer. Positive ion spectra were recorded by spraying sample mixtures from metal-coated borosilicate capillaries using a Harvard syringe pump at 10 μL/min.

Photolysis Experiments. Kinetic measurements on the photo-induced NO release from **1**−**3** were carried out with a Cary 50 Varian spectrophotometer equipped with a fiber optics probe to detect the absorbance values at a fixed wavelength. The lens fitted on the top of the fiber was dipped into the solution of the [(L)Fe(NO)](ClO$_4$)$_2$ complex (prepared in dark) in MeCN. Tungsten lamps of different light intensity (25, 60, and 100 W) were used. The lamps were fixed at a distance of 5 cm from the sample solution. Continuous scanning of the absorbance values at 535 nm was performed in case of **2** and **3** since both nitrosyls exhibit maximum spectral changes at this wavelength. The pseudo first-order conditions were maintained by the use of dilute solutions ($\sim 0.3 \times 10^{-3}$ M, solvent in excess). Data collection began as soon as the light source was turned on. The observed NO-off rate constant (K_{NO}) values were calculated by fitting the kinetic traces to the equation $A(t) = A_\infty + (A_0 - A_\infty)\{\exp(-K_{NO}t)\}$, where A_0 and A_∞ are the initial and final absorbances, respectively, at the fixed wavelength.

X-ray Data Collection and Structure Solution and Refinement. Violet plates of **2**·MeCN were obtained from a saturated solution of the complex in MeCN via slow diffusion of Et_2O at 0 °C. Red plates of **3**·1.75MeCN were grown from a dilute MeCN solution of the complex via diffusion of Et_2O at 0 °C. Diffraction data for both complexes were collected at 91 K on a Bruker SMART 1000 system. Mo Kα (0.710 73 Å) radiation was used, and the data were corrected for absorption. The structures were solved by direct methods (standard SHELXS-97 package). Machine parameters, crystal data, and data collection parameters for all the complexes are summarized in Table 1 , while selected bond distances and angles are listed in Table 2. Complete crystallographic data for [(PcPy$_3$)Fe(NO)](ClO$_4$)$_2$·MeCN (**2**·MeCN) and [(MePcPy$_3$)Fe(NO)](ClO$_4$)$_2$·1.75MeCN (**3**·1.75MeCN) are available in the Supporting Information.

Table 1. Summary of Crystal Data and Intensity Collection and Structural Refinement Parameters for [(PcPy$_3$)Fe(NO)](ClO$_4$)$_2$·MeCN (**2**·MeCN) and [(MePcPy$_3$)Fe(NO)](ClO$_4$)$_2$·1.75MeCN (**3**·1.75MeCN)

formula	$C_{22}H_{23}Cl_2FeN_7O_{10}$	$C_{24.50}H_{27.25}Cl_2FeN_{7.75}O_{10}$
mol wt	672.22	717.04
crystal color, habit	violet plate	red plate
T, K	93 (2)	93 (2)
crystal system	orthorhombic	monoclinic
space group	*Pbca*	*I*2/*a*
a, Å	7.6984 (13)	14.665 (5)
b, Å	22.015 (4)	18.429 (6)
c, Å	30.754 (5)	21.460 (7)
α, deg	90	90
β, deg	90	93.221 (7)
γ, deg	90	90
V, Å^3	5212.2 (15)	5791 (3)
Z	8	8
d_{calcd}, g cm^{-3}	1.713	1.645
abs coeff, μ, mm^{-1}	0.856	0.777
GOF[a] on F^2	0.796	1.118
R1,[b] %	5.18	6.83
wR2,[c] %	10.31	19.17

[a] GOF = $[\sum w(F_o^2 - F_c^2)^2]/(M - N)]^{1/2}$ (M = number of reflections, N = number of parameters refined). [b] R1 = $\sum||F_o| - |F_c||/\sum|F_o|$. [c] wR2 = $[\sum w(F_o^2 - F_c^2)^2]/[\sum w(F_o)^2]]^{1/2}$.

