Inorg. Chem. **2002**, *41*, 2560–2564

Tripodal Pseudopeptides with Three Histidine or Cysteine Donors: Synthesis and Zinc Complexation†

Michael Gelinsky, Raphael Vogler, and Heinrich Vahrenkamp*

Institut für Anorganische und Analytische Chemie der Universität Freiburg, Albertstr. 21, D-79104 Freiburg, Germany

Received December 11, 2001

Peptide coupling of benzene-1,3,5-carboxylic acid with 3 equiv of histidine ethyl ester or cysteine ethyl ester has yielded the tripodal pseudopeptide ligands THB and H_3TCB. Likewise, the combination of tris(carboxyethyl)-nitromethane with 3 equiv of cysteine ethyl ester gave the tripod H_3TCM. With zinc salts, the pseudopeptides form the insoluble compounds $(THB)_2Zn_5Cl_{10}$, $Zn_3(TCB)_2$, and $Zn_3(TCM)_2$ which are likely to be coordination polymers. Solution studies of THB with potentiometric methods have identified the complex species $[(THB)_2Zn]^{2+}$, $[(THB)Zn–OH_2]^{2+}$, and $[(THB)Zn–OH]^{+}$. The pK_a of the zinc-bound water molecule is 6.2, making the (THB)Zn complex a viable model of carbonic anhydrase.

Introduction

The search for ligands mimicking the environment of metals in biological systems has resulted in a tremendous upsurge in the study of tripodal systems of all kinds. As a rule, these offer heterocyclic nitrogen donors as a replacement for histidine in proteins, carboxylate or phenolate in place of aspartate, glutamate, and tyrosinate, and thioethers or sometimes thiolates as replacements for cysteine.[2] The use of the "correct" amino acids as donors in tripodal ligands or the construction of tripodal pseudopeptides is still very rare. In our field, coordination chemistry related to the biological functions of zinc, it seems to have been applied only once so far.[3−6]

Even the biomimetic chemistry with realistic cysteine or histidine substitutes, that is, tripods with three imidazole or alkanethiol functions, is not well developed. There are a few tris(alkanethiol) tripods, but not much coordination chemistry of them has been described.[7−10] Among the tris(imidazole) tripods, the P(im)$_3$ system has been used for two decades.[11−14] More sophisticated tris(imidazole) systems based on central NX_3,[15] RCX_3,[16] benzene,[17] and cyclohexane[18] anchors have been applied in bioinorganic and bioorganic chemistry. The most sophisticated tris(imidazole) ligands for a biomimetic zinc chemistry so far are Reinaud's substituted calixarenes.[19−22]

Our own contributions to the chemistry of C_3 symmetrical tripods consist mainly of the use of tris(pyrazolyl)borates for zinc model complexes.[23] We have approximated tris-

† Zinc complexes of amino acids and peptides, 16. Previous communication, see ref. 1.

* To whom correspondence should be addressed. E-mail: vahrenka@uni-freiburg.de.

