New Selectivity in Peptide Hydrolysis by Metal Complexes. Platinum(II) Complexes Promote Cleavage of Peptides Next to the Tryptophan Residue

Natalia V. Kaminskaia and Nenad M. Kostić*

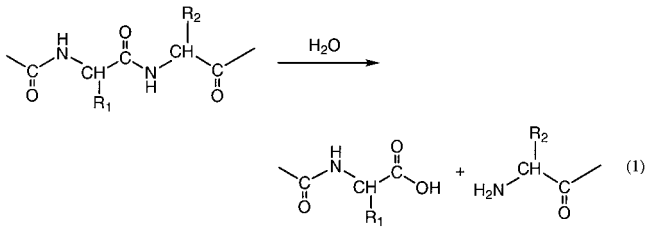
Department of Chemistry, Iowa State University, Ames, Iowa 50011-3111

Received December 12, 2000

Tryptophan-containing *N*-acetylated peptides AcTrp-Gly, AcTrp-Ala, AcTrp-Val, and AcTrp-ValOMe bind to platinum(II) and undergo selective hydrolytic cleavage of the *C*-terminal amide bond; the *N*-terminal amide bond remains intact. In acetone solution, bidentate coordination of the tryptophanyl residue via the C(3) atom of indole and the amide oxygen atom produces complexes of spiro stereochemistry, which are characterized by ^{1}H, ^{13}C, and ^{195}Pt NMR spectroscopy, and also by UV−vis, IR, and mass spectroscopy. Upon addition of 1 molar equiv of water, these complexes undergo hydrolytic cleavage. This reaction is as much as 10^4-10^5 times faster in the presence of platinum(II) complexes than in their absence. The hydrolysis is conveniently monitored by ^{1}H NMR spectroscopy. We report the kinetics and mechanism for this reaction between *cis*-$[Pt(en)(sol)_2]^{2+}$, in which the solvent ligand is water or acetone, and AcTrp-Ala. The platinum(II) ion as a Lewis acid activates the oxygen-bound amide group toward nucleophilic attack of solvent water. The reaction is unimolecular with respect to the metal−peptide complex. Because the tryptophanyl fragment AcTrp remains coordinated to platinum(II) after cleavage of the amide bond, the cleavage is not catalytic. Added ligand, such as DMSO and pyridine, displaces AcTrp from the platinum(II) complex and regenerates the promoter. This is the first report of cleavage of peptide bonds next to tryptophanyl residues by metal complexes and one of the very few reports of organometallic complexes involving metal ions and peptide ligands. Because these complexes form in nonaqueous solvents, a prospect for cleavage of membrane-bound and other hydrophobic proteins with new regioselectivity has emerged.

Introduction

Selective cleavage of peptides and proteins (eq 1) is an important procedure in biochemistry and molecular biology. The half-life for the hydrolysis of amide bonds is 350−500 years at room temperature and pH 4−8.[1] Because uncatalyzed hydrolysis of peptides is extremely slow, relatively fast methods of artificial cleavage are needed. Traditionally, sequence analysis has been done with a small number of proteolytic enzymes, but among them only trypsin is highly regioselective.[2] Requirements for the traditional applications are changing, and new challenges



(1)

are emerging. New cleaving agents are needed for the following tasks: sequencing of large or blocked proteins; preparation of semisynthetic proteins; structural mapping and functional analysis of protein domains; analysis of protein interactions with other proteins and with nucleic acids; elucidation of the protein folding process; analysis of nonnative and partially folded protein states; development of new therapeutic agents; and others. The particular conditions (pH, temperature, and solvent) under which proteolytic enzymes are active may be incompatible with the proteins or processes under investigation.

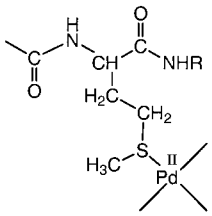
For all the aforementioned reasons, chemical (nonenzymatic) reagents are needed in biochemical practice. Only cyanogen bromide, however, is used routinely.[1] It cleaves proteins on the carboxylic side of methionine residues and, because these residues are relatively rare, usually produces long fragments. Although cyanogen bromide is very useful, it has several shortcomings. It is volatile and toxic, requires harsh conditions, and often produces incomplete cleavage. There are also reagents, such as *N*-bromosuccinimide and BNPS skatole, that are used for tryptophan-selective cleavage.[2,3] Cleavage by the *N*-bromosuccinimide requires harsh conditions that might result in substrate degradation.[2] Reactions with BNPS skatole are only effective if high excess of the cleaver is present.[3]

Transition-metal complexes are beginning to be applied to hydrolytic cleavage of regular amide bonds that are not activated specifically for fast reaction.[5−11] Several cobalt(III) complexes bind to peptides and facilitate their hydrolysis, but they cleave only the *N*-terminal amino acid.[4] Clever kinetic studies with these complexes revealed some mechanistic features of hydrolysis, but they did not result in new methods for analytical

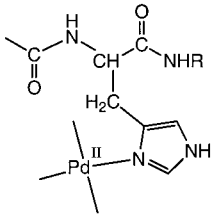
(1) Radzicka, A.; Wolfenden, R. *J. Am. Chem. Soc.* **1996**, *118*, 6105.
(2) Ramachandran, K. L.; Witkop, B. *Methods Enzymol.* **1976**, *11*, 283.
(3) Fontana, A. *Methods Enzymol.* **1972**, *25*, 419.
(4) Croft, L. R. *Handbook of Protein Sequence Analysis*, 2nd ed.; Wiley: Chichester, 1980.
(5) Sutton, P. A.; Buckingham, D. A. *Acc. Chem. Res.* **1987**, *20*, 357 and references threin.
(6) Chin J. *Acc. Chem. Res.* **1991**, *24*, 145 and references therein.
(7) Chin, J.; Banaszczyk, B.; Jubian, V.; Kim, J. H.; Mrejem, K In *Bioorganic Chemistry Frontiers*; Dugas, H., Ed.; Springer-Verlag: Berlin, 1991; Vol. 2 and references therein.
(8) Dixon, N. E.; Sargeson, A. M. In *Zinc Enzymes*; Spiro, T. G., Ed.; Wiley: New York, 1983; Chapter 7.
(9) Suh, J. *Acc. Chem. Res.* **1992**, *25*, 273 and references therein.
(10) Fife, T. H. *Acc. Chem. Res.* **1993**, *26*, 325.
(11) Fife, T. H. *Perspect. Bioinorg. Chem.* **1991**, *l*, 43.

10.1021/ic0014033 CCC: $20.00
Published on Web 04/12/2001

biochemistry because practical applications usually require cleavage of internal amide bonds. Research in this laboratory showed that amide bonds (both internal and terminal) near histidine and methionine residues in peptides and proteins can be cleaved with several palladium(II) aqua complexes, which bind to the side chains of methionine (**1**) and histidine (**2**).[12−19] After this initial binding, the reaction proceeds by two activation



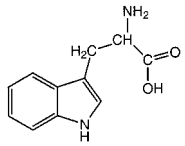
1



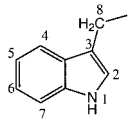
2

pathways. In the external-attack mechanism, a free water molecule attacks the amide bond that is activated by the coordination of the oxygen atom to the metal ion. In the internal-attack mechanism, the aqua or hydroxo ligand is "delivered" to the amide bond held near the metal ion but not bonded to that ion. Molecular-dynamics calculations showed that different complexes of palladium(II) prefer one or the other mechanism.[19b] Therefore, regioselectivity of cleavage depends on the mode of attachment of substrate to the metal complex and on the proximity of the attachment site to the scissile amide bond. The multistep chemical synthesis required for the covalent attachment of an iron(III) complex that is otherwise a fast cleaver of proximate amide bonds in proteins[20−22] is an intricate procedure, whereas binding of palladium(II) aqua complexes to peptides and proteins is spontaneous. Whereas cleavage by iron complexes requires addition of hydrogen peroxide and ascorbic acid, cleavage by palladium(II) reagents requires only water, present in the solution.

While methionine and histidine side chains are well-known ligands for transition metals, there are no precedents for tryptophan coordination to metals in biological systems. Tryptophan (**3**) is a widely distributed amino acid.[23−25] Indole, a part of its side chain (**4**), is an electron-rich aromatic group with characteristic chemical reactivity. Tryptophan is the most hydrophobic of amino acid side chains.[26] It plays important roles



3



4

in the stabilization of various proteins by stacking with other aromatic groups, such as tyrosine and phenylalanine, and in the formation of the electron-transfer pathways in certain enzymes.[27−30]

In contrast to alkylamines and nitrogen-containing heterocycles such as pyridine, the lone pair of electrons on the indole nitrogen atom is a part of the π-electron system and is not readily available for donation to metal ions.[31] The N(1)H group in indole nucleus has pK_a of 16.82 and can be deprotonated only by very strong bases.[32] Therefore, complexes of indole with alkali metals and Grignard reagents contain ionic metal−nitrogen bonds.[33,34] Tryptophan binds to methylmercury as a bidentate ligand, via the deprotonated indole nitrogen and also the amino or the carboxylate groups.[35] Despite the high pK_a values, substitution of the N(1) proton in indole takes place under mild conditions and at low CH_3Hg^+ concentrations in water-containing ethanol as a solvent. The kinetics and mechanism of this reaction, and such reactions with other metals, have not been studied.

