

# Mimicking the binding of glutamate to zinc in thermolysin and carboxypeptidase: the synthesis of $[\eta^3\text{-(HCO}_2\text{)}\text{Bp}^{\text{Bu}^t, \text{Pr}^i}]\text{ZnCl}$ by insertion of $\text{CO}_2$ into a B–H bond of the bis(pyrazolyl)hydroborato zinc complex $[\text{Bp}^{\text{Bu}^t, \text{Pr}^i}]\text{ZnCl}$

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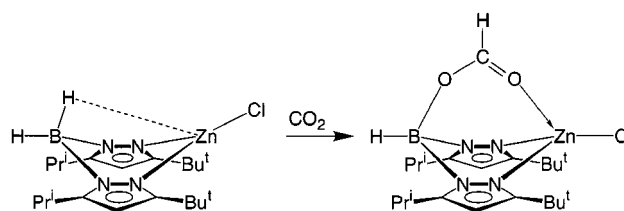
Insertion of  $\text{CO}_2$  into one of the B–H bonds of the bis(pyrazolyl)hydroborato complex  $[\text{Bp}^{\text{Bu}^t, \text{Pr}^i}]\text{ZnCl}$  yields  $[\eta^3\text{-(HCO}_2\text{)}\text{Bp}^{\text{Bu}^t, \text{Pr}^i}]\text{ZnCl}$ ; the carboxylate group of the generated [NNO] donor ligand mimics 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.<sup>1</sup> 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.<sup>1</sup> In this regard, we have reported synthetic analogues of (i) carbonic anhydrase based on tris(pyrazolyl)hydroborato<sup>2,3</sup> and tris(imidazolyl)-phosphine<sup>4</sup> ligands, (ii) liver alcohol dehydrogenase based on a bis(thioimidazolyl)(pyrazolyl)hydroborato ligand,<sup>5</sup> (iii) bacteriophage T7 lysozyme and bovine 5-aminolevulinic acid dehydratase based on a bis(pyrazolyl)(thioalkoxide)hydroborato ligand,<sup>6</sup> and (iv) thermolysin based on a bis(pyrazolyl)-(alkoxide)hydroborato ligand.<sup>7</sup> 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.<sup>1,8</sup> 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.<sup>9–11</sup> 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.<sup>12</sup>

Our previous studies have demonstrated that [NNO] donor arrays, namely  $[\eta^3\text{-(R}_2\text{CHO)Bp}^{\text{Bu}^t, \text{Pr}^i}]$ , may be constructed by insertion of  $\text{R}_2\text{C=O}$  into a B–H bond of bis(pyrazolyl)hydroborato complexes.<sup>6,7</sup> An important consequence of attaching the nitrogen and oxygen donors to a tetrahedral boron center is that facial binding of the  $[\eta^3\text{-(R}_2\text{CHO)Bp}^{\text{Bu}^t, \text{Pr}^i}]$  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.<sup>13</sup> However, a drawback of the  $[\eta^3\text{-(R}_2\text{CHO)Bp}^{\text{Bu}^t, \text{Pr}^i}]$  ligand is that the O-donor is

based on an alkoxide, rather than carboxylate, functionality. Significantly, therefore, we have discovered that a more biomimetically relevant carboxylate group (albeit aberrated) may be introduced by insertion of  $\text{CO}_2$  into one of the B–H bonds of  $[\text{Bp}^{\text{Bu}^t, \text{Pr}^i}]\text{ZnCl}$ <sup>14</sup> (Scheme 1).<sup>15</sup> Spectroscopic evidence for the formation of an  $[\text{HCO}_2]$  group is provided by the observation of signals at  $\delta$  7.21 and 168.2 in the <sup>1</sup>H and <sup>13</sup>C NMR spectra, respectively, both of which exhibit a <sup>1</sup>J<sub>C–H</sub> coupling constant of 224 Hz.<sup>16</sup> Moreover, the  $[\text{HCO}_2]$  moiety is also characterized by two absorptions in the IR spectrum attributable to  $\nu(\text{C–O})$  at 1650 and 1336  $\text{cm}^{-1}$ ,<sup>17</sup> which have been confirmed by the shifts observed for the <sup>13</sup>C-labelled analogue. In addition to the spectroscopic data, convincing evidence for the nature of  $[\eta^3\text{-(HCO}_2\text{)}\text{Bp}^{\text{Bu}^t, \text{Pr}^i}]\text{ZnCl}$  was obtained by single crystal X-ray diffraction, as illustrated in Fig. 1.<sup>18</sup>



