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Thioether Complexes of Palladium(II) and Platinum(II) as Artificial Peptidases. Residue-Selective Peptide Cleavage by a Palladium(II) Complex

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We report the synthesis and characterization of perchlorate salts containing the following three novel complex cations each with a bidentate thioether ligand: binuclear *cis*-$[Pt(CH_3SCH_2CH_2CH_2SCH_3)(\mu\text{-}OH)]_2^{2+}$, mononuclear *cis*-$[Pt(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$, and mononuclear *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$. Despite their analogous compositions, the mononuclear Pt(II) and Pd(II) complexes differ in the selectivity with which they promote the hydrolysis of polypeptides. The complex *cis*-$[Pt(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ promotes slow but selective cleavage of Met–Pro peptide bonds at pH 2.0. The selectivity of the complex *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ is pH-dependent. At pH 2.0, this Pd(II) complex promotes residue-selective hydrolysis of the X–Y bond in X-Y-Met and X-Y-His sequences; the rate is enhanced when residue Y is proline. At pH 7.0, this kinetic preference becomes sequence-selective in that the Pd(II) complex exclusively cleaves the X–Pro bond in X-Pro-Met and X-Pro-His sequences. The enhanced reactivity of the X–Pro amide group is attributed to the high basicity of its carbonyl oxygen atom. Binding of the metal(II) atom enhances the electrophilicity of the carbonyl carbon atom and promotes nucleophilic attack by a solvent water molecule. The bidentate thioether ligand disfavors the formation of hydrolytically unreactive complexes, allowing the Pd(II) complex to promote the cleavage reaction.

Introduction

Selective Hydrolysis of Peptides and Proteins. Many bioanalytical and biochemical techniques involve the selective cleavage of peptide bonds. Proteomic applications require the digestion of proteins into fragments suitable for mass spectrometric analysis.[1] In footprinting and folding studies, local protein structures can be determined by the regioselective cleavage of peptide bonds in solvent-exposed segments.[2] In protein semisynthesis, natural proteins are selectively hydrolyzed to give long fragments containing terminal amino and carboxylic groups, which are then reconnected to form new proteins. Site-specific cleavage is also needed for the removal of fusion tags from bioengineered fusion proteins.[3,4]

Unactivated amide groups are extremely unreactive toward hydrolysis. For instance, the N-acetylated dipeptide AcGly–Gly at room temperature and in the pH range between 4.0 and 8.0 has a half-life of several hundred years.[5−7] To make this reaction proceed at practical rates and regioselectively is a formidable task.

Proteolytic enzymes, also called peptidases, can catalyze nonselective or selective protein cleavage. Nonselective processes are involved in protein degradation, apoptosis, and antigen processing, while selectivity is necessary in the regulation of the cell cycle, signal transduction, and transcription. Although some proteolytic enzymes are selective, react with turnover, and require only mild conditions, they also have disadvantages: they cleave themselves as well as the intended substrates, and they remain in digests as proteinaceous contaminants. For these reasons, chemical

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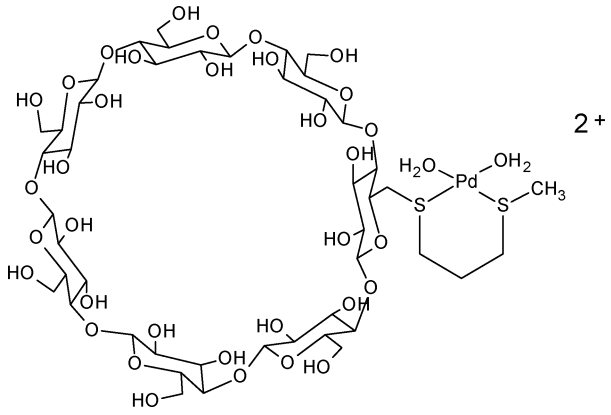
reagents for peptide and protein cleavage are much needed. The use of cyanogen bromide and other chemical reagents has been limited by poor selectivity, incomplete cleavage, toxicity, and the need for harsh reaction conditions.[8] These reagents are usually applied in a large molar excess over the substrate, and they produce chemically altered protein fragments such as covalently modified amino acid side chains.

New chemical proteases that efficiently and selectively hydrolyze peptides and proteins should be capable of binding to particular amino acid residues or short sequences and promoting the hydrolysis of specific peptide bonds under mild conditions. After the reaction, these reagents should be easily removable from the cleaved fragments. Certain transition-metal complexes have the desired properties, but few of them cleave peptides or proteins regioselectively.[9−28]

Complexes of Palladium(II) and Platinum(II) as Synthetic Peptidases. Our laboratory has achieved residue-selective and even sequence-selective hydrolysis of many peptides and various proteins by simple Pd(II) and Pt(II) complexes containing at least two aqua ligands.[29−36] When a nucleophilic side chain displaces one of the aqua ligands, the metal(II) atom becomes anchored to the substrate. In the hydrolytically reactive complexes, the metal(II) atom acts as a Lewis acid and activates a proximate amide group toward nucleophilic attack by a solvent water molecule. Methionine and histidine side chains are anchors for the Pd(II) reagents. The residue-selective cleavage in weakly acidic solutions occurs at the X−Y bond in X-Y-Met-Z and X-Y-His-Z sequences, provided that residues X, Y, and Z have noncoordinating side chains.[31−33] Only methionine side chains act as anchors for the Pt(II) reagents, and the residue selectivity is different. These reagents cleave Met−Z bonds.[30]

Both Pd(II) and Pt(II) ions can be removed from the cleaved fragments by the addition of a strong chelating agent, such as sodium diethyldithiocarbamate; the pristine fragments can then be further analyzed or used.[9,37] Because the combined abundance of histidine and methionine residues in proteins is only ca. 5.5%, the fragments are larger than those produced by trypsin and therefore more useful in modern biochemical practice.[38]

In an effort to mimic the multiple functions of an enzyme, we combined the ability of β-cyclodextrin to recognize and bind aromatic side chains and the ability of palladium(II) aqua complexes to cleave a polypeptide backbone. A reagent containing these two moieties selectively cleaved the X−Pro bond in an X-Pro-Y sequence having phenylalanine as residue Y.[35] The organic and inorganic moieties in the new reagent were connected with a bidentate thioether ligand, as shown below. This new Pd(II)−cyclodextrin conjugate reacted not only sequence-selectively but also relatively fast, more rapidly than $[Pd(OH_2)_4]^{2+}$. In this study, we investigate the possible effects of the bidentate thioether ligand on the rate and selectivity of hydrolytic cleavage. We compare analogous thioether complexes of Pd(II) and Pt(II) ions because, surprisingly, these two metal ions show different selectivities in protein cleavage.[34]