Tryptophan, like other α-amino acids, readily forms familiar chelates via its amino and carboxylate groups,[36,37] but we are interested in unusual coordination involving the indole group. Bidentate coordination to palladium(II) through the N(1) and C(2) atoms occurs in binuclear complexes with N,C-bridging.[38] The tautomer **5**, in which a hydrogen atom has moved from N(1) to C(3), is named 3H-indolenine or simply indolenine. When the C(3) atom in **5** bears a substituent R, two enantiomeric forms (R and S) can coexist. Palladium(II) complexes of

5

3-alkylindolenine coordinated through the nitrogen atom have recently been isolated and characterized by X-ray crystallography and spectroscopic methods.[39] Binuclear complexes of palladium(II) and indole-3-acetic acid, a compound similar to tryptophan, have been reported.[40] Cyclopalladation in these mixed-ligand complexes yields an interesting spiro structure with the C(3) atom at the junction.

We have recently discovered that palladium(II) aqua complexes hydrolytically cleave tryptophan-containing peptides.[41]

(12) Korneeva, E. N.; Ovchinnikov, M. V.; Kostić, N. M. *Inorg. Chim. Acta* **1996**, *243*, 9.
(13) Parac, T. N.; Kostić, N. M. *J. Am. Chem. Soc.* **1996**, *118*, 51.
(14) Parac, T. N.; Kostić, N. M. *J. Am. Chem. Soc.* **1996**, *118*, 5946.
(15) Zhu, L.; Qin, L.; Parac, T. N.; Kostić, N. M. *J. Am. Chem. Soc*. **1994**, *116*, 5218.
(16) Zhu, L.; Kostić, N. M. *Inorg. Chem.* **1992**, *31*, 3994.
(17) Zhu, L.; Kostić, N. M. *J. Am. Chem. Soc.* **1993**, *115*, 4566.
(18) Zhu, L.; Kostić, N. M. *Inorg. Chim. Acta* **1994**, *217*, 21.
(19) (a) Burgeson, I. E.; Kostić, N. M. *Inorg. Chem.* **1991**, *30*, 4299. (b) Parac, T. N.; Ullmann, G. M.; Kostić, N. M. *J. Am. Chem. Soc.* **1999**, *121*, 3127.
(20) Rana, T. M.; Meares, C. F. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10578.
(21) Rana, T. M.; Meares, C. F. *J. Am. Chem. Soc*. **1991**, *113*, 1859.
(22) Rana, T. M.; Meares, C. F. *J. Am. Chem. Soc*. **1990**, *112*, 2457.
(23) Fontana, A.; Toniolo, C. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Ed.; New York, 1976; pp 309−449.
(24) Hayaishi, O. In *Biochemical and Medical Aspects of Tryptophan Metabolism*; Hayaishi, O., Ed.; New York, 1980; pp 15−29.
(25) Wurtman, R. J. In *Biochemical and Medical Aspects of Tryptophan Metabolism*; Hayaishi, O., Ed.; New York, 1980; pp 31−46.
(26) Nozaki, Y.; Tanford, C. *J. Biol. Chem*. **1971**, *246*, 2211.
(27) Burley, S. K.; Petsko, G. A. *Science* **1985**, *229*, 23.
(28) Nordlund, P.; Eklund, H. *J. Mol. Biol*. **1993**, *232*, 123.
(29) Pelletier, H.; Kraut, J. *Science* **1992**, *258*, 1748.
(30) Mauro, J. M.; Fishel, L. A.; Hazzard, J. T.; Meyer, T. E.; Tollin, G.; Cusanovich, M. A.; Kraut, J. *Biochemistry* **1988**, *27*, 6243.
(31) Remers, W. A. In *Heterocyclic Compounds*; Houlihan, W. J., Ed.; New York, 1972; pp 1−226.
(32) Yagil, G. *J. Phys. Chem*. **1967**, *71*, 1034.
(33) Reinecke, M. G.; Sebastian, J. F.; Johnson, H. W.; Pyun, C. *J. Org. Chem*. **1971**, *36*, 3091.
(34) Reinecke, M. G.; Sebastian, J. F.; Johnson, H. W.; Pyun, C. *J. Org. Chem*. **1972**, *37*, 3066.
(35) Corbeil, M. C.; Beauchamp, A. L. *Can. J. Chem.* **1988**, *66*, 2458.
(36) Aoki, K.; Yamazaki, H. *J. Chem. Soc., Dalton Trans*. **1987**, 2017.
(37) Masuda, H., Sugimori, T.; Odani, A.; Yamauchi, O. *Inorg. Chim. Acta* **1991**, *180*, 73.
(38) Robson, R. *Inorg. Chim. Acta* **1982**, *57*, 71.
(39) Yamauchi, O.; Takani, M.; Toyoda, K.; Masuda, H. *Inorg. Chem.* **1990**, *29*, 1856.
(40) Takani, M.; Masuda, H.; Yamauchi, O. *Inorg. Chim. Acta* **1995**, *235*, 367.
(41) Kaminskaia, N. V.; Johnson, T. W.; Kostić, N. M. *J. Am. Chem. Soc.* **1999**, *121*, 8663.

To understand this unprecedented reaction better, we studied the formation and structure of palladium(II) and platinum(II) complexes with indole-containing ligands that resemble tryptophan and dipeptides.[42] Now we turn to complexes of platinum(II) with tryptophan-containing peptides. Unexpectedly, platinum(II) complexes turned out to be much more effective than the analogous palladium(II) complexes in promoting hydrolytic cleavage of peptide bonds adjacent to the tryptophanyl residue. We present here the first examples of the coordination of platinum(II) to the indole side chain of tryptophan. This coordination is followed by the tryptophan-selective cleavage of peptides. We report the kinetics and mechanism of these useful, new reactions.

Experimental Procedures

Chemicals. The deuterium-containing compounds D_2O and $DClO_4$ and the salts $K_2[PdCl_4]$, $PdCl_2$, and $AgClO_4 \cdot H_2O$ were obtained from Sigma Chemical Co. and Aldrich Chemical Co. Acetone-d_6, methanol-d_4, and dimethylformamide-d_7 were obtained from Cambridge Isotope Laboratories. Indole-3-acetamide, indole-3-acetic acid, indole-3-acetic acid ethyl ester, *N*-acetyl-5-hydroxy-tryptamine, *N*-(3-indolylacetyl)-L-alanine, *N*-(3-indolylacetyl)-L-valine, *N*-acetyl-L-tryptophanamide, *N*-acetyl-L-tryptophan, *N*-acetyl-L-tryptophan ethyl ester, L-tryptophan methyl ester, and also the dipeptides Trp-Ala, Trp-Gly, Trp-Val, and Trp-ValOMe were obtained from Sigma Chemical Co. and Aldrich Chemical Co. Dipeptide Ala-Trp was obtained from Bachem Chemical Co. Platinum(II) complexes *cis*-$[Pt(cod)Cl_2]$, *cis*-$[Pt(cod)Cl_2]$ (in which cod is 1,5-cyclooctadiene), and *cis*-$[Pt(en)Cl_2]$ were obtained from Aldrich Chemical Co. Anhydrous $AgBF_4$, $Ag(CF_3SO_3)$, and $AgClO_4$ (caution, strong oxidant!) were obtained from Aldrich Chemical Co. The ligands ethane-1,2-diamine (en) and 1,5-dithiacyclooctane (dtco) were obtained from Aldrich Chemical Co. All the chemicals were of reagent grade.

Palladium(II) and Platinum(II) Complexes. The palladium(II) complexes *cis*-$[Pd(en)Cl_2]$ and *cis*-$[Pd(dtco)Cl_2]$ were prepared by the published procedures.[43−45] The chloro ligands were displaced by the solvent ligands (sol) by stirring solutions of these complexes and 2 equiv of $AgBF_4$, $Ag(CF_3SO_3)$, $AgClO_4$ (caution, strong oxidant!), or $AgClO_4 \cdot H_2O$ in acetone-d_6 at 25 °C in the dark for 1 and 8 h in the case of palladium(II) and platinum(II) complexes, respectively. The solid AgCl and AgI were filtered off in the dark, and a fresh solution of the complex containing the solvent ligands was used in further experiments. The coordinated solvent (sol) is acetone-d_6 or H_2O/D_2O. The salts *cis*-$[Pd(en)(sol)_2](ClO_4)_2$ and *cis*-$[Pt(en)(sol)_2](ClO_4)_2$ had absorption maxima at 360 and 330 nm, respectively, as reported before.[43] The complex *trans*-$[Pd(PhCN)_2Cl_2]$ was prepared by the published procedure.[46]

Substrates for Hydrolysis. The substrates for hydrolysis were the *N*-acetylated dipeptides AcTrp-Ala, AcTrp-Gly, AcTrp-Val, and AcTrp-ValOMe. They were prepared by acetylation of the respective dipeptides by a standard procedure, with acetic anhydride in glacial acetic acid as the solvent.[12−19]

One-Dimensional Proton, Carbon-13, and Nitrogen-15 NMR Spectra. These spectra were recorded with Varian VXR-300, Bruker DRX-400, Bruker DRX-500, and AC-200 spectrometers. The chemical shifts (δ) in the ^1H and ^{13}C spectra are given in ppm downfield from the methyl resonance of the solvent, which was acetone-d_6. The chemical shifts in ^{15}N spectra are given in ppm downfield and upfield from the resonance (at 0.00 ppm) of external standard, which was the saturated solution of $^{15}NH_4NO_3$ in D_2O. The internal reference in kinetic experiments monitored by ^1H NMR spectra was tetramethylsilane. The quality of the ^{13}C and the ^{15}N spectra was improved by their acquisition in narrow windows; usually 3000−20000 scans were taken. These spectra were recorded with and without proton decoupling. In quantitative experiments, in which accurate relative intensities were needed, the coupling was preserved. The ^1H and ^{13}C resonances were integrated with estimated errors of ± 5 and 10%, respectively. Concentrations of the compounds were determined on the basis of these integrals and the known initial concentrations of reagents. The rates and rate constants were calculated from the known concentrations of the reactants and products, with an estimated error of 10−20%.

