HHS Public Access

Author manuscript

J Am Chem Soc. Author manuscript; available in PMC 2017 July 13.

Published in final edited form as:

J Am Chem Soc. 2016 June 15; 138(23): 7200–7211. doi:10.1021/jacs.6b00747.

Insight into the FeMoco of nitrogenase from synthetic iron complexes with sulfur, carbon, and hydride ligands

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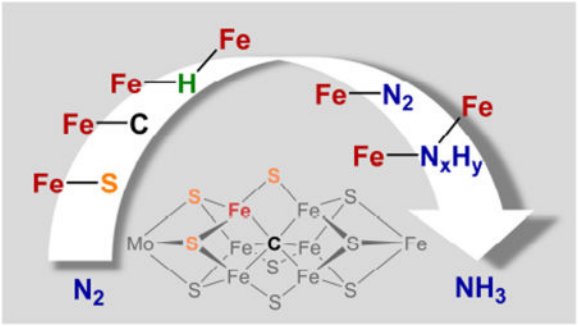
Abstract

Nitrogenase enzymes are used by microorganisms for converting atmospheric N_2 to ammonia, which provides an essential source of N atoms for higher organisms. The active site of the molybdenum-dependent nitrogenase is the unique carbide-containing iron-sulfur cluster called the iron-molybdenum cofactor (FeMoco). On the FeMoco, N_2 binding is suggested to occur at one or more iron atoms, but the structures of the catalytic intermediates are not clear. In order to establish the feasibility of different potential mechanistic steps during biological N_2 reduction, chemists have prepared iron complexes that mimic various structural aspects of the iron sites in FeMoco. This reductionist approach gives mechanistic insight, and also uncovers fundamental principles that could be used more broadly for small molecule activation. Here, we review recent results and highlight directions for future research. In one direction, synthetic iron complexes have now been shown to bind N_2, break the N-N triple bond, and produce ammonia catalytically. Carbon and sulfur based donors have been incorporated into the ligand spheres of Fe-N_2 complexes to show how these atoms may influence the structure and reactivity of the FeMoco. Hydrides have been incorporated into synthetic systems, which can bind N_2, reduce some nitrogenase substrates, and/or reductively eliminate H_2 to generate reduced iron centers. Though some carbide-containing iron clusters are known, none yet have sulfide bridges or high-spin iron atoms like the FeMoco.

Graphical Abstract



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The authors declare no competing financial interest.

1. INTRODUCTION

Nitrogen is an essential element for all life, and the supply of biologically available nitrogen limits the productivity of many terrestrial and marine ecosystems.[1–3] The most plentiful source of nitrogen is N_2, but this molecule has low reactivity. Biological fixation of N_2 to ammonia,[4–8] a bioavailable source of nitrogen, is performed by diazotrophic microorganisms.[9–11] These specialized microorganisms can be free-living or in symbiotic relationships with certain plants (*e.g.* legumes) and animals (*e.g.* termites).[12,13] Before the invention of the Haber-Bosch process for industrial ammonia production, most nitrogen atoms in living organisms originated from these microorganisms.[14,15] They have the only type of enzymes that can perform the key N_2-reducing reaction, and these are termed *nitrogenase* enzymes. Elucidation of the mechanism of biological N_2 fixation by nitrogenases is a great challenge in bioinorganic chemistry. Chemists aim to explain the mechanism of N_2 reduction in nature, and are also excited about the opportunity to harness the power of nitrogenase for other applications.

N_2 reduction by nitrogenases occurs at metal clusters, with the most well-characterized being the iron-molybdenum cofactor (FeMoco) (Fig. 1).[6,16,19–21] FeMoco contains seven Fe and one Mo that are connected by bridging sulfides and arranged around a central carbide. The biosynthesis of FeMoco from two Fe_4S_4 clusters, an additional S atom, and a C atom initially forms an Fe_8S_9C carbide cluster as an intermediate.[22,23] Substitution of an apical Fe for Mo/homocitrate is followed by the transfer of the cluster to the apo form of the catalytic MoFe protein.

Alternative nitrogenases use V or Fe in place of Mo, and are less efficient at N_2 fixation than the Mo-dependent enzyme.[24–26] X-ray structures of the alternative nitrogenases are not yet available, but biochemical and genetic studies indicate that the structures of their active site cofactors are similar to the FeMoco.[24,25,27] Spectroscopic studies further support the idea that the iron-vanadium cofactor (FeVco) contains an interstitial carbide like the FeMoco.[28,29] The shared cluster type in various nitrogenases, and studies on the activity and spectroscopy of variants of the FeMo enzyme that derive from point mutations,[30–33] indicate that the belt iron sites (indicated in red in Fig. 1) are the site of N_2 binding and reduction.[6,32,34]

Well-defined synthetic “model” systems are often used to provide insight into the feasibility of different mechanisms and structures within enzymes.[35,36] However, the chemistry of iron complexes in a coordination environment like the Fe sites in the FeMoco was unknown until a number of recent advances. Here we discuss the implications of recent progress in synthetic iron complexes supported by ligand scaffolds that coordinate to iron through carbon and/or sulfur, as well as iron hydrides that are relevant to the mechanism of N_2 reduction.[6] Reviews discussing N_2 reduction by Mo systems, by Fe systems with P and N ligands, and by other metals are available elsewhere.[37–44]

2. COORDINATION CHEMISTRY OF FEMOCO

Recent attention in the nitrogenase modeling community has focused on the belt Fe sites (see Fig. 1), each of which has pseudotetrahedral 3S-1C coordination in the resting state. X-ray crystal structures are known for nitrogenases from both *Azotobacter vinelandii* and *Clostridium pasterianum* to resolutions of better than 1.1 Å, and the atomic positions in the FeMoco in each were determined to high precision (~0.02 Å).[16,45] The Fe-S bond lengths are all the same within ± 0.03 Å, suggesting that none of them are protonated. The FeMoco is mixed-valent in the resting state with both Fe^{2+} and Fe^{3+} centers, which couple to give an $S = 3/2$ ground state.[6,7] A combination of X-ray absorption spectroscopy and computations show that the oxidation states are $Fe^{2+}_3Fe^{3+}_4Mo^{3+}$ in the resting state.[17,46,47] Spatially-resolved anomalous dispersion refinement of the structure recently confirmed this picture.[18] These data suggest the oxidation state assignments indicated in Figure 1, and the three sites with higher electron density are on the side of the cofactor that abuts two positively charged arginine residues. Thus, even though the FeMoco has near three-fold symmetry in the core bonding, there is asymmetry imposed by the local polarity in the protein.

Though this detailed characterization of the resting state geometric and electronic structure is a major achievement, the binding of substrates occurs only after conversion of the FeMoco to other, more reduced forms that have not been structurally characterized.[6] The cofactor is connected to the protein through only two linkages, and there are many precedents for structural rearrangements in synthetic iron-sulfur clusters,[48,49] and therefore the Fe-C-S cluster structure is likely to rearrange during the catalytic cycle. Binding of N_2 to Fe sites is implicated most strongly (see above), so most chemists have considered that breaking or elongation of the bonds to belt Fe atoms could accompany or precede substrate binding. Consequently, N_2 binding proposals include either elongation/dissociation of an Fe-C bond (*e.g.* **1**, Fig. 2),[50–53] or Fe-S bond cleavage with protonation of a sulfide (*e.g.* **2**, Fig. 2).[54–58] Supporting **1**, extended X-ray absorption fine structure (EXAFS) and nuclear resonance vibrational spectroscopy (NRVS) studies indicate the lengthening of Fe-C bond in FeMoco upon binding of propargyl alcohol.[59] In Fe-phosphine model complexes (described below) Fe-C bond elongation has been observed upon reduction and N_2 binding.[60,61] Supporting **2**, Fe–S bond cleavage in FeMoco is experimentally supported by the observation of sulfide replacement by CO in a structure of CO-treated nitrogenase,[62] by Fe–S cleavage in smaller Fe–S clusters,[63,64] and by observation of Fe-S cleavage with N_2 binding in a Fe model complex.[65] Another plausible geometry for N_2 binding is *endo* coordination, where N_2 is positioned close to three additional iron atoms (**3**, Fig. 2).[6,66] Other hypotheses for N_2 binding modes include η^2 coordination and bridging modes.[57,66] End-on binding of N_2 has been proposed on the basis of ENDOR studies of species trapped during N_2 reduction by nitrogenase, which show coupling to only one type of N environment.[67]

N_2 reduction by Mo-dependent nitrogenase always releases at least one equivalent of H_2 per N_2 reduced.[68,69] The dependence of the N_2 reduction rate on $[N_2]$ suggests the limiting stoichiometry in eq 1 (P_i = inorganic phosphate-containing products).[7,68,70] Note that this stoichiometric reaction is highly exergonic because of the consumption of so much ATP.[71,72]

The overall energy efficiency of N_2 reduction is low, which reflects the challenging kinetic problems for performing this multi-step reaction under ambient conditions.

Mechanistic data on Mo-nitrogenase are commonly rationalized using the Thorneley–Lowe kinetic scheme, which was derived from global fitting of data from kinetic studies on the enzymatic reaction.[70,73–76] In this scheme, intermediates are named E_n, where n represents the number of electrons added to the resting state. Importantly, the kinetic data indicate that N_2 binding does not take place until 3–4 electrons have been added. A series of EPR/cryoannealing studies confirm this model, and specifically show that E_4 can reversibly bind N_2 to form a spectroscopically observed intermediate.[77,78] Figure 3 shows a version of the Thorneley-Lowe scheme with binding of N_2 at the E_4 state.

One frustrating aspect of nitrogenase research is that none of the redox potentials for E_n transformations are known.[79] The electrons are supplied by the “Fe protein” which has an iron-sulfur cluster whose potential is influenced by the binding of ATP, ADP, and the catalytic protein.[80] The Fe protein is the only reductant known that leads to N_2 reduction, and addition of the Fe protein in the absence of substrates inevitably leads to production of H_2, rendering the electrochemistry irreversible.[7] However, the use of the same re-ductant for all eight electrons is an important constraint, implying that there is not excessive charge buildup on the cofactor.[6,81,82] This feature has traditionally been explained by assuming that one proton is added per electron, as shown in Fig. 3. This is an example of proton-coupled electron transfer (PCET), where the negative charge of each electron is balanced by the positive charge on a proton.[83,84]

ENDOR studies suggest that the E_4 state (presumably protonated to give E_4H_4) has two bridging hydrides, implying that there are two additional protons that reside on the sulfide bridges.[85–91] One potential structure for E_4H_4 that satisfies the experimental constraints is shown as **4** in Figure 2.[88] Interestingly, the $4H^+/4e^-$ can be lost from E_4 as two molecules of H_2 to regenerate the resting state, even in frozen solution.[87,90] The fact that H_2 is a competitive inhibitor of N_2 reduction by the enzyme, that D_2 can be incorporated into certain substrates,[7,78] and that higher N_2 pressures lead to higher concentrations of $E_4H_2N_2$,[77] support the idea that reversible H_2 loss is an integral part of binding of N_2 by nitrogenase.[6,70,77,78] Although this reductive elimination of H_2 “wastes” two reducing equivalents, it might be the only way under biological constraints to create a reduced iron site that can binds N_2 strongly.[55,77] Fryzuk and Quadrelli have reviewed the ability of hydrides to store electrons for N_2 activation without formal reduction of the metal.[81,82] Interestingly, the reductive elimination of H_2 does not occur until N_2 binding, as shown by the fact that H_2/D_2 exchange only occurs in the presence of N_2.[7]

After N_2 binding, multistep delivery of electrons and protons to N_2 could give a range of potential intermediates. Two mechanistic pathways, distal and alternating,[34] are commonly invoked as possibilities (Fig. 4), and are distinguished by the location of protons on the N_2 unit and by the timing of ammonia release.[6] An alternating pathway for N_2 reduction on iron is supported by computational, spectroscopic, and trapping studies on the enzyme.[34,92–97] The alternating mechanism implicates hydrazine ($H_2N–NH_2$) and diazene (HN=NH) complexes as intermediates, consistent with the observations that these two molecules are

substrates for nitrogenase.[93,95–97] Though Fig. 4 shows intermediates with only one iron center, many of the N_xH_y species along the alternating pathway could be bridging (see below), so more than one iron center could well be involved in the reduction. In addition, hybrid mechanisms could involve intermediates from both alternating and distal pathways, by addition of protons or transfer of H atoms in intermediates.[61,98,99]

Nitrogenases are capable of reducing a variety of other unsaturated compounds including CO and CO_2.[100] Vanadium-dependent nitrogenase is especially efficient for reductive coupling of CO to make C-C bonds,[101,102] a process related to Fischer-Tropsch synthesis.[103] Even isolated cofactors display the ability to form C-C bonds from CO.[104] The coordination chemistry of FeMoco with CO and CO_2 is relevant to understanding the mechanism behind these potentially useful processes and aids the study of CO inhibition of the enzyme, and has been discussed in detail elsewhere.[62,105]

3. SYNTHETIC IRON-SULFUR CLUSTERS

Our description of synthetic compounds will start with the most obvious feature of FeMoco: that it is an iron-sulfur (Fe-S) cluster. Fe-S clusters are found in a range of different kinds of enzymes and electron-transfer proteins.[106,107] In order to understand their properties, Fe-S clusters have been synthesized, often through assembly from simple starting materials.[48,49,108] Artificial clusters of many sizes and topologies are now accessible, and heterometals can be incorporated into clusters. However, no Fe-S cluster with a carbide has yet been synthesized by chemists.

