Galactose Oxidase models: ^{19}F NMR as a powerful tool to study the solution chemistry of tripodal ligands in the presence of copper(II)†

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In copper(II) complexes of tripodal ligands, the protonation state of the phenol moiety, and its position (axial *vs.* equatorial), are easily assessed by ^{19}F NMR.

Galactose Oxidase (GO) is a copper(II) enzyme that catalyses the oxidation of primary alcohols into the corresponding aldehydes, with concomitant reduction of dioxygen into hydrogen peroxide. This two-electron chemistry is promoted by a single copper atom, working in synergy with a tyrosyl radical from the protein.[1] The mechanism by which the radical is generated is of crucial interest, and it has been shown that mixing metal-free apo-GO with copper(I or II) in the presence of O_2 affords the mature Cu^{II}-radical enzyme.[2] With the aim of a better understanding of radical cofactors' formation, we have recently studied the solution chemistry of tripodal ligands involving one phenol group, such as HL^{NO2} (Fig. 1) under various copper(II), base, and dioxygen conditions.[3] In particular, we have evidenced acid–base and redox equilibria by the mean of UV-vis and EPR spectroscopies.

New techniques to study the solution chemistry of GO models[4] are of major interest: no tool enables the discrimination in the phenol position (axial *vs.* equatorial), and dynamic information is generally missing. In this context, we present herein how powerful ^{19}F NMR spectroscopy is. Compared to more classical techniques, it offers unique advantages, such as a lack of interfering background signals, a high sensitivity, distribution of resonances over a wide spectral width, and access to dynamic information. To get all of these, labelling of the tripodal ligands should be judiciously carried out: the fluorine atom must be close enough to the paramagnetic copper centre to be sensitive to protonation–complexation processes occurring on the phenolate moiety. It must also be far enough to avoid dramatic line broadening in the NMR spectrum. To take in account these constraints, labelling has been realized by replacing one pyridine by a 6-fluoroquinoline group. This strategy provides a huge advantage compared to an easier *para*-phenol labelling: the redox and acid–base properties of the phenol can still be tuned. Moreover, the basicity of the nitrogen is poorly affected by the fluorine atom when incorporated at the 6-position of a quinoline (this is not the case when it is incorporated at the 2-position of the pyridine). The solution chemistry of HLq^{NO2} (Fig. 1) is explored in this paper.

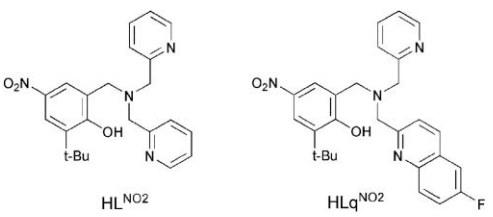

Fig. 1 Formulas of the tripodal ligands.

HLq^{NO2} was obtained by reductive amination of 3-*tert*-butyl-2-hydroxy-5-nitro-benzaldehyde in the presence of 6-fluoro-quinolin-ylmethyl)-pyridin-2-ylmethyl-amine and sodium borohydride. The precursor amine was obtained from reaction between the picolyl-amine and the 6-fluoro-quinoline-2-carbaldehyde prepared according to F. Huet *et al.*[5] When one equivalent of $Cu(ClO_4)_2{\cdot}6H_2O$ and HLq^{NO2} were mixed in acetonitrile, complex $[(HLq^{NO2})Cu]^{2+}$ (**1H**)‡ was obtained (Fig. 2). The crystal cell consists of two

