Cobalt(III) Complexes with Carboxamido-*N* and Sulfenato-*S* or Sulfinato-*S* Ligands Suggest that a Coordinated Sulfenate-*S* is Essential for the Catalytic Activity of Nitrile Hydratases

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A bis(sulfenato-*S*)Co^{III} complex and its corresponding bis(sulfinato-*S*) complex were prepared. Their catalytic nitrile hydration activity was tested. Whereas the former catalyzes the hydration of CH_3CN under mild acidic conditions, the latter is inactive. A mechanism for this reaction is proposed, in relation with nitrile hydratase activity.

Introduction

Nitrile hydratases (NHases) are non-heme iron or non-corrinoid cobalt-containing enzymes that catalyze the hydration of nitriles to amides.[1] NHases are used for the industrial production of acrylamide[2] and for the removal of nitriles from waste streams.[3] Both active and inactive forms of the iron NHases have recently been characterized by X-ray diffraction and ENDOR (Electron Nuclear Double Resonance) spectroscopy. The coordination sphere of the metal centre is very unusual. For the active form of the NHase from *Rhodococcus* sp.R312, the structure at 2.65 Å resolution showed that iron is bound to three cysteinthiolates, two amido nitrogen atoms from peptide bonds of the protein main chain,[4] and an axial hydroxide or water molecule as the sixth ligand.[5] For the inactive form of the NHase from *Rhodococcus* sp.N-771, the structure at 1.7 Å resolution revealed that two of the coordinated sulfur atoms belong to sulfenic (Cys-SOH) and sulfinic (Cys-SO_2H) moieties, and one nitric oxide ligand occupies the sixth position.[6] The cysteine-sulfinate and cysteine-sulfenate ligands are assigned to the post-translational modification of former cysteines. The iron and cobalt NHases have similar metal sites, according to EXAFS data.[7] A very recent study of a Co-substituted Fe-NHase showed that the catalytic activity is correlated to the post-translational oxidation of a cysteine.[8] This oxidation of one cysteine into cysteine-sulfinate has been established by mass spectrometry, but this does not exclude the existence of a former relatively unstable cysteine-sulfenate at the enzyme active site.

All these data did not suggest a role for the sulfenato-*S* and/or sulfinato-*S* ligands in NHase catalysis. The study of relevant mimetic complexes, however, should yield clues to this question. Several mimetic Fe^{III} and Co^{III} model compounds of NHases have recently been synthesized.[9–12] Some of them have been oxidized by H_2O_2 to give the sulfinato-*S* species[10a][11a][11d] and one gave an inert η^2-sulfenato, sulfinato-*S* derivative.[12b] Only one cobalt mimetic complex has been reported to have nitrile hydratase activity.[11b] The structure of this presumably hydroxo complex $[Co(PyPS)(OH)]^{2-}$ is not known (although it is assumed to contain one pyridine, two carboxamido-*N* and one thiolato ligands in a plane, and the second thiolate in an axial position). It allowed conversion of acetonitrile into acetamide at 50 °C and pH = 9.5 (18 turnovers). This reaction probably involves the nucleophilic property of the hydroxide ligand.

We have reported the first example of the oxidation of a carboxamido-*N*, thiolato-Fe^{III} complex $[FeN_2S_3]^{2-}$ by dioxygen to give a sulfinato-*O* species $[FeN_2S_2OSO]^{2-}$ raising the question of *S*- vs. *O*-coordination to the metal.[9c] To investigate which factors could control the eventual oxidation of the bound in-plane thiolates in Co-NHases, we have synthesized a tetradentate eleven-atom ligand $H_4N_2S_2$, containing two aliphatic thiols and two aliphatic amides **1** (H_4L).[13] The derived square-planar complex $[Co^{III}L]^-$ (**2**) was characterized by X-ray diffraction.[9b] Cyanides and isocyanides were the only ligands found able to give hexacoordinated complexes upon bis-axial coordination, as shown by spectroscopic data together with an EXAFS study.[9b] Such a coordination allows oxygenation of the in-plane thiolates to take place by air or hydrogen peroxide. We have thus reported the preparation of the disulfenato compound $Na[Co(L\text{-}N_2SOSO)(t\text{BuNC})_2]$ (**3**).[9a] We now report the preparation of the corresponding disulfinato derivative $(Me_4N)[Co(L\text{-}N_2SO_2SO_2)(t\text{BuNC})_2]$ (**4**). Whereas **3** exhibits nitrile hydration catalytic activity, **4** is inactive.

