Superoxide dismutase-like activity of cobalt(II) complexes based on a sugar platform

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A SOD-like activity evaluated by a modified McCord–Fridovich test was evidenced for two Co(II) complexes built from "glycoligands" using a sugar platform derived from D-galactose and D-galactal and functionalized by three 2-picolyl groups.

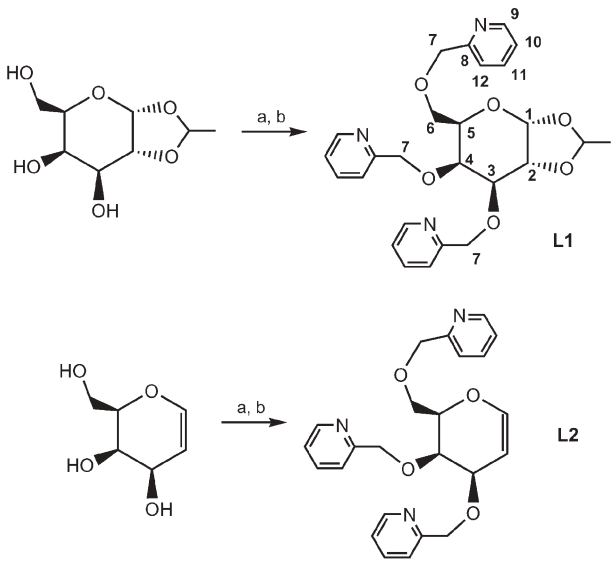
Synthesis of ligands for metallic cations with a controlled geometry and a high level of modularity is of interest. The strategy we have initiated in our group consists in taking advantages from sugar scaffold diversity to tailor a wide family of polydentate ligands by appending desired Lewis bases at selected positions around sugar cycles.

Although sugars and their derivatives are important biomolecules, their interactions with transition metal cations are far less documented than are metal cations interactions with amino-acids or nucleic acids. Recently, however, there has been a renewed interest in this topic.[1,2,5] Sugars functionalized by a unique ligand and related complexes have been recently described.[1–4] The original strategy we are presenting here consists in the design of a polydentate chelating claw onto a sugar scaffold, this scaffold being taken as a distribution frame. No transition metal complex derived from such a strategy have been structurally characterized so far. Such an approach is inspired from 'scaffold mimetics' developed in the field of peptidomimetics,[6] but with a reversed aim. Instead of targeting a guest-molecule designed to interact with a given host-site, we generate a host for cationic transition metals. Sugars are an interesting alternative to other platforms currently used in coordination chemistry such as cyclams, phenyl or cyclohexyl rings, peptides, cyclodextrins or calixarenes. With a simple sugar scaffold, it is possible to easily control the regio- and the stereoselectivity of functionalization. We are testing this strategy in order to prepare complexes with superoxide dismutase-like (SOD-like) activity, as such an activity is of pharmaceutical interest for protection against oxidative stress.[7]

Herein, we present the first two structurally defined cobalt(II) complexes of this series, derived from two ligands **L1** 3,4,6-tri-*O*-(2-picolyl)-1,2-*O*-ethylidene-α-D-galactopyranose and **L2** 3,4,6-tri-*O*-(2-picolyl)-D-galactal.† Galactose-ethylidene and galactal were chosen for two main reasons: (a) the all-*cis* equatorial/axial/equatorial sequence (at positions 3, 4 and 5 of the pyranose ring) provides a three finger-claw favourable to chelation;[2] (b) these structures can be further functionalized (after deprotection of the ethylidene moiety for **L1** and through the double bond for **L2**). Syntheses are depicted in Scheme 1.[8]

Both compounds **1** and **2** were crystallized as $[Co^{II}(\mathbf{L}i)](PF_6)_2$ (i = 1,2) by slow evaporation of an ethanol/acetone solution.[8–10] Their single-crystal X-ray structures were solved (see Fig. 1).

The metal environments are distorted N_3O_3 octahedra, with 3 pyridines and 3 ether groups from the sugar moiety. The stereochemical environments of the Co^{II} are Δ in **1** and Λ in **2**. In the latter, the double bond induces a strain by flattening the six membered ring, as can be seen from the puckering amplitude (0.655(4)Å for **1**, 0.493(6)Å for **2**).



