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- [10] Crystal structure analysis: General: Rigaku AFC7S diffractometer,  $T = 23^\circ\text{C}$ ,  $\text{MoK}\alpha$  radiation ( $\lambda = 0.71069 \text{ \AA}$ ). The structures were solved by direct methods (SIR92 for **1** and SHELXS-86 for **2**) and refined by full-matrix least-squares methods on  $F^2$  by using SHELXL-93. Hydrogen atoms, except for those of water molecules, were placed at calculated positions but were not refined. The absolute structures for **1** and **2** were determined on the basis of the known  $R$  configuration of the asymmetric carbon atom in the L-cys ligand and were confirmed by the Flack parameters ( $-0.10(6)$  for **1** and  $0.03(3)$  for **2**). **1**:  $\text{C}_7\text{H}_{23}\text{AgCoN}_7\text{O}_9\text{S}$ , crystal dimensions  $0.10 \times 0.15 \times 0.15 \text{ mm}$ , orthorhombic, space group  $P2_12_12_1$ ,  $a = 8.037(4)$ ,  $b = 8.056(3)$ ,  $c = 26.648(6) \text{ \AA}$ ,  $V = 1725.4(9) \text{ \AA}^3$ ,  $Z = 4$ ,  $\rho_{\text{calc}} = 2.110 \text{ g cm}^{-3}$ ,  $\mu = 22.75 \text{ cm}^{-1}$ ,  $\omega/2\theta$  scan mode,  $2\theta_{\text{max}} = 55.0$ , 2298 reflections collected, 2296 independent reflections, 1815 observed reflections ( $F > 4\sigma(F)$ ), 235 parameters, empirical absorption corrections based on  $\psi$  scans, max./min. transmission 0.992/0.882,  $R_1 = 0.054$  ( $F > 4\sigma(F)$ ),  $wR_2 = 0.155$  (all data), residual electron density  $3.04/-1.24 \text{ e \AA}^{-3}$  (peaks larger than  $1.0 \text{ e \AA}^{-3}$  were found in the vicinity of heavy atoms). **2**:  $\text{C}_7\text{H}_{26.33}\text{AgCoN}_7\text{O}_{10.67}\text{S}$ , crystal dimensions  $0.13 \times 0.14 \times 0.60 \text{ mm}$ , trigonal, space group  $R3$ ,  $a = 24.115(3)$ ,  $c = 8.417(3) \text{ \AA}$ ,  $V = 4238(1) \text{ \AA}^3$ ,  $Z = 8$ ,  $\rho_{\text{calc}} = 1.812 \text{ g cm}^{-3}$ ,  $\mu = 18.63 \text{ cm}^{-1}$ ,  $\omega/2\theta$  scan mode,  $2\theta_{\text{max}} = 55.0$ , 2398 reflections collected, 2170 independent reflections, 2076 observed reflections ( $F > 4\sigma(F)$ ), 268 parameters, empirical absorption corrections based on  $\psi$  scans, max./min. transmission 0.997/0.916,  $R_1 = 0.038$  ( $F > 4\sigma(F)$ ),  $wR_2 = 0.116$  (all data), residual electron density  $0.84/-0.70 \text{ e \AA}^{-3}$ . Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-153429 (**1**) and CCDC-153430 (**2**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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- [12] The 2:1 reaction of  $\Delta_L\text{-[Co(L-cys-N,S)(en)]}_2(\text{ClO}_4)$  with  $\text{AgClO}_4$  in water gave the 2:1 adduct  $\Delta_L\Delta_L\text{-[Ag[Co(L-cys-N,S)(en)]}_2(\text{ClO}_4)_3 \cdot 4\text{H}_2\text{O}$  (**4**). X-ray analysis demonstrated that in the complex cation of **4**, the central  $\text{Ag}^{\text{I}}$  ion is coordinated by two thiolate S atoms of two octahedral  $\Delta_L\text{-[Co(L-cys-N,S)(en)]}_2^+$  units to form an S-bridged  $\text{Co}^{\text{III}}\text{Ag}^{\text{I}}\text{Co}^{\text{III}}$  trinuclear structure ( $\text{Ag-S } 2.393(1)$ ,  $\text{Co-S } 2.263(1) \text{ \AA}$ ;  $\text{S-Ag-S } 172.39(6)^\circ$ ). The corresponding 2:1 adduct  $\Delta_L\Delta_L\text{-[Ag[Co(L-cys-N,S)(en)]}_2(\text{ClO}_4)_3 \cdot 4\text{H}_2\text{O}$  (**5**) was prepared by using  $\Delta_L\text{-[Co(L-Hcys-N,S)(en)]}_2(\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$  instead of  $\Delta_L\text{-[Co(L-cys-N,S)(en)]}_2(\text{ClO}_4)$ . It was confirmed that **5** also has an S-bridged  $\text{Co}^{\text{III}}\text{Ag}^{\text{I}}\text{Co}^{\text{III}}$  trinuclear structure, from its elemental and plasma emission spectral analyses, electronic absorption and CD spectra, and molar conductivity in water ( $303 \text{ \Omega cm}^2 \text{ mol}^{-1}$ ), which is in good agreement with that of **4** ( $307 \text{ \Omega cm}^2 \text{ mol}^{-1}$ ).
- [13] In agreement with this assumption, a noticeable difference was observed in the solid-state CD spectra (Nujol paste) of **2** and **3**, though their CD spectra in water are essentially identical; **2** exhibits a major negative CD band at 521 nm in the solid state, while the corresponding negative band of **3** appears at much longer wavelength of 551 nm.
- [14] As indicated by one of the referees, the solid-state structures of **1** and **2** would not be retained in solution; conversion to monomers and/or oligomers would result from cleavage of Ag-S and Ag-O bonds. This is suggested by the fact that the CD spectra of **1** and **2** in water are very similar to those of **4** and **5**, respectively.
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## Diiron Azadithiolates as Models for the Iron-Only Hydrogenase Active Site: Synthesis, Structure, and Stereoelectronics\*\*

