

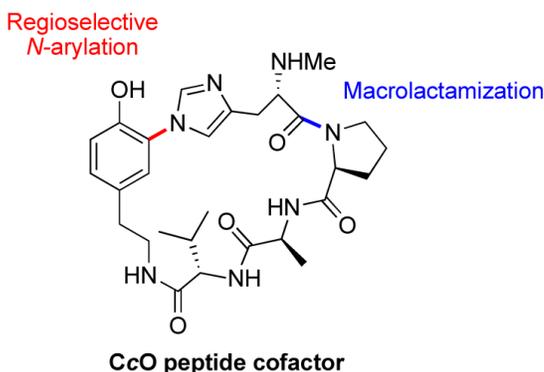
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Synthesis of a Cyclic Pentapeptide Mimic of the Active Site His-Tyr Cofactor of Cytochrome *c* Oxidase

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Abstract



Arylboronic acid-based technology provides a mild, regioselective and non-toxic *N*-arylation procedure for accessing the unusual *N*-arylated side chain histidine found in the active site of cytochrome *c* oxidase (CcO). The *N*-arylated histidine is elaborated to the complete cytochrome *c* oxidase cyclic pentapeptide cofactor. Molecular modeling of the cofactor provides insight into the dynamic character of the *N*-aryl bond.

Introduction

Cytochrome *c* oxidase (CcO) is the terminal electron acceptor of the mitochondrial electron transport chain and is responsible for the four-electron reduction of dioxygen to water.¹ This reaction drives the translocation of four protons across the inner mitochondrial membrane, establishing an electrochemical gradient that drives ATP synthesis.² The active site of CcO consists of a binuclear center (heme *a*₃ and Cu_B) and a peptide cofactor, the latter of which is of particular interest to our research program. This cofactor was discovered and characterized through X-ray crystallography, revealing an unusual post-translational modification: an *N*-arylation linking *N*₁ of the His²⁴⁰ side chain to the εC of Tyr²⁴⁴ (bovine heart numbering).³ The macrocyclic pentapeptide, formed with the intervening residues Pro, Glu and Val, is part of an α-helix that, along with distal His residues 290 and 291, is bound to Cu_B, providing a rigid binding site for the metal (Figure 1).⁴ A detailed understanding of this unusual cross-linkage is necessary to fully define the reaction mechanism of CcO.

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Supporting Information Available: Synthetic procedures, complete spectroscopic data, 1H, 11B and 13C NMR spectra for all new compounds, LCMS and CIF data for **24** are available free of charge via the Internet at <http://pubs.acs.org>.

Of the four electrons needed in the catalytic cycle, it is accepted that two electrons arise from the redox cycle of heme a_3 (Fe^{II} to Fe^{IV}) and one electron from the oxidation of Cu_B . It has been postulated that the His-Tyr crosslink plays a role in stabilizing either a Tyr radical or tyrosinate, allowing the cofactor to provide a proton^{5,6,7} and the fourth electron⁸ required to cleave the dioxygen bond and produce water. Furthermore, the His-Tyr cross-link appears to create a kink in the α -helix, placing the phenol directly at the edge of the K-channel and, thus, facilitating proton delivery to the active site.⁹

Previously, this laboratory synthesized models of the CcO active site incorporating a His-phenol crosslink and a pyridyl appendage that, together with the histidine main chain nitrogen, act as substitutes for the distal histidine ligands to Cu_B (Figure 1).¹⁰ However, these His-phenol mimics could not be expected to properly portray the effects, both electronic and structural, of the entire cyclic pentapeptide found in the native enzyme.

Electron delocalization of a tyrosine-based radical species onto the histidine imidazole through the unique C-N bond of the cofactor could be attenuated by the dihedral angle between the two aryl rings which, in turn, would be dictated by the energetics of the cyclic pentapeptide structure. In addition, the stereochemistry imparted by the biaryl dihedral angle is important for mimicking through-space interactions between the tyrosine-phenol and Cu_B . Thus, it seemed prudent to expand our synthetic efforts toward a cyclic pentapeptide structure that incorporates the cofactor.

Herein is presented the synthesis of macrocyclic pentapeptide **1** (Scheme 1). The native Glu has been replaced by Ala for reasons that will be discussed, and the C-terminal functionality has been removed. Hence, this model system incorporates a tyramine unit (abbreviated “tyr” to contrast with the standard 3-letter abbreviation “Tyr” for the amino acid tyrosine). Macrolactamization was executed at the His – Pro bond, utilizing the turn-inducing preference of proline to facilitate cyclization. The linear pentapeptide was assembled via coupling of the C-terminus of tripeptide **A** to the terminal amino group on *N*-arylated histidine fragment **B**.

Results and Discussion

Although there are many methods in the literature for *N*-arylation, nearly all involve the use of base and heat, conditions that are expected to be unacceptable for peptide substrates. Of the remaining methods, the substrate scope rarely incorporates substituted imidazoles, which consistently afford diminished yields due to regioselectivity issues. This laboratory previously described the regioselective *N*-arylation of histidine with activated tyrosine and phenol substrates using aryllead(IV) tricarboxylates under mild conditions.¹⁰ We envisioned expanding this methodology to include the His-Tyr and His-tyr systems needed to complete the synthesis of the fully formed cyclic pentapeptide mimic of CcO.

Commercially available Boc-Tyr-OH was mono-iodinated, benzyl protected (**2**) and protected on the phenol functionality to afford compound (**3**) (Scheme 2) by procedures developed in our laboratory for another project.¹¹ Treatment with $\text{PdCl}_2(\text{PPh}_3)_2$ and hexabutyliditin under mild conditions provided stannylated compound (**4**). Transmetalation to the aryllead(IV) tricarboxylate (**5**) was accomplished under mercury catalysis at 30 °C. This material is highly light sensitive and must be handled under inert atmosphere; it is stable in the dark at 0 °C for several months.

With the suitably protected and activated tyrosine derivative prepared, *N*-arylations with the histidine coupling partner were examined. As with previous models, we chose to employ a histidine residue bearing both *N*-methyl and *N*-protection groups on the main chain nitrogen. Previous work had identified a facile route to Bn-MeHis-OMe and Me-His-OMe (Scheme 3).¹² The former compound easily suffered ester exchange to afford allyl ester **6**, while the latter

compound proved to be an adequate starting material for bis-carbamate protected compound **7** and main chain carbamate protected **8**.