Experimental Procedures

Chemicals. Distilled water was demineralized and purified to a resistivity higher than 17 MΩ cm. Palladium sponge, $Na_2[PdCl_4]$, piperidine, diethyldithiocarbamic acid sodium salt, α-cyano-4-

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hydroxycinnamic acid, human angiotensin II, and *N*,*N*-diisopropylethylamine were obtained from Sigma-Aldrich Chemical Co. Methyl phenyl sulfone was obtained from Lancaster Synthesis Inc. Acetonitrile of HPLC grade, dichloromethane, *N*,*N*-dimethylformamide (DMF), and diethyl ether were obtained from Fisher Scientific Co. Silver perchlorate monohydrate, 1,3-bis(methylthio)propane, trifluoroacetic acid, and $K_2[PtCl_4]$ were obtained from Alfa Aesar. The *N*-α-Fmoc-Ala-Wang resin, 2-(1-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, and *N*-hydroxybenzotriazole were obtained from Calbiochem-Novabiochem Corp. The N-acetylated peptide Ac-Lys-Ala-Tyr-Asp-Pro-His-Ala-Ala-Arg-Ala, designated as Pro-His peptide, was custom-ordered from Sigma Genosys.

Peptide Synthesis. The N-acetylated peptides Ac-Ala-Lys-Phe-Gly-Met-Ala-Tyr-Arg-Gly (termed Met-Ala peptide), Ac-Ala-Lys-Ala-Gly-Met-Pro-Ala-Tyr-Arg-Ala (termed Met-Pro peptide), Ac-Lys-Ala-Tyr-Asp-Pro-Met-Ala-Ala-Arg-Ala (termed Pro-Met peptide), and Ac-Lys-Gly-Gly-Ala-Ser-Pro-Phe-Ala-Ala-Arg-Ala (termed Pro-Phe peptide) were synthesized by a standard manual Fmoc solid-phase procedure[8,39] and purified by reverse-phase high-performance liquid chromatography (HPLC) on a C18 preparative column as described previously.[32] The purity, as examined by analytical HPLC, was higher than 99.5%. The respective measured and calculated molecular masses were 1043.16 and 1043.23 Da for Met-Ala peptide, 1078.95 and 1078.28 Da for Met-Pro peptide, 1136.62 and 1136.31 Da for Pro-Met peptide, and 1074.75 and 1075.21 Da for Pro-Phe peptide.

NMR Spectroscopy. The ^{1}H NMR spectra were recorded in water at 25.0 °C with a Bruker DRX500 spectrometer and externally referenced to the methyl signal of sodium 2,2-dimethyl-2-silapentane-5-sulfonate in aqueous solutions. Because peptide cleavage is very slow at room temperature, we safely assumed that the sample composition remained unchanged during the NMR measurements. TOCSY (total correlation spectroscopy) spectra were acquired in aqueous solution for reactions involving Met-Ala peptide. ROESY (rotating-frame Overhauser enhancement spectroscopy) resolved the assignment of the two alanine and two glycine residues in the peptide designated Met-Ala. A mixing time of 100 ms was used in TOCSY experiments. The spin-lock field strength during mixing was 6.0 kHz in TOCSY experiments and 2.5 kHz in ROESY experiments. The water signal was suppressed by incorporating WATERGATE in the pulse sequences.[40] The spectra for characterizing the bidentate thioether complexes were recorded in the temperature interval of 278−318 K.

HPLC Separations. The digest components were separated by a Hewlett-Packard 1100 HPLC system containing an autosampler and a multiwavelength detector set at 215, 280, and 350 nm. Absorption at 215 nm is common to all peptides and proteins; absorption at 280 nm results from aromatic residues and bound Pd(II) or Pt(II) ions; and absorption at 350 nm is diagnostic of Pd(II) and Pt(II) ions. In the reverse-phase separations, analytical Supelco Discovery Bio Wide Pore C18 and C5 columns (sized 25 cm × 4.6 mm, beads of 5 μm) were used. The eluting solvent A was 0.10% (v/v) trifluoroacetic acid in water, and solvent B was 0.080% (v/v) trifluoroacetic acid in acetonitrile. In a typical run, the percentage of solvent B in the eluent was kept at 0% for 5 min after the injection of the sample and then raised gradually to 45% over a 35-min period. The flow rate in all analytical runs was 1.0 mL/min.

Mass Spectrometry. The MALDI-TOF experiments were performed with a Bruker Proflex instrument. The samples were prepared by a standard dried-droplet procedure: 1.0 μL of the sample of interest was mixed with 9.0 μL of a saturated solution of the matrix (α-cyano-4-hydroxycinnamic acid) in a 2:1 (v/v) mixture of 0.10% (v/v) trifluoroacetic acid in water and 0.080% (v/v) trifluoroacetic acid in acetonitrile. Each spectrum consisted of 100 scans. For the sake of clarity, molecular masses are reported only for the peptide fragments free of the Pd(II) and Pt(II) complexes unless otherwise specified, although the peptide fragments bearing the metal ions were also observed in the MALDI spectra. A solution of bradykinin and α-cyano-4-hydroxycinnamic acid was used as an external standard. The measured molecular mass of a given fragment was compared with the value calculated by PAWS software, obtained from ProteoMetrics, LLC. Excellent agreement conclusively identified each peptide.

The presence of Pd(II) and Pt(II) ions bound to the peptide or its fragment(s) was established not only by the matching molecular masses of the labeled peptides but also by the isotopic distributions. Diagnostic for Pd: 1.0% ^{102}Pd, 11.1% ^{104}Pd, 22.3% ^{105}Pd, 27.3% ^{106}Pd, 26.5% ^{108}Pd, and 11.8% ^{110}Pd. Diagnostic for Pt: 0.8% ^{192}Pt, 33.0% ^{194}Pt, 33.8% ^{195}Pt, 25.2% ^{196}Pt, and 7.2% ^{198}Pt.

The inductively coupled plasma mass spectrometry (ICP-MS) experiments were performed with an HP 4500 instrument having a Babington nebulizer, nickel cones, and a quadrapole mass analyzer to count ^{195}Pt atoms. The samples were prepared by dissolving a known mass of *cis*-$[Pt(CH_3SCH_2CH_2CH_2SCH_3)(\mu_2\text{-}OH)]_2(ClO_4)_2$ in 5 mL of fuming nitric acid. The solution was heated just below the boiling point for 4 h. The sample was then diluted with water to an appropriate concentration for analysis. A blank solution containing the same concentration of nitric acid was used in background comparisons.