(1) Vogler, R.; Vahrenkamp, H. *Eur. J. Inorg. Chem.* **2002**, 761.
(2) For a recent review and references see: Moberg, C. *Angew. Chem.* **1998**, *110*, 260; *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 248.
(3) Severin, K.; Beck, W.; Trojandt, G.; Polborn, K.; Steglich, W. *Angew. Chem.* **1995**, *107*, 1570; *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1449.
(4) Trojandt, G.; Polborn, K.; Steglich, W.; Schmidt, M.; Nöth, H. *Tetrahedron Lett.* **1995**, *36*, 857.
(5) Trojandt, G.; Herr, U.; Polborn, K.; Steglich, W. *Chem.—Eur. J.* **1997**, *3*, 1254.
(6) Herr, U.; Spahl, W.; Trojandt, G.; Steglich, W.; Thaler, F.; van Eldik, R. *J. Bioorg. Med. Chem.* **1999**, *7*, 699.
(7) Kolomyjec, C.; Whelan, J.; Bosnich, B. *Inorg. Chem.* **1983**, *22*, 2343.
(8) Barbaro, P.; Bianchini, C.; Scapacci, G.; Masi, D.; Zanello, P. *Inorg. Chem.* **1994**, *33*, 3180.
(9) Nanda, K. K.; Sinn, E.; Addison, A. W. *Inorg. Chem.* **1996**, *33*, 1.
(10) Glaser, M.; Spies, H.; Berger, R.; Hahn, F. E.; Lugger, T.; Johannsen, B. *Inorg. Chim. Acta* **1997**, *257*, 143.
(11) Slebocka-Tilk, H.; Cocho, J. L.; Frakman, Z.; Brown, R. S. *J. Am. Chem. Soc.* **1984**, *106*, 2421 and references therein.
(12) Kimblin, C.; Murphy, V. J.; Hascall, T.; Bridgewater, B. M.; Bonanno, J. B.; Parkin, G. *Inorg. Chem.* **2000**, *39*, 967.
(13) Kläui, W.; Piefer, C.; Rheinwald, G.; Lang, H. *Eur. J. Inorg. Chem.* **2000**, 1549.
(14) Sorell, T. N.; Allen, W. E.; White, P. S. *Inorg. Chem.* **1995**, *34*, 952.
(15) Chen, S.; Richardson, J. F.; Buchanan, R. M. *Inorg. Chem.* **1994**, *33*, 2376.
(16) Jairam, R.; Potvin, P. G. *J. Org. Chem.* **1992**, *57*, 4136.
(17) Dr. A. Wander A.-G., Br. Patent 1,007,334; *Chem. Abstr.* **1966**, *64*, 5104.
(18) Metzger, A.; Lynch, V. M.; Anslyn, E. V. *Angew. Chem.* **1997**, *109*, 911; *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 865.
(19) Sénèque, O.; Rager, M. N.; Duprat, A.; Reinaud, O. *J. Am. Chem. Soc.* **2000**, *122*, 6183.
(20) Rondelez, Y.; Sénèque, J.; Rager, M. N.; Duprat, A.; Reinaud, O. *Chem.—Eur. J.* **2000**, *6*, 4218.
(21) Le Clainche, L.; Giorgi, M.; Reinaud, O. *Inorg. Chem.* **2000**, *39*, 3436.
(22) Sénèque, O.; Giorgi, M.; Reinaud, O. *J. Chem. Soc., Chem. Commun.* **2001**, 984.

10.1021/ic011263c CCC: $22.00
Published on Web 04/05/2002

(imidazole) and tris(thiolate) systems by using benzimidazole[24−27] and thioimidazole[28] derived tripods. Our interest in histidine and cysteine as donors in zinc complexes is, however, long standing: all previous papers in this series on zinc complexes of amino acids and peptides[1] have dealt with ligands containing histidine and/or cysteine. Several of them were on peptides with two histidine[1,29−31] or cysteine[30−33] constituents.

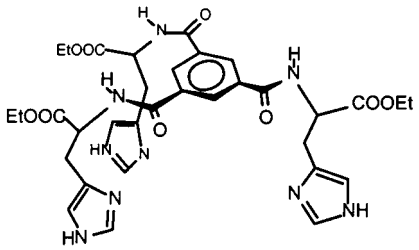
So far, we have not worked on pseudopeptides, that is, molecules containing amino acids attached by peptide bonds to nonbiological spacers. The advantages of tripod ligands for a biomimetic zinc chemistry have now induced us to do so. As before in this series, we have focused on histidine and cysteine as donors for zinc. The objects of this initial study were C_3 symmetrical tris(histidine) and tris(cysteine) ligands based on benzene or trialkylmethane cores. Three of the four target ligands could be obtained, and some of their basic zinc complex chemistry could be explored.

Results and Discussion

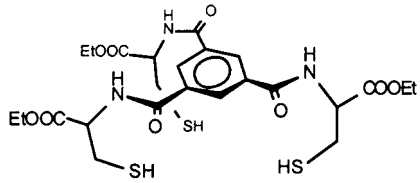
Ligand Synthesis. Benzene-1,3,5-tricarboxylic acid (trimesic acid) and tris(carboxyethyl)nitromethane were chosen as trifunctional centers for the construction of the C_3 symmetrical tripods. The amino acids to be attached to them had to be protected such that histidine-imidazole and cysteine-thiolate were their only donor functions. Thus, the amino acid reagents were histidine ethyl ester and *S*-tritylated cysteine ethyl ester. Peptide coupling by the carbonyldiimidazole (CDI) method was applied to attach the amino acids to the benzene ring. Thereby, ligand THB was obtained in one synthetic step. The formation of H_3TCB required the subsequent removal of the trityl groups with ethanethiol. Both tripods resulted analytically pure after HPLC.

