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N_2 Binding to an Iron-Sulfur-Carbon Site

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Abstract

Nitrogenases are found in some microorganisms, and these enzymes convert atmospheric N_2 to ammonia, thereby providing essential nitrogen atoms for higher organisms. Some nitrogenases reduce atmospheric N_2 at the FeMoco, a sulfur-rich iron-molybdenum cluster[1–5]. The iron centers that are coordinated to sulfur and carbon atoms in FeMoco have been proposed as the substrate binding sites, based on kinetic and spectroscopic studies[5,6]. Studies on the enzyme indicate that iron atom Fe6 and possibly also adjacent belt iron sites are involved.[5–8] In the resting state, the central Fe sites (including Fe6) have identical environments consisting of three sulfides and a carbide. Addition of electrons to the resting state causes the FeMoco to react with N_2, but the geometry and bonding environment of N_2-bound species remain unknown[5]. In this manuscript, we describe a synthetic complex with a sulfur-rich coordination sphere that, upon reduction, breaks an Fe-S bond and binds N_2. The product is the first synthetic Fe–N_2 complex in which iron has bonds to sulfur and carbon atoms, providing a model for N_2 coordination in the FeMoco. Our results demonstrate that breaking an Fe-S bond is a chemically reasonable route to N_2 binding in the FeMoco, and show structural and spectroscopic details for weakened N_2 on a sulfur-rich iron site.

N_2 binding to FeMoco is believed to take place at an iron center with three sulfur ligands following Fe-C bond elongation or dissociation (I to II, Fig. 1A)[9–15]. These sites could accommodate N_2 binding by breaking an Fe-S or an Fe-C bond, but data on the enzyme do not yet distinguish between these possibilities. The likely enzymatic intermediates, iron-N_2 species supported either solely by sulfur, or by sulfur and carbon ligands, are unprecedented in isolated coordination complexes. Here, we focus on an alternative hypothesis where one of the Fe-S bonds at the active site is broken upon reduction/protonation to expose the N_2

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Supplementary Information is available in the online version of the paper.

Author Contributions I.Č. designed the iron-sulfur-carbon system for N_2 binding, performed the laboratory experiments, and analyzed data. B.Q.M. collected and interpreted crystallographic data. E.B. interpreted solid-state (SQUID) magnetic data. D.J.V. collected and fit EPR data. P.L.H. supervised the research, and I.Č. and P.L.H. wrote the manuscript.

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binding site (**I** to **III**, Fig. 1A)[16,17]. N_2 would thus bind at a pseudotetrahedral S,S,C-bound iron site. The feasibility of Fe-S bond cleavage in FeMoco is experimentally supported by the loss of this S atom in the structure of CO-inhibited nitrogenase[7], and by the observation of Fe-S cleavage upon protonation in smaller FeS clusters[18,19]. Other N_2 binding hypotheses include side-on binding, bridging, and *endo* coordination where N_2 is positioned close to three additional iron atoms and opposite to a sulfur atom (**IV**, Fig. 1A)[5,11,17].

Iron-N_2 complexes supported solely by sulfur, or by sulfur and carbon supporting ligands, are likely N_2-bound species in the nitrogenase catalytic cycle, but they are experimentally unprecedented. Though chemists have prepared complex iron-sulfur clusters inspired by the multimetallic structure of FeMoco, N_2 does not bind to any known synthetic iron-sulfur cluster[20]. A number of well-defined iron complexes with B, N, and P supporting ligands are known to activate N_2, and Peters has established P- and C-based systems capable of performing catalytic reduction of N_2 to ammonia[14,15,21–24]. A few iron-N_2 complexes have thioether/thiolate donors on the same iron center, and each is additionally supported by P- or N- donors[25–27]. To the best of our knowledge, there are no examples of terminal N_2 complexes of any metal having immediate ligand environments similar to those in **II**-**IV**, which hinders scientists' ability to predict the behavior of the FeMoco.

