JOC Notes

Synthesis of Cytochrome *c* Oxidase Models Bearing a Tyr^{244} Mimic

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Abstract: A close structural analogue of the metal-free cytochrome *c* oxidase active site has been synthesized. This model has a proximal imidazole tail and three distal imidazole pickets attached to a porphyrin. One distal imidazole is cross-linked to a phenol, mimicking Tyr^{244}. The strategy behind the successful synthesis of this regioisomerically pure model involved discovering the best sequence to introduce the phenol-substituted imidazole and employing a fluorinated substituent.

Cytochrome *c* oxidase is the terminal enzyme in the respiratory chains of mitochondria and aerobic bacteria.[1a−e] One of the three His coordinating CuB in cytochrome *c* oxidase (C*c*O) is cross-linked to a phenol residue from tyrosine. This phenol that our previous CcO models lacked is thought to play a key role in the $4H^+$, $4e^-$ reduction of O_2 to H_2O and has been the subject of model studies.[2a−j,3a−c] Recently, this Tyr^{244}-His^{240} moiety has been studied in some non-heme models,[2a−j] where other organic components were missing (heme, proximal imidazole). To obtain a closer analogue of the enzyme active site, we have designed **1**, which has one of the distal imidazole rings N-substituted with a phenol (Figure 1).

Two regioisomers are presented: *N*-methylimidazole rings may lie either on *cis meso*-positions 10 and 15 which makes the porphyrin asymmetric (**1-*cis***) or on *trans meso*-positions 10 and 20 which makes the porphyrin symmetric (**1-*trans***). The free base porphyrins **1-*cis*** and **-*trans*** share many structural features with our previous reported models, such as $\alpha_3\beta$-atropisomerism, two distal *N*-methyl-substituted imidazoles, and a proximal CF_3Ph-imidazole tail (**1^{F}**) or a nonsubstituted H-tail (**1^{H}**).[4a−c] Herein, we describe the synthesis of **1^{F}-*cis***, the closest structural analogue of the metal-free cytochrome *c* oxidase active site. Its key feature is a phenol-containing imidazole **2** in the distal area.

The synthesis of **2** starts from the key intermediate **4** which we described previously.[3d] A new procedure using the H_2WO_4/H_2O_2 couple[5] was adopted to desulfurize **4**. This led to **5** with an improved yield and a simple workup compared with the earlier $HNO_3/NaNO_2$ method.[3] The desired phenoxy-substituted imidazole **2** was obtained after acid hydrolysis of **5** and subsequent formation of the acid chloride **6**. The synthesis of **2** requires nine steps and gives an 8% overall yield starting from 2-aminophenol (Scheme 1).

Although the synthetic route to **1** appears simple, the first challenge in its preparation relies on developing methods to introduce two different imidazoles onto the α_3 face of the porphyrin **3** in good yield. Since the three distal amines in **3** are not identical, we envisioned that the introduction of one or two imidazoles would lead to two regioisomers. The second challenge is separation of the very polar porphyrin regioisomers.

We developed two synthetic routes to **1** starting from either of two porphyrin synthons $\alpha_3A\beta F_3T$ (**3^{F}**)[4a−c] or $\alpha_3A\beta T$ (**3^{H}**):[4a,b] (1) introduction of the phenol substituted imidazole **2** first and subsequently the two simple imidazoles **7**, the "[1 + 2] approach" (route A, Scheme 2); (2) introduction of the two simple imidazoles **7** first and subsequently the phenol-substituted imidazole **2**, the "[2 + 1] approach" (route B, Scheme 2).

These two strategies have advantages and disadvantages regarding (a) yields of introducing the first imidazole leading to **8$_{mono}$** ([1 + 2] approach) or **9$_{bis}$** ([2 + 1] approach), (b) byproduct formation and recycling, (c) yields of introducing the second imidazole, (d) chromatographic separation of polar porphyrin intermediates from the polar porphyrin starting materials, and (e) chromatographic separation of regioisomers of polar porphyrins.

All of these issues are compared for each approach in order to determine which offers the best compromise to these problems and gives an optimum pathway to obtain **1**.

