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# The first oligopeptide derivative of 1′-aminoferrocene-1-carboxylic acid shows helical chirality with antiparallel strands†

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**The unnatural organometallic amino acid 1′-aminoferrocene-1-carboxylic acid (Fca) induces a turn structure in a tetrapeptide with anti-parallel strands which is stabilized by two intra-molecular hydrogen bonds in the solid state and in solution.**

Turn structures are important secondary structural elements in proteins. In addition to their classification, they have become important targets in medicinal chemistry.[1–4] Metal complexes may serve as turn mimetics in peptides. This aspect was first realized by Herrick and coworkers, who proposed the use of ferrocene-1,1′-dicarboxylic acid as a turn mimetic.[5] Subsequently, structural work has been carried out by several groups, in particular those of Hirao and Kraatz.[6–12] We have recently investigated the influence of charge in the turn inducing metal unit on structure and stability of metallocene peptides **1** by exchanging the neutral ferrocene for the cobaltocenium cation.[13]

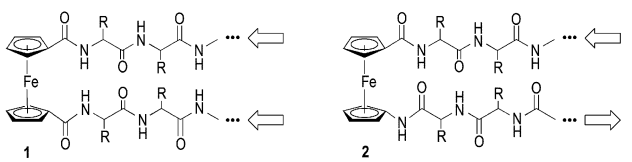
One important consequence of the symmetrical nature of complexes **1** is that only *parallel* peptide strands can be formed (Scheme 1). Natural peptide turns, however, will always result in *anti-parallel* peptide strands. This feature can be realized in compounds of type **2**, which incorporate the unnatural amino acid 1′-aminoferrocene-1-carboxylic acid (Fca) coupled with natural amino acids. Several papers on the synthesis of Fca and derivatives were published.[14–18] In this paper, we report the preparation of the first oligopeptide of Fca (**5**). Structural work in solution by CD spectroscopy and in the solid state by X-ray crystallography indeed confirms an *anti-parallel* orientation of the peptide strands with intra-molecular hydrogen bonds.

Compound **5** was synthesized starting from Boc-Fca-OH **3** (see Scheme 2 and ESI)‡ §. Coupling to the dipeptide H-Ala-Ala-OMe yields the protected tripeptide Boc-Fca-Ala-Ala-OMe **4**. Boc-deprotection was achieved by gaseous HCl in ethyl acetate. Subsequent coupling to Boc-Ala-OH yields the tetrapeptide Boc-Ala-Fca-Ala-Ala-OMe **5**, which was purified by preparative thin layer chromatography.

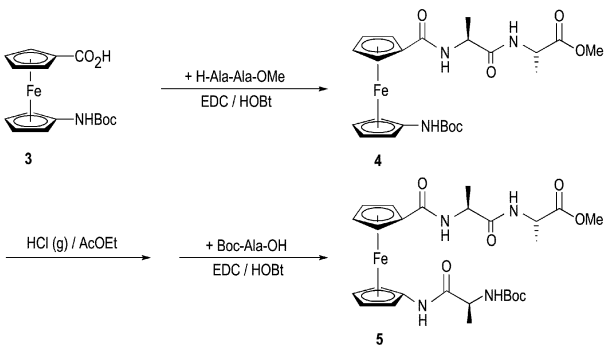
The mass spectrum (EI, 70 eV) of **5** shows the $M^+$ peak at $m/z$ = 572 (36% intensity), this peak is also prominent in the ESI MS ($m/z$ = 573, $[M+H]^+$). The $^1$H NMR spectrum (DMSO, approx. 15 mM) is in accordance with the proposed structure, which is further supported by the number and chemical shift of the amide protons, which appear at 9.60 ppm (s, $Fca_{NH}$), and the three $Ala_{NH}$ at 8.57, 7.74, and 7.23 ppm (all d). Some of these amide hydrogen atoms are involved in hydrogen bonding, as shown in the solution IR spectrum ($CH_2Cl_2$, approx. 2 mM) of **5**. In addition to a relatively sharp band at 3436 $cm^{-1}$, which is characteristic of a free NH bond, there are three broad bands below 3400 $cm^{-1}$ which indicate hydrogen bonding.[13,19,20] Compound **5** shows a reversible one-electron oxidation at +97 mV *vs.* $Fc/Fc^+$ (see ESI). This is in the expected potential range for Fca derivatives and an unambiguous indication that the electroactive metallocene is incorporated in the peptide.

