DOI: 10.1002/ejic.201000658

Spectroscopic and Electrochemical Investigations into the Interactions of Metal Ions with a Ferrocenoyl–Histidine Peptide Conjugate

Lan-Ya Cheng,[a,b] Yi-Tao Long,*[b] He Tian,[b] and Heinz-Bernhard Kraatz*[a]

Keywords: Cyclic voltammetry / NMR spectroscopy / N ligands / Metal recognition / Chiral induction

A new ferrocene–peptide conjugate Fc-[His(DNP)-Gly-OMe]$_2$ (**2**) was synthesized and characterized spectroscopically. The conjugate displays the familiar 1,2′-*P* helical Herrick conformation in solution and exhibits a reversible one-electron oxidation at 380 mV versus Fc/Fc$^+$. The interactions of the metal ions Na^+, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} with conjugate **2** were probed by using a range of techniques, including cyclic voltammetry, and NMR and CD spectroscopies. Electrochemical studies showed that the system exhibits a rare cathodic potential shift upon the addition of metal ions, which followed the order Cu^{2+} (–436 mV) > Fe^{2+} (–284 mV) > Zn^{2+} (–270 mV) > Cd^{2+} (–238 mV) > Mg^{2+} (–180 mV) > Na^+ (–75 mV). The formation of a 1:1 metal complex was postulated on the basis of CV and NMR spectroscopic titration experiments. The complexes themselves were characterized by electrospray mass spectrometry. NMR spectroscopic studies show that the His imidazole is the site of metal coordination. As is confirmed by CD spectroscopy, metal coordination itself results in conformational changes of the ferrocene core that are dependent on the particular metal ion and its coordination preferences. Whereas *P*-helicity of the conjugate is maintained during the interactions with Mg^{2+}, Zn^{2+}, and Cd^{2+}, *M*-helicity is adopted for Fe^{2+} and Cu^{2+}.

Introduction

The ferrocenyl (Fc) group has proven itself as a useful scaffold for the design of well-defined peptide structural motifs, including β-sheets, γ-turns, and other more complex foldamer structures.[1] The separation between the two cyclopentadienyl rings is ideal to support hydrogen-bonding interactions between the peptide substituents on the two Cp rings that help to stabilize structural motifs. The H-bonding interactions, however, are controlled to some degree by the particular amino acid. Most bis(peptide)-substituted Fc conjugates display two H-bonding interactions involving the conformation that locks the central Fc core into a specific axial conformation.[2] Generally, for most L-amino acids, a 1,2′-*P*-helical arrangement is preferred in solution and in the solid state. The CD spectra of such conjugates exhibit a characteristic signal with a positive Cotton effect in the Fc-based absorption region from λ = 440–480 nm. The corresponding D-amino acids give rise to a 1,5′-*M*-helical conformation with the corresponding negative CD signal in this region.[2b,2d,2e,2n]

Whereas Fc–peptide conjugates have been explored to probe cell uptake and targeting in medicinal applications,[3] and used as part of biosensor platforms,[4] their coordination chemistry with metal ions and their effects on the structure of the conjugate and the stereochemistry of the Fc core remains largely unexplored. Metzler-Nolte and coworkers recently reported the structure of an Fc–cysteine derivative in which the two Cys sulfur atoms coordinate to an Fe–carbonyl fragment.[5] Hirao et al. reported a Pd^{2+} complex of an Fc–peptide–pyridine conjugate in which a Pd^{2+} ion is coordinated to the two pyridine ligands.[6] Results on a cyclic Fc–His derivative show that alkali metals can coordinate presumably to the amide carbonyl groups, giving rise to significant changes in the redox potential of the Fc core.[7] A similar coordination behavior was observed for the interaction of alkali metal ions with cyclic Fc–peptides in which the metal ions coordinate to the amide C=O oxygen atom.[8]

Peptides can readily interact with metal ions through the peptide backbone, involving the O or N site of the amide group.[9] While such interactions are pH-dependent and generally show poor selectivity to metal ions, peptide-involving functional side groups, such as His, are expected to enhance the metal selectivity. The study of these compounds is of growing interest in an attempt to understand the role of metal ions in peptide interactions and aggregations.[10] In addition, they are being exploited in sensor surfaces for Cu recognition. For example, the tripeptide Gly–Gly–His readily binds to Cu^{2+} ions at ultralow concentrations through the imidazole group.[11]

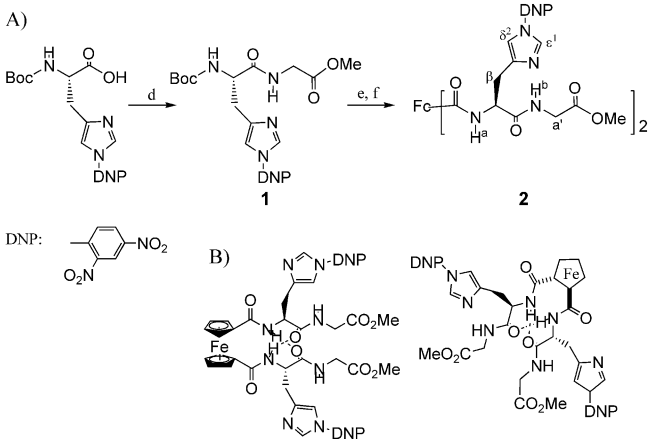
[a] Department of Chemistry, The University of Western Ontario, London, Ontario N6A 5B7, Canada
Fax: +1-519-661-3022 x81561
E-mail: hkraatz@uwo.ca
[b] Department of Chemistry, East China University of Science and Technology,
Shanghai 200237, China
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejic.201000658.

Here, we focus on the use of a histidine-containing Fc conjugate and probe its interactions with a range of metal ions. Histidine plays a critical role in many metalloproteins and provides a coordination site for metals such as Fe, Cu, and Zn. We will provide evidence that in a bis(peptide)–Fc conjugate, metal coordination to the imidazole of the His moiety influences the stereochemistry of the central core and also gives rise to significant changes in the redox properties of the Fc group.

