Dalton Transactions

COMMUNICATION

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Cite this: Dalton Trans., 2020, 49, 23

Received 19th October 2019, Accepted 26th November 2019 DOI: 10.1039/c9dt04098f

rsc.li/dalton

Facile hydrogen atom abstraction and sulfide formation in a methyl-thiolate capped iron-sulfur-carbonyl cluster⁺

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The interest in methyl group C-H bond activation near or bound to iron-containing clusters is of key biological importance, due to the broad relevance of radical SAM reactions. Specifically, such processes are implicated in the biogenesis of the interstitial carbide found in the nitrogenase FeMoco active site. In this work, we find that the diamagnetic, methyl-thiolate capped iron-carbonyl cluster anion $[(CH_3S)Fe_3(CO)_9]^-$ (1) undergoes facile C-H hydrogen atom abstraction upon treatment with TEMPO. The process leads to (i) eradication of the CH3 moiety, (ii) formation of a sulfide bridge, and (iii) cluster dimerization-thereby generating the 'dimer of trimers' cluster [K(benzo-15-crown-5)2]2[(SFe2(CO)12)2Fe(CO)2] (2). In contrast, the corresponding isopropyl variant $[Fe_3(S^iPr)(CO)_9]^-$ (3) does not react with TEMPO. Mass spectrometry confirmed the presence of TEMPOH, as well as CO oxidation vis a vis CO₂ and 2,2,6,6-tetramethylpiperidine. GC-MS measurements of the headspace reveal that the ultimate fate of the methyl carbon is likely incorporation into multiple products-one of which may be a volatile low mass hydrocarbonrather than carbon/carbide incorporation.

The biogenesis of the M cluster in nitrogenase has garnered much interest since the identification of the interstitial carbide in 2011.^{1,2} The unique motif of a 6-coordinate C⁴⁻ bound to the six iron centers present in FeMoco/FeVco is the only known example in biological systems,³ and few examples exist in chemical systems.⁴⁻⁷ The Nif class of enzymes performs the transformations necessary to generate the nitrogenase cofactor. NifS and NifU, the first enzymes in the series, convert cysteinal thiolates to inorganic [Fe₂S₂] and [Fe₄S₄] clusters.⁸ Ribbe and coworkers have elucidated much of the mechanism of carbide insertion through a clever fusion protein of NifEN-B, a construct which combines the scaffold

protein NifEN with the radical *S*-adenosyl methionine (SAM) enzyme NifB.⁹

In other work, it was shown that carbide formation requires two equivalents of SAM; one for methyl group donation (which eventually becomes the carbide), and a second for radical abstraction of a hydrogen atom.¹⁰ The initial binding site of the methyl group was also traced by HPLC and GCMS, revealing that the methyl group first binds to a sulfide in the native enzyme.¹¹ Furthermore, it was observed that R group transfer occurs before any radical reactions, likely through a simple S_N2-type reaction. The subsequent radical abstraction of a hydrogen atom then allows for a radical-electron induced rearrangement of the iron clusters.¹² This provides a stimulus for investigating the reactivity of methyl substituted ironsulfur clusters with a radical initiator. Relatedly, oxidation of NifEN-B with indigo disulfonate resulted in cessation of all methyl transfer activity, suggesting that the sulfide must be sufficiently anionic and/or poised at a sufficiently negative potential for the reaction to proceed.

We recently reported reduced clusters of general formula K $[S_lFe_m(CO)_n]$ (l = 1-4, m = 2-4, n = 7-12) that could serve as non-enzymatic models for both the methyl substitution (S_N2) and subsequent radical reaction. These clusters have the advantages of being significantly reduced, as well as having an alkali cation that is not susceptible to radical chemistry.¹³ Specifically, the cluster K₂[SFe₃(CO)₉] (**1a**) was chosen, due to its versatility in substituting R groups using common electrophiles that can be installed on the S^{2–} 'bridgehead'. This approach makes it a suitable—albeit not entirely biomimetic—model system for investigating –SCH₃ reaction mechanisms associated with iron-containing clusters.

