A Journal of the Gesellschaft Deutscher Chemiker
Angewandte Chemie
GDCh
International Edition
www.angewandte.org

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To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.202108135

Link to VoR: https://doi.org/10.1002/anie.202108135

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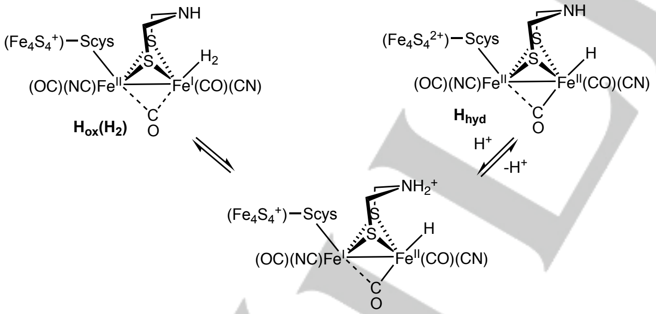
Surprising Condensation Reactions of the Azadithiolate Cofactor

Fanjun Zhang,* Casseday P. Richers, Toby J. Woods, and Thomas B. Rauchfuss*[a]

Dedication: Memory of Ralf Steudel, scholar and friend

Abstract: Azadithiolate, a cofactor found in all [FeFe]-hydrogenases, is shown to undergo acid-catalyzed rearrangement. $Fe_2[(SCH_2)_2NH](CO)_6$ self-condenses to give $Fe_6[(SCH_2)_3N]_2(CO)_{17}$. The reaction, which is driven by loss of NH_4^+, illustrates the exchange of the amine group. X-ray crystallography reveals that three $Fe_2(SR)_2(CO)_x$ butterfly subunits interconnected by the aminotrithiolate $[N(CH_2S)_3]^{3-}$. Mechanistic studies reveal that $Fe_2[(SCH_2)_2NR](CO)_6$ participate in a range of amine exchange reactions, enabling new methodologies for modifying the adt cofactor. . $Ru_2[(SCH_2)_2NH](CO)_6$ also rearranges, but proceeds further to give derivatives with Ru-alkyl bonds $Ru_6[(SCH_2)_3N][(SCH_2)_2NCH_2]S(CO)_{17}$ and $[Ru_2[(SCH_2)_2NCH_2](CO)_5]_2S$, featuring Ru-alkyl bonds.

Azadithiolate ($[HN(CH_2S)_2]^{2-}$) is a cofactor found in all [FeFe]-hydrogenases.[1] The thiolates glue together the two $Fe(CN)(CO)_x$ centers of the active site, and the amine group functions as a proton relay, facilitating formation and deprotonation of dihydrogen ligands (Scheme 1).



Scheme 1. Selected states of the [FeFe] hydrogenase active site, illustrating the proton-relay function of the azadithiolate cofactor.[2]

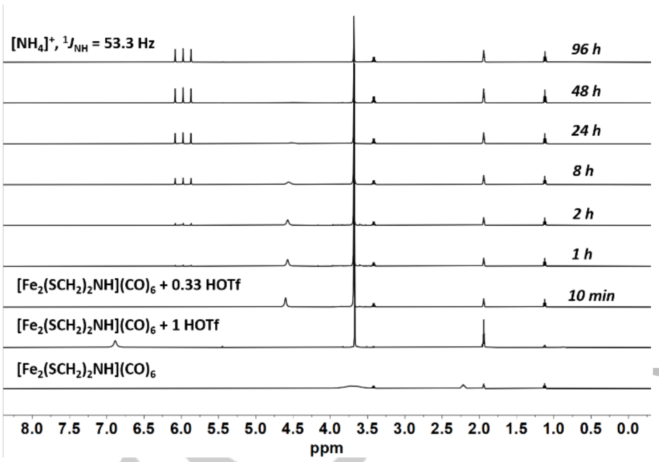


Figure 1. 500 MHz NMR spectra for **1**, $[H\mathbf{1}]^+$ and conversion of **1** to **2** (CD_3CN solution).