Results and Discussion

Preparation and Characterization of Fc[CO-His(DNP)-Gly-OMe]$_2$ (2)

A brief overview of the synthetic strategy leading to peptide conjugate **2** is shown in Scheme 1, essentially following well-documented coupling strategies, which involve 1,*n*′-ferrocenedicarboxylic acid [Fc(COOH)$_2$], *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU)/*N*′-[3-(dimethylamino)propyl]-*N*-ethylcarbodiimide (EDC) as coupling reagent, and the deprotected dipeptide Boc-His(DNP)-Gly-OMe (**1**; DNP = 2,4-dinitrophenol).[2]



Scheme 1. (A) Synthesis of the ferrocene conjugate Fc[CO-His(DNP)-Gly-OMe]$_2$ (**2**) by cross-coupling with the dipeptide Boc-His(DNP)-Gly-OMe under suitable conditions (DNP = 2,4-dinitrophenol). (a) HOBT, HBTU, TEA, H-Gly-OMe; (b) TFA/CH_2Cl_2 (1:1); (c) Fc(COOH)$_2$, HOBT, EDC. (B) "Herrick" motif showing the 1,2′-arrangement of the two peptide substituents and the interstrand H-bonding interactions between the NH^a and His CO on opposite strands.

The Fc–peptide conjugate **2** was purified by column chromatography and obtained as a yellow–orange solid in 40% yield. Time-of-flight mass spectrometry measurements show a strong [M + H]$^+$ peak at *m*/*z* = 1023.1920. Compound **2** has a good solubility in most organic solvents and was characterized spectroscopically in CD_3CN solution. At room temperature, compound **2** displays two signals assigned to the two amide protons (10 mM) at δ = 8.23 (NH^a) and 7.62 ppm (NH^b). The position of these two amide signals is indicative of hydrogen-bonding interactions.[2d,2e] To evaluate if intra- or intermolecular interactions are responsible for the observed chemical shifts of the amide protons, the NMR spectroscopic properties of conjugate **2** were investigated at various temperatures ranging from 290 to 320 K and at concentrations ranging from 1 to 20 mM. The H-bonding interactions are temperature-sensitive and, at higher temperatures, H-bonding interactions are weakened. This is readily detected by NMR spectroscopy. If H-bonding interactions are present, an increase in the temperature will weaken these interactions resulting in an upfield shift of the resonances assigned to the amide protons. At low concentrations (below the self-association concentration of the peptide conjugate), an upfield shift indicates the presence of intramolecular H-bonding. A study of the concentration dependence of the chemical shift will allow an evaluation which of the amide protons are involved in intermolecular H-bonding interactions. Downfield shifts of the amide proton with increasing concentration indicate the involvement of intermolecular H-bonding interactions. Plots showing the effects of varying the temperature and the concentrations on the chemical shifts of NH^a and NH^b are shown in Figure 1. Several findings are noteworthy. At a concentration of 1 mM, the chemical shift of the His NH^a proton is affected more by changes in the temperature compared with that of the Gly NH^b proton. The $\Delta\delta/T$ values of the amide protons are –6 ppb/K for NH^a and –3.2 ppb/K for NH^b. Temperature coefficients larger than 4 ppb/K indicate the involvement of the amide protons in H-bonding.[2b,12]

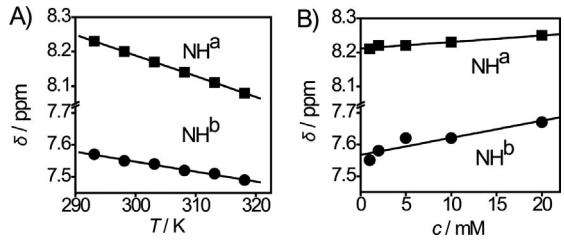


Figure 1. Effects of temperature and concentration on the chemical shift of the amide protons of compound **2** in CD_3CN solution. (A) VT NMR plots of the resonances for NH^a and NH^b at 293.15, 298.15, 303.15, 308.15, 313.15, and 318.15 K ([conjugate **2**] = 1 mM, CD_3CN). (B) VC NMR plots of the resonances of NH^a and NH^b at 1, 2, 5, 10, and 20 mM concentrations in CD_3CN.

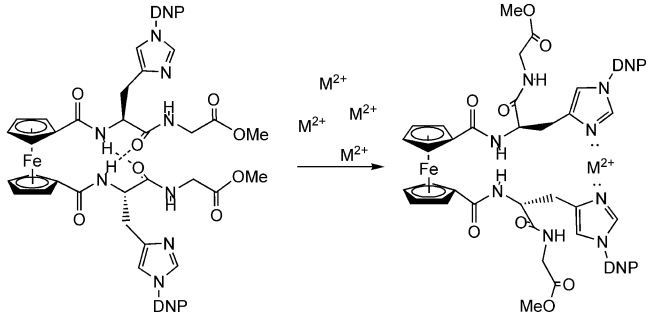
Although both amide signals exhibit concentration dependence, the effect of concentration on the chemical shift of NH^b is stronger. Increasing the concentration will favor intermolecular interactions, therefore suggesting that the Gly NH^b moiety is involved in intermolecular H-bonding interactions. These spectroscopic results are compatible with the Fc-proximal NH^a being involved in intramolecular cross-strand H-bonding interactions, presumably giving rise to "Herrick"-type H-bonding, which is characteristic for many bis(peptide)-substituted 1,*n*′-Fc conjugates. This leaves the distal Gly NH^b moiety free to engage in intermolecular interactions. The "Herrick" pattern locks the Fc core into a 1,2′-*P*-helical conformation.[2d] The conformation of compound **2** was studied by CD spectroscopy in acetonitrile solution at 1 mM. The CD plot of compound **2** in the range of 300–600 nm is shown in Figure S25 in the

Supporting Information. The system exhibits a CD band in the Fc region with a positive Cotton effect and a maximum absorbance at λ = 480 nm, indicating a *P*-helical conformation around the Fc group. The properties of the Fc-centered CD signal are similar to those of a wide range of bis(peptide)-substituted Fc derivatives derived from L-amino acids,[2d,2e] but differences are noticeable in the band at λ = 350 nm showing a negative CD signal. This band is rationalized largely by transitions involving the Cp and the Fe and is significantly more intense compared with other Fc–peptide conjugates, presumably due to contributions from the DNP-protecting group on the imidazole ring of His moiety, which proved to be useful in this context.[2p]

The redox properties of conjugate **2** were tested in acetonitrile solution with cyclic voltammetry (CV) by using tetrabutylammonium perchlorate (TBAP) as the supporting electrolyte. Figure S26 shows a typical voltammogram of conjugate **2** at a scan rate of 100 mV. The system displays a single one-electron oxidation with a halfwave potential ($E_{1/2}$) of 380(5) mV (vs. Fc/Fc^+), a peak separation (ΔE) of 89(5) mV at 100 mV, and a linear relationship between the peak potential ip_a and the square-root of the scan rate. In addition, the peak current ratio ip_a/ip_c is close to unity (Table S1).

