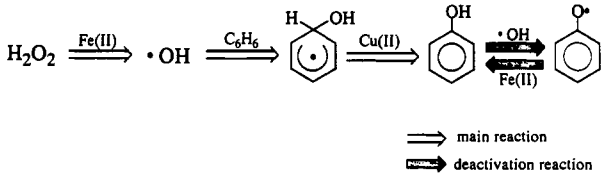
Scheme II



In conclusion, the minimal reaction scheme for the Fenton's oxidation of benzene is outlined in Scheme II. The main deactivation reaction is the reaction of hydroxyl radical with phenol, followed by reaction of the resulting phenoxy radical with Fe(II) ion. The direct reaction of hydroxyl radical with Fe(II) ion causes the slight decrease (ca. 4% at 20 mM of Fe(II) ion) in the yield of phenol (Table 6).

Experimental Section

Materials. All chemicals used were commercial and reagent grade and were used without further purification. Hydrogen peroxide (35 wt %) was obtained from Katayama Kagaku Kogyou.

Analysis. Phenol, hydroquinone, and catechol were determined by reversed-phase HPLC using a JASCO Intelligent HPLC Pump (880-PU) and a JASCO Intelligent UV/vis detector (875-UV) coupled with a Shimadzu Chromatopac C-R6A. The separation column was a Cica-MERCK Hibar Lichrosorb RP-18 (5 μm), and the eluent was a mixture of acetonitrile (14 vol %) and a 50 mM phosphate buffer (86 vol %, pH 3.5). Biphenyl was determined by GLC. Hydrogen proxide (titanium sulfate method)[20] and iron(II) ions (*o*-phenanthroline method) were determined spectroscopically by use of a JASCO UVIDEC 610 spectrophotometer.

Oxidation of Benzene. The standard procedure for the Fenton's oxidation of benzene was as follows: Into a 100-mL Erlenmeyer flask with a rubber stopper equipped with four glass tubings was placed 25 mL of a solution that was 50 mM H_2SO_4, 10 mM $FeSO_4$, and 30 mM $CuSO_4$. After air was purged from the flask with N_2 through two glass tubings fitted to the rubber-stopper, 0.75 mL (8.4 mmol) of benzene was added through the third tubing using a microfeeder (Azuma Denki Kogyo, MF-2). With a magnetic bar stirring at 1000–1100 rpm, 100 mM H_2O_2 in 50 mM H_2SO_4 were added dropwise using another microfeeder (Azuma Denki Kogyo, MF-2) with a rate of 0.2759 mL min^{-1}. After addition of the precalculated amount of H_2O_2 at 25 °C, the reaction mixture was allowed to stand for 30 min with stirring. After evaporating the remaining benzene with a stream of N_2, 5 mL of the reaction mixture was diluted to 50 mL with 25 mM EDTA. An aliquot of the diluted reaction mixture was subjected to HPLC analysis.

Oxidation of Phenol. Into a 100-mL Erlenmeyer flask with rubber-stopper was added 25 mL of a solution that was 50 mM H_2SO_4, 5 mM phenol, 20 mM $FeSO_4$, and 30 mM $CuSO_4$. After air was purged from the flask with N_2, 100 mM H_2O_2 in 50 mM H_2SO_4 was added dropwise using the microfeeder with a magnetic stir bar. The reaction mixture was allowed to stand at 25 °C for 30 min and was subjected to HPLC analysis after dilution with 50 mM EDTA.

Registry No. C_6H_6, 71-43-2; H_2O_2, 7722-84-1; $CuSO_4$, 7758-98-7; $Fe(ClO_4)_2$, 13933-23-8; $FeSO_4$, 7720-78-7; C_6H_5-C_6H_5, 92-52-4; C_6H_5OH, 108-95-2; HOC_6H_4-*o*-OH, 120-80-9; HOC_6H_4-*p*-OH, 123-31-9; HO, 3352-57-6; H_2, 1333-74-0.

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(20) Erlenmayer, H.; Zell, R.; Brintziger, H.; Prijs, B. *Helv. Chim. Acta* **1964**, *47*, 792.

Bipyridyl Amino Acid–Metal Complexes and Their Characterization by Electrospray Mass Spectrometry

Stephen R. Wilson,* Arfa Yasmin, and Yunhui Wu

Department of Chemistry, New York University, Washington Square, New York, New York 10003

Received July 16, 1992

The syntheses of bipyridylalanine and related bipyridyl-functionalized peptide derivatives are described. The copper complexes of several peptide–bipyridyls were prepared and characterized. Application of electrospray ionization mass spectrometry to the structure determination of cationic bipyridyl Cu^+ complexes **2** and **3**, as well as peptide–metal complexes **18–22**, was explored.

