Impact of Ligands and Media on the Structure and Properties of Biological and Biomimetic Iron-Sulfur Clusters

Shun Ohta[a] and Yasuhiro Ohki[b,c,*]

[a] Department of Frontier Materials Chemistry, Graduate School of Science and Technology, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan

[b] Department of Chemistry, Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602, Japan.

[c] PRESTO, Japan Science and Technology Agency (JST), 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan

* Corresponding author.

E-mail address: ohki@chem.nagoya-u.ac.jp (Y. Ohki)

Contents

Abstract

Iron-sulfur clusters are inorganic centers that are widely distributed in nature, mediating electron-transfer processes and transformation reactions. Their properties and functions arise from the different amino acid residues on the iron centers, some weak interactions within the protein matrices, and the extent of exposure to the medium. Studies on biomimetic model clusters have elucidated the molecular basis of the effects of iron-bound ligands and solvents on the synthesis and properties of iron-sulfur clusters. This review summarizes the effects of ligands and media on the properties and structure of both biological and biomimetic iron-sulfur clusters.

Keywords iron · sulfur · cluster · biomimetic · model compound

Highlights

- Influence of ligands and hydrogen bonds for biological iron-sulfur clusters.
- Effect of ligands and media on the synthesis of biomimetic iron-sulfur clusters.
- Ligand effects on the properties and structures of biomimetic iron-sulfur clusters.

Abbreviations: CoA, coenzyme A; Cys, cysteine; Fd, ferredoxin; HiPIP, high-potential iron sulfur protein; His, histidine; Asp, aspartate; Glu, glutamate; Ser, serine; NHE, normal hydrogen electrode; HMQC, heteronuclear multiple quantum correlated spectroscopy; NMR, nuclear magnetic resonance; *Pf*, *pyrococcus furiosus*; DPOR, dark-operative protochlorophyllide oxidoreductase; MEcPP, 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate; HMBPP, (*E*)-1-hydroxy-2-methyl-but-2-enyl-4-diphosphate; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; HMPA, hexamethylphosphoric triamide; FNR, fumarate and nitrate reduction; Nif, proteins for nitrogenase biosynthesis; Isc, proteins for iron-sulfur cluster synthesis; TMS, $SiMe_3$; Tip, 2,4,6-$^{i}Pr_3C_6H_2$; Dmp, 2,6-$(mesityl)_2C_6H_3$; Mes, mesityl (2,4,6-$Me_3C_6H_2$); DME, 1,2-dimethoxyethane (ethyleneglycol-dimethylether); THF, tetrahydrofuran; SCE, saturated calomel electrode; Gly, glycine; Ala, alanine; NHC, N-heterocyclic carbene; Cp, η^5-C_5H_5; Eind, 1,1,3,3,5,5,7,7-octaethyl-s-hydrindacen-4-yl; Tbt, 2,4,6-tris[bis(trimethylsilyl)methyl]phenyl; Dxp, 2,6-$(xylyl)_2C_6H_3$; Dpp, 2,6-$Ph_2C_6H_3$; TEMPO, 2,2,6,6-tetramethylpiperidin-1-yl-oxyl.

1. Introduction

Iron-sulfur clusters are inorganic centers that are abundant in nature, and indispensable for various biological processes such as electron transfer, enzymatic transformation reactions, iron/sulfur storage, and the regulation of gene expression [1, 2]. A potential relevance of iron-sulfur clusters to the origin of life has also been proposed [3]. As summarized in Fig. 1, the core compositions of currently known biological iron-sulfur clusters are [2Fe-2S], [3Fe-4S], [4Fe-3S], [4Fe-4S], and [8Fe-7S]. The [2Fe-2S] clusters **1**–**3** contain an Fe_2S_2 rhombus, and they exist in two oxidation states under physiological conditions, i.e., $[2Fe\text{-}2S]^{2+}$ and $[2Fe\text{-}2S]^{+}$. The triangular [3Fe-4S] cluster **4** usually appears in the $[3Fe\text{-}4S]^{+}$ or $[3Fe\text{-}4S]^{0}$ oxidation states in proteins, while the all-ferrous $[3Fe\text{-}4S]^{2-}$ state can be generated upon applying very low potentials [4]. The [4Fe-4S] clusters **6**–**8** feature a cubic core and occur predominantly in the $[4Fe\text{-}4S]^{3+}$, $[4Fe\text{-}4S]^{2+}$, and $[4Fe\text{-}4S]^{+}$ states, although an all-ferrous $[4Fe\text{-}4S]^{0}$ cluster is formed in the presence of titanium(III) citrate in the Fe protein of the nitrogenase from *Azotobacter vinelandii* [5] and the 2-hydroxyglutaryl-CoA dehydratase from *Acidaminococcus fermentans* [6]. The [4Fe-3S] clusters $\mathbf{5}^{\mathbf{Red}}/\mathbf{5}^{\mathbf{Ox}}$ and the [8Fe-7S] clusters $\mathbf{9}^{\mathbf{Red}}/\mathbf{9}^{\mathbf{Ox}}$ (henceforth denoted as the P-cluster) have been discovered relatively recently. These represent iron-sulfur clusters unique to the O_2-tolerant [NiFe] hydrogenase [7–10] and nitrogenase [11–17] enzymes, respectively, and their structures depend on their oxidation state.

Proteins incorporating iron-sulfur clusters are termed ‘iron-sulfur proteins’, in which the iron atoms are supported by the amino acid residues of the protein backbone. Cysteinyl thiolate (SCys; Fig. 1) is the most common ligand for complexing iron, found in various proteins such as ferredoxins (Fds) [18–20] and high-potential iron sulfur proteins (HiPIPs) [21, 22]. Other non-cysteinyl residues such as the imidazole moiety of histidine (His) [23–31], the carboxylate groups of aspartate (Asp) [32, 33] and glutamate (Glu) [34–36], as well as the amide groups of peptides [8, 14, 15, 17] occasionally bind to iron. Histidine ligation, for example, is found in the [2Fe-2S] clusters of MitoNEET (**2**) [23, 24]

and in Rieske proteins (**3**) [25, 26], as well as in some [4Fe-4S] clusters (**7**) of hydrogenases [27–30] and 4-hydroxybutyryl-CoA dehydratase [31]. The coordination of amide groups of peptides to iron occurs in the oxidized form of the [4Fe-3S] cluster of O_2-tolerant [NiFe] hydrogenase ($\mathbf{5^{Ox}}$) [8] and the P-cluster ($\mathbf{9^{Ox}}$) [14, 15, 17]. These non-cysteinyl ligands are expected to change the redox potentials of these clusters relative to those of cysteine-bound clusters. The electrochemical properties may furthermore be significantly impacted by hydrogen bonding between the iron-sulfur clusters and the peptides or water (see Section 2.1).

This review provides an overview of the effects of ligands and media on the chemistry of iron-sulfur clusters, a field that has been developed by complementary biochemical and inorganic approaches. The following sections deal with the influence of hydrogen bonding and amino acid residues on the properties of clusters in proteins, before the effect of ligands and media on the synthesis, properties, and structures of biomimetic clusters are discussed.

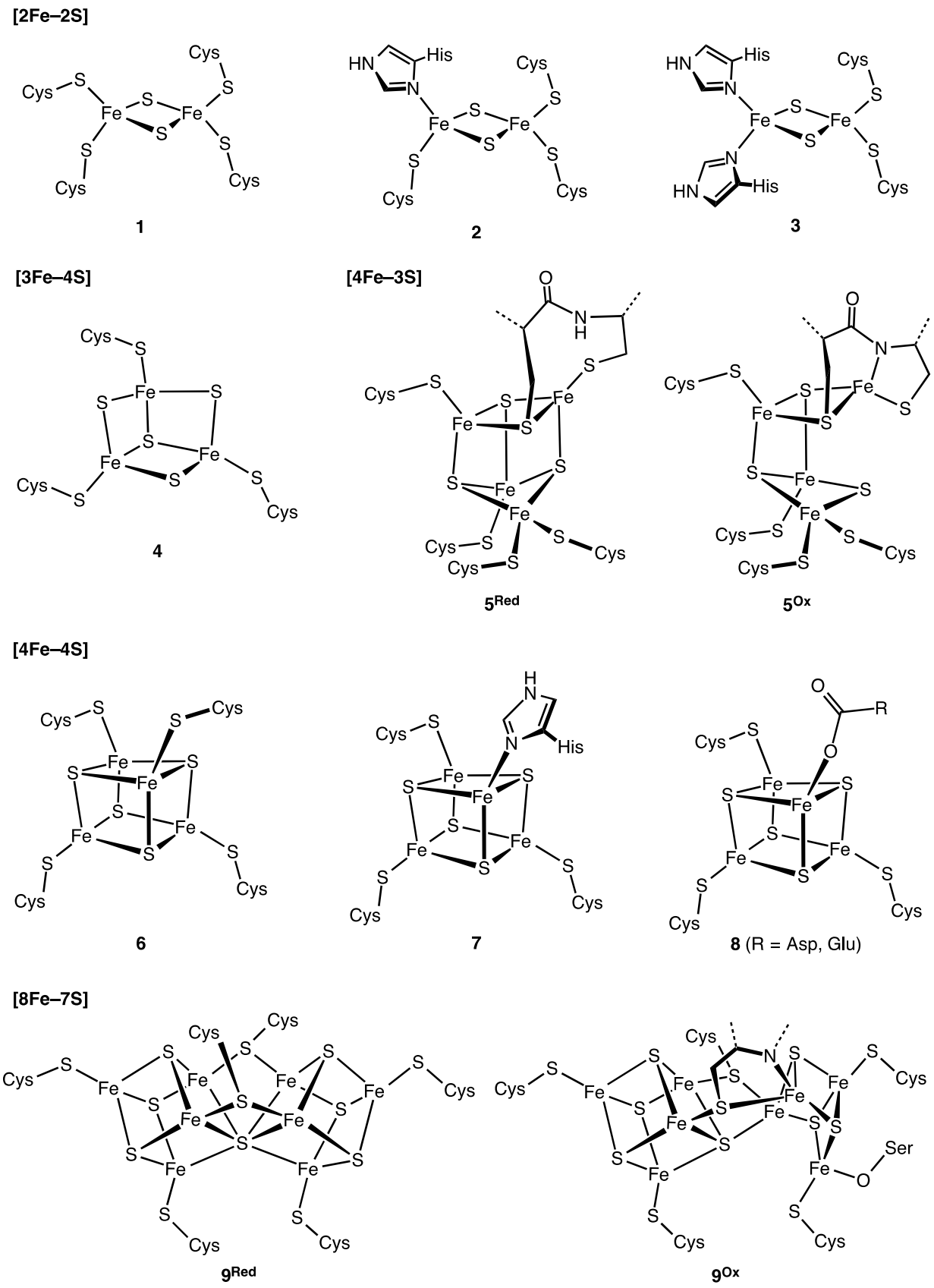

Fig. 1. Structures of the [2Fe-2S], [3Fe-4S], [4Fe-3S], [4Fe-4S], and [8Fe-7S] clusters. Cys = cysteine, His = histidine, Asp = aspartate, Glu = glutamate, Ser = serine.

2. Influence of Ligands and Hydrogen Bonding on the Properties of Biological Iron-Sulfur Clusters

Redox reactions are crucial for various cellular processes, and iron-sulfur clusters are frequently involved as mediators. The [2Fe-2S], [3Fe-4S], and [4Fe-4S] clusters are ubiquitous electron transfer cofactors, and they cover a wide range of redox potentials (–700 to 450 mV vs. normal hydrogen electrode (NHE)) [37]. The breadth of this range of redox potentials has been attributed to the variety of ligands that can be accommodated around the cluster cores and to the extent of hydrogen bonding with peptides and water. These effects are the subject of this section, while the electron-transfer properties of iron-sulfur clusters have been reviewed elsewhere [37–39].

2.1. The Importance of Hydrogen Bonding

Although ferredoxins (Fds) and high-potential iron sulfur proteins (HiPIPs) incorporate very similar cysteine-supported [4Fe-4S] cores, the oxidation states operating in the electron-transfer processes are different, i.e., $[4Fe\text{-}4S]^{2+/1+}$ for Fds and $[4Fe\text{-}4S]^{3+/2+}$ for HiPIPs [40]. As a consequence, the redox potentials of Fds (–300 to –700 mV vs. NHE) and HiPIPs (+450 to +100 mV vs. NHE) are distinctly different [37]. This difference is mainly attributed to the ease of access of water to the cluster cores, which leads to the formation of O–H···S hydrogen bonds and additional hydrogen bonding between clusters and peptide backbones [21], although effects of cluster spin topology and hydrophobic interactions have also been proposed [39, 41]. Protein crystal structures of HiPIPs have revealed the presence of hydrophobic cavities around their [4Fe-4S] cores, which preclude contacts with water [42–47]. Conversely, the [4Fe-4S] clusters in Fds are more exposed to the surface of proteins, rendering interaction with water more feasible [38]. The restricted access of water to the [4Fe-4S] cluster of HiPIPs has been supported by estimated H/D exchange rates, which were obtained from ^{1}H–^{15}N HMQC and ^{19}F NMR measurements on the native form of *Chromatium*

vinosum and its mutant [48]. A comparison of the resonance Raman spectra in D_2O of the HiPIP from *Chromatium vinosum* with the Fds from *Clostridium pasteurianum* and *Clostridium acidiurici* indicated that an H/D exchange around the cluster in HiPIPs requires partial unfolding of the protein in order to provide access for water [49]. A solvent model based on protein-dipole Langevin-dipole electrostatic calculations also suggested the importance of water accessibility on the redox properties [50]. The effect of water on the redox potentials of iron-sulfur clusters has also been discussed in the context of sulfur K-edge X-ray absorption spectroscopy and theoretical calculations [51, 52].

The correlation between the redox potentials of [4Fe-4S] clusters and the number of hydrogen bonds between the clusters and the peptide backbone has been discussed in a review [21], wherein the authors also addressed the difficulty to fully explain the wide range of redox potentials only based on hydrogen bonding. On average, five and eight N–H···S hydrogen bonds are observed for HiPIPs and Fds, respectively, whereby the higher number of hydrogen bonds in latter is able to efficiently stabilize the reduced and more negatively charged states. This effect has been demonstrated using backbone-engineered HiPIPs that were designed to prevent hydrogen bonding between the peptides and the cysteinyl sulfur atoms attached to the cluster [53].

2.2. Representative non-Cysteinyl Ligands and their Roles

As previously mentioned, the iron atoms in iron-sulfur clusters are occasionally supported by non-cysteinyl residues such as histidine, aspartate, glutamate, and amide group of peptides. Relative to typical, cysteine-supported clusters, these ligands should induce different redox properties and stabilizing effects for the iron-sulfur clusters.

2.2.1. Histidine Ligation

Histidine contains an imidazole moiety, i.e., a five-membered nitrogen-containing heterocycle that usually binds to iron via the lone pair on the nitrogen atom. In this case, the imidazole moiety serves as a charge-neutral ligand and is thus less electron-donating compared to the anionic cysteinyl thiolate. This results in more positive redox potentials of histidine-supported iron-sulfur clusters relative to those of cysteine-supported clusters. For instance, the redox potentials of [2Fe-2S] clusters in Rieske proteins with two imidazoles (+490 to –100 mV vs. NHE) are significantly higher at neutral pH than those of cysteine-supported clusters in Fds (–300 to –460 mV vs. NHE) [39]. The [2Fe-2S] cluster in MitoNEET is supported by three cysteines and one histidine, and its midpoint potential has been reported as *ca*. 0 mV vs. NHE [54]. Another feature of the imidazole moiety is its ability to release the proton from the N–H group, the ease of which is characterized by its p*K*a values (*e.g.* 7.7 and 9.1 for the oxidized Rieske protein from the bovine mitochondrial cytochrome bc_1 complex [55]). The reversible deprotonation/protonation behavior modulates the redox potential depending on the pH value, as found for Rieske proteins from the *Rhodobacter sphaeroides*, *Thermus thermophilus*, and *Burkholderia* sp. strain LB400 [56], while the redox potentials are also influenced by the degree of coupling between cluster oxidation state and histidine protonation state. The deprotonated anionic imidazole exhibits a higher electron-donating ability relative to the neutral imidazole, which leads to more negative cluster potentials. Indeed, the reduction potential of Rieske proteins from *Rhodobacter sphaeroides* is –134±6 mV vs. NHE at high pH (deprotonated form), and 308±3 mV vs. NHE at low pH (protonated form) [56].

Some histidine-supported [4Fe-4S] clusters can be found in the electron-transfer chains of hydrogenase enzymes [27–30], which catalyze the reversible oxidation of H_2. In these enzymes, histidine-ligated [4Fe-4S] clusters are located close to the surface of proteins and are denoted *distal*-[4Fe-4S] clusters, as they are distant from the Ni-Fe or Fe-Fe active sites, which are deeply buried in the protein matrices. In the electron-transfer chain of [NiFe] hydrogenase, three iron-sulfur

clusters, as well as the Ni-Fe active site are involved (Fig. 2). In order to ensure the reversibility of the enzymatic reaction, the electron transfer needs to be bidirectional, thus requiring a switching system for the direction of the electron flow. One of the possible solutions proposed, based on structural analogues [57], is the deprotonation/protonation behavior of the histidine-supported *distal*-[4Fe-4S] cluster. However, this possibility has not yet been well supported by biochemical studies, although the protein crystal structures of [NiFe] hydrogenases from *Desulfovibrio gigas*, *Desulfovibrio fructosovorans*, and *Desulfovibrio Vulgaris Miyazaki F* have revealed *distal*-[4Fe-4S] clusters with N–H groups oriented toward the outside of the protein to enable reversible deprotonation [27, 58, 59]. Based on a mutagenesis experiment on the [NiFe] hydrogenase from *Desulfovibrio fructosovorans*, the replacement of histidine with cysteine on the *distal*-[4Fe-4S] cluster results in a reduced electron transfer rate [60].

