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# Interaction of Molybdocene Dichloride with Cysteine-Containing Peptides: Coordination, Regioselective Hydrolysis, and Intramolecular Aminolysis

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Reactions of the organometallic compound molybdocene dichloride ($Cp_2MoCl_2$, Cp = $\eta^5$-cyclopentadienyl) with the cysteine-containing peptides L-cysteinylglycine (Cys-Gly), *N*-acetyl-L-cysteine (AcCys), glycyl-L-cysteine (Gly-Cys), glycyl-L-cysteinylglycine (Gly-Cys-Gly), and $\gamma$-L-glutamyl-L-cysteinylglycine (glutathione, GSH) have been studied in aqueous solution in the pH range 2–9. The dipeptides Cys-Gly and Gly-Cys and the acetylated amino acid AcCys form 1:1 and 2:1 complexes of composition $[Cp_2Mo(\text{peptide-}S)(OH_{(2)})]^{n+/-}$ and $[Cp_2Mo(\text{peptide-}S)_2]^{n+/-}$ as well as the chelates $[Cp_2Mo(\text{AcCys-}S,O)]$, $[Cp_2Mo(\text{Gly-Cys-}S,O)]^+$, and $[Cp_2Mo(\text{Cys-Gly-}S,N)]$ with the $Cp_2Mo^{2+}$ unit binding to the deprotonated thiolate group and the free amino or carboxylate group of the cysteine residue. Upon treatment of Gly-Cys-Gly and the naturally occurring tripeptide GSH with $Cp_2MoCl_2$ at elevated temperature, release of free glycine was observed. The $Cp_2Mo^{2+}$ entity coordinates to the thiolate group of GSH and mediates regioselective hydrolysis of the Cys-Gly peptide bond by intramolecular metal hydroxide activation. $Cp_2Mo^{2+}$-promoted hydrolysis of GSH was followed at pD 7.4 and 5.2 and 40 and 60 °C. By contrast, the Cys–Gly bond in $[Cp_2Mo(\text{Gly-Cys-Gly-}S,N)]$ is cleaved by intramolecular aminolysis at pD $\geq$ 7.4 and 60 °C leading to glycine and the $Cp_2Mo^{2+}$ complex of the 2,5-diketopiperazine derivative *cyclo*-(Gly-Cys). Chelating coordination of the $Cp_2Mo^{2+}$ moiety to the thiolate group and to the deprotonated amide nitrogen of the tripeptide changes the configuration of the peptide bond from (preferred) trans to cis, thus enabling nucleophilic attack of the primary amino group at the Cys–Gly bond. The reaction product $[Cp_2Mo\{\textit{cyclo}\text{-(Gly-Cys)}\}]\cdot 2H_2O$ has been characterized by X-ray crystallography.

## Introduction

With the half-life for the hydrolysis of peptides being 250−600 years around physiological pH,[1] peptide bonds are not easily cleaved under mild conditions. The ability of metal ions to promote amide hydrolysis by Lewis acid or metal hydroxide activation is well-known, and metal complexes that hydrolyze amides under mild conditions have attracted considerable interest as potential artificial peptidases for applications in biochemistry and molecular biology.[2] However, the majority of studies on metal-catalyzed or metal-mediated hydrolysis of amides have been performed with activated amides such as *p*-nitroanilides or amino acid esters as peptide models.[2] By contrast, metal complexes that promote the hydrolytic cleavage of *unactivated* amides or peptides are rather rare,[3] and despite some remarkable progresses in this area,[4−12] there is still a great need for efficient cleaving agents. An even more formidable task than the search for general cleaving agents is the development of selective ones that cleave peptides residue-specifically. This

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challenge has been met by several groups: Kostic and co-workers achieved selective hydrolysis at histidine, methionine, and cysteine residues with Pt and Pd complexes.[4] The site-specificity is based on the high affinity of Pt and Pd for sulfur and imidazole nitrogen of the amino acid side chains that anchor the catalyst and orient it in close contact to the proximate peptide bond. Recently, selective hydrolysis at tryptophan residues upon Pt−C binding to the indole ring of tryptophan has been discovered by the same author.[13] Other examples for residue-specific peptide cleavage are the selective hydrolysis of serine-containing peptides by zinc salts reported by Komiyama[14] and the selective hydrolysis of the N-terminal peptide bond by Co(III) complexes[15] as well as the site-selective protein cleavage mediated by certain Fe(II),[16−19] Ni(II),[20] and Cu(II)[21] complexes.

The organometallic compound molybdocene dichloride, $Cp_2MoCl_2$ (Cp = $\eta^5$-cyclopentadienyl), which has raised interest due to its antitumor activity,[22] has been found to catalyze the hydrolytic cleavage of amino acid esters[23] as well as of activated and unactivated phophodi- and phosphomonoesters.[24,25] Very recently, the $Cp_2Mo^{2+}$-mediated hydration of nitriles was reported.[26] The reaction yielded exclusively amides without further hydrolysis to the corresponding carboxylic acids taking place. The only exception was found to be 2-hydroxyacetamide that was converted into glycolic acid. However, 2-hydroxyacetamide is highly susceptible to hydrolysis due to the intramolecular hydrogen bond between the hydroxy group and the carbonyl oxygen which activates the amide group for nucleophilic attack.

The ability of $Cp_2MoCl_2$ to efficiently cleave robust phosphate diesters prompted us to explore the possibility to cleave amide bonds of peptides with $Cp_2MoCl_2$ despite the obvious stability of amides in the presence of $Cp_2MoCl_2$ as observed during nitrile hydration.[26] We reasoned that $Cp_2Mo^{2+}$-mediated amide hydrolysis might become feasible, when the reaction could proceed via an intramolecular pathway, i.e., when donor atoms of amino acid side chains of a peptide serve as anchors and position the $Cp_2Mo^{2+}$ unit in close proximity of a scissile bond.

An obvious choice for a docking site for $Cp_2Mo^{2+}$ is the cysteine side chain. As expected for the soft metal center, coordination of $Cp_2Mo^{2+}$ to thiolates is well established[27] and the chelate complex [$Cp_2Mo$(AcCys-*S*,*O*)] and the 2:1 complex [$Cp_2Mo$(Cys-*S*)$_2$] with monodentate coordination of cysteine to $Cp_2Mo^{2+}$ were reported by Harding and co-workers.[28,29] Harding and co-workers have also investigated the formation of adducts between $Cp_2MoCl_2$ and the naturally occurring tripeptide glutathione ($\gamma$-L-glutamyl-L-cysteinyl-glycine, GSH).[29,30] However, the potential function of $Cp_2Mo^{2+}$ as a promotor of peptide hydrolysis at elevated temperature has not been studied before.

Here we report on the reaction of $Cp_2MoCl_2$ with cysteine-containing di- and tripeptides and show that coordination of the $Cp_2Mo^{2+}$ unit to the thiol group of X-Cys-Y peptides assists release of the amino acid at the carboxyl end of the cysteine residue.

## Experimental Section

**Materials and General Methods.** Molybdocene dichloride was purchased from Aldrich and used as supplied. Gly-Cys-Gly and Gly-Cys were obtained from Bachem, and Cys-Gly, GSH, AcCys, Gly, glycinamide, and Gly pNA, from Sigma. $D_2O$, DCl, and NaOD were purchased from Deutero.

All reactions were carried out under a nitrogen atmosphere by using standard Schlenk techniques. $D_2O$ and $H_2O$ were degassed and purged with nitrogen for at least 20 min prior to use.

$^1$H NMR spectra were recorded on a Varian Mercury spectrometer at 200.13 MHz using sodium 3-(trimethylsilyl)propanesulfonate (TSP) as internal reference.[31] $^{13}$C NMR measurements were performed on a Bruker DRX 500 spectrometer. $^{13}$C NMR chemical shifts were referenced to dioxane as an internal reference ($\delta_{dioxane}$ = 67.19 in $D_2O$). $^{13}$C peak assignments were made by $^1$H−$^{13}$C 2D correlation spectra. The pD values of $D_2O$ solutions were measured by use of a glass electrode and addition of 0.4 to the pH meter reading.[32,33] Thin-layer chromatography (TLC) was performed on silica gel-coated Merck silica plates (methanol:water:pyridine = 80:20:4) and was visualized with ninhydrin spray reagent.

**Binding Studies and Peptide Cleavage Studies.** In a typical experiment 7 $\mu$mol of $Cp_2MoCl_2$ was dissolved in 700 $\mu$L of $D_2O$ by sonication for at least 1 h, and the solution was kept at room temperature under nitrogen for a couple of hours to ensure complete hydrolysis of the chloride ligands. This way green-brown solutions of pD 2.5−3 were obtained. After addition of the peptide the pD value was adjusted to the required value with 1 M DCl or NaOD. $^1$H NMR spectra were recorded 30 min after initial adjustment of


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the pD and after that in appropriate time intervals. In the course of the reactions the pD dropped slowly with time. Usually, within the first 30 min of the reaction a drop in pD of 0.2−0.5 units was observed. Fluctuations in pD were generally less pronounced at pD ≤ 4 and at pD ≥ 8.

Peptide cleavage was followed by monitoring the relative intensities of the $H_\alpha$ resonances of free glycine and of the adducts of the intact peptides. The error in integrating the resonances was estimated at ±5%. Small amounts of brownish precipitates that occasionally occurred when the solution was kept at pD ≤ 7 were ignored.

