

Protonation of the Hydroxide Ligand in a Synthetic Analogue of Carbonic Anhydrase, $[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{ZnOH}$: Inhibition of Reactivity Towards CO_2

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An essential step in the mechanisms of action of a large variety of zinc enzymes, such as carbonic anhydrase, involves reversible proton transfer which serves to interconvert the aqua and hydroxide forms of the active sites, $[\text{LZn}-\text{OH}_2]^{2+}$ and $[\text{LZn}-\text{OH}]^+$.^{1,2} Surprisingly, however, examples of this transformation for which both partners have been isolated and structurally characterized are unknown. In this paper, we report the synthesis and structural characterization of a monomeric zinc aqua complex, which is obtained by protonation of the hydroxide form of a synthetic analogue of carbonic anhydrase, and also demonstrate that protonation inhibits reactivity towards CO_2 .

Carbonic anhydrase, one of the most extensively studied zinc enzymes, possesses an active site that is composed of a tetrahedral zinc center which is coordinated to a water molecule and three histidine donors [NNN] from the protein backbone.¹ The best small molecule synthetic analogues which structurally mimic this $\{[\text{NNN}]\text{ZnOH}_n\}$ ($n = 1, 2$) coordination environment are the tris-(pyrazolyl)hydroborato and tris(imidazolyl)phosphine complexes $[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{ZnOH}$,³ $[\text{Tp}^{\text{Ar}, \text{Me}}]\text{ZnOH}$ ($\text{Ar} = \text{C}_6\text{H}_4\text{Pr}^i$),⁴ and $\{[\text{Pim}^{\text{Pr}, \text{Bu}^t}]\text{ZnOH}\}(\text{ClO}_4)$.^{5,6} To date, however, reactions of these hydroxide complexes with protic reagents have not yielded isolable zinc aqua complexes, a principal problem being that the incipient aqua ligand is readily, and irreversibly,⁷ displaced by the counterion.⁸ Indeed, Vahrenkamp has used this method extensively for preparing a variety of $[\text{Tp}^{\text{RR}}]\text{ZnX}$ complexes; for example, the reaction of $[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{ZnOH}^3$ with $p\text{-TolS}(\text{O})_2\text{OH}$ yields $[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{ZnOS}(\text{O})_2\text{Tol}$.^{8c}

In view of the above formation of $[\text{Tp}^{\text{RR}}]\text{ZnX}$ complexes in reactions of $[\text{Tp}^{\text{RR}}]\text{ZnOH}$ with HX , it is significant that the hydroxide ligand in $[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{ZnOH}$ may be reversibly protonated to give a zinc aqua derivative $\{[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{Zn}(\text{OH}_2)\}^+$. Specifically, $(\text{C}_6\text{F}_5)_3\text{B}(\text{OH}_2)^{9-11}$ is capable of protonating $[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{ZnOH}$ to give an aqua complex $\{[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{Zn}(\text{OH}_2)\}[\text{HOB}(\text{C}_6\text{F}_5)_3]$ in which the water molecule is *not* displaced by the counterion (Scheme 1). The importance of employing the $[(\text{C}_6\text{F}_5)_3\text{BOH}]^-$ counterion to stabilize the zinc aqua moiety is underscored by

(1) See, for example: (a) Holm, R. H.; Kennepohl, P.; Solomon, E. I. *Chem. Rev.* **1996**, *96*, 2239–2314. (b) Lipscomb, W. N.; Sträter, N. *Chem. Rev.* **1996**, *96*, 2375–2433. (c) Kimura, E.; Koike, T.; Shionoya, M. *Struct. Bonding* **1997**, *89*, 1–28. (d) Lindskog, S. *Pharmacol. Ther.* **1997**, *74*, 1–20.

(2) For calculations of the pK_a of zinc aqua complexes in model systems, see: Bertini, I.; Luchinat, C.; Rosi, M.; Sgamellotti, A.; Tarantelli, F. *Inorg. Chem.* **1990**, *29*, 1460–1463.