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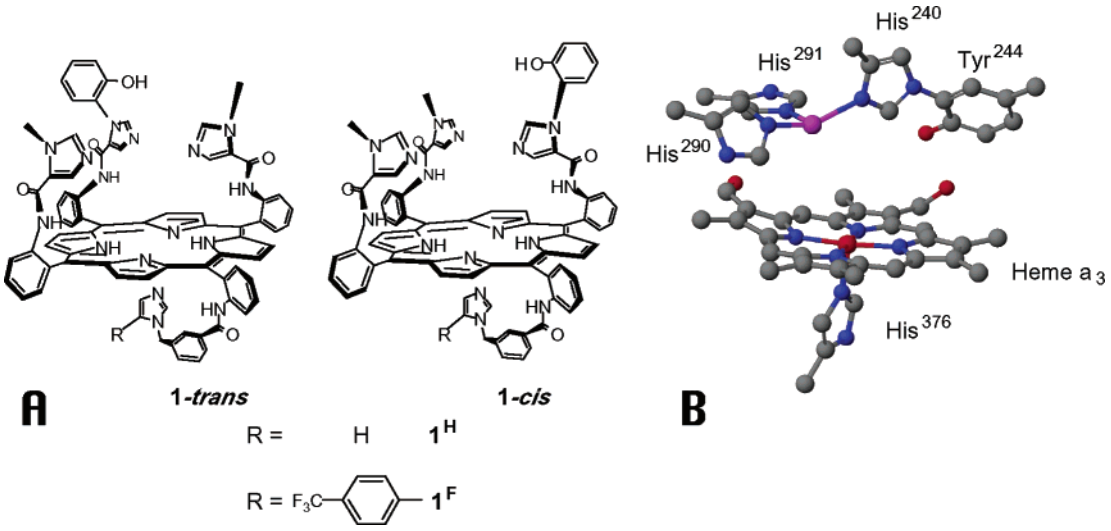
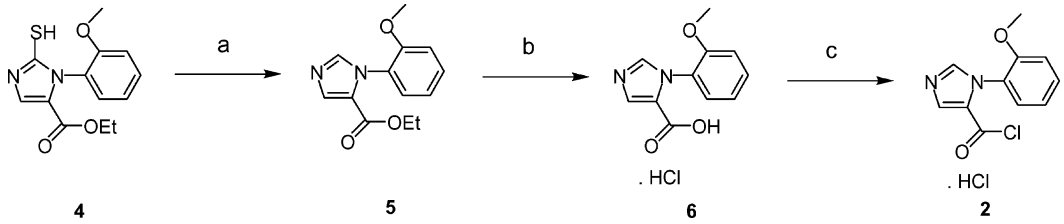


FIGURE 1. (A) Chemical structure of the target models. (B) Heme a_3/Cu_B of bovine cytochrome *c* oxidase.[1e] The C atoms are gray, the N atoms are blue, the O and Fe atoms are red, and Cu is pink.

SCHEME 1[a]



[a] Reagents and conditions: (a) H_2WO_4, H_2O_2, $Et_2NC_6H_6$, rt, 5 h, 95%; (b) HCl, 100 °C, 92%; (c) $(COCl)_2$, CH_3CN, cat. DMF, 4 h, rt, quantitative.

