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Modeling Zinc Enzyme Inhibition with Functional Thiolate Ligands

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The blocking of zinc enzymes by thiolate-containing inhibitors was modeled by treating $Tp^{Ph,Me}Zn–OH$ with functional thiols. The latter were chosen such that they contain an additional donor function (COOH, COOR, NH_2, NHR, OH) in a position favorable for chelation. Of them, mercapto carboxylic acid esters were incorporated as thiolates. The corresponding mercapto carboxylic acids, however, used only their carboxylate function for coordination. Various mercapto amines, mercapto alcohols, and mercaptophenol were exclusively converted to thiolate ligands. The two modes of inhibitor attachment, terminal or chelating, were observed equally frequently. As a rule, they occur as alternatives for similar ligands. In case of 2-mercaptophenol they coexist in the crystalline state and in solution. Hydrogen bonding, both intra- and intermolecular, seems to be a decisive factor determining the inhibitor attachments. Its persistence in solution is underlined by the observation that $Tp^{Ph,Me}Zn–$hydroxythiophenolates are methylated about 2 orders of magnitude slower than $Tp^{Ph,Me}Zn–SPh$ itself.

Introduction

Many widespread diseases, like arthritis, multiple sclerosis, high blood pressure, and even cancer, have a relation to the action of zinc-containing enzymes.[1−3] Likewise many potent toxins, like snake venoms, the anthrax lethal factor, or botulinus toxin, contain zinc enzymes as the active ingredients.[4−6] Accordingly, the controlled inhibition of the related enzymes by drug substances is of high significance in medical research.

As the inhibitor should bind to the active center of the enzyme, it ought to be a good ligand for zinc. Furthermore the assumption that an efficient enzyme−inhibitor complex is a transition state analogue (i.e. its constitution and geometry resemble that of the transition state of the zinc-catalyzed enzymatic reaction[7−9]) suggests that a chelating bidentate nature of the ligand is favorable for inhibition. This assumption and empirical work along the same lines have made hydroxamates,[3,10] carboxylates,[2,3] and β-ketoenolates[3,11] preferred objects of related research. Until today, however, the clinical success of the resulting drugs has been limited.

Another attractive zinc-binding group is the thiolate function. Not only are the thiolates good ligands for zinc but also are most of the zinc-containing matrix metalloproteases "turned on" by the cysteine switch which consists of the removal of a side chain bound to zinc through cysteinate.[1,3] Accordingly, many thiolates and recently also chelating ligands containing one thiol or thione function have been tested as inhibitors for matrix metalloproteases and toxins.[3,12−16]

Our approach to this field is that of coordination chemistry with simple ligands. The "Freiburg enzyme model" is the group of TpZn−OH complexes, particularly $Tp^{Ph,Me}Zn−OH$

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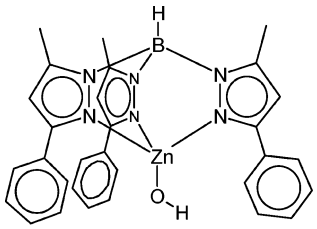
(**1**). It could be shown that the attachment of hydroxamates or α-ketoalkoxides to the TpZn unit creates "transition state analogues" related to the action of matrix metalloproteases or class II aldolases,[9,17] and a structure correlation analysis of such TpZn-chelate complexes led to a proposal for the geometric pathway of zinc enzyme-catalyzed hydrolyses.[9,18]

In the past 15 years we have tackled the interactions between zinc complexes and drug substances sporadically,[19−23] and we have combined the TpZn unit with some pharmaceutically relevant substrates.[17,24−27] We have now come back to these studies with emphasis on enzyme inhibition. One purpose of this work is collecting information on the structure and bonding of enzyme/inhibitor interactions using the small molecule approach. More important, however, is our intention to use the coordination chemist's knowledge to find, apply in the models, and then propose to the drug researcher new types of chelating ligands which may be suitable as zinc-binding groups in drugs.

From these studies we have so far published the work with some new hydroamates[28] and an extensive investigation of chelating keto compounds.[29] Some of our work is related to and complemented by similar work by Cohen and co-workers, who in recent years have used the enzyme model **1** to test interactions between zinc and hydroxamates, thiolates, pyridinones, and chelating Schiff bases[30−34] and who have extended their studies to in vitro testing with zinc-containing proteases.[15]



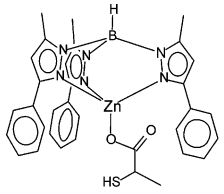
1

This paper reports our work with thiol-containing "inhibitors". All thiols used contained one additional function which is suitable for chelation and in some cases may become a competing donor after deprotonation. The purpose of the work was to find out which factors control monodentate or chelating coordination and the structure and stability of the inhibitor complexes. We wanted to learn how strong the preference of thiolate over the competing donors is for zinc coordination and what the role of hydrogen bonding is in the zinc inhibitor interactions. For this purpose the functional thiols were reacted with complex **1**, the enzyme model.

Results and Discussion

General Remarks. This paper reports 11 new complexes. Six of them can be classified as TpZn complexes with monodentates thiolate ligands and the other five as species containing O,O, S,O, or S,N chelating ligands. Nine of the complexes were characterized by structure determinations. An inspection of the drawings of the molecular structures has shown that they do not convey any important information that is not already represented by the formula drawings. Hence it was decided to present all structural details in the Supporting Information and to focus the discussion of the structures on general aspects in a separate chapter with tabular material; see below.

