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Unsymmetrical 1,*n*′-disubstituted ferrocenoyl peptides: convenient one pot synthesis and solution structures by CD and NMR spectroscopy

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We present a systematic study on unsymmetrical amino acid derivatives of 1,1′-ferrocene dicarboxylic acid. A convenient and general one pot synthetic procedure is presented in which the unsymmetrical amino acid derivatives are readily separated by column chromatography. The following ferrocene derivatives Fe[C_5H_4-CO-Aaa_1-OMe][C_5H_4-CO-Aaa_2-OMe] with different amino acids were prepared and their solution structures studied by NMR and CD spectrosocopy (Aaa_1 = Aaa_2 = Phe, **1**; Aaa_1 = Aaa_2 = DPhe, **1a**; Aaa_1 = Aaa_2 = Ala, **2**; Aaa_1 = Phe, Aaa_2 = Ala, **3**; Aaa_1 = DPhe, Aaa_2 = Ala, **4**; Aaa_1 = Aaa_2 = Pro, **5**; Aaa_1 = Aaa_2 = Gly, **6**; all amino acids are pure L enantiomers except where stated otherwise). NMR spectroscopy in $CDCl_3$ confirms intramolecular hydrogen bonds for the disubstituted derivatives **1–4** and **6**. CD spectra were recorded for all derivatives in CH_2Cl_2. They clearly show a *P* helical isomer of the ferrocene moiety for the L amino acid derivatives **1–3** and *M* helicity for the DPhe derivative **1a**. The racemic derivative **6** shows only intermolecular interactions, while the Pro derivative **5**, which is devoid of amide protons, most likely exists in an "open conformation". Most importantly, the unsymmetrical mixed D,L derivative **4** has only a weak CD signal in the ferrocene region which is interpreted as an equilibrium between the *P* and *M* helical isomers with a slight excess of the latter.

Introduction

The bioorganometallic chemistry of ferrocene has grown rapidly in recent years. Nucleic acids, carbohydrates, peptides and other biomolecules have been substituted with ferrocene and their properties investigated.[1] Fundamental questions of hydrogen bond formation, conformational issues of the ferrocene moiety, and their interdependence have been addressed in structural studies of amino acid derivatives of ferrocene carboxylic acid.[1,2] For amino acid derivatives of 1,1′-ferrocene dicarboxylic acid, Herrick and coworkers proposed a 1,2′ conformation which is stabilized by two intramolecular hydrogen bonds as shown in Scheme 1a.[3] This "Herrick conformation" was later confirmed to be the dominant arrangement for many derivatives by X-ray crystallographic studies in the solid state and spectroscopic investigations in solution. It follows that for L amino acids on both rings, the ferrocene moiety shows a *P* helical arrangement of the substituents as the energetically favourable conformation, as drawn schematically in Scheme 1a. This arrangement we term L,*P*,L stereoisomer. CD spectroscopy is a powerful tool for the elucidation of metallocene chirality in solution.[4–7] For the ferrocene *P* isomer, a strong positive band around 480 nm is observed. Conversely, the *M* helical enantiomer, which forms with D amino acids, shows a negative band at about 480 nm (D,*M*,D enantiomer).[4] A different conformation with one hydrogen bond has been observed for the phenylalanine derivative in the solid state (Scheme 1b, "van Staveren conformation").[8] Finally, an "open conformation" is feasible if no hydrogen bonds between substituents on the two different cyclopentadienyl (Cp) rings form, Scheme 1c. In this case, no stable helical isomer is preferred for the metallocene core.

So far, almost exclusively symmetrical peptides have been prepared and studied, and all studies have used amino acids of the same chirality on both Cp rings, including some cyclic derivatives.[9,10] An interesting question arises as to what structures will form in an *unsymmetrical* derivative which has different amino acids on the two Cp rings. In particular, if the amino acids are of opposite chirality, *i.e.* one D and one L amino acid, which helical chirality will be induced on the metallocene core? As an extension of our previous synthetic and structural studies on ferrocene peptide conjugates[6–8] we present here a systematic study of unsymmetrical 1,*n*′ amino acid derivatives of 1,1′-ferrocene dicarboxylic acid. Only two examples of unsymmetrical peptides derived from 1,1′-ferrocene dicarboxylic acid have been reported in the literature so far. The first account, published by Kimura and coworkers, unfortunately gives no experimental details on the synthesis.[11] The other example reported by Kraatz *et al.* describes the isolation of the activated intermediates Fe[C_5H_4-CO-Pro_n-OMe][C_5H_4-CO-OBt], n = 3 or 4, by column chromatography.[12,13] Although compounds of this type would be ideal precursors for unsymmetrically substituted ferrocene peptides, the method seems to be limited to this particular case, and no general synthetic procedure is yet available.

Results and discussion

Synthesis

Following the Kraatz procedure, H-Aaa-OMe (Aaa = Ala or Phe) were reacted with activated 1,1′-ferrocene dicarboxylic acid. However, only small amounts of the desired intermediates Fe[C_5H_4-CO-Aaa-OMe][C_5H_4-CO-OBt] were isolated, together with symmetrically disubstituted products Fe[C_5H_4-CO-

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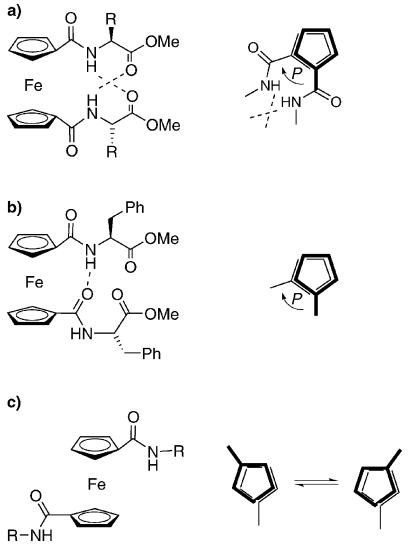


Scheme 1 (a) Left: The preferred "Herrick conformation" of bis (amino acid) 1,*n*′ ferrocene dicarboxylic acid amide derivatives, stabilized by two symmetrical intramolecular interstrand hydrogen bonds. Right: Schematic drawing of the *P* helical arrangement of the metallocene in the "Herrick conformation". (b) The "van Staveren conformation" found in the crystal structure of the bis(phenylalanine) derivative **1** has only one intramolecular hydrogen bond, but shows a *P* helical arrangement. (c) The "open conformation" has no intramolecular hydrogen bond. Consequently, *M*- and *P*-helical isomers easily interconvert.

