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Amino acid conjugates of 1,1′-diaminoferrocene. Synthesis and chiral organization†

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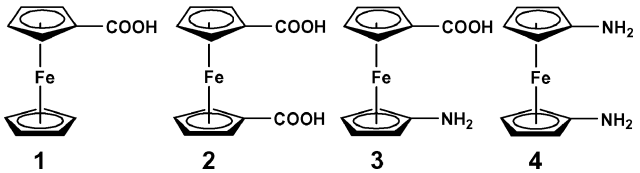
1,1′-Bis(*tert*-butoxycarbonylamino)ferrocene (**6**), a protected derivative of 1,1′-diaminoferrocene, has been synthesized by a very convenient method and serves as a synthon for 1,1′-diaminoferrocene. Its structure in solid state and in solution has been studied by NMR and X-ray crystallography. 1,1′-Bis(*tert*-butoxycarbonylamino)ferrocene serves as starting material for the synthesis of amino acid conjugates of L- and D-alanine. The structures of these bioconjugates have been studied by NMR and CD spectroscopy and X-ray crystallography and reveal that the chiral organization of the podant amino acid chains is controlled by the chirality of the attached amino acid. The substituents engage in strong intramolecular H-bonding generating 14-membered H-bonded rings, a motif previously unrealized in ferrocene–amino acid and peptide conjugates.

Introduction

Controlling the secondary structures of peptides is of great importance in designing functional peptidic materials[1,2] and understanding the function of proteins and enzymes.[3] Among various means, use of molecular scaffolding has attracted great attention in achieving specific secondary structures.[4] In this context, ferrocene (Fc) derivatives **1–3** are widely used as a redox active scaffold. Having the cyclopentadienyl rings separated by 3.3 Å, allows effective intramolecular interstrand H-bonding in peptide conjugates of disubstituted Fc, where the peptide strands are on the two different cyclopentadienyl (Cp) rings. Hydrogen bonding imposes a specific secondary structure onto the bioconjugates, first described by Herrick and co-workers.[5] The particular choice of Fc-scaffold will influence the ability for forming H-bonded assemblies. For example, conjugates of **1** often give rise to one dimensional H-bonded chains, whereas **2** and **3** can give rise to H-bonded β-sheet like structures or even engage in chiral helical arrangements.[6–12] It is also observed that, based on the torsional twist about the Cp(centroid)–Fe–Cp(centroid) axis, conformational enantiomerization is possible for the case of 1,1′-disubstituted ferrocene[13] and the conformations can be stabilized by intramolecular hydrogen bonding between the two peptides strands, when the substituents are peptides, as described by Hirao using **2** as a scaffold.[10] Given the general difficulty in preparing mono- and diaminoferrocene derivatives in sufficiently high yields,[14–17] peptide derivatives of 1,1′-diaminoferrocene (**4**) are completely absent from the literature. We set out to develop a synthetic procedure that circumvents the use of the explosive 1,1′-diazoferrocene.[18]



Here, we report the convenient synthesis of 1,1′-bis(*tert*-butoxycarbonylamino)ferrocene (**6**), a convenient synthon of 1,1′-diaminoferrocene (**4**). The bis-Boc derivative is compatible with the peptide synthesis protocol, as is demonstrated by the preparation of its first amino acid conjugates. To test the chiral organization pattern of the Fc conjugate, L- and D-alanine conjugates were prepared. Importantly, the systems exhibit a previously unknown H-bonding motif for Fc–peptide conjugates.

