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A pair of highly biotolerated diamagnetic and paramagnetic iron(II) complexes displaying electroneutrality†
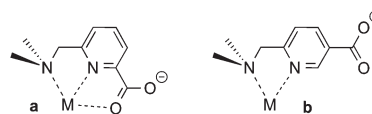
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A pair of structurally analogous macrocyclic iron(II) complexes with a magnetic off-on relationship is reported that exhibit electroneutrality at neutral pH and high stability in physiological media. This has been achieved by external charge compensation using nicotinate pendent arms. No contact toxicity was observed for cells up to 4 mM for the low-spin and 2 mM for the high-spin complex. These results are a necessary precursor to the future design of turn-on probes with elevated biotolerance.

Coordination compounds play an increasingly important role as molecular probes, contrast agents or tracers in the biomedical imaging arena (MRI, PET). Their biotolerance is a pre-eminent requisite for reliable results in research, and safety in clinical imaging.[1,2] Well tolerated coordination compounds in biomedical applications would benefit from the availability of electroneutral versions. This would (a) maximize the complex' stability by coulombic compensation, (b) minimize the Lewis-acid character of those species with open coordination shells, and (c) reduce its osmolality. Electrocompensation in coordination compounds employing multipodal ligands is largely achieved by the use of carboxylates (often acetates) that coordinate the metal center (ZnEDTA, GdDOTA);[3] fewer cases of "external" charge compensation are known. For medium-soft to soft metal centers, multipodal ligands with softer, non-oxygenated coordinating sites have to be chosen to assure highest complex strength.[4,5] In the particular case of iron(II) centers, coulombic compensation should also increase the ligand field exercised by N6 ligands and thus reinforce the low-spin state, if such a state is indeed the aim as in our case. However, a straightforward way of partial coulombic compensation, let alone attainment of electroneutrality, in complexes com-

prising oxophobic transition metals is not obvious as most imine-displaying multi-podal ligands do not exhibit any negative charges.

We reported on a first example of a smart (turn-on) probe (a magnetogenic probe) irreversibly switching from a diamagnetic to a paramagnetic state when reacted with a chemical analyte or an enzyme.[6] The design of such probes requires the availability of a pair of structurally related iron(II) chelates which respectively adopt a low-spin and a high-spin state and exhibit high stability in water.[7] We already disclosed the increased biotolerance of a high-spin iron(II) complex of a pentadentate, macrocyclic ligand displaying two pendent tetrazolylmethyl arms.[8] These tetrazolyl units exhibit a pKa close to 5.5 and thus should reliably compensate the metal center's two charges at physiological pH. However, we were yet unable to create a low-spin analog of this complex by the incorporation of a third pendent arm of imine nature, likely because of the increased hardness known for tetrazolate ligands, and also for a less than optimal steric approach. Indeed, electroneutral, low-spin iron(II) complexes that are binary and thus may exhibit high stability in competitive solvents, are a relative rarity. While most porphyrin or phthalocyanine complexes do not apply[7] (they almost always display two fairly labile, monodentate ligands in their apical positions,[9,10] the recently reported clathrochelates (macrobicyclic boron-capped tris-dioximes) are eletroneutral, low-spin iron(II) complexes of impressive stability.[11] However, it is not obvious how to derive a high-spin version thereof. We therefore decided to retain the demonstrated capacity of 1,4,7-tripicolyl-1,4,7-triazacyclononane (TPTACN) (picolyl = 2-pyridylmethyl) to render a ferrous metal center low-spin[12–14] and compensate the two positive charges by the inclusion of carboxylates displayed on the periphery of the complex.

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Coordination compounds with two types of picoline-pendent arms showing carboxylate appendices have so far

been reported: picolyl-2-carboxylates ("picolinates"; **a**) and picolyl-3-carboxylates ("nicotinates"; **b**). The complexes displaying picolinates mainly show lanthanide centers because the ligand exploits the bidentate nature of the pendent arm.[15–18] In an exception to this rule, a Cu(II) complex has recently been reported where the claimed coordination motif **a** should be accompanied by a non-negligible induction of strain.[19] By contrast, another Cu(II) complex displays the monodentate nature of its single picolinate as expected for a transition-metal complex; the stability of the complex also appears to benefit from the charge compensation contributed by the non-coordinated carboxylate.[20] Nicotinate-containing coordination compounds are rare. One case comprising a tripodal ligand displaying a single nicotinate has been reported where the resultant Cu(I) complex can be predicted to be electroneutral.[21] More recently, Bryce, Hambley and co-workers conducted a systematic study on progressive charge reduction in a cobalt(III) complex exploiting one to three nicotinate-pendent arms in order to achieve better cell penetration.[22] Herein, we report on a pair of structurally analogous, binary iron(II) complexes that show high stability in physiological media and low contact toxicity in cell culture while adopting a high-spin and a low-spin state, respectively. The complexes' underlying ligands represent the first instance of macrocyclic multidentate ligands displaying nicotinates.

