Porphyrin Complexes

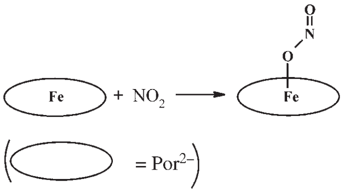
DOI: 10.1002/anie.200502409

Reactions of Nitrogen Oxides with Heme Models: Spectral Characterization of an Elusive Five-Coordinate $\mathrm{Fe^{III}}$(porphyrin) Nitrito Intermediate[**]

Tigran S. Kurtikyan and Peter C. Ford**

The roles of nitrogen dioxide (NO_2) and of the nitrite ion (NO_2^-) in mammalian biology are drawing increasing attention.[1–4] NO_2 is a strong oxidant and nitrating species that initiates destructive pathways in living systems.[1] Several disease states have been suggested to be connected with exogenously and endogenously formed NO_2.[2,3] Likely endogenous sources of NO_2 are the reactions of nitric oxide (NO) with oxyglobins and with the superoxide ion (O_2^-), which leads to coordinated and free peroxynitrite ($ONOO^-$),[5,6] subsequent decay of which affords both the nitrate ion and NO_2.[7] Furthermore, there is increasing evidence that NO_2 may also be formed through enzymatic pathways with the participation of heme peroxidases.[1a] NO_2 has been shown to be a product of the reaction of NO and nitrate, as mediated by a heme center.[8] With regard to nitrite, the NO_2^- ion is ubiquitous in mammalian tissues and fluids and is the largest reservoir of accessible NO equivalents in the cardiovascular system.[4a,b] Recent studies have suggested that NO_2^- itself is either a vasodilator or forms the known vasodilator NO through reaction with heme centers.[4c] Clearly, there is a continuing incentive to elucidate the interactions of these ligands with heme models to understand the fundamental chemistry relevant to their biological properties.

Reported herein are studies of the reaction of NO_2 with ferroheme models $\mathrm{Fe^{II}}$(por) (por = *meso*-tetraphenylporphyrinato dianion (TPP) or *meso*-tetra-*p*-tolylporphyrinato dianion (TTP)) in porous, sublimed layers. In an earlier study,[9] it was shown that the reaction of Fe(tpp) leads to the formation of the nitrato complex Fe(tpp)(η^2-O_2NO). Herein we demonstrate FTIR and UV/Vis spectroscopic evidence that manifest the formation of the elusive five-coordinate nitrito complex Fe(por)(η^1-ONO) [Eq. (1)] as an intermediate in the first stage of this reaction. Notably, the only well-characterized iron porphyrin complexes of NO_2 are those with sterically protecting porphyrins (e.g. "picket fence" porphyrin)[10] and the nitro–nitrosyl complexes Fe(por)(NO_2)(NO) that are stable only under NO.[11] No five-coordinate nitrito complexes of the type $\mathrm{Fe^{III}}$(por)(NO_2) (or $\mathrm{Fe^{III}}$(por)(ONO)) have been characterized previously, even with picket fence porphyrins.



(1)

The introduction of small increments of NO_2 gas into a cryostat, at room temperature, that contains sublimed layers of Fe(ttp) (see Experimental Section) leads to the appearance of three new IR spectroscopic bands at $\tilde{\nu}$ = 1528, 901, and 751 cm^{-1} that have their isotope counterparts at $\tilde{\nu}$ = 1495, 878, and 747 cm^{-1} when $^{15}NO_2$ is used (Figure 1). For the analogous complex with Fe(tpp), only small deviations from these values (1–2 cm^{-1}) are seen (see Table 1), but the last band is masked by an intense TPP band and can be registered only in the difference spectra. The intensity growth of these bands correlates directly with the addition of each portion of NO_2 ($^{15}NO_2$) (Figure 2) and demonstrates the formation of a new species that clearly contains a coordinated NO_2 ligand. This new complex is fairly stable in the solid state at room temperature, but decomposes under vacuum within a few days to give mostly the nitrosyl complex Fe(tpp)(NO) with a strong ν(NO) band at $\tilde{\nu}$ = 1677 cm^{-1}.