Table 2. Selected Bond Distances [Å] and Bond Angles [deg]

	complex **2**	complex **3**
Fe−N1	1.968(6)	1.961(4)
Fe−N2	1.868(5)	1.888(4)
Fe−N3	1.987(5)	1.985(4)
Fe−N4	1.984(5)	1.983(4)
Fe−N5	1.971(6)	1.988(4)
Fe−N6	1.680(6)	1.678(4)
N6−O2	1.147(7)	1.147(5)
C7−O1	1.247(8)	1.234(6)
C7−N2	1.329(8)	1.337(6)
N1−Fe−N2	81.7(2)	82.06(17)
N1−Fe−N3	166.2(2)	166.82(17)
N1−Fe−N4	94.6(2)	97.42(17)
N1−Fe−N5	97.1(2)	94.55(16)
N1−Fe−N6	98.1(3)	97.30(19)
N2−Fe−N3	84.4(2)	84.83(17)
N2−Fe−N4	87.6(2)	88.51(17)
N2−Fe−N5	87.8(2)	87.40(17)
N2−Fe−N6	178.4(3)	178.49(19)
N3−Fe−N4	84.3(2)	83.61(16)
N3−Fe−N5	82.8(2)	83.41(16)
N3−Fe−N6	95.8(2)	95.77(18)
N4−Fe−N5	166.7(2)	166.69(17)
N4−Fe−N6	93.9(2)	92.94(18)
N5−Fe−N6	90.7(2)	91.29(18)
Fe−N6−O2	177.3(5)	177.6(4)

Results and Discussion

Synthesis and Characterization of [(L)Fe(NO)](ClO$_4$)$_2$ Complexes. As described in a previous paper,[12] complex **1**

can be conveniently synthesized by passing NO gas through a methanolic solution of $[(PaPy_3)Fe(MeOH)](ClO_4)_2$ (formed in situ by mixing $PaPy_3H$, $[Fe(DMF)_6](ClO_4)_3$, and NEt_3) at 45 °C. This method, however, did not work in the case of $PcPy_3H$ and $MePcPy_3H$ ligands presumably due to slow "loading" of the nonconjugated carboxamide moieties. It appears that the complexation of the carboxamido nitrogen to the Fe(III) center is facilitated by the conjugation present in the pyridine-2-carboxamide portion of $PaPy_3H$. Since the carboxamide group in both $PcPy_3H$ and $MePcPy_3H$ ligands is not conjugated with the pyridine ring, coordination of these ligands to the Fe(III) center is somewhat more intricate. Tuftlund and co-workers have reported a similar N_5 ligand, namely *N*-(2-pyridylmethyl)-3-bis(2-pyridylmethyl)amine)-propanamide, that also contains a nonconjugated carboxamide moiety.[17] Interestingly, Fe(II) or Fe(III) complexes of this ligand have not been structurally characterized. The researchers concluded that "the coordination of the carboxamide moiety is unfavorable" with this ligand in case of iron; only the two pyridine nitrogens and the tertiary amine coordinate to the metal center.[17]

To ensure that the $PcPy_3^-$ and $MePcPy_3^-$ ligands bind Fe(III) in pentadentate fashion in the present work, the methanol and ethanol adducts, $[(PcPy_3)Fe(MeOH)](ClO_4)_2$ and $[(MePcPy_3)Fe(EtOH)](ClO_4)_2$, were first synthesized and their spectral parameters were compared to the $[(PaPy_3)Fe(MeOH)](ClO_4)_2$ precursor. The two alcohol adducts exhibit their carbonyl stretching frequency (ν_{CO}) at 1634 cm^{-1}. Since this value is close to the ν_{CO} value of $[(PaPy_3)Fe(MeOH)](ClO_4)_2$ (1645 cm^{-1}), we were confident of the fact that both $PcPy_3^-$ and $MePcPy_3^-$ are properly coordinated to the Fe(III) centers of the two precursors $[(PcPy_3)Fe(MeOH)](ClO_4)_2$ and $[(MePcPy_3)Fe(EtOH)](ClO_4)_2$. These precursors have been valuable in the syntheses of the target nitrosyls **2** and **3**.

