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1′-Acetylferrocene amino acid esters and amides. A simple model for parallel β-helical peptides

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ABSTRACT

In this study we present a synthesis and conformational analysis of 1′-acetylferrocene amino acid derivatives of type Ac–Fn–CO–AA–Y (Fn=ferrocene-1,1-diyl; AA=Gly, Ala or Val; Y=OMe or NHMe) as a simple model for parallel β-helical peptides. Derivatives with only one amino acid adopt a reduced number of total conformations and allow a more exact analysis of intramolecular hydrogen bonds (IHB) close to the ferrocene unit. Conformational analysis of these bioconjugates was performed by a combination of spectroscopic techniques (IR, NMR and CD) and corroborated by solution-phase DFT calculations. The investigation of ester conjugates **1**–**3** indicates the coexistence of non-bonded (an open forms) and hydrogen bonded NH_a group forming a 7-membered ring (γ-turn). The amide derivatives **4**–**6** with an additional NH_b hydrogen bond donor are mostly constituted of conformers with a 10-membered ring (β-turn) as a single IHB pattern or the β-turn accompanied by a 7-membered ring (γ-turn) containing NH_a group. The exchange of the amino acid side-chain does not significantly affect the conformational properties and IHB pattern of the studied conjugates **1**–**6**.

1. Introduction

A great number of ferrocene–peptide bioconjugates have been prepared to mimic highly ordered conformations of peptides and develop new biomaterials. Ferrocene is well suited for a peptidomimetic. Due to the specific distance between the two cyclopentadienyl rings (ca. 3.3 Å) and their inherent flexibility to rotate relative to each other, ferrocene is a reliable organometallic scaffold for construction of ordered structures via intramolecular hydrogen bonds (IHB) between the two peptide chains linked to these rings. These compounds might adopt structures similar to those in natural peptides mimicking natural peptide turns, but with ferrocene acting as a turn inducer. Furthermore, the ferrocene nucleus in these bioconjugates is able to act as a redox centre and an electrophore in receptors and biomaterials.[1,2]

Symmetrically substituted bioconjugates of ferrocene-1,1′-dicarboxylic acid [(Fcd), type I, Fig. 1] have been extensively studied by Herrick, Hirao, Kraatz and others.[3] The first symmetrical bioconjugates of ferrocene-1,1′-diamine [(Fcda), type II, Fig. 1] were reported by Kraatz.[4] Ferrocene-1,1′-dicarboxylic acid and ferrocene-1,1′-diamine have been used as turn-inducing scaffolds in the

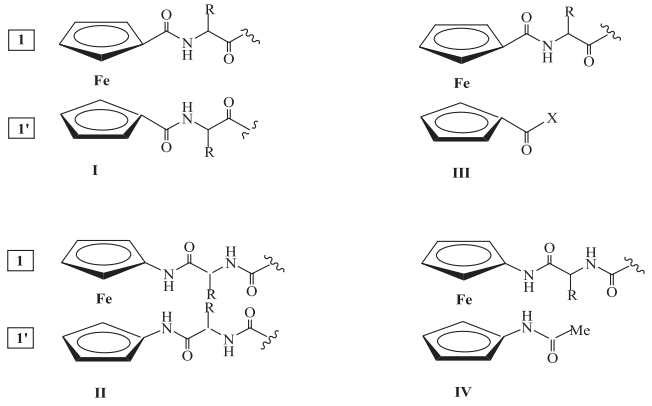

Fig. 1. Ferrocene-containing symmetrical double-strand oligopeptides (**I**,**II**) and desymmetrized single-strand oligopeptides **III** (X=Me, NHMe) and **IV**.