Ohki, Tatsumi, and coworkers reported several examples of iron-sulfur clusters that have a topology reminiscent of FeMoco but with a central sulfide (Fig. 5).[109–111] Fe_8S_7 cluster **6** was formed from the reaction of coordinatively unsaturated dinuclear Fe^{2+} complex **5** with S_8 in toluene.[109] In this remarkable self-assembly process, some Fe^{2+} centers in the starting material are oxidized to give a $Fe^{2+}{}_5Fe^{3+}{}_3$ cluster. The Fe centers in **6** are arranged in two Fe_4S_3 units that are connected by three thiolate bridges and a central μ^6-sulfide. A $Mo_2Fe_6S_9$ cluster which has two cuboidal $MoFe_3(\mu^3\text{-}S)_3$ units joined via a central μ^6-S atom was reported by Holm.[112] Selectively joining $MoFe_3S_3$ and Fe_4S_3 subclusters in structures similar to FeMoco remains a challenge. It is likely that comparison of symmetrical and unsymmetrical clusters would provide insight into the role of Mo in the FeMoco.

A related Fe_8-sulfur cluster (**8**) that contains an oxygen atom as the central atom has been synthesized by the reaction of the coordinatively unsaturated dinuclear iron(II) thiolate/alkoxide complex **7** with water and sulfur in toluene (Fig. 5).[111] The oxygen atom in this cluster of five Fe^{2+} and three Fe^{3+} forms a bridge between two Fe_4S_3 fragments. One of the exciting aspects of this cluster is the presence of two coordinatively unsaturated Fe atoms that are weakly bound to mesityl rings. Though this cluster does not react with 1 atm of N_2, its reduced forms have not yet been reported and might have heightened N_2 reactivity.

Clusters related to **6** but having one of the bridging thiolates replaced with an alkoxide or an amide are also known.[109,111] Although they do not contain Mo, the synthesis of clusters in Fig. 5 demonstrates the stability of cluster structures similar to FeMoco. They are further

relevant to the proposed structure of the cofactor in all- iron nitrogenase, and to the Fe_8S_9C cluster that is formed during biosynthesis of FeMoco.[22,23]

Despite remarkable achievements in the synthesis of complex Fe-S clusters, N_2 binding to an iron-sulfide cluster has never been reported.[48,49,113–115] Recently, a synthetic iron-sulfide cluster was combined with cofactor-deficient nitrogenase, which formed an artificial enzyme that reduced acetylene, but not N_2.[116] Pairing of synthetic and biological components has substantial promise as a strategy for identifying the essential components of the nitrogenase mechanism.

4. Fe-N_2 COMPLEXES WITH SULFUR LIGANDS

It is well-known that the σ interaction of N_2 with metals is weak, and that π-backbonding from filled metal *d* orbitals into the π* orbitals of N_2 is most influential.[38] Iron-N_2 complexes have been isolated in a number of systems where the iron atom has the +2 oxidation state or lower.[39,40] However, only a few synthetic complexes are known that have both sulfur and N_2 ligands on the same iron center (Fig. 6).[117] Since these resemble feasible structures of crucial N_2-bound species in nitrogenase catalytic cycle, they have been important topics of inquiry.

The Peters group reported a series of thioether ligated Fe-N_2 complexes with tetradentate ligands that are conceptually related to their previous tris(phosphine) complexes.[118] A ligand with one thioether and two phosphine donors yielded the cationic paramagnetic ($S = 1$) complex **10** (Fig. 6). The N_2 ligand in **10** is lost under reduced pressure, and this lability is consistent with its high N-N stretching frequency of 2156 cm^{-1}. Reduction of **10** to with potassium/graphite (KC_8), Na(Hg), or $CoCp_2$ resulted in N_2 loss, cleavage of the S-C(alkyl) bond, bridging S, and/or thioether dissociation. These undesired reactions illustrate common difficulties that arise during attempts to stabilize N_2 complexes using sulfur-based ligands.

When a ligand with two thioethers and one phosphine arm was used, the resulting iron(II) complex did not bind N_2. However, partial reduction of this complex with 0.5 equiv of $Cr(C_6H_6)_2$ resulted in the mixed valent species **11** and **12**, which have bridging N_2 (Fig. 6).[118] Compounds **10–12** have N_2 bound to Fe that is ligated by sulfur donors, and their distorted trigonal-bipyramidal geometries model the potential binding mode **1** in FeMoco (Fig. 2).

Interestingly, a mononuclear Fe-N_2 species **13** with two thioether donors can be obtained by introduction of a hydride ligand at the iron center (Fig. 6).[118] Since metal-N_2 interactions are so dependent on backbonding, it is reasonable that the more electron rich Fe in **13** is better able to bind N_2. A similar iron-N_2 hydride complex **14** was obtained using a ligand with a single thioether donor. Thus, it appears that strong-field hydride ligands can facilitate N_2 binding to Fe, corresponding to one possible role for hydrides in the FeMoco.[119]

Thiolate donors, which are negatively charged, are better suited to model the anionic sulfides in FeMoco. Peters recently described the first example of a thiolate-iron-N_2 species, with $Fe^{1+}Fe^{1+}$, $Fe^{1+}Fe^{2+}$, and $Fe^{2+}Fe^{2+}$ in binuclear thiolate bridged iron complexes that are additionally coordinated by phosphine and silyl donors (**15**, Fig. 7).[120] The anionic bis(N_2)

species produces 1.8 ± 0.3 equiv of ammonia when treated with excess KC_8 and $[H(OEt_2)_2]BAr^F{}_4$, and also can catalyze the disproportionation of N_2H_4 to NH_3 and N_2. Furthermore, the same ligand scaffold enables formation of $Fe^{1+}Fe^{2+}$ and $Fe^{2+}Fe^{2+}$ iron-hydride species having an Fe-N_2 fragment (**16**, Fig. 7).

In order to create an environment consisting only of donors present in the FeMoco, the bis(thiolate) ligand platform in **17** was introduced (Fig. 8).[65] Reduction of tris(thiolate) iron(II) complex **17** to a formal iron(0) oxidation state at low temperature resulted in the loss of one thiolate, and formation of a terminal N_2 complex **18** that features partial coordination of the central arene with Fe-C distances of 2.04 and 2.24 Å (Fig. 8). The iron in **18** thus has close coordination of two S and one C, as in the proposed N_2 binding mode **2** for FeMoco (Fig. 2). Breaking an Fe-S bond concomitant with N_2 binding during the formation of **18** suggests that breaking an Fe–S bond is a chemically reasonable route to N_2 binding in FeMoco. Furthermore, **18** has a quite activated N_2 ligand, as evidenced by N–N stretching frequency of 1880 cm^{-1}. This shows that thiolates give electron rich, high-spin iron centers that backbond well into the π^* orbital of N_2, though N_2 is lost readily from **18** at room temperature.

Future work is necessary to advance the chemistry of biomimetic complexes with S donors. First, it will be advantageous in the future to incorporate carbides and additional iron atoms in a complex with a S-dominated ligand sphere. In addition, use of sulfides rather than thiolates is a challenge that will require careful cluster design. Ideally, it will be possible to design S/C ligand spheres that enable direct comparison of hypotheses **1** and **2** (Fig. 2 above).

5. CARBIDE COMPLEXES, AND Fe-N_2 COMPLEXES WITH CARBON LIGANDS

The carbide in the FeMoco comes from the methyl group of the sulfonium ion *S*-adenosylmethionine, which is thought to be transferred to a cluster sulfur, and subsequently subjected to abstraction of H atoms.[121–123] The carbide is neither exchanged nor lost from the cofactor during catalysis.[124] The fact that there is cellular machinery devoted to the synthesis of this unusual cage structure implies that the carbide may play a functional role in N_2 reduction. Some possibilities for this functional role include enforcement of an appropriate geometry in the active site, stabilization of the cluster (note that carbides are present in steel[125]),[124] transient formation/cleavage of Fe-C bonds,[50–53] and reversible formation of C-H bonds. Recent computational studies propose that the carbide could even engage in covalent bonding with the N atoms during the N_2 reduction.[126,127]

Synthetic iron carbide clusters are also known, though in these cases the carbides come from very different sources.[128–144] In a six-iron example, the Fe_6 cluster $[Me_4N]_2[Fe_6C(CO)_{16}]$ (**19**, Fig. 9a) was studied in the early 1970's as a model of possible intermediates in the iron-catalyzed Fischer-Tropsch process.[129,130] In collaboration with DeBeer, we recently reported more extensive crystallographic characterization of **19**, as well as X-ray emission studies that elucidated the electronic structure of the iron-carbide core and demonstrated the delocalized orbitals that give rise to Fe-C bond-ing.[145] Oxidation of **19** leads to related Fe_5

and Fe_4 carbide/carbonyl clusters.[134,142] One transformation with particular relevance to the FeMoco mechanism is that $[Fe_4C(CO)_{12}]^{2-}$ (**21**, Fig. 9b) can be oxidized in the presence of H_2 to give neutral $HFe_4(CH)(CO)_{12}$ (**22**).[134,136,139,146] This product can also be synthesized through double protonation of the cluster.[134,140] The oxidative addition of H_2 is the microscopic reverse of reductive elimination from a hydride-iron cluster, and suggests that reversible formation of C-H bonds in the FeMoco should be considered as a possibility.

It should be borne in mind that there are several differences between these synthetic carbide clusters and the FeMoco. First, none of these carbide clusters have the trigonal prismatic geometry of the FeMoco. Second, the π-acidic CO ligands in the synthetic iron-carbide clusters stabilize Fe^{1+} and Fe^0 oxidation levels, as opposed to Fe^{3+} and Fe^{2+} centers that are present in the enzyme. Finally, the strong-field CO ligands in the synthetic clusters make them diamagnetic, which contrasts to the sulfide-supported, high-spin iron sites in the enzyme.[47] Thus, the behavior of these CO-supported clusters may not be representative of the FeMoco environment. The synthesis of iron-carbide clusters in weak-field environments (preferably with S donors) is needed in order to predict the chemical behavior of Fe atoms in the FeMoco.

In the above carbonyl clusters, the carbide is derived from reduction of a coordinated CO, and thus it is probable that new synthetic strategies will be needed for non-carbonyl clusters. Several μ-carbido complexes with iron are known, although their chemistry has not been explored extensively.[147–153] The diamagnetic tetra-phenylporphyrinato complex **20** with a linear Fe=C=Fe bridge was theoretically predicted and synthesized in the early 1980's (Fig. 9a).[147,148,154] The carbide in **20** originates from CI_4 in a one-step reaction, suggesting that this route might be applicable to other carbides in non-carbonyl environments.