**Preparation of [$Cp_2Mo${*cyclo*-(Gly-Cys)}]·2$H_2O$ (7).** $Cp_2MoCl_2$ (20.8 mg, 0.07 mmol) was dissolved in degassed and $N_2$-saturated water (7 mL) by sonication for 2.5 h. After addition of Gly-Cys-Gly (16.5 mg, 0.07 mmol) the pH was adjusted to 8.9 by 1 M NaOH. The reaction mixture was heated at 60 °C for 2 d and then kept at room temperature. After 3 d, dark red, cubic crystals of [$Cp_2Mo${*cyclo*-(Gly-Cys)}]·2$H_2O$ were obtained. Yield: 10.4 mg (0.025 mmol, 36%). $^1$H NMR (200 MHz, $D_2O$): $\delta$ 2.09 (dd, $^2J$(H−H) = 12.4 Hz, $^3J$(H−H) = 12.4 Hz, 1 H, Cys-$CH_2$), 3.15 (dd, $^3J$(H−H) = 4.1 Hz, $^2J$(H−H) = 12.4 Hz, 1 H; Cys-$CH_2$), 3.74 (d, $^2J$(H−H) = 15.9 Hz, 1 H; Gly-$CH_2$), 3.88 (d, $^2J$(H−H) = 15.9 Hz, 1 H; Gly-$CH_2$), 5.40 (s, 5 H, Cp), 5.42 (s, 5 H, Cp) (the Cys-$H_\alpha$ proton is not detectable because of the presence of the water signal). Selected IR data (cm$^{-1}$): 3353 (s, br), 3206 (sh), 2956 (m), 2889 (m), 1632 (s), 1574 (s), 1560 (m), 1539 (m), 1410 (m), 1294 (m), 1414 (m), 1004 (m), 826 (m), 652 (m), 447 (m). Anal. Calcd for $C_{15}H_{20}MoN_2O_4S$: C, 42.9; H, 4.8; N, 6.7. Found: C, 42.7; H, 4.5; N, 6.3.

**X-ray Analysis.** Crystal data were collected at room temperature on an Enraf-Nonius-Kappa CCD diffractometer[34] using graphite-monochromated Mo Kα radiation ($\lambda$ = 0.710 69 Å). For data reduction and cell refinement the programs DENZO and SCALEPACK were used.[35] The structure was solved by conventional Patterson methods and subsequent Fourier syntheses and refined by full-matrix least squares on $F^2$ using the SHELXTL PLUS and SHELXL-93 programs.[36] The scattering factors were those given in the SHELXTL PLUS program. All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were generated geometrically and given fixed isotropic thermal parameters. The Flack parameter[37] converged to 0.11(4). Graphics were produced with SHELXTL PLUS; Cremer−Pople ring-puckering parameters[38] were calculated with PLATON.[39] Crystallographic data and details of refinement are reported in Table 1.

**Table 1.** Crystal and Structure Refinement Data for [$Cp_2Mo${*cyclo*-(Gly-Cys)}]·2$H_2O$ (**7**)

| | |
|---|---|
| formula | $C_{15}H_{20}MoN_2O_4S$ |
| fw | 420.33 |
| cryst habit | red cube |
| cryst dimens (mm) | 0.20 × 0.23 × 0.25 |
| cryst system | monoclinic |
| space group | $P2_1$ |
| $a$ (Å) | 7.457(1) |
| $b$ (Å) | 10.416(1) |
| $c$ (Å) | 11.070(1) |
| $\beta$ (deg) | 109.63(1) |
| $V$ (Å$^3$) | 809.9(2) |
| Z | 2 |
| $F$(000) | 428 |
| $\lambda$ (Å) | 0.710 69 |
| $\rho_{calcd}$ (g cm$^{-3}$) | 1.724 |
| $\mu$(Mo Kα) (mm$^{-1}$) | 0.960 |
| $\theta$ limits (deg) | 3.50−28.50 |
| reflcns collcd/unique | 7439/2099 ($R_{int}$ = 0.039) |
| obsd reflcns[a] | 1824 |
| params | 208 |
| R1[a,b] | 0.026 |
| wR2[a,c] | 0.053 |
| Δ/$\rho$ max/min (e Å$^{-3}$) | 0.381/−0.368 |

[a] Observation criterion: $I > 2\sigma(I)$. [b] $R1 = \sum||F_o| - |F_c||/\sum|F_o|$. [c] $wR2 = [\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2]^{1/2}$.

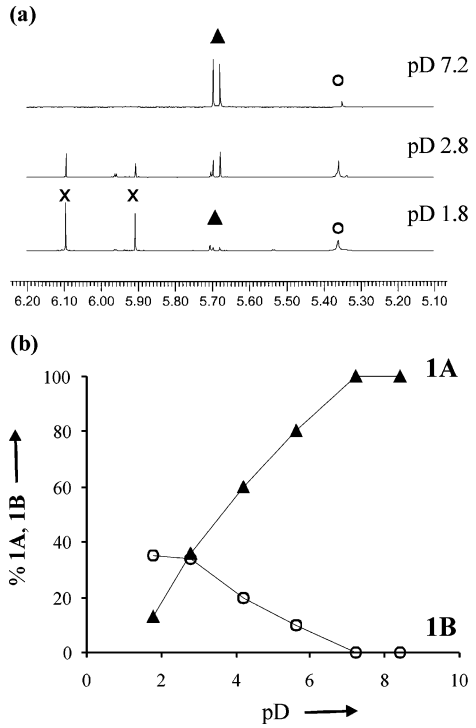


**Figure 1.** (a) Cp resonances in the $^1$H NMR spectra (5.1−6.2 ppm) of the equimolar reaction of $Cp_2MoCl_2$(aq) and AcCys at different pD values. Spectra were recorded 30 min after mixing. Key: (×) $Cp_2MoCl_2$(aq) species; (▲) **1A**; (○) **1B**. (b) pD dependence of the product distribution in the 1:1 reaction of $Cp_2MoCl_2$(aq) with AcCys at room temperature. Key: (▲) **1A**; (○) **1B**.

## Results and Discussion

The aqueous chemistry of $Cp_2MoCl_2$ is characterized by rapid replacement of the chloride ligands by water, while the Cp−Mo ligation is remarkably resistant toward protolysis.[40] In contrast to related metallocenes, release of the Cp ligands is negligible at pH 2−7 so that reactions can be conveniently monitored by $^1$H NMR spectroscopy, with product species being easily distinguished by their Cp resonances. The diaqua species $[Cp_2Mo(H_2O)_2]^{2+}$ with $pK_{a1}$ = 5.5 and $pK_{a2}$ = 8.5 is prone to dimerization and formation of hydroxo-bridged species.[40] The number, nature, and percentages of the species present in an aqueous solution of $Cp_2MoCl_2$ strongly depend on pH and concentration. All

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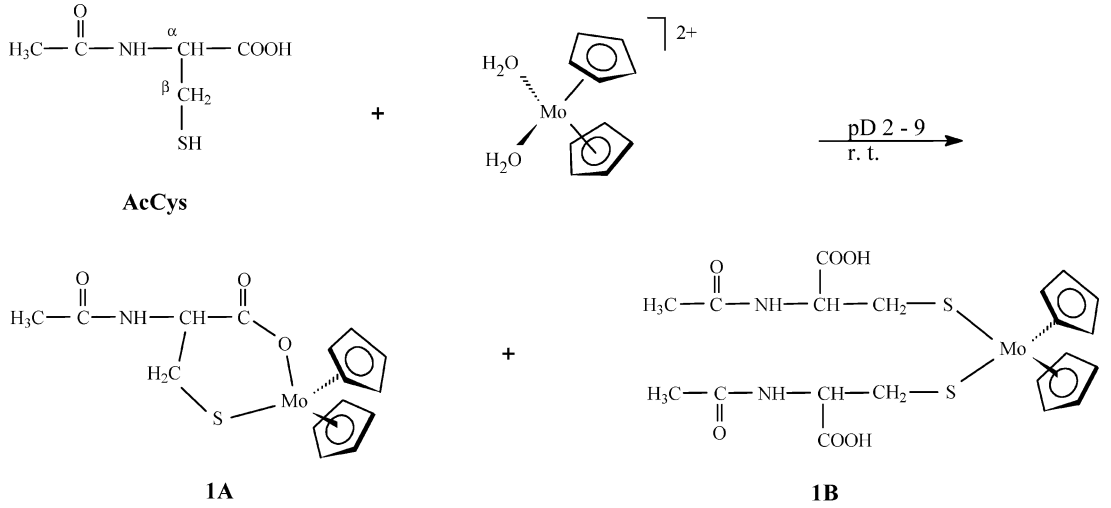
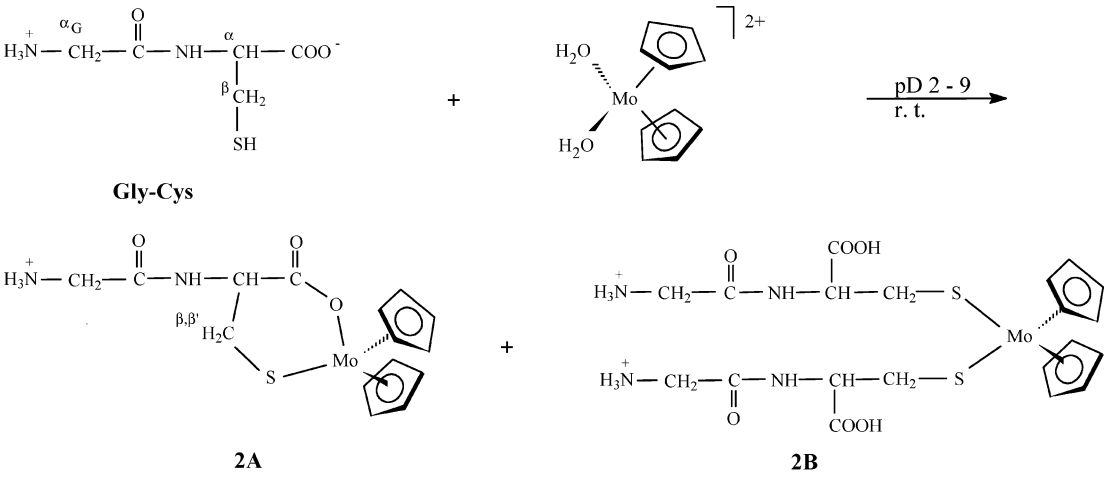
**Scheme 1.** Formation of $Cp_2Mo^{2+}$ Complexes with AcCys




