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Ferrocene-dipeptide conjugates derived from aminoferrocene and 1-acetyl-1′-aminoferrocene: synthesis and conformational studies

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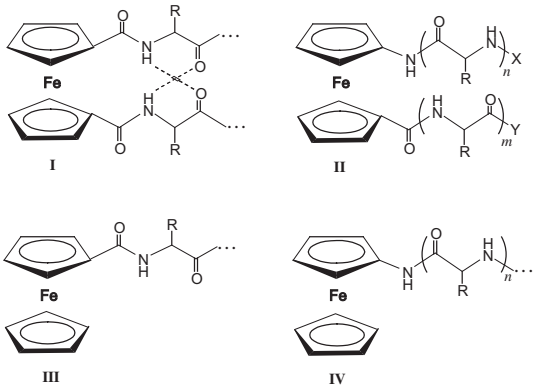
ABSTRACT

In this study we present the synthesis and conformational analysis of mono- and disubstituted ferrocene bioconjugates bearing dipeptide chains (Boc-AA-AA-Fn-X, AA=Gly, L-Ala, L-Val). The conformational preferences of novel aminoferrocene derived conjugates with X=H, as well as their 1-acetyl analogues (X=COMe), were investigated by spectroscopic techniques (IR, NMR and CD) and corroborated by DFT calculations. Chirally organized structures, stabilized through intrachain hydrogen bonds, prevail in solution when X=H. The resulting 10-membered hydrogen-bond ring is destabilized by heteroannular introduction of an acetyl group when X=COMe.

1. Introduction

Turns are common structural features of peptides and proteins, involving three (γ-turn) or four (β-turn) consecutive residues stabilized by intramolecular hydrogen bonds (IHBs), playing a critical role in protein folding.[1] Molecular scaffolds composed of short peptides have attracted much attention in design of peptide secondary structure mimetics applicable in pharmacology, asymmetric catalysis and molecular biotechnology.[2–6] In this context, ferrocenes are recognized as molecular templates with the potential to mimic natural turn inducers in proteins (e.g., Proline, Pro) as the almost free rotating cyclopentadienyl rings are separated by about 3.3 Å, which is an appropriate distance for intramolecular hydrogen bonding between podand peptide chains.[2,5]

Bioconjugates of ferrocene-1,1′-dicarboxylic acid (Fcd, **I**)[6–11] and 1′-aminoferrocene-1-carboxylic acid (Fca, **II**)[12–18] with various L-, D- and β-amino acids have been thoroughly studied (Scheme 1). Detailed structural analysis of type **I** conjugates revealed the presence of two strong IHBs forming 10-membered rings (β-turn) in the solid state as well as in solution. Our group extensively studied type **II** conjugates and showed that, unlike the previously mentioned Fcd-derivatives characterized by parallel peptide strands, these bioorganometallics are the first truly ferrocene turn mimetics maintaining an antiparallel orientation of the peptide chains. Fca bioconjugates of type **II** can be divided into two subgroups with distinct conformational preferences. Desymmetrized



Scheme 1. Peptidomimetics derived from ferrocene-1,1′-dicarboxylic acid (**I**), 1′-aminoferrocene-1-carboxylic acid (**II**—constructed from various L- and D-amino acids, *m*, *n*=0, 1, 2…; X=Ac, Boc; Y=OMe, Me), ferrocenecarboxylic acid (**III**) and aminoferrocene (**IV**).

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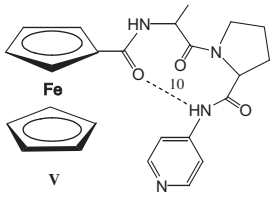
bisamides composed of Fca and a single α-amino acid attached at the *C*-terminal side of Fca, i.e., 'short' oligoamides, form a library of several conformational isomers in solution. Symmetrically substituted polyamides (Scheme 1, n=m=1,2) with Fca located in between two α-amino acids, i.e., 'long' oligoamides, are well described by a single conformer, which is highly stabilized by two IHBs forming 9- and 11-membered rings in solution as well as in the solid state.

An intrinsic property of both type **I** and type **II** peptidomimetics is the helical chirality around the ferrocene moiety, which has been amply demonstrated by X-ray crystallographic analyses. The chiral arrangement of the ferrocene core arises from intramolecular hydrogen bonding between the two podand chiral peptide chains.[19] CD spectroscopy is the method of choice to determine the helical chirality of the ferrocene unit in such systems in solution. Fcd bioconjugates of type **I** containing L-amino acids are characterized in the solid state by two IHBs resulting in *P*-helicity of the ferrocene subunit. Strong positive Cotton effects ($M_\theta \sim 5000$ deg cm^2 dmol^{-1}) around the UV/Vis absorption maximum of the ferrocene chromophore ($\lambda_{max} \approx 455$ nm) verify that same chiral arrangement is maintained in solutions of non-coordinating solvents. In contrast, bioorganometallics derived from D-amino acids are characterized by negative CD signals and *M*-helicity. In the case of type **II** 'long' oligoamides, strong CD absorptions ($M_\theta \sim 10{,}000$ deg cm^2 dmol^{-1}) revealed right-handed conformation of the ferrocene unit when L-amino acids were used and vice versa. In type **II** conjugates composed of D-Ala and L-Ala the metallocene core chirality is determined by the configuration of the first amino acid attached to the Fca amino group.[13] As a result of interconverting energetically accessible conformations of *P*- and *M*-helicity, the chirooptical properties of 'short' oligoamides cannot be easily described because increasing size of the amino acid side chain or even the small changes in solvent polarity can promote some conformers over others and reverse the sign of Cotton effect. In all cases the intensity of CD signal is about one order of magnitude lower than those for 'long' oligoamides ranging from M_θ=−1000 to +1000 deg cm^2 dmol^{-1}.