Aaa-OMe]$_2$. Obviously, simple amino acids like H-Phe-OMe or H-Ala-OMe react faster with the activated ferrocene diacid than the Pro oligomers used by Kraatz and coworkers.[12,13] Consequently, the activated intermediates are consumed more rapidly and their isolation is more difficult.

We therefore changed our strategy to a one-pot procedure. First, 1,1′-ferrocene dicarboxylic acid was activated with 1 equivalent of HBTU and reacted with a mixture of 0.5 eq. of H-Phe-OMe and 0.5 eq. of H-Ala-OMe (Scheme 2). In a second step this procedure was repeated, again activation with 1 eq. of HBTU was followed by reaction with a mixture of 0.5 eq. of H-Phe-OMe and 0.5 eq. of H-Ala-OMe. At least with simple amino acids, the second carboxylic acid function seems to react faster once the first acid has been substituted. Therefore, the yield of the undesired symmetrical products increases if 1 eq. of the first amino acid, *e.g.* H-Ala-OMe, is used for the first step and 1 eq. of the other amino acid, H-Phe-OMe, for the second reaction step. Working in two steps and using an equimolar mixture of both amino acids in each step optimizes the yield of the desired unsymmetrical product.

As expected, three compounds were obtained in this reaction: Fe[C_5H_4-CO-Phe-OMe]$_2$ **1**, Fe[C_5H_4-CO-Ala-OMe]$_2$ **2** and Fe[C_5H_4-CO-Phe-OMe][C_5H_4-CO-Ala-OMe] **3**. The separation of the three-component mixture by column chromatography was rather simple.[14,15] Peptide **1**, a derivative of the aromatic amino acid Phe, and peptide **2**, a derivative of the aliphatic amino acid Ala, separated very well in the mobile phase (ethyl acetate: hexane = 5 : 5). The R_f value of the unsymmetrical derivative **3** (R_f = 0.14), with one aromatic and one aliphatic amino acid substituent, fell midway between the R_f values of peptides **1** (R_f = 0.20) and **2** (R_f = 0.06).

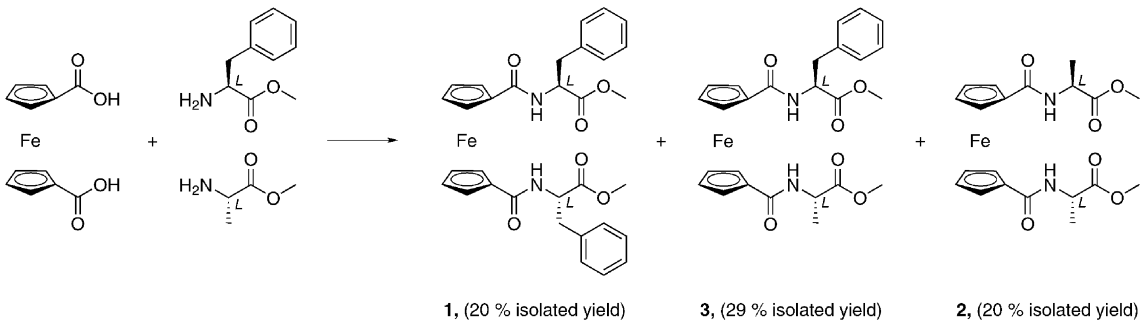
Encouraged by the success of this methodology we repeated the reaction with one D- and one L-amino acid, namely with H-DPhe-OMe and H-Ala-OMe (Scheme 3). Again three products were formed, Fe[C_5H_4-CO-DPhe-OMe]$_2$ **1a**, Fe[C_5H_4-CO-DPhe-OMe][C_5H_4-CO-Ala-OMe] **4** and Fe[C_5H_4-CO-Ala-OMe]$_2$ **2**. Compound **1a** is the enantiomer of **1**. Compound **4** is the first unsymmetrical derivative of 1,1′-ferrocene dicarboxylic acid with amino acids of opposite chirality. In this experiment, purification of the three component mixture was more difficult. The chromatographic properties of the unsymmetrical derivative **4** are much closer to **2** than to **1a**. Therefore, the last fractions of **4** were contaminated by **2**, decreasing the isolated yield of pure **4**. In addition, pure **2** could not be isolated from this experiment.

For comparison, ferrocene peptides **5–7** were prepared (Scheme 4). The disubstituted derivatives were obtained by simply mixing one equivalent of ferrocene dicarboxylic acid and two equivalents of H-Pro-OMe in the case of **5**,[12] or two equivalents of H-Gly-OMe in the case of **6**.[16] The monosubstituted ferrocene peptide **7** was prepared by coupling ferrocene monocarboxylic acid with one equivalent of H-Phe-OMe.[8]