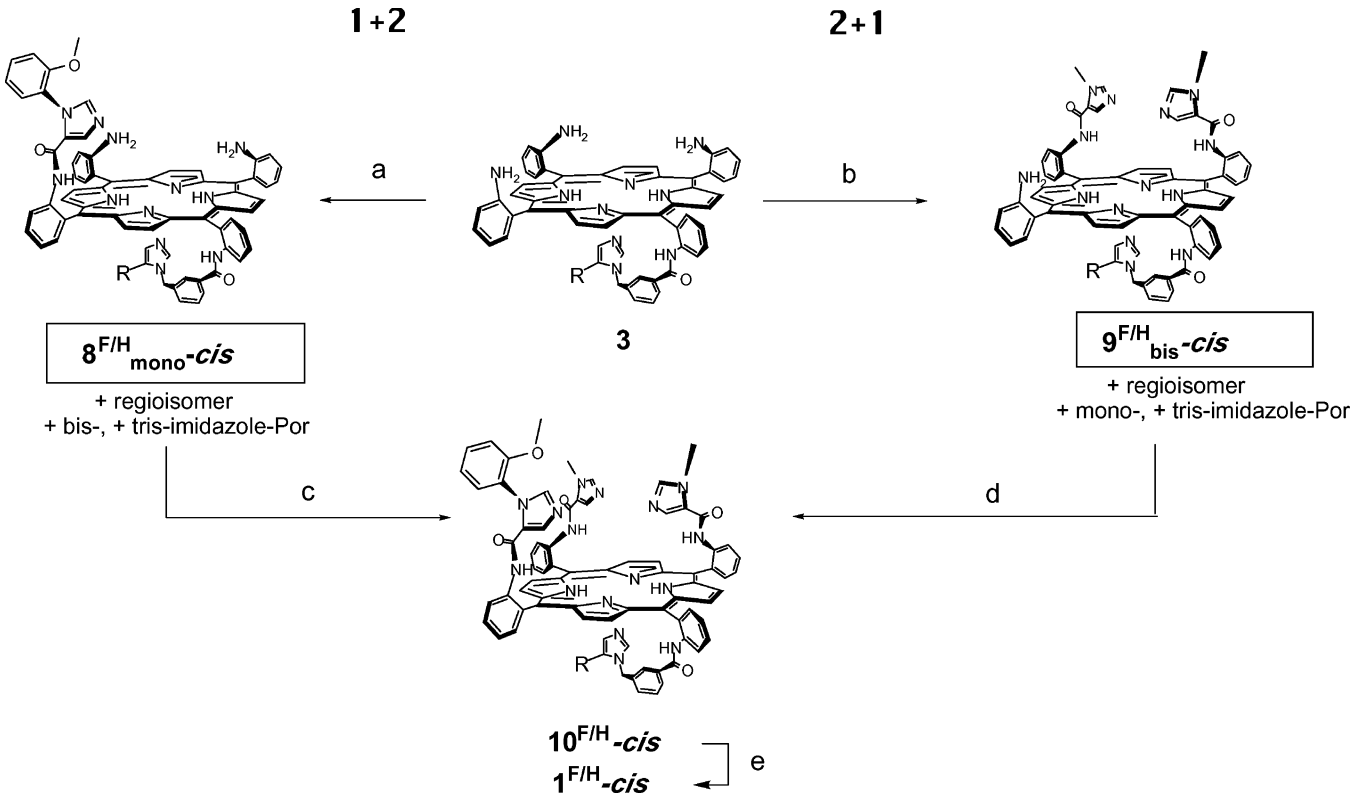
In the first step of the [1 + 2] approach (route A), the target intermediate is the monoimidazole porphyrin $\mathbf{8}_{\mathbf{mono}}$ which is obtained from reaction of *N*-methoxyphenyl-containing imidazole **2** with **3** (Scheme 2). A survey of the **2**/**3** ratio vis-à-vis dilution conditions showed that a large excess of picket **2** leads to high conversion; however, the byproducts $\mathbf{8}_{\mathbf{bis}}$ and $\mathbf{8}_{\mathbf{tris}}$ were obtained in 17% and 14% yield, respectively, while the yield of the target $\mathbf{8}_{\mathbf{mono}}$ is only 21%. Since $\mathbf{8}_{\mathbf{bis-tris}}$ are not useful for the preparation of **1**, fewer equivalents of **2** were added. This led to a low conversion (20%) but to a more satisfactory ratio mono/bis/tris (4:1:1). However, this approach is unsatisfactory because purification is difficult: the R_f values of $\mathbf{8}_{\mathbf{mono}}$ and the recovered starting material **3** are so close, 0.55 and 0.60, respectively, that it makes the isolation of $\mathbf{8}_{\mathbf{mono}}$ difficult. This became even more troublesome when fewer equivalents of **2** were used.

In the first step of the [2 + 1] approach (route B), the target intermediate is the bis imidazole porphyrin species $\mathbf{9}_{\mathbf{bis}}$ obtained from reaction between **7** and **3** (Scheme 2). The intermediate $\mathbf{9}_{\mathbf{bis}}$ is obtained together with the mono- and tris-condensation products $\mathbf{9}_{\mathbf{mono}}$ and $\mathbf{9}_{\mathbf{tris}}$. In contrast to the [1 + 2] approach where two useless byproducts $\mathbf{8}_{\mathbf{bis}}$ and $\mathbf{8}_{\mathbf{tris}}$ are formed, the useful intermediate $\mathbf{9}_{\mathbf{mono}}$ can be recycled and reacted again with **7** to give $\mathbf{9}_{\mathbf{bis}}$. Therefore, in an effort to conserve the porphyrin synthon **3**, the first step in the [2 + 1] approach appears to be more practical than the one in the [1 + 2] because only a single side-product porphyrin ($\mathbf{9}_{\mathbf{tris}}$) is formed whereas two are generated from the [1 + 2] approach ($\mathbf{8}_{\mathbf{bis}}$ and $\mathbf{8}_{\mathbf{tris}}$). Moreover, the [2 + 1] approach is more satisfactory in terms of isolating the target porphyrin from the statistical mixture, compared with the [1 + 2] approach because the R_f values of bis(*N*-methylimidazole)porphyrins $\mathbf{9}_{\mathbf{bis}}$ are very different from the starting material **3** in contrast to what we found with monomethoxyphenyl-containing-imidazole porphyrins $\mathbf{8}_{\mathbf{mono}}$.