**AcCys** **1A** **1B**

**Scheme 2.** Formation of $Cp_2Mo^{2+}$ Complexes with Gly-Cys




**Gly-Cys** **2A** **2B**

**Table 2.** $^1$H NMR Chemical Shifts in ppm of Gly-Cys Complexes Formed with the $Cp_2Mo^{2+}$ Cation in $D_2O$

| | Gly-Cys (pD 5.2) | **2A** (pD 5.2) | **2B** (pD 5.2) |
|---|---|---|---|
| Cp | | 5.70 (s), 5.68 (s) | 5.36 (s) |
| Cys-C$H_\alpha$ | 4.44 (t) | 4.29 (dd) | 4.23 (dd) |
| Cys-C$H_\beta$ | 2.94 (d) | 2.77 (dd, $H_\beta$), 2.29 (dd, $H_\beta$)[a] | 2.65 (d) |
| Gly-C$H_\alpha$ | 3.89 (s) | 3.79 (d) | 3.87 (s) |

[a] $^3J(H_\beta-H_\alpha) = 2.9$ Hz; $^2J(H_{\beta'}-H_\alpha) = 11.4$ Hz; $^2J(H_\beta-H_{\beta'}) = 13.2$ Hz.

**Table 3.** $^{13}$C NMR Chemical Shifts in ppm of Gly-Cys Complexes Formed with the $Cp_2Mo^{2+}$ Cation in $D_2O$[a]

| | Gly-Cys (pD 7.2) | **2A** (pD 7.2) | **2B** (pD 7.2) |
|---|---|---|---|
| Cp | | 102.4 | 97.8 |
| Cys-$C_\alpha$ | 57.5 | 58.8 | 57.2 |
| Cys-$C_\beta$ | 27.4 | 28.8 | 38.3 |
| Gly-$C_\alpha$ | 41.4 | 41.3 | 40.5 |
| *C*=*O* | 167.6 | 167.2 | 167.1 |
| *C*OO$^-$ | 176.3 | 181.4 | 176.8 |

[a] Assignment made by $^1$H−$^{13}$C 2D correlation spectra.

experiments described in this work were carried out with freshly prepared, 10 mM solutions of $Cp_2MoCl_2$ in the absence of oxygen. Under these conditions dissolution of $Cp_2MoCl_2$ in $D_2O$ gave greenish-brown solutions of pD 2.5−3 whose $^1$H NMR spectra featured two singlets at 6.1 and 5.9 ppm in a 3:1 ratio. Raising the pD to 7 resulted in a single NMR-detectable species whose Cp ligands gave rise to a singlet at 6.0 ppm. A detailed NMR spectroscopic study on the solution behavior of $Cp_2MoCl_2$ in water has been provided by Kuo et al.[40,41] They attributed the resonance at 6.1 ppm to the monomeric diaqua species $[Cp_2Mo(H_2O)_2]^{2+}$, while the signal at 6.0 ppm could be assigned to the hydroxo-bridged dimer $[(Cp)_2Mo(\mu\text{-}OH)_2Mo(Cp)_2]^{2+}$.[41] For the sake of simplicity the notation $Cp_2MoCl_2$(aq) will be used throughout the manuscript for any species resulting from aquation of $Cp_2MoCl_2$ at the respective pD value.

In the course of this work, reactions of $Cp_2MoCl_2$ with peptides of type X-Cys, Cys-X, and X-Cys-X have been studied in the pH range $2 \leq pH \leq 9$. First, interactions of $Cp_2MoCl_2$(aq) with glycyl-L-cysteine (Gly-Cys), *N*-acetyl-L-cysteine (AcCys), and L-cysteinylglycine (Cys-Gly) leading to 2:1 complexes as well as to S,O- and S,N-chelates will be described. These binding modes are all incompatible with hydrolytic activity. After the discussion of the dipeptide complexes, we will describe the reaction behavior of $Cp_2MoCl_2$(aq) toward X-Cys-Gly tripeptides which involves $Cp_2Mo^{2+}$-mediated release of glycine.

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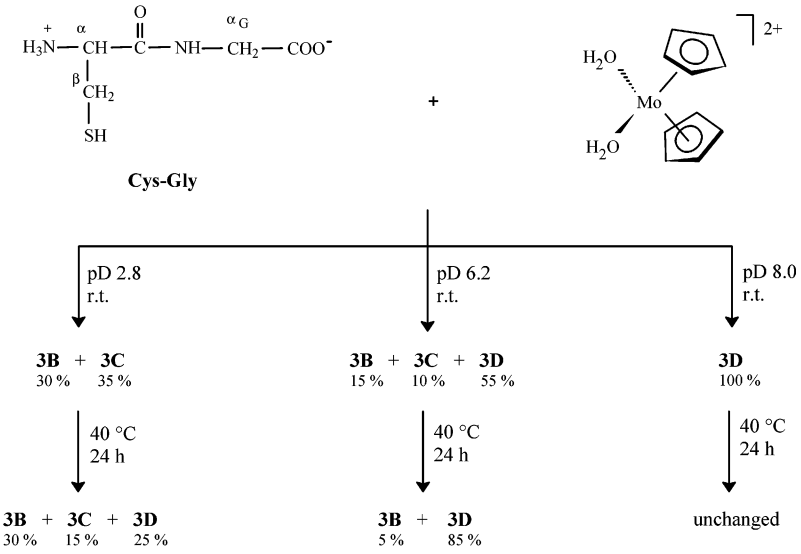
**Scheme 3.** Reaction Behavior of $Cp_2Mo^{2+}$ toward Cys-Gly

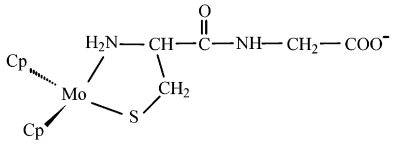
**(a) Summary of reactions of Cys-Gly with $Cp_2Mo^{2+}$ at different pD values**



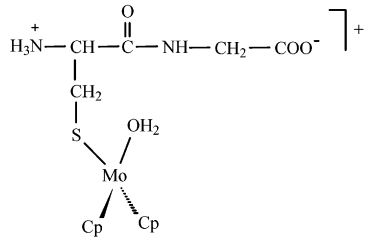

**(b) Structures of the product complexes**

$[Cp_2Mo(Cys\text{-}Gly\text{-}S)_2]$

**3B**



**3D**



**3C**

**Binding of $Cp_2Mo^{2+}$ to Gly-Cys and AcCys.** The dipeptide Gly-Cys and the acetylated amino acid AcCys represent very simple models for a cysteine positioned at the carboxyl terminus of a protein. Formation of adducts is immediately observed in the NMR spectrum, when Gly-Cys or AcCys are mixed with a $Cp_2MoCl_2$(aq) solution in a 1:1 ratio at room temperature. Two types of $Cp_2Mo^{2+}$ complexes, **A** and **B**, whose relative concentrations change with pD are easily detected by their Cp resonances (shown for AcCys in Figure 1a).

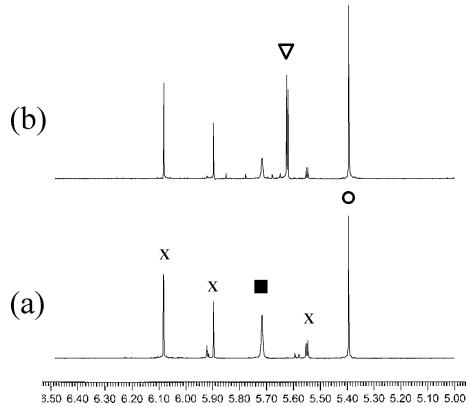



**Figure 2.** Cp resonances in the $^1$H NMR spectrum (5.2−6.4 ppm) of an equimolar mixture of Cys-Gly and $Cp_2MoCl_2$(aq) at pD 2.8. Spectra were recorded (a) directly after mixing and (b) after 24 h at 40 °C. Key: (×) $Cp_2MoCl_2$(aq) species; (○) **3B**; (■) **3C**; (▽) **3D**.