Interactions with Metal Ions

The behavior of conjugate **2** in the presence of metal ions was probed by CV. Since the conjugate contains His residues it can be expected that metal ions can coordinate to the imidazole groups of the two proximal His residues (Scheme 2).[9a] Metal coordination to the imidazole groups is expected to influence the redox properties of the Fc group and, in addition, should further be detectable by a shift in the resonance of the imidazole protons in the ^1H NMR spectrum.



Scheme 2. Proposed interaction of conjugate **2** with transition metal ions involving the two His residues.

First, the effects on the electrochemical properties were investigated. Figure 2 shows CV plots of conjugate **2** in the presence of 2 equiv. of the metal ions Na^+, Mg^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, and Cu^{2+}. A shift to lower potential was observed for all metal ions. The cathodic shift decreases in the order Cu^{2+} (–403 mV) > Zn^{2+} (–268 mV) > Fe^{2+} (–254 mV) > Cd^{2+} (–242 mV) > Mg^{2+} (–137 mV) > Na^+ (–40 mV), indicating the Fc component with the various metals are more easily oxidized in the order $Cu^{2+} > Zn^{2+} > Fe^{2+} > Cd^{2+} > Mg^{2+} > Na^+$. For the most part, cation coordination to an Fc host is expected to result in an anodic shift of the redox potential, making the Fc group more difficult to oxidize, whereas anions can cause cathodic shifts.[13] There are few examples showing similar cathodic shifts. Beer and co-workers reported an Fc–crown-ether conjugate with a thioether linker, which showed an anodic potential shift when Na^+ ions were added, but displayed a surprising cathodic potential shift when K^+ ions were added.[13b] This was ascribed to the formation of a 1:1 K^+ complex. Another example of a cathodic shift is observed for the alkali metal complex of a cyclic 1,1′-FcCO–His conjugate (FcCO-His-OMe), in which cyclization is achieved through the ε-N atom of the imidazole.[7] Metal binding occurs presumably through the oxygen atom of the Fc-C=O moiety, which translates into the slight conformational changes in the Fc core. Another interesting example is a Pd^{2+} complex of $Fc[CO\text{-}L\text{-}Ala\text{-}L\text{-}Pro\text{-}NHPy]_2$, which has the metal ion complexed through the C-terminal pyridine groups. Upon coordination, the Fc group displays a cathodic shift of about 20 mV versus the free Fc–peptide.[6]

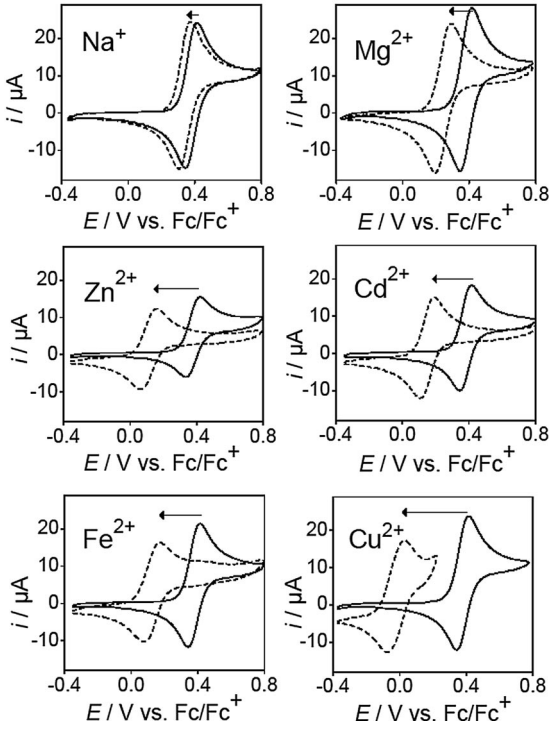


Figure 2. The cyclic voltammograms of conjugate **2** in acetonitrile before (——) and after (-----) the addition of metal perchlorate salts. The addition of metal salts caused cathodic shifts ([conjugate **2**] = 1 mM, acetonitrile, 1 M TBAP was added as supporting electrolyte; glassy carbon working electrode, Pt wire counter electrode, Ag/AgCl reference electrode; Fc/Fc^+ was used as an external reference).

A titration experiment in which metal ions were added to a solution of conjugate **2** was carried out, and the cathodic shift as a function of added metal ions was measured. The cathodic shift decreases in the order Cu^{2+} (–436 mV) > Fe^{2+} (–284 mV) > Zn^{2+} (–270 mV) > Cd^{2+} (–238 mV) > Mg^{2+} (–180 mV) > Na^{+} (–75 mV) when 4 equiv. of the metal ions were added. The collective result of this study is shown in Figure 3. The results clearly demonstrate that cathodic shifts increase significantly upon the addition of 1 equiv. of metal ion. The addition of excess metal ions does not affect the redox potential significantly, and further addition of metal ions shows negligible changes. This suggests that the Fc conjugate **2** binds to a single metal ion, which gives rise to a cathodic shift. The effect of introducing water to the solution was also considered, but the shifts caused by water are significantly smaller than the effects of the added metal ions (see Figures S27 and S28 in the Supporting Information).

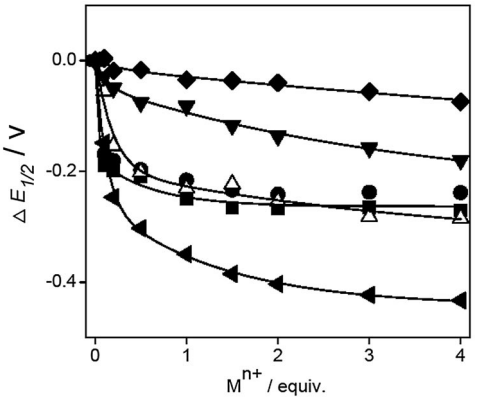


Figure 3. Effects of metal ion addition on the half-wave potential of compound **2** (1 mM in acetonitrile). The following metal ions were added as their perchlorate salts: ♦ = Na^{+}; ● = Zn^{2+}; ■ = Cd^{2+}; ▼ = Mg^{2+}; Δ = Fe^{2+}; ◂ = Cu^{2+}.