For this work, we designed bis(thiolate) ligand $\mathbf{L}^{2-}$, which offers only sulfur and carbon based coordination sites (indicated by yellow and grey spheres in Fig. 1B). Our approach was guided by the proposed binding mode **III** in Fig. 1A, which requires the presence of two coordinating sulfur atoms. These are provided by two chelating arylthiolate donors with bulky 2,4,6-triisopropylphenyl groups shielding the S sites. A central aromatic ring connects the two arylthiolate arms and additionally provides potential carbon based attachment sites[28]. Although carbide is electronically different than the arene ring in $\mathbf{L}^{2-}$, each could provide flexible bonding for stabilization of various intermediates during ammonia production[14,15].

Iron(II) ions were installed in the ligand framework by treating $\mathbf{LH_2}$ with iron(II) bis(bis(trimethylsilyl)amide) in tetrahydrofuran (THF), which yielded the bright yellow, high spin iron(II) complex $LFe(THF)_2$ (**1**, Fig. 2A). Its crystal structure reveals that it is four-coordinate, and that all Fe-C distances are at least 2.59 Å (Fig. 2B). Reduction of **1** to iron(I) with potassium graphite (KC_8) results in the formation of brown-yellow **2**, with close Fe-C distances (2.04 – 2.12 Å) indicating η^6-binding of the central arene ring (Fig. 2A, 2C). Comparison of the molecular structures of **1** and **2** reveals that rotation of the arylthiolate arms enables the central aryl ring to move closer to the iron atom. Compound **2** has a rhombic EPR (electron paramagnetic resonance) spectrum with $\mathbf{g}$ = [2.180, 2.020, 1.989] and a solution magnetic moment of 2.1 μ_B, which indicate a low-spin ($S = 1/2$) iron(I) center.

Encouraged by the ability of the ligand to stabilize low-valent iron sites, we further reduced the iron site to the iron(0) oxidation state. Reduction of a brown-yellow solution of **2** with one equivalent of KC_8 under one atmosphere of N_2 at −70 °C resulted in an immediate color change to deep red. After addition of 18-crown-6 to sequester potassium cations, dark red-brown crystals of **3** grew at −40 °C. X-ray diffraction analysis shows that **3** is $[LFeN_2]$

$[K(18\text{-crown-6})(THF)_2]_2$ (Fig. 2A and 3A). In **3**, N_2 is bound as a terminal ligand at a pseudotetrahedral iron(0) site, which is further bound to two S atoms and the arene of the supporting ligand. The closest Fe-C distance in **3** is 2.04 Å, and there is a second carbon atom within bonding distance (Fe-C = 2.24 Å), indicating asymmetric η^2 coordination of the arene. The potassium cations do not bind to the N_2 ligand.

The new N_2 complex **3** provides a structural model of the pseudotetrahedral S,S,C supported N_2 binding mode **III** proposed for FeMoco (Fig. 1A). It is compared to the experimental structures of resting state FeMoco and CO-inhibited FeMoco in Fig. 3B[2,7]. In the fourth coordination site that has labile S and CO ligands in nitrogenase structures[7], **3** contains an N_2 ligand. The Fe-S bond distances in **3** (2.32–2.35 Å) are somewhat longer than the Fe-S bonds in resting state FeMoco (2.25–2.27 Å), due to either the lesser negative charge of the thiolate or the greater steric hindrance. Remarkably, the Fe-C distance in **3** at 2.04 Å is very close to the Fe6-carbide distance of 2.01 Å in FeMoco structures. Overall, the relatively simple ligand $\mathbf{L^{2-}}$ is capable of arranging appropriate atoms around iron and imparting a geometry that resembles the likely active iron site in FeMoco structures. However, the electronic structure of the iron(0) complex **3** may be different than the iron site in the N_2-binding form of the FeMoco (for which the structure and iron oxidation state are unknown).