Synthesis of *cis*-$[PdCl_2(CH_3SCH_2CH_2CH_2SCH_3)]$. *cis*-$[PdCl_2(CH_3SCH_2CH_2CH_2SCH_3)]$ was synthesized by a modification of a published procedure.[41,42] To a round-bottomed flask were added 1,3-bis(methylthio)propane (1.00 mmol, 138.8 μL) and water (0.50 mL) with stirring. A solution of $Na_2[PdCl_4]$ (1.00 mmol, 294.1 mg) in water (4.0 mL) was added with a dropping funnel, over a period of 30 min. The reaction mixture was refluxed for 4 h, cooled to room temperature, and stored overnight at 4 °C. The orange solid product was filtered off, washed with cold water and ethanol, and dried under vacuum. Yield: 0.27 g, 87%. Mp: 238 °C. UV−vis absorption maxima in water. That MALDI mass spectra did not show the intact dichloro compound is evidence for the lability of the chloro ligands. Molecular masses. Calcd for $[PdCl(CH_3SCH_2CH_2CH_2SCH_3)]$: 275.62, 276.63, 277.63, 279.62, and 281.63 Da. MALDI: 275.05, 276.00, 277.05, 279.05, and 281.05 Da. Calcd for $[Pd(CH_3SCH_2CH_2CH_2SCH_3)]$: 240.18, 241.18, 242.17, 244.16, and 246.17 Da. Measd: 240.30, 241.32, 242.36, 244.30, and 246.20 Da. The isotopic distribution was diagnostic of Pd.

Synthesis of *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2](ClO_4)_2$. A solution of silver perchlorate monohydrate (0.20 mmol, 45.1 mg) in water (3.0 mL) was added to *cis*-$[PdCl_2(CH_3SCH_2CH_2CH_2SCH_3)]$ (0.10 mmol, 31.4 mg), and the mixture was stirred at 60 °C for 2 h in the dark. The mixture was filtered, and the yellow supernatant containing *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2](ClO_4)_2$ was collected. UV−vis absorption maxima in water: 365 nm ($\epsilon \approx 1290$ M^{-1} cm^{-1}) and 261 nm ($\epsilon \approx 10\,640$ M^{-1} cm^{-1}). That MALDI mass spectra did not show the intact diaqua compound is evidence

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for the lability of the aqua ligands. MALDI molecular masses. Calcd: 240.18, 241.18, 242.18, 244.18, and 246.18 Da. Measd: 240.00, 241.05, 241.99, 244.00, and 246.06 Da. The isotopic distribution was diagnostic of Pd. ^{1}H NMR (D_2O): δ 2.360(s), 2.445(s), 2.515(m), 2.575(m), 2.642(m), and 2.829(m). ^{13}C NMR (D_2O): δ 19.8, 19.9, 22.1, 30.9, and 34.0.

Synthesis of *cis*-[PtCl$_2$(CH$_3$SCH$_2$CH$_2$CH$_2$SCH$_3$)]. *cis*-[PtCl$_2$(CH$_3$SCH$_2$CH$_2$CH$_2$SCH$_3$)] was synthesized by a modification of a published procedure.[41,42] To a round-bottomed flask were added 1,3-bis(methylthio)propane (1.00 mmol, 138.8 μL) and water (2.0 mL) with stirring. A solution of K_2[PtCl$_4$] (0.5 mmol, 207.5 mg) in water (4.0 mL) was added with a dropping funnel over a period of 30 min. The solution was refluxed for 4 h. A yellow solid was collected by vacuum filtration, washed with ethanol and cold water, and recrystalized from acetonitrile. Yield: 0.13 g, 65%. Mp: 238°C. UV−vis absorption maxima in water. MALDI mass spectrometry detected both the dichloro and monochloro species. Molecular masses. Calcd for [PtCl$_2$(CH$_3$SCH$_2$CH$_2$CH$_2$SCH$_3$)]: 406.15, 407.15, 408.15, and 409.15 Da. MALDI: 405.96, 406.96, 407.96, and 408.98 Da. Calcd for [PtCl(CH$_3$SCH$_2$CH$_2$CH$_2$SCH$_3$)]: 365.69, 366.99, 367.69, and 369.70 Da. Measd: 365.96, 366.97, 367.95, and 368.93 Da. The isotopic distribution was diagnostic of Pt.

Synthesis of *cis*-[Pt(CH$_3$SCH$_2$CH$_2$CH$_2$SCH$_3$)(H$_2$O)$_2$](ClO$_4$)$_2$. A solution of silver perchlorate monohydrate (0.16 mmol, 36.1 mg) in water (3.5 mL) was added to *cis*-[PtCl$_2$(CH$_3$SCH$_2$CH$_2$CH$_2$SCH$_3$)]-(0.080 mmol, 32.2 mg), and the mixture was stirred at 60 °C for 2 h in the dark. The mixture was filtered, and the yellow supernatant, containing *cis*-[Pt(CH$_3$SCH$_2$CH$_2$CH$_2$SCH$_3$)(H$_2$O)$_2$](ClO$_4$)$_2$, was collected. UV−vis maxima in water. That MALDI mass spectra did not show the intact diaqua compound is evidence for the lability of the aqua ligands. MALDI molecular masses. Calcd: 330.24, 331.24, 332.24, and 334.24 Da. Measd: 330.02, 331.00, 332.63, and 334.84 Da. The isotopic distribution was diagnostic of Pt. ^{1}H NMR (D_2O): δ 2.289(s), 2.44(s), 2.47(m), 2.56(m), 2.92(m), and 3.01(m).

Synthesis of *cis*-[Pt(CH$_3$SCH$_2$CH$_2$CH$_2$SCH$_3$)(μ_2-OH)]$_2$(ClO$_4$)$_2$. A solution of silver perchlorate monohydrate (0.60 mmol, 135.2 mg) in water (2.0 mL) was added to *cis*-[PtCl$_2$(CH$_3$SCH$_2$CH$_2$CH$_2$-SCH$_3$)] (0.30 mmol, 120.7 mg). The mixture was gently heated to 50 °C while stirring for 2 h in the dark and subsequently filtered. The yellow supernatant was stored at 4 °C, and yellow crystals of *cis*-[Pt(CH$_3$SCH$_2$CH$_2$CH$_2$SCH$_3$)(μ_2-OH)]$_2$(ClO$_4$)$_2$ were filtered off after several days. Yield: 8.1 mg, 6%. Elem anal. Calcd: C, 13.50; H, 2.95; S, 14.42; Pt, 74.7. Found: C, 13.54; H, 2.86; S, 14.38; Pt, 75.6 (by ICP-MS). UV−vis maximum: 344 nm. *cis*-[Pt(CH$_3$SCH$_2$-CH$_2$CH$_2$SCH$_3$)(μ_2-OH)]$_2$(ClO$_4$)$_2$ was insoluble in benzene, hexane, acetone, ethanol, methanol, pyridine, acetonitrile, water, dichloromethane, and nitrobenzene; it was only slightly soluble in DMF and DMSO, perhaps owing to their coordinating ability. IR (cm^{-1}): 3450(sh), 3336(b,w), 1416(s).