Results and Discussion

The addition of 50% aq. H_2O_2 to a solution of the square-planar complex $[Co^{III}L]^-$ (**2**)[9b] in EtOH, in the

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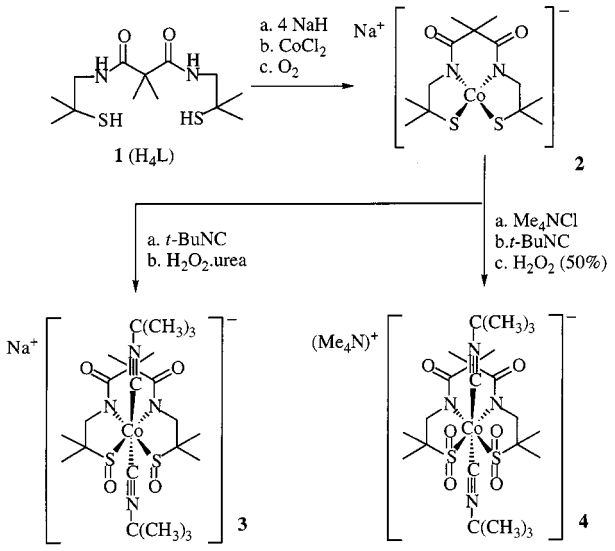
presence of excess *t*BuNC and one equivalent of Me_4NCl, gave the yellow disulfinato complex $(Me_4N)[Co(L\text{-}N_2SO_2\text{-}SO_2)(t\text{BuNC})_2]$ (**4**) (Scheme 1). Single crystals of **4** suitable for X-ray analysis were formed by slow diffusion of Et_2O into an EtOH solution in the presence of a small amount of H_2O. The X-ray structure of **4** is shown in Figure 1.



Scheme 1. Synthetic scheme for complexes **3** and **4**

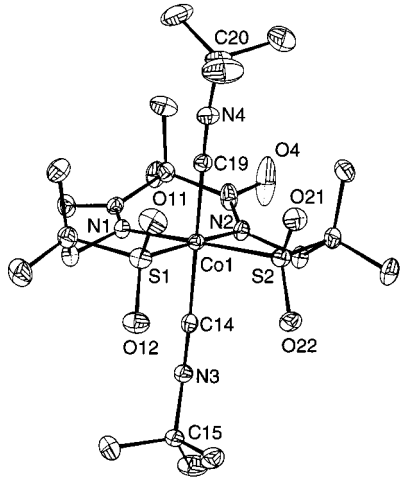


Figure 1. CAMERON representation (ellipsoid at 30% probability level) of **4**; H atoms are omitted for clarity; selected bond lengths (Å) and angles (°): Co(1)−S(1) 2.2114(9), Co(1)−S(2) 2.2255(9), Co(1)−N(1) 1.962(3), Co(1)−N(2) 1.954(3), Co(1)−C(14) 1.877(3), Co(1)−C(19) 1.867(3), C(14)−N(4) 1.149(4), C(19)−N(4) 1.143(4), S(1)−O(11) 1.449(3), S(1)−O(12) 1.470(3), S(2)−O(21) 1.452(3), S(2)−O(22) 1.472(3); S(1)−Co(1)−S(2) 95.56(4), S(1)−Co(1)−N(1) 86.87(8), N(1)−Co(1)−N(2) 90.9(1), S(1)−Co(1)−N(1) 86.87(8), S(2)−Co(1)−N(2) 86.65(8), C(14)−Co(1)−C(19) 174.7(1), Co(1)−C(19)−N(4) 175.0(3), Co(1)−C(14)−N(3) 176.5(3), C(14)−N(3)−C(15) 166.5(3), C(19)−N(4)−C(20) 173.6(3)