A series of ^1H NMR spectroscopic titrations was carried out, in which the chemical shifts of conjugate **2** were monitored as a function of added metal ion. The ^1H NMR spectra for the titrations of Cd^{2+} to a solution of conjugate **2** in CD_3CN are shown in Figure 4. It is clearly seen that the signals of the imidazole protons δ^2 and ε^1 are significantly affected by the addition of the Cd^{2+} ions. The amide proton is also affected by the addition of metal ions. Similar results were observed on the addition of the other transition metal ions to a solution of conjugate **2**, indicating that metal coordination involves the imidazole group of His. A plot of the chemical shift change ($\Delta\delta$) versus the equivalents of Cd^{2+} added to the solution is shown in Figure 5. Essentially, the results are comparable with our CV titration experiments and show that the chemical shift is affected until 1 equiv. of metal ion is added. Addition of excess metal ion does not cause any significant changes of the chemical shifts. It can be assumed that transition metals prefer to bind to conjugate **2** involving the formation of a chelate with 1:1 stoichiometry. This would presumably involve N-coordination of the imidazole group. This is supported by our NMR spectroscopic titration experiments.

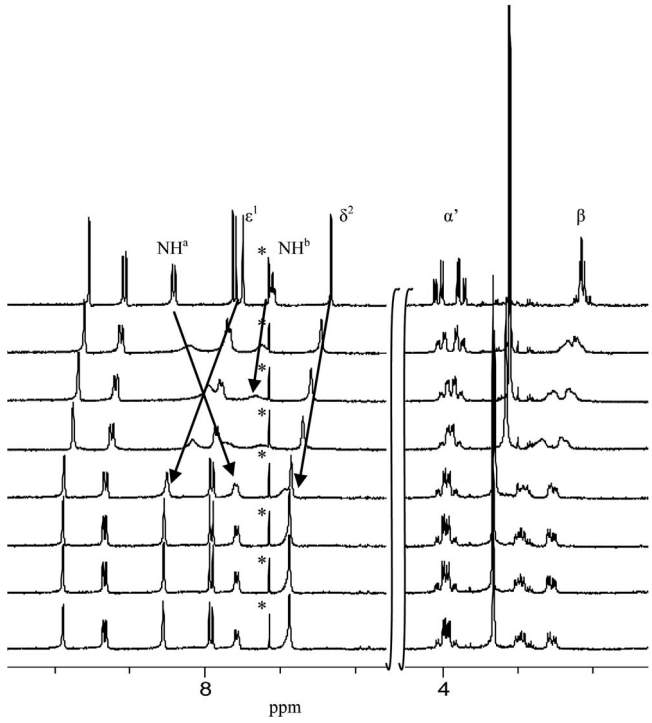


Figure 4. ^1H NMR spectroscopic titration experiments of Cd^{2+}. Cd^{2+} was added at 0, 0.2, 0.4, 0.6, 1.0, 1.2, 1.4, and 2.0 equiv. shown from top to bottom ([conjugate **2**] = 1 mM, CD_3CN; the asterisk indicates residual $CHCl_3$).

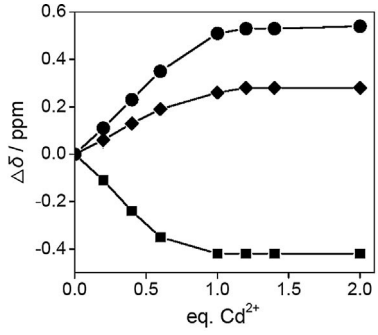


Figure 5. Changes in the chemical shifts of the two His protons ε^1 and δ^2 as a function of added Cd^{2+} to a 1 mM solution of conjugate **2** in CD_3CN. ● = ε^1 of His, ♦ = δ^2 of His, ■ = NH_a. Note that after the addition of 1 equiv. of Cd^{2+}, no additional chemical shift changes are observed.

There are some additional interesting changes noticeable in the ^1H NMR spectra shown in Figure 5, presumably related to the structural rigidity of the metal complex. Before metal addition, the two diastereotopic β-protons are observed as a single multiplet, which upon addition of Cd^{2+} splits into two separate multiplets; this indicates enhanced conformational rigidity upon metal coordination to the His imidazole. This is a reasonable assumption, since side-chain rotation will not be restricted for a metal complex. Additional ^1H NMR spectroscopic titrations can be found in

Figures S12–S17 in the Supporting Information. The addition of Na^+ ions to **2** did not cause any visible change during the titration, which is comparable with the electrochemical results. In the case of Fe^{2+} and Cu^{2+}, the paramagnetic nature of the hexaqua ion essentially causes line broadening.

Our spectroscopic and electrochemical studies suggest the formation of a 1:1 complex between the conjugate **2** and metal ions, such as Zn^{2+}, Cd^{2+}, Fe^{2+}, Mg^{2+}, and Cu^{2+}. Next we carried out additional experiments focused on evaluating the stoichiometry of the complex in more detail. For this purpose, we carried out studies by electrospray time-of-flight mass spectrometry at the ratio of 4:1 of metal ions/conjugate **2**. A typical mass spectrum of the solution of **2**·Cd^{2+} displays a peak at m/z = 1133.2 assigned to $[M + Cd - H]^+$ and 1233.2 assigned to $[M + Cd + 2\ CH_3CN + H_2O - H]^+$. In both cases, the mass spectrum shows an isotope envelope indicative of a Cd complex. The $[M + Cd - H]^+$ cluster was analyzed further by high-resolution MS, and the experimental result, together with a theoretical isotope pattern of the molecular ion $[M + Cd - H]^+$ at m/z = 1133.0789, is shown in Figure 6. Similar MS results were obtained for Zn^{2+}, Fe^{2+}, and Cu^{2+} and are summarized in Table 1 (see also the Supporting Information).

