

# ChemComm

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

*Accepted Manuscripts* are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

## COMMUNICATION

Unexpected explanation for the enigmatic acid-catalysed reactivity of  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  clusters<sup>†</sup>

Cite this: DOI: 10.1039/x0xx00000x

Ahmed Alwaaly,<sup>a</sup> Ian Dance<sup>\* b</sup> and Richard A. Henderson<sup>\* a</sup>Received 00th January 2012,  
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

**Density functional calculations show that Fe-S clusters undergo unexpected large structural changes when protonated at S. Protonation of prototypical cubanoid  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  to  $[\text{Fe}_4\text{S}_3(\text{SH})\text{X}_4]^-$  ( $\text{X} = \text{Cl}, \text{SR}, \text{OR}$ ) results in formation of doubly-bridging SH, severance of one Fe-S bond, and creation of a three-coordinate Fe. These findings explain previously enigmatic results concerning the reactivity of these clusters, including the rates of protonation,  $\text{pK}_a$  data, and the kinetics of acid-catalysed ligand substitution.**

Cubanoid clusters  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  occur widely in biology ( $\text{X} =$  cysteine), and accordingly synthetic analogues of these clusters ( $\text{X} =$  halide, Saryl, Salkyl or phenolate) have been investigated in great detail since the first preparations by Holm *et al* in 1972<sup>1-5</sup>. It has been shown that substitution of the terminal ligands,  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-} + \text{Y}^- \rightarrow [\text{Fe}_4\text{S}_4\text{X}_3\text{Y}]^{2-} + \text{X}^-$ , is acid-catalysed, and kinetic investigations have sought to identify the origin of the acid catalysis.<sup>6-8</sup> These kinetic studies have allowed determination of the  $\text{pK}_a$  of the clusters, the rates of protonation, and the effect that protonation has on the rates of substitution. The  $\text{pK}_a$  values all fall in a relatively narrow range (17.9 - 18.8), and are the same as those for  $[\text{Fe}_2\text{S}_2\text{X}_4]^{2-}$  clusters.<sup>6-8</sup> This is an unexpected result, because  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  contains only triply-bridging S ( $\mu_3\text{-SFe}_3$ ), while  $[\text{Fe}_2\text{S}_2\text{X}_4]^{2-}$  contains only doubly-bridging S ( $\mu\text{-SFe}_2$ ), and these structurally different S atoms would be expected to have different basicities. In addition, the rates of protonation of  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  are considerably less than the diffusion-controlled limit even for thermodynamically-favourable reactions ( $k \leq 1 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ ),<sup>9</sup> and show no significant primary isotope effect.<sup>10, 11</sup> Finally, protonation of the cluster increases the rate of substitution of  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  irrespective of the nature of X. Thus there are four general features concerning the reactivity of these protonated clusters that are unexplained: (a) why and how is substitution catalysed by protons? (b) why is protonation of S slower than expected? (c) why is there no isotope effect, and,

most enigmatically, (d) why are the measured  $\text{pK}_a$  values so similar? Clearly, something fundamental about the protonation chemistry of these clusters is not understood.

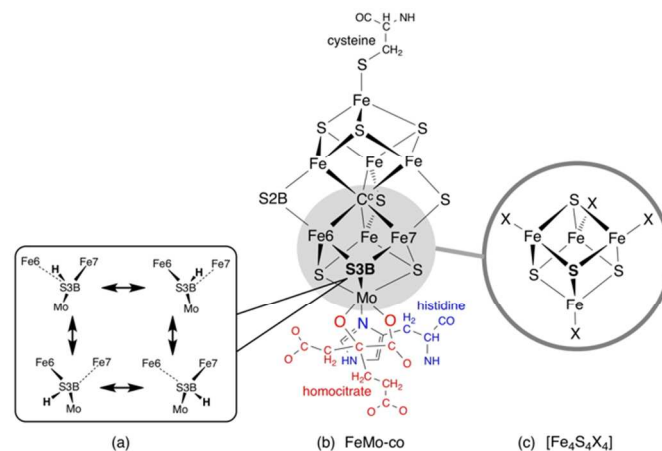


Fig 1. The structure of FeMo-co (b), the four trigonal pyramidal conformers of S3B-H after protonation of S3B and their interconversions (a), and the structural congruence of  $[\text{Fe}_4\text{S}_4\text{X}_4]$  and the section of FeMo-co containing S3B (encircled).

