Coordination compounds as building blocks: simple synthesis of Ru^{II}-containing amino acids and peptides

Dennis J. Hurley, Jeffrey R. Roppe and Yitzhak Tor*

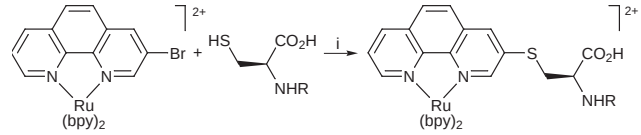
Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California 92093-0358, USA. E-mail: ytor@ucsd.edu

Received (in Corvallis, OR, USA) 15th February 1999, Accepted 12th April 1999

Ru^{II}-containing amino acids and short peptides are obtained in high yields *via* direct displacement reactions of $[(bpy)_2Ru(3\text{-bromo-}1,10\text{-phenanthroline})]^{2+}(PF_6^-)_2$ with amino acids and peptides that contain thiolate and phenolate nucleophiles.

Metal-containing amino acids and peptides are key components for the study of photo-induced energy– and electron–transfer processes,[1–3] as well as for the development of chemosensors and labeling reagents.[4,5] These organic–inorganic hybrid molecules have been constructed *via* three predominate approaches: (i) coordination of metal complexes to donor-containing natural amino acids (*e.g.* histidine),[6,7] (ii) conjugation of complexes to peptide termini,[2,3] and (iii) synthesis of chelator-containing amino acids followed by metal coordination.[8–12] These routes typically require the exposure of peptides to reactive metal precursors or necessitate the multistep synthesis of new amino acids. Although productive, these methods are elaborate and do not always provide full control over the final structure and absolute configuration at the metal center. Here we report a simple one-step synthesis of metal-containing amino acids and peptides *via* the direct reaction between nucleophile-containing amino acids (*e.g.* cysteine, tyrosine) and functionalized coordination compounds. Reactions of $[(bpy)_2Ru(3\text{-bromo-}1,10\text{-phenanthroline})]^{2+}(PF_6^-)_2$ **1** with free or *N*-protected amino acids, as well as short peptides, provide the Ru^{II}-containing amino acids in a single step with predetermined stereochemistry at the metal center.

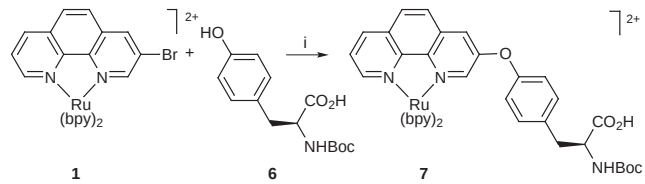
We have previously reported that functionalized tris-chelate complexes (*e.g.* **1**) are excellent substrates for palladium-mediated cross-coupling reactions[13,14] and nucleophilic aromatic substitutions.[15] This unprecedented reactivity has been attributed to the increased electrophilicity of the 3-bromo-1,10-phenanthroline ring upon metal coordination.[15] Thus, when **1** is reacted with L-cysteine **2** in the presence of a weak base (*e.g.* Na_2CO_3) a smooth reaction takes place within 1–3 h giving the desired modified amino acid **4** in good yields (Scheme 1, Table 1).† Identical reaction conditions can be utilized to incorporate the octahedral complex into *N*-protected amino acids. Thus, reacting **1** and *N*-Boc-L-cysteine **3** in DMF–H_2O (1 : 1) at slightly elevated temperatures (45–55° C) affords the protected derivative **5**. Similarly, deprotonation of the phenolic side chain in *N*-Boc-L-tyrosine **6** followed by a reaction with **1** gives the expected metal-containing amino acid **7** (Scheme 2).



Scheme 1 *Reagents and conditions*: i, Na_2CO_3, H_2O–DMF (1 : 1), 55 °C, 1 h.