Because of the difficulty in designing appropriate clusters, simpler carbon-based donors have been used to make mononuclear complexes. One compelling choice of *C*-donor is the *N*-heterocyclic carbene (NHC), which can form stable complexes with iron.[155] However, NHC-stabilized Fe-N_2 complexes are rare.[156] Peters reported that a two-coordinate $(CAAC)_2Fe$ complex **23** [CAAC = cyclic (alkyl)(amino)carbene] binds N_2 at low temperature to form a putative three-coordinate N_2 complex **24** (Fig. 10).[157] It can be reduced to Fe(-I) in the heterobimetallic bridging N_2 complex **25**, which has a low N-N stretching frequency of 1850 cm^{-1}. Three-coordinate Fe-N_2 complexes are rare and have previously been observed using bulky β-diketiminate ligands.[158–160] Treatment of **25** with silyl chlorides functionalizes N_2 to give iron silyldiazenido complexes **26**. Catalytic reduction of N_2 to ammonia can be achieved using KC_8 and $[H(OEt2)2]BAr^F{}_4$ catalyzed by **23** at −95 °C, with yields of up to 3.3 ± 1.1 equivalents of NH_3 per iron.[157] Thus, the electron-rich CAAC ligands and the low coordination number are particularly effective for N_2 activation. Other Fe-N_2 complexes supported by chelating NHC ligand frameworks are also known (*e.g.* **27a–c**, Fig. 11), but have not been studied as extensively.[161–164]

Alkyl ligands have also been used as carbide mimics. In a systematic set of studies, Peters described trigonal pyramidal complexes of a tris(phosphine) platform with different alkyl ligands placed in one axial position (Fig. 12). First, the Fe^{1+} complex **28** was reduced to give an Fe^0 complex with N_2 binding *trans* to the carbon ligand (**29**, Fig. 12a).[60] Upon reduction

and N_2 binding, the Fe–C distance in **28** (2.153(2) Å) elongated to 2.254(5) Å. Treatment of the complex with a silyl triflate at low temperature affords four-coordinate Fe diazenido complex **30**, with Fe–C = 2.116(1) Å. These show the great flexibility of the Fe–C bond, supporting model **1** for N_2 binding in FeMoco (Fig. 2).

A related system with aryl linkers gave N_2 complexes having three different oxidation states (**31**, Fig. 12b).[61] Fe-C bond lengthening from 2.081(3) Å in the cationic to 2.165(2) Å in the anionic complex again showed the flexibility of the Fe-C bond, and the anionic complex is capable of catalytic ammonia production (47 equiv/Fe).[165]

Ohki and coworkers reported a coordinatively unsaturated iron complex **32**, from solutions of which diiron-N_2 complex **33** crystalizes under N_2 atmosphere (Fig. 13).[166] Complex **33** contains a Fe-N_2 moiety in a solely carbon-based ligand environment, with Cp*, NHC, and an alkyl donor. The N_2 in **33** is held weakly, as indicated by reversion to **32** upon dissolving, and by a high N-N stretching frequency of 2126 cm^{-1}.

Further examples of C-ligated Fe-N_2 complexes include bis(imino)pyridine stabilized complexes with alkyl and aryl ligands[167–169] and also bis-cyclometalated CPC and CNC pincer supported complexes,[170,171] as well as N_2 complexes stabilized by η^6-interaction with arenes.[171–173] In the long run, all of these complexes will add to our knowledge about the influence of donor power and geometry on N_2 activation by iron.

6. Fe-H COMPLEXES WITH SULFUR AND N_2 LIGANDS

As described above, the E_4H_4 state of nitrogenase has been shown by ENDOR spectroscopy to have two H atoms with direct bonds to iron (Fe-hydrides).[86–89] In principle, these hydrides could be either terminal (Fe-H) or bridging (Fe-H-Fe), and *ab initio* studies of a simplified model have evaluated the relative energies of these different binding modes.[174] The ENDOR spectra of E_4H_4 show an intrinsic **T** tensor (coupling between the unpaired spin and the ^1H spin) that is rhombic.[6,86] Analogous ENDOR experiments on isolable Fe-H compounds with terminal hydrides showed an axial tensor, while bridging hydrides gave a rhombic tensor.[6,175,176] This combination of experiments validated the idea that that the signals from the FeMoco-hydride ("E_4") are most consistent with *bridging* hydrides in E_4H_4.[6] However, the active form might have transient terminal hydrides, as is currently believed for hydrogenase enzymes based on synthetic modeling studies.[177]

Most isolated iron-hydride species have low-spin electronic configurations, whereas the weak-field environment of the iron atoms in the FeMoco is electronically different. This motivates the preparation of new iron hydrides in weak-field ligand coordination environments, particularly those having sulfur-based ligands. We developed low-coordinate $Fe(\mu\text{-}H)_2Fe$ dimers that have weak-field lig-and environments, and these react with nitrogenase-relevant substrates such as alkynes, CO_2, cyanide, and azide (but only react with N_2 upon irradiation).[178–180] Murray recently reported $Fe_3(\mu\text{-}H)_3$ clusters stabilized by a trinucleating β-diketiminate scaffold, which react with CO_2 but not other nitrogenase substrates.[181] However, none of the above Fe-hydride species contained S donors.

Iron-hydride-thiolate complexes without CO or P ligands are ra-re.[182,183] Qu and coworkers recently reported diiron hydrides in a sulfur and carbon rich environment, which are bridged by bis(thiolate) and stabilized by Cp* ligands (**34** and **35**, Fig. 14).[183]

A recent report from our group introduced the sulfide and hydride bridged diiron complex **36** (Fig. 15a), which models a potential coordination mode for hydrides in FeMoco as depicted in **4** (Fig. 2 above).[182] Hydride complex **36** reacted with CO_2 to give bridging formate complex **37**, which is relevant to CO_2 reduction by nitrogenase.[184–186] Complex **16b** from above,[120] which has a terminal hydride (Fig. 15b), also reduces CO_2 to give a thiolate bridged formate (**38**). Therefore, both bridging and terminal hydrides appear to be active in S-supported Fe-H complexes, at least for CO_2 reduction.

Formation of N_2 complexes within a iron-sulfide-hydride framework is particularly relevant to the proposed reductive elimination of two hydrides from E_4H_4. Reduction of **36** by 2 e^- under N_2 resulted in the formation of a diiron(0)–N_2 complex.[182] Liberation of H_2 is observed, suggesting a parallel to the reductive elimination in the Thorneley-Lowe scheme. However, **36** has only one hydride and therefore cannot reductively eliminate H_2 in an intramolecular reaction. In addition, the low yields of the N_2 complex and H_2 (~25%) makes the significance of H_2 evolution in this system uncertain.

Studies of N_2 binding to Fe complexes with *multiple* hydrides are more relevant to N_2 binding from **4** (Fig. 2). Though well-precedented with other metals,[81,82] such studies are rare with Fe complexes.[187–189] The Peters group recently reported the mixed-valent $Fe^{2+}(\mu\text{-}H)_2Fe^{1+}$ complex **40**, which displays 10^6-fold enhancement of N_2 binding affinity over $Fe^{2+}(\mu\text{-}H)_2Fe^{2+}$ species **39** (Fig. 16).[119] Treatment of **39** with excess KC_8 and $[(Et_2O)_2H]\text{-}BAr^F{}_4$ resulted in production of 1.4 ± 0.5 mol equiv NH_3. This is the first example of bridging hydrides in an Fe-N_2 complex, which is relevant to N_2 binding by E_4H_4. The authors suggest a model in which the role of hydrides may be to increase the ligand field at iron, stabilizing a low-spin electronic configuration that is preferred for N_2 binding. However, many iron-N_2 complexes with highly activated N_2 ligands have been observed in high-spin complexes of iron, so the need for low-spin Fe is not clear.[65,158–160,190] Overall, more studies are needed, particularly on S-supported systems, to draw firmer conclusions about the dependence of N_2 binding and H_2 elimination on the spin state of iron.

7. Fe-N_xH_y COMPLEXES WITH SULFUR LIGANDS

N_2 binding and reduction is now well established with iron complexes in P and N ligand environments. Recent highlights include cleavage of N_2 to two nitrides (**41**) and catalytic systems for conversion of N_2 to ammonia by mononuclear model complexes, the most successful being **42** (Fig. 17).[61,157,191,192,41,44,165] Peters has shown that phosphine ligands provide excellent platforms for functionalization of N_2 on iron,[60,193,194] and enable characterization of potential N_2 reduction intermediates Fe-N_xH_y,[98,99,165,194–196] including systems that feature a coordinated hydride.[40,42,43] Ammonia production by tripodal trisphosphine ligands with an axial donor (Si, C, or B) increases in the order Si < C < B.[165] The higher degree of Fe-heteroatom bond flexibility in **42** could be crucial for stabilizing the variety of N_2 reduction intermediates in the catalytic cycle.[61,192,193,197] In a stoichiometric

study using the B-based ligand, an Fe≡N–NH_2 intermediate was characterized that models the distal mechanism for N_2 reduction (Fig. 4).[98] Subsequent study with a Si-based ligand postulated a pathway for N_2 reduction that includes Fe=N–NH_2 and Fe-NH_2NH_2 species.[99] However, sulfur-supported complexes are needed to more closely resemble the iron environment in the FeMoco.

Sellmann and coworkers characterized hydrazine, diazene, and NH_3 complexes using multidentate thioether/thiolate ligands.[198,199] The *trans*-N_2H_2 ligand in complex **43** is bound to two iron centers and stabilized by hydrogen bonding interactions with S-ligands, a scenario that is feasible in FeMoco (Fig. 18). However, no N_2 complex was accessible in this system. More generally, no reported system with S donors has yet been observed to bind N_2 *and* to bind partially reduced N_xH_y species. Additionally, none of these S-based systems has been reported to perform N_2 reduction, though some are capable of catalytic reduction of hydrazine.[200,201]

In a notable contribution, Qu and coworkers used a thiolate-bridged diiron system that binds *cis*-diazene (**44**), and this complex can be protonated to give N_2H_3 species **45** (Fig. 18).[58] Upon treatment with 2 equivalents of a reducing reagent and protonation, **45** is transformed into the bridging amide complex **46**. These reactions are relevant because transformation of N_2 to ammonia might be mediated by at least two neighboring Fe atoms through a series of N_xH_y bridged intermediates.[66] This Cp*/thiolate supported system is capable of hydrazine reduction as well.[58]

Binding of nitrogenase-relevant N_xH_y intermediates to Fe complexes with sulfides, rather than thiolates, is rare. Holland and coworkers reported a diiron sulfide species that is coordinatively unsaturated, to which N_2H_4 and a substituted hydrazido ligand can bridge in a μ-η^1:η^1 fashion (**47**, Fig. 18).[202,203] Complexes of substituted hydrazines have greater stability in this system,[203] and one mixed-valence system was studied using ENDOR for comparison of hyperfine parameters to nitrogenase intermediates.[204] However, this diiron sulfide system has not yet been observed to bind N_2 without loss of sulfide. More model complexes are needed that have the combination of sulfur rich ligand environments, N_2 binding, and the ability to stabilize various Fe-N_xH_y intermediates.

8. CONCLUSION AND OUTLOOK

At the turn of the century, synthetic modeling of nitrogenase was based on molybdenum, because of its demonstrated ability to reduce N_2 to ammonia.[37] However, emerging spectroscopy, crystallography, and biosynthesis of the enzyme has suggested iron binding of N_2 instead. The broader acceptance of the iron hypothesis has crucially depended on advances in the understanding of synthetic iron complexes. Most notably, it has been shown that iron species are capable of reducing N_2 to NH_3, including several cases with catalytic reduction of N_2 noted above. Equally influential has been the realization that other hypothetical features of FeMoco intermediates, such as high-spin iron-hydrides, iron-diazenes and iron-hydrazines are feasible structures in the presence of sulfides and other sulfur-based donors. Spectroscopic studies on these species provide signatures for use in

identifying intermediates, and their reactivity shows what reactions are reasonable to expect on the FeMoco.