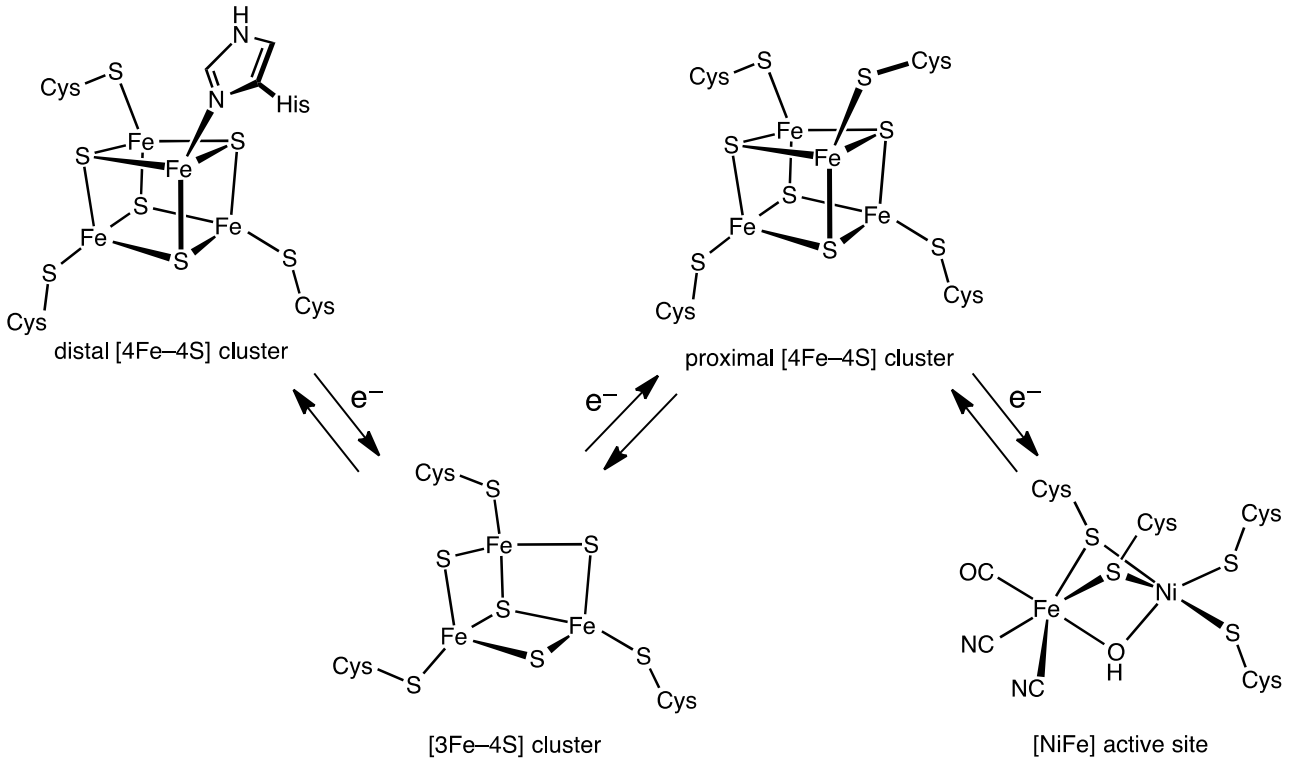

Fig. 2. Metal centers in the electron-transfer chain of [NiFe] hydrogenase [27–30].

2.2.2. Carboxylate Ligation

In some proteins, [4Fe-4S] clusters are supported by three cysteinyl thiolate groups and one carboxylate from aspartate or glutamate. Aspartate-bound [4Fe-4S] clusters can mediate electron transfer in ferredoxin from *Pyrococcus furiosus* (*Pf* Fd) [32] and in the NB protein of the dark-operative protochlorophyllide oxidoreductase (DPOR) from *Rhodobacter capsulatus* [33]. While the redox potential of the *Pf* Fd protein is –368 mV vs. NHE, the mutation of aspartate to cysteine results in a negative shift to –426 mV vs. NHE [61]. This result indicates that carboxylate-bound [4Fe-4S] clusters can accept electrons from cysteine-supported [4Fe-4S] clusters. This is, for example, the case for DPOR (Fig. 3), where the aspartate-bound [4Fe-4S] cluster in the NB protein accepts an electron from the cysteine-supported [4Fe-4S] cluster in the L-protein [62]. The very same electron transfer process is hampered upon replacement of aspartate with cysteine [33].

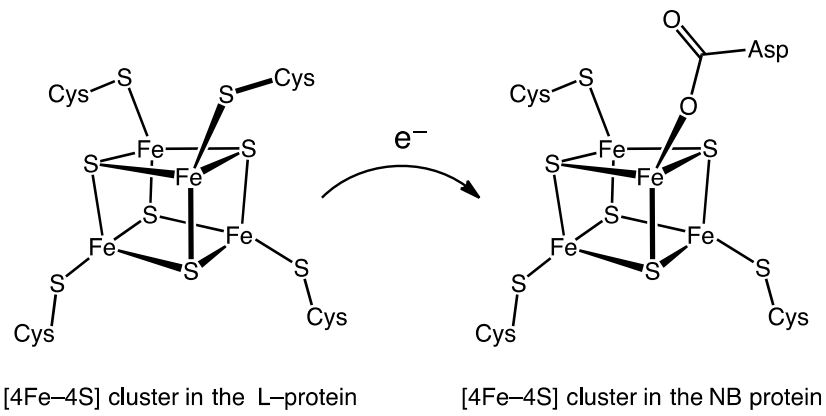

Fig. 3. Electron-transfer process in dark-operative protochlorophyllide oxidoreductase (DPOR) [62].

IspG is an enzyme for the conversion of 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate (MEcPP) into (*E*)-1-hydroxy-2-methyl-but-2-enyl-4-diphosphate (HMBPP) in the presence of protons and electrons (Fig. 4a), which is one of the intermediary steps for the biosynthesis of isoprenoid [34–36]. The active site of IspG is a [4Fe-4S] cluster bearing a glutamate (Fig. 4b). This glutamate residue has

been proposed to dissociate, thus opening the iron site for substrate (MEcPP) binding [63]. This hypothesis was based on the crystal structure of IspG co-crystallized with MEcPP [64] and the results of isotope exchange and spectroscopic studies [34, 65, 66]. Moreover, the deprotonated glutamate was proposed to mediate the reversible deprotonation/protonation of the iron-bound substrate [64]. Hence, the glutamate in IspG probably serves as both a leaving group and as an acid-base catalyst.

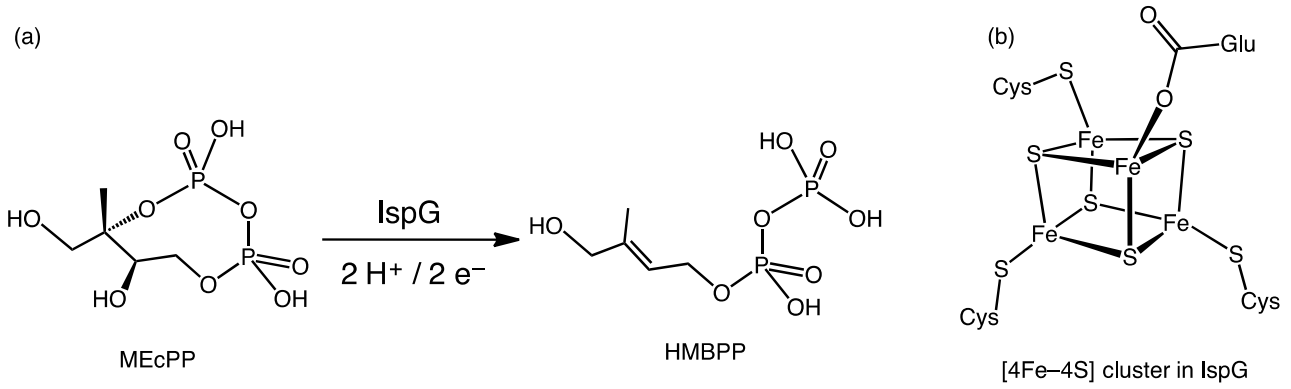

Fig. 4. (a) IspG-catalyzed biosynthesis of HMBPP from MEcPP [34–36]. (b) Structure of the [4Fe-4S] cluster in IspG.

2.2.3. Peptide Amide Group as a Supporting Ligand

Coordination of a peptide amide group to iron has been found in the oxidized forms of the [4Fe-3S] cluster $\mathbf{5}^{\mathbf{Ox}}$ and the [8Fe-7S] cluster $\mathbf{9}^{\mathbf{Ox}}$ (Fig. 1). The [4Fe-3S] cluster **5** in the O_2-tolerant [NiFe] hydrogenase is located in spatial proximity to the Ni-Fe active site, and protein crystallographic analyses have suggested redox-dependent changes in its structure [8]. The binding of a deprotonated peptide amide group to an iron center occurs in the ferricyanide-oxidized form $\mathbf{5}^{\mathbf{Ox}}$, which corresponds to a $[4Fe\text{-}3S]^{5+}$ state with one ferrous and three ferric iron centers. Interestingly, cluster **5** exists in three oxidation states, i.e., $[4Fe\text{-}3S]^{3+/4+/5+}$, and all of these fall within a narrow range of *ca.* 200 mV [67, 68]. This is possibly due to the additional coordination of the peptide amide group in the $\mathbf{5}^{\mathbf{Ox}}$ state, which may induce a negative shift of the redox potential of $[4Fe\text{-}3S]^{4+/5+}$. Compared to the

cysteine-supported [4Fe-4S] cluster in **6**, the [4Fe-3S] cluster in **5** contains two more cysteinyl thiolates and one less core sulfur atom, which may endow **5** with the structural flexibility required for the formation of $\mathbf{5^{Ox}}$.

One of the iron atoms in the [8Fe-7S] core of the P-cluster is able to accommodate a peptide amide group in its two-electron oxidized form ($\mathbf{9^{Ox}}$), which is accompanied by coordination of a serinate oxygen to another iron atom and cleavage of two Fe–S bonds with the central sulfur atoms of the core. The reversibility of this structural change has been confirmed by protein crystallographic analyses [14, 15, 17], while its relevance to the electron-transfer properties of the P-cluster still remains to be determined.

3. Effect of Ligands and Media on the Synthesis of Biomimetic Iron-Sulfur Clusters

Iron-sulfur clusters that are structurally relevant to biological clusters, the so-called biomimetic or synthetic analogues [69, 70], have been successfully synthesized since the 1970s [71]. Elucidation of their spectroscopic, magnetic, and electronic properties, as well as their structures and reactivity may provide insight into the properties and functions of iron-sulfur proteins. It is thus not surprising that these compounds receive substantial interest from inorganic/coordination chemists. The spontaneous assembly of iron atoms in the presence of sulfur reagents is a common method for the synthesis of iron-sulfur clusters. The following section starts with an overview of the established synthetic routes to conventional iron-sulfur clusters, before addressing the controlled synthesis of non-conventional iron-sulfur clusters. The electronic and steric properties of the ligands surrounding the iron centers, as well as the stoichiometry and reaction media represent important factors for the manipulation of the composition and the yield of the products.

3.1. Synthesis of Iron-Sulfur Clusters via Salt Metathesis Reactions in Polar Solvents

The synthetic methods developed in early studies involve the reaction of iron halides (usually $FeCl_2$ or $FeCl_3$) with thiolates (^-SR) and sulfide sources (e.g. HS^-, S^{2-}, or elemental sulfur) in polar organic solvents such as methanol and *N*,*N*-dimethylformamide (DMF). The most common class of biomimetic [4Fe-4S] clusters is the thiolate-coordinated $[4Fe\text{-}4S]^{2+}$ cluster **10** (Fig. 5a) [71], which was first reported in 1972 in the form of $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ [72]. The first established synthetic protocol for **10** was the reaction of $FeCl_3$ with NaSR, NaSH, and NaOMe in methanol [73]. Slightly modified reactions of $FeCl_x$ (x = 2, 3) with NaSR and elemental sulfur also afford **10** [74, 75]. The one-electron reduced form of **10**, $[Fe_4S_4(SR)_4]^{3-}$, can be obtained from $FeCl_2$, alkane-thiolates NaSR (R = Me, Et, CH_2Ph, ^{t}Bu), and NaSH in DMF [76]; however, this method is not applicable to aryl-thiolates.

While the first [2Fe-2S] cluster, $[Fe_2S_2\{(SCH_2)_2C_6H_4\}_2]^{2-}$, was synthesized using the bidentate dithiolate $\{(SCH_2)_2C_6H_4\}^{2-}$ in a one-pot reaction between $FeCl_3$, Na_2(dithiolate), NaSH, and NaOMe [77, 78], the biomimetic $[2Fe\text{-}2S]^{2+}$ cluster **11** containing monodentate thiolates is usually generated by the assembly reaction between $FeCl_3$, NaSR (R = Ph, *p*-tolyl), and elemental sulfur in methanol (Fig. 5b) [79]. Anionic iron-thiolate complexes such as $[Fe(SR)_4]^{2-}$ (R = Ph, Et), $[Fe(SR)_4]^-$ (R = Me, Et), and $[Fe_2(SEt)_6]^{2-}$ have also been reported as useful precursors for the preparation of **11** in acetonitrile in the presence of elemental sulfur (Fe:S = 1:1) [80–82]. The analogous reaction of $[Fe(SEt)_4]^{2-}$ in acetone using a slight excess of elemental sulfur (Fe:S = 1:1.4) furnished linear $[3Fe\text{-}4S]^+$ cluster **12** as the major product (Fig. 5c) [80, 81]. The formation of a linear [3Fe-4S] cluster under physiological conditions has been proposed for the Beef heart aconitase at high pH (>9.5) [83], and an anaerobically isolated pyruvate formate-lyase-activating enzyme has been suggested to contain a linear [3Fe-4S] cluster as a minor component (*ca*. 10% of the total amount of Fe) [84].

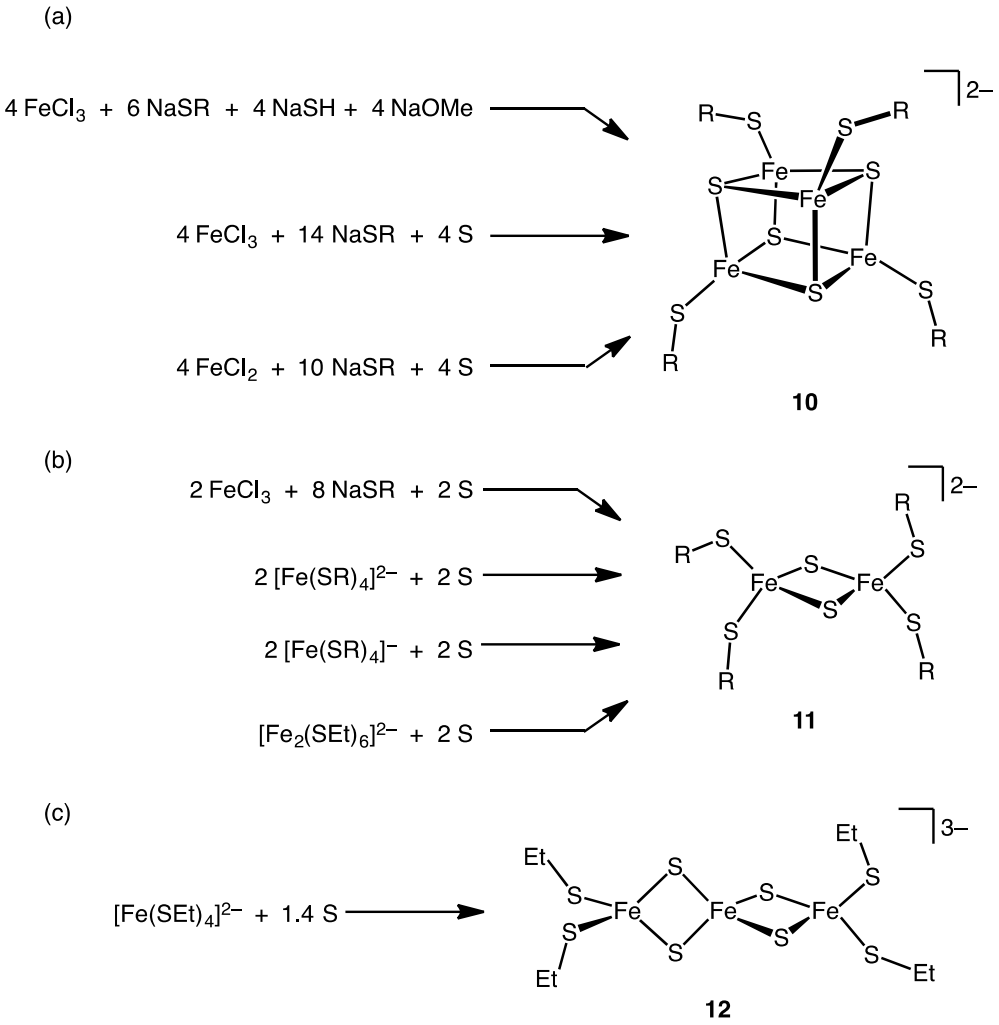

Fig. 5. Synthesis of (a) $[4Fe\text{-}4S]^{2+}$ cluster **10** [72–75], (b) $[2Fe\text{-}2S]^{2+}$ cluster **11** [79–82], and (c) linear $[3Fe\text{-}4S]^{+}$ cluster **12** [80, 81].

Some biologically irrelevant, thiolate-supported iron-sulfur clusters have been synthesized through analogous protocols (Fig. 6). For example, the [6Fe–9S] cluster **13** (R = ^{t}Bu), in which six iron atoms are arranged in a coplanar fashion, was initially obtained as a byproduct of the reaction between $FeCl_3$, Li_2S, and LiS^{t}Bu (4:4:6) in methanol in the presence of an excess of LiOMe [85]; the yield of this reaction would later be improved to 70% by applying an improved stoichiometry of reactants (Fig. 6a) [85, 86]. A more versatile route to **13** (R = Me, Et, CH_2Ph) is the addition of Na_2S_2 to $[Fe(SR)_3]$, which is available as a dark-green precipitate from the reaction between $FeCl_3$ and NaSR

(1:3) in methanol [87, 88]. Cluster **13** is also available by heating a mixture of *in-situ*-generated $[Fe(SEt)_4]^{2-}$ with 1.5 equiv. of elemental sulfur in acetonitrile [81].

The so-called prismane-type [6Fe-6S] cluster **14** is prepared from FeX_2 (X = Cl, Br, I), NaSPh, $[Et_4N]X$ (X = Cl, Br, I), and elemental sulfur (Fig. 6b) [89, 90], while one-electron-oxidized forms of **14** are available from a mixture of metallic iron, elemental sulfur, I_2, and I^- [91], or from the chemical oxidation of $[Fe_4S_4X_4]^{2-}$ (X = Cl, Br) using a ferrocenium cation [92]. Although thiolate-capped prismanes **14** (X = SR) are available from the substitution of halides with thiolates, their thermal instability restricts their isolation to low temperatures (*ca*. –20 ºC) [93]. At ambient temperature, thiolate-capped **14** gradually degrades to afford a more robust [4Fe-4S] cluster. Similarly, thermolysis of halide-capped **14** in various polar organic solvents furnishes a [4Fe-4S] cluster [90].

