Ammonia formation by a thiolate-bridged diiron amide complex as a nitrogenase mimic

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Although nitrogenase enzymes routinely convert molecular nitrogen into ammonia under ambient temperature and pressure, this reaction is currently carried out industrially using the Haber-Bosch process, which requires extreme temperatures and pressures to activate dinitrogen. Biological fixation occurs through dinitrogen and reduced N_xH_y species at multi-iron centres of compounds bearing sulfur ligands, but it is difficult to elucidate the mechanistic details and to obtain stable model intermediate complexes for further investigation. Metal-based synthetic models have been applied to reveal partial details, although most models involve a mononuclear system. Here, we report a diiron complex bridged by a bidentate thiolate ligand that can accommodate HN=NH. Following reductions and protonations, HN=NH is converted to NH₃ through pivotal intermediate complexes bridged by N₂H₃⁻ and NH₂⁻ species. Notably, the final ammonia release was effected with water as the proton source. Density functional theory calculations were carried out, and a pathway of biological nitrogen fixation is proposed.

N itrogen fixation has been the subject of extensive investigation for the past century. A successful artificial fixation known as the Haber-Bosch process is routinely practised on a large scale, producing about 160 million tonnes of ammonia per year, which is essentially used as fertilizer for one-third of Earth's population. The extreme conditions necessary for this process (200– 400 atm and 400–600 $^{\circ}$ C) make it very energy-consuming; indeed, it consumes over 1% of the world's annual energy supply. In contrast, nitrogenases in some microorganisms produce ammonia from dinitrogen at ambient temperature and pressure. Understanding the fixation mechanisms of the FeMo-cofactor, the active site of these enzymes, would provide insights into designing and optimizing artificial fixation.

Elucidating the detailed mechanism of enzymatic nitrogen fixation has long been a challenge for scientists^{1,2}. Based on the known [Fe₇MoS₉C] core structure of the FeMo-cofactor^{3,4}, as well as experimental and theoretical studies, two general pathways for dinitrogen reduction to ammonia have been formalized by Hoffman and Dean: the distal (that is, $M^n - N \equiv N \rightarrow M^{n+3} \equiv N + NH_3$ $\rightarrow M^n + NH_3$) and alternating $(M^n - N \equiv N \rightarrow M^n - NH = NH \rightarrow$ $M^n - NH_2 - NH_2 \rightarrow M^n + 2 NH_3$) mechanisms^{5,6}. Two main assumptions have been proposed for dinitrogen binding and reduction on the FeMo-cofactor: one involves dinitrogen binding to the molybdenum atom⁷⁻¹², and the other suggests the participation of one or more iron atoms of the metallocluster^{13,14}.

Recent site-directed mutagenesis studies have suggested a high probability that the 'belt' iron atoms of the FeMo-cofactor are the site of dinitrogen binding and reduction^{6,15–17}. In addition, some density functional theory (DFT) calculations have proposed that the transformation of dinitrogen to ammonia is mediated by the two 'belt' iron atoms through a series of N_xH_y bridged intermediates^{18,19} (Fig. 1a). Although the coordination chemistry of dinitrogen and reduced dinitrogen species with iron has seen a growth spurt in the past decade^{13,14}, most iron-based synthetic models involve the single iron system. Only a few examples of model complexes with two or more iron centres have been described to date. Peters and co-workers successfully synthesized a number of N_xH_y bridged diiron complexes supported by Fe[PhBPR₃] (R = Ph or CH₂Cy) scaffolds^{20,21}. More recently, the Holland group reported a unique tetra-iron nitride complex with β -diketiminate auxiliary ligands, which can be reduced by dihydrogen to release ammonia²². Additionally, some thiolate- and sulfido-bridged diiron model complexes bearing N₂H₂ and PhNNH₂ ligands have also been reported by Sellmann^{23,24}, Holland^{25,26} and colleagues.

Structural and functional mimicking of nitrogenase with synthetic diiron models bearing thiolate ligands has been one of the fields researched by our group. To this end, a series of diazene bridged diiron complexes supported by the monodentate thiolate ligand $[Cp^{+}Fe(\mu-SEt)_{2}(\mu-\eta^{1}:\eta^{1}-HN=NH)FeCp^{+}]^{n+}$ ($Cp^{+}=\eta^{5}-C_{5}Me_{5}$, $\eta^{-5}-C_{5}Me_{4}H$, n = 0, 1), were prepared and spectroscopically characterized clearly²⁷. Catalytic cleavage of the N–N bond of hydrazine was realized using the phenylhydrazine complex $[Cp^{+}Fe(\mu-SEt)_{2}(\mu-\eta^{1}:\eta^{1}-PhN=NH)FeCp^{+}]$ as catalyst^{28,29}.