(3) Alsasser, R.; Trofimenko, S.; Looney, A.; Parkin, G.; Vahrenkamp, H. *Inorg. Chem.* **1991**, *30*, 4098–4100.

(4) Ruf, M.; Vahrenkamp, H. *Inorg. Chem.* **1996**, *35*, 6571–6578.

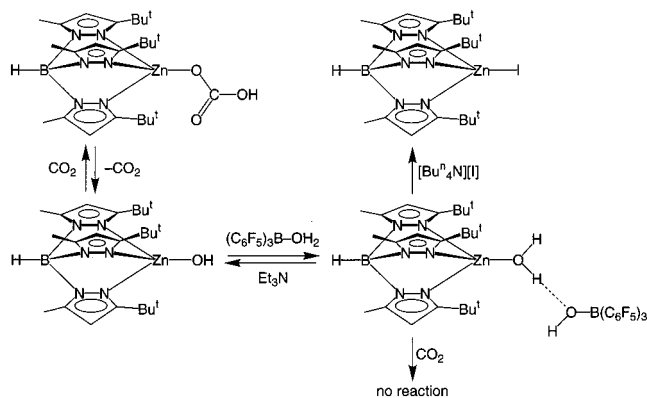
(5) Kimblin, C.; Allen, W. E.; Parkin, G. *J. Chem. Soc., Chem. Commun.* **1995**, 1813–1815.

(6) An excellent functional model is provided by Kimura's triazacyclododecane complex $\{(\text{tacd})\text{Zn}(\text{OH})_2(\text{ClO}_4)_2(\text{HClO}_4)\}$. See: Kimura, E.; Shiota, T.; Koike, T.; Shiro, M.; Kodama, M. *J. Am. Chem. Soc.* **1990**, *112*, 5805–5811.

(7) The irreversibility refers to the reaction of $[\text{Tp}^{\text{RR}}]\text{ZnX}$ with H_2O , since $[\text{Tp}^{\text{RR}}]\text{ZnOH}$ may, in certain circumstances, be regenerated from $[\text{Tp}^{\text{RR}}]\text{ZnX}$ by reaction with OH^- sources. See, for example: Parkin, G. *Adv. Inorg. Chem.* **1995**, *42*, 291–393.

(8) (a) Hartmann, U.; Vahrenkamp, H. *Chem. Ber.* **1994**, *127*, 2381–2385. (b) Ruf, M.; Weis, K.; Vahrenkamp, H. *J. Chem. Soc., Chem. Commun.* **1994**, 135–136. (c) Brandsch, T.; Schell, F.-A.; Weis, K.; Ruf, M.; Müller, B.; Vahrenkamp, H. *Chem. Ber./Recueil* **1997**, *130*, 283–289. (d) Ruf, M.; Weis, K.; Brasack, I.; Vahrenkamp, H. *Inorg. Chim. Acta* **1996**, *250*, 271–281. (e) Ruf, M.; Weis, K.; Vahrenkamp, H. *Inorg. Chem.* **1997**, *36*, 2130–2137. (f) Hikichi, S.; Tanaka, M.; Moro-oka, Y.; Kitajima, N. *J. Chem. Soc., Chem. Commun.* **1992**, 814–815.

Scheme 1



the fact that the coordinated water is displaced by addition of $[\text{Bu}^n, \text{N}][\text{I}]$ to give $[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{ZnI}$.¹² The use of $(\text{C}_6\text{F}_5)_3\text{B}(\text{OH}_2)$ as an acid to obtain a stable zinc aqua complex is also noteworthy since it has been reported that treatment of both $[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{ZnOH}$ and $[\text{Tp}^{\text{Cum}, \text{Me}}]\text{ZnOH}$ with the alternative acid, HClO_4 , results in hydrolytic destruction of the tris(pyrazolyl)borato ligand.^{8c} The formation of $\{[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{Zn}(\text{OH}_2)\}^+$ is, as expected, reversible, and subsequent treatment with Et_3N regenerates $[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{ZnOH}$.