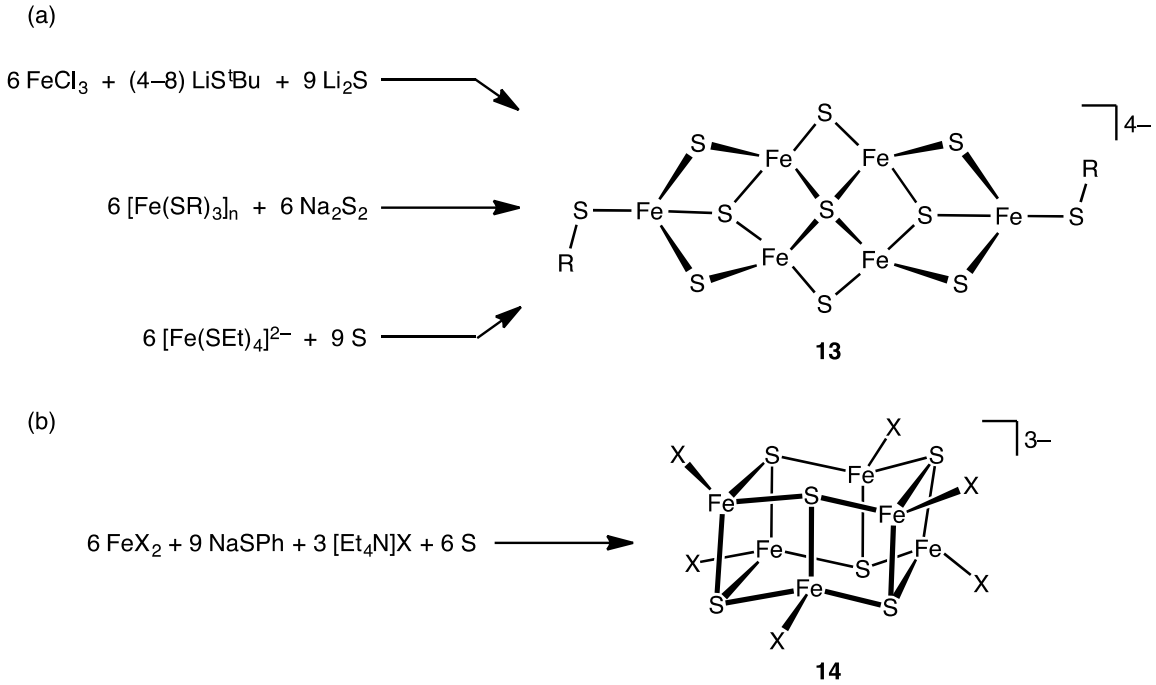

Fig. 6. Synthesis of (a) [6Fe-9S] cluster **13** [81, 85–88] and (b) [6Fe-6S] cluster **14** [89, 90].

Some examples of core conversions between conventional iron-sulfur clusters have been reported, e.g. the dimerization of two $[2Fe\text{-}2S]^{2+}$ clusters to generate a cubic $[4Fe\text{-}4S]^{2+}$ cluster. This

dimerization is slow at ambient temperature in aqueous solutions mixed with dimethyl sulfoxide (DMSO), DMF, hexamethylphosphoric triamide (HMPA), or methanol [94, 95]. However, it proceeds even in aprotic solvents at elevated temperatures, as evident from e.g. the formation of $[Fe_4S_4(SEt)_4]^{2-}$ upon heating a saturated acetonitrile solution of $[Fe_2S_2(SEt)_4]^{2-}$ to 80 ºC [81]. Electrochemical reduction represents an alternative to couple two [2Fe-2S] clusters, as exemplified by the reduction of $[Fe_2S_2(SPh)_4]^{2-}$ in acetonitrile to furnish the corresponding $[4Fe\text{-}4S]^{2+}$ cluster [94]. The corresponding retro-dimerization, i.e., the dissociation of a [4Fe-4S] cluster into two [2Fe-2S] clusters, has been postulated as a key process for O_2-sensing in some proteins, such as the fumarate and nitrate reduction (FNR) protein, which are involved in the control of various gene expressions, [96–98] and the A-type iron-sulfur cluster assembly protein ^{Nif}IscA from *Azotobacter vinelandii* [99]. In the presence of pyridine, the all-ferric $[4Fe\text{-}4S]^{4+}$ cluster **15** (for its synthesis, see the next section) [100, 101] splits into two [2Fe-2S] clusters (**16**). Coupling of **16** was subsequently achieved either by removing the pyridine with $B(C_6F_5)_3$, or by chemical reduction [102] (Fig. 7). Disassembly of the [4Fe-4S] core was observed only in the all-ferric $[4Fe\text{-}4S]^{4+}$ cluster, indicating the importance of the all-ferric state for the [4Fe-4S] to [2Fe-2S] core conversion in proteins, even though this state is relatively uncommon, possibly due to its short lifetime and ESR silence. In contrast to the thiolate ligands in the abundant biological and synthetic [4Fe-4S] clusters, the amide ligands in cluster **15** are strongly electron-donating and able to stabilize high oxidation states. A review for the structural conversions of iron sulfur clusters was recently published [103].

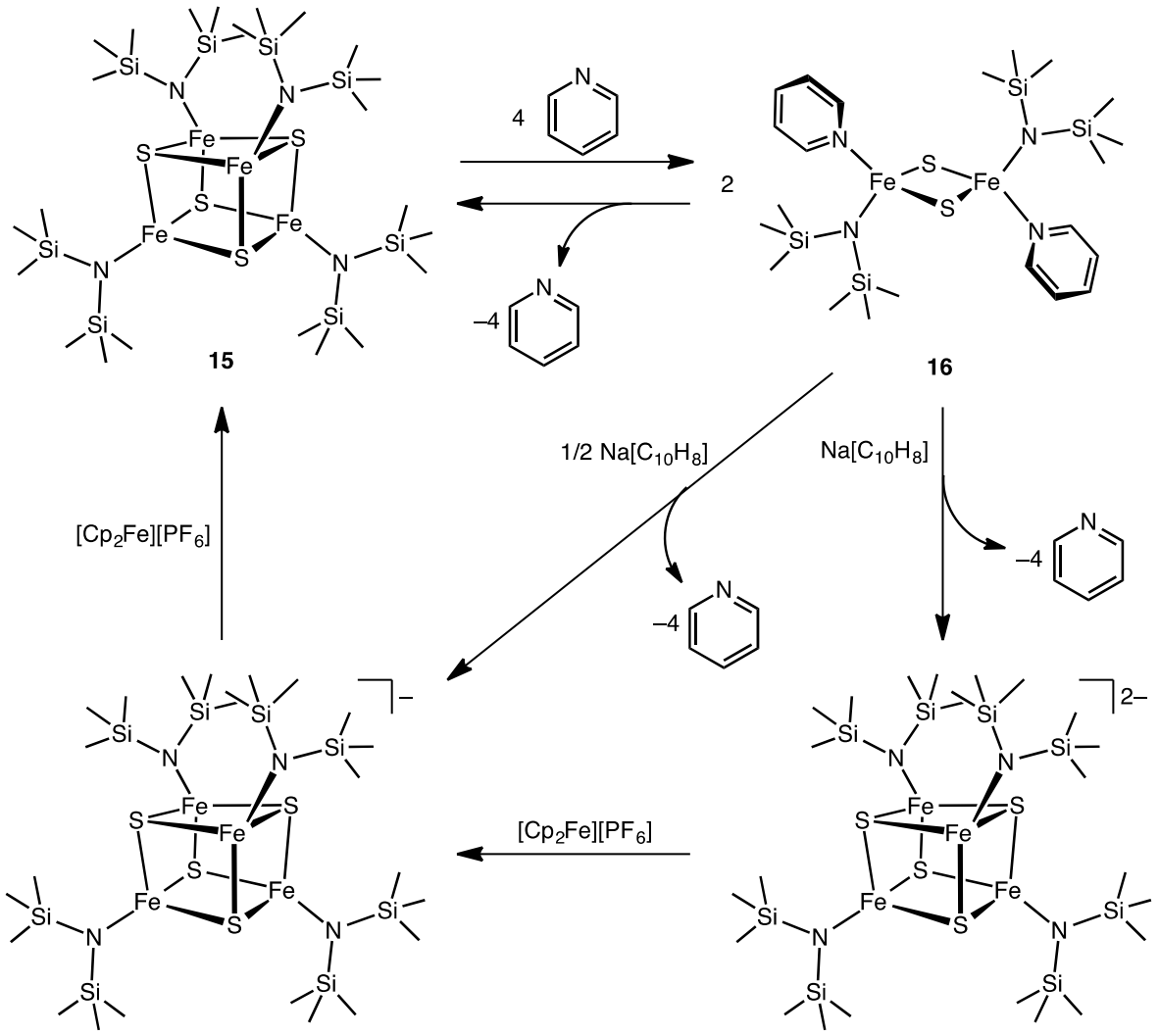

Fig. 7. Core conversions between [4Fe-4S] and [2Fe-2S] clusters [102].

3.2. Controlling the Nuclearity in Synthetic Iron-Sulfur Clusters using Bulky Ligands and Non-Polar Solvents

The general synthetic method described in the previous section is based on ionic reactants such as iron halides and alkali metal thiolates and sulfides, which are dissolved in a polar organic solvent. Although such salt metathesis reactions are useful to synthesize conventional iron-sulfur clusters, charge-neutral reactions employing bulky ligands in non-polar organic solvents are able to afford new classes of iron-sulfur clusters [104, 105], such as high-nuclearity iron-sulfur clusters reproducing the [8Fe-7S] core of the P-cluster (**9** in Fig. 1), or structural analogues of the nitrogenase FeMo-cofactor (Fig. 8), which mediates biological N_2 fixation [106–111].

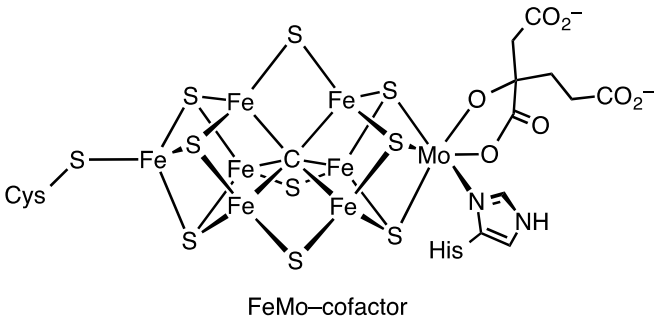

Fig. 8. Structure of the nitrogenase FeMo-cofactor [106–111]. Cys = cysteine, His = histidine.

In contrast to iron halides, which are usually subjected to salt metathesis reactions in polar solvents for ligand exchange, $Fe\{N(SiMe_3)_2\}_2$ [112, 113] is soluble in non-polar organic solvents and its amide ligands function as a base. The addition of thiols as Brønsted acids to $Fe\{N(SiMe_3)_2\}_2$ leads thus to the replacement of amides with thiolates with concomitant liberation of $HN(SiMe_3)_2$ [113–119]. This feature has been applied to the synthesis of iron-sulfur clusters from $Fe\{N(SiMe_3)_2\}_2$, thiols, elemental sulfur, and additives in non-polar organic solvents such as toluene (Fig. 9). The first successful example of such a synthetic approach was the preparation of [4Fe-4S] cluster **17**, which is supported by two thiolate and two thiourea ligands [116]. A modification of the stoichiometry of this reaction led to the discovery of cluster **18a**, whose [8Fe-7S] core represents that of the reduced form of the nitrogenase P-cluster (**9**$^{\mathbf{Red}}$; Fig. 1) [120, 121]. Derivatives of **18a**, carrying multiple thiolate ligands instead of thiourea and/or amide ligands, have also been synthesized to provide better structural analogues of the P-cluster (Fig. 10) [121]. In addition, some trinuclear clusters (**19**) have been prepared from using appropriate ratios of $Fe\{N(SiMe_3)_2\}_2$, HSR (R = *p*-tolyl, adamantyl), and elemental sulfur [122]. The addition of elemental sulfur to $Fe\{N(SiMe_3)_2\}_2$ furnished all-ferric [4Fe-4S] cluster **15** together with the tris-amide complex $Fe\{N(SiMe_3)_2\}_3$ [100]. Cluster **15** is susceptible to a reductive desulfurization of the cubic [4Fe-4S] core by PR_3 (R = Me, Et), and the resulting phosphine-sulfides ($S{=}PR_3$) are incorporated in the generated [8Fe-7S] clusters **18b** and **18c**

[123]. This reductive fusion of two [4Fe-4S] clusters could proceed through the generation of a corner-voided [4Fe-3S] species, in which three vacant iron atoms capture one of the corner sulfur atoms of the [4Fe-4S] cube to generate the central μ_6-sulfur atom of the [8Fe-7S] core.

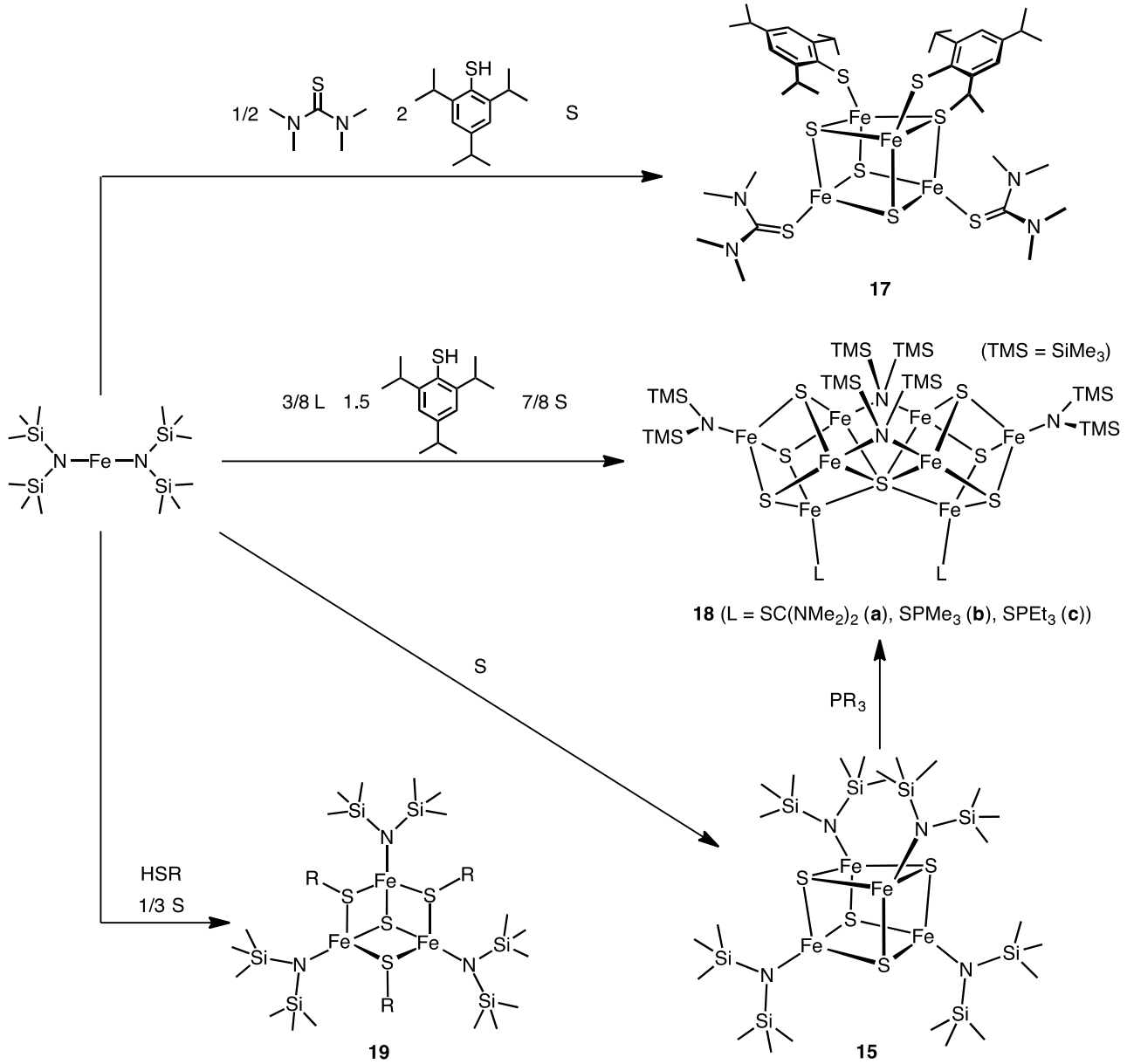

Fig. 9. Synthesis of iron sulfur clusters **15** [100], **17** [116], **18** [120, 121, 123], and **19** [122] from $Fe\{N(SiMe_3)_2\}_2$.

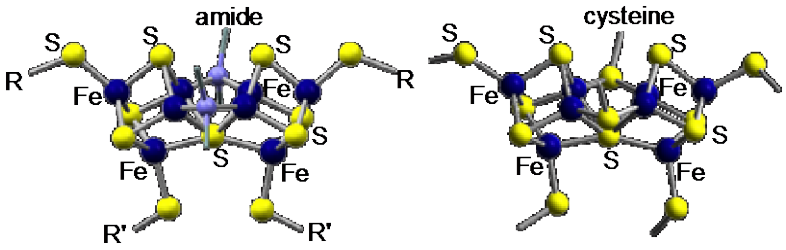

Fig. 10. Structures of one of the P-cluster models (left), and the reduced form of the native P-cluster $\mathbf{9^{Red}}$ (right). R = 2,4,6-{$CH(SiMe_3)_2$}$_3C_6H_2$, R' = $(C_6H_5)Fe(C_5H_5)$, amide = $N(SiMe_3)_2$.

The aforementioned charge-neutral reaction protocol based on $Fe\{N(SiMe_3)_2\}_2$ has been extended to the synthesis of structural mimics of the FeMo-cofactor (Fig. 8). Monomeric or dimeric Fe(II) complexes $[Fe(SR)(SR')]_n$ (n = 1, 2) with bulky thiolates were obtained from the addition of bulky thiols HSR and HSR' to $Fe\{N(SiMe_3)_2\}_2$ in toluene [119, 124]. A subsequent addition of elemental sulfur afforded iron-sulfur clusters, as exemplified by the synthesis of [8Fe-7S] cluster **20** from $[(TipS)Fe]_2(\mu\text{-}SDmp)_2$ (Tip = 2,4,6-^{i}Pr$_3C_6H_2$, Dmp = 2,6-(mesityl)$_2C_6H_3$) (Fig. 11) [124]. From the one-pot reaction of $Fe\{N(SiMe_3)_2\}_2$ with HSTip, HSDmp, and elemental sulfur, another [8Fe-7S] cluster (**21**) with a bridging amide ligand was obtained together with **20**. A remarkable structural feature common to clusters **20** and **21** is the trigonal-prismatic arrangement of six iron atoms encapsulating a central μ_6-sulfur atom, which makes the inorganic core resemble that of the FeMo-cofactor (Fig. 12).