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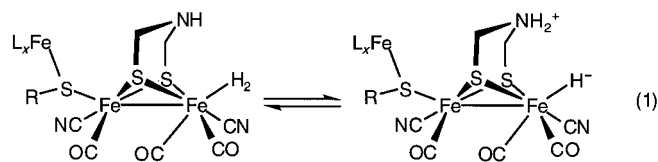
The active site of the Fe-only hydrogenases ( $\text{FeH's}$ )<sup>[1,2]</sup> resemble archetypal  $[\text{Fe}_2(\text{SR})_2(\text{CO})_6]$  derivatives first reported by Reihlen in 1928<sup>[3]</sup> and feature four unusual ligands: CO,  $\text{CN}^-$ ,  $[(\text{RS})_3\text{Fe}_4\text{S}_4(\text{SR})]$ , and a unique organic dithiolate cofactor. Recent work has suggested that this dithiolate is

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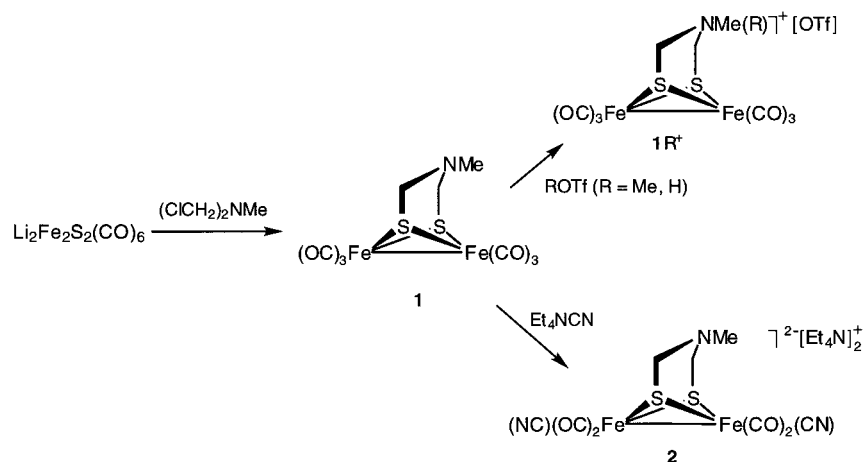
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$\text{HN}(\text{CH}_2\text{S}^-)_2$ .<sup>[4]</sup> An amine-containing cofactor could participate in proton transfer required in the heterolytic processing of  $\text{H}_2$  [Eq. (1)]. Indeed, classical organometallic studies have shown that ligand-appended, noncoordinating amines facilitate the heterolytic activation of dihydrogen,<sup>[5]</sup> a key facet of the chemistry of the hydrogenases.<sup>[6]</sup> Herein we report diiron carbonyl derivatives of the *N*-methyl analogue of the proposed cofactor.



Treatment of  $\text{Li}_2[\text{Fe}_2\text{S}_2(\text{CO})_6]$ <sup>[7]</sup> with  $(\text{ClCH}_2)_2\text{NMe}$  afforded  $[\text{Fe}_2\{(\text{SCH}_2)_2\text{NMe}\}(\text{CO})_6]$  (**1**) as red crystals in 50% yield (Scheme 1). The NMR and infra-red (IR) spectroscopic analysis indicate that the amine is not coordinated. In the  $^1\text{H}$  NMR spectrum the resonance signal for  $\text{CH}_2$  splits at  $-60^\circ\text{C}$  consistent with slowed folding of the  $\text{FeS}_2\text{C}_2\text{N}$  ring, however, only one methyl resonance signal was observed  $> -80^\circ\text{C}$ . Protonation of **1** in  $\text{Et}_2\text{O}$  solution with HOTf (OTf = trifluoromethanesulfonate) precipitated orange  $[\text{Fe}_2\{(\text{SCH}_2)_2\text{N}(\text{H})\text{Me}\}(\text{CO})_6]^+$  (**1**· $\text{H}^+$ ). Solutions of **1**· $\text{H}^+$  in MeCN are fully deprotonated by aniline but not by 4-cyanoaniline, thus placing its  $\text{pK}_a$  between 10.6 and 7.6, which is lower than that typical for a tertiary amine ( $\text{pK}_a$  18).<sup>[8]</sup> In the IR spectrum, the  $\nu_{\text{CO}}$  pattern for **1**· $\text{H}^+$  resembles that for **1**, but the bands are shifted  $15\text{ cm}^{-1}$  to higher energy, which may be relevant to the IR spectroscopic signatures of the  $\text{FeH}^+\text{s}$ .<sup>[9]</sup> The  $\nu_{\text{CO}}$  pattern is identical for **1**· $\text{H}^+$  and **1**· $\text{Me}^+$ , (an orange solid from  $\text{MeOTf} + \mathbf{1}$ ), indicating that the observed shifts in the  $\nu_{\text{CO}}$  bands for **1**· $\text{H}^+$  are not the result of proton–CO interactions nor does protonation occur at other sites, such as, the Fe–Fe bond.<sup>[10]</sup> Protonation has no effect on the  $\nu_{\text{CO}}$  bands in the *N*-methylpiperidine-4-thiolate  $[\text{Fe}_2(\text{S}-4\text{-C}_5\text{H}_9\text{NMe})_2(\text{CO})_6]$  because the amine is some distance from the dimetal center.<sup>[11]</sup>



Scheme 1.

