THE INFLUENCE OF THE PROTECTING GROUP ON THE CONFORMATIONAL PROPERTIES OF FERROCENE CONJUGATES WITH VALINE

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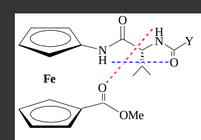
FERROCENE PEPTIDOMIMETICS

I. INTRODUCTION

The biochemical properties of D-amino acids, as well as peptides containing D-amino acids, are of great interests because of their toxic, protective, or signalling effect. [1] To date, very few ferrocene peptides based on D-amino acids have been studied. [2, 3]

Here, we synthesized the bioconjugates Boc-D-Val-NH-Fn-COOMe (**1**) and Ac-D-Val-NH-Fn-COOMe (**2**) (Ac = acetyl, Boc = *tert*-butoxycarbonyl). To determine whether the branched isopropyl side chain of valine and the protecting groups affect the intramolecular hydrogen bonding (Figure 1), i.e., their conformational behaviour, we performed a detailed spectroscopic analysis (IR, NMR and CD), followed by a crystallographic and DFT study.

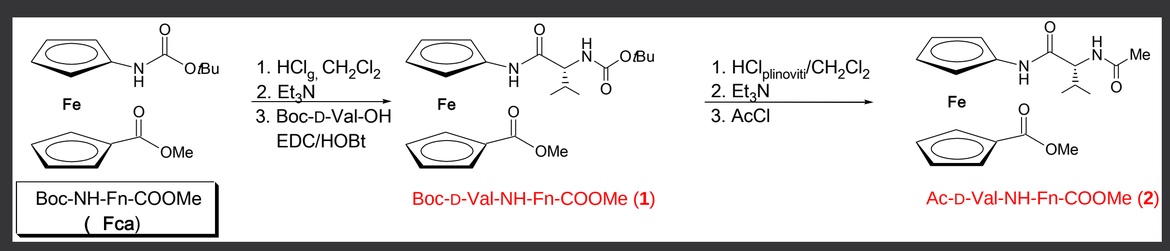


1 (Boc-D-Val-NH-Fn-COOMe); Y = OtBu
2 (Ac-D-Val-NH-Fn-COOMe) Y = Me

Figure 1. Bioconjugates **1** and **2**
(possible intramolecular bonds are marked with red and blue line)

II. RESULTS

• SYNTHESIS OF FERROCENE PEPTIDES 1 AND 2



• IR SPECTROSCOPY

Owing to their high sensitivity to hydrogen bonding, the associated and free amide vibrations are separated in the IR spectra. Therefore, the distinct bands at ~ 3430 cm^{-1} and ~ 3250-3300 cm^{-1} in solution state IR spectra of all the examined peptides indicate the presence of both free and associated NH groups. As can be seen from the Figure 2., free amide band of peptides **1** and 2 is more noticable than associated amide band. Ac-protected peptide **2** shows stronger associated band than its Boc-analogue **1**. Since the relative intensities of free and associated amide bands of the examined peptides are maintained during dilution (from $c = 5\times10^{-2}$ M to 3×10^{-3} M), the intramolecular engagement of hydrogen-bonded amide groups is strongly supported .

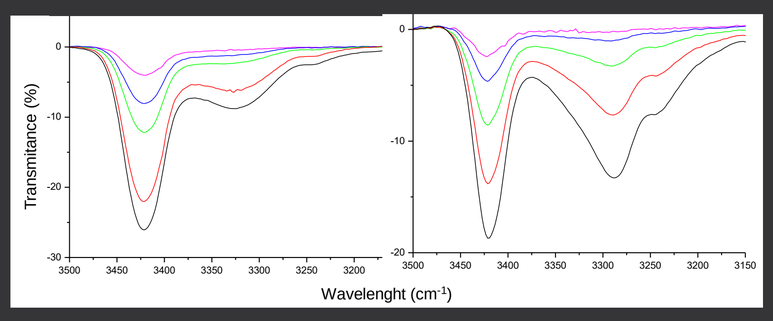


Figure 2. The NH stretching vibrations of **1** (*left*) and **2** (*right*) in IR spectra [CH_2Cl_2]

• CD SPECTROSCOPY

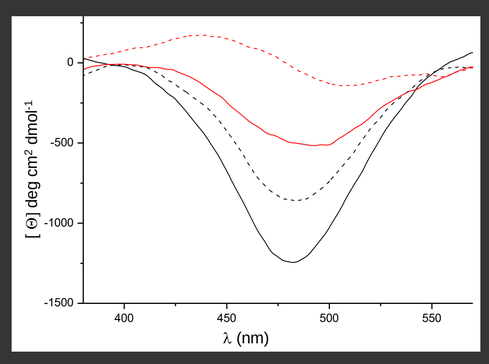


Figure 3. The Cotton effects in chirality-organized ferrocene peptide **1** (black) and **2** (red) in solution [$c = 5\times10^{-3}$M (solid line)] and with 20 % DMSO added (dashed line)

