Zinc Complexes of Amino Acids and Peptides, 7[◇]

# Solution Behavior and Zinc Complexation of Dipeptides Made up Solely from Histidine and Cysteine☆

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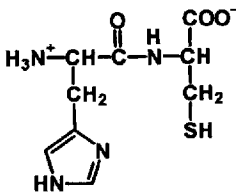
The eight possible types of dipeptides made up from histidine and cysteine, namely the unprotected ones H-His-Cys-OH (**1**) and H-Cys-His-OH (**2**), the *N*-protected ones Ac-His-Cys-OH (**3**) and Ac-Cys-His-OH (**4**), the *C*-protected ones H-His-Cys-OEt (**5**) and H-Cys-His-$NH_2$ (**6**), and the fully protected ones Ac-His-Cys-OEt (**7**) and Ac-Cys-His-OEt (**8**) were prepared analytically pure. Their acid-base behavior and zinc complexation in solution were studied potentiometrically. In all cases 1:1 (zinc:peptide) complexes are the dominant species near neutral pH. 1:2 complexes could only be detected for **4**, **6**, **7**, and **8**, and precipitations occurred in basic media. From equimolar mixtures of the peptides and zinc salts (chloride, iodide, perchlorate) solid complexes were obtained upon addition of a base. They always contain coordinating anions (chloride, iodide, or trifluoroacetate which was introduced with the peptide). They have the composition LZnX for L = **5**–**8** and $L_4Zn_5X_2$ for L = **1**–**4**. It is proposed that all complex species observed contain tetrahedral zinc, that they are monomers in dilute solution and thiolate-bridged oligomers in the solid state, and that the peptides as a rule occupy at least three coordination sites.

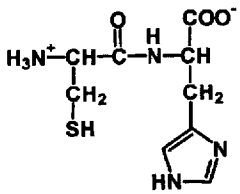
Cysteine and histidine are the most prominent amino acids for the coordination of zinc in proteins, their thiolate and imidazole donor functions being ideally suited for the moderately soft Lewis acid $Zn^{2+}$ in low coordination numbers[2–4]. While catalytically active zinc centers in metallo enzymes bear at least one additional oxygen donor (aspartate, glutamate, water, or the substrate), exclusive *N* and *S* coordination is realized in most structural zinc centers in enzymes[2,3] or the zinc finger proteins[5,6]. It is for this biological significance that the coordination chemistry of zinc with the amino acids cysteine and histidine and their derivatives draws its justification.

The earliest preparative studies of cysteine- and histidine-containing zinc complexes date back to the fifties[7,8], and the first review mentioning them appeared in 1973[9]. The lack of crystallization of the resulting species has made potentiometric investigations of solution equilibria an important method for this subject, having again been applied quite early to the simple systems[10] and appearing in tabulations[11] and review articles[4]. However, while the zinc coordination of the two simple amino acids and their derivatives is well-investigated and while small peptides containing one of them have been used to a reasonable degree for this purpose[4,9,12–14], very few reports exist on the coordination chemistry of peptides containing cysteine *and* histidine, and to our knowledge only one such dipeptide, benzoyl-Cys-His-OMe, has been used for zinc complexation[15].

In continuation of our systematic studies[1,10,13,14,16] we therefore chose the series of the eight possible types of Cys/His dipeptides **1**–**8** for an investigation of their zinc complex chemistry. This series consists of four pairs being either completely unprotected (**1**, **2**), only *N*-protected (**3**, **4**), only *C*-protected (**5**, **6**), or fully protected (**7**, **8**), always having the free thiol and imidazole functions available for coordination. Depending on the degree of protection, these dipeptides are potentially tetradentate (**1**, **2**), tridentate (**3**–**6**), or bidentate (**7**, **8**).



H-His-Cys-OH
**1**
Ac-His-Cys-OH
**3**
H-His-Cys-OEt
**5**
Ac-His-Cys-OEt
**7**



H-Cys-His-OH
**2**
Ac-Cys-His-$OH_2$
**4**
H-Cys-His-$NH_2$
**6**
Ac-Cys-His-OEt
**8**

## Peptide Syntheses

The cysteine/histidine combination is the most difficult one among dipeptides, due to the trifunctional nature of

[◇] Part 6: Ref.[1].

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both constituents. It therefore took some effort to obtain the eight dipeptides, specifically the unprotected ones **1** and **2**, in analytically pure form. When necessary, we used the Boc (*tert*-butoxycarbonyl) group to protect the histidine imidazole and the Trt (trityl) group to protect the cysteine thiol function, using in addition cystine instead of cysteine in the early stages of the peptide syntheses. Starting from histidine, cystine, histidine ethyl ester, and *N*-acetylcysteine and using the established procedures for protection and coupling[17], we prepared the following dipeptide derivatives as the immediate precursors of the dipeptides **1**–**8** (Z = benzyloxycarbonyl):

[Boc-His(Boc)-Cys-OEt]$_2$ (Ac-His-Cys-OEt)$_2$
(H-Cys-His-OMe)$_2$ (Z-Cys-His-NH$_2$)$_2$
Ac-Cys(Trt)-His-OEt

From these the protecting groups were removed according to the sequences described in the experimental part. In general, trifluoroacetic acid was used to remove Boc groups, dithioerythritol was used to cleave the cystine S–S bonds, and alkaline hydrolysis was used to remove ester groups. Of the four *C*-protected dipeptides **5**–**8**, **6** is the only one bearing an amide instead of an ester function. The reason for this is that H-Cys-His-OEt proved to be somewhat labile towards ester hydrolysis during the potentiometric titrations.

After the deprotection steps the resulting peptides **1**–**8** were purified by HPLC using the reversed-phase adsorbent Nucleosil 7 C18 and eluting with acetonitrile/water mixtures containing 0.1% trifluoroacetic acid (HTFA). They were isolated as analytically pure colorless powders containing at least 1.5 equivalents of HTFA per equivalent of peptide. The HTFA content was verified by $^{19}$F-NMR spectroscopy ($\delta = -73.5 \pm 2$) and quantified by potentiometric titrations.

### Solution Equilibria

The eight dipeptides **1**–**8** contain variable numbers of acid/base functions and donor sites for metal complexation. The method of potentiometric titration was used to quantify their behavior in solution and to establish their mutual interrelations. The experimental procedures used correspond to those applied by us to the related derivatives of the single amino acids histidine and cysteine[10] which can also serve as points of reference.

Table 1 lists the p$K_a$ values for the four dissociable functions in the dipeptides. The imidazole function (p$K \approx 6-7$) and the thiol function (p$K \approx 8$) are available in all eight cases, while the carboxylic acid function (p$K \approx 2.5$) and the amino function (p$K \approx 9$) are blocked each in four cases.