Crystallographic analysis of **1** reveals the familiar  $[\text{Fe}_2(\text{SR})_2(\text{CO})_6]$  framework but the details of the azadithiolate strap are noteworthy (Figure 1). Two conformers cocrystallize; in the dominant (60%) conformer the methyl group is *axial*, which is normally disfavored.<sup>[12]</sup> For this axial conformer, the sum of angles at nitrogen is  $352^\circ$  versus  $337^\circ$  for the minor (equatorial) isomer. The C–S distance is  $1.86(8)\text{ \AA}$  (average of the two conformers) versus  $1.821(5)\text{ \AA}$  in the related propanedithiolate.<sup>[13]</sup>

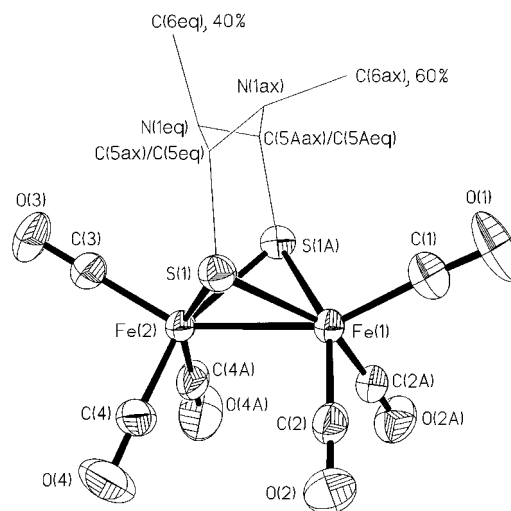


Figure 1. Structure of the axial (atoms labeled ax) and equatorial (eq) conformers of **1**. Selected bond lengths [ $\text{\AA}$ ] and angles [ $^\circ$ ] for the axial conformer: Fe(1)–Fe(2) 2.4924(7), Fe(1)–S(1) 2.2600(7), S(1)–C(5) 1.88(2), N(1)–C(5) 1.40(2), Fe(2)–S(1)–Fe(1) 66.96(2).

Density functional theory (DFT) calculations<sup>[14]</sup> were carried out on the axial and equatorial conformers of **1** and the cyclic model molecules  $\text{CH}_2(\text{SCH}_2)_2\text{NR}$  ( $\text{R} = \text{H}$  and  $\text{CH}_3$ ). Stabilization of the axial conformer of **1** and the models can be explained in the context of the anomeric effect, which is classically invoked to explain axial–equatorial equilibria in 1-oxo-3-azacyclohexanes.<sup>[15]</sup> Calculations confirm that the structure of **1** results from a balance between the anomeric effect, which favors the axial methyl, and a competing steric repulsion between  $(\text{CH}_3)_{\text{ax}}$  and the carbonyl ligand lying beneath ( $\text{H}\cdots\text{C} = 2.47\text{ \AA}$ ;  $\text{H}\cdots\text{O} = 2.57\text{ \AA}$ ). This unfavorable nonbonding interaction is offset somewhat by a flattening of the amine, which further enhances the anomeric interaction (Figure 2). In all cases, the elongation of the C–S bond provides an easy gauge of the energetic significance of the anomeric effect (Table 1, note DFT calculated distances are slightly elongated). Replacing Me by H in **1** relieves much of the steric strain such that the axial form displays a relative stability of  $5.7\text{ kcal mol}^{-1}$ .

Treatment of **1** with  $\text{Et}_4\text{NCN}$  following our previous protocol<sup>[16]</sup> afforded the dicyanide complex  $(\text{NET}_4)_2[\text{Fe}_2\{(\text{SCH}_2)_2-$

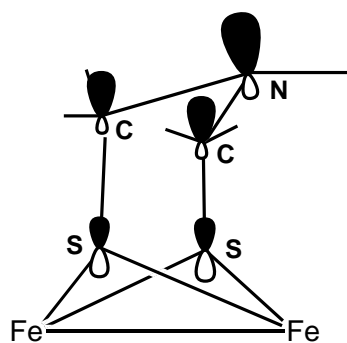


Figure 2. Orbital interactions associated with the anomeric effect in the axial conformer of  $[\text{Fe}_2\{(\text{SCH}_2)_2\text{NMe}\}(\text{CO})_6]$ , as indicated by DFT calculations.