Scheme 1 Synthetic pathways to **L1** and **L2** and atom labels for the NMR spectra. (a) NaH (1.2 eq./OH) DMF (b) 2-picolylchloride.HCl, NaH (1.2 eq.) DMF ; rt 17 h. Purification : SiO_2, AcOEt/MeOH (9/1).

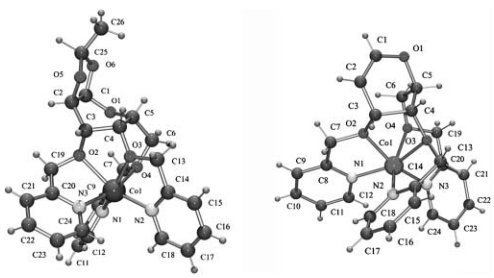


Fig. 1 Ball and stick drawing of the cationic moiety of **1** (on the left) and **2** (on the right).

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The electronic spectra of **1** and **2** were recorded in acetone. A low intensity d–d band was observed in the visible region,[9,10] consistent with a six-coordinate species in a symmetry close to octahedral,[11,12] as in the solid state.

Behaviour upon superoxide addition on **1** was studied in anhydrous DMSO, which allows the preparation of stable solutions of superoxide (*ca.* 5 10^{-3} mol L^{-1}).[13] A new band at 350 nm (sh) was recorded, in the region reported for cobalt(II) superoxo species.[14] It indicates that superoxide has entered the coordination sphere of cobalt.

1 and **2** were tested for superoxide dismutase-like activity, through a modified McCord–Fridovich assay. This test is based on kinetic competition for reaction of superoxide with the putative SOD-mimic or ferricytochrome *c*.[15,16] Because of the very low solubility in water of **1** and **2**, the test was modified and conducted in phosphate buffer (50 mM, pH 7.8)/DMSO (60/40).[17] **1** and **2** were found to inhibit the reduction of ferricytochrome *c* by the superoxide anion (Fig. 2).[18] The IC_{50} are reported below, along with the recalculated kinetics constant, k_{McCF}.[19–21] The activities reported are 0.2% of that of the Cu–Zn SOD (the most efficient one).

Thus, polyfunctionalized ligands based on galactose derivatives were synthesized and the corresponding Co^{II} complexes fully described, with structures showing pseudo-octahedral coordination sphere N_3O_3. They were shown to inhibit the reduction of ferricytochrome *c* by superoxide in the McCord–Fridovich assay, with IC_{50} (with [cytcFe^{III}] = 22μM) of *ca.* 2 μM. They are among the quite rare Co(II) reported complexes with a SOD-like activity.[22,23]

Such low molecular weight complexes displaying SOD-like activity, even if they are less active than enzymes and even in the case of stoichiometric scavenger,[18] have a potential use as pharmaceuticals as they lack the major enzyme drawbacks.[24,25] **1** and **2** are of special interest because of their straightforward synthesis. Moreover, their structures make these compounds quite attractive as they are easy to be further functionalized in order to modulate properties such as hydro/lipophilicity. Such properties are important pharmacological parameter with regard to the delivery to cells. As in **1**, (see Fig. 1) the sugar cycle is dividing space into two well-separated regions: under the sugar, space devoted to chelation and above, space that will be devoted to further functionalization. Due to the spatial separation of the two regions, such a functionalization should not disturb the chelation half. Such a kind of easily modulable complex is of great interest to tailor families of compounds with tunable properties.