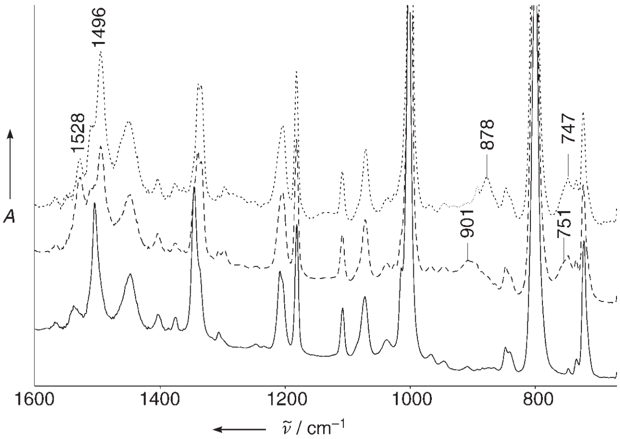


Figure 1. FTIR spectra of Fe(ttp) sublimed layers (solid line) after short (≈30 s) exposure to NO_2 (5×10^{-2} mmHg) followed by evacuation (dashed line)). The same procedure with $^{15}NO_2$ (dotted line).

---

[*] Prof. Dr. T. S. Kurtikyan
Research Institute of Applied Chemistry (ARIAC)
375005 Yerevan (Armenia)
and
Molecule Structure Research Centre (MSRC) NAS
375014 Yerevan (Armenia)
Fax: (+37) 410-28-2667
E-mail: tkurt@msrc.am
Prof. Dr. P. C. Ford
Department of Chemistry and Biochemistry
University of California
Santa Barbara, CA 93106 (USA)
Fax: (+1) 806-893-4120
E-mail: ford@chem.ucsb.edu

[**] This work was supported by the U.S. Civilian Research and Development Foundation (CRDF Grant No. AC2-2520-TB-03) and by the US Department of Energy (DE-FG02-04ER15506). We thank M. E. Hakobyan for providing us with pure samples of iron(II) porphyrins.

Table 1: IR spectroscopic data (cm^{-1}) of nitrito and nitrito–nitrosyl complexes of Fe(por).[a] The data for the complexes with ^{15}N-substituted nitrogen oxides are given in the parenthesis.

Complexes	ν(N=O)	ν(N-O)	δ(O-N=O)	ν(N=O)
Fe(ttp)(ONO)	1528(1496)	901(878)	751(747)	
Fe(ttp)(ONO)(NO)	1507(≈1472)	934(914)	–	1886(1851)
Fe(tpp)(ONO)	1527(1495)	900(877)	–	
Fe(tpp)(ONO)(NO)	1508(≈1474)	936(915)	–	1887(1851)

[a] por = *meso*-tetraphenylporphyrinato dianion (TPP) or *meso*-tetra-*p*-tolylporphyrinato dianion (TTP).

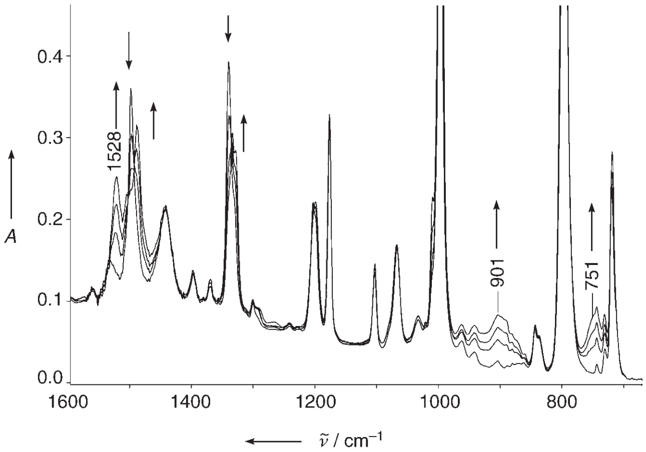
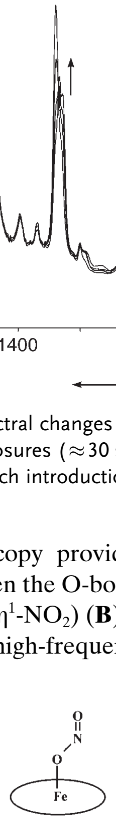
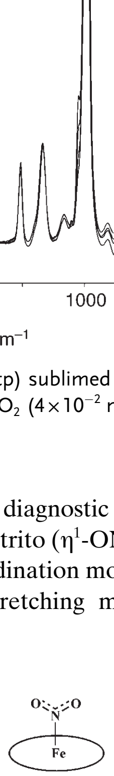


Figure 2. FTIR spectral changes in Fe(ttp) sublimed layer after sequential short time exposures (≈30 s) to NO_2 (4×10^{-2} mmHg) and evacuation after each introduction.


