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How Useful Is Ferrocene as a Scaffold for the Design of β-Sheet Foldamers?**

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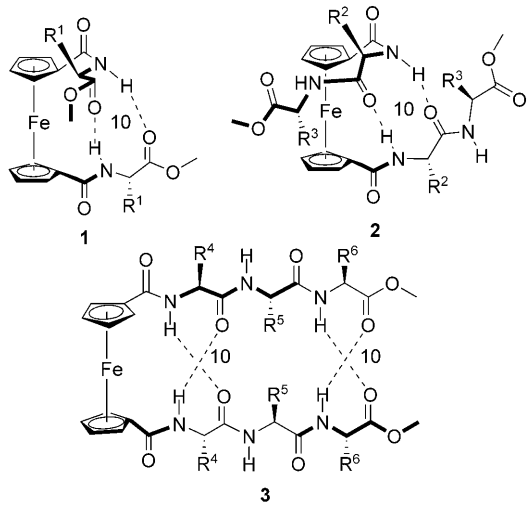
The design of structurally well-defined synthetic peptide foldamers is motivated to some degree by the aim to study mechanisms of protein folding[1] or biochemical processes,[2] to generate molecules with potential biological applications,[3] or to generate novel materials.[4] Foldamers adopting β-sheet-like conformations have held a particular fascination and have tremendous potential as model systems in furthering our understanding of diseases, including Alzheimer's and Huntington's diseases. Non-natural scaffolds have been particularly important for obtaining foldamers with a defined, stable β-sheet-like structure.[5,6] Ferrocene (Fc) has been investigated as a molecular scaffold that supports β-sheet-like interactions between two podant peptide chains that are linked to the two parallel cyclopentadienyl rings.[7] 1,*n*′-disubstituted Fc-conjugates of amino acids exhibit a distinct non-proteinic "cross-strand" H-bonding pattern, in which a 10-membered H-bonded ring is formed (compound **1** in Scheme 1). This result suggests that simple extension might result in a foldamer that possesses an extended β-sheet-like structure. However, extending the two substituents by one amino acid each to form a dipeptide, as illustrated by compound **2** in Scheme 1, shows the two additional amino acids pointing in opposite directions, while the H-bonding pattern of the proximal amino acids is maintained. Modification of the two C termini of the peptide conjugate by a N-heterocycle yields a γ turn motif, in which the peptide strands are engaged in intrastrand H-bonding,[8] thus raising serious concerns of the true value of the Fc scaffold to support an extended β-sheet-like structure.

The problem appears to be with the colinear alignment of the peptide chains and potential steric congestion about the Fc core. Forcing peptides into a specific conformation by cyclization has led to a H-bonding pattern that, although not identical, bears some resemblance to what is observed in proteinic β-sheets, in terms of the formation of H-bonded rings and peptide dihedral angles. On an intermolecular level, H-bonding interactions form associated conjugates into unprecedented supramolecular assemblies.[9] The key to this success was the use of glycine (Gly) as a flexible amino acid that can accommodate a wide range of dihedral angles proximal to the Fc core. Based on these results, we hypothesized that if Gly is chosen as proximal amino acid followed by an amino acid with high β-sheet propensity, such as valine (Val) or isoleucine (Ile),[10] it might be possible to form acyclic Fc-peptide foldamers that support extended H-bonding interactions between the two pendant peptide substitutents (**3** in Scheme 1). Based on these considerations, we selected the sequences Gly-Val-Cys (Cys = cysteine) and Gly-Ile-Cys and coupled them to 1,1′-Fc-dicarboxylic acid through the *N*-terminal Gly residues. Herein, the results of our investigations are presented, which demonstrate that these foldamers exhibit a stable H-bonding interaction with an alternating β-sheet-like conformation of the peptide backbone.



Scheme 1. H-bonding and conformations of disubstituted ferrocene-peptide conjugates with the different lengths of peptide strands.