In this Article, the bidentate benzene-1,2-dithiolate (bdt) ligand was used in constructing diiron model complexes bearing $N_x H_y$ species (Fig. 1b). The choice of the bdt ligand was based on the following considerations: (1) its redox non-innocent character^{30,31} (that is, bdt can act as either a dianionic or a π -radical monoanionic ligand), and (2) it supports variable coordination geometries^{32–36}. These properties mean that the bdt ligand can potentially provide variable Fe–S scaffolds in one complex, allowing this complex to support the binding and transformation of different $N_x H_y$ species. Here, we report the synthesis, interconversion and ammonia-releasing reaction of bdt-bridged diiron $N_x H_y$ complexes, through which a reduction sequence of the $N_x H_y$ species was established.

Results and discussion

Synthesis, characterization and interconversion of diiron N_xH_y , complexes. The diiron precursor $[Cp^*Fe(\mu-\eta^2:\eta^4-bdt)FeCp^*]$ (1) was prepared in a similar manner as the recently reported triply

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Figure 1 | Hypothetical diiron sites of ammonia production from dinitrogen. a,b, Possible routes are shown for the FeMo-cofactor (a) and our diiron model complex (b).

thiolate-bridged diiron complexes $[Cp^*Fe(\mu-SR)_2FeCp^*]$ (R = Et, Ph)37. Treatment of [Cp*FeCl]2 with 1 equiv. of Li2(bdt) in tetrahydrofuran (THF) from -78 °C to room temperature produced 1 as red-black crystals. The solid-state structure of 1 is shown in Supplementary Fig. S1. The most distinctive structural feature of 1 is that it is not symmetrical; the bdt ligand leans towards one iron centre, and binds to the two iron centres in a μ - η^2 : η^4 manner: to one iron centre through the two thiolates (in an η^2 fashion) and to the other through the two thiolates and their two adjacent carbons (η^4). The distances C23–C24 and C25–C26 in the benzene ring are found to be noticeably shorter than the others (Supplementary Table S15). This is characteristic of the π -radical monoanionic bdt ligand³⁰, indicating an electron transfer from the bdt ligand to the metal centres in order to stabilize the highly electron-deficient diiron core. We assume that this electrondeficient nature of 1 would make it a good candidate as a nitrogenase model complex, so as to accommodate the electrondonating $N_x H_y$ species (Fig. 2).

The room-temperature addition of 8 equiv. of hydrazine to a red-purple THF solution of $[Cp^*Fe(\mu-\eta^2:\eta^4-bdt)FeCp^*]$ (1)

resulted in the production of the dark green [Cp*Fe(μ - η^2 : η^2 -bdt) $(\mu - \eta^{1}: \eta^{1} - HN = NH)FeCp^{*}$ (2a) and the release of ammonia. Crystals of 2a suitable for X-ray diffraction analysis were not obtained owing to its high air sensitivity and good solubility in most solvents. One resonance for Cp^{*} methyl protons ($\delta = 1.27$ ppm) and two for bdt protons ($\delta = 6.31$ and 6.77 ppm) are shown in the ¹H NMR spectrum (in deuterated THF-d₈) of 2a, indicating the symmetrical geometry of 2a. A N₂H₂ resonance is noted at 15.29 ppm, which splits into an apparent doublet (${}^{1}J_{NH} = 68.4 \text{ Hz}$) when 2a is prepared using ${}^{15}N_2H_4$. The broadness of the doublets precludes the resolution of higher-order N-H and H-H coupling. The ¹⁵N NMR spectrum (Fig. 3) shows an AA'XX' splitting pattern centred at 349.87 ppm, indicative of two equivalent sp²-hybridized nitrogen atoms. This is similar to that observed in the related side-on N₂H₂ species, *cis*-[Fe(DMeOPrPE)₂(η^2 -N₂H₂)]³⁸. The infrared spectrum of 2a shows the ν (N–H) band at 3,242 cm⁻¹ which shifts to 3,236 cm⁻¹ in the sample of ¹⁵N-2a.