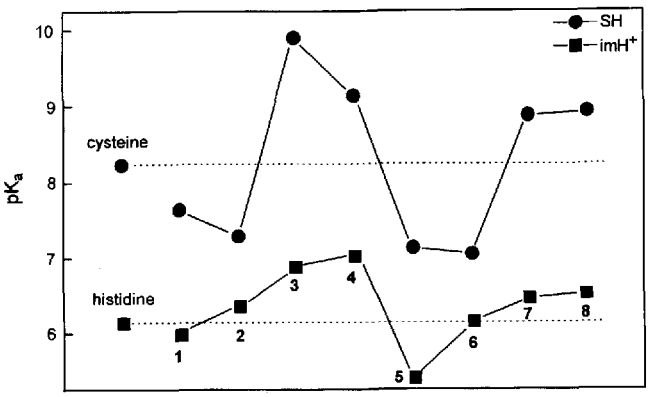
The unprotected amino acids cysteine and histidine have carboxylate p$K_a$ values of 1.82 and 1.77, respectively, which are raised to 3.08 and 2.94 in their *N*-acetylated derivatives. The carboxylate p$K_a$ values of **1**–**4** lie between these extremes. The spatial (**1**, **2**) or chemical (**3**, **4**) removal of the stabilizing $NH_3^+$ functions raises the p$K_a$ values with respect to those of the free amino acids, but the fact that distant stabilizing functions are still present ($NH_3^+$ in **1** and **2**, im-H$^+$ in **1**–**4**) limits the rise. Likewise, in comparison with the $NH_3^+$ p$K_a$ value of cysteine (10.36), those of **2** and **6** with *N*-terminal cysteine are lowered by about 1 unit. In comparison with the $NH_3^+$ p$K_a$ value of histidine (9.24) those of **1** and **5** with *N*-terminal histidine are not typically changed. This is attributed to the fact that the nearest acid/base function in **1** and **5** (imidazole) is uncharged and that that in **2** and **6** (thiolate) is charged when the $NH_3^+$ function is deprotonated. In the same context it is noticeable that among the eight peptides H-His-Cys-OH (**1**) has the highest acidity of the carboxylic acid function and the highest basicity of the amino function. This indicates that this peptide prefers a conformation which minimizes the distance between these functions in solution.

Table 1. p$K_\alpha$ values of the dipeptides

| Acid | COOH | imH$^+$ | SH | $NH_3^+$ |
|---|---|---|---|---|
| HHisCysOH (**1**) | 2.33(10) | 5.98(4) | 7.64(3) | 9.89(2) |
| HCysHisOH (**2**) | 2.74(3) | 6.37(1) | 7.30(1) | 9.48(1) |
| AcHisCysOH (**3**) | 2.85(6) | 6.82(4) | 9.83(3) | -- |
| AcCysHisOH (**4**) | 2.81(10) | 7.02(8) | 9.13(6) | -- |
| HHisCysOEt (**5**) | -- | 5.45(14) | 7.17(12) | 8.84(8) |
| HCysHisNH$_2$ (**6**) | -- | 6.13(1) | 7.03(1) | 9.26(1) |
| AcHisCysOEt (**7**) | -- | 6.48(2) | 8.89(2) | -- |
| AcCysHisOEt (**8**) | -- | 6.53(3) | 9.00(2) | -- |

Figure 1 is a graphical representation of the p$K_a$ variations of the thiol and imidazole functions along the series. The reference points are the corresponding p$K_a$ values of cysteine (8.24) and histidine (6.14). In general, both functions are more acidic for the *N*-unprotected peptides (**1**, **2**, **5**, **6**) and less acidic for the *N*-protected peptides (**3**, **4**, **7**, **8**). Conversely, the effect of changing the C terminus (protected vs. unprotected) is less significant. This indicates that the charge at the N terminus is the dominating mediator for both the SH and the imH$^+$ acidities which to our opinion is again a conformational effect.

Figure 1. p$K_a$ values for the imidazole and thiol functions in the peptides **1**–**8**




For the evaluation of the complexation equilibria potentiometric titrations were performed for 1 mM solutions of the peptides in the presence of ca. 0.3, 0.6, or 1.2 equivalents of $Zn(NO_3)_2$. Under these conditions precipitation of

zinc compounds in basic media was minimized (it was, however, observed for **1**–**3**), and the titration curves could be analyzed unambiguously. Figures 2 and 3 show two typical species distributions.

Figure 2. Species distribution in solutions of Ac-Cys-His-OH (**4**) and $Zn(NO_3)_2$ (1.6:1)

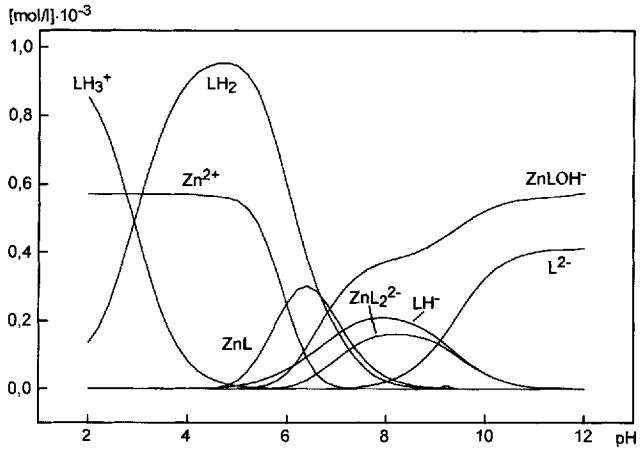
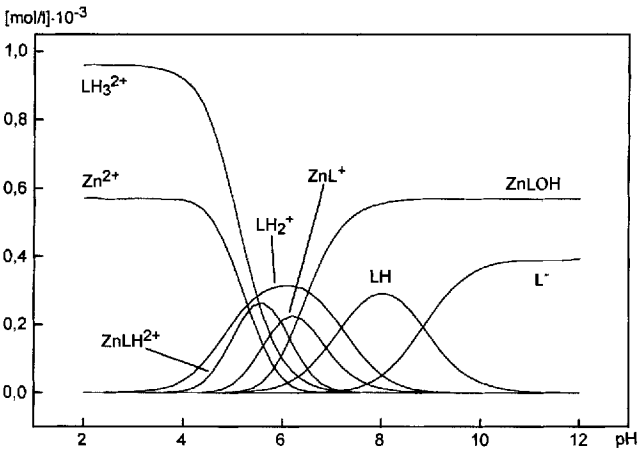



Figure 3. Species distribution in solutions of H-His-Cys-OEt (**5**) and $Zn(NO_3)_2$ (1.7:1)




The species observed in solution are the peptides with varying degrees of protonation, the 1:1 complexes ZnL and ZnLH, the singly deprotonated 1:1 complexes ZnLOH, and the 1:2 complexes $ZnL_2$. It must be noted that this notation neglects the charges of the species which are correctly assigned in Figures 2 and 3 and which can be derived by evaluating the charge of the fully protonated ligand $LH_n$ for which $n = 4$ for **1** and **2**, $n = 3$ for **3**–**6**, and $n = 2$ for **7** and **8**. The notation ZnLOH corresponds to a species with $n = 1$ to which we assign a zinc complex with a deprotonated aqua ligand rather than with a peptide ligand deprotonated at the NH function. Table 2 lists the overall stability constants β for the observed species ZnL ($\beta_{(110)}$), $ZnL_2$ ($\beta_{(120)}$), ZnLH ($\beta_{(111)}$), and ZnLOH ($\beta_{(11-1)}$), according to the following definitions:

$$\beta_{(110)} = \frac{[ZnL]}{[Zn][L]} \qquad K_{ZnL} = \frac{[ZnL]}{[ZnL][L]}$$

$$\beta_{(120)} = \frac{[ZnL_2]}{[Zn][L]^2} \qquad K_{ZnL_2} = \frac{[ZnL_2]}{[ZnL][L]}$$

$$\beta_{(111)} = \frac{[ZnLH]}{[Zn][L][H]} \qquad K_{ZnLH} = \frac{[ZnLH]}{[ZnL][H]} = \frac{[ZnLH]}{[Zn][LH]}$$

$$\beta_{(11-1)} = \frac{[ZnLOH][H]}{[Zn][L]} \qquad K_{ZnLOH} = \frac{[ZnLOH][H]}{[ZnL]}$$