As part of a program to develop new methods for peptide cross-linking, we are studying the use of a bipyridyl metal template, where the geometric requirements of coordination might be used to constrain a peptide to a well-defined conformation.[1] Metal binding sites in proteins are well-known to stabilize structure as well as to participate in catalytic enzyme function. Cross-linking of synthetic peptides with metals and specific derivatization of peptides with bipyridyl groups has only recently been reported in the literature.[2] Recent reports of bipyridylalanine complexes by Imperiali[3] have prompted us to report our work in this area.[4]

Results and Discussion

Two different strategies have been explored to incorporate the bipyridyl ligand into a peptide. The first involves direct introduction of the bipyridyl (bpy) group onto a cysteine thiol or at the terminus of preformed peptides. The second route involves the preparation of a *new amino acid* containing the bipyridyl (bpy) side chain for use in peptide synthesis.

Electrospray ionization mass spectrometry (ESI-MS) has recently revolutionized the mass measurement of biological molecules.[5] We have also shown that ESI-MS is quite

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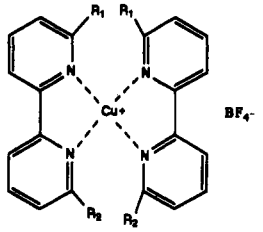
(3) Imperiali, B.; Fisher, S. L. *J. Am. Chem. Soc.* **1991**, *113*, 8527.

(4) Presented in part at the National Organic Symposium, Minneapolis, MN, June 1991.

0022-3263/92/1957-6941$03.00/0

useful for organic and organometallic compounds.[6] The discovery that ESI-MS is suitable for characterization of molecular ions for charged metal complexes has had a great impact on this project. (Strictly speaking, the term cation mass in preference to molecular ion is to be used, since the *cation* is the species whose mass is measured). We have determined in this work that ESI-MS is uniquely suited to the structure determination of peptide–metal complexes *in solution*.

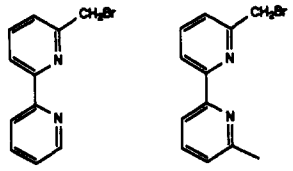
Our initial model systems were the known[7] cationic bipyridyl–copper(I) complexes 1–3. The X-ray structure[8]



1. $R_1 = R_2 = H$ $(bpy)_2Cu^+$ BF_4^-
2. $R_1 = H$, $R_2 = CH_3$ $(mbp)_2Cu^+$ BF_4^-
3. $R_1 = R_2 = CH_3$ $(dmbp)_2Cu^+BF_4^-$

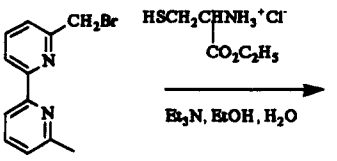
of 3 reveals near-ideal tetrahedral geometry, which might prove useful in peptide structure stabilization. Sterically hindered complexes 2 and 3 are stable to chromatography and could be readily identified by NMR and their molecular ions by ESI-MS, vida infra.

Preparation of Peptide Ligands. Thiol Alkylation Studies. The incorporation of a bipyridine ligand into a presynthesized peptide began with alkylation at a sulfhydryl group of cysteine. Our target alkylating agents were the known 6-(bromomethyl)-2,2′-bipyridine (4)[10] and 6-(bromomethyl)-6-methyl-2,2′-bipyridine (5).[11]



4 5

Alkylation of L-cysteine ethyl ester hydrochloride with bpy-bromide 5 yields the S-alkylated product[12] 6 (eq 1). The amino group in compound 6 was converted to amide 7 by reaction with acetic anhydride. In a similar manner, reduced-somatostatin bisbipyridyl compound 8 as well as two nonpeptide bis-bipyridyl models 9 and 10 were also prepared.



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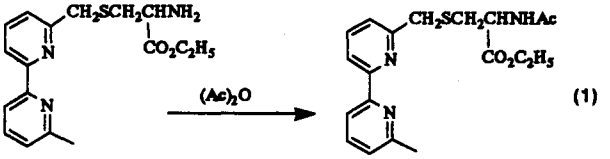
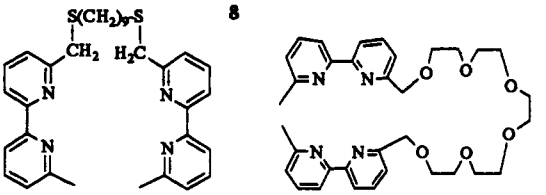


(1)

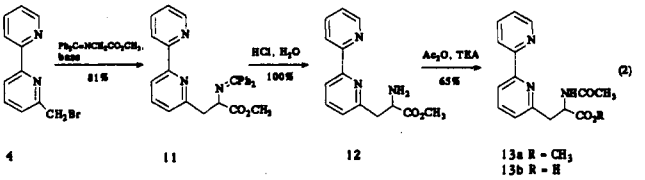
6 7

8



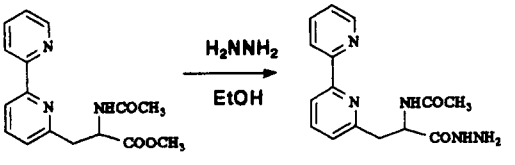
9 10

Bpy-Alanine Synthesis. Synthesis of the peptide building block bipyridylalanine 12[3,4] by the phase-transfer method of O'Donnell[13] was followed by acetylation giving ligand 13a (abp) (eq 2). Peptides containing bipyridylalanine 12 could be made by using the azide coupling method. The acetylated bipyridylalanine methyl ester 13a was converted to the corresponding hydrazide 14 followed by coupling with *S*-benzyl- or *S*-benzoyl-L-cysteine methyl ester to give dipeptides 15a or 15b (eq 3).