Many of the initial advances in N_2 reduction have been enabled by N and P based ligands, even though FeMoco iron sites use S and C donors. Thus, the next stage in nitrogenase modeling is to incorporate S and C donors into the model iron complexes. Although preparation of an exact model of the FeMoco would be an amazing synthetic achievement, perfect structural fidelity is not needed for deep insight. For example, analogous systems with and without S and C ligands enable us to understand the influence of these ligand types on the FeMoco reactivity and spectroscopy. Further, there is a need for systems that allow observation of the elementary steps that link N_xH_y species. These use proton-coupled electron transfer (PCET),[83,205] which has not yet been studied in the context of nitrogen reduction. The addition of single electrons and protons as part of the Thorneley-Lowe scheme suggests that PCET is crucial for nitrogenase catalysis, as noted above.[6,7,24] There have only been a few studies on PCET for any iron-sulfur clusters.[64,206–208] Studies on PCET to N_2, N_2H_2, N_2H_4, and other fragments of relevance to N_2 reduction are needed,[99,209] because such reactions are involved in the part of the nitrogenase mechanism spanning the E_4 to E_8 levels. We also note that systems that engage in PCET often involve hydrogen bonds,[210] but to our knowledge there are not yet any examples of hydrogen bonding to an N_2 unit in a transition-metal complex.

Another priority is the understanding of reversible H_2 loss with N_2 binding. Reductive elimination of H_2 simultaneously generates an open site and two reducing equivalents on the metal, both of which contribute to N_2 binding ability.[81,82] Interestingly, the available evidence on nitrogenase indicates that H_2 loss, though reversible, occurs only upon addition of N_2. This observation suggests that the enzyme might generate an iron-H_2 complex that undergoes an associative ligand exchange reaction to bind N_2. This idea was also recently suggested by the kinetics of H_2 loss upon photolyzing nitrogenase.[88] The reductive elimination of H_2 provides an ideal way for the FeMoco to store reducing equivalents while leaving the iron sites in reasonable oxidation states. Though two electrons are “wasted” when H_2 is lost, the need for nitrogen fixation makes this a worthwhile bargain of thermodynamics for kinetics. (*In vivo*, it is also likely that the “lost” H_2 can be reabsorbed for recovery of some of the energy.) Synthetic iron compounds that exchange H_2 and N_2 have been studied in phosphine-containing systems,[211,212] and should be extended to sulfur-containing environments. Also, a biomimetic strategy of H_2 loss may enable chemists to forego the use of very strong acids and reducing agents during the catalytic reduction of N_2.[165]

This Perspective has highlighted these and other areas in which synthetic chemistry will continue to enrich our understanding of the fascinating process of reducing N_2.

Acknowledgments

Nitrogen research in the Holland lab has been generously supported by the National Institutes of Health (GM065313).

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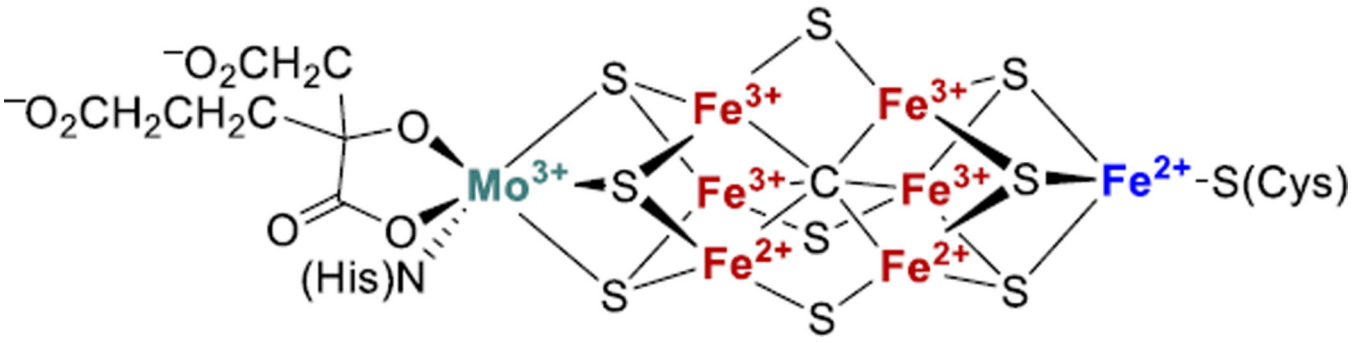


Figure 1.

Structure of the resting state FeMoco from high-resolution X-ray crystallography.[16] The oxidation states of the iron atoms shown here are from X-ray absorption and anomalous dispersion studies.[17,18] The belt iron sites are colored red.

$$N_2 + 8\,H^+ + 16\,MgATP + 8\,e^- \rightarrow 2\,NH_3 + H_2 + 16\,MgADP + 16\,P_i \quad (1)$$

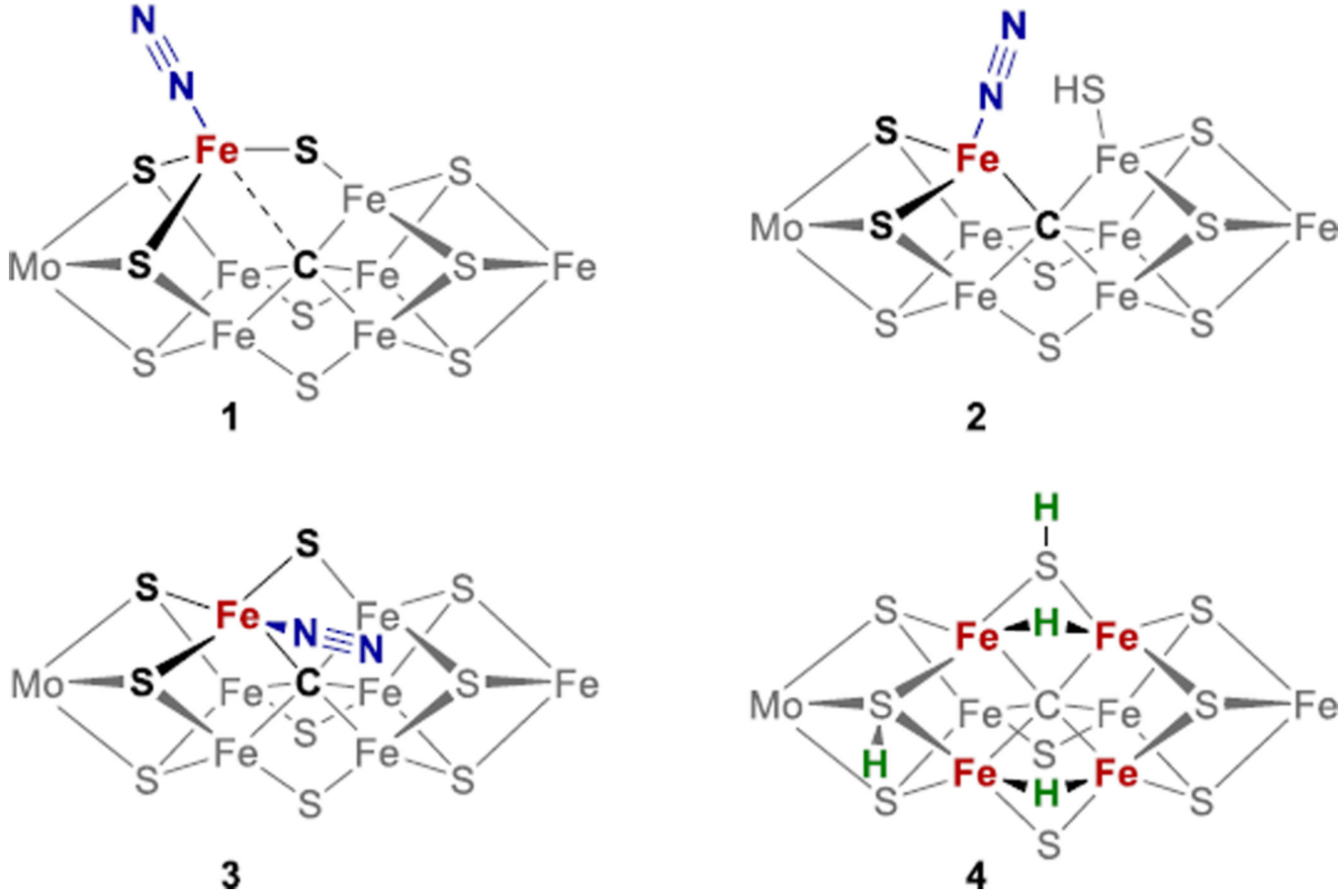


Figure 2.
Possible FeMoco intermediates during reduction of N_2. Additional potential protonation sites and cluster charges are not shown.

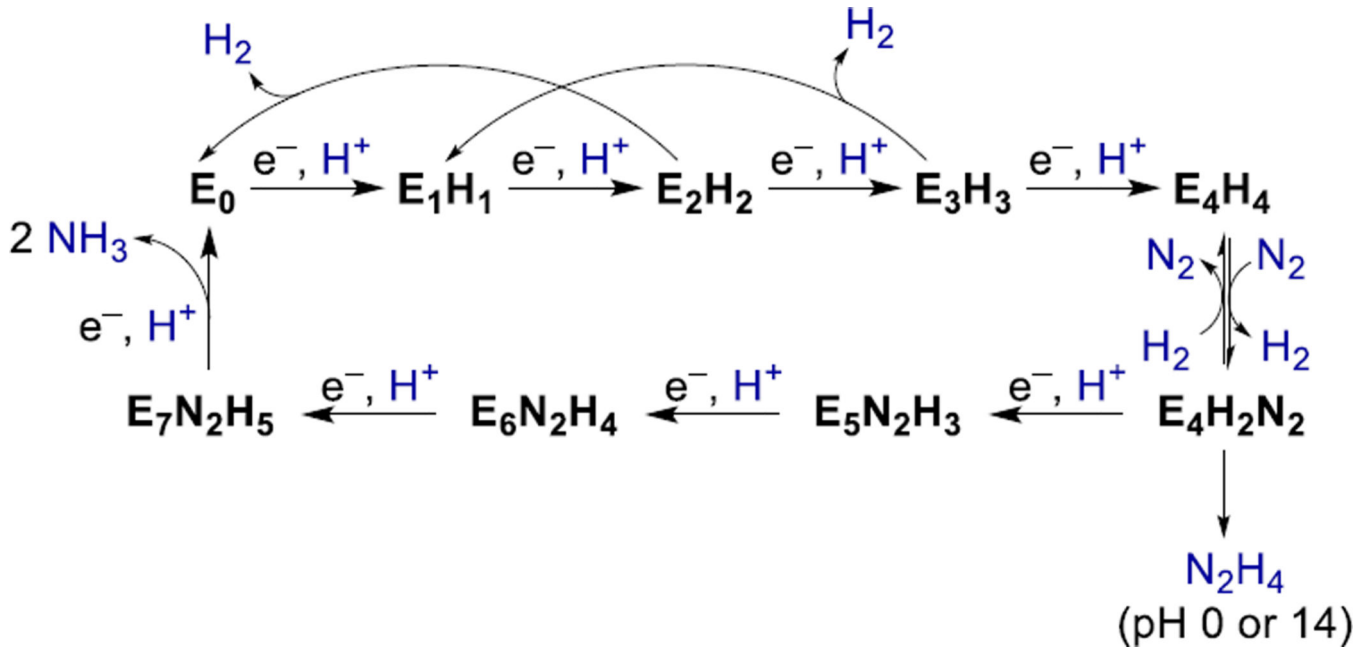


Figure 3.
Thorneley-Lowe scheme for N_2 reduction.