Recent theoretical investigations<sup>12</sup> of the protonation of the active site of the enzyme nitrogenase<sup>13-17</sup> have indicated the answer. Fig 1(b) shows the active site of nitrogenase, FeMo-cofactor (FeMo-co), which is an  $\text{Fe}_7\text{MoS}_9\text{C}$  cluster<sup>18</sup> ligated to the surrounding protein through a cysteine and a histidine residue. In the cluster core, the triply-bridging sulfur atom S3B is proposed as the entry point of the multiple protons required for the catalytic hydrogenation reactions effected by FeMo-co,<sup>19</sup> and DFT calculations showed that  $\mu_3\text{-S3B}$  becomes doubly-bridging  $\mu\text{-S3B-H}$  upon protonation.<sup>12</sup> Protonation causes substantial elongation of one of the two Fe-S3B bonds in FeMo-co. The S3B-H function in FeMo-co has four different stable conformations (Fig 1 (a)), each with one long (*ca* 3Å) S---Fe

distance, and each conformation has threefold pyramidal coordination of S3B (to H, Fe and Mo). Tetrahedral fourfold coordination of S3B (H, 2Fe, Mo) is calculated to be 7–10 kcal mol<sup>-1</sup> less stable.<sup>12</sup> This suggests that  $\mu_3$ -S in other metal clusters may also change to  $\mu$ -SH on protonation. A recent search of the Cambridge Structural Database yielded no unambiguous occurrences of  $(\mu_3\text{-SH})\text{M}_3$ , an absence noted also by Kuwata and Hidai.<sup>20</sup> To investigate the consequences of protonating  $\mu_3$ -S we undertook a density functional simulation with the simple cubanoid clusters  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  which are iso-structural with the relevant part of FeMo-co (see Fig 1 (c)) and found that this system elongates one S-Fe bond on protonation, similar to that observed with FeMo-co. Herein, we also present a qualitative description of the mechanisms for the acid-catalysed substitution reactions of  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  and  $[\text{Fe}_2\text{S}_2\text{X}_4]^{2-}$ . These mechanisms are supported by DFT optimisations of all reactants, intermediates, and products. For reaction steps involving charged species in acetonitrile solution, such as protonation/deprotonation by  $\text{HNEt}_3^+ / \text{NEt}_3$ , dissociation of  $\text{X}^-$ , and association of  $\text{PhS}^-$  (or  $\text{PhSH} + \text{NEt}_3$ ), the transition states in acetonitrile solution are difficult to calculate accurately, and no attempt is made to calculate rate constants for comparison with experiment. Rather, our contribution is the recognition of the cluster core disruption associated with protonation of  $\mu_3$ -S in a metal cluster, permitting rationalisation of a number of enigmatic observations, and providing a consistent basis for understanding the mechanisms of proton-catalysed substitution reactions of iron-sulfur clusters.

The key structural result from our calculations is that protonation changes  $\mu_3$ -S in all  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  ( $\text{X} = \text{halide, thiolate or alkoxide}$ ) to  $\mu$ -SH in  $[\text{Fe}_4\text{S}_3(\text{SH})\text{X}_4]^-$ . Fig 2 shows the two conformations calculated for  $[\text{Fe}_4\text{S}_3(\text{SH})\text{X}_4]^-$ . The SH function has pyramidal trigonal stereochemistry, and the Fe<sup>a</sup>--S distance has extended from ca 2.3 Å to at least 2.85 Å in the most stable electronic states (see ESI). The two isomers are designated *endo* and *exo*, signifying the SH direction relative to the long S--Fe vector. The structural change associated with protonation of  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  is calculated to be reversible.