Derivatives of Mercaptopropionic Acid. Both 2- and 3-mercaptopropionic acid were applied as the free acids and in the form of esters. The free acids showed sluggish reactions with **1**, the isolable products of which were the carboxylate complexes **2** and **3** in low to medium yields. The structure determination of **3** and the observation of the SH resonances in the ^{1}H NMR spectra confirmed that in both complexes the thiol function is not involved in any interaction with the metal. There is a thermodynamic interpretation of this observation in the pK_a values of the functional groups, which for the COOH function are 5−8 units lower than for the SH function. But one might have expected that the high affinity between zinc and sulfur might overcome this by chelation or the formation of dinuclear complexes such that both thiolate and carboxylate are bound to zinc. Yet this was not observed here, although we previously observed the latter for homocysteine.[35]



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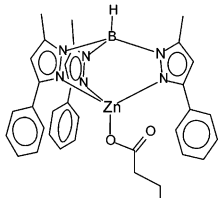

3

The esters of both mercaptopropionic acids underwent smooth reactions with **1**, resulting in the thiolate complexes **4** and **5** in good yields. The IR bands for the ester carbonyl in **4** (1687 cm^{-1}) and **5** (1737 cm^{-1}) indicated different bonding patterns. The structure determinations confirmed this by revealing the striking difference between the two compounds. Only in **4**, where a favorable five-membered chelate

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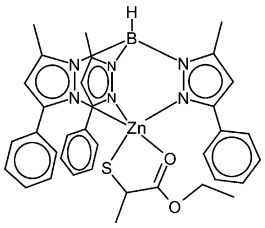
ring can be formed, does the carbonyl function act as a fifth donor for zinc.



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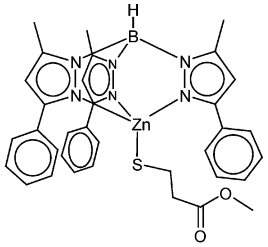
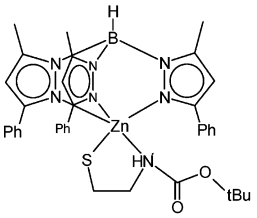


5

Our findings for the mercaptopropionic acids complement those by Cohen for similar functionalized aliphatic and aromatic thiols.[30,33] In his case three out of four structures showed the predicted chelation, while 2-mercaptobenzoate was bound with a noncoordinating SH group even though chelation should be favorable. In view of these findings it must be stated that the mode of attachment of thiol-functionalized carboxylate inhibitors to zinc in enzymes cannot be predicted and that structure−activity correlations based on a presumed mode of attachment[3,12,16,36] should be viewed with some reservation.

***β*-Mercaptoamines.** It has been known for a long time that aminoethanethiolates are very good chelators for zinc[37] (although the structure of the basic 1:2 complex was reported only recently[38]), and we have reported corresponding results for the TpZn moiety.[39] We now wanted to test whether a reduction of the basicity of the amine nitrogen would make it unable to coordinate to the TpZn unit, thereby making the *β*-aminothiolates monodentate. For this purpose we chose *N*-Boc-aminoethanethiol and 2-mercaptoaniline. Their reactions with **1** yielded complexes **6** and **7**.



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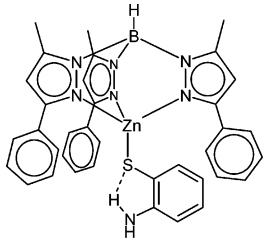


7

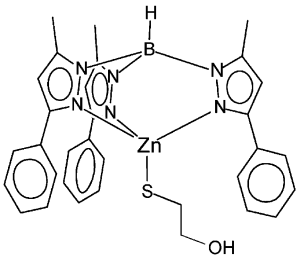
The structural assignment of **6** rests on its ^{1}H NMR spectrum in which the resonances for the two CH_2 groups and the NH proton are shifted upfield by 1−2 ppm relative to those of the free *N*-Boc-aminoethanethiol. This corresponds to the experience that embedding between the three phenyl rings of $Tp^{Ph,Me}$ causes upfield shifts, the magnitude of which for **6** indicates that the aminoethane fragment is close to the zinc ion. This points to a chelating mode of the *N*-Boc-aminoethanethiolate ligand, although it does not prove it. Support for this assignment comes, however, from the structure of the corresponding complex of cysteine ethyl ester, where the chelating amine nitrogen is of similarly reduced basicity.[39]

Complex **7** was characterized by a structure determination. Unlike in **6** the amino group does not coordinate to zinc despite the favorable conditions for chelation. Instead it gets involved in a hydrogen bond with sulfur. A similar observation was made by Cohen for the $Tp^{Ph,Me}$Zn complex of 2-mercaptobenzamide[33] where, however, the dispositions of the amine and thiolate functions are unfavorable for a chelating attachment of the N,S ligand.

With respect to enzyme inhibition these results with amine−thiolates again produce a caveat. Hydrogen bonding is omnipresent in proteins, and as the comparison of **6** and **7** shows, it can overcome the chelating tendency of the amine function. Even though this may be of advantage for the stability of the enzyme−inhibitor complex, it sheds doubts on the attempts to correlate structure and function.

***β*-Mercapto Alcohols.** There has been some success with derivatives of *β*-mercapto alcohols as inhibitors for matrix methalloproteases, and it was suggested that they bind to zinc as S,O chelators.[13,14] However, both we[39] and Cohen[30] had observed that hydroxyethanethiolate and the methylated derivatives thereof are bound to zinc in a monodentate fashion with the hydroxy group in an anti position with respect to the thiolate. We now came back to this group of thiolates with the question whether a change of the reaction conditions or a variation of substituents might induce chelation.