The approach for the attachment of the amino acids to the nitromethane center was very much the same. The amino acid ethyl esters were coupled with tris(carboxyethyl)-nitromethane by the dicyclohexyl carbodiimide (DCC) method. For histidine, the coupling was incomplete, and only impure products resulted. For cysteine, both the coupling and the removal of the trityl group worked, and the tripod H_3TCM was obtained in good yields.

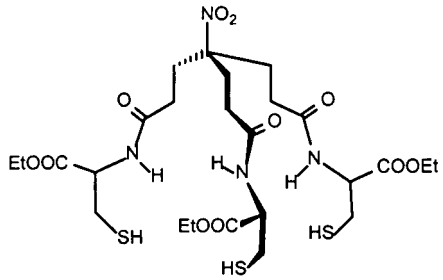
The identity of all three tripods was verified by ESI-MS. They were characterized by their NMR spectra (see Experimental Section). Ligand THB is soluble in water and was hence suitable for a solution study with potentiometric methods. Ligands H_3TCB and H_3TCM are not soluble enough for studies in aqueous solution. Chemical reactions of all three ligands could be performed conveniently in ethanolic solutions.



THB



H_3TCB



H_3TCM

Isolated Zinc Complexes. Ligand THB and all zinc salts yielded insoluble precipitates to which simple compositions could not be assigned. A typical example is compound **1**, $(THB)_2Zn_5Cl_{10}$, whose composition is deduced from elemental analyses but cannot be backed by NMR data due to its insolubility. For the 5 zinc ions in **1**, there are 6 imidazole donors and 10 chloride ligands, allowing the prediction that in this coordination polymer there is chloride bridging and/or the additional coordination of carbonyl donors, cf. the structure of $Zn(GlyHis)_2$.[34]

Both cysteine tripods, after deprotonation, yielded simple zinc complexes with zinc salts of noncoordinating anions. H_3TCB and $Zn(ClO_4)_2$ formed **2**; H_3TCM and $Zn(NO_3)_2$ formed **3**. Compounds **2**, $Zn_3(TCB)_2$, and **3**, $Zn_3(TCM)_2$, are again insoluble and therefore probably polymeric. The fact that six thiolate donors are available for three zinc ions correlates well with the coordination requirements: if each thiolate is bridging, each zinc ion can be tetrahedrally coordinated, as expected in a sulfur environment. The 3:2

(23) Vahrenkamp, H. *Acc. Chem. Res.* **1999**, *32*, 589.
(24) Gregorzik, R.; Hartmann, U.; Vahrenkamp, H. *Chem. Ber.* **1994**, *127*, 2117.
(25) Hartmann, U.; Gregorzik, R.; Vahrenkamp, H. *Chem. Ber.* **1994**, *127*, 2123.
(26) Burth, R.; Vahrenkamp, H. *Z. Anorg. Allg. Chem.* **1998**, *624*, 381.
(27) Müller-Hartmann, A.; Vahrenkamp, H. *Eur. J. Inorg. Chem.* **2000**, 2371.
(28) Tesmer, M.; Shu, M.; Vahrenkamp, H. *Inorg. Chem.* **2001**, *40*, 4022.
(29) Förster, M.; Brasack, I.; Duhme, A. K.; Nolting, H. F.; Vahrenkamp, H. *Chem. Ber.* **1996**, *129*, 347.
(30) Gockel, P.; Gelinsky, M.; Vogler, R.; Vahrenkamp, H. *Inorg. Chim. Acta* **1998**, *272*, 115.
(31) Gockel, P.; Vogler, R.; Gelinsky, M.; Meissner, A.; Albrich, H.; Vahrenkamp, H. *Inorg. Chim. Acta* **2001**, *323*, 16.
(32) Meissner, A.; Haehnel, W.; Vahrenkamp, H. *Chem.—Eur. J.* **1997**, *3*, 261.
(33) Vogler, R.; Gelinsky, M.; Guo, L. F.; Vahrenkamp, H. *Inorg. Chim. Acta*, in press.
(34) Förster, M.; Vahrenkamp, H. *Chem. Ber.* **1995**, *128*, 541.