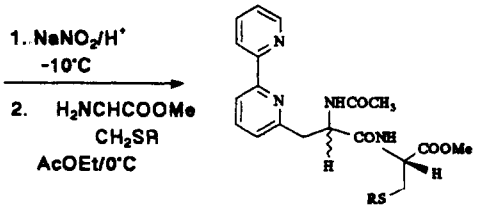


(2)

4 11 12 13a R = CH_3, 13b R = H



13a 14



(3)

15a R = Bn
15b R = Bz

(5) (a) Fenn, J. B. et al. *Science* **1989**, *246*, 64. (b) Fenn, J. B. et al. *Mass Spectrom. Rev.* **1990**, *9*, 37. (c) Smith, R. D.; Loo, J. A.; Edwards, C. G.; Barinaga, C. J.; Udseth, H. D. *Anal. Chem.* **1990**, *62*, 882. (d) Jardine, I. *Nature* **1990**, *345*, 747.

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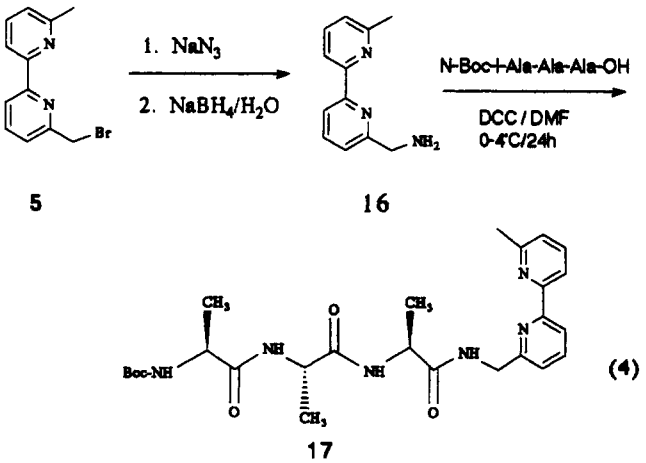
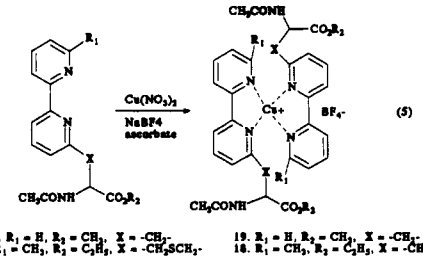
(13) O'Donnell, M. J.; Bruder, W. A. *Tetrahedron Lett.* **1982**, 4255.

In addition, the bipyridyl group itself could be introduced on the C-terminus of peptides. Amino bipyridyl compound **16** was prepared by treating bromide **5** with sodium azide under PTC conditions followed by reduction with $NaBH_4$.[14] Peptide bond formation was simply achieved by treating **16** with *N*-Boc-L-$(Ala)_3$-OH in DCC/DMF (eq 4). The ^{1}H-NMR spectrum and a peak



at m/z = 513 in the FAB-MS spectrum of **17** indicated the formation of a tripeptide containing the bipyridyl ligand.

Copper Complex Formation. With new ligands **7–10**, **13a**, **15a**, **15b**, and **17** in hand we prepared the metal complexes. The reaction of bipyridyl ligands with copper nitrate followed by reduction of the Cu^{2+} complex with ascorbate had been reported for complexes **1–3**. In a similar manner, copper complexes **18** and **19** could be formed by reaction of the respective ligands **7** and **13a** with $Cu(NO_3)_2$ in aqueous CH_3OH (1:1), followed by reduction with ascorbate. Conversion of **7** into complex **18** and **13a** into complex **19** proceeds in 72% and 30% yields, respectively (eq 5).



13a, R_1 = H, R_2 = CH_3; X = $-CH_2-$. 19, R_1 = H, R_2 = CH_3, X = $-CH_2-$.
7, R_1 = CH_3; R_2 = C_2H_5, X = $-CH_2SCH_2-$. 18, R_1 = CH_3, R_2 = C_2H_5, X = $-CH_2SCH_2-$.

Copper complexes **2**, **3**, **18**, and **19** could be purified by flash chromatography or preparative TLC and are air stable. In a similar way, we have also prepared and purified copper complexes of **9** and **10**. Related Zn^{2+} and Ru^{2+} complexes as well as copper complexes of ligands **15a,b** and **17** were prepared and characterized by ESI-MS as described below.