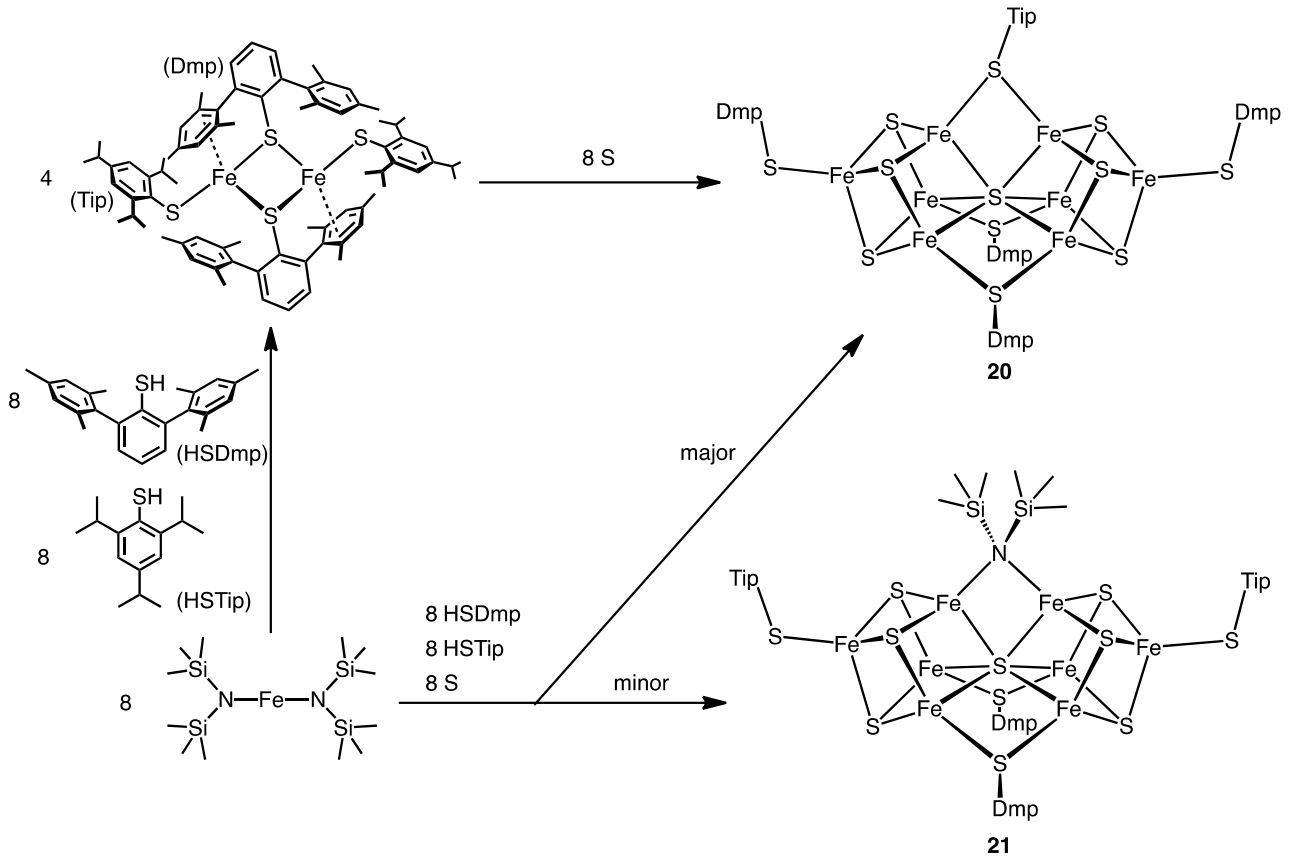

Fig. 11. Synthesis of [8Fe-7S] clusters **20** and **21** [124].

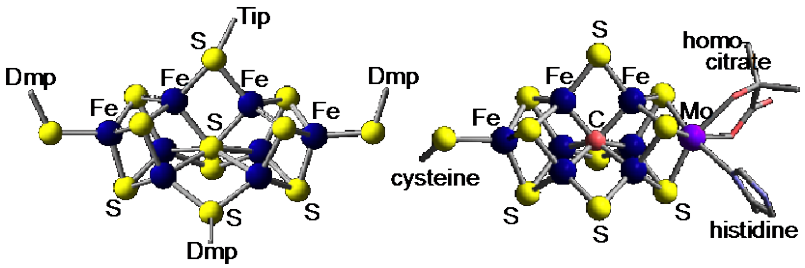

Fig. 12. Structures of cluster **20** (left) and the nitrogenase FeMo-cofactor (right). Only a part of homocitrate is shown for clarity. Tip = 2,4,6-$^{i}Pr_3C_6H_2$, Dmp = 2,6-(mesityl)$_2C_6H_3$.

For the successful synthesis of FeMo-cofactor analogues, several other important factors and ligand effects need to be considered. During spontaneous assembly reactions that furnish iron-sulfur clusters, the number of iron atoms should increase in a stepwise manner, and the termination of assembly should occur when the product becomes stable. For example, the dianionic [4Fe-4S]

clusters $[Fe_4S_4(SR)_4]^{2-}$ are a representative class of robust and thermodynamically stable iron-sulfur clusters. Accordingly, one central requirement, if one wishes to synthesize clusters with more than four iron atoms, is to avoid forming stable [4Fe-4S] clusters. This condition can be fulfilled via a destabilization of the [4Fe-4S] cores by modifying their oxidation states. So far, more than 70 examples have been reported for $[Fe_4S_4(SR)_4]^{2-}$ clusters in the $[4Fe\text{-}4S]^{2+}$ state [71]. In comparison, less than 10 examples are known for $[Fe_4S_4(SR)_4]^{-}$, and isolated examples for $[Fe_4S_4(SR)_4]^{0}$ still remain elusive [57, 125, 126], which suggests that the $[Fe_4S_4(SR)_4]^{0}$ cluster in the $[4Fe\text{-}4S]^{4+}$ state should be unstable. Reactions of $[Fe(SR)(SR')]_n$ (n = 1, 2) with elemental sulfur (Fig. 11) are targeting such $[Fe_4S_4(SR)_4]^{0}$ clusters, where elemental sulfur works not only as the source of the [4Fe-4S] core sulfur atoms, but also as an oxidant to induce the reductive elimination of disulfides from thiolate ligands on iron. The steric effects imparted by the bulky thiolate ligands are important to control the nuclearity of the cluster products, as encapsulation of the cluster core by bulky substituents leads to kinetic stabilization and thus terminates the assembly process. Non-polar organic solvents (e.g. toluene in Fig. 11) are also needed to prevent the generation of ionic species, which can be formed via intermolecular electron transfer and/or disproportionation reactions to furnish $[Fe_4S_4(SR)_4]^{2-}$ and other degradation products.

This charge-neutral synthetic protocol is not limited to $Fe\{N(SiMe_3)_2\}_2$. The iron mesityl complex Fe_2Mes_4 (Mes = mesityl) represents a suitable alternative precursor [127–129], which affords [8Fe-7S] cluster **22** upon reaction with the bulky thiol HSDmp and elemental sulfur (Fig. 13a) [130]. The bridging SMes ligand in cluster **22** is formed through insertion of a sulfur atom into an Fe–C(Mes) bond. The incorporation of a water-derived oxygen atom in the center of the Fe–S core is achieved by using the alkoxide/thiolate precursor $\{(Ph_3CO)Fe\}_2(\mu\text{-}SDmp)_2$, which is obtained from the successive treatment of $Fe\{N(SiMe_3)_2\}_2$ with a stoichiometric amount of HSDmp and $HOCPh_3$ (Fig. 13b) [131]. This precursor reacts with elemental sulfur and H_2O to afford [8Fe-6S-O] cluster **23**.

The central oxygen atom bridges four out of the six inner iron atoms. Two of the inner iron atoms interact weakly with the aromatic rings of the bridging SDmp ligands, which approach the outside of the core comparable to external substrates. This coordination mode of inner iron atoms may be reminiscent of the substrate-bound mode of the FeMo-cofactor. Although the binding mode in the FeMo-cofactor is still not clear, the central iron atoms have been hypothesized to capture N_2, CO, hydrazine, and alkynes [111, 132–137]. One of the proposed ways to open the reaction site(s) at the inner iron atom(s) is the cleavage of the Fe–μ_6-C bond(s) [138–143].

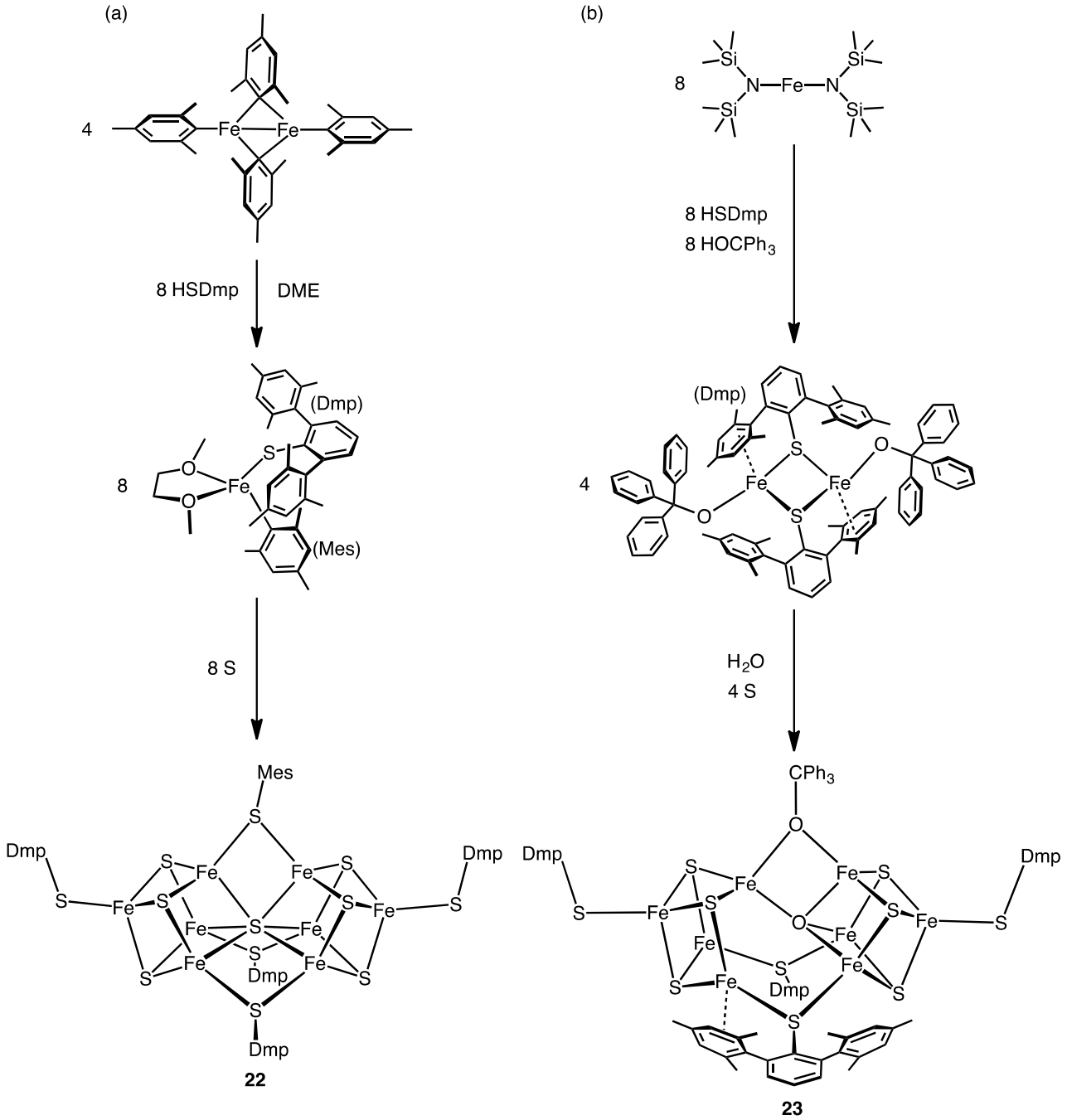

Fig. 13. Synthesis of (a) [8Fe-7S] cluster **22** [130] and (b) [8Fe-6S-O] cluster **23** [131].

Prior to the synthesis of structural mimics of nitrogenase clusters, the charge-neutral iron-sulfur cluster synthetic method was applied to the phosphine-supported Fe(II) complexes $Fe(PR_3)_2X_2$ (R = Me, Et, ^{i}Pr, ^{n}Bu, X = SPh, Cl, Br, I) (Fig. 14). The $SiMe_3$ groups of $(Me_3Si)_2S$ may act a scavengers for the (pseudo)halides of $Fe(PR_3)_2X_2$ in THF, leading to the generation of Me_3SiX and sulfides under concomitant formation of iron-sulfur clusters **24**–**26** [144–147]. The stoichiometry and the presence of additives are key factors that determine the structure of the products, *i.e.* treatment of $Fe(PR_3)_2X_2$ (R = Me, Et, ^{n}Bu, X = SPh, Cl, Br, I) with 1 equiv. of $(Me_3Si)_2S$ results in the formation of the basket-type [6Fe-6S] cluster **24** [144, 145], while the analogous reaction of $Fe(P^{i}Pr_3)_2Cl_2$ with $(Me_3Si)_2S$ and an *N*-heterocyclic carbene furnishes the all-ferrous [4Fe-4S] cluster **25** [146], and addition of 1.5 equiv. of $(Me_3Si)_2S$ to $Fe(PEt_3)_2Cl_2$ affords the [7Fe-6S] cluster **26** [146, 147].

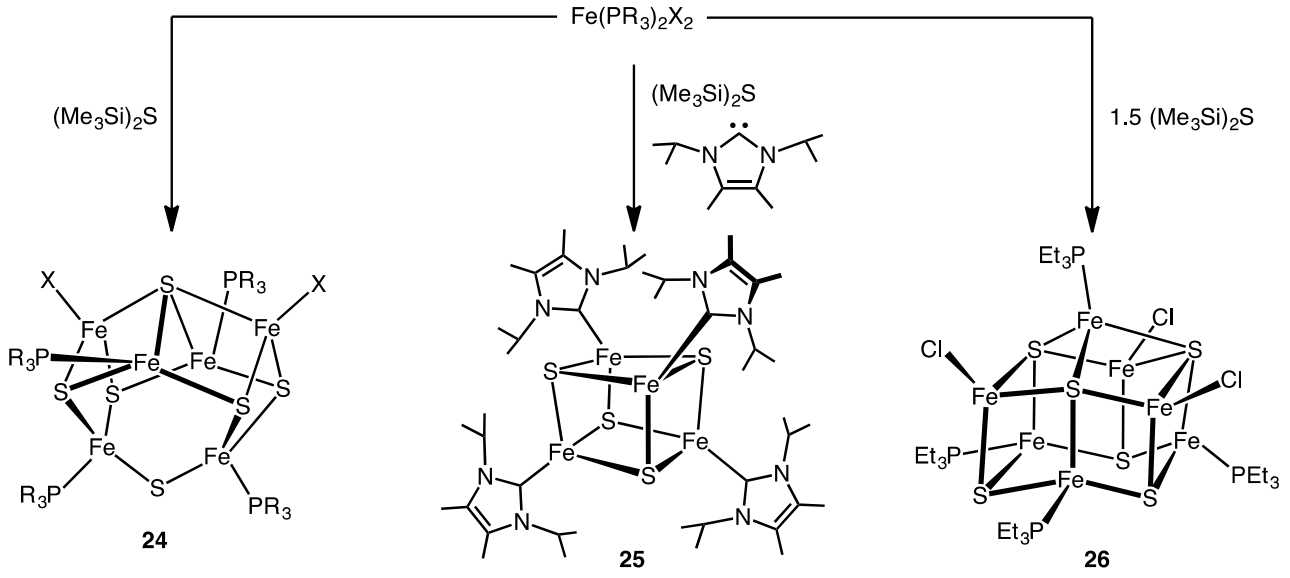

Fig. 14. Synthesis of basket type [6Fe-6S] cluster **24** (R = Me, Et, ^{n}Bu, X = SPh, Cl, Br, I) [144, 145], all-ferrous [4Fe-4S] cluster **25** [146], and [7Fe-6S] cluster **26** [146, 147].

This synthetic method, i.e., employing $(Me_3Si)_2S$ as a sulfurization agent, has also been extended

to the synthesis of ionic clusters, as demonstrated by the selective synthesis of iron-imide-sulfur cubanes $[Fe_4(\mu_3\text{-}N^tBu)_n(\mu_3\text{-}S)_{4-n}Cl_4]^z$ (n/z = 3/1–, 2/2–, 1/2–; Fig. 15) [148, 149]. The μ-sulfido/μ-imido complex **27**, which is a precursor for $[Fe_4(\mu_3\text{-}N^tBu)_2(\mu_3\text{-}S)_2Cl_4]^{2-}$, was obtained from the reaction of $Fe_2(N^tBu)_2Cl_2(NH_2{}^tBu)_2$ with $(Me_3Si)_2S$ in the presence of chloride anions, while the μ-sulfido/μ-amide precursor **28** was prepared from the successive treatment of $Fe\{N(SiMe_3)_2\}_2$ with $NH_2{}^tBu$ and elemental sulfur. One of these cubane clusters, $[Fe_4(\mu_3\text{-}N^tBu)(\mu_3\text{-}S)_3Cl_4]^{2-}$ with the [4Fe-3S-N] core (Fig. 15, bottom right), displays a structural analogy to the [4Fe-3S-C] subunit of the FeMo-cofactor.

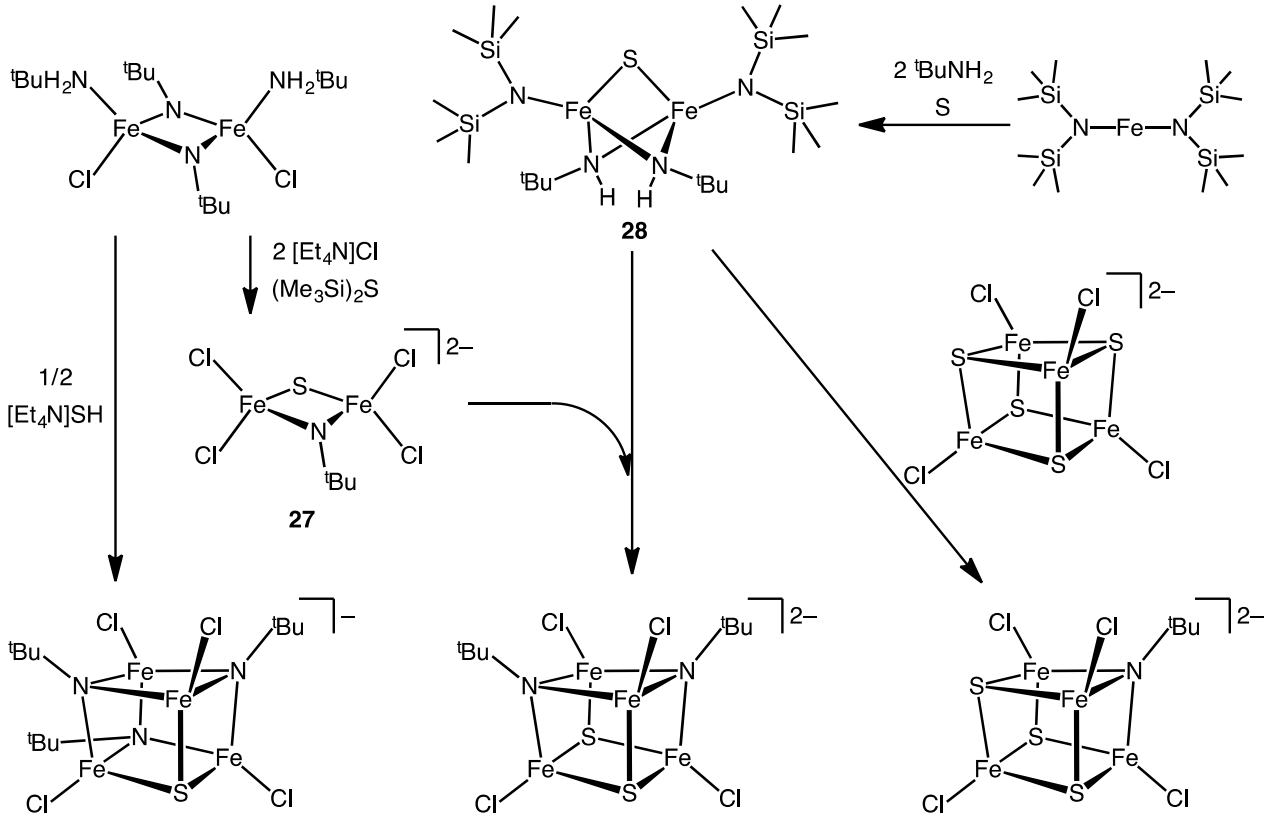

Fig. 15. Synthesis of iron-imide-sulfur clusters [148, 149].