The molecular structure of $\{[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{Zn}(\text{OH}_2)\}[\text{HOB}(\text{C}_6\text{F}_5)_3]$ has been determined by X-ray diffraction (Figure 1), thereby allowing important details of the bonding to be ascertained. Of particular note, the Zn–O bond [1.937(2) Å] is significantly longer than that in the parent hydroxide $[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{ZnOH}$ [1.850(8) Å],^{3,13} thus providing excellent evidence that the hydroxide ligand has been protonated.¹⁴ Correspondingly, the B–O bond [1.502(3) Å] is substantially shortened upon deprotonation relative to that in the aqua complex $(\text{C}_6\text{F}_5)_3\text{B}(\text{OH}_2)$ [1.608(3) Å],^{9b,15} and is comparable to that in the isolated $[(\text{C}_6\text{F}_5)_3\text{BOH}]^-$ anion (1.49 Å).^{16–18}

The structural study also indicates that $\{[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{Zn}(\text{OH}_2)\}[\text{HOB}(\text{C}_6\text{F}_5)_3]$ exists as a hydrogen-bonded ion pair, with an $\text{O}\cdots\text{O}$ separation of 2.480(3) Å.¹⁹ In accord with the zinc aqua

(9) $(\text{C}_6\text{F}_5)_3\text{B}(\text{OH}_2)$ is prepared by addition of 1 equiv of H_2O to $(\text{C}_6\text{F}_5)_3\text{B}$ in pentane^{9a} and its nature has been determined by X-ray diffraction.^{9b} It should be noted that the hydrated derivative $[(\text{C}_6\text{F}_5)_3\text{B}(\text{OH}_2)]\cdot 2\text{H}_2\text{O}$ has also been reported.^{9c,d} (a) Bradley, D. C.; Harding, I. S.; Keefe, A. D.; Motevalli, M.; Zheng, D. H. *J. Chem. Soc., Dalton Trans.* **1996**, 3931–3936. (b) Harlan, C. J.; Bergquist, C.; Bridgewater, B. M.; Norton, J. R.; Parkin, G., unpublished results. (c) Siedle, A. R.; Lamanna, W. M.; Newmark, R. A.; Stevens, J.; Richardson, D. E.; Ryan, M. *Makromol. Chem., Macromol. Symp.* **1993**, *66*, 215–224. (d) Danopoulos, A. A.; Galsworthy, J. R.; Green, M. L. H.; Cafferkey, S.; Doerrer, L. H.; Hursthouse, M. B. *J. Chem. Soc., Chem. Commun.* **1998**, 2529–2530.

(10) The acidic nature of $[(\text{C}_6\text{F}_5)_3\text{B}(\text{OH}_2)]\cdot 2\text{H}_2\text{O}$ has been previously reported.^{10a–c} Furthermore, in situ generated $(\text{C}_6\text{F}_5)_3\text{B}(\text{OH}_2)$ has also been used as a proton acid. Specifically, (i) addition of H_2O to a mixture of $(\text{Bu}^t)_2\text{bpy}-\text{PtMe}_2$ and $(\text{C}_6\text{F}_5)_3\text{B}$ has been reported to give $[(\text{Bu}^t)_2\text{bpy}]\text{Pt}(\text{Me})\{[\text{HOB}(\text{C}_6\text{F}_5)_3]\}^+$,^{10b} and (ii) traces of water causes $(\text{C}_6\text{F}_5)_3\text{B}$ to polymerize isobutylene.^{10c} (a) References 9c and 9d. (b) Hill, G. S.; Manojlovic-Muir, L.; Muir, K. W.; Puddephatt, R. J. *Organometallics* **1997**, *16*, 525–530. (c) Shaffer, T. D.; Ashbaugh, J. R. *J. Polym. Sci., Part A: Polym. Chem.* **1997**, *35*, 329–344.

(11) For a review of the applications of $\text{B}(\text{C}_6\text{F}_5)_3$, see: Piers, W. E.; Chivers, T. *Chem. Soc. Rev.* **1997**, *26*, 345–354.

(12) $[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{ZnI}$ has been previously described. See: Looney, A. Ph.D. Thesis, Columbia University, 1993.