The first attempts at forming the desired His-Tyr coupled product followed our previously successful route in forming His-phenol model systems; namely, subjecting Bn-MeHis-OMe and aryllead(IV) reagent **5** to Cu(OAc)₂ catalysis. Unfortunately, unacceptably low yields of desired product **9** were obtained by this route, with the main byproduct being the loss of lead from the tyrosine residue (Scheme 4).

Additionally, simple base (LiOH) hydrolysis of methyl ester **9** surprisingly was accompanied by a more rapid (5 min at 0 °C) benzyl ester hydrolysis, leading to an inseparable mixture of the benzyl-deprotected *N*-arylated histidine and *N*-arylated histidine diacid. Consequently, non-hydrolytic methods for affecting the selective cleavage of a methyl ester in the presence of a benzyl ester were attempted. Reaction of the *N*-arylated histidine with Na₂S (3.0 equiv) in DMPU, with or without Ti(*i*-OPr)₄, left only unreacted substrate after 24 h. Hydrolysis attempts with the highly nucleophilic TMSOK in either Et₂O or DCM were unsuccessful. One possible way to avoid this selective deprotection problem involved changing the methyl ester protection to an allyl protection on the histidine. However, the reaction of allyl ester **6** with **5** under Cu(OAc)₂ catalysis also gave low yields of desired product **10**. Allyl deprotection conditions using the standard palladium method adventitiously removed the *N*-terminal Boc group of **10**. Under the more exotic ruthenium conditions, the biaryl system of **10** underwent degradation.¹³ It has recently been shown that ruthenium can insert into *N*-aryl bonds, which supports this degradation pathway.¹⁴

To explain the low arylation yields we postulated that the metal binding ability of the histidine residue, bearing both the strongly chelating imidazole ring and the main chain alkylamine in Bn-MeHis-OMe, sequestered the reactive lead species. Alternatively, this highly active metal binding site could bind spent Pb(OAc)₂, thus decreasing the nucleophilic character of the imidazole and making it an unsuitable substrate for reaction with the aryllead(IV) triacetate. Thus, we looked to decrease the metal binding ability of this residue by switching the benzyl protection for the electron withdrawing –Cbz protection group and compound **8** (Scheme 4).¹⁵ In addition, we chose to eliminate the C-terminus of tyrosine, which would bypass the ester differentiation problems and, it was hoped, improve the *N*-arylation yields (Table 1). To this end, aryllead(IV) tyramine derivative **14**, comparable to tyrosine derivative **5** was prepared (Scheme 5).

Commercially available tyramine-HCl was nitrogen protected and iodinated to give **11** under the conditions developed for the synthesis of **3**, then subjected to phenol protection under standard conditions to give **12**.¹⁶ Stannylation (**13**) and subsequent plumbation yielded aryllead (IV) coupling partner **14** (Scheme 5).

Although the tyramine aryllead(IV) triacetate could be prepared in improved yield over the tyrosine system, the *N*-arylation yields were uniformly low (Table 1, entries 1-4). Variations in copper source and the addition of DBU, which has been used to increase yields in certain aryllead coupling reactions,¹⁷ did not lead to increased recovery of desired product. Therefore, more traditional methods for *N*-arylation, employing aryl iodides, were explored; however, no product was observed for any of these reactions (entries 5, 6).¹⁸

Lam-Chan coupling procedures have been used for the arylation of simple imidazoles with arylboronic acids.¹⁹ However, such protocols require the use of two equivalents of boronic acid and struggle with regioselectivity issues on substituted imidazole substrates. Nonetheless, we next attempted to produce a tyramine coupling partner functionalized with a boronic acid group.

A variety of methods were screened to affect the Miyaura reaction on iodide **12**, including both microwave and conventional heating as well as variations in ligand design.^{20,21,22} Optimal conditions included the use of pinacol borane rather than the traditional bis-pinacol borane and DCBP as a ligand to afford crystalline aryl boronic ester **17** (Scheme 6). Oxidative cleavage of the boronate ester with NaIO₄ provided arylboronic acid **18** in 88% yield.

Unfortunately, Lam-Chan type coupling with CuI in MeOH provided no product (Table 1, entry 7). A procedure by Collman *et al.* for the *N*-arylation of imidazoles with arylboronic acids, originally based on the work of Lam-Chan, was adopted for our needs.²³ Collman reported high yields and mild conditions using a catalytic amount of a novel copper system (in the form [Cu(OH)TMEDA]₂Cl₂) under aerobic conditions, albeit with modest regioselectivity for substituted imidazoles. Additionally, as in the aryllead case, a 40% excess of arylboronic acid is needed with respect to the imidazole. However, in our hands the optimal reaction conditions afforded 61% of the *N*-arylated histidine **16** (Table 1, entry 8) as a single regioisomer using a 1/1 ratio of boronic acid to histidine.²⁴ It should be noted that this stoichiometry represents an unusual finding in that commonly a 2/1 ratio of boronic acid to histidine is optimal. The use of 4 Å molecular sieves, as employed by Collman, afforded variable yields and were, therefore, not used.

Much of the same arylboronic acid chemistry can be achieved with the easily prepared and highly crystalline trifluoroborate salts.²⁵ Attempts to by-pass the arylboronic ester in synthesizing the trifluoroborate salt **19** from **12** failed,²⁶ but proceeded from **17** by treatment with KHF₂.²⁷ *N*-arylation reactions failed with the trifluoroborate salt (Table 1, entry 9). However, **19** could be easily recrystallized and hydrolyzed to **18** for incorporation into the optimized procedure.²⁸

With a viable His-tyr dipeptide fragment in hand (**B** in Scheme 1), tripeptide Boc-Pro-Ala-Val-OH **22** (serving as fragment **A** in Scheme 1) was prepared easily on a large scale (Scheme 7). Alanine was chosen to replace the glutamic acid residue of the mammalian CcO structure to avoid any additional problems with side chain protection groups. It was believed that this replacement would not have an impact on the ring conformation and C-N bond rotation energetics. Dipeptide **20** was prepared in high yield using an HOBt/EDC coupling of Boc-Ala-OH with H-Val-OMe-HCl,²⁹ which was then nitrogen deprotected under standard conditions and coupled with Boc-Pro-OH to provide protected tripeptide **21**. Hydrolysis revealed *N*-protected tripeptide **22**.