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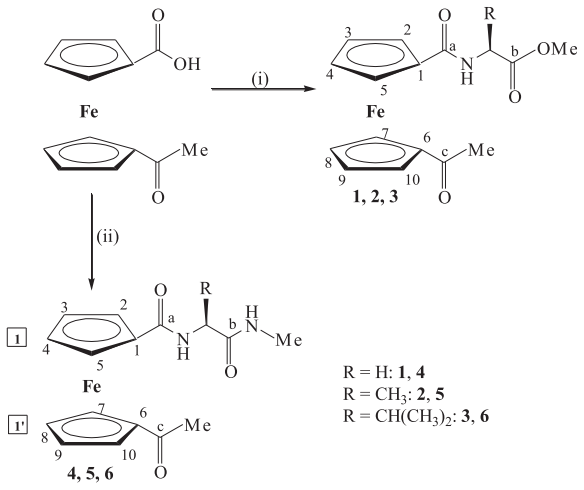
synthesis of conjugates with the parallel orientation of peptide chains. Two very strong IHB between two amino acid substituents at each cyclopentadienyl ring are typical for these bioconjugates. For example, systems derived from ferrocene-1,1′-dicarboxylic acid resulted in symmetrically substituted bioconjugates characterized by 2 equiv 10-membered IHB forming 'Herrick conformation', i.e., β-turns. Alternative arrangements are also possible. For example, a single H-bonding interaction between the substituents is also known as a γ-turn or 'van Staveren conformation'.[3] The absence of any H-bonding interaction between the substituents is usually described as the open form or 'Xu-conformation'.[3] In the ferrocene-1,1′-diamine conjugates two IHB forming a 14-membered ring usually connect the peptide strands.[4]

The combined use of CD, NMR and IR spectroscopy, together with molecular modelling, have already proved valuable in the research of hydrogen bonding interactions in desymmetrized derivatives synthesized from ferrocene-1,1′-dicarboxylic acid and ferrocene-1,1′-diamine with only one amino acid substituent.[5a,b] Conformational analysis of desymmetrized derivatives MeNHCO–Fn–CO–AA–Y (AA=Gly, L-Ala and L-Val, Y=OMe, NHMe, type III, Fig. 1) elucidated individual conformational preferences in solution with respect to the size of the amino acid side-chains. It was shown that solutions of these compounds in weakly coordinating solvents (like CH_2Cl_2) contained an ensemble of conformers without any preference for a specific one. However, an increase in the steric demand of the amino acid side-chain resulted in a smaller number of conformers.[5a]

In weakly coordinating solvents, as well as in DMSO solution, conjugates Boc–AA–NH–Fn–NHAc (AA=Gly, L-Ala and L-Val, type IV, Fig. 1) are described as an ensemble of conformers with IHB involving the NH groups nearest to the ferrocene unit.[5b] The steric demand of the amino acid side-chain in these conjugates barely affects the number of energetically accessible conformations. In our recent studies on desymmetrized conjugates of ferrocene-1,1′-dicarboxylic acid and amino acids/peptides, we have performed conformational analyses of compounds MeOC–Fn–CO–$(AA)_2$–OMe (AA=Gly; L-Ala, type III, Fig. 1).[5c] X-ray analysis demonstrated that self-assembly processes (including N–H···O, C–H···O hydrogen bonds and π···π interactions) prevailed in the Ala conjugate in the solid state. A thorough spectroscopic investigation of these bioconjugates in nonpolar solvents (CH_2Cl_2, $CDCl_3$) and in DMSO-d_6 demonstrated that formation of intra- and interchain weak and medium IHB prevailed. These IHB included the amide nitrogen remote from Fc as the hydrogen bond donor. The oxygen atom from the 1-FcCO or 1′-FcCO groups, and/or the nitrogen atom near to the Fc acted as the hydrogen bond acceptor. These conformers were also accompanied by open forms. The same experiments revealed that amide proton NH_a was not involved in hydrogen bonds. All of the experimental findings were corroborated by DFT calculations. CD spectroscopy of all desymmetrized chiral derivatives revealed that (*P*)-helical conformations predominate in solution.[5c] Furthermore, the change in the number of potential hydrogen bond donor/acceptor atoms considerably changed the conformers' distribution of MeOC–Fn–CO–AA–OMe when compared with MeNH–CO–Fn–CO–AA–NHMe.