The limited solubility of $[(PcPy_3)Fe(MeOH)](ClO_4)_2$ and $[(MePcPy_3)Fe(EtOH)](ClO_4)_2$ in alcoholic media requires their conversion into the corresponding MeCN adducts before the reaction with NO. Interestingly, the rate of solvent (the sixth ligand) exchange is much faster with $[(PaPy_3)Fe(MeOH)](ClO_4)_2$ than that with either $[(PcPy_3)Fe(MeOH)](ClO_4)_2$ or $[(MePcPy_3)Fe(EtOH)](ClO_4)_2$ (5 min compared to 20 h). However, once the MeCN adducts are formed (as indicated by the appearance of the strong absorption band at 535 nm), NO reacts rapidly with both $[(PcPy_3)Fe(MeCN)](ClO_4)_2$ and $[(MePcPy_3)Fe(MeCN)](ClO_4)_2$ to afford the desired NO complexes $[(PcPy_3)Fe(NO)](ClO_4)_2$ (**2**) and $[(MePcPy_3)Fe(NO)](ClO_4)_2$ (**3**) in good yields (50−60%).

Structures of the Complexes. $[(PcPy_3)Fe(NO)](ClO_4)_2\cdot$MeCN (2·MeCN). The structure of $[(PcPy_3)Fe(NO)]^{2+}$ (the cation of **2**) is shown in Figure 2, and the selected bond distances and angles are listed in Table 2. The mode of coordination of the pentadentate monoanionic ligand $PcPy_3^-$ to the Fe(III) center is very similar to that noted in $[(PaPy_3)Fe(NO)]^{2+}$ (cation of **1**) and related complexes;[12,18,26] the three pyridine nitrogens and the tertiary amine nitrogen reside in the equatorial plane while the carboxamido nitrogen occupies an axial position that is trans to the bound NO.

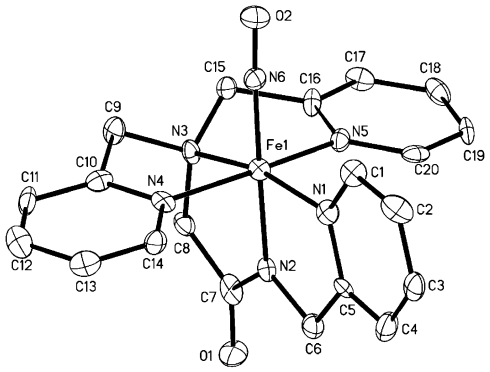

Figure 2. Thermal ellipsoid (probability level 50%) plot of $[(PcPy_3)Fe(NO)]^{2+}$ (cation **2**) with the atom-labeling scheme. H atoms are omitted for the sake of clarity.

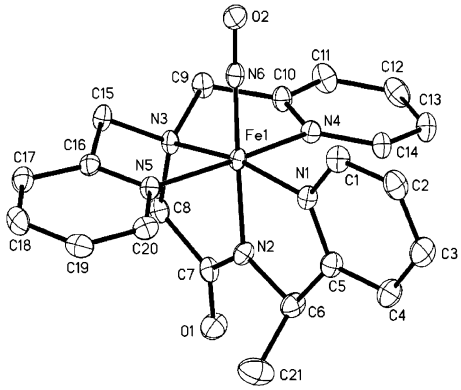

Figure 3. Thermal ellipsoid (probability level 50%) plot of $[(MePcPy_3)Fe(NO)]^{2+}$ (cation **3**) with the atom-labeling scheme. H atoms are omitted for the sake of clarity.

The Fe−N(O) bond distance (1.680(6) Å) of **2** lies within the range of other structurally characterized $\{Fe-NO\}^6$ complexes (1.6−1.7 Å) including **1** (Fe−N(O) distance = 1.677(2) Å). This short Fe−N(O) distance and the almost linear Fe−N−O angle (177.3(5)°) are typical of $\{Fe-NO\}^6$ nitrosyls. Although the average $Fe-N_{py}$ (1.974(5) Å) and $Fe-N_{amine}$ (1.987(5) Å) distances of **2** are very similar to those noted for **1** ($Fe-N_{py}$ = 1.981(2) Å and $Fe-N_{amine}$ = 1.972(2) Å), the $Fe-N_{amido}$ distance (1.868(5) Å) of **2** is noticeably shorter than that of **1** ($Fe-N_{amido}$ = 1.9009(19) Å). The shortening of the N_{amido} bond (∼0.033 Å) results from the loss of conjugation of the carboxamido nitrogen with the pyridine ring.