Complex **8** had been obtained by us[39] by crystallization from dichloromethane/methanol and by Cohen[30] from benzene/pentane. It came as a surprise that recrystallization from hot acetonitrile produced crystals of **8′**. In solution the ^{1}H NMR data for **8′** are identical with the previously observed data for for **8**. But in the crystal there is a syn-arrangement of the hydroxy and thiolate functions which goes along with the O−H···S hydrogen bond (O···S 2.99 Å). Thereby the hydrogen bond arrangement in **8′** is fully analogous to that in **7**.



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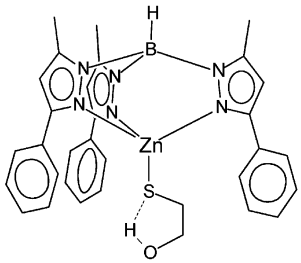

8'

After the surprise with **8′** we applied 1-thioglycerol, assuming that its second hydroxy function would strengthen the tendency to form intra- or intermolecular hydrogen bonds. It was another surprise that the resulting thiolate complex **9** bears no resemblance at all to **8** or **8′**. **9** was difficult to crystallize. The crystals which were finally obtained from

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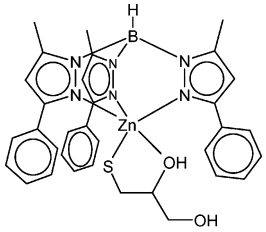
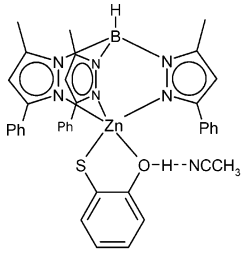
hot dichloromethane contained both the solvent and 3-methyl-5-phenylpyrazole (resulting from decomposition of the Tp ligand) hydrogen bonded to the two OH groups, with an additional N−H···S hydrogen bond between the pyrazole and the thiolate sulfur. The surprise was the fact that in these crystals the hydroxyethanethiolate fragment of the thioglycerol acts as a chelate ligand, unlike in all previously crystallized 2-hydroxyethanethiolate complexes of the TpZn unit.[30,39] The pure compound **9** seems to be a chelate complex too, according to its ^{1}H NMR spectrum which again shows large high-field shifts for the SCH_2 and CH protons compared to those of free thioglycerol.



9

As a result, it can be stated that the β-hydroxythiolates just like the carbalkoxy- and the β-aminothiolates before can be induced to act both as monodentate and as chelating ligands for the TpZn unit. Further research is necessary to learn how far hydrogen bonding determines either of the alternatives and how this can be controlled.

Hydroxythiophenols. The simplest of these, 2-mercaptophenol, is a rather strong acid. Previously we had observed that in its reactions with TpZn−OH complexes the Tp ligands are hydrolyzed, resulting in untractable reaction products. This problem was overcome now by dilution techniques, and the reaction with **1** produced the expected thiolate complex in good yields. Its ^{1}H NMR spectrum at room temperature was inconclusive, showing a multiplet structure for the four thiophenolate protons at 5.72−6.42 ppm. Thus, the first structural information was obtained from crystals grown from acetonitrile. In these crystals both modes of attachment of the 2-hydroxythiophenolate ligand are realized at the same time: bidentate in **10** and monodentate in **11**.



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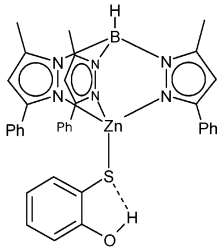
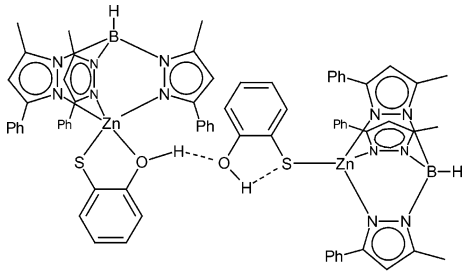


11

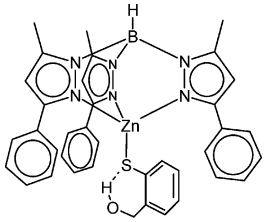
In **10** a cocrystallized acetonitrile molecule is attached to the OH function by a hydrogen bond. Assuming that the absence of this hydrogen bond would render **10** unstable compared to **11**, we recrystallized the compound from benzene. Solvent-free crystals resulted, the ^{1}H NMR spectrum of which is identical with that of the CH_3CN-solvated species. In the crystals, however, both the bidentate and the monodentate attachment of the 2-hydroxythiophenolate are realized again, this time in the form of **10′**/**11′**. In principle, **10′** and **11′** show the same bonding pattern as **10** and **11**, i.e., with an external hydrogen bond for **10′** and an internal one for **11′**. The difference is that this time the hydrogen bond from the OH function of **10′** involves the attachment of the OH function of **11′**, thereby making **10′**/**11′** an unsymmetrically ligand-bridged dimer.