Table 2. Stability constants (log β) for the zinc complex species in solution

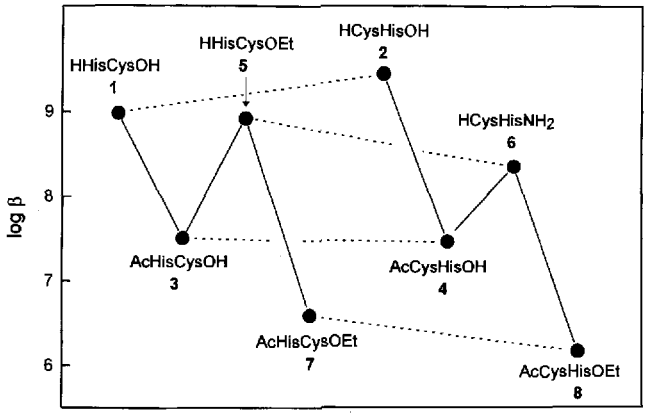
| Ligand | ZnL | $ZnL_2$ | ZnLH | ZnLOH |
|---|---|---|---|---|
| HHisCysOH (**1**) | 9.00(3) | -- | 15.35(3) | -- |
| HCysHisOH (**2**) | 9.41(6) | -- | 15.34(3) | -- |
| AcHisCysOH (**3**) | 7.52(6) | -- | 12.80(8) | -- |
| AcCysHisOH (**4**) | 7.43(6) | 12.97(8) | -- | 0.56(6) |
| HHisCysOEt (**5**) | 8.36(10) | -- | 14.31(12) | 2.00(20) |
| $HCysHisNH_2$ (**6**) | 8.90(6) | 16.33(9) | 13.75(6) | 2.87(5) |
| AcHisCysOEt (**7**) | 6.17(2) | 12.42(3) | -- | -0.98(12) |
| AcCysHisOEt (**8**) | 6.57(6) | 11.72(9) | -- | 0.20(7) |

In all cases the 1:1 complexes ZnL are observed. Their stabilities are quite high as can be seen by comparison of the log β values for the H-His-Cys ligands **1** (9.00) and **5** (8.36) with that for H-His-OMe (4.45), or of those for the H-Cys-His ligands **2** (9.41) and **6** (8.90) with that for H-Cys-OEt (7.42). The 1:2 complexes $ZnL_2$ could be identified only for **4**, **6**, **7**, and **8**. Their gain in stability with respect to the 1:1 complexes amounts to about 5 log units except for **8** where it is 7.5 log units. Only the latter value corresponds to a significant gain which is comparable with those observed in going from the ZnL to the $ZnL_2$ complexes of cysteine and cysteine ethyl ester. For all dipeptides **1**–**8** at least one ZnLH or ZnLOH complex is observed. They are pH-dependent variations of the ZnL species, and their existence underlines the preference for the 1:1 composition. The fact that the ZnLH species is observed for all four *N*-unprotected peptides and only for one *N*-protected peptide seems to indicate that the amino function is the protonated one in ZnLH.

Figure 4 depicts the stability relations between the eight ZnL complexes. The peptides being related only by exchanging His and Cys are connected by dotted lines. It is obvious that the free amino function (*N*-unprotected peptides) is of prime importance for high stabilities. It allows formation of the favorable five- or six-membered chelate rings with *N,S* or *N,N* coordination. In contrast, despite the negative charge provided by the carboxylate in peptides **3** and **4**, the resulting six- and seven-membered chelate rings with *S,O* and *N,O* coordination provide significantly less stabilization. And finally the fully protected peptides **7** and **8** offer no favorable chelation and hence provide the lowest stabilities.

It should be noticed, however, that even the relatively low ZnL stabilities for **7** and **8** are still considerable. This becomes obvious by comparison with the log $\beta_{(ZnL)}$ values for

Figure 4. Overall stability constants (log β) of the ZnL complexes of peptides **1**–**8**




glycine (5.19[11]) or *N*-acetylcysteine (4.90[10]) which both can form chelate rings with one or two negative charges. This indicates that the 10-membered chelate rings generated by coordination of **7** or **8** must be relatively strain-free. Along the same line it should be noted that the ZnL complexes of **1** and **2** are the most stable zinc peptide complexes reported until today.

Compared to the dramatic decrease in complex stability upon blocking of the N terminus, the corresponding effect upon blocking of the C terminus is less pronounced. It amounts to log β changes between 0.5 (**2**/**6**) and 1.35 (**3**/**7**). It is strong enough, though, to point to the fact that in addition to the thiolate and imidazole functions the carboxylate function is also a coordinating unit in the *C*-unprotected peptides.

### Isolated Complexes

The solution equilibrium studies revealed that the complex species ZnL exist in neutral or weakly basic solutions and that $ZnL_2$ complexes have a much smaller probability of formation. This was borne out by the preparative studies which failed to produce $ZnL_2$ compounds but yielded variations of the ZnL composition in all cases. The preparative procedure consisted in the combination of the zinc salt and the peptide, normally in ethanol, and inducing precipitation of the product by adding triethylamine as the reagent for the consumption of the equivalents of acid present. The stoichiometric ratio Zn/L was of little influence on the outcome of the reaction, but purer products were obtained by using a 1:1 ratio. $ZnCl_2$ and $ZnI_2$ were used as zinc salts of coordinating anions, $Zn(ClO_4)_2 \cdot 6\ H_2O$ was meant to provide zinc ions without coordinating anions. It turned out, however, that the trifluoroacetate (TFA) introduced with the peptides, acted as the coordinating anion in this case.

Using the *C*-protected peptides **5**–**8**, we found a simple verification of the ZnL composition by isolation of the complexes LZnX:

| **5** · ZnX | **6** · ZnX | **7** · ZnX | **8** · ZnX |
|---|---|---|---|
| **15b**: X = I | **16b**: X = I | **17a**: X = Cl | **18a**: X = Cl |
| **15c**: X = TFA | **16c**: X = TFA | **17b**: X = I | **18b**: X = I |
| | | **17c**: X = TFA | **18c**: X = TFA |

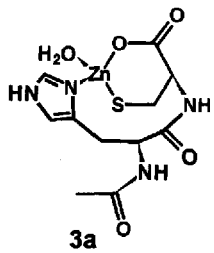
Using the *C*-unprotected peptides **1**–**4**, we could expect products of the composition ZnL. However, in all cases the isolated complexes had the composition $L_4Zn_5X_2$ which can be written as 4 ZnL · $ZnX_2$:

| $(\mathbf{1})_4Zn_5X_2$ | $(\mathbf{2})_4Zn_5X_2$ | $(\mathbf{3})_4Zn_5X_2$ | $(\mathbf{4})_4Zn_5X_2$ |
|---|---|---|---|
| **11b**: X = I | **12a**: X = Cl | **13b**: X = I | **14b**: X = I |
| **11c**: X = TFA | **12b**: X = I | **13c**: X = TFA | **14c**: X = TFA |
| | **12c**: X = TFA | | |

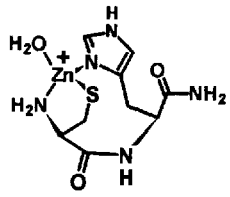
All complexes **11**–**18** have a low solubility in all common solvents, thereby indicating their oligomeric nature. They could, however, be subjected to $^1$H-NMR spectroscopy in hot DMSO (see Experimental) which confirmed the presence of the intact ligands. $^{19}$F-NMR spectroscopy confirmed the presence of trifluoroacetate in all complexes **c**. In the IR spectra (see Experimental) the S–H or O–H bands of the free peptides are missing, as expected. Assuming monodentate coordination by the anions, it can therefore be concluded that the peptides provide at least three donor functions in complexes LZnX and at least four donor functions for the ZnL constituents of complexes $L_4Zn_5X_2$. Attempts to crystallize any one of the isolated complexes for a structure determination remained unsucessful.

### Conclusions

The high stabilities of the ZnL complexes in solution point to favorable chelating properties of the peptide ligands L, the low solubility of the isolated complexes points to their multidentate nature. This allows us to propose constitutional assignments with a reasonable degree of certainty. We propose tridentate coordination in dilute solution as exemplified by **3a** and **6a** containing **3** and **6** as ligands. Dilute solution conditions (1 mM) prevail during the potentiometric titrations, thereby reducing the probability of intermolecular coordination of the various donor functions and of thiolate bridging.




