# New 1,4,7-triazacyclononane-based functional analogues of the Fe/Cu active site of cytochrome *c* oxidase: structure, spectroscopy and electrocatalytic reduction of oxygen

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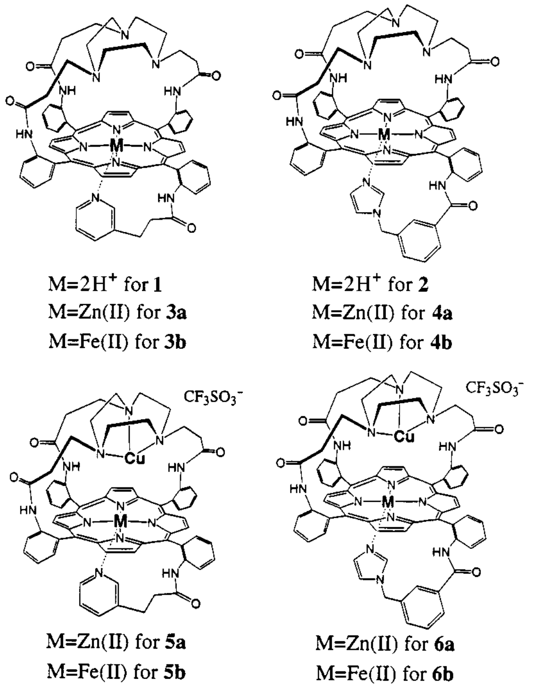
**Two new covalently linked functional model compounds for the $Fe_{a3}/Cu_B$ active sites of heme-copper oxidases have been prepared and characterized by novel synthetic methodologies; the X-ray structure of the Zn form and the electrocatalytic reduction of $O_2$ by the Fe/Cu forms are reported.**

Cytochrome *c* oxidase (C*c*O) is a membrane-bound metalloenzyme which catalyzes the $4e^-$, $4H^+$ reduction of $O_2$ to $H_2O$.[1] Toxic intermediates such as $H_2O_2$ and $HO_2^-$ are not released during this exergonic reaction. The energy produced by the $O_2$ reduction is coupled to proton translocation, contributing to the protomotive force which drives conversion of ADP to ATP.[2] Recent X-ray structures of bacterial[3] and mammalian[4] forms of this enzyme have initiated increased activity among biomimetic chemists.[5] There are several unresolved issues concerning the mechanism of $O_2$ reduction at the heme $a_3/Cu_B$ bimetallic center: (a) the role of the $Cu_B$ and Tyr residue in $O_2$ binding and activation, (b) the nature of the peroxy-intermediate, and (c) the possible involvement of an imidazole ligand in the proton pumping mechanism.[1,6]

The construction of covalently-linked functional model compounds which closely resemble the $Fe_{a3}/Cu_B$ active site is an important part of the effort to resolve these issues. We have previously reported the synthesis and catalytic activity of two such model compounds prepared by a congruent multiple Michael addition methodology.[7] Of these, the 1,4,7-triazacyclononane- (TACN) 'capped' Co/Cu and *N,N′,N″*-tribenzyl-tris(aminoethyl)amine- (TBTren) 'capped' Fe/Cu complexes have shown $4e^-$, $4H^+$ electrocatalytic reduction of $O_2$ to $H_2O$ at physiological pH. Prompted by these encouraging results we have developed a more flexible synthetic strategy[8] and prepared two new TACN-capped porphyrins, **1** and **2** (Scheme 1), with different covalently attached axial ligands.

Herein, we describe a new methodology for the selective incorporation of Zn(II) or Fe(II) into these new ligands. We present $^1H$ NMR analysis of the free base, Zn(II), Fe(II), Zn(II)/Cu(I), and Fe(II)/Cu(I) forms as a useful tool for partial elucidation of the conformations of these complexes in solution. Furthermore, we report the crystal structure of the pyridine-tailed Zn(II) complex—the first structural characterization within this family of compounds. Finally we present preliminary results on the electrocatalytic reduction of $O_2$ to $H_2O$ by these new Fe/Cu model compounds.