Table 1. Selected results for DFT calculations on the axial (ax) and equatorial (eq) conformers of  $\text{CH}_2(\text{SCH}_2)_2\text{NR}$  and  $[\text{Fe}_2\{(\text{SCH}_2)_2\text{NR}\}(\text{CO})_6]$ .

Compound (conformation at N)	Relative $E$ [kcal mol $^{-1}$ ]	C–S [Å]	C–N [Å]	$\Sigma \chi$ at N [°]
$\text{CH}_2(\text{SCH}_2)_2\text{NH}$ (ax)	0	1.883	1.444	336.0
$\text{CH}_2(\text{SCH}_2)_2\text{NH}$ (eq)	+7.3	1.860	1.448	337.4
$\text{CH}_2(\text{SCH}_2)_2\text{NMe}$ (ax)	0	1.894	1.440	343.6
$\text{CH}_2(\text{SCH}_2)_2\text{NMe}$ (eq)	+6.9	1.853	1.454	336.1
$[\text{Fe}_2\{(\text{SCH}_2)_2\text{NH}\}(\text{CO})_6]$ (ax)	0	1.907	1.429	338.1
$[\text{Fe}_2\{(\text{SCH}_2)_2\text{NH}\}(\text{CO})_6]$ (eq)	+5.7	1.878	1.437	341.8
$[\text{Fe}_2\{(\text{SCH}_2)_2\text{NMe}\}(\text{CO})_6]$ (ax)	0	1.925	1.422	350.3
$[\text{Fe}_2\{(\text{SCH}_2)_2\text{NMe}\}(\text{CO})_6]$ (eq)	+0.8	1.866	1.445	335.7
$[\text{Fe}_2\{(\text{SCH}_2)_2\text{NMe}\}(\text{CO})_6]$ (planar)	+3.6	1.963	1.405	360.0

$\text{NMe}\}(\text{CO})_4(\text{CN})_2]$  (**2**; Scheme 1). Only the axial conformer of **2** crystallizes (Figure 3). Again the nitrogen approaches planarity ( $\Sigma \chi = 347^\circ$ ), and the C–S bond is elongated (1.869(3) Å). Noteworthy is the similarity in the IR spectrum of **2** and the reduced H-cluster from *D. desulfuricans*.<sup>[4]</sup> The  $\nu_{\text{C-X}}$  (X = O, N) bands for the model are at 2075, 2032, 1963, 1922, 1884, 1875  $\text{cm}^{-1}$  while for the reduced enzyme, these bands occur at 2079, 2041, 1965, 1940, 1916, 1894  $\text{cm}^{-1}$ .

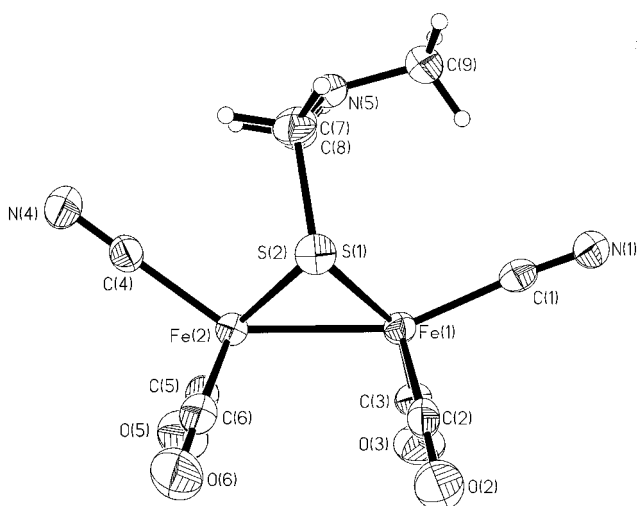


Figure 3. Structure of **2** (as  $\text{Et}_4\text{N}^+$  salt), selected bond lengths [Å] and angles [°]: Fe(1)–Fe(2) 2.5090(6), Fe(1)–C(2) 1.745(3), Fe(1)–C(3) 1.751(3), Fe(1)–C(1) 1.940(3), C(7)–N(5) 1.424(4), Fe(1)–S(1) 2.2871(9); Fe(1)–S(2)–Fe(2) 66.49(2).

