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# Incorporation of the Unnatural Organometallic Amino Acid 1′-Aminoferrocene-1-carboxylic Acid (Fca) into Oligopeptides by a Combination of Fmoc and Boc Solid-Phase Synthetic Methods

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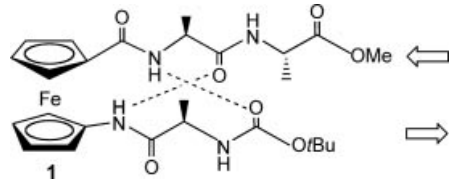
The organometallic amino acid 1′-aminoferrocene-1-carboxylic acid (Fca) was incorporated internally into a peptide sequence by solid-phase methods combining natural Fmoc-protected amino acids and Boc-Fca-OH to give the pentapeptide Boc-Fca-Ala-Gly-Val-Leu-$NH_2$ (**2**) and the octapeptide Ac-Val-Gly-Ala-Fca-Ala-Gly-Val-Leu-$NH_2$ (**3**). Compound **3** was found to have a helically ordered structure by NMR and CD spectroscopy, which is stabilized by intramolecular hydrogen bonding in an antiparallel β-sheet-like arrangement.


Turn structures are ubiquitous secondary structural elements in proteins that have become important targets in medicinal chemistry.[1–4] Metal complexes, in particular ferrocene, may serve as turn mimetics in peptides.[5] The use of ferrocene-1,1′-dicarboxylic acid as a turn inducer was described by several groups, particularly those of Herrick, Hirao and Kraatz.[6–14] Ferrocene derivatives bearing *parallel* podand peptide strands were shown to induce helically ordered structures which are controlled by strong intramolecular hydrogen bonds as shown in the solid state by X-ray crystallography studies as well as in solution by IR, $^1$H NMR and CD spectroscopy.[7–10,15,16]

In previous work we investigated the role of 1′-aminoferrocene-1-carboxylic acid (Fca) as a turn mimetic in the tetrapeptide Boc-Ala-Fca-Ala-Ala-OMe (**1**) which contains *antiparallel* peptide strands stabilized by two intramolecular hydrogen bonds in both, solution and solid phases (Scheme 1).[17] All derivatives of ferrocene-1,1′-dicarboxylic acid and Fca so far were prepared by stepwise synthesis in solution. Solid-phase peptide synthesis (SPPS) using orthogonal protecting groups and linkers is the standard method for the synthesis of small to medium-sized peptides. Twenty years ago, a few papers were published dealing with the application of SPPS to metallocenes. Ferrocenylalanine (Fer) and cymantrenylalanine (Cym) were incorporated into biologically active peptides (enkephalin, bradykinin and substance P). Most work was carrid out on enkephalin which was transformed into [Fer$^4$,Leu$^5$] or [Cym$^4$,Leu$^5$] analogues by replacement of the phenylalanine side chain (Phe$^4$) with Fer or Cym, thus preserving the aromatic character of this residue.[18–21] It is worth to mention SPPS of a "ferrocenophanic" conformationally constrained analogue of substance P.[22] Very recently, SPPS of *N*-terminated cobaltocenium-NLS peptides[23] and metallocenoyl (M = Fe, Co) tripeptides[24] with antibacterial activity were described by one of us. In all peptides described up to the present day, the organometallic group is part of the side chain or linked to the *N*-terminus. We therefore set out to incorporate a metallocene such as Fca into the peptide backbone by SPPS. In this paper we describe for the first time an SPPS procedure for constitutive insertion of a ferrocene derivative into the peptide chain back bone.




Scheme 1. The antiparallel orientation of peptide strands in tetrapeptide **1** is induced by the unnatural amino acid 1′-aminoferrocene-1-carboxylic acid (Fca) and stabilized by intramolecular hydrogen bonds.