10′/11′

After these observations were made, the ^{1}H NMR spectra were recorded again at variable temperatures. Indeed, after cooling of the sample below −10 °C, the multiline multiplet for the C_6H_4 unit became structured, showing separate multiplets for six protons in the range 5.57−6.36 ppm which partly overlap with resonances of the $Tp^{Ph,Me}$ ligand. More importantly, two additional multiplets, each with the intensity for one proton, evolved in the unobscured regions at 7.27 and 7.63 ppm. Their equal intensities indicate that at low temperatures in solution, too, **10** and **11** exist in a 1:1 ratio, either as separate entities or as the **10′**/**11′** dimer. This observation was made for solutions of acetonitrile-containing **10**/**11** and of solvent-free **10′**/**11′**. To our knowledge, it is the first time that the coexistence of such isomers has been observed.

In comparison to 2-mercaptophenol, 2-(hydroxymethyl)-thiophenol contains a CH_2 group between the phenyl ring and the OH function. Geometrical inspection shows that its zinc-bound thiolate cannot be oriented in a way that makes a bidentate attachment favorable. It therefore was expected to bind in the monodentate fashion. This was borne out by the synthesis of **12** and its structure determination. While the CH_2OH unit is not suitable for chelation, it is very favorable for O−H···S hydrogen bonding involving an O···S distance of 3.09 Å, just like in **8′**. Thus, all three thiolate complexes in this group, **10**−**12**, have hydrogen bonds as structure-determining features again.



12

While the solid-state structures underline the importance of the hydrogen bonds, their persistence in solution was not evident from the available data. We therefore applied a chemical test to verify their existence. The test reaction is the alkylation with methyl iodide which converts the TpZn−SR complexes to TpZn−I and the corresponding thioether CH_3SR.[40] It has been observed in several cases that the involvement of the thiolate sulfur in an O−H···S or N−H···S hydrogen bond reduces the rate of alkylation significantly.[41−43] Expecting the same effect here, we chose complexes **10**/**11** and **12** for alkylations, knowing the rate of alkylation of their hydrogen bond free parent complex $Tp^{Ph,Me}$Zn−SPh.[40]

Using conditions such as those for the methylation of the thiophenolate complex, **10**/**11** and **12** were treated with a 10-fold amount of CH_3I in $CDCl_3$. The intensities of the ^{1}H NMR signals for the methyl groups of the resulting methylthioethers were taken as measures of the concentrations. From their log plots the k_{obs} values were obtained according to $\ln(1 - I_t/I_\infty) = -k_{obs}t$. The k_{obs} values at 300 K for 0.03 M solutions of the complexes were 7.4×10^{-6} s^{-1} for **10**/**11** and 3.9×10^{-6} s^{-1} for **12**. The corresponding value for $Tp^{Ph,Me}$Zn−SPh, obtained from a 0.014 M solution, is 3.6×10^{-4} s^{-1}.[40] Thus, we are observing that the hydrogen-bonded thiophenolates are roughly 2 orders of magnitude less reactive than their parent complex. We take this as a clear indication that the hydrogen bonds in complexes **7**−**12** exist in solution as well as in the solid state.

Structural Comparisons. Early on the 3-substituted pyrazolylborate ligands, particularly the t-Bu-substituted one,[44] were termed "tetrahedral enforcers", supposing that the pocket created by the three 3-substiuents has room for only one additional donor. Later on it turned out that such TpM complexes with two additional donors are not uncommon, particularly when those two donors belong to a chelating ligand. It was found that the coordination geometry of the metals in the TpM(X)(Y) complexes varies over the whole range between square pyramidal and trigonal bipyramidal, never reaching the two ideal cases due to the restraints imposed by the Tp ligands. Nevertheless we could exploit this variation in a structure correlation analysis leading to a proposal for the geometric pathway of zinc-enzyme-catalyzed hydrolyses.[18]

The 11 molecular structures determined in this work represent the full range of alternatives represented above. Six of them contain monodentate thiolate ligands. Their geometrical details, the essential ones of which are listed in Table 1, are remarkably uniform. They are pseudotetrahedral: the small byte of the Tp ligand leads to large S−Zn−N angles with an average value of 122°. For each complex these angles vary little, the extreme case being **12** (115−129°). Likewise there is only a small spread of the Zn−N bond lengths (average 2.06 Å), and the Zn−S bond lengths vary by 0.02 Å only.

Table 1. Zinc Coordination in the Pseudotetrahedral $Tp^{Ph,Me}$Zn−SR Complexes (Bond Lengths in Å, Angles in deg)

param	**5**	**7**	**8′**	**11**	**11′**	**12**
Zn−N1	2.116(4)	2.050(3)	2.042(2)	2.045(4)	2.055(2)	2.044(2)
Zn−N2	2.066(4)	2.102(3)	2.082(2)	2.036(4)	2.077(2)	2.072(2)
Zn−N3	2.047(4)	2.080(3)	2.068(2)	2.073(4)	2.047(2)	2.062(2)
Zn−S	2.227(1)	2.234(1)	2.228(1)	2.231(3)	2.248(1)	2.230(1)
S−Zn−N1	126.3(1)	126.5(1)	125.7(1)	120.7(1)	125.7(1)	129.0(5)
S−Zn−N2	121.1(1)	125.5(1)	124.3(1)	124.7(1)	116.1(1)	115.3(5)
S−Zn−N3	122.8(1)	118.4(1)	121.2(1)	123.9(1)	116.7(1)	124.9(5)