Compound **4** is a distorted octahedral Co^{III} complex with an N_2S_2 equatorial plane containing carboxamido-*N* and sulfinato-*S* ligands and two axial isocyanido ligands. Water molecules help to stabilize the crystal lattice through hydrogen bonding to the oxygen atoms of the sulfinato-*S* and carboxamido-*N* ligands. The presence of one Me_4N^+ cation per cobalt in the unit cell of **4** confirms the oxidation state as cobalt(III). The Co−N bond lengths are similar to those reported for carboxamido-*N* ligands in hexacoordinated Co^{III} complexes,[11] but are 0.08 Å longer than those found in the parent square-planar complex **2**.[9b] The Co−S bond lengths (2.22 Å) are similar to those reported for structurally characterized Co^{III}-sulfinato complexes.[10a,11a,11d] The Co−*C*-isocyanido bonds are 0.04 Å longer than the only reported values (1.83−1.79 Å) for Co^{III}−*C*-isocyanido cyclopentadienyl compounds.[14] The isocyanides are almost linearly coordinated to the metal, with a Co−C−N angle of 176°, a C−N−C angle of 173° and a C−N bond length of 1.14 Å, which confirms a C≡N triple bond. The IR spectrum (KBr pellet) of **4** shows the characteristic coordinated carboxamido-*N* ν_{CO} frequency at 1565 cm^{-1} and strong absorptions at 1195, 1065 cm^{-1} corresponding to the ν_{SO} stretching vibrations of coordinated *S*-sulfinates.[9b] The strong asymmetric ν_{CN} stretching frequencies of the isocyanides are increased from 2140 cm^{-1} to 2215 cm^{-1} upon coordination, indicating a higher bond order in **4** than in the free ligand, fitting the C≡N triple bond description and a prevailing σ-donor character of the isocyanide ligands rather than a potential π-acceptor contribution.

It is noteworthy that the overall geometries of $[Co(L\text{-}N_2SOSO)(t\text{BuNC})_2]^-$ (**3**) and $[Co(L\text{-}N_2SO_2SO_2)(t\text{BuNC})_2]^-$ (**4**) are very similar. The two complexes have comparable Co−N and Co−S bond lengths, but the Co−C bonds are longer in **4** than in **3** suggesting that the sulfinato (SO_2) group is a stronger-field ligand than the sulfenato (SO) group.

Yellow solutions of **4** in polar solvents (alcohols, DMSO, DMF, H_2O) are stable in air and the complex behaves as a hexacoordinated species with a diamagnetic low spin Co^{III} (Evans method). The electrospray mass spectrum exhibits a peak at m/z = 591 ($[Co(L\text{-}N_2SO_2SO_2)(t\text{BuNC})_2]^-$). The structural and spectral characteristics of **4** are similar to those of a Co^{III}-sulfinato complex reported very recently.[10a]

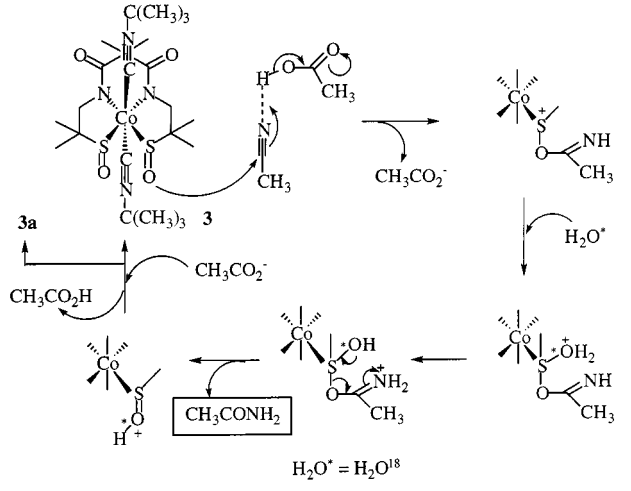
The testing of the hydration catalytic activity of compounds **3** and **4** was conducted in a mixture of CH_3CN/H_2O under various conditions of temperature and pH. The reaction is slow and could be followed by ^1H NMR spectroscopy using 1,3,5-trimethoxybenzene as internal reference for turnover number determination. Only **3** led to the formation of acetamide; no trace could be detected with **4**. Under alkaline conditions (aq. $NaHCO_3$, pH = 9), no conversion of CH_3CN was observed. At pH = 7 and 20 °C, acetamide was formed (2 turnovers) and **3** remained unchanged (^1H NMR spectroscopy) after 24 h. Varying the temperature did not improve the turnover number. Acidic pH = 4.8 (1 M HOAc/NaOAc buffer) at 4 °C gave the best

result (50 turnovers). Other acidic conditions (0.5 M HOAc/NaOAc buffer, pH = 4.8; 0.4 M HOAc; 0.03 M HCl; or 0.5 M H_3PO_4/KH_2PO_4 buffer, pH = 2.1) gave a less efficient reaction.