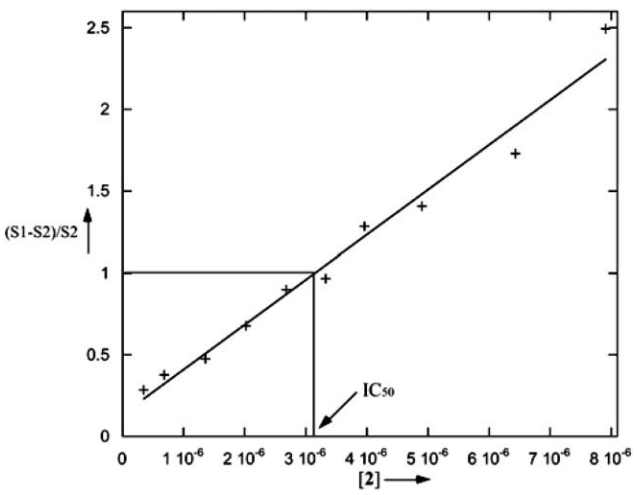

Fig. 2 McCord–Fridovich assay performed on **2** ([**2**] mol L^{-1}). The reduction of ferricytochrome *c* was followed at 550 nm. *S*1 corresponds to the slope of the kinetic trace for cytcFe^{III} reduction before introduction of the complex and *S*2 to the slope recorded after addition of the putative SOD-mimic. (*S*1 − *S*2)/*S*2 is equal to 1 at the IC_{50} value.

Table 1 SOD-like activity of **1** and **2** measured by the McCord–Fridovich assay (with [cytcFe^{III}] = 22μM)

Complex	IC_{50}/μM	k_{McCF}/mol^{-1} L s^{-1}
1	2.5	2.3 10^6
2	3	1.9 10^6

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Notes and references

† **L1 and L2.** The typical protocols for **L1** and **L2** are described here in the case of **L1**. 1,2-*O*-ethylidene-α-D-galactopyranose (3 g, 14 mmol), was dissolved in DMF (50 mL) and NaH was added (2.3 g). 2-picolylchloride.HCl, deprotected by NaH (2.8 g, 1.2 eq.) in DMF (50 mL) was slowly added over an hour. After 17 h, the solvent was evaporated. CH_2Cl_2(100 mL)/H_2O(100 mL) was added and the resulting aqueous phase was extracted by CH_2Cl_2. The organic phases were dried over anhydrous Na_2SO_4. The product was obtained as an orange oil that was purified on SiO_2 (AcOEt/EtOH, 9/1). **Characterizations for L1**: Yield 60%. Microanalysis calcd ($C_{26}H_{29}N_3O_6$): C65.12, H6.10, N8.76; measd: C64.79, H6.19, N8.79%. MS-ES: *m*/*z*(%): 502.2 (100) [*M* + Na], 480.2 (4.5) [*M* + H]. RMN ^{1}H (360 MHz, $CDCl_3$): δ (ppm) = 8.53–8.47 (m, 3H, H9s); {7.72–7.60 (m, 3H); 7.55–7.35 (m, 3H) and 7.19–7.11 (m, 3H) (H10s, H11s, H12s)}; {5.65 (d, *J* = 4.3 Hz) and 5.55 (d, *J* = 4.5 Hz) (H1a and H1b)}; {5.40 (q, *J* = 4.8 Hz) and 5.18 (q, *J* = 4.9 Hz) (H13a and H13b)}; 5.1–4.56 (6d, *J* = 13.3 to 12.8 Hz, 6H, H7s); {4.47 (dd, *J* = 7 Hz, 4.3 Hz, H2a); 4.25–4.12 (m, H2b, H4, H5), (3H)}; 3.8–3.7 (m, 3H, H3 and H6); {1.39 (d, *J* = 4.9 Hz) and 1.37 (d, *J* = 4.8 Hz) (3H, H14)}. RMN ^{13}C (90.5 MHz, $CDCl_3$): δ = 158.7–158.1 (C8s); 149.1, 149.0, 148.9, 148.0 (C9s); 136.7 (C11s); 122.5, 121.5 (C10s and C12s); (100.8 and 100.2) (C13a and C13b), (98.2 and 97.7) (C1a and C1b); 81.5 (C3); 78.5 (C2a); {77.00; 75.6; 74.3; 74.0; 73.8; 72.9; 72.7; 72.5 (C2b, C4, C5, C7s)}; 68.9 (C6); (21.3 and 20.9) (C14a and C14b). Some peaks are split into two ((a) and (b)), due to the presence of two diastereoisomers, that were not separable by chromatography, arising from the ethylidene moiety. CH_2 protons (H7s) from the picolyl moiety are diastereotopic and thus appear as 2 close doublets. (Labels: as usual for sugar moiety, as indicated in scheme for picolyl moieties). IR (NaCl): *ν* (cm^{-1}) = 1591.0 (*ν*C=N), 1571.6 (*ν*C=C), 1115.9 (br) (δ_{py}), 759.9 (δ_{py}). **Characterizations for L2**: Yield: 60%. Microanalysis calcd ($C_{24}H_{25}N_3O_4(H_2O)$) : C65.89, H6.22, N 9.60; measd: C 65.7, H 5.81, N 9.13%. MS-ES: *m*/*z* (%): 442.2 (100) [*M* + Na]$^+$. RMN ^{1}H (400 MHz, $CDCl_3$): δ (ppm) = 8.46 (m, 3H, H9s); {7.59 (bm, 3H), 7.48 (d, *J* = 7.9 Hz, 1H), 7.37 (t, *J* = 8.2 Hz, 2H), 7.1 (m, 3H) (H10s, H11s, H12s)}; 6.37 (dd, *J* = 6.2 Hz, *J* = 1.2 Hz, H1); {[4.99 (d, *J* = 13.3 Hz, 1H), 4.77 (d, *J* = 13.3 Hz, 1H) (H7a)], 4.73 (s, 2H, H7b), [4.64 (d, *J* = 13.3 Hz, 1H), 4.57 (d, *J* = 13.3Hz, 1H) (H7c)]}; 4.88 (ddd, *J* = 6.2 Hz, *J* = 2.9 Hz, *J* = 1.0 Hz, 1H, H2); 4.29 (m, 2H, H3 and H5); 4.12 (m, 1H, H4) {3.89 (dd, *J* = 10.3 Hz, *J* = 7.2 Hz, 1H), 3.81 (dd, *J* = 10.3 Hz, *J* = 5 Hz, 1H) (H6)}; RMN ^{13}C (100 MHz, $CDCl_3$): δ (ppm) = 158.5, 158.1 (C8s); 149.0, 148.8, 148.7 (C9s); 144.3 (C1); 136.7, 136.6 (C11s); {122.3–121.2 (C10s, C12s)}, 99.7 (C2), {(75.4, 74.3, 74.1, 72.4, 71.9, 71.3, 69.0) (C3, C4, C5, C6, C7s)}. CH_2 protons (H7a and H7c) from the picolyl moiety are diastereotopic and appear as 2 close doublets. H6s are diastereotopic. (Labels: as usual for sugar moiety, as indicated in scheme for picolyl moieties). IR (NaCl) : *ν*