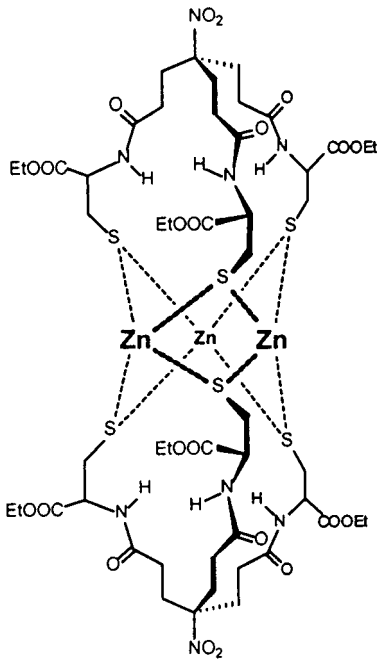


Figure 1. Molecular representation of $Zn_3(TCM)_2$ (**3**).

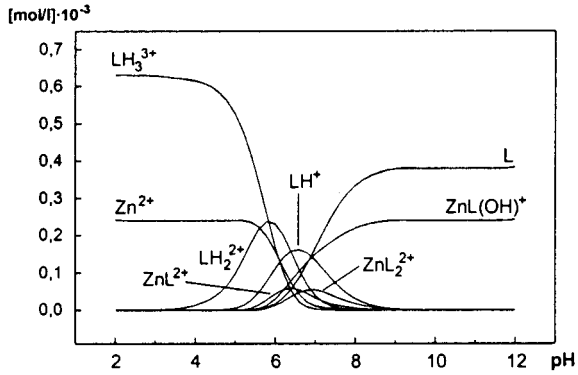


Figure 2. Species distribution in a solution of THB and $Zn(NO_3)_2$ at a ratio of 2.6:1.

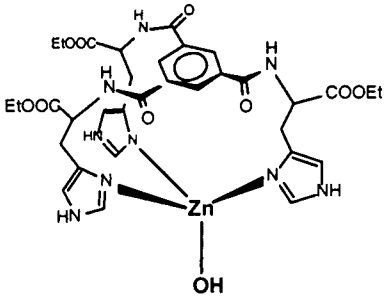
stoichiometry also allows a simple molecular representation which is given in Figure 1 for **3** and which can be reproduced, although with some strain due to the tetrahedral nature of zinc, by molecular models.

Potentiometric Titrations. Ligand THB, being soluble in water, could be subjected to potentiometric titrations in the absence and presence of zinc nitrate. Its three imidazole donor functions give rise to the three pK_a values of 5.78(4), 6.35(4), and 6.92(5) which are within the range spanned by the values for H−His−OMe (5.41)[35] and Ac−His−OH (7.11)[35] and can also be compared with those of tripeptides with histidine at both termini like Ac−HisProHis−OEt (5.95 and 6.97).[30]

For mixtures of THB and zinc nitrate, the potentiometric titrations yield a species distribution as depicted in Figure 2. Both a ZnL and a ZnL_2 species are detected near neutral pH which disappear in the basic region, leaving $LZnOH^+$ as the only zinc complex in solution. Thus, although there is also some precipitation above pH 6, in the dilute solutions of the potentiometric measurements, the typical mononuclear zinc complexes can be identified.

The thermodynamic stabilities of the zinc complexes are high. For ZnL, the log β value is 4.28(3); for ZnL_2, it is 8.11(10). In comparison, the ZnL complexes of Ac−His−NH_2 and Ac−HisProHis−NH_2 have log β values of 1.96[35] and 3.29,[30] respectively, and the ZnL_2 complexes of the latter two ligands have log β values of 5.74 and 7.10, respectively. Both for the ZnL and for the ZnL_2 species, ligand THB forms the most stable complexes, and in the whole series, the ZnL_x stabilities rise proportionally to the number of the imidazole donors present. This is strong evidence that ligand THB uses all its histidine units for coordination to one zinc ion. The species distribution and the stability constants for the $Zn(NO_3)_2$/THB system are quite similar to those for Potvin's tripodal tris(imidazolylalkyl)amine ligands,[16] while Steglich's tripodal "peptide bundle" with three histidine donors makes an even more stable ZnL complex.[6]