Because of their extraordinary efficiency and in view of the great interest in hydrogen as a renewable fuel, the diiron azadithiolates have been intensely studied.[3] Its biosynthesis is currently the focus of analysis.[4] This interest is motivated by the possibility that Fe_2(adt) modules would be the basis for synthetic catalysts for hydrogen evolution or fuel cells.[5] To exploit this molecular machinery,[6] however, it is essential to understand the strengths and vulnerabilities of this cofactor.

Most reports on diiron azadithiolates focus on their tertiary amine analogues, e.g., $Fe_2[(SCH_2)_2NR](CO)_6$ (R = alkyl, aryl).[7] According to the Cambridge Structural Database, hundreds of crystal structures of such diiron azadithiolates have been characterized crystallographically. It is however the *secondary* amine (R = H) that occurs naturally. In this report we show that the natural azadithiolate is particularly susceptible to unexpected reactivity. This behavior is relevant to the design of bioinspired catalysts for hydrogen evolution and oxidation.[8]

In parallel with the biology, synthetic adt complexes are well known to undergo reversible protonation at the amine (eq 1).

$$\underset{(\mathbf{1})}{Fe_2[(SCH_2)_2NH](CO)_6} \underset{-H^+}{\overset{H^+}{\rightleftharpoons}} \underset{([H\mathbf{1}]^+)}{[Fe_2[(SCH_2)_2NH_2](CO)_6]^+} \quad (1)$$

This amine/ammonium equilibrium is key to functional models.[9] Both protonated and unprotonated states have been well characterized in models[10] as well as in vitro enzyme studies.[2] We have re-examined the simplest model, $Fe_2[(SCH_2)_2NH](CO)_6$ (**1**), since the results are most fundamental. Using ^1H NMR and IR spectroscopies, we confirm that solutions of **1** and its conjugate acid $[Fe_2[(SCH_2)_2NH_2](CO)_6]^+$ ($[H\mathbf{1}]^+$), exhibit the expected behavior. The surprise is that *a mixture of [**1**] and [H**1**]$^+$ is unstable.*

The acid-catalyzed reactions of **1** were initially chararacterized by ^1H NMR spectroscopy. Addition of small amounts of HOTf to a MeCN solution of **1** resulted in one set of CH_2 signals (δ 4.5) because of rapid proton exchange between **1** and $[H\mathbf{1}]^+$. Over the course of hours, however, a 1:1:1 triplet signal appears at δ 5.98 (Figure 1). This triplet

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10.1002/anie.202108135

signal ($^1J_{NH}$ = 53.3 Hz) is assigned to $[NH_4]^+$, as confirmed by addition of authentic $[NH_4]OTf$ to the reaction mixture. Concomitant with the formation of $[NH_4]OTf$, a red-orange solid precipitated from the solution. Subsequent experiments showed that this solid is a mixture of two compounds, $[Fe_6[(SCH_2)_3N]_2(CO)_{17}]$ (**2**) and its CO adduct $[Fe_6[(SCH_2)_3N]_2(CO)_{18}]$ (**2**(CO)). Under optimized conditions, the addition of 0.33 equiv of HOTf to an MeCN solution of **1** gave **2** and **2**(CO) in a combined 85% yield. Because it is more soluble in MeCN, **2**(CO) can be separated from **2**.

The FT-IR spectra of **2** and **2**(CO) (THF solution, ν_{CO} ~ 2071 - 1989 cm^{-1}) resemble that for **1**. This result suggests that the reaction does not alter the oxidation state of the Fe centers. On the basis of the analysis given below, **2** is proposed to form according to the stoichiometry in eq 2.

$$H^+ + 3\ \underset{(\mathbf{1})}{Fe_2[(SCH_2)_2NH](CO)_6} \xrightarrow{-CO} \underset{(\mathbf{2})}{Fe_6[(SCH_2)_3N]_2(CO)_{17}} + NH_4^+ \quad (2)$$

The conversion of a secondary amine to a tertiary amine under mild conditions is characteristic of some strained amines.[11] The structure of **2** was established by X-ray crystallography (Figure 2). The secondary amine groups have condensed with loss of ammonia, generating an ensemble of three $Fe_2(SR)_2$ butterfly groups. The compound is stitched together with a pair of the unprecedented aza*tri*thiolate ligand $N(CH_2S^-)_3$. One thiolate has displaced one CO of one $Fe_2(SR)_2$ subcluster, a motif seen in $[Fe_6[(SCH_2)_3CMe]_2(CO)_{17}]$.[12] The cluster has a plane of symmetry through Fe1, Fe2, Fe5, and Fe6.