Platinum-195 NMR spectra. These spectra were recorded with an AC-200 spectrometer at 43 MHz using 5-mm tubes. The chemical shifts are given in ppm upfield from the resonance (at −1628 ppm) of external standard, which was the saturated solution of $K_2[PtCl_4]$ in 1.0 M NaCl in D_2O at 296 K. The following parameters were chosen: 90° pulse P1 = 8.7 μs and delay D1 = 1.0 s.

Two-dimensional NMR Experiments. Two-dimensional heteronuclear shift correlation spectroscopy (HETCOR) was done with the standard Bruker pulse programs. The ^1H-^{13}C HETCOR spectra were obtained with the pulse sequence inv4gs and the following parameters: 90° pulses P1 = 12.2 μs and P3 = 10.0 μs and delays D1 = 1.80 s and D2 = 3.57 s. The ^1H-^{15}N HETCOR spectra were obtained with the same sequences, using the following parameters: 90° pulses P1 = 9 μs and P3 = 38 μs; and delays D1 = 1.75 s, and D2 = 3.45 ms. The ^1H-^{13}C HETCOR experiments optimized on long-range couplings were done with the pulse program inv4gslplrnd. The coupling was preserved during the acquisition. There were 128 free-induction decays (FID) of 1024 data points, with four scans for each point. The NOESY spectra were obtained using the standard pulse sequence noesyst. There were 512 FIDs of 2048 data points, with eight scans for each point. The repetition time was 8.3 s, and spectral width was 4500 Hz in both dimensions. The ROESY spectra were obtained using the standard pulse sequence roesyst. There were 256 FIDs of 2048 data points, with 16 scans for each point. The repetition time was 8.3 s, and spectral width was 4500 Hz in both dimensions.

Other Spectra. Ultraviolet−visible spectra were recorded with a Perkin-Elmer Lambda 18 spectrophotometer at 296 K. Infrared spectra of solutions were recorded with a Nicolet Magna-IR 560 spectrometer using sodium chloride cells with 0.1-mm spacers. Electrospray ionization (ESI) experiments were done with a Finnigan TSQ700 triple quadrupole mass spectrometer fitted with a Finnigan ESI interface. Samples were introduced directly into the electrospray interface through an unheated fused-silica capillary with a 50 μm v.d. and 190 μm o.d.

Kinetics of Reactions. The solvent in all the reactions was acetone-d_6. The temperature was 313 ± 0.5 K, unless stated otherwise. The initial concentrations were 0.094 M for the palladium(II) or platinum(II) complex and 0.0094 M for the derivative of indole, unless stated otherwise. The binding of the indole derivatives to various metal complexes was followed by ^1H NMR spectroscopy. In a typical experiment, to a solution of freshly prepared complex was added the optional chemical, such as D_2O or trifluoroacetic acid, and finally the solution of the *N*-acetylated peptide to start the reaction. The ^1H NMR acquisition began in less than one minute.

The observed rate constants for the relatively fast reactions were determined over at least seven half-lives. The rate constants for the relatively slow reactions were determined from the initial rates, when these reactions were only 3−5% complete.

Composition of the Reaction Mixtures. The reactants were indole-3-acetamide, indole-3-acetic acid, indole-3-acetic acid ethyl ester, *N*-acetyl-5-hydroxy-tryptamine, *N*-(3-indolylacetyl)-L-alanine, *N*-(3-indolylacetyl)-L-valine, *N*-acetyl-L-tryptophanamide, *N*-acetyl-L-tryptophan ethyl ester, L-tryptophan methyl ester, and the dipeptides AcTrp-Ala, AcTrp-Gly, AcTrp-Val, AcTrp-ValOMe, and AcAla-Trp. All of these compounds were detected by both ^1H and ^{13}C NMR spectroscopy. Proton and carbon-13 NMR data (δ in ppm) for all the substrates are given in the Supporting Information. Some of these reactants bind to the platinum(II) complexes and give final products, ^1H and ^{13}C chemical shifts of which are shown in Tables 1 and 2. The chemical shifts could deviate from the stated values by 0.20 ppm or less, depending on the composition of the reaction mixture and other conditions.

Products of Cleavage Reactions. Each peptide was cleaved into two moieties: the amino acids Gly, Ala, Val, and valine methyl ester (ValOMe) became free in solution, whereas AcTrp remained bound to

(42) Kaminskaia, N. V.; Ullmann, G. M.; Fulton, D. B.; Kostić, N. M. *Inorg. Chem.* **2000**, *39*, 5004.
(43) Hohmann, H.; Van Eldik, R. *Inorg. Chim. Acta* **1990**, *174*, 87.
(44) Drexler, C.; Paulus, H.; Elias, H. *Inorg. Chem.* **1991**, *30*, 1297.
(45) Watt, G. W.; Cude, W. A. *Inorg. Chem.* **1968**, *7*, 335.
(46) Holden, J. R.; Baenziger, N. C. *Acta Cryst.* **1956**, *9*, 194.

Table 1. Proton Chemical Shifts (in ppm) for the Several Identified Products of Peptide Binding to *cis*-$[Pt(en)(sol)_2]^{2+}$ Complex in Which the Solvent Ligand Is Water or Acetone; Atom Numbering Is Shown in Structure **4**

peptide[a]	N(1)H	C(2)H	C(4)H	C(5)H	C(6)H	C(7)H	C(8)H_2	amide-NH[b]	other
indole-3-acetamide	10.80 br	8.55 s	8.12 d	7.44 t	7.37 t	7.58 d	4.70 d, 3.52 d	9.15 s, 8.90 s	
N-(3-indolylacetyl)-L-alanine	11.12 br, 11.05 br	8.58 s, 8.38 s	8.15 d, 8.11 d	7.45 t	7.37 t	7.57 d	3.60 d, 3.55 d, 4.72 d, 4.62 d	9.65 br, 9.85 br	4.60 q, 1.50 d, 1.48 d,
N-(3-indolylacetyl)-L-valine	11.10 br, 11.05 br	8.50 s, 8.44 s	8.13 br dd	7.45 t	7.37 t	7.61 d	4.81 d, 4.68 d, 3.72 d, 3.65 d	9.75 br, 9.50 br	4.58 d, 4.54 d, 2.10 m, 0.99 m
N-acetyl-L-tryptophanamide	10.45 br	7.51 s	7.81 d	7.15 t	7.11 t	7.48 d	3.65 m	9.00 s, 9.40 s, 9.30 s, 9.45 s[c]	7.45 br, 5.12 m, 1.85 s
AcTrp-Gly	10.45 br	7.51 d	7.70 d	7.12 t	7.07 t	7.45 d	3.63 d	9.80 br[c]	7.47 br, 5.22 t, 4.18 d, 1.77 s
AcTrp-Ala	10.42 br	7.53 s	7.73 d	7.15 t	7.10 t	7.50 d	3.65 d	9.75 br[c]	7.27 br, 5.22 t, 4.55 q, 1.83 s, 1.53 d
AcTrp-Val	10.40 br	7.50 s	7.70 d	7.14 t	7.10 t	7.45 d	3.65 d	9.68 br s, 9.71 br s[c]	7.47 br, 5.30 m, 4.48 m, 2.0 br, 1.84 s, 0.96 m
AcTrp-ValOMe	10.47 br	7.48 s	7.66 d	7.13 t	7.08 t	7.49 d	3.62 d	9.70 br s, 9.74 br s[c]	5.40 t, 4.48 d, 2.0 br, 1.79 s, 3.70 s 1.0 m
AcTrp	n.d.	8.05 low	7.74 d	7.41 t	7.48 t	7.58 d	3.30 m		7.80 br, 5.30 m, 2.54 s

[a] Ac is *N*-acetyl group. [b] Amide group is bound to Pt(II) via the oxygen atom. [c] For the major product.

Table 2. Carbon-13 Chemical Shifts (in ppm) for the Four Products of Peptide Binding to *cis*-$[Pt(en)(sol)_2]^{2+}$ Complex in Which the Solvent Is Water or Acetone; Atom Numbering Is Shown in Structure **4**

peptide	C(2)	C(3)	C(4a)	C(4)	C(5)	C(6)	C(7)	C(7a)	C(8)	C(O)NH	α-CH	other
indole-3-acetamide	119.0	72.0	133.0	122.5	129.0	123.5	114.5	141.0	35.0	192.0		
N-(3-indolylacetyl)-L-alanine	120.0, 118.0	71.5	132.0	122 br	128.5	123.5	114.0	140.0	37.0 34.0	188.0	50.0	16.0, 18.0, 173.0
N-acetyl-L-tryptophanamide	119.0	69.5	129.0	122.3	126.5	124.5	112.5	137.5	17.5	190.0	56.0	175.0[a], 32.0
N-acetyl-L-tryptophan	117.0	69.3	138.7	120.0	126.4	123.0	114.0	146.0	17.0	210.0[b]	56.3	172[a], 171.0[a], 24.3

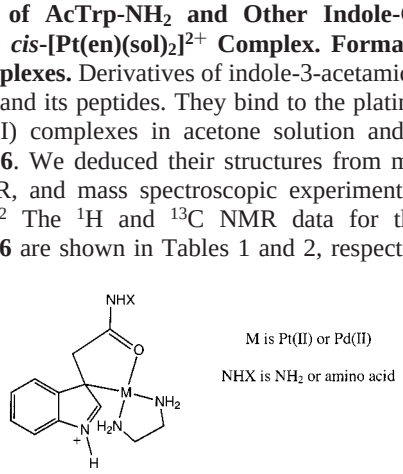
[a] Carbonyl carbon in the CH_3CO group. [b] Carboxylate group bound to Pt(II).

the metal complex. The free amino acids were identified by their ^1H NMR chemical shifts. Proton NMR data (δ in ppm) in acetone-d_6 for Gly, α-CH_2 4.19 br s; for Ala, α-CH 4.45 q, and CH_3 1.71 d; for Val, α-CH 4.28 d, β-CH 2.15 m, and CH_3 1.15 m; for ValOMe, α-CH 4.35 d, β-CH 2.22 m, CH_3 (side chain) 1.14 m, and CH_3 (ester) 3.78 s. Proton NMR data (δ in ppm) in acetone-d_6 for AcTrp coordinated to *cis*-$[Pt(en)(sol)_2]^{2+}$: N(1)H not observed, C(2)H 8.05 m (very weak owing to the exchange), C(4)H 7.72 d, C(5)H 7.40 t, C(6)H 7.47 t, C(7)H 7.55 d, C(8)H 3.28 m, amide NH 7.60 br s, α-CH 5.35 t, and CH_3 2.40 s.