**3a**

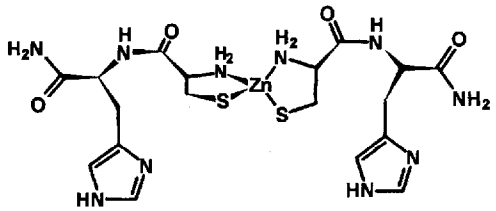



**6a**

In concentrated solution or in the solid state oligomerization seems to occur. For this situation it is more difficult to make constitutional proposals due to the multitude of possibilities. We assume, however, that zinc is tetrahedrally coordinated in all cases because this is the standard situation for N/S ligand sets[2,3]. For ligands **1**, **2**, **3**, and **6** which can form favorable N,S or O,S chelate rings the ligation pattern in the oligomers should be just like it is in **3a** and **6a** except that the "dangling" donor provides the bridging function. For ligands **4**, **5**, **7**, and **8** which do not favor terminal thiolate coordination due to chelation the possibility of thiolate bridging must be considered. This would in-

crease the number of donor functions per peptide by one, which could be accounted for by replacement of the ligating water molecules. Alternatively, with coordinating anions, less potent donor functions, e.g. carboxylate, might stay unused.

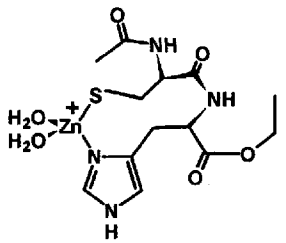
The preference of the peptides to be tridentate can also explain why $ZnL_2$ complexes are not favored. In going from a ZnL to a $ZnL_2$ complex either the first L has to be converted from tridentate to bidentate or the second L has to come in monodentate. There is only one favorable case for this, namely $ZnL_2$ with L = H-Cys-His-$NH_2$ (**6**). In this case $ZnL_2$ is a neutral $ZnN_2S_2$ complex with two five-ring chelate ligands, one of the most stable situations in zinc complex chemistry. The exceptionally high stability of the resulting complex **6b** confirms this.



**6b**

We cannot offer a good explanation for the 4:5:2 composition of complexes **11**–**14** other than that they are 4:1 composities of ZnL and $ZnX_2$. A neutral unit ZnL implies two negative charges on L which is easily realized for L = **1**–**4**. It also requires the provision of four donor functions by $L^{2-}$ which is no problem for **1** and **2** and which implies bidentate carboxylate or bridging thiolate for **3** and **4**. Why the stoichiometric ratio $ZnL/ZnX_2$ is persistently 4:1 in these compounds remains an open question.

Compared to the situation in proteins most of the peptides used here are still "unnatural": **1**–**6** contain either terminal amino or terminal carboxylate functions which due to their ligating and chelating properties cause too high complex stabilities. Only **7** and **8** which form the least stable ZnL complexes possess the "natural" unsupported thiolate and imidazole donors. However, as pointed out above, the stabilities of their ZnL complexes are still considerable despite the presence of 10-membered chelate rings as in **8a**.



**8a**

Thus, the dipeptides investigated here are still only milestones on the way to an understanding of zinc complexation by proteins. The favorable interaction between cysteine and histidine in the fully protected peptides **7** and **8** can, however, be taken as a preview of the preorganization of the polypeptides causing the site specificity of zinc ion incorporation.

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## Experimental

The general working and measuring methods are described in ref.[16], the HPLC procedures in ref.[14], the procedures for potentiometric measurements in ref.[10]. The amino acid starting materials (optically pure L amino acids) and the zinc salts were obtained commercially.

The peptide syntheses started from histidine, cystine, histidine ethyl ester, and *N*-acetylcysteine. Standard protecting and coupling techniques[17] were used to convert these to the immediate precursors of the peptides **1**–**8**, viz. the S–S dimers [Boc-His(Boc)-Cys-OEt]$_2$, (Ac-His-Cys-OEt)$_2$, (H-Cys-His-OMe)$_2$ and (Boc-Cys-His-$NH_2$)$_2$ and to Ac-Cys(Trt)-His-OEt. For the following procedures the abbreviations HTFA = trifluoroacetic acid and DTE = dithioerythritol are used. The HPLC eluents always contained 0.1% HTFA causing all peptides to be isolated as HTFA adducts. Their precise HTFA content was determined by potentiometric titration and confirmed by elemental analysis. All elemental analyses are summarized in Table 4.

*Peptides* **1**: A solution of 5.67 g (5.84 mmol) of [Boc-His(Boc)-Cys-OEt]$_2$ in 50 ml of methanol was treated with 1.17 g (7.60 mmol) of DTE. The pH was adjusted to 8 with 0.2 M NaOH and the mixture stirred for 4 h. Then 50 ml of 1 M NaOH was added, and stirring was continued for 2 h. Then the mixture was acidified to pH 2 with 1 M HCl. The solvent was removed in vacuo, the residue taken up in 50 ml of methanol, the solution filtered and the filtrate evaporated to dryness again. The residue was treated five times with 10 ml of ether each in an ultrasound bath. The remaining colorless powder consisted of 4.01 g (96%) of Boc-His-Cys-OH which was spectroscopically pure. – 4.87 g (13.57 mmol) of Boc-His-Cys-OH was treated at 0 °C with 16 ml of anhydrous HTFA. The mixture was stirred for 20 h during which time it was allowed to warm up to room temp. All volatile components were removed in vacuo. HPLC (water/acetonitrile, gradient 98/2···90/10) yielded 1.98 g (30%) of **1** · 2 HTFA as a colorless, very hygroscopic powder of m.p. 109 °C.

**2**: A solution of 5.86 g (6.88 mmol) of (H-Cys-His-OMe)$_2$ · 4 HBr in 16 ml of methanol was treated with a solution of 0.25 g (10.8 mmol) of sodium in 10 ml of methanol. After 5 min the resulting precipitate was filtered and dried in vacuo. The remaining colorless powder consisted of 3.46 g (98%) of (H-Cys-His-OH)$_2$ which was spectroscopically pure. – A solution of 2.00 g (3.89 mmol) of (H-Cys-His-OH)$_2$ in 50 ml of water was treated with a solution of 1.37 g (8.90 mmol) of DTE in 20 ml of water. The pH was adjusted to 8.5 with 2 M NaOH, and the resulting solution was stirred under nitrogen for 4 h at room temp. The mixture was acidified to pH 3 with HTFA and freeze-dried. The resulting raw product was washed 3 times each with 10 ml of diethyl ether, then dissolved in 7 ml of water. HPLC (water/acetonitrile, 100:0, for 10 min followed by 80:20) yielded 0.68 g (68%) of **2** as a colorless powder of m.p. 68 °C.

**7**: A solution of 3.35 g (3.80 mmol) of (Ac-His-Cys-OEt · HTFA)$_2$ in 30 ml of methanol was treated with 0.76 g (4.94 mmol) of DTE. The pH was adjusted to 8 with 0.2 M NaOH and the mixture stirred for 3 h at room temp. Then the mixture was acidi-

fied with HTFA to pH 2 and the solvent removed in vacuo. The remaining oil was dissolved in methanol, the solution filtered and the filtrate evaporated to dryness again. HPLC (water/acetonitrile, 85:15) yielded 2.56 g (65%) of **7** · 1.5 HTFA · $H_2O$ as a colorless, very hygroscopic powder of m.p. 89°C.