The formation of **2** can be rationalized according to the steps in Scheme 2. An early intermediate results from nucleophilic attack of **1** on its conjugate acid. This reaction would generate a $Fe_2(\mu\text{-}SCH_2NH_2)$ group with a nucleophilic primary amine. In principle, **1** would polymerize, but **2**(CO) forms instead (Scheme 2). Decarbonylation of **2**(CO) gives **2**. The nucleophilic attack of an amine toward the adt cofactor in **1** was examed independently: treating **1** with 2 equiv of benzyl amine gave $[Fe_2[(\mu\text{-}SCH_2)_2NCH_2Ph]CO)_6]$ (45% yield, room temperature, 24 h).

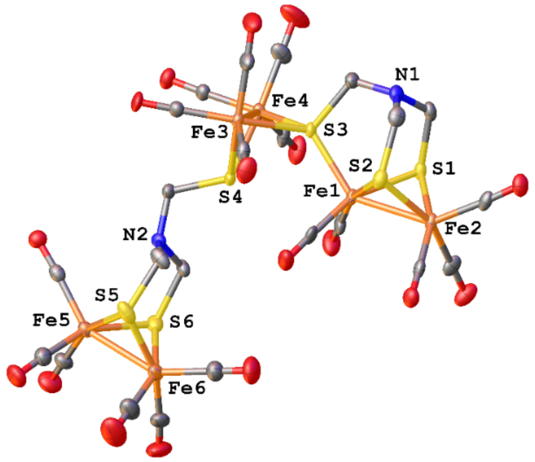
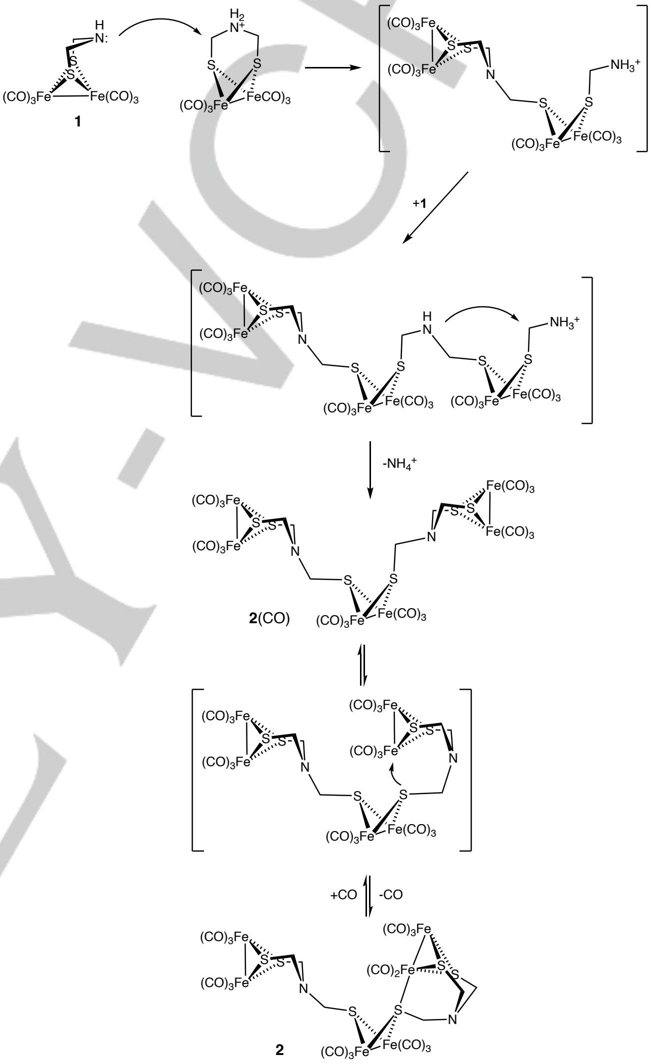