A


B

IR spectroscopy provides a diagnostic tool for differentiation between the O-bound nitrito (η^1-ONO) (**A**) and the N-bound nitro (η^1-NO_2) (**B**) coordination modes of the NO_2^- ligand.[12] The high-frequency stretching modes are much more separated for the O-bound than for the N-bound species. Indeed, for the nitro–nitrosyl complex, Fe(tpp)(NO_2)(NO), the asymmetric and symmetric ν(NO) modes of coordinated NO_2 are located at 1440 and 1300 cm^{-1}. Other six-coordinate porphyrin nitro complexes also show similar bands. In sublimed films of five-coordinate Co(tpp)(NO_2), these bands are located at 1468 and 1282 cm^{-1}.[13] However, on addition of a sixth ligand (piperidine (Pip) or pyridine (Py)) the bands shift to $\tilde{\nu}$ = 1437 and 1308 cm^{-1} and to $\tilde{\nu}$ = 1439 and 1310 cm^{-1}, respectively,[14] which are close to the values observed for Fe(tpp)(NO_2)(NO).

On the other hand, the ruthenium complex Ru(tpp)(ONO)(NO), for which the X-ray crystal structure has revealed nitrito coordination, shows IR spectroscopic bands at $\tilde{\nu}$ = 1522 and 930 cm^{-1}.[15,11c] Another example is the (thermally unstable) nitrite–nitrosyl manganese analogue Mn(tpp)(ONO)(NO), which was identified[16] in the course of NO disproportionation on the sublimed Mn(tpp) layers. It displays isotope-sensitive IR spectroscopic bands at $\tilde{\nu}$ = 1480 and 971 cm^{-1}, which were assigned to the coordinated nitrito ligand.[16] A third example is a metastable compound that was recently prepared[17] by photolysis of Fe(tpp)(NO_2)(NO) in a low-temperature KBr pellet. The photoproduct was concluded to be the nitrite–nitrosyl complex Fe(tpp)(ONO)(NO) on the basis of extensive DFT calculations and the observation of N-isotope-sensitive IR spectroscopic bands at $\tilde{\nu}$ = 1507 and 934 cm^{-1} that were assigned to the ν(N=O) and ν(N-O) stretching modes of the Fe-O-N=O moiety.[17]

The analogy between these spectral data and our observations supports the formation of the nitrito complex Fe(por)(η^1-ONO) at the initial stage of reaction between NO_2 and Fe(por). However, in the above studies only two bands were reported for coordinated nitrito ligands, although the ONO fragment should display three IR spectroscopic active normal modes. The failure to observe the third mode can now be explained in terms of the intense porphyrin band in the vicinity of $\tilde{\nu}$ = 750 cm^{-1} in TPP complexes, which masks the low-intensity δ(ONO) deformation expected for the coordinated nitrito ligand. In contrast, Fe(ttp) is relatively free in this spectral range, so an isotope-sensitive band was found for Fe(ttp)(η^1-ONO) in the region where the δ(ONO) was expected.[12]

The IR spectra also rule out an η^2-O_2N chelate structure. In such complexes the $\nu_a(NO_2)$ and $\nu_s(NO_2)$ stretching vibrations are separated only by about 200 cm^{-1} in contrast to the ≈600 cm^{-1} separation of the high-frequency bands observed in the present work.[12]

Additional information regarding the nature of this complex can be drawn from porphyrin vibrational modes that reveal regular changes depending on the spin and oxidation state of axial complexes of Fe(tpp).[18] The band at $\nu \approx 1350$ cm^{-1}, which represents a porphyrin core mode ($\nu(C_a$-$C_m)$ mixed with some $\nu(C_m$-phenyl)) and a porphyrin core deformation mode near $\tilde{\nu}$ = 450 cm^{-1}, is sensitive to the spin state of the metal complexes. For the intermediate-spin state Fe(tpp), these bands lie at $\tilde{\nu}$ = 1346 and 464 cm^{-1}. Upon coordination with NO_2, they shift to $\tilde{\nu}$ = 1341 and 434 cm^{-1}, respectively. This is consistent with a change to the high-spin state for the new, five-coordinate Fe(ttp)(ONO) complex. The axial complexes of Fe(ttp) also have such electronic structure-sensitive bands. For this porphyrin ligand, the aforementioned bands are disposed initially at $\tilde{\nu}$ = 1346 and 455 cm^{-1} and correspondingly shift upon nitrito-complex formation to $\tilde{\nu}$ = 1340 and 428 cm^{-1}. In the case of Fe(ttp), there is another structure-sensitive band at $\tilde{\nu}$ = 1505 cm^{-1} that undergoes a shift to $\tilde{\nu}$ = 1495 cm^{-1} upon the formation of Fe(ttp)(η^1-ONO).