Electrospray Ionization MS. We have used the new technique of ESI-MS to further study these complexes. Salts and cationic metal complexes[15,16] can be injected *directly in solution* into the ESI mass spectrometer. For example, a solution of 50 μg/mL of complex **2** in methanol is injected at 1.5 μL/min to provide the spectrum shown in Figure 1a. The cation $(mbp)_2Cu^+$ at m/e = 403 and an ion showing loss of one ligand at m/e = 233 are the only peaks in the spectrum. Similarly, complex **3** provides the

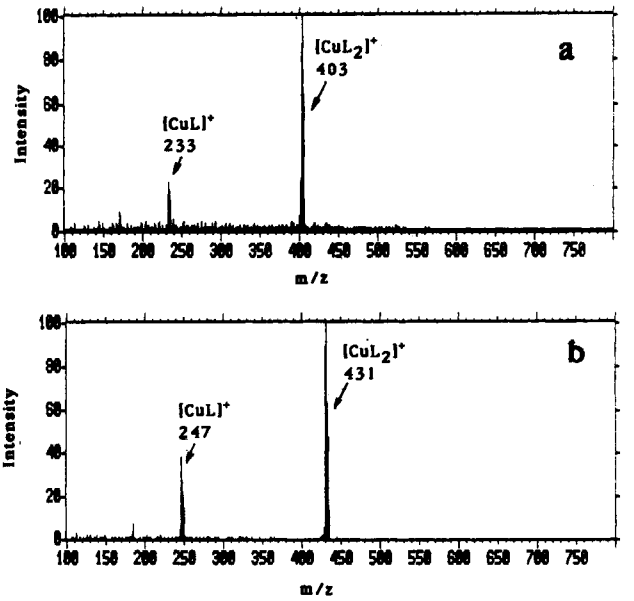


Figure 1. (a) Electrospray spectrum of complex **2**, $((mbp)_2Cu^+BF_4^-$ in methanol. $[CuL]^+$ = 233, $[CuL_2]^+$ = 403. (b) Electrospray spectrum of complex **3**, $(dmbp)_2Cu^+BF_4^-$ in methanol. $[CuL]^+$ = 247, $[CuL_2]^+$ = 431.

spectrum shown in Figure 1b which shows cation $(dmbp)_2Cu^+$ at m/e = 431 and the M – ligand ion $(dmbp)Cu^+$ at m/e = 247. These spectra are typical of the incredibly clean spectra we see for cationic complexes.

Analysis of the (bpy-Cys) complex **18** and amino acid (abp) complex **19** by ESI-MS likewise provides the expected cations 809 and 661, respectively (Figure 2a and Figure 2b). Besides a typical $[M^+$ – ligand] ion, peaks at 300 [protonated ligand] and 322 [ligand + Na^+] may be seen in Figure 2b.

Other complexes have been characterized using ESI-MS, including divalent complexes $(bpy)_2Zn^{2+}$ (m/e = 188, Figure 3, see supplementary material) and **13b** $(bpy)_2Ru^{2+}$ (m/e = 349, Figure 4, see supplementary material).[17] Bear in mind that doubly-charged cations appear at $^1/_2$ their formula weight. Most interesting from the point of view of the potential use of bipyridyl metal templates is the observation that cyclic copper(I) complexes of **9** may be prepared and their cation observed at m/e = 619 (Figure 5, supplementary material). We have previously reported[6] the ESI-MS spectrum of [**10** + Cu^+] which shows the cation as the only peak in the spectrum.

The ESI-MS spectrum showed (Figures 6 and 7, see supplementary material) that dipeptides **15a** and **15b** formed 2M + Cu^+ (**20** and **21**) in methanol in the presence of Cu^+ as well as a major ion at M + Cu^+. The ESI-MS spectrum also showed (Figure 8, see supplementary material) that both 2M + Cu^+ **22** and M + Cu^+ complexes were formed when $Cu(CH_3CN)_4BF_4$ was added to the alcoholic solution of the tripeptide **17**. Reduced-somatostatin bis-bipyridyl compound **8** does not cleanly form a complex.

Conclusions

We have shown that bipyridyl amino acid building blocks can be prepared and their cationic metal complexes prepared, isolated, and purified. The new technique of ESI-MS has been demonstrated to be extremely helpful

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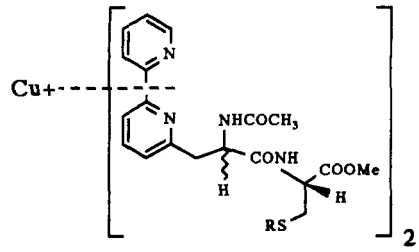
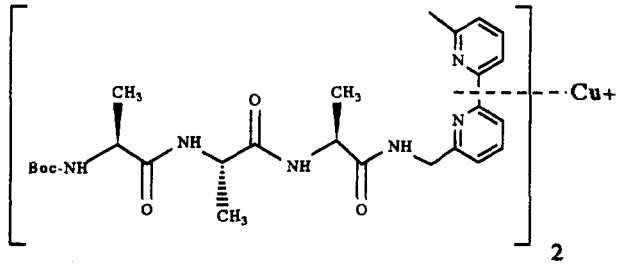
(17) Compound **13b** was obtained by treating **13a** with 1 equiv of 1 N NaOH in H_2O/dioxane for 30 min. Complex $[(13b)(bpy)_2Ru]^{2+}\cdot 2Cl^-$ was made according to the literature. Sprintschnik, G.; Sprintschnik, H. W.; Kirsch, P. P.; Whitten, D. G.; *J. Am. Chem. Soc.* **1977**, *99*, 4947.