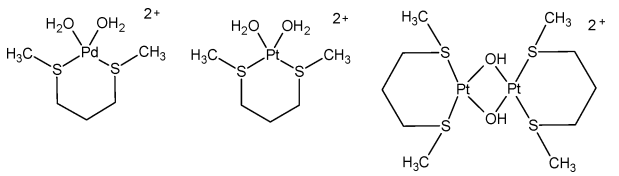
Study of Hydrolysis. Reactions were carried out in 2.0-mL glass vials. A 5.0 mM stock solution of each peptide in water was prepared. In a typical experiment involving 10 mol equiv of the metal complexed to 1 mol equiv of methionine or histidine residues, 200 μL of a 5.0 mM peptide solution was mixed with 20.0 μL of a 500 mM solution of either *cis*-[Pd(CH$_3$SCH$_2$CH$_2$CH$_2$SCH$_3$)-(H$_2$O)$_2$]$^{2+}$ or *cis*-[Pt(CH$_3$SCH$_2$CH$_2$CH$_2$SCH$_3$)(H$_2$O)$_2$]$^{2+}$, 50.0 μL of a solution containing 25.0 mg/mL of phenyl methyl sulfone, and 730 μL of water. For reactions in which the pH was in the interval of 5.0−7.0, phosphate buffer replaced the water.

The pH was measured with a Fisher Accumet instrument and an Aldrich Ag/AgCl reference electrode and was adjusted by careful addition of either 1.00 M HClO$_4$, 1.00 M NaOH, or 0.500 M trisodium phosphate with stirring. The reaction mixture was kept in a dry bath at 60 ± 1 °C. After the reaction was complete, the pH remained within ±0.10 of the initial value. Possible "background" cleavage was ruled out in control experiments in which the conditions were kept the same except that the Pd(II) or Pt(II) reagent was absent.

For the kinetic measurements, 100-μL samples were periodically taken from the reaction mixture, an excess of solid sodium diethyldithiocarbamate was added to form an insoluble compound of Pd(II) or Pt(II), the precipitate was filtered off, and the clear solution was immediately subjected to reverse-phase HPLC separation. Because the cleavage is very slow at room temperature and the Pd(II) or Pt(II) reagent was removed, the species distribution in the chromatogram matched that in the digest at the time of sampling. To compensate for a possible error in the injection volume and evaporation, the areas under the chromatographic peaks were integrated and normalized to the peak of methyl phenyl sulfone, the internal standard. The error of this integration was estimated at 5%. The plots of the peak areas of the cleavage fragments versus time were fitted to a first-order rate law with SigmaPlot, version 5.0, obtained from SPSS Inc. The stated errors in the rate constants correspond to 2 standard deviations, i.e., a confidence limit greater than 95%. This conservative reporting reduces the tendency for overinterpreting differences among rate constants. Representative chromatograms are shown in Figure S1 in the Supporting Information. Cleaved fragments were collected from analytical HPLC separations, dried with a stream of air, dissolved in 10.0 μL of water, and analyzed by MALDI-TOF mass spectrometry.

Results and Discussion

New Bidentate Thioether Complexes of Pd(II) and Pt(II). The new mononuclear complexes shown below were prepared simply by Ag$^+$-assisted aquation of the corresponding known chloro complexes (see Figure S2 in the Supporting Information). Because this one-step reaction is practically complete, the aqua complexes prepared in situ can be used immediately as hydrolytic reagents. It is advantageous for practical applications to avoid isolation. In the absence of elemental analysis, the composition of these simple complexes is determined by spectroscopy and the well-established methods of preparation. Binding of the bidentate ligand to the Pd(II) and Pt(II) ions causes downfield shifts of the ^{1}H NMR methyl signal by 0.245 and 0.174 ppm, respectively. The proton NMR spectra of both mononuclear diaqua complexes contained two sets of relatively broad signals: two singlets, corresponding to the methyl groups, and four multiplets, corresponding to the methylene groups. As the temperature was raised from 278 to 318 K, the methyl singlets began to coalesce for both of the mononuclear diaqua complexes, indicating that two configurational isomers were interconverting by inversion at the chiral sulfur atoms as shown in Figures S3 and S4 in the Supporting Information.



The hydroxo-bridged binuclear complex is formed in low yield upon concentration of a basic solution of the corre-

Chart 1. Polypeptides Used as Substrates for Cleavage by Metal Complexes

abbreviation	sequence
Met-Ala	Ac-Ala-Lys-Phe-Gly-Met-Ala-Tyr-Arg-Gly
Pro-Met	Ac-Lys-Ala-Tyr-Asp-Pro-Met-Ala-Ala-Arg-Ala
Met-Pro	Ac-Ala-Lys-Ala-Gly-Met-Pro-Ala-Tyr-Arg-Ala
Pro-Phe	Ac-Lys-Gly-Gly-Ala-Ser-Pro-Phe-Ala-Ala-Arg-Ala
Pro-His	Ac-Lys-Ala-Tyr-Asp-Pro-His-Ala-Ala-Arg-Ala
His-Pro	Val-Tyr-Ile-His-Pro-Phe

sponding mononuclear complex. An infrared spectrum showed three bands corresponding to the bending and stretching modes of the hydroxo ligands. This evidence for the doubly bridging hydroxo ligands agrees with the published spectra of similar complexes.[43−47]

Polypeptide Substrates and Hydrolytic Reaction Conditions. The substrates to be cleaved include five synthetic polypeptides and the bioactive polypeptide angiotensin II, designated as His-Pro, shown in Chart 1. With the exception of the control peptide Pro-Phe, all of the sequences contained a methionine or histidine anchoring residue in a position appropriate for MALDI mass spectrometric analysis. All peptides except human angiotensin II were acetylated at the N terminus, to prevent binding of the Pd(II) or Pt(II) atom and to render the polypeptides more similar to proteins. Coordinating anions, such as acetate and chloride, can inhibit cleavage by Pd(II) and Pt(II) reagents and were therefore excluded from all reactions.[41] The pH was carefully chosen to avoid both background cleavage in strongly acidic solutions and the formation of hydroxo-bridged species in basic solutions. Control experiments ruled out significant background cleavage at pH values between 1.8 and 7.2.

