DALTON COMMUNICATION

Mimicking the binding of glutamate to zinc in thermolysin and carboxypeptidase: the synthesis of [η^3-$(HCO_2)Bp^{Bu^t,Pr^i}$]ZnCl by insertion of CO_2 into a B–H bond of the bis(pyrazolyl)hydroborato zinc complex [Bp^{Bu^t,Pr^i}]ZnCl

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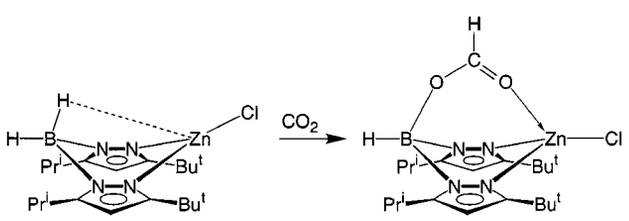
Insertion of CO_2 into one of the B–H bonds of the bis(pyrazolyl)hydroborato complex [Bp^{Bu^t,Pr^i}]ZnCl yields [η^3-$(HCO_2)Bp^{Bu^t,Pr^i}$]ZnCl; the carboxylate group of the generated [NNO] donor ligand mimics the glutamate residues at the active sites of thermolysin and carboxypeptidase.

With approximately 300 zinc enzymes known today, the importance of zinc in biology is widely recognized.[1] As a result, developing the bioinorganic chemistry of zinc is of considerable importance. One approach to understanding the role of zinc in biological systems involves designing synthetic analogues of the active sites of zinc enzymes, many of which consist of tetrahedral zinc centers that are bound to the protein by a combination of nitrogen-, oxygen-, and sulfur-donors of histidine, glutamate, aspartate, and cysteine residues.[1] In this regard, we have reported synthetic analogues of (*i*) carbonic anhydrase based on tris(pyrazolyl)hydroborato[2,3] and tris(imidazolyl)-phosphine[4] ligands, (*ii*) liver alcohol dehydrogenase based on a bis(thioimidazolyl)(pyrazolyl)hydroborato ligand,[5] (*iii*) bacteriophage T7 lysozyme and bovine 5-aminolevulinate dehydratase based on a bis(pyrazolyl)(thioalkoxide)hydroborato ligand,[6] and (*iv*) thermolysin based on a bis(pyrazolyl)-(alkoxide)hydroborato ligand.[7] In this paper, we describe the construction of a tridentate [NNO] ligand in which the O-donor is a carboxylate moiety and, as such, provides an improved synthetic analogue for thermolysin and carboxypeptidase.

Thermolysin and carboxypeptidase are two closely related zinc enzymes that are responsible for catalyzing the hydrolysis of peptide bonds specifically, thermolysin is an endopeptidase with a particular preference for peptide bonds on the amino side of hydrophobic residues, while carboxypeptidase is an exopeptidase that displays selectivity towards C-terminal amino acid residues.[1,8] In addition to their similar function, the active sites of these enzymes bear a close resemblance, with the zinc centers of each being bound to the protein by a combination of one glutamate and two histidine residues.[9–11] The similarity is further emphasized by the fact that the glutamate residue of each enzyme is capable of binding in both a uni- and bi-dentate manner.[12]

Our previous studies have demonstrated that [NNO] donor arrays, namely [η^3-$(R_2CHO)Bp^{Bu^t,Pr^i}$], may be constructed by insertion of $R_2C{=}O$ into a B–H bond of bis(pyrazolyl)hydroborato complexes.[6,7] An important consequence of attaching the nitrogen and oxygen donors to a tetrahedral boron center is that facial binding of the [η^3-$(R_2CHO)Bp^{Bu^t,Pr^i}$] ligand is ensured, in contrast to the biomimetically irrelevant 'T-shaped' binding that has been observed with differently constructed [NNO] ligands, such as bis(pyrazolylethyl) ethers.[13] However, a drawback of the [η^3-$(R_2CHO)Bp^{Bu^t,Pr^i}$] ligand is that the O-donor is based on an alkoxide, rather than carboxylato, functionality. Significantly, therefore, we have discovered that a more biomimetically relevant carboxylato group (albeit aberrated) may be introduced by insertion of CO_2 into one of the B–H bonds of [Bp^{Bu^t,Pr^i}]ZnCl[14] (Scheme 1).[15] Spectroscopic evidence for the formation of an [HCO_2] group is provided by the observation of signals at δ 7.21 and 168.2 in the ^{1}H and ^{13}C NMR spectra, respectively, both of which exhibit a $^1J_{C-H}$ coupling constant of 224 Hz.[16] Moreover, the [HCO_2] moiety is also characterized by two absorptions in the IR spectrum attributable to ν(C–O) at 1650 and 1336 cm^{-1},[17] which have been confirmed by the shifts observed for the ^{13}C-labelled analogue. In addition to the spectroscopic data, convincing evidence for the nature of [η^3-$(HCO_2)Bp^{Bu^t,Pr^i}$]ZnCl was obtained by single crystal X-ray diffraction, as illustrated in Fig. 1.[18]