4. Ligand Effects on the Properties and Structure of Biomimetic Iron-Sulfur Clusters

The properties of iron-sulfur clusters in proteins can be modified via the ligands on the iron centers and the extent of the interactions between the clusters and the peptides/media (cf. Section 2).

Similarly, the properties and structures of biomimetic clusters can be modulated by the ligands on the iron atoms. The topics to be discussed in the following sections are (a) the effects of intramolecular N–H···S hydrogen bonding, (b) the electron-donating properties of the ligands, (c) the extent of steric shielding offered by bulky ligands, (d) the structural confinement by multi-dentate ligands, and (e) the substituent effect of ligands on the solubility and stability. It should be noted that similar topics have been addressed in part by previous reviews [70, 71, 150–153].

4.1. Electronic Effects of Cluster Ligands

Hydrogen bonding in iron-sulfur proteins, where sulfur atoms of cysteine and/or cluster cores interact with hydrogen atoms of peptides or water, affects the redox potentials of clusters. This effect has been modeled for biomimetic iron-sulfur clusters, by employing thiolate ligands with intramolecular hydrogen bonding units (Fig. 16). In $[4Fe\text{-}4S]^{2+}$ clusters that bear *o*-(acylamino)benzenethiolate ligands **29** and **30**, hydrogen bonding between the N–H moieties and thiolate sulfur atoms was observed [154], which resulted in a positive shift of the redox potential for the $[4Fe\text{-}4S]^{2+/1+}$ process (for **29** (R = CH_3): –0.83 V vs. saturated calomel electrode (SCE) in acetonitrile) relative to that of the benzenethiolate-supported cluster (–1.0 V vs. SCE). The magnitude of the potential shift depends on the number of hydrogen bonds, and the potentials of clusters incorporating **30** are thus more positively shifted than those with **29**. Oligopeptide-containing cysteinate ligands **31** and **32** also form N–H···S hydrogen bonds within [4Fe-4S] clusters [155, 156]. These ligands feature characteristic –Cys–Gly–Ala– and –Cys–Gly–NHC_6H_4-*p*-X (Gly = glycine, Ala = alanine, X = H, OMe, F, Cl, CN) sequences, wherein the amide groups of Ala or NHC_6H_4-*p*-X interact with the sulfur atom of Cys, which induce a positive shift of the redox potential relative to that of alkanethiolate-supported clusters. An analogous effect of N–H···S hydrogen bonding has been observed for the $[2Fe\text{-}2S]^{3+/2+}$ processes of biomimetic [2Fe-2S] clusters bearing ligands **29** or **30**

[154].

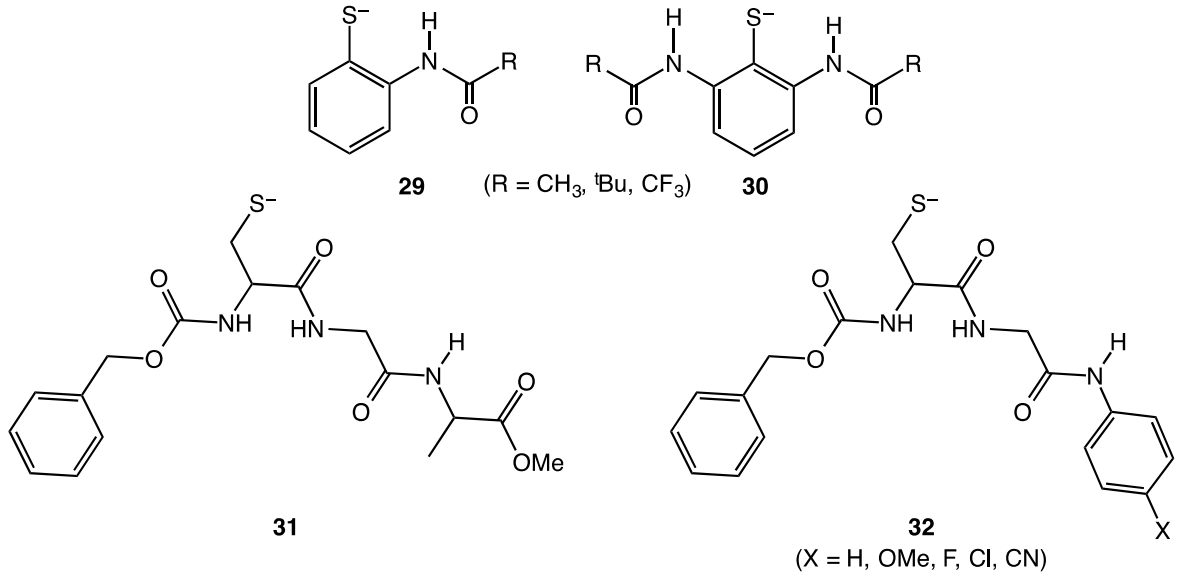

Fig. 16. Thiolate ligands that form intramolecular N–H···S hydrogen bonds [154–156].

The electronic properties of ligands on iron allow the stabilization of five oxidation states for [4Fe-4S] clusters ($[4Fe\text{-}4S]^{4+/3+/2+/1+/0}$). As previously described (cf. Section 3), $[4Fe\text{-}4S]^{2+}$ represents the most stable state when supported by thiolates or halides, while its one-electron reduced form ($[4Fe\text{-}4S]^{1+}$) is accessible under reducing conditions. The formation of the lowest oxidation state ($[4Fe\text{-}4S]^{0}$) is possible in the presence of π-acceptor ligands such as phosphines and cyanides (Fig. 17). For example, phosphine-stabilized $[4Fe\text{-}4S]^{1+}$ cluster **33** (R = ^{t}Bu, Cy, ^{i}Pr) was prepared by the replacement of the chloride ions in $[Fe_4S_4Cl_4]^{2-}$ with phosphines under concomitant spontaneous $1e^-$ reduction. A further reduction in the presence of sodium acenaphthalenide generated the $[4Fe\text{-}4S]^{0}$ cluster $[Fe_4S_4(PR_3)_4]^0$ (**34**) [157, 158]. However, this class of $[4Fe\text{-}4S]^{0}$ clusters gradually releases part of the phosphines at ambient temperature, which leads to oligomerizations that afford $[Fe_8S_8(PCy_3)_6]$ (**35**) or $[Fe_{16}S_{16}(PR_3)_8]$ (R = ^{t}Bu, ^{i}Pr). The first structurally characterized example of a $[4Fe\text{-}4S]^{0}$ cluster was $[Fe_4S_4(CN)_4]^{4-}$ (**36**), which was obtained after substitution of the phosphine ligands in **34** with CN^-, followed by a reduction with benzophenone ketyl radical anion [159].

N-heterocyclic carbenes (NHCs), which display stronger σ-donating and weaker π-accepting abilities than phosphines, can also be used as supporting ligands for $[4Fe\text{-}4S]^0$ cluster **25** [146]. This cluster was synthesized from the reaction of **35** with an excess of NHC or from the chloride/sulfide exchange between $(^{i}Pr_3P)_2FeCl_2$ and $(Me_3Si)_2S$, followed by the addition of NHC.

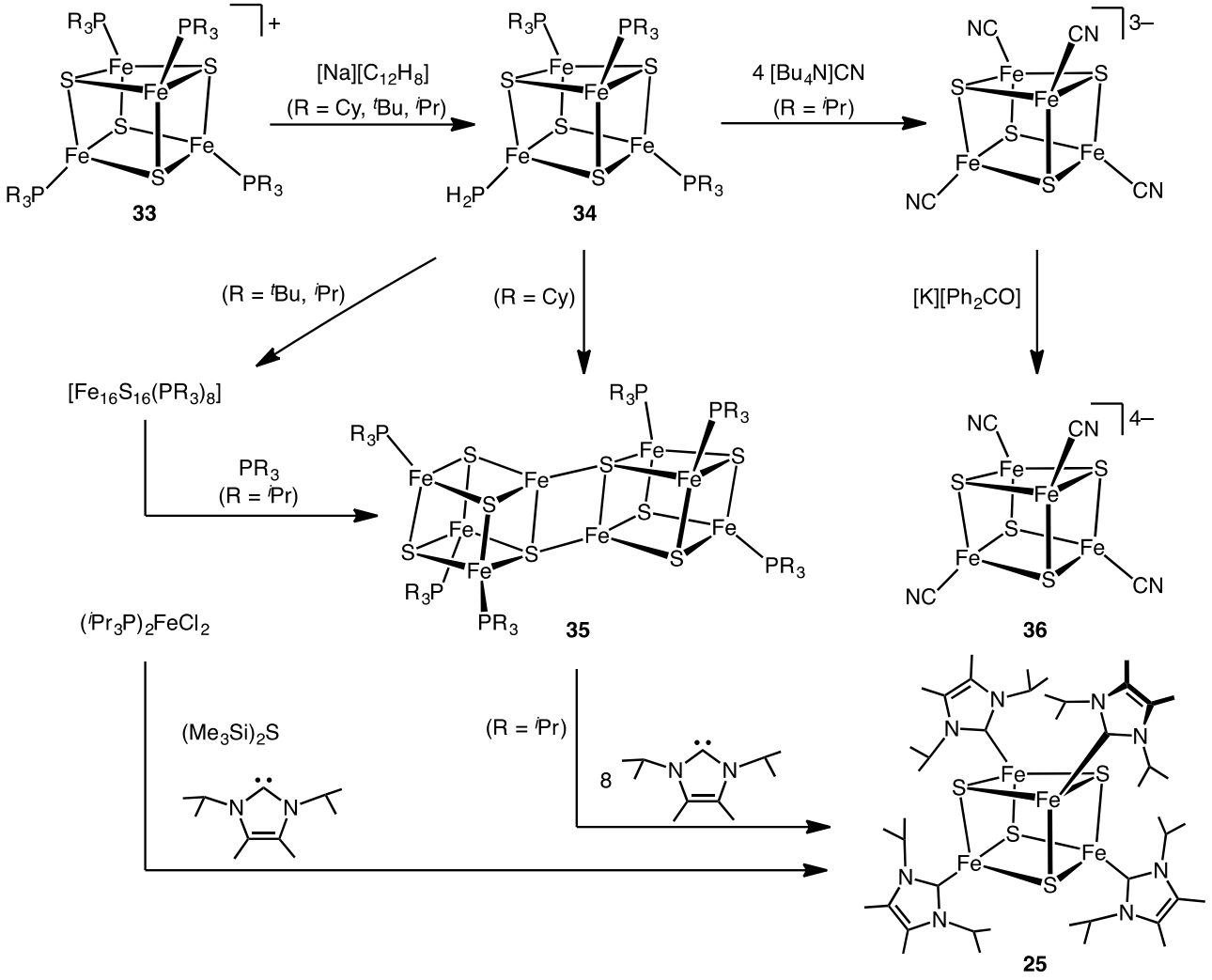

Fig. 17. Synthesis of $[4Fe\text{-}4S]^{1+/0}$ clusters supported by phosphines, cyanides, or *N*-heterocyclic carbenes [146, 157–159].

The stabilization of the $[4Fe\text{-}4S]^{4+/3+}$ oxidation states has been achieved with bulky amide or thiolate ligands. Amide-supported **15** represents an example of an all-ferric $[4Fe\text{-}4S]^{4+}$ cluster, synthesized by treatment of $Fe\{N(SiMe_3)_2\}_2$ with elemental sulfur (Fig. 9) [100] or by addition of NaSH to a THF solution of $FeCl\{N(SiMe_3)_2\}_2(THF)$ [101]. An important factor to stabilize the all-ferric $[4Fe\text{-}4S]^{4+}$

state of cluster **15** is the strong electron donation from the amide ligands. The amide nitrogen atom adopts a planar sp^2 geometry to locate a lone pair of electrons in a *p*-orbital, which can efficiently interact with a *d*-orbital on iron to form a π-type interaction leading to additional electron donation to the iron center. The steric shielding imposed by the bulky amide ligands may also contribute to the kinetic stabilization of the all-ferric form.

One-electron reduction of **15** in the presence of sodium naphthalenide or NaSH furnishes the $[4Fe\text{-}4S]^{3+}$ cluster $[Fe_4S_4\{N(SiMe_3)_2\}_4]^-$ [57, 101], while further reduction to the $[4Fe\text{-}4S]^{2+}$ state is also possible in the presence of Na_2S. The $[4Fe\text{-}4S]^{3+}$ cluster $[Fe_4S_4\{N(SiMe_3)_2\}_4]^-$ serves as a versatile precursor for a series of thiolate-supported $[4Fe\text{-}4S]^{3+}$ clusters (**37**) (Fig. 18) [57, 126], which represent the oxidized form of high-potential iron-sulfur proteins (HiPIPs). The reaction in Fig. 18 shows an acid-base type reaction between the $Fe–N(SiMe_3)_2$ groups and the bulky thiols, where the amide ligand accepts a proton from the thiol. However, the first isolated thiolate-supported $[4Fe\text{-}4S]^{3+}$ cluster, $[Fe_4S_4(STip)_4]^-$ (Tip = 2,4,6-^{i}Pr$_3$C$_6$H$_2$), was synthesized via chemical oxidation of $[Fe_4S_4(STip)_4]^{2-}$ with $[Cp_2Fe][PF_6]$ (Cp = η^5-C_5H_5) [125]. The electrochemical properties of the series of clusters **37** indicate that the steric shielding imposed by the bulky thiolates leads to a negative shift of the $[4Fe\text{-}4S]^{3+/2+}$ redox potentials, rendering the reduction of the $[4Fe\text{-}4S]^{3+}$ clusters more difficult. For example, the $[4Fe\text{-}4S]^{3+/2+}$ redox potentials of **37** were found at –0.21 V (R = Ph), –0.53 V (R = Tip), and –0.82 V (R = Dmp) (all potentials vs. $Ag/AgNO_3$ in THF; for the structures, see: Fig. 18) [126]. Similarly, upon incorporation of bulky thiolates, the $[4Fe\text{-}4S]^{2+/1+}$ redox potentials were also observed to shift negatively. The crystallographically determined molecular structures of **37** suggest that some extremely bulky thiolates such as SDmp, SEind, and STbt (Fig. 18) nearly encapsulate the [4Fe-4S] core, thus hindering contact between the anion **37** and the counter-cation. Consequently, a less efficient electrostatic interaction by ion-pairing is expected for **37** when bulky thiolates are incorporated. Accordingly, the $[4Fe\text{-}4S]^{3+/2+}$ redox potentials are negatively shifted and

the reduction of **37** becomes more difficult when bulky thiolates are employed. This steric effect of bulky thiolates is relevant for the stabilization of the hydrophobic cavities in the [4Fe-4S]$^{3+}$ cluster $[Fe_4S_4(SCys)_4]^-$ in the oxidized form of HiPIPs. As discussed in Section 2.1, the exposure of the [4Fe-4S] cluster in HiPIPs to water and the degree of N–H···S hydrogen bonding with the peptide backbone are limited, which results in less efficient charge neutralization. The [4Fe-4S]$^{2+}$ form $[Fe_4S_4(SCys)_4]^{2-}$ in HiPIPs is thus less stable than the cluster in Fds, which is more exposed to water and reveals more E–H···S hydrogen bonds (E = O or N).

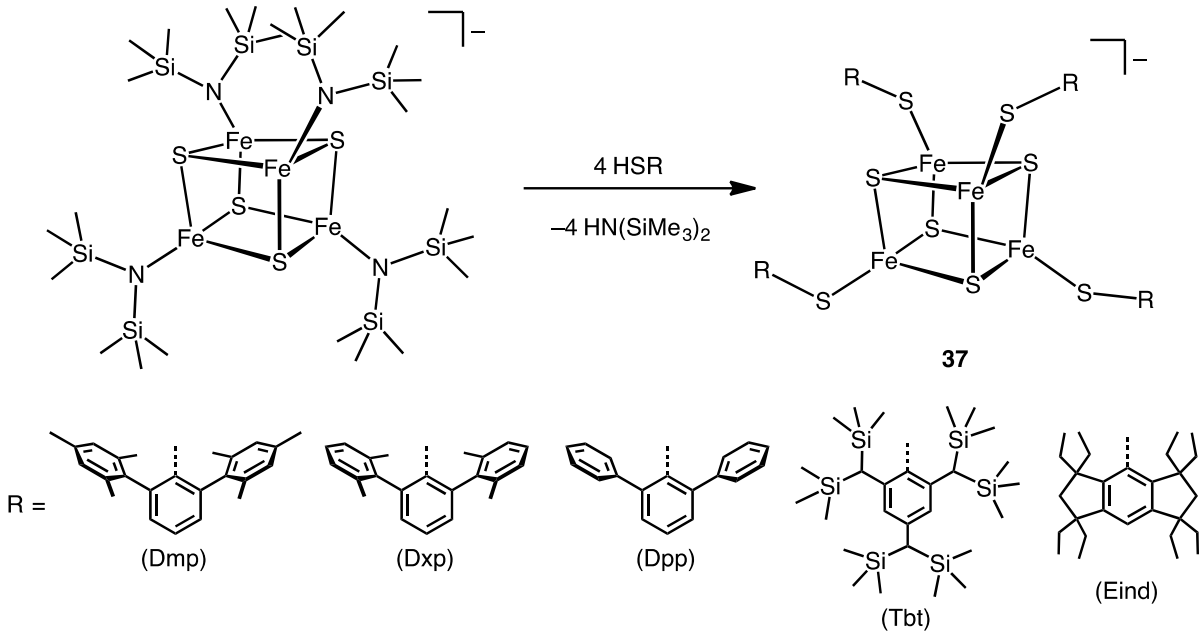

Fig. 18. Reactions of $[Fe_4S_4\{N(SiMe_3)_2\}_4]^-$ with bulky thiols [57, 126].