Results and discussions

1,1′-Bis(carbonylazido)ferrocene (**5**) was synthesized from 1,1′-ferrocenedicarboxylic acid (**2**) by treatment with ethyl chloroformate, followed by reaction with sodium azide. The reaction of the carbonylazido compound **5** with *tert*-butanol at 80 °C for 8 hours cleanly leads to the desired bis-Boc-protected compound **6** in 72% yield (Scheme 1). The material is stable in solution and in the solid state and does not decompose in solution even after several months. Compound **6** was characterized spectroscopically and by single-crystal X-ray analysis. The ^{1}H NMR spectrum shows the two carbamide NHs upfield from typical amides at δ 6.06. Similarly, the ^{13}C NMR spectrum exhibits a resonance at δ 153.2, typical for the Boc carbonyl. The IR spectrum shows a strong absorption for the C=O group at 1695 cm^{-1}. Single crystals of compound **6** were obtained by slow diffusion of hexane into a chloroform solution. The X-ray diffraction study (Table 1, ‡) revealed the presence of two independent molecules occupying general positions and one molecule with its iron atom [Fe(3)] lying on an inversion centre in the asymmetric unit. The three rotamers show the two substituents at different rotational angles with respect to the Cp–Fe–Cp vector (70°, 144°, 180°) with an approximate 1,2′ (70°) and 1,3′ (144°, 180°) conformation. All three rotamers are linked *via* intermolecular N(H)···O=C bonding. In one of the rotamers, strong intramolecular H-bonding is present [see Fig. 1, ferrocene Fe(1)]. The ^{1}H NMR spectrum provides an indication for the presence of more than one rotamer even in

† Electronic supplementary information (ESI) available: crystal packing for compound **6**, temperature-dependent NMR studies for compound **7** and SWV spectra and structural data for compounds **6**, **7** and **8**. See http://dx.doi.org/10.1039/b506178d

‡ CCDC reference numbers 265455–265457. See http://dx.doi.org/10.1039/b506178d for crystallographic data in CIF or other electronic format.

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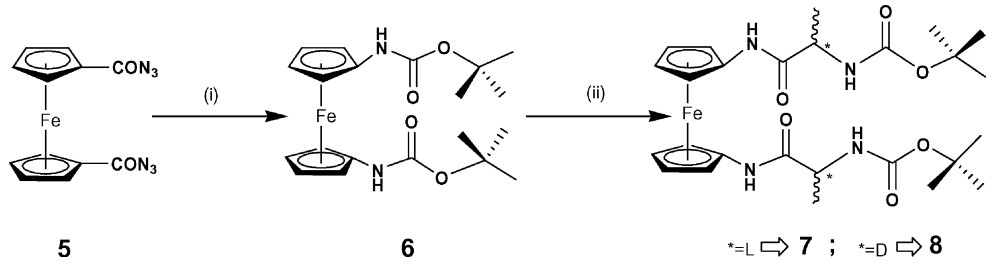


Scheme 1 Synthesis of 1,1′-bis(Boc-amino)ferrocene (**6**) *via* 1,1′-bis(carbonylazido)ferrocene (**5**), and its amino acid derivatives with L-Ala (**7**) and D-Ala (**8**). (i) Reflux in *t*-BuOH, 80 °C, 8 h; (ii) a) TFA, b) TEA, c) Boc-Ala-OH activated by EDC/HOBt.