Selective deprotection of the *N*-Boc functionality of **16** in the presence of the -MOM group was performed (10% impurity of MOM deprotected material)³⁰ prior to standard HOBt/EDCI coupling with **22**, which afforded the linear pentapeptide **23** in 83% yield (Scheme 8, 58% overall for the 2 steps). Other methods, such as CIP³¹ or PyBOP/HOAt, resulted in significant byproduct formation.

Standard LiOH hydrolysis of the methyl ester in **23** was accomplished in high yield. This crude salt was subjected to TFA deprotection of the remaining -Boc group, again in the presence of the -MOM functionality, to provide the linear pentapeptide as a free carboxylic acid/ammonium salt, which was taken on without purification to the cyclization reaction. At this stage, a variety of activation agents were screened in an attempt to cyclize this 21-membered macrocycle. Among them, PyBOP/HOAt and EDC/HOBt provided only minimal product after 2 d of reaction, whereas EDC/pentafluorophenol (Pfp) provided the ring-closed intermediate at an exceptional rate. This material was fully MOM deprotected in high yield, providing the free phenol macrocyclic pentapeptide **24**, as confirmed by LCMS and single crystal X-ray analysis (Figure 2). At this point, the Cbz group was removed with Parr hydrogenolysis to

provide the complete pentapeptide **1**, which includes the cytochrome *c* oxidase peptide cofactor.

Modeling

Our goal was to synthesize a compound that more closely resembled the natural peptide system in CcO as regards to the global conformation and corresponding rotational freedom around the novel C-N bond linking the two aryl side chains (biaryl dihedral angle, BDA), in full recognition of the significant contributions likely made by both metals, their corresponding ligand systems and the polypeptide structure of the native protein.

Of all the compounds studied in this laboratory, the crystal structure of compound **24** exhibits a BDA magnitude most like the native system. However, the sign is opposite (-40° , synthetic; $+44^\circ$, native). It is compelling to propose that this similarity in BDA magnitude represents the natural tendency of the bicyclic system³³ based on sound physical organic chemistry principles, and that the sign is dictated by second sphere forces (heme, metals, polypeptide backbone). By contrast, the dihedral angles (ϕ, ψ) defining the cyclic peptide backbone are found to be quite different from those of the native system (Table 2).

The barrier to rotation around the unusual C-N bond was probed through computational studies.³⁴ Driving the dihedral from -40° to $+40^\circ$ over 10° increments and minimizing the energy at each step provided the ensemble of structures shown in Figure 3. The barrier to rotation about this bond was 12.6 kcal/mol, low enough so that in solution there is no expectation of atropisomer formation. The dynamic nature of this biaryl system has a two-fold impact. First, the lack of atropisomers simplifies the purification process of the complete cyclic pentapeptide. Second, it has been proposed that the conformational changes between the oxidized and reduced forms of the enzyme are implicated in proton pumping.³⁵ A flexible biaryl system is required for this conformational mobility.

Conclusion

Synthesis of a pentapeptide containing the unusual amino acid cofactor of cytochrome *c* oxidase has been presented. Key to the synthesis was an *N*-arylation procedure, which improved the stoichiometry and regiocontrol over previous methods. The biaryl dihedral angle is found to be almost identical in magnitude to that found in the natural system, while opposite in sign. Continued synthetic efforts to append a copper binding site to the histidine residue of the cyclic pentapeptide are ongoing and will be reported in due course.

Experimental Section

(S)-Methyl 2-((Benzyloxycarbonyl)(methyl)amino)-3-(1-(5-(2-(*tert*-butoxycarbonylamino)ethyl)-2-(methoxymethoxy)phenyl)-1*H*-imidazol-4-yl)propanoate (**16**)

A solution of histidine derivative **8** (0.51 g, 1.61 mmol, 1.0 equiv) in DCM (6.4 mL, 0.1 *M*) was added to freshly prepared boronic acid **18** (0.52 g, 1.61 mmol, 1.0 equiv). Copper catalyst $[\text{Cu}(\text{OH})\text{TMEDA}]_2\text{Cl}_2$ (0.074 g, 0.161 mmol, 0.1 equiv) was added to the reaction under N_2 , which turned the solution dark blue. A drying tube (CaSO_4) was put in place to allow diffusion of dry air into the reaction mixture, which turned the solution a pale aqua color. After stirring for 36 h the reaction was concentrated to a green oil and loaded onto a column for purification with 1:4 ethyl acetate/hexanes followed by ethyl acetate. 0.59 g (61%) was recovered as a clear oil. 1.0 : 0.9 dr (Cbz rotomers on main chain nitrogen); ^1H NMR (500 MHz, CDCl_3): δ (ppm) 7.68 (d, $J = 14.0$ Hz, 1H), 7.23 – 7.29 (m, 5H), 7.19 (dd, $J = 1.0, 8.5$ Hz, 1H), 7.13 (d, $J = 8.5$ Hz, 1H), 7.04 (d, $J = 9.5$ Hz, 1H), 6.978 (s, 1H), [6.88 (s, 1H)], 5.11 (s, 2H), 5.06 – 5.08 (m, 2H), 5.01 – 5.03 (m, 1H), [4.92 – 4.93 (m, 1H)], 4.62 (bs, 1H), 3.74 (s, 3H), [3.64 (s, 3H)],

3.33 (s, 3H), [3.32 (s, 3H)], 3.305 – 3.34 (m, 2H), 3.06 – 3.19 (m, 2H), 2.91 (s, 3H), [2.90 (s, 3H)], 2.76 (d, $J = 4.0$ Hz, 2H), 1.41 (s, 9H); ^{13}C NMR (125 MHz, CDCl_3): δ (ppm) 171.4, 155.7, 137.9, 136.4, 133.4, 128.9, 128.9, 128.3, 127.7, 127.4, 125.6, 116.6, 116.5, 95.1, 79.2, 67.1, 59.5, 56.2, 52.2, 52.1, 41.5, 35.1, 28.2; HRMS for $\text{C}_{31}\text{H}_{41}\text{N}_4\text{O}_8$ [M+H] calcd., 597.2919, found, 597.3001 (Error = 13.73 ppm); $[\alpha]_D^{28}$ (c 0.2, MeOH); IR (thin film) 1642, 1519, 1404, 1344, 1145, 1039, 990 cm^{-1} .

