Modeling the active sites of bacteriophage T7 lysozyme, bovine 5-aminolevulinate dehydratase, and peptide deformylase: synthesis and structural characterization of a bis(pyrazolyl)(thioalkoxy)hydroborato zinc complex, $[(Ph_2CHS)Bp^{Bu^t,Pr^i}]ZnI$

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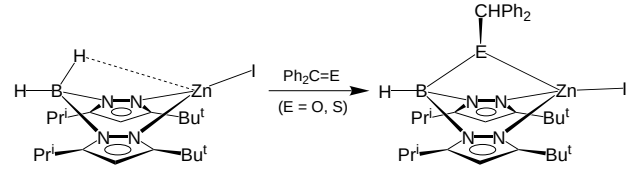
Insertion of thiobenzophenone into a B–H bond of $[Bp^{Bu^t,Pr^i}]ZnI$ achieves the synthesis of $[(Ph_2CHS)Bp^{Bu^t,Pr^i}]ZnI$, a complex in which the *in situ* generated [NNS] donor ligand models the binding of the histidine and cysteine residues at the active sites of bacteriophage T7 lysozyme, bovine 5-aminolevulinate dehydratase and peptide deformylase.

Zinc is essential to all forms of life.[1,2] Of the trace metals, its abundance in biological systems is second only to iron, with an average human containing *ca.* 3 g of zinc. Correspondingly, there are many biological functions of zinc and a large number of zinc enzymes are known. Interestingly, the active sites of many of these enzymes exhibit a common structural motif which comprises a pseudo-tetrahedral zinc center to which a single water molecule is attached, with the three remaining sites being occupied by a combination of nitrogen, oxygen and sulfur donors provided by histidine, glutamate, aspartate and cysteine residues of the protein backbone.[3] Despite the overall similarity in the structure of the active sites of these enzymes, however, each performs a different function. It is, therefore, important to understand the manner in which a $[N_xO_yS_z]$ donor array modifies the chemistry of a tetrahedral zinc center. A first step to achieve such an objective involves the synthesis and structural characterization of well defined complexes with a series of appropriate $[N_xO_yS_z]$ donor ligands. For this reason, we are actively studying synthetic analogues of zinc enzymes which differ in the ligand complement, *e.g.* carbonic anhydrase [NNN],[4–6] thermolysin [NNO],[7] and liver alcohol dehydrogenase [NSS].[8] In this paper, we describe the construction of a [NNS] ligand which provides a model of the groups that bind zinc at the active sites of bacteriophage T7 lysozyme, bovine 5-aminolevulinate dehydratase and peptide deformylase.

Bacteriophage T7 lysozyme is a zinc enzyme which destroys bacteria by cleaving polysaccharide components within their cell walls.[9,10] X-Ray diffraction studies of a mutant lysozyme (AK6) reveal that the active site is located in a cleft within the protein which is *ca.* 22–26 Å long and 10–11 Å deep. The tetrahedral zinc center of the active site is bound to the protein backbone *via* one sulfur and two nitrogen donors of cysteine (Cys-130) and histidine (His-17 and His-122) residues; the fourth site is occupied by a water molecule.[10] Bovine 5-aminolevulinate dehydratase[11] and peptide deformylase[12] are enzymes that are related to T7 lysozyme by virtue of the common coordination of one cysteine and two histidine donors to the zinc center of the active site. In order to mimic the tetrahedral binding of zinc to the protein backbone of these enzymes, a [NNS] ligand which presents the nitrogen and sulfur donors as a facial, rather than T-shaped, array is required. For this purpose, we have focused attention on the construction of ligands in which the donor groups are linked to a common tetrahedral center, since we have previously noted that such attachment serves to enforce facial binding to zinc.[7,8] Significantly, a monomeric zinc complex containing a facially tridentate [NNS] donor ligand, namely $[(Ph_2CHS)Bp^{Bu^t,Pr^i}]ZnI$, may indeed be constructed by insertion of thiobenzophenone into a B–H of $[Bp^{Bu^t,Pr^i}]ZnI$[13] (Scheme 1).



Scheme 1

The molecular structure of $[(Ph_2CHS)Bp^{Bu^t,Pr^i}]ZnI$ (Fig. 1) has been determined by X-ray diffraction.[14] Of most importance, the diffraction study demonstrates that (*i*) $[(Ph_2CHS)Bp^{Bu^t,Pr^i}]ZnI$ exists as a well defined monomeric complex, and (*ii*) the [NNS] ligand supports a pseudo-tetrahedral zinc center that is structurally related to the active sites of T7 lysozyme,[15] 5-aminolevulinate dehydratase,[16] and peptide deformylase.[12]

The significance of isolating $[(Ph_2CHS)Bp^{Bu^t,Pr^i}]ZnI$ is further underscored by noting that other attempts to use tridentate [NNS] ligands to support monomeric tetrahedral zinc complexes of the type [NNS]ZnX have not been very successful. For example, Vahrenkamp has used *N*-(2-mercaptoethyl)picolylamine (MEPAH) in an attempt to provide a [NNS] environment pertinent to zinc enzymes.[17] Unfortunately, however, the zinc chemistry derived from MEPAH was found to be very complex, with polymeric