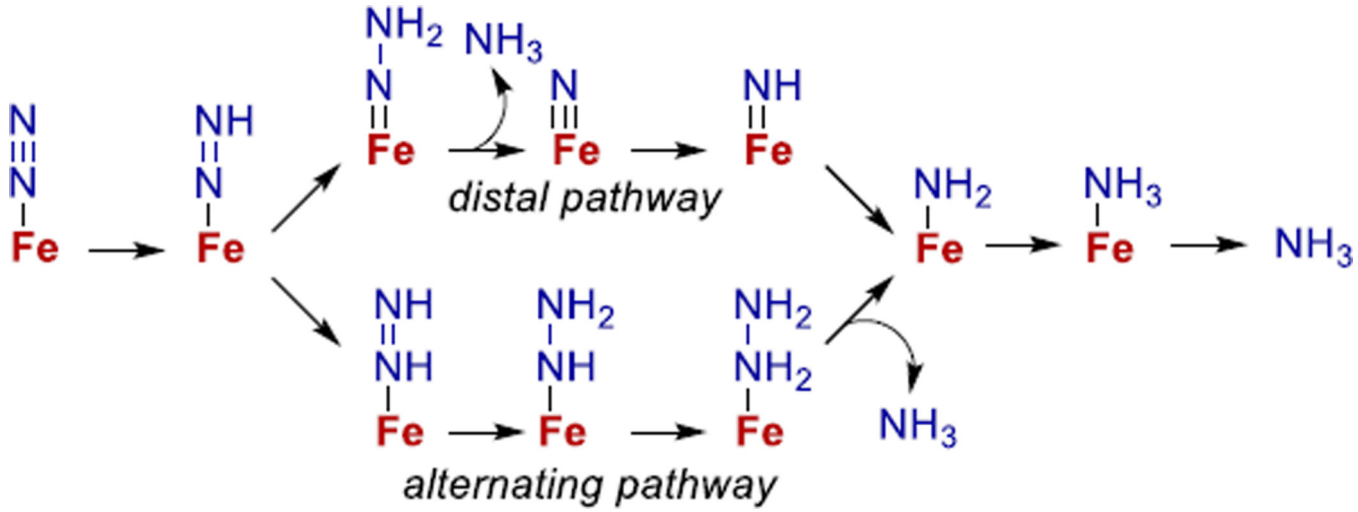


Figure 4.

Potential intermediates in N_2 reduction on iron. All steps except NH_3 release include input of H^+ and e^-.

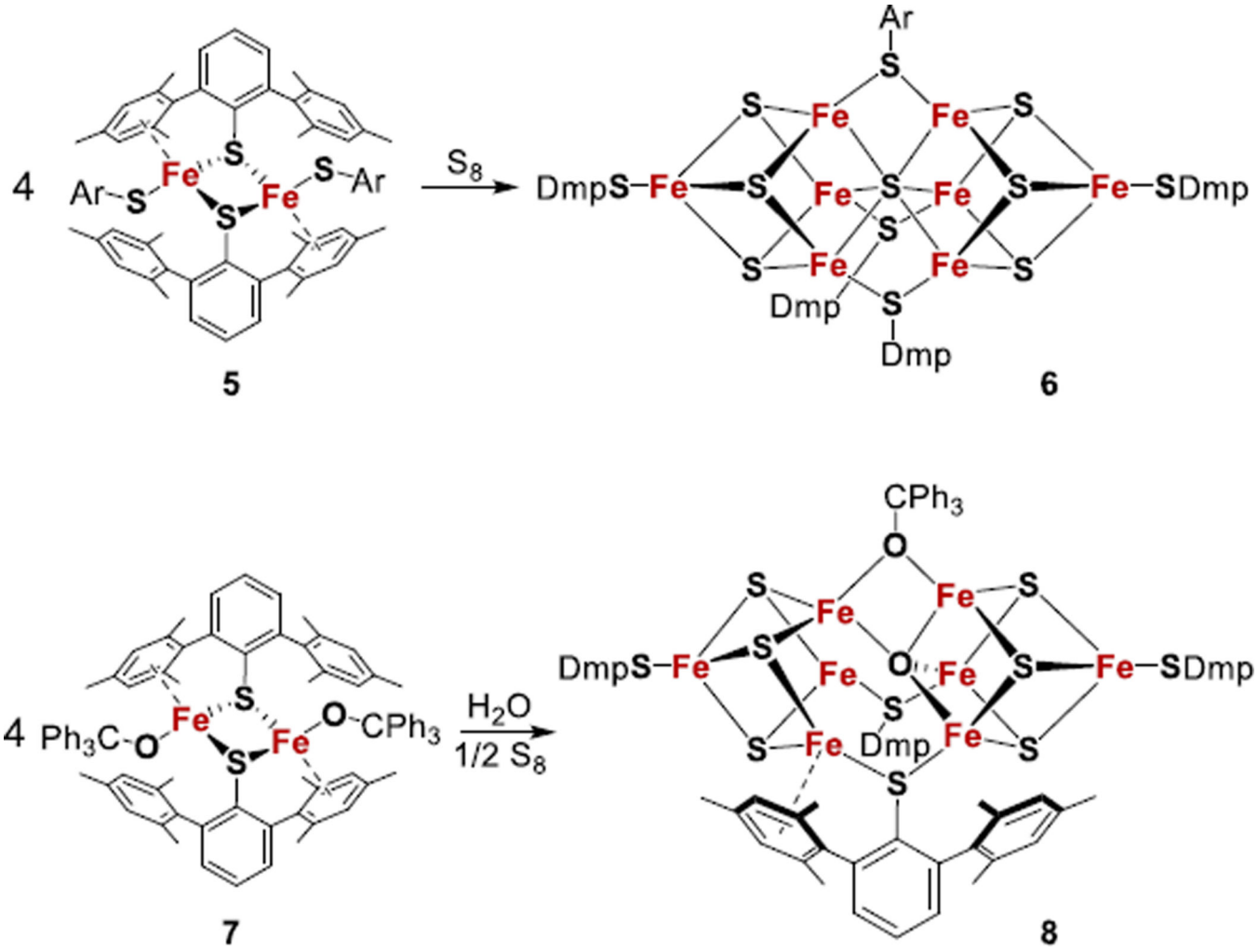


Figure 5.

Structures of synthetic iron-sulfur clusters. Dmp = 2,6-(mesityl)$_2$C$_6$H$_3$. Ar = 2,4,6-^{i}Pr$_3$C$_6$H$_2$.

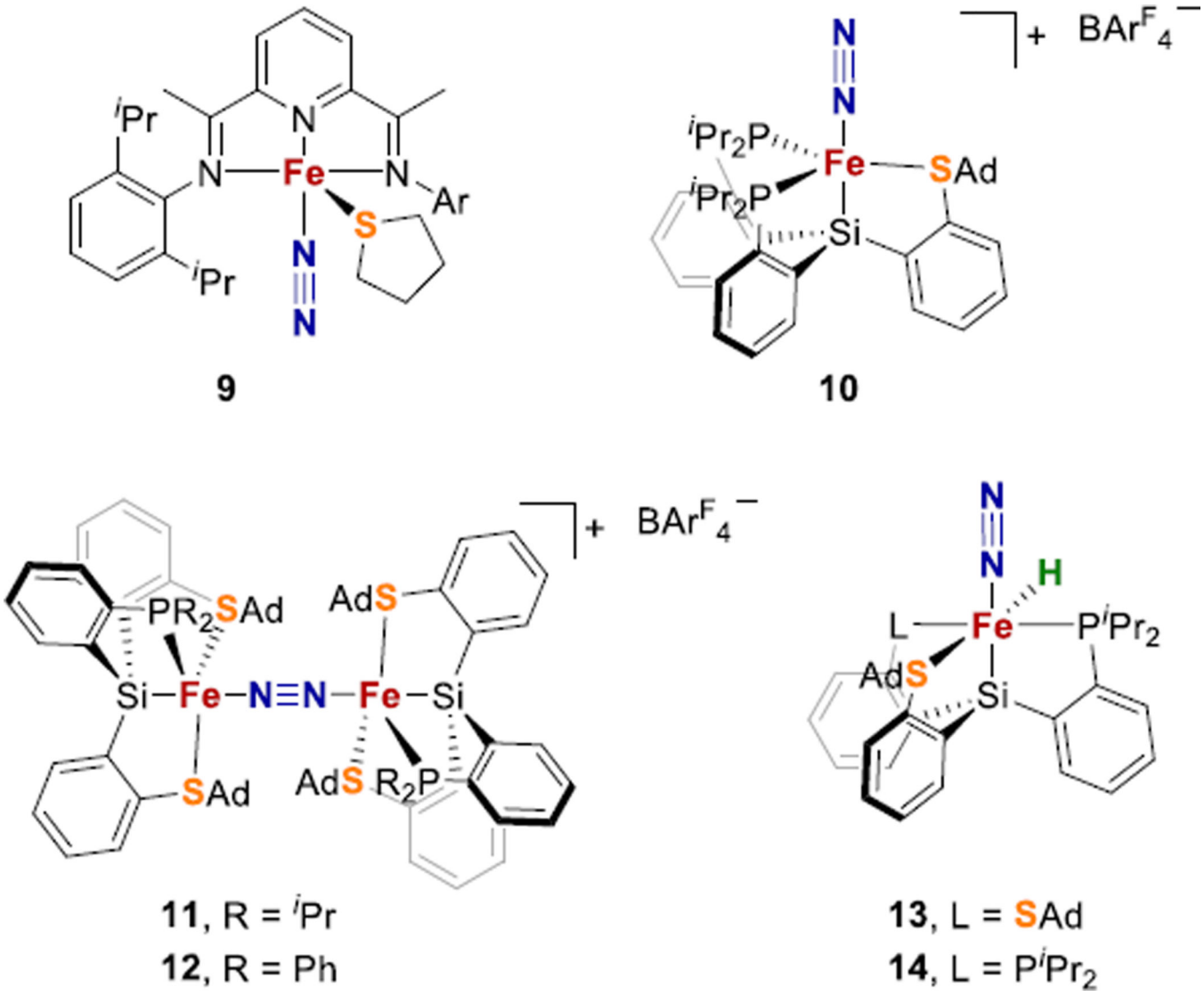


Figure 6.
Fe-N_2 complexes with thioether ligands. $BAr^F{}_4^-$ = tetrakis(3,5-trifluoromethylphenyl)borate.

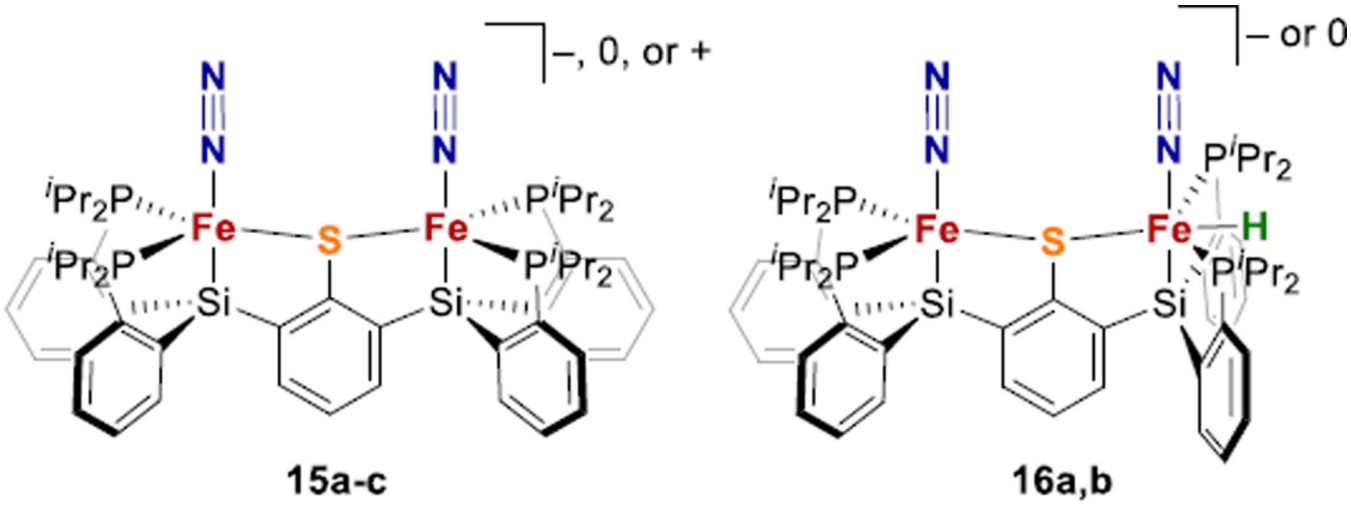


Figure 7.
N_2 binding to iron using a thiolate donor.

