High Arenophilicity and Water Tolerance in Direct Derivatization of Peptides and Proteins by Metal π-Coordination

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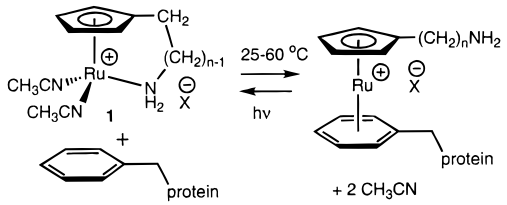
Site-specific modification of proteins leads to invaluable structural and reactivity information for biochemical and medicinal studies and to new biotherapeutics.[1] Modification frequently depends on reaction of electrophilic reagents with nucleophilic atoms of the protein.[1] Classic examples include reaction of BrCN at S,[2a] RNCS at N,[2b] and electrophilic halogens at the tryptophan π-system.[2c] The organic reagents which react with tryptophan or tyrosine react much more slowly or not at all with the less electron-rich phenylalanine. Moreover, the same electrophilic or oxidizing reagents also enter into unwanted side reactions with nucleophilic S- or N-donors.[1] Here we show that metal π-complexation to the arene-containing amino acid phenylalanine can succeed even in the face of S- and N-donor ligands found in methionine, cystine, or histidine, leaving these residues unchanged. Metal π-complexation to amino acids and dipeptides has been studied in nonaqueous solvents,[3] and metals have been covalently attached to proteins indirectly, by using organic ligands which are attached to mercapto or amino sites.[4] In contrast, here we report the attachment of a metal directly to an arene ring in a protein in water. Our strategy for reversible derivatization of aryl amino acids exploits the capability of $CpRu^+$ and Cp^*Ru^+ fragments to bind to arenes,[3,5] providing air-stable products from which arene subsequently can be released on photolysis. We have made chelates **1**, which unlike $CpRu^+$ and Cp^*Ru^+ derivatives are new compounds featuring a bound amino ligand,[6] a nucleophile or water-solubilizing group locked by coordination until release by an incoming aryl amino acid. A series of experiments culminating with reaction of the small protein secretin at 10^{-4} M concentrations demonstrate high arenophilicity and water tolerance in rapid room-temperature reactions, promising conditions for applications to proteins.



Scheme 1 outlines the preparation of homologous chelates **1a**,**b**. Without purification of intermediates, thallium salts **2** were formed in 56−60% overall yield from salts $Br(CH_2)_nNH_3Br$ by monoalkylation of CpLi (2 equiv),[6d−8] protection of the amino nitrogen, and deprotonation of the resulting cyclopentadiene mixture using TlOEt.[7b] Metathesis between **2** and $[Cl(\mu\text{-}Cl)Ru(\eta^6\text{-arene})]_2$ and exchange to the noncoordinating PF_6^- anion[5a] provided sandwich complexes **3**, which were deprotected by hydrogenolysis.[5e,9] Photolysis of **4** in CH_3CN produced **1**. The rate of reaction was greater for the longer side chain ($n = 3$) and for the smaller arene.[10a] Conversely, arene complexation to **1** in CD_3NO_2 is about 2 times faster for the shorter side chain, comparisons which may indicate that the chelate ring in **1a** is more strained.[10b]

Arenes (Scheme 2) of widely disparate steric demand, such as 1,3-dimethylbenzene (**5a**) and 1,4-di-*tert*-butylbenzene (**5b**) readily opened chelate **1a** under similar conditions (CD_3NO_2 or CD_3OD, 60 °C, 1−6 h), to give sandwich complexes **6**. Amino acid derivatives **5c**−**h** could also be used, to afford **6c**−**h**. These results and the ones below show that all functional groups present in proteins except for thiols are tolerated.[11] As seen for other η^6-arene complexes, the coordination of the metal to the arene shifted the arene proton resonances upfield by approximately 1 ppm and shifted the C_5H_4R proton resonances downfield by about 1 ppm.[8] The η^6-arene complexes could be analyzed by MALDI-MS, showing the robust nature of the Ru−arene linkage even on irradiation by the laser.[12]

Recognition of arene rings in proteins will succeed only if coordination of the metal to other strong ligands such as amines

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(8) Full preparative details and characterization of new compounds, including NMR and mass spectra for secretin complexation experiments appear in Supporting Information.

(9) Amino protection and deprotection were necessary: the Tl salt corresponding to **2a** could be made, but metathesis with $[(arene)RuCl(\mu\text{-}Cl)]_2$ was much slower, and other species, perhaps involving N-coordination by Tl, were present.