Although **3** gives a slow reaction under the conditions studied, the hydration mechanism deserves interest. At variance with the known[11b] complex $[Co(PyPS)(OH)]^{2-}$, which promotes the conversion of acetonitrile into acetamide (18 turnovers) only at 50 °C and pH = 9.5 (Tris buffer), probably through a Co^{III}-bound hydroxide ligand, **3** does not catalyze nitrile hydration in basic conditions (pH = 9). The reaction works better in mildly acidic conditions, although **3** undergoes a slow decomposition under these conditions, probably due to the protonation of the carboxamido-*N* ligands. Compound **3** has no free coordination site to allow either water or acetonitrile binding to Co^{III} and our results suggest that a general acid catalysis could be required for nitrile hydration catalyzed by **3**. The absence of catalytic activity of **4** points to a possible role of the nucleophilicity of the coordinated *S*-sulfenates in **3**. The oxygen atom of S-bound sulfenates has been shown to exhibit nucleophilic properties.[15]

When 30% $H_2{}^{18}O/H_2{}^{16}O$ was used for the hydration of acetonitrile in a 1 M HOAc/NaOAc buffer (pH = 4.8) at 4 °C for 17 h, complex **3** gave the complexes $[Co(L\text{-}N_2S^{18}OS^{16}O)(t\text{BuNC})_2]^-$ (**3a**) and $[Co(L\text{-}N_2S^{18}OS^{18}O)(t\text{BuNC})_2]^-$ (**3b**) characterized by electrospray mass spectrometry (*m*/*z* = 561, 563 respectively). This indicates that an exchange of oxygen atom occurs at the coordinated sulfenates during the hydration catalysis. That this exchange occurs during the actual hydration catalysis is demonstrated by the absence of any ^{18}O incorporation into **3** when acetonitrile is absent in exactly the same conditions.

Our observations, together with the known nucleophilic properties of coordinated *S*-sulfenates,[15] lead us to propose a tentative mechanism for the formation of complexes **3a** and **3b** during the hydration of CH_3CN catalyzed by **3** (Scheme 2). Complex **3** displays nucleophilic catalysis, assisted by general acid catalysis by the HOAc/NaOAc buffer.



Scheme 2. Tentative mechanism for the hydration of CH_3CN catalyzed by **3**

Conclusion

This study has shown the importance of coordinated sulfenates (SO) for nitrile hydration catalysis by a carboxamido-*N* Co^{III} complex. The corresponding sulfinato (SO_2) derivative is inactive under the same conditions. Our results suggest that in the case of Fe or Co NHases, the post-translational oxidation of metal-bound cysteines into sulfenic moieties, is a crucial step for nitrile hydration. Although the hydration reaction catalyzed by **3** appears to be of low efficiency due to its intermolecular character, this situation could be overcome by a proximity reaction within the enzyme active site. The conjunction of nucleophilic and general acid catalyses might explain the enzyme uncommon route for nitrile hydration.

Experimental Section

Complex 3: This compound was prepared according to our previously reported procedure[9a] with some slight modifications. *t*BuNC (51 mg, 0.6 mmol) was added to a deep green solution of Na[CoL]·DMF (**2**)[9b] (46 mg, 0.1 mmol) in DMF (0.5 mL) and acetone (1 mL) cooled to −10 °C. The resulting pink solution was stirred for 10 min. and then solid H_2O_2·urea (56 mg, 0.6 mmol) was added. After 1 h at −10 °C, acetone (1 mL) was added and the orange precipitate was filtered, rinsed with acetone, and dried under vacuum to afford $Na[Co^{III}(L\text{-}N_2SOSO)(t\text{BuNC})_2]$·urea (**3**) (55 mg, 85%). − ^1H NMR (250 MHz, [D$_6$]DMSO): δ = 5.44 (s, 4 H, urea), 3.60 (d, *J* = 13.3 Hz, 2 H, NCH_AH_B), 3.09 (d, 2 H, NCH_AH_B), 1.39 (s, 18 H, *t*BuNC), 1.28 (s, 6 H, CH_3), 1.17 (s, 6 H, CH_3), 1.12 (s, 6 H, CH_3).