The proposed structures of the $Cp_2Mo^{2+}$ complexes are depicted in Schemes 1 and 2. Complex **1A** has already been identified as the S,O-chelate of AcCys by Waern and Harding, who studied the reaction of $Cp_2MoCl_2$ with AcCys under acidic conditions (pD 2).[29] NMR data of the analogous Gly-Cys complex **2A** are listed in Table 2. S,O-chelation in **2A** similar to **1A** agrees with the following observations: (i) The $H_\alpha$ and $H_\beta$ signals exhibit pronounced upfield shifts. (ii) The resonance of the diastereotopic methylene protons that appears as doublet in the spectrum of the free ligand splits into two four-line signals separated by 0.48 ppm indicative of the absence of rotation around the $C_\alpha-C_\beta$ bond due to chelation. Furthermore, the chelating coordination mode is in accordance with the large value for the $^2J(H_\alpha-H_{\beta'})$ coupling constant (Table 2). (iii) The signals of **2A** show no pD-dependent shifts around pD 3, where a free carboxylate group would become protonated. This is evidence that the carboxylate group participates in the metal coordination. (iv) S,O-coordination is further corroborated by $^{13}$C NMR data (Table 3). Compared with the free dipeptide, a downfield shift of 5.1 ppm is observed for the carboxylate carbon in **2A** at pD 7.4. The $C_\beta$ resonance is shifted by 1.4 ppm to lower field. The signal sets of species **B** are assigned to the complexes $[Cp_2Mo(AcCys\text{-}S)_2]$ (**1B**) and $[Cp_2Mo(Gly\text{-}Cys\text{-}S)_2]$ (**2B**) for the following reasons: (i) Relative intensities and chemical shifts of the $^1$H NMR signals of **1B** (Supporting

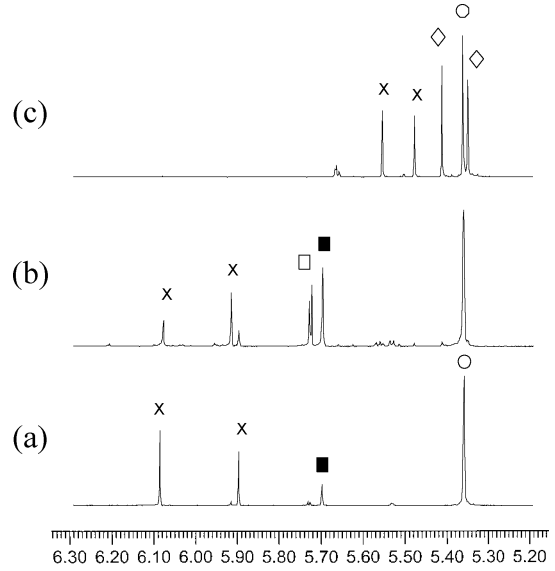



**Figure 3.** Aromatic regions of the $^1$H NMR spectra of an equimolar mixture of Gly-Cys-Gly and $Cp_2MoCl_2$(aq) recorded 3 h after mixing at room temperature at (a) pD 2, (b) pD 4, and (c) pD 9. Key: (×) $Cp_2MoCl_2$(aq) species; (○) **4B**; (■) **4C**; (□) **4E**; (◇) **4F**.

**Table 4.** $^1$H NMR Chemical Shifts in ppm of Cys-Gly Complexes Formed with the $Cp_2Mo^{2+}$ Cation in $D_2O$

| | Cys-Gly (pD 2.8) | **3B** (pD 2.8) | **3C** (pD 2.8) | **3D** (pD 2.8) |
|---|---|---|---|---|
| Cp | | 5.40 (s) | 5.72 (s) | 5.63 (s), 5.62 (s) |
| Cys-C$H_\alpha$ | 4.29 (t) | 4.15 (t) | 4.15 (t) | 4.45 (dd) |
| Cys-C$H_\beta$ | 3.11 (d) | 2.72 (d), 2.66 (d) | 2.61 (d) | 2.76 (dd), 1.99 (dd) |
| Gly-C$H_\alpha$ | 4.04 (s) | 4.06 (s) | 4.06 (s) | 4.03 (s), 4.02 (s) |

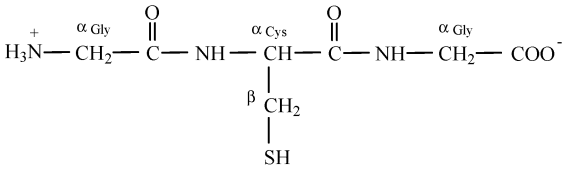
Information) and **2B** (Table 2) are consistent with two monodentate ligands binding through the thiolate sulfur. (ii) As shown in Figure S1, the $H_\alpha$ resonance moves downfield in acidic solution due to protonation of the carboxyl group indicating that the carboxyl oxygens are not involved in metal binding.[42] (iii) In line with the proposed 2:1 composition, the concentrations of **1B** and **2B** grow, when an excess of the ligands is applied. For example, $Cp_2MoCl_2$(aq) completely reacts to **1B** at pD 7.2 in the presence of 3 equiv of AcCys, while the S,O-chelate is no longer observed (Figure S2). The $C_\beta$ resonance of **2B** appears 10.9 ppm downfield from that of the free peptide (for pD 7.2), while the chemical shift of the carboxylate carbon of **2B** and free Gly-Cys are coincident (Table 3).

The variation of product distribution with pD has been studied in the range $2 \le pD \le 9$ and is shown for AcCys in Figure 1b. Coordination of the $Cp_2Mo^{2+}$ entity to AcCys starts to take place at pD 2, and in neutral solution all $Cp_2MoCl_2$(aq) applied is bound. **1A** predominates in neutral solution, while at pD 2 almost exclusively **1B** is formed.

No changes in the NMR spectra occur, when reaction mixtures at $pD \ge 4$ are heated to 40 or 60 °C overnight. However, keeping more acidic solutions at 40 °C for 24 h results in significant decomposition of the $Cp_2Mo^{2+}$ complexes as evident from the appearance of intractable, brownish precipitates.

(42) The notations **1B** and **2B** are used for the 2:1 complex irrespective of the protonation state of the noncoordinating carboxyl group. Likewise, for all other compounds having ionizable functional groups the respective compound number used in the text covers all protonation states of the noncoordinating groups.

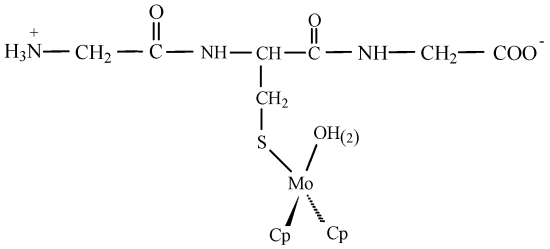
**Chart 1**



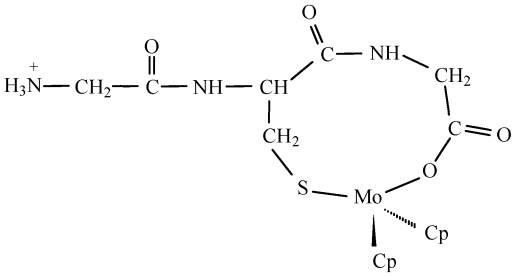
**Gly-Cys-Cys**

$[Cp_2Mo(Gly\text{-}Cys\text{-}Gly\text{-}S)_2]$

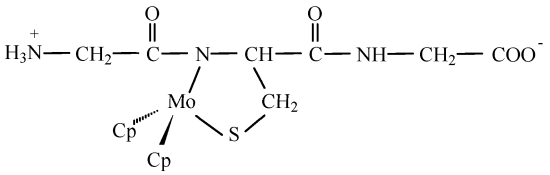
**4B**



**4C**



**4E**



**4F**

**Binding of $Cp_2Mo^{2+}$ to Cys-Gly.** When the dipeptide Cys-Gly whose cysteine residue possesses a primary amino group is added to an aqueous solution of $Cp_2MoCl_2$ at pD 2.8, new Cp resonances indicate the formation of two main products designated **3B** and **3C** immediately after incubation at room temperature. When the reaction mixture is kept at 40 °C for 24 h, the concentration of **3C** is decreased and a third species designated **3D** is detected (Figure 2). The yield of **3D** and the rate of its formation increases with increasing pD. The reaction behavior of $Cp_2MoCl_2$(aq) toward Cys-Gly that has been studied at pD 2.8, 6.2, and 8.0 is summarized in Scheme 3.

Complete NMR data for **3B**, **3C**, and **3D** are listed in Table 4. By analogy with the complexes obtained with AcCys and Gly-Cys, the signal set **3B** is assigned to the 2:1 complex $[Cp_2Mo(Cys\text{-}Gly\text{-}S)_2]$. The 0.16 ppm downfield shift of the

**Table 5.** $^1$H NMR Chemical Shifts in ppm of Gly-Cys-Gly Complexes Formed with the $Cp_2Mo^{2+}$ Cation in $D_2O$

| | | | | | | 4F | |
|---|---|---|---|---|---|---|---|
| | Gly-Cys-Gly (pD 2) | **4B** (pD 2) | **4C** (pD 2) | Gly-Cys-Gly (pD 7.4) | **4E** (pD 7.4) | cis | trans |
| Cp | | 5.36 (s) | 5.71 (s) | | 5.74 (s), 5.72 (s) | 5.42 (s) | 5.37 (s) |
| Cys-C$H_\alpha$ | 4.65 (t) | 4.45 (dd) | 4.45 (dd) | 4.65 (t) | 4.49 (dd) | [a] | [a] |
| Cys-C$H_\beta$ | 2.97 (d) | 2.61 (m) | 2.51 (d) | 2.97 (d) | 2.52 (m), 2.26 (dd) | 3.25 (d, br), 2.27 (dd) | 2.87 (dd), 2.20 (dd) |
| $H_2$N-Gly-C$H_\alpha$ | 3.90 (s) | 3.90 (s) | 3.90 (s) | 3.92 (s) | 3.90 (d) | 3.73 (s) | 3.73 (s) |
| HO-Gly-C$H_\alpha$ | 4.02 (s) | 4.02 (s) | 4.02 (s) | 3.83 (s) | 3.53 (s) | 3.60 (s) | 3.60 (s) |

[a] Signal coincident with HOD signal.