20 R = Bn
21 R = Bz



22

in characterization of such compounds.

Experimental Section

General. General synthetic procedures are described in detail in ref 18. ESI-MS spectra were obtained either from Rockefeller University or on a Vestec Model M-200 electrospray mass spectrometer. Spectra were taken about 15–30 min after the sample preparation by electrospraying the sample solution at 3–5 μL/min. Unless specified, all the spectra were done with the following instrument settings: needle voltage, 2.0–2.4 kV; electrospray chamber temperature, 45–60 °C; nozzle voltage, 200 V; block temperature, 245–250 °C; lens temperature, 120 °C; repeller voltage, 20 V; electrospray chamber pressure, 1.4–1.5 × 10^{-1}.

Complexes 2 and 3. Prepared according to ref 9. Reported X-ray parameters for **3** matched those of the literature.[9] Compound **2** showed a cation at 403 and [M – ligand]$^+$ at 233 in the ESI spectrum (Figure 1a) in CH_3OH. Compound **3** showed a cation at 431 and [M – ligand]$^+$ at 247 in the ESI spectrum (Figure 1b) in CH_3OH.

Synthesis of 6-[(*O*-Ethylcystein-*S*-yl)methyl]-6′-methyl-2,2′-bipyridine (6). 1-Cysteine ethyl ester hydrochloride (0.070 g, 0.38 mmol) was dissolved in ethanol–water (10 mL of a 2:1 mixture) and stirred at rt under Ar. Triethylamine (116 mg, 1.14 mmol) was added, the reaction mixture was stirred for 15 min, and then bromide **5** (100 mg, 0.38 mmol) in CH_2Cl_2 (1 mL) was added. The reaction mixture was stirred for 24 h at rt. Usual workup gave a yellow viscous product, which was chromatographed (2% MeOH–CH_2Cl_2) to yield 64 mg (50%) of compound **6**. TLC: R_f = 0.67 (5% MeOH–CH_2Cl_2). HRMS: calcd for $C_{17}H_{21}N_3O_2S$, 331.1355, obsd 331.1366. ^{1}H NMR: δ 8.30 (d, J = 7.5 Hz, 1 H), 8.21 (d, J = 7.5 Hz, 1 H), 7.78 (t, J = 7.5 Hz, 1 H), 7.68 (t, J = 7.5 Hz, 1 H), 7.37 (d, J = 7.8 Hz, 1 H), 7.15 (d, J = 7.5 Hz, 1 H), 4.14 (q, J = 7.2 Hz, 2 H), 3.93 (s, 2 H), 3.7 (dd, J = 8.1, 4.5 Hz, 1 H), 2.99 (dd, J = 13.8, 4.5 Hz, 1 H), 2.75 (dd, J = 13.8, 7.8 Hz, 1 H), 2.62 (s, 3 H), 1.79 (s, 2 H), 1.20 (t, J = 7.2 Hz, 3 H). ^{13}C NMR: δ 173.9, 157.8, 155.8, 155.4, 137.6, 136.9, 123.2, 122.7, 119.3, 118.2, 61.1, 54.0, 38.1, 36.6, 24.6, 14.1.

Synthesis of 6-[(*N*-Acetyl-*O*-ethylcystein-*S*-yl)methyl]-6′-methyl 2,2′-bipyridine (7). 6-[(*O*-Ethylcystein-*S*-yl)methyl]-6′-methyl-2,2′-bipyridine **6** (20 mg, 0.060 mmol) was dissolved in 5 mL of acetic anhydride, and the reaction mixture was stirred under Ar at rt for 12 h. The volatiles were removed under reduced pressure, and the remaining reaction mixture was neutralized with anhydrous K_2CO_3 and extracted with $CHCl_3$. Usual workup and chromatography (5% MeOH–CH_2Cl_2) gave 17 mg (75%) of compound **7**. TLC: R_f = 0.38 (5% MeOH–CH_2Cl_2). IR: 3410, 3040, 2925, 1740, 1670, 1570, 1500, 1440, 1370, 1310, 1250, 1210 cm^{-1}. ^{1}H NMR: δ 8.29 (d, J = 7.8 Hz, 1 H), 8.22 (d, J = 7.8 Hz, 1 H), 7.79 (t, J = 7.5 Hz, 1 H), 7.74 (t, J = 7.5 Hz, 1 H), 7.32 (d, J = 7.8 Hz, 1 H), 7.18 (d, J = 7.5 Hz, 1 H), 7.03 (d, J = 6.6 Hz, 1 H), 4.90 (dd, J = 12.3, 6 Hz, 1 H), 4.17 (q, J = 7.2 Hz, 2 H), 3.93 (s, 2 H), 3.03 (m, 2 H), 2.63 (s, 3 H), 1.96 (s, 3 H), 1.2 (t, J = 7.2 Hz, 3 H). ^{13}C NMR: δ 170.8, 169.8, 157.9, 157.6, 156.2, 155.3, 137.7, 137.0, 123.3, 122.7, 119.7, 118.3, 61.6, 52.6, 38.0, 33.4, 24.6, 23.0, 14.0. HRMS: calcd for $C_{19}H_{23}N_3O_3S$ 373.1434, obsd, 373.146.