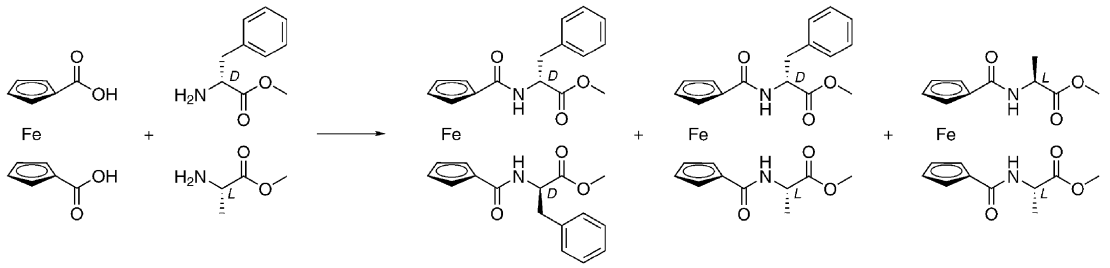
Structures in solution

In order to investigate the solution structure, CD spectra (CH_2Cl_2) of ferrocene peptides **1–7** were recorded. Relevant data for all compounds are compiled in Table 1. In Fig. 1 the CD spectra of **1** and **1a** are shown. Bands between 300–600 nm are characteristic for metal-centred transitions. Molar ellipticities M_θ were calculated in order to facilitate a straightforward comparison between different compounds. The M_θ values for these metal-centred transitions are in the order of 0.5–10 deg mM^{-1} cm^{-1}. Much stronger bands ($M_\theta \approx$ 25–100 deg mM^{-1} cm^{-1}) are observed for bands originating from the amino acids between 180–300 nm.[17] The strong signal at about 480 nm indicates a chiral ferrocene moiety produced by hydrogen bonding interactions between the two chiral peptide strands.[4,18] The enantiomers **1** and **1a** have identical CD spectra but opposite sign.

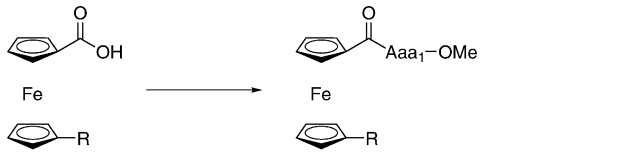
The CD spectra of **1–3**, that contain only L-amino acids, are very similar as shown in Fig. 2. This result can be taken as a strong indication that **1–3** have similar structures in solution, namely the "Herrick conformation" with two symmetrical intramolecular hydrogen bonds and *P*-helical chirality of the



Scheme 2 Reaction conditions: (a) 1,1′-ferrocene dicarboxylic acid (1 eq.), HBTU (1 eq.), HOBt (1 eq.), DIPEA (4 eq.), H-Phe-OMe (0.5 eq.) and H-Ala-OMe (0.5 eq.), CH_2Cl_2, 0 °C → r. t., 6 h; (b) HBTU (1 eq.), HOBt (1 eq.), DIPEA (4 eq.), H-Phe-OMe (0.5 eq.) and H-Ala-OMe (0.5 eq.), r. t., 16 h.



Scheme 3 Reaction conditions: (a) 1,1′-ferrocene dicarboxylic acid (1 eq.), HBTU (1 eq.), HOBt (1 eq.), DIPEA (4 eq.), H-D-Phe-OMe (0.5 eq.) and H-Ala-OMe (0.5 eq.), CH_2Cl_2, 0 °C → r. t., 6 h; (b) HBTU (1 eq.), HOBt (1 eq.), DIPEA (4 eq.), H-D-Phe-OMe (0.5 eq.) and H-Ala-OMe (0.5 eq.), r. t., 16 h.



Scheme 4 Reaction conditions for **5** or **6**: 1,1′-ferrocene dicarboxylic acid (1 eq.), HBTU (2 eq.), HOBt (2 eq.), DIPEA (4 eq.) and H-Aaa-OMe (2 eq.), CH_2Cl_2, 0 °C → r. t., 16 h. Reaction conditions for **7**: ferrocene carboxylic acid (1 eq.), HBTU (1 eq.), HOBt (1 eq.), DIPEA (2 eq.) and H-Phe-OMe (1 eq.), CH_2Cl_2, 0 °C → r. t., 16 h.

ferrocene moiety.[4,6,7,18] The solid state structure of **1** shows a "van Staveren conformation" with only one intramolecular hydrogen bond. It has been argued that packing forces are responsible for a different conformation in the crystal of **1**, but in solution the more stable "Herrick conformation" is obtained.[8] A symmetrical solution conformation for **1**, as well as for **2**, is further supported by symmetrical NMR ($CDCl_3$) spectra. In addition, all amide protons in the ^{1}H NMR spectra of **1–3** show resonances above **7** ppm, indicating their involvement in hydrogen bonds in nonpolar solvents.

CD spectra of the unsymmetrical ferrocene peptides **3** and **4** are shown in Fig. 3. While **3** displays the characteristic spectrum for a "Herrick conformation" (see above), the CD signals of **4** are much weaker. The amide protons of both compounds show resonances above **7** ppm in the ^{1}H NMR spectrum ($CDCl_3$), characteristic for hydrogen bonding. Both findings can be explained if it is assumed that **4** gives in solution a diastereomeric mixture of the "Herrick conformer" with chirality D,*P*,L favourable for the L amino acid and the "Herrick conformer" with chirality D,*M*,L favourable for the D amino acid. Since a weak negative CD signal is obtained at about 480 nm, there must be a small excess of the later species. However, variable temperature ^{1}H NMR ($CDCl_3$, 230–300 K) of **4** reveals only one set of signals and not a mixture of conformers. Variable temperature CD spectroscopy did not show any significant changes in the CD spectra in the small temperature window available (278–308 K). Also, addition of 10% (v : v) of MeOH to the solutions did not change the appearance of the CD spectra of **3** or **4**. By measuring the spectra at different concentrations, intermolecular interactions could also be ruled out as the source of the weak signals in the CD spectrum of **4**.

The CD spectra of ferrocene peptides **5–7** are shown in Fig. 4 and compared with compound **4**. Qualitatively, the molar ellipticity of the metallocene-derived bands for all compounds **4–7** is much lower than for the other compounds in the study. The derivative Fe[C_5H_5-CO-Pro-OMe]$_2$ **5** cannot form intramolecular hydrogen bonds since it has no amide protons and consequently in the solid state the "open conformer" was found.[12] However, the CD spectrum of **5** is qualitatively different from that of **4**. This finding seems to rule out an "open conformation" for compound **4** in CH_2Cl_2 solution.