The compounds synthesized represent a novel family of photo- and redox-active Ru^{II}-containing amino acids. Their absorption spectra show intense bands in the UV region due to the overlapping π–π* transitions of the bipyridine and phenanthroline ligands (Table 1). The presence of a phenanthroline moiety with a heteroatom-containing substituent results in the appearance of lower energy bands between 320–350 nm (Table 1).[15] The tris-chelated Ru^{II} centers in **4**, **5** and **7** give rise to the typical visible metal to ligand charge transfer (MLCT) bands that are centered around 450 nm. Upon excitation at this wavelength, all the Ru^{II}-containing amino acids emit intensely at 600–615 nm (Table 1). Cyclic and square-wave voltammetry show the expected $Ru^{III/II}$ couple at $E_{1/2} \cong +1.0$ V *vs.* Ag/Ag+ (Table 1).‡

Mild substitution reaction conditions can be employed for the incorporation of octahedral Ru^{II} complexes into short peptides. Thus, reacting glutathione **8** (γ-L-glutamyl-L-cysteinylglycine)

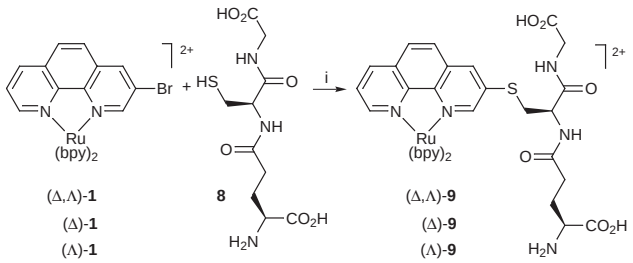


Scheme 2 *Reagents and conditions*: i, Na_2CO_3, H_2O–DMF (1 : 1), 55 °C, 1 h.

Table 1 Preparation, spectral and electrochemical data for metal-containing amino acids and peptides

Product	Yield (%)[a]	MS[b]	λ/nm (ε/10^4 M^{-1} cm^{-1})[c]	$E_{1/2}(Ru^{III/II})$[d]/V	λ_{em}[e]/nm
4	74–87	858.2 (M^+), 356.6 (M^{2+})	242 (4.3), 286 (7.5), 338 (1.7), 450 (1.5)	0.964	612
5	76–85	958 (M^+), 407 (M^{2+})	246 (4.1), 286 (6.9), 344 (1.8), 450 (1.5)	1.000	611
7	72	1018 (M^+), 436 (M^{2+})	234 (4.4), 286 (6.7), 320 (1.5), 446 (1.5)	0.992	601
9	58–63[f]	898.0 (MH^+), 449.5 (MH_2^{2+})	246 (4.0), 286 (7.1), 343 (1.6), 450 (1.4)	0.968	612
10	82–90	1043 (M^+)	246 (4.3), 286 (7.6), 344 (1.9), 448 (1.6)	0.996	614

[a] Isolated yields of chromatographed complexes. *N*-Boc protected derivatives were isolated as their PF_6^- salt. Unprotected derivatives were HPLC purified and isolated as the $CF_3CO_2^-$ salt. [b] Electrospray ionization mass spectra. [c] Measured in MeCN (for **5**, **7** and **10**) and MeOH (for **4**, **9**). [d] Measured in MeCN containing 0.1 M $Bu_4N^+PF_6^-$ *vs.* Ag/Ag^+. Under these conditions $E_{1/2}$ = 0.084 V for Fc/Fc^+. [e] Metal-centered emission taken in water upon excitation at 450 nm. [f] Individual yields for HPLC-purified products: **Δ,Λ-9** (60–63%), **Δ-9** (60%) and **Λ-9** (58%).



Scheme 3 *Reagents and conditions*: i, Na_2CO_3, H_2O–DMF (1 : 1), 65 °C, 1 h.

with the racemic complex **1** in a mixture of DMF–water gives the desired substituted peptide **9**, as a mixture of the two epimeric products (Scheme 3). The use of functionalized coordination compounds as building blocks permits the incorporation of coordination compounds with predetermined absolute configuration at the metal center.[16] Thus, reacting glutathione **8** with the enantiomerically-pure complexes **Λ-1** and **Δ-1** gives the diastereomerically-pure metal-containing peptides Λ-**9** and Δ-**9**, respectively (Scheme 3). The CD spectra of the various products clearly reveal the opposite absolute configuration at the two epimeric metal-containing peptides Λ-**9** and Δ-**9** (Fig. 1).[16]