Table 2. Zinc Coordination in the Five-Coordinate $Tp^{Ph,Me}$Zn−Chelate Complexes (Bond Lengths in Å, Angles in deg)

param	**3**	**4**	**9**	**10**	**10′**
Zn−N1	2.040(3)	2.071(5)	2.158(4)	2.041(4)	2.108(2)
Zn−N2	2.079(3)	2.172(5)	2.086(4)	2.095(4)	2.105(2)
Zn−N3	2.020(3)	2.083(5)	2.078(4)	2.214(5)	2.143(2)
X(axial)[a]	O2	O1	O1	O2	O2
Y(equatorial)[a]	O1	S	S	S2	S2
Zn−X	2.567(3)	2.332(4)	2.384(4)	2.415(5)	2.443(2)
Zn−Y	1.923(3)	2.260(2)	2.257(2)	2.245(2)	2.279(1)
N−Zn−X[b]	169.6(1)	170.3(2)	166.6(2)	171.9(1)	163.0(1)
N−Zn−Y[c]	126.7(1)	132.9(2)	129.3(1)	132.1(1)	135.1(1)
% TBP[d]	78	89	95	88	65

[a] Classifying the complexes as trigonal bipyramidal (TBP). [b] Largest angle. [c] Second largest angle. [d] According to our adaption of Holmes' method for TpZn(X)(Y) complexes.[18]

All five five-coordinate zinc complexes which were structurally characterized here contain chelating coligands. As Table 2 shows, their geometries vary to a larger degree. The ZnL_5 polyhedron of each of them can be set up as a trigonal bipyramid, and then its deformation toward a square pyramid can be described and quantified. One axial position of the trigonal bipyramid is always occupied by the non-sulfur donor of the chelating ligand, in this case always an O atom. This axial zinc−ligand bond is always the longest bond in the molecule, which in case of **3** is 0.64 Å longer than the other Zn−coligand bond and hence represents a rather weak interaction.

As a rule, the Zn−N bond trans to the long Zn−X bond is also the longest of the three Zn−N bonds, as is to be expected for trigonal-bipyramidal coordination. The angle defined by these two bonds is the largest angle in the molecule, in this case ranging from 163 to 172°. Except for the Zn−S bond lengths, which are again remarkably uniform, the Zn−L bond lengths in this group of complexes vary much more than those in the other group. There are three reasons for this: (i) the distinction of the Zn−N bonds between axial and equatorial; (ii) the varying degree of bonding interactions in the weak Zn−X bonds; (iii) the variation of the complex geometries between trigonal-bipyramidal and square-pyramidal. The latter can be read in a rough approximation from the difference between the largest and the second largest angle (ideally 180°/120° for TBP and ca. 140°/140° for SP). We have quantified it by our adaption of Holmes' dihedral angle summation method[45] for TpZn complexes.[18] As Table 2 shows, four of the five complexes are close to being TBP,

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and only **10′** is roughly halfway between TBP and SP. This observation corresponds to the general trend for TpZn(X)(Y) complexes.[18] In the present case it also represents the dominance of the Zn−S interaction, which only leaves a weak interaction, i.e., a position on the TBP axis, for the fifth zinc−ligand bond.

Conclusions

The major observation of this work is the variability of the zinc coordination for each of the potentially bidentate ligands. In each of the four groups of ligands employed a small variation of the ligand composition or even of the reaction conditions has brought about the switch from monodentate to chelating coordination. In the extreme case of **10**/**11** and **10′**/**11′** the two coordination modes coexist in the solid state and even in solution. Considering that the TpZn−SR complexes belong to the best investigated ones in TpZn chemistry, this variability, which was unexpected for us, must be called remarkable.

The most noticeable factor distinguishing and probably controlling the attachment of the thiolates is the presence of hydrogen bonds. In most of the complexes with a monodentate thiolate coordination there is an intramolecular hydrogen bond from an OH or NH function to the thiolate sulfur. Likewise, a characteristic feature of the chelate complexes with OH-containing chelate ligands is the involvement of the OH in an external hydrogen bond. These phenomena certainly call for a more detailed investigation.

With respect to enzyme inhibition this work raises a 2-fold caveat. On one side the model complexes may be of little relevance for the enzymatic situation, where the hydrogen-bonding pattern can exert its influence on the binding of the inhibitor in an unpredictable manner. On the other side the bonding patterns proposed as a result of enzyme−inhibitor studies may be wrong due to insufficient consideration of the factors outlined in the work by ourselves and our competitors. Actually, in contrast to the wealth of structural information on the model complexes, this information on real enzyme−inhibitor complexes is unpleasantly poor.

During this work we have also been reminded again of the limitations of such model studies. The complex enzymatic situation, exemplified here by the hydrogen bonds, cannot be reproduced with the presently used Tp ligands yet. Nevertheless, the experienced coordination chemist can advance the field by proposing new ligand types for enzyme inhibitors, as we believe to have done in this and the preceding paper.[29] Furthermore, we have started a new generation of Tp ligands with hydrogen-bonding substituents.[46,47] As our activities in the field of biomimetic zinc chemistry have come to an end, it is only left to us to express our optimism that our successors will continue making significant contributions to this area of the life sciences from the field of inorganic chemistry.

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Experimental Section

The general experimental procedures were as described previously.[48] The starting complex **1** was prepared as published.[39] The organic reagents were obtained commercially. In the ^{1}H NMR spectra in $CDCl_3$ the resonances of the $Tp^{Ph,Me}$ ligands in all complexes are observed at practically identical positions: $\delta = 2.5$ [s, 9H, Me(pz)], 6.2 [s, 3H, H(pz)], 7.2−7.4 [m, 9H, Ph(3,4,5)], 7.5−7.7 [m, 6H, Ph(2,6)]. Hence only the ^{1}H NMR resonances of the coligands are reported here.