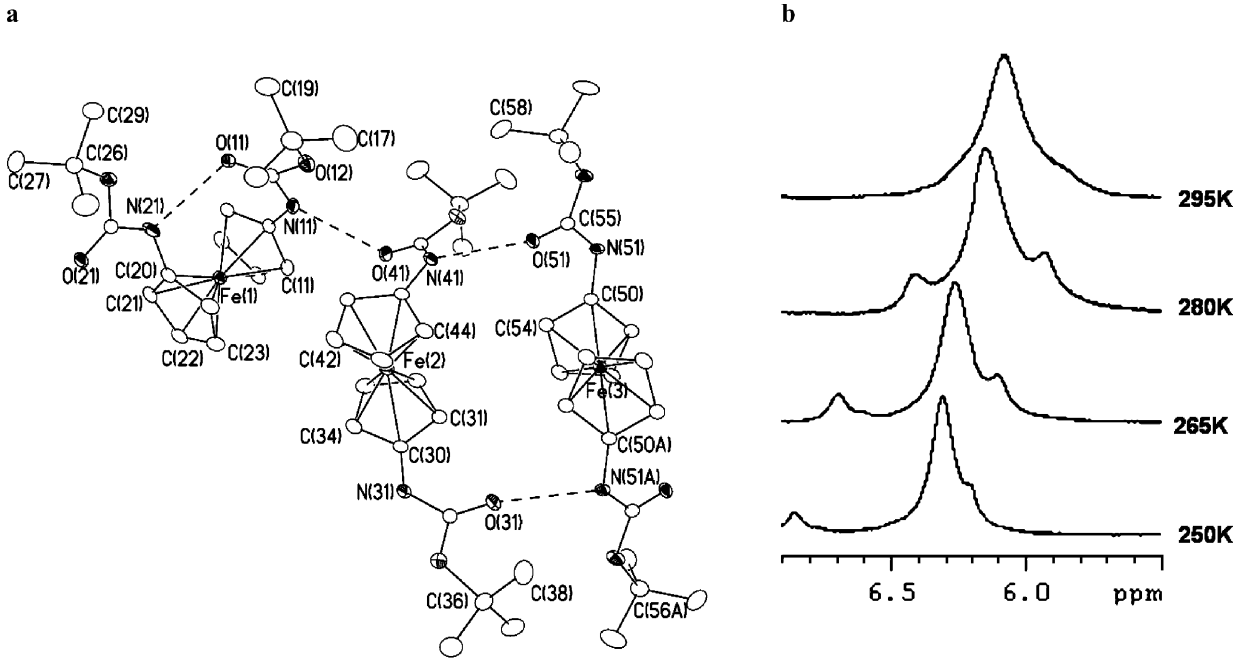


Fig. 1 (a) ORTEP diagram of 1,1′-bis(*tert*-butoxycarbonylamino)ferrocene (**6**) showing three rotamers. Intra- and intermolecular hydrogen bonds are indicated by dotted lines. Relevant bond distances [Å] and angles [°]: Fe(3)–C(Cp)$_{av}$ 1.99(6); N(31)–C(30) 1.509(4); N(31)–C(35) 1.417(4); N(41)–C(40) 1.423(4); N(41)–C(45) 1.471(4); C(35)–O(31) 1.203(4); C(35)–O(32) 1.411(4); C(45)–O(41) 1.217(3), C(45)–O(42) 1.384(3); Cp/Cp twist angle 0.3(1); β_{av} 4.7(2) [A: $1-x$, $1-y$, $-z$]. (b) Partial ^{1}H NMR spectrum recorded at different temperatures showing the amide N–H region of compound **6**, indicating the presence of at least three distinct rotamers in solution at low temperatures and facile interconversion between the rotamers at high temperatures.

Table 1 Summary of crystallographic data for 1,1′-bis(*tert*-butoxycarbonylamino)ferrocene (**6**), 1,1′-bis(*tert*-butoxycarbonyl-L-alanine-amido)-ferrocene (**7**) and 1,1′-bis(*tert*-butoxycarbonyl-D-alanine-amido)ferrocene (**8**)

	6	**7**	**8**
Chemical formula	$C_{20}H_{28}FeN_2O_4$	$C_{26}H_{38}FeN_4O_6$	$C_{26}H_{38}FeN_4O_6$
FW	416.29	558.45	558.45
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/n$	$C2$	$C2$
a (Å)	10.81160(10)	15.4790(3)	15.490(3)
b (Å)	11.80650(10)	9.3420(3)	9.3390(19)
c (Å)	40.9023(4)	11.0400(3)	11.036(2)
α (°)	90.00	90.00	90.00
β (°)	91.0490(4)	117.071(2)	117.09(3)
γ (°)	90.00	90.00	90.00
V (Å^3)	5220.19(8)	1421.53(7)	1421.3(5)
Z	10	2	2
D_{calcd} (g cm^{-3})	1.324	1.305	1.305
T (K)	173(2)	173(2)	173(2)
λ (Å)	0.71073	0.71073	0.71073
μ (mm^{-1})	0.748	0.575	0.575
Measured reflections	17111	7467	2796
Unique reflections	10080	4039	2795
Absolute structure parameter	n.a.	−0.002(12)	0.026(17)
$R\,[I > 2\,\sigma(I)]^a$	0.0476	0.0346	0.0393
wR (all data)^b	0.1136	0.0753	0.0810
GOOF on F^2	1.022	1.069	1.058

^{a} $R_1 = [\Sigma \| F_o | - | F_c \|]/[\Sigma | F_o |]$. ^{b} $wR_2 = \{[\Sigma w(F_o^2 - F_c^2)^2]/[\Sigma w(F_o^2)^2]\}^{1/2}$; $w = [\sigma(F_o^2) + (aP)^2 + (bP)]^{-1}$ where $P = [\mathrm{Max}(F_o^2,0) + 2\,F_c^2]/3$.

solution. At 250 K, the ^{1}H NMR spectrum shows the presence of three N–H signals of different intensities. Upon increasing the temperature to 295 K, the signals merge to a single peak, indicating rapid interconversion of the rotamers on the NMR time scale (Fig. 1b).