As we know the stoichiometry of our Fc–peptide metal complexes, it is of interest to evaluate if metal coordination will cause any structural changes in the Fc conjugate. For this purpose, we used CD spectroscopy. The 1,*n*′-bis(peptide)–Fc conjugates display a characteristic CD signal in the Fc region of λ = 400–500 nm that is linked to the axial chirality of the Fc group and its substitution pattern.[2b,2d,2e] Figure 7 shows the CD spectra of the Fc–peptide conjugate **2** in the presence of various metal ions in 1 mM acetonitrile solution. The spectrum of conjugate **2** is characterized by a negative CD signal at λ = 350 nm, a negative shoulder at $\lambda \approx$ 420 nm, and a positive signal at λ = 490 nm, indicative of a *P*-helical arrangement of the Fc core. This is in line with the results from the ^1H NMR spectroscopic studies of conjugate **2**, which indicated that the proximal amide protons are involved in intramolecular H-bonding interactions.

As expected, the addition of Na^+ ions does not cause any significant changes in the CD spectrum of compound **2**, whereas the addition of all other metal ions caused significant changes. Two changes are particularly noteworthy. Firstly, for all metal ions the signal at λ = 330 nm has a positive Cotton effect and experiences a hypsochromic shift, and its intensity is affected by the specific metal ion. Secondly, the Fc-based signal remains positive on addition of Mg^{2+}, Zn^{2+}, and Cd^{2+} ions, indicating that the helical chirality of the Fc core retains *P*-helicity. This would suggest that metal coordination to the two imidazoles of the conjugate does not alter the conformation around the Fc core. It should be noted that all three metal ions can accommodate a tetrahedral coordination environment, which presumably does not cause any significant steric stress on the Fc core upon coordination in a bidentate fashion. In contrast, the addition of Fe^{2+} and Cu^{2+} ions appears to cause significant

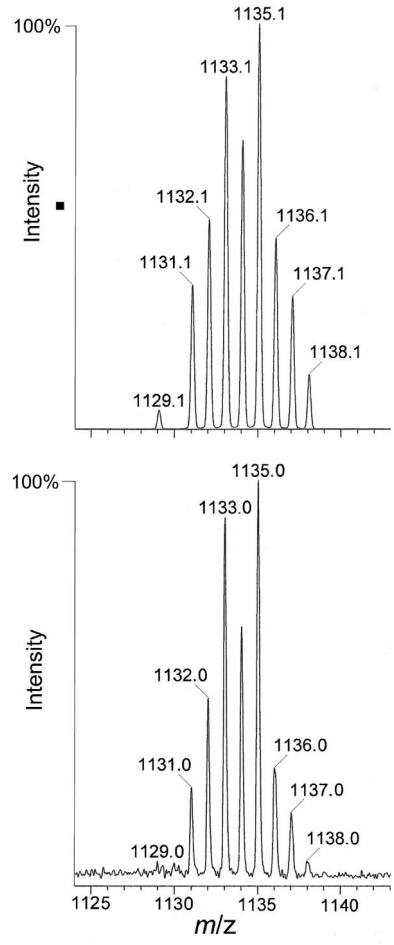


Figure 6. Partial ES-Tof-MS results showing the experimental and theoretical isotopic pattern of the Cd^{2+} complex of conjugate **2**, assigned to $[M + Cd - H]^+$. Top: calculated molecular isotopic distribution for $[M + Cd - H]^+$; bottom: observed partial spectrum.

Table 1. Results of the high-resolution electrospray mass spectrometry analysis of acetonitrile solutions of ferrocene conjugate **2** in the presence of Fe^{2+}, Zn^{2+}, Cu^{2+}, and Cd^{2+}.

Complex compostion	Mass	Calcd. mass
$[M+Fe - H]^+$	1077.1110	1077.1149
$[M+Zn - H]^+$	1085.1067	1085.1091
$[M+Cu]^+$	1085.1165	1085.1174
$[M+Cd - H]^+$	1133.0789	1133.0789

conformational changes in the structure of the Fc core and a change to an *M*-helical arrangement is indicated by the CD signal with a negative Cotton effect at $\lambda \approx$ 470 nm for both complexes. Presumably, coordination of the two imidazole ligands to the metal ions is responsible for the changes in the Fc conformation. The disposition of the two imidazoles around the metal ion is expected to be 90° due to the preference of the Fe^{2+} and Cu^{2+} ions for octahedral to distorted octahedral (or square-pyramidal to square-planar) environments. This in turn might influence the structure of the Fc core and distort the Cp rings away from a 1,2′-*P* helical conformation towards a more open *M*-helical structure.

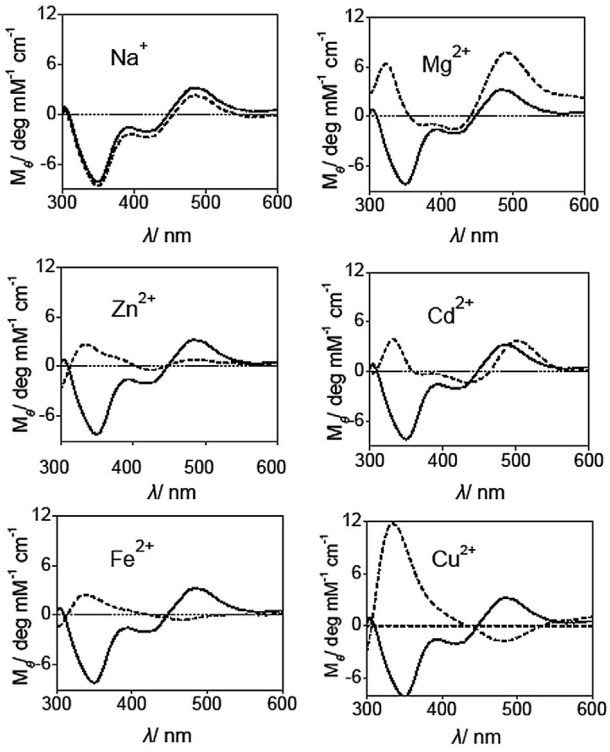


Figure 7. CD spectra of the six metal complexes prepared with 2 equiv. of metal ions with compound **2** (dashed line), compared with compound **2** (1 mM; solid line) in acetonitrile.

Conclusion

A novel disubstituted ferrocene–peptide histidine conjugate has been successfully synthesized, and its coordination chemistry with a range of metal ions was studied. Our studies show that the metal ions coordinate to the His imidazoles. Furthermore, metal coordination to the imidazoles influences the helicity of the central Fc group. For Mg^{2+}, Zn^{2+}, and Cd^{2+}, the Fc maintains *P*-helicity, whereas for Fe^{2+} and Cu^{2+} the Fc group is clearly *M*-helical. Changes in the Fc helicity are thought to be the result of differences in the coordination environments between metals preferring a tetrahedral to an octahedral (square-planar) geometry. Presumably, a tetrahedral geometry is more amenable to a *P*-helicity.