Cys-$H_\alpha$ signal of **3D** compared with that of the uncoordinated dipeptide and the pronounced upfield shift of the $H_\beta$ resonance suggest a coordination mode involving S,$N_{amino}$ chelation for **3D** as shown in Scheme 3b. (Monodentate binding to the thiolate group in **1B** and **2B** shifts the Cys-$H_\alpha$ resonance to higher field.) The large shift difference between the two diastereotopic methylene protons is also in accordance with a chelating binding mode for **3D** (Table 4). For further corroboration of S,$N_{amino}$ chelation the reaction has been carried out in $H_2O$ at pH 8. (At this pH only **3D** is formed, Scheme 3a.) After evaporation of the solvent, the residue has been dissolved in DMSO-$d_6$ to monitor the amino protons. The amino protons that give a broad signal at 8.27 ppm in the case of the free dipeptide appear at 7.89 ppm. The 0.38 ppm upfield shift of the amino proton signal agrees with the replacement of an ammonium proton by $Cp_2Mo^{2+}$. The signal set **3C** belongs to a complex having a 1:1 composition. Because the glycine $H_\alpha$ resonance remains unaffected by the metal coordination, macrochelation through thiolate sulfur and carboxylate oxygen can be ruled out as possible binding mode. The NMR data for **3C** (Table 4) rather suggest an adduct of type $[Cp_2Mo(Cys\text{-}Gly\text{-}S)(OH_2)]^+$ with unidentate S-coordination (Scheme 3b).

**Coordination of $Cp_2Mo^{2+}$ to Glycyl-L-cysteinylglycine (Gly-Cys-Gly).** In X-Cys-Gly tripeptides the cysteine residue is flanked by two peptide bonds as is generally the case in proteins. Figure 3 shows the aromatic regions of the $^1$H NMR spectra recorded 3 h after incubation of equimolar amounts of Gly-Cys-Gly and $Cp_2MoCl_2$(aq) at different pD values.

Overall, in the range $2 \le pD \le 9$ four $Cp_2Mo^{2+}$ complexes are formed that are depicted in Chart 1 (see Table 5 for complete NMR data). At pD 2, two of the four species are present in 10 and 40% yield. The major species can be identified as the 2:1 complex $[Cp_2Mo(Gly\text{-}Cys\text{-}Gly\text{-}S)_2]$ (**4B**) for the following reasons: (i) The chemical shifts of the Cp resonances agree with those of **1**−**3B**. (ii) The upfield shift of the Cys-$H_\beta$ signal indicates S-coordination. (iii) The glycine resonances coincide with those of the free tripeptide (i.e. no coordination occurs at the terminal amino and carboxylate group). (iv) The relative signal intensities are in accordance with a 2:1 composition. (v) **4B** becomes the only species present in solution, when 2 equiv of the tripeptide is applied. The minor species at pD 2 is assigned to the 1:1 complex **4C** with monodentate S-coordination.

The third species designated **4E** becomes visible around pD 4. **4E** is assumed to be the analogue of the GSH macrochelate reported by Harding et al.[30] Evidence for macrochelation through the thiol group of the cysteine residue and the carboxylate group of the C-terminal glycine in **4E** comes from the pronounced upfield shift of the Gly-$H_\alpha$ resonance ($\Delta\delta = 0.3$) in addition to the shift of the Cys-$H_\beta$ signal. The fourth species designated **4F** starts to form around neutral pD. For **4F** coordination through the thiolate S and the deprotonated amide nitrogen is suggested, since the chemical shifts of the Cp and Cys-$H_\beta$ proton agree well with those observed for the structurally characterized S,$N_{amide}$ chelate **7** (see below). As will be discussed further below, two isomers of **4F** differing in the configuration of the metalated peptide group are present in solution. At neutral pD **4B**,**C**,**E**,**F** coexist with the ratio **4C**:**4B**:**4E**:**4F** being 1:1.5:1.5:2. In alkaline solution (pD 9), only **4B** (30%) and **4F** (40%) are observed as reaction products.

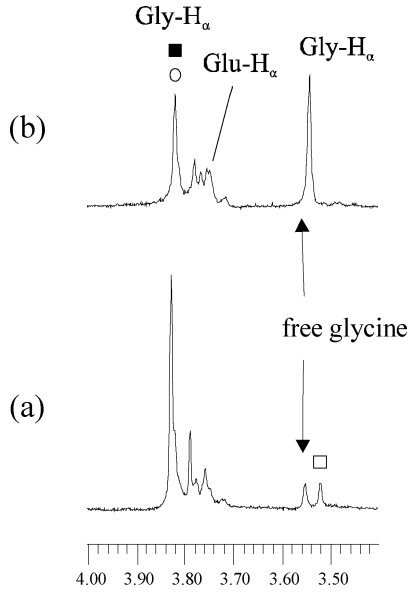


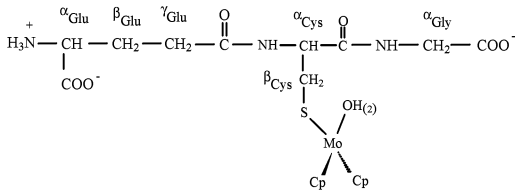

**Figure 4.** $Cp_2Mo^{2+}$-mediated cleavage of the Cys−Gly bond in GSH. Shown is the region of the Gly-$H_\alpha$ resonances in the NMR spectrum after (a) 1 h and (b) 48 h at 60 °C (pD 4.5). Key: (○) **5B**; (■) **5C**; (□) **5E**. The coexistence of **5B**,**C** is concluded from the Cp resonances (not shown).

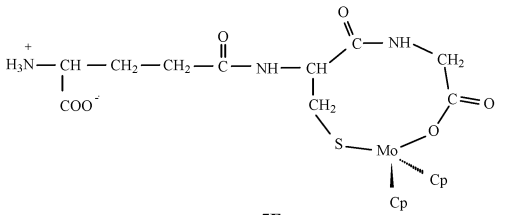
**Cleavage of X-Cys-Y Peptides: $Cp_2Mo^{2+}$-Mediated Hydrolysis of the Cys-Gly Peptide Bond of GSH.** The capability of $Cp_2MoCl_2$ to cleave peptides at cysteine residues was studied by using the naturally occurring tripeptide GSH. When an equimolar solution of GSH and $Cp_2MoCl_2$(aq) is kept at 60 °C for 1 h, a new resonance at 3.54 ppm emerges in the NMR spectrum at pD ≥ 4.5 besides resonances attributable to **B**-, **C**-, **E**-, and **F**-type adducts (Figure 4, Chart 2). This signal can be unambiguously assigned to "free" glycine resulting from peptide hydrolysis. The identity of the glycine resonance has been confirmed by addition of a genuine sample of the amino acid, and to rule out a

**Chart 2**

$[Cp_2Mo(\gamma\text{-Glu-Cys-Gly-}S)_2]$

**5B**

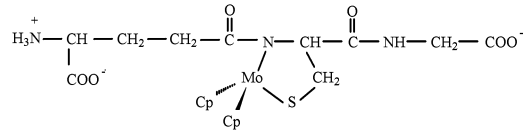




**5C**



**5E**



**5F**

**Table 6.** $^1$H NMR Chemical Shifts in ppm of $[Cp_2Mo(\gamma\text{-Glu-Cys-}S,O)]$ (**6A**) in $D_2O$ (pD 5.2)

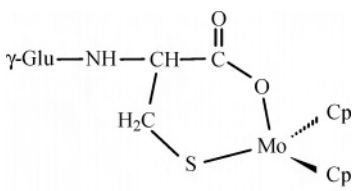
| | |
|---|---|
| Cp | 5.70 (s), 5.68 (s) |
| Cys-C$H_\alpha$ | 4.21 (t) |
| Cys-C$H_\beta$ | 2.72 (dd), 2.39 (dd) |
| Glu-C$H_\alpha$ | 3.77 (t) |
| Glu-C$H_\beta$ | 2.16 (q) |
| Glu-C$H_\gamma$ | 2.53 (t) |

$Cp_2Mo^{2+}$−tripeptide adduct whose Gly-$H_\alpha$ signal accidentally coincides with that of glycine, the pD dependence of the putative glycine resonance has also been checked. The formation of free glycine was further corroborated by TLC.

Release of glycine from GSH has been followed in detail at pD 5.2 and 7.4 (60 °C). Within 20 h, 40 and 25% GSH are hydrolyzed in acidic and neutral solution, respectively. Besides the tripeptide adducts of type **5B**,**C**,**E**,**F**, a new $Cp_2Mo^{2+}$ complex is observed in yield identical with that of glycine in the reaction mixtures. By comparison with the chemical shifts of the Cp and Cys-$H_\alpha$ resonances of **1A** and **2A**, the NMR data for this species can be assigned to the dipeptide complex $[Cp_2Mo(\gamma\text{-Glu-Cys-}S,O)]^+$, **6A** (Table 6, Chart 3).