Cyclic Pentapeptide, *N*-Cbz protection-TFA salt (**24**)

Linear pentapeptide **23** (0.23 g, 0.26 mmol, 1.0 equiv) was dissolved in a 1/1 mixture of THF/ H_2O (5.2 mL, 0.05 M) and cooled to 0 °C. After the solution reached 0 °C, LiOH (0.033 g, 0.79 mmol, 3.0 equiv) was added and stirred for 1 h, at which point the ice bath was removed and the solution was allowed to reach rt where it was kept for an additional 2 h. The THF was removed *in vacuo* and the aqueous mixture was loaded onto a 50 mL Sephadex C18 solid phase extraction column (preconditioned by running a column volume (cv) of MeOH followed by one cv of H_2O). The sample was eluted with 3 cvs of H_2O followed by 3 cvs of MeOH. The sample eluted immediately with MeOH over ~ 20 mL. The organic layer contained only the desired acid salt as determined by LCMS. These fractions were lyophilized to provide the linear pentapeptide lithium carboxylate salt. This salt was dissolved in TFA (2.6 mL, 0.1 M) at rt and stirred for 4 min. At this time, 100 mL of DCM was added and the solution was evaporated *in vacuo*. The compound was evaporated two additional times with DCM to provide the bis-TFA salt as a yellow oil. Some (10%) of this material was free phenol (-MOM), deprotected under these conditions and was carried through as a mixture. For R = MOM; HRMS for $\text{C}^{38}\text{H}_{50}\text{N}_7\text{O}_8$ [M+H] calcd., 732.37154, found, 732.3782 (Error = 9.09 ppm). To the oil was added DMF (6.6 mL, 0.05 M). The solution was cooled to 0 °C and EDC (0.05 g, 0.26 mmol, 1.0 equiv) and pentafluorophenol (Pfp) (0.24 g, 1.32 mmol, 5.0 equiv) in DMF (3.3 mL) were added. This mixture was allowed to react for 30 min. At this point the pH of the solution was increased with the addition of DIPEA (0.44 mL, 2.64 mmol, 10.0 equiv). The reaction was periodically monitored by LCMS, which indicated that cyclization proceeded rapidly (little starting material remained after 15 min) and was complete after 3 h. An LCMS of the crude reaction mixture revealed only the presence of product (a variable mixture of MOM protected and deprotected) with Pfp as the only other identifiable product. Purification was accomplished with a C18 Sephadex column (prepared as described previously for isolating the carboxylate salt). The reaction mixture was diluted with water (50 mL) and loaded onto the column. This was eluted with 3 cvs of H_2O followed by 3 cvs of MeOH. This first separation is used to remove all DMF. After the organics were removed a second purification was necessary. The crude product was loaded with 20% MeOH/ H_2O and eluted in 10 mL portions of 50, 60, 70, 80, 90, 100% (20 mL) MeOH/ H_2O . The desired material eluted between the 80 and 90% MeOH/ H_2O gradient. The purity was analyzed by LCMS analysis; see chromatograph in Supporting Information Part 2. The macrocyclic pentapeptide (0.030 g, 0.036 mmol), as a variable mixture of MOM protected and MOM deprotected material, was stirred in a 1:1 TFA/DCM (1 mL) mixture. After 2 h the mixture was concentrated *in vacuo* and any excess TFA was removed by evaporation with DCM. X-ray quality crystals were grown from a H_2O /DMSO/ CH_3CN solvent system, which confirmed the connectivity of compound **24**. Data for R = H; Due to the multiple conformations in solution, see ^1H and ^{13}C NMR spectra for purity. HRMS for $\text{C}_{36}\text{H}_{46}\text{N}_7\text{O}_7$ [M+H], calcd., 688.3453, found, 688.3402 (Error = 7.41 ppm); $[\alpha]_D^{28}$ (c 3.3, DMSO).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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- (33). DFT calculations on simple model systems (Pratt DA, Pesavento RP, van der Donk WA. *Org. Lett* 2005;7:2735–2738. [PubMed: 15957934] put the BDA at 42.5°, very close to the native enzyme BDA. Most x-ray structures, including our own previous work, suffer from intermolecular hydrogen bonds that likely perturb the fundamental BDA; in our laboratory we have documented BDAs ranging from –25.7° to –36.3°. By contrast, the structure of **24** appears devoid of serious intermolecular interactions. Hence, this molecule affords the best experimental evidence for the intrinsic BDA of the biaryl cofactor.
- (34). Macromodel v9.0.014 was used for the calculations. The Amber* force field was employed, dielectric = 80, with a PRCG minimization method.
- (35). Qin L, Liu J, Mills DA, Proshlyakov DA, Hiser C, Ferguson-Miller S. *Biochemistry* 2009;48:5121–5130. [PubMed: 19397279]