The methylation of $K_2[SFe_3(CO)_9]$ with CH_3I proceeded in a straightforward manner in THF at 0 °C to afford the monoanionic product $K[(CH_3S)Fe_3(CO)_9]$ (1). After treatment with excess benzo-15-crown-5, dark red X-ray quality crystals were obtained (Fig. 1). The resulting bond metrics were similar to those previously observed in $NEt_4[(CH_3S)Fe_3(CO)_9].^{14}$ Importantly, the lack of easily dissociable protons or reactive C–H



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[†]Electronic supplementary information (ESI) available. CCDC 1944881–1944883. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c9dt04098f



Fig. 1 ORTEP diagrams of [K(benzo-15-crown-5)₂][S(CH₃)Fe₃(CO)₉] (1, 30% thermal ellipsoids) and [K(benzo-15-crown-5)₂][S(C₃H₇Fe₃(CO)₉] (3, 50% thermal ellipsoids) at 100 K; counterions are omitted for clarity.

moieties in the K-crown counterion (unlike NEt_3H^+ or NEt_4^+) provided an ideal cluster for methyl C-H activation studies.

Since the starting material $K[(CH_3S)Fe_3(CO)_9]$ (1) contains multiple Fe centers capable of forming bridging hydrides, it was important to select a relatively mild H-atom abstraction reagent. Thus 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) was selected due to its mild O-H bond formation strength.¹⁵ Reactions of the methylated cluster 1 with TEMPO in THF generated a red-brown color over the course of 2 hours. Following extraction of species in Et₂O (identified as the anionic fragment [HFe₃CO₉]⁻, m/z = 420), the insoluble material was dissolved in MeCN, with excess benzo-15-crown-5 for crystallization. The product was identified as [K(benzo-15-crown-5)₂]₂ $[(SFe_2(CO)_{12})_2Fe(CO)_2]$ (2) by single crystal X-ray diffraction (Fig. 2). Cluster 2 reveals two key transformations: (i) S-CH₃ bond breakage to generate the inorganic sulfide, and (ii) the combination of two Fe₃ units (and extrusion of one Fe center) to form an S₂Fe₅ framework. Additionally, two electron oxidation of the Fe centers was observed (one electron from each progenitor cluster of 1). The same product was obtained regardless of solvent identity (THF, MeCN, FPh) or temperature (-78 °C or ambient temperature). The bond metrics of the dianion 2 were similar to that observed previously in the PPh₄⁺ and NEt_4^+ formulation of 2.^{16,17} which were synthesized by an entirely different route.

This process bears some resemblance to the biosynthesis of the FeMoco active site, wherein the addition of SAM causes



Fig. 2 ORTEP diagram (50% thermal ellipsoids) of $[K(benzo-15-crown-5)_2]_2[(SFe_2(CO)_{12})_2Fe(CO)_2]$ (2) at 100 K; counterions are omitted for clarity.

oxidation of the SAM-motif bound $[Fe_4S_4]^{1+}$ in NifB to an oxidized EPR silent intermediate;⁹ in this process, S²⁻ and C⁴⁻ are ultimately generated as charged ligands, implying oxidation of the iron centers *en route* to the intermediate L cluster. Unfortunately, the incorporation of any carbon (or carbide) motif to the cluster was not observed in this work, although it was demonstrated that a radical electron can aid in the fusion of two iron–sulfur units.

It has been speculated that after the first hydrogen atom abstraction, the subsequent transformations to a carbide proceed by acid–base chemistry.⁸ To test this hypothesis in our system, the same reaction was performed using two equivalents of a non-coordinating base (lutidine) at -78 °C (to prevent any deprotonation event prior to TEMPO addition. However, no changes in reaction outcome were observed.

Attempts using NMR to characterize the fate of the carbon atom using isotopically labeled ¹³CH₃I and CD₃I proved unviable, as the reaction mixture exhibited paramagnetism. We deemed it likely that any products generated from a C₁ source not incorporated into a cluster could be gaseous at room temperature. Thus, headspace GC measurements of the reaction mixture were pursued (Fig. S1[†]), revealing two major peaks. CO_2 was identified as the first species ($R_f = 9 \text{ min}$) by comparison to an internal standard, and is attributed to oxidation of CO by a TEMPO derivative, namely TEMPOH (m/z = 157, Fig. S3[†]). TEMPOH was presumed to participate in proton donation and hydride formation as suggested by detection of the cluster fragment $[HFe_3CO_9]^-$ in the reaction mixture (m/z =420, Fig. S4[†]). The generated TEMPO⁻ (m/z = 156, Fig. S3[†]) then induced CO oxidation to form CO₂, which is further supported by the detection of 2,2,6,6 tetramethylpiperidine (m/z =140, 142, Fig. S3[†]).