Peptide Fe[C_5H_5-CO-Gly-OMe]$_2$ **6** gives in solution a racemic mixture of the *P* and *M* helical "Herrick conformers", since no source of chirality is present. This finding is confirmed by the X-ray single crystal structure of **6**.[16] The chemical shift of the amide protons in the ^{1}H NMR ($CDCl_3$) of **6**, at 7.94 ppm, confirm intramolecular hydrogen bonding in solution. The CD spectrum of **6** shows only very weak signals that could be a measure of intramolecular interactions (Fig. 4). The CD spectrum of the monosubstituted derivative Fe[C_5H_5-CO-Phe-OMe][C_5H_5] **7** also shows bands in the ferrocene region but is different from the spectrum of **6**, as shown in Fig. 4. Compound **7** has only one peptide strand and only intermolecular hydrogen bonds are possible, as shown by the solid state structure.[8] For **7**, very similar spectra were obtained at two different concentrations (1 mM and 10 mM), as well as in CH_2Cl_2 and CH_3CN, thus making intermolecular interactions as the source of the CD bands unlikely. Based on those

Table 1 CD data (CH_2Cl_2) of ferrocene peptides 1–7

Compound	λ_{max}/nm (M_θ/deg mM^{-1} cm^{-1})			
1	311(+7.9)	355(−6.4)	415(−2.0)	481(+5.6)
1a	312(−7.8)	355(+6.6)	416(+2.1)	482(−5.7)
2	310(+10.0)	356(−4.8)	416(−1.7)	484(+5.5)
3	311(+9.4)	355(−5.9)	415(−1.9)	482(+5.7)
4	—[a]	351(+0.6)	416(+0.4)	476(−0.5)
5	315(+1.6)	366(−0.2)	—[a]	470(+0.6)
6	308(+0.3)	—[a]	—[a]	479(+0.1)
7	340(−0.5)	410(−0.1)	460(+0.1)	502(−0.1)

[a] Not observed.

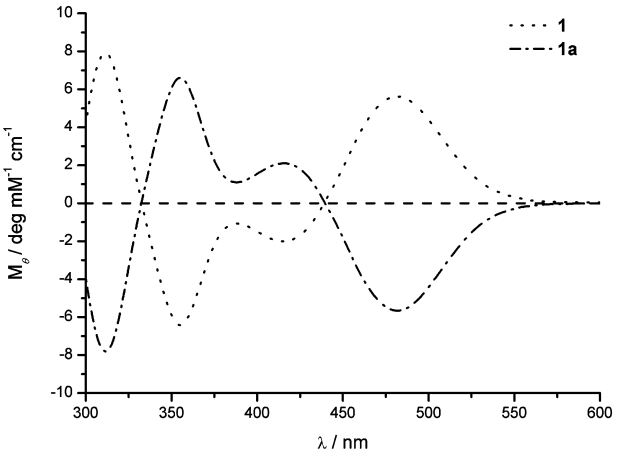


Fig. 1 CD spectra (CH_2Cl_2) of ferrocene peptides **1** and **1a**.

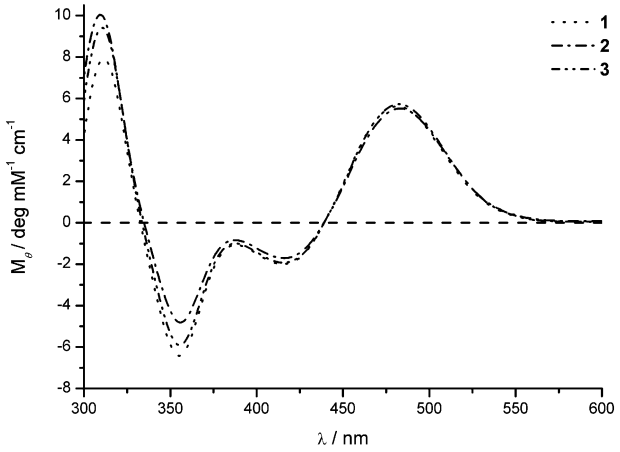


Fig. 2 CD spectra (CH_2Cl_2) of ferrocene peptides **1–3**.

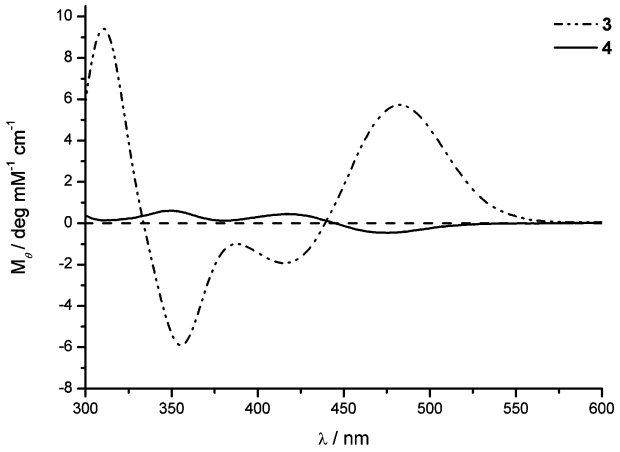


Fig. 3 CD spectra (CH_2Cl_2) of ferrocene peptides **3** and **4**.

differences in the CD spectra, the CD spectrum of **4** seems to indicate intra- rather than intermolecular interactions.

Conclusion

We present a general synthetic scheme for the preparation of unsymmetrical amino acid derivatives of 1,1′ ferrocene dicarboxylic acid. As an example, Ala and Phe substituents were chosen. In a one-pot procedure, mixtures of the symmetrical and unsymmetrical products were obtained. Separation of unsymmetrical ferrocene peptide derivatives with Ala and Phe substituents was possible with column chromatography, since the polarity of the parent amino acids[15] as well as their chromatographic mobility[14] differ significantly. Using this strategy, it has been possible to prepare ferrocene derivatives with different amino acids on each ring, *i.e.* Ala and Phe in compound **3**. Moreover, we were able to prepared a diastereomer of **3** by replacing the naturally occurring L-Phe with D-Phe, yielding **4**.