## Experimental Section

**1**: A THF solution of  $\text{Li}_2[\text{Fe}_2\text{S}_2(\text{CO})_6]$ <sup>[7, 17]</sup> (0.120 g, 0.349 mmol; 20 mL) at  $-78^\circ\text{C}$  was treated with a THF solution of  $(\text{CH}_2\text{Cl})_2\text{NCH}_3$  (0.054 g, 0.419 mmol; 5 mL).<sup>[18]</sup> After 2 h, the red solution was treated with  $\text{NEt}_3$  (0.10 mL), and allowed to warm to room temperature. Red crystals grew from cold hexane solutions, yield 0.071 g (51%). Elemental analysis (%) calcd for  $\text{C}_9\text{H}_7\text{Fe}_2\text{NO}_6\text{S}_2$ : C 26.96, H 1.76, N 3.49; found: C 27.07, H 1.89, N 3.27;  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{CN}$ ,  $25^\circ\text{C}$ ):  $\delta = 3.31$  (s, 4H;  $\text{NCH}_2\text{S}$ ), 2.12 (s, 1H;  $\text{NCH}_3$ ); IR (MeCN):  $\tilde{\nu}_{\text{CO}} = 2074, 2037, 1996 \text{ cm}^{-1}$ .

Protonation of **1**: An orange MeCN solution of **1** (0.043 g, 0.11 mmol; 10 mL) lightened upon acidification with one equivalent of  $\text{HO}_3\text{SCF}_3$ , IR (MeCN):  $\tilde{\nu}_{\text{CO}} = 2090, 2053, 2017 \text{ cm}^{-1}$ . Treatment of  $\mathbf{1} \cdot \text{H}^+$  with  $4\text{-H}_2\text{NC}_6\text{H}_4\text{CN}$  (0.013 g, 0.107 mmol) resulted in no change. Treatment of  $\mathbf{1} \cdot \text{H}^+$  with  $\text{PhNH}_2$  (0.010 mL, 0.107 mmol) gave free **1** as indicated by both the color change and the IR spectrum.

Methylation of **1**: A solution of **1** (0.035 g, 0.087 mmol) in MeCN (10 mL) was treated with  $\text{CH}_3\text{O}_3\text{SCF}_3$  (0.015 mL, 1.31 mmol). After 30 min, the IR bands for the starting material had disappeared. IR (MeCN):  $\tilde{\nu}_{\text{CO}} = 2090, 2053, 2018 \text{ cm}^{-1}$ .

**2**: A solution of **1** (0.10 g, 0.25 mmol) in  $\text{CH}_3\text{CN}$  (10 mL) was treated with a solution of  $\text{Et}_4\text{NCN}$  (0.073 g, 0.49 mmol) in MeCN (5 mL), causing gas evolution. After 1 h, the reaction solution was concentrated to  $\sim 1$  mL, and the red product was precipitated with  $\text{Et}_2\text{O}$ , yield: 0.080 g (50%). Elemental analysis (%) calcd for  $\text{C}_{25}\text{H}_{47}\text{Fe}_2\text{N}_5\text{O}_4\text{S}_2$ : C 45.67, H 7.20, N 10.65; found: C 45.74, H 7.23, N 10.52;  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{CN}$ ,  $25^\circ\text{C}$ ):  $\delta = 3.42$  (s, 4H;  $\text{NCH}_2\text{S}$ ), 3.16 (q, 16H;  $\text{NCH}_2\text{CH}_3$ ), 2.47 (s, 3H;  $\text{NCH}_3$ ), 1.22 (t, 24H;  $\text{NCH}_2\text{CH}_3$ ); IR (MeCN):  $\tilde{\nu}_{\text{CN}} = 2075, 2032$ ;  $\tilde{\nu}_{\text{CO}} = 1963, 1922, 1884, 1875 \text{ cm}^{-1}$ .