Initial Binding of *cis*-[Pt($CH_3SCH_2CH_2CH_2SCH_3$)(H_2O)$_2$]$^{2+}$ to a Methionine-Containing Polypeptide in Acidic Solution. Because Pt(II) reagents anchor to the thioether group of methionine, we chose the nonapeptide Met-Ala to study the initial binding of *cis*-[Pt($CH_3SCH_2CH_2CH_2SCH_3$)(H_2O)$_2$]$^{2+}$ to substrates. ROESY ^{1}H NMR spectra of this nonapeptide (see Figure S5 in the Supporting Information) allowed the unambiguous assignment of the two glycine residues and the two alanine residues. A mixture of the Met-Ala peptide and a 10-fold excess of *cis*-[Pt($CH_3SCH_2CH_2CH_2SCH_3$)(H_2O)$_2$]$^{2+}$ was incubated at 60 °C for 24 h and examined by ^{1}H NMR spectroscopy. In this and other studies, an excess of the metal complex was used to ensure its complete binding to the peptide. The aliphatic region in the one-dimensional spectrum and a part of the amide-to-aliphatic region in the TOCSY spectrum are shown in Figure 1. A comparison of the free peptide (Figure 1a) and the reaction mixture (Figure 1b) shows the disappearance of the cross-peaks between the amide NH and the side-chain

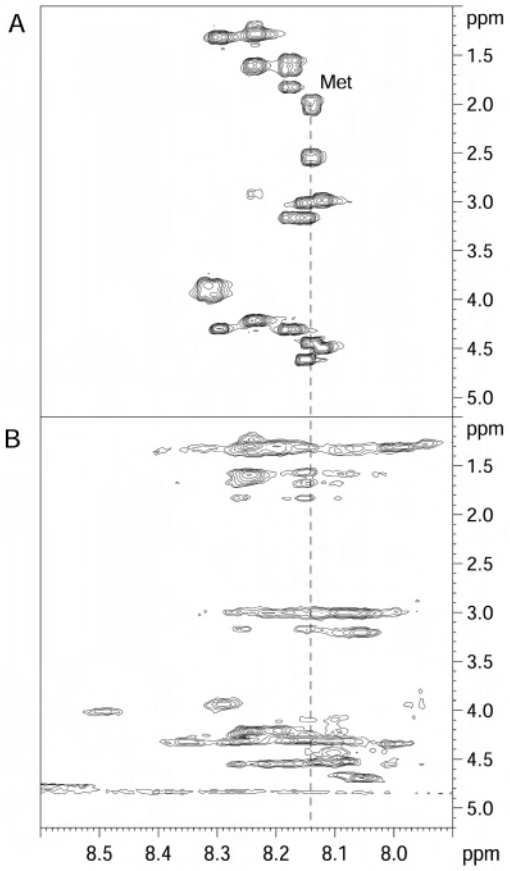


Figure 1. TOCSY ^{1}H NMR spectra of the NH-to-aliphatic region of the polypeptide designated as Met-Ala at pH 2.0 and room temperature (a) free and (b) after 24 h of incubation at 60 °C with a 10-fold excess of *cis*-[Pt($CH_3SCH_2CH_2CH_2SCH_3$)(H_2O)$_2$]$^{2+}$. The vertical dashed line shows the presence of the Met signal in panel a and its absence in panel b.

protons within methionine, evidence for the binding of the Pt(II) atom to the deprotonated amide nitrogen in methionine. The predominant species at pH 2.0 is therefore complex **2**, in which the anchoring methionine residue acts as a bidentate ligand (see Scheme 1). The pK_a for the first NH group "upstream" (toward the amino terminus) of the methionine residue bearing a Pt(II) atom was estimated at 1.8, consistent with the NMR results showing the presence of mostly complex **2** in the reaction mixture.[30] The two bidentate ligands are inert to substitution and render complex **2** unreactive toward hydrolysis. Indeed, an HPLC analysis of a 10:1 mixture of *cis*-[Pt($CH_3SCH_2CH_2CH_2SCH_3$)(H_2O)$_2$]$^{2+}$ and Met-Ala peptide showed a persistent peak corresponding to the Pt(II) complex with the intact peptide and no appearance of the peptide fragments. A MALDI mass spectrum of the reaction mixture after 1 week of incubation at 60 °C confirmed this composition (see Table 1).

Methionine-Guided Sequence-Selective Polypeptide Cleavage by *cis*-[Pt($CH_3SCH_2CH_2CH_2SCH_3$)(H_2O)$_2$]$^{2+}$ in Acidic Solution. The decapeptide Met-Pro contains a tertiary amide group in the salient part of its sequence. When a mixture of Met-Pro peptide and a 10-fold excess of *cis*-[Pt($CH_3SCH_2CH_2CH_2SCH_3$)(H_2O)$_2$]$^{2+}$ was kept at pH 2.0 and 60 °C for 7 days, HPLC and MALDI analyses showed an incomplete cleavage of the Met−Pro bond (see Table 1). The Pt(II) reagent formed mostly the unreactive complex **2**

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Scheme 1

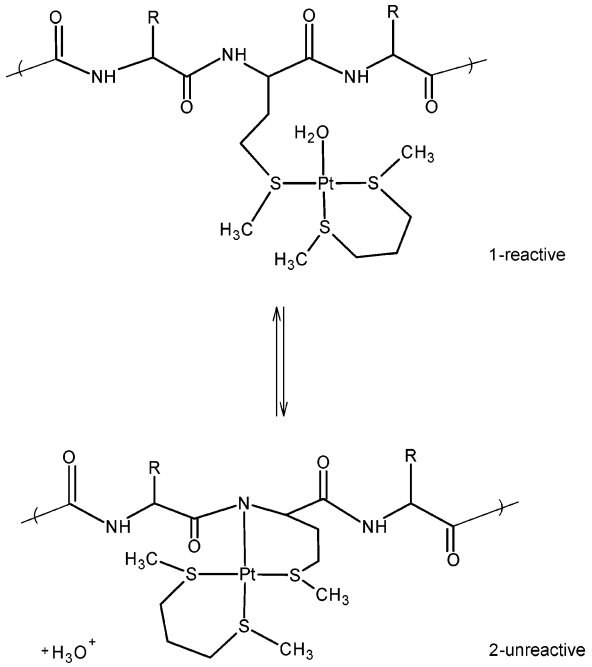


Table 1. MALDI Mass Spectrometric Identification of Fragments of the Polypeptide Designated as Met-Ala and the Polypeptide Designated as Met-Pro upon Hydrolytic Cleavage by *cis*-$[Pt(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ at pH 2.0

peptide substrate	polypeptide fragment	molecular mass (Da) obsd	calcd
Met-Ala	AKFGMAYRG	1042.84	1043.23
	AKFGMAYRG + Pt(II) reagent	1370.09	1371.48
Met-Pro	AKAGM	519.61	519.64
	PAYRA	578.39	577.66

at pH 2.0, but a small amount of the hydrolytically reactive complex **1** was responsible for the cleavage (see Scheme 1). At pH values higher than 2.0, *cis*-$[Pt(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ formed only unreactive complexes with either Met-Ala peptide or Met-Pro peptide, and neither polypeptide was detectably cleaved.