Suitably protected dipeptides, Boc-Val-Cys(Bn)-OMe (**4**) (Boc = *tert*-butoxycarbonyl, Bn = benzyl) and Boc-Ile-Cys(Bn)-OMe (**6**) and tripeptides Boc-Gly-Val-Cys(Bn)-OMe (**5**) and Boc-Gly-Ile-Cys(Bn)-OMe (**7**) were synthesized by the standard solution peptide-coupling method using HBTU as a coupling reagent. The target compounds Fc[CO-Gly-Val-Cys(Bn)-OMe]$_2$ (**8**) and Fc[CO-Gly-Ile-Cys(Bn)-OMe)]$_2$ (**9**) were synthesized by coupling the corresponding tripeptides to ferrocenedicarboxylic acid and characterized

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spectroscopically (see Supporting Information). The ^{1}H NMR spectra of compounds **8** and **9** in $CDCl_3$ show the Gly and Cys amide NH proton resonance signals downfield from that of the Val residue in **8** (Ile in compound **9**). Importantly, the chemical shifts of the amide NH proton signals of Gly and Cys in both compounds are concentration-independent, whereas those of Val and Ile are concentration-dependent, suggesting the involvement of these amide protons in intermolecular H-bonding (Figure 1), which is confirmed by single crystal X-ray structural studies of the two compounds (see below).

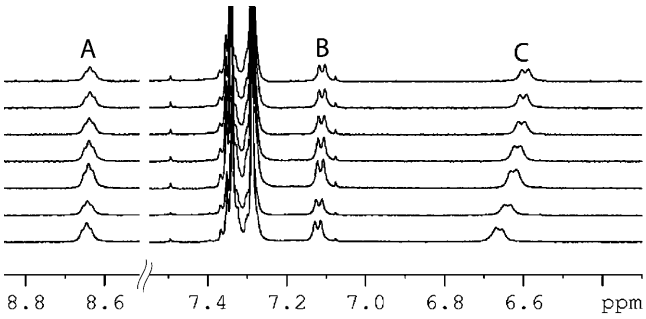

Figure 1. ^{1}H NMR spectra in $CDCl_3$ solution of compound **8** showing the amide regions. Concentration of solutions (mM) from bottom to top; 2.00, 1.60, 1.33, 1.14, 1.00, 0.98 and 0.80. Peak A corresponds to Gly-NH, B to Cys-NH and C to Val-NH.

Circular dichroism (CD) spectroscopy has been useful in evaluating the axial stereochemistry of the Fc group in Fc conjugates. A positive Cotton effect of the Fc centered transition indicates *P*-helicity, whereas a negative Cotton effect indicates *M*-helicity.[7c] The CD spectra of compounds **8** and **9** recorded in MeCN solution are presented in Figure 2, clearly showing a positive Cotton effect for the Fc-based transition at around 480 nm, which is an indication of a rigid *P*-helical structure about the Fc core. The negative signal around 225 nm indicates the presence of a sheet-like conformation in both compounds.

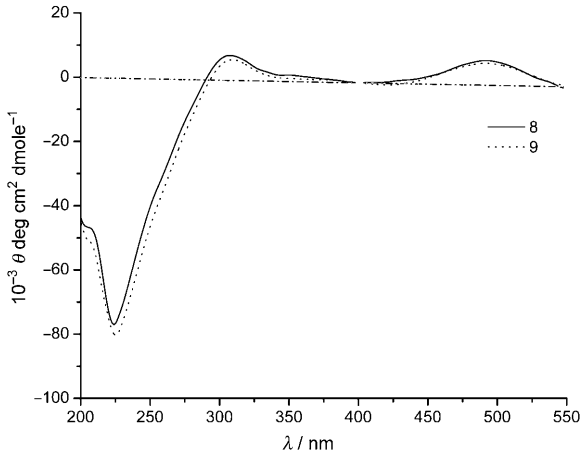

Figure 2. CD spectra of compounds **8** and **9** in CH_3CN solution at 25 °C, showing the presence of *P*-helical arrangement of the ferrocene core and the signature of β-sheet conformations of the two peptide substitutents.

These results were supported by two single-crystal X-ray diffraction studies of compounds **8** and **9**. Suitable crystals were obtained by slow solvent evaporation from chloroform at 0 °C. Both compounds crystallize in the space group $P2_12_12_1$. The molecular structures are shown in Figure 3.