Synthesis of Complex 18. Compound **7** (17 mg, 0.045 mmol) was dissolved in 1:1 methanol–water (5 mL) by slight heating, and then $Cu(NO_3)_2{\cdot}6H_2O$ (5 mg, 0.022 mmol) was added. A green color indicated the formation of a Cu^{2+} complex. The reaction mixture was stirred for 10 min, and then $NaBF_4$ (5 mg, 0.045 mmol) was added, followed by the addition of sodium ascorbate (22.4 mg, 0.113 mmol) to reduce the Cu^{2+} complex to a Cu^+ complex. The red solution was stirred for 1 h at rt and then concentrated and chromatographed (5% MeOH–CH_2Cl_2) to yield 13 mg (72%) of compound **18**. TLC: R_f = 0.10 (2% MeOH–CH_2Cl_2). UV–vis: (EtOH) λ_{max} = 312, 425 nm. ESI-MS (CH_3OH):

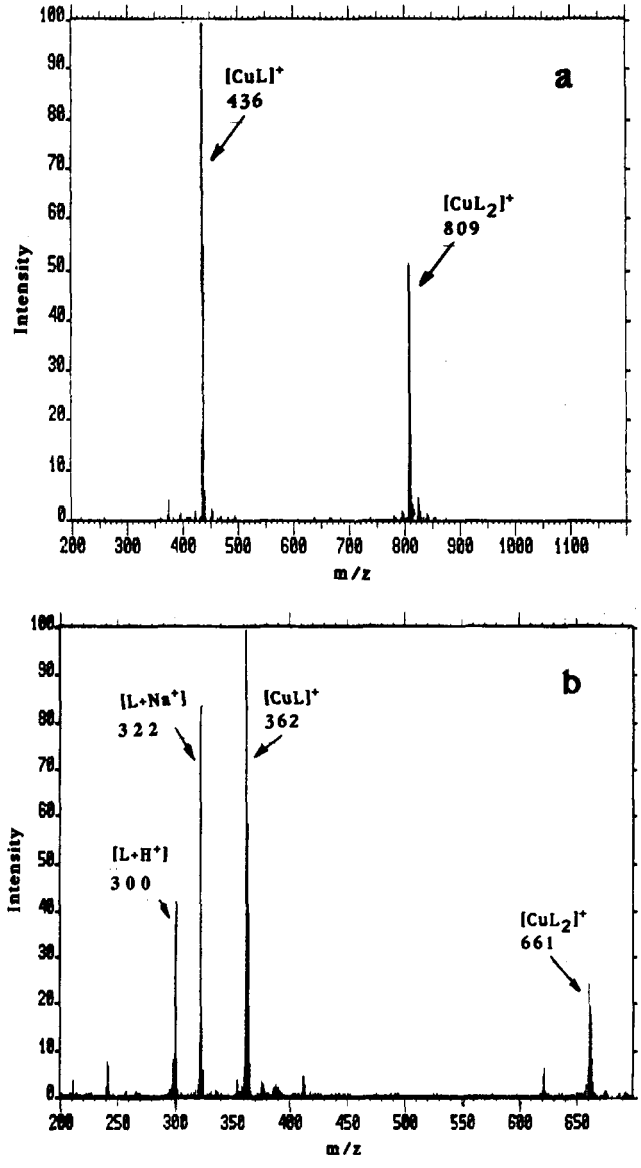


Figure 2. (a) Electrospray spectrum of complex **18** ((bpy-Cys)$_2$Cu$^+$BF$_4^-$ in methanol. [CuL]$^+$ = 436, [CuL$_2$]$^+$ = 809, where L = compound **7**, (bpy-Cys). (b) Electrospray spectrum of complex **19** (abp)$_2$Cu$^+$BF$_4^-$ in methanol. [L + H$^+$] = 300, [L + Na$^+$] = 322, [CuL]$^+$ = 362, [CuL$_2$]$^+$ = 661, where L = compound **13a** (abp).

(18) Wilson, S. R.; Digrandi, M. J. *J. Org. Chem.* **1991**, *56*, 4766.

m/z = 809 [M^+], 436 [M^+ – ligand] (Figure 2a.)