From the highly resolved IR spectral data, one can conclude that the initial products of the reaction of the ferroheme model compounds, Fe(por), with NO_2 are O-coordinated and are five-coordinate Fe(por)(η^1-ONO) complexes rather than bisligated, six-coordinate Fe(por)(η^1-ONO)$_2$ complexes. During the course of adding NO_2 in portions (beginning from very low values), one would expect to observe additional IR spectroscopic bands if both five- and six-coordinate structures are formed, but this was not the case. Furthermore, formation of Fe(por)(η^1-ONO)$_2$ would involve oxidation either of the iron to give a Fe^{IV} species or of the porphyrin ring to give the porphyrin radical. For the latter, an intense π cation radical marker band in the vicinity of $\tilde{\nu} = 1300\ \text{cm}^{-1}$[19] should be observed as was seen for the bisnitro complex Co^{III}(tpp$^{\cdot+}$)(NO$_2$)$_2$,[20] which was obtained in sublimed layers. No such band was seen in the spectra reported herein.

The reaction of Fe(ttp)(ONO) with NO was investigated through preparation of a sample of the layered Fe(ttp)(ONO) material as described above, followed by cooling to 77 K and introducing excess NO. At 77 K there was no apparent reaction, but heating to 150 K led to the appearance of new bands at $\tilde{\nu} = 1886\ \text{cm}^{-1}$, 1507, and 934 cm^{-1}, whereas the bands at $\tilde{\nu} = 1528$, 901, and 757 cm^{-1}, which were assigned to Fe(ttp)(ONO), disappeared (Figure 3, bottom curve). The former bands have their isotope counterparts at $\tilde{\nu} = 1851$, ≈ 1472, and 914 cm^{-1} when ^{15}NO interacts with layers containing Fe(ttp)(O^{15}NO). Notably, the product spectra are very similar to those reported[17] for metastable species obtained when a low temperature KBr pellet, which contained Fe(tpp)(NO)(NO$_2$), was photolyzed. The photochemical product displayed IR spectroscopic bands at $\tilde{\nu} = 1507$ and 934 cm^{-1} that were assigned to the ν(N=O) and ν(N-O) nitrito stretching modes of the nitrito–nitrosyl complex Fe(tpp)(ONO)(NO). The ν(N≡O) mode of this transient species was not reported owing to overlap with the nitrosyl band of the starting material, although difference spectra indicate that the photoproduct nitrosyl band shifts to higher frequencies. The coincidence of the spectral data reported herein for the product of the NO reaction with Fe(ttp)(ONO) and the metastable photoproduct implies the formation of an analogous species, namely the η^1-ONO complexes. Thus, we conclude that the low-temperature reaction of NO with Fe(ttp)(ONO) is a simple addition to give Fe(ttp)(η^1-ONO)(NO), which supports the view that Fe(por)(ONO) is a five-coordinate nitrito complex.

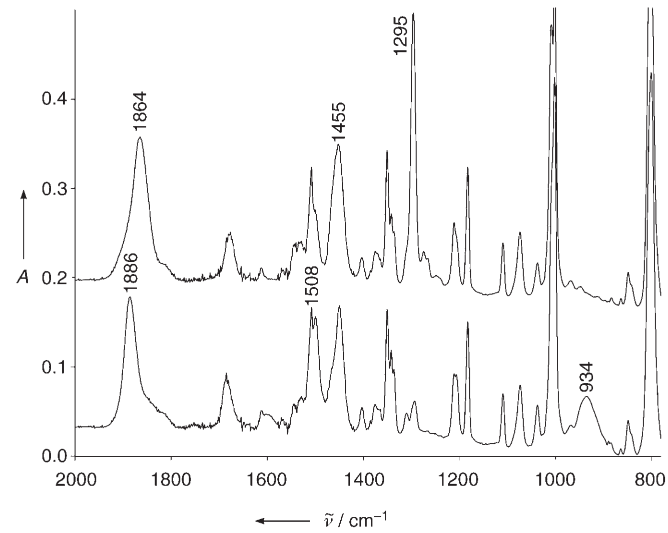
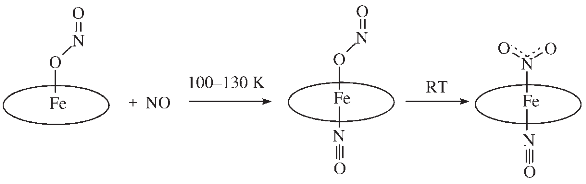