To obtain more insight into the coordination geometry of the N₂H₂ ligand, one-electron oxidation of 2a was performed with $Fc \cdot PF_6$ (Fc = Ferrocene). The solid-state structure of $[Cp^*Fe(\mu \eta^2:\eta^2$ -bdt)(μ - $\eta^1:\eta^1$ -HN=NH)FeCp*][PF_6] (2a[PF_6]) is shown in Fig. 4a. The N₂H₂ ligand coordinates the [Fe₂S₂] scaffold in a cis fashion. The long Fe-Fe distance of 3.176(2) Å is indicative of the absence of a bonding interaction between the two iron centres. The N-N bond distance of 1.312(4) Å is similar to those found in the previously reported diiron complexes bearing an end-on bridging N2H2 ligand (1.28-1.37 Å)^{20,21,23,24,27,39}. This distance is closer to the value of an $N(sp^2)-N(sp^2)$ double bond. The shortened Fe-N bond distances of 1.827(3) Å and 1.826(3) Å in $2a[PF_6]$ suggest a strong interaction between the iron orbitals and the system of diazene. Unlike complex 1, the carbon-carbon distances of the benzene ring in 2a[PF₆] are fairly consistent, indicating a dianionic character of the bdt ligand. The Mössbauer spectrum of 2a[PF₆] was recorded with isomer shift and quadrupole



Figure 2 | Synthesis of diiron N_xH_y complexes and their methyl-substituted analogues. Reagents and conditions: (i) 1 equiv. Li₂(bdt), THF, -78 °C to room temperature, 45%; (ii) for **2a**, 8 equiv. anhydrous hydrazine, THF, room temperature, 2 h, 88%; for **2b**, 8 equiv. methyl hydrazine, THF, room temperature, 3 h, 68%; (iii) 1 equiv. Fc·PF₆, CH₂Cl₂, room temperature, 1 h, 85% for **2a**[PF₆] and 83% for **2b**[PF₆]; (iv) for **3a**[BPh₄], 1 equiv. Lut·HBPh₄ (Lut = 2,6-lutidine), THF, -20 °C, 3 h, 89%; for **3b**[BPh₄], 1 equiv. Lut·HBPh₄, CH₂Cl₂, -50 °C to room temperature, 3 h, 71%; (v) 2 equiv. CoCp₂, 2 equiv. Lut·HBPh₄, THF, -78 °C to room temperature, 85% for **4a**[BPh₄] and 56% for **4b**[BPh₄].





splitting to be 0.29 and 0.74 mm s⁻¹, respectively (Supplementary Fig. S58). Complexes **2a**[**PF**₆] and ¹⁵N-**2a**[**PF**₆] were further characterized by electrospray ionization high-resolution mass spectrometry (ESI-HRMS) and infrared spectrometry.

According to the spectroscopic data discussed above, we assign the N_2H_2 ligand in 2a and $2a[PF_6]$ to the diazene ligand, rather than the hydrazido(2–) ligand presented in some mono-iron sideon N_2H_2 complexes^{38,40}. Considering the multimetal structure of the FeMo-cofactor, the end-on *cis* bridging diazene species, corresponding to 2a and $2a[PF_6]$, may be a possible intermediate in biological nitrogen fixation.

The addition of the proton source Lut-HBAr^F₄ (Lut = 2,6-lutidine, BAr^F₄ = tetrakis(3,5-bis(trifluoromethyl)phenyl)borate anion) and Lut-HBPh₄, to the THF solution of **2a** at -30 °C and -20 °C, respectively, resulted in the N₂H₃⁻ complexes [Cp*Fe(μ - $\eta^1:\eta^2$ -bdt)(μ - $\eta^1:\eta^2$ -NH₂-NH)FeCp*][BAr^F₄] (**3a**[BAr^F₄]) and [Cp*Fe(μ - $\eta^1:\eta^2$ -bdt)(μ - $\eta^1:\eta^2$ -NH₂-NH)FeCp*][BPh₄] (**3a**[BPh₄]). Protonation-induced electron transfer occurs in this reaction. By transferring two electrons from the diiron centre to the protonated diazene ligand, the formal valences of the two iron atoms rise from $Fe^{II}-Fe^{II}$ in 2 to $Fe^{III}-Fe^{III}$ in 3⁺. Both $3a[BAr_{4}^{F}]$ and $3a[BPh_{4}]$ are stable in the solid state, but convert to $2a^+$ and $4a^+$, probably through an intermolecular proton transfer in solution at room temperature, as evidenced by ¹H NMR spectroscopy. The solidstate structure of 3a[BAr^F₄] reveals that a μ - η^1 : η^2 N₂H₃⁻ ligand bridges the diiron centre (Fig. 4b). Comparatively, the coordination manner of the bdt ligand changes from μ - η^2 : η^2 in 2 to μ - η^1 : η^2 in $3a[BAr_{4}^{F}]$. Meanwhile, the Fe-Fe distance of $3a[BAr_{4}^{F}]$ is pulled close to 2.6990(11) Å and falls into the range of a bonding interaction. We believe that it is the binding flexibility of the bdt ligand, including the apparent synergy between its binding mode and the Fe-Fe interaction, that allows for the observed transformation. The N-N bond length of 1.402(5) Å is longer than that in $2a[PF_6]$ (1.312(4) Å) after protonation, within the range of the N-N single bond lengths. This distance is close to the N-N lengths observed in hydrazido-borane complexes $[(SiP_3^R)Fe(\eta^1 N_2H_3B(C_6F_5)_3)$] (R = Ph and *i*-Pr)⁴¹ and slightly longer than that of the mono-iron $N_2H_3^-$ complex [(PhBP^{Ph}₃)Fe(η^2 -N₂H₃)(CO)]³⁹. The Fe-N distances (Fe2-N1 1.892(5) Å, Fe1-N1 1.907(4) Å, Fe2-N2 1.937(5) Å) indicate the sp³-hybridization of nitrogen atoms in the $N_2H_3^-$ ligand.