Another criteria for comparing the [1 + 2] and [2 + 1] approaches is the separation of regioisomeric intermediates. Regardless of the type of imidazole introduced, separation of regioisomers is more facile with monoimidazole porphyrins than with bis-imidazole porphyrins. Therefore, the [1 + 2] approach is superior to the [2 + 1] approach for cleanly separating the regioisomers.[6] Also, we found that the nature of the tail appears to influence the ease of regioisomer separation. For example, regioisomer separation was found to be more straightforward with the CF_3Ph-tailed (F) than with H-tailed porphyrins (H), especially with the bis-imidazole porphyrin species $\mathbf{9}_{\mathbf{bis}}$. Finally, we found that the ratio of *cis*/*trans* compounds obtained was about 2:1 for the mono- as well as the bis-porphyrins. It should also be noted that in contrast to the ***-trans*** regioisomers, the ***-cis*** regioisomers of $\mathbf{8}_{\mathbf{mono}}$ or $\mathbf{9}_{\mathbf{bis}}$ are chiral, but we made no attempt to resolve the enantiomers.

The second step in either the [1 + 2] or the [2 + 1] approach starts with a pure regioisomer of mono- or bis-imidazole porphyrin and introduces the second, different imidazole. It is worth mentioning that when a mixture of regioisomers $\mathbf{8}_{\mathbf{mono}}$***(cis + trans)*** or $\mathbf{9}_{\mathbf{bis}}$ ***(cis + trans)***

SCHEME 2. Direct Introduction of Two Different Types of Imidazoles onto the α_3 Face of the α_3AβTail Porphyrin 3: [1 + 2] and [2 + 1] Approaches[a]



[a] Reagents and conditions: (a) **2**, CH_3CN, rt, 30 min, 21%; (b) **7**, CH_3CN, $Et_2NC_6H_5$, rt, 30 min, 25%; (c) (1) HCl/Et_2O, $CH_3CN-THF$, rt, 10 min, (2) **7**, CH_3CN, $Et_2NC_6H_5$, rt 30 min, 60%; (d) 1) HCl/Et_2O, $CH_3CN-THF$, rt, 10 min, (2) **2**, CH_3CN, $Et_2NC_6H_5$, rt 30 min, 35−40%; (e) (1) BBr_3, CH_3Cl_2, −78 °C, 30 min, (2) 0 °C, 2 h, (3) CH_3OH, rt 30 min, 80−85%.

was used for this second condensation reaction, regioisomer separation of the tris-imidazole porphyrin **10** was tedious. As we had shown that regioisomer separation is more difficult with bis-imidazole porphyrins than with mono-imidazole porphyrins, it was not surprising to find that regioisomer separation of the more polar tris-imidazole species **10** was even more difficult than with $\mathbf{9_{bis}}$ species, even when an F-tail was present. Because of this problem, we always performed the regioisomer separation of each intermediate $\mathbf{8_{mono}}$ or $\mathbf{9_{bis}}$ prior any condensation reaction leading to porphyrin **10**. We also discovered that the second condensation reaction could not be performed using the same experimental conditions as those described for the first step; otherwise, low yields of target molecule **10** are obtained together with a large amount of unidentified fractions. Optimum conditions required a homogeneous solution of the porphyrins, obtained by using cosolvents such as dichloromethane or tetrahydrofuran. Also the porphyrin mixture must be acidified with 3 to 4 equiv of HCl before adding the acyl chlorides in order to reprotect the nucleophilic imidazole nitrogen in intermediates $\mathbf{8_{mono}}$ or $\mathbf{9_{bis}}$ which were no longer protonated after workup ($NaHCO_3$) and chromatography (NH_3-saturated eluent). As was pointed out earlier,[4b] the formation of unidentified fractions may be explained by the fact that unprotected imidazoles quaternize by reaction with acyl chloride to produce oligomeric acyl imidazolium species.[4b] During the condensation, some minimum equivalent of base was added allowing the desired transformation to occur. After taking these precautions, the yields of **10** from $\mathbf{8_{mono}}$ ([1 + 2] approach) or $\mathbf{9_{bis}}$ ([2 + 1] approach) rose to 70% and 35−40%, respectively. Without preliminary acid treatment of porphyrins the yields dropped to 18% and 9%, respectively. In fact, better yields in the second condensation reaction were always obtained from the [1 + 2] approach compared with from the [2 + 1] approach. This confirms our assumption that the more nonprotected imidazoles that are present in the starting material (up to three in $\mathbf{9_{bis}}$ in the [2 + 1] approach, up to two in $\mathbf{8_{mono}}$ in the [1 + 2] approach) the lower are the yields in the second condensation reaction. Alternatively, when the condensation reaction in acetic acid/sodium acetate system was run following our earlier procedures, only 30% yield was obtained.[4b]