In recent years, the fluorenylmethoxycarbonyl (Fmoc) group has largely replaced the original Merrifield *tert*-butoxycarbonyl (Boc) group as the temporary *N*-terminal protecting group because it may be easily removed under mild basic conditions. Boc removal, on the other hand, requires strong acid which is incompatible with sensitive com-

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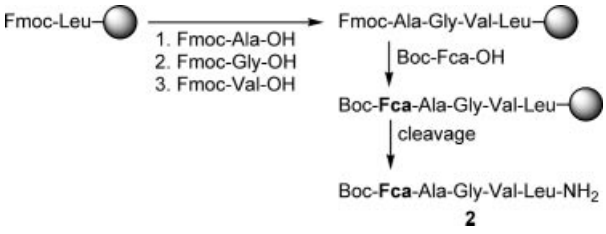
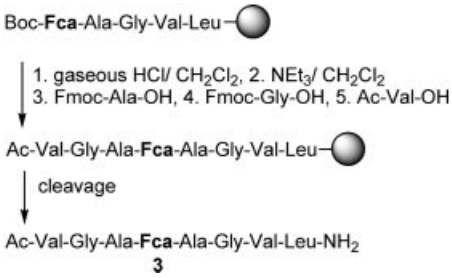
pounds, for instance some organometallic complexes.[25,26] For this reason, we originally planned to synthesize Fca-containing oligopeptides according to the standard Fmoc approach.[27] However, *N*-Fmoc-Fca-terminated oligopeptides turned out to be very unstable. Therefore, a combination of Boc-Fca-OH and Fmoc-Aaa-OH had to be chosen, thereby reintroducing the classical Merrifield method to the synthesis of Fca-containing peptides by solid-phase synthesis.

Pentapeptide **2** was obtained by standard Fmoc SPPS, starting from Fmoc-Leu loaded TentaGel S resin with the base-labile HMB linker, Fmoc-Val-OH, Fmoc-Gly-OH and Fmoc-Ala-OH.[27] Subsequent coupling to Boc-Fca-OH, cleavage from the resin by concentrated ammonia in methanol and thin layer chromatography (TLC) purification gave Boc-Fca-Ala-Gly-Val-Leu-$NH_2$ (**2**) (Scheme 2).




Scheme 2. Solid-phase synthesis of the Fca-containing pentapeptide **2**.

Octapeptide **3** was prepared starting from pentapeptide **2** attached to the solid support. The temporary Boc-protecting group masking the α-amino group of Fca was removed by gaseous HCl in $CH_2Cl_2$ during 1 h. The resulting ammonium salt was converted by the action of 10% $NEt_3/CH_2Cl_2$ into the corresponding amino species which was coupled with Fmoc-Ala-OH, Fmoc-Gly-OH and Ac-Val-OH to give octapeptide Ac-Val-Gly-Ala-Fca-Ala-Gly-Val-Leu-$NH_2$ (**3**) (Scheme 3). The product was cleaved from the resin by concd. $NH_3$ in MeOH and purified by TLC. The yields of both compounds after TLC purification (14% for **2** and 23% for **3**, respectively) were in the expected range for SPPS procedures, given that no effort was made for optimization in these initial experiments. The purity of both peptides **2** and **3** was confirmed by analytical HPLC (see Supporting Information).




Scheme 3. Solid-phase synthesis of octapeptide **3** with the organometallic amino acid Fca incorporated internally.

Evidence for the proposed composition of **2** and **3** was obtained from ESI-MS where peaks at $m/z$ = 1391.7 $[2 \times M + Na]^+$ (**2**) and $m/z$ = 876.5 $[M + Na]^+$ (**3**) were observed. The $^1H$ NMR spectra confirmed the proposed structures, too. Due to the limited solubility of the metallocene peptides, all spectra were measured in $[D_6]$DMSO (approx. 10 mM), which competes as a hydrogen-bond acceptor. First of all, the correct number of amide proton signals confirms the number of amino acids, i.e. six for **2** and nine for **3**, both including the *C*-terminal amide with the intensity of 2 H. Upon closer inspection, the $^1H$ NMR spectrum of **2** shows two signals at $\delta > 8$ ppm, whereas **3** has five amide signals at $\delta > 8$ ppm. Intermolecular hydrogen bonding is unlikely to occur in DMSO at mM concentrations. Thus, these signals are indicative of relatively strong intramolecular hydrogen bonds between the peptide strands.[14] From this analysis it follows for **2** that a structure similar to the one in Scheme 1, but with a Boc group on the ferrocene amide moiety, is stabilized by only two hydrogen bonds, one being between Gly-NH and the Boc group carbonyl oxygen atom. Much stronger stabilization prevails in **3**, with about five intramolecular hydrogen bonds. This situation is indeed similar to an antiparallel β-sheet-like protein structure.