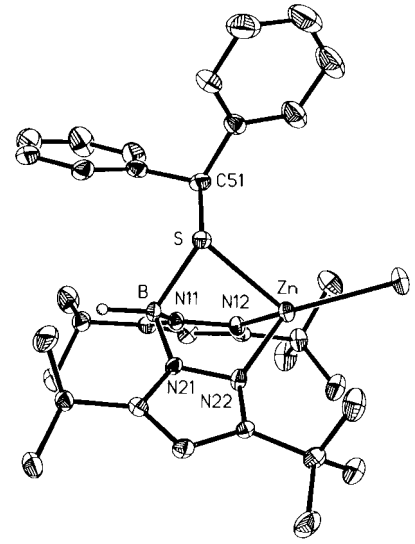


Fig. 1 Molecular structure of $[(Ph_2CHS)Bp^{Bu^t,Pr^i}]ZnI$. Selected bond lengths (Å) and angles (°): Z–N(12) 2.031(5), Zn–N(22) 2.040(5), Zn–S 2.460(2), Zn–I 2.4869(8); N(12)–Zn–N(22) 94.6(2), N(12)–Zn–S 88.61(14), N(22)–Zn–S 84.00(14), N(12)–Zn–I 125.63(13), N(22)–Zn–I 129.34(13), S–Zn–I 121.71(5).

$[(MEPA)ZnX]_n$ (X = Cl, Br), trinuclear $[(MEPA)_4Zn_3]X_2$ (X = BF_4, ClO_4, NO_3), dinuclear $[(MEPA)Zn(OAc)]_2$, and other complexes being isolated; in no instance was a monomeric tetrahedral [NNS]ZnX complex strucurally characterized.[18] Undoubtedly, one of the principal reasons for the difficulty in isolating mononuclear [NNS]ZnX complexes is associated with the propensity of sulfur to act as a bridge between metal centers.[19] In view of the difficulty associated with isolating such complexes, the *in situ* construction of a [NNS] donor ligand by elaboration of a [NN] donor ligand at a metal center therefore provides a useful method of synthesis of other [NNS]ZnX derivatives.[20]

In addition to $[(Ph_2CHS)Bp^{Bu^t,Pr^i}]ZnI$, the alkoxide analogue $[(Ph_2CHO)Bp^{Bu^t,Pr^i}]ZnI$ has also been synthesized (Scheme 1) and structurally characterized by X-ray diffraction.[21] Other than the difference in Zn–O *vs.* Zn–S bond lengths, the coordination environment about zinc is similar for $[(Ph_2CHS)Bp^{Bu^t,Pr^i}]ZnI$ and $[(Ph_2CHO)Bp^{Bu^t,Pr^i}]ZnI$. The geometry at the chalcogen in each case is distinctly pyramidal, with the $[Ph_2CH]$ alkyl substituent being displaced from the B–E–Zn plane, so that both molecules are chiral. The barriers to enantiomer interconversion are, however, considerably different for the two derivatives. For example, whereas the thioalkoxide complex $[(Ph_2CHS)Bp^{Bu^t,Pr^i}]ZnI$ exhibits two sets of resonances in the 500 MHz ^1H NMR spectrum at −50 °C for the two diasterotopic pyrazolyl groups, the corresponding ^1H NMR spectrum of the alkoxide analogue $[(Ph_2CHO)Bp^{Bu^t,Pr^i}]ZnI$ shows only a single set due to chemical exchange as a result of isomerization. The barrier for enantiomer interconversion, is, therefore, considerably lower for the alkoxide complex than for the thioalkoxide derivative.[22] Enantiomer interconversion for $[(Ph_2CHS)Bp^{Bu^t,Pr^i}]ZnI$ is, however, observed to occur rapidly on the NMR timescale at ambient temperature,[23] with an activation barrier ($\Delta G^{\ddagger}$) of 14.5(3) kcal mol^{-1} (1 cal = 4.184 J) at 25 °C.[24,25] Two possible mechanisms for enantiomer interconversion include (*i*) cleavage of the E → Zn dative bond, rotation about the B–E bond, followed by re-coordination, and (*ii*) pyramidal inversion at E. Although we have no evidence to distinguish between these mechanisms, if enantiomer interconversion were to proceed *via* a common mechanism, the observation that the exchange is more facile for the alkoxide than for the thioalkoxide suggests that the mechanism may involve direct inversion at the chalcogen, since barriers to inversion are typically lower for second row (*i.e.* O), as opposed to third row (*i.e.* S), elements.[22,26] Some support for this notion is provided by the observation that the $[Ph_2CH]$ substituent is much closer to planarity with the B–E–Zn plane in the alkoxide than in the thioalkoxide derivative, as judged by the respective sum of bond angles at O (336°) and S (288°). In contrast, however, if enantiomer interconversion were to require dissociation of the E → Zn dative bond, then the exchange would be expected to be more facile for the complex with the weaker bond, *i.e.* the thioalkoxide derivative.

In summary, the insertion of thiobenzophenone into a B–H bond of $[Bp^{Bu^t,Pr^i}]ZnI$ results in the synthesis of $[(Ph_2CHS)Bp^{Bu^t,Pr^i}]ZnI$. Importantly, the [NNS] ligand so obtained is capable of sustaining a monomeric tetrahedral geometry about zinc and resembles the histidine and cysteine residues that bind zinc at the active sites of T7 lysozyme, 5-aminolevulinate dehydratase and peptide deformylase.

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