2. A solution of 2-mercaptopropionic acid (37 mg, 31 μL, 0.35 mmol) in methanol (20 mL) was dropped slowly into a stirred solution of **1** (200 mg, 0.35 mmol) in dichloromethane (40 mL). After it was stirred for 2 h, the solution was filtered. The filtrate was evaporated in vacuo until a precipitate formed. This was filtered off and dried in vacuo. A 60 mg (26%) amount of **2** remained as a colorless powder, mp 110 °C (dec). Attempts at recrystallization led to decomposition.

Anal. Calcd for $C_{33}H_{33}BN_6O_2SZn$ ($M_r = 653.95$): C, 60.61; H, 5.09; N, 12.85; S, 4.90. Found: C, 59.63; H, 5.31; N, 12.58; S, 5.23. IR (KBr): 2548 m (BH), 1684 m, 1621 m (CO). ^{1}H NMR ($CDCl_3$): $\delta = 1.11$ [d, $J = 7.0$ Hz, 3H, CH_3], 1.95 [d, $J = 5.7$ Hz, 1H, SH], 3.10 [q, $J = 5.8$ Hz, 1H, CH].

3. A solution of 3-mercaptopropionic acid (108 mg, 88 μL, 1.02 mmol) in dichloromethane (20 mL) was added slowly to a stirring solution of **1** (577 mg, 1.02 mmol). After the solution was stirred for 3 h, the volume was reduced to half in vacuo. The resulting precipitate was filtered off, dried in vacuo, and crystallized from hot acetonitrile. A 346 mg (52%) amount of **3** was obtained as colorless crystals, mp 148 °C.

Anal. Calcd for $C_{33}H_{33}BN_6O_2SZn$ ($M_r = 653.95$): C, 60.61; H, 5.09; N, 12.85; S, 4.90. Found: C, 59.43; H, 5.47; N, 12.87; S, 4.84. IR (KBr): 2542 m (BH), 1615 s (CO). ^{1}H NMR ($CDCl_3$): $\delta = 1.46$ [t, $J = 8.2$ Hz, 1H, SH], 2.05 [t, $J = 7.2$ Hz, 2H, CH_2], 2.26 [m, 2H, CH_2].

4. A solution of 2-mercaptopropionic acid ethyl ester (52 mg, 50 μL, 0.39 mmol) in methanol (10 mL) was dropped slowly into a stirring solution of **1** (219 mg, 0.39 mmol) in dichloromethane. After 4 h of stirring, the volume was reduced to one-third in vacuo. The resulting precipitate was filtered off, washed with methanol, dried in vacuo, and crystallized by slow evaporation from dichloromethane/ethanol. A 231 mg (87%) amount of **4** resulted as colorless crystals, mp 192 °C.

Anal. Calcd for $C_{35}H_{37}BN_6O_2SZn{\cdot}CH_2Cl_2$ ($M_r = 681.98 + 84.93$): C, 56.38; H, 5.13; N, 10.96; S, 4.18. Found: C, 56.64; H, 5.55; N, 11.37; S, 4.10. IR (KBr): 2532 m (BH), 1687 s (CO). ^{1}H NMR ($CDCl_3$): $\delta = 0.58$ [t, $J = 7.1$ Hz, 3H, CH_3(ester)], 0.94 [d, $J = 7.1$ Hz, 3H, CH_3], 2.50 [q, $J = 7.1$ Hz, 1H, CH], 3.05 [q, $J = 7.1$ Hz, 2H, CH_2(ester)], 5.29 [s, 2H, CH_2Cl_2].

5. This was made in the same way as **4** from 3-mercaptopropionic acid methyl ester (59 mg, 55 μL, 0.49 mmol) and **1** (278 mg, 0.49 mmol). Yield: 278 mg (85%) of **5** as colorless crystals, mp 170 °C.

Anal. Calcd for $C_{34}H_{35}BN_6O_2SZn{\cdot}CH_2Cl_2$ ($M_r = 667.96 + 84.93$): C, 55.84; H, 4.95; N, 11.16; S, 4.26. Found: C, 55.66; H, 5.12; N, 12.04; S, 4.15. IR (KBr): 2544 m (BH), 1737 s (CO). ^{1}H NMR ($CDCl_3$): $\delta = 1.23$ [t, $J = 7.2$ Hz, 2H, CH_2], 1.53 [t, $J = 7.2$ Hz, 2H, CH_2], 3.39 [s, 3H, OCH_3], 5.29 [s, 2H, CH_2Cl_2].

6. This was made in the same way as **4** from *N*-Boc-aminoethylmercaptan (73 mg, 0.41 mmol) and **1** (232 mg, 0.41 mmol). Yield: 199 mg (67%) of **6** as a colorless powder, mp 202 °C.