Complex 4: Me_4NCl (16 mg, 0.15 mmol) and *t*BuNC (60 mg, 0.72 mmol) were added successively to a deep green solution of Na[CoL]·DMF (**2**)[9b] (54 mg, 0.12 mmol) in degassed EtOH (1 mL) cooled to −10 °C. The resulting pink solution was stirred under Ar for 10 min. and an aq. 50% solution of H_2O_2 (310 mg, 5.44 mmol) was then added dropwise. The orange solution was stirred at −10 °C for 3 h and kept in a refrigerator overnight. The precipitate (NaCl) was filtered off and rinsed with EtOH (95%, 1 mL). Et_2O (≈ 20 mL) was added to the combined yellow solution and kept at 4 °C overnight. The resulting yellow crystals were filtered and dried under vacuum to afford $(Me_4N)[Co^{III}(L\text{-}N_2SO_2SO_2)(t\text{BuNC})_2]\cdot H_2O$ (**4**) (70 mg, 96%). − $C_{27}H_{54}CoN_5O_7S_2$ (683.81): calcd. C 47.42, H 7.96, N 10.24, S 9.38; found C 47.17, H 7.54, N 10.11, S 9.26. − UV/Vis (MeOH) [λ_{max}, nm (ε, $m^{-1}\,cm^{-1}$)]: 400 (sh), 312 (20000), 208 (13000). − IR (KBr): $\tilde{\nu}$ = 2215 (s, ν_{CN}), 1565 (s, ν_{CO}), 1195 and 1065 (s, ν_{SO}) cm^{-1}. − ^1H NMR (250 MHz, [D$_6$]DMSO): δ = 7.75 (br. s, 2 H, D_2O exchangeable, H_2O), 3.18 (s, 4 H, NCH_2), 1.39 (s, 18 H, *t*BuNC), 1.31 [s, 12 H, $(CH_3)_4N^+$], 1.27 (s, 6 H, CH_3), 1.18 (s, 12 H, CH_3). − Electrospray MS (MeOH): *m*/*z* (%) = 591 [M − Me_4N^+]$^-$.

Crystal Data for 4·H_2O: $C_{27}H_{54}CoN_5O_7S_2$, *M* = 683.81, yellow crystals, monoclinic, space group $P2_1/n$, *a* = 11.621(7), *b* = 16.845(6), *c* = 18.468(4) Å, *Z* = 4, β = 79.64(3)° *V* = 3556(3) Å^3, D_c = 1.28 g·cm^{-3}, *T* = 295 K, Mo-K_α radiation (λ = 0.71069 Å),

7606 reflections were measured, 4631 of which were used in all calculations (R_{int} = 0.02). The structure was solved by SHELXS-86[16] and refined by least-squares analysis using anisotropic thermal parameters, H atoms were introduced in calculated positions, with $F_o > 3\sigma(F_o)$, the programs CRYSTALS[17] and CAMERON[18] were used, R = 0.0479, R_W = 0.0574.

Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-161812. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Typical Procedure for the Catalytic Hydration of CH_3CN: A mixture of Na[Co^{III}(L-N_2SOSO)(tBuNC)$_2$]·urea (**3**) (5 mg, 0.008 mmol) and 1,3,5-trimethoxybenzene (7 mg, 0.042 mmol) in CH_3CN (0.5 mL) and a HOAc/NaOAc buffer (1 M, pH = 4.8, 0.5 mL) was stirred at 4 °C for 17 h. After lyophilization, [D_6]Me_2SO was added to the mixture and the presence of 0.144 mmol of acetamide was determined (18 turnovers) by ^{1}H NMR spectroscopy. The turnover number increases with the reaction time. 42 Turnovers and 50 turnovers were found after 3 and 6 days, respectively.