The time-dependent development of "free" glycine at 60 °C and under physiological conditions at 40 °C is displayed

**Chart 3**



**6A**

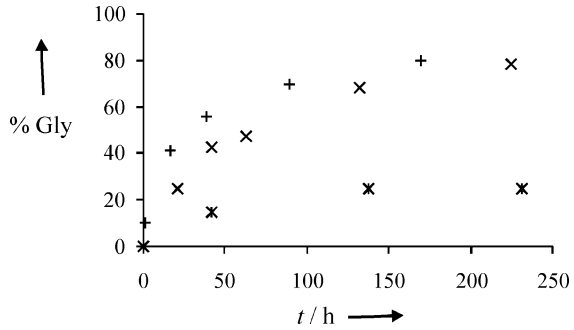


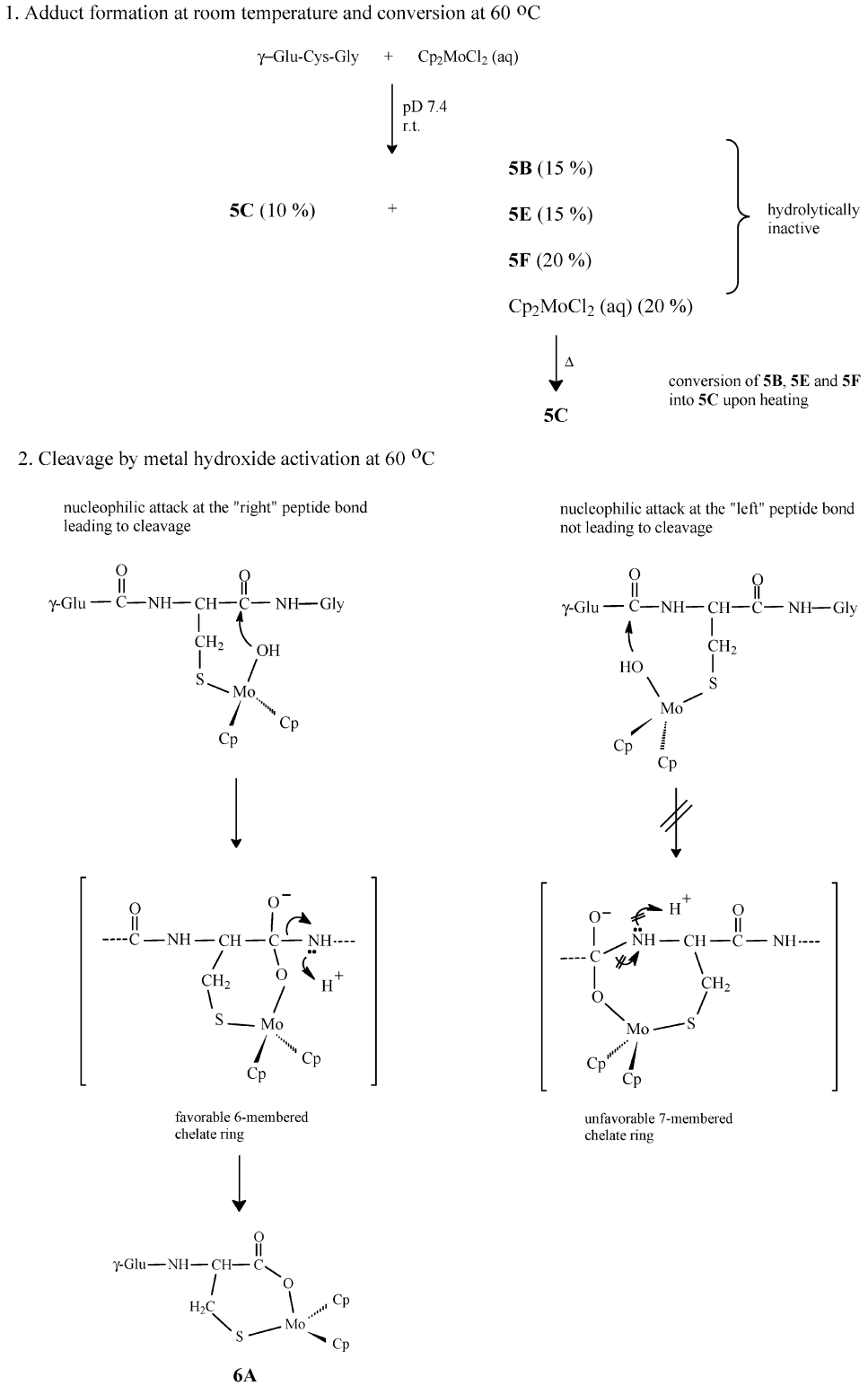

**Figure 5.** Plot of the percentage of free glycine versus time during the equimolar reaction of GSH and $Cp_2MoCl_2$(aq) at pD 5.2 and 60 °C (+), at pD 7.4 and 60 °C (×), and at pD 7.4 and 40 °C (*).

in Figure 5. In light of the half-life of 250−600 y reported for the hydrolytic cleavage of peptide bonds around neutral pH,[1] the occurrence of significant amounts of hydrolysis products of GSH at pD 7.4 within 24 h is remarkable. In accordance with the normally extreme slow rate of peptide cleavage, no release of glycine is observed under the same conditions in the absence of $Cp_2MoCl_2$(aq). Interestingly, the dipeptide complex **6A** and glycine are the only hydrolysis products of GSH. The NMR spectra do not feature any signals attributable to glutamic acid, complexes of the dipeptide Cys-Gly (**3A**−**C**), or cysteine. Likewise, only glycine can be detected when the reaction mixture is analyzed by TLC. Clearly, the $Cp_2Mo^{2+}$-mediated cleavage of the tripeptide into dipeptide and amino acid is regioselective.

As will be discussed further below, no hydrolysis is observed when amides such as glycinamide that lack the anchoring thiol group are treated with $Cp_2MoCl_2$(aq). This indicates an intramolecular metal-mediated reaction. The putative reaction pathway is presented in Scheme 4. In general, metal-promoted hydrolyses proceed either via metal−hydroxide or Lewis-acid activation; i.e., the metal ion provides $OH^-$ as efficient nucleophile at moderate pH or activates the substrate for nucleophilic attack by polarization. Consequently, complexes **5B**,**E**,**F** should be hydrolytically inactive, while in the case of **5C** intramolecular attack of the Mo−OH group at the carbonyl group can lead to release of glycine and formation of **6A**. The trapping of $Cp_2Mo^{2+}$ in hydrolytically inactive **5B**,**E**,**F** is the limiting factor of the hydrolysis reaction. In the neutral equilibrium mixture at room temperature the percentage of **5C** is only 10%. Upon being heated to 60 °C other adducts can be converted into **5C**. In particular, **5C** can be generated from **5B** and unreacted $Cp_2MoCl_2$(aq) so that about 80% of the GSH become hydrolyzed with time. At 40 °C, the overall yield of hydrolysis products is limited to 25% (Figure 5). Further hydrolysis with longer reaction times is not achieved because conversion of **5B**,**F** into **5C** is inefficient at moderate temperature.

There are several aspects that can contribute to the regioselectivity of the peptide cleavage: (i) different susceptibility of the two (nonidentical) peptide groups toward nucleophilic attack, (ii) the tetrahedral intermediate of the reaction; (iii) the resulting hydrolysis product. The first step of amide hydrolysis involves attack of the nucleophile leading

**Scheme 4**




to a tetrahedral intermediate. In Scheme 4 the tetrahedral intermediates that are associated with $Cp_2Mo^{2+}$-mediated hydrolysis of the two peptide bonds of the cysteine residue are depicted: interaction of the $Cp_2Mo^{2+}$ moiety anchored by the thiolate group with the "right" and "left" peptide bond gives intermediates with six- and seven-membered chelate rings, respectively. Hydrolysis of the Cys−Gly bond of GSH involves the more favorable intermediate (six-membered chelate ring) and leads to the chelate complex **6A** as a favorable hydrolysis product (already preformed in the intermediate), while cleavage of the γ-Glu−Cys bond would be associated with a less favorable intermediate (seven-membered chelate ring) and would require disruption of the intermediate chelate ring.