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Table 3. Crystallographic Details

param	**3**	**4**	**5**	**7**	**8′**	**9**	**10/11**	**10′/11′**	**12**
formula	$C_{33}H_{33}BN_6O_2SZn$	$C_{35}H_{37}BN_6O_2SZn{\cdot}CH_2Cl_2$	$C_{34}H_{37}BN_6O_2SZn{\cdot}CH_2Cl_2$	$C_{36}H_{34}BN_7SZn$	$C_{32}H_{33}BN_6OSZn$	$C_{33}H_{35}BN_6O_2SZn{\cdot}C_{10}H_{10}N_2{\cdot}CH_2Cl_2$	$C_{36}H_{33}BN_6OSZn{\cdot}0.5CH_3CN$	$C_{36}H_{33}BN_6OSZn$	$C_{37}H_{35}BN_6OSZn$
MW	653.9	682.0 + 84.9	668.0 + 84.9	673.0	625.9	656.0 + 158.2 + 84.9	674.0 + 20.5	674.0	688.0
space group	$P2_1/c$	$P2_1/n$	$P\bar{1}$	$Pna2_1$	$P2_1/n$	$P2_1/n$	$P\bar{1}$	$P\bar{1}$	$P\bar{1}$
Z	4	4	2	8	4	4	4	4	2
a (Å)	9.79(1)	16.416(3)	11.782(2)	19.734(3)	11.144(2)	15.403(4)	11.47(2)	12.957(4)	10.929(2)
b (Å)	23.55(2)	12.451(2)	11.803(2)	16.457(3)	9.833(2)	12.149(3)	16.18(2)	14.346(4)	12.250(2)
c (Å)	13.77(1)	18.142(3)	15.942(3)	20.290(3)	27.873(5)	24.212(7)	19.50(3)	19.033(6)	13.455(3)
α (deg)	90	90	71.171(3)	90	90	90	104.46(3)	98.031(6)	75.945(3)
β (deg)	98.91(2)	97.270(3)	76.224(3)	90	95.084(4)	97.798(5)	93.72(3)	100.448(6)	78.960(3)
γ (deg)	90	90	60.919(3)	90	90	90	96.61(3)	99.935(5)	76.853(3)
V (Å^3)	3139(5)	3678(1)	1825(1)	6589(2)	3042(1)	4489(2)	3465(9)	3373(2)	1684(1)
d_{calcd} (g cm^{-3})	1.38	1.38	1.37	1.36	1.37	1.33	1.33	1.33	1.36
μ(Mo Kα) (mm^{-1})	0.89	0.91	0.92	0.85	0.91	0.76	0.81	0.83	0.83
R1 (obsd reflcns)	0.050	0.092	0.073	0.040	0.043	0.070	0.047	0.040	0.034
wR2 (all reflcns)	0.139	0.289	0.248	0.248	0.126	0.200	0.131	0.124	0.101

Anal. Calcd for $C_{37}H_{42}BN_7O_2SZn$ (M_r = 725.05): C, 61.29; H, 5.84; N, 13.52; S, 4.42. Found: C, 60.94; H, 5.97; N, 13.24; S, 4.63. IR (KBr): 2534 m (BH), 1608 vs (CO). ^{1}H NMR ($CDCl_3$): δ = 0.90 [t, J = 5.8 Hz, 2H, CH_2], 1.34 [s, 9H, t-Bu], 2.25 [t, J = 5.7 Hz, 2H, CH_2], 4.40 [s, 1H, NH].

7. A solution of 2-mercaptoaniline (66 mg, 57 μL, 0.35 mmol) in methanol (15 mL) was added slowly to a stirring solution of **1** (300 mg, 0.53 mmol) in dichloromethane (30 mL). After the solution was stirred for 5 h, the volume was reduced to one-third in vacuo. The resulting precipitate was filtered off, washed with 10 mL of methanol, and crystallized from dichloromethane/methanol by slow evaporation. A 253 mg (71%) amount of **7** resulted as colorless crystals, mp 161 °C.

Anal. Calcd for $C_{36}H_{34}BN_7SZn$ (M_r = 672.98): C, 64.25; H, 5.09; N, 14.57; S, 4.76. Found: C, 64.00; H, 5.16; N, 14.46; S, 4.94. IR (KBr): 2548 s (BH). ^{1}H NMR ($CDCl_3$): δ = 4.20 [broad singlet, 2H, NH_2], 6.04−6.53 [m, 4H, C_6H_4].

8′. A solution of 2-mercaptoethanol (27 mg, 24 μL, 0.35 mmol) in methanol (20 mL) was added with stirring to a solution of **1** (200 mg, 0.35 mmol) in dichloromethane (30 mL). After the solution was stirred for 1 h, the volume was reduced to half in vacuo. The resulting precipitate was filtered off. To remove traces of methanol it was dissolved in a few milliliters of acetonitrile and then evaporated to dryness again, and this procedure was repeated twice. Crystallization from hot acetonitrile yielded 134 mg (61%) of **8′** as colorless crystals, mp 204 °C.

Anal. Calcd for $C_{32}H_{33}BN_6OSZn$ (M_r = 625.92): C, 61.41; H, 5.31; N, 13.43; S, 4.12. Found: C, 61.32; H, 5.26; N, 13.47; S, 5.31. IR (KBr): 3452 w (OH), 2553 m (BH). ^{1}H NMR ($CDCl_3$): δ = 1.18 [t, J = 5.9 Hz, 2H, SCH_2], 2.63 [t, J = 5.9 Hz, 2H, OCH_2].

9. This was made in the same way as **2** from 1-thioglycerol (36 mg, 30 μL, 0.34 mmol) and **1** (194 mg, 0.34 mmol). Yield: 145 mg of **9** as a colorless powder, mp 163 °C.