Molecular Modeling and Graphics. Structures of complexes **6** and **7** were simulated with the program Hyperchem,[47] using either steepest-descent or Fletcher−Reeves algorithms. The structure, saved as a PDB file, was visualized with the molecular-graphics program RasMol2.[48]

Results and Discussion

Binding of AcTrp-NH_2 and Other Indole-Containing Ligands to *cis*-$[Pt(en)(sol)_2]^{2+}$ Complex. Formation of the Spiro Complexes. Derivatives of indole-3-acetamide resemble tryptophan and its peptides. They bind to the platinum(II) and palladium(II) complexes in acetone solution and form new complexes **6**. We deduced their structures from many NMR, UV−vis, IR, and mass spectroscopic experiments described elsewhere.[42] The ^1H and ^{13}C NMR data for the selected complexes **6** are shown in Tables 1 and 2, respectively.



6

Evidence from ^1H and ^{13}C NMR Spectroscopy. This study concerns the coordinating abilities of actual tryptophan and its peptides. Tryptophan can coordinate to transition metals via its amino and carboxylic acid groups and form well-known chelate complexes.[36] Esters Trp-OMe and Trp-OEt bind only via the amino group in aqueous solution, whereas *N*-acetyl-L-tryptophan ethyl ester does not coordinate at all. Because we are mostly interested in cleavage of the tryptophanyl peptides, we chose *N*-acetyl-L-tryptophanamide (AcTrp-NH_2), the simplest tryptophanyl compound that contains two amide bonds, as a model for binding studies. Our preliminary experiments showed that it undergoes very slow hydrolysis, which does not interfere with the relatively fast coordination to the metal complex. The half-lives for the hydrolysis and coordination at 296 K are 15 and 0.3 h, respectively.

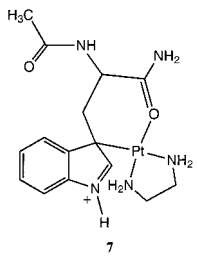
Reaction of *N*-acetyl-L-tryptophanamide and *cis*-$[Pt(en)(sol)_2]^{2+}$ in acetone solution gives one major product, identified by ^1H, ^{13}C, ^{15}N, and ^{195}Pt NMR spectroscopy. Because resonances of both the indole group and the backbone are shifted, we conclude that both of them coordinate to the platinum(II) ion. The coordination mode of the amide groups in the tryptophan-derived product is judged from the ^1H and ^{13}C NMR spectra, which were assigned by two-dimensional correlation (^1H-^1H and ^1H-^{13}C COSY) and NOESY or ROESY spectroscopy. Both amide protons of the *C*-terminus shift downfield upon binding, from 6.90 and 6.35 ppm in the free AcTrp-NH_2 to 9.00 and 9.40 ppm in the product; see Table 1. The ^{13}C resonance of the *C*-terminal carbonyl oxygen shifts downfield from 170.0 ppm in the free AcTrp-NH_2 to 190.0 ppm in the product, see Table 2. The ^{13}C resonances of the *N*-acetyl group, however, are not significantly affected by the coordination. These findings are consistent with the direct binding of

(47) Hypercube, Inc. *HyperChem*, release 4.5; May 1995.
(48) Sayle, R. *RasMol* v2.5, a molecular visualisation program; updated October, 1994.
(49) Cooney, M.; Gommans, L. H. P.; Main, L.; Nicholson, B. K. *J. Organomet. Chem.* **1988**, *349*, 197.
(50) Fairlie, D. P.; Woon, T. C.; Wickramasinghe, W. A.; Willis, A. C. *Inorg. Chem.* **1994**, *33*, 6425.

the *C*-terminal carbonyl oxygen to the platinum(II) ion, similar to that in structure **6**.[49−51] The ^{1}H-^{15}N HETCOR experiments ruled out formation of the iminol species and the deprotonation of the amide nitrogen.

Upon coordination to platinum(II), the tryptophan N(1)H resonance remains but is shifted from 10.08 ppm in free *N*-acetyl-L-tryptophanamide to 10.45 ppm in the product; see Table 1. The ^{1}H-^{15}N HETCOR experiments established that the resonance at 10.45 ppm belongs to the tryptophan N(1)H, and not to the hydroxylic proton of an iminol tautomer. The C(3) resonance, characteristic of 3H-indolenine,[39,40] is absent from the ^{1}H spectrum of the product; this tautomer, too, can be ruled out. The multiplet of C(8)H_2 at 3.18 ppm and also the α-CH resonance at 4.65 ppm for free *N*-acetyl-L-tryptophanamide are both shifted downfield upon coordination, because of their proximity to the aromatic phenyl ring.[52] Downfield shift of the α-CH resonance is consistent with the coordination of the C-terminal carbonyl oxygen to the metal ion. The signals of the C(2)H at 7.15 ppm and of C(4)H at 7.64 ppm shift upon coordination downfield, to 7.51 and 7.81 ppm, respectively. All the other proton resonances of the indole group are also shifted downfield in the product, but to lesser extents; see Table 1.

The ^{13}C NMR resonance of C(3) at 110.0 ppm in the spectrum of free *N*-acetyl-L-tryptophanamide is characteristic of aromatic carbons. Upon coordination to the platinum, this resonance moves to 69.5 ppm. This upfield shift indicates that the aromaticity at C(3) is lost and that this atom has become tetrahedral.[39,40] The ^{13}C resonance of C(7a) moves downfield upon coordination, as reported before,[40,50,51] but this change in chemical shift is smaller than that expected for the 3H-indolenine structures because the N(1) atom in the coordinated ligand is protonated. In this respect the coordinated AcTrp-NH_2 resembles compound **6**. The presence of the proton at N(1) in both compounds causes the ^{13}C resonance of C(2) to sit far upfield of the position that ^{13}C resonances have in similar complexes in which the indole nitrogen is not protonated.[39,40] The α-CH resonance moves downfield consistently upon the coordination of the C-terminal carbonyl oxygen to the metal ion. The allylic C(8) resonance in free *N*-acetyl-L-tryptophanamide at 23.0 ppm shifts upfield to 17.5 ppm upon coordination, as the double bond moves further away from this carbon atom. In complex **6**, however, the C(8) resonance moves slightly downfield upon coordination. We attribute this difference in the changes in chemical shift to a difference in stereochemistry: the six-membered chelate formed by AcTrp-NH_2 versus the more rigid five-membered chelate in **6**. All the ^{13}C and ^{1}H chemical shifts agree with the reported values.[39,40,53,54] All the aforementioned spectroscopic properties of the tryptophanyl product resemble those of complex **6**. We, therefore, concluded that *N*-acetyl-L-tryptophanamide forms complex **7**, as indole-3-acetamide forms the complex **6**.



7

Evidence from ^{15}N and ^{195}Pt NMR Spectroscopy. Direct observation of ^{15}N nuclei did not yield reliable one-dimensional spectra because of the low sensitivity and the low natural abundance of this isotope. Therefore, the ^{15}N chemical shifts were obtained by ^{1}H-^{15}N HETCOR spectroscopy. Free *N*-acetyl-L-tryptophanamide has resonances at 104.0, 126.5, and 131.0 ppm for the *C*-terminal amide, the *N*-terminal amide, and the indole N(1), respectively. The resonance at 104.0 ppm is split in the proton direction by the two inequivalent protons attached to the N atom. Upon coordination all three resonances move downfield, to 117.0, 132.5, and 135 ppm, respectively. Evidently, the *C*-terminal resonance at 104.0 ppm is affected the most, as expected for the structure **7**. The downfield shifts and the splitting patterns in the two-dimensional ^{1}H-^{15}N spectra support this structure.[55]

Platinum-195 chemical shift greatly depends on the ligands and allows identification of the coordinated atoms.[56] The shift, however, also depends on the solvent and temperature.[57,58] For instance, the temperature dependence of the chemical shift of $[PtCl_6]^{2-}$ in aqueous solution is 1.1 ppm/K, and that of $[PtCl_4]^{2-}$, the external reference in our experiments, is similar.[56,58] Our determinations of the temperature dependences for several complexes are given in Figure S1 and Table S1 in the Supporting Information. The ^{195}Pt chemical shift for *cis*-$[Pt(en)(sol)_2]^{2+}$ markedly depends on temperature, both intrinsically and because of the temperature-dependent interaction between the acetone (sol) molecules in the coordination sphere and in the solvent.[56] That the ^{195}Pt chemical shifts of complexes **6** and **7** depend relatively little on temperature is consistent with the absence of the solvent ligands in the coordination sphere.