Scheme 1

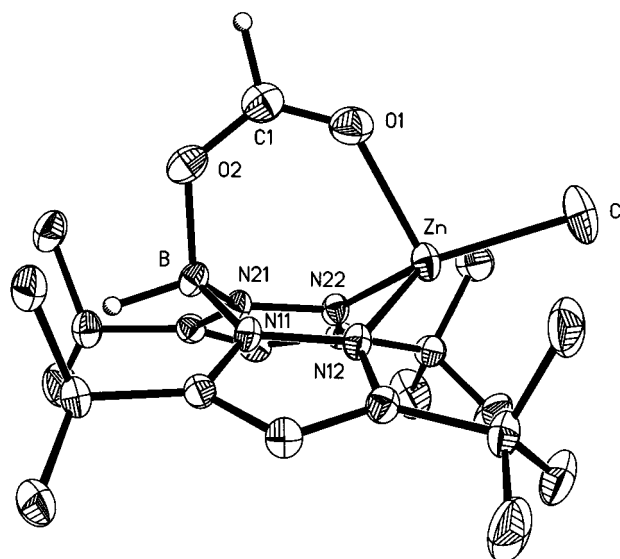


Fig. 1 Molecular structure of  $[\eta^3\text{-(HCO}_2\text{)}\text{Bp}^{\text{Bu}^t, \text{Pr}^i}]\text{ZnCl}$ . Selected bond lengths (Å) and angles (°): Zn–N12 2.016(3), Zn–N22 2.004(3), Zn–O1 2.065(4), Zn–Cl 2.165(2), C1–O1 1.209(6), C1–O2 1.278(6), B–N11 1.551(5), B–N21 1.542(5), B–O2 1.514(6); N11–Zn–N22 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<sup>a</sup>

	$[\eta^3\text{-(HCO}_2\text{)Bp}^{\text{Bu}^t\text{,Pr}^t}\text{]ZnCl}$	$[\eta^3\text{-(MeO)Bp}^{\text{Bu}^t\text{,Pr}^t}\text{]ZnMe}^b$	Thermolysin	Carboxypeptidase
$d(\text{Zn–N})/\text{\AA}$	2.004(3)	2.036(6)	2.08, <sup>c</sup> 1.9 <sup>d</sup>	2.2 <sup>e</sup>
$d(\text{Zn–N})/\text{\AA}$	2.016(3)	2.101(6)	2.10, <sup>c</sup> 2.0 <sup>d</sup>	2.2 <sup>e</sup>
$d(\text{Zn–O})/\text{\AA}$	2.065(4)	2.182(5)	2.08, <sup>c</sup> 1.9 <sup>d</sup>	2.2 <sup>e</sup>

<sup>a</sup> Only data for active sites with unidentate glutamate are listed. <sup>b</sup> Data taken from ref. 7. <sup>c</sup> Data taken from ref. 10a. <sup>d</sup> Data cited in ref. 10c. <sup>e</sup> Data cited in ref. 11.

The compound  $[\eta^3\text{-(HCO}_2\text{)Bp}^{\text{Bu}^t\text{,Pr}^t}\text{]ZnCl}$  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.<sup>10a,c,11</sup> 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  $[\eta^3\text{-(HCO}_2\text{)Bp}^{\text{Bu}^t\text{,Pr}^t}]$  ligand represents a notable advance in modeling thermolysin and carboxypeptidase. For comparison purposes, the Zn–O and Zn–N bond length data for  $[\eta^3\text{-(HCO}_2\text{)Bp}^{\text{Bu}^t\text{,Pr}^t}\text{]ZnCl}$  and the enzymes are summarized in Table 1. The significance of the construction of the  $[\eta^3\text{-(HCO}_2\text{)Bp}^{\text{Bu}^t\text{,Pr}^t}]$  ligand is further highlighted by the fact that, despite much effort in developing biomimetic [NNO] ligands,<sup>8e,13,19–21</sup> structurally characterized examples of mononuclear tetrahedral zinc complexes of tridentate [NNO] ligands remain rare, regardless of the nature of the O-donor.<sup>22</sup> For example, rather than yielding a monomeric tetrahedral complex, the [NNO] donor ligand  $\text{HN}\{\text{CH}_2(2\text{-HOC}_6\text{H}_4)\}\{\text{CH}_2(2\text{-C}_5\text{H}_4\text{N})\}$  (HSALAMP), gives the dinuclear octahedral complex  $[\text{Zn}(\text{SALAMP})(\text{NO}_3)]_2$ , in which the phenoxide moiety bridges the two zinc centers.<sup>20</sup>