The second gaseous species ($R_f = 27 \text{ min}$) proved more perplexing. The peak height of this gas was roughly five times greater than that of CO₂. Solvent effects, particularly the incorporation of cyanide from MeCN, were excluded by performing the same experiment in fluorobenzene (Fig. S2[†]), which afforded the same pattern of gases and elution times. Headspace MS performed in CI mode (Fig. 3) revealed the m/zvalues for the two unknowns as 40 and 55. To further investigate these peaks, the deuterium labeled complex $K[(CD_3S)]$ $Fe_3(CO)_9$ was prepared. While the peak at m/z = 55 remained unchanged, the peaks centered near m/z = 40 showed a different distribution of peak ratios. This indicates that deuterium was incorporated into the species corresponding to m/z =40, and that this species originated from the isotopically labeled methyl group. Several reports have investigated the products and kinetics of methyl radicals with alkenes to form small carbon units.¹⁸⁻²² An unambiguous result would have been a quantitative shift of all features near m/z = 40 to m/z =42 or 44. However, since the shift in features occurred in a non-uniform manner, a direct assignment is not possible. It is likely that there is no single isotopic product that is generated from the methyl group, possibly due to H/D scrambling via solvent/radical processes. In fact, examination of the mass



Fig. 3 Mass chromatograms of the headspace of the reaction between TEMPO and $K[(CD_3S)Fe_3(CO)_9]$ (top) and $K[(CH_3S)Fe_3(CO)_9]$ (bottom). Notably the peaks centered near m/z = 40 show significant changes.

spectra of the reaction mixture (Fig. S3 and S4[†]) reveals multiple products.

Lastly, we sought to determine the selectivity of the TEMPO-driven reaction for the methyl group. Thus we investigated H-atom abstraction using a cluster with just a single α -proton, namely the isopropyl analog K[(ⁱPrS)Fe₃(CO)₉] (3), whose X-ray structure is also depicted in Fig. 1. Intriguingly, reaction of 3 with TEMPO in any solvent, (THF, MeCN, FPh) and at any temperature (-78 °C to ambient) provided no evidence of any reaction. In fact, X-ray quality crystals of 3 were recovered in nearly quantitative yield from the reactions with TEMPO. Such reactivity appears counter-intuitive, as the slightly bulkier isopropyl R group could stabilize any organic radical intermediate. The inertness of 3 towards TEMPO suggests that the electronics of the Fe centers in the cluster is not the primary driving force of C-H atom abstraction. Rather, the driving force of the reaction is likely the peculiar susceptibility of the methyl C-H σ^* orbital to TEMPO abstraction, as well as (in this case) the irreversible formation of gaseous byproducts.

In conclusion, we have demonstrated facile C-H atom abstraction from a methylthiolate-capped iron-carbonyl cluster (1); the isopropyl variant (3) is unreactive with TEMPO. H-atom abstraction from CH_3S^- ultimately results in the formation of an inorganic sulfide, which is incorporated to the product (2). Each starting cluster undergoes a one-electron oxi-



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Scheme 1 Transformation of the bare potassium cluster [(SCH₃) Fe₃(CO)₉]⁻ (1) to the fused iron cluster [S₂Fe₅(CO)₁₄]²⁻ (2). Mass spectrometry was utilized to detect the TEMPO byproducts TEMPOH and 2,2,6,6-tetramethylpiperidine. CO₂ was detected by GC and was matched to an internal standard.

dation and radical-assisted coupling of clusters, resulting in the S₂Fe₅ framework of the product dianion (2). This loosely resembles the SAM-mediated fusion of [Fe₄S₄] clusters that ultimately comprise the nitrogenase active site. CO oxidation has been robustly characterized by identification of each TEMPO and cluster intermediate *en route* to CO_2 (Scheme 1). This result is of particular importance for future radical reactions attempting to generate a carbide, where utilization an iron-sulfur cluster not supported by carbonyls could minimize undesired side reactions. The fate of the methyl-carbon in this system is incorporation into multiple isotopic products, rather than carbon/carbide incorporation into the iron-sulfur-carbonyl cluster. Further mechanistic details in this system and non-carbonyl iron-sulfur clusters will be needed to shed further insight into the carbide insertion process during the formation of FeMoco.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors acknowledge funding from the National Science Foundation (CHE-1808311) and the Welch Foundation (F-1822). We also thank Dr Vince Lynch for assistance in X-ray data collection and analysis.

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