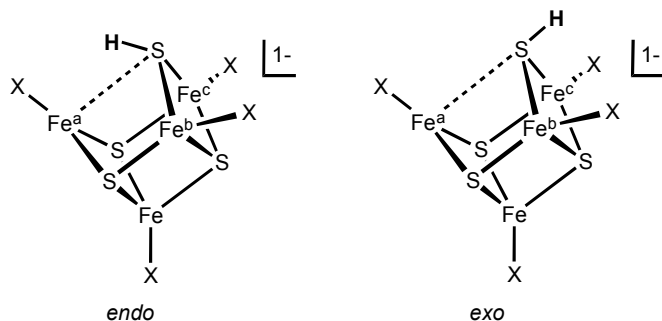


Fig 2. *Endo* and *exo* isomers of  $[\text{Fe}_4\text{S}_3(\text{SH})\text{X}_4]^-$ .

This result suggests simple explanations for the enigmatic reactivity observed with these clusters. The unexpectedly slow rate of proton transfer to  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  is now more understandable, because the measured or estimated rate constant does not correspond to the simple transfer of a proton from acid to  $\mu_3$ -S, but rather is a proton transfer step coupled to elongation / cleavage of an Fe-S bond. For the same reason there is no significant isotope effect (when using deuterated acid). Moreover, it is now clear that the similarity in the  $\text{p}K_a$ s of  $\mu_3$ -SH and  $\mu$ -SH is illusory. The apparent  $\text{p}K_a$  values

measured for protonation of  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  (containing only  $\mu_3$ -S) are, in fact, not true  $\text{p}K_a$ s because the process being measured is protonation of a  $\mu_3$ -S coupled to cleavage of an Fe-S bond. In contrast, the  $\text{p}K_a$ s measured for  $[\text{Fe}_2\text{S}_2\text{X}_4]^{2-}$  (containing only  $\mu$ -S) are true  $\text{p}K_a$ s since the reaction only involves protonation of a  $\mu$ -S (*vide infra*).

Furthermore, the reason why protonation of a core  $\mu_3$ -S labilises substitution of any terminal ligand in these clusters now becomes clear. Protonation of  $\mu_3$ -S results in one Fe atom in  $[\text{Fe}_4\text{S}_3(\text{SH})\text{X}_4]^-$  becoming under-coordinated, with essentially planar three-coordination, and, consequently, this Fe becomes more susceptible to binding of nucleophiles than is the four-coordinate Fe in  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$ . Fig 3 shows a generic mechanism that accounts qualitatively for all kinetic data on the protonation and substitution reactions of  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$ . Both the *endo* and *exo* conformers of  $[\text{Fe}_4\text{S}_3(\text{SH})\text{X}_4]^-$  facilitate the substitution reaction. A key aspect of the experimental substitution kinetics is the rate dependence on the concentration of the nucleophile Y (Y is shown as PhSH in Fig 3). When  $\text{X} = \text{Cl}$  or  $\text{Br}$  the observed rate is dependent on  $[\text{Y}]$  because the prior X dissociation step is fast, while for other  $\text{X} = \text{SR, OR}$ , substitution is independent of  $[\text{Y}]$  (see ESI) because dissociation of X is slower and rate-determining. This enhanced lability of Cl, Br, relative to other X, occurs also in the uncatalysed substitution reactions.<sup>21, 22</sup>