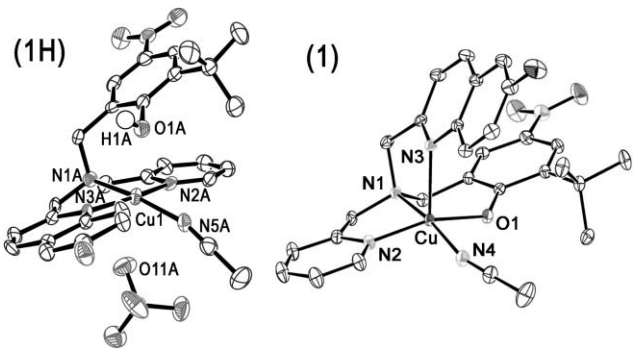

Fig. 2 Structures of **1H** and **1** (ORTEP view: ellipsoids drawn at the 50% probability level). Hydrogen atoms, except H1A, are omitted. Selected bond lengths [Å] and angles [°]: for **1H**, the cell consists of two crystallographically independent subunits (only one, arbitrary chosen, is shown): Cu1–O1A 2.363(3), Cu1–O11A 2.615(4), Cu1–N1A 2.008(4), Cu1–N2A 2.002(4), Cu1–N3A 2.038(4), Cu1–N5A 1.996(4), N2A–Cu1–N1A 83.86(16), N1A–Cu1–N3A 82.24(16), N5A–Cu1–N1A 169.83(17), N2A–Cu1–N3A 165.20(14), N5A–Cu1–N2A 91.85(16), N5A–Cu1–N3A 101.03(16); Cu2–O1B 2.637(4), Cu2–O21B 2.650(4), Cu2–N1B 1.994(3), Cu2–N2B 1.976(4), Cu2–N3B 1.985(4), Cu2–N5B 1.972(4), N2B–Cu2–N1B 84.67(15), N3B–Cu2–N1B 81.90(14), N5B–Cu2–N1B 166.45(17), N2B–Cu2–N3B 166.55(14), N5B–Cu2–N2B 93.79(17), N5B–Cu2–N3B 99.46(16). For **1**, Cu–O1: 1.905(1), Cu–N1: 2.053(1), Cu–N2: 1.965(1), Cu–N3: 2.229(1), Cu–N4 2.015(2), O1–Cu–N1: 94.27(5), O1–Cu–N2: 161.74(5), O1–Cu–N3: 93.18(5), O1–Cu–N4: 83.78(6), N1–Cu–N2: 83.53(6), N1–Cu–N3: 82.62(5), N1–Cu–N4: 168.67(5), N2–Cu–N3: 104.46(5), N2–Cu–N4: 94.82(6), N3–Cu–N4: 108.60(5).

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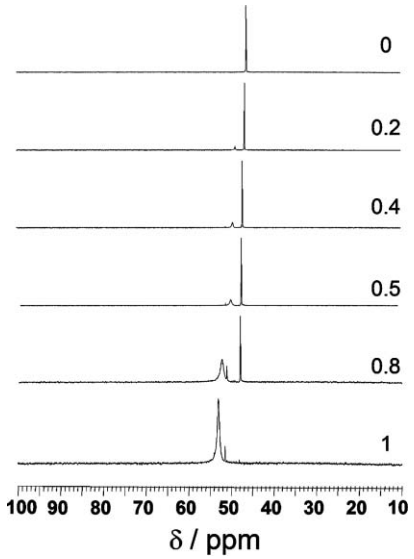


Fig. 3 Titration of HLq^{NO2} (80 mM) with copper(II) perchlorate: ^{19}F NMR spectra recorded in (CD_3CN : CH_3CN) (1 : 4) at 293 K, the numbers correspond to the molar equivalents of copper added. *Intensities are normalized.*

distinct complexes in which the Cu^{2+} ion resides within an octahedral geometry. An exogenous acetonitrile, the pyridine, the *quinoline* and the tertiary amine nitrogens occupy the *equatorial* positions. The *phenol* oxygen and one perchlorate oxygen atom weakly coordinate in *axial* positions. The use of one equivalent of copper(II), one equivalent of NEt_3 and HLq^{NO2} affords the phenolate copper complex $[(Lq^{NO2})Cu]^+$ (**1**)‡ in which the copper atom is within a square pyramidal geometry ($\tau = 0.12$, Fig. 2):[6] the *phenolate* moiety occupies an *equatorial* position, and the *quinoline* coordinates in an *axial* position.

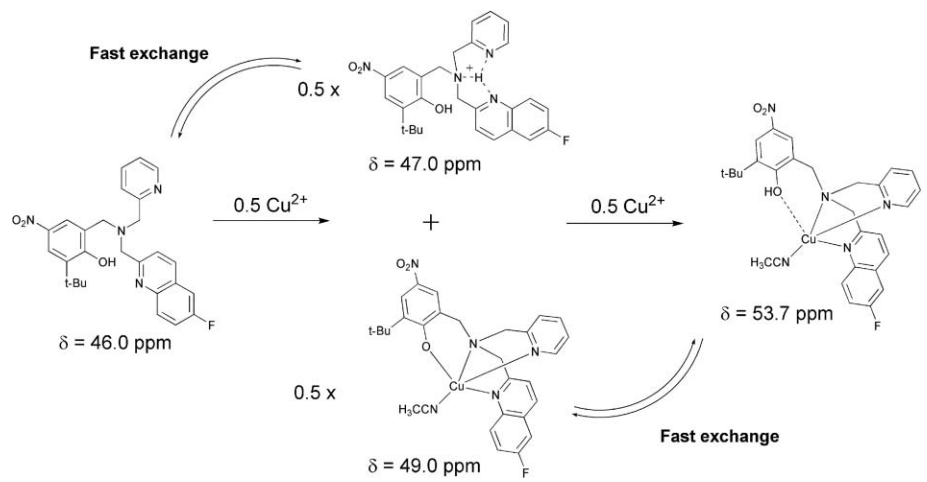
The remarkable feature is thus the different arrangement of the chelating groups around the copper center of **1** compared to **1H**: in **1H** the Cu···OH(Ar) bond is weak and the phenol occupies the more labile, *i.e.* axial, position.[7] In the deprotonated complex **1**, the Cu···O^-(Ar) bond is stronger, and isomerization may be explained by steric hindrances[8] around the quinoline nitrogen, and weak π-stacking between the phenolate and quinoline rings.