(10) (a) Times for completion: **1a** from **4a**−η^6-benzene, 8 h; from **4a**−η^6-mesitylene, > 4 d; **1b** from **4b**−η^6-mesitylene, 2 d. (b) We have not yet been able to grow crystals of **1a**,**b** suitable for X-ray diffraction.

(11) Presumably, thiol interference could be avoided by S-alkylation or oxidization prior to complexation; see for example ref 1a, pp 63−90.

(12) (a) The MALDI-TOF laser used produces pulses of 337-nm light, and the reported λ_{max} for $CpRu(\eta^6\text{-benzene})PF_6$ is 325 nm.[12b] In some experiments, small losses of the Ru fragment may have occurred; this did not occur at all while acquiring electrospray-MS data on secretin complex **6k**. (b) McNair, A. M.; Schrenk, J. L.; Mann, K. R. *Inorg. Chem.* **1984**, *23*, 2633−2640.

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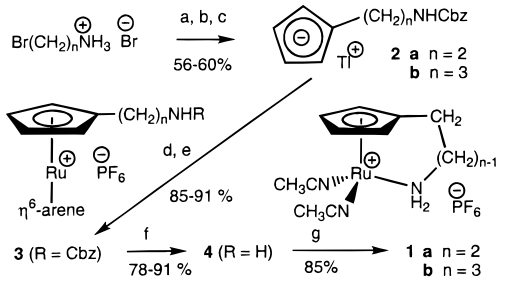
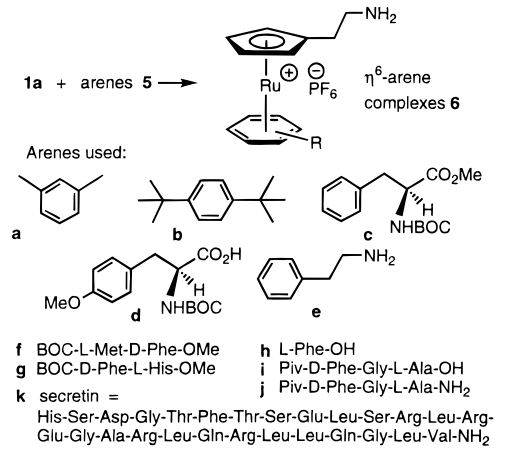
Scheme 1[a]



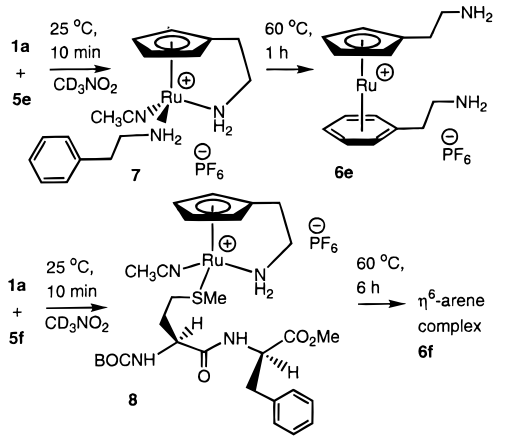
[a] (a) 2 CpLi, THF, 5 °C, 20 h; (b) Cbz-Cl, *N*-methylmorpholine, Et_2O, 25 °C, 1.5 h; (c) TlOEt, Et_2O, 25 °C, 0.5 d; (d) **2** + 0.5 $[Cl(\mu\text{-}Cl)Ru(\eta^6\text{-arene})]_2$ (arene = benzene or mesitylene), CH_3CN, 25 °C, 5 h; (e) KPF_6, H_2O; (f) H_2 (40 psig), 10% Pd/C, 3−8 h; (g) Pyrex-filtered UV light, CH_3CN, time indicated in ref 10a.

Scheme 2[a]



[a] Piv = *N*-pivaloyl.