Selected ^{195}Pt chemical shifts are compiled in Table S2 in the Supporting Information. The resonances for all the complexes containing the ligand ethylenediamine are relatively broad (∼ 20 ppm) because of the unresolved coupling to ^{14}N nuclei and fast quadrupolar relaxation.[59] Upon addition of *N*-acetyl-L-tryptophanamide, the ^{195}Pt resonance of *cis*-$[Pt(en)(sol)_2]^{2+}$ at −1890 ppm moves upfield, to −2301 ppm, as expected for entering ligands that are stronger electron donors than acetone and water. This evidence is consistent with the carbon and the amide-oxygen coordination in structure **7**.[60] The ^{195}Pt chemical shift for the complex with vitamin C (Table S2) is higher than that for complex **7** because the negatively charged oxygen in vitamin C is a stronger *σ*-donor than the electroneutral amide oxygen in **7**.[61] Upon addition of 3.0 M water and 0.1 M $HClO_4$ and incubation at 296 K for 1 h, the resonance of the complex **7** at −2301 ppm moves to −2307 ppm. This upfield movement is consistent with the coordination of the negatively charged carboxylate, a product of the hydrolysis of *N*-acetyl-L-tryptophanamide, to the platinum(II) ion. There are no ^{195}Pt satellites in the ^{13}C and ^{15}N NMR spectra, even for nuclei directly bonded to platinum, presumably because of the fast relaxation induced by the ^{14}N nuclei. Others, too, noted the lack of the ^{195}Pt

(51) Woon, T. C.; Fairlie, D. P. *Inorg. Chem.* **1992**, *31*, 4069.
(52) Zhang, F.; Yajima, T.; Odani, A.; Yamauchi, O. *Inorg. Chim. Acta* **1998**, *278*, 136.
(53) Myers, W. H.; Sabat, M.; Harman, W. D. *J. Am. Chem. Soc.* **1991**, *113*, 6682.
(54) Hodges, L. M.; Moody, M. W.; Harman, W. D. *J. Am. Chem. Soc.* **1994**, *116*, 7931.
(55) Watson, A. A.; Fairlie, D. P. *Inorg. Chem.* **1995**, *34*, 3087.
(56) *NMR and the Periodic Table*; Harris, R., Mann, B., Eds.; Academic Press: New York 1978; p 249.
(57) Pesek, J. J.; Mason, W. R. *J. Magn. Reson.* **1977**, *25*, 519.
(58) Freeman, W.; Pregosin, P. S.; Sze, S. N.; Venanzi, L. M.. *J. Magn. Reson.* **1976**, *22*, 473.
(59) Schwederski, B. E.; Lee, H. D.; Margerum, D. W. *Inorg. Chem.* **1990**, *29*, 3569.
(60) Kirvan, G. E.; Margerum, D. W. *Inorg. Chem.* **1985**, *24*, 3017.
(61) Hollis, L. Stern, E. W.; Amudsen, A. R.; Miller, A. V.; Doran, S. L. *J. Am. Chem. Soc.* **1987**, *109*, 3596.

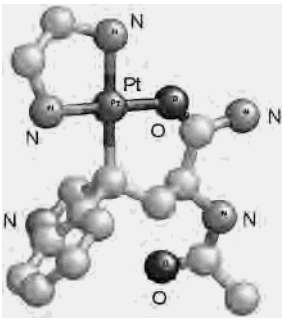


Figure 1. Structure of the R,S diastereomer of complex **7**, built and optimized with the program HyperChem and drawn with the program RasMol2.

Table 3. Molecular Masses and the Molecular Peaks in Electrospray Ionization Mass Spectra

complex	structure	mass	*m*/*z*
cis-[Pt(en)(indole-3-acetamide)]$^{2+}$	**6**	429	429
cis-[Pt(en)(*N*-(3-indolylacetyl)-L-alanine)]$^{2+}$	**6**	501	500
cis-[Pt(en)(*N*-acetyl-L-tryptophanamide)]$^{2+}$	**7**	500	499
cis-[Pt(en)(AcTrp-Ala)]$^{2+}$	**7**	572	571

satellites in similar complexes.[62] We managed to detect broad ^{195}Pt satellites for C(2)H in the ^{1}H NMR spectra of complexes **6**. Our coupling constant, ^3J = 55.8 Hz, agrees well with reported values.[63]

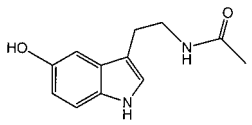
Evidence from UV, IR, and Mass Spectroscopy. The characteristic UV absorption spectra of tryptophan derivatives are sensitive to changes in substitution and to coordination of the indole group to metals.[31] Upon binding of *N*-acetyl-L-tryptophanamide to *cis*-[Pt(en)(sol)$_2$]$^{2+}$ the absorption bands near 280 nm decrease and eventually disappear, while those at 220 nm decrease in intensity but remain. These findings are consistent with the proposed structure, **7**.[31,39,64] The d−d absorption band at 330 nm in *cis*-[Pt(en)(sol)$_2$]$^{2+}$ shifts to 325 nm upon formation of the complex **7**, as the weaker donor, acetone or water, is replaced by the stronger one, indole.[65]

Substituents on indole affect the N(1)−H IR stretching frequency.[31] We confirmed that in free *N*-acetyl-L-tryptophanamide ν_{NH} = 3601 cm^{-1} (ϵ = 400).[32,66] Upon coordination this band decreases, and a very broad band centered at 3552 cm^{-1} appears. A similar shift of vibrational bands accompanies the formation of complexes analogous to **6**. Upon coordination a new band, corresponding to the protonated imine group in complex **7**, >C=NH$^+$-, appeared at ca. 1632 cm^{-1}.[31] A similar band was observed at 1650 cm^{-1} for complex **6**. Infrared spectra also support structure **7**.

In the mass spectrum of the tryptophan-containing complex, the main feature, at *m*/*z* 499 (Table 3) fits the molecular formula [Pt(en)(C$_{10}$H$_9$N$_2$O)] and the overall charge +1, i.e., the deprotonated complex **7**. In the NMR spectra, however, all the protons in **7** were clearly observed and assigned. Although the complex **7** in solution is not deprotonated, it becomes deprotonated upon electrospray ionization. This is a well-known phenomenon; in fact, data in Table 3 show that the similar complexes of indole-3-acetamide and its derivatives also deprotonate in the electrospray experiment. The proton is most probably removed from the indole nitrogen in **7**, because this site has the lowest pK_a value, ca. 8−9. The product would be the 3H-indolenine complex **5**.[67]

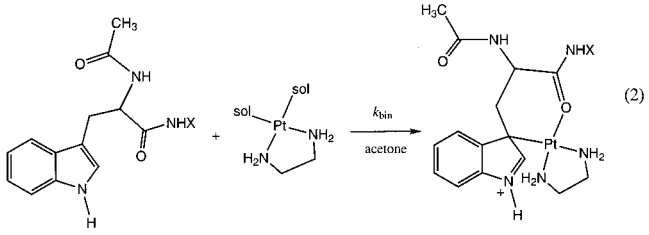
Molecular-Mechanics Simulation and Diastereomerism of 7. Spectroscopic evidence clearly supports structure **7** for the complex formed from *N*-acetyl-L-tryptophanamide and *cis*-[Pt(en)(sol)$_2$]$^{2+}$. Despite much effort, crystals suitable for X-ray diffraction could not be obtained. Structure **7** was built by the program Hyperchem,[46] optimized geometrically, and imported into the program RasMol2. The result is shown in Figure 1.[47] Because the C(3) atom in *N*-acetyl-L-tryptophanamide is prochiral, complex **7** can exist in R,S and S,S diastereomeric forms, distinguishable by ^{1}H NMR spectroscopy. Indeed, major (95%) and a minor (5%) species were observed in the ^{1}H NMR spectra. Resonances for the latter were partially masked by those for the former.

Size of the Chelate Ring. Binding of *N*-acetyl-L-tryptophanamide to the platinum(II) ion via the tryptophan C(3) atom and the amide oxygen atom in the *N*-terminal acetyl group is not observed, presumably because the resulting seven-membered chelate ring would be unfavorable. Indeed, *N*-acetyl-5-hydroxytryptamine (**8**) does not detectably coordinate to *cis*-[Pt(en)(sol)$_2$]$^{2+}$ at 296 K. Upon raising the temperature to 313 K the complex between **8** and *cis*-[Pt(en)(sol)$_2$]$^{2+}$ starts to form but slowly, with the rate constant of 4.7 × 10^{-3} min^{-1}. The respective rate constant for *N*-acetyl-L-tryptophanamide coordination at 313 K is 3.1 × 10^{-1} min^{-1}, more than a hundred times greater. The hydroxyl group in **8** is too far from the ligand atoms to alter the coordination rate. Therefore, the great difference in the rate constants for *N*-acetyl-5-hydroxytryptamine and *N*-acetyl-L-tryptophanamide can be attributed to the formation of the seven-membered versus the six-membered chelate rings.