Compound **6** is a convenient starting material for the preparation of amino acid bioconjugates. The syntheses of the L-Ala and D-Ala conjugates 1,1′-bis(*tert*-butoxycarbonyl-L-alanineamido)ferrocene (**7**) and 1,1′-bis(*tert*-butoxycarbonyl-D-alanineamido)ferrocene (**8**) were readily achieved after deprotection of the amino group, followed by coupling with the corresponding Ala derivatives in the presence of a carbodiimide under inert atmosphere, to prevent the oxidation of the diaminoferrocene formed *in situ* (see Scheme 1). Compounds **7** and **8** were characterized by spectroscopic methods. Both compounds display a single broad absorption in the visible region with λ_{max} = 448 nm due to the Fc-based d–d transition, which experiences a bathochromic shift compared to the Boc-protected **6**. In the IR, a new signal appears at 1671 cm^{-1} due to the presence of the Fc-amide C=O, in addition to the signal due to the Boc C=O group at 1575 cm^{-1}. Similarly, the ^{13}C NMR spectra each exhibit two resonances in the carbonyl region at δ 171.7 (Fc-amide C=O) and at δ 155.6 (Boc C=O). The corresponding NH signals are observed at δ 9.00 for the Fc–NH–CO group and at δ 5.11 for the Boc-protected Ala. The α-H of Ala is observed as a multiplet at δ 4.36, while the Ala-CH_3 is observed upfield at δ 17.6.

The compounds readily crystallize from chloroform solution by slow diffusion of hexanes to produce yellow-orange needles. Two single crystal X-ray diffraction studies of these compounds were carried out showing that the compounds crystallize in the space group C_2 (Table 1).‡ A graphical representation of compound **8** is shown in Fig. 2. The Cp rings in both ferrocenoyl groups are coplanar [angle between Cp planes: 2.8(2)°]. The amides and Cp rings are twisted by 18.9(2)°. Importantly, the molecules maximize their H-bonding through formation of intermolecular and intramolecular N(H) · · · O=C-bonds. Two intramolecular H-bonds [2.805(6) Å] are formed between the amide groups connected to the Cp rings and the adjacent carbonyl of the Boc causing *M*-helicity of the compound.

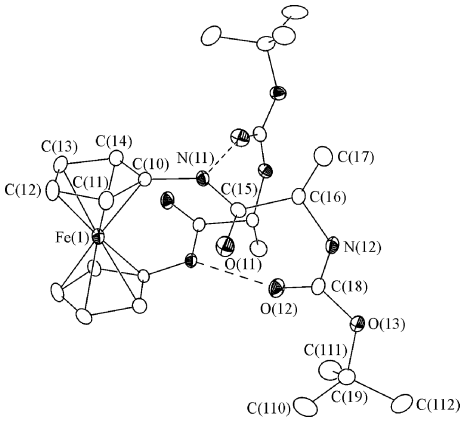
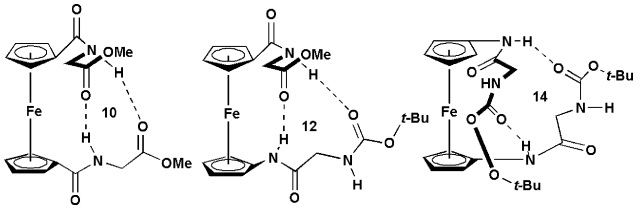


Fig. 2 ORTEP diagram of the X-ray single crystal structure of **8** (30% probability) showing intramolecular hydrogen bonds. Relevant bond distances (Å) and angles (°): Fe–C(Cp)$_{av}$ 2.040(5); N(11)–C(10) 1.419(4); N(11)–C(15) 1.349(3); C(15)–O(11) 1.224(3); C(15)–C(16) 1.536(4); C(16)–N(12) 1.445(3); Cp/Cp* twist angle 3.43(9); ct(Cp)–Fe–ct(Cp*) 159.7(9); β 19.08(9). [Symmetry transformation used to generate equivalent atoms, *: $-x$, y, $-z$. The labelling of the starred atoms has been omitted for clarity, ct = centroid].