(13) It is also noteworthy that the Zn–O bond length in $\{[\text{Tp}^{\text{Bu}^t, \text{Me}}]\text{Zn}(\text{OH}_2)\}^+$ is longer than the values in dinuclear $\{[\text{Tp}^{\text{RR}}]\text{Zn}\}$ complexes with bridging $[\text{H}_2\text{O}_2]$ moieties [1.871(6) – 1.916(6) Å]. See: Ruf, M.; Weis, K.; Vahrenkamp, H. *J. Am. Chem. Soc.* **1996**, *118*, 9288–9294.

(14) The Zn–OH₂ bond length in the five-coordinate tris(benzimidazolylmethyl)amine complex $\{[\text{BIMA}]\text{Zn}(\text{OH}_2)_2\}(\text{MeSO}_3)_2$ is 2.011(3) Å. See ref 8c.

(15) Note that longer B–O bonds are observed in the hydrogen-bonded species $[(\text{C}_6\text{F}_5)_3\text{B}(\text{OH}_2)]\cdot 2\text{H}_2\text{O}$ [1.577(1) Å]^{15a} and $(\text{C}_6\text{F}_5)_3\text{B}(\text{OH}_2)\cdot \text{dioxane}\cdot \text{CH}_2\text{Cl}_2$ [1.565(3) Å].^{15b} (a) Reference 9d. (b) Janiak, C.; Braun, L.; Scharmann, T. G.; Girsdsies, F. *Acta Crystallogr.* **1998**, *C54*, 1722–1724.

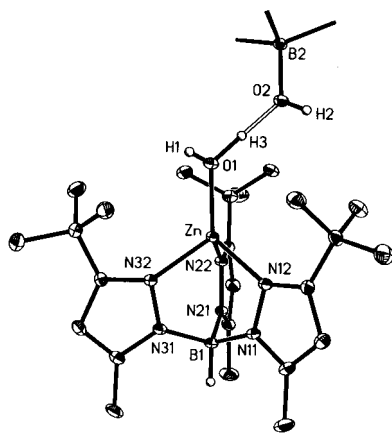


Figure 1. Molecular structure of $\{[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{Zn}(\text{OH}_2)\}[\text{HOB}(\text{C}_6\text{F}_5)_3]$ (for clarity, only a portion of the anion is shown). Selected bond lengths (Å) and angles (deg): Zn–O(1) 1.937(2), Zn–N(12) 2.023(2), Zn–N(22) 2.007(2), Zn–N(32) 2.025(2), O(1)–H(1) 0.78(4), O(1)–H(3) 1.09(4), O(2)–H(2) 0.78(4), O(2)–H(3) 1.39(4), O(1)···O(2) 2.480(3), B(2)–O(2) 1.502(3); O(1)–Zn–N(12) 123.01(8), O(1)–Zn–N(22) 119.16(9), O(1)–Zn–N(32) 119.87(9).

formulation of the structure, the bridging hydrogen is much more displaced towards the zinc oxygen than towards the boron oxygen [O(1)–H(3) = 1.09(4) Å and O(2)–H(3) 1.39(4) Å].²⁰ The interaction also persists in benzene solution, with the $[\text{H}_3\text{O}_2]$ moiety being characterized by ν_{OH} absorptions at 3660, 3637, and 3445 cm^{-1} in the IR spectrum, of which the lowest energy signal is attributed to the hydrogen-bonded interaction.

The existence of a hydrogen-bonding interaction is a clear indication of the acidic nature of the coordinated water molecule in the $\{[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{Zn}(\text{OH}_2)\}^+$ cation. In this regard, it is pertinent to note that the zinc water ligand at the active site of carbonic anhydrase also participates in a hydrogen bond with Thr-199;²¹ the hydrogen-bonding interaction within $\{[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{Zn}(\text{OH}_2)\}[\text{HOB}(\text{C}_6\text{F}_5)_3]$ thus has analogies to that of the enzyme active site.

