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CYTOCHROME *C* OXIDASE ACTIVE SITE MIMICS: NEW LIGANDS FOR COPPER AND AN UNEXPECTED OXIDATIVE C-C BOND FORMATION‡

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Abstract – Ligands that mimic the unusual active site polypeptide of cytochrome *c* oxidase have been prepared in overall good yield; structures of the corresponding Cu(II) complexes are secured by single crystal x-ray analysis. The key step in the synthesis of the ligands is the coupling of a suitable organolead(IV) phenol derivative with the ε-N of the histidine imidazole ring. In the process of preparing crystals of a tridentate ester ligand an unusual oxidative C-C bond-forming reaction occurs that affords the imidazotetrahydropyridine ring system.

Cytochrome *c* oxidase (C*c*O) is the terminal enzyme in the electron transport chain, catalyzing the reduction of dioxygen to water. This redox reaction is coupled to proton translocation across the inner mitochondrial membrane, creating an electrochemical proton gradient that serves as the driving force for ATP synthesis.[1] While C*c*O has been studied for decades, the detailed mechanism of dioxygen reduction is still unknown.[2] The active site of C*c*O, centered on the heme a_3/Cu_B bimetallic center, is unique in the copper binding site (Figure 1). Posttranslational modification[3] of the 200 kDa enzyme produces a new C-N bond between His_{240} and Tyr_{244},[4] *i* and *i*+4 residues along an α-helical section of subunit 1.[5] Thus, a rigid binding site is constructed for Cu_B, which is ligated to two distal histidine residues together with His_{240}, in close proximity heme a_3. The side chain cross-linked Tyr_{244} has been proposed to play an important role in proton and electron transfer to the iron-bound oxygen molecule, but direct evidence has been lacking.

‡ This paper is dedicated to Professor Steve Weinreb for his many contributions to heterocyclic chemistry

Our work in this area stems from an interest in understanding the intricacies of the biological O_2 reduction reaction. For this purpose we successfully constructed compound (**1**) as a model system of the active site His-Tyr system and explored some of the basic properties of the new biaryl construction.[6] Key to the success of this synthetic work was the use of aryllead(IV) reagents for the formation of the desired C-N bond under mild conditions.[7] However, to explore the role of the copper-polypeptide interaction required the construction of a more elaborate set of compounds that would function as ligands for the metal. To that end we prepared compounds (**2**-**5**), which incorporate one (**2** and **3**) and two (**4** and **5**) pyridine units. It was expected that these donor residues would act as additional ligating elements (together with the cross-linked imidazole and sp^3 nitrogens) for copper, allowing us to explore the relationship of the metal to the phenol (tyrosine) functionality.

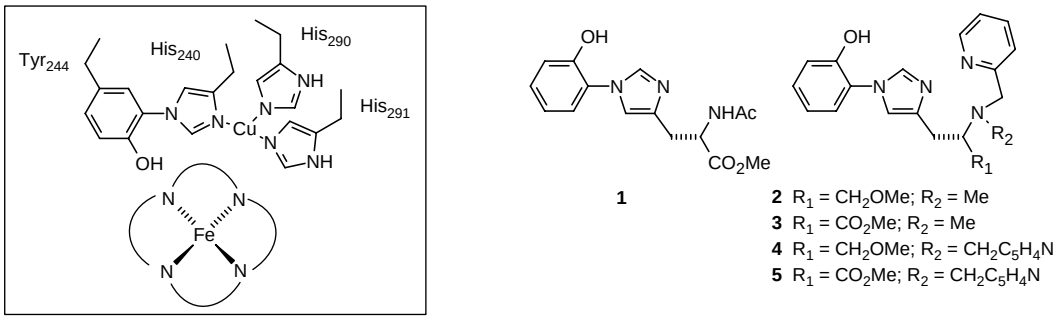


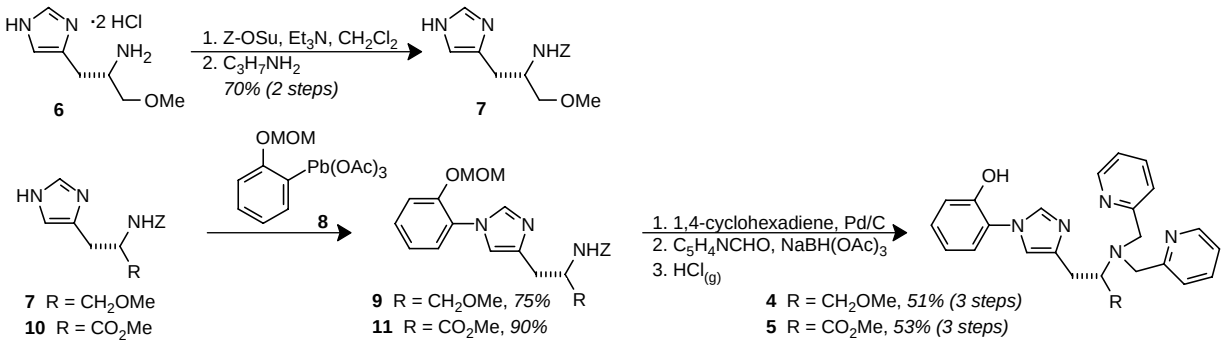
Figure 1. Schematic depiction of the heme a_3/Cu_B active site of cytochrome *c* oxidase and C*c*O active site mimics