All compounds with amide hydrogen atoms form intramolecular hydrogen bonds as shown by their ^{1}H NMR spectra in $CDCl_3$. As expected, the Pro derivative **5** which is devoid of any amide hydrogens exists in an "open conformation" in solution. CD spectroscopy suggests a "Herrick conformation" with *P* helical chirality of the metallocene for the symmetrical Phe (**1**) and Ala (**2**) derivatives from a strong positive band around 480 nm. As expected, the enantiomeric D-Phe derivative **1a** has opposite *M* helicity with a negative band around 480 nm, whereas no preference is observed for the racemic Gly derivative **6**.[16] The CD spectrum of the unsymmetrical Phe, Ala derivative **3**, in which both amino acids posses the same

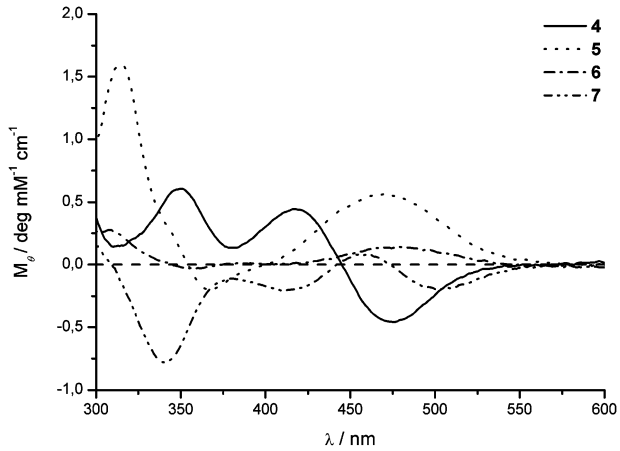


Fig. 4 CD spectra (CH_2Cl_2) of ferrocene peptides **4–7**. Note the different scale of the *y* axis compared to Figs. 1–3.

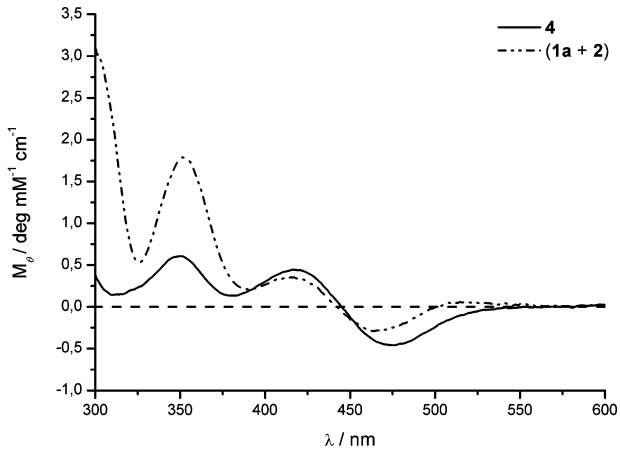


Fig. 5 Comparison of the CD spectrum of **4** with the sum of the CD spectra (**1a** + **2**). Note the different scale of the *y* axis compared to Figs. 1–3.

L chirality, is almost identical to the CD spectra of **1** and **2**. A strong positive band around 482 nm again suggests *P* helicity of the metallocene. The unsymmetrical D-Phe, Ala derivative **4**, on the other hand, has only a very weak negative band at 476 nm. It seems that the helicity-inducing power of the bulkier Phe amino acid is slightly bigger than that of Ala. It is likely that at least two diastereomers exist in solution in equilibrium, but there is a slight excess of the *M* helical diastereomer which is obviously induced by the chirality of the D amino acid. This is nicely illustrated by the fact that the CD spectrum of **4** is almost identical with the sum of the spectra (**1a** + **2**) in the ferrocene bands above 400 nm, see Fig. 5. The resulting CD spectrum (**1a** + **2**) in fact shows that Phe induces slightly stronger bands in the ferrocene moiety than Ala.

Using the general synthetic methodology presented herein, almost any combination of amino acids and peptides on 1,1′-ferrocene dicarboxylic acid can be prepared. Indeed, most other diacids should also be amendable to this procedure. With these compounds in hand, the subtle effects of such parameters as size, hydrophilicity and chirality on the structure and hydrogen bonding pattern of ferrocenoyl peptides can be studied. Further work along those lines is already in progress in our laboratory.

Experimental

General remarks

Reactions were carried out in ordinary glassware and chemicals used without further purification. All chemicals were

obtained from Aldrich, Iris, Fluka or Novabiochem. Pure L amino acid methyl esters were used except for Phe where indicated. NMR spectra were determined on a Bruker AM 360 spectrometer, ^{1}H at 360.14 MHz and ^{13}C at 90.56 MHz. Chemical shifts, δ/ppm, indicate a downfield shift from tetramethylsilane, TMS, the internal standard. Coupling constants, J, are given in Hz (absolute values). Individual peaks are marked as: singlet (s), doublet (d), triplet (t) or multiplet (m). Mass spectra were measured on a Mat 8200 instrument. Only characteristic fragments with possible composition are given in parentheses. Infrared spectra were recorded on a Bruker Equinox55 FT-IR spectrometer as KBr discs. Wavenumbers ν are given in cm^{-1}. UV/Vis spectra were measured on a Varian CARY 100 instrument in 1 cm quartz Suprasil cells thermostated at 20 °C. Absorption maxima, λ_{max}, and molar absorption coefficients, ε_{max}, are given in nm and M^{-1} cm^{-1}, respectively. CD spectra were recorded on a JASCO J-810 spectropolarimeter in 1 cm quartz Suprasil cells under argon thermostated at 20 °C. Ellipticity maxima, θ_{max}, are given in nm. Molar ellipticity coefficients, M_θ, were calculated as $M_\theta = 100\ \theta/c \times l$, where ellipticity θ is in deg, concentration c in mol l^{-1} and pathlength l in cm, thus giving deg mM^{-1} cm^{-1} for M_θ.[17] Stock solutions were obtained by accurately weighting *ca.* 5 mg of substance on an analytical balance and dissolving this amount in 5 ml of CH_2Cl_2 in a graded analytical flask. Typically, concentrations of 1.5–2.5 mM were used for the measurements.