One of the most important notions concerned with the mechanism of action of carbonic anhydrase is that the coordinated

(16) For example, $[\text{Cp}^*\text{Ta}(\text{Me})(\text{OH})][(\text{C}_6\text{F}_5)_3\text{BOH}]$ [1.49(1) Å],^{16a} $[\text{Et}_3\text{NH}][(\text{C}_6\text{F}_5)_3\text{BOH}]$ [1.487(3) Å],^{16b,c} and $[\text{K}(\text{dibenzo-18-crown-6})][(\text{C}_6\text{F}_5)_3\text{BOH}][(\text{C}_6\text{F}_5)_3\text{B}(\text{OH}_2)] \cdot \text{H}_2\text{O} \cdot \text{MeCHO}$.^{16d} (a) Schaefer, W. P.; Quan, R. W.; Bercaw, J. E. *Acta Crystallogr.* **1993**, C49, 878–881. (b) Siedle, A. R.; Newmark, R. A.; Lamanna, W. M.; Huffman, J. C. *Organometallics* **1993**, 12, 1491–1492. (c) The bond length for $[\text{Et}_3\text{NH}][(\text{C}_6\text{F}_5)_3\text{BOH}]$ is erroneously attributed to $[\text{Et}_3\text{NH}][(\text{C}_6\text{H}_5)_3\text{BOH}]$ due to a typographic error (Huffman, J. C., personal communication). (d) Reference 9d.

(17) $[(\text{C}_6\text{F}_5)_3\text{BOH}]^-$ is also known to act as a coordinating anion capable of binding to other centers, but with concomitantly longer bond lengths than in the noncoordinating anion, for example, $(\text{Bu}^i\text{bpy})\text{Pt}(\text{Me})\{[\text{HOB}(\text{C}_6\text{F}_5)_3]\}$ [1.526(3) Å]^{17a} and $[\text{CpIr}(\text{COD})\text{H}][\{(\text{C}_6\text{F}_5)_3\text{B}\}_2(\mu\text{-OH})]$ [1.566(6) and 1.564(6) Å].^{17b} (a) Reference 10b. (b) Reference 9d.

(18) Other structurally characterized complexes with $[(\text{C}_6\text{F}_5)_3\text{B}-\text{O}]$ bonds include the adducts $[(\text{C}_6\text{F}_5)_3\text{B}-\text{OC}(\text{X})\text{Ph}]$: X = H [1.610(8) Å], Me [1.576(5) Å], OEt [1.594(6) Å], NPr_2 [1.52(1) Å]. See: Parks, D. J.; Piers, W. E.; Parvez, M.; Atencio, R.; Zaworotko, M. J. *Organometallics* **1998**, 17, 1369–1377.

(19) For a review of hydrogen bonding involving $[\text{H}_3\text{O}_2]^-$, see: Ardon, M.; Bino, A. *Struct. Bonding* **1987**, 65, 1–28.

(20) For some dinuclear $\{[\text{Tp}^{\text{RR}}]\text{Zn}\}$ complexes with bridging $[\text{H}_3\text{O}_2]$ moieties, see ref 13.

water is deprotonated prior to reaction with CO_2 .^{1,22} However, such a proposal has not been demonstrated by direct comparison of the reactivity of a pair of structurally characterized $[\text{LZn}(\text{OH})]$ and $[\text{LZn}(\text{OH}_2)]^+$ complexes with coordination environments that mimic well the active site of carbonic anhydrase.^{23,24} The isolation of both $[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{ZnOH}$ and its conjugate acid $\{[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{Zn}(\text{OH}_2)\}[\text{HOB}(\text{C}_6\text{F}_5)_3]$, therefore, provides a unique opportunity to study such a proposition in a well-defined system. Significantly, whereas $[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{ZnOH}$ is in rapid equilibrium with the bicarbonate derivative $[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{ZnOC}(\text{O})\text{OH}$ in the presence of CO_2 ,²⁵ its conjugate acid $\{[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{Zn}(\text{OH}_2)\}[\text{HOB}(\text{C}_6\text{F}_5)_3]$ does not react with CO_2 under comparable conditions.²⁶ Such direct comparison provides an excellent demonstration that deprotonation of the zinc-bound water is indeed an essential step in the mechanism of action of carbonic anhydrase.