The formation of a stable S,O-chelate as hydrolysis product requires stoichiometric amounts of $Cp_2MoCl_2$. It should be

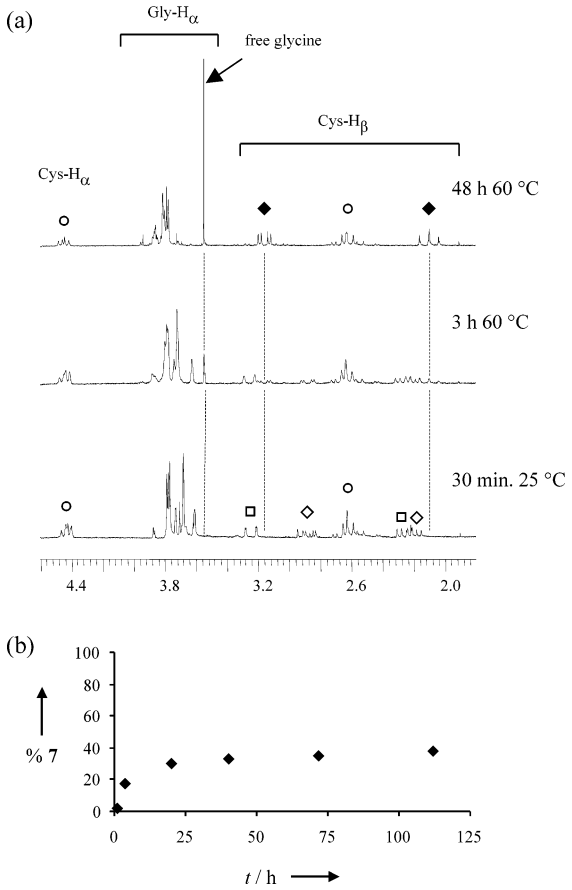



**Figure 6.** (a) $^1$H NMR spectra (2.0−4.4 ppm) of the reaction of $Cp_2MoCl_2$(aq) and Gly-Cys-Gly at pD 8.9 and 60 °C. Key: (○) **4B**; (□) cis isomer of **4F**; (◇) trans isomer of **4F**; (◆) **7**. (b) Plot of the formation of **7** versus time.

noted that to date only a few highly efficient, *true catalysts* for peptide cleavage have been reported.[10,13] The rate acceleration provided by $Cp_2MoCl_2$ is comparable to that achieved with Ce(IV) salts. Komiyama has recently shown that stoichiometric amounts of Ce(IV) ions that are anchored by carboxylate groups give 19−62% hydrolysis of dipeptides within 72 h at pH 8 and 50 °C.[6]

**Cleavage of X-Cys-Y Peptides: $Cp_2Mo^{2+}$-Assisted Intramolecular Aminolysis of the Cys−Gly Peptide Bond of Gly-Cys-Gly.** In contrast to GSH where cysteine forms a peptide bond with the carboxyl group at the $\gamma$-C atom of glutamic acid, Gly-Cys-Gly has regular peptide linkages. When Gly-Cys-Gly was reacted with $Cp_2MoCl_2$ at pD ≥ 7 and room temperature and when the resulting solution containing **4B**,**F** was heated at 60 °C on a NMR scale, conversion of **4F** into the $Cp_2Mo^{2+}$ complex of the 2,5-diketopiperazine derivative *cyclo*-(Gly-Cys) and free glycine was observed (Figure 6, Scheme 5). **4B** remained essentially unchanged. The time dependence of the reaction that has been followed over 5 d is plotted in Figure 6b. The identity of the reaction product, [$Cp_2Mo${*cyclo*-(Gly-Cys-*S*,$N_{amide}$)}] (**7**), was confirmed by X-ray analysis after preparation and isolation on a preparative scale (see below).

**Reaction Pathway and Role of $Cp_2Mo^{2+}$.** Two possible reaction pathways that could account for the diketopiperazine formation are presented in Scheme 6. Pathway a involves (metal-promoted) hydrolytic cleavage of Gly-Cys-Gly into Gly-Cys and Gly and subsequent intramolecular condensation between the terminal carboxyl and amino group. The slow conversion of dipeptides into diketopiperazines is a well-known reaction in peptide chemistry[44] and is explained by the thermodynamic stability of the six-membered ring. Komiyama et al. found that this reaction can be catalyzed by lanthanide ions that bind and activate the carboxylate group for the intramolecular nucleophilic attack by the primary amino group.[6b] In pathway b cyclization occurs via intramolecular aminolysis of the C-terminal peptide bond. Some tripeptides spontaneously decompose to cyclic peptides and free amino acids under mild conditions.[45] The reaction that is initiated by nucleophilic attack of the primary amino group at the peptide bond is a transpeptidation and requires the amide group to adopt the cis configuration instead of the usually preferred trans configuration. Consequently, transformation of tripeptides into cyclic dipeptides via intramolecular aminolysis is specifically observed for tripeptides containing amino acids that readily form cis amide bonds, like glycine and proline.[45]

The following experiments and observations lead to the conclusion that **7** must be formed by $Cp_2Mo^{2+}$-assisted, intramolecular aminolysis: (i) In the absence of $Cp_2MoCl_2$, Gly-Cys-Gly is stable in neutral and alkaline solution. When the tripeptide was heated for 2 d at pD 9 and 60 °C, neither formation of *cyclo*-(Gly-Cys) nor release of glycine was observed. Hence, generation of the diketopiperazine is clearly induced by $Cp_2Mo^{2+}$. (ii) **7** is formed at the same rate at which glycine is released (Supporting Information). This suggests a one-step pathway implying an intramolecular transpeptidation according to Scheme 6b rather than a two-step pathway comprising peptide hydrolysis followed by cyclization of the linear dipeptide (Scheme 6a). (iii) No cyclization could be detected when the dipeptides Gly-Cys and Cys-Gly were reacted with $Cp_2MoCl_2$ at 60 °C and pD 9.

What is the role of the $Cp_2Mo^{2+}$ entity in the cyclization? In the NMR spectra shown in Figure 6 two sets of resonances are observed for **4F** (Table 5). It is proposed that these two signal sets originate from two isomers with cis and trans configuration of the metalated peptide group. The relative intensities of the signals correspond to a ratio of 3:2. This ratio remains constant over longer reaction times at room temperatures or at elevated temperature. By comparison with the NMR data for **7** having a cis configuration (cf. Experimental Section for chemical shifts) the major species is associated with the cis configuration of the metalated peptide group while the minor species is assumed to be the trans isomer. It seems clear that in **4F** electronic activation of the substrate cannot account for the conversion of Gly-Cys-Gly into *cyclo*-(Gly-Cys). Instead, $Cp_2Mo^{2+}$ can be proposed to play a structural role by generating the cis amide bond

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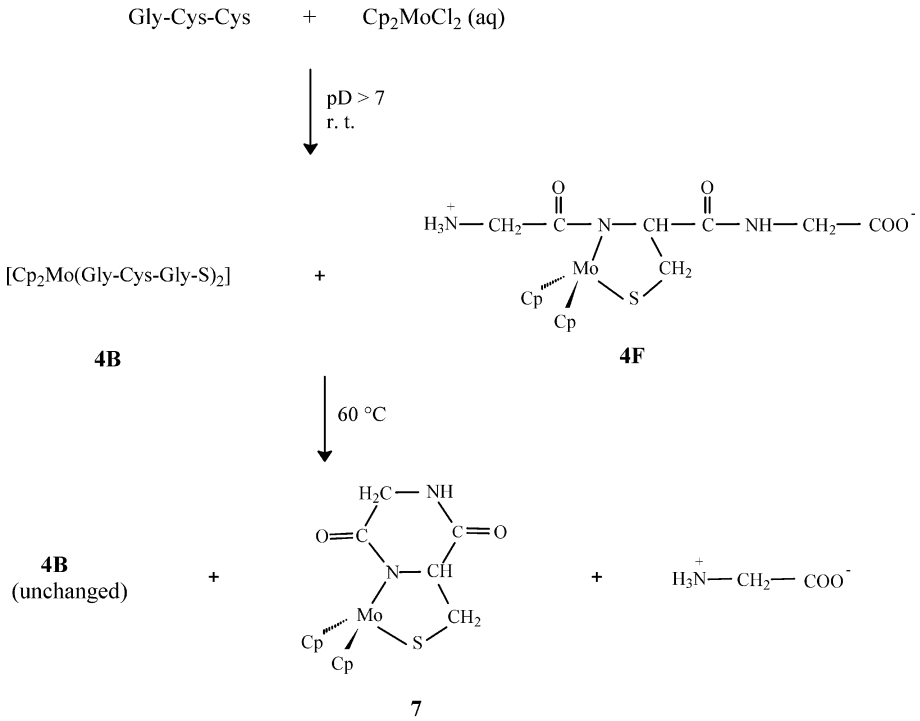
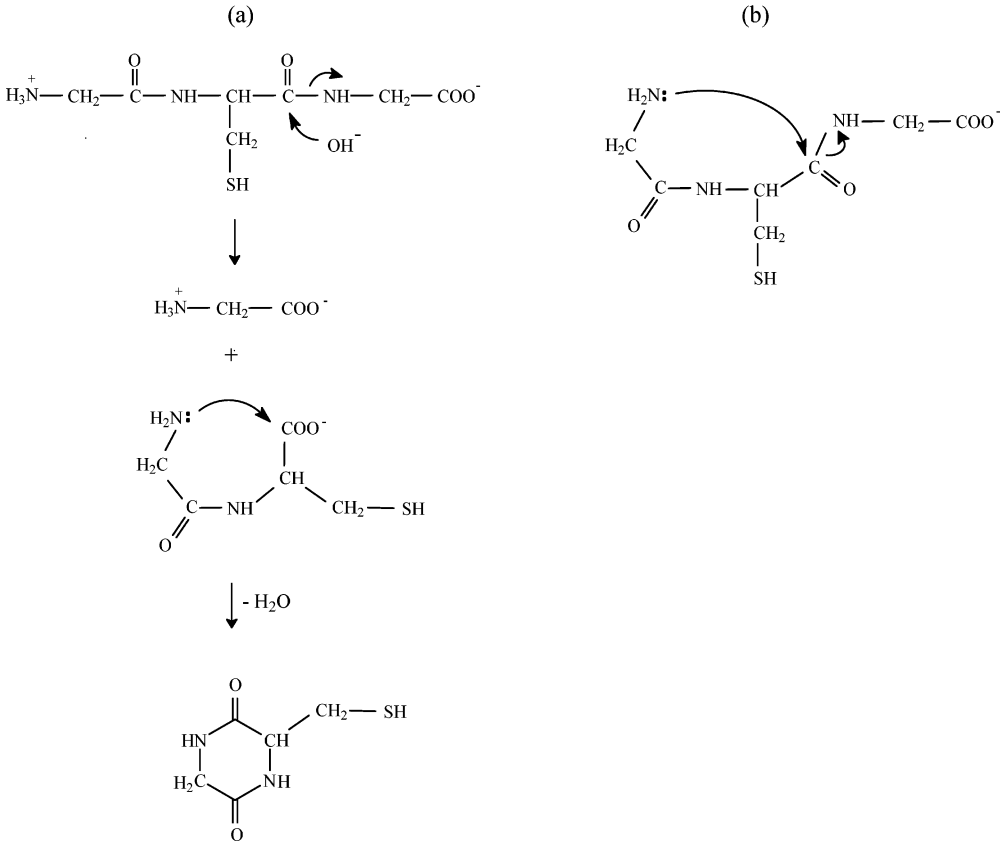
**Scheme 5**