The difference in reactivity between the two peptides at pH 2.0 can be attributed to the greater reactivity of the tertiary amide group (in Met-Pro) than the secondary amide group (in Met-Ala). Because proline residues have the most basic carbonyl oxygen atom in proteins, this atom preferentially binds to the Pt(II) atom. Thus activated by the Lewis acid, the X−Pro group is especially susceptible to nucleophilic attack by a solvent water molecule, resulting in hydrolysis.[33]

This study with the Pt(II) reagent at pH 2.0 shows an increased hydrolytic reactivity of X−Pro groups. Although the lower reactivity of the Pt(II) complexes in comparison with Pd(II) complexes makes the rate and yield of cleavage relatively low, the reaction is sequence-selective in that the cleavage site depends not only on the location of the anchoring residue (methionine) but also on the residue following it (proline).

Initial Binding of *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ to a Methionine-Containing Polypeptide in Acidic Solution. A mixture of the polypeptide Met-Ala and a 10-fold excess of *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ was prepared at pH 2.0. The modes of binding were studied by ^{1}H NMR spectroscopy at room temperature immediately after mixing and after 24 h of incubation at 60 °C. The aliphatic region in the one-dimensional spectrum and a part of the amide-to-aliphatic region in the TOCSY spectrum are shown in Figure 2. Upon addition of *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ to the Met-Ala peptide, the methionine amide NH signal at 8.14 ppm shifted to 8.32 ppm, evidence for binding of the Pd(II) atom to the methionine thioether side chain (see Figure 2a,b). Furthermore, the amide NH signals of the two glycine residues at 8.30 ppm and the two alanine residues at 8.30 ppm, which overlap in the TOCSY spectrum of the free peptide, each split into multiple signals upon binding of the Pd(II) atom to the methionine side chain. TOCSY evidence shows this binding to occur immediately upon mixing of *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ and the substrate, before heating. Because the subsequent cleavage reaction occurs in hours, the initial binding can

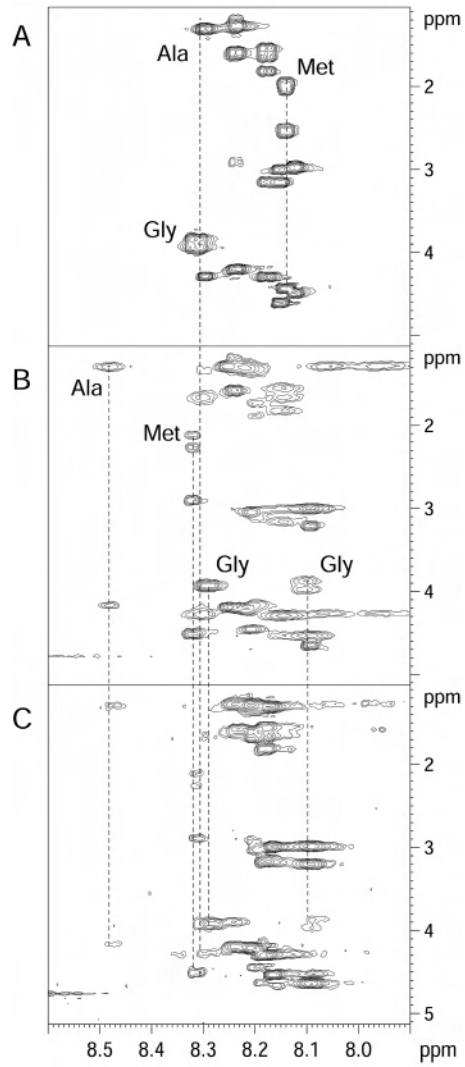
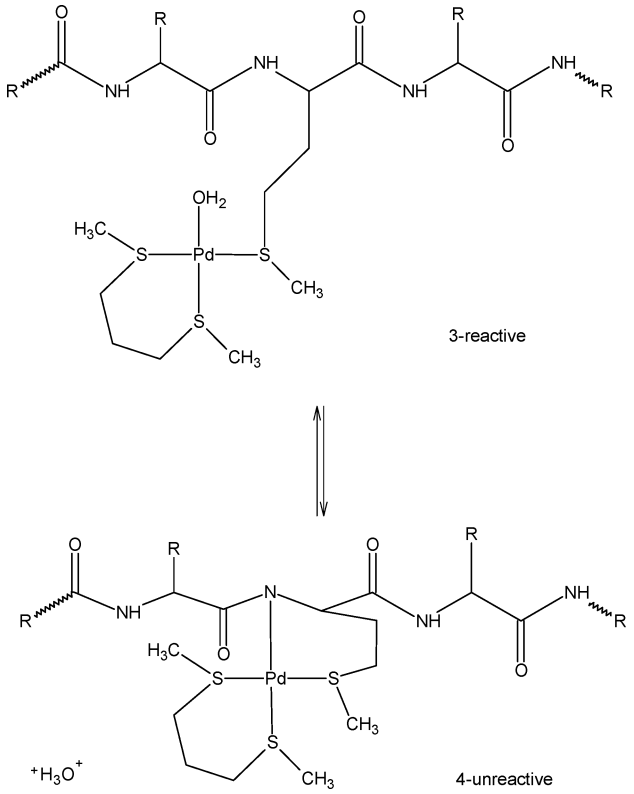


Figure 2. TOCSY ^{1}H NMR spectra of the NH-to-aliphatic region of polypeptide designated as Met-Ala at pH 2.0 and room temperature (a) free, (b) immediately after the addition of a 10-fold excess of *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$, and (c) after 24 h of incubation at 60 °C with a 10-fold excess of *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$. Vertical dashed lines connect amide-to-side chain cross-peaks of residues within each panel and clearly distinguish the shifts of signals.

Scheme 2



justifiably be neglected in fitting the cleavage kinetics to a first-order rate law.