Circular dichroism (CD) is a method of choice for studying the secondary structure, folding behaviour and binding properties of proteins. The former studies on ferrocene peptides revealed that the introduction of ferrocene moiety into the chiral peptide surrounding induces CD activity in the region of ferrocene-based transitions around 480 nm, owing to the intramolecular hydrogen bonding between two peptide strands. The CD data of peptides **1** and **2** indicated ordered structures in solution state and reduction of intensity of Cotton effect for 59% in peptide **1**, i.e. for 79 % in peptide **2** (Fig. 3).

• NMR SPECTROSCOPY

An evaluation of the individual hydrogen-bonding contacts and the strength of the individual hydrogen bonds were examined by NMR spectroscopy. In order to acquire more insight into the conformational space of the novel bioorganometallics, the concentration-, temperature- and solvent-dependent NMR measurements were performed. Amide protons next to ferrocene core are shifted downfiled (coloured black on graphs), suggesting their involvement in formation of hydrogen bond inside peptide chain (Figures 4-6).

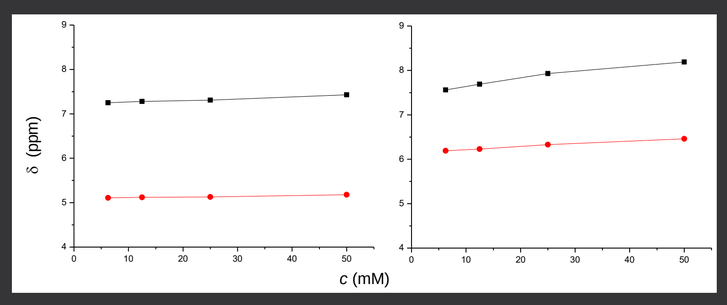


Figure 4. Graph of concentration dependance of chemical shifts of NH-groups of peptides **1** (*left*) and **2** (*right*)

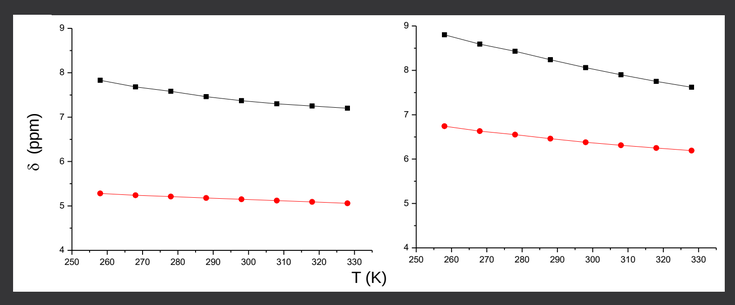


Figure 5. Graph of temperature dependance of chemical shifts of NH-groups of peptides **1** (*left*) and **2** (*right*)

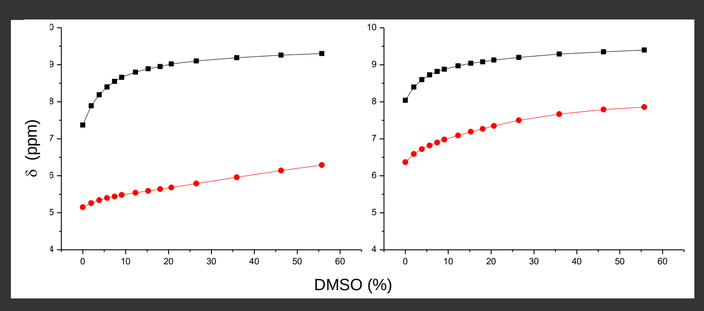


Figure 6. DMSO-titration of chlorofrom solution of peptides **1** (*left*) and **2** (*right*)