The most significant observation is the detection of a $LZnOH^+$ species which can be described as resulting from deprotonation of $LZn(OH_2)$ which is conventionally depicted as ZnL. From its log β value of −1.92(6), the pK_a of the zinc-bound water molecule can be calculated according to pK_a = log $\beta(ZnLOH^+)$ − log $\beta(ZnL^{2+})$ as 6.20. This is an unusually low value, being equaled only by the value obtained with Steglich's histidine-containing "peptide bundle".[6] In contrast, Potvin's tris(imidazolylalkyl)amine−zinc−aqua complexes yield pK_a's around 8[16] which can be considered normal for low-coordinate zinc in aqueous solution.[36] The fact that the histidine-terminal tripods generate such an acidic $Zn-OH_2$ species must have to do with the presence of the intact histine-ester attachments "beyond" the imidazole donors. The hydrophobic environment provided by them accounts for a significant pK_a depression,[6] just as it is observed with highly substituted tris(pyrazolyl)borate ligands.[23] The low pK_a also corresponds to a coordination number of zinc of not more than four, as experienced by ourselves[23] and others[36] before, lending support to the formulation of LZnOH as given here.



Altogether, the ZnL complex of THB with its tris(histidine) zinc coordination and its ability to form a Zn−OH species at neutral pH joins Steglich's "peptide bundle" zinc complexes[6] as "correct" models of the zinc enzyme carbonic

(35) Gockel, P.; Vahrenkamp, H.; Zuberbühler, A. D. *Helv. Chim. Acta* **1993**, *76*, 511.

(36) Bertini, I.; Luchinat, C.; Rosi, M.; Sgamelotti, A.; Tarantelli, F. *Inorg. Chem.* **1990**, *29*, 1460.

anhydrase[37] or a large group of matrix metalloproteases.[38,39] For the zinc−aqua function in these enzymes, pK_a values at or slightly above 7 are reported. Steglich's and our model complexes have even lower pK_a's; that is, they yield even higher concentrations of the Zn−OH species at neutral pH. It remains to be tested whether this can be exploited in terms of catalytic activities for CO_2 hydration or hydrolytic cleavages. To achieve this, the problems of isolating mononuclear zinc complexes or handling the complex solutions at pH values above 7 will have to be overcome.

Experimental Section

The general experimental procedures[40] and our techniques of peptide synthesis[30] were as described previously. Starting materials were obtained commercially. HPLC separations were performed on a commercial preparative scale column filled with the reversed phase material Nucleosil 7C18 by Macherey und Nagel, which allowed runs on a multigram scale. The trifluoroacetic acid (HTFA) contents of the products were determined by the potentiometric titrations; they were found to vary somewhat for otherwise identical preparations.

Ligand THB. Trimesic acid (630 mg, 3.00 mmol) in dimethylformamide (25 mL) was treated under vigorous stirring with carbonyldiimidazole (1.460 g, 9.00 mmol) in small portions. After stirring for 1 h, triethylamine (1.82 g, 18.0 mmol) and H−His−OEt·2HCl (2.305 g, 9.00 mmol) were added, and the mixture stirred overnight and then for 1 h at 40 °C. After filtration, the solvent was removed in vacuo and the residue mixed with 5 mL of ethanol and evaporated to dryness again (this step was repeated three times to remove all DMF). A 3.72 g portion of crude THB remained as a colorless oil. Purification by chromatography with ethanol over alumina was followed by HPLC with water/acetonitrile (84:16) containing 0.5% of HTFA. Final freeze-drying left 2.64 g (77%) of THB·3.9HTFA as a colorless powder, mp 61 °C.

Anal. Calcd for $C_{33}H_{39}N_9O_9 \cdot 3.9C_2HF_3O_2$ (M_r = 705.73 + 444.69): C, 42.60; H, 3.76; N, 10.96. Found: C, 42.58; H, 3.73; N, 11.26. IR (KBr): 3272w, 3151m (NH), 1741s (ester), 1670vs (amide I), 1540s (amide II), 1204vs (CF_3). ^{1}H NMR (DMSO-d_6): δ 1.15 [t, J = 7.1 Hz, 9H, Et], 3.26 [m, 6H, $C_\beta H_2$], 4.11 [q, J = 7.1 Hz, 6H, Et], 4.81 [m, 3H, $C_\alpha H$], 7.41 [s, 3H, im], 8.45 [s, 3H, C_6H_3], 8.97 [s, 3H, im], 9.25 [d, J = 7.6 Hz, 3H, NH−His]. ESI-MS: m/z 706.7 (M + H, 100%), 353.9 (M + 2H, 83%), 236.3 (M + 3H, 6%).