Finally, it is worth noting that in contrast to the extensively studied insertion of CO<sub>2</sub> into M–X bonds (M = metal; X = H, C, O or N),<sup>23</sup> well defined illustrations of insertion of CO<sub>2</sub> into B–X bonds are rare. For example, although insertion of CO<sub>2</sub> into B–H bonds is preceded,<sup>24,25</sup> none of the products has been structurally characterized. Thus,  $[\eta^3\text{-(HCO}_2\text{)Bp}^{\text{Bu}^t\text{,Pr}^t}\text{]ZnCl}$  also represents the first structurally characterized example of a complex derived from insertion of CO<sub>2</sub> into a B–H bond.

In summary, CO<sub>2</sub> insertion into a B–H bond has allowed the synthesis of  $[\eta^3\text{-(HCO}_2\text{)Bp}^{\text{Bu}^t\text{,Pr}^t}\text{]ZnCl}$ , a complex in which the carboxylate donor mimics the glutamate residues at the active sites of thermolysin and carboxypeptidase. As such,  $[\eta^3\text{-(HCO}_2\text{)Bp}^{\text{Bu}^t\text{,Pr}^t}\text{]ZnCl}$  is an improved synthetic analogue for these enzymes.

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## Notes and References

- For reviews on zinc enzymes, see, B. L. Vallee and D. S. Auld, *Biochemistry*, 1990, **29**, 5647; B. L. Vallee and D. S. Auld, *Acc. Chem. Res.*, 1993, **26**, 543; I. Bertini, C. Luchinat, W. Maret, M. Zeppezauer (Editors), in *Progress in Inorganic Biochemistry and Biophysics*, Birkhauser, Boston, 1986, vol. 1; B. L. Vallee and D. S. Auld, in *Matrix Metalloproteinases and Inhibitors*, eds. H. Birkedal-Hansen, Z. Werb, H. G. Welgus and H. E. van Wart, Gustav Fischer Verlag, New York, 1992, pp. 5–19; B. L. Vallee and D. S. Auld, *Biochemistry*, 1993, **32**, 6493; J. E. Coleman, *Annu. Rev. Biochem.*, 1992, **61**, 897; W. N. Lipscomb and N. Sträter, *Chem. Rev.*, 1996, **96**,