$[(MePcPy_3)Fe(NO)](ClO_4)_2\cdot$1.75MeCN (3·1.75MeCN). The structure of $[(MePcPy_3)Fe(NO)]^{2+}$, the cation of complex **3**, is shown in Figure 3 and the selected bond distances and angles are displayed in Table 2. Structurally, **3** is similar to **2**, with the exception of the elongation of the C7−N2 bond (1.338(6) Å in **3** versus 1.329(8) Å in **2**), and the shortening of the C7−O1 bond (1.234(6) Å in **3** versus 1.247(8) Å in **2**). The extra methyl group at position C6 of **3** thus gives rise to a shorter C═O bond (more double bond character) and a longer C−N bond (more single bond character) when compared to **2**. The Fe−N(O) (1.678(4) Å) bond distance is

(26) (a) Patra, A. K.; Afshar, R. K.; Rowland, J. M.; Olmstead, M. M.; Mascharak, P. K. *Angew. Chem., Int. Ed.* **2003**, *42*, 4517−4521. (b) Patra, A. K.; Rowland, J. M.; Marlin, D. S.; Bill, E.; Olmstead, M. M.; Mascharak, P. K. *Inorg. Chem.* **2003**, *42*, 6812−6823.

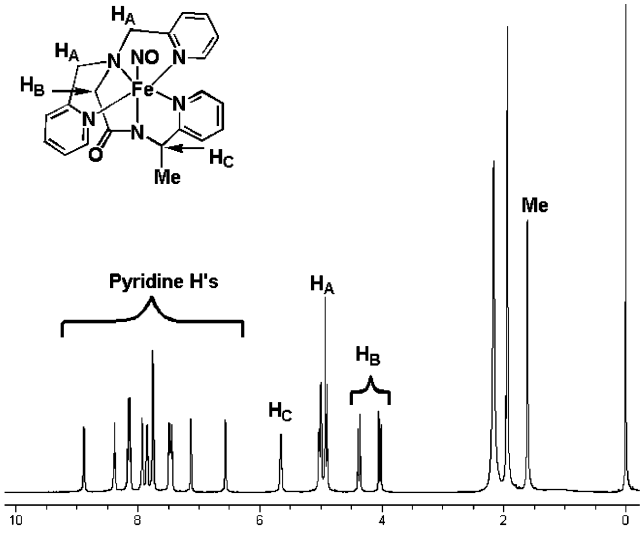

Figure 4. ^{1}H NMR (500 MHz) spectrum of $[(MePcPy_3)Fe(NO)](ClO_4)_2$ (**3**) in CD_3CN at 295 K. The two peaks around 2 ppm are due to solvent and water.

similar to **2** (1.6880(6) Å) while the Fe−N_{amido} (1.888(4) Å) bond length lies between that of **1** (1.9009(19) Å) and **2** (1.868(5) Å).

Spectroscopic Properties. The two nitrosyls of the present work display strong NO band (ν_{NO}) at 1897 cm^{-1} (value for **2**) and 1918 cm^{-1} (value for **3**). These ν_{NO} values are within the range (1822−1937 cm^{-1}) expected for {Fe−NO}^{6} type complexes.[12,26,27] Both complexes also exhibit strong carbonyl stretching frequency (ν_{CO}) at 1622 cm^{-1} (value for **2**) and 1628 cm^{-1} (value for **3**) The red shift of ν_{CO} of the corresponding free ligands (1660 cm^{-1} for $PcPy_3H$ and 1666 cm^{-1} for $MePcPy_3H$) confirm coordination of the deprotonated carboxamido nitrogen to the Fe(III) center in these complexes. The ^{1}H NMR spectra of complexes **2** and **3** (prepared and run in the dark) indicate an $S = 0$ ground state (Figure 4). Interestingly, the C8 protons (protons of the CH_2 unit between the tertiary amine and carbonyl group) appear as a singlet in the ^{1}H NMR spectrum of **2** (Figure S1, Supporting Information), while in case of **3**, these protons give rise to doublet of doublets (peak marked with H_B in Figure 4, $J = 170.5$ Hz).