Our first publication in this area describes the synthesis of compounds (**2** and **3**) together with solid state structures of the corresponding copper complexes and physical data that relates to the question of the copper/tyrosine interaction through the imidazole ring of the histidine residue.[8] In this publication we present the synthesis of compounds (**4** and **5**), and the solid state structure of the Cu(II) complex of **4**. In addition, we present evidence of a novel oxidative coupling reaction of **3** which could have implications to the biosynthesis of the key His/Tyr C-N bond formation of C*c*O.

Initial investigations into the alkylation of histidine with 2-(chloromethyl)pyridine for the preparation of **4** and **5** were not successful; reactions resulted in low yields of material that was difficult to purify. However, the use of reductive amination as the cornerstone reaction led to the successful synthesis of the desired compounds, as shown in Scheme 1. The synthesis of the tetradentate ether was completed in six steps with an overall yield of 27%. Known histidine derivative (**6**)[9] was reacted with two equivalents of *N*-(benzyloxycarbonyloxy)succinimide to give the corresponding biscarbamate which, without isolation, was selectively deprotected with propylamine to yield **7**.[10] Compound (**7**) was coupled to triacetoxy[(2-methoxymethoxy)phenyl]lead (**8**) to afford compound (**9**) in 75% yield.[7] Subsequent deprotection under transfer hydrogenation conditions provided the free amine,[11] which was subjected to direct hydride-induced reductive amination with 2-pyridine carboxaldehyde.[12] Finally, stirring the

reaction product at room temperature in a CH_2Cl_2 solution saturated with $HCl_{(g)}$ afforded the free phenol product (**4**) as a multiple hydrochloride salt.

Following the synthesis of **4**, the production of methyl ester **5** was straightforward. Commercial Z-His-OMe (**10**) was subjected to aryllead(IV) coupling with **8** to give **11**, which afforded **5** following the reaction sequence developed for the preparation of **4** (reductive removal of the Z-protection group, exhaustive reductive amination with 2-pyridine carboxaldehyde and removal of the phenol protection group).



Scheme 1. Synthesis of ligands **4** and **5**.

