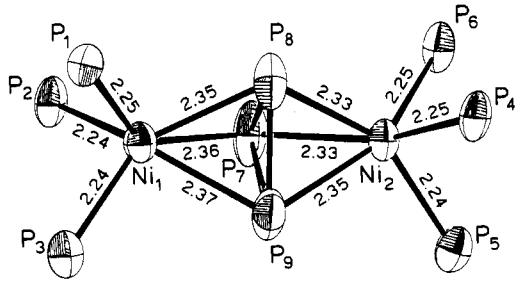

Figure 2. Inner skeleton of $[LNi(P_3)NiL]^{2+}$. Values of P_n–Ni–P_m angles: 89–96° (n, m = 1–6), 53–55° (n, m = 7–9).

phosphorus atoms of L and with the p_π orbitals of the P_3 molecule. If the latter are considered to provide 3 electrons and the former to provide 6 electrons, the total number of 18 electrons is attained for complex **1.** By the same approach, the number of 33 electrons is obtained for the nickel derivatives, which has never been reported for triple-decker sandwich compounds.[5,6] The presence of an odd number of electrons is confirmed by the value of the magnetic moment which corresponds to one unpaired electron for dimer.

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References and Notes

(1) A. P. Ginsberg and W. e. Lindsell, *J. Am. Chem. Soc.*, **93,** 2083 (1971).
(2) G. Schmid and H. P. Kempny, *Z. Anorg. Allg. Chem.*, **432,** 160 (1977).
(3) G. L. Simon and L. F. Dahl, *J. Am. Chem. Soc.*, **95,** 2175 (1973).
(4) P. Dapporto, S. Midollini, and L. Sacconi, *Inorg. Chem.*, **14,** 1643 (1975); P. Dapporto, S. Midollini, A. Orlandini, and L. Sacconi, *ibid.*, **15,** 2768 (1976); C.Mealli, S. Midollini, and L. Sacconi, *ibid.*, in press.
(5) J. W. Lauher, M. Elian, R. H. Summerville, and R. Hoffmann, *J. Am. Chem. Soc.*, **98,** 3219 (1976).
(6) H. Werner, *Angew, Chem., Int. Ed. Engl.*, **16,** 1 (1977).

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Catalytic Irreversible Inhibition of Mammalian Ornithine Decarboxylase (E.C. 4.1.1.17) by Substrate and Product Analogues

Sir:

The diamine putrescine and the polyamines spermidine and spermine which are derived from it have been implicated in the regulation of growth processes.[1] In attempts to delineate the still controversial roles of these bioamines, several reversible inhibitors of the pyridoxal phosphate (PLP)-dependent enzyme L-ornithine carboxylyase (ODC, E.C. 4.1.1.17) which catalyzes the conversion of L-ornithine to putrescine, have been prepared.[2] A new and elegant approach to specific, irreversible enzyme inactivation is to design inhibitors possessing latent reactive groupings which are unmasked at the enzyme's active site as a result of the normal catalytic turnover.[3] Such known inhibitors are analogues of the normal enzyme substrate, but, less obviously, in view of the microscopic reversibility principle, they may conceptually be analogues of the product. This communication discloses that, not only the ornithine analogues **1,** but also the putrescine analogues 5-hexyne-1,4-diamine (**2**) and *trans*-hex-2-en-5-yne-1,4-diamine (**3**) are irreversible inactivators of ODC, and that, in each case, the mechanism of inhibition demands activation of the inhibitor by the target enzyme.

Incubation of the enzyme preparation, obtained from the livers of thioacetamide-treated rats[4] at pH 7 with **1a, 1b, 1c, 2,** and **3** in each case resulted in a time-dependent loss of enzyme activity which followed pseudo-first-order kinetics for at least two half-lives (Table I). Over longer time periods, the semilogarithmic plots deviated from linearity.[5] However, incubation with **1a,** the most efficient inhibitor among the ornithine analogues, or **2** at 0.1 mM concentration resulted in 95% inactivation of ODC after 10 min. Prolonged (24 h) dialysis of enzyme previously inactivated by **1a** or **2** against a buffer solution containing phosphate (30 mM), pyridoxal phosphate (0.1 mM), and dithiothreitol (5 mM) (conditions where the native enzyme is stable) did not lead to regeneration of enzyme activity, thus demonstrating the irreversibility of the process. That the inhibition of ODC is active site directed is shown by the protective effects of the natural substrate L-ornithine, of a competitive inhibitor 2-methylornithine[6] and of putrescine, the product of decarboxylation, against induced inactivation. The presence of dithiothreitol (5 mM) in the preincubation medium and the absence of lag time before the onset of inhibition rule out the possibility of inhibition via an affinity labeling mode by a diffusible alkylating species.