General synthetic procedure

1,1′-Ferrocene dicarboxylic acid (274 mg, 1 mmol) was dissolved in CH_2Cl_2 (40 ml) and cooled to 0 °C with an ice bath. HBTU (379 mg, 1 mmol), HOBt × H_2O (153 mg, 1 mmol) and DIPEA (700 µl, 4 mmol) were added and stirring was continued for 30 min. A mixture of phenylalanine methyl ester hydrochloride (107.9 mg, 0.5 mmol) and alanine methyl ester hydrochloride (69.8 mg, 0.5 mmol) was added and stirring was continued for 6 hours. After this period HBTU (379 mg, 1 mmol), HOBt × H_2O (153 mg, 1 mmol) and DIPEA (700 µl, 4 mmol) were added again and stirring was continued for 30 min. A mixture of phenylalanine methyl ester hydrochloride (107.9 mg, 0.5 mmol) and alanine methyl ester hydrochloride (69.8 mg, 0.5 mmol) was added and stirring was continued over night. After this period CH_2Cl_2 (40 ml) was added to the reaction mixture and it was washed with $NaHCO_3$ (sat. aq, 3 × 80 ml), citric acid (10% aq, 3 × 80 ml) and water (80 ml). The organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure to yield an orange solid as crude product.

Reaction I. H-Phe-OMe and H-Ala-OMe were used in the general synthetic procedure. The crude product was subjected to flash column chromatography (85 g, ∅ = 3 cm, ethyl acetate: hexane = 5:5). The orange solids **1** (R_f = 0.20, 120 mg, 20%), **3** (R_f = 0.14, 150 mg, 29%) and **2** (R_f = 0.06, 90 mg, 20% yield) were isolated.

Reaction II. H-DPhe-OMe and H-Ala-OMe were used in the general synthetic procedure. The crude product was subjected to flash column chromatography (85 g, ∅ = 3 cm, ethyl acetate: hexane = 7:3). The orange solids **1a** (R_f = 0.58, 120 mg, 20%) and **4** (R_f = 0.41, 120 mg, 23%) could be isolated. Derivative **2** (R_f = 0.33) could not be isolated in a pure form in this experiment.

Fe[C$_5$H$_4$-CO-Phe-OMe]$_2$, 1. M_r ($C_{32}H_{32}N_2O_6Fe$) = 596.45; MS (EI): *m/z* 596 (100) [M]$^+$, 434 (9) [M − CH(CH$_2$Ph)CO$_2$Me]$^+$, 271 (27) [M − 2 CH(CH$_2$Ph)CO$_2$Me]$^+$; HRMS (EI): *m/z* exp. 596.1613, calc. 596.1610; ^{1}H NMR ($CDCl_3$): δ 7.73 (d, 2 H, NH, J = 8.5), 7.33–7.20 (m, 10 H, H_{Ar}), 5.04 (ddd, 2 H, $C_\alpha H$, J = 10.5, 8.5 and 5.0), 4.80–4.79 (m, 2 H, $H_{\alpha,Fc}$), 4.68–4.67 (m, 2 H, $H_{\alpha,Fc}$), 4.51–4.49 (m, 2H, $H_{\beta,Fc}$), 4.30–4.29 (m, 2H, $H_{\beta,Fc}$), 3.85 (s, 2H, OCH_3), 3.22 (dd, 2 H, $C_\beta H$, J = 14.0 and 5.0), 2.98 (dd, 2 H, $C_\beta H$ J = 14.0 and 10.5); ^{13}C NMR ($CDCl_3$): δ 175.4 (CO_{ester}), 170.3 (CO_{amide}), 136.7 ($C_{i,Phe}$), 128.9 ($C_{m,Phe}$), 128.6 ($C_{o,Phe}$), 127.0 ($C_{p,\ Phe}$), 75.8 ($C_{i,\ Fc}$), 71.8 ($C_{\alpha,Fc}$), 71.3 ($C_{\alpha,Fc}$), 70.3 ($C_{\beta,Fc}$), 69.9 ($C_{\beta,Fc}$), 53.9 (C_α), 52.8 (OCH_3), 37.0 (C_β); IR (KBr): ν_{max} 3368 ($NH_{valence}$), 1746 (CO_{ester}), 1634 (CO_{amide}), 1538 (NH_{def}); UV/Vis (CH_2Cl_2): $\lambda_{max}(\varepsilon)$ 438 (249), 341 (411); CD (CH_2Cl_2): θ_{max} (M_θ) 481 (5.6), 415 (−2.0), 355 (−6.4), 311 (7.9).

Fe[C$_5$H$_4$-CO-DPhe-OMe]$_2$, 1a. This compound was isolated in reaction II and is an enantiomer of **1**. The composition and purity were confirmed by ^{1}H NMR ($CDCl_3$) and and CD spectra.

Fe[C$_5$H$_4$-CO-Ala-OMe]$_2$, 2. M_r ($C_{20}H_{24}N_2O_6Fe$) = 444.26; MS (EI): *m/z* 444 (100) [M]$^+$, 342 (15) [M − Ala-OMe]$^+$, 271 (23) [M − 2 CH(Me)CO$_2$Me]$^+$; HRMS (EI): *m/z* exp. 444.0985 and calc. 444.0984; ^{1}H NMR ($CDCl_3$): δ 7.74 (d, 2 H, NH, J = 8.0 Hz), 4.91–4.90 (s, 2 H, $H_{\alpha,Fc}$), 4.86 (pseudo-quintet, 2 H, $C_\alpha H$), 4.76–7.75 (m, 2 H, $H_{\alpha,Fc}$), 4.56–4.54 (m, 2 H, $H_{\beta,Fc}$), 4.35 –4.33 (m, 2 H, $H_{\beta,Fc}$), 3.81 (s, 3 H, CH_3), 1.40 (d, 3 H, $C_\beta H$), J = 7.5 Hz); ^{13}C NMR ($CDCl_3$): D 176.3 (CO_{ester}), 170.0 (CO_{amide}), 75.7 ($C_{i,Fc}$), 71.9 ($C_{\alpha,Fc}$), 71.3 ($C_{\alpha,Fc}$), 70.3 ($C_{\beta,Fc}$), 70.1 ($C_{\beta,Fc}$), 52.7 (C_α), 51.5 (CH_3), 16.7 (C_β); IR (KBr): ν_{max} 3328 ($NH_{valence}$), 1744 (CO_{ester}), 1633 (CO_{amide}), 1533 (NH_{def}); UV/Vis (CH_2Cl_2): λ_{max} (ε) 437 (251), 340 (410); CD (CH_2Cl_2): θ_{max} (M_θ) 484 (+5.5), 416 (−1.8), 356 (−4.8), 310 (+10.0).