Both target iron(II) complexes **1** and **2** are equipped with two nicotinate pendent arms and a third arm that is either coordinating (**1**) or not (**2**) (Scheme 1). Researchers have sought to establish such an "asymmetric" substitution pattern on TACN since the early 90s.[23–25] We chose one of these strategies by preparing the orthoamide of TACN (**6**, Scheme 2). **6** was obtained according to literature protocols.[26–28] It can be monoalkylated by benzyl chloride or picolyl chloride, respect-
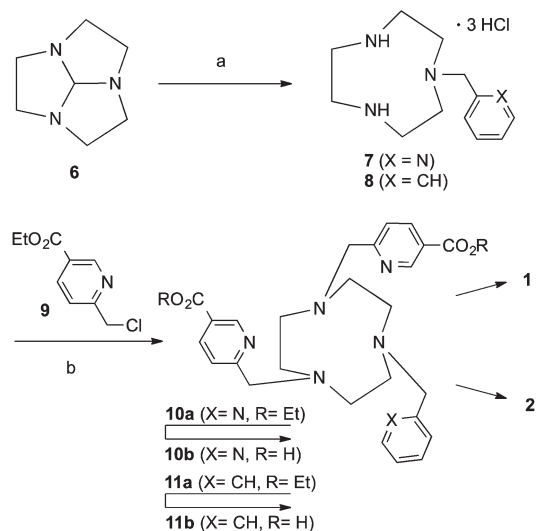
Scheme 2 Synthesis.

ively, and the resulting ammonium ions subsequently hydrolyzed to give intermediates **7**[29] and **8**[23]. Their dialkylation with **9**[30–32] yields the protected versions **10a** and **11a** of the hexadentate and pentadentate ligands. Exhaustive purification is followed by hydrolysis under acidic conditions leading to the target ligands **10b** and **11b** which are reacted with $Fe(BF_4)_2 \cdot 6H_2O$ to furnish the respective iron(II) complexes **1** and **2**.

Complex **1** was obtained as a dark-red powder that could be recrystallized in the form of very dark-colored crystals. X-ray diffraction analysis revealed a structure (Fig. 1 and Table S01†) displaying a coordination motif very similar to the parent compound **5**.[12,13] The presence of two counterions in the unit cell lead to the conclusion that the complex crystallized in its double protonated form. Nonetheless, the uniform iron-nitrogen distances around 2.0 Å prove the complex to be low-spin in the solid state, a fact that is confirmed for the solution state (water) by its proton NMR entering into the habitual 0 to 10 ppm interval (Fig. S01†). Complex **2** was obtained in the form of dark-yellow needles that did not produce diffraction
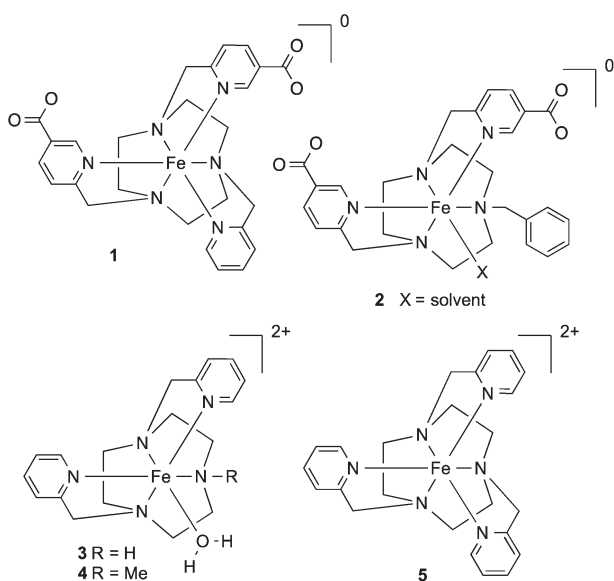
Scheme 1 Electroneutral ferrous chelates **1** and **2**, and reference compounds.
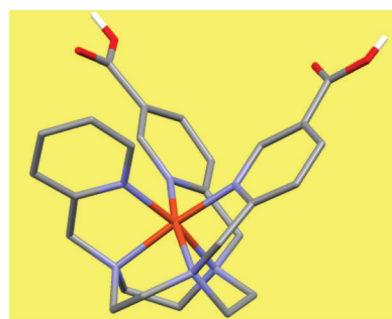
Fig. 1 Structure of **1**$^{2+}$ deduced from X-ray diffraction data. Hydrogens omitted for clarity, except the carboxylate hydrogens.

data of sufficient quality to derive a structure. During unit mass analysis *via* electrospray ionization (compare to high-resolution mass analysis, Fig. S02 and S03†), both complexes generate fairly weak signals in pure form and cannot be observed at all during their formation in the reaction medium.