Table I. Kinetic Constants for the Irreversible Inhibition of Rat Liver ODC[a]

Compd	K_I, μM[c]	τ_{50}, min[c]	k_{inact}, s^{-1}[c]
1a	39	3.1	3.7×10^{-3}
1b	*d*	*d*	
1c	8700	29	0.4×10^{-3}
2	2.3	9.7	1.2×10^{-3}
[^{2}H]-**2**	4.3	9.6	1.2×10^{-3}
3	1	5	2.3×10^{-3}

[a] The composition of the stock solution of ODC follows: proteins (16 mg/mL), sodium phosphate buffer (30 mM, pH 7.1), dithiothreitol (5 mM), pyridoxal phosphate (0.1 mM). The specific activity of this stock solution was 0.12 nmol of CO_2/min per mg of protein. [b] For a typical experiment 320 μL of this stock solution were mixed at time 0 with 80 μL of a solution of inhibitor in water and incubated at 37 °C. At different times 50-μL aliquots were transferred into a 1-mL assay medium containing sodium phosphate (30 mM, pH 7.1), dithiothreitol (5 mM), pyridoxal phosphate (0.1 mM), L-ornithine (0.081 μmol), and DL-[1-^{14}C]ornithine (0.043 μmol, 58 Ci/mol, Amersham) in a closed vessel in which a filter paper moistened with 50 μL of hyamine hydroxide (1 M) was fitted. The reaction was allowed to proceed for 60 min at 37 °C and then terminated by the addition of 0.5 mL of 40% trichloroacetic acid. After an additional 30 min the CO_2 absorbed on the filter paper was counted in a standard scintillation cocktail. [b] Partially purified preparations of similar specific activity have been used by others[2b] in their assessment of potential irreversible inhibitors of ODC. [c] K_I (apparent dissociation constant), τ_{50} (half-life or $t_{1/2}$ at infinite concentration of inhibitor), and k_{inact} (inactivation rate constant) were calculated according to the method of Kitz and Wilson.[8] [d] No saturation kinetics were apparent with **1b;** at a concentration of 0.1 mM, $t_{1/2}$ is 22 min.

Further evidence for the involvement of the enzyme's active site in the inhibitory process comes from the observed saturation effect (Table I) on the rate of inactivation (demonstrated by plotting $t_{1/2}$ as a function of $1/I$ according to Kitz and Wilson[8]). Moreover, with both **1a** and **2,** the inhibitory activity resides with only one optical isomer ((−)-**1a** and (−)-**2**), the other isomer being essentially inactive.[9]

When the rate of inhibition induced by 4-deuterio-5-hexyne-1,4-diamine ([^{2}H]**2**) was compared with that observed with **2,** no kinetic isotope effect on the inactivation rate constant was observed, but rather a primary kinetic isotope effect on the apparent Michaelis constant is measured ($K_{H/D}$ = 1.9). Proton abstraction hence must occur, but is not rate limiting. Presumably, the rate-determining step involves covalent linkage of transformed inhibitor to the enzyme.

A straightforward mechanism (Scheme I) can be considered for the inactivation of ODC by **1a, 1b,** and **1c.** The first steps

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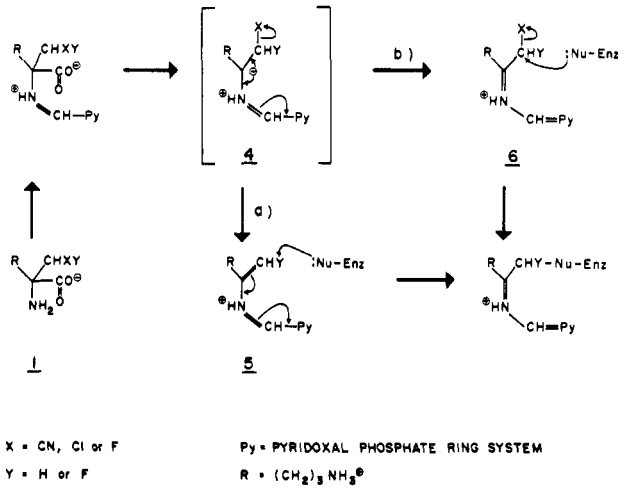
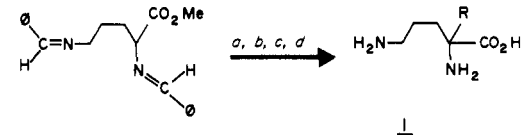
Scheme I