In contrast to the successful ligand exchange between amides and thiolates in the reactions of $[Fe_4S_4\{N(SiMe_3)_2\}_4]^-$ with thiols (Fig. 18), the analogous reaction of the all-ferric cluster **15** with HSDmp is accompanied by a one-electron reduction via release of one thiolate ligand, furnishing a [4Fe-4S]$^{3+}$ cluster upon treatment with THF [57] (*vide infra*, Fig. 21). The generation of all-ferric [4Fe-4S]$^{4+}$ clusters supported by extremely bulky thiolates has been demonstrated by electrochemical measurements of $[Fe_4S_4(SR)_4]^-$ (R = Dmp or Eind), whereas their isolation was unsuccessful, most

likely reflecting their short lifetime in solution [126].

4.2. Ligands for the Structural Control of Clusters

As described in Section 2, the iron sites of [4Fe-4S] and [2Fe-2S] clusters in proteins are sometimes inequivalent and supported by non-cysteinyl residues. Such iron-sulfur clusters with different iron sites are called site-differentiated clusters, for which synthetic approaches of analogues often require chelating ligands for the selective incorporation of ligand sets. One example for such a [4Fe-4S] cluster synthesis is a typical self-assembly reaction in the presence of two iron-binding ligands X and Y, which affords multiple cluster products $[Fe_4S_4(X)_n(Y)_{4-n}]^{2-}$ that are not easily separated. Furthermore, an inter-cluster ligand exchange reaction between $[Fe_4S_4Cl_4]^{2-}$ and $[Fe_4S_4(SPh)_4]^{2-}$ in DMF has been reported to afford an equilibrium mixture of $[Fe_4S_4Cl_n(SPh)_{4-n}]^{2-}$ [160], which indicates the difficulty in controlling the ligand distribution around the [4Fe-4S] core.

For the synthesis of 3:1 site-differentiated [4Fe-4S] clusters, tridentate thiolate ligands **38**–**40** (Fig. 19) [161–164], as well as some other tridentate thiolate ligands [165–167], have been designed. A common feature among ligands **38**–**40** is the central benzene ring, which is alternately substituted with thiolate-containing and non-thiolate chains (**38**: thioether, **39** and **40**: ethyl). These chains orient in alternate directions with respect to the central benzene ring to minimize the steric congestion, locating the three thiolate moieties of **38**–**40** at the positions suitable for binding three iron sites of the [4Fe-4S] cluster. Thus, a chelate effect and the higher acidity of the aryl-thiol moieties in the protonated form of **38** relative to that of alkane-thiols facilitate the ligand exchange reaction with $[Fe_4S_4(SEt)_4]^{2-}$ via concomitant elimination of HSEt (Fig. 20) [162]. Subsequent treatment of **41** with pivaloyl chloride results in the replacement of the SEt ligand with a chloride to furnish **42** [162], which serves as a versatile precursor for a series of 3:1 site-differentiated [4Fe-4S] clusters containing a phenolate, cyclic triamine, imidazole, and phosphine ligand at the unique iron site [71]. In a similar

manner, 3:1 site-differentiated [4Fe-4S] clusters supported by **39** or **40** have been synthesized [161, 163, 164], including carboxylate-bound [4Fe-4S] clusters modeling those in *Pf* Fd [32] and the NB protein of *Rhodobacter capsulatus* DPOR [33]. The crystallographic analysis of these clusters revealed a η^1-coordination of the carboxylate ligands to the unique iron [164].

The displacement of iron from the 3:1 site-differentiated [4Fe-4S] cluster **41** was achieved by treatment with chelating agents, furnishing a triangular [3Fe-4S] cluster **43** modeling those in ferredoxins and aconitase (Fig. 20) [168, 169]. The structural confinement imposed by the tridentate thiolates facilitates the preparation and increases the stability of the [3Fe-4S] core, while analogous triangular [3Fe-4S] clusters with conventional terminal thiolates have not yet been isolated. An electrochemical analysis of the triangular [3Fe-4S] cluster in solution revealed three oxidation states, $[3Fe\text{-}4S]^{1+/0/1-}$; the $[3Fe\text{-}4S]^{1+}$ and $[3Fe\text{-}4S]^{0}$ states also occur naturally in proteins [169].

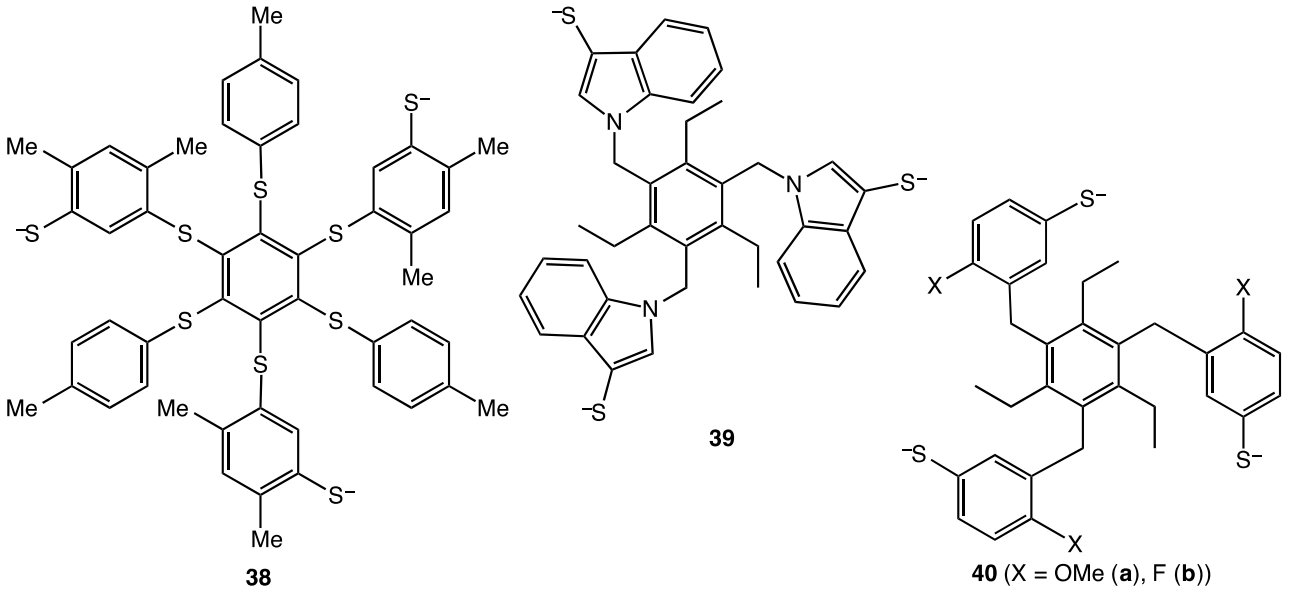

Fig. 19. Tridentate thiolate ligands for the synthesis of 3:1 site-differentiated [4Fe-4S] clusters [161–164].

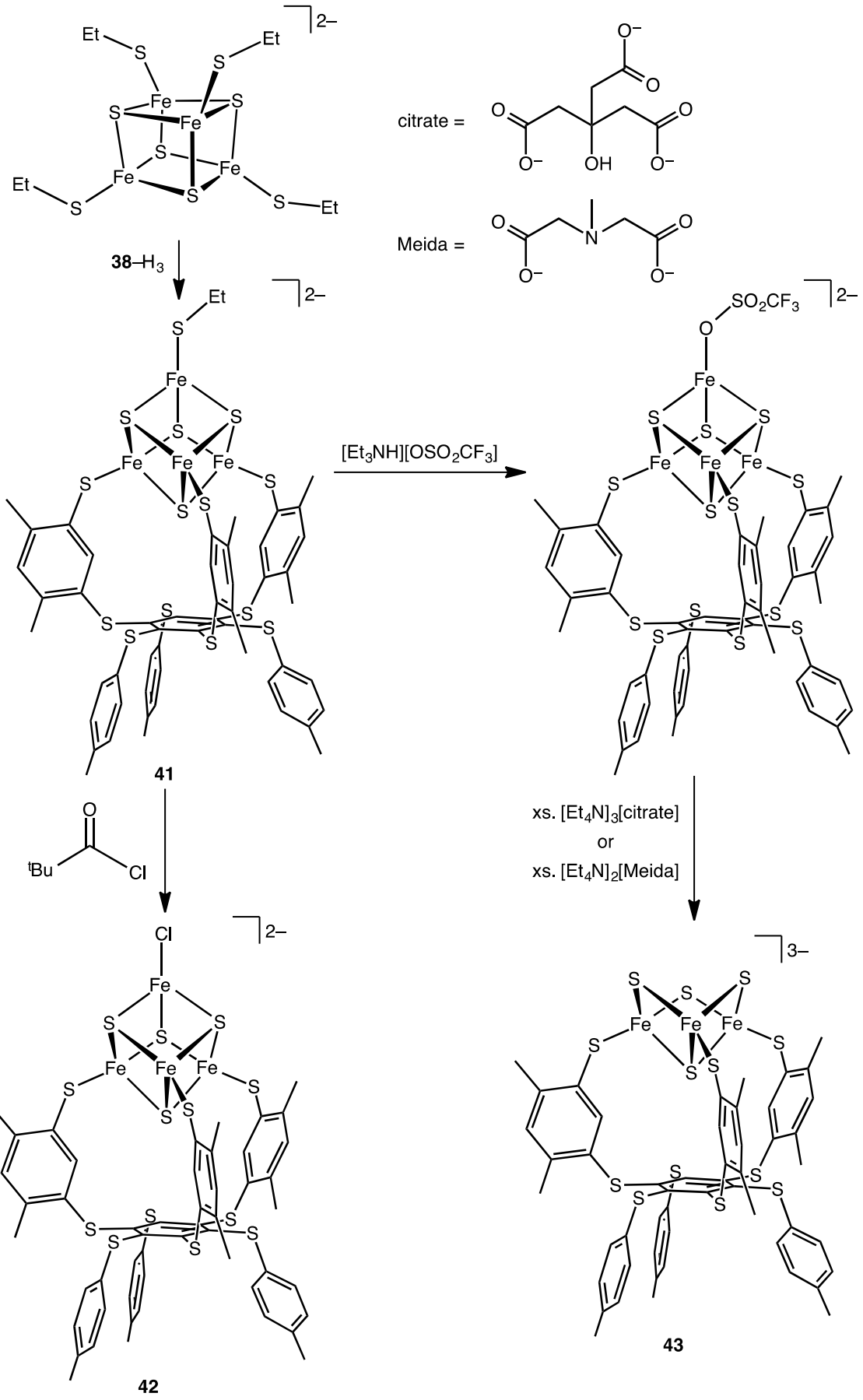

Fig. 20. Synthesis of 3:1 site-differentiated [4Fe-4S] clusters **41** and **42** [162] and a [3Fe–4S] cluster

43 [168, 169].

A recent study demonstrated that steric hindrance is an alternative way to synthesize 3:1 site-differentiated [4Fe-4S] clusters. As previously discussed (cf. Section 4.1), addition of 4 equiv. of HSDmp to a toluene solution of amide-supported $[4Fe\text{-}4S]^{4+}$ cluster **15**, followed by a treatment with THF afforded **44** (Fig. 21) [57]. The labile THF ligands on iron were subsequently replaced by a tetramethylimidazole to furnish imidazole-supported cluster **45**, which is a structural analogue of the *distal*-[4Fe-4S] cluster in hydrogenases [27–30]. The one-electron reduced form of **45** has been synthesized from a ligand displacement reaction between $[Fe_4S_4(SDmp)_4]^-$ and tetramethylimidazole. Therein, the one-electron reduction occurs via elimination of one of the thiolate ligands, most likely in the form of a half-disulfide molecule (1/2 DmpS–SDmp). These ligand displacement reactions indicate that steric repulsion among bulky ligands on the [4Fe-4S] core may be a driving force to open one of the iron sites for binding a different ligand.

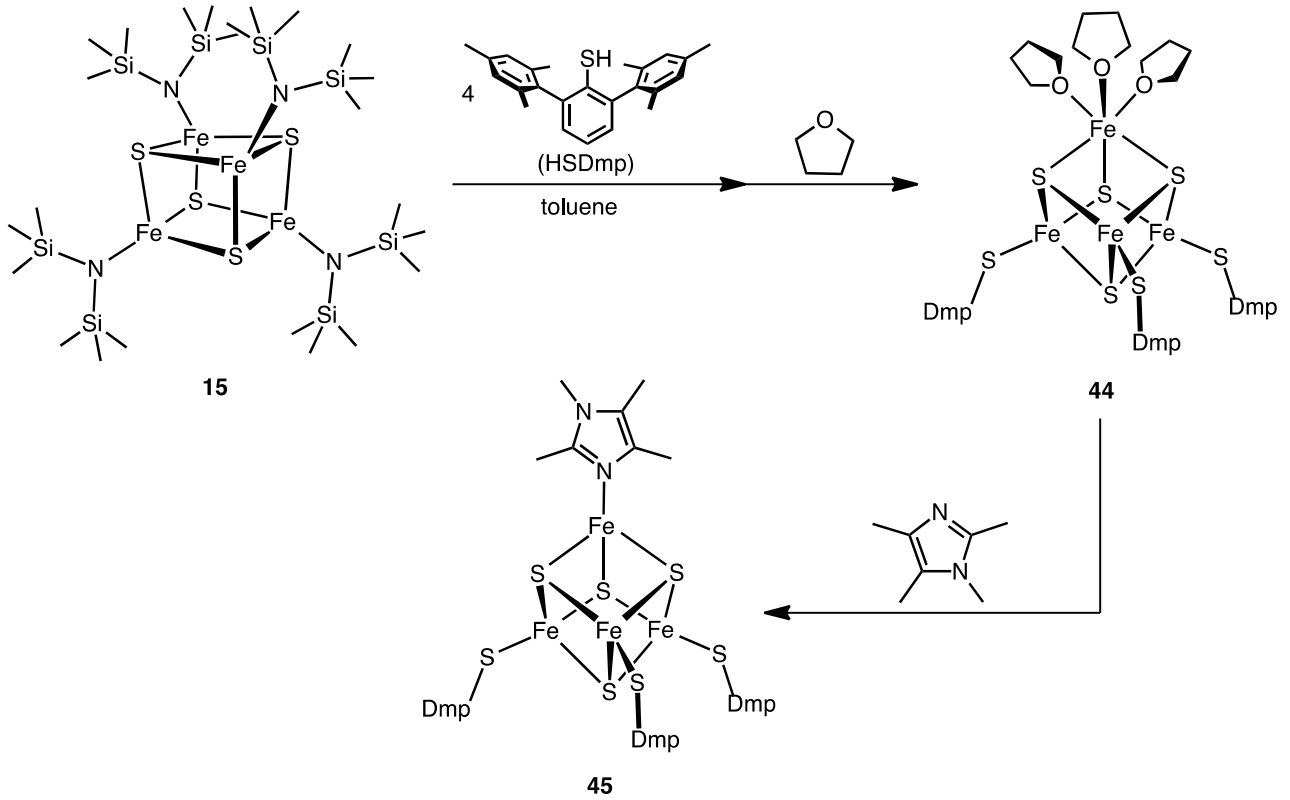

Fig. 21. Synthesis of **45**, which serves as a model for the *distal*-[4Fe-4S] clusters in hydrogenases [57].

The steric hindrance imposed by bulky bidentate ligands may be a key factor for the selective synthesis of site-differentiated [2Fe-2S] clusters, which could model those in Rieske proteins supported by two histidine residues. The first site-differentiated [2Fe-2S] cluster supported by N- and S-donor ligands, **46** (Fig. 22), was synthesized by a successive ligand exchange reaction of $[Fe_2S_2Cl_4]^{2-}$ with a bidentate amide **47** and *o*-xylyldithiolate [170]. After incorporation of **47** via replacement of chlorides in $[Fe_2S_2Cl_4]^{2-}$, the fused six-membered rings of the indole moieties in **47** orient toward the other iron site of the [2Fe-2S] cluster to kinetically stabilize mono-substituted $[Fe_2S_2(\mathbf{47})Cl_2]^{2-}$ for the successful synthesis of unsymmetric cluster **46**. Thus, some reactions using less sterically demanding diamide ligands have resulted in the formation of symmetric [2Fe-2S] clusters **48** and **49** supported by two bidentate or tridentate ligands [171–173]. Diamide ligand **50**, with two benzimidazole moieties, was employed as a supporting ligand for a mixed-valent (ferrous/ferric) and *N*-protonated model of the Rieske [2Fe-2S] cluster, which underwent proton-coupled electron-transfer in the presence of TEMPO (2,2,6,6-tetramethylpiperidin-1-yl-oxyl) [174]. Through a stepwise ligand exchange of chlorides in $[Fe_2S_2Cl_4]^{-}$ with bidentate biphenyl-dithiolate and benzimidazolyl-benzenethiolate ligands, a model [2Fe-2S] cluster of MitoNEET supported by three thiolates and one imidazole was recently synthesized [175].

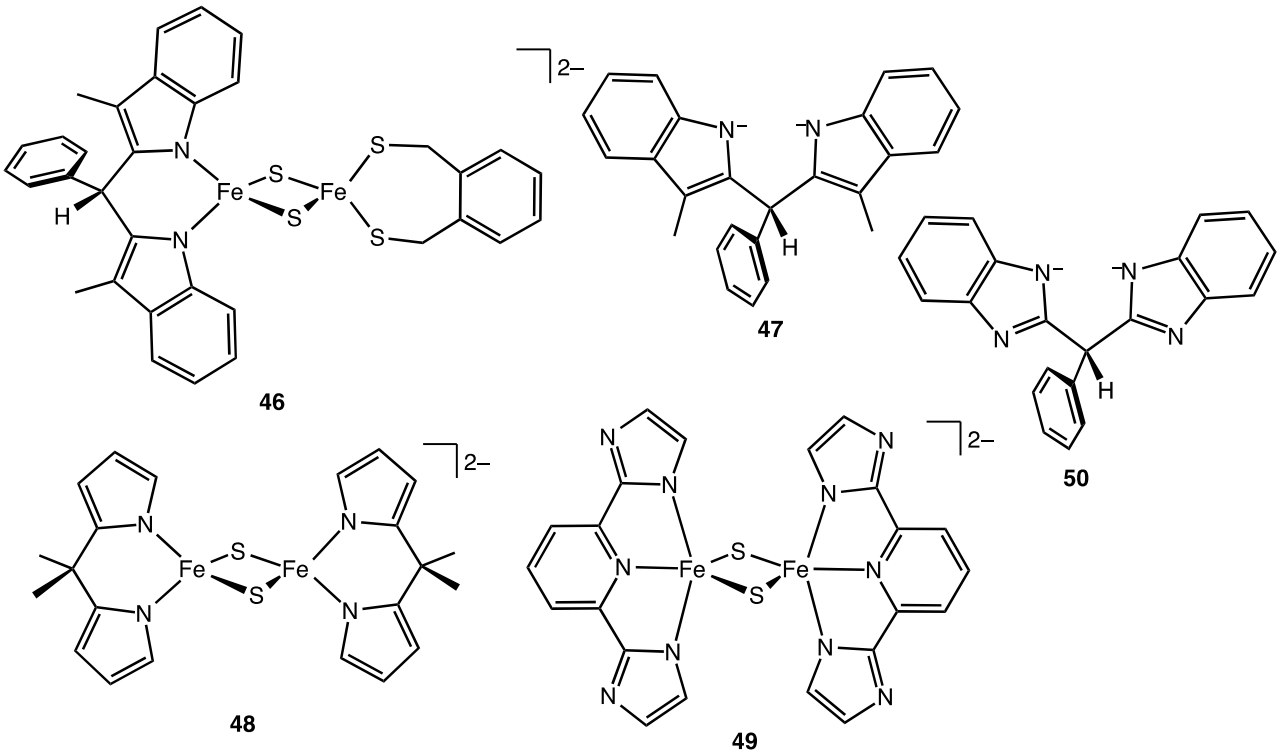

Fig. 22. A synthetic analogue of the Rieske-type [2Fe–2S] cluster **46** with a bidentate amide ligand **47** [170], symmetric [2Fe-2S] clusters **48** and **49** supported by two bidentate or tridentate ligands [171, 172], and another diamide ligand **50** with two benzimidazole moieties [174].