Photolability of the Bound NO. In the solid state, both **2** and **3** are stable and quite resistant to damage by light. Solutions of these nitrosyls in MeCN can be stored in the dark for weeks (as confirmed by their ^{1}H NMR spectra). However, when such solutions are exposed to visible light (50-W tungsten lamp), rapid color changes are observed. For example, when a solution of **3** in MeCN is exposed to light, the color of the solution changes from red to purple. The changes in the absorption spectrum are shown in Figure 5. Much like the behavior of **1** described in our earlier

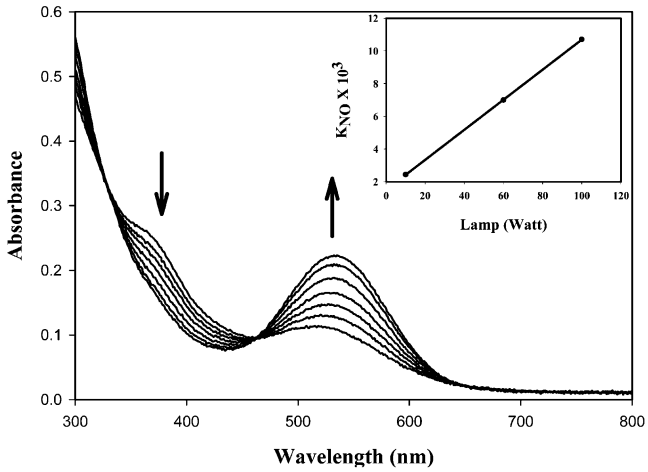

Figure 5. Conversion of $[(MePcPy_3)Fe(NO)](ClO_4)_2$ (**3**), $\lambda_{max} = 505$ nm, to $[(MePcPy_3)Fe(MeCN)](ClO_4)_2$, $\lambda_{max} = 535$ nm, in MeCN under illumination with a 50-W tungsten lamp ($t_{1/2} = 90$ s). Concentration of **3**: 0.15 mM. The scans were recorded at intervals of 30 s.

Table 3. Values of K_{NO} of Complexes **1**−**3** in MeCN with Varying Light Intensities

complex	light intensity (W)	$K_{NO} \times 10^3$ (s^{-1})
$[(PaPy_3)Fe(NO)]^{2+}$ (**1**)	25	1.73 ± 0.01
	60	5.60 ± 0.01
	100	10.20 ± 0.02
$[(PcPy_3)Fe(NO)]^{2+}$ (**2**)	25	2.10 ± 0.03
	60	7.90 ± 0.01
	100	11.60 ± 0.02
$[(MePcPy_3)Fe(NO)]^{2+}$ (**3**)	25	2.43 ± 0.02
	60	7.00 ± 0.02
	100	10.70 ± 0.03

report,[12,26b] $[(MePcPy_3)Fe(NO)]^{2+}$ (cation of **3**) is converted into $[(MePcPy_3)Fe(MeCN)]^{2+}$ upon illumination. The isosbestic points at 323, 463, and 644 nm illustrate the clean conversion of the NO-bound complex into the MeCN-bound species. The MeCN adduct is quite stable and a back reaction does not occur to reform **3** in the dark. However, **3** can be synthesized by bubbling excess NO through the solution of $[(MePcPy_3)Fe(MeCN)]^{2+}$ in MeCN. Thus, binding of NO to the Fe(III) center is reversible. Very similar behavior has been noted with **2**. In MeCN, **2** displays isosbestic points at 320, 462 nm, and 650 nm (Figure S2, Supporting Information) during the $[(PcPy_3)Fe(NO)]^{2+} \rightarrow [(PcPy_3)Fe(MeCN)]^{2+}$ transformation.

A pseudo-first-order behavior has been noted in the photolysis of **2** and **3** in MeCN. The value of the NO off rate constant, K_{NO}, increases linearly with the intensity of light (Figure 5). Complexes **2** and **3** release NO slightly faster than **1** (Table 3). However, the stability of $[(PaPy_3)Fe(NO)](ClO_4)_2$ (**1**) in solvents such as DMF or water is greater than that of either **2** or **3**. The latter two nitrosyls are rapidly converted into the corresponding solvated species even in the absence of light. It is therefore evident that the conjugation in the pyridine-2-carboxamido moiety of $PaPy_3^-$ provides extra stability to **1** toward solvolysis. This is supported by the fact that while $[(PaPy_3)Fe(MeCN)](ClO_4)_2$ has been isolated in crystalline state,[18] the MeCN-bound species derived from **2** or **3** have not been isolated in pure

