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Synthesis of Nitric Oxide Reductase Active Site Models Bearing Key Components at Both Distal and Proximal Sites

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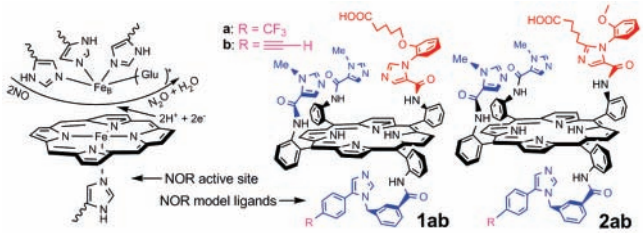
ABSTRACT



Porphyrins 1ab and 2ab were successfully synthesized from *cis*-α_2-bisimidazole-β-imidazole-tail porphyrins and two newly synthesized imidazole pickets containing an aliphatic ester chain following a [2+1] approach. The four compounds possess a distal trisimidazole set, a distal carboxylic acid, and a proximal imidazole, which constitute all the key features of the coordination environment of the active site in Bacterial Nitric Oxide Reductase (NOR) and make them the closest synthetic NOR model ligands to date.

The success in modeling cytochrome *c* oxidase (C*c*O) chemistry[1] aroused our interest in biomimetic studies of nitric oxide reductase (NOR), a distant member of the superfamily of cytochrome oxidases. NOR, a membrane-bound enzyme, is involved in bacterial denitrification, the sequential reduction of nitrate to dinitrogen; it catalyzes the $2e^-$ reduction of nitric oxide to nitrous oxide with the formation of a N−N bond.[2] Structurally, C*c*O and NOR have many similar features, as well as some significant differences. Both active sites are bimetallic with a monohistidine ligated five-coordinate heme and a trisimidazole ligated distal metal. However, Fe_B occupies the distal position in NOR instead of Cu_B in C*c*O. Also, a tyrosine residue in C*c*O is missing in NOR, while a glutamic acid residue is postulated to coordinate to the Fe_B center in NOR.

So far, few synthetic models have been developed to study NOR. A diiron porphyrin lacking the glutamic acid mimic and the proximal histidine group was able to bind NO to form dinitrosyl complexes, but did not lead to N_2O formation.[3] Recently, a closer model featuring a porphyrin bearing

(1) (a) Collman, J. P.; Herrmann, P. C.; Boitrel, B.; Zhang, X. M.; Eberspacher, T. A.; Fu, L.; Wang, J. L.; Rousseau, D. L.; Williams, E. R. *J. Am. Chem. Soc.* **1994**, *116*, 9783. (b) Collman, J. P.; Fu, L.; Herrmann, P. C.; Zhang, X. *Science* **1997**, *275*, 949. (c) Collman, J. P.; Rapta, M.; Broring, M.; Raptova, L.; Schwenninger, R.; Boitrel, B.; Fu, L.; L'Her, M. *J. Am. Chem. Soc.* **1999**, *121*, 1387. (d) Collman, J. P.; Sunderland, C. J.; Boulatov, R. *Inorg. Chem.* **2002**, *41*, 2282. (e) Collman, J. P.; Sunderland, C. J.; Berg, K. E.; Vance, M. A.; Solomon, E. I. *J. Am. Chem. Soc.* **2003**, *125*, 6648. (f) Collman, J. P.; Decréau, R. A.; Zhang, C. *J. Org. Chem.* **2004**, *69*, 3546. (g) Collman, J. P.; Devaraj, N. K.; Decréau, R. A.; Yang, Y.; Yan, Y. L.; Ebina, W.; Eberspacher, T. A.; Chidsey, C. E. D. *Science* **2007**, *315*, 1565. (h) Decréau, R. A.; Collman, J. P.; Yang, Y.; Yan, Y.; Devaraj, N. K. *J. Org. Chem.* **2007**, *72*, 2794.