After incubation of the reaction mixture for 24 h at 60 °C, the TOCSY spectrum showed a minor decrease in the intensity of the cross-peaks between the amide NH and the α-CH, β-CH, and γ-CH side-chain resonances in methionine (see Figure 2c). Evidently, there are multiple binding modes during the reaction at pH 2.0. The decrease in intensity of the cross-peaks corresponds to an increase in the ratio of complex **4** to complex **3** (see Scheme 2). Our previously reported reagents *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and $[Pd(H_2O)_4]^{2+}$ at pH 2.0 predominantly bind to the thioether group and the deprotonated amide nitrogen of methionine residues, with the loss of both aqua ligands. The new reagent *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$, however, binds only to the thioether group, with the loss of one aqua ligand, to form complex **3**. This difference in the initial anchoring can be attributed to the steric bulk of the bidentate thioether ligand, which disfavors bidentate coordination of the methionine residue. Evidently, the anchoring mode of Pd(II) reagents can be controlled by the choice of ancillary ligands.

To determine whether the bidentate thioether ligand remains coordinated to the Pd(II) ion during the cleavage reaction, we compared the ^{1}H NMR spectra of free $CH_3SCH_2CH_2CH_2SCH_3$, *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$, and the reaction mixture with the Met-Ala polypeptide after 24 h at 60 °C (see the Supporting Information, Figure S6). The methyl singlet occurred at 2.115 ppm in the free thioether ligand and at 2.360 ppm in the Pd(II)-bound ligand. The spectrum of the reaction mixture showed only the singlet at 2.360 ppm, unaffected by binding of the Pd(II) atom to the peptide. The absence of the 2.115 ppm signal over time showed that the bidentate thioether ligand remained bound to the Pd(II) ion throughout the cleavage reaction. Furthermore, MALDI spectra at the end of the cleavage reaction showed only the mass of the $[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ species. The free thioether was not observed in the MALDI mass spectra, although its detection was possible.

Table 2. HPLC Separation and MALDI Mass Spectrometric Identification of the Fragments of the Polypeptide Designated as Met-Ala and the Polypeptide Designated as Pro-Met upon Hydrolytic Cleavage by *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ at pH 2.0

peptide substrate	elution time (min)	polypeptide fragment	molecular mass (Da)	
			obsd	calcd
Met-Ala	21.3	AKF	407.49	407.42
	18.3	GMAYRG	654.76	655.16
Pro-Met	16.2	KAYD	538.58	539.07
	18.4	PMAARA	616.60	616.76

Table 3. HPLC Separation and MALDI Mass Spectrometric Identification of the Fragments of the Polypeptide Designated as Pro-His and Angiotensin II (the Polypeptide Designated as His-Pro) upon Hydrolytic Cleavage by *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ at pH 2.0

peptide substrate	elution time (min)	peptide fragment	molecular mass (Da)	
			obsd	calcd
Pro-His	15.2	KAYD	538.58	539.07
	13.7	PHAARA	623.71	622.70
His-Pro	*a*	VY + Pd(II) reagent	387.11	387.75
	18.4	IHPF + Pd(II) reagent	620.01	620.04

[a] Fragment VY is insoluble in water and acetonitrile and could not be separated from the reaction mixture and was therefore analyzed without separation.

In cleavage reactions with the *cis*-$[Pd(en)(H_2O)_2]^{2+}$ reagent, the ethylenediamine ligand detaches from the Pd(II) ion in the formation of a hydrolytically reactive complex because the hard and relatively strong Brønsted base amine has only a moderate affinity for the relatively soft Pd(II) acid and because protonation by H^+ ions in solution assists detachment of the amine.[32] In contrast, the soft and very weak Brønsted base thioether has an affinity for the Pd(II) ion and cannot be protonated under the reaction conditions, and it therefore remains bound. This chelating ligand in *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ ensures the predominance of the hydrolytically reactive complex **3** in the reaction conditions at pH 2.0. The ancillary ligands thus control the cleavage reactivity of the Pd(II) complex.

Methionine-Guided and Residue-Selective Polypeptide Cleavage by *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ in Acidic Solution. The results in Tables 2 and 3 show that the new reagent selectively cleaved the Phe−Gly bond in the polypeptide designated as Met-Ala, the Tyr−Ile bond in angiotensin II (designated as His-Pro), the Asp−Pro bond in the polypeptide Pro-His, and the Asp−Pro bond in the polypeptide Pro-Met, while the polypeptide designated as Pro-Phe remained intact because it lacks anchoring methionine or histidine residues. Evidently, the selectivity of Pd(II) reagents for the X−Y bond in X-Y-Met and X-Y-His sequences is unaffected by the ancillary ligands. Because binding of the Pd(II) atom to the methionine side chain is very fast, a first-order fitting in Figure 3 is justified. The decrease of the rate constant with increasing pH, seen in

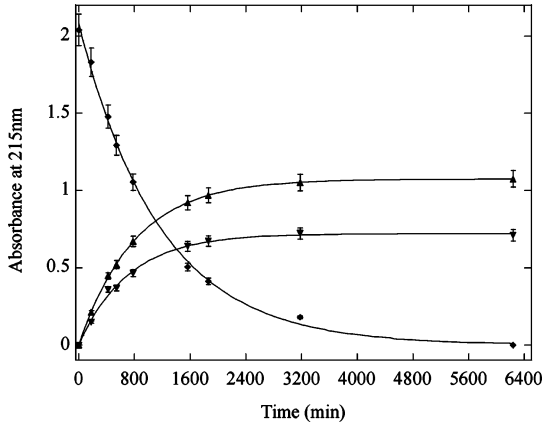


Figure 3. Kinetics of the cleavage of the polypeptide designated as Met-Ala by *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$, where ◆ indicate the disappearance of the substrate, ▲ indicate the appearance of the fragment GMAYRG, and ▼ indicate the appearance of the fragment AKF. The error bars indicate a confidence limit greater than 95%.

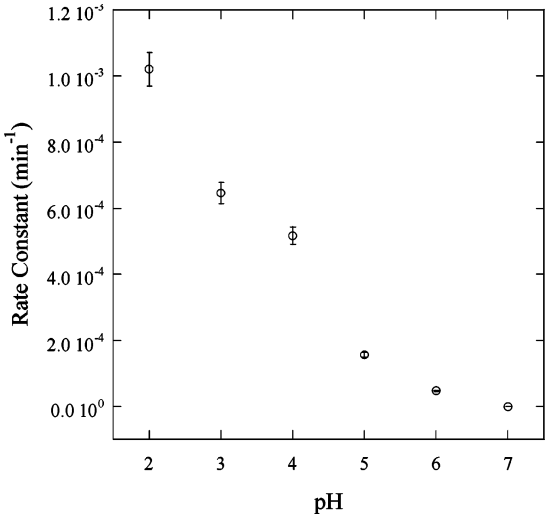


Figure 4. Dependence on the pH of the rate constant for the cleavage of the polypeptide designated as Met-Ala by the complex *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$. The error bars indicate a confidence limit greater than 95%.