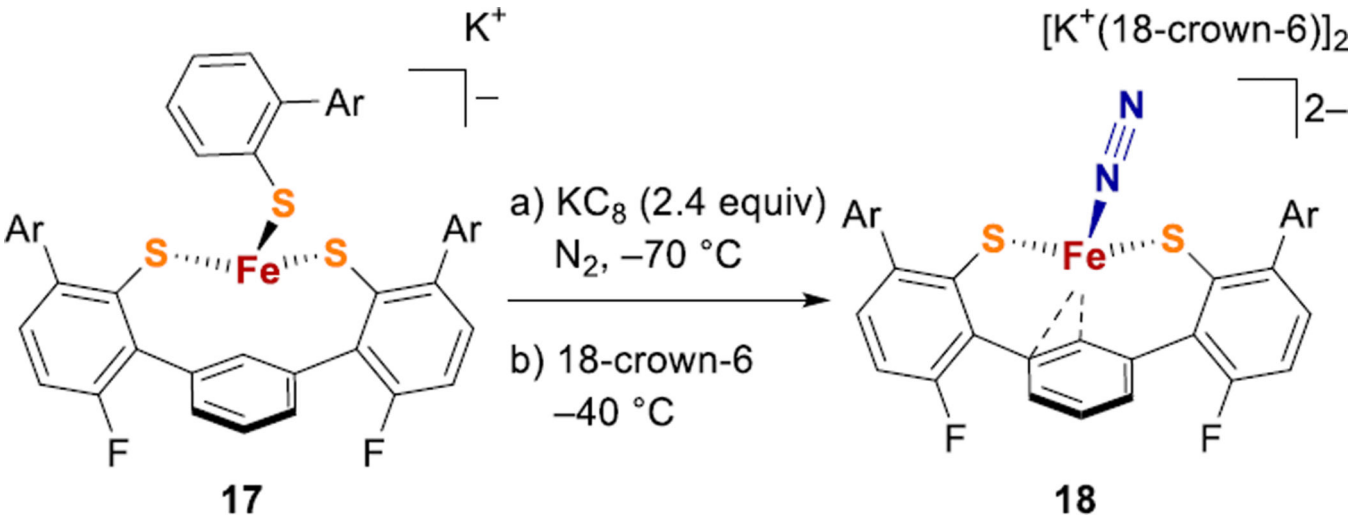


Figure 8.

N_2 binding to an iron–sulfur–carbon site. Ar = 2,4,6-^{i}Pr$_3$C$_6$H$_2$.

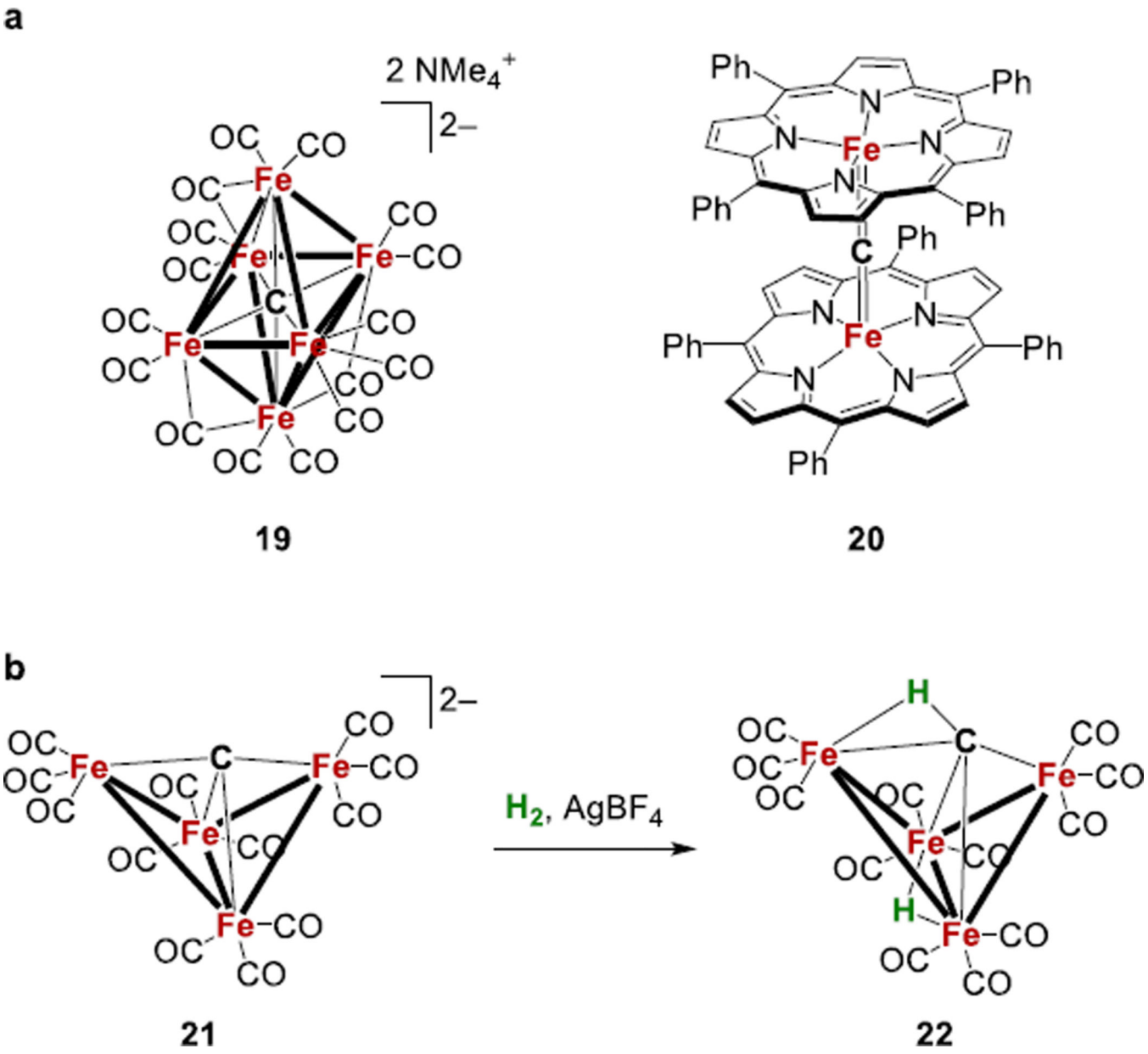


Figure 9.
Structures and reactivity of synthetic iron-carbide complexes.

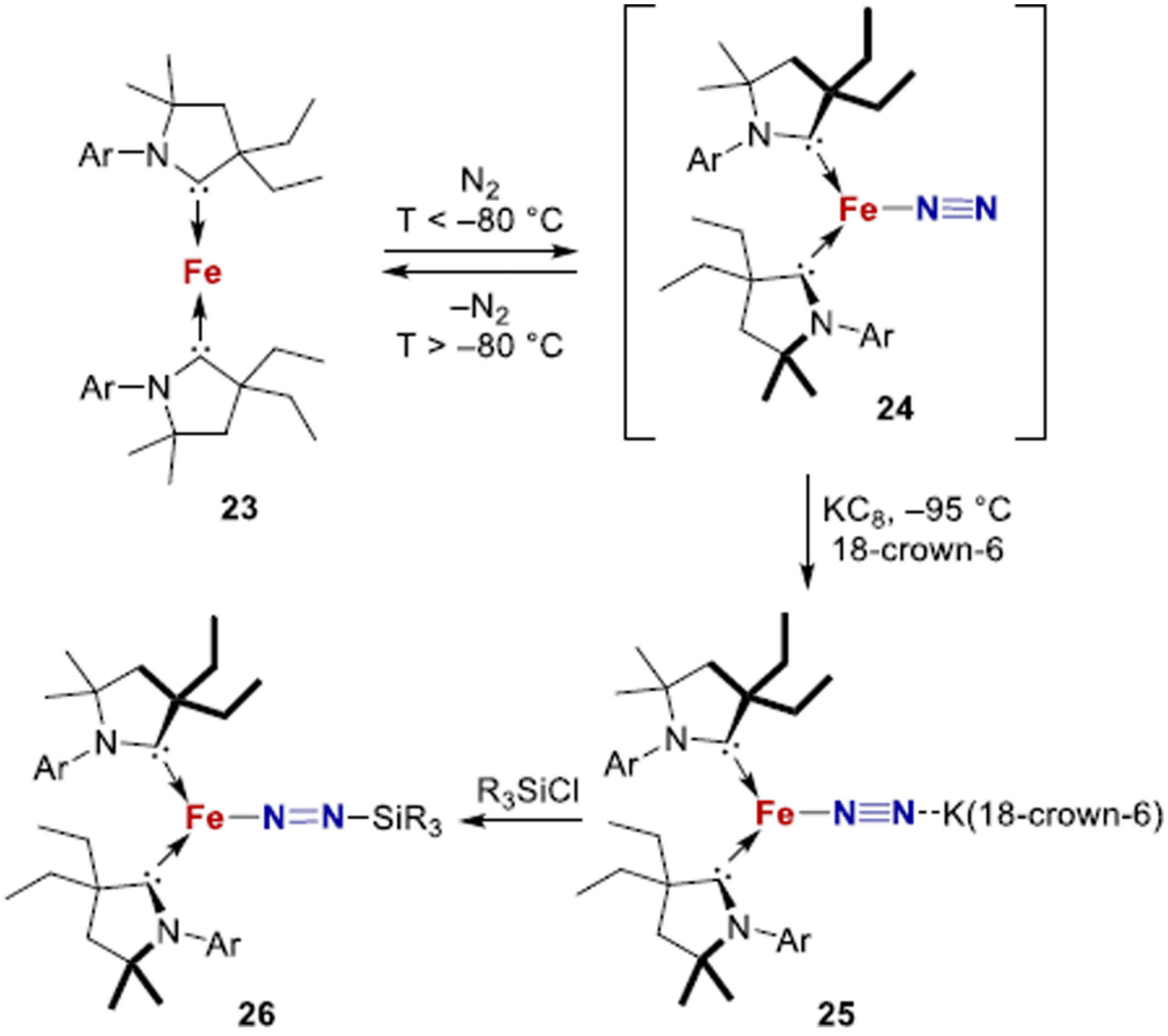


Figure 10.

N_2 binding and functionalization using CAAC ligands. Ar = 2,6-$^{i}Pr_2C_6H_3$. R = Me, Et.