The UV-vis spectrum of **1H** in CH_3CN is characterized by copper(II) d–d transitions at around 630 nm, in agreement with a square planar, or elongated octahedral geometry, around the metal. The electronic spectrum of **1** dissolved in CH_3CN, as well as the transmittance spectrum of its single crystals, shows a phenolate-to-copper CT transition at 510 nm: We can therefore conclude that the *equatorial* position of the *phenolate* group is preserved in solution.

When 0 to 0.5 molar equivalent of Cu^{2+} are progressively added to HLq^{NO2} in CH_3CN, the very sharp resonance of the free ligand at 46.0 ppm (given relative to C_6F_6 used as external reference, $\delta_{C6F6} = -162.17$ ppm *vs.* $CFCl_3$) progressively shifts towards 47.0 ppm, value attributed to the protonated $(H_2Lq^{NO2})^+$ species (Fig. 3). This reflects a fast equilibrium between HLq^{NO2} and $(H_2Lq^{NO2})^+$, as expected for a simple acid–base process. Simultaneously, a new broader peak, corresponding to **1** (an identical peak is obtained by dissolving single crystals of **1** in CD_3CN), appears at 49.0 ppm ($W_{1/2} = 25$ Hz). Its chemical shift does not change, but its intensity increases up to 0.5 molar equivalent of copper added: once the copper is chelated by parts of the available amount of ligand, the remaining free ligand HLq^{NO2} acts as a base and deprotonates the weakly coordinating phenol of **1H**, affording **1** (Scheme 1).

Addition of 0.5 to 1 molar equivalent of Cu^{2+} to HLq^{NO2} results in the progressive disappearance of the $(H_2LqNO_2)^+$ resonance (without change in chemical shift). From 0.5 to 0.8 molar equivalent of Cu^{2+} added, the 49.0 ppm resonance of **1** broadens, and progressively shifts. At exactly 1 molar equivalent of Cu^{2+} added, this peak sharpens ($\delta = 53.7$ ppm, $W_{1/2} = 250$ Hz), and becomes identical to that of single crystals of **1H** dissolved in CH_3CN. Broadening from 0.5 to 0.8 molar equivalent of Cu^{2+} added shows that **1H** and **1** are in fast equilibrium. Complexation of Cu^{2+} by $(H_2LqNO_2)^+$ induces its deprotonation: the proton is transferred to the phenolate of **1** according to Scheme 1.

We found remarkable the difference of line width between **1H** and **1**. It cannot be attributed to the concentration of paramagnetic Cu^{2+} in solution, as shown by the titration of **1H** by NEt_3 (at constant Cu^{2+} concentration, see ESI†). The fluorine atom is thus much more sensitive to Cu^{2+} paramagnetism (largest line width) in **1H** than in **1**. This is interpreted by shorter Cu–F and Cu–N3 distances in **1**, in agreement with the X-ray structural analysis. This



Scheme 1 Reaction of HLq^{NO2} with Cu^{2+} (0–1 equivalent added).

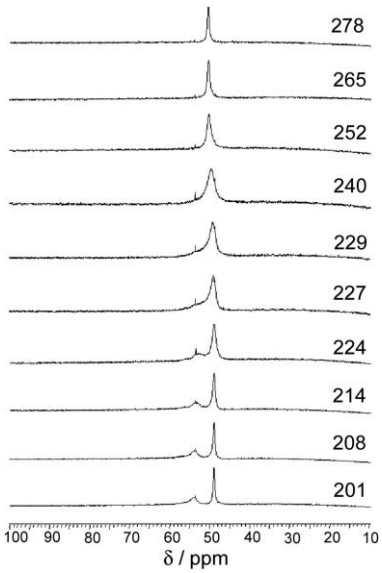


Fig. 4 Variable temperature ^{19}F NMR spectra of a (1 : 1) mixture of **1H** and **1** (total = 2 mM) recorded in (CD_3CN : C_2H_5CN) (1 : 4). *Intensities are normalized*, temperature (in K) as indicated at right.

nicely demonstrates the efficiency of ^{19}F NMR in sensing the position, axial or equatorial, of the quinoline group.