In summary, protonation of the zinc hydroxide complex $[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{ZnOH}$ by $(\text{C}_6\text{F}_5)_3\text{B}(\text{OH}_2)$ yields the aqua derivative $\{[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{Zn}(\text{OH}_2)\}^+$, a transformation that results in a lengthening of the Zn–O bond by ca. 0.1 Å. The protonation is reversible and treatment of $\{[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{Zn}(\text{OH}_2)\}^+$ with Et_3N regenerates $[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{ZnOH}$. Consistent with the notion that the catalytic hydration of CO_2 by carbonic anhydrase requires deprotonation of the coordinated water molecule, $\{[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{Zn}(\text{OH}_2)\}^+$ is inert towards CO_2 , whereas $[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{ZnOH}$ is in rapid equilibrium with the bicarbonate complex $[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{ZnOC}(\text{O})\text{OH}$.

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Supporting Information Available: Experimental and crystallographic information (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(21) In addition, the zinc-bound water is also part of a hydrogen-bonding network involving additional water molecules which mediate a proton shuttle to His-64, prior to proton transfer to the surroundings. See: (a) Denisov, V. P.; Jonsson, B.-H.; Halle, B. *J. Am. Chem. Soc.* **1999**, 121, 2327–2328. (b) Toba, S.; Colombo, G.; Merz, K. M., Jr. *J. Am. Chem. Soc.* **1999**, 121, 2290–2302. (c) Christianson, D. W.; Fierke, C. A. *Acc. Chem. Res.* **1996**, 29, 331–339 and references therein. (d) Liang, Z.; Xue, Y.; Behravan, G.; Jonsson, B.-H.; Lindskog, S. *Eur. J. Biochem.* **1993**, 211, 821–827. (e) Merz, K. M., Jr. *J. Mol. Biol.* **1990**, 214, 799–802. (f) Eriksson, A. E.; Jones, A. T.; Liljas, A. *Proteins* **1988**, 4, 274–282. (g) Xue, Y.; Liljas, A.; Jonsson, B.-H.; Lindskog, S. *Proteins* **1993**, 17, 93–106. (h) Håkansson, K.; Carlsson, M.; Svensson, L. A.; Liljas, A. *J. Mol. Biol.* **1992**, 227, 1192–1204.

(22) Zhang, X.; Hubbard, C. D.; van Eldik, R. *J. Phys. Chem.* **1996**, 100, 9161–9171.

(23) The pH dependence of the kinetics of hydration of CO_2 by certain macrocyclic zinc complexes, nevertheless, suggests that the reactions proceed via initial deprotonation of a zinc-bound water. See, for example: (a) Zhang, X.; van Eldik, R. *Inorg. Chem.* **1995**, 34, 5606–5614. (b) Zhang, X.; van Eldik, R.; Koike, T.; Kimura, E. *Inorg. Chem.* **1993**, 32, 5749–5755.

(24) NMR spectroscopic data has been suggested to provide evidence for deprotonation of a zinc-bound water molecule in the tris(benzimidazolyl)amine complex $\{[\text{BIMA}]\text{Zn}(\text{OH}_2)\}^{2+}$.^{24a} The latter complex is not, however, strictly four-coordinate since the amine nitrogen atom also coordinates to the zinc center.^{24b} (a) Nakata K.; Uddin M. K.; Ogawa, K.; Ichikawa, K. *Chem. Lett.* **1997**, 991–992. (b) Reference 8c.

(25) Looney, A.; Han, R.; McNeill, K.; Parkin, G. *J. Am. Chem. Soc.* **1993**, 115, 4690–4697.

(26) Since we do not observe any lifetime broadening for $\{[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{Zn}(\text{OH}_2)\}^+$ in the presence of CO_2 , we can estimate that its reactivity towards CO_2 is at least a factor of 10^2 less than that of $[\text{Tp}^{\text{Bu}^i,\text{Me}}]\text{ZnOH}$.