Figure 2. Structure of **2** (50% probability thermal ellipsoids). Hydrogen atoms are omitted for clarity. Selected distances (Å): Fe1-Fe2, 2.5388(11); Fe3-Fe4, 2.5095(11); Fe5-Fe6, 2.482(2); Fe5-Fe6A, 2.615(10); Fe1-S1, 2.2595(15); Fe1-S2, 2.2678(17); Fe1-S3, 2.2598(15); Fe2-S1, 2.2509(16); Fe2-S2, 2.2691(16); Fe3-S3, 2.2654(15); Fe4-S3, 2.2602(15); Fe3-S4, 2.2736(16); Fe4-S4, 2.2651(16); Fe5-S5, 2.2529(18); Fe5-S(6), 2.2541(18); Fe6-S5, 2.199(4); Fe6-S6, 2.303(4).

Indeed, THF solutions of **2** react readily with 1 atm of CO concomitant with a color change from red to bright orange. The ^1H NMR spectrum of the resulting solution shows the expected 2:2:4:4 pattern for the CH_2 groups associated with the equatorial-axial isomer of **2**(CO).



Scheme 2. Proposed pathway for formation of **2** from **1**.

The lability of the amine group in **1** defines a new way to modify adt cofactors by amine exchange. Thus, solutions of **1** were treated with anilinium salts (2 equiv) to give $Fe_2[(SCH_2)_2NPh](CO)_6$ (**3**) over the course of 48 h at room temperature (eq 3).

$$PhNH_2 + \underset{(\mathbf{1})}{Fe_2[(SCH_2)_2NH](CO)_5L} \xrightarrow{H^+} \underset{\substack{(\mathbf{3},\ L = CO)\\(\mathbf{4},\ L = PMe_3)}}{Fe_2[(SCH_2)_2NPh](CO)_5L} + NH_4^+ \quad (3)$$

$$PhNH_2 + \underset{(\mathbf{5})}{Fe_2[(SCH_2)_2NMe](CO)_5L} \xrightarrow{H^+} Fe_2[(SCH_2)_2NPh](CO)_6 + MeNH_3^+ \quad (4)$$

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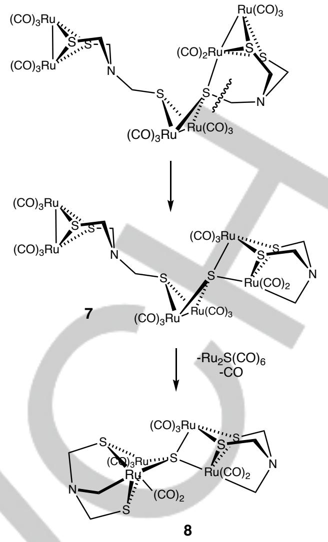
The driving force is the formation of the ammonium salt of the more basic amine. An analogous reaction cleanly converts $[Fe_2[(SCH_2)_2NMe](CO)_6$ (**5**) to **3** (eq 4). This result is significant because the tertiary amines like **5** are more easily prepared than is **1**.

CO-substituted derivatives of **1** are poor substrates for amine exchange. For the complexes $Fe_2[(SCH_2)_2NH](CO)_5(PR_3)$ (R = Me, Ph), only PMe_3 derivative exchanged with aniline and that reaction proceeded slowly.

Examination of the literature on diiron azadithiolates suggests that condensations related to **1** → **2** may have been observed but overlooked.[13] For example, ^1H NMR studies show that treatment of $[Fe_2[(SCH_2)_2NH](CO)_5(PMe_3)]$ with HOTf generates a 1:1:1 signal (J = 58 Hz) at δ 6.01.[14] Originally assigned to $[Fe_2[(SCH_2)_2NH_2](CO)_5(PMe_3)]OTf$, this signal matches that for NH_4OTf.

Amine-exchange was also attempted with $[Fe_2[(SCH_2)_2NH](CN)_2(CO)_4]^{2-}$, which more closely resembles the $[2Fe]_H$ active site of the [FeFe]-hydrogenase. Treatment of the $(NEt_4)^+$ salt with HOTf initially gave $[Fe_2[(SCH_2)_2NH_2](CN)_2(CO)_4]^-$. Within minutes at room temperature, this ammonium complex tautomerized to the known isomeric hydrides.