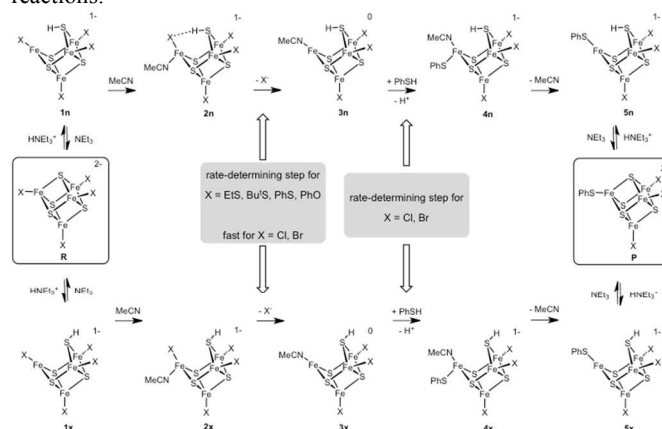


Fig 3. The proposed mechanism for acid-catalysed substitution of  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  to form  $[\text{Fe}_4\text{S}_4\text{X}_3(\text{SPh})]^{2-}$  in MeCN, passing through either *endo* or *exo*  $[\text{Fe}_4\text{S}_3(\text{SH})\text{X}_4]^-$ .<sup>23</sup> The rate-limiting step is dependent on X, as marked. This mechanism is consistent with all kinetic data. The structures of all intermediates have been confirmed by DFT optimisation. There are no significant energy differences between the *endo* and *exo* pathways.

Key features of the mechanism in Fig 3 are: (1) protonation of  $\mu_3$ -S generates a three-coordinate Fe site which is primed to be the site of substitution because it is susceptible to attack by a nucleophile; (2) coordination of acetonitrile (present as solvent) to the three-coordinate Fe prior to dissociation of  $\text{X}^-$ ; (3) maintenance of the three-coordinate Fe after dissociation of  $\text{X}^-$ , in intermediate 3.

What about the acid-catalysed substitution reactions of the terminal chloro-ligands in  $[\text{Fe}_2\text{S}_2\text{Cl}_4]^{2-}$ , and related complexes  $[\text{Cl}_2\text{FeS}_2\text{VS}_2\text{FeCl}_2]^{3-}$  and  $[\text{S}_2\text{MS}_2\text{FeCl}_2]^{2-}$  ( $\text{M} = \text{Mo or W}$ ), which contain only  $\mu$ -S ligands? Kinetic studies show that substitution is faster upon protonation of these ' $\mu$ -S only' clusters, and the rate of protonation of  $\mu$ -S is similar to that observed for  $\mu_3$ -S in cubanoid

clusters.<sup>24, 25</sup> Protonation of clusters containing only  $\mu$ -S would be expected to form  $\mu$ -SH, without severance of an Fe-S bond, and this has been confirmed with DFT calculations. Therefore an acid-catalysed substitution mechanism involving three-coordinate Fe is not available in these systems. What is the explanation for the slow rate of protonation of ' $\mu$ -S only' clusters? Calculations reveal that protonation of  $[\text{Fe}_2\text{S}_2\text{Cl}_4]^{2-}$  at  $\mu$ -S causes considerable geometrical change. Energy minimisation, after addition of a proton in approximately planar  $\text{Fe}_2$ -SH stereochemistry, results in elongation of the Fe-Fe distance with concomitant bending of the H-S bond out of the  $\text{Fe}_2\text{S}$  plane: this is a type of 'scissors' reconfiguration in which Fe-S-Fe angle increases as the H-S-Fe angles decrease. The Fe-SH distances are *ca* 0.2 Å larger than the corresponding Fe-S distances. The significant result here is that protonation of  $\mu$ -S to form  $\mu$ -SH, while not involving Fe-S cleavage, does involve substantial structural changes, resulting in slow proton transfer.<sup>25</sup>

