DALTON COMMUNICATION

A zinc thiolate species which mimics aspects of the chemistry of the Ada repair protein and matrix metalloproteinases: the synthesis, structure and reactivity of the tris(2-mercapto-1-phenylimidazolyl)hydroborato complex [Tm^{Ph}]ZnSPh †

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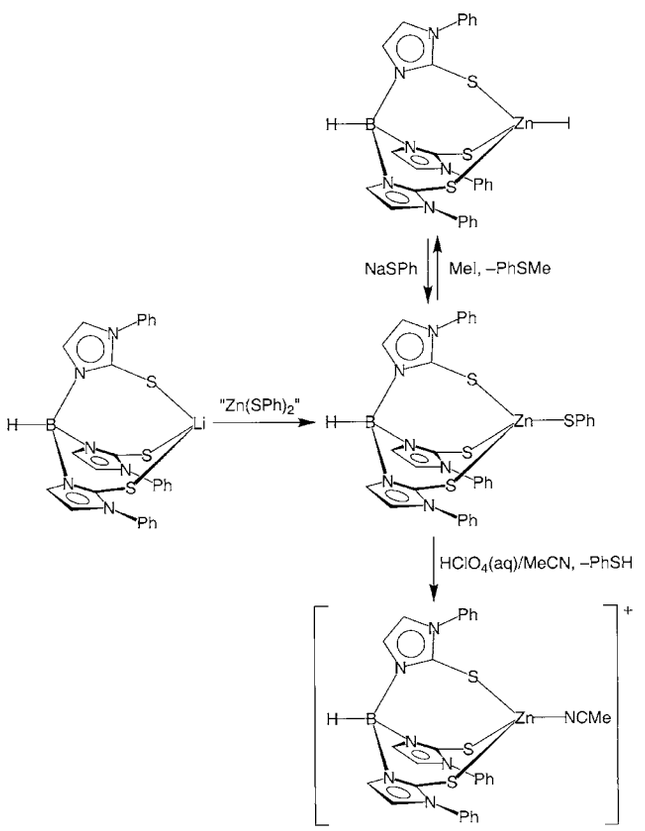
The tris(2-mercapto-1-phenylimidazolyl)hydroborato ligand, [Tm^{Ph}], has been used to prepare the zinc phenylthiolate derivative, [Tm^{Ph}]ZnSPh, which provides a good structural model for zinc enzymes that possess [Zn(Cys)$_4$] motifs; furthermore, the reactivity of the thiolate linkage in [Tm^{Ph}]ZnSPh mimics the chemistry of the Ada protein and the activation mechanism of matrix metalloproteinases.

The active sites of the majority of zinc enzymes feature a tetrahedrally coordinated zinc center that is attached to the protein backbone by three amino acid residues and a water molecule, *i.e.* [{XYZ}Zn^{II}–OH$_2$], where X, Y, and Z are three protein residues.[1,2] While the mechanisms of action of these zinc enzymes center around the role of the coordinated water, a growing number of zinc proteins and enzymes have recently been discovered in which the activity centers on the reactivity of a zinc–thiolate linkage. For example, alkylation of zinc–cysteine thiolates has been proposed to be a step in the mechanisms of action of the Ada DNA repair protein, methionine synthase, methanol–coenzyme M methyltransferase, farnesyl–protein transferase and geranylgeranyl–protein transferase.[3] As a result, the alkylation of zinc thiolate complexes has received considerable attention, most notably by Lippard,[4] Vahrenkamp,[5] and Darensbourg.[6] In addition to zinc–cysteine thiolate alkylation, the reactivity of this moiety is also of relevance to the mechanism of action of matrix metalloproteinases (matrixins), an important group of zinc enzymes responsible for degradation of extracellular matrix components.[7] In this paper, we address aspects of zinc thiolate chemistry of relevance to the Ada repair protein and matrix metalloproteinases.