- 2375; R. H. Holm, P. Kennepohl and E. I. Solomon, *Chem. Rev.*, 1996, **96**, 2239.
- R. Alsfasser, S. Trofimenko, A. Looney, G. Parkin and H. Vahrenkamp, *Inorg. Chem.*, 1991, **30**, 4098; A. Looney, R. Han, K. McNeill and G. Parkin, *J. Am. Chem. Soc.*, 1993, **115**, 4690.
- Bis- and tris-(pyrazolyl)hydroborato ligands are represented by the abbreviations  $[\text{Bp}^{\text{R,R}'}]$  and  $[\text{Tp}^{\text{R,R}'}]$ , with the 3- and 5-alkyl substituents listed respectively as superscripts. See, S. Trofimenko, *Chem. Rev.*, 1993, **93**, 943; G. Parkin, *Adv. Inorg. Chem.*, 1995, **42**, 291.
- C. Kimblin, W. E. Allen and G. Parkin, *J. Chem. Soc., Chem. Commun.*, 1995, 1813.
- C. Kimblin, T. Hascall and G. Parkin, *Inorg. Chem.*, 1997, **36**, 5680.
- P. Ghosh and G. Parkin, *Chem. Commun.*, 1998, 413.
- C. Dowling and G. Parkin, *Polyhedron*, 1996, **15**, 2463.
- Several studies concerned with modeling the function of these enzymes have been reported, although the zinc complexes used have not been structurally characterized as tetrahedral [NNO]ZnX derivatives. See, for example, (a) J. T. Groves and R. M. Dias, *J. Am. Chem. Soc.*, 1979, **101**, 1033; (b) K. Ogino, K. Shindo, T. Minami, W. Tagaki and T. Eiki, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1101; (c) C. C. Tang, D. Davalian, P. Huang and R. Breslow, *J. Am. Chem. Soc.*, 1978, **100**, 3918; (d) D. S. Sigman and C. T. Jorgenson, *J. Am. Chem. Soc.*, 1972, **94**, 1724; (e) A. Schepartz and R. Breslow, *J. Am. Chem. Soc.*, 1987, **109**, 1814.
- For structures of carboxypeptidase, see, (a) D. C. Rees, M. Lewis and W. N. Lipscomb, *J. Mol. Biol.*, 1983, **168**, 367; (b) M. F. Schmid and J. R. Herriott, *J. Mol. Biol.*, 1976, **103**, 175; (c) D. W. Christianson and W. N. Lipscomb, *Acc. Chem. Res.*, 1989, **22**, 62.
- For structures of thermolysin, see, (a) M. A. Holmes and B. W. Matthews, *J. Mol. Biol.*, 1982, **160**, 623; (b) D. R. Holland, D. E. Tronrud, H. W. Pley, K. M. Flaherty, W. Stark, J. N. Jansonius, D. B. McKay and B. W. Matthews, *Biochemistry*, 1992, **31**, 11 310; (c) D. R. Holland, A. C. Hausrath, D. Juers and B. W. Matthews, *Protein Science*, 1995, **4**, 1955; (d) B. W. Matthews, *Acc. Chem. Res.*, 1988, **21**, 333.
- W. R. Kester and B. W. Matthews, *J. Biol. Chem.*, 1977, **252**, 7704.
- Thus, carboxypeptidase A exhibits bidentate glutamate binding,<sup>9a</sup> whereas carboxypeptidase B exhibits unidentate co-ordination.<sup>9b</sup> Thermolysin has likewise been reported to crystallize with either uni- or bi-dentate co-ordination, depending upon pH.<sup>10c</sup>
- C. Dowling, V. J. Murphy and G. Parkin, *Inorg. Chem.*, 1996, **35**, 2415.
- $[\text{Bp}^{\text{Bu}^t\text{,Pr}^t}\text{]ZnCl}$  is synthesized by metathesis of ZnCl<sub>2</sub> with  $\text{Ti}[\text{Bp}^{\text{Bu}^t\text{,Pr}^t}]$ , see, C. Dowling, P. Ghosh and G. Parkin, *Polyhedron*, 1997, **16**, 3469.
- A solution of  $[\text{Bp}^{\text{Bu}^t\text{,Pr}^t}\text{]ZnCl}$  (0.25 g, 0.56 mmol) in C<sub>6</sub>H<sub>6</sub> (ca. 10 mL) in an ampoule was treated with CO<sub>2</sub> [ca. 2 atm (1 atm = 101 325 Pa)] and stirred overnight at 50 °C. The volatile components were removed *in vacuo* and the residue was washed with pentane (5 mL) to give  $[\eta^3\text{-(HCO}_2\text{)Bp}^{\text{Bu}^t\text{,Pr}^t}\text{]ZnCl}$  as a white solid (0.16 g, 59%) (Found: C, 51.9; H, 7.5; N, 11.6. Calc. for C<sub>21</sub>H<sub>36</sub>BClN<sub>4</sub>O<sub>2</sub>Zn: C, 51.7; H, 7.4; N, 11.5%). IR data (cm<sup>-1</sup>, KBr pellet): 2586 [ν(B–H)], 1650 and 1336 [ν(C–O)]. <sup>1</sup>H NMR, (C<sub>6</sub>D<sub>6</sub>): δ 1.07 [d, <sup>3</sup>J<sub>H–H</sub> = 7, 6 H, CH(CH<sub>3</sub>)<sub>2</sub>], 1.19 [d, <sup>3</sup>J<sub>H–H</sub> = 7, 6 H, CH(CH<sub>3</sub>)<sub>2</sub>], 3.33 [sept, <sup>3</sup>J<sub>H–H</sub> = 7, 2 H, CH(CH<sub>3</sub>)<sub>2</sub>], 1.54 [s, 18 H, C(CH<sub>3</sub>)<sub>3</sub>], 5.98 [s, 2 H, C<sub>3</sub>N<sub>2</sub>H], 7.21 [s, 1 H, HCO<sub>2</sub>], BH not located. <sup>13</sup>C NMR (C<sub>6</sub>D<sub>6</sub>): δ 22.4 [q, <sup>1</sup>J<sub>C–H</sub> = 127, 2 C, CH(CH<sub>3</sub>)<sub>2</sub>], 23.4 [q, <sup>1</sup>J<sub>C–H</sub> = 127, 2 C, CH(CH<sub>3</sub>)<sub>2</sub>], 26.9 [d, <sup>1</sup>J<sub>C–H</sub> = 129, 2 C, CH(CH<sub>3</sub>)<sub>2</sub>], 30.8 [q, <sup>1</sup>J<sub>C–H</sub> = 126, 6 C, C(CH<sub>3</sub>)<sub>3</sub>], 32.2 [s, 2 C, C(CH<sub>3</sub>)<sub>3</sub>], 100.5 [d, <sup>1</sup>J<sub>C–H</sub> = 175, 2 C, C<sub>3</sub>N<sub>2</sub>H], 158.9 [s, 2 C, C<sub>3</sub>N<sub>2</sub>H], 165.0 [s, 2 C, C<sub>3</sub>N<sub>2</sub>H], 168.2 [d, <sup>1</sup>J<sub>C–H</sub> = 224 Hz, 1 C, HCO<sub>2</sub>].
- Coupling in the <sup>1</sup>H NMR spectrum was observed using enriched <sup>13</sup>CO<sub>2</sub>.
- G. B. Deacon and R. J. Phillips, *Coord. Chem. Rev.*, 1980, **33**, 227.