Anal. Calcd for $C_{33}H_{35}BN_6O_2SZn{\cdot}H_2O$ (M_r = 655.95 + 18.02): C, 58.81; H, 5.53; N, 12.47; S, 4.76. Found: C, 58.58; H, 5.56; N, 12.26; S, 4.72. IR (KBr): 3441 s (H_2O), 2545 m (BH). ^{1}H NMR ($CDCl_3$): δ = 1.05 [d, J = 5.4 Hz, 2H, SCH_2], 1.34 [broad, 1H, OH], 1.52 [s, 2H, H_2O], 1.86 [d, J = 5.3 Hz, 1H, OH], 2.78 [m, 2H, OCH_2], 2.79 [m, 1H, CH].

Crystallization of 9. A 131 mg (0.20 mmol) amount of **9** was dissolved in 15 mL of hot dichloromethane, upon which a precipitate formed. This was filtered off and the filtrate subjected to very slow evaporation through a rubber cap. A 35 mg (27%) amount of **9**·methylphenylpyrazole·CH_2Cl_2 resulted as colorless crystals, mp 172 °C.

Anal. Calcd for $C_{33}H_{35}BN_6O_2SZn{\cdot}C_{10}H_{10}N_2{\cdot}CH_2Cl_2$ (M_r = 655.95 + 158.20 + 84.93): C, 58.78; H, 5.27; N, 12.46; S, 3.57. Found: C, 58.03; H, 5.41; N, 12.09; S, 3.81.

10/11. A solution of 2-hydroxythiophenol (25 mg, 37 μL, 0.36 mmol) in methanol (50 mL) was dropped with stirring over a period of 8 h into a solution of **1** (201 mg, 0.36 mmol) in dichloromethane (250 mL). After 12 h of stirring the volume was reduced to 40 mL in vacuo. The resulting precipitate was filtered off, washed with methanol, and dried in vacuo. Crystallization from hot acetonitrile yielded 190 mg (76%) of **10**/**11** as colorless crystals, mp 160 °C.

Anal. Calcd for $C_{36}H_{33}BN_6OSZn{\cdot}0.5CH_3CN$ (M_r = 673.96 + 20.53): C, 63.99; H, 5.01; N, 13.11; S, 4.62. Found: C, 63.81; H, 5.02; N, 13.09; S, 4.65. IR (KBr): 2545 m (BH). ^{1}H NMR ($CDCl_3$): δ = 1.99 [s, 1.5H, CH_3CN], 5.72−6.42 [m, 3H, H3, H4, H5 of C_6H_4]. The signal for H6 of C_6H_4 is hidden under the Tp's phenyl signals. For low-temperature NMR, see text.

10′/11′. The raw precipitate of **10**/**11** obtained above was dissolved in benzene (10 mL) and this solution evaporated to dryness again. This step was repeated twice to remove traces of methanol. Crystallization from hot benzene produced 198 mg (82%) of **10′**/**11′** as yellowish crystals, mp 176 °C.

Anal. Calcd for $C_{36}H_{33}BN_6OSZn$ (M_r = 673.96): C, 64.16; H, 4.94; N, 12.47; S, 4.76. Found: C, 64.01; H, 5.05; N, 12.47; S, 4.96.

12. A solution of 2-(hydroxymethyl)thiophenol (74 mg, 61 μL, 0.53 mmol) in methanol (15 mL) was added dropwise with stirring to a solution of **1** (300 mg, 0.53 mmol) in dichloromethane (30 mL). After the solution was stirred for 2 h the volume was reduced to half in vacuo. Addition of 10 mL of methanol produced a precipitate which was filtered off, washed with methanol, and dried in vacuo. Crystallization from methanol/dichloromethane by slow evaporation yielded 288 mg (79%) of **12** as colorless crystals, mp 152 °C.

Anal. Calcd for $C_{37}H_{35}BN_6OSZn$ (M_r = 687.99): C, 64.59; H, 5.13; N, 12.22; S, 4.66. Found: C, 63.69; H, 5.28; N, 12.04; S, 4.58. IR (KBr): 2458 m (BH). ^{1}H NMR ($CDCl_3$): δ = 3.40 [t, J = 7.0 Hz, 1H, OH], 4.49 [d, J = 7.0 Hz, 2H, OCH_2], 5.92 [m, 2H, C_6H_4], 6.44 [m, 1H, C_6H_4], 6.73 [m, 1H, C_6H_4].

Kinetic Measurements. The general procedures were as before.[40] The apparatus and the solutions were thermostated to 300.0 K. A 10.0 mg amount of each of **10**/**11** and **12** (14.7 and 14.5 μmol, respectively) was dissolved in 0.50 mL of $CDCl_3$, and 20.6 mg (9.0 μL, 145 μmol) each of methyl iodide were added. The intensity of the methyl resonance of the resulting methylthioester was recorded automatically at 5 min intervals. Data were recorded for 4 days for **10**/**11** and for 8 days for **12**, which is for more than 6

half-lives. The log plots for the determination of the k_{obs} values had correlation coefficients >0.99.

Structure Determinations. Crystals of **12** were obtained by recrystallization from hot acetonitrile. All other crystals were taken as obtained from the preparations. The data sets were obtained with a Bruker AXS Smart CCD diffractometer at 230 K and subjected to empirical absorption corrections. The structures were solved with direct methods and refined anisotropically using the SHELXTL program suite.[49] Table 3 lists the crystallographic data.

(49) SHELXTL program package of the Bruker Smart CCD diffractometer, version 5.1, 2002.

(50) Keller, E. *SCHAKAL 99 for Windows*; Universität Freiburg: Freiburg, Germany, 2006.

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Supporting Information Available: Nine X-ray crystallographic files in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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