**3**: 1.39 g (2.68 mmol) of **7** · 1.5 HTFA · $H_2O$ was dissolved in a 1:1 mixture of 30 ml of methanol and 30 ml of 1 M NaOH and the solution stirred at room temp. for 4 h. Then the mixture was acidified to pH 3 with 1 M HCl and the solvent removed in vacuo. The residue was suspended in methanol, the suspension filtered and the filtrate evaporated to dryness again. HPLC (water/acetonitrile, 92:8) yielded 0.96 g (69%) of **3** · 1.5 HTFA · EtOH as a colorless powder of m.p. 179°C.

**8**: 3.89 g (6.82 mmol) of Ac-Cys(Trt)-His-OEt was treated with 20 ml of ethanthiol, followed by 20 ml of HTFA. The mixture was stirred for 2.5 h, and then the solvent was removed in vacuo. The remaining solid was suspended in 50 ml of water and the suspension extracted 3 times each with 20 ml of diethyl ether. The combined organic layers were washed 3 times each with 20 ml of water and then dried in vacuo. HPLC (water/acetonitrile, 90:10) yielded 2.01 g (59%) of **8** · 1.5 HTFA as a colourless crystalline solid of m.p. 99°C.

**4**: 0.68 g (1.36 mmol) of Ac-Cys-His-OEt · 1.5 HTFA was dissolved in a 1:1 mixture of 20 ml of methanol and 20 ml of 1 M NaOH and the solution stirred at room temp. for 5 h. Then the mixture was acidified to pH 3 with 1 M HCl and the solvent removed in vacuo. The residue was suspended in methanol, the suspension filtered and the filtrate evaporated to dryness again. HPLC (water/acetonitrile, 92:8) yielded 0.48 g (75%) of **4** · 1.5 HTFA as a colorless crystalline solid of m.p. 91°C.

**5**: 8.94 g (9.20 mmol) of [Boc-His(Boc)-Cys-OEt]$_2$ was treated with 25 ml of HTFA at 0°C. During 15 h the stirred mixture was allowed to warm to room temp. Then the solvent was removed in vacuo. The remaining oil was triturated with dry diethyl ether and then dried in vacuo. The remaining colorless powder consisted of 9.81 g (97%) of (H-His-Cys-OEt · 2 HTFA)$_2$ which was spectroscopically pure. – A solution of 1.38 g (1.34 mmol) of (H-His-Cys-OEt · 2 HTFA)$_2$ in 30 ml of methanol was treated with 0.27 g (1.75 mmol) of DTE. The pH was adjusted to 8 with 0.2 M NaOH, and the mixture was stirred for 4 h at room temp. It was then acidified with HTFA to pH 2 and the solvent removed in vacuo. The remaining solid was dissolved in methanol, the solution filtered and the filtrate evaporated to dryness again. HPLC (water/acetonitrile, 90/10) yielded 0.84 g (61%) of **5** · 2.5 HTFA as a colorless, very hygroscopic powder of m.p. 82–84°C.

**6**: 3.66 g (4.69 mmol) of (Z-Cys-His-$NH_2$)$_2$ (Z = benzyloxycarbonyl) was suspended in 15 ml of glacial acetic acid and the suspension treated with a solution of 15 ml of HBr in glacial acetic acid (33%). After 16 h the resulting precipitate was collected by filtration, washed 3 times each with 40 ml of diethyl ether and dried for 4 d with KOH. The resulting light yellow powder consisted of 3.61 g (92%) of (H-Cys-His-$NH_2$)$_2$ · 4 HBr of m.p. 178°C. – A solution of 1.00 g (1.20 mmol) of (H-Cys-His-$NH_2$)$_2$ · 4 HBr in 20 ml of water was treated with 0.23 g (1.45 mmol) of DTE. The pH was adjusted to 8.5 with 2 M NaOH, and the mixture was stirred for 4 h at room temp. It was then acidified with HTFA to pH 3, and the solvent was removed in vacuo. The remaining solid was washed 3 times each with 10 ml of diethyl ether. HPLC (water/acetonitrile, 100:0) yielded 0.41 g (70%) of **6** · 2 HTFA as a colorless powder of m.p. 76°C.

Relevant IR data of the peptides [ν(OH), ν(NH), ν(SH), amide bands, in KBr, $\tilde{\nu}$ in $cm^{-1}$]: **1**: 3423, 3239, 3147, 2652, 1677, 1635, 1558, 1543. **2**: 3391, 3147, 3043, 2642, 1662, 1561, 1532. **3**: 3300, 3154, 3029, 2700, 1638, 1545. **4**: 3290, 3153, 3042, 2639, 1673, 1542. **5**: 3421, 3390, 3146, 3037, 2669, 1737, 1673, 1558, 1543. **6**: 3394, 3149, 3024, 2632, 1672, 1543. **7**: 3408, 3286, 3166, 3140, 3045, 2648, 1666, 1644, 1531. **8**: 3275, 3153, 3039, 2642, 1671, 1542.

$^1$H-NMR data of the peptides (calibrated on the residual proton resonance of the deuterated solvent): **1** (DMSO, 300 K): δ = 2.77–2.99 (m, 2H, $H_\beta$), 3.18–3.23 (m, 2H, $H_\beta$), 4.25–4.31 (m, 1H, $H_\alpha$); 4.46–4.56 (m, 1H, $H_\alpha$); 7.44 (s, 1H, 5-H), 8.84 (d, $^3J$ = 7.5 Hz, 1H, NH), 8.97 (s, 1H, 2-H). **2** ($D_2O$, 300 K): δ = 3.13 (d, $^3J$ = 6.0 Hz, 2H, $H_\beta$Cys), 3.19 (dd, $^3J$ = 8.7, $^2J$ = 15.0 Hz, 1H, $H_\beta$His), 3.45 (dd, $^3J$ = 5.3, $^2J$ = 15.0 Hz, 1H, $H_\beta$His), 4.30 (t, $^3J$ = 6.0 Hz, 1H, $H_\alpha$Cys), 4.82–4.90 (m, 1H, $H_\alpha$His), 7.41 (s, 1H, 5-H), 8.68 (d, $^3J$ = 1.2 Hz, 1H, 2-H).