Porphyrinato–Zn(II) complexes **3a** and **4a** are diamagnetic, redox-inactive analogues of the porphyrinato–Fe(II) complexes. These are easily prepared by reaction of the free base with an excess of zinc acetate, followed by basic-alumina filtration using a solvent saturated with $NH_3$(g). Selective introduction of Fe(II) into the porphyrin is achieved by the reaction of the free base porphyrin with excess $FeBr_2$ without using external base, followed by basic-alumina filtration.[9] Final Zn(II)/Cu(I) and Fe(II)/Cu(I) complexes **5** and **6** are prepared by the reaction of the appropriate porphyrinato complex with copper(I) triflate.[10]



M=2H$^+$ for **1**
M=Zn(II) for **3a**
M=Fe(II) for **3b**

M=2H$^+$ for **2**
M=Zn(II) for **4a**
M=Fe(II) for **4b**

M=Zn(II) for **5a**
M=Fe(II) for **5b**

M=Zn(II) for **6a**
M=Fe(II) for **6b**

**Scheme 1**

The $^1H$ NMR spectra of **2** and its Zn(II) complex **4a** are compared in Fig. 1. The desired $\alpha_3\beta$ symmetry of the porphyrin core can be deduced from the pattern of β-pyrrolic and phenyl resonances.[11] The broad, upfield-shifted $CH_2$ resonances of the TACN ligand confirm the flexible structure of the cap as well as its location above the porphyrin ring. Introduction of Zn(II) into the porphyrin results in significant upfield shifts for the axial ligand resonances: signals *ortho* to the coordinated nitrogen are shifted by $>3.5$ ppm, reflecting the close proximity to the porphyrin ring. The two *ortho* proton resonances of the axial base in the Fe(II)CO forms[9] are at higher field than in the Zn(II)

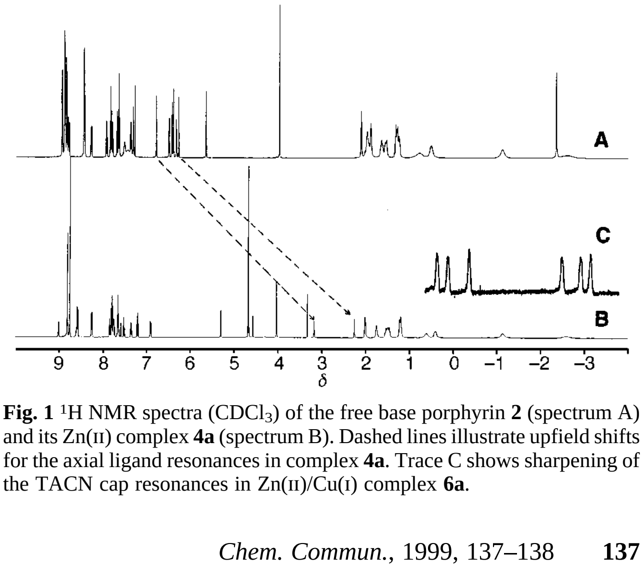


**Fig. 1** $^1H$ NMR spectra ($CDCl_3$) of the free base porphyrin **2** (spectrum A) and its Zn(II) complex **4a** (spectrum B). Dashed lines illustrate upfield shifts for the axial ligand resonances in complex **4a**. Trace C shows sharpening of the TACN cap resonances in Zn(II)/Cu(I) complex **6a**.