In order to get dynamic information on the **1** to **1H** interconversion, a variable temperature experiment has been undertaken on an equimolar mixture of **1H** and **1** (Fig. 4). A broad resonance is observed down to 226 K, while at lower temperatures, the spectra consist of two distinct resonances corresponding to **1H** and **1**. At the coalescence temperature T_C = 226 K, according to eqn. (1),[9] a rate constant $k = 3000 \pm 100$ s^{-1} could be calculated for the equilibrium eqn. (2). This rate constant is large, indicating that proton transfer and/or molecular rearrangement (isomerization) is extremely rapid.

$$k = \frac{\pi \times \Delta v}{2^{1/2}} \quad (1)$$

$$(\mathbf{1H})_A + (\mathbf{1})_B \overset{k}{\rightleftharpoons} (\mathbf{1})_A + (\mathbf{1H})_B \quad (2)$$

(A and B represent two distinct complexes that interconvert)

In conclusion, a very efficient fluorine labelling has been realized. The protonation state of both the ligand and copper(II) complexes, as well as the position (axial *vs.* equatorial) of the phenol, could be directly assessed by ^{19}F NMR. Its use should be extended to many other systems. Dual ^{19}F labelling is in progress to separate the effects on the chemical shift of the protonation state from the positioning of the phenol. ^{19}F NMR could thus be considered as powerful tool, giving access to unprecedented information. Its use should be extended to many other systems. Dual ^{19}F labelling is currently in progress to separate the effects on the chemical shift of the protonation state from the axial *vs.* equatorial positioning of the phenol.

Notes and references

‡ Crystals were mounted on a Kappa CCD Nonius diffractometer equipped with graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) and a cryostream cooler. *Crystal data for* **1H**: $C_{60}H_{65}N_{11}O_{23}F_2Cl_4Cu_2$, M_w = 1615.11, blue prism (0.2 × 0.2 × 0.2 mm), triclinic, space group $P\bar{1}$, a = 11.938(3), b = 17.472(3), c = 17.886(3) Å, α = 73.2(2), β = 81.81(2), γ = 75.46(2)°, V = 3447.4(12) Å^3, Z = 2, D_c = 1.556 g cm^{-3}, T = 150 K, μ(Mo-Kα) = 0.863 mm^{-1}. 44288 reflections were collected and corrected for Lorentz and polarization effects. The crystal was twinned and the two components were separated using the EvalCCD software package with the following twin law [−1 0 0, −0.627 1 −0.5, 0 0 −1] corresponding to a two fold axis along the [0 1 0] direction. The structure was solved by direct methods and refined by full matrix least-squares, based on F^2, using the SHELXL program[10] through the WinGX software.[11]. The refined fractional contributions of two individuals were 0.724(1), 0.276(1). All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were generated on idealized positions, riding on the carrier atoms with isotropic thermal parameters. The hydrogen atom bonded to O1A was found in Fourier map and was added. It is refined riding on the carriers atoms with isotropic thermal parameters. For the three acetonitrile molecules (ligands and solvent) hydrogen atoms were generated on idealized position riding on the carriers atoms with isotropic thermal parameters. For water molecule, it is not possible to place hydrogen atoms. Final refinement with 921 variables led to $R1$ = 0.093 (34789 reflections, $F \geqslant 2\sigma(F)$), w$R2$ = 0.27, goodness of fit S = 1.12, max/min residual peaks were 1.70/−1.20 e Å^3. *Crystal data for* **1**: $C_{29}H_{29}N_5O_7FClCu$, M_w = 677.57, blue block (0.35 × 0.3 × 0.14 mm), triclinic, space group $P\bar{1}$, a = 10.459(4), b = 11.722(4), c = 13.077(3) Å, α = 112.29(2), β = 96.2(3), γ = 94.69(3)°, V = 1466.4(9) Å^3, Z = 2, D_c = 1.534 g cm^{-3}, T = 150 K, μ(Mo-Kα) = 0.896 mm^{-1}. 38897 reflections were collected and corrected for Lorentz and polarization effects. Crystal structural solution (direct method) and refinement (by full-matrix least squares on F) was performed using the teXsan analysis package.[12] Non-hydrogen atoms were refined with anisotropic thermal parameters, while the other hydrogen atoms were generated on idealized positions, riding on the carrier atoms with isotropic thermal parameters. Of 8500 unique reflections (R_{int} = 0.082), 6880 were observed ($F \geqslant 2\sigma(F)$) and used in the full-matrix least-squares refinement of 424 variables. R = 0.039, R_w = 0.049, goodness of fit S = 1.23, max/min residual peaks were 0.57/−0.76 e Å^3. CCDC 287166 (**1**), 600813 (**1H**). For crystallographic data in CIF or other electronic format see DOI: 10.1039/b605852c

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