Next, we designed a compound (**5**) intended to test the idea that Fe-S bond dissociation could provide a coordination site for N_2 binding (**I** to **III** in Fig. 1A). The bis(thiolate) complex **1** reacted with thiolate **4** to give the iron tris(thiolate) complex **5** (Fig. 2A). This orange high-spin iron(II) complex contains three S ligands, like Fe6 in the FeMoco resting state (**I** in Fig. 1). The interaction of iron with the central arene ring is weak, with the closest Fe-C distance at 2.48 Å (Fig. 2D). Thus we view this site as three-coordinate and unsaturated, which is supported by the reversible binding of one THF molecule at low temperature (Fig. 2E and Supplementary Information show the X-ray crystal structure of **5**·THF and temperature-dependent UV-vis spectra).

The tris(thiolate) iron(II) site in **5**/**5**·THF was reduced to the iron(0) oxidation level with just over two equivalents of KC_8, under conditions otherwise equivalent to those used for reduction of the iron(I) bis(thiolate) complex **2** (Fig. 2A). This yielded the same N_2 complex **3** described above, and 1.0 equiv. of free thiolate was produced. Reduction thus causes an Fe-S bond to break concomitant with N_2 binding, as in the proposed pathway for N_2 binding to FeMoco in Fig. 1A (**I** to **III**). We note that tris(thiolate) **5** contains all the nearby atoms to support alternative binding modes **II** and **IV** in Fig. 1A, but Fe-S dissociation takes place instead.

We return to describe the further characterization of **3**, which gives insight into potential properties of N_2 after binding at FeMoco. Though complex **3** is very thermally sensitive, it was possible to isolate pure samples of **3** in >80% yield from reduction of **5** at low temperature and washing the crystals with cold butane at −70 °C. Analysis of these crystals by Mössbauer spectroscopy confirms the presence of a single iron species. Infrared spectroscopy (IR) analysis of single crystals of **3** revealed a strong N-N stretching band at 1880 cm^{-1}. These frequencies are the lowest observed for any Fe-N_2 complex with a terminal N_2 ligand[23], which shows that the thiolates are powerful electron donors that enable substantial backbonding into the N_2 π* orbitals. The N_2 ligand in **3** exchanges with free $^{15}N_2$ (giving an ^{15}N-^{15}N stretching band at 1813 cm^{-1}) at −70 °C in the solid state. Samples of **3** kept at room temperature for a few hours lack the N_2 stretching vibration, further demonstrating the lability of N_2. The lability suggests that the Fe-N_2 interaction, though strong as judged by IR spectroscopy, may be compensated with tighter binding to the arene ring.

Compound **3** has a high spin ($S = 1$) electronic configuration, as determined by SQUID (superconducting quantum interference device) magnetometry on a crystalline sample. This experimental observation was confirmed with density functional theory calculations on a truncated model of **3**. Optimization with $S = 1$ gave a model close to the experimental geometry, but optimization with $S = 0$ gave significantly different bond lengths and angles, and a Gibbs free energy (ΔG°) that was higher by 37 kJ/mol (see Supplementary Information). High spin iron(0) dinitrogen complexes are rare, and have been seen mainly in cases where high symmetry makes the frontier orbitals nearly degenerate[29,30]. To our knowledge, **3** is the first high spin iron complex that contains both S and N_2 ligands[25,26], and shows that high-spin iron (as expected in the weak-field sulfur-dominated environment of iron atoms in the FeMoco) can activate N_2 substantially.

The preparation of an iron-N_2 complex with a sulfur-rich environment provides structural and spectroscopic precedents for FeMoco-N_2 binding, and also gives insight into the nitrogenase mechanism. Reduction of complex **5** breaks an Fe-S bond as in the hypothetical conversion of **I** to **III** in the FeMoco (Fig. 1), and binds N_2 in a form where the N-N bond is greatly weakened. In this way, the results support the idea that the sulfur-rich iron site in the FeMoco is particularly well-suited for N_2 activation, and that Fe-S bonds can be easily broken upon reduction to allow binding of N_2.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Einsle O, et al. Nitrogenase MoFe-Protein at 1.16 Å Resolution: A Central Ligand in the FeMo-Cofactor. Science. 2002; 297:1696–1700. [PubMed: 12215645]
2. Spatzal T, et al. Evidence for Interstitial Carbon in Nitrogenase FeMo Cofactor. Science. 2011; 334:940. [PubMed: 22096190]
3. Lancaster KM, et al. X-ray Emission Spectroscopy Evidences a Central Carbon in the Nitrogenase Iron-Molybdenum Cofactor. Science. 2011; 334:974–977. [PubMed: 22096198]