The UV spectra of both complexes in water (Fig. S04 and S05†) exhibit high-energy transitions between 340 and 440 nm that are assigned to metal-to-ligand charge transfer bands (MLCT), the one for **2** being of significantly lower intensity (ε = 1650 *vs.* 4700). This is a first line of evidence arguing in favor of the low-spin state for sample **1** and a high-spin state for sample **2**.[33] These spin states are also in congruence with colors observed in solution for the two complexes. Beyond the MLCT band, one also observes a band below 300 nm (weak for complex **2**) that is habitually attributed to metal-independent π–π^* transitions within the pyridine rings. The evolution (or its absence) of these UV spectra was then studied with variation of the pH in both directions away from neutrality. Complex **1** does not show significant spectral evolution when being brought from neutral pH to pH 13 (Fig. S06†). This supports the notion that the complex already exists as an electroneutral species at pH 7 and no further deprotonation can occur; no immediate degradation appears to take place when being brought to this high pH. By contrast, when being acidified, **1** clearly suffers conversion to another species in view of the significant change of its MLCT band (an isosbestic point is observed); this can be explained with the protonation of one carboxylate. When complex **2** is brought to high pH by use of NaOH (Fig. S07†), a spectral change is observed that can be rationalized with the oxidation to iron(III) and the deprotonation of one inner sphere water molecule to yield the hydroxide complex.[34] Acidification of a sample of **2** leads to a significant change of the UV spectrum, too. Here, the absence of isosbestic points indicates a mixture of two or more protonation species, present concomitantly and in varying proportions; replacement of the inner-sphere monodentate ligand by another component of the medium may also occur.

Next, the spin states of **1** and **2** in water were determined. The method introduced by Evans[14,35,36] furnished susceptibility values $\chi_m(T)$ of 0.07 emu for **1** and 3.60 emu for **2** (Fig. S09, S10, Table S02†), thus confirming the desired low-spin state for **1** and high-spin state for **2** in aqueous solution. The spin-state can also be indirectly deduced from the determination of the longitudinal relaxation times (T_1) of bulk water in aqueous samples of both complexes using a habitual NMR spectrometer (Table S03†) and with a 3 T MRI instrument (Fig. S11†). While the sample of **1** (4.0 mM) yielded a value of 2.2 s, thus approaching that of pure water, the sample for **2** (4.0 mM) gave 0.5 s, in congruence with previously reported data. These data provide independent confirmation of spin states already established above. The relaxivities derived from these values (Fig. 2) clearly demonstrate the distinct off-on relationship in terms of magnetism that this structurally analoguous pair of macrocylic complexes exhibits. Indeed, the favorable relaxivity observed for the high-spin chelate **2** is obtained by rendering the ligand penta-dentate
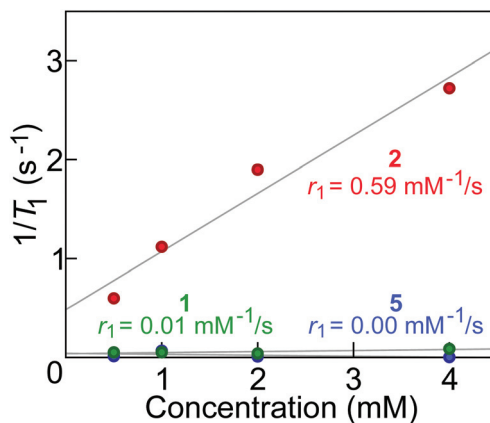

Fig. 2 Off-on relationship of relaxtivities of complexes **1** and **2**.

thus allowing for fast and reversible interaction with the solute in the first coordination sphere.