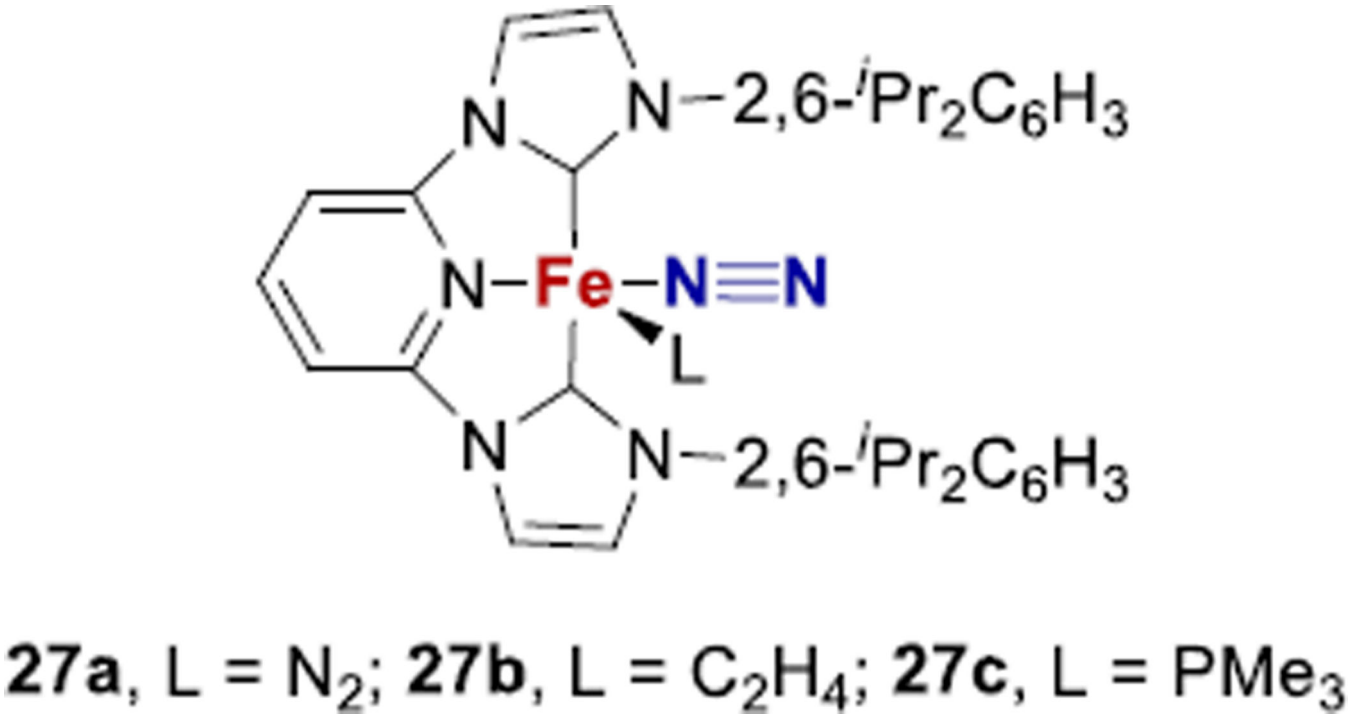


Figure 11.
N_2 binding to Fe using NHC ligands.

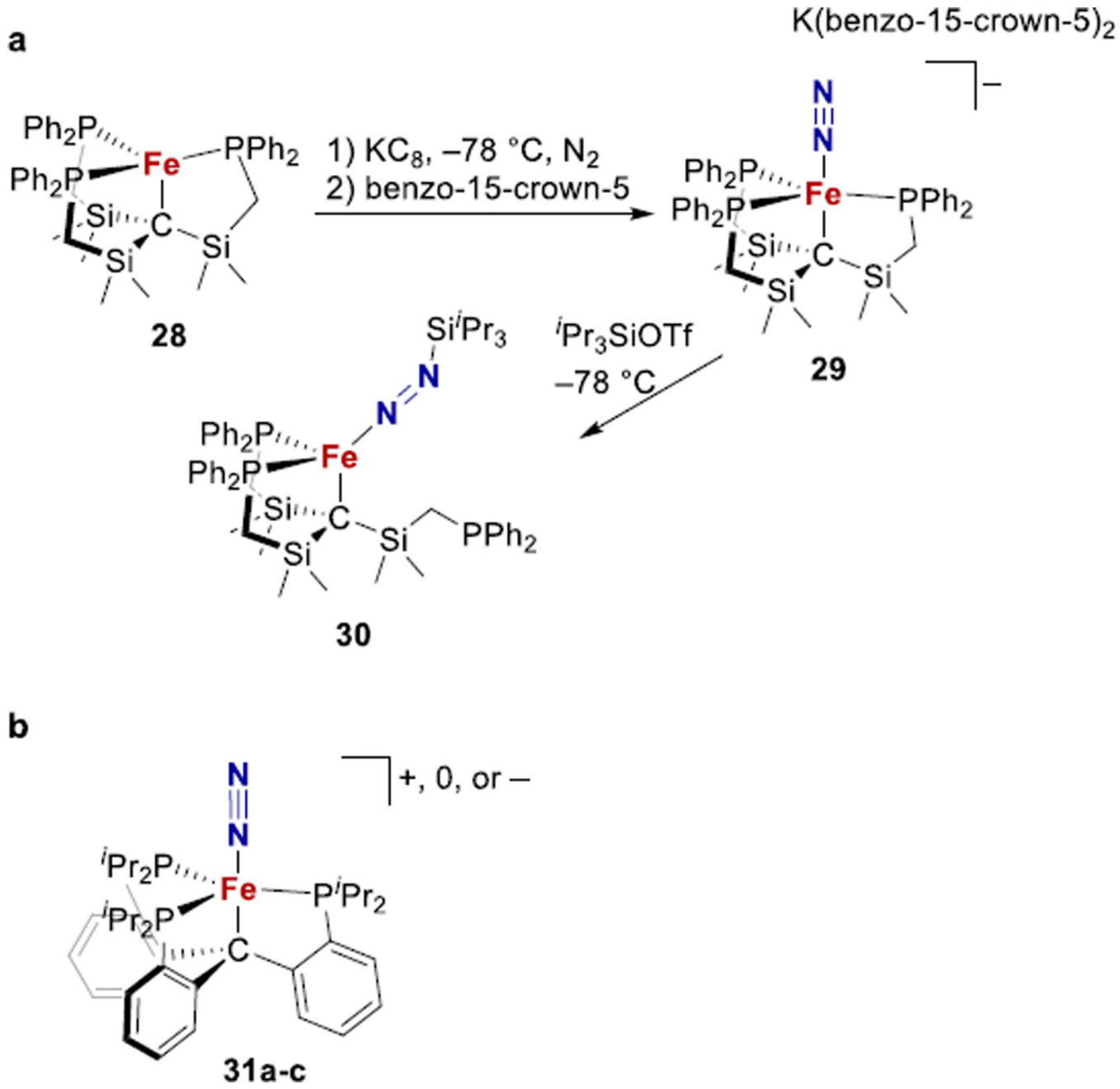


Figure 12.
Fe-N_2 complexes with trans carbon and N_2 ligands.

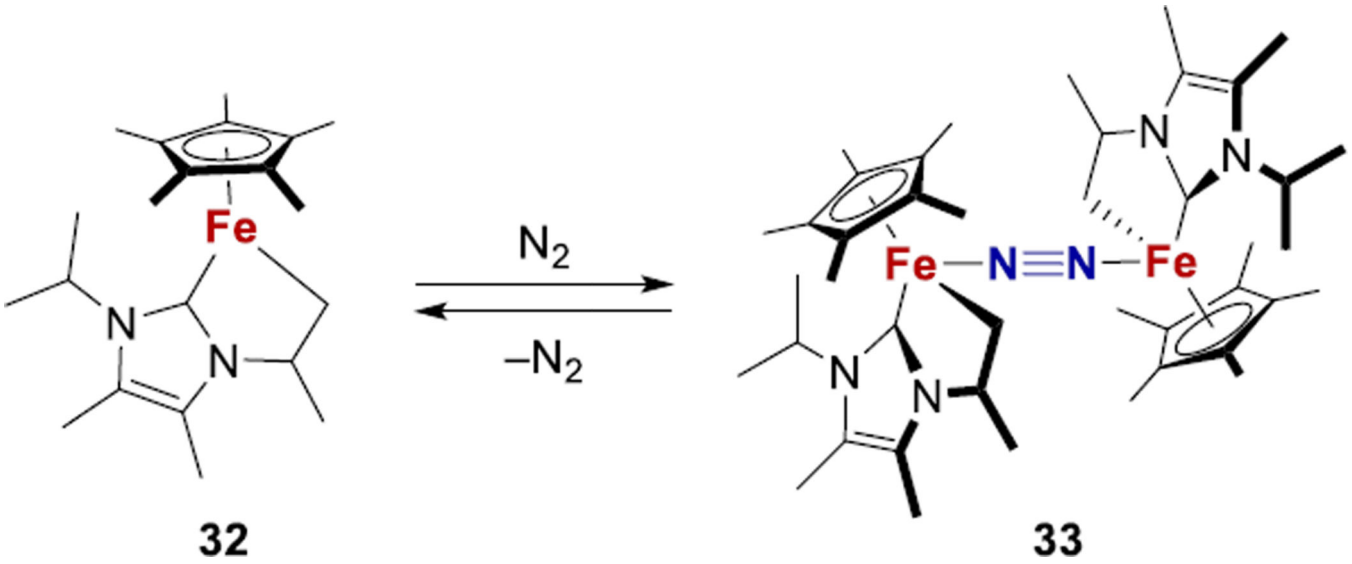
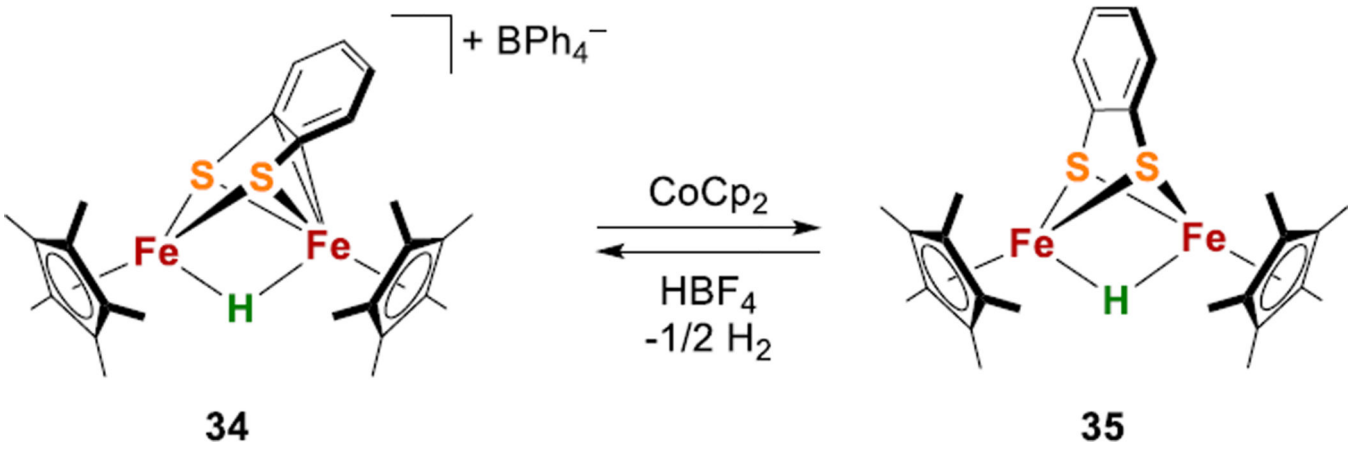


Figure 13.
N_2 binding to Fe using multiple carbon-based ligands.



34 35

Figure 14.
Bridging iron hydrides in sulfur- and carbon-rich environment.