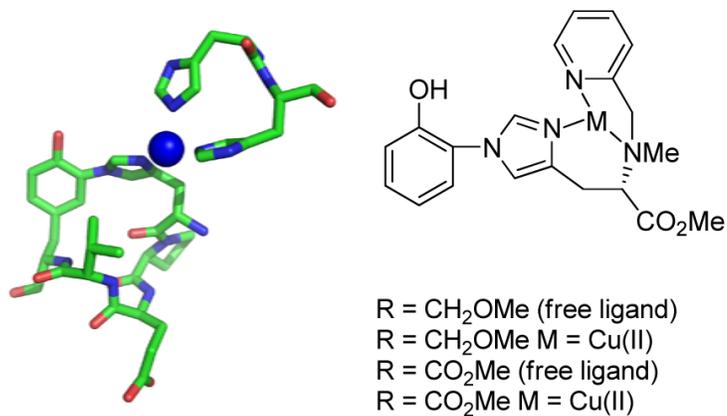
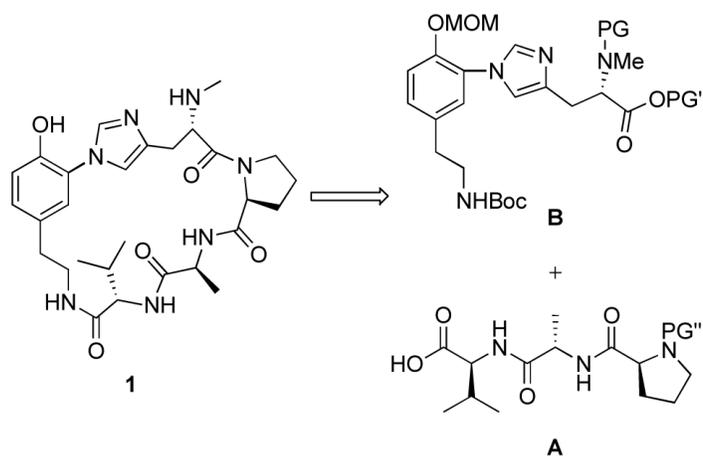
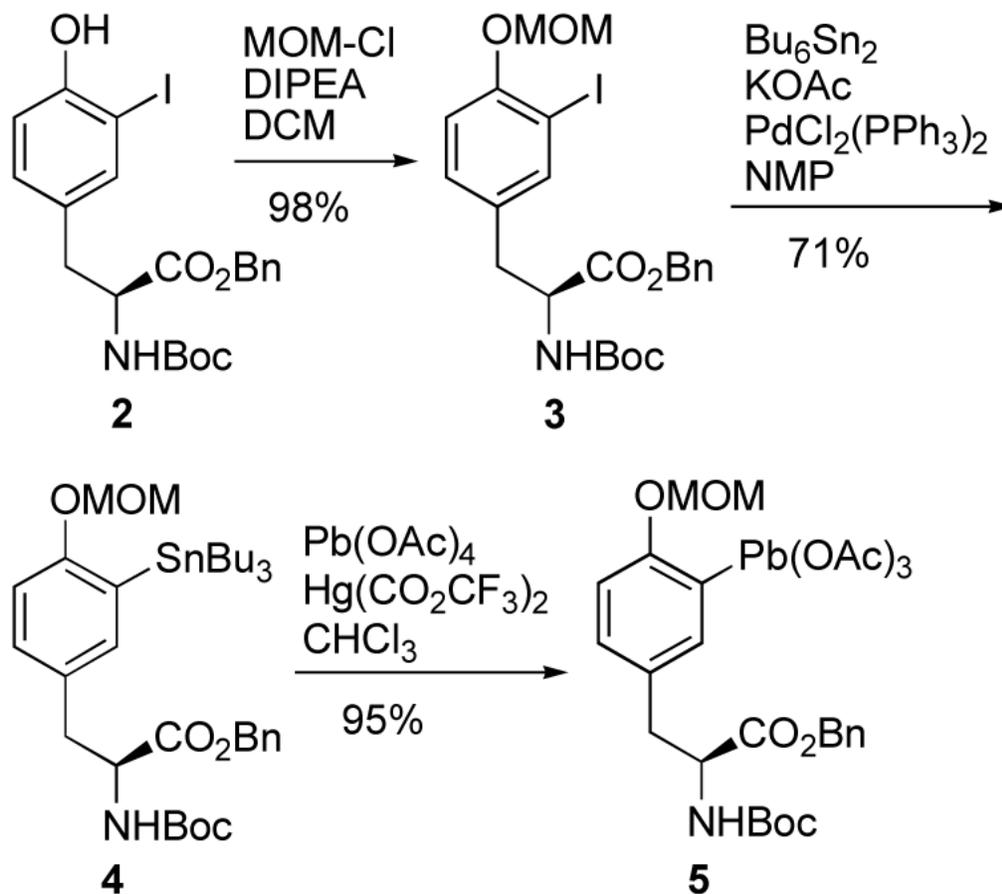


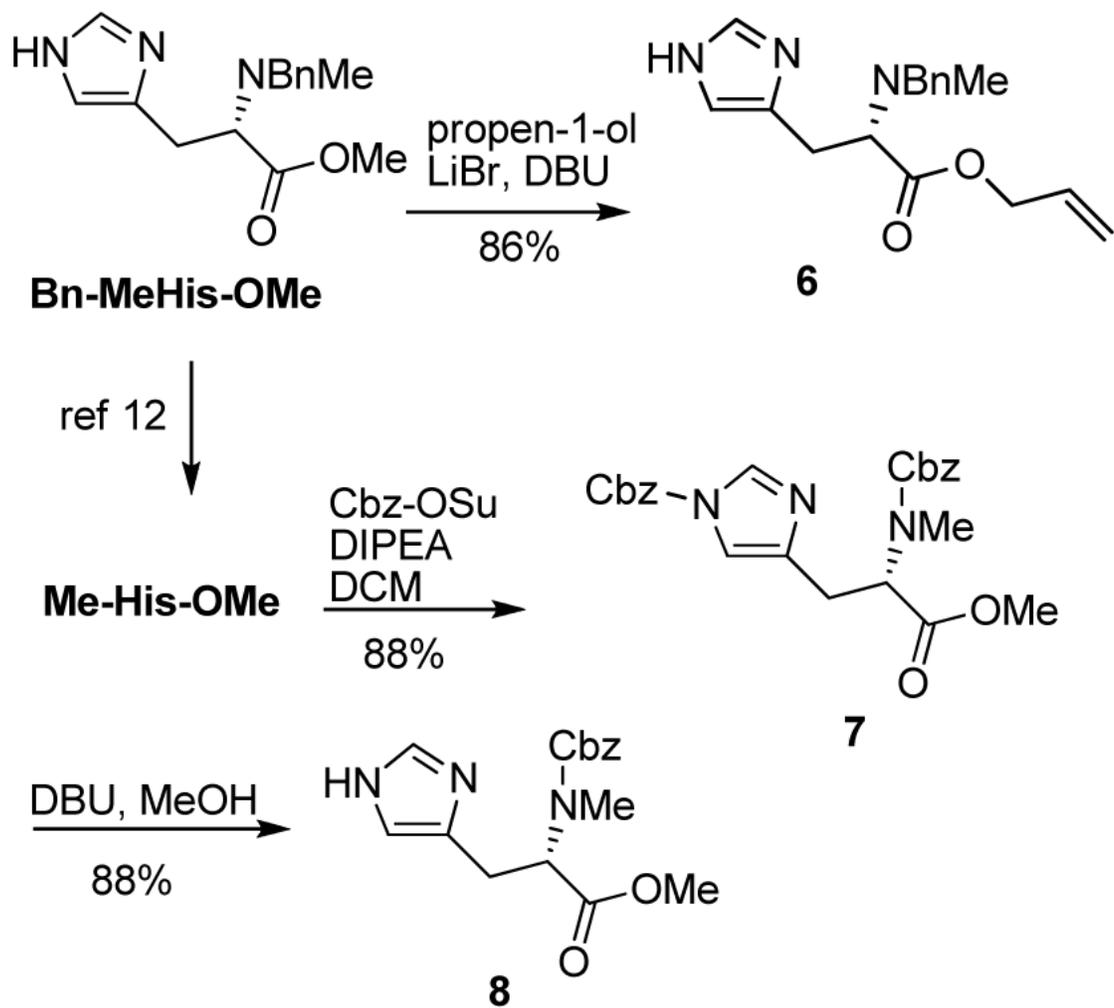
Figure 1. Left – Cu_B of CcO Bound to the Peptide Cofactor; Right – Previously Synthesized Cofactor Mimics.