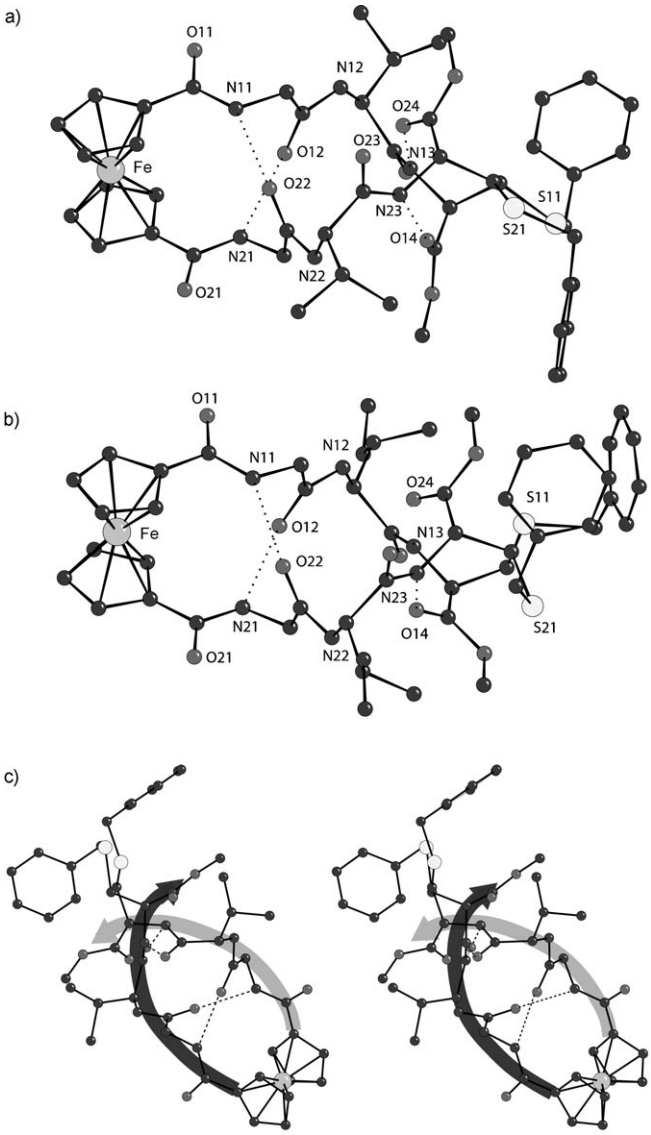

Figure 3. Molecular structures of a) compound **8** and b) compound **9** as obtained from single crystal X-ray diffraction studies, showing the extended sheet-like conformation. Hydrogen atoms are omitted for clarity. c) Stereoview of compound **8,** highlighting the two peptide backbones.

The crystal structures of compound **8** and **9** exhibit a Herrick-type H-bonding pattern, which is typical for 1,*n*′-Fc-peptide conjugates.[7a,c] The two peptide chains do not diverge but are aligned parallel with respect to each other, allowing for additional H-bonding interactions between the amide NH of Cys on one strand with the ester C=O of the other strand (see Figure 3 and Supporting Information). The H-bond lengths involving the Gly-NHs are shorter, which is compatible with the downfield shift of the NH resonances in the

^{1}H NMR spectrum. The longer H-bonding interactions involving the Cys-NH may be due to the steric repulsion between the bulky protecting groups on Cys. It is important to note that ROESY studies clearly show that the solid state structures are maintained in solution ($CDCl_3$). Thus for compound **8**, ROE correlations are observed between protons of the ester OMe and the Val-CH_3, the ester OMe and the phenyl Hs, and between the β-H of Cys and the phenyl Hs (see Figure S11 and Figure S12 in Supporting Information). These ROE signals correspond well with the solid state structure. The distance between two S atoms in a single molecule are 6.059 Å and 5.255 Å for **8** and **9**, respectively, which is outside the sum of the van der Waals radii of sulfur (1.85 Å).

A second set of H-bonding contacts exist that link molecules on an intermolecular level, which again matches the findings in the solution ^{1}H NMR spectroscopy. Each molecule is linked to two other molecules through intermolecular H-bonding (O11···N22* = 2.869 Å, and N12···O21** = 3.064(4) Å in **8** and O11···N22* = 2.926(4) Å and N12···O21** = 2.894(4) Å in **9**) to form the sheet like continuous assembly (Figure 4 and Supporting Information). The intermolecular H-bonding interactions between head-to-tail-connected ferrocene peptides results in the formation of a 14-membered H-bonded ring as are found in antiparallel β-sheet-like structures. Again, it is important to stress that the observed H-bonding network in both compounds is significantly different to proteinic structural motifs.