The Ru_2 analogue of **1** also undergoes acid-catalyzed rearrangement, but with interesting differences. When treated with substoichiometric quantities of HOTf, solutions of $Ru_2[(SCH_2)_2NH](CO)_6$[15] (**6**) efficiently yielded NH_4OTf. The reaction proceeds at a convenient rate at 35 °C: over the course of 3 days, the color of the yellow solution deposited an orange solid precipitate. ESI-MS indicated an Ru_6 complex with a formula analogous to that of **2**. The FT-IR spectrum shows several bands ranging from 2107 to 1950 cm^{-1}, a pattern quite different from that of **2** and **2**(CO), suggesting a new bonding motif. This puzzle was solved by X-ray crystallography. Analogous to **2**, $Ru_6[(SCH_2)_3N][(SCH_2)_2NCH_2]S(CO)_{17}$ (**7**) consists of three Ru_2S_2 centers and 17 CO ligands. Compound **7**, however, features one inorganic sulfur (μ_4-S), the result of cleavage of a C-S bond and formation of an Ru-alkyl bond. This oxidative addition results in a mixed valence $[Ru(I)]_4[Ru(II)]_2$ species. The $[Ru(II)]_2$ pair can be identified by the long (3.2925(1) Å) nonbonding metal-metal separation between these face-sharing octahedral centers. The Ru-Ru bonds remain intact for other two of Ru_2 subunits. It is logical to suggest that **7** arises via an intermediate like **2** (Scheme 3).



Scheme 3. Proposed pathway for formation of **7** and **8** from Ru analogue of **2**.

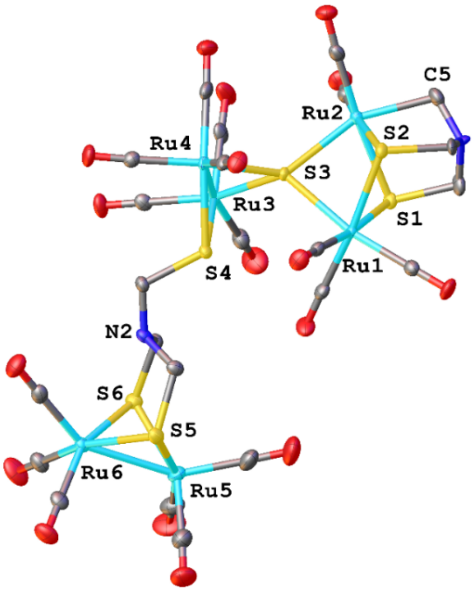
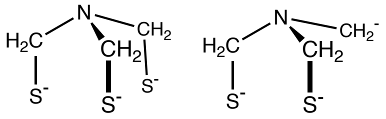


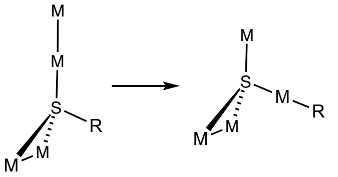
Figure 3. Structure of **7** (50% probability thermal ellipsoids). Hydrogen atoms are omitted for clarity. Selected distances (Å): Ru1-Ru2, 3.2925(1); Ru3-Ru4, 2.6721(4); Ru5-Ru6, 2.6924(4); Ru1-S1, 2.4278(9); Ru1-S2, 2.4387(9); Ru1-S3, 2.4602(8); Ru2-S1, 2.4505(9); Ru2-S2, 2.4615(9); Ru2-S3, 2.4686(9); Ru3-S3, 2.3981(8); Ru3-S4, 2.4078(9); Ru4-S4, 2.3970(9); Ru4-S3, 2.4034(9); Ru5-S5, 2.3738(10); Ru5-S6, 2.3917(9); Ru6-S5, 2.3865(9); Ru6-S6, 2.3908(9).

The results on Fe_6 and Ru_6 polyclusters establish precedents for two new simple tripodal ligands. These are aza*tri*thiolaate and methyleneazadithiolate, respectively $N(CH_2S^-)_3$ and $N(CH_2S^-)_2(CH_2^-)$:



Like azaditiolate itself, these new ligands require stabilization by a metal template since the free tri- and dithiols would be impossible to isolate otherwise.