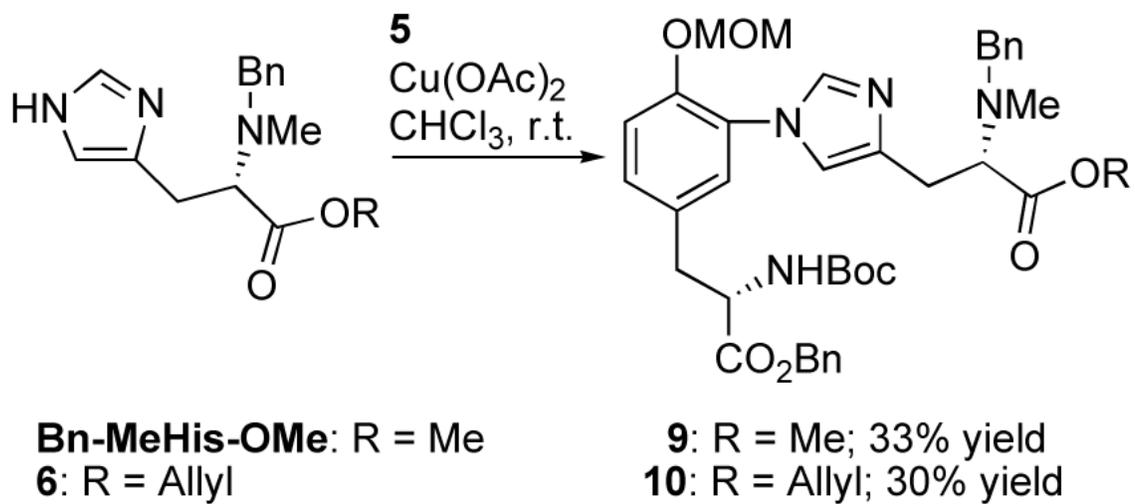
Scheme 1.
Retrosynthesis for the CcO Cyclic Pentapeptide Model.



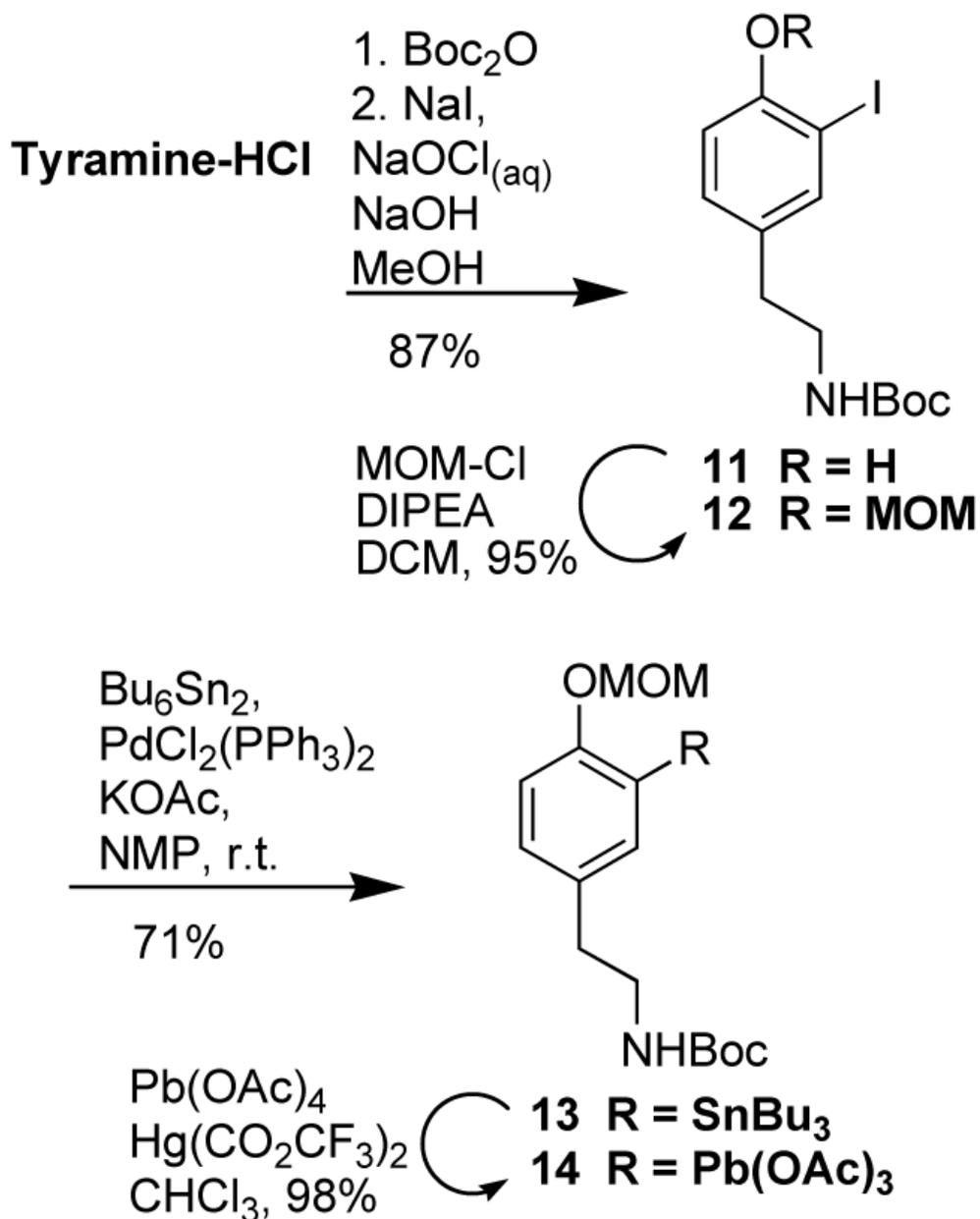
Scheme 2.
Synthesis of Tyrosine Aryllead(IV) triacetate.