A comparison between [1 + 2] and [2 + 1] approaches is relevant for the first and most difficult step of the synthetic scheme. With porphyrins bearing F-tail, al-

(6) For all compounds presented here, successful separation of regioisomeric porphyrins depends on using thin silica layer (500 μm), saturating the eluent with ammonia and low loading of porphyrin. These criteria became more crucial for the separation of regioisomeric intermediates of $\mathbf{9^F_{bis}}$ extremely dependent on ammonia (with H-tail porphyrins, the separation of regioisomers of $\mathbf{9^H_{bis}}$ is not possible).

(7) (a) Differential protection of the amines using our previously reported protective groups was attempted. The purification of mono- and bis-trifluoroacetamido or mono- and bis-trityl porphyrins is even more troublesome than the purification of mono- and bis-imidazole porphyrins; for that reason, these protective groups were not used here. (b) Given the difficulties to introduce only one or two imidazoles, we tried the selective deprotection of one phenol among three available in $\mathbf{8_{tris}}$. Eventually, some target mono-phenolic compound was obtained, but the yields were low and hardly reproducible, and mostly the tris-deprotected species was obtained.

though the separation of regioisomeric intermediates is less straightforward with [2 + 1] ($\mathbf{9_{bis}}$) rather than with [1 + 2] ($\mathbf{8_{mono}}$), the [2 + 1] approach was preferred because it was easier to control the introduction of the first kind of picket, the possibility to recycle one byproduct and the efficient separation of target from the statistical mixture.[7a] However, with porphyrins bearing the H-tail, the separation of regioisomeric intermediates in the [2 + 1] approach was not possible; therefore, only the [1 + 2] approach was considered.

The final step of our synthetic scheme is deprotection of the phenol in $\mathbf{10^F}$-***cis***. This was achieved using 18−20 equiv of BBr_3 at low temperature (−78 to 0 °C) and gave $\mathbf{1^F}$-***cis*** in 85% yield. When more equivalents of BBr_3 are used or when the reaction is run at room temperature for a longer time, several decomposition products are observed together with a lower yield of **1**. Except for this point, this last step in the synthetic scheme is less difficult and chromatographic separation is reasonable.[7b] However, the target $\mathbf{1^F}$-***cis*** appears to be more air- and light-sensitive than the other porphyrin intermediates described above. This free base porphyrin is a good model for the natural CcO active site because all of the key groups are present: porphyrin, proximal base, three distal imidazoles including one imidazole linked to a phenol-protected residue.

Finally, it is interesting to note that the ^{1}H NMR spectrum of the chiral mono- and bis-imidazole-bearing porphyrins (***cis***) changes noticeably with respect to the symmetric porphyrins (***trans***). Because *cis*-porphyrins presented here are chiral, the methylene protons in the proximal tail are diastereotopic as shown by their ^{1}H NMR signals. But after introduction of the second type of imidazole, bringing more symmetry to the system, the benzyl CH_2 signal of *cis*-porphyrins again appears as a singlet.

$\mathbf{1^F}$-***cis*** is to be used for mechanistic and biomimetic catalytic studies of reduction of O_2 to H_2O, after insertion of Fe and Cu. Larger amounts of $\mathbf{1^F}$-***cis*** will need to be prepared in the future for which we intend to bring improvements to these methods.

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Supporting Information Available: Experimental procedures and characterization data for new compounds **1**−**2**, **6**, and **8**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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