(2) (a) Tavares, P.; Pereira, A. S.; Moura, J. J. G.; Moura, I. *J. Inorg. Biochem.* **2006**, *100*, 2087. (b) Wasser, I. M.; Vries, S. D.; Moenne-Loccoz, P.; Schröder, I.; Karlin, K. D. *Chem. Rev.* **2002**, *102*, 1201. (c) Hendriks, J.; Gohlke, U.; Saraste, M. J. *Bioenerg. Biomembr.* **1998**, *30*, 15. (d) Zumft, W. G. *Microbiol. Mol. Biol. Rev.* **1997**, *61*, 533. (e) Averill, B. A. *Chem. Rev.* **1996**, *96*, 2951.

(3) (a) Ju, T. D.; Woods, A. S.; Cotter, R. J.; Moenne-Loccoz, P.; Karlin, K. D. *Inorg. Chim. Acta* **2000**, *297*, 362. (b) Wasser, I. M.; Ju, T. D.; Huang, H. W.; Moenne-Loccoz, P.; Karlin, K. D. *J. Inorg. Biochem.* **2001**, *86*, 483. (c) Wasser, I. M.; Martens, C. F.; Verani, C. N.; Rentschler, E.; Huang, H. W.; Moenne-Loccoz, P.; Zakharov, L. N.; Rheingold, A. L.; Karlin, K. D. *Inorg. Chem.* **2004**, *43*, 651. (d) Wasser, I. M.; Huang, H. W.; Moenne-Loccoz, P.; Karlin, K. D. *J. Am. Chem. Soc.* **2005**, *127*, 3310.

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trisimidazole picket and a carboxylic acid moiety at the distal site showed that the presence of a carboxylic acid moiety not only helped stabilize Fe_B coordination at the distal binding site, but also mediated the redox properties of both the heme Fe and Fe_B centers.[4] However, to more faithfully mimic the active site of NOR, a proximal histidine residue should be included in the model. Although spectroscopic studies indicate that the proximal histidine dissociates from heme iron after its binding of NO,[5] histidine binding also prevents NO association with the metal center on the proximal side. Also, recoordination of the detached proximal histidine upon reduction of the diiron center and concomitant loss of the bridging oxo ligand should stabilize the reduced heme iron center. Moreover, recent studies on Fe^{II} porphyrin NO complexes suggested that a six-coordinate heme-nitrosyl intermediate with an imidazole axial ligand is beneficial for the N−N bond forming step in the proposed mechanism.[6] Such a six-coordinate species was detected in the reduction of NO to N_2O by a ba_3-type heme-copper oxidase from resonance Raman spectroscopy.[7]

In this report, we disclose the synthesis of NOR ligands **1ab** and **2ab** which feature a porphyrin with three imidazole pickets and a carboxylic acid moiety at the distal site and an imidazole ligand at the proximal position; both the distal ligand set and the proximal ligand are covalently attached to the porphyrin (Figure 1). Porphyrins **1**−**2a** have a proximal

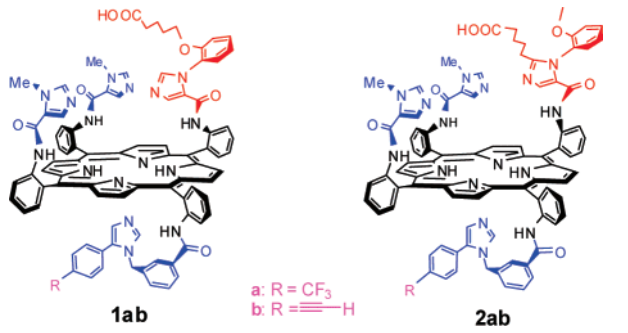


Figure 1. NOR ligands **1ab** and **2ab**.

CF_3Ph imidazole tail, while **1**−**2b** possess a terminal alkyne moiety on the tail. The former is to be used as a comparison to our first-generation NOR model[4] for spectroscopic and NO reactivity studies, whereas the latter is to be covalently attached onto electrode surfaces by Sharpless Click Chemistry[8] in order to conduct electrocatalytic studies of $2e^-$ reduction of NO to N_2O under rate-limiting electron flux.