(27) (a) Grapperhaus, C. A.; Patra, A. K.; Mashuta, M. S. *Inorg. Chem.* **2002**, *41*, 1039−1041. (b) Chatel, S.; Chauvin, A.-S.; Tuchagues, J.-P.; Leduc, P.; Bill, E.; Chottard, J.-C.; Mansuy, D.; Artaud, I. *Inorg. Chim. Acta* **2002**, 336, 19−28. (c) Schweitzer, D.; Ellison, J. J.; Shoner, S. C.; Lovell, S.; Kovacs, J. A. *J. Am. Chem. Soc.* **1998**, *120*, 10996−10997. (d) Scheidt, W. R.; Lee, Y. J.; Hatano, K. *J. Am. Chem. Soc.* **1984**, *106*, 3191−3198. (e) Nappier, T. E.; Feltham, R. D.; Enemark, J. H.; Kruse, A.; Cooke, M. *Inorg. Chem.* **1975**, *14*, 806−812.

and solid form so far. Solutions of $[(PcPy_3)Fe(MeCN)]^{2+}$ and $[(MePcPy_3)Fe(MeCN)]^{2+}$ in MeCN also change color slowly in air. For example, exposure to air causes the purple color of these two MeCN-adducts to turn yellow (in case of $[(PcPy_3)Fe(MeCN)]^{2+}$) and red (in case of $[(MePcPy_3)Fe(MeCN)]^{2+}$) within 24 h. The nature of the species in such solution is unknown at this time.

S-Nitrosylation of Thiols using 1−3. S-Nitrosylation of thiol (Cys) group(s) in proteins activate several biochemical processes such as bronchodilation in human airways, vasorelaxation of blood vessels, and regulation of blood flow.[1,2] The nitrosylated proteins also serve as compartments for NO storage or as a transporter of NO.[28,29] Recently, studies have also shown that nitrosylation of specific thiols by NO can modulate the enzymatic activity of several cellular enzymes.[30−32] These in vitro studies utilized NO donors that consisted of S-nitrosylated compounds such as S-nitrosoglutathione (GSNO) and S-nitroso-*N*-acetyl-penicillamine (SNAP), which transfer NO to available vicinal thiol(s) present in the protein. The addition of the S-nitrosylated thiols (RSNOs) to proteins (P−SH) afford the corresponding nitrosylated proteins (P−SNO) as well as the S-thiolate adduct (P−SSR).[33] To find out whether the new complexes **1**−**3** can transfer NO to compounds containing −SH group, we have examined the reactions of these nitrosyls with four such compounds, namely *N*-acetyl-penicillamine (NAP), *N*-acetyl-cysteine-methyl ester (NACE), 3-mercaptopropionic acid (MP), and glutathione (GSH). The addition of any of these $\{FeNO\}^6$ complexes to these compounds with −SH group in MeCN resulted in rapid formation of the corresponding SNOs, which were identified by mass spectrometry following separation by HPLC (Table 4). This S-nitrosylation reaction is rapid (complete within 5 min) at room temperature *in the absence of light*. To our knowledge, there is no example of an iron nitrosyl that transfers NO to a −SH group *at ambient temperature*. Compounds such as RBS, RRS, RRE, and SNP all require heat or intense light to release NO. For example, in a control reaction, prolonged heating (24 h) of a solution of RBS and GSH in water at 45 °C afforded only 6.0% of GSNO. Likewise, SNP yielded only 3.4% of GSNO under the same condition. In contrast, complex **1** affords 90% GSNO at 25 °C within 5 min (Table 4). Among the three nitrosyls, **3** exhibits the lowest capacity to transfer NO to all the substrates presumably due to its instability in solution. Nevertheless, significant extent of S-nitrosylation is observed with this nitrosyl and the products can be easily identified by HPLC and mass spectrometry

Table 4. Yields of Nitrosothiols Obtained in S-Nitrosylation of Thiols with **1**−**3** in MeCN or Water as Determined by High- Pressure Liquid Chromatography (HPLC)

complex	thiol[a]	% yield (SNO)
$[(PaPy_3)Fe(NO)](ClO_4)_2$ (**1**)	NAP	91.4
	MP	92.8
	NACE	80.7
	GSH[b]	90.0
$[(PcPy_3)Fe(NO)](ClO_4)_2$ (**2**)	NAP	34.0
	MP	65.1
	NACE	83.8
	GSH[b]	81.7
$[(MePcPy_3)Fe(NO)](ClO_4)_2$ (**3**)	NAP	30.2
	MP	62.2
	NACE	83.6
	GSH[b]	73.4

[a] NAP = *N*-acetylpenicillamine, MP = 3-mercaptopropionic acid, NACE = *N*-acetyl-cysteine methyl ester, and GSH = glutathione. [b] Reaction performed in water.