Scheme 3



(e.g., lysine), thioethers and disulfides (methionine and cystine), and histidine does not interfere. A series of control experiments using **1a**,**b** showed conclusively that η^1-S or η^1-N coordination is kinetically favored but that arene coordination is thermodynamically preferred. For example (Scheme 3), when **1a** was mixed with phenylethanamine (**5e**) in CD_3NO_2 at 25 °C, within 10 min **1a** and the free amine had disappeared, and 1 mol of CH_3CN and a new complex, formulated as **7**, were observed. Particularly diagnostic spectral features for **7** include four slightly broad one-proton multiplets between δ 3.70 and 4.12 ppm, consistent with the maintenance of three σ-donor ligands on Ru and the formation of a chiral complex in which all four C_5H_4R protons are inequivalent. A complex similar to **7** from $[Cp^*Ru(CH_3CN)_3]^+TfO^-$ and tryptamine had been seen to decompose.[5f] In contrast, warming the mixture containing **7** at 60 °C for 1 h led to its disappearance and formation of **6e**, revealed especially well by downfield shifts of the C_5H_4R proton resonances (δ 5.29 and 5.39 ppm, each t, J = 2 Hz, 2H) and upfield shifts of the arene proton resonances (δ 6.05−6.22 ppm, m, 5H). BOC-Met-Phe-OMe and BOC-Phe-His-OMe behaved similarly, initially giving η^1-complexes such as **8** at 25 °C, which converted to η^6-arene complexes (e.g, **6f**) within 6 h at 60 °C. While being a significant demonstration of the arenophilicity of the Ru fragment, these conversions may only serve as models for proteins in which the strong S- or N-donor is close to Phe, and intramolecular migration of the Ru fragment is feasible. That an S-donor need *not* be connected to Phe was shown by mixing **1a**, BOC-Met-OMe, and BOC-Phe-OMe in a ratio of 1:1:1. Within minutes at 25 °C, an η^1-S complex formed; warming at 60 °C for 1 h led to η^6-arene complex **6c**. Tolerance of disulfides was shown by similar results from a mixture of **1a** and unprotected cystine and Phe (molar ratio 1:1:1).

The high arenophilicity of the complexes prompted binding studies of **1a** with the gastrointestinal hormone secretin[13] (mass 3039 daltons). Secretin (**5k**) contains 27 residues, including a unique Phe, along with N-terminal His, four Arg, one Asp, and two Glu, all containing potentially interfering functional groups. Secretin (0.1 or 0.25 mg) and an internal standard were dissolved in deoxygenated CD_3OD or D_2O in a 5-mm NMR tube.[8] After acquisition of the ^1H NMR spectrum, chelate **1a** was added as a solution in CD_3OD.[14] Optimum conditions for consumption of secretin, monitored by the upfield shift of the Phe C_6H_5 proton resonances, appeared to require only 3.3 to 10 equiv of **1a**.[15] Preliminary indications point to a remarkable and useful solvent effect that is, whereas the reactions in CD_3OD require mild heating at 60 °C to be completed within 5 h, similar reactions in D_2O are completed within 8 h *at room temperature*, promising for applications to protein chemistry. The resulting ruthenated protein **6k** (yield 70−100% as determined by NMR integration[8]) could be purified by reversed-phase HPLC, analyzed by MALDI-MS or electrospray-MS, and subjected to automated Edman degradation, which by lack of an HPLC peak for the phenylthiohydantoin of Phe further verified that the Phe residue had been covalently modified. Finally, liberation of arene from sandwich complexes **6** by irradiation in water−acetonitrile mixtures is possible.[16]

In summation, others have studied either π-complexation to small amino acid derivatives in nonaqueous solvents[3] or attachment of preformed π-complexes to biomolecules using organic chemistry.[4e] In distinct contrast, we have shown that direct π-complexation of a protein in aqueous medium is possible, even in the presence of other coordinating groups. The implications of enhanced reactivity of **1** in water and further applications involving the amino-containing side chain are under investigation.

Acknowledgment. We thank Minh Huynh for preparing a number of peptides and John Nemmers of Finnigan Corporation for electrospray-MS data on **6k**. Engelhardt Corp. gave $RuCl_3 \cdot xH_2O$.

Supporting Information Available: Full preparative details and spectral data for new compounds, and representative NMR and MS spectra for **6k** (29 pages print/PDF). See any current masthead page for ordering information and Web access instructions.

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(14) The amount of CD_3OD thus added (25−75 μL) is small compared to the amount of buffered D_2O (1 mL). The triflate analogue **1a**-OTf, showing better solubility in CD_3OD, was used in some of these experiments.[8]

(15) After complexation was complete and before HPLC purification, 3-mercaptopropionic acid (ca. 20 mol per mol of **1a** used) was added to scavenge any excess metal which might be loosely bound to the protein.[8]

(16) Pyrex-filtered light from a 450-W Hg lamp was used on (η^6-benzene)Ru(η^5-$C_5H_4CH_2CH_2NH_2$)$^+$ Cl^- in D_2O−CD_3CN (1:3) for 32 h to form benzene (91% yield by NMR integration) and **1a** (presumed to have the Cl^- counterion).[8]