Table 3. Preparative details for the zinc complexes

| | Peptide | | EtOH | $NEt_3$ | | Zinc salt $ZnX_2$ | | | EtOH | Product | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nr. | mg | mmol | ml | mg | mmol | X | mg | mmol | ml | Nr. | mg | % |
| **1** | 185 | 0.381 | 25 | 154 | 1.524 | I | 122 | 0.381 | 5 | **11b**•2EtOH•2$H_2O$ | 105 | 79 |
| **1** | 185 | 0.381 | 25 | 154 | 1.524 | $ClO_4$(·6$H_2O$) | 142 | 0.381 | 5 | **11c**•2EtOH•2$H_2O$ | 95 | 73 |
| **2** | 239 | 0.440 | 15 | 178 | 1.759 | Cl | 60 | 0.440 | 5 | **12a**•2EtOH•3$H_2O$ | 126 | 91 |
| **2** | 239 | 0.440 | 15 | 178 | 1.759 | I | 140 | 0.440 | 5 | **12b**•2EtOH•4$H_2O$ | 137 | 88 |
| **2** | 239 | 0.440 | 15 | 178 | 1.759 | $ClO_4$(·6$H_2O$) | 164 | 0.440 | 5 | **12c**•2EtOH•4$H_2O$ | 137 | 89 |
| **3** | 122 | 0.236 | 15 | 60 | 0.589 | I | 75 | 0.236 | 10 | **13b**•4$H_2O$ | 74 | 85 |
| **3** | 122 | 0.236 | 15 | 60 | 0.589 | $ClO_4$(·6$H_2O$) | 88 | 0.236 | 10 | **13c**•4$H_2O$ | 73 | 85 |
| **4** | 67 | 0.142 | 10 | 36 | 0.355 | I | 45 | 0.142 | 5 | **14b**•4$H_2O$ | 47 | 90 |
| **4** | 67 | 0.142 | 10 | 36 | 0.355 | $ClO_4$(·6$H_2O$) | 53 | 0.142 | 5 | **14c**•4$H_2O$ | 39 | 76 |
| **5** | 180 | 0.314 | 20 | 111 | 1.099 | I | 100 | 0.314 | 10 | **15b** | 114 | 76 |
| **5** | 180 | 0.314 | 20 | 111 | 1.099 | $ClO_4$(·6$H_2O$) | 117 | 0.314 | 10 | **15c** | 117 | 77 |
| **6** | 62 | 0.114 | 15 | 40 | 0.400 | I | 37 | 0.114 | 10 | **16b** | 29 | 57 |
| **6** | 62 | 0.114 | 15 | 40 | 0.400 | $ClO_4$(·6$H_2O$) | 43 | 0.114 | 10 | **16c** | 31 | 63 |
| **7** | 180 | 0.348 | 20[a] | 35[b] | 0.870 | Cl | 47 | 0.348 | 10[a] | **17a** | 25 | 17 |
| **7** | 150 | 0.280 | 20 | 71 | 0.700 | I | 89 | 0.280 | 10 | **17b**•$H_2O$ | 97 | 64 |
| **7** | 150 | 0.280 | 20 | 71 | 0.700 | $ClO_4$(·6$H_2O$) | 104 | 0.280 | 10 | **17c**•$H_2O$ | 102 | 70 |
| **8** | 350 | 0.701 | 15[a] | 70[c] | 1.753 | Cl | 96 | 0.701 | 10[a] | **18a**•$H_2O$ | 247 | 79 |
| **8** | 210 | 0.421 | 15 | 106 | 1.051 | I | 134 | 0.421 | 10 | **18b** | 149 | 68 |
| **8** | 210 | 0.421 | 20 | 106 | 1.051 | $ClO_4$(·6$H_2O$) | 157 | 0.421 | 5 | **18c** | 118 | 56 |

[a] Water was used as solvent instead of EtOH. – [b] 0.2 M aqueous NaOH was used instead of $NEt_3$. – [c] 1 M aqueous NaOH was used instead of $NEt_3$.

**3** (DMSO, 300 K): δ = 1.16 (t, $^3J$ = 6.9 Hz, 3H, $CH_3$ ethoxy), 1.82 (s, 3H, $CH_3$ acetyl), 2.70–3.10 (m, 4H, $H_\beta$), 3.09 (q, $^3J$ = 6.9 Hz, 2H, $CH_2$ ethoxy), 4.35–4.45 (m, 1H, $H_\alpha$), 4.60–4.71 (m, 1H, $H_\alpha$), 7.33 (s, 1H, 5-H), 8.23 (d, $^3J$ = 8.3 Hz, 1H, NH), 8.24 (d, $^3J$ = 7.7 Hz, 1H, NH), 8.95 (s, 1H, 2-H).

**4** (MeOH, 300 K): δ = 2.01 (s, 3H, $CH_3$ acetyl), 2.71–2.92 (m, 2H, $H_\beta$), 3.11–3.41 (m, 2H, $H_\beta$), 4.36–4.42 (m, 1H, $H_\alpha$), 4.73–4.80 (m, 1H, $H_\alpha$), 7.35 (s, 1H, 5-H), 8.78 (s, 1H, 2-H).

**5** (DMSO, 300 K): δ = 1.19 (t, $^3J$ = 7.1 Hz, 3H, $CH_3$ ethoxy), 2.80–2.88 (m, 2H, $H_\beta$), 3.13–3.21 (m, 2H, $H_\beta$), 4.16 (q, $^3J$ = 7.1 Hz, 2H, $CH_2$ ethoxy), 4.23–4.29 (m, 1H, $H_\alpha$), 4.51–4.60 (m, 1H, $H_\alpha$), 7.38 (s, 1H, 5-H), 8.22 (d, $^3J$ = 16.6 Hz, 1H, NH), 8.85 (s, 1H, 2-H).

**6** ($D_2O$, 300 K): δ = 3.12–3.15 (m, 2H, $C_\beta$Cys), 3.28–3.34 (m, 2H, $C_\beta$His), 4.26–4.32 (m, 1H, $C_\alpha$Cys), 4.74–4.82 (m, 1H, $C_\alpha$His), 7.40 (s, 1H, 5-H), 8.68 (d, $^3J$ = 1.2 Hz, 1H, 2-H).