Figure 3. FTIR spectra of sublimed layer containing Fe(ttp)(ONO) after introducing 2 mm NO into cryostat at 77 K, warming to 140 K and recooling (bottom curve); after additional warming to 240 K (upper curve) and recooling.

Upon warming the initially formed nitrite–nitrosyl complex, isomerization to the more stable nitro–nitrosyl form (Fe(por)(NO$_2$)(NO))[10,11] as characterized by IR spectroscopic bands at $\tilde{\nu} = 1864$ {ν(N≡O)}, ≈ 1455 {ν_a(NO$_2$)}, and 1295 cm^{-1} {ν_s(NO$_2$)} was observed [Figure 3, upper curve, Eq. (2)]. For both the nitrite–nitrosyl complex and its nitro–nitrosyl isomer (formed upon warming), the spin-sensitive bands appear at the higher frequencies characteristic of the low-spin iron state.



(2)

Formation of the five-coordinate nitrito complex Fe(por)(η^1-ONO) upon initial reaction of NO$_2$ with Fe(por) is also supported by changes in the UV/Vis spectra (Figure 4). The reaction was first monitored by FTIR spectroscopy (by using CaF$_2$ plates) and then the electronic absorption spectrum of the same sample was recorded. The spectral changes observed are characteristic of conversion from the ferrous into the ferric state, that is, going from Fe(ttp) to Fe(ttp)(ONO). Furthermore, despite the difference in media (solid vs. solution), the electronic spectrum of the latter is quite similar to that obtained by Scheidt and co-workers[21] at the first stage of the reaction between [Fe(tpp)(OClO$_3$)] and NO$_2^-$ in CH$_2$Cl$_2$. Although the spectrum obtained under these conditions was reasonably assigned as likely being due to an iron(III) nitrite complex, the exact nature of this complex was

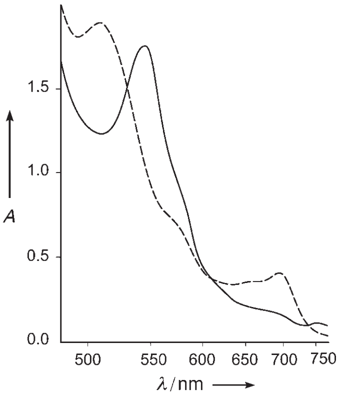


Figure 4. UV/Vis spectrum of Fe(ttp) sublimed layer (solid line) after short time exposure to a NO$_2$ atmosphere (4×10^{-2} mmHg, dashed line). Spectrum was recorded after FTIR spectroscopic measurement of the same sample evidencing formation of the nitrito complex Fe(ttp)(ONO).

not clear owing to uncertainty regarding stoichiometry (one or two nitrites), mode of ligation (O or N bound), and whether solvent is coordinated. However, the electronic spectral similarities with the thin-film experiments strongly suggest that the unstable species seen in the earlier solution study[21] was indeed the five-coordinate nitrito complex Fe(tpp)(η^1-ONO) characterized here.

Continued addition of NO_2 increments to the layered nitrite complex Fe(por)(η^1-ONO) at room temperature led to the formation of the known nitrato complexes Fe(por)(η^2-O_2NO). This is consistent with a previous study[9] that found that interaction of NO_2 gas with a sublimed layer of Fe(tpp) gave Fe(tpp)(η^2-O_2NO). Notably, the high-frequency ν(N=O) band at $\tilde{\nu} \approx 1530\ \text{cm}^{-1}$ for this complex is disposed in the same spectral range as the ν(N=O) of O-coordinated nitrito ligand. This coincidence is likely the reason that the intermediacy of the nitrito complexes in such reactions was not detected in the previous study.