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[1] S. Nagashima, M. Nakasato, N. Dohmae, M. Tsujimura, K. Takio, M. Odaka, M. Yohda, N. Kamiya, I. Endo, *Nature Struct. Biol.* **1998**, *5*, 347−351.
[2] M. Kobayashi, T. Nagasawa, H. Yamada, *Trends Biotechnol.* **1992**, *10*, 402−408.
[3] J. M. K. Wyatt, C. J. Knowles, *Int. Biodeterior. Biodegrad.* **1995**, *35*, 227−248.
[4] W. Huang, J. Jia, J. Cumming, M. Nelson, G. Schneider, Y. Lindqvist, *Structure* **1997**, *5*, 691−699.
[5] P. E. Doan, M. J. Nelson, H. Jin, B. M. Hoffman, *J. Am. Chem. Soc.* **1996**, *118*, 7014−7015;
[6] S. Nagashima, M. Nakasato, N. Dohmae, M. Tsujimura, K. Takio, M. Odaka, M. Yohda, N. Kamiya, I. Endo, *Nature Struct. Biol.* **1998**, *5*, 347−351.
[7] B. A. Brennan, G. Alms, M. J. Nelson, L. T. Durney, R. C. Scarrow, *J. Am. Chem. Soc.* **1996**, *118*, 9194−9195.
[8] N. Masaki, H. Nakayama, M. Odaka, M. Yohda, K. Takio, I. Endo, *FEBS Letters* **2000**, *465*, 173−177.
[9] [9a] L. Heinrich, Y. Li, J. Vaisserman, J. C. Chottard, *Eur. J. Inorg. Chem.* **2001**, 1407−1409. − [9b] L. Heinrich, Y. Li, K. Provost, A. Michalowicz, J. Vaisserman, J. C. Chottard, *Inorg. Chim. Acta* **2001**, *318*, 117−126. − [9c] L. Heinrich, Y. Li, J. Vaisserman, G. Chottard, J. C. Chottard, *Angew. Chem. Int. Ed.* **1999**, *38*, 3526−3528.
[10] [10a] M. Rat, R. Alves de Sousa, J. Vaisserman, D. Mansuy, I. Artaud, *J. Inorg. Biochem.* **2001**, *in press.* − [10b] S. Chatel, M. Rat, S. Dijols, P. Leduc, J. P. Tuchagues, D. Mansuy, I. Artaud, *J. Inorg. Biochem.* **2000**, *80*, 239−246. − [10c] I. Artaud, S. Chatel, A. S. Chauvin, D. Bonnet, M. A. Kopf, P. Leduc, *Coord. Chem. Rev.* **1999**, *190*, 577−586.
[11] [11a] L. A. Tyler, J. C. Noveron, M. M. Olmstead, P. K. Mascharak, *Inorg. Chem.* **2000**, *39*, 357−362. − [11b] J. C. Noveron, M. M. Olmstead, P. K. Mascharak, *J. Am. Chem. Soc.* **1999**, *121*, 3553−3554. − [11c] J. C. Noveron, R. Herradora, M. M. Olmstead, P. K. Mascharak, *Inorg. Chim. Acta* **1999**, *285*, 269−276. − [11d] L. A. Tyler, J. C. Noveron, M. M. Olmstead, P. K. Mascharak, *Inorg. Chem.* **1999**, *38*, 616−617. − [11e] J. C. Noveron, M. M. Olmstead, P. K. Mascharak, *Inorg. Chem.* **1998**, *37*, 1138−1139.
[12] [12a] J. Shearer, I. Y. Kung, S. Lovell, W. Kaminsky, J. A. Kovacs, *J. Am. Chem. Soc.* **2001**, *123*, 463−468. − [12b] I. Kung, D. Schweitzer, J. Shearer, W. Taylor, H. L. Jackson, S. Lovell, J. A. Kovacs, *J. Am. Chem. Soc.* **2000**, *122*, 8299−8300. − [12c] J. Shearer, I. Y. Kung, S. Lovell, J. A. Kovacs, *Inorg. Chem.* **2000**, *39*, 4998−4999. − [12d] J. J. Ellison, A. Nienstedt, S. C. Shoner, D. Barnhart, J. A. Cowen, J. A. Kovacs, *J. Am. Chem. Soc.* **1998**, *120*, 5691−5700. − [12e] S. C. Shoner, D. Barnhart, J. A. Kovacs, *Inorg. Chem.* **1995**, *34*, 4517−44518. − [12f] D. Schweitzer, J. J. Ellison, S. C. Shoner, S. Lovell, J. A. Kovacs, *J. Am. Chem. Soc.* **1998**, *120*, 10996−10997.
[13] L. Heinrich, J. C. Chottard, Y. Li, *Synth. Comm.* **2001**, *31* (9), 47−57.
[14] [14a] J. Doherty, J. Fortune, A. R. Manning, F. S. Stephens, *J. Chem. Soc., Dalton Trans.* **1984**, 1111−1116. − [14b] Y. Wakatsuki, K. Aoki, H. Yamazaki, *J. Chem. Soc., Dalton Trans.* **1986**, 1193−1199.
[15] J. D. Lydon, E. Deutsch, *Inorg. Chem.* **1982**, *21*, 3180−3185.
[16] G. M. Sheldrick, *SHELXS-86, Program for Crystal Structure Solution,* University of Göttingen, **1986**.
[17] D. J. Watkin, C. K. Prout, J. R. Carruthers, P. W. Betteridge, Crystals Chemical Crystallography Laboratory, University of Oxford, *10*, **1996**.
[18] D. J. Watkin, C. K. Prout, L. J. Pearce, *CAMERON,* Crystallography Laboratory, University of Oxford, **1996**.

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