Crystals suitable for an X-ray single crystal study were grown by slow diffusion of pentane into a chloroform solution of **5** ($c$ = 10 mg $ml^{-1}$). An ORTEP plot of **5** is shown in Fig. 1. Inspection of the structural parameters reveals a rather unstrained molecule. Two intra-molecular hydrogen bonds form between (N1)H and O52 and (N51)H and O2, respectively. The N–H–O bond angle deviates only slightly from linearity, and N–O distances around 2.9 Å indicate fairly strong hydrogen bonds. The angle $\omega$[13] between the substituents on the Cp rings is 60.7°, which resembles the typical 1,2′ positioning of amino acid substituents in related structures. One interesting feature of compound **5** is the axial chirality of the ferrocene group. It is forced into a *P*-helical arrangement in all molecules in the crystal. This preferred helical conformation is induced by the enantiomerically pure L-Ala amino acids which were used in the synthesis of **5**. Hirao and coworkers also observed a *P*-helical conformation in their L-amino acid derivatives of ferrocene-1,1′-dicarboxylic acid.[6,9]

In order to investigate the conformation of **5** in solution, CD spectra of **5** were recorded in $CH_3CN$ (Fig. 2). A strong Cotton effect (spectrum a in Fig. 2) at about 480 nm is indicative of a chiral conformation at the ferrocene moiety.[21] A positive signal, as observed for **5**, correlates with a *P*-helical arrangement, as was previously shown by Hirao and coworkers for derivatives **1**.[8,9] We expect that scission of the hydrogen bonds would result in rotation




**Scheme 1** The parallel (**1**) *vs.* anti-parallel (**2**) orientation in ferrocene amino acid conjugates is indicated by arrows. Arrows point from *C*- to *N*-terminus of the peptide.




**Scheme 2** Synthesis of the tetrapeptide Boc-Ala-Fca-Ala-Ala-OMe **5**.

† Electronic supplementary information (ESI) available: experimental details, full analytical data, plot of the cyclic voltammogram, plot of crystal packing. See http://www.rsc.org/suppdata/cc/b4/b407771g/

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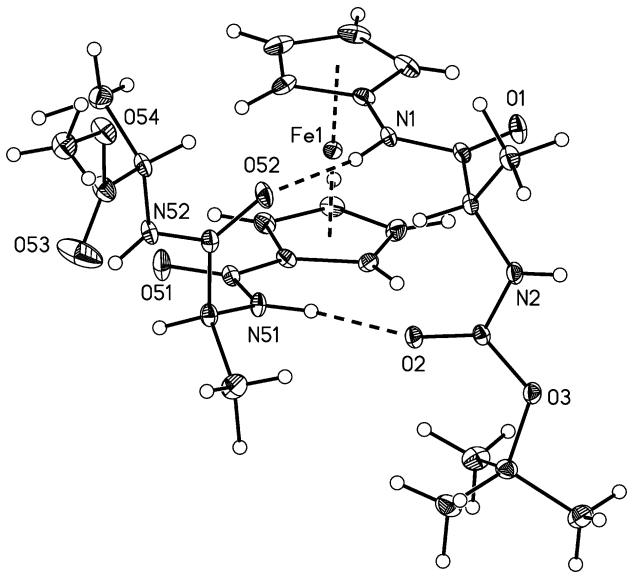


**Fig. 1** ORTEP plot of the X-ray single crystal structure of **5** (50% probability) with intra-molecular hydrogen bonds. Selected hydrogen bond distances (Å) and angles(°): N1–O52 2.812(3), N51–O2 2.914(3), N1–H1N–O52 169(3), H51–H51N–O2 157(3).