Scheme II. Synthesis of Ornithine Analogues I



	R	RX	Overall Yield,[e,f] %	Mp,[g]
1a	CHF_2	F_2CHCl	60	183
1b	CH_2Cl	$ClCH_2Br$	65	140
1c	CH_2CN	$NCCH_2Cl$	12[h]	260

[a] $LiN[CH(CH_3)_2]_2$. [b] RX. [c] 1 M HCl, room temperature, then 6 M HCl, reflux. [d] When R = CH_2CN, stepwise hydrolyses are required; 1 M HCl for removal of the benzylidene groups and NEt_3 in H_2O for cleavage of the methyl ester. [e] Not optimized. [f] Isolated as the monohydrochloride salt; **1a** crystallizes with 1 mol of H_2O. [g] Instantaneous from Kofler hot bank. [h] Low yield due to competitive formation of the corresponding piperidone derivative during the basic hydrolysis of the methyl ester.

involve enzymatic decarboxylation of the inhibitor to generate the intermediate carbanionic species **4** which can either induce the elimination of the leaving group X to produce the conjugated imine **5** (path a) or lead to formation of the tautomeric imine **6** in which the leaving group X is now in an activated allylic position (path b). The reactive imines **5** or **6** could then alkylate a nucleophilic residue (Nu in Scheme I) at or near the active site to covalently bind the inhibitor to the enzyme.[10] This process finds support first in the fact that α-amino acid decarboxylases are known in some cases (for example 3,4-dihydroxyphenylalanine[11] and histidine[12] decarboxylases) to decarboxylate the α-methyl analogue of their substrate, and second in the now well-documented irreversible inactivation of PLP-dependent enzymes catalyzing α-hydrogen abstraction from α-amino acids, such as transaminases and racemases, by substrate analogues bearing a halogen atom on the β-carbon atom.[13]

The mechanism by which **2** and **3** irreversibly inactivate ODC can be rationalized on the basis of the microscopic reversibility principle. It is proposed that, if these compounds can replace putrescine in the active site,[14] then the proton abstraction implicit in the reverse reaction should lead to propargylic anion formation, yielding an allene on protonation. Such an allene, being conjugated to the pyridoxaldimine function, should be a reactive Michael acceptor which could alkylate a nucleophilic residue in the active site.[15]

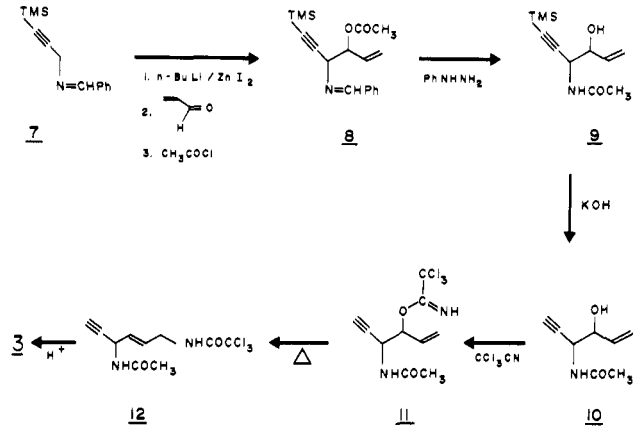
As expected from the postulated mechanisms of action, **1a** and **2** are specific inhibitors of ODC in vitro. When tested at millimolar concentrations, **1a** and **2** had no irreversible inhibitory effect on glutamic acid decarboxylase and aromatic amino acid decarboxylase. As **2** is an amine, it was also verified that it lacks inhibitory activity toward GABA-transaminase and diamine oxidase.

The synthesis of the ornithine analogues **1**[16] is depicted in Scheme II and relies on the reaction of the Schiff base methyl ester synthon[17] with bifunctional alkylating agents, followed by appropriate deprotection.