- 18  $[\eta^3\text{-}(\text{HCO}_2)\text{Bp}^{\text{Bu},\text{Pr}}]\text{ZnCl}$ :  $\text{C}_{21}\text{H}_{36}\text{BClN}_4\text{O}_2\text{Zn}$ , triclinic,  $P\bar{1}$  (no. 2),  $a = 9.800(3)$ ,  $b = 9.856(4)$ ,  $c = 13.742(3)$  Å,  $\alpha = 74.87(3)$ ,  $\beta = 83.04(2)$ ,  $\gamma = 83.95(3)^\circ$ ,  $U = 1268.1(7)$  Å<sup>3</sup>,  $Z = 2$ ,  $\mu = 1.096$  mm<sup>-1</sup>,  $T = 293(2)$  K,  $R1 = 0.0558$  for 3224 reflections. CCDC reference number 186/1038.
- 19 A. Abufarag and H. Vahrenkamp, *Inorg. Chem.*, 1995, **34**, 3279; A. Abufarag and H. Vahrenkamp, *Inorg. Chem.*, 1995, **34**, 2207.
- 20 S. S. Tandon, S. Chander, L. K. Thompson, J. N. Bridson and V. McKee, *Inorg. Chim. Acta*, 1994, **219**, 55.
- 21 T. C. Higgs and C. J. Carrano, *Inorg. Chem.*, 1997, **36**, 291; T. C. Higgs and C. J. Carrano, *Inorg. Chem.*, 1997, **36**, 298.
- 22 For example, only one mononuclear tetrahedral zinc complex of a tridentate [NNO] ligand is listed in the Cambridge Structural Database (Version 5.14). See, E. J. Corey, P.-W. Yuen, F. J. Hannon and D. A. Wierda, *J. Org. Chem.*, 1990, **55**, 784.
- 23 W. Leitner, *Coord. Chem. Rev.*, 1996, **153**, 257 and refs. therein; A. Behr, *Carbon Dioxide Activation by Metal Complexes*, VCH, Weinheim, 1988.
- 24 The first report of CO<sub>2</sub> insertion into a B-H bond of which we are aware involves the reaction of NaBH<sub>4</sub> with CO<sub>2</sub> to give, depending upon conditions, Na<sub>x</sub>[BO(OCH<sub>3</sub>)(O<sub>2</sub>CH)]<sub>x</sub> and Na[HB(O<sub>2</sub>CH)<sub>3</sub>].<sup>24a</sup> However, these compounds were neither spectroscopically nor structurally authenticated. More recently, [(biL)(Ph<sub>3</sub>P)Cu][BH<sub>4</sub>] was reported to react with CO<sub>2</sub> in the presence of PPh<sub>3</sub> to give [(biL)(Ph<sub>3</sub>P)<sub>2</sub>Cu][HB(O<sub>2</sub>CH)<sub>3</sub>] and [(biL)(Ph<sub>3</sub>P)<sub>2</sub>Cu][H<sub>2</sub>B(O<sub>2</sub>CH)<sub>2</sub>] (biL = 1,10-phenanthroline and 3,4,7,8-tetramethyl-1,10-phenanthroline), which were spectroscopically characterized.<sup>24b</sup> (a) T. Wartik and R. K. Pearson, *J. Inorg. Nucl. Chem.*, 1958, **7**, 404; (b) G. L. Monica, G. A. Ardizzoia, F. Cariati, S. Cenini and M. Pizzotti, *Inorg. Chem.*, 1985, **24**, 3920.
- 25 For an example of CO<sub>2</sub> insertion into a B-N bond, see, A. Meller and A. Ossko, *Monatsh. Chem.*, 1972, **103**, 577.

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