CD spectra (in DMSO) were recorded in order to further elucidate the hydrogen bonding situation (Figure 1). The strong Cotton effect in the CD spectrum of octapeptide **3** at about 480 nm is evidence for a chiral conformation at the ferrocene core, similar to our results on the tetrapeptide **1**. The strong intramolecular hydrogen bonding is retained even in DMSO, which confirms our NMR analysis above. A positive signal, as observed for **3**, correlates with a (*P*)-helical arrangement as shown by Hirao[9,10] and by us.[14,17] Compound **2** lacks one pendant peptide strand and, as explained above, the secondary structure is far less stabilized. Consequently, the absence of a Cotton effect in the CD spectrum of **2** may be readily explained by the lack of strong intramolecular hydrogen bonding.

In conclusion, we have prepared and characterized two peptides containing the organometallic amino acid Fca as part of the backbone by SPPS, namely pentapeptide **2** and octapeptide **3**. Our method is unique because it combines Fmoc and Boc procedures. The total decomposition of Fmoc-Fca-Ala-Gly-Val-Leu-resin upon removal of the protecting group led us to terminate this peptide with Boc-Fca which is a convenient intermediate either for further elongation of the peptide chain by Fmoc-protected amino acids to yield **3** or for its successfully cleavage from the resin (for **2**). The target molecules of previously reported SPPS with metallocenes were: (*i*) bioconjugates obtained by replacement of one or two Phe "internal" subunits by metallocenylalanine or (metallocene-1,1′-diyl)bis(alanine),[18–22] (*ii*) *N*-metallocenoyl terminated peptide sequences.[23,24] In this paper, we show that it is possible to incorporate the unnatural ferrocene amino acid Fca into the peptide backbone by SPPS. To this end, a suitable combination of Fmoc and Boc SPPS strategies, which are usually seen as orthogonal, had to be adopted. The two cyclopentadienyl rings in ferrocene may rotate almost freely against each other. This gives ferrocene-based turn mimetics a unique degree of structural flexibility compared to all known organic systems. Indeed, we obtained evidence for an antiparallel β-

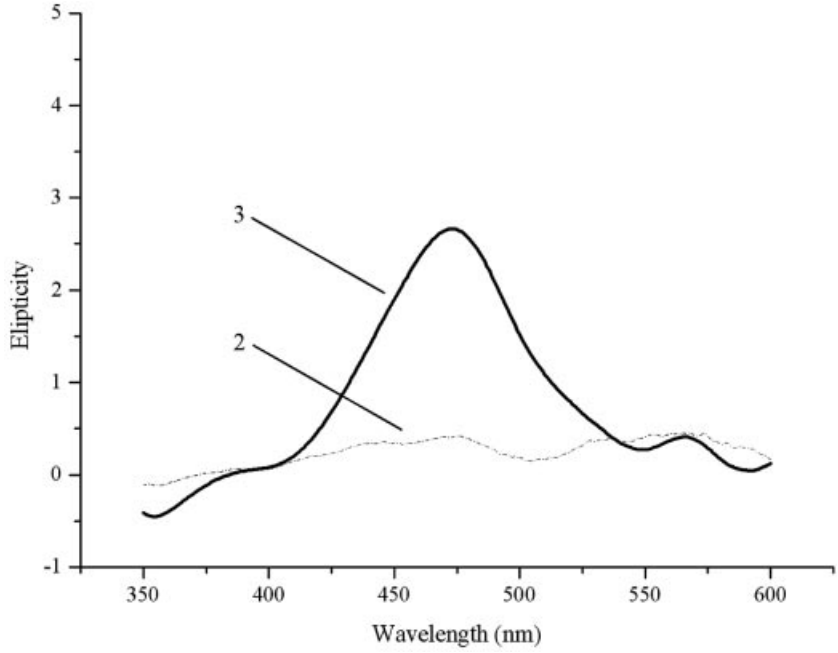



Figure 1. CD spectra of **2** and **3** (1 mM in DMSO).

sheet-like arrangement for the first time with an organometallic turn inducer. Further work in our groups will explore the possibilities inherent in such biomimetic organometallic systems.[28–31]

**Supporting Information** (see footnote on the first page of this article): Synthetic details and spectroscopic data including HPLCs for compounds **2** and **3**.

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