(cm^{-1}) = 1592.9 (νC=N), 1571.9 (νC=C), [1151.2–1085.5] (br, δ_{py}), 761.4 (δ_{py}). **Synthesis of 1 and 2**. The typical protocols for **1** and **2** are described here in the case of **1**. **L1** (100 mg, 0.21 mmol) was dissolved in 3 mL absolute EtOH. A blue solution of $CoCl_2$, $6H_2O$ (57 mg, 0.24 mmol) in 3 mL absolute EtOH was added dropwise. The solution turned red, then blue and a precipitate appeared. It was redissolved by addition of 5 mL acetone and a clear blue solution was obtained. NH_4PF_6 (150 mg, 0.92 mmol) in absolute EtOH (2 mL). Slow evaporation afforded 110 mg of red crystals. Yield 64%. For characterizations see refs. 9,10. **Crystal data for 1**: $C_{26}H_{29}CoN_3O_6.P_2F_{12}$, M_r = 828.39, red prisms, crystal size 0.30 × 0.29 × 0.22 mm^3, orthorhombic, space group $P2_12_12_1$, a = 10.139(1), b = 16.795(2), c = 18.882(3) Å, V = 3215.3(7) $Å^3$, T = 298K, Z = 4, ρ_{calc} = 1.711 g cm^{-3}, μ(MoKα) = 7.49 cm^{-1}, F(000) = 1676; a total of 26171 reflections up to $h(-11/11)$, $k(-18/18)$, $l(-20/20)$ in the range $3 < \theta < 25$, of which 4616 were unique (R_{int} = 0.0310) and 4124 observed with $Fo > 4\sigma(Fo)$, 141 parameters, $R1_{obs}$ = 0.037, $wR2_{obs}$ = 0.103, $R1_{all}$ = 0.043, GOF = 1.060, Flack parameter 0.008(16), max./min residual electron density 0.39/−0.28 e/$Å^3$. Distances [Å] and angles [°]: Co1-O2 2.244(3), Co1-O3 2.034(3), Co1-O4 2.115(3), Co1-N1 2.097(3), Co1-N2 2.090(3), Co1-N3 2.057(3), O2-Co1-N1 77.0(1), O3-Co1-N2 76.5(1), O4-Co1-N3 77.6(1), O2-Co1-O3 75.6 (1), O2-Co1-O4 86.1(1), O3-Co1-O4 81.2(1), N1-Co1-N2 100.3(1), N1-Co1-N3 97.6(1), N2-Co1-N3 113.8(1). The sugar moiety is in a boat (^{3,6}B) conformation (QT = 0.655(4)Å, $\theta 2$ = 81.9(4)° and $\varphi 2$ = 115.5(4)°). CCDC 264230. See http://dx.doi.org/10.1039/b508893c for crystallographic data in CIF or other electronic format. **Crystal data for 2:** $C_{24}H_{25}CoN_3O_4.P_2F_{12}$, M_r = 768.34, red prisms, crystal size 0.72 × 0.34 × 0.20 mm^3, monoclinic, space group $P2_1$, a = 9.233(2), b = 10.440(2), c = 15.360(3) Å, b = 90.17(1)°, V = 1480.6(5) $Å^3$, T = 298 K, Z = 2, ρ_{calc} = 1.723 g cm^{-3}, μ(MoKα) = 8.01 cm^{-1}, F(000) = 774; a total of 12063 reflections up to $h(-10/10)$, $k(-11/11)$, $l(-17/17)$ in the range $3 < \theta < 30$, of which 4276 were unique (R_{int} = 0.0403) and 3229 observed with $Fo > 4\sigma(Fo)$, 423 parameters, $R1_{obs}$ = 0.040, $wR2_{obs}$ = 0.090, $R1_{all}$ = 0.058, GOF = 0.910, Flack parameter 0.023(18), max./min. residual electron density 0.35/−0.27 e/$Å^3$. Distances [Å] and angles [°]: Co1-O2 2.069(3), Co1-O3 2.164(3), Co1-O4 2.132(3), Co1-N1 2.061(4), Co1-N2 2.099(5), Co1-N3 2.042(4), O2-Co1-N1 76.9(2), O3-Co1-N2 77.6(2), O4-Co1-N3 78.3(1), O2-Co1-O3 79.3(1), O2-Co1-O4 84.1(1), O3-Co1-O4 77.4(1), N1-Co1-N2 98.7(2), N1-Co1-N3 107.8(2), N2-Co1-N3 105.4(2). The sugar moiety is in a halfboat (^{5}H$_4$) conformation (QT = 0.493(6)Å, $\theta 2$ = 50.8(8)° and $\varphi 2$ = −161.4(9)°). CCDC 264231. See http://dx.doi.org/10.1039/b508893c for crystallographic data in CIF or other electronic format. **McCord–Fridovich assay:** Superoxide anion was supplied to the system by the xanthine–xanthine oxidase system. Because of the low solubility of complexes **1** and **2** in water, a modified McCord–Fridovich assay has been used using (DMSO/phosphate buffer (50 mM, pH 7.8)) (40/60). It was validated by several checks (see below). To check that neither DMSO nor the tested compounds inhibit the production of superoxide by xanthine oxidase, the rate of conversion of xanthine to urate was determined by measuring the increase in absorbance at 290 nm over a two minute period with and without DMSO, with and without the tested compounds. No inhibition of this conversion has been recorded. For a typical protocol see ref. 13. Activities were measured probing the ferricytochrome c reduction (ΔDO = 0.025 min^{-1} in the absence of the putative SOD mimic) with and without the putative SOD mimic. To ensure the reliability of the assay in conditions (DMSO/phosphate buffer (50 mM, pH 7.8)) (40/60), IC_{50} values of a Mn(II) complex soluble in both conditions were measured in both conditions (conventional phosphate buffer and DMSO modified procedure). IC_{50} values obtained were close, which validates the measurements in (DMSO/phosphate buffer) (40/60). DMSO appeared to improve the assay (improved linearity of the trace at 550 nm and over a longer period) which could be due to the higher dioxygen solubility in DMSO with regard to water. IC_{50} value represents the concentration of putative-SOD mimic that induces 50% inhibition of the reduction of cytochrome c. They were determined as previously described (see ref. 13).