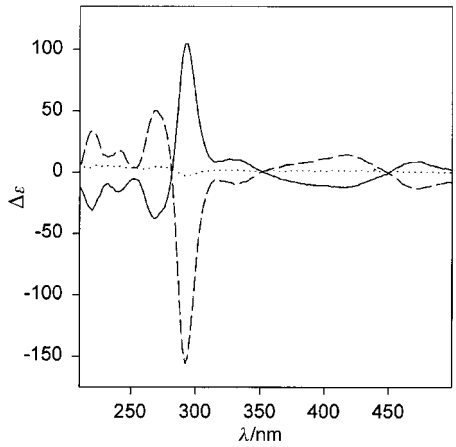
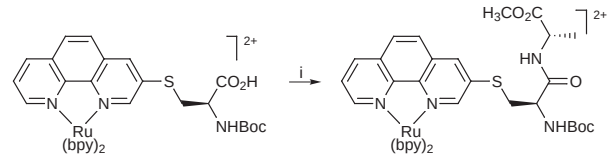


Fig. 1 CD spectra in MeOH of Λ-**9** (solid), Δ-**9** (dashed) and Λ,Δ-**9** (dotted).

Protected metal-containing amino acids such as **5** and **7** are useful building blocks for peptide synthesis. Their condensation with other suitably protected amino acids provides short metal-containing peptides. Thus the reaction of **5** with L-alanine methyl ester in the presence of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate ($BOPPF_6$), *N*-hydroxysuccinimide and DMAP proceeds rapidly at room temperature to give the fully protected dipeptide **10** in high yield (Scheme 4, Table 1). As is evident from the data presented in Table 1, the short metal-containing peptides **9** and **10** exhibit photophysical and electrochemical characteristics similar to their metal-containing precursors.

The direct incorporation of metal complexes into amino acids and peptides provides a facile new route for the synthesis of novel photo- and redox-active biomolecules. Modifying the side-chains of naturally-occurring amino acids places the polypyridine Ru^{II} complexes in close proximity to the peptide backbone. These derivatives are therefore useful building blocks for the study of photophysical processes in peptides and proteins. We are currently exploring these directions *via* the construction of metallated peptides with well-defined secondary structures.



Scheme 4 *Reagents and conditions*: i, L–alanine methyl ester, $BOPPF_6$, *N*-hydroxysuccinimide, DMAP, DMF, 10 min at room temp.

We gratefully acknowledge the National Institutes of Health (GM58447) for financial support.

Notes and references

† Representative procedures: A solution of L-cysteine (20 mg, 0.13 mmol) and Na_2CO_3 (40 mg, 0.38 mmol) in degassed water (1 ml) is added to a degassed solution of **1** (40 mg, 0.042 mmol) in DMF (1 ml). The reaction mixture is kept at 55 °C for 1 h. It is then diluted with water (10 ml) and washed with CH_2Cl_2 (3 × 50 ml). The aqueous phase is evaporated to dryness and the dark red residue is HPLC-purified on a C8 semi-preparative reversed-phase column using 0.1% TFA in MeCN–0.1% TFA in H_2O (1 : 7.3) as eluent to afford **4** (34 mg, 87% yield) after lyophilization. The more lipophilic *N*-Boc protected metal-containing amino acids are chromatographed on silica gel using 0.5% saturated aq. KNO_3 in 10–15% H_2O in MeCN. In these cases the products contain a carboxylate anion as an internal counterion. The free carboxylic acid derivative can be obtained by dissolving the *N*-protected derivative in dilute aqueous HPF_6 followed by extraction into CH_2Cl_2.The organic phase is then dried (Na_2SO_4), filtered and evaporated to yield the desired product. All new compounds have been fully characterized by UV–VIS spectroscopy, ESI MS, IR and ^1H NMR as well as cyclic and squarewave voltammetry.

‡ The *N*-protected derivatives showed reversible cyclic voltammograms ($\Delta E_p \cong 60$–70 mV, $i_a \cong i_c$) while the free amines gave irreversible CV, and their $E_{1/2}$ values were determined using squarewave voltammetry.

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Communication 9/01272I