In addition, the carboxylic acid moiety in **1ab** is on the ortho position of the phenyl ring that is *N*-substituted to a distal imidazole, while in **2ab**, the moiety is on the C-2 position of a distal imidazole. The former may favor an orientation parallel to the porphyrin plane, while the latter will possibly point upward, which might provide a different coordination environment for Fe_B after metalating these free bases. The four porphyrins possess all the key features of the coordinating environment of the active site of the native NORs and represent the best available free-base NOR ligands.

Given the similarities between the actives sites of C*c*O and NOR, modification of our previous C*c*O models is a promising strategy to provide functional NOR models. Since the *cis*-α_2-bisimidazole-β-tail-porphyrins **5ab** were previously synthesized in our laboratory,[1f,h] and attaching a phenol-containing imidazole onto them led to free-base C*c*O models,[1f] the key is to synthesize new imidazole pickets containing a carboxylic acid moiety and to attach them onto **5ab** (Scheme 3) to afford free-base NOR models.

The synthesis of new imidazole pickets starts from the key intermediate **8**, which was described previously.[1f] The desired alkyl chain carboxylic acid moiety can be introduced onto either the C-2 position of the imidazole (position 1) or the ether linkage on the benzene ring (position 2) (Figure 2). Before the imidazole picket is attached onto a porphyrin,

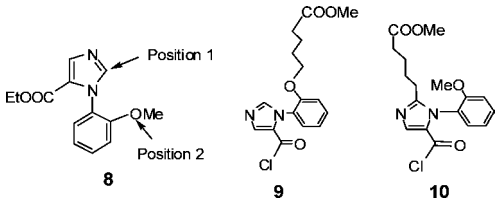


Figure 2. Two types of imidazole pickets **9** and **10** containing an alkyl chain ester and their key precursor **8**.

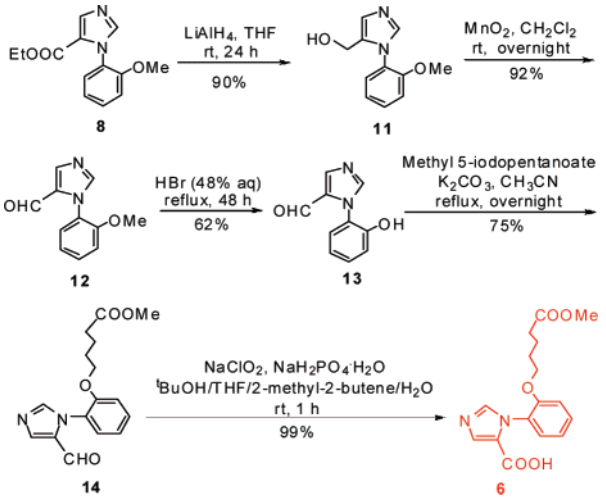
the carboxylic acid needs to be protected as an ester to avoid its interference in the amide-forming reaction. Since there are two ester groups (COOEt and the aliphatic chain ester) in the same imidazole picket, it is necessary to differentiate them and selectively convert the COOEt to COOH while keeping the aliphatic chain ester.

We chose to convert the ethyl ester group of **8** to an aldehyde, then introduce the aliphatic chain ester and oxidize the aldehyde to afford the desired ester-containing imidazole acid.[9] Therefore, compound **8** was reduced to alcohol **11** by $LiAlH_4$ in THF (Scheme 1). Oxidation of **11** in the presence of excess MnO_2 in CH_2Cl_2 afforded the aldehyde **12**. These two steps gave very good yields (>90%). Transformation of the phenyl methyl ether of **12** to phenol was first tried with BBr_3. However, the yield was very low (10−13%) even with over 10 equiv of BBr_3 and a prolonged reaction time (room temper-

(4) (a) Collman, J. P.; Yan, Y. L.; Lei, J. P.; Dinolfo, P. H. *Org. Lett.* **2006**, *8*, 923. (b) Collman, J. P.; Yan, Y. L.; Lei, J. P.; Dinolfo, P. H. *Inorg. Chem.* **2006**, *45*, 7581.
(5) Praneeth, V. K. K.; Näther, C.; Peters, G.; Lehnert, N. *Inorg. Chem.* **2006**, *45*, 2795.
(6) Pinakoulaki, E.; Ohta, T.; Soulimane, T.; Kitagawa, T.; Varotsis, C. *J. Am. Chem. Soc.* **2005**, *127*, 15161.
(7) Moeenne-Loccoz, P.; de Vries, S. *J. Am. Chem. Soc.* **1998**, *120*, 5147.
(8) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.