4. Wiig JA, Hu Y, Lee CC, Ribbe MW. Radical SAM-Dependent Carbon Insertion into the Nitrogenase M-Cluster. Science. 2012; 337:1672–1675. [PubMed: 23019652]
5. Hoffman BM, Lukoyanov D, Yang ZY, Dean DR, Seefeldt LC. Mechanism of Nitrogen Fixation by Nitrogenase: The Next Stage. Chem Rev. 2014; 114:4041–4062. [PubMed: 24467365]
6. Seefeldt LC, Hoffman BM, Dean DR. Mechanism of Mo-Dependent Nitrogenase. Annu Rev Biochem. 2009; 78:701–722. [PubMed: 19489731]
7. Spatzal T, Perez KA, Einsle O, Howard JB, Rees DC. Ligand binding to the FeMo-cofactor: Structures of CO-bound and reactivated nitrogenase. Science. 2014; 345:1620–1623. [PubMed: 25258081]
8. Yandulov DV, Schrock RR. Catalytic Reduction of Dinitrogen to Ammonia at a Single Molybdenum Center. Science. 2003; 301:76–78. [PubMed: 12843387]
9. Holland PL. Low-coordinate iron complexes as synthetic models of nitrogenase. Can J Chem. 2005; 83:296–301.
10. MacBeth CE, Harkins SB, Peters JC. Synthesis and characterization of cationic iron complexes supported by the neutral ligands $NP^{i\text{-}Pr}_3$, $NArP^{i\text{-}Pr}_3$, and $NS^{t\text{-}Bu}_3$. Can J Chem. 2005; 83:332–340.
11. Dance I. Ramifications of C-centering rather than N-centering of the active site FeMo-co of the enzyme nitrogenase. Dalton Trans. 2012; 41:4859–4865. [PubMed: 22395244]
12. Hinnemann B, Nørskov JK. Chemical Activity of the Nitrogenase FeMo Cofactor with a Central Nitrogen Ligand: Density Functional Study. J Am Chem Soc. 2004; 126:3920–3927. [PubMed: 15038746]
13. George SJ, et al. EXAFS and NRVS Reveal a Conformational Distortion of the FeMo-cofactor in the MoFe Nitrogenase Propargyl Alcohol Complex. J Inorg Biochem. 2012; 112:85–92. [PubMed: 22564272]
14. Creutz SE, Peters JC. Catalytic Reduction of N_2 to NH_3 by an Fe–N_2 Complex Featuring a C-Atom Anchor. J Am Chem Soc. 2014; 136:1105–1115. [PubMed: 24350667]
15. Anderson JS, Rittle J, Peters JC. Catalytic conversion of nitrogen to ammonia by an iron model complex. Nature. 2013; 501:84–87. [PubMed: 24005414]
16. Kästner J, Blöchl PE. Ammonia Production at the FeMo Cofactor of Nitrogenase: Results from Density Functional Theory. J Am Chem Soc. 2007; 129:2998–3006. [PubMed: 17309262]
17. Schimpl J, Petrilli HM, Blöchl PE. Nitrogen Binding to the FeMo-Cofactor of Nitrogenase. J Am Chem Soc. 2003; 125:15772–15778. [PubMed: 14677967]
18. Alwaaly A, Dance I, Henderson RA. Unexpected explanation for the enigmatic acid-catalysed reactivity of $[Fe_4S_4X_4]^{2-}$ clusters. Chem Commun. 2014; 50:4799–4802.
19. Saouma CT, Morris WD, Darcy JW, Mayer JM. Protonation and Proton-Coupled Electron Transfer at S-Ligated [4Fe-4S] Clusters. Chem Eur J. 2015; 21:9256–9260. [PubMed: 25965413]
20. Lee SC, Lo W, Holm RH. Developments in the Biomimetic Chemistry of Cubane-Type and Higher Nuclearity Iron–Sulfur Clusters. Chem Rev. 2014; 114:3579–3600. [PubMed: 24410527]
21. Ung G, Peters JC. Low-Temperature N_2 Binding to Two-Coordinate L_2Fe^0 Enables Reductive Trapping of $L_2FeN_2^-$ and NH_3 Generation. Angew Chem Int Ed. 2015; 54:532–535.
22. Rodriguez MM, Bill E, Brennessel WW, Holland PL. N_2 Reduction and Hydrogenation to Ammonia by a Molecular Iron-Potassium Complex. Science. 2011; 334:780–783. [PubMed: 22076372]
23. Hazari N. Homogeneous iron complexes for the conversion of dinitrogen into ammonia and hydrazine. Chem Soc Rev. 2010; 39:4044–4056. [PubMed: 20571678]
24. Danopoulos AA, Wright JA, Motherwell WB. Molecular N_2 complexes of iron stabilised by *N*-heterocyclic 'pincer' dicarbene ligands. Chem Commun. 2005:784–786.
25. Takaoka A, Mankad NP, Peters JC. Dinitrogen Complexes of Sulfur-Ligated Iron. J Am Chem Soc. 2011; 133:8440–8443. [PubMed: 21574618]
26. Bart SC, Lobkovsky E, Bill E, Wieghardt K, Chirik PJ. Neutral-Ligand Complexes of Bis(imino)pyridine Iron: Synthesis, Structure, and Spectroscopy. Inorg Chem. 2007; 46:7055–7063. [PubMed: 17655227]