Ligand H_3TCB. Trimesic acid (630 mg, 3.00 mmol) in dimethylformamide (20 mL) and THF (10 mL) was treated under vigorous stirring with carbonyldiimidazole (1.460 g, 9.00 mmol) in small portions. After stirring for 1 h, triethylamine (910 mg, 9.00 mmol) and H−Cys(Trt)−OEt·HCl (3.85 g, 9.00 mmol) were added, and the mixture was stirred overnight and then for 1 h at 40 °C. After filtration, the solvents were removed in vacuo, and the residue was dissolved in 20 mL of ethyl acetate, washed five times with water, dried over Na_2SO_4, filtered, and evaporated to dryness. A 3.90 g (98%) portion of tritylated H_3TCB remained as a yellow oily solid. This product was dissolved with ice cooling in ethanethiol (15 mL) and HTFA (15 mL). After 2 h of stirring, the mixture was filtered and the filtrate evaporated to dryness. The oily residue was extracted three times with diethyl ether and then dried in vacuo. A 1.35 g (75%) portion of crude H_3TCB remained. Purification by HPLC as described previously with water/acetonitrile (60:40) and freeze-drying resulted in pure H_3TCB·0.1HTFA as a pale yellow powder, mp 190 °C.

Anal. Calcd for $C_{24}H_{33}N_3O_3S_3 \cdot 0.1C_2HF_3O_2$ (M_r = 603.74 + 11.40): C, 47,25; H, 5.42; N, 6.83. Found: C, 47.68; H, 5.39; N, 6.83. IR(KBr): 3445m, 3234w (NH), 1747vs (ester), 1645vs (amide I), 1558s (amide II). ^{1}H NMR (DMSO-d_6): δ 1.21 [t, J = 7.1 Hz, 9H, Et], 2.26 [t, J = 8.2 Hz, 3H, SH], 2.96 [m, 6H, $C_\beta H_2$], 4.15 [q, J = 7.1 Hz, 6H, Et], 4.60 [m, 3H, $C_\alpha H$], 8.52 [s, 3H, C_6H_3], 9.15 [d, J = 6.5 Hz, 3H, NH−Cys]. ESI-MS: m/z 642.8 (M + K, 35%), 626.7 (M + Na, 100%), 604.7 (M + H, 26%).

Ligand H_3TCM. Tris(2-carboxyethyl)nitromethane (832 mg, 3.00 mmol), triethylamine (910 mg, 9.00 mmol), and H−Cys(Trt)−OEt·HCl (3.852 g, 9.00 mmol) in DMF (25 mL) were cooled to 5 °C. Hydroxybenzotriazole (HOBT, 1.216 g, 9.00 mmol) and dicyclohexylcarbodiimide (DCC, 1.857 g, 9.00 mmol) were added with stirring in small portions. After stirring overnight, the mixture was filtered and the filtrate evaporated to dryness. The residue was dissolved in ethyl acetate and washed subsequently with two portions of aqueous acetate buffer, $NaHCO_3$ solution, water, and NaCl solution. After drying over Na_2SO_4 and filtration, the solution was evaporated to dryness. A 4.00 g (95%) portion of crude tritylated H_3TCM remained as a yellow oil. This oil was dissolved with ice cooling in ethanethiol (15 mL) and HTFA (15 mL) with ice cooling. After 2 h of stirring, the mixture was filtrated and the filtrate evaporated to dryness. The oily residue was extracted three times with diethyl ether and then dried in vacuo. A 1.29 g (63%) portion of crude H_3TCM remained as a yellow oil. HPLC as described with water/acetonitrile (60:40) and freeze-drying resulted in pure H_3TCM·0.4HTFA as a pale yellow solid, mp 50 °C.

Anal. Calcd for $C_{25}H_{42}N_4O_{11}S_3 \cdot 0.4C_2HF_3O_2$ (M_r = 670.82 + 45.61): C, 43.25; H, 5.96; N, 7.82. Found: C, 43.76; H, 5.89; N, 7.84. IR (KBr): 3368w (NH), 1739vs (ester), 1656vs (amide I), 1539vs (amide II), 1205s (CF_3). ^{1}H NMR (DMSO-d_6): δ 1.19 [t, J = 7.0 Hz, 9H, Et], 2.15 [m, 12H, CH_2CH_2], 2.26 [t, J = 8.6 Hz, 3H, SH], 2.78 [m, 6H, $C_\beta H_2$], 4.11 [q, J = 7.0 Hz, 6H, Et], 4.42 [m, 3H, $C_\alpha H$], 8.33 [d, J = 7.8 Hz, 3H, NH−Cys]. ESI-MS: 709.9 (M + K, 78%), 693.8 (M + Na, 100%), 671.3 (M + H, 49%).