The cyclovoltammogram for **1** in water *vs.* SCE shows one single reversible wave and furnishes a redox potential of 0.56 V (Table 1). For **2** an irreversible wave is observed at all investigated scan rates (25 mV to 20 V s^{-1}); it yields a potential of 0.34 V. The irreversibility can most probably be attributed to rearrangements occurring in the coordination sphere of the electrogenerated iron(III) center.[37] These potentials are systematically lower than those observed for the corresponding low-spin and high-spin *cationic* reference complexes **5** [Fe (TPTACN)] (0.85 V; value extrapolated to SCE reference from 0.35 V reported for Fc+/Fc reference in MeCN[12]) and **4** [Fe (DPTACN-Me)] (0.50 V).[38] This shift of the redox potential to lower values indicates somewhat lower oxidation stability compared to non-carboxylated complexes and may be attributed to the reduced challenge of establishing a single positive charge starting from an electroneutral species compared to a third one starting from a species carrying already two positive charges.

Table 1 Electrochemistry

Sample[b]	$E_{1/2}$[a] (V)
1	0.56
2	0.34
4	0.50
5	0.85[b]

Sample[c]	Conductivity (mS cm^{-1})
Distilled water	0.0084
1 × 2 BF$_4$ (4 mM)	1.221
HBF$_4$ (8 mM)	1.242
5 × 2 BF$_4$ (4 mM)	2.721

[a] 1 mM at 20 °C *vs.* Standard calomel electrode; for more details see the ESI. [b] Obtained by extrapolation of the value determined against Fc+/Fc. [c] Conditions: 20 °C; pH 7 (adjusted by NaOH 1 M solution); calibrated to KCl solution (10 mM).

More far-reaching support for the notion of electroneutrality at neutral pH may be obtained by determining the specific conductivity of **1** (Table 1). Indeed, conductimetry has already been considered early on as a useful means for characterizing coordination compounds, but in those days studies in water were excluded for fear of "complex hydrolysis or lack of solubility".[39] A more recent report demonstrates electroneutrality of a lanthanide chelate in aqueous solution by conductimetry.[40] Thus, an aqueous sample containing $[\mathbf{1} \times 2 \text{ HBF}_4]$ (crystals; 4 mM) was adjusted with NaOH to pH 7 and compared to a reference sample containing hydrotetrafluoroboric acid (HBF_4) at the same concentration (8 mM), also adjusted to pH 7 by use of NaOH. Both gave almost identical conductivites (1.221 mS cm^{-1} (**1**); 1.242 mS cm^{-1} (HBF_4)). By contrast, analysis of a sample of cationic reference complex **5** ($[\text{Fe}^{\text{II}}(\text{TPTACN})]^{2+} \times 2 \text{ BF}_4$ at 4 mM), also adjusted to neutral pH *via* NaOH titration, furnished a conductance 2.2 times higher (2.721 mS cm^{-1}), thus confirming the electroneutral quality of **1** at physiological pH, one of the prime objectives of this study.

Finally, complexes **1** and **2** were tested for their contact toxicity in a classic cell viability test (Fig. 3). While low-spin complex **1** does not show any contact toxicity up to 4 mM concentration in the culture medium, even high-spin complex **2** (powder) shows appreciable biotolerance with an LD50 of 4 mM. These data bode well for the employment of nicotinate pendent arms in future magnetogenic probes responding to an enzyme activity of one's choice and thus destined for biological applications.

In conclusion, a pair of structurally analogous macrocyclic iron(II) complexes is presented that exhibit electroneutrality at neutral pH and high stability in physiological media. This instance of an electroneutral low-spin iron(II) complex has been made possible through the incorporation of nicotinate pendent arms. They allow for external charge compensation of the iron center without impeding the experimenter's ability to render the iron center low-spin. Both complexes are highly tolerated by mammalian cells at concentrations up to 4 mM for the low-spin and 2 mM for the high-spin complex. They exhibit a marked off/on magnetic relationship in aqueous solution. The results herein are currently transferred to the exploration of molecular candidates for a responsive, magnetogenic probe operating *in vivo*.

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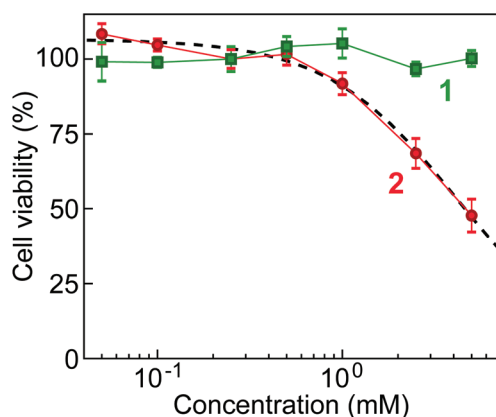
Fig. 3 MTS cell viability test over 24 h for **1** and **2**.

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