Synthesis of 1,9-Bis[(6′-methyl-2,2′-bipyridin-6-yl)-methylene]dithiononane (9). 1,9-Nonanedithiol (18.9 mg, 0.095 mmol) in benzene (5 mL) and DBU (36.2 mg, 0.24 mmol) was stirred at rt. Compound **5** (50 mg, 0.19 mmol) in benzene (1 mL) was added, and the reaction mixture was stirred for 12 h. A white precipitate of DBU·HBr appeared as the reaction proceeded. The reaction was quenched by the addition of water, and the mixture was extracted with benzene. Usual workup and chromatography (2% MeOH–CH_2Cl_2) gave 39 mg (75%) of product. FAB MS analysis indicated a mixture of **9** and the related disulfide "dimer" 1-[(6′-methyl-2,2′-bypyridin-6-yl)methylene]dithiononane-9-thiol disulfide, which could not be separated chromatographically. TLC: R_f = 0.27 (2% MeOH–CH_2Cl_2). FAB-MS: 557 (M + H, compound **9**), 747 (M + H, "dimer"). ^{1}H NMR: δ 8.2 (d, J = 7.8 Hz, 2 H), 8.13 (d, J = 7.8 Hz, 2 H), 7.70 (t, J = 7.5 Hz, 2 H), 7.60 (t, J = 7.5 Hz, 2 H), 7.31 (d, J = 7.5 Hz, 2 H), 7.10 (d, J = 7.8 Hz, 2 H), 3.80 (s, 4 H), 2.56 (s, 6 H), 2.45 (m, 4 H), 1.54 (s, 4 H), 1.21 (s, 10 H). ^{13}C NMR: δ 158.4, 155.7, 155.6, 155.5, 137.4, 136.9, 123.1, 122.6, 119.0, 118.1, 38.1, 31.5, 29.3, 29.1, 28.8, 28.4, 24.6.

Synthesis of Complexes of 9. The mixture of compound **9** and its "dimer" (20 mg, 0.035 mmol) was dissolved in 1:1 methanol–water (5 mL), and then $Cu(NO_3)_2{\cdot}6H_2O$ (6 mg, 0.035 mmol) was added. A green color indicated the formation of the Cu^{2+} complex. The reaction mixture was stirred for 10 min, and then $NaBF_4$ (8 mg, 0.078 mmol) was added, followed by the addition of 4 mg (0.18 mmol) of sodium ascorbate. The red solution was stirred for 1 h at rt and then concentrated and chromatographed (2% MeOH–CH_2Cl_2) to yield 14 mg (70%) of the complex. UV–vis (EtOH) λ_{max} = 310, 425 nm. TLC: R_f = 0.41 (5% MeOH–CH_2Cl_2). ESI-MS (CH_3OH): [M^+] = 619 (complex of **8**) and [M^+] = 809 ("dimer" complex).

***N*-(Diphenylmethylene)-∂-(2,2′-dipyridyl-6-methylene)-glycine Methyl Ester (11).** To a solution of *N*-(diphenylmethylene)glycine methyl ester (8.1 g, 32 mmol) and 6-(bromomethyl)-2,2′-dipyridyl (4.1 g, 16 mmol) **4** in CH_2Cl_2 (25 mL) was added a cooled solution of n-$Bu_4N^+HSO_4^-$ (8.5 g, 25 mmol) in 20 mL of 10% NaOH. The resulting mixture was stirred at rt for 24 h. Usual workup and chromatography (5:2 hexane–ethyl acetate) gave 5.5 g (81%) of **11** as a yellow oil which crystallized upon cooling. Mp: 90.5–91.5 °C NMR ($CDCl_3$): δ 3.39, 3.56, 4.79 (ABX, 3 H), 3.78 (s, 3 H), 6.71–8.65 (m, 17 H). Anal. Calcd for $C_{27}H_{23}N_3O2$: C, 76.94; H, 5.50; N, 9.97. Found: C, 76.71; H, 5.58; N, 9.81.

∂-(2,2′-Dipyridyl-6-methylene)glycine Methyl Ester Hydrochloride (12). To a cooled solution of **11** (2.11 g, 5 mmol) in 30 mL of ether was added 15 mL of 1 N HCl dropwise over 30 min. The reaction mixture was stirred at rt for about 24 h. The aqueous phase was separated and evaporated to give 1.84 g (100%) of compound **12** as an oil. NMR (D_2O): δ 3.65 (t, 2 H), 3.77 (s, 3 H), 4.88 (t, 1 H), 7.62 (d, 1 H), 8.08 (t, 2 H), 8.21 (d, 1 H), 8.62 (d, 1 H), 8.70 (t, 1 H), 8.86 (d, 1 H).

***N*-Acetyl-∂-(2,2′-dipyridyl-6-methylene)glycine Methyl Ester (13a).**[17] To a cooled solution of **12** (1.9 g, 5 mmol) in DMF (15 mL) and TEA (2.09 mL, 15 mmol) was added a mixture of Ac_2O (0.47 mL) and TEA (0.70 mL) in DMF (6 mL). The combined solution was stirred in an ice bath for 15 min and then at rt for 5 h. Usual workup gave 0.98 g (65%) of compound **13a** as a yellow oil which solified upon cooling, mp 97–99 °C. ESI-MS (5% AcOH–CH_3OH): m/z = 300 (M + H^+), 322 (M + Na^+). NMR ($CDCl_3$): δ 1.96 (s, 3 H), 3.69 (s, 3 H), 3.36, 3.52 and 5.05 (ABX, 3 H), 6.94 (d, 1 H), 7.13 (d, 1 H), 7.30 (t, 1 H), 7.73 (t, 1 H), 7.82 (t, 1 H), 8.28 (t, 2 H), 8.66 (d, 1 H). ^{13}C NMR ($CDCl_3$, ppm): 23.17, 38.34, 50.83, 119.08, 120.88, 123.86, 137.34, 148.57, 149.75, 155.36, 155.93, 156.25, 169.77, 172.28. IR (CH_2Cl_2): 3434 (weak), 1746, 1676. Anal. Calcd for $C_{16}H_{17}N_3O_3{\cdot}{}^1/_4H_2O$: C, 63.25; H, 5.81; N, 13.83. Found: C, 63.44; H, 5.86; N, 13.76.