The new results are also relevant to the formation of $M_4(\mu_4$-S) clusters from thiols, an old reaction that is always vaguely described.[16] In the present study, the conversion of **6** to **7** provides detailed snapshots of the pathway by which polymetallic ensembles effect this prevalent reaction. The thiolate initially bridges a pair of metals (e.g., **6**), a third metal attacks the remaining lone pair on sulfur to give a μ_3-SR ligand (e.g., **2**) and, finally, C-S oxidative addition ensues, giving the μ_4-sulfide concomitant with a metal-alkyl bond (eq 5).



(5)

Although the conversion of **6** is efficient, the yield of **7** is modest. The low yield is due to the fact that **7** reacts further. Indeed, other clusters can be detected by thin-layer chromatography of the reaction mixture. We purified a further species in this sequence of reactions, $[Ru_2[(SCH_2)_2NCH_2](CO)_5]_2S$ (**8**). The structure of this cluster was also elucidated by X-ray crystallography. This compact cluster is clearly derived from **7**. It features a two $Ru_2[(SCH_2)_2NCH_2](CO)_5$ species joined by μ_4-S. The compound features four Ru(II) centers, consistent with the nonbonding $Ru\cdots Ru$ distances. The two-dimensional ^1H-^{13}C NMR spectrum (HSQC) shows six cross-peaks, indicating that the C_2-symmetric $[Ru_2(SCH_2)_2NCH_2]_2S$ core is rigidly chiral.

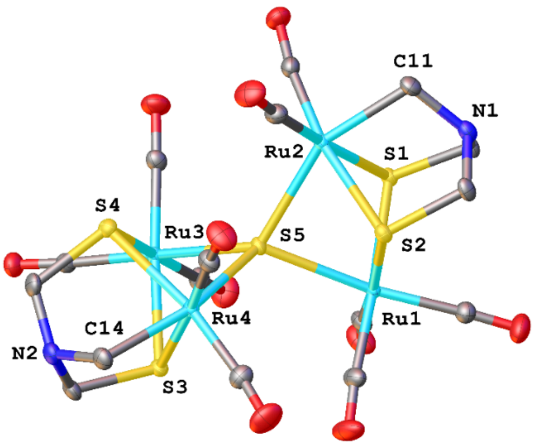


Figure 4. Structure of **8** (50% probability thermal ellipsoids). Hydrogen atoms are omitted for clarity. Selected distances (Å): Ru1-Ru2, 3.2982(4); Ru3-Ru4, 3.2588(3); Ru1-S1, 2.4145(3); Ru1-S2, 2.4306(3); Ru2-S1, 2.4393(3); Ru2-S2, 2.4455(3); Ru2-C11, 2.151(1); Ru3-S3, 2.4374(3); Ru3-S4, 2.4302(3); Ru4-S4, 2.4431(4); Ru4-S3, 2.4628(3); Ru4-C14, 2.150(1).

The robustness of the adt cofactor *in vivo* underscores the advantage of site-isolation provided by the protein.[1] For studies on biomimetic catalysts our results indicate that the azadithiolate in $Fe_2[(SCH_2)_2NR](CO)_6$ is a highly manipulable functional group.[17] Finally the results with ruthenium show how thiophilic platinum group metals may not be well suited for thiolate-supported catalysts because they tend to abstract sulfur and self-destruct.

Acknowledgments

This research was supported by the U.S. National Institutes of Health.

Keywords: cofactors • hydrogenase • iron • carbonyls • thiolate

[1] H. Land, M. Senger, G. Berggren, S. T. Stripp, *ACS Catalysis* **2020**, *10*, 7069-7086.

[2] J. A. Birrell, V. Pelmenschikov, N. Mishra, H. Wang, Y. Yoda, K. Tamasaku, T. B. Rauchfuss, S. P. Cramer, W. Lubitz, S. DeBeer, *J. Am. Chem. Soc.* **2020**, *142*, 222-232.

[3] T. B. Rauchfuss, *Acc. Chem. Res.* **2015**, *48*, 2107-2116.