8

Binding of the Tryptophanyl Peptides to *cis*-[Pt(en)(sol)$_2$]$^{2+}$. Dipeptides such as AcTrp-Gly, AcTrp-Ala, AcTrp-Val, and AcTrp-ValOMe can be considered derivatives of *N*-acetyl-L-tryptophanamide in which NHX represents the *C*-terminal amino acid. They also coordinate to *cis*-[Pt(en)(sol)$_2$]$^{2+}$ in acetone, according to eq 2. The resulting complexes **9** are structurally similar to complex **7**, as results in Tables 1 and 3 show. At ambient temperature the complexes **9** hydrolyze relatively rapidly. To inhibit hydrolysis and accumulate complex **9** for study, a mixture of AcTrp-Ala and *cis*-[Pt(en)(sol)$_2$]$^{2+}$ was kept for 15 min at 313 K, cooled to 233 K, and examined by



(2)

9

NHX is Gly, Ala, Val, or ValOMe

(62) Nagao, N.; Kobayashi, T.; Takayama, T.; Koike, Y.; Ono, Y.; Watanabe, T.; Mikami, T.; Suzuki, M.; Matumoto, T.; Watabe, M. *Inorg. Chem.* **1997**, *36*, 4195.
(63) Goggin, P. L.; Goodfellow, R. J.; Knight, J. R.; Norton, M. J.; Taylor, B. F. *J. Chem. Soc., Dalton Trans.* **1973**, 2220.
(64) Hinman, R. L.; Whipple, E. B. *J. Am. Chem. Soc.* **1962**, *84*, 2534.
(65) Storhoff, B. N.; Huntley, C. L., Jr. *Coord. Chem. Rev.* **1977**, *23*, 1.
(66) Katritzky, A. R.; Ambler, A. P. In *Physical Methods in Heterocyclic Chemistry*; Katritzky, A. R., Ed.; Academic Press: New York, 1963; Vol. 2.

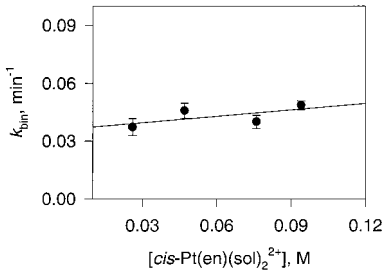


Figure 2. Observed rate constant for binding of *N*-acetyl-L-tryptophanamide to *cis*-$[Pt(en)(sol)_2]^{2+}$ is virtually independent of the initial concentration of *cis*-$[Pt(en)(sol)_2]^{2+}$. Initial concentration of *N*-acetyl-L-tryptophanamide was 0.0094 M, the solvent was acetone-d_6, and temperature was 296 K.

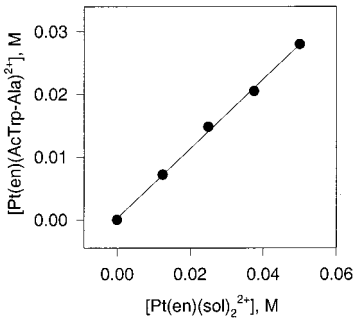


Figure 3. The maximum concentration of complex **9** depends on initial concentration of *cis*-$[Pt(en)(sol)_2]^{2+}$. Initial concentration of AcTrp-Ala was 0.0094, the solvent was acetone-d_6, and temperature was 323 K.

Table 4. Observed Rate Constants (k_{bin}) for Ligand Binding to *cis*-$[Pt(en)(sol)_2]^{2+}$ in Acetone-d_6 Solution at 296 K[a]

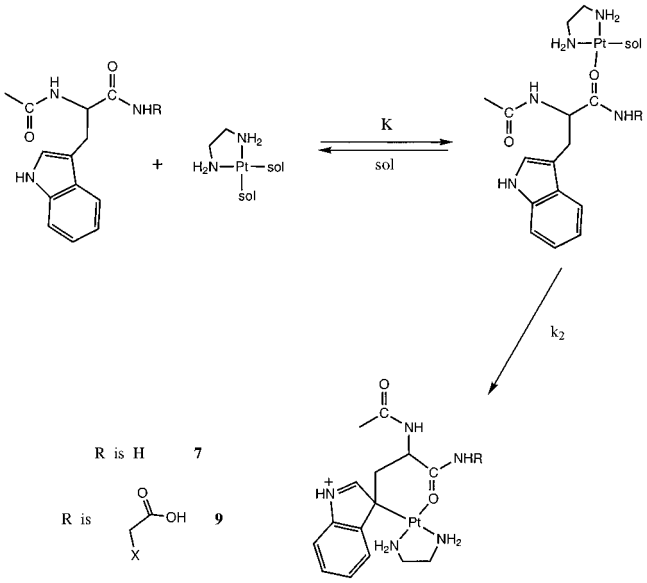
ligand	$k_{bin} \times 10^3$, min^{-1}
indole-3-acetamide[b]	120 ± 12
N-(3-indolylacetyl)-L-alanine[b]	125 ± 18
N-(3-indolylacetyl)-L-leucine[b]	45.7 ± 3.9
N-acetyl-L-tryptophanamide	48.6 ± 10.0
AcTrp-Ala	10.0 ± 2.0

[a] Initial concentrations of *cis*-$[Pt(en)(sol)_2]^{2+}$ and the ligand were 0.094 and 0.0094 M, respectively. [b] From ref 42.

^{195}Pt NMR spectroscopy at this lower temperature. Two resonances were found: one for the starting *cis*-$[Pt(en)(sol)_2]^{2+}$, at −1937 ppm, and the other for the product, the complex **9**, at −2338 ppm. Extrapolation of these chemical shifts with the temperature coefficients in Table S1 in the Supporting Information gave the chemical shifts of −1863 and −2301 ppm at 296 K, respectively. The ^{195}Pt chemical shift of complex *cis*-[Pt(en)(AcTrp-Ala)]$^{2+}$ at 296 K exactly matches that of complex **7**. Given the great dependence of ^{195}Pt chemical shift on the ligation, this full agreement suggests that the donor atoms in the complexes **7** and **9** are the same.

Kinetics of Formation of the Complexes 7 and 9. Because hydrolysis of the complex **7** is slow, we could study its formation with sufficient accuracy. As Figure 2 shows, the rate of *N*-acetyl-L-tryptophanamide coordination to *cis*-$[Pt(en)(sol)_2]^{2+}$ is almost independent of the concentration of the platinum(II) complex. Similar independence was observed previously for the coordination of indole-3-acetamide, an analogue of tryptophanamide.[42] The rate constants for binding, k_{bin}, in Table 4 reflect stereochemical factors. The first two ligands form five-membered chelate rings, whereas the last two ligands form six-membered chelate rings, which may be somewhat less favorable. Between these last two, the smaller ligand (the tryptophan derivative) binds approximately five times more rapidly than the dipeptide does. The linear dependence in Figure 3 is consistent with similar findings for the coordination of indole-3-acetamide to the same platinum(II) complex.[42]

Scheme 1. Stepwise Formation of the Chelate Ring via the Amide Oxygen Atom and the C(3) Atom of Indole



Mechanism of Formation of the Complexes 7 and 9. The mechanism in Scheme 1 is consistent with the zeroth order in the platinum(II) complex, evident in Figure 2. Initial unidentate coordination of the substrate via the amide oxygen atom is followed by the closure of the chelate ring. Under the pre-equilibrium approximation, eq 3, the observed rate constant for the formation of the chelate is given in eq 4. If the equilibrium constant *K* is relatively large, eq 4

$$k_{-1} + k_1[Pt(en)(sol)_2^{2+}] \gg k_2 \tag{3}$$

$$k_{obs} = \frac{Kk_2[Pt(en)(sol)_2^{2+}]}{K[Pt(en)(sol)_2^{2+}] + 1} \tag{4}$$

reduces to eq 5, from which the concentration of the platinum(II) complex is absent. Clearly, eq 5 agrees with the evidence in Figure 2.

$$k_{obs} = k_2 \tag{5}$$

Careful examination of the ^{1}H NMR spectra reveals that the Pt(II)-bound amide oxygen atoms are more abundant than the Pt(II)-bound C(3) atoms. This finding supports the mechanism in Scheme 1, via the unidentate intermediate. The equilibrium constant *K* was determined by integrating the amide proton resonances in free and O-bound unidentate *N*-acetyl-L-tryptophanamide. The value of $K = 1000$ M^{-1} agrees with precedents.[68]

Hydrolytic Cleavage of the Dipeptides in the Presence of *cis*-$[Pt(en)(sol)_2]^{2+}$. Products of the Hydrolytic Cleavage. In the presence of the platinum(II) complex all four dipeptides were cleaved according to eq 6. Only the *C*-terminal amide bond is hydrolyzed, while the *N*-terminal bond remains intact. The rate constant is k_{hyd}. The formation of the leaving amino acid (Gly,

(67) Longworth, J. W.; Rahn, R. O.; Shulman, R. G. *J. Chem. Phys.* **1966**, *45*, 2930.
(68) Kaminskaia, N. V.; Kostić, N. M. *Inorg. Chem.* **1998**, *37*, 4302.