27. Creutz SE, Peters JC. Diiron Bridged-Thiolate Complexes That Bind N_2 at the $Fe^{II}Fe^{II}$, $Fe^{II}Fe^{I}$, and $Fe^{I}Fe^{I}$ Redox States. J Am Chem Soc. 2015; 137:7310–7313. [PubMed: 26039253]
28. Ellison JJ, Ruhlandt-Senge K, Power PP. Synthesis and Characterization of Thiolato Complexes with Two-Coordinate Iron(II). Angew Chem Int Ed Engl. 1994; 33:1178–1180.
29. Suess DLM, Peters JC. H–H and Si–H Bond Addition to $Fe{\equiv}NNR_2$ Intermediates Derived from N_2. J Am Chem Soc. 2013; 135:4938–4941. [PubMed: 23472709]
30. Moret ME, Peters JC. Terminal Iron Dinitrogen and Iron Imide Complexes Supported by a Tris(phosphino)borane Ligand. Angew Chem Int Ed. 2011; 50:2063–2067.

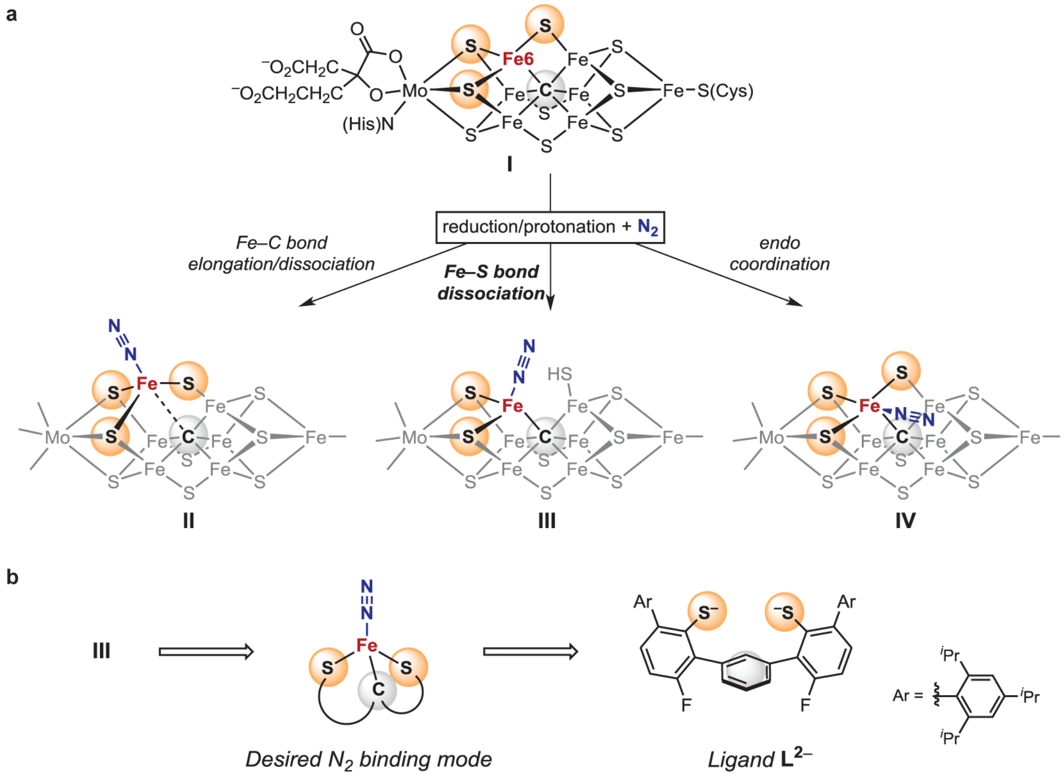

Figure 1. N_2 binding to iron in sulfur and carbon rich environments

a, Schematic representations of FeMoco and three potential N_2 binding modes. Potentially protonated sulfur ligands are not specified. **b**, Ligand design for a synthetic sulfur-carbon site.