The presence of an intermediate O-coordinated nitrito ligand has been proposed previously [8,22] as a reasonable interpretation of kinetic and spectral data for various transformations that take place in the course of the NO_x interaction with heme models in solids and in solution. The demonstration herein that such five-coordinate iron-porphyrin nitrito complexes are formed from the reaction of Fe(por) and NO_2 at room temperature confirms these suggestions.

In summary, the data reported herein indicate that the first stage of the reaction of NO_2 with the ferrous porphyrins Fe(por) leads to the formation of the previously unknown five-coordinate ferric nitrito complex Fe(por)(η^1-ONO). The mechanism of subsequent transformations upon the addition of new NO_2 increments, which leads to the eventual formation of nitrato complexes, is now under detailed investigation.

Experimental Section

The ferrous porphyrinates Fe(por) are very sensitive to oxygen and are readily oxidized to Fe^{III} derivatives. For this reason the more stable hexacoordinate complexes, Fe(por)$(B)_2$, with nitrogen bases (por = TPP or the *meso*-tetra-*p*-tolylporphyrinato dianion; B is Py or Pip) were used as the precursors. These were synthesized by a known procedure.[23] The low-temperature sublimate was prepared[24] by placing a Fe(por)$(B)_2$ sample in a Knudsen cell and heating to about 470 K under high vacuum ($P = 3\times10^{-5}$ Torr). Evacuation for 3 h resulted in the complete elimination of the coordinated axial ligands B, as monitored by measurement of the pressure at the outlet of the cryostat. Liquid nitrogen was then poured into the cryostat, and the Knudsen cell was heated to 520 K, at which temperature sublimation of Fe(por) onto the KBr or CaF_2 substrate occurred. In the latter case, CaF_2 plates were also used as the optical windows of the cryostat. The metal–aryl porphyrins layers obtained in this manner by sublimation onto a low-temperature (77 K) surface are spongelike[25a,b] and have high microporosity.[25c] Potential reactants easily diffuse across these layers, and adducts thus formed can be studied spectroscopically without solvent interference.

Sublimation was typically carried out over periods of 0.3–2.0 h to build up layers of thickness sufficient for UV/Vis and IR spectral studies. The Fe(por) sublimed layer was then heated to room temperature under dynamic vacuum, and small increments of NO_2 gas were introduced for a short-time period followed by a period of pumping out. The FTIR and UV/Vis spectra were measured after each NO_2 introduction.

NO_2 ($^{15}NO_2$), obtained by oxidation of NO (^{15}NO) with excess pure dioxygen, was dried over P_2O_5 and then was purified by fractional distillation until a pure white solid was obtained. ^{15}NO with 98.5% enrichment was purchased from the Institute of Isotopes, Republic of Georgia.