We have previously reported the use of tris(2-mercapto-1-arylimidazolyl)hydroborato ligands to emulate the [S$_3$] coordination environment provided by three cysteine protein residues in zinc enzymes, as illustrated by the complexes [Tm^{Ph}]ZnX (X = I, NO$_3$), {[Tm^{Ph}]Zn(NCMe)}$^+$, [Tm^{Mes}]ZnX (X = Cl, I; Mes = mesityl) and {[Tm^{Mes}]Zn(HOMe)}$^+$.[8] In order to investigate the reactivity of the zinc–thiolate linkage in a sulfur rich environment, we have now prepared the phenylthiolate complex [Tm^{Ph}]ZnSPh by the sequences illustrated in Scheme 1. Thus, [Tm^{Ph}]ZnSPh may be obtained by either (i) treatment of [Tm^{Ph}]ZnI with NaSPh or (ii) treatment of [Tm^{Ph}]Li with "Zn(SPh)$_2$".[9]

The molecular structure of [Tm^{Ph}]ZnSPh has been determined by single crystal X-ray diffraction (Fig. 1),§ thereby confirming the mononuclear and tetrahedral nature of the complex. Interestingly, despite the prominent role that the tetrahedral Zn[S$_4$] motif plays in influencing the structure of zinc enzymes,[1] only *one* mononuclear species with such a motif is listed in the Cambridge Structural Database,[10] namely [Zn(SPh)$_4$]$^{2-}$;[11] other thiolate complexes with Zn[S$_4$] motifs are known, but are oligomeric due to the proclivity of thiolate groups to bridge zinc centers.[12] Riordan has, nevertheless, recently reported the phenylthiolate derivative [PhB(CH$_2$SBu^t)$_3$]ZnSPh,[13] which has a similar [S$_3$]Zn–SPh motif to that of [Tm^{Ph}]ZnSPh, but a more detailed comparison with [Tm^{Ph}]-ZnSPh is not possible since it has not been structurally characterized by X-ray diffraction.



Scheme 1

In view of the fact that mononuclear complexes with the Zn[S$_4$] motif are rare, [Tm^{Ph}]ZnSPh represents a good structural model for enzymes with this feature, such as the Ada repair protein, which acts by a mechanism involving cysteine thiolate demethylation of DNA.[14] Isolation of [Tm^{Ph}]ZnSPh thus permits alkylation of a zinc thiolate in a Zn[S$_4$] coordination sphere to be modeled, as illustrated by its rapid reaction with MeI to give PhSMe[15] and [Tm^{Ph}]ZnI[16] at room temperature (Scheme 1).

† Electronic supplementary information (ESI) available: experimental details, NMR data and selected bond lengths and angles. See http://www.rsc.org/suppdata/dt/b0/b007886g/

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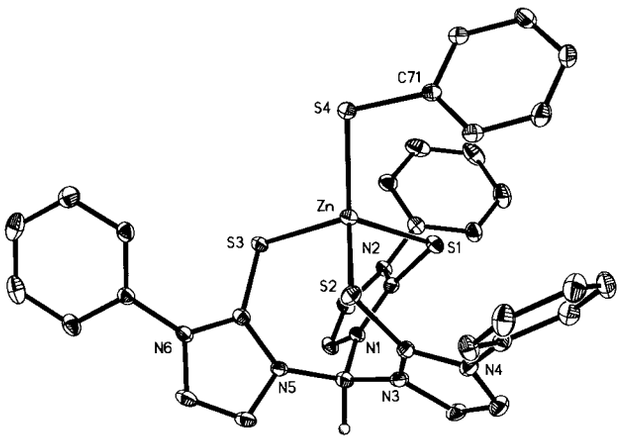

Fig. 1 Selected bond lengths (Å) and angles (°) for [Tm^{Ph}]ZnSPh: Zn–S(1) 2.375(1), Zn–S(2) 2.372(1), Zn–S(3) 2.361(1), Zn–S(4) 2.258(1); S(1)–Zn–S(2) 105.34(4), S(1)–Zn–S(3) 105.81(4), S(1)–Zn–S(4) 114.81(4), S(2)–Zn–S(3) 104.78(4), S(2)–Zn–S(4) 113.57(4), S(3)–Zn–S(4) 111.69(4).