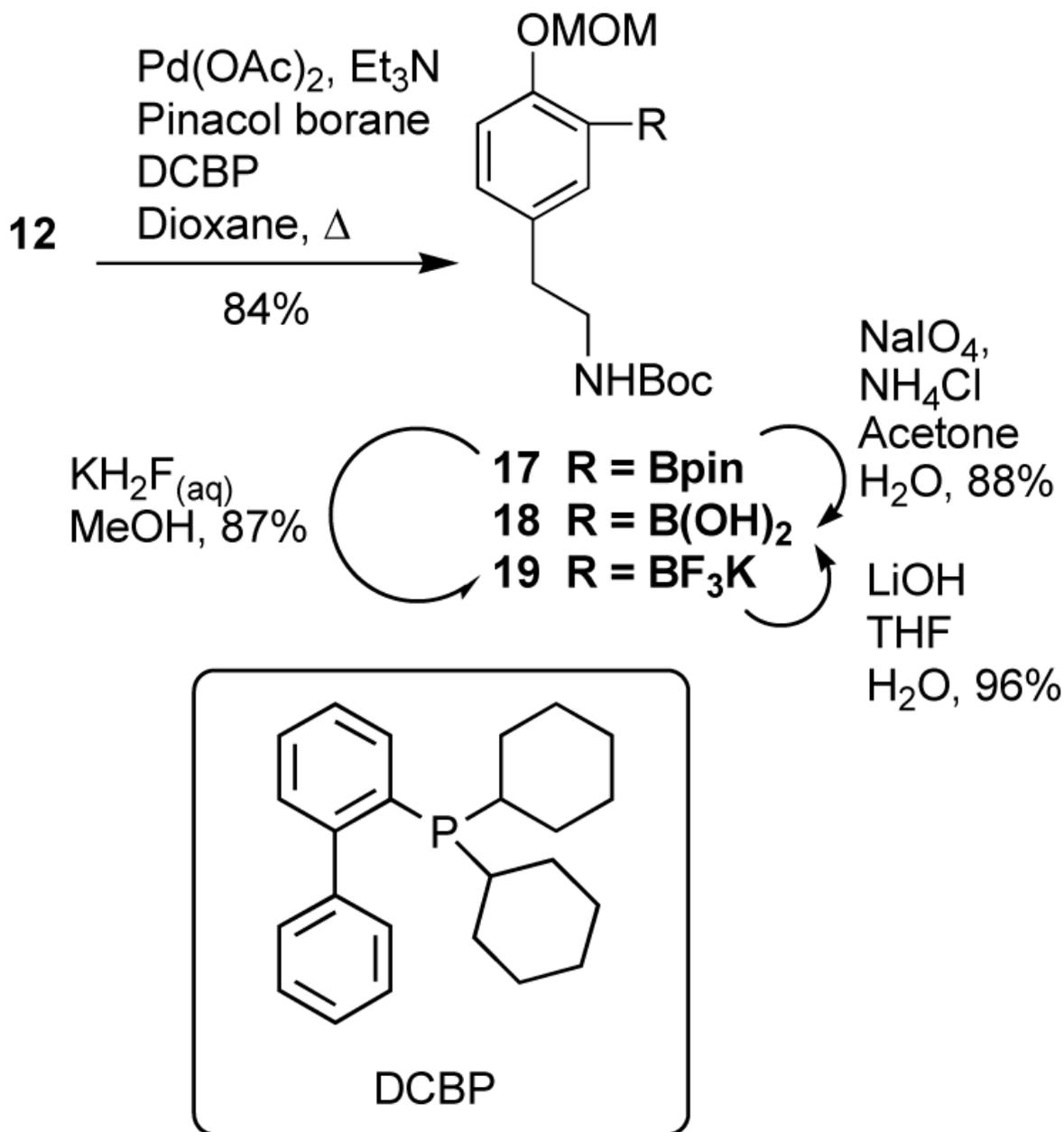
Scheme 3.
Synthesis of a suitably protected histidine coupling partner.



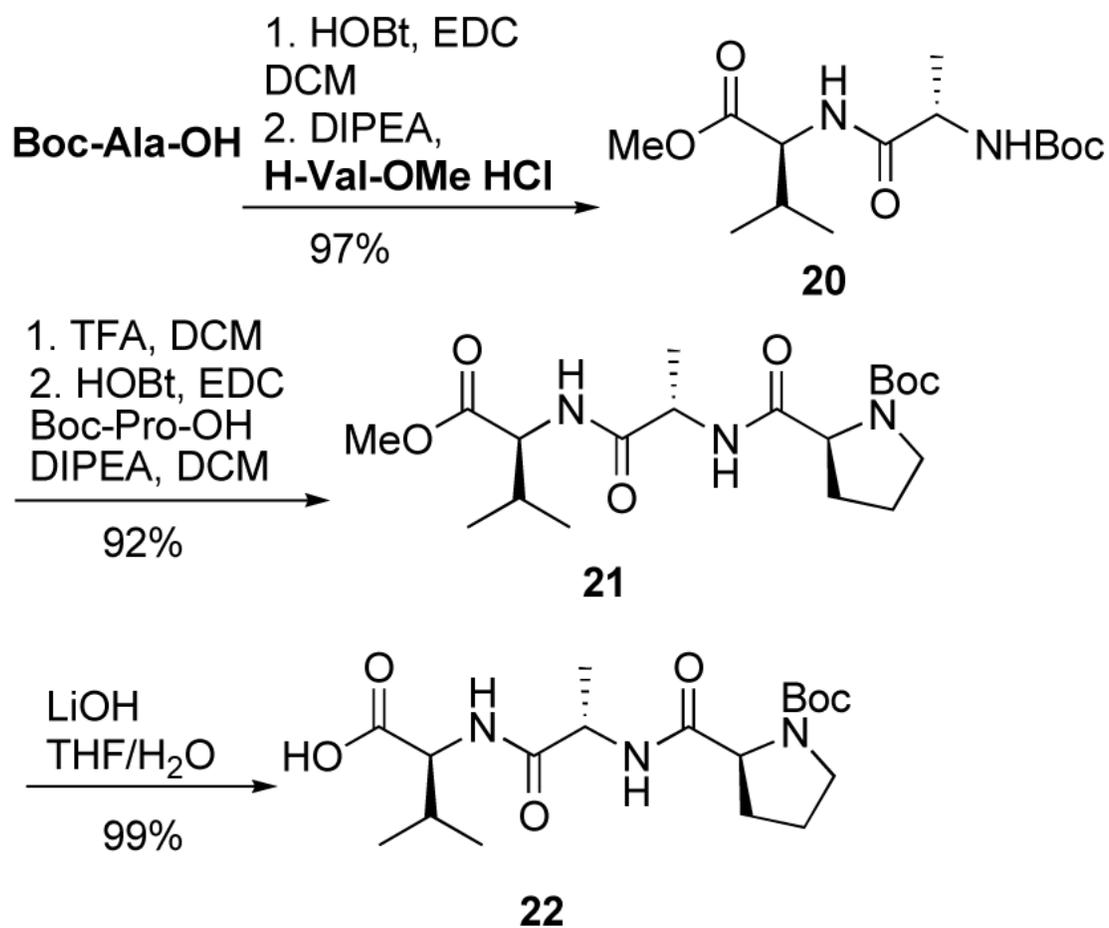
Scheme 4.
N-Arylation with Tyrosine Aryllead(IV) triacetate.