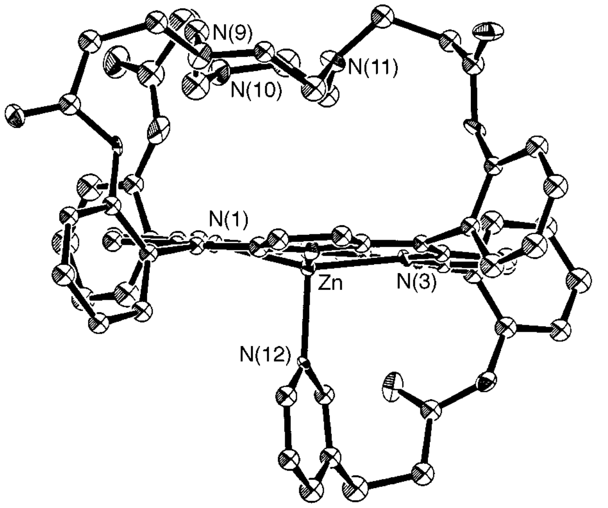


**Fig. 2** Thermal ellipsoid plot of the molecular structure of **3a**. Selected bond lengths (Å) and bond angles (°): Zn–N(1) 2.064(9), Zn–N(2) 2.075(8), Zn–N(3) 2.095(8), Zn–N(4) 2.095(8), Zn–N(12) 2.109(9); N(1)–Zn–N(2) 88.1(3), N(1)–Zn–N(3) 159.0(4), N(1)–Zn–N(12) 100.4(4), N(2)–Zn–N(3) 89.2(3), N(2)–Zn–N(4) 160.2(4), N(2)–Zn–N(12) 101.9(3), N(3)–Zn–N(4) 87.5(3), N(3)–Zn–N(12) 100.5(4), N(4)–Zn–N(12).

complexes, which is consistent with stronger coordination of the axial ligand to Fe(II). The high-field portion of the spectrum for the Zn(II)/Cu(I) complex **6a** (Fig. 1, trace C) shows a significant sharpening of the TACN cap resonances, consistent with a single conformation in solution.

The geometry of the empty-cap porphyrinato–Zn(II) complex **3a** was further eluciated by single crystal X-ray diffraction.[12] The thermal-ellipsoid plot is shown in Fig. 2 with key bond distances and bond angles. The zinc atom shows an expected distorted square-pyramidal geometry with the four porphyrin nitrogens in equatorial positions [average Zn–N distance of 2.074(9) Å] and a pyridine ligand occupying an apical position [Zn–$N_{pyr}$ distance of 2.109(9) Å]. The Zn atom is displaced 0.37 Å from the porphyrin plane, which shows a moderate degree of ruffling similar to those of related Zn and Fe porphyrin complexes.[13] The TACN cap is in a chair conformation which is probably preferred for efficient crystal packing. This conformation must change upon coordination of Cu(I) in order to achieve effective binding to all three nitrogen ligands. Other bond distances and bond angles are similar to those of the related compounds and will be analyzed in detail elsewhere.

In their reduced forms, Fe(II)/Cu(I) complexes **5b** and **6b** show UV–VIS spectra identical to those of the Fe(II)–porphyrinato precursors.[9] On the other hand, incorporation of Cu(I) (as the triflate salt) has a significant effect on the complexes' reactivities toward $O_2$, as well as their solubilities in organic solvents. Reversible binding of $O_2$ to the emtpy-cap Fe(II) complexes becomes irreversible with the FeCu complexes, resulting in the formation of an apparent peroxo–Fe(III) ($O_2^{2-}$) Cu(II) intermediate [$\lambda_{max}$ 422 nm (Soret)]. In dry toluene or acetononitrile a 'peroxo-signature' around 800 $cm^{-1}$ can be detected by IR spectroscopy. Addition of protic solvent (*e.g.* methanol) or exposure to the atmosphere results in complete disappearance of this vibrational mode, indicating a fast decomposition of the peroxo-species in the presence of protons.

The electrocatalytic reduction of $O_2$ by these Fe/Cu complexes have been studied by rotating graphite disc–platinum ring voltammetry.[14] Both new complexes show predominantly 4e$^-$ reduction of $O_2$ to $H_2O$, with a negligible production of peroxide, as determined by the comparison of the ring and disc currents. The half-wave potential for the electroreduction of $O_2$ is 45 mV more positive for the imidazole-tailed complex **6b** in comparison with the pyridine-tailed complex **5b**. These results show that our new Fe/Cu complexes are functional models of the C*c*CO $Fe_{a3}/Cu_B$ active site, and together with our related studies,[7] represent a prime example of fine-tuning their catalytic properties by small structural changes in the porphyrin ligand. We consider these new robust, capsule-like Fe/Cu complexes to be ideal model compounds for solution catalytic studies and for the isolation and structural characterization of various forms of the cytochrome *c* oxidase $Fe_{a3}/Cu_B$ active site.