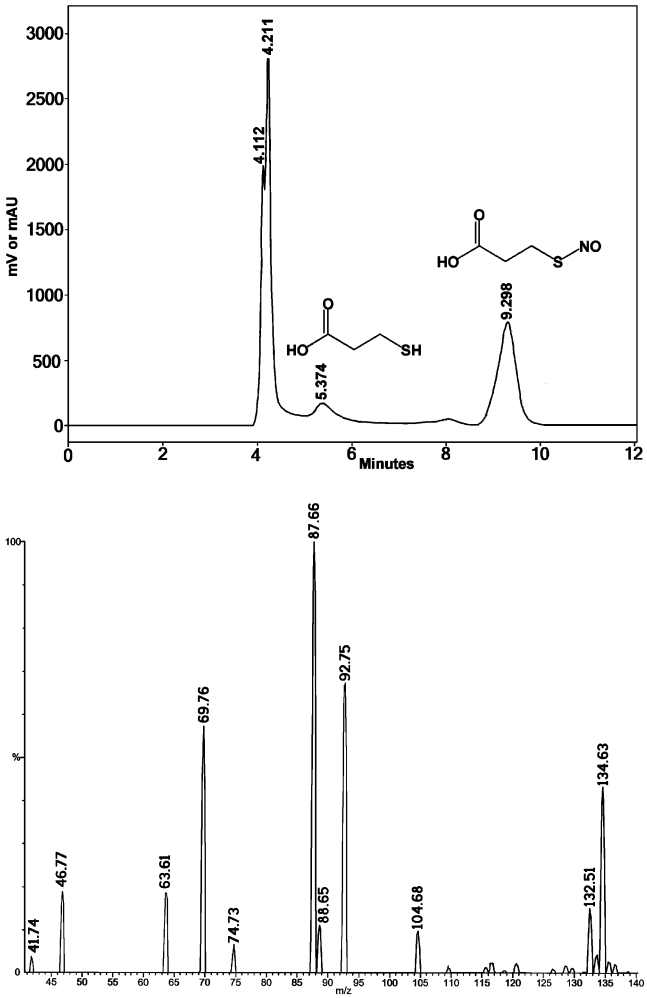

Figure 6. (Top) HPLC trace of a reaction mixture containing **3** and 3-mercaptopropionic acid (1:1) in MeCN in the absence of light at 25 °C. The iron-containing species give rise to the first peak in the chromatogram. (Bottom) MS trace of the nitrosothiol (retention time of 9.298 min) that shows a peak at 134, which corresponds to mass of the parent ion.

(Figure 6). The high NO donor capacity of **1**−**3** at ambient temperature is noteworthy. At this time, we are studying their utility as nitrosylating agents for proteins.

Since all the nitrosyls of the present study (**1**−**3**) show greater stability in MeCN solutions, we have further explored the nitrosylation reactions in MeCN. These nitrosyls ef-

(28) Moncada, S.; Palmer, M. J.; Higgs, E. A. *Pharm. Rev.* **1991**, *43*, 109−142.
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(31) Mannick, J. B.; Schonhoff, C. M. *Arch. Biochem. Biophys.* **2002**, *408*, 1−6.
(32) Foster, M. W.; McMahon, T. J.; Stamler, J. S. *Trends Mol. Med*. **2003**, *9*, 160−168.
(33) (a) Srivastava, S.; Dixit, B. L.; Ramana, K. V.; Chandra, A.; Chandra, D.; Zacarias, A.; Petrash, J. M.; Bhatnagar, A.; Srivastava, S. K. *Biochem. J*. **2001**, *358*, 111−118. (b) Chandra, A.; Srivastava, S.; Petrash, J. M.; Bhatnagar, A.; Srivastava, S. K. *Biochemistry* **1997**, *36*, 15801−15809.