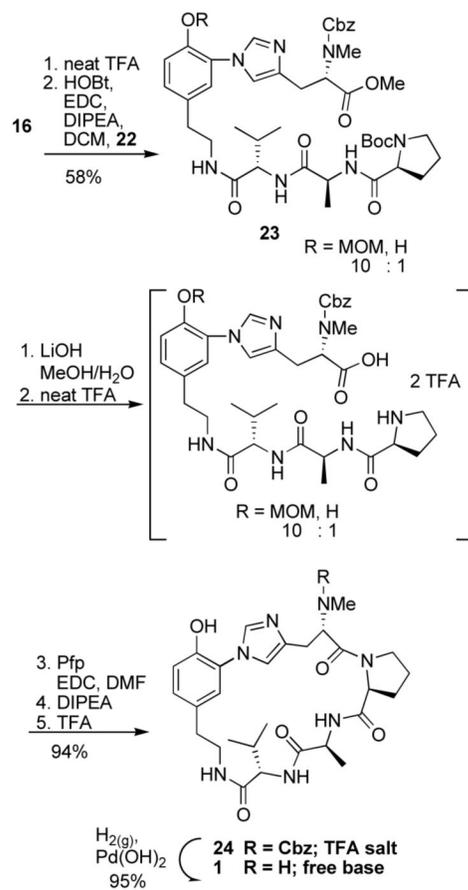
Scheme 5.
 Synthesis of Tyramine Aryllead(IV) tricarboxylate.



Scheme 6.
 Synthesis of Tyramine Boronic Ester, Acid and Trifluoroborate



Scheme 7.
Synthesis of Tripeptide 2.



Scheme 8.
Synthesis of Cytochrome *c* Oxidase Peptide Cofactor 1.

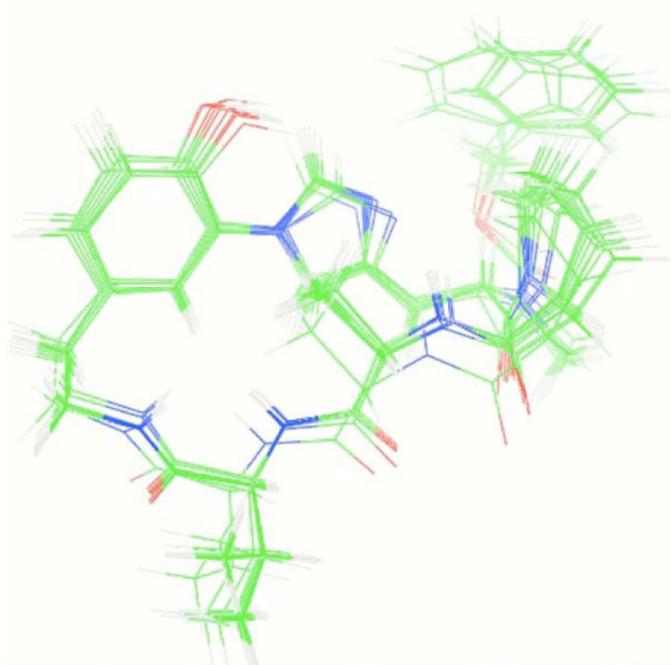


Figure 3.
Ensemble of structures minimized about the biaryl bond.

Table 1

Optimizing the *N*-arylation.

Entry	tyr	R	Cu source	Solvent	Product	Yield (%) ^a
1	14	Bn	Cu(OAc) ₂	DCM	15	19 ^b
2	14	Cbz	CuI	DCM	16	0 ^b
3	14	Cbz	Cu(OAc) ₂	DCM	16	41
4	14	Cbz	Cu(OAc) ₂	DCM	16	25 ^{b,c}
5	12	Cbz	CuI	DMF	16	0 ^{b,d}
6	12	Cbz	CuI	DMF	16	0 ^{b,e}
7	18	Cbz	CuI	MeOH	16	trace ^b
8	18	Cbz	[Cu(OH)TMEDA] ₂ Cl ₂	DCM	16	61 ^f
9	19	Cbz	[Cu(OH)TMEDA] ₂ Cl ₂	DMSO	16	0 ^f

^a Isolated yield.^b Reaction performed under an inert atmosphere of Ar(g) or N₂(g).^c DBU was added to the reaction.^d 2-Hydroxybenzaldehyde phenylhydrazine added to the reaction.^e 2-Acetylcyclohexanone added to the reaction.

^fReaction performed under an atmosphere of dry air (drying tube with CaCO₃).

Table 2 $(\phi\psi)$ Angles of Native and Synthetic Peptide.

	Native peptide ^a		Synthetic peptide ^b	
	ϕ	ψ	ϕ	ψ
His	-68	-51	-170	77
Pro	-51	-34	-73	-14
Glu/Ala	-57	-39	-65	142
Val	-69	-29	-69	-37
Tyr/tyr	-77	-26	- ^c	- ^c
BDA^d	44 °		-40 °	

^aData from crystal structures give in reference 4.^bData from crystal structure.^cThe absence of a C-terminus on the synthetic peptide eliminates the ability to report these angles.^dBiaryl dihedral angle.