Scheme 1

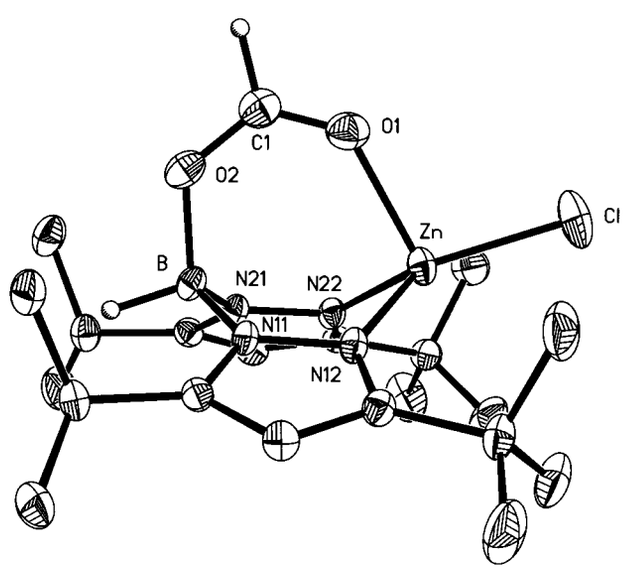


Fig. 1 Molecular structure of [η^3-$(HCO_2)Bp^{Bu^t,Pr^i}$]ZnCl. Selected bond lengths (Å) and angles (°): Zn–N12 2.016(3), Zn–N22 2.004(3), Zn–O1 2.065(4), Zn–Cl 2.165(2), C1–O1 1.209(6), C1–O2 1.278(6), B–N11 1.551(5), B–N21 1.542(5), B–O2 1.514(6); N11–Zn–N22 95.6(1), N12–Zn–O1 96.4(1), N22–Zn–O1 95.9(1), N12–Zn–Cl 127.5(1), N22–Zn–Cl 129.9(1), O1–Zn–Cl 102.2(1)

Table 1 Comparison of Zn–N and Zn–O bond lengths in thermolysin and carboxypeptidase with their synthetic analogues[a]

	$[\eta^3\text{-}(HCO_2)Bp^{Bu^t,Pr^i}]ZnCl$	$[\eta^3\text{-}(MeO)Bp^{Bu^t,Pr^i}]ZnMe$[b]	Thermolysin	Carboxypeptidase
d(Zn–N)/Å	2.004(3)	2.036(6)	2.08,[c] 1.9[d]	2.2[e]
d(Zn–N)/Å	2.016(3)	2.101(6)	2.10,[c] 2.0[d]	2.2[e]
d(Zn–O)/Å	2.065(4)	2.182(5)	2.08,[c] 1.9[d]	2.2[e]

[a] Only data for active sites with unidentate glutamate are listed. [b] Data taken from ref. 7. [c] Data taken from ref. 10*a*. [d] Data cited in ref. 10*c*. [e] Data cited in ref. 11.

The compound $[\eta^3\text{-}(HCO_2)Bp^{Bu^t,Pr^i}]ZnCl$ is the first structurally characterized tetrahedral zinc complex of a tridentate [NNO] ligand in which the O-donor is a carboxylate group. The most interesting aspect of the structure is, therefore, concerned with its resemblance to the active sites of thermolysin and carboxypeptidase, specifically in their unidentate glutamate forms.[10*a*,*c*,11] Thus, as in the enzymes, the [NNO] bis(pyrazolyl)(carboxylato)hydroborato ligand binds to a distorted tetrahedral zinc center in a facially tridentate manner. Importantly, since the O-donor is a carboxylate group, the $[\eta^3\text{-}(HCO_2)Bp^{Bu^t,Pr^i}]$ ligand represents a notable advance in modeling thermolysin and carboxypeptidase. For comparison purposes, the Zn–O and Zn–N bond length data for $[\eta^3\text{-}(HCO_2)Bp^{Bu^t,Pr^i}]ZnCl$ and the enzymes are summarized in Table 1. The significance of the construction of the $[\eta^3\text{-}(HCO_2)Bp^{Bu^t,Pr^i}]$ ligand is further highlighted by the fact that, despite much effort in developing biomimetic [NNO] ligands,[8*e*,13,19–21] structurally characterized examples of mononuclear tetrahedral zinc complexes of tridentate [NNO] ligands remain rare, regardless of the nature of the O-donor.[22] For example, rather than yielding a monomeric tetrahedral complex, the [NNO] donor ligand $HN\{CH_2(2\text{-}HOC_6H_4)\}\{CH_2\text{-}(2\text{-}C_5H_4N)\}$ (HSALAMP), gives the dinuclear octahedral complex $[Zn(SALAMP)(NO_3)]_2$, in which the phenoxide moiety bridges the two zinc centers.[20]

Finally, it is worth noting that in contrast to the extensively studied insertion of CO_2 into M–X bonds (M = metal; X = H, C, O or N),[23] well defined illustrations of insertion of CO_2 into B–X bonds are rare. For example, although insertion of CO_2 into B–H bonds is precedented,[24,25] none of the products has been structurally characterized. Thus, $[\eta^3\text{-}(HCO_2)Bp^{Bu^t,Pr^i}]$-ZnCl also represents the first structurally characterized example of a complex derived from insertion of CO_2 into a B–H bond.

In summary, CO_2 insertion into a B–H bond has allowed the synthesis of $[\eta^3\text{-}(HCO_2)Bp^{Bu^t,Pr^i}]ZnCl$, a complex in which the carboxylate donor mimics the glutamate residues at the active sites of thermolysin and carboxypeptidase. As such, $[\eta^3\text{-}(HCO_2)Bp^{Bu^t,Pr^i}]ZnCl$ is an improved synthetic analogue for these enzymes.

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