In the present study we decided to investigate simplified peptidomimetics with a reduced number of hydrogen bond donors and acceptors **1**–**6** shown in Scheme 1. The main focus was on the IHB that might be formed in the immediate proximity of the ferrocene unit between the hydrogen bond donor and acceptor atoms of the substituents connected to the cyclopentadienyl rings, thus more realistically mimicking the turns observed in natural peptides. In these bioconjugates, the 1′-acetyl and amino acid ester carbonyl group in **1**–**6** are potential hydrogen bond acceptors, and one (**1**–**3**) or two (**4**–**6**) amido groups are potential hydrogen bond donors and/or acceptors. A combined spectroscopic (IR, NMR CD) and computational study was performed in order to shed more light on the conformational preferences in solution with respect to steric demand of the amino acid side-chain together with the decreasing of the number of NH groups in these 'simplified' models.



Scheme 1. Synthesis of ferrocene amino esters and amides: (i) 1. HOBt/EDC, CH_2Cl_2, 2. L-AA–OMe·HCl, NEt_3, CH_2Cl_2; (ii) 1. HOBt/EDC, CH_2Cl_2, 2. L-AA–NHMe·HCl, NEt_3, CH_2Cl_2. The atom enumeration is shown for each reaction product.

2. Results and discussion

The syntheses of esters **1**–**3** and amides **4**–**6** are depicted in Scheme 1. The starting compound 1′-acetylferrocene-1-carboxylic acid was prepared according to a previously described procedure.[6] Activation of the acid with HOBt/EDC (HOBt=1-hydroxybenzo-triazole, EDC=*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydro-chloride) in CH_2Cl_2 and coupling with Gly–OMe, L-Ala–OMe, and L-Val–OMe (obtained from AA–OMe·HCl by treatment with NEt_3) resulted in the formation of esters **1**–**3** (59–65%). Coupling of AA–NHMe (AA=Gly, L-Ala, L-Val) to 1′-acetylferrocene-1-carboxylic acid using the HOBt/EDC procedure resulted in the formation of the desired amides **4**–**6** (50–57%).

Hydrogen bonding is one of the major factors that governs the overall structure and functionality of organic and biomolecules, playing also an important role in molecular recognition processes and interactions between molecules. A combined use of experimental (IR, NMR and CD spectroscopy) and theoretical methods (DFT calculations) can provide a wealth of information on hydrogen bonding and molecular structures in solution of ferrocene–peptide bioconjugates.

The position of absorption band above 3400 cm^{-1} corresponds to the non-hydrogen bonded NH functionalities while a signal at lower values (below 3400 cm^{-1}) indicates the presence of intra- or intermolecular hydrogen bonded species.[5,7] Absorptions of NH stretching vibrations in the IR spectra of esters **1**–**3** measured in KBr are found around 3300 cm^{-1} providing evidence of hydrogen bonding interactions. The additional band around 3450 cm^{-1} shows the presence of non-bonded NH groups (Table 1). It was observed that an increase in steric hindrance in derivatives with the bulkier amino acid side-chain increases the intensity of absorption band corresponding to the associated NH groups. The IR spectra of amides **4**–**6** measured in KBr were characterized by the following bands: 3496, 3379, 3347 cm^{-1} for **4**, 3496, 3304 cm^{-1} for **5** and 3295 cm^{-1} for **6** (Table 1), respectively. A much larger contribution of associated NH groups in the amides **4**–**6** than in the esters **1**–**3** was observed. The IR spectra of esters **1**–**3** and amides **4**–**6** were also measured in CH_2Cl_2 solution ($c \approx 10^{-2}$ M) (Table 1). A