Table 4. Analytical data

| Compound | Formula (mol.mass) | | Analyses C | H | N | Zn |
|---|---|---|---|---|---|---|
| **1**•2HTFA | $C_9H_{14}N_4O_3S•2C_2HF_3O_2$ | calcd. | 32.11 | 3.32 | 11.52 | |
| | (258.3 + 228.1) | found | 31.82 | 3.43 | 11.10 | |
| **2**•2HTFA | $C_9H_{14}N_4O_3S•2C_2HF_3O_2$ | calcd. | 32.11 | 3.32 | 11.52 | |
| | (258.3 + 228.1) | found | 31.42 | 3.37 | 11.21 | |
| **3**•1.5HTFA•EtOH | $C_{11}H_{16}N_4O_4S•1.5C_2HF_3O_2•C_2H_6O$ | calcd. | 37.14 | 4.58 | 10.83 | |
| | (300.3 + 171.0 + 46.1) | found | 37.63 | 4.49 | 11.03 | |
| **4**•1.5HTFA | $C_{11}H_{16}N_4O_4S•1.5C_2HF_3O_2$ | calcd. | 35.67 | 3.74 | 11.89 | |
| | (300.3 + 171.0) | found | 35.68 | 3.79 | 12.05 | |
| **5**•2.5HTFA | $C_{11}H_{18}N_4O_3S•2.5C_2HF_3O_2$ | calcd. | 33.63 | 3.62 | 9.81 | |
| | (286.4 + 285.1) | found | 33.16 | 3.63 | 9.29 | |
| **6**•2HTFA | $C_9H_{15}N_5O_2S•2C_2HF_3O_2$ | calcd. | 32.17 | 3.53 | 14.43 | |
| | (257.3 + 228.1) | found | 32.16 | 3.73 | 13.50 | |
| **7**•1.5HTFA•$H_2O$ | $C_{13}H_{20}N_4O_4S•1.5C_2HF_3O_2•H_2O$ | calcd. | 37.14 | 4.58 | 10.83 | |
| | (328.4 + 171.0 + 18.0) | found | 37.39 | 4.41 | 10.00 | |
| **8**•1.5HTFA | $C_{13}H_{20}N_4O_4S•1.5C_2HF_3O_2$ | calcd. | 38.48 | 4.34 | 11.22 | |
| | (328.4 + 171.0) | found | 38.25 | 4.33 | 11.09 | |
| **11b**•2EtOH•2$H_2O$ | $C_{40}H_{64}I_2N_{16}O_{16}S_4Zn_5$ | calcd. | 27.71 | 3.72 | 12.92 | 18.85 |
| | (1734.0) | found | 27.71 | 3.59 | 12.23 | 18.05 |
| **11c**•2EtOH•2$H_2O$ | $C_{44}H_{64}F_6N_{16}O_{20}S_4Zn_5$ | calcd. | 30.97 | 3.78 | 13.13 | 19.16 |
| | (1706.2) | found | 30.73 | 4.03 | 13.16 | 19.29 |
| **12a**•2EtOH•3$H_2O$ | $C_{40}H_{66}Cl_2N_{16}O_{17}S_4Zn_5$ | calcd. | 30.62 | 4.24 | 14.28 | 20.83 |
| | (1569.1) | found | 30.71 | 4.09 | 14.22 | 20.30 |
| **12b**•2EtOH•4$H_2O$ | $C_{40}H_{68}I_2N_{16}O_{18}S_4Zn_5$ | calcd. | 27.14 | 3.87 | 12.66 | 18.47 |
| | (1770.0) | found | 27.19 | 3.45 | 12.11 | 18.11 |
| **12c**•2EtOH•4$H_2O$ | $C_{44}H_{68}F_6N_{16}O_{22}S_4Zn_5$ | calcd. | 30.33 | 3.93 | 12.86 | 18.76 |
| | (1742.3) | found | 29.75 | 3.85 | 12.80 | 18.98 |
| **13b**•4$H_2O$ | $C_{44}H_{64}I_2N_{16}O_{20}S_4Zn_5$ | calcd. | 28.63 | 3.49 | 12.14 | 17.71 |
| | (1846.0) | found | 28.33 | 3.24 | 11.45 | 17.40 |
| **13c**•4$H_2O$ | $C_{48}H_{64}F_6N_{16}O_{24}S_4Zn_5$ | calcd. | 31.71 | 3.55 | 12.33 | 17.98 |
| | (1818.3) | found | 31.32 | 3.38 | 12.49 | 17.88 |
| **14b**•4$H_2O$ | $C_{44}H_{64}I_2N_{16}O_{20}S_4Zn_5$ | calcd. | 28.63 | 3.49 | 12.14 | 17.17 |
| | (1846.0) | found | 28.94 | 3.28 | 11.96 | 17.33 |
| **14c**•4$H_2O$ | $C_{48}H_{64}F_6N_{16}O_{24}S_4Zn_5$ | calcd. | 31.71 | 3.55 | 12.33 | 17.98 |
| | (1818.3) | found | 31.91 | 3.56 | 13.02 | 17.41 |
| **15b** | $C_{11}H_{17}IN_4O_3SZn$ | calcd. | 27.66 | 3.59 | 11.73 | 13.69 |
| | (477.6) | found | 27.71 | 3.40 | 10.84 | 13.99 |
| **15c**•$H_2O$ | $C_{13}H_{19}F_3N_4O_6SZn$ | calcd. | 32.41 | 3.98 | 11.98 | 13.69 |
| | (481.8) | found | 31.79 | 4.00 | 11.92 | 13.88 |
| **16b** | $C_9H_{14}IN_5O_2SZn$ | calcd. | 24.10 | 3.15 | 15.61 | 14.57 |
| | (448.6) | found | 26.08 | 3.29 | 15.57 | 14.90 |
| **16c** | $C_{11}H_{14}F_3N_5O_4SZn$ | calcd. | 30.39 | 3.25 | 16.11 | 15.04 |
| | (434.7) | found | 28.57 | 3.50 | 16.81 | 14.90 |
| **17a** | $C_{13}H_{19}ClN_4O_4SZn$ | calcd. | 36.46 | 4.47 | 13.08 | 15.27 |
| | (428.2) | found | 37.02 | 4.45 | 12.47 | 15.66 |
| **17b**•$H_2O$ | $C_{13}H_{21}IN_4O_5SZn$ | calcd. | 29.04 | 3.94 | 10.42 | 12.16 |
| | (537.7) | found | 29.57 | 3.61 | 10.08 | 12.50 |
| **17c**•$H_2O$ | $C_{15}H_{21}F_3N_4O_7SZn$ | calcd. | 34.40 | 4.04 | 10.70 | 12.48 |
| | (523.8) | found | 34.87 | 4.06 | 11.48 | 12.78 |
| **18a**•$H_2O$ | $C_{13}H_{21}ClN_4O_5SZn$ | calcd. | 34.99 | 4.74 | 12.56 | 14.65 |
| | (446.2) | found | 35.07 | 4.18 | 12.41 | 15.15 |
| **18b** | $C_{13}H_{19}N_4O_4SZn$ | calcd. | 30.05 | 3.69 | 10.78 | 12.58 |
| | (519.7) | found | 29.82 | 3.76 | 10.08 | 13.18 |
| **18c** | $C_{15}H_{19}F_3N_4O_6SZn$ | calcd. | 35.62 | 3.79 | 11.08 | 12.93 |
| | (505.8) | found | 35.54 | 4.04 | 11.45 | 13.24 |

**7** (DMSO, 300 K): δ = 1.19 (t, $^3J$ = 7.0 Hz, 3H, $CH_3$ ethoxy), 1.84 (s, 3H, $CH_3$ acetyl), 2.73–3.18 (m, 4H, $H_\beta$), 4.11 (q, $^3J$ = 7.0 Hz, 2H, $CH_2$ ethoxy), 4.40–4.50 (m, 1H, $H_\alpha$), 4.61–4.73 (m, 1H, $H_\alpha$), 7.34 (s, 1H, 5-H), 8.25 (d, $^3J$ = 7.7 Hz, 1H, NH), 8.42 (d, $^3J$ = 7.1 Hz, 1H, NH), 8.98 (s, 1H, 2-H).

**8** (DMSO, 300 K): δ = 1.15 (t, $^3J$ = 7.1 Hz, 3H, $CH_3$ ethoxy), 1.87 (s, 3H, $CH_3$ acetyl), 2.54–2.81 (m, 2H, $H_\beta$), 3.01–3.24 (m, 2H, $H_\beta$), 4.09 (q, $^3J$ = 7.1 Hz, 2H, $CH_2$ ethoxy), 4.28–4.38 (m, 1H, $H_\alpha$), 4.53–4.62 (m, 1H, $H_\alpha$), 7.39 (s, 1H, 5-H), 8.10 (d, $^3J$ = 7.9 Hz, 1H, NH), 8.56 (d, $^3J$ = 7.8 Hz, 1H, NH), 8.98 (s, 1H, 2-H).

*Zinc Complexes. – General Preparative Procedure:* Under nitrogen the peptide-HTFA adduct was dissolved in ethanol, and a 3- to 4-fold excess of triethylamine was added to the mixture followed by a solution of the zinc salt in ethanol. Upon stirring for 1–2 h a cloudy precipitate formed which was collected on a low-porosity paper filter and washed five times with cold ethanol. After drying for 3 d in vacuo, the resulting complex remained as a colorless, non-hygroscopic powder which was analytically pure and had a melting point around 200 °C. Table 3 gives the details for the preparations.

Relevant IR data of the complexes [ν(NH), amide bands, in KBr, $cm^{-1}$]: **11b**: 3270, 3136, 1615, 1510. **11c**: 3381, 3292, 1650, 1615. **12a**: 3246, 3135, 1673, 1616, 1525. **12b**: 3412, 3252, 3136, 1673, 1620, 1551. **12c**: 3258, 3138, 1674, 1619, 1508. **13b**: 3303, 3140, 1645, 1524. **13c**: 3303, 3149, 1650, 1638, 1531. **14b**: 3300, 3137, 1639, 1528. **14c**: 3386, 3134, 1642, 1531. **15b**: 3273, 3136, 1672, 1580, 1538. **15c**: 3305, 3154, 1674, 1583, 1542. **16b**: 3412, 3266, 1671, 1560, 1541. **16c**: 3418, 3307, 1639, 1602, 1528. **17a**: 3307, 3110, 1656, 1521. **17b**: 3507, 3307, 1656, 1524. **17c**: 3504, 3306, 3139, 1661, 1530. **18a**: 3307, 1655, 1531. **18b**: 3324, 1655, 1639, 1531, 1525. **18c**: 3316, 3149, 1657, 1531.