Complex 19 Formation. $Cu(NO_3)_2$ (0.5 equiv) and excess $NaBF_4$ were added to a solution of compound **13a** (1 equiv) in 1:1 methanol–water. After the solution was stirred for 10 min, ascorbate (slightly excess) was added, and the reaction mixture immediately became dark red. Usual workup and preparative TLC (silica, CH_3OH) gave the complex **19** as dark red oil. ESI-MS (CH_3OH): m/z = 661. NMR ($CDCl_3$): δ 1.96 (s, 3 H), 3.38, 3.51, and 4.98 (ABX, 3 H), 7.29–8.72 (m, 7 H). UV (CH_3OH): λ_{max} = 300 nm (ε 18654), 449 nm (ε 3429).

Dipeptides 15a and 15b. The acyl azide coupling method[19] was used to produce **15a** and **15b** in ~28% yield respectively from abp hydrazide **14** (prepared from abp **13a** in 68% yield) and *S*-benzyl or *S*-benzoyl-L-cysteine methyl ester hydrochloride. Dipeptide **15a**. MP: 144–5 °C. ESI-MS (CH_3OH, m/z): 494 (M + H^+), 516 (M + Na^+), 532 (M + K^+), 556 (M + Cu^+), 1050 (2M + Cu^+). Anal. Calcd for $C_{26}H_{28}N_4O_4S{\cdot}{}^1/_2H_2O$: C, 62.25; H, 5.83; 11.17. Found: C, 62.72; H, 6.04; N, 10.75. Dipeptide **15b**. Mp: 59–61 °C. ESI-MS (CH_3OH, m/z): 508 (M + H^+), 530 (M + Na^+), 546 (M + K^+), 570 (M + Cu^+), 1078, (2M + Cu^+). Anal. Calcd for $C_{26}H_{26}N_4O_5S{\cdot}{}^1/_2H_2O$: C, 60.57; H, 5.28; N, 10.87. Found: C, 60.69; H, 5.37; N, 10.20.

Tripeptide 17. A cooled mixture of Boc-$(Ala)_3$-OH,[18] 6-(aminomethyl)-6′-methyl-2,2′-bipyridyl[14] (**16**), and DCC in DMF was kept overnight in the refrigerator. After filtration and concentration, the residue was purified on preparative TLC (1:1 CH_3OH–CH_3COOEt) to give 90 mg (59%) of **17** as a light yellow solid, mp 175–7 °C. ESI-MS (CH_3OH): 576 (M + Cu^+), 1090 (2M + Cu^+), 514 (M + H^+), 536 (M + Na^+), 552 (M + K^+). Anal. Calcd for $C_{26}H_{36}N_6O_5$: C, 60.92; H, 7.08; N, 16.39. Found: C, 62.68; H, 7.49; N, 14.16.

Synthesis of Bis(6-methyl-2,2′-bipyridyl-6′-methylene)-*S*,*S*′-somatostatin (8). To a solution of cyclic somatostatin (3 mg, 1.8 μmol, Bachem Corp.) in deionized water (0.6 mL) was added a solution of DTT (6 mg, 38.9 μmol, Sigma) in trizma buffer (6 mL, pH = 8.3). After 1 h a mixture of **5**·2HBr (78 mg, 184 μmol), TEA (51 μL, 366 μmol, Aldrich), and ethanol (6 mL) was added to the above solution. After 4.5 h, the reaction mixture was acidified with acetic acid to pH ~4 and lyophilized. The crude product was redissolved in H_2O, and extracted with $CHCl_3$ to remove unreacted **5** and its hydrolysis product 6-(hydroxymethyl)-6′-methyl-2,2′-bipyridine and again lyophilized. ESI-MS: [M + $4H_3O^+$] = 520.

In Situ Metal Complexation/ESI. Peptide metal complexes could be prepared and characterized by ESI-MS without isolation. In some cases the transition-metal complexes appeared not to be stable to chromatography. A solution of ligand (10^{-3}–10^{-4} M) in water, acetonitrile, or methanol was treated with about 0.5 equiv of $Cu(CH_3CN)_4BF_4$ or $Cu(CH_3CN)_4PF_6$ and allowed to react at room temperature for 10–30 min. ESI-MS spectra were obtained in the usual way by slow (4–5 μL/min) infusion of the solution into the instrument. Spectra represent average of 1–2 min after reaching a stable ESI current.

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Supplementary Material Available: ESI-MS spectra of **9**, $[bpy]_2Zn^{2+}$, $[bpy]_2$[**13b**]Ru^{2+}, **20–22** (Figures 3–8) as well as the 300-MHz NMR spectra of **11–13a, 15a,b,** and **17** (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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