The metal complexes of our Fc–His conjugate also gives rise to cathodic potential shifts of the Fc signal. This demonstrates the 1,*n*′-disubstituted Fc–histidine conjugates are a good scaffolds to recognize the metal ions through electrochemical observation, and different chiral changes, affected by different metal chelation, will show different shifts in potential.

Experimental Section

General: All syntheses were carried out in air unless otherwise indicated. CH_2Cl_2 (BDH; ACS grade), used for synthesis, was dried with CaH_2 and distilled. Ferrocenedicarboxylic acids were synthesized from ferrocene according to literature procedures.[14,15] Boc-His(DNP)-OH·IPA (IPA = 2-propanol), H-Gly-OMe·HCl, HOBT·H_2O, and EDC·HCl were obtained from Advanced ChemTech. $Cd(ClO_4)_2·6H_2O$, $Zn(ClO_4)_2·6H_2O$, $Cu(ClO_4)_2·6H_2O$, and $Fe(ClO_4)_2·xH_2O$ were bought from Aldrich; $Mg(ClO_4)_2·6H_2O$ and $NaClO_4$ were bought from Alfa–Aesar. All chemicals were used without further purification. All ^{1}H NMR spectroscopic experiments were carried out with a Varian Nova 400M NMR spectrometer at 25 °C, except the VT NMR experiments. All ^{1}H NMR spectroscopic titrations (except the VC NMR spectroscopic experiment) and CV titration experiments were carried out at an Fc conjugate concentration of 1 mM in acetonitrile. The metal concentration was changed by the addition of aliquots of metal salt solutions in [D]acetonitrile by using microliter syringes. Mass spectrometry was carried out with a Finnigan MAT 8200 mass spectrometer. A JASCO J-810 CD spectrometer was used to evaluate the solution conformation of the Fc–peptide conjugate **2**; 1 mm cuvettes were used for these studies and a solution of conjugate **2** in freshly distilled acetonitrile (1 mM concentration). A 0.1 M metal perchlorate solution was prepared in dry, distilled acetonitrile. The metal salt solution was added in 0.2 μL aliquots. and the resulting concentrations were adjusted.

Synthesis of Boc-His(DNP)-Gly-OMe (1): Boc-His(DNP)-OH·IPA (1.44 g, 3 mmol) was dissolved in distilled CH_2Cl_2 (50 mL). The solution was cooled in an ice bath, and solid HOBT (1.2 eqiuv., 0.49 mg, 3.6 mmol) and HBTU (1.36 g, 3.6 mmol) were added immediately, followed by the addition of triethylamine (TEA; 0.75 mL). After stirring for 0.5 h, H-Gly-OMe·HCl (0.38 g, 3 mmol) in distilled CH_2Cl_2 (30 mL), together with TEA (0.75 mL), was added to the stirred, activated mixture of Boc-His(DNP)-OH after 10 min. The ice bath was removed and the stirring continued at r.t. overnight. The reaction solution was then dried in vacuo and purified on a silica column by using a solvent gradient (CH_2Cl_2 to 2% methanol/CH_2Cl_2). The desired peptide was obtained as a yellow powder (1.26 g, 85.4%). R_f = 0.24 (CH_2Cl_2/MeOH, 91:9). ^{1}H NMR ($CDCl_3$): δ = 8.84 (d, J = 2.4 Hz, 1 H, H-DNP), 8.57 (m, 1 H, H-DNP), 7.78 (d, J = 8.8 Hz, 1 H, H-DNP), 7.62 (s, 1 H, $H^{\varepsilon 1}$), 7.00 (br. s, 1 H, NH^a), 6.96 (s, 1 H, $H^{\delta 2}$), 6.18 (br. s, 1 H, NH^b), 4.54 (br. s, 1 H, CH_α), 3.99 (m, 2 H, CH_2-ester), 3.70 (s, 3 H, $COOCH_3$), 3.12 (m, 2 H, CH_2-imidazole), 1.47 (s, 9 H, H-Boc) ppm. ^{13}C NMR ($CDCl_3$): δ = 171.9, 170.4, 147.1, 144.6, 140.3, 136.7, 135.4, 129.8, 128.4, 121.5, 118.1, 80.4, 54.5, 52.4, 47.4, 41.3, 30.3, 28.5, 8.92 ppm. TOF MS (ES+): found for $C_{20}H_{24}N_6O_9$ 492.1625.

Synthesis of Fc[CO-His(DNP)-Gly-OMe]$_2$ (2): Boc-His-Gly-OMe (0.98 g, 2 mmol) was dissolved in distilled CH_2Cl_2 (20 mL). TFA (20 mL) was added slowly, and the solution was stirred at r.t. for 20 min and then dried under vacuum. The residue was then dissolved in CH_2Cl_2 (40 mL), and TEA was added to the solution (ca. 2 mL). After 5 min, the clear solution was again concentrated to dryness and divided into portions. $Fc(COOH)_2$ (0.14 g, 0.5 mmol) was dissolved in distilled CH_2Cl_2 (30 mL). Solid HOBT·H_2O (0.18 g, 1.2 mmol) and EDC·HCl (0.21 g, 1.1 mmol) were added, and the reaction mixture was stirred overnight. The reaction solution was then cooled in an ice bath, and the deprotected peptide (0.49 g,1.2 mmol) dissolved in CH_2Cl_2 was added, followed by the addition of TEA (0.6 mL). Stirring was continued at r.t. for 4 d. The reaction solution was then dried under vacuum and purified chromatographically by using a gradient eluent from chloroform to chloroform/methanol (93:7). The desired product was obtained as a brown powder (0.20 g, 39.1%.). ^{1}H NMR ($CDCl_3$, 298 K, c = 10 mM): δ = 8.74 (m, 2 H, H-DNP), 8.50 (m, 2 H, H-DNP), 8.47 (d, J = 7.4 Hz, 2 H, NH^a), 7.72 (m, 2 H, H-DNP), 7.63 (s, 2 H,