Experimentally, the rate of the reaction between  $[\text{Fe}_2\text{S}_2\text{Cl}_4]^{2-}$  and PhSH in the presence of  $\text{HNEt}_3^+$  is independent of the concentration of PhSH (see ESI). We propose the associative mechanism shown in Fig 4 for proton-catalysed substitution of  $[\text{Fe}_2\text{S}_2\text{Cl}_4]^{2-}$ . The weakened coordination of Fe upon protonation of S (Fe-S extension by 0.2 Å) increases the susceptibility of Fe to incoming ligands. Coordination of solvent acetonitrile (intermediate 7) is coupled with dissociation of  $\text{Cl}^-$  (8), followed by coordination and deprotonation of PhSH (9), dissociation of acetonitrile (10), and deprotonation of  $\mu$ -SH. This sequence of steps is analogous to that proposed above for  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  (Fig 3). We expect that these mechanistic concepts for acid-catalysed substitution of  $[\text{Fe}_2\text{S}_2\text{Cl}_4]^{2-}$  will apply to other ' $\mu$ -S only' Fe-S clusters.

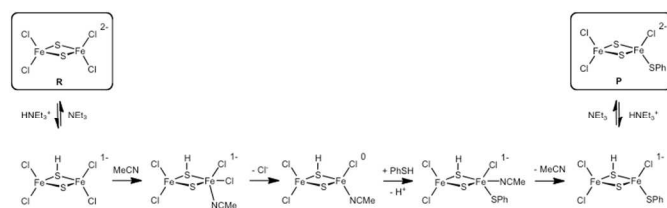


Fig 4. The mechanism proposed for acid-catalysed substitution of  $[\text{Fe}_2\text{S}_2\text{Cl}_4]^{2-}$ , for instance by PhSH.

Until now it has reasonably been assumed that during the substitution and protonation reactions of cubanoid  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  the cluster framework remains robust and essentially inflexible. However, the results presented in this paper indicate that protonation of  $\mu_3$ -S in  $[\text{Fe}_4\text{S}_4\text{X}_4]^{2-}$  is coupled to a Fe-S bond elongation. This disruption of the cluster framework affects its reactivity, and in particular primes the cluster to nucleophilic attack by generating an under-coordinated Fe site. A new and subtle meaning now attaches to the name 'acid-labile sulfide' used in the early period of research on biological FeS clusters.<sup>26, 27</sup> The  $\mu_3$ -SM<sub>3</sub> structural motif pervades synthetic and natural metal sulfide clusters,<sup>4, 28-33</sup> and transient cluster disruption, induced by protonation, may be a widespread phenomenon.

This research was undertaken with the assistance of resources provided at the National Computational Facility at the Australian National University through the National Computational Merit Allocation Scheme supported by the Australian Government. Ahmed Alwaaly acknowledges the Iraqi Ministry of Higher Education and Scientific Research for financial support.

## Notes and references

<sup>a</sup> School of Chemistry, Newcastle University, Newcastle upon Tyne NE1 7RU, UK. E-mail: richard.henderson@ncl.ac.uk.

<sup>b</sup> School of Chemistry, University of New South Wales, Sydney 2052, Australia. E-mail: i.dance@unsw.edu.au

† Electronic supplementary information (ESI) available: All kinetic data relevant to this paper; computational procedures, and optimised structures for  $[\text{Fe}_4\text{S}_3(\text{SH})\text{X}_4]^-$  and reaction intermediates. See DOI: 10.1039/c000000x/

1 T. Herskovitz, B. A. Averill, R. H. Holm, J. A. Ibers, W. D. Phillips and J. F. Weiher, *Proceedings of the National Academy of Sciences of the United States of America*, 1972, **69**, 2437-2441.

2 R. H. Holm, S. Ciurli and J. A. Weigel, *Prog. Inorg. Chem.*, 1990, **38**, 1-74.

3 H. Beinert, R. H. Holm and E. Munck, *Science*, 1997, **277**, 653-659.

4 D. C. Rees, *Annu. Rev. Biochem.*, 2002, **71**, 221-246.

5 P. Venkateswara Rao and R. H. Holm, *Chemical Reviews*, 2004, **104**, 527-560.

6 R. A. Henderson, *Chem. Rev.*, 2005, **105**, 2365-2437.

7 R. A. Henderson, *Coord. Chem. Rev.*, 2005, **249**, 1841-1856.

8 R. A. Henderson, *Bioinorg. React. Mech.*, 2012, **8**, 1-27.

9 R. A. Henderson and K. E. Oglieve, *Journal of the Chemical Society, Dalton Transactions*, 1999, 3927-3934.

10 A. J. Dunford and R. A. Henderson, *Journal of the Chemical Society, Dalton Transactions*, 2002, 2837-2842.

11 J. Bell, A. J. Dunford, E. Hollis and R. A. Henderson, *Angewandte Chemie International Edition*, 2003, **42**, 1149-1152.