Complex 1. THB·3.9HTFA (293 mg, 0.26 mmol) in ethanol (16 mL) was treated under stirring with 7 mL (1.4 mmol) of a 0.2 M solution of sodium ethoxide in ethanol. $ZnCl_2$ (35 mg, 0.26 mmol) in ethanol (5 mL) was added with stirring. The resulting precipitate was filtered off, washed with ethanol, and dried in vacuo. A 103 mg (97%) portion of **1** remained as a colorless powder, mp 270 °C (dec).

Anal. Calcd for $C_{66}H_{78}Cl_{10}N_{18}O_{18}Zn_5$ (M_r = 2092.93): C, 37.88; H, 3.76; N, 12.05; Zn, 15.62. Found: C, 38.16; H, 3.67; N, 11.93; Zn, 15.53. IR (KBr): 3446m (NH), 1733vs (ester), 1654vs (amide I), 1541s (amide II).

Complex 2. H_3TCB·0.1HTFA (123 mg, 0.20 mmol) in ethanol (20 mL) was treated with triethylamine (88 μL, 64 mg, 0.63 mmol) and acetonitrile (5 mL) to give a clear solution. $Zn(ClO_4)_2 \cdot 6H_2O$ (114 mg, 0.30 mmol) in ethanol (10 mL) was added with stirring. The resulting precipitate was filtered off, washed with ethanol, and dried in vacuo. A 92 mg (65%) portion of **2** remained as a colorless powder, mp 230 °C (dec).

Anal. Calcd for $C_{48}H_{60}N_6O_{18}S_6Zn_3$ (M_r = 1397.60): C, 41.25; H, 4.33; N, 6.01; Zn, 14.04. Found: C, 39.71; H, 4.80; N, 5.91; Zn, 13.81. IR (KBr): 3382s (NH), 1733s (ester), 1654vs (amide I), 1524s (amide II).

(37) Botrè, F.; Gros, G.; Storey, B. T.; Eds. *Carbonic Anhydrase*; VCH Publishers: Weinheim, Germany, 1991.
(38) Christianson, D. W. *Adv. Protein Chem.* **1991**, *42*, 281.
(39) Vallee, B. L.; Auld, D. S. *Acc. Chem. Res.* **1993**, *26*, 543.
(40) Förster, M.; Burth, R.; Powell, A. K.; Eiche, T.; Vahrenkamp, H. *Chem. Ber.* **1993**, *126*, 2643.

Complex 3 was synthesized as described in a previous paragraph from $H_3TCM \cdot 0.4HTFA$ (248 mg, 0.35 mmol), sodium methoxide (5.9 mL = 1.2 mmol of a 0.2 M solution), and $Zn(NO_3)_2 \cdot 6H_2O$ (154 mg, 0.52 mmol). Yield 45 mg (17%) of **3** as a colorless powder, mp 200 °C (dec).

Anal. Calcd for $C_{48}H_{64}N_6O_{20}S_6Zn_3$ (M_r = 1531.77): C, 39.21; H, 5.13; N, 7.32; Zn, 12.81. Found: C, 39.52; H, 5.27; N, 6.62; Zn, 12.64. IR (KBr): 3445s (NH), 1733s (ester), 1655s (amide I), 1540vs (amide II).

Potentiometric Titrations. The apparatus used, the experimental details, the calibration techniques, and the titration procedures were as described before.[30,35] The common anion in all solutions was nitrate (KNO_3, $Zn(NO_3)_2$, HNO_3), the ionic strength was 0.1, and all measurements were done at 25 ± 0.1 °C. The starting concentrations were the following: THB, 1.040; additional acid, 0.424−2.306; Zn, 0.244−0.489; NaOH, 0.202 mol/L. Eight titrations were performed and analyzed. Zuberbühler's TITFIT program[41] including the evaluation procedures for precipitate formation[42] was used for the computations.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. We thank Mrs. F. Bitgül for technical assistance and Dr. J. Wörth for mass spectra.

IC011263C

(41) Zuberbühler, A. D.; Kaden, T. A. *Talanta* **1982**, *29*, 201.
(42) Kaden, T. A.; Schaller, K. H.; Sigel, H. *Inorg. Chem.* **1986**, *25*, 1313.