Density functional theory calculations were carried out using generalized gradient approximation (GGA), as implemented in the ADF program.<sup>[19]</sup> The formalism is based upon the local spin-density approximation characterized by the electron gas exchange ( $X\alpha$  with  $\alpha = 2/3$ ) together with Vosko–Wilk–Nusair parameterization for correlation.<sup>[20]</sup> Nonlocal corrections have been added for the exchange energy<sup>[21]</sup> and the correlation energy.<sup>[22]</sup> For first-row atoms, the 1s shell was frozen and described by a single Slater function. For iron and sulfur the frozen core composed of the 1s and 2s/p shells was also modeled by a minimal Slater basis. The Slater basis set for the valence shell of all nonmetal atoms is of triple- $\zeta$  quality and is supplemented with a polarization function. The 3s and 3p shells of iron are described by a double- $\zeta$  Slater basis, the 3d and 4s shells by a triple- $\zeta$  basis, and the 4p shell by a single orbital.

Crystals of **1** and **2** were mounted on glass fibers using Paratone-N (Exxon) and were analyzed on a Siemens Platform/CCD automated diffractometer at 193 K. Data were processed with SHELXTL. The structures were solved using direct methods and refined using full matrix least-squares on  $F^2$  using the program SHELXL-93. Hydrogen atoms were fixed in idealized positions with thermal parameters 1.2 times those of the attached carbon atoms. Data were corrected for absorption on the basis of integration.

Crystal data for **1**:  $\text{C}_9\text{H}_7\text{Fe}_2\text{NO}_6\text{S}_2$ ,  $M = 400.98$ , monoclinic,  $C2/m$ ;  $a = 16.246(2)$ ,  $b = 10.0990(14)$ ,  $c = 8.8964(12)$  Å;  $\beta = 92.086(2)^\circ$ ;  $V = 1458.7(3)$  Å $^3$ ;  $Z = 4$ ;  $\rho_{\text{calcd}} = 1.826 \text{ Mg m}^{-3}$ ,  $F(000) = 800$ , 120 parameters:  $R1 = 0.0322$ ,  $wR2 = 0.0715$ ,  $\text{GOF} = 1.035$  for all 1410 data ( $I > 2\sigma(I)$ ); max./min. residual electron density:  $0.494/-0.348 \text{ e}^- \text{ Å}^{-3}$ .

Crystal data for **2**:  $\text{C}_{25}\text{H}_{47}\text{Fe}_2\text{N}_5\text{O}_4\text{S}_2$ ;  $M = 657.50$ ; orthorhombic,  $P2_12_12_1$ ;  $a = 11.9403(18)$ ,  $b = 14.043(2)$ ,  $c = 18.862(3)$  Å;  $V = 3162.8(8)$  Å $^3$ ;  $Z = 4$ ;  $\rho_{\text{calcd}} = 1.381 \text{ Mg m}^{-3}$ ,  $F(000) = 1392$ , 352 parameters:  $R1 = 0.0390$ ,  $wR2 = 0.0722$ ,  $\text{GOF} = 0.916$  for all 5791 data ( $I > 2\sigma(I)$ ); max./min. residual electron density:  $0.327/-0.265 \text{ e}^- \text{ Å}^{-3}$ .

Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-155386 (**1**) and CCDC-155387 (**2**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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## An Enzyme Assay Using pM\*\*

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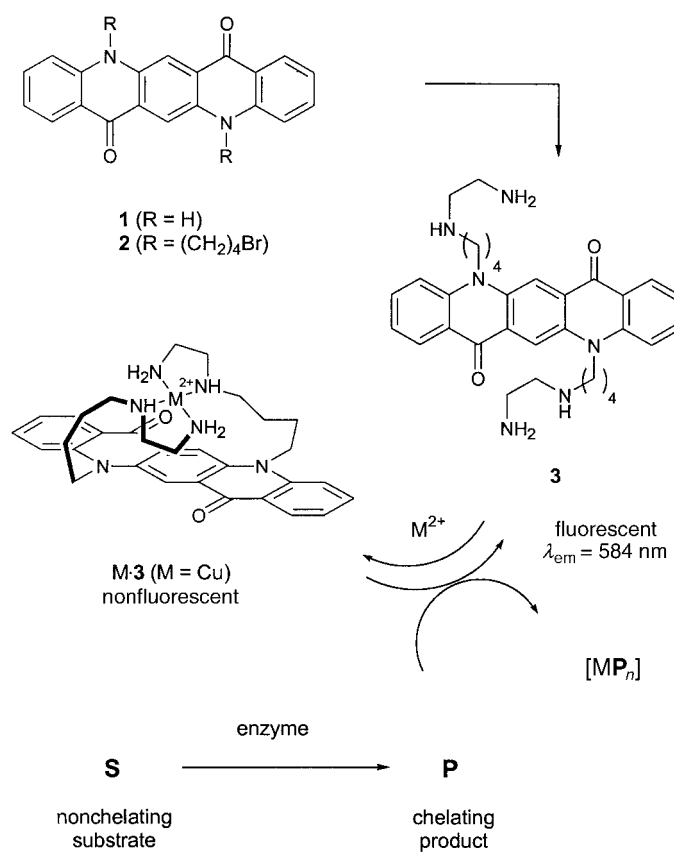
The development of new catalytic methods, in particular enzymatic processes, is increasingly being followed with combinatorial and evolutionary methods, whereby sensitive assays for catalysis play an essential role.<sup>[1–3]</sup> Most enzyme assays are based on chromogenic or fluorogenic substrates.<sup>[1, 4, 5]</sup> In many cases, however, it is desirable to measure the reaction of a well-defined substrate of interest, and not

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that of a different fluorogenic or chromogenic derivative. Such direct detection can be achieved with instrumental methods, such as HPLC and mass spectrometry,<sup>[6]</sup> by using product-selective fluorescent sensors,<sup>[7]</sup> or with indirect colorimetric assays based on substrate- or product-binding proteins, such as cat-ELISAs (catalytic enzyme-linked immunosorbent assays)<sup>[8]</sup> or the QUEST method (“querying for enzymes using the three-hybrid system”).<sup>[9]</sup> More simply, one can also measure spectrophotometrically a physico-chemical parameter that responds to the progress of the reaction, such as measuring the temperature by IR thermography,<sup>[10]</sup> or, for reactions releasing acids or bases, the pH value of the reaction medium with pH indicators.<sup>[11]</sup> Herein we report a new enzyme assay based on following the evolution of pM (−lg[M], where M is free metal ions) as a function of reaction time. The change in pM is recorded using an orange fluorescent metal-sensor derived from quinacridone.

The orange fluorescent sensor **3**, which is obtained from quinacridone (**1**) via dibromide **2**, responds to the presence of small concentrations of Cu<sup>2+</sup> by an almost quantitative quenching of its fluorescence ( $\lambda_{em(max)} = 558$  nm). The effect can be explained by formation of a macrocyclic chelate M·**3**, in which energy transfer quenching occurs due to the proximity of the quenching metal ion to the fluorophore (Scheme 1).<sup>[12]</sup> The complex M·**3** contains a 17-membered ring. The chelate effect induced by such a large ring is expected to be weak. Indeed the macrocycle is readily broken by addition of excess metal ion, leading to the formation of an M<sub>2</sub>·**3** complex. This suggests that other weak metal chelators,



Scheme 1. Principle of the fluorescence enzyme assay using pM.