2 was prepared by alkylation of the anion derived from $TMSC{\equiv}CCH_2N{=}CHPh$ (**7**) with $Br(CH_2)_3N{=}CHPh$ (bp 120 °C at 0.05 mm), followed by acid hydrolysis (6 M HCl).[18,19]

The synthesis of **3** is shown in Scheme III. The anion derived from the protected propargylamine **7** underwent a regioselective 1,2 addition to acrolein, in the presence of ZnI_2, the resulting alcoholate being trapped with acetyl chloride to afford the aldimine **8**.[20] Treatment of crude **8** with $PhNHNH_2$ caused cleavage of the aldimine function with a concomitant O- to N-acyl shift leading to the allylic alcohol **9** (mp 116 °C, 29% from **3**). The trimethylsilyl group was removed to give **10**

Scheme III



(mp 94 °C, 88%) which, when treated with CCl_3CN as described by Overman,[21] afforded the allylic trichloroacetimidate **11**. **11** was not purified but was subjected to thermal rearrangement[21] in refluxing *m*-xylene to give the trichloroacetamide **12** (mp 158–159 °C, 40%). Removal of the amide functions was achieved with 3 M HCl and the diamine **3** purified as the dihydrochloride (mp 175 °C dec, 70%).

In conclusion, **1**, **2**, and **3** are the first reported irreversible inhibitors of ODC and are likely to be useful tools for assessing the biological roles of polyamines.[22] The new approach for the specific irreversible inhibition of decarboxylases by analogues of the substrate α-amino acid possessing an α-methyl group substituted by halogen atoms has proven to be of a general nature and its extension to the irreversible inactivation of other α-amino acid decarboxylases will be the subject of other reports. That the microscopic reversibility principle can be used to advantage in the design of catalytic irreversible inhibitors has been hitherto unexploited. In this connection we have also observed what appears to be a mechanistically identical inhibition of glutamic acid decarboxylase by 4-aminohex-5-ynoic acid.[23]

References and Notes

(1) (a) P. S. Mamont, P. Böhlen, P. P. McCann, P. Bey, F. Schuber, and C. Tardif, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 1626 (1976); (b) D. H. Russell, "Polyamines in Normal and Neoplastic Growth", Raven Press, New York, N.Y., 1973.
(2) (a) For a review, see H. G. Williams-Ashman, A. Corti, and B. Tardolini, *Ital. J. Biochem.*, **25**, 6 (1976); (b) N. Relyea and R. R. Rando, *Biochem. Biophys. Res. Commun.*, **67**, 392 (1975); (c) P. Bey, C. Danzin, V. van Dorssaeler, P. Mamont, M. J. Jung, and C. Tardif, *J. Med. Chem.*, **21**, 50 (1978).
(3) R. R. Rando, *Science*, **185**, 320 (1974).
(4) M. Ono, H. Inoue, F. Suzuki, and Y. Takeda, *Biochem. Biophys. Acta*, **284**, 285 (1972).
(5) P. Leff and W. G. Bardsley, *Biochem. Soc. Trans.*, **5**, 778 (1977). An al-