Figure 4, is caused by the progressive conversion of the reactive complex **3** to the unreactive complex **4**, in which the deprotonated amide nitrogen is coordinated to the Pd(II) ion. The published pK_a values of the related compounds *cis*-$[Pt(CH_3CH_2SCH_2CH_2CH_2SCH_2CH_3)(H_2O)_2]^{2+}$, *cis*-$[Pd(en)(H_2O)_2]^{2+}$, and *cis*-$[Pt(en)(H_2O)_2]^{2+}$ are >3.0, 5.6, and 5.8, respectively.[48−50] The values of 5.6 and 5.8 suggest that Pd(II) and Pt(II) complexes of the same composition will have similar pK_a values. Therefore, the pK_a of *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ can be estimated at 3.0, indicating that within the pH range in the hydrolysis experiments both *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ and *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)(OH)]^{+}$ are present. As the pH is raised, the more labile aqua complex is converted into the less labile hydoxo complex, and the rate constant for hydrolysis decreases. Because the pK_a of the first NH group "upstream" of a histidine-anchored Pd(II) atom has been estimated at ca. 2.0, an increase in the pH is expected to favor the unreactive complex **4**.[51−57] As Table 4 shows, the cleavage by *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ at pH 2.0 is significantly accelerated when the anchoring residue is preceded by a proline residue. In that case, hydrolysis is completed in less than 1 day.

Table 4. HPLC Separation and MALDI Mass Spectrometric Identification of the Fragments of the Polypeptide Designated as Pro-Met and the Polypeptide Designated as Pro-His upon Hydrolytic Cleavage by *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ at pH 7.0 and the Rate Constants for the Disappearance of the Polypeptide at pH 2.0

peptide substrate	elution time (min)	peptide fragment	molecular mass (Da) obsd	molecular mass (Da) calcd	rate constant (min^{-1})
Pro-Met	15.6	KAYD	539.07	539.07	4.37×10^{-3}
	21.3	PMAARA	618.15	616.76	
Pro-His	15.4	KAYD	538.99	539.07	2.21×10^{-3}
	13.9	PHAARA	623.20	622.70	

Methionine-Guided and Histidine-Guided Sequence-Selective Polypeptide Cleavage by *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ in Neutral Solution. Remarkably, the selectivity of *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ depends on the pH of the reaction mixture. At pH 2.0, the cleavage is residue-selective as explained in the preceding subsection. At pH 7.0, however, the cleavage becomes sequence-selective in that it occurs only at the X−Pro bonds in X-Pro-Met and X-Pro-His sequences. The substrates lacking a Pro-Met or Pro-His, namely, angiotensin II and the polypeptides designated as Pro-Phe and Met-Ala, remained intact in the presence of *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ at pH 7.0. For residue-selective cleavage in weakly acidic solutions, it is enough that the substrate contains methionine or histidine as an anchor. For sequence-selective cleavage in neutral solutions, it is necessary that the anchoring residue be preceded by proline.

This useful change in selectivity can be attributed to the effect of pH on the predominant species in solution. At pH 2.0, the predominant species is the hydrolytically reactive complex **3**. At pH 7.0, the predominant species is the hydrolytically unreactive complex **4,** and complex **3** is only a minor species. Its low concentration is sufficient for cleavage only at the most reactive peptide bonds, namely, X−Pro bonds in the X-Pro-His and X-Pro-Met sequences.

Conclusions

Complexes *cis*-$[Pt(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ and *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ are new reagents for the selective hydrolysis of peptides. These complexes

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are prepared in situ by two straightforward ligand substitution reactions. The simplicity of this synthetic method lends to the practicality of using *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ and *cis*-$[Pt(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ as reagents for the cleavage of peptides. Although these complexes are structurally similar to the established reagents *cis*-$[Pt(en)(H_2O)_2]^{2+}$ and *cis*-$[Pd(en)(H_2O)_2]^{2+}$, the thioether-containing reagents differ in reactivity from the ethylenediamine-containing reagents. The new reagent *cis*-$[Pt(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ cleaves with the same selectivity as *cis*-$[Pt(en)(H_2O)_2]^{2+}$ in a weakly acidic solution but more slowly because of the steric bulk of the thioether ligand. The new reagent *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ cleaves with the same selectivity as *cis*-$[Pd(en)(H_2O)_2]^{2+}$ in a weakly acidic solution but more slowly, again for steric reasons. Unlike *cis*-$[Pd(en)(H_2O)_2]^{2+}$, however, the new reagent also cleaves in neutral solution. The difference in hydrolytic activity at pH 7 results from a difference in the mode of Pd(II)−substrate binding. While *cis*-$[Pd(en)(H_2O)_2]^{2+}$ loses its ethylenediamine ligand and forms unreactive multidentate complexes with the substrate, *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ retains its thioether ligand at pH 7.0 and forms a reactive as well as an unreactive complex with the substrate. The substrate in the reactive complex then becomes selectively cleaved. Both *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ and our previously reported Pd(II)−cyclodextrin conjugate[35] sequence-selectively cleave peptides at pH 7.0, and both require the presence of a proline residue near the site of cleavage. The two reagents, however, differ in sequence-selectivity; the Pd(II)−cyclodextrin conjugate cleaves the X−Pro bond of X-Pro-Y sequences, where Y is phenylalanine, while *cis*-$[Pd(CH_3SCH_2CH_2CH_2SCH_3)(H_2O)_2]^{2+}$ cleaves the X−Pro bond of X-Pro-Met and X-Pro-His sequences. Although their selectivity is different, the two reagents show similar rates of hydrolysis at pH 7.0, with cleavage being nearly complete after 48 h.

Acknowledgment. This work was funded by the National Science Foundation through Grant CHE316868. We thank Dr. D. Bruce Fulton for help in recording and interpreting NMR spectra, Dr. Robert S. Houk and Cory Gross for help with ICP-MS analysis of Pt, and Dr. Nebojša Milović for helpful discussions and advice.

Supporting Information Available: Six figures showing the HPLC chromatograms of a hydrolytic reaction mixture, the synthetic reaction scheme for the Pd(II) and Pt(II) mononuclear complexes, the ^{1}H NMR spectra of the mononuclear Pd(II) and Pt(II) complexes at different temperatures, a ROESY NMR spectrum of a peptide, and the ^{1}H NMR spectra of the thioether ligand and its complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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