Fe[C$_5$H$_4$-CO-Phe-OMe][C$_5$H$_4$-CO-Ala-OMe], 3. M_r ($C_{26}H_{28}N_2O_6Fe$) = 520.36; MS (EI): *m/z* 520 (100) [M]$^+$, 358 (58) [M − CH(CH$_2$Ph)CO$_2$Me]$^+$, 271 (43) [M − CH(CH$_2$Ph)CO$_2$Me- − CH(Me)CO$_2$Me]$^+$; HRMS (EI): *m/z* exp. 520.1298 and calc. 520.1297; ^{1}H NMR ($CDCl_3$): δ 7.82 (d, 1 H, NH, J = 8.5), 7.73 (d, 1 H, NH, J = 8.5), 7.28–7.16 (m, 5 H, H_{Ar}), 5.00 (ddd, 2 H, $C_{\alpha,Phe}H$, J = 10.5, 8.5 and 4.5), 4.92–4.84 (m, 2 H, $H_{\alpha,Fc}$ and $C_{\alpha,Ala}$), 4.82–4.80 (m, 1 H, $H_{\alpha,Fc}$), 4.74–4.72 (m, 1 H, $H_{\alpha,Fc}$), 4.69–4.67 (m, 1 H, $H_{\alpha,Fc}$), 4.53–4.50 (m, 2 H, $H_{\beta,Fc}$), 4.33–4.29 (m, 2 H, $H_{\beta,Fc}$), 3.86 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3), 3.15 (dd, 1 H, $C_{\beta,Phe}H$, J = 13.8 and 4.8), 2.84–2.75 (dd, 1 H, $C_{\beta,Phe}H$, J = 10.8 and 10.5), 1.45 (d, 3 H, $C_{\beta,Ala}H$, J = 7.5); ^{13}C NMR ($CDCl_3$): D 176.4 (CO_{ester}), 175.3 (CO_{ester}), 170.3 (CO_{amid}) 170.0 (CO_{amid}), 136.7 ($C_{i,Phe}$), 128.9 ($C_{m,Phe}$), 128.6 ($C_{o,Phe}$), 127.0 ($C_{p,\ Phe}$), 75.9 ($C_{i,Fc}$), 75.8 ($C_{i,Fc}$), 71.9 (2 C, $C_{\alpha,Fc}$), 71.3 (2 C, $C_{\alpha,Fc}$), 70.3 (3 C, C,Fc), 70.2 ($C_{\beta,Fc}$), 53.9 ($C_{\alpha,Phe}$), 52.7 (2 C, $C_{\alpha,Ala}$ and OCH_3), 47.8 (OCH_3), 37.0 ($C_{\beta,Phe}$), 16.9 ($C_{\beta,Ala}$); IR (KBr): ν_{max} 3373 ($NH_{valence}$), 1728 (CO_{ester}), 1652 (CO_{amide}), 1535 (NH_{def}); UV/Vis (CH_2Cl_2): λ_{max} (ε) = 438 (243), 341 (400); CD (CH_2Cl_2): θ_{max} (M_θ) = 482 (+5.7), 415 (−1.9), 355 (−5.9), 311 (+9.4).

Fe[C$_5$H$_4$-CO-DPhe-OMe][C$_5$H$_4$-CO-Ala-OMe], 4. M_r ($C_{26}H_{28}N_2O_6Fe$) = 520.36. MS (EI): *m/z* 520 (100) [M]$^+$, 358 (24) [M − CH(CH$_2$Ph)CO$_2$Me]$^+$, 271 (34) [M − CH (CH$_2$Ph)CO$_2$Me-CH(Me)CO$_2$Me]$^+$; HRMS (EI): *m/z* exp. 520.1298 and calc. 520.1297; ^{1}H NMR ($CDCl_3$): 7.38 (d, 1 H, NH, J = 7.0), 7.33–7.22 (m, 6 H, H_{Ar} and NH), 4.75–4.68 (m, 3 H, $H_{\alpha,Fc}$ and $C_{\alpha,Phe}H$), 4.64–4.61 (m, 2 H, $H_{\alpha,Fc}$), 4.49 (pseudo-quintet, 1 H, $C_{\alpha,Ala}H$, J = 7.0), 4.43–4.36 (m, 4 H, $H_{\beta,Fc}$) 3.82 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3), 3.13 (dd, 1 H, $C_{\beta,Phe}H$, J = 14.0 and 5.5), 3.17 (dd, 1 H, $C_{\beta,Phe}H$, J = 14.0 and 9.0), 1.52 (d, 3 H, $C_{\beta,Ala}H$, J = 7.0); ^{13}C NMR ($CDCl_3$): 174.4 (CO_{ester}), 173.4 (CO_{ester}), 170.3 (CO_{amid}), 170.0 (CO_{amid}), 136.9 ($C_{i,Phe}$), 129.1 ($C_{m,Phe}$), 128.6 ($C_{o,Phe}$), 127.0 ($C_{p,Phe}$), 76.5 (2 C, $C_{i,Fc}$), 71.6 ($C_{\alpha,Fc}$), 71.5 ($C_{\alpha,Fc}$), 71.4 ($C_{\alpha,Fc}$), 71.3 ($C_{\alpha,Fc}$), 71.1

($C_{\beta,Fc}$), 70.6 ($C_{\beta,Fc}$), 70.3 ($C_{\beta,Fc}$), 70.0 ($C_{\beta,Fc}$), 54.3 ($C_{\alpha,Phe}$), 52.6 (2 C, $C_{\alpha,Ala}$ and OCH_3), 48.7 (OCH_3), 37.0 ($C_{\beta,Phe}$), 17.2 ($C_{\beta,Phe}$); UV/Vis (CH_2Cl_2): λ_{max} (ε) 436 (219), 339 (334); CD (CH_2Cl_2): θ_{max} (M_θ) 476 (−0.5), 416 (+0.4), 351 (+0.6).