ternative explanation may be the presence of two isozymes. See M. F. Obenrader and W. F. Prouty, *J. Biol. Chem.*, **252**, 2860 (1977).
(6) M. M. Abdel-Monom, N. E. Newton, and C. E. Weeks, *J. Med. Chem.*, **17**, 447 (1974).
(7) R. R. Rando, *Biochem. Pharm.*, **23**, 2328 (1974).
(8) R. Kitz and I. B. Wilson, *J. Biol. Chem.*, **237**, 3245 (1962).
(9) (+)-**1a** ($[\alpha]^{20}_D$ +7° (*c* 0.48, MeOH)) and (−)-**1a** ($[\alpha]^{20}_D$ −10° (*c* 0.74, MeOH)) were obtained via resolution of DL-3-amino-3-difluoromethyl-2-piperidone with the enantiomeric binaphthylphosphoric acids as described by J. Jacques, C. Fouquet, and R. Viterbo, *Tetrahedron Lett.*, 4617 (1971). (±)-**2** was converted to the 6-phthalimido derivative which was partially resolved using (+)-camphorsulfonic acid. The free amines were then regenerated using hydrazine and the dihydrochlorides recrystallized to afford the individual isomers $[\alpha]_D$ +21° (*c* 0.78, MeOH) and $[\alpha]_D$ −25° (*c* 0.26, MeOH).
(10) Path a is more likely than path b, as a fluorine atom and a cyano group undergo E_2 elimination more easily than S_N2 substitution.
(11) W. Lovenberg, H. Weissbach, and S. Udenfriend, *J. Biol. Chem.*, **237**, 89 (1962).
(12) G. Kahlson, E. Rosenberg, and R. Thunberg, *J. Physiol.*, **169**, 467 (1963).
(13) R. B. Silvermann and R. H. Abeles, *Biochemistry*, **15**, 4718 (1976). For a review, see R. H. Abeles and A. L. Maycock, *Acc. Chem. Res.*, **9**, 313 (1976). Decarboxylases and PLP-dependent enzymes catalyzing α-hydrogen abstraction from α-amino acids generate similar carbanionic intermediates during their catalytic steps.
(14) Relyea and Rando[2b] have shown that *trans*-1,4-diaminobut-2-ene (dehydroputrescine) has an affinity for ODC more than 10 000 times that of putrescine itself and have suggested that this is due, in part, to a preferential binding of the extended conformer.
(15) The acetylene–allene rearrangement has been proposed in other cases to account for the irreversible inhibition of PLP-dependent enzymes, induced by the acetylenic analogue of the normal amine substrate. See (a) R. H. Abeles and C. T. Walsh, *J. Am. Chem. Soc.*, **95**, 6124 (1973); (b) R. C. Hevey, J. Babson, A. L. Maycock, and R. H. Abeles, *ibid.*, **95**, 6125 (1973); (c) R. R. Rando and J. de Mairena, *Biochem. Pharm.*, **23**, 463 (1974); (d) M. J. Jung and B. W. Metcalf, *Biochem. Biophys. Res. Commun.*, **67**, 301 (1975).
(16) NMR and IR spectra, as well as elemental analyses unless stated otherwise, of all new compounds described herein were in agreement with the proposed structure.
(17) P. Bey and J. P. Vevert, *Tetrahedron Lett.*, 1455 (1977).
(18) B. W. Metcalf and P. Casara, *Tetrahedron Lett.*, 3337 (1975). 4-Deuterio-5-hexyne-1,4-diamine was synthesized using $TMSC{\equiv}CCD_2N{=}CHPh$, which had been obtained from $TMSC{\equiv}CCH_2N{=}CHPh$ (**7**) by two sequential treatments with *n*-BuLi and CH_3OD. The $TMSC{\equiv}CCD_2N{=}CHPh$ thus obtained contained 6% H in the 1 position (as estimated by ^1H NMR). The 4-protio compound was not detected in the 4-deuterio-5-hexyne-1,4-diamine, presumably owing to the kinetic isotope effect during the proton abstraction step, prior to alkylation of $TMSC{\equiv}CCD_2N{=}CHPh$ with $Br(CH_2)_3N{=}CHPh$.
(19) We thank Dr. M. W. Gittos for the purification of the dihydrochloride of **2** (mp 170 °C).
(20) The 1,2 addition apparently proceeds with a high degree of stereoselectivity as only one of the possible diastereomers of the amide **9** was isolated. The configuration of this isomer is under investigation.
(21) L. E. Overman, *J. Am. Chem. Soc.*, **98**, 2901 (1976).
(22) In culture of rat-hepatoma cells,[1a] **1a** has been shown to inactivate ODC, to deplete putrescine and spermidine, and to drastically decrease cell proliferation: P. S. Mamont, M. C. Duchesne, J. Grove, and P. Bey, *Biochem. Biophys. Res. Commun.*, in press.
(23) B. W. Metcalf, M. J. Jung, B. Lippert, P. Casara, and C. Danzin, the 173rd National Meeting of the American Chemical Society, New Orleans, La., March 20–25, 1977, Abstracts of Papers; M. J. Jung, B. W. Metcalf, P. Bey, P. Lippert, C. Danzin, P. Böhlen, and P. J. Schechter, the 2nd Joint Conference of the American Chemical Society–Chemical Institute of Canada, Montreal, May 29–June 7, 1977, Abstracts of Papers.