A hexagonal [3Fe–3S] cluster was synthesized by using a triethylbenzene-capped tris(β-diketiminate)cyclophane template ligand [176]. The single crystal X-ray analysis revealed the planar arrangement of inorganic core, which was controlled by the cyclophane ligand. The hexagonal conformation corresponds to the originally proposed structure for the 3-Fe site of Ferredoxin I from *Azotobacter vinelandii* [177, 178], while it was later replaced to a triangular [3Fe–4S] cluster **4** [179, 180].

4.3. Ligands for the Enhancement of the Solubility of Clusters

The synthesis and manipulation of biomimetic iron-sulfur clusters in solution usually require polar organic solvents such as DMF, DMSO, and CH_3CN. The use of bulky amide/thiolate ligands can

increase the solubility of these clusters in low-polarity or non-polar solvents (Fig. 7, 9, 11, 13, 18, and 21). Charge neutralization is another promising strategy to increase the solubility of such clusters in non-polar solvents (Fig. 7, 9, 11, 13, 14, and 21). In aqueous media, however, two major difficulties are associated with dissolving and handling iron-sulfur clusters: the low solubility of conventional biomimetic iron-sulfur clusters, and the gradual hydrolysis of thiolate-supported iron-sulfur clusters, *e.g.*, Fe–SR + H_2O $\rightarrow$ Fe–OH + HSR, in the presence of a large excess of water. To overcome these difficulties, a thiolate ligand consisting of a carboxylate-terminated amphiphilic dendron **51** (Fig. 23) was designed and synthesized [181]. The dendrimer unit of **51** partially encapsulates the [4Fe-4S] core and provides a hydrophobic cavity for the cluster, while maintaining the solubility of the cluster in 15% Me_2SO/H_2O. The redox potentials of the $[4Fe\text{-}4S]^{2+}$ clusters surrounded by dendron units vary by up to 500 mV, depending on the generation of the dendrimer from two to four [182]. Studies on the stability of [4Fe-4S] clusters in aqueous media show that biomimetic $[Fe_4S_4(SR)_4]^{2-}$ clusters (R = CH_2CH_2OH, $CH_2CH_2COO^-$, *p*-$C_6H_4CH_2OH$, or cyclodextrin derivatives) are stable for 12 h in 60% Me_2SO/H_2O, but degrade within the same period when dissolved in 20–40% Me_2SO/H_2O [183]. The most stable cluster contains β-cyclodextrin-substituted dithiolate **52** [184], and a similar β-cyclodextrin-thiolate has also been synthesized [185].

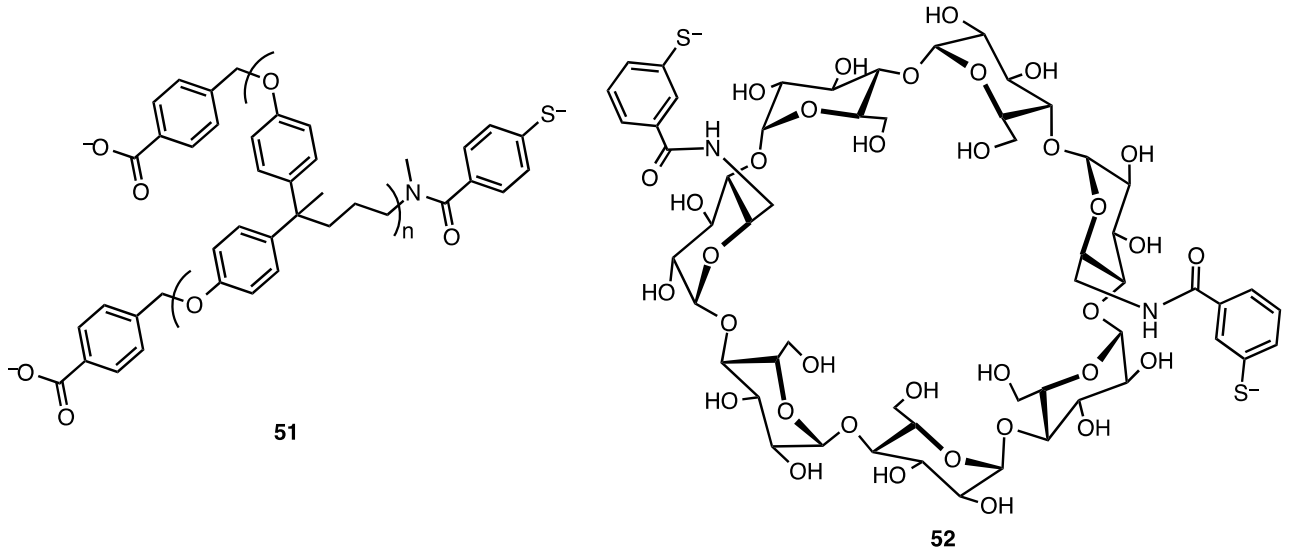

Fig. 23. Thiolate ligand **51** with a dendron unit [181] and an example of a cyclodextrin-based

dithiolate ligand (**52**) [184].

5. Summary

Over the past decades, biochemical and inorganic studies on iron-sulfur clusters have revealed the importance of ligands and media for the manipulation of their electrochemical properties, stability, and reactivity.

The electronic properties of both biological and synthetic iron-sulfur clusters are modulated by the following factors (cf. Sections 2 and 4): (a) the extend of E–H···S hydrogen bonding (E = O, N) between the sulfur atoms of the iron-sulfur clusters and the media, peptides, or intramolecular hydrogen bonding units leads to the stabilization of relatively reduced and negatively charged clusters; (b) the steric shielding of biomimetic clusters imposed by bulky ligands prevents the efficient charge neutralization and leads to the stabilization of less negatively charged (relatively oxidized) clusters; (c) the electron-donating/accepting properties of the ligands on the iron centers, particularly those of non-biological ligands serving as π-acceptors (phosphines, cyanides) or strong π-donors (amides), affect the stable oxidation states of the resultant clusters.

Self-assembly type reactions using iron chlorides, thiolates ($^{-}$SR), and sulfide sources (usually HS^{-} or S^{2-}) in polar organic solvents are versatile for the synthesis of biomimetic [2Fe-2S] and [4Fe-4S] clusters. Moreover, some analogous reactions furnish non-conventional and high-nuclearity iron-sulfur clusters (cf. Section 3.1). For the selective incorporation of ligands around the cluster cores, steric factors need to be considered; this is exemplified by the use of structurally confined tridentate thiolate ligands for the synthesis of site-differentiated [4Fe-4S] clusters and triangular [3Fe-4S] clusters, and the use of sterically demanding bidentate amide ligands for Rieske [2Fe-2S] cluster models (cf. Section 4.2). For the synthesis of large iron-sulfur clusters mimicking the P-cluster or the FeMo-cofactor of nitrogenase, charge-neutral reactions in non-polar organic solvents turned

out to be useful (cf. Section 3.2).

Biochemical and inorganic studies on iron-sulfur clusters complement each other; thus, further discoveries of new structures and functions of clusters in proteins, *e.g.* the [4Fe-3S] cluster **5** in the O_2-tolerant [NiFe] hydrogenase (Fig. 1), and the investigation of new synthetic iron-sulfur clusters with unprecedented ligands and media, should facilitate further advances in this field. Of particular relevance are interdisciplinary studies employing both biological and inorganic approaches. These may be able to unveil the secrets of complex metal-sulfur clusters such as those in nitrogenases and CO dehydrogenases, which mediate the conversion of small molecules.

Acknowledgements

This work was financially supported by grants-in-aids for Scientific Research (16H04116, 15K13655) and Scientific Research on Innovative Areas (15H00936) from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT), as well as the PRESTO program of the Japan Science and Technology Agency.

Declaration of interest

The authors declare no conflict of interest including financial, personal, and other relationships with other people or organizations.

References

[1] D.C. Johnson, D.R. Dean, A.D. Smith, M.K. Johnson, Annu. Rev. Biochem. 74 (2005) 247–281.

[2] C. Ayala-Castro, A. Saini, F.W. Outten, Microbiol. Mol. Biol. Rev. 72 (2008) 110–125.

[3] C. Huber, G. Wächtershäuser, Science 281 (1998) 670–672.

[4] J.L.C. Duff, J.L.J. Breton, J.N. Butt, F.A. Armstrong, A.J. Thomson, J. Am. Chem. Soc. 118 (1996) 8593–8603.

[5] S.J. Yoo, H.C. Angove, B.K. Burgess, M.P. Hendrich, E. Münck, J. Am. Chem. Soc. 121 (1999) 2534–2545.

[6] M. Hans, W. Buckel, E. Bill, J. Biol. Inorg. Chem. 13 (2008) 563–574.

[7] J. Fritsch, P. Scheerer, S. Frielingsdorf, S. Kroschinsky, B. Friedrich, O. Lenz, C.M.T. Spahn, Nature 479 (2011) 249–252.

[8] Y. Shomura, K.-S. Yoon, H. Nishihara, Y. Higuchi, Nature 479 (2011) 253–256.

[9] J. Fritsch, O. Lenz, B. Friedrich, Nature Rev. Microbiol. 11 (2013) 106–114.

[10] W. Lubitz, H. Ogata, O. Rüdiger, E. Reijerse, Chem. Rev. 114 (2014) 4081–4148.

[11] J. Kim, D.C. Rees, Nature 360 (1992) 553–560.

[12] M.K. Chan, J. Kim, D.C. Rees, Science 260 (1993) 792–794.

[13] H. Schindelin, C. Kisker, J.L. Schlessman, J.B. Howard, D.C. Rees, Nature 387 (1997) 370–376.

[14] J.W. Peters, M.H.B. Stowell, S.M. Soltis, M.G. Finnegan, M.K. Johnson, D.C. Rees, Biochemistry 36 (1997) 1181–1187.

[15] S.M. Mayer, D.M. Lawson, C.A. Gormal, S.M. Roe, B.E. Smith, J. Mol. Biol. 292 (1999) 871–891.

[16] F.A. Tezcan, J.T. Kaiser, D. Mustafi, M.Y. Walton, J.B. Howard, D.C. Rees, Science 309 (2005) 1377–1380.

[17] C.P. Owens, F.E.H. Katz, C.H. Carter, V.F. Oswald, F.A. Tezcan, J. Am. Chem. Soc. 138 (2016) 10124–10127.

[18] M. Bruschi, F. Guerlesquin, FEMS Microbiol. Rev. 54 (1988) 155–176.

[19] H. Matsubara, K. Saeki, Adv. Inorg. Chem. 38 (1992) 223–280.

[20] Z. Dauter, K.S. Wilson, L.C. Sieker, J. Meyer, J.-M. Moulis, Biochemistry 36 (1997) 16065–16073.

[21] J.A. Cowan, S.M. Lui, Adv. Inorg. Chem. 45 (1998) 313–350.

[22] C.A. Kerfeld, A.E. Salmeen, T.O. Yeates, Biochemistry 37 (1998) 13911–13917.

[23] M.L. Paddock, S.E. Wiley, H.L. Axelrod, A.E. Cohen, M. Roy, E.C. Abresch, D. Capraro, A.N. Murphy, R. Nechushtai, J.E. Dixon, P.A. Jennings, Proc. Natl. Acad. Sci. USA 104 (2007) 14342–14347.

[24] J. Lin, T. Zhou, K. Ye, J. Wang, Proc. Natl. Acad. Sci. USA 104 (2007) 14640–14645.

[25] H. Bönisch, C.L. Schmidt, G. Schäfer, R. Ladenstein, J. Mol. Biol. 319 (2002) 791–805.

[26] L.M. Hunsicker-Wang, A. Heine, Y. Chen, E.P. Luna, T. Todaro, Y.M. Zhang, P.A. Williams, D.E. McRee, J. Hirst, C.D. Stout, J.A. Fee, Biochemistry 42 (2003) 7303–7317.

[27] A. Volbeda, M.-H. Charon, C. Piras, E.C. Hatchikian, M. Frey, J.C. Fontecilla-Camps, Nature 373 (1995) 580–587.

[28] A. Volbeda, E. Garcin, C. Piras, A.L. de Lacey, V.M. Fernandez, E.C. Hatchikian, M. Frey, J.C. Fontecilla-Camps, J. Am. Chem. Soc. 118 (1996) 12989–12996.

[29] J.W. Peters, W.N. Lanzilotta, B.J. Lemon, L.C. Seefeldt, Science 282 (1998) 1853–1858.

[30] J.C. Fontecilla-Camps, A. Volbeda, C. Cavazza, Y. Nicolet, Chem. Rev. 107 (2007) 4273–4303.

[31] B.M. Martins, H. Dobbek, I. Çinkaya, W. Buckel, A. Messerschmidt, Proc. Natl. Acad. Sci. USA 101 (2004) 15645–15649.

[32] Y. Hu, S. Faham, R. Roy, M.W.W. Adams, D.C. Rees, J. Mol. Biol. 286 (1999) 899–914.

[33] N. Muraki, J. Nomata, K. Ebata, T. Mizoguchi, T. Shiba, H. Tamiaki, G. Kurisu, Y. Fujita, Nature 465 (2010) 110–114.

[34] M. Lee, T. Gräwert, F. Quitterer, F. Rohdich, J. Eppinger, W. Eisenreich, A. Bacher, M. Groll, J. Mol. Biol. 404 (2010) 600–610.

[35] I. Rekittke, T. Nonaka, J. Wiesner, U. Demmer, E. Warkentin, H. Jomaa, U. Ermler, FEBS Lett. 585 (2011) 447–451.

[36] W. Wang, E. Oldfield, Angew. Chem. Int. Ed. 53 (2014) 4294–4310.

[37] D.W. Bak, S.J. Elliott, Curr. Opin. Chem. Biol. 19 (2014) 50–58.

[38] P.J. Stephens, D.R. Jollie, A. Warshel, Chem. Rev. 96 (1996) 2491–2513.

[39] J. Liu, S. Chakraborty, P. Hosseinzadeh, Y. Yu, S. Tian, I. Petrik, A. Bhagi, Y. Lu, Chem. Rev. 114 (2014) 4366–4469.

[40] R. Cammack, Adv. Inorg. Chem. 38 (1992) 281–322.

[41] A. Dey, C.L. Roche, M.A. Walters, K.O. Hodgson, B. Hedman, E.I. Solomon, Inorg. Chem. 44 (2005) 8349–8354.

[42] C.W. Carter Jr., J. Kraut, S.T. Freer, R.A. Alden, J. Biol. Chem. 249 (1974) 6339–6346.

[43] I. Rayment, G. Wesenberg, T.E. Meyer, M.A. Cusanovich, H.M. Holden, J. Mol. Biol. 228 (1992) 672−686.

[44] L. Liu, T. Nogi, M. Kobayashi, T. Nozawa, K. Miki, Acta Cryst. D58 (2002) 1085−1091.

[45] S.S. Mansy, Y. Xiong, C. Hemann, R. Hille, M. Sundaralingam, J.A. Cowan, Biochemistry 41 (2002) 1195−1201.

[46] A. González, S. Benini, S. Ciurli, Acta Cryst. D59 (2003) 1582−1588.

[47] M. Stelter, A.M.P. Melo, G.O. Hreggvidsson, S. Hjorleifsdottir, L.M. Saraiva, M. Teixeira, M. Archer, J. Biol. Inorg. Chem. 15 (2010) 303−313.

[48] D. Li, A. Agarwal, J.A. Cowan, Inorg. Chem. 35 (1996) 1121–1125.

[49] G. Backes, Y. Mino, T.M. Loehr, T.E. Meyer, M.A. Cusanovich, W.V. Sweeney, E.T. Adman, J. Sanders-Loehr, J. Am. Chem. Soc. 113 (1991) 2055–2064.

[50] R. Langen, G.M. Jensen, U. Jacob, P.J. Stephens, A. Warshel, J. Biol. Chem. 267 (1992) 25625–25627.

[51] A. Dey, F.E. Jenney Jr., M.W.W. Adams, E. Babini, Y. Takahashi, K. Fukuyama, K.O. Hodgson, B. Hedman, E.I. Solomon, Science 318 (2007) 1464−1468.

[52] S. Niu, T. Ichiye, J. Am. Chem. Soc. 131 (2009) 5724–5725.

[53] D.W. Low, M.G. Hill, J. Am. Chem. Soc. 122 (2000) 11039–11040.

[54] D.W. Bak, J.A. Zuris, M.L. Paddock, P.A. Jennings, S.J. Elliott, Biochemistry 48 (2009) 10193–10195.

[55] T.A. Link, Biochim. Biophys. Acta 1185 (1994) 81–84.

[56] Y. Zu, M.M.-J. Couture, D.R.J. Kolling, A.R. Crofts, L.D. Eltis, J.A. Fee, J. Hirst, Biochemistry 42 (2003) 12400–12408.

[57] Y. Ohki, K. Tanifuji, N. Yamada, M. Imada, T. Tajima, K. Tatsumi, Proc. Natl. Acad. Sci. USA 108 (2011) 12635–12640.

[58] A. Volbeda, L. Martin, C. Cavazza, M. Matho, B.W. Faber, W. Roseboom, S.P.J. Albracht, E. Garcin, M. Rousset, J.C. Fontecilla-Camps, J. Biol. Inorg. Chem. 10 (2005) 239–249.

[59] H. Ogata, S. Hirota, A. Nakahara, H. Komori, N. Shibata, T. Kato, K. Kano, Y. Higuchi, Structure 13 (2005) 1635–1642.

[60] S. Dementin, V. Belle, P. Bertrand, B. Guigliarelli, G. Adryanczyk-Perrier, A.L. De Lacey, V.M. Fernandez, M. Rousset, C. Léger, J. Am. Chem. Soc. 128 (2006) 5209–5218.

[61] P.S. Brereton, M.F.J.M. Verhagen, Z.H. Zhou, M.W.W. Adams, Biochemistry 37 (1998) 7351−7362.