In contrast with the previously described 1,1′-disubstituted conjugates, ferrocenes bearing only one peptide chain (**III**, **IV**) exhibit different conformational preferences mainly driven by intermolecular hydrogen bonding. Generally, a highly organized assembly, sometimes ordered in a helical arrangement[20] is usually observed in the solid state.[21] The first example of monosubstituted Fc-peptide conjugate containing β-turn structure was presented by Kraatz and co-workers.[22] X-ray crystallographic analysis of type **III** conjugate bearing tripeptide chain (-L-Pro-L-Pro-L-Phe-OH) revealed a strong intramolecular hydrogen bond between C=O (adjacent to the ferrocene unit) and NH_{Phe}. Recently Hirao[23] found that introduction of minimum-sized heterochiral peptide chain (-L-Ala-D-Pro-NH-4-Py or -D-Ala-L-Pro-NH-4-Py) into type **III** conjugate led to the formation of a β-turn-like structure (Scheme 2). Detailed spectroscopic analysis using NMR, FTIR and CD techniques revealed that a chirally organized structure achieved via intramolecular hydrogen bonding is likely to be preserved in solution of these conjugates.



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Scheme 2. β-turn found in ferrocene peptide conjugates bearing a one dipeptide chain of the heterochiral sequence (-L-Ala-D-Pro-NH-4-Py or -D-Ala-L-Pro-NH-4-Py).

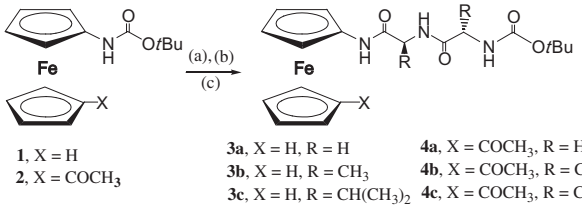
So far, there is only one paper dealing with the conformational preferences of bioconjugates derived from aminoferrocene and natural amino acids (**IV**, n=1, AA=Gly, Leu, Phe, Val, Cys, Tyr).[24] An intermolecular H-bonding network connects molecules of Boc-Gly-NH-Fc in the solid state resulting in the formation of linear chains. ^{1}H NMR spectroscopic study of these compounds suggests the absence of strong intramolecular hydrogen bonds in solution. In addition, the CD spectra of chiral derivatives display only weak signals originating from metal-centred transitions.

The scarce literature date available for type **IV** conjugates, particularly minimum-sized peptide derivatives with the ability to form stable intrachain hydrogen bonds, prompted us to investigate the conformational preferences in Boc-AA_2-AA_1-NH-Fc ($AA_{1,2}$=Gly, Ala, Val) dipeptides. The synthesis of previously reported Boc-$(Ala)_2$-NH-Fc is included here for comparison.[13] To examine the influence of an additional hydrogen-bond accepting group on the conformational properties of subclass **IV**, we extended our work on peptides of type **II** (n=2, AA=Gly, Ala, Val, m=0, Y=Me). To accomplish this task a combined experimental and theoretical investigation has been performed with CD, IR and NMR spectroscopic techniques in combination with DFT calculations.

2. Results and discussion

2.1. Synthesis of ferrocene derivatives

N-Boc-protected aminoferrocene (**1**) and 1-acetyl-1′-aminoferrocene (**2**) were prepared by previously described procedures.[13,25] Deprotection of **1** and **2** was performed by action of gaseous hydrochloric acid in ethyl acetate. The resulting amine hydrochlorides were treated with an excess of triethylamine to give free amines. Boc-AA_2-AA_1-OH ($AA_{1,2}$=Gly, Ala or Val) were activated using HOBt/EDC protocol [HOBt=1-hydroxybenzo triazole, EDC=*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride] and coupled to free amines giving **3a** (91%), **3b** (81%), **3c** (75%), **4a** (62%), **4b** (64%) and **4c** (81%), respectively (Scheme 3).



1, X = H
2, X = $COCH_3$

3a, X = H, R = H
3b, X = H, R = CH_3
3c, X = H, R = $CH(CH_3)_2$

4a, X = $COCH_3$, R = H
4b, X = $COCH_3$, R = CH_3
4c, X = $COCH_3$, R = $CH(CH_3)_2$

Scheme 3. Syntheses of Fca peptides: (a) $HCl_{(g)}$/EtOAc, (b) NEt_3, CH_2Cl_2, (c) 1. HOBt/EDC, CH_2Cl_2, 2. Boc-AA-AA-OH, CH_2Cl_2.

2.2. IR, NMR and CD studies

Due to their flexibility, most linear peptides are usually present in solution as ensembles containing a large number of different conformers including hydrogen bonded and hydrogen bond free structures. To establish the conformational preferences of the ferrocene-containing dipeptides in solution we undertook IR, NMR and CD spectroscopic study. The solution IR spectra of compounds **3a**–**c** and **4a**–**c** display signals due to hydrogen-bonded NH groups below 3400 cm^{-1} together with signals above 3440 cm^{-1} belonging to free NH groups (Table 1). Since no significant change in the integrated areas of these two bands was seen over a concentration range of 50 to 0.5 mM, the possibility of intermolecular hydrogen bonds can be ruled out (Fig. 1). Furthermore, almost equal intensity of free and hydrogen-bonded NH bands suggests the approximately same population of free and bonded NH groups. The IR spectra in