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8 See notes above.
9 **1**. Yield 64%. Microanalysis calcd: C37.7, H3.53, N5.07, F27.52, Co7.11; measd: C37.48, H3.42, N5.01, F27.01, Co6.61%. MS-ES (+): m/z (%) = 480.1 (100) $[M\text{-}Co + H]^+$, 502.1 (45) $[M\text{-}Co + Na]^+$, 269 (100) $[M]^{2+}$, IR (KBr pellet): ν (cm^{-1}) = 1612.5 (C=N), 1571.8 (C=C), 1165.0, 1122.4, 1090.9 ($C\text{–}H_{Ar}$, δ_{py}) 772.1 (δ_{py}), 839.1 and 558.2 (PF_6^-) (strong bands only). UV/vis (acetone): λ_{max} (ε mol L^{-1} cm^{-1}): 520 (50). χT = 2.79 cm^3 mol^{-1} K at rt, corresponding to S = 3/2 and g = 2.44, in agreement with large CSO for Co(II) high spin compounds. CCDC 264230.
10 **2.** Yield 70%. Microanalysis calcd: C37.50, H3.28, N5.47, F29.68, Co7.67; measd: C37.36, H3.17, N5.43, F29.07, Co7.45%. MS-ES: m/z (%) = 442.2 (100) $[M\text{-}Co + Na]^+$, 239.1 (7) $[M]^{2+}$; IR (KBr pellet): ν (cm^{-1}) = 1611.8 (C=N), 1574.2 (C=C), 1099.8, 1086.2, 1070.2, 1056.8 ($C\text{–}H_{Ar}$, δ_{py}) 768.4 (δ_{CHpy}), 838.3 and 558.1 (PF_6^-) (strong bands only). UV/vis (acetone): λ_{max} (ε mol L^{-1} cm^{-1}): 510 (50). CCDC 264231.
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17 This test requires several preliminary checks.[13,26–28] For a typical protocol see footnote 1 in text and ref. 13.
18 This SOD-*like* activity has been evaluated by the McCord and Fridovich assay. This assay can provide positive results in the case of both a catalytic scavenger and a stoichiometric scavenger.[13,27] The values reported here are at the upper limit for evidence of catalytic activity.[13] Further experiment, such as pulsed radiolysis, could provide definitive proof for catalytic activity (*true* SOD-activity). However, this assay remains relevant, as it provides a stationary state of superoxide concentration, close to what is usually encountered *in vivo*. To finish with, $CoCl_2$ under the same conditions displayed an IC_{50} of *ca.* 40 μM, indicating a positive effect of the complexation, probably associated with a lowering of the Co(III)/Co(II) potential.
19 IC_{50} are hardly comparable from article to article, as they are dependent both on the nature of UV-visible probe (P, usually cytcFe(III) or NitroBlue Tetrazolium) used and on its concentration.[29] For comparison sake, a kinetic constant can be derived from the IC_{50}, labeled here k_{McCF}, as at the IC_{50}* k_{McCF} = [P]* k_P[29] where P is the UV-vis probe and k_P is the kinetic constant of the UV-vis probe P with the superoxide anion (k_{cyte} = 2.6 10^5mol L^{-1} s^{-1})[21].
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