(9) Our attempts to selectively hydrolyze an imidazolyl carboxylic acid ethyl ester in the presence of an aliphatic *tert*-butyl ester were unsuccessful. In our control experiments, the *tert*-butyl ester was always hydrolyzed first.

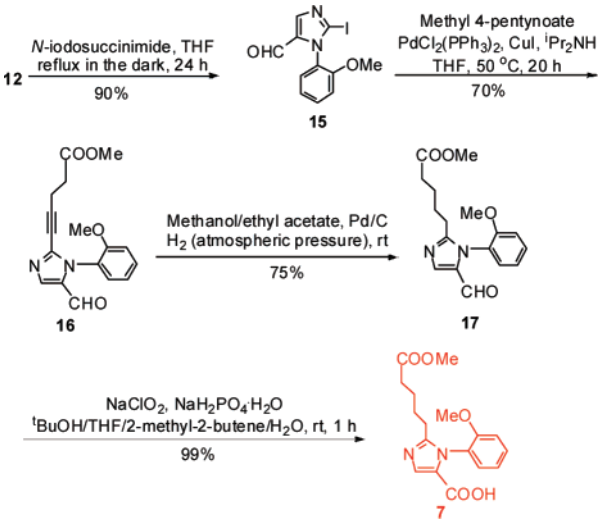
Scheme 1. Synthesis of *O*-Alkylated Imidazole Acid **6**



ature, 72 h). A possible reason is that the complex formed by the coordination of BBr_3 with N atoms in compound **12** became nearly insoluble, which may prevent further attack by BBr_3 on the O atom of the ether moiety. The fact that more than half of the starting material was usually recovered is consistent with this explanation. The methyl ether was successfully removed to yield phenol **13** in 62% yield when refluxing **12** in 48% HBr aqueous solution. Compound **14** was easily obtained by refluxing **13** and methyl 5-iodopentanoate in the presence of excess K_2CO_3 in dry acetonitrile. Subsequent oxidation of **14** with $NaClO_2/NaH_2PO_4$ led quantitatively to the formation of pure imidazole acid **6**.

Synthesis of the second imidazole picket containing an aliphatic chain ester on the C-2 position of the imidazole ring is described in Scheme 2. Refluxing **12** with 2 equiv of