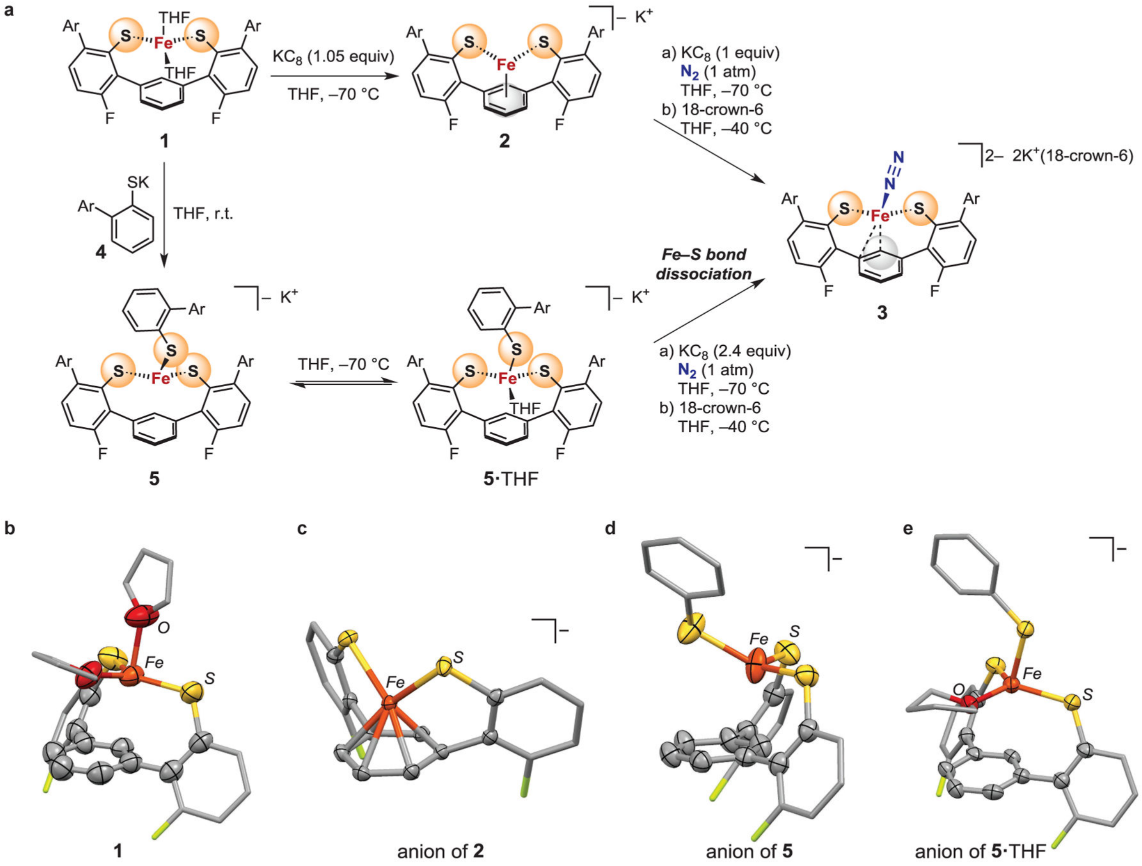

Figure 2. N_2 binding at an iron-sulfur-carbon site through Fe-S bond cleavage

a, Reactions of synthetic iron-sulfur sites leading to N_2-binding. The bottom pathway shows Fe-S cleavage with N_2 binding. Ar = 2,4,6-triisopropylphenyl. **b–e**, Molecular structures of the synthetic mononuclear iron-sulfur sites presented here. Hydrogen atoms and Ar groups are omitted for clarity.