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Existence and Stability of a Simple Sulfide of Iron(III)

Sir:

The existence of a simple sulfide of iron(III) has been debated periodically for several years.[1-8] The central difficulties that have produced much of the debate are, first, the apparent instability of the compound and second, the lack of a good method for characterizing insoluble amorphous or microcrystalline materials of this type. In view of the possible geological significance of iron(III) sulfide[9] and its relation[7] to iron-sulfur minerals (pyrite, marcasite) that are a major problem in the utilization of many coals, we have undertaken a detailed study of the chemical and physical properties of this material. We report here a synthesis, infrared characterization, and Mössbauer study with particular emphasis on the thermal stability of the compound. Previous reports[4,5] on the synthesis of Fe_2S_3 have casually mentioned Mössbauer measurements, but details (center shifts, quadrupole splittings, thermal effects) were not given.

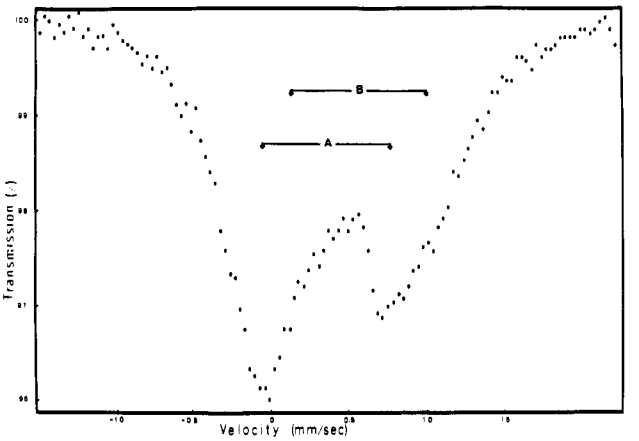

Figure 1. Mössbauer spectrum of iron(III) sulfide at 78 K (bar indicates error).

It is claimed that Fe_2S_3 can be obtained from iron(III) alkoxides and H_2S in organic solvents,[4] from ammoniacal solutions of iron(III) tartrates and HS^- ion,[4] from H_2S and $Fe_2O_3{\cdot}H_2O$[8] or suspensions of $Fe(OH)_3$ at pH 9.5,[5] and by titrating $FeCl_3$ with Na_2S.[10] The first two methods give products that are heavily contaminated and, importantly, all previous preparations apparently were carried out at room temperature, or higher.

We have prepared[11] iron(III) sulfide in an amorphous form by treating stoichiometric quantities of aqueous Fe(III) with aqueous Na_2S at 0 °C or below. Examination of the material with a scanning electron microscope equipped with an energy dispersive x-ray spectrometer confirmed the fact that sodium and chloride were not present and that the atomic ratio of Fe:S is 1:1.5. Thus at least on an empirical basis, the material prepared is Fe_2S_3. The Mössbauer spectrum[12] of the compound at 78 K is shown in Figure 1; this spectrum is basically different from that of any of the known iron sulfides or compounds such as $NaFeS_2$.[6] The spectrum was analyzed by using a nonlinear least-square fitting program assuming Lorentzian line shapes. The best fit was obtained with two doublets with the following center shifts (CS) and quadrupole splittings (QS): doublet A, CS = 0.35 ± 0.06 mm/s, ΔE_Q = 0.82 ± 0.06 mm/s; doublet B, CS = 0.51 ± 0.12 mm/s, ΔE_Q = 0.88 ± 0.12 mm/s. These results indicate that there are two different environments for the iron ions in the compound, both with a symmetry lower than cubic. The observed CS is consistent with iron(III) or low-spin iron(II).[13] In the latter case the ground state is nonmagnetic, as in pyrite, FeS_2.[14]

In order to characterize fully the charge state of the iron ion, Mössbauer measurements were carried out at 4.2 K. A six-line pattern with relatively broad line widths was obtained, as shown in Figure 2. The hyperfine magnetic field at the ^{57}Fe nucleus is 253 ± 6 kOe.[15] The presence of a magnetic splitting at 4.2 K completely excludes the presence of iron(II), confirming the formation of the compound as a sulfide of iron(III). It is to be noted that the presence of a similar hyperfine magnetic field for the two sites of iron excludes the possibility of having two different compounds. The value observed for the splitting is much smaller than that expected for high-spin iron(III),[16] indicating a high degree of covalency. In order to determine the type of order in this compound, measurements

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