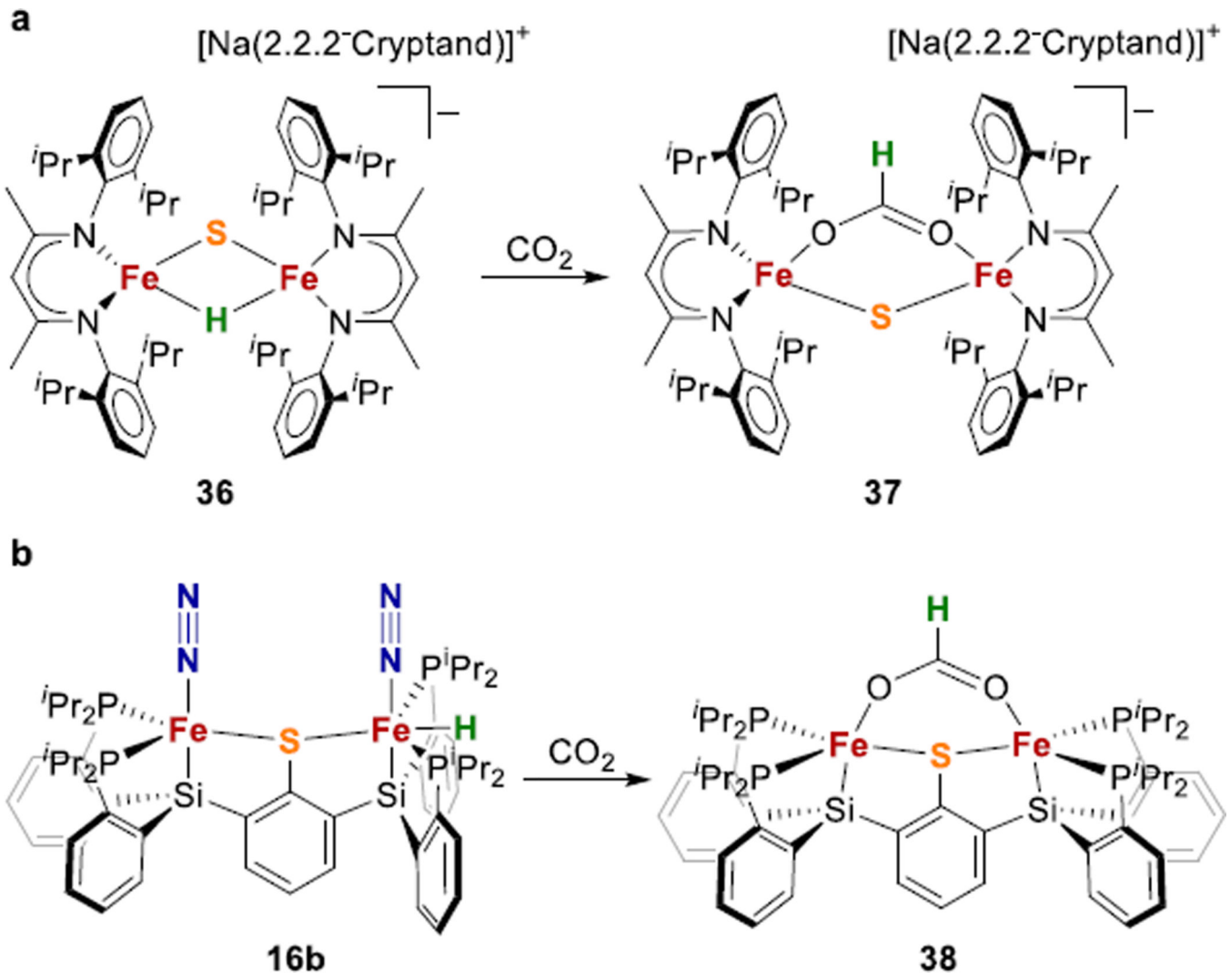


Figure 15.
Iron hydrides with sulfur ligands that reduce CO_2.

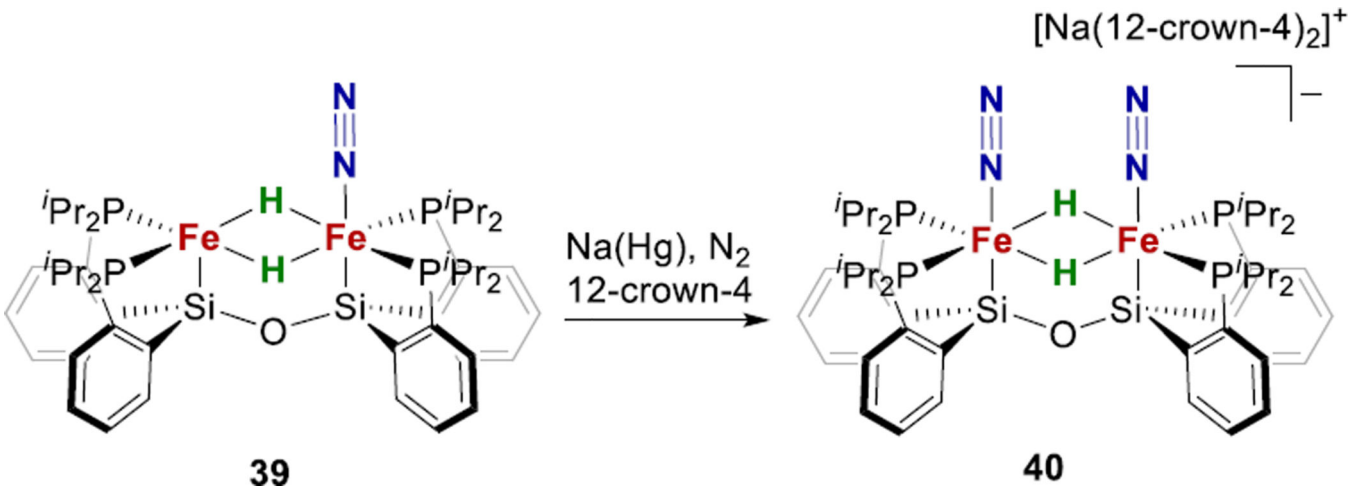
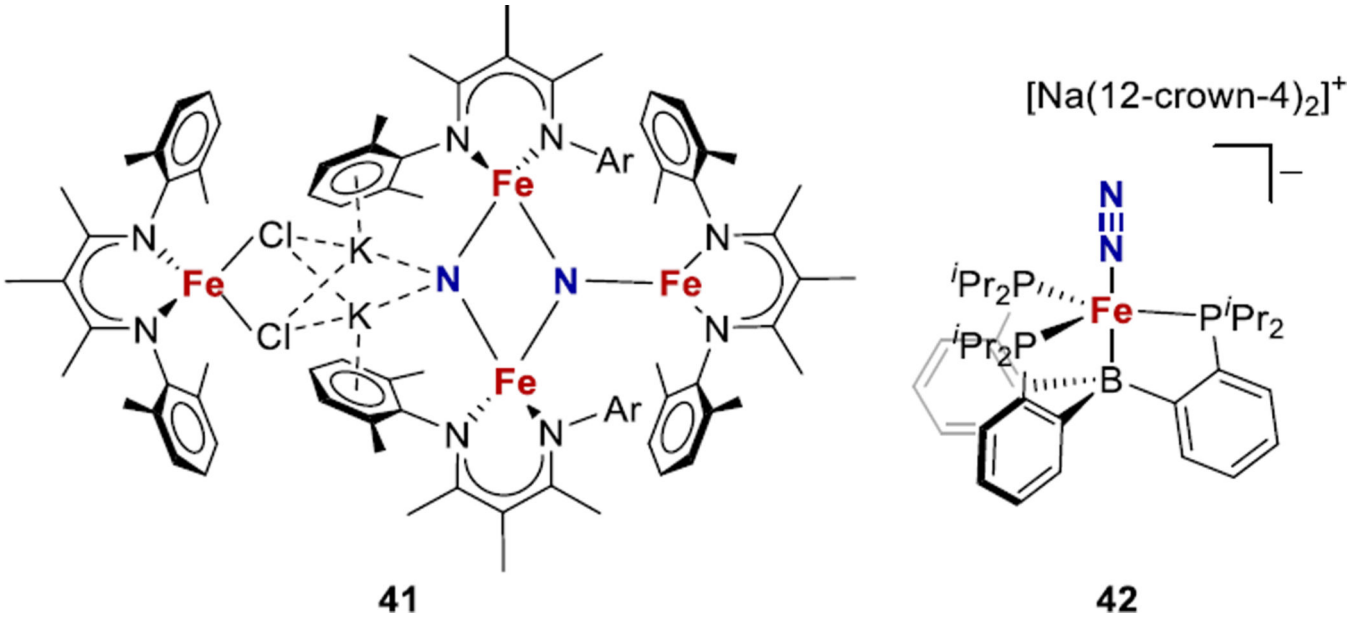


Figure 16.
Binding of N_2 in the presence of bridging hydrides.



41 42

Figure 17.
Recent examples of N_2 activation on iron with N- and P-based supporting ligands.

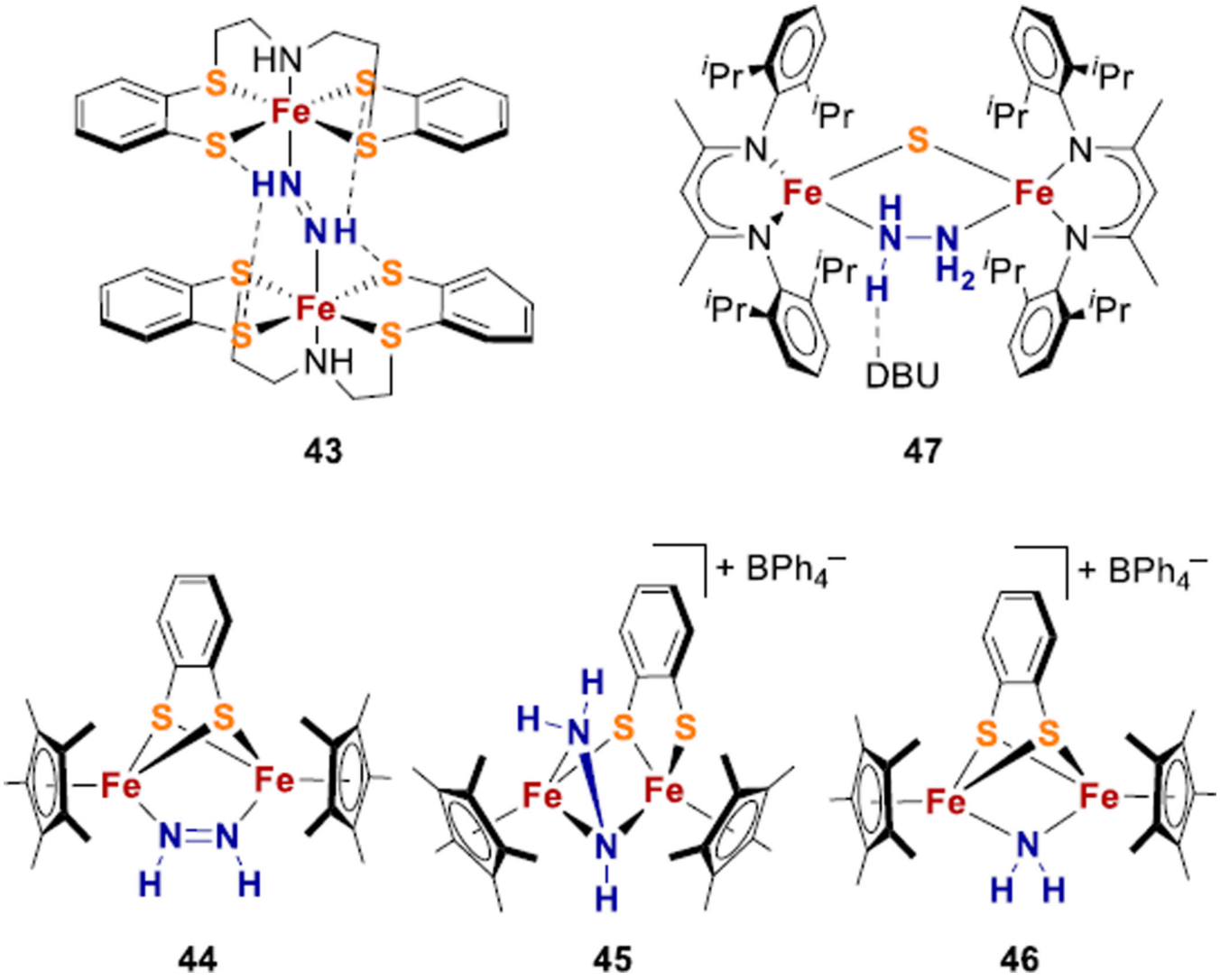


Figure 18.

Fe-N_xH_y species in sulfur-rich environments. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.