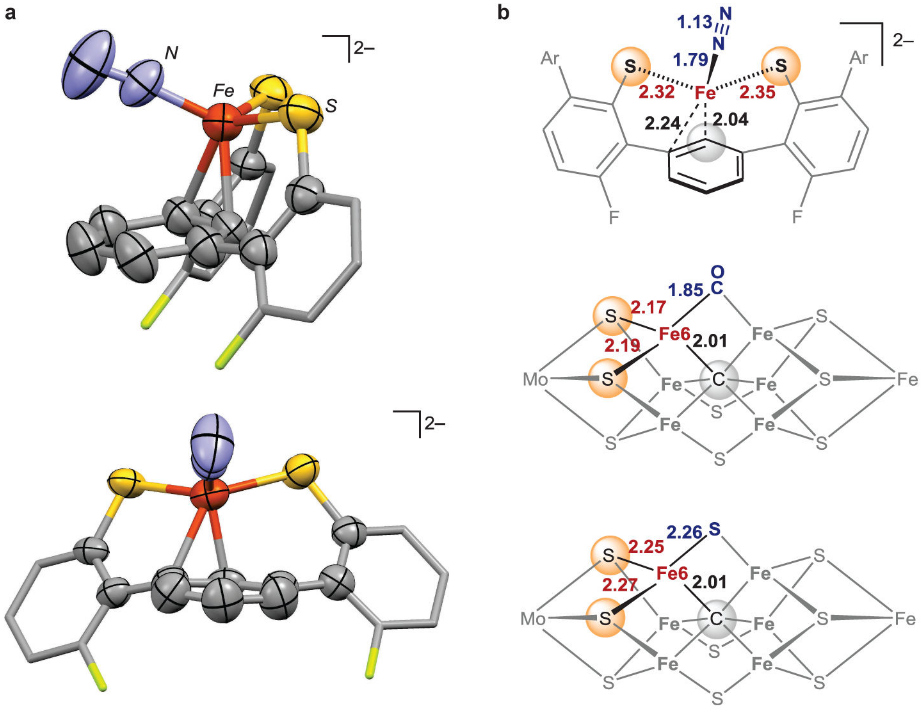

Figure 3. Iron-N_2 complex supported by sulfur and carbon ligands

a, Two views of the molecular structure of the anionic part of **3**. Hydrogen atoms and Ar groups are omitted. **b**, Comparison of geometric parameters with CO-inhibited FeMoco[7] and resting state FeMoco[2]. All distances are reported in Ångstrøms.