Scheme 2. Synthesis of *C*-Alkylated Imidazole Acid **7**

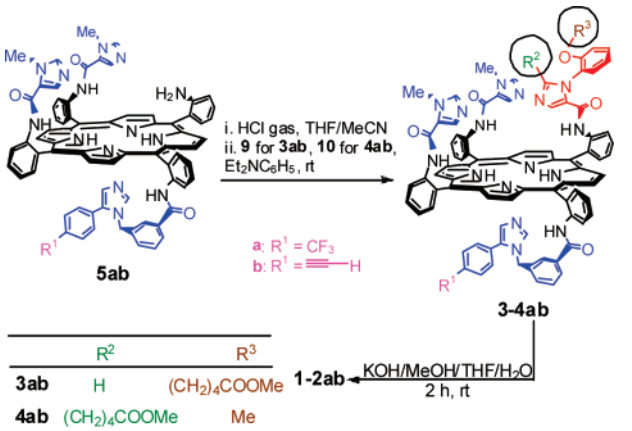


N-iodosuccinimide in dry THF resulted in the formation of an iodoimidazole **15** in 90% yield. First, a Pd-catalyzed coupling reaction between **15** and an alkylzinc iodide reagent [$IZn(CH_2)_4COOMe$] was tried to produce compound **17**. Although it has been reported that such selective carbofunctionalization of the imidazole moiety at the C-2 atom worked pretty well and afforded desired products in excellent yields,[10] in our hands, the starting material was always recovered cleanly without any reaction. Finally, a Pd-catalyzed Sonogashira coupling reaction was used to react the iodoimidazole **15** with methyl 4-pentynoate yielding **16** in 70% yield. Subsequent hydrogenation of the alkyne moiety under atmospheric H_2 pressure in the presence of Pd/C led to **17** in 75% yield.[11a] Oxidation of the aldehyde group of **17** by $NaClO_2/NaH_2PO_4$ afforded the imidazole acid **7** in nearly quantitative yield.

The imidazole acids **6** and **7** can be easily converted to their corresponding acyl chlorides **9** and **10** by reaction with oxalyl chloride or thionyl chloride under anhydrous conditions. While the acyl chloride **9** precipitated during the reaction and could be separated as a pure solid in high yield, the acyl chloride **10** remained as an oil. Attempts to precipitate **10** from various solvent mixtures such as diethyl ether and acetonitrile failed.

Synthesis of α_3-trisimidazole-β-tail-porphyrins **3ab** and **4ab** followed the [2+1] strategy formerly developed in our group (Scheme 3).[1f] Freshly prepared acyl chloride **9** reacted

Scheme 3. Synthesis of NOR Models **1ab** and **2ab** following a [2+1] Approach



with acid protected *cis*-α_2-bisimidazole-β-tail-porphyrins **5ab** and led to **3ab** without any other byproduct. The [2+1] products have almost the same polarity as their corresponding starting materials, which made it impossible to monitor the progress of the reaction by TLC. Therefore, an excess of **9** (>10 equiv) was added to ensure good conversion of **5ab**. Separation of the reaction mixture that usually contains the product and the unreacted starting material was achieved by rotating disk chromatography (chromatotron). Though only

(10) Evans, D. A.; Bach, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1326.

(11) (a) About 10−15% of alcohol was obtained, which was very easily converted to aldehyde **17** with MnO_2 in CH_2Cl_2. (b) After removing all excess thionyl chloride, the residue was washed with anhydrous ether (2 × 1 mL), then left under vacuum overnight.

one band could be observed, the last part of the fraction was collected separately and it turned out to be the clean [2+1] product.

The syntheses of **4ab** were not as straightforward as that of **3ab**. The reaction of **5a** with freshly prepared acyl chloride **10** from oxalyl chloride led to **4a** (25%) along with several polar fractions. Characterization of one of these (compound **2c**, structure shown in the Supporting Information) shows that it bears an oxamic acid picket. This inefficient synthesis was due to difficulties in working up the acyl chloride precursor **10**, which forms an oil always containing some oxalyl chloride. When **10** was obtained from the reaction of **7** with thionyl chloride, it could be more easily worked up than when prepared from oxalyl chloride.[11b] As a result, the reaction of a cleaner version of **10** with **5ab** led mainly to the expected product of **4ab** along with unreacted starting material and a very small amount of a polar fraction. Surprisingly, the [2+1] products **4ab** were less polar than their starting materials **5ab**, and it was possible to monitor the progress of the reaction by TLC and to separate **4ab** from **5ab** with the chromatotron.

The methyl esters were saponified to afford free bases **1ab** and **2ab** in high yields. The inseparable mixture of **3a**, **5a** and **3b**, **5b** can be easily separated after saponification of **3ab** to **1ab**, since the acid products **1ab** are much more polar than the remaining compounds **5ab**. Thus, the unreacted valuable *cis*-bisimidazole porphyrin synthon can be cleanly recovered. Porphyrin compounds **1**−**4ab** were purified and characterized based on the standards of porphyrinoid and porphyrin chemistry established previously.[12]