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9 Iron(II)–porphyrin **3b**: MS(LSIMS$^+$): *m/z* 1152.3 (MH$^+$) for $C_{67}H_{60}N_{12}O_4Fe$. UV–VIS (MeCN): $\lambda_{max}$ 445 (Soret), 565 nm. $^1H$ NMR ($CDCl_3$): $\delta$ 48–54 (four broad peaks characteristic of β-pyrrolic protons on a paramagnetic five-coordinate iron ($S$ = 2) porphyrin with $\alpha_3\beta$ symmetry. $^1H$ NMR ($CDCl_3$ under CO atmosphere, axial ligand resonances only): $\delta$ 6.15(d, 1H), 5.22(m, 1H), 1.52(d, 1H), 1.37(s, 1H).

Iron(II)–porphyrin **4b**: MS(LSIMS$^+$): *m/z* 1204.3 (MH$^+$) for $C_{70}H_{61}N_{13}O_4Fe$. UV–VIS (MeCN): $\lambda_{max}$ 444 (Soret), 566 nm. $^1H$ NMR ($CDCl_3$): $\delta$ 48–54 (m, see above); $^1H$ NMR ($CDCl_3$ under CO atmosphere, axial ligand resonances only): $\delta$ 7.24(d, 1H), 6.95(m, 1H), 6.62(d, 1H), 4.68(s, 1H), 3.62(s, 2H), 3.37(s, 1H), 2.05(s, 1H), 2.31(s, 1H). Full analysis by 1D and 2D $^1H$ NMR spectroscopy of Zn(II) and Fe(II) complexes **3** and **4** will be published elsewhere.

10 MS (LSIMS$^+$): *m/z* 1224.9 (MH$^+$) for $C_{68}H_{60}N_{12}O_4ZnCu$ (for Zn/Cu complex **5a**); *m/z* 1274.1 for $C_{70}H_{61}N_{13}O_4ZnCu$ (for Zn/Cu complex **6a**); *m/z* 1217.2 (MH$^+$) for $C_{68}H_{60}N_{12}O_4FeCu$ (for Fe/Cu complex **5b**); *m/z* 1266.3 for $C_{70}H_{61}N_{13}O_4FeCu$ (for Fe/Cu complex **6b**). The spectra match the calculated isotope distributions exactly.

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12 *Crystal data*: $ZnC_{67}H_{60}N_{12}O_4\cdot 3CHCl_3$, $M$ = 1520.80, triclinic, space group $P\bar{1}$, $a$ = 13.3435(8), $b$ = 13.5214(8), $c$ = 20.850(1) Å, $\alpha$ = 103.096(1), $\beta$ = 91.287(1), $\gamma$ = 111.723(1)°, $V$ = 3380.1(3) Å$^3$, $Z$ = 2, $D_c$ = 1.494 g cm$^{-3}$, $T$ = −146 °C, $\mu$ = 0.779 mm$^{-1}$, $R(R_w)$ = 0.098(0.112). All C atoms have been refined isotopically. CCDC 182/1090. See http://www.rsc.org/suppdata/cc/1999/137/ for crystallographic files in .cif format.

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14 (*a*) T. Geiger and F. C. Anson, *J. Am. Chem. Soc.*, 1981, **103**, 7489; (*b*) J. Koutecky and V. G. Levich, *Zh. Fiz. Khim.*, 1956, **17**, 203; (*c*) Half-wave potentials (*vs.* SSCE) for the reduction of $O_2$ in air-saturated phosphate buffer (pH = 7) at a graphite disk electrode coated with catalyst and rotated at 200 rpm are −95 and −50 mV for **5b** and **6b**, respectively. Number of electrons involved in the reduction of $O_2$ as estimated from the slopes of Koutecky–Levich plots[14b] are $n_{app}$ = 3.89 for **5b** and $n_{app}$ = 3.92 for **6b**.

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