$H^{\varepsilon 1}$), 7.45 (t, J = 6.2 Hz, 2 H, NH^b), 7.04 (s, 2 H, $H^{\delta 2}$), 4.93 (m, 2 H, CH_α), 4.78 (m, 2 H, H-Fc), 4.73 (m, 2 H, H-Fc), 4.43 (m, 2 H, H-Fc), 4.27 (m, 2 H, H-Fc), 3.99 (m, 4 H, CH_2-ester), 3.58 (s, 6 H, $COOCH_3$), 3.09 (d, J = 7.3 Hz, 4 H, CH_2-imidazole) ppm. ^{13}C NMR ($CDCl_3$, 298 K, c = 10 mM): δ = 173.5, 170.7, 170.4, 147.0, 140.7, 136.7, 135.4, 129.6, 128.4, 121.4, 118.2, 77.2, 76.4, 72.1, 71.6, 70.6, 70.4, 53.6, 52.4, 41.7, 29.7 ppm. ^1H NMR (CD_3CN, 298 K, C = 1 mM): δ = 8.77 (s, 1 H, H-DNP), 8.76 (s, 1 H, H-DNP), 8.545 (m, 2 H, H-DNP), 8.53 (d, J = 7.8 Hz, 2 H, NH^a), 7.80 (m, 2 H, H-DNP), 7.74 (s, 2 H, $H^{\varepsilon 1}$), 7.55 (t, J = 5.7 Hz, 2 H, NH^b), 7.16 (s, 2 H, $H^{\delta 2}$), 4.81 (m, 2 H, CH_α), 4.73 (m, 2 H, H-Fc), 4.68 (m, 2 H, H-Fc), 4.43 (m, 2 H, H-Fc), 4.34 (m, 2 H, H-Fc), 3.95 (m, 4 H, CH_2-ester), 3.55 (s, 6 H, $COOCH_3$), 3.07 (m, 4 H, CH_2-imidazole) ppm. ^{13}C NMR (CD_3CN, 298 K, c = 1 mM): δ = 174.1, 170.5, 169.9, 168.6, 166.5, 147.1, 130.0, 128.8, 121.5, 110.4, 76.8, 71.8, 71.5, 71.0, 69.9, 53.5, 52.0, 41.4, 37.0, 32.8 ppm. TOF MS (ES+): calcd. for $C_{42}H_{38}FeN_{12}O_{16}$ $[M + H]^+$ 1023.1956; found 1023.1920. $C_{42}H_{38}FeN_{12}O_{16}$ (1022.67): calcd. C 49.33, H 3.75, N 16.44; found C 49.60, H 3.87, N 16.66.

Electrochemical Studies: All electrochemical experiments described here make use of a glassy carbon working electrode (diameter 3 mm), Pt wire as counter electrode, and Ag wire as pseudo-reference electrode. The glassy carbon electrode was polished with 0.05 μm Al_2O_3, sonicated in MilliQ water for 1 min to fully remove any absorbed Al_2O_3, rinsed in MilliQ water, and dried under N_2. The Pt wire was sonicated and rinsed with ethanol and MilliQ water, dried under N_2, and flamed by using a propane torch until glowing red. The Ag wire was sonicated and rinsed with ethanol and MilliQ water, and polished with sand paper. All electrochemical data were reported relative to the Fc/Fc^+ redox couple. Acetonitrile was dried with CaH_2 and freshly distilled prior to use. TBAP (0.1 M in acetonitrile) was used as the supporting electrolyte. Conjugate **2** was studied at concentrations of 1 mM in the supporting electrolyte solution. Since most of the metal salts contain water, it was decided to probe the effect of water since it is known that the addition of water to an Fc–peptide conjugate can result in shifts to lower potentials;[16] however, no significant effects were observed. The metal salts were added in 0.1 equiv. portions to the analyte solution.

Supporting Information (see footnote on the first page of this article): CV, CD spectroscopy, ^1H, g-cosy, VC, and VT NMR spectra of conjugate **2**, CV titration plots and ^1H NMR titration spectra of conjugate **2** with metal ions, CV plots and ^1H NMR spectra of conjugate **2** with introducing H_2O effect, and mass spectra of conjugate **2** and its metal complexes.

Acknowledgments

The authors thank Natural Sciences and Engineering Research Council of Canada and the China Scholarship Council for financial support in the form of a scholarship to L.-Y. C. We acknowledge the financial support of the Shanghai Shuguang Project (07SG36) and funding from the Ministry of Health (Grant 2009ZX10004-301). We also want to acknowledge discussions with Dr. Sanela Martic.

[1] a) L. Barisic, M. Dropucic, V. Rapic, H. Pritzkow, S. I. Kirin, N. Metzler-Nolte, *Chem. Commun.* **2004**, 2004–2005; b) L. Barisic, M. Cakic, K. A. Mahmoud, Y.-n. Liu, H.-B. Kraatz, H. Pritzkow, S. I. Kirin, N. Metzler-Nolte, V. Rapic, *Chem. Eur. J.* **2006**, *12*, 4965–4980; c) S. Chowdhury, K. A. Mahmoud, G. Schatte, H. B. Kraatz, *Org. Biomol. Chem.* **2005**, *3*, 3018–3023; d) S. Chowdhury, G. Schatte, H. B. Kraatz, *Angew. Chem. Int. Ed.* **2006**, *45*, 6882–6884; e) S. Chowdhury, G. Schatte, H. B. Kraatz, *Angew. Chem. Int. Ed.* **2008**, *47*, 7056–7059.