Nucleophilic character of zinc phenylthiolates has literature precedence. For example, Lippard has previously investigated model chemistry of relevance to the Ada protein by studying the reactivity of the tetrathiolate $[Zn(SPh)_4]^{2-}$ towards $(MeO)_3PO$.[4] Significantly, Lippard observed that $[Zn(SPh)_4]^{2-}$ is alkylated by $(MeO)_3PO$ to form PhSMe, $(MeO)_2PO_2^-$, and $[Zn(SPh)_3]^-$, and concluded that the reaction proceeded *via* initial heterolytic dissociation generating an incipient thiolate anion. In contrast, Vahrenkamp has studied the reactivity of [Tp$^{RR'}$]ZnSR towards MeI and has postulated that the reactions occur without prior dissociation of RS^-,[5] while Carrano has formed a similar conclusion for reactions of the chelated sulfur ligand of $[HC(pz^{Me}_2)(CMe_2S)]ZnX$[17] and a series of other scorpionate zinc thiolates.[18] Darensbourg, however, has studied methylation of a chelated thiolate ligand with MeI and has concluded that the issue of whether alkylation occurs at a zinc–bound thiolate or a dissociated thiolate is still a matter of debate.[6]

The Zn–S bond length of [Tm^{Ph}]ZnSPh [2.258(1) Å] is comparable to the mean terminal Zn–SPh bond length [2.29 Å] for complexes listed in the Cambridge Structural Database, and also that of the Zn–SEt bond length [2.203(3) Å] in the related tris(pyrazolyl)hydroborato complex [Tp^{Ph}]ZnSEt.[19] These bond lengths are, however, noticeably shorter than the Zn–SPh bond length in $[Zn(SPh)_4]^{2-}$ [2.35 Å].[11,20] The latter observation may be of relevance to the different mechanisms that have been proposed in the literature for the alkylation of zinc–thiolate groups. Specifically, the long Zn–SPh bond length in $[Zn(SPh)_4]^{2-}$ would be expected to promote an alkylation mechanism involving PhS^- dissociation, as proposed by Lippard.[4] Conversely, the shorter Zn–SR bond length in [Tp$^{RR'}$]ZnSR derivatives would be expected to inhibit RS^- dissociation and thus promote a mechanism involving direct reaction at the Zn–SR moiety, as proposed by Vahrenkamp.[5] Thus, it is evident that the different ligand environments influence the mechanism of the reactivity of Zn–SR moieties. It should also be recognized that the mechanism is likely to be strongly influenced by the nature of the alkylating agent, *e.g.* MeI *versus* $(MeO)_3PO$.

While we presently have no evidence in our system to address whether phenylthiolate alkylation proceeds *via* initial dissociation or by direct reaction at the Zn–SPh moiety, we have performed DFT calculations (B3LYP)[21] using Jaguar[22] to address other aspects of the reaction between [Tm^{Ph}]ZnSPh and MeI. For example, MeI reacts selectively with the phenylthiolate sulfur atom of [Tm^{Ph}]ZnSPh, as opposed to reacting with the "thione" sulfurs of the [Tm^{Ph}] ligand. This observation is particularly noteworthy when it is considered that the Zn–SPh "thiolate" bond length [2.258(1) Å] is shorter than the average Zn–S "thione" bond length [2.37 Å] associated with the L_2X[23] donor [Tm^{Ph}] ligand. Significantly, the calculations indicate that the HOMO possesses a large degree of [PhS] sulfur lone pair character, with the sulfur lone pairs of the thione groups being located at lower energies. As such, the thiolate sulfur atom would be expected to possess greater nucleophilicity. In addition to this factor, the calculations indicate that the thiolate sulfur bears a greater negative charge,[24] which would thereby also contribute to its greater nucleophilicity.[25]

The DFT calculations also indicate that the alkylation is strongly exothermic, with $\Delta E_{calc} = -17.1$ kcal mol^{-1}. Consideration of the individual calculated Zn–SPh (75.1 kcal mol^{-1}), Zn–I (80.4 kcal mol^{-1}), Me–SPh (71.4 kcal mol^{-1}), and Me–I (59.5 kcal mol^{-1}) bond dissociation energies[26] indicates that the exothermicity of the reaction is associated with the fact that the Zn–I bond is stronger than the Zn–SPh bond, whereas the converse is true for the Me–I and Me–SPh bonds.