[62] R. Sarma, B.M. Barney, T.L. Hamilton, A. Jones, L.C. Seefeldt, J.W. Peters, Biochemistry 47 (2008) 13004–13015.

[63] F. Rohdich, F. Zepeck, P. Adam, S. Hecht, J. Kaiser, R. Laupitz, T. Gräwert, S. Amslinger, W. Eisenreich, A. Bacher, D. Arigoni, Proc. Natl. Acad. Sci. USA 100 (2003) 1586–1591.

[64] I. Rekittke, H. Jomaa, U. Ermler, FEBS Lett. 586 (2012) 3452–3457.

[65] Y. Xiao, D. Rooker, Q. You, C.L. Freel Meyers, P. Liu, ChemBioChem 12 (2011) 527–530.

[66] Y.-L. Liu, F. Guerra, K. Wang, W. Wang, J. Li, C. Huang, W. Zhu, K. Houlihan, Z. Li, Y. Zhang, S.K. Nair, E. Oldfield, Proc. Natl. Acad. Sci. USA 109 (2012) 8558–8563.

[67] M.-E. Pandelia, W. Nitschke, P. Infossi, M.-T. Giudici-Orticoni, E. Bill, W. Lubitz, Proc. Natl. Acad. Sci. USA 108 (2011) 6097–6102.

[68] M.M. Roessler, R.M. Evans, R.A. Davies, J. Harmer, F.A. Armstrong, J. Am. Chem. Soc. 134 (2012) 15581–15594.

[69] R.H. Holm, E.I. Solomon, Chem. Rev. 104 (2004) 347–348.

[70] S. Groysman, R.H. Holm, Biochemistry 48 (2009) 2310–2320.

[71] P.V. Rao, R.H. Holm, Chem. Rev. 104 (2004) 527–559.

[72] T. Herskovitz, B.A. Averill, R.H. Holm, J.A. Ibers, W.D. Phillips, J.F. Weiher, Proc. Natl. Acad. Sci. USA 69 (1972) 2437–2441.

[73] B.A. Averill, T. Herskovitz, R.H. Holm, J.A. Ibers, J. Am. Chem. Soc. 95 (1973) 3523–3534.

[74] G. Christou, C.D. Garner, J. Chem. Soc. Dalton Trans. (1979) 1093–1094.

[75] F. Bonomi, M.T. Werth, D.M. Kurtz Jr., Inorg. Chem. 24 (1985) 4331–4335.

[76] K.S. Hagen, A.D. Watson, R.H. Holm, Inorg. Chem. 23 (1984) 2984–2990.

[77] J.J. Mayerle, R.B. Frankel, R.H. Holm, J.A. Ibers, W.D. Phillips, J.F. Weiher, Proc. Natl. Acad. Sci. USA 70 (1973) 2429–2433.

[78] J.J. Mayerle, S.E. Denmark, B.V. DePamphilis, J.A. Ibers, R.H. Holm, J. Am. Chem. Soc. 97 (1975) 1032–1045.

[79] J.G. Reynolds, R.H. Holm, Inorg. Chem. 19 (1980) 3257–3260.

[80] K.S. Hagen, R.H. Holm, J. Am. Chem. Soc. 104 (1982) 5496–5497.

[81] K.S. Hagen, A.D. Watson, R.H. Holm, J. Am. Chem. Soc. 105 (1983) 3905–3913.

[82] S. Han, R.S. Czernuszewicz, T.G. Spiro, Inorg. Chem. 25 (1986) 2276–2277.

[83] M.C. Kennedy, T.A. Kent, M. Emptage, H. Merkle, H. Beinert, E. Münck, J. Biol. Chem. 259 (1984) 14463–14471.

[84] C. Krebs, T.F. Henshaw, J. Cheek, B.H. Huynh, J.B. Broderick, J. Am. Chem. Soc. 122 (2000) 12497–12506.

[85] G. Christou, R.H. Holm, M. Sabat, J.A. Ibers, J. Am. Chem. Soc. 103 (1981) 6269–6271.

[86] G. Christou, M. Sabat, J.A. Ibers, R.H. Holm, Inorg. Chem. 21 (1982) 3518–3526.

[87] G. Henkel, H. Strasdeit, B. Krebs, Angew. Chem. Int. Ed. Engl. 21 (1982) 201–202.

[88] H. Strasdeit, B. Krebs, G. Henkel, Inorg. Chem. 23 (1984) 1816–1825.

[89] M.G. Kanatzidis, W.R. Dunham, W.R. Hagen, D. Coucouvanis, J. Chem. Soc. Chem. Commun. (1984) 356–358.

[90] M.G. Kanatzidis, W.R. Hagen, W.R. Dunham, R.K. Lester, D. Coucouvanis, J. Am. Chem. Soc. 107 (1985) 953–961.

[91] W. Saak, G. Henkel, S. Pohl, Angew. Chem. Int. Ed. Engl. 23 (1984) 150–151.

[92] D. Coucouvanis, M.G. Kanatzidis, W.R. Dunham, W.R. Hagen, J. Am. Chem. Soc. 106 (1984) 7998–7999.

[93] M.G. Kanatzidis, A. Salifoglou, D. Coucouvanis, Inorg. Chem. 25 (1986) 2460–2468.

[94] J. Cambray, R.W. Lane, A.G. Wedd, R.W. Johnson, R.H. Holm, Inorg. Chem. 16 (1977) 2565–2571.

[95] K.S. Hagen, J.G. Reynolds, R.H. Holm, J. Am. Chem. Soc. 103 (1981) 4054–4063.

[96] A.S. Fleischhacker, P.J. Kiley, Curr. Opin. Chem. Biol. 15 (2011) 335–341.

[97] J.C. Crack, J. Green, A.J. Thomson, N.E. Le Brun, Curr. Opin. Chem. Biol. 16 (2012) 35–44.

[98] G. Unden, S. Nilkens, M. Singenstreu, Dalton Trans. 42 (2013) 3082–3087.

[99] D.T. Mapolelo, B. Zhang, S.G. Naik, B.H. Huynh, M.K. Johnson, Biochemistry 51 (2012) 8071–8084.

[100] Y. Ohki, Y. Sunada, K. Tatsumi, Chem. Lett. 34 (2005) 172–173.

[101] C.R. Sharp, J.S. Duncan, S.C. Lee, Inorg. Chem. 49 (2010) 6697–6705.

[102] K. Tanifuji, S. Tajima, Y. Ohki, K. Tatsumi, Inorg. Chem. 55 (2016) 4512–4518.

[103] R.H. Holm, W. Lo, Chem. Rev. 116 (2016) 13685–13713.

[104] Y. Ohki, K. Tatsumi, Z. Anorg. Allg. Chem. 639 (2013) 1340–1349.

[105] Y. Ohki, Bull. Chem. Soc. Jpn. 87 (2014) 1–19.

[106] J.B. Howard, D.C. Rees, Chem. Rev. 96 (1996) 2965–2982.

[107] O. Einsle, F.A. Tezcan, S.L.A. Andrade, B. Schmid, M. Yoshida, J.B. Howard, D.C. Rees, Science 297 (2002) 1696–1700.

[108] D.C. Rees, F.A. Tezcan, C.A. Haynes, M.Y. Walton, S. Andrade, O. Einsle, J.B. Howard, Phil. Trans. R. Soc. A 363 (2005) 971–984.

[109] T. Spatzal, M. Aksoyoglu, L. Zhang, S.L.A. Andrade, E. Schleicher, S. Weber, D.C. Rees, O. Einsle, Science 334 (2011) 940.

[110] K.M. Lancaster, M. Roemelt, P. Ettenhuber, Y. Hu, M.W. Ribbe, F. Neese, U. Bergmann, S. DeBeer, Science 334 (2011) 974–977.

[111] B.M. Hoffman, D. Lukoyanov, Z.-Y. Yang, D.R. Dean, L.C. Seefeldt, Chem. Rev. 114 (2014) 4041−4062.

[112] R.A. Andersen, K. Faegri Jr., J.C. Green, A. Haaland, M.F. Lappert, W.-P. Leung, K. Rypdal, Inorg. Chem. 27 (1988) 1782–1786.

[113] Y. Ohki, S. Ohta, K. Tatsumi, Inorg. Synth. 35 (2010) 137–143.

[114] J.J. Ellison, K. Ruhlandt-Senge, P.P. Power, Angew. Chem. Int. Ed. Engl. 33 (1994) 1178–1180.

[115] F.M. MacDonnell, K. Ruhlandt-Senge, J.J. Ellison, R.H. Holm, P.P. Power, Inorg. Chem. 34 (1995) 1815–1822.

[116] M. Harmjanz, C. Junghans, U.-A. Opitz, B. Bahlmann, S. Pohl, Z. Naturforsch. B51 (1996) 1040–1048.

[117] R. Hauptmann, R. Kliß, J. Schneider, G. Henkel, Z. Anorg. Allg. Chem. 624 (1998) 1927–1936.

[118] T. Komuro, H. Kawaguchi, K. Tatsumi, Inorg. Chem. 41 (2002) 5083–5090.

[119] S. Ohta, Y. Ohki, Y. Ikagawa, R. Suizu, K. Tatsumi, J. Organomet. Chem. 692 (2007) 4792–4799.

[120] Y. Ohki, Y. Sunada, M. Honda, M. Katada, K. Tatsumi, J. Am. Chem. Soc. 125 (2003) 4052–4053.

[121] Y. Ohki, M. Imada, A. Murata, Y. Sunada, S. Ohta, M. Honda, T. Sasamori, N. Tokitoh, M. Katada, K. Tatsumi, J. Am. Chem. Soc. 131 (2009) 13168–13178.

[122] R. Pryadun, R.H. Holm, Inorg. Chem. 47 (2008) 3366–3370.

[123] Y. Ohki, K. Tanifuji, N. Yamada, R.E. Cramer, K. Tatsumi, Chem. Asian. J. 7 (2012) 2222–2224.

[124] Y. Ohki, Y. Ikagawa, K. Tatsumi, J. Am. Chem. Soc. 129 (2007) 10457–10465.

[125] T. O'Sullivan, M.M. Millar, J. Am. Chem. Soc. 107 (1985) 4096−4097.

[126] K. Tanifuji, N. Yamada, T. Tajima, T. Sasamori, N. Tokitoh, T. Matsuo, K. Tamao, Y. Ohki, K. Tatsumi, Inorg. Chem. 53 (2014) 4000–4009.

[127] B. Machelett, Z. Chem. 16 (1976) 116–117.

[128] H. Müller, W. Seidel, H. Görls, J. Organomet. Chem. 445 (1993) 133–136.

[129] A. Klose, E. Solari, C. Floriani, A. Chiesi-Villa, C. Rizzoli, N. Re, J. Am. Chem. Soc. 116 (1994) 9123–9135.

[130] T. Hashimoto, Y. Ohki, K. Tatsumi, Inorg. Chem. 49 (2010) 6102–6109.

[131] S. Ohta, Y. Ohki, T. Hashimoto, R.E. Cramer, K. Tatsumi, Inorg. Chem. 51 (2012) 11217–11219.

[132] J. Schimpl, H.M. Petrilli, P.E. Blöchl, J. Am. Chem. Soc. 125 (2003) 15772–15778.

[133] B.M. Barney, H.-I. Lee, P.C. Dos Santos, B.M. Hoffman, D.R. Dean, L.C. Seefeldt, Dalton Trans. (2006) 2277–2284.

[134] B.M. Hoffman, D.R. Dean, L.C. Seefeldt, Acc. Chem. Res. 42 (2009) 609–619.

[135] T. Spatzal, K.A. Perez, O. Einsle, J.B. Howard, D.C. Rees, Science 345 (2014) 1620–1623.

[136] I. Čorić, B.Q. Mercado, E. Bill, D.J. Vinyard, P.L. Holland, Nature 526 (2015) 96–99.

[137] I. Čorić, P.L. Holland, J. Am. Chem. Soc. 138 (2016) 7200–7211.

[138] J. Vela, S. Stoian, C.J. Flaschenriem, E. Münck, P.L. Holland, J. Am. Chem. Soc. 126 (2004) 4522–4523.

[139] J. Vela, J. Cirera, J.M. Smith, R.J. Lachicotte, C.J. Flaschenriem, S. Alvarez, P.L. Holland, Inorg. Chem. 46 (2007) 60–71.

[140] S.J. George, B.M. Barney, D. Mitra, R.Y. Igarashi, Y. Guo, D.R. Dean, S.P. Cramer, L.C. Seefeldt, J. Inorg. Biochem. 112 (2012) 85–92.

[141] J. Rittle, J.C. Peters, Proc. Natl. Acad. Sci. USA 110 (2013) 15898–15903.

[142] J.S. Anderson, J. Rittle, J.C. Peters, Nature 501 (2013) 84–87.

[143] S.E. Creutz, J.C. Peters, J. Am. Chem. Soc. 136 (2014) 1105–1115.

[144] B.S. Snyder, R.H. Holm, Inorg. Chem. 27 (1988) 2339–2347.

[145] M.S. Reynolds, R.H. Holm, Inorg. Chem. 27 (1988) 4494–4499.

[146] L. Deng, R.H. Holm, J. Am. Chem. Soc. 130 (2008) 9878–9886.

[147] I. Noda, B.S. Snyder, R.H. Holm, Inorg. Chem. 25 (1986) 3851–3853.

[148] X.-D. Chen, J.S. Duncan, A.K. Verma, S.C. Lee, J. Am. Chem. Soc. 132 (2010) 15884–15886.

[149] X.-D. Chen, W. Zhang, J.S. Duncan, S.C. Lee, Inorg. Chem. 51 (2012) 12891–12904.

[150] L.L. Tan, R.H. Holm, S.C. Lee, Polyhedron 58 (2013) 206–217.

[151] S.C. Lee, W. Lo, R.H. Holm, Chem. Rev. 114 (2014) 3579–3600.

[152] P. Zanello, Coord. Chem. Rev. 280 (2014) 54–83.

[153] P. Zanello, Coord. Chem. Rev. 306 (2016) 420–442.

[154] N. Ueyama, Y. Yamada, T. Okamura, S. Kimura, A. Nakamura, Inorg. Chem. 35 (1996) 6473–6484.

[155] N. Ueyama, T. Terakawa, M. Nakata, A. Nakamura, J. Am. Chem. Soc. 105 (1983) 7098–7102.

[156] R. Ohno, N. Ueyama, A. Nakamura, Inorg. Chem. 30 (1991) 4887–4891.

[157] C. Goh, B.M. Segal, J. Huang, J.R. Long, R.H. Holm, J. Am. Chem. Soc. 118 (1996) 11844–11853.

[158] H.-C. Zhou, R.H. Holm, Inorg. Chem. 42 (2003) 11–21.

[159] T.A. Scott, C.P. Berlinguette, R.H. Holm, H.-C. Zhou, Proc. Natl. Acad. Sci. USA 102 (2005) 9741–9744.

[160] D. Coucouvanis, M. Kanatzidis, E. Simhon, N.C. Baenziger, J. Am. Chem. Soc. 104 (1982) 1874–1882.

[161] C. Walsdorff, W. Saak, S. Pohl, J. Chem. Soc. Dalton Trans. (1997) 1857–1861.

[162] T.D.P. Stack, R.H. Holm, J. Am. Chem. Soc. 110 (1988) 2484–2494.

[163] T. Terada, T. Wakimoto, T. Nakamura, K. Hirabayashi, K. Tanaka, J. Li, T. Matsumoto, K. Tatsumi, Chem. Asian. J. 7 (2012) 920–929.

[164] T. Terada, K. Hirabayashi, D. Liu, T. Nakamura, T. Wakimoto, T. Matsumoto, K. Tatsumi, Inorg. Chem. 52 (2013) 11997–12004.

[165] M.A. Whitener, G. Peng, R.H. Holm, Inorg. Chem. 30 (1991) 2411–2417.

[166] J.E. Barclay, M.I. Diaz, D.J. Evans, G. Garcia, M.D. Santana, M.C. Torralba, Inorg. Chim. Acta 258 (1997) 211–219.

[167] G.P.F. van Strijdonck, J.A.E.H. van Haare, P.J.M. Hönen, R.C.G.M. van den Schoor, M.C. Feiters, J.G.M. van der Linden, J.J. Steggerda, R.J.M. Nolte, J. Chem. Soc. Dalton Trans. (1997) 449–461.

[168] J. Zhou, R.H. Holm, J. Am. Chem. Soc. 117 (1995) 11353–11354.

[169] J. Zhou, Z. Hu, E. Münck, R.H. Holm, J. Am. Chem. Soc. 118 (1996) 1966–1980.

[170] J. Ballmann, A. Albers, S. Demeshko, S. Dechert, E. Bill, E. Bothe, U. Ryde, F. Meyer, Angew. Chem. Int. Ed. 47 (2008) 9537–9541.

[171] J. Ballmann, X. Sun, S. Dechert, E. Bill, F. Meyer, J. Inorg. Biochem. 101 (2007) 305–312.

[172] M.G.G. Fuchs, S. Dechert, S. Demeshko, U. Ryde, F. Meyer, Inorg. Chem. 49 (2010) 5853–5858.

[173] A. Albers, S. Demeshko, S. Dechert, E. Bill, E. Bothe, F. Meyer, Angew. Chem. Int. Ed. 50 (2011) 9191–9194.

[174] A. Albers, S. Demeshko, S. Dechert, C.T. Saouma, J.M. Mayer, F. Meyer, J. Am. Chem. Soc. 136 (2014) 3946–3954.

[175] M. Bergner, S. Dechert, S. Demeshko, C. Kupper, J.M. Mayer, F. Meyer, J. Am. Chem. Soc. 139 (2017) 701–707.

[176] Y. Lee, I.-R. Jeon, K.A. Abboud, R. García-Serres, J. Shearer, L.J. Murray, Chem. Commun. 52 (2016) 1174–1177.

[177] D. Ghosh, W. Furey Jr., S. O'Donnell, C.D. Stout, J. Biol. Chem. 256 (1981) 4185–4192.

[178] D. Ghosh, S. O'Donnell, W. Furey Jr., A.H. Robbins, C.D. Stout, J. Mol. Biol. 158 (1982) 73–109.

[179] C.D. Stout, J. Biol. Chem. 263 (1988) 9256–9260.

[180] C.D. Stout, J. Mol. Biol. 205 (1989) 545–555.

[181] A.K. Sharma, N. Kim, C.S. Cameron, M. Lyndon, C.B. Gorman, Inorg. Chem. 49 (2010) 5072–5078.

[182] C.B. Gorman, J.C. Smith, J. Am. Chem. Soc. 122 (2000) 9342–9343.

[183] W. Lo, T.A. Scott, P. Zhang, C.-C. Ling, R.H. Holm, J. Inorg. Biochem. 105 (2011) 497–508.

[184] W. Lo, P. Zhang, C.-C. Ling, S. Huang, R.H. Holm, Inorg. Chem. 51 (2012) 9883–9892.

[185] Y. Kuroda, Y. Sasaki, Y. Shiroiwa, I. Tabushi, J. Am. Chem. Soc. 110 (1988) 4049–4050.

A figure for Table of Contents