To locate the $N_2H_3^-$ protons, ¹⁵N-3a[BPh₄] was synthesized by protonation of ¹⁵N-2a. The ¹H NMR spectrum (-50 °C, THF-d₈) of $3a[BPh_{1}]$ shows three distinct protons ($\delta = 10.24$, 5.51 and 4.10 ppm) for the $N_2H_3^-$ ligand. They all split into doublets ${}^{(1)}_{NH} = 80$ Hz for $\delta = 10.24$ ppm; ${}^{11}_{J_{NH}} = 84$ Hz for $\delta = 5.51$ and 4.10 ppm) following ${}^{15}N$ labelling. The ${}^{15}N{}^{1}H{}$ NMR spectrum (-50 °C, THF-d₈, Fig. 3b) reveals two resonances at 111.58 and 33.05 ppm, which split into a doublet (${}^{1}J_{\rm NH} = 80.0$ Hz) and triplet $(^{1}J_{\rm NH} = 84.0 \text{ Hz})$, respectively, on turning the proton decoupler off, implicating the presence of an -NH nitrogen atom and an $-NH_2$ nitrogen atom. N–N coupling is also observed (${}^{1}J_{NN} = 9.4$ Hz). Correlated information from the ¹H-¹⁵N heteronuclear single quantum correlation (HSQC) spectrum $(-50 \degree C, THF-d_8)$ suggests that the proton assigned at 10.24 ppm attaches to the nitrogen assigned at 111.58 ppm, while protons assigned at 5.51 and 4.10 ppm connect the nitrogen assigned at 33.05 ppm (Supplementary Fig. S19). All these NMR experiments confirm that complex $3a[BPh_4]$ contains an $N_2H_3^-$ ligand. Complexes $3a[BPh_4]$, $3a[BAr_4^F]$ and their ¹⁵N-labelled congeners were further characterized by ESI-HRMS and infrared spectrometry.

Although $N_2H_3^{-}$ is believed to be an important intermediate in the reduction of N_2 to NH_3 , iron $N_2H_3^-$ complexes were not synthesized until recently. Tyler and co-workers prepared the first iron(II) $N_2H_3^-$ complex $[Fe(DMeOPrPE)_2(\eta^2-N_2H_3)]^+$ by either protonation of $[Fe(DMeOPrPE)_2(\eta^2-N_2H_2)]$ or deprotonation of $[Fe(DMeOPrPE)_2(\eta^2-N_2H_4)]^{2+}$ (ref. 40). The first iron $N_2H_3^{-}$ complexes characterized by X-ray crystal diffraction analysis were the zwitterionic iron(II) hydrazido-borane complexes $[(SiP_{3}^{R})Fe(\eta^{1} N_2H_3B(C_6F_5)_3)$] (R = Ph and *i*-Pr)⁴¹. Another $N_2H_3^-$ iron complex $[(PhBP^{Ph}_{3})Fe(\eta^{2}-N_{2}H_{3})(CO)]$, reported by the Peters group, was achieved by addition of hydrazine to [(PhBPPh_3)Fe(Me)] at low temperature, followed by treatment with CO (ref. 39). These complexes show two different coordination geometries of the $N_2H_3^{-}$ ligand, η^1 and η^2 , on the single iron centres. In contrast, the case we report here represents the coordination of the $N_2H_3^$ ligand on diiron centres. This μ - η^1 : η^2 coordination of the N₂H₃⁻ ligand in $3a^+$ offers a new possible mode for $N_2H_3^-$ binding to the FeMo-cofactor.