Proteolytic cleavage of zinc–thiolate groups is of relevance to the mechanism of action of matrix metalloproteinases, a class of enzymes that are essential for embryonic development, wound healing, bone and growth development, and other physiological remodeling processes.[7] The activity of matrix metalloproteinases is controlled by secreting the enzymes in inactive proenzyme forms, $[(His)_3Zn(Cys)]$, and activation is achieved by cleavage of the cysteine thiolate residue from the zinc center. Several mechanisms exist for activating the proenzyme, one of which involves proteolytic cleavage of the cysteine thiolate residue.[7]

A chemical model for this activation mechanism is provided by the reactivity of [Tm^{Ph}]ZnSPh towards H^+. Specifically, treatment of [Tm^{Ph}]ZnSPh with $HClO_4$ in acetonitrile results in the rapid elimination of PhSH at room temperature and formation of $\{[Tm^{Ph}]Zn(NCMe)\}(ClO_4)$, as illustrated in Scheme 1.[27] While a more biologically relevant transformation would yield a zinc–aqua species, the formation of a zinc–acetonitrile complex is not surprising given the paucity of simple tetrahedral zinc-aqua complexes. Nevertheless, the above transformation is of relevance since it clearly demonstrates that proteolytic cleavage of a Zn–SPh moiety is facile. In this regard, it is worth noting that the reverse type of reaction, namely Zn–SPh bond formation, has also been observed to be facile. For example, [Tp^{Cum,Me}]ZnOH reacts with PhSH to give [Tp^{Cum,Me}]ZnSPh.[5] Thus, on the basis of the facility of these Zn–SPh bond cleavage and formation transformations, it seems likely that a proteolytic cleavage mechanism for activating matrix metalloproteinases could be reversible. As such, it provides a means of not only activating the enzyme, but also deactivating it.

In summary, the zinc phenylthiolate derivative [Tm^{Ph}]ZnSPh provides a good structural model for zinc enzymes that possess $[Zn(Cys)_4]$ motifs. Furthermore, the reactivity of the thiolate linkage in [Tm^{Ph}]ZnSPh towards MeI mimics the chemistry of the Ada protein, while the reactivity towards H^+ mimics the mechanism of activation of matrix metalloproteinases.

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Notes and references

§ Crystal data for [Tm^{Ph}]ZnSPh: $C_{33}H_{27}BN_6S_4Zn$, $M = 712.03$, triclinic, $a = 9.9840(10)$, $b = 10.0007(10)$, $c = 17.7216(16)$ Å, $\alpha = 80.440(2)$, $\beta = 78.784(2)$, $\gamma = 67.801(2)°$, $T = 238(2)$ K, space group $P\bar{1}$, $Z = 2$, μ(Mo-Kα) = 1.064 cm^{-1} ($\lambda = 0.71073$ Å), 7110 reflections measured, $R1 = 0.0524$. CCDC reference number 186/2223. See http://www.rsc.org/suppdata/dt/b0/b007886g/ for crystallographic files in .cif format.

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26 For comparison, the experimentally reported Me–I [26*a*] and Me–SPh [26*b*] bond energies are 56 kcal mol^{-1} and 67.4 kcal mol^{-1}, respectively. Furthermore, the calculated Me–I bond length in MeI (2.180 Å) is in close agreement with experimentally determined value (2.139 Å).[26*c*] (*a*) R. T. Sanderson, *Chemical Bonds and Bond Energy*, 2nd edn, Academic Press, New York, 1976; (*b*) S. W. Benson, *Chem. Rev.*, 1978, **78**, 23; (*c*) S. L. Miller, L. C. Aamodt, G. Dousmanis, C. H. Townes and J. Kraitchman, *J. Chem. Phys.*, 1952, **20**, 1112.
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