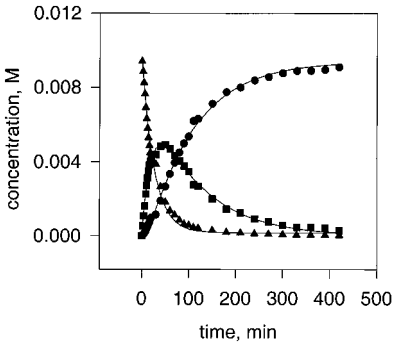
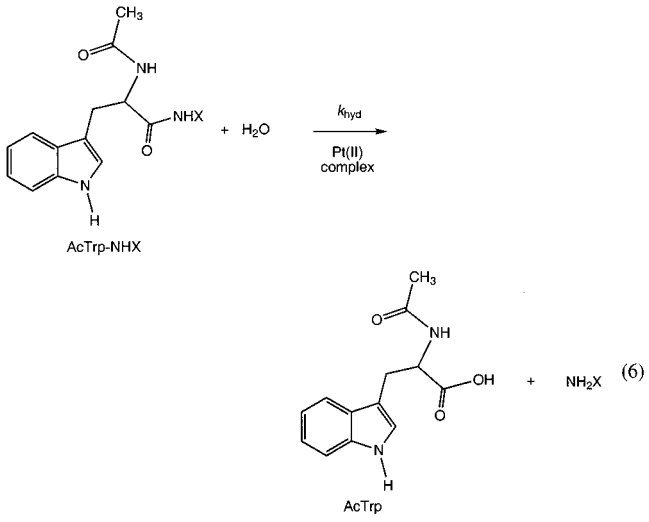


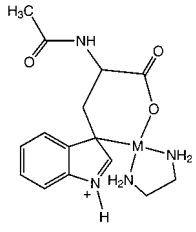
Figure 4. Time course of hydrolysis of AcTrp-Ala in the presence of *cis*-[Pt(en)(sol)$_2$]$^{2+}$. The substrate is free AcTrp-Ala (▲), the intermediate **9** is *cis*-[Pt(en)(AcTrp-Ala)]$^{2+}$ (■), and the products are free alanine and *cis*-[Pt(en)(AcTrp)]$^{+}$, formed in equimolar amounts (●). Initial concentrations of AcTrp-Ala and *cis*-[Pt(en)(sol)$_2$]$^{2+}$ were 0.0094 and 0.094 M, respectively; the solvent was acetone-d_6; and temperature was 313 K.

Ala, Val, or ValOMe) was monitored by ^{1}H NMR spectroscopy. When each reaction mixture was spiked with the pure amino acid, no new resonances appeared, and the resonance of this hydrolytic product grew. These findings confirmed the reaction in eq 6.



(6)

The platinum(II) ion remains bound to *N*-acetyl-L-tryptophan after the cleavage as *cis*-[Pt(en)(AcTrp)]$^{2+}$, complex **10**. Its NMR properties are given in Tables 1 and 2. This complex was also prepared independently, from free *N*-acetyl-L-tryptophan and *cis*-[Pt(en)(sol)$_2$]$^{2+}$ in acetone-d_6 solution. Its ^{1}H and ^{13}C NMR spectra matched those of the product of the dipeptide cleavage.



10

The hydrolysis in the presence of the platinum(II) complex is enhanced over the reactions in the absence of catalysts as much as 10^4-10^5-fold. Dipeptide AcTrp-Ala is well suited for kinetic studies because its hydrolysis in the presence of

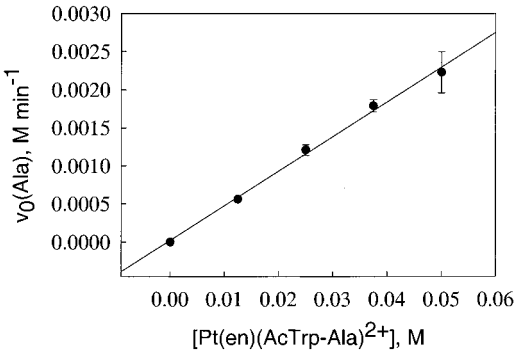


Figure 5. Initial rate of cleaving AcTrp-Ala, calculated from the appearance of free alanine, is directly proportional to the maximum concentration of complex **9**. Initial concentration of AcTrp-Ala was 0.0094 M, the solvent was acetone-d_6, and temperature was 323 K.

Table 5. Observed Rate Constants[a] (k_{hyd}) for the Hydrolysis of *N*-Acetylated Tryptophanyl Peptides in the Presence of *cis*-[Pt(en)(sol)$_2$]$^{2+}$ in Acetone-d_6 Solution at 313 K[b]

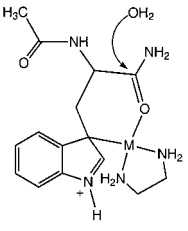
peptide	$k_{hyd} \times 10^3$, min^{-1}
AcTrp-Gly	300 ± 20
AcTrp-Ala	62.9 ± 10
AcTrp-Val	3.8 ± 0.7
AcTrp-ValOMe	3.7 ± 0.8

[a] From the initial rates of appearance of free *C*-terminal amino acid. [b] The initial concentrations of *cis*-[Pt(en)(sol)$_2$]$^{2+}$ and the peptides were 0.094 and 0.0094 M, respectively.

platinum(II) is relatively fast. Because ^{1}H resonances for the free dipeptide, complex **9**, and free alanine do not overlap much, the NMR spectra could be integrated with sufficient accuracy.

Intermediate in the Hydrolysis Reaction. As Figure 4 shows, four species are observed in the hydrolytic reactions. The kinetic profile of complex **9** shows it to be an intermediate in the reaction. Indeed, Figure 5 shows that the rate of hydrolysis is directly proportional to the concentration of complex **9**. Complexes **9** evidently are the reactive, true intermediates in the hydrolysis reactions.

The dipeptides in complexes **9** act as bidentate ligands. Since ethylenediamine remains coordinated during hydrolysis, all four coordination sites on platinum(II) are occupied by relatively inert ligands. The role of the platinum(II) ion in the hydrolysis is to activate the substrate toward nucleophilic attack by the solvent water, as shown in **11**.



11

Kinetic Effects of the Steric Bulk. As Table 5 shows, hydrolysis is strongly inhibited by the steric bulk of the amide substituent X in the leaving amino acid. The bulkier the substituent X, the greater the shielding of the amide oxygen from the platinum(II) complex, and the slower the formation of the hydrolytically active intermediate **9**.[42] The bulky substituents X in this intermediate shield the amide carbon from the nucleophilic attack by water in the hydrolysis step, shown in **11**.

Kinetic Effects of Water and Acid. As Figure S2 in the Supporting Information shows, added water somewhat inhibits

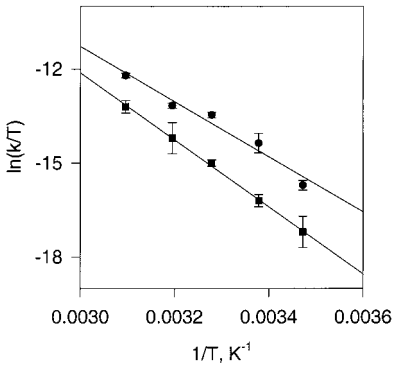


Figure 6. Dependence on temperature of the rate constants k_{bin}, for the binding of AcTrp-Ala to *cis*-[Pt(en)(sol)$_2$]$^{2+}$ (●), and k_{H_2O}, for the subsequent hydrolysis of complex **9** (■). Initial concentrations of AcTrp-Ala and *cis*-[Pt(en)(sol)$_2$]$^{2+}$ were 0.0094 and 0.094 M, respectively, and the solvent was acetone-d_6.

hydrolysis. Although this may at first appear suspicious, it is easily explained in terms of Scheme 2. Water is, of course, essential for the hydrolysis step (k_{H_2O}), but it somewhat inhibits the preceding binding step:[68] the rate constant k_{bin} is $(3.8 \pm 0.14) \times 10^{-2}$ and $(2.29 \pm 0.11) \times 10^{-2}$ min^{-1} in the presence of 0.20 and 3.50 M added H_2O, respectively. When the rate of formation of the complex **9** is lowered upon addition of water, so is its maximal concentration, as NMR spectra showed. This partial suppression of the reactive intermediate causes a decrease in the rate of hydrolysis.

Effects of added base could not be studied because the platinum(II) catalyst was converted into insoluble compounds.[69] Acids added to relatively high concentrations caused fast "background" hydrolysis of the dipeptides. Fortunately, variation of acid concentration over a relatively narrow range gave useful kinetic results: the rate constant k_{bin} was $(3.8 \pm 0.2) \times 10^{-2}$, $(7.0 \pm 0.3) \times 10^{-2}$, and $(6.22 \pm 0.3) \times 10^{-2}$ min^{-1} when the concentration of added $DClO_4$ was 1.0×10^{-4}, 0.10, and 0.30 M, respectively. The values for k_{H_2O} were $(1.3 \pm 0.1) \times 10^{-2}$, $(1.1 \pm 0.3) \times 10^{-2}$, and $(1.2 \pm 0.3) \times 10^{-2}$ min^{-1} when the concentration of added $DClO_4$ was 1.0×10^{-4}, 0.10, and 0.30 M, respectively. Clearly, neither the initial binding step nor the subsequent cleavage step depends on acid concentration. These findings are consistent with Schemes 1 and 2. The platinum(II) complex provides neither the general base catalyst nor the nucleophile. It activates the coordinated amide group toward nucleophilic attack by free water.