Fe[C_5H_4-CO-Pro-OMe]$_2$, 5[12]. M_r ($C_{24}H_{28}FeN_2O_6$) = 496.33; MS (EI): m/z 496 (100) $[M]^+$, 368 (10) [M − Pro-OMe]$^+$; ^{1}H NMR ($CDCl_3$): 4.86 (br. s, 4 H, $H_{\alpha,Fc}$), 4.60–4.57 (m, 2 H, $C_\alpha H$), 4.47 (br. s, 4 H, $H_{\beta,Fc}$), 3.94–3.68 (m, 10 H, $C_\delta H$ and OCH_3) 2.27–1.94 (m, 8 H, $C_\beta H$ and $C_\gamma H$);^{13}C NMR ($CDCl_3$): 172.9 (CO_{ester}), 168.8 (CO_{amide}), 76.4 ($C_{i,Fc}$), 72.4 ($C_{\alpha,Fc}$), 72.3 (2 C, $C_{\alpha,Fc}$ and $C_{\beta,Fc}$) 71.2 ($C_{\beta,Fc}$), 60.2 (C_α), 52.1 (OCH_3), 48.4 (C_δ), 28.7 (C_γ), 25.6 (C_γ); UV/Vis (CH_2Cl_2): λ_{max} (ε) 446 (270), 341 (347); CD (CH_2Cl_2): θ_{max} (M_θ) 470 (+0.6), 366 (−0.2), 315 (+1.6).

Fe[C_5H_4-CO-Gly-OMe]$_2$, 6. 1,1′-Ferrocene dicarboxylic acid (137 mg, 0.5 mmol) was dissolved in CH_2Cl_2 (40 ml) and cooled to 0 °C with an ice bath. HBTU (379 mg, 1 mmol), HOBt × H_2O (153 mg, 1 mmol) and DIPEA (700 µl, 4 mmol) were added and stirring was continued for 30 min. Then glycine methyl ester hydrochloride (125.0 mg, 1 mmol) was added and the stirring was continued for 16 hours. After this period CH_2Cl_2 (40 ml) was added to the reaction mixture and it was washed with $NaHCO_3$ (sat. aq, 3 × 80 ml), citric acid (10% aq, 3 × 80 ml) and water (80 ml). The organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was subjected to flash column chromatography (40 g, ∅ = 2.5 cm, ethyl acetate: hexane = 9 : 1). Orange solid, 283 mg (68.0%, R_f = 0.23). M_r ($C_{18}H_{20}FeN_2O_6$) = 416.21; MS (EI): m/z 416 (100) $[M]^+$; HRMS (EI): m/z exp. 416.0670 and calc. 416.0671; ^{1}H NMR ($CDCl_3$): 7.94 (t, 2 H, NH, J = 6.0), 4.81 (t, 4 H, $H_{\alpha,Fc}$, J = 2.0), 4.46 (t, 4 H, $H_{\beta,Fc}$, J = 2.0), (d, 4 H, $C_\alpha H$, J = 6.3) 3.82 (s, 6 H, OCH_3); ^{13}C NMR ($CDCl_3$): 173.5 (CO_{ester}), 170.4 (CO_{amide}), 75.5 ($C_{i,Fc}$), 71.6 ($C_{\alpha,Fc}$), 70.5 ($C_{\beta,Fc}$), 52.8 (OCH_3), 40.9 (C_α); UV/Vis (CH_2Cl_2): λ_{max} (ε) 438 (228), 338 (454); CD (CH_2Cl_2): θ_{max} (M_θ) 479 (+0.1), 308 (+0.3).

Fe[C_5H_4-CO-Phe-OMe][C_5H_5], 7. M_r ($C_{21}H_{21}FeNO_3$) = 391.24; MS (EI): m/z 391 (100) $[M]^+$, 213 (32) [M − Phe-OMe]$^+$; ^{1}H NMR ($CDCl_3$): 7.37–7.19 (m, 5 H, H_{Ar}) 6.05 (d, 1 H, NH, J = 8.0), 5.06–5.01 (m, 1 H, $C_\alpha H$), 4.64–4.62 (m, 2 H, $H_{\alpha,Fc}$), 4.60–4.58 (m, 2 H, $H_{\alpha,Fc}$), 4.34–4.31 (m, 2 H, $H_{\beta,Fc}$), 4.12 (s, 5 H, H_{Cp}), 3.77 (s, 3 H, OCH_3), 3.23 (dd, 1 H, $C_\beta H$, J = 14.0 and 6.0), 3.16 (dd, 1 H, $C_\beta H$, J = 14.0 and 6.0); ^{13}C NMR ($CDCl_3$): 172.3 (CO_{ester}), 168.8 (CO_{amide}), 136.0 ($C_{i,Phe}$), 129.2 ($C_{m,Phe}$), 128.7 ($C_{o,Phe}$), 127.2 ($C_{p,Phe}$), 75.3 ($C_{i,Fc}$), 70.5 (2 C, $C_{\alpha,Fc}$), 69.7 (5 C, Cp), 68.3 ($C_{\beta,Fc}$), 68.0 ($C_{\beta,Fc}$), 52.7 (C_α), 52.3 (OCH_3), 38.0 (C_β); UV/Vis (CH_2Cl_2): λ_{max} (ε) 438 (199), 341 (295), 302 (1000); CD (CH_2Cl_2): θ_{max} (M_θ) 502 (−1.3), 460 (+0.6), 410 (−1.3), 340 (−5.2).

Abbreviations. Aaa: any amino acid; DIPEA: *N,N*-diisopropylethylamine; HBTU: *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate; HOBt: 1-hydroxybenzotriazole.

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