Further reduction and protonation of **3a**[**BPh**₄] was carried out. The addition of 2 equiv. of CoCp₂ to the THF solution of **3a**[**BPh**₄] at -78 °C, followed by the addition of 2 equiv. of Lut·HBPh₄, afforded brown [Cp*Fe(μ - η^2 : η^2 -bdt)(μ -NH₂)FeCp*][BPh₄] (**4a**[**BPh**₄]) with the release of ammonia. The released ammonia was collected and quantified, showing an 80% yield by ¹H NMR



Figure 4 | Oak Ridge thermal ellipsoid plot (ORTEP) diagrams, with the thermal ellipsoids shown at a 50% probability level. a, 2a[PF₆]. b, 3a[BAr^F₄]. c, 4a[BPh₄]. d, 2b. e, 3b[BPh₄]. f, 4b[BPh₄]. For each panel, anions, one THF molecule and hydrogen atoms on carbons are omitted for clarity. See Supplementary Information for more details.

in DMSO-d₆ as its NH₄Cl salt after vacuum transfer of the reaction volatiles into an ether solution of HCl. The crystal structure of $4a[BPh_4]$ indicates that a bridging NH_2^- ligand binds to the diiron centres (Fig. 4c). The Fe-N distances of 1.924(3) and 1.932(3) Å are as expected for coordination of sp^3 -hybridized nitrogen to iron, and similar to those observed in the two bridging NH₂⁻ diiron complexes synthesized to date, 2.049(8) and 2.034(9) Å for $[{(PhBP^{Ph}_{3})Fe}_{2}(\mu-NH_{2})_{2}]^{20}$ and 1.996(2) Å for $[{(Ar)_{2}C_{6}H_{3}}]$ $Fe_{2}(\mu-NH_{2})_{2}^{42}$. The Fe-Fe distance of 2.4423(6) Å suggests the interaction of the two iron atoms. The ¹H NMR spectrum (THF-d₈) reveals a solution structure similar to that observed in the solid state. An $\rm NH_2^-$ resonance is noted at 8.84 ppm and splits into a doublet upon ¹⁵N labelling. Its ¹⁵N NMR spectrum (THF-d₈, Fig. 3c) contains a triplet at 76.38 ppm (${}^{1}J_{\text{NH}} = 72.0 \text{ Hz}$) that becomes singlet upon decoupling. The correlation of the proton resonance and the ¹⁵N resonance in the ¹H-¹⁵N HSQC spectrum confirms the presence of the NH_2^- ligand (Supplementary Fig. S26). Complexes $4a[BPh_4]$ and ${}^{15}N-4a[BPh_4]$ were further characterized by ESI-HRMS and infrared spectrometry.

In the conversion of $3a[BPh_4]$ to $4a[BPh_4]$, the coordination of the bdt ligand shifts from μ - η^1 : η^2 back to μ - η^2 : η^2 . Thus, three coordination manners for the bdt ligand, including μ - η^2 : η^4 π -radical monoanionic for 1, μ - η^2 : η^2 dianionic for 2a, $2a[PF_6]$ and $4a[BPh_4]$, and μ - η^1 : η^2 dianionic for $3a[BPh_4]$ and $3a[BAr_4^F]$, are observed during the conversion of 1 to $4a[BPh_4]$. It follows that the bdt ligand can shift the coordination according to the nature of the N_xH_y species, which, to some extent, mimics the flexibility of the FeMo-cofactor.

DFT investigations of the reaction from $3a^+$ to $4a^+$. Evidently, two reductive protonation steps, as well as the release of one ammonia molecule, are involved in the transformation of $3a[BPh_4]$ to $4a[BPh_4]$. We reduced the amount of reductant or proton source in an attempt to analyse the details of this multistep reaction. However, complicated products were obtained, and no signals corresponding to $[Cp^*Fe(\mu-bdt)(N_2H_4)FeCp^*]^+$ and $[Cp^*Fe(\mu-bdt)(NH)FeCp^*]^+$ were observed by the ESI-HRMS spectroscopy. We posited that the steps involved in the conversion of $3a[BPh_4]$ to $4a[BPh_4]$ are energetically downhill with low activation energy barriers. As a result, it is difficult to capture the intermediates along the reaction until the formation of $4a[BPh_4]$.