$^1$H-NMR data of the complexes (calibrated on the residual proton resonance of the deuterated solvent) (the complexes **11b**, **11c**, **12a**, **12b** and **12c** were not soluble in any deuterated solvent): **13b** (DMSO, 350 K): δ = 1.86 (s, 3H, $CH_3$ acetyl), 2.75–3.17 (m, 2 · 2H, $H_\beta$), 4.08–4.24 (m, 1H, $H_\alpha$), 4.42–4.62 (m, 1H, $H_\alpha$), 7.08 (s, 1H, 5-H), 7.70–7.88 (m, 2 · 1H, NH), 8.05 (s, 1H, 2-H).

**13c** (DMSO, 350 K): δ = 1.86 (s, 3H, $CH_3$ acetyl), 2.75–3.17 (m, 2 · 2H, $H_\beta$), 4.03–4.22 (m, 1H, $H_\alpha$), 4.41–4.68 (m, 1H, $H_\alpha$), 7.09 (s, 1H, 5-H), 7.71–7.90 (m, 2 · 1H, NH), 8.07 (s, 1H, 2-H).

**14b** (DMSO, 350 K): δ = 1.86 (s, 3H, $CH_3$ acetyl), 2.59–2.73 (m, 2H, $H_\beta$), 3.10–3.51 (m, 2H, $H_\beta$), 4.20–4.35 (m, 1H, $H_\alpha$), 4.40–4.61 (m, 1H, $H_\alpha$), 6.96–7.02 (m, 1H, 5-H), 7.53–7.61 (m, 1H, NH), 7.76–7.84 (m, 1H, NH), 7.91–7.98 (m, 1H, 2-H).

**14c** (DMSO, 350 K): δ = 1.86 (s, 3H, $CH_3$ acetyl), 2.61–2.73 (m, 2H, $H_\beta$), 2.92–3.21 (m, 2H, $H_\beta$), 4.19–4.31 (m, 1H, $H_\alpha$), 4.40–4.60 (m, 1H, $H_\alpha$), 6.96–7.01 (m, 1H, 5-H), 7.58–7.66 (m, 1H, NH), 7.76–7.84 (m, 1H, NH), 7.92–7.96 (m, 1H, 2-H).

**15b** (DMSO, 350 K): δ = 1.18 (t, $^3J$ = 7.3 Hz, 3H, $CH_3$ ethoxy), 2.58–3.15 (m, 2 · 2H, $H_\beta$), 3.98–4.12 (m, 3H, $CH_2$ ethoxy and $H_\alpha$), 4.29–4.49 (m, 1H, $H_\alpha$), 7.08 (s, 1H, 5-H), 7.93 (s, 1H, 2-H).

**15c** (DMSO, 350 K): δ = 1.19 (t, $^3J$ = 6.4 Hz, 3H, $CH_3$ ethoxy), 2.60–3.12 (m, 2 · 2H, $H_\beta$), 3.87–4.18 (m, 3H, $CH_2$ ethoxy and $H_\alpha$), 4.25–4.41 (m, 1H, $H_\alpha$), 7.05 (s, 1H, 5-H), 7.90 (s, 1H, 2-H).

**16b** (DMSO, 350 K): δ = 2.57–3.18 (m, 2 · 2H, $H_\beta$), 4.31–4.60 (m, 2 · 1H, $H_\alpha$), 6.97 (s, 1H, 5-H), 7.02–7.12 (m, 1H, NH), 7.91 (s, 1H, 2-H).

**16c** (DMSO, 350 K): δ = 2.49–3.17 (m, 2 · 2H, $H_\beta$), 4.25–4.66 (m, 2 · 1H, $H_\alpha$), 6.97 (s, 1H, 5-H), 7.02–7.11 (m, 1H, NH), 7.91 (s, 1H, 2-H).

**17a** (DMSO, 350 K): δ = 1.16 (t, $^3J$ = 6.6 Hz, 3H, $CH_3$ ethoxy), 1.85 (s, 3H, $CH_3$ acetyl), 2.62–2.97 (m, 2 · 2H, $H_\beta$), 4.06 (q, $^3J$ = 6.6 Hz, 2H, $CH_2$ etoxy), 4.12–4.64 (m, 2 · 1H, $H_\alpha$), 6.91 (s, 1H, 5-H), 7.56–7.64 (m, 1H, NH), 7.68 (s, 1H, 2-H), 7.77–7.86 (m, 1H, NH).

**17b** (DMSO, 350 K): δ = 1.17 (t, $^3J$ = 7.1 Hz, 3H, $CH_3$ ethoxy), 1.85 (s, 3H, $CH_3$ acetyl), 2.78–3.18 (m, 2 · 2H, $H_\beta$), 4.07 (q, $^3J$ = 7.1 Hz, 2H, $CH_2$ ethoxy), 4.17–4.28 (m, 1H, $H_\alpha$), 4.37–4.52 (m, 1H, $H_\alpha$), 7.03 (s, 1H, 5-H), 7.65–7.75 (m, 1H, NH), 7.85 (s, 1H, 2-H), 7.85–7.94 (m, 1H, NH).

**17c** (DMSO, 350 K): δ = 1.16 (t, $^3J$ = 7.0 Hz, 3H, $CH_3$ ethoxy), 1.86 (s, 3H, $CH_3$ acetyl), 2.78–3.14 (m, 2 · 2H, $H_\beta$), 4.06 (q, $^3J$ = 7.0 Hz, 2H, $CH_2$ ethoxy), 4.13–4.22 (m, 1H, $H_\alpha$), 4.33–4.48 (m, 1H, $H_\alpha$), 7.02 (s, 1H, 5-H), 7.69–7.78 (m, 1H, NH), 7.83 (s, 1H, 2-H), 7.83–7.90 (m, 1H, NH).

**18a** (DMSO, 350 K): δ = 1.17 (t, $^3J$ = 7.1 Hz, 3H, $CH_3$ ethoxy), 1.86 (s, 3H, $CH_3$ acetyl), 2.60–2.71 (m, 2H, $H_\beta$), 3.01–3.20 (m, 2H, $H_\beta$), 4.08 (q, $^3J$ = 7.1 Hz, 2H, $CH_2$ ethoxy), 4.25–4.49 (m, 2 · 1H, $H_\alpha$), 7.10–7.30 (m, 2 · 1H, 5-H, NH), 7.64–7.74 (m, 1H, NH), 7.88 (s, 1H, 2-H).

**18b** (DMSO, 300 K): δ = 1.17 (t, $^3J$ = 7.2 Hz, 3H, $CH_3$ ethoxy), 1.85 (s, 3H, $CH_3$ acetyl), 2.60–2.80 (m, 2H, $H_\beta$), 3.04–3.16 (m, 2H, $H_\beta$), 4.08 (q, $^3J$ = 7.2 Hz, 2H, $CH_2$ ethoxy), 4.14–4.34 (m, 2 · 1H, $H_\alpha$), 7.13 (s, 1H, 5-H), 7.66 (d, $^3J$ = 6.3 Hz, 1H, NH), 7.97 (s, 1H, 2-H), 8.05 (d, $^3J$ = 9.1 Hz, 1H, NH).

**18c** (DMSO, 300 K): δ = 1.16 (t, $^3J$ = 7.1 Hz, 3H, $CH_3$ ethoxy), 1.86 (s, 3H, $CH_3$ acetyl), 2.59–2.78 (m, 2H, $H_\beta$), 3.00–3.20 (m, 2H, $H_\beta$), 4.08 (q, $^3J$ = 7.1 Hz, 2H, $CH_2$ ethoxy), 4.20–4.41 (m, 2 · 1H, $H_\alpha$), 7.13 (s, 1H, 5-H), 7.67 (d, $^3J$ = 6.9 Hz, 1H, NH), 7.97 (s, 1H, 2-H), 8.02–8.10 (m, 1H, NH).

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☆ Dedicated to Professor *Max Herberhold* on the occasion of his 60th birthday.


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