[2] a) R. S. Herrick, R. M. Jarret, T. P. Curran, D. R. Dragoli, M. B. Flaherty, S. E. Lindyberg, R. A. Slate, L. C. Thornton, *Tetrahedron Lett.* **1996**, *37*, 5289–5292; b) T. Moriuchi, A. Nomoto, K. Yoshida, A. Ogawa, T. Hirao, *J. Am. Chem. Soc.* **2001**, *123*, 68–75; c) D. R. van Staveren, T. Weyhermüller, N. Metzler-Nolte, *Dalton Trans.* **2003**, 210–217; d) D. R. van Staveren, N. Metzler-Nolte, *Chem. Rev.* **2004**, *104*, 5931–5985; e) S. I. Kirin, H. B. Kraatz, N. Metzler-Nolte, *Chem. Soc. Rev.* **2006**, *35*, 348–354; f) T. Moriuchi, T. Nagai, T. Hirao, *Org. Lett.* **2005**, *7*, 5265–5268; g) T. Moriuchi, T. Nagai, T. Hirao, *Org. Lett.* **2006**, *8*, 31–34; h) K. Heinze, M. Beckmann, *Eur. J. Inorg. Chem.* **2005**, 3450–3457; i) S. I. Kirin, U. Schatzschneider, X. De Hatten, T. Weyhermüller, N. Metzler-Nolte, *J. Organomet. Chem.* **2006**, *691*, 3451–3457; j) J. Lapic, D. Siebler, K. Heinze, V. Rapic, *Eur. J. Inorg. Chem.* **2007**, 2014–2024; k) K. Heinze, D. Siebler, *Z. Anorg. Allg. Chem.* **2007**, *633*, 2223–2233; l) M. C. Semenic, D. Siebler, K. Heinze, V. Rapic, *Organometallics* **2009**, *28*, 2029–2037; m) S. Djakovic, D. Siebler, M. C. Semenic, K. Heinze, V. Rapic, *Organometallics* **2008**, *27*, 1447–1453; n) S. I. Kirin, D. Wissenbach, N. Metzler-Nolte, *New J. Chem.* **2005**, *29*, 1168–1173; o) X. Hatten, T. Weyhernuller, N. Metzler-Nolte, *J. Organomet. Chem.* **2004**, *689*, 4856–4867; p) M. Kawai, U. Nagai, Y. Inai, H. Yamamura, R. Akasaka, S. Takagi, Y. Miwa, T. Taga, *Pept. Sci.* **2005**, *80*, 186–198.

[3] a) F. Noor, R. Kinscherf, G. A. Bonaterra, S. Walczak, S. Woelfl, N. Metler-Nolte, *ChemBioChem* **2009**, *10*, 493–502; b) N. Metzler-Nolte, *Chimia* **2007**, *61*, 736–741; c) J. T. Chantson, M. V. V. Falzacappa, S. Crovella, N. Metzler-Nolte, *ChemMedChem* **2006**, *1*, 1268–1274.

[4] a) K. A. Mahmoud, H.-B. Kraatz, *Chem. Eur. J.* **2007**, *13*, 5885–5895; b) K. Kerman, K. A. Mahmoud, H.-B. Kraatz, *Chem. Commun.* **2007**, 3829–3831; c) K. A. Mahmoud, J. H. Luong, *Anal. Chem.* **2008**, *80*, 7056–7062; d) K. A. Mahmoud, S. Hrapvic, J. H. Luong, *ACS Nano* **2008**, *2*, 1051–1057; e) K. Kerman, H.-B. Kraatz, *Analyst* **2009**, *134*, 2400–2404.

[5] X. Hatten, E. Bothe, K. Merz, I. Huc, N. Metzler-Nolte, *Eur. J. Inorg. Chem.* **2008**, 4530–4537.

[6] T. Moriuchi, K. Yoshida, T. Hirao, *J. Organomet. Chem.* **2001**, *637–639*, 75–79.

[7] S. Chowdhury, G. Schatte, H.-B. Kraatz, *Eur. J. Inorg. Chem.* **2006**, 988–993.

[8] H. Huang, L. Mu, J. He, J.-P. Cheng, *J. Org. Chem.* **2003**, *68*, 7605–7611.

[9] a) H. Sigel, R. B. Martin, *Chem. Rev.* **1982**, *82*, 385–426; b) N. I. Jakab, A. Jancso, T. Gajda, B. Gyurcsik, A. Rockenbauer, *J. Inorg. Biochem.* **2008**, *102*, 1438–1448; c) I. N. Jakab, O. Lorincz, A. Jancso, T. Gajda, B. Gyurcsik, *Dalton Trans.* **2008**, 6987–6995.

[10] For example: a) J. Shearer, V. A. Szalai, *J. Am. Chem. Soc.* **2008**, *130*, 17826–17835; b) D. F. Raffa, R. Gomez-Balderas, P. Brunelle, G. A. Rickard, A. Rauk, *J. Biol. Inorg. Chem.* **2005**, *10*, 887–902; c) M. A. Zoroddu, S. Medici, M. Peana, *J. Inorg. Biochem.* **2009**, *103*, 1214–1220; d) V. Minicozzi, S. Morante, G. C. Rossi, F. Stellato, N. Christian, K. Jansen, *Int. J. Quantum Chem.* **2008**, *108*, 1992–2015.

[11] a) E. Chow, J. J. Gooding, *Electroanalysis* **2006**, *18*, 1437–1448; b) W. R. Yang, D. Jaranillo, J. J. Gooding, D. B. Hibbert, R. Zhang, G. D. Willett, K. J. Fisher, *Chem. Commun.* **2001**, 1982–1983.

[12] E. S. Stevens, N. Sugawara, G. M. Bonora, C. Toniolo, *J. Am. Chem. Soc.* **1980**, *102*, 7048–7050.

[13] a) S. R. Bayly, P. D. Beer, G. Z. Chen, in: *Ferrocenes: Ligands, Materials and Biomolecules* (Ed.: P. Stepnicka), Wiley, New York, **2008**; b) P. D. Beer, J. P. Danks, D. Hesek, J. F. McAleer, *J. Chem. Soc., Chem. Commun.* **1993**, 1735–1737; c) A. Chesney, M. R. Bryce, A. S. Batsanov, J. A. K. Howard, L. M.

Goldenberg, *Chem. Commun.* **1998**, 677–678; d) F. Zapata, A. Caballero, A. Espinosa, A. Tarraga, P. Molina, *Inorg. Chem.* **2009**, *48*, 11566–11575; e) F. Zapata, A. Caballero, A. Espinosa, A. Tarraga, P. Molina, *J. Org. Chem.* **2009**, *74*, 4787–4796; f) A. Caballero, A. Espinosa, A. Tarraga, P. Molina, *J. Org. Chem.* **2008**, *73*, 5489–5497.

[14] M. Rosenblum, R. B. Woodward, *J. Am. Chem. Soc.* **1958**, *80*, 5443–5449.

[15] A. Sonada, I. Moritani, *J. Organomet. Chem.* **1971**, *26*, 133–140.

[16] M. Baker, H.-B. Kraatz, J. W. Quail, *New J. Chem.* **2001**, *25*, 427–433.

Received: June 15, 2010
Published Online: October 11, 2010