Received: July 11, 2005
Revised: October 7, 2005

Keywords: heme · IR spectroscopy · iron · nitrogen oxides · porphyrinoids

---

[1] a) M. Kirsch, H.-G. Korth, R. Sustmann, H. de Groot, *Biol. Chem.* **2002**, *383*, 389–399; b) S. Pfeiffer, B. Mayer, B. Hemmens, *Angew. Chem.* **1999**, *111*, 1824–1844; *Angew. Chem. Int. Ed.* **1999**, *38*, 1714–1731.
[2] M. G. Espey, S. Xavier, D. D. Thomas, K. M. Miranda, D. A. Wink, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 3481–3486.
[3] S. Pfeiffer, A. Lass, K. Schmidt, B. Mayer, *FASEB J.* **2001**, *15*, 2355–2364.
[4] a) N. S. Bryan, T. Rassaf, R. E. Maloney, C. M. Rodriguez, F. Saijo, J. R. Rodriguez, M. Feelisch, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 4308–4313; b) T. Rassaf, M. Feelisch, M. Kelm, *Free Radical Biol. Med.* **2004**, *36*, 413–422; c) K. Cosby, K. S. Partovi, J. H. Crawford, R. P. Patel, C. D. Reiter, S. Martyr, B. K. Yang, M. A. Waclawiw, G. Zalos, X. Xu, K. T. Huang, H. Shields, D. B. Kim-Shapiro, A. N. Schechter, R. O. Cannon, M. T. Gladwin, *Nat. Med.* **2003**, *9*, 1498–1505.
[5] M. P. Dohle, J. W. Hoekstra, *J. Inorg. Biochem.* **1981**, *14*, 351–358.
[6] a) S. Herold, M. Exner, T. Nauser, *Biochemistry* **2001**, *40*, 3385; b) S. Herold, *FEBS Lett.* **1999**, *443*, 81.
[7] J. L. Bourassa, E. P. Ives, A. L. Marqueling, R. Shimanovich, J. T. Groves, *J. Am. Chem. Soc.* **2001**, *123*, 5142–5143.
[8] T. S. Kurtikyan, G. M. Gulyan, G. G. Martirosyan, M. D. Lim, P. C. Ford, *J. Am. Chem. Soc.* **2005**, *127*, 6216–6224.
[9] T. S. Kurtikyan, T. G. Stepanyan, M. E. Akopyan, *Russ. J. Coord. Chem.* **1999**, *25*, 721–725.
[10] G. R. A. Wyllie, W. R. Scheidt, *Chem. Rev.* **2002**, *102*, 1067–1089.
[11] a) T. Yoshimura, *Inorg. Chim. Acta* **1984**, *83*, 17–21; b) M. K. Ellison, C. E. Schulz, W. R. Scheidt, *Inorg. Chem.* **1999**, *38*, 100–108; c) T. S. Kurtikyan, G. G. Martirosyan, I. M. Lorkovic′, P. C. Ford, *J. Am. Chem. Soc.* **2002**, *124*, 10124–10129.
[12] K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, 3rd ed., Wiley, New York, **1978**, pp. 244–247.
[13] T. S. Kurtikyan, T. G. Stepanyan, A. V. Gasparyan, *Russ. J. Coord. Chem.* **1997**, *23*, 563–567.
[14] T. G. Stepanyan, M. E. Akopyan, T. S. Kurtikyan, *Russ. J. Coord. Chem.* **2000**, *26*, 453–457.
[15] K. M. Miranda, X. Bu, I. M. Lorkovic, P. C. Ford, *Inorg. Chem.* **1997**, *36*, 1992.
[16] G. G. Martirosyan, A. S. Azizyan, T. S. Kurtikyan, P. C. Ford, *Chem. Commun.* **2004**, 1488–1489.
[17] J. Lee, A. Y. Kovalevsky, I. Novozhilova, K. Bagley, P. Coppens, G. B. Richter-Addo, *J. Am. Chem. Soc.* **2004**, *126*, 7180–7181.
[18] H. Oshio, T. Ama, T. Watanabe, J. Kincaid, K. Nakamoto, *Spectrochim. Acta Part A* **1984**, *40*, 863–870.
[19] E. T. Shimomura, M. A. Phillippi, H. M. Goff, W. F. Sholz, C. A. Reed, *J. Am. Chem. Soc.* **1981**, *103*, 6778–6780.
[20] T. S. Kurtikyan, T. H. Stepanyan, *Russ. Chem. Bull.* **1998**, *47*, 695–698.
[21] M. J. Finnegan, A. G. Lappin, W. R. Scheidt, *Inorg. Chem.* **1990**, *29*, 181–185.

[22] M. D. Lim, I. M. Lorkovic, K. Wedeking, A. W. Zanella, S. M. Massick, P. C. Ford, *J. Am. Chem. Soc.* **2002**, *124*, 9737 – 9743.

[23] L. M. Epstein, D. K. Straub, C. Maricondi, *Inorg. Chem.* **1967**, *6*, 1720 – 1722.

[24] K. Nakamoto, T. Watanabe, T. Ama, M. W. Urban, *J. Am. Chem. Soc.* **1982**, *104*, 3744 – 3745.

[25] a) M. P. Byrn, C. J. Curtis, Y. Hsiou, S. I. Khan, P. A. Sawin, S. K. Tendick, A. Terzis, C. E. Strouse, *J. Am. Chem. Soc.* **1993**, *115*, 9480 – 9497; b) M. P. Byrn, C. J. Curtis, S. I. Khan, P. A. Sawin, R. Tsurumi, C. E. Strouse, *J. Am. Chem. Soc.* **1990**, *112*, 1865 – 1874; c) T. S. Kurtikyan, A. V. Gasparyan, G. G. Martirosyan, G. H. Zhamkochyan, *J. Appl. Spectrosc.* **1995**, *62*, 62 – 66. (Russ.)