To access the whole conversion process from $3a[BPh_4]$ to 4a[BPh₄], DFT model calculations were performed. The computed profile of the free energy in solution and gas phase is shown in Fig. 5 (see Supplementary section 'Experimental' for computational details). In the calculations, the Cp^{*} ligand was modelled by Cp (Cp = η^5 -C₅H₅), which is generally adopted in computations of the metallocene system for the sake of CPU processing time. The cationic species $[CpFe(\mu-\eta^1;\eta^2-bdt)(\mu-\eta^1;\eta^2-NH_2-NH)FeCp]^+$ (3am⁺) and $[CpFe(\mu - \eta^2: \eta^2-bdt)(\mu - NH_2)FeCp]^+$ (4am⁺) were taken as model compounds for the cationic parts of $3a[BPh_4]$ and $4a[BPh_4]$, respectively. In the following and Fig. 5, the superscripts in the labelling of stationary points indicate the charge and spin multiplicity of corresponding species. For example, '3am^{[2],} denotes the neutral species 3am with spin multiplicity of 2, and labelling '3am^{+[1]}' represents species 3am with charge of +1 and multiplicity of 1. As shown in Fig. 5, the reduction of starting model complex $3am^{+[1]}$ (S = 0) is exergonic by 9 kcal mol⁻¹ in solution, leading to $3am^{[2]}(S=1/2)$.

The protonation of $3am^{+[1]}$, which leads to dicationic species with either the NH···NH₃ or NH₂-NH₂ moiety, is computed to be endergonic by more than 68 kcal mol⁻¹ in the gas phase and more than 21 kcal mol⁻¹ in solution (Supplementary Fig. S62). This suggests an energetically unfavourable protonation of $3am^{+[1]}$ in comparison with its reduction leading to $3am^{[2]}$. The protonation of $3am^{[2]}$ favours $6^{+[2]}$ (S = 1/2) with an NH···NH₃ moiety rather than $9^{+[2]}$ with an H₂N-NH₂ moiety, because the former is significantly lower in free energy than the latter (by 20 kcal mol⁻¹ in solution). The conversion of $3am^{[2]}$ to $6^{+[2]}$ is exergonic by 14 kcal mol⁻¹ in solution and 29 kcal mol⁻¹ in the



Figure 5 | Computed minimum free-energy pathway for the transformation of 3am⁺ to 4am⁺. TPSSTPSS/TZVP relative free energies in solution (ΔG_R) are in kcal mol⁻¹. The data in parentheses are the corresponding relative free energies in the gas phase. The energy shown is the reaction free energy including that of all species involved in the corresponding reaction. See Supplementary section 'Experimental' for computational details and Figs S61-63 for a more detailed energy profile.

gas phase (see the $\Delta\Delta G_{\rm R}$ of this conversion in Fig. 5). The release of NH₃ from $6^{+[2]}$ yields $7^{+[2]}$ and the isolated NH₃ molecule. This step is almost isoenergetic ($\Delta\Delta G_{\rm R} = -1$ kcal mol⁻¹ in solution) due to the very weak binding between the released NH₃ and the NH moiety in $6^{+[2]}$. These results suggest that the cleavage of the N–N bond to liberate NH₃ most probably occurs through HN…NH₃ rather than the H₂N–NH₂ moiety.

HN-W bond to hold to hold the H13 most probably occurs through HN-W bond to hold the H2N-NH2 moiety. The 7^{+[2]} could undergo reduction and subsequent protonation to give **8**^{+[1]} (S=0) with a relative free energy in solution of -54 kcal mol⁻¹. The reduction of 7^{+[2]} leads to neutral species 7^[1]. This step is exergonic by 20 kcal mol⁻¹ in solution and 11 kcal mol⁻¹ in the gas phase. The subsequent protonation of 7^[1] is also exergonic by 11 kcal mol⁻¹ and 21 kcal mol⁻¹ in solution and the gas phase, respectively. The **8**^{+[3]} (S=1) species has almost the same energy (-53 kcal mol⁻¹) as that of **8**^{+[1]} gives the final product **4am**^{+[1]} (S=0). Such an isomerization is exergonic ($\Delta\Delta G_R = -2$ kcal mol⁻¹ in solution and -4 kcal mol⁻¹ in the gas phase) and occurs through a transition state with a free energy barrier of 6 kcal mol⁻¹ in solution (Supplementary Fig. S59), suggesting a feasible process. As shown in Fig. 5, the whole process (transformation of **3am**^{+[1]} to **4am**^{+[1]}) was computed to be exergonic by 56 kcal mol⁻¹ in solution and 64 kcal mol⁻¹ in gas phase. The current DFT results suggest that the ammonia release was mainly derived from the unbridging NH₂ of **3am**⁺.