FT-IR spectroscopy is another powerful technique in examining the secondary structure of peptides.[11] Absorptions in the amide I region offer a characteristic signature that allows us to distinguish an α helix, a β sheet and a random coil. The FT-IR spectrum of compound **8** in the solid state exhibits strong amide I bands at 1634 and 1680 cm^{-1} and an amide II band at 1531 cm^{-1}, which is the characteristic peak pattern for a sheet conformation. The absorption at 1680 cm^{-1} is particularly indicative of sheet characteristics.[11,12] The IR absorptions of compound **9** (1638 cm^{-1}, 1679 cm^{-1}, and 1533 cm^{-1}) are similar to those of compound **8** (see Figure S1 in Supporting Information), again providing a consistent picture for a sheet-like structure of this Fc foldamer in the solid state. In the amide A region, strong absorptions are observed at 3294 cm^{-1} for **8** and 3282 cm^{-1} for **9**, which is compatible with H-bonding of the NH group.

In conclusion, we have presented the first example of an acyclic Fc-peptide foldamer that clearly exhibits an extended H-bonded structure. In contrast to previous work, it is possible to prevent the two podant peptide chains from diverging using a very flexible Gly proximal to the Fc core. The Ramachandran plot of the foldamer clearly shows that Gly supports a large range of dihedral angles and provides sufficient flexibility for the system to adopt a peptide structure that is dictated by subsequent addition of "high β-sheet-propensity" amino acids.

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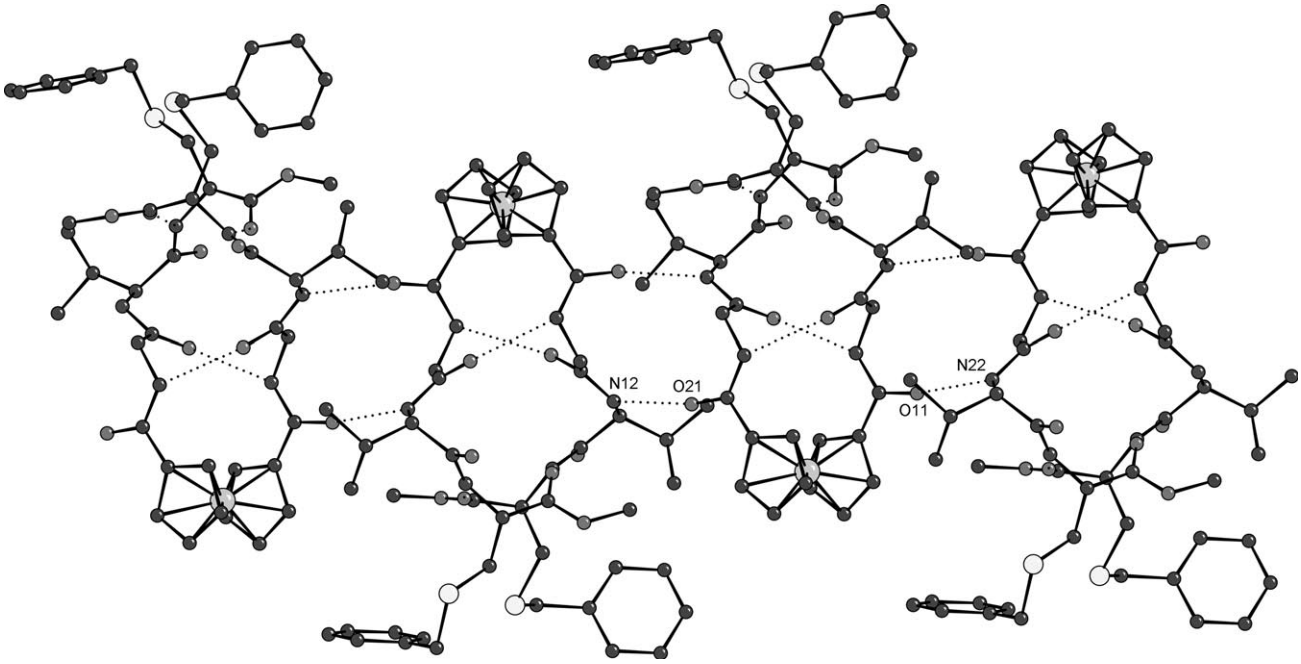

Figure 4. Intermolecular arrangement of **8** showing the formation of a sheet-like assembly. Intermolecular H-bond lengths: O11···N22* 2.869(4) Å, N12···O21** 3.064(4) Å. Symmetry operations: * = $-x+1/2, -y, z-1/2$, ** = $-x+1/2, -y, z+1/2$. Compound **9** shows an identical supramolecular assembly. Intermolecular H-bond lengths: O11···N22* 2.926(4) Å, and N12···O21** 2.894(4) Å. Symmetry operations: * = $-x+1, y+1/2, -z+1/2$, ** = $-x+1, y-1/2, -z+1/2$. Hydrogen atoms are omitted for clarity.

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