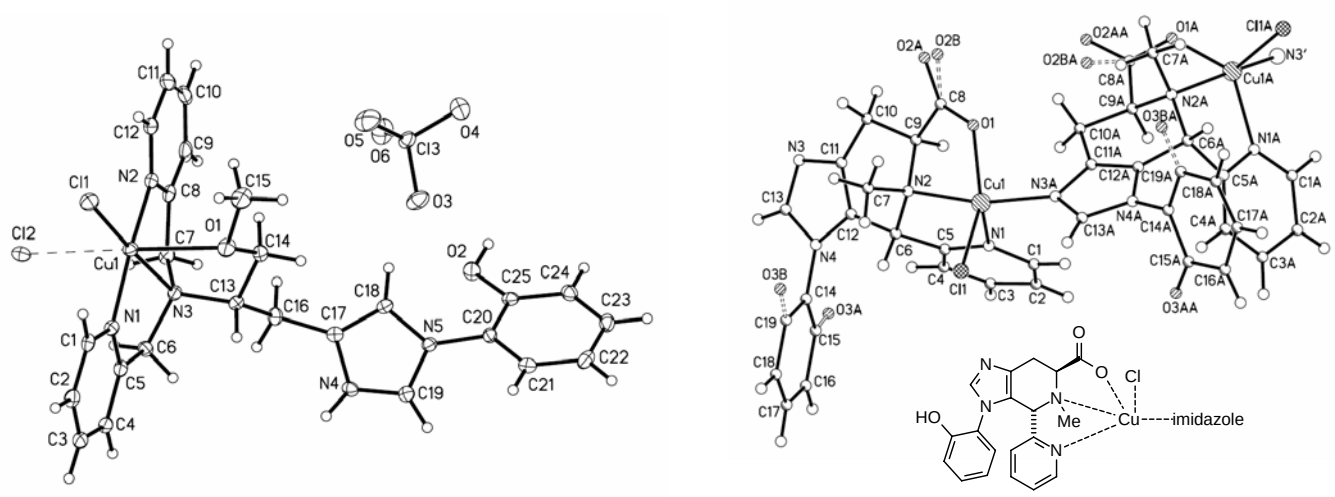


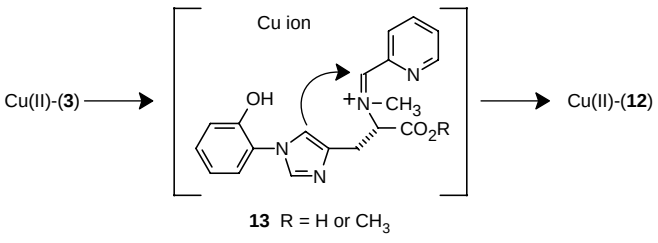
Figure 2. Single crystal X-Ray determination of Cu(II)-(**4**) (left) and Cu(II)-(**12**), with chemical structure of Cu(II)-**12**.

Blue crystals of the copper(II) complex of **4** were grown by slow evaporation of a methanol/acetonitrile mixture of the ligand and $Cu(ClO_4)_2$ plus added chloride ion; the resulting structure was determined by single crystal x-ray analysis is shown in Figure 2. While it was anticipated that the Cu(II) atom would be 5-coordinate with chloride as one ligand, the coordination of the methyl ether unit rather than the imidazole ring came as a surprise. The ether is only loosely associated with the metal (2.43 Å); it appears

that the depicted collection of 5-membered rings incorporating copper is preferable, at least in the solid state, to the presence of a 6-membered ring that would necessarily be formed should the imidazole nitrogen be involved in chelation. Studies on the solution structure of Cu(II)-(**4**) will be initiated shortly.

Gram quantities of crystalline ligand-metal complexes were required for the mechanistic studies related to C*c*O; only crystalline material gave assurance of the required 1:1 ligand-to-metal ratio. Therefore, the development of efficient methods for the large scale crystallization of these complexes became a focus of keen interest. Since chloride is known to be a strong ligand for Cu(II), experiments were conducted to determine the best sources of this halide.

Treatment of ligand (**3**) (tridentate ester) with $CuCl_2$, $Cu(ClO_4)_2$/NaCl, $Cu(ClO_4)_2$/Et_3NHCl and $Cu(ClO_4)_2$/H_4NCl all produced the desired material, which could be obtained as needle-like crystals by slow evaporation from methanol.[8] However, robust cubic crystals formed readily from the methanol solution when Amberlite IRA-410 (Cl^- form) was employed as the chloride source. Indeed, isolation of these crystals and subjecting the mother liquors to further crystallization afforded the well-known needle-like material that had already been identified as Cu(II)-(**3**).[8] Single crystal x-ray analysis of the cubic crystalline material afforded the structure shown in Figure 2, in which a new C-C bond is evident. This new bond links C-6, the methylene generated in the reductive amination reaction, and C-12, the imidazole carbon adjacent to the key C-N bond between the heterocycle and phenol (x-ray numbering), to give Cu(II)-(**12**). The structure of this new fused ring system, a tetrahydroimidazopyridine, shows some disorder around the phenol and original histidine methyl ester unit, which now exists as the free acid complexed directly to the copper atom. As with all the structures observed in these studies, the central copper atom is 5-coordinate, now relying on a single chloride together with the carboxylate of the histidine residue and three nitrogen donors (histidine amine and pyridine from one molecule and imidazole from the adjacent molecule in the crystal lattice).



Scheme 2. Proposed mechanism for the formation of Cu(II)-(**12**).

The cyclization of histidine with an aldehyde via the classic Pictet-Spengler reaction readily affords the spinacine ring system.[13] However, to invoke such a reaction mechanism requires an unforeseen oxidation of tertiary amine Cu(II)-(**2**) to iminium ion (**13**), which is then subject to attack by the electron-rich imidazole ring.

Although we know of no ongoing studies on the biosynthesis of the His/Tyr side chain coupling reaction in C*c*O, it is reasonable to speculate that an electron-poor phenol derivative is generated as an acceptor to the electron-rich nitrogen of the imidazole ring held in close proximity by the *i* to *i*+4 α-helix relationship of the two residues. Indeed, such a mechanism has been suggested for the biosynthesis of the Tyr/Cys cross-link at the active site of galactose oxidase, in which a thioether bond (analogous to the C-N bond in C*c*O) is formed in the final processing steps to the fully functional enzyme.[14] In the suggested mechanism the copper-phenolate species allows for oxidation and activation of the phenol ring toward attack of the cysteine side chain sulfur atom.

By analogy, we can speculate that copper mediates the oxidation of the histidine amine to corresponding iminium ion (**13**),[15] which is in proper position to form the new fused bicyclic system. The reasons for the uniqueness of the resin-bound chloride source to promote this reaction, the changes in the oxidation state of the copper ion and the timing of the methyl ester to acid transformation are currently under study and will be disclosed in due course.

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