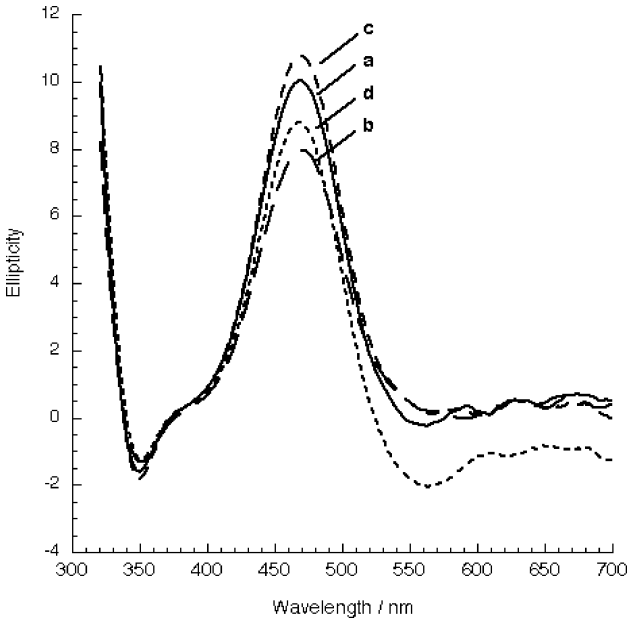


**Fig. 2** CD spectra (1 mM) of **5** at room temperature in $CH_3CN$ (a), at 50 °C (b), at room temperature after heating to 50 °C for one hour (c), and 24 h after addition of 20% (v/v) of MeOH to the $CH_3CN$ solution (d, the baseline for this spectrum is slightly shifted because it originates from a different set of measurements).

of the two Cp rings and hence loss of the CD signal at 480 nm. Upon heating to 50 °C, there is a decrease in intensity in this band of only 20%, which is regained upon cooling to room temperature (spectra b and c in Fig. 2). Even addition of 20% of MeOH to the $CH_3CN$ solution does not result in cleavage of the hydrogen bonds even after 24 h (spectrum d). In NMR spectroscopy of proteins, this is considered a strong criterion for a very stable hydrogen bond.[22] Obviously, the two intra-molecular hydrogen bonds form a very stable conformation.

In conclusion, we have prepared and characterized the first oligopeptide derivative derived from the unnatural amino acid 1′-aminoferrocene-1-carboxylic acid Fca and L-alanine. Typical peptide coupling reactions are possible at the *C*- as well as the *N*-terminus of Fca. A solid state structure of the tetrapeptide **5** confirms that i) a turn structure is induced by Fca, ii) an *anti-parallel* orientation of the two peptide strands persists, which is iii) stabilized by two intra-molecular hydrogen bonds, resulting in a *P*-helical conformation of the metallocene. Compound **5** is not only one of the few real organometallic amino acids.[23–25] Unlike all previous metallocene turn structures, it is also a truly organometallic turn mimetic. As such it is capable of replacing natural amino acids in turn structures while maintaining an *anti-parallel* orientation of the peptide strands. In addition, it offers the possibility of including an electrochemically active group into the peptide backbone. Further synthetic and structural investigations into Fca conjugates with amino acids are in progress in our groups.

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## Notes and references

‡ The usual nomenclature for amino acids and peptides is applied for this new amino acid.

§ *Crystal data for* **5**: Bruker SMART-CCD diffractometer, SHELX-97 programs. Mo-$K_\alpha$ radiation ($\lambda$ = 0.71073 Å). $C_{31}H_{48}FeN_4O_7$, $M$ = 644.58, orthorhombic, space group $P2_12_12_1$, $a$ = 11.2239(5) Å, $b$ = 14.8020(7) Å, $c$ = 18.6314(8) Å, $V$ = 3095.3(2) Å$^3$, $Z$ = 4, $T$ = 103(2) K, $\mu$(Mo-$K_\alpha$) = 0.540 mm$^{-1}$, 27588 reflections measured, 9445 unique ($R_{int}$ = 0.0484) which were used in all calculations. Hydrogen atoms except some of the methyl groups were located and refined isotropically. 445 parameters, no restraints. $R1$ = 0.0461 (all data) and $wR2(F^2)$ = 0.1174 (all data). Flack parameter is −0.03(1), in agreement with the absolute configuration (L) of the amino acids used. The compound crystallizes with a solvent molecule (probably pentane), which is severely disordered and could not be refined satisfactorily. The data were therefore corrected using the SQUEEZE routine in PLATON (A. L. Spek, see CIF file). CCDC 239828. See http://www.rsc.org/suppdata/cc/b4/b407771g/ for crystallographic data in .cif or other electronic format.

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