Table 6. Activation Parameters in Acetone-d_6 Solution for Binding of AcTrp-Ala to *cis*-[Pt(en)(sol)$_2$]$^{2+}$ and for Subsequent Hydrolysis of the Coordinated AcTrp-Ala[a]

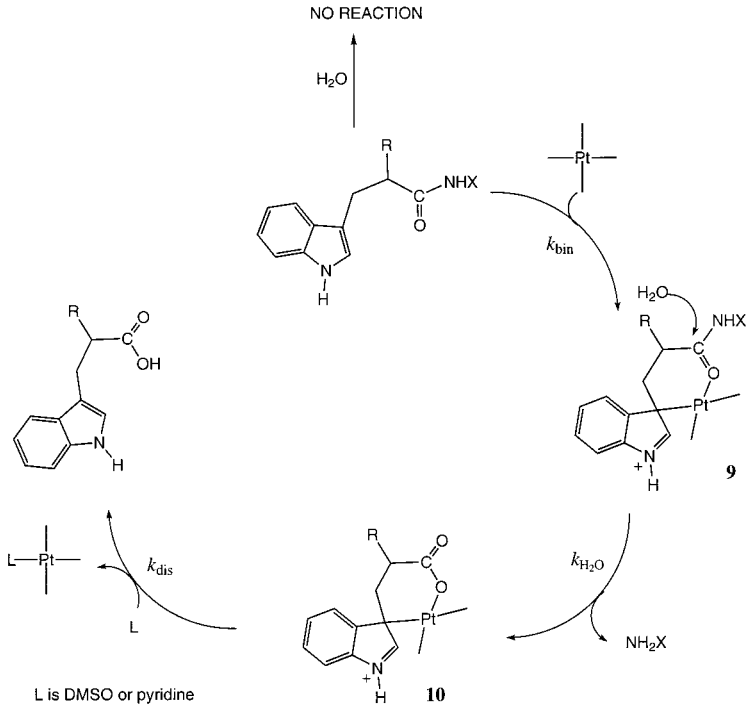
rate constant	$\Delta S^{\ddagger}$, J K^{-1} mol^{-1}	$\Delta H^{\ddagger}$, kJ mol^{-1}	$\Delta G^{\ddagger}$ at 296 K, kJ mol^{-1}
k_{bin}	-72.0 ± 26.6	73.05 ± 8.06	94.4 ± 15.9
k_{H_2O}	-22.4 ± 8.3	92.2 ± 2.5	98.4 ± 5.0

[a] Initial concentrations of *cis*-[Pt(en)(sol)$_2$]$^{2+}$ and AcTrp-Ala were 0.094 and 0.0094 M, respectively.

Activation Parameters for Binding and Subsequent Cleavage. Results of the experiments in Figure 6 are given in Table 6. In the binding step, the activation parameters for the dipeptide and indole-3-acetamide[42] are similar. In particular, negative $\Delta S^{\ddagger}$ for both ligands is diagnostic of ordering in the transition state as the chelate ring is closed.[70] In the hydrolysis step, the $\Delta H^{\ddagger}$ value agrees with those reported for similar reactions,[70] and negative $\Delta S^{\ddagger}$ is consistent with the bimolecular process shown in **11** and Scheme 2.

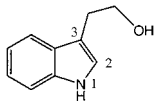
Overall Mechanism for Peptide Hydrolysis. The mechanism for the hydrolysis of the tryptophanyl peptides promoted by platinum(II) is shown in Scheme 2. The observed rate constant k_{hyd} in eq 6 is a composite of the binding (k_{bin}) and hydrolysis (k_{H_2O}) steps. In organic solvents such as acetone, the tryptophanyl peptide coordinates to platinum(II) as a bidentate ligand, via the indole group and the amide oxygen. Electron withdrawal by platinum(II) makes the amide carbon more susceptible toward nucleophilic attack by water, a stoichiometric amount of which is added to acetone. This attack results in the hydrolytic cleavage of the peptide into two fragments. Since the tryptophan-containing fragment remains coordinated to

Scheme 2. Overall Mechanism for the Hydrolysis of the Tryptophanyl Peptides Catalyzed by Platinum(II) Complexes



platinum(II), hydrolysis of the peptides is noncatalytic. When, however, a ligand such as DMSO or pyridine (L) is added to the reaction mixture, the tryptophan-containing fragment is displaced, and platinum(II) is regenerated. This regeneration raises the possibility of catalytic turnover, but more research will be needed to achieve it.

All the rate constants in Scheme 2 were determined for the cleavage of AcTrp-Ala promoted by *cis*-$[Pt(en)(sol)_2]^{2+}$ at 313 K: $k_{bin} = (3.8 \pm 0.2) \times 10^{-2}$ min^{-1}, $k_{H_2O} = (1.3 \pm 0.3) \times 10^{-2}$ min^{-1}, and $k_{dis} > 1$ M^{-1} min^{-1}. The second-order rate constant for the hydrolysis of **9** is $(6.9 \pm 1.5) \times 10^{-2}$ M^{-1} min^{-1}. Because hydrolysis of complex **9** is slower than its formation, this complex is detected by NMR spectroscopy. Because displacement of AcTrp from complex **10** is relatively fast in the presence of DMSO or pyridine, only the lower limit for k_{dis} could be set. In the absence of this added ligand, complex **10** slowly degrades to unknown products. Their identification by NMR spectroscopy is precluded by selective deuteriation of the indole group in acetone-d_6. For example, in complex **10** the C(2) proton is completely replaced by deuterium after 1 h at 313 K. This isotope exchange seems to be assisted by the carbonyl group in **10**. To test this hypothesis, we examined tryptophol (**12**), a compound that does not detectably bind to



12

platinum(II). In acetone-d_6 solution, in the presence of as little as 0.010 M $DClO_4$, selective exchange of the C(2) proton is completed in several minutes at room temperature. Because this exchange does not require platinum(II), we believe that the hydroxyl group in **12** is responsible for this selective deuteriation. Carboxylate in **10** perhaps acts similarly.

Prospects of Cleavage by Other Promoters. Besides *cis*-$[Pt(en)(sol)_2]^{2+}$, we tested several other platinum(II) and palladium(II) complexes as promoters of hydrolytic cleavage of tryptophanyl peptides. Platinum and palladium form similar complexes with these peptides.[41,42] As Table S3 in the Supporting Information shows, *cis*-[Pd(en)(AcTrp-Ala)]$^{2+}$ and *cis*-[Pd(en)(*N*-acetyl-L-tryptophanamide)]$^{2+}$ have similar structures.[42] Their ^{1}H NMR resonances are broadened because of exchange processes. The extent of binding to palladium(II) and platinum(II) is very similar. Because binding to palladium(II) is fast[71] ($t_{1/2} < 0.5$ min^{-1} at 296 K), its kinetics could not be followed by NMR spectroscopy. As Table 7 shows, various promoters differ in their hydrolytic activity. These differences in reactivity, the sensitivity of the tryptophanyl coordination to solvent, and inhibition by the steric bulk can be used to achieve regioselective cleavage of longer peptides, which contain multiple anchoring sites for metal complexes. This will be the goal of our future research.

Table 7. Observed Rate Constants[a] for Hydrolytic Cleavage of AcTrp-Ala Promoted by Various Metal Complexes in Acetone-d_6 Solution at 313 K[b]

promoter	$k_{obs} \times 10^3$, min^{-1}
cis-$[Pt(en)(sol)_2]^{2+}$	62.9 ± 10
cis-$[Pt(cod)(sol)_2]^{2+}$	2.3 ± 0.2
cis-$[Pt(bpy)(sol)_2]^{2+}$	0.8 ± 0.2
cis-$[Pd(en)(sol)_2]^{2+}$	0.25 ± 0.05[c]

[a] From the initial rates of appearance of free alanine. [b] The initial concentrations of promoter metal complex and AcTrp-Ala were 0.094 and 0.0094 M, respectively. [c] From ref 41.

Conclusions

Previous studies in this laboratory showed that peptides and proteins can be selectively cleaved in aqueous solutions by palladium(II) complexes bonded to the side chains of methionine and histidine. In some cases even catalytic turnover was achieved. Very recently,[41] and again in this study, we showed that cleavage is possible also next to a tryptophan residue. Because this residue is a poor ligand and cannot compete with water for binding to the metal complex, the new reaction is done in acetone containing little water, only the amount necessary for the hydrolysis step. Therefore, the overall selectivity of peptide cleavage can be altered by changing the solvent. If a platinum promoter is present in stoichiometric excess, cleavage will occur near histidine and methionine in aqueous solution but near tryptophan in acetone solution. Platinum(II) and palladium(II) complexes hold promise for cleaving hydrophobic or membrane-bound proteins, which require the use of nonaqueous solutions.

Acknowledgment. This work was supported by the National Science Foundation, through Grant CHE-9816522. We are grateful to Dr. D. Bruce Fulton for help in recording the NMR spectra.

Supporting Information Available: NMR spectral properties of the reactants; Tables S1−S3, showing temperature-dependence coefficients of ^{195}Pt chemical shifts, selected ^{195}Pt NMR chemical shifts, and ^{1}H NMR data for palladium(II) complexes, respectively; Figures S1 and S2, showing temperature dependence of ^{195}Pt resonance and reaction inhibition by water, respectively. This material is available free of charge via the Internet at http://pubs.acs.org.

IC0014033

(69) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 5th ed.; Wiley: New York, 1988; p 924.

(70) (a) Espenson, J. H. *Chemical Kinetics and Reaction Mechanisms*, 2nd ed.; McGraw-Hill: New York, 1994; p 303. (b) Reeves, M. E.; Thornalley, P. J. *Biochem. Soc. Trans.* **1993**, *21*(2), 169(S). (c) Slebocka-Tilk, H.; Pescorla, C. G.; Shirin, S. *J. Am. Chem. Soc.* **1997**, *119*, 10969.

(71) (a) Romeo, R.; Scolaro, L. M.; Nastasi, N.; Arena, G. *Inorg. Chem.* **1996**, *35*, 5087. (b) Wendt, O. F.; Kaiser, N. K.; Elding, L. I. *J. Chem. Soc., Dalton Trans.* **1997**, *24*, 4733. (c) Moullet, B.; Zwahlen, C.; Frey, U.; Gervasio, G.; Merbach, A. E. *Inorg. Chim. Acta* **1997**, *261*, 67.