• DFT CALCULATIONS

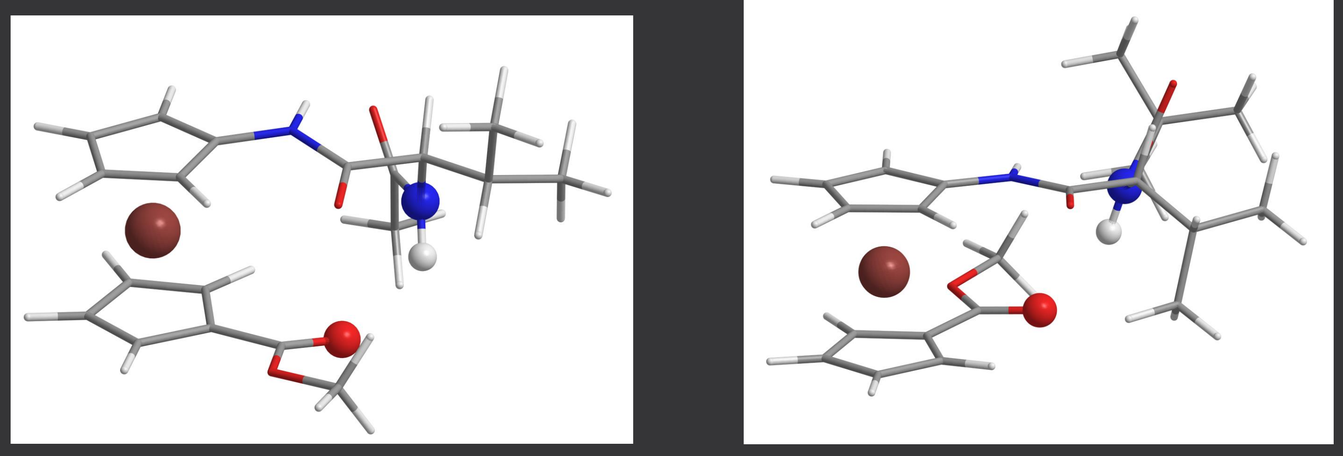
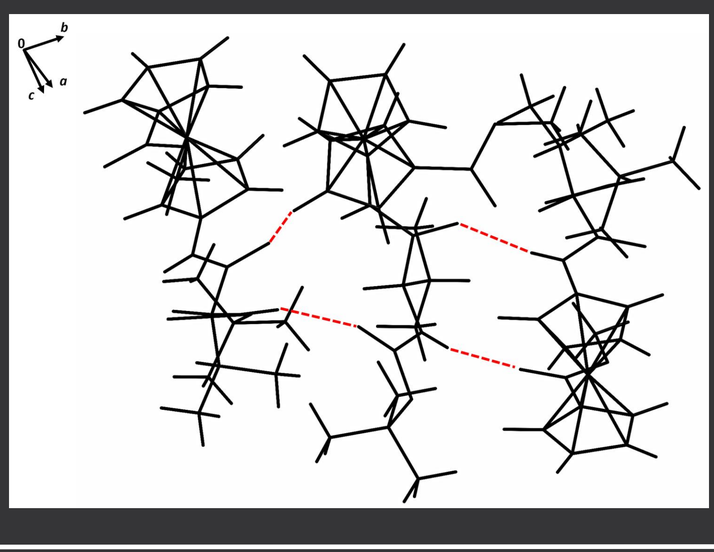


Figure 7. The most stable conformers of **1** (left) and **2** (right) optimized at B3LYP-D3 level of theory (6-311+G(d,p) and LanL2DZ for Fe, SMD model for chloroform).

• CRYSTAL STRUCTURE OF 1



III. CONCLUSIONS

- IR spectra of the ferrocene peptides **1** and **2** show the the presence of both free and associated NH groups, considering slightly increase of associated band of Ac-protected peptide 2
- Since the observed resonances of NH groups next to ferrocene core are shifted downfield ($\delta \gtrsim 7$ ppm), their participation in HB is supported
- The participation of amide protons in IHBs was studied by temperature- and solvent-dependant NMR spectra and DMSO titration connectivities revealing the involvement of both NH_{Fn} protons in IHB bonds.
- The right-handed structures and negative Cotton effect arose if the D-amino acid was proximal to ferrocene core; CD activity (M_q ~ 1000 deg cm^2 $dmol^{-1}$) can be considered as an indicator of a organized chiral environment of ferrocene peptides **1** and **2.** Reduction of intensity of Cotton effect for 59% in peptide 1, i.e. for 79 % in peptide 2, indicates very weak IHB
- Crystal structure of peptide **1** suggests the presence of intermolecular HB.
- Although the turn structure was not observed in the solid state, the DFT calculations suggest the presences of 9-memberd ring ($NH_{Val}\cdot\cdot\cdot OCOOMe$) in the most stable conformers of the both examined compounds.

IV. REFERENCES

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[3] M. Kovačević, I. Kordin, S. Roca, K. Molčanov, Y. Shen, B. Adhikari, H-B. Kraatz, L. Barišić, *Chem. Eur. J.* 23 (2017) 10372.

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