Methyl hydrazine experiment. To corroborate the reaction pathway from $3a[BPh_4]$ to $4a[BPh_4]$ proposed by the DFT calculation, we studied the reaction of 1 with methyl hydrazine, in which the two nitrogen atoms are differentiated by different substitutions, together with the subsequent reductive protonation process (Fig. 2). The methyl diazene complex Cp*Fe(μ - η^2 : η^2 -bdt) $(\mu - \eta^{1}: \eta^{1} - MeN = NH)FeCp^{*}$ (2b) can be obtained in a similar manner by the addition of methyl hydrazine into the THF solution of 1. Oxidation of 2b with Fc·PF₆ afforded [Cp*Fe(μ - η^2 : η^2 bdt)(μ - η^1 : η^1 -MeN=NH)FeCp*][PF_6] (**2b**[PF_6]). They show the same cis coordination manner as the diazene analogues, with N-N bond distances of 1.292(10) and 1.305(4) Å for 2b (Fig. 4d) and **2b**[PF₆], respectively. Protonation of **2b** gives [Cp*Fe(μ - η^1 : η^2 bdt)(μ - η^{1} : η^{2} -NH₂-NMe)FeCp*][BPh₄] (**3b**[BPh₄], Fig. 4e). The methyl group attaches to the bridging nitrogen atom and two hydrogen atoms located at the terminal nitrogen atom. This implies that the protonation might take place at the =NH nitrogen atom rather than at the =NMe nitrogen atom of the MeN=NH ligand in 2b. The N-N bond distance of 1.447(4) Å is ~ 0.04 Å longer than that of 3a[BAr^F₄]. Taken together, the unchanged coordination pattern, similar geometry and spectroscopic data of 2b, 2b[PF₆] and 3b[BPh₄], compared with their unsubstituted analogues, suggest that steric hindrance does not significantly influence the coordination and reactivity of the nitrogenous ligands. Thus, it is reasonable to believe that the reductive protonation of **3b**[**BPh**₄] proceeds through the same pathway as **3a**[**BPh**₄].

The addition of 2 equiv. of $CoCp_2$ to the THF solution of **3b**[**BPh**₄] at -78 °C, followed by the addition of 2 equiv. of Lut-HBPh₄, resulted in a mixture of **4a**[**BPh**₄] and the NHMe⁻ complex [Cp*Fe(μ - η^2 : η^2 -bdt)(μ -NHMe)FeCp*][BPh₄] (**4b**[**BPh**₄]), with a ratio of 1:4. A relatively pure sample of **4b**[**BPh**₄] was obtained in 56% yield (Fig. 4f). This offers further experimental evidence that the ammonia released in the reaction of **3a**[**BPh**₄] to **4a**[**BPh**₄] was mainly derived from the unbridging NH₂ of the N₂H₃⁻ ligand, which is consistent with the DFT calculation results.

Ammonia release from complex 4a[BPh₄] in water. In the studies that have been reported to date, some well-defined iron dinitrogen

Table 1 | Ammonia production from complex 4a[BPh₄] in the presence of reducing agent and proton source.

Entry	Reducing agent	E _{1/2} (V)*	Proton source	Yield of NH ₃ (%)
1	CoCp ₂ (2 equiv.)	-1.15	Lut·HBPh ₄ (2 equiv.)	46
2	CoCp ₂ (6 equiv.)		Lut·HBPh ₄ (6 equiv.)	51
3	CoCp ₂ (2 equiv.)		H ₂ O (50 equiv.)	98
4	CrCp ₂ (2 equiv.)	-0.88	H ₂ O (50 equiv.)	8
5	CoCp* ₂ (2 equiv.)	-1.87	H ₂ O (50 equiv.)	96
6	$CrCp_{2}^{*}$ (2 equiv.)	-1.35	$H_2^{-}O$ (50 equiv.)	93
7	CoCp ₂ (2 equiv.)		CH ₃ OH (50 equiv.)	15
8	None		H ₂ O (50 equiv.)	Trace
9	CoCp ₂ (2 equiv.)		None	4

Reaction conditions: **4a[BPh₄]** (0.2 mmol), THF (6 ml), room temperature, 12 h. The NH₄⁺ was quantified by integration of the NH₄⁺ resonance with respect to an internal reference of ferrocene (see Supplementary section 'Experimental' for details). *Electrochemical data ($E_{1/2}$) of reductant in MeCN versus Ag/Ag⁺ in 0.1 M AgNO₃ (ref. 50).

and nitride complexes have shown the ability to produce ammonia by the addition of a hydrogen atom source and/or reductant^{22,43–49}. Although no example of ammonia release from an iron amide complex has been reported, it is believed to be the intermediate at the late stage of the dinitrogen reduction route. Studies were therefore carried out to examine the competence of $4a[BPh_4]$ in producing ammonia.