ficiently transfer NO to substrates containing −SH group (Table 4). However, the reaction takes a very different course when the corresponding thiolates are used as the substrates. For example, the reaction of **1** and NACE gives rise to the corresponding nitrosothiol SNACE in ∼80% yield. In contrast, when deprotonated NACE (a thiolate) is used, one does not obtain any nitrosothiol; instead, the {Fe−NO}^{6} complex **1** is quantitatively converted to the corresponding {Fe−NO}^{7} species [($PaPy_3$)Fe(NO)](ClO_4). The same result is obtained with aromatic thiolates. The addition of (Et_4N^+)-(*p*-$ClC_6H_4S^-$) to a solution of **1** in MeCN in the absence of light and oxygen affords the {Fe−NO}^{7} species [($PaPy_3$)-Fe(NO)](ClO_4) within minutes (as evidenced by the appearance of a band at 476 nm in the absorption spectrum).[26b] The thiolate substrate in each case causes reduction of the iron center according to eq 1

$$\{Fe{-}NO\}^6 + (Et_4N^+)(p\text{-}ClC_6H_4S^-) \rightarrow \{Fe{-}NO\}^7 + p\text{-}ClC_6H_4S{-}SC_6H_4Cl\text{-}p \quad (1)$$

and no NO transfer is observed. This result demonstrates that the {Fe−NO}^{7} species are not NO transfer agents. The reduction of Fe(III) complexes by thiols has been studied by various groups.[34] The reaction 1 however is the first example of a thiolate reducing a designed non-heme {Fe−NO}^{6} complex to the corresponding {Fe−NO}^{7} species. The other product of this reaction is the disulfide, which results from the coupling of the thiyl radicals.[35]

The reactions of **1**−**3** with thiols in MeCN deserve further discussion. Since the NO transfer occurs in the absence of light, it is evident that these reactions do not proceed simply via loss of NO from the nitrosyls followed by addition of NO to the RSH molecules. Indeed, reactions of purified NO gas with thiols such as NACE in MeCN afford mostly disulfide even in the absence of dioxygen. This behavior is in sharp contrast to the reaction of **1** with NACE and MP in MeCN when the corresponding RSNOs are obtained in ∼90% yield. Only a trace of disulfide is obtained in these reactions.[36] Since the other product in these reactions is the corresponding [($PaPy_3$)Fe(MeCN)]$^{2+}$ (an Fe(III) species), it is evident that a bimolecular nucleophilic attack by RSH occurs at the coordinated NO moiety.[37] We are currently performing more experiments to elucidate the mechanism of this NO transfer reaction by **1**−**3**.

Conclusion

The three {Fe−NO}^{6} nitrosyls, namely [($PaPy_3$)Fe(NO)]-(ClO_4)$_2$ (**1**), [($PcPy_3$)Fe(NO)](ClO_4)$_2$ (**2**), and [($MePcPy_3$)-Fe(NO)](ClO_4)$_2$ (**3**), are photolabile and rapidly release NO upon illumination with visible light. The rates of NO release from these nitrosyls show minor changes upon alteration of the pyridine/carboxamide portion of the ligand frame although removal of conjugation in this portion of the ligand results in reduced stability of the metal complex. These nitrosyls are efficient NO donors to compounds with −SH group and the S-nitrosylation reaction occurs rapidly at room temperature in the absence of light. When thiolates are used as the substrates, the {Fe−NO}^{6} nitrosyls are reduced to the corresponding {Fe−NO}^{7} species and no nitrosothiols are obtained.

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Supporting Information Available: ^{1}H NMR spectrum of **2** (Figure S1) and changes in the absorption spectrum of **2** in MeCN upon illumination (Figure S2) (PDF); and X-ray crystallographic data (in CIF format) and tables for the structure determination of complexes [($PcPy_3$)Fe(NO)](ClO_4)$_2$·MeCN (**2**·MeCN) and [($MePcPy_3$)Fe(NO)](ClO_4)$_2$·1.75MeCN (**3**·1.75MeCN). This material is available free of charge via the Internet at http://pubs.acs.org.

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(34) For example, see Ellis, K. J.; Lappin, A. G.; McAuley, A. *J. Chem. Soc., Dalton Trans.* **1975**, 1930−1934 and references therein.

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(37) The same trend is observed in the reaction of **1** and GSH in water. In the absence of light and dioxygen, one obtains ∼90% GSNO and only a trace of disulfide.