[4] R. D. Britt, G. Rao, L. Tao, *Nature Rev. Chem.* **2020**, *4*, 542-549.

[5] S. Gao, W. Fan, Y. Liu, D. Jiang, Q. Duan, *Int. J. Hydrogen Energy* **2020**, *45*, 4305-4327.

[6] J. T. Kleinhaus, F. Wittkamp, S. Yadav, D. Siegmund, U.-P. Apfel, *Chem. Soc. Rev.* **2021**, *50*, 1668-1784.

[7] a. S. Dey, A. Rana, S. G. Dey, A. Dey, *ACS Catalysis* **2013**, *3*, 429-436; b. Y.-L. Li, Y. Wu, J. Wei, J. Wei, B. Xie, L.-K. Zou, J. Cheng, Z. Wang, J. He, M.-L. Wu, P.-H. Zhao, *Polyhedron* **2017**, *135*, 231-236; c. M. E. Ahmed, S. Dey, M. Y. Darensbourg, A. Dey, *J. Am. Chem. Soc.* **2018**, *140*, 12457-12468.

[8] F. Möller, S. Piontek, R. G. Miller, U.-P. Apfel, *Chem. Eur. J.* **2017**, *24*, 1471-1493.

[9] a. F. Gloaguen, *Inorg. Chem.* **2016**, *55*, 390-398; b. G. Eilers, L. Schwartz, M. Stein, G. Zampella, L. de Gioia, S. Ott, R. Lomoth, *Chem. Eur. J.* **2007**, *13*, 7075-7084.

[10] a. F. Wang, M. Wang, X. Liu, K. Jin, W. Dong, L. Sun, *Dalton Trans.* **2007**, 3812-3819; b. M. E. Carroll, B. E. Barton, T. B. Rauchfuss, P. J. Carroll, *J. Am. Chem. Soc.* **2012**, *134*, 18843-18852.

[11] a. X. E. Hu, *Tetrahedron* **2004**, *60*, 2701–2743; b. I. D. G. Watson, A. K. Yudin, *J. Org. Chem.* **2003**, *68*, 5160-5167.

[12] W. Zhong, G. Zampella, Z. Li, L. De Gioia, Y. Liu, X. Zeng, Q. Luo, X. Liu, *J. Organomet. Chem.* **2008**, *693*, 3751-3759.

[13] F. Wang, M. Wang, X. Liu, K. Jin, W. Dong, G. Li, B. Åkermark, L. Sun, *Chem. Commun.* **2005**, 3221-3223.

[14] Z. Wang, J. H. Liu, C. J. He, S. Jiang, B. Åkermark, L. C. Sun, *J. Organomet. Chem.* **2007**, *692*, 5501-5507.

[15] a. C. Sommer, C. P. Richers, W. Lubitz, T. B. Rauchfuss, E. J. Reijerse, *Angew. Chem. Int. Ed.* **2018**, *57*, 5429-5432; b. C. Sommer, C. P. Richers, W. Lubitz, T. B. Rauchfuss, E. J. Reijerse, *Angew. Chem.* **2018**, *130*, 5527-5530.

[16] a. M. D. Curtis, S. H. Druker, *J. Am. Chem. Soc.* **1997**, *119*, 1027-1036; b. L. Linford, H. G. Raubenheimer, *Adv. Organomet. Chem.* **1991**, *32*, 1-119; c. H. Vahrenkamp, *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 322-329.

[17] Y. Kim, J. Heo, D. Kim, S. Chang, S. Seo, *Nature Commun.* **2020**, *11*, 4761.

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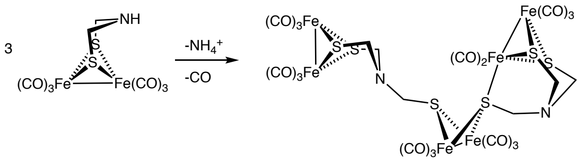

Azadithiolate (adt), key cofactor of the [FeFe]-hydrogenase machinery, rearranges readily via novel condensation pathway. For FeFe adt's, the condensation gives aza*trit*hiolates, but the RuRu adt's go farther, giving *methylene*azadithiolates.