Model **1** is a racemic mixture that has ^{1}H NMR features reminiscent of earlier racemic C*c*O models.[1f,h,12b] However, model **2**, which is structurally quite similar to **1**, is characterized by a distinct ^{1}H NMR spectrum with a split in the signals of N−Me, OMe(ether), OMe (ester), and to a lesser extent the alkynyl and the NH protons. This was unexpected given the high degree of purity of our models and precursor synthons.[11] We suggest that there are several conformers present due to the rotation of two functional groups, the alkyl chain and the methoxyphenyl ring, which is reminiscent of the phenomenon encountered in our previous C*c*O model containing one methoxyphenyl ring that ended up having several conformers.[14] The ratio of the split peaks is always 1:1 for the spectra obtained with different NMR solvents and on NMR instruments with different magnet field (500 and 600 MHz), suggesting the presence of two sets of conformers with almost the same free energy. This complex phenomenon involving several conformers in a racemic mixture is currently being studied by ROESY and COSY NMR, Gaussian and Spartan calculations, and also through the spectroscopic study of simpler models bearing parts of **2** in order to disentangle the contribution of each component in this process. A thorough spectroscopic analysis of these complex models (**1** and **2**) and their metalated versions will be reported in future work.

The proton signals of both the alkyl ester and alkyl acid chain in compounds **1**−**4ab** moved upfield (0.5−2.0 ppm) compared to those in the free imidazole pickets **6** and **7**, implying that they are actually suspended over the porphyrin plane instead of rotated away from the porphyrin.

Free bases **1ab** and **2ab** possess all the key groups—porphyrin, proximal imdazole, three distal imidazoles including one imidazole containing an aliphatic carboxylic acid—and represent the closest metal-free models for the natural NOR active site reported to date. These are to be metalated with Fe at both porphyrin and the distal site and will be examined as functional NOR models for biomimetic and mechanistic studies of reduction of NO to N_2O. Spectroscopic characterization of the diiron porphyrins and investigation of interaction between diiron porphyrins and O_2 and NO are currently in progress and will be reported later.

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Supporting Information Available: Synthetic procedures and characterization including ^{1}H NMR, ^{13}C NMR, LRMS, HRMS, and HPLC-MS of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) (a) Collman, J. P.; Broring, M.; Fu, L.; Rapta, M.; Schwenninger, R.; Straumanis, A. *J. Org. Chem.* **1998**, *63*, 8082. (b) Collman, J. P.; Broring, M.; Fu, L.; Rapta, M.; Schwenninger, R. *J. Org. Chem.* **1998**, *63*, 8084. (c) Collman, J. P.; Brauman, J. I.; Doxsee, K. M.; Halbert, T. R.; Bunnenberg, E.; Linder, R. E.; Lamar, G. N.; Delgaudio, J.; Lang, G.; Spartalian, K. *J. Am. Chem. Soc.* **1980**, *102*, 4182. (d) Collman, J. P.; Gagne, R. R.; Reed, C. A.; Halbert, T. R.; Lang, G.; Robinson, W. T. *J. Am. Chem. Soc.* **1975**, *97*, 1427. (e) Collman, J. P.; Decréau, R. A. *Org. Lett.* **2005**, *7*, 975.

(13) (a) Careful analyses of the purity of imidazole and porphyrin synthons in the syntheses of models **2ab** were carried out by ^{1}H NMR, ^{13}C NMR, and HPLC-MS. Only the C-2 regioisomer was found in the iodoimidazole precursor **15** and the subsequent synthons **16**, **17**, and **7**. Substitution at C-4 vs C-2 in imidazoles is known to trigger significant changes in their ^{1}H and ^{13}C NMR spectra, which is well documented in the literature.[13b] Moreover, these analyses did not reveal the presence of trans-regioisomer contaminant in the *cis*-bisimidazole porphyrin precursor **5ab**. (b) O'Connell, J. F.; Parquette, J.; Yelle, W. E.; Wang, W.; Rapoport, H. *Synthesis* **1988**, 767.

(14) Collman, J. P.; Decreau, R. A.; Costanzo, S. *Org. Lett.* **2004**, *6*, 1033.