The addition of 2 equiv. of CoCp₂ and 2 equiv. of Lut·HBPh₄ to the THF solution of 4a[BPh₄] (0.2 mmol) produced NH₃ (46%) and H₂ (50% based on Lut·HBPh₄) (Table 1, entry 1). According to the ¹H NMR spectrum, 38% of the reactant 4a[BPh₄] remained in the reaction residues. Increasing the amount of CoCp2 and Lut·HBPh4 (6 equiv. to 4a[BPh₄]) led to no significant improvement in ammonia production (entry 2). We believe that the low yield of ammonia stemmed from the competitive reduction of protons to H₂, which is also observed with nitrogenase enzymes. We speculated that the weaker acidic proton source may inhibit the production of H₂, thus improving the yield of ammonia. Indeed, when waterwhich is the proton source of natural enzymes-was used in a 50 equiv. amount in combination with 2 equiv. of CoCp₂ at room temperature, an excellent yield of ammonia (up to 98%) was detected (entry 3). Only a trace amount or even no ammonia was detected in the control experiments (entries 8 and 9). Other reductants were also examined. When CrCp₂ was used, only an 8% yield of ammonia was obtained as a result of its lower reducing ability (entry 4). Stronger reductants (CoCp*2 and CrCp*2) were effective, giving good yields of ammonia (entries 5 and 6). When methanol, a proton source less acidic than water, was used in the reaction with CoCp₂, only a poor yield of ammonia was obtained (entry 7). The CO adduct of $4a[BPh_4]$, $[Cp^*Fe(CO)(\mu - \eta^1 : \eta^2 - bdt)(\mu - NH_2)$ FeCp*][BPh₄] (5[BPh₄]) (Supplementary section 'Experimental' and Table S5, Figs S9, S28, S45, S56), is not efficient in releasing ammonia, giving only a 12% yield. No H₂ was detected in any case when water was used as the proton source.



Conclusion

In summary, we have designed and synthesized a new diiron complex, $[Cp^*Fe(\mu-\eta^2:\eta^4-bdt)FeCp^*]$ (1). This $[Fe_2S_2]$ scaffold of

1 was found to be very effective in stabilizing nitrogenous donors, due in part to the binding flexibility of the bdt ligand, as evidenced by the fact that a series of diiron N_xH_y species containing $cis-\eta^1:\eta^1$ diazene, $\eta^1: \eta^2$ -hydrazido(1-) and amide bridges have been synthesized and characterized. Following stepwise protonation and reduction, the transformation of coordinated N₂H₂ to NH₂⁻ (through an N₂H₃⁻ intermediate followed by the final release of ammonia) was realized at diiron sites. DFT calculations were carried out to gain insight into the mechanism of the reaction $3a^+$ to $4a^+$. We assume that the first reductive protonation occurs at the unbridging NH_2 of the $N_2H_3^-$ ligand. After releasing NH_3 , the resulting NH⁻ bridged complex is converted to the NH₂ bridged complex through the second reductive protonation. These results provide effective evidence for a diiron mechanism for biological nitrogen fixation and show that N₂H₂, N₂H₃⁻ and NH₂ are very probably the species produced during the N2 reduction process within natural enzymes. In addition, a reduction route different from both alternating and distal mechanisms, $\mathrm{HN}{=}\mathrm{NH} \rightarrow \mathrm{HN}{-}\mathrm{NH}_2 \rightarrow \mathrm{NH}(+\mathrm{NH}_3) \rightarrow \mathrm{NH}_2 \rightarrow \mathrm{NH}_3, \quad \text{is} \quad \mathrm{pre-}$ sented. Further studies are under way to investigate the reactivity of this kind of [Fe₂S₂] scaffold towards N₂ and to elucidate how N₂ is converted to ammonia at diiron sites.

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Author contributions

J.Q. and B.W. supervised the project. J.Q. and Ya.L. conceived and designed the experiments. Ya.L., Yi.L., D.Y. and P.T. performed the experiments. Y.Lu., L.L. and S.C. performed the computational studies. J.Q., Ya.L., B.W. and F.C. co-wrote the paper. J.Z. and Y.Z. analysed the data. All authors discussed the results and commented on the manuscript.

Additional information

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Competing financial interests

The authors declare no competing financial interests.