12 I. Dance, *Inorg. Chem.*, 2013, **52**, 13068-13077.

13 B. K. Burgess and D. J. Lowe, *Chem. Rev.*, 1996, **96**, 2983-3011.

14 J. B. Howard and D. C. Rees, *Chem. Rev.*, 1996, **96**, 2965-2982.

15 O. Einsle, F. A. Tezcan, S. L. A. Andrade, B. Schmid, M. Yoshida, J. B. Howard and D. C. Rees, *Science*, 2002, **297**, 1696-1700.

16 R. Y. Igarashi and L. C. Seefeldt, *Crit. Rev. Biochem. Mol. Biol.*, 2003, **38**, 351-384.

17 D. C. Rees, F. A. Tezcan, C. A. Haynes, M. Y. Walton, S. Andrade, O. Einsle and J. A. Howard, *Phil. Trans. Roy. Soc. A*, 2005, **363**, 971-984.

18 T. Spatzal, M. Aksoyoglu, L. Zhang, S. L. A. Andrade, E. Schleicher, S. Weber, D. C. Rees and O. Einsle, *Science*, 2011, **334**, 940.

19 I. Dance, *Chem Commun (Camb)*, 2013, **49**, 10893-10907.

20 S. Kuwata and M. Hidai, *Coord. Chem. Rev.*, 2001, **213**, 211-305.

21 R. A. Henderson and K. E. Oglieve, *J. Chem. Soc., Dalton Trans.*, 1993, 1467.

22 R. A. Henderson and K. E. Oglieve, *Journal of the Chemical Society, Chemical Communications*, 1994, 377-379.

23 The structure of *endo*- $[\text{Fe}_4\text{S}_3(\text{SH})\text{X}_4]^-$  suggested another associative mechanistic pathway in which SH hydrogen bonds with the leaving ligand X (after binding of the incoming ligand) and facilitates its dissociation as XH. This mechanism is not consistent with the kinetic data: see ESI.

24 K. L. C. Gronberg and R. A. Henderson, *Journal of the Chemical Society, Dalton Transactions*, 1996, 3667-3675.

25 K. Bates, B. Garrett and R. A. Henderson, *Inorg. Chem.*, 2007, **46**, 11145-11155.

26 R. Malkin and J. C. Rabinowitz, *Biochemistry*, 1966, **5**, 1262-1268.

- 27 L. H. Jensen, *Annual Review of Biochemistry*, 1974, **43**, 461-474.
- 28 I. G. Dance and K. J. Fisher, *Prog. Inorg. Chem.*, 1994, **41**, 637-803.
- 29 P. Feng, X. Bu and N. Zheng, *Acc. Chem. Res.*, 2005, **38**, 293-303.
- 30 S. Dehnen and M. Melullis, *Coordination Chemistry Reviews*, 2007, **251**, 1259-1280.
- 31 M. N. Sokolov and P. A. Abramov, *Coordination Chemistry Reviews*, 2012, **256**, 1972-1991.
- 32 O. Fuhr, S. Dehnen and D. Fenske, *Chemical Society Reviews*, 2013, **42**, 1871-1906.
- 33 Y. Mizobe and H. Seino, in *Handbook of Chalcogen Chemistry: New Perspectives in Sulfur, Selenium and Tellurium (2)*, The Royal Society of Chemistry, 2013, vol. 2, pp. 7-24.