**Scheme 6.** Possible Pathways for the Conversion of Gly-Cys-Gly into a Diketopiperazine: (a) Hydrolytic Cleavage of the Tripeptide into Dipeptide and Glycine and Subsequent Intramolecular Condensation of the Dipeptide; (b) Intramolecular Aminolysis[a]




[a] Cf. text for possible roles of the metal ion.

required for the cyclization to occur. Once the cis configuration is adopted, the cyclization is driven by the thermodynamic stability of the six-membered diketopiperazine ring. Metal ion coordination to tertiary amides has been shown to assist cis−trans isomerization by lowering the energy barrier of rotation.[46,47] However in **4F**, the metal ion coordinates to the *deprotonated* amide nitrogen of a secondary amide. In this case, metal coordination reinforces the

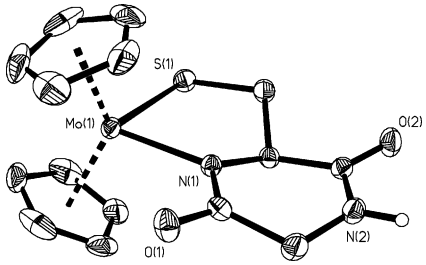



**Figure 7.** Molecular structure of [$Cp_2Mo${*cyclo*-(Gly-Cys)}]·$2H_2O$ (**7**). Except for the amide proton, hydrogen atoms are omitted for clarity. Thermal ellipsoids are drawn at the 30% level.

**Table 7.** Selected Bond Lengths (Å), Angles (deg), and Torsional Angles (deg) for [$Cp_2Mo${*cyclo*-(Gly-Cys)}]·$2H_2O$ (**7**)

| | | | |
|---|---|---|---|
| Mo1−Cp(center) | 2.002(4) | Mo1−Cp(center) | 1.986(5) |
| Mo1−N1 | 2.178(3) | Mo1−S1 | 2.455(1) |
| N1−C3 | 1.333(5) | C3−O1 | 1.247(5) |
| N2−C5 | 1.324(6) | C5−O2 | 1.250(5) |
| | | | |
| Cp−Mo1−Cp | 134.8(2) | N1−Mo1−S1 | 79.82(9) |
| Mo1−S1−C1 | 100.0(1) | Mo1−N1−C3 | 121.3(2) |
| C3−N1−C2 | 118.7(3) | C5−N2−C4 | 123.4(3) |
| | | | |
| $\omega$(C2−C5−N2−C4) | −1.74(6) | $\omega$(C4−C3−N1−C2) | 1.8(5) |
| $\psi$(N1−C2−C5−N2) | 25.9(5) | $\psi$(N2−C4−C3−N1) | 22.1(6) |
| $\phi$(C3−N1−C2−C5) | −25.5(5) | $\phi$(C5−N2−C4−C3) | −22.1(6) |

partial C−N double bond character and thus increases the energy barrier of rotation.[43] However, the formation of the cis isomer may be rationalized with the steric bulk of the $Cp_2Mo^{2+}$ moiety that affects the conformation of the peptide group.

**Structural Features of the Cleavage Product.** Figure 7 gives a view of the molecular structure of [$Cp_2Mo${*cyclo*-(Gly-Cys)}]·$2H_2O$ (**7**), and selected bond lengths and angles are listed in Table 7. The $Cp_2Mo^{2+}$ unit coordinates to the thiolate sulfur and to one deprotonated amide nitrogen of the diketopiperazine ring so that a five-membered chelate ring is formed. The structural features of the $Cp_2Mo^{2+}$ moiety are $d$(Cp(center)−Mo−Cp′(center)) = 2.002(4) and 1.986(5) Å and ∠(Cp(center)−Mo−Cp′(center)) = 134.8(2)° and are thus unexceptional.[48,49] As expected, the metalated and the uncoordinated amide groups are both planar with the C−N−C−O torsion angles being 176.1(4)° (C2−N1−C3−O1) and 179.7(4)° (C4−N2−C5−O2). The maximum deviation of any atom of the two peptide groups from the respective least-squares plane is 0.003 Å. The torsional angles $\omega$, $\phi$, and $\psi$ that are normally quoted for peptide structures are given in Table 7.

The C−O and C−N bond lengths remain unaffected by coordination of $Cp_2Mo^{2+}$; for both amide groups of the diketopiperazine C−O and C−N bond distances are equal, respectively. However, replacement of the amide hydrogen by the bulky $Cp_2Mo^{2+}$ moiety decreases the C−N−C bond angle by ∼5° (C3−N1−C2 = 118.7(3)°; C5−N2−C4 = 123.4(3)°). The six-membered diketopiperazine ring adopts the boat conformation with the Cremer−Pople ring-puckering parameters[38] being $Q$ = 0.323(5) and $\theta$ = 86.0(8) (expected $\theta$ value for pure boat conformation: 90°). The dihedral angle of the fold in the diketopiperazine ring as calculated between the least-squares planes formed by the atoms C2/C5/N2/C4 and C4/C3/N1/C2 is 25.1(2)°. Since diketopiperazines are quite abundant in nature and since they represent key intermediates in the asymmetric synthesis of amino acid derivatives,[50] their conformation has attracted some interest and structural,[51−53] spectroscopic,[54] and theoretical studies[55−60] have been reported. Planar,[51,52] boat,[53] and twisted boat[52] conformations were found by X-ray analyses with the dihedral angle of the folded six-membered rings ranging up to 40°. Theoretical calculations reported for *cyclo*-(Gly-Gly) indicated the boat conformer to be the most stable conformer.[55,59] Cyclic peptides have been widely used to study interactions of metal ions with peptide side chains because they are devoid of terminal amino and carboxylate groups. However, to the best of our knowledge, **7** is the first example of a metal complex of a cysteine-containing cyclic dipeptide that has been characterized by X-ray analysis. In fact, we are aware of only one study on metal coordination to *cyclo*-(X-Cys) peptides.[61]

**Role of the Anchoring Cysteine Side Chain for Peptide Hydrolysis.** To evaluate the role of the cysteine side chain, peptides devoid of the anchoring thiol group have been reacted with $Cp_2MoCl_2$(aq). In contrast to the facile cleavage of X-Cys-Gly into X-Cys and Gly, cleavage of peptides that do not provide a suitable docking site is not mediated by $Cp_2Mo^{2+}$. Neither glycinamide nor the activated amide glycine-*p*-nitroanilide (Gly pNA), which is more susceptible to hydrolysis due to the good leaving group *p*-nitroanilide, is cleaved in the presence of 1 equiv of $Cp_2MoCl_2$(aq) near neutral pH after 24 h at 60 °C. The only $Cp_2Mo^{2+}$ complex identified in the reaction mixture is the hydroxo-bridged dimer $[(Cp)_2Mo(\mu\text{-}OH)_2Mo(Cp)_2]^{2+}$ that is ineffective as a catalyst because of the saturated coordination sphere of Mo. Cleavage of Gly pNA is likewise not observed at pD 3.7 (48 h, 60 °C) when 70% of the added $Cp_2MoCl_2$ exist as the monomeric diaqua species $[Cp_2Mo(H_2O)_2]^{2+}$. Evidently, for peptide hydrolysis an amino acid side chain is required that provides a docking site for the $Cp_2Mo^{2+}$ unit.

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## Conclusions

Monodentate coordination of the organometallic $Cp_2Mo^{2+}$ unit by cysteine residues of peptides leads to rapid, regioselective cleavage of the Cys−X peptide bond around neutral pH. The presence of the thiol group that serves as anchor and brings the Mo−OH group in close contact to the scissile peptide bond is an indispensable prerequisite for $Cp_2Mo^{2+}$-mediated peptide cleavage. Glycine peptides that lack the side chain are not hydrolyzed. The high affinity of Mo for thiols results in a stable product complex which prevents a catalytic reaction. However, it might also guarantee highly selective cysteine coordination and efficient anchoring of the cleaving agent by cysteine residues when longer oligopeptides are used. This point will be addressed in future work.

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**Supporting Information Available:** NMR spectra of the reaction of $Cp_2MoCl_2$ with AcCys, complete $^1H$ and $^{13}C$ NMR data for **1A**,**B**, pD-dependent $^1H$ NMR chemical shifts of compound **1A**, kinetics of the conversion of **4F** into **7**, and a crystallographic file in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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