Thus, the two podand amino acid substituents have the same arrangement as was observed for L- and D-amino acid conjugates of ferrocenedicarboxylic acid **2**[9] and for the conjugates of ferrocene amino acid **3**.[12] Importantly, for amino acid conjugates of compound **4**, intramolecular NH · · · O=C H-bonding results in the formation of a previously unobserved 14-membered H-bonded ring. Thus, the nature of the parent Fc derivative (**2–4**) appears to direct the size of the H-bonded ring, as depicted in Scheme 2. The hydrogen bonded ring size in peptide conjugates increases from 10 for the derivatives of **2**,[5] to 12 for the derivatives of **3**[12] to 14 for the derivatives of **4**. This is an important result, which may allow the use of ferrocenediamine as a scaffold in the rational design of structurally well-defined peptide assemblies.



Scheme 2 The intramolecular H-bonding pattern in amino acid conjugates of disubstituted Fc derivatives results in the formation of 10-membered rings for conjugates of **2**, 12-membered rings for conjugates of **3** and novel 14-membered rings for conjugates of **4**.

In addition, the molecules are engaged in intermolecular NH · · · O=C hydrogen bonds [2.825(6) Å, see Fig. 3], showing a honeycomb structure, which contrasts the helical arrangement for ferrocenedicarboxylic acid derivatives observed by Hirao and co-workers.[7] We decided to study the H-bonding ability of compounds **7** and **8** in solution. Variable temperature ^{1}H NMR spectroscopy at various concentrations allows the detection of both inter- and intramolecular H-bonding (see Fig. 4). Upon increasing the temperature, intermolecular H-bonding is weakened [−6.9 ppb K^{-1} for N(12)], while the intramolecular H-bonding, responsible for stabilizing the particular helicity about the Fc core exhibits a positive temperature behaviour (+4.2 ppb K^{-1}) (Fig. 4), which may indicate that as the intermolecular

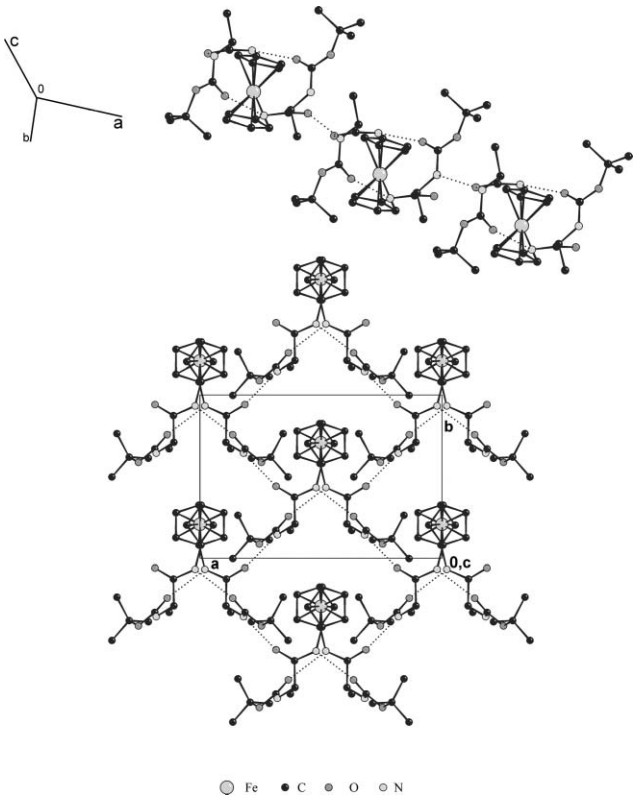


Fig. 3 Crystal structure of **8**, showing intra- and intermolecular H-bonding and hydrophobic interactions between ferrocene moieties and *tert*-butyl groups, viewed along the *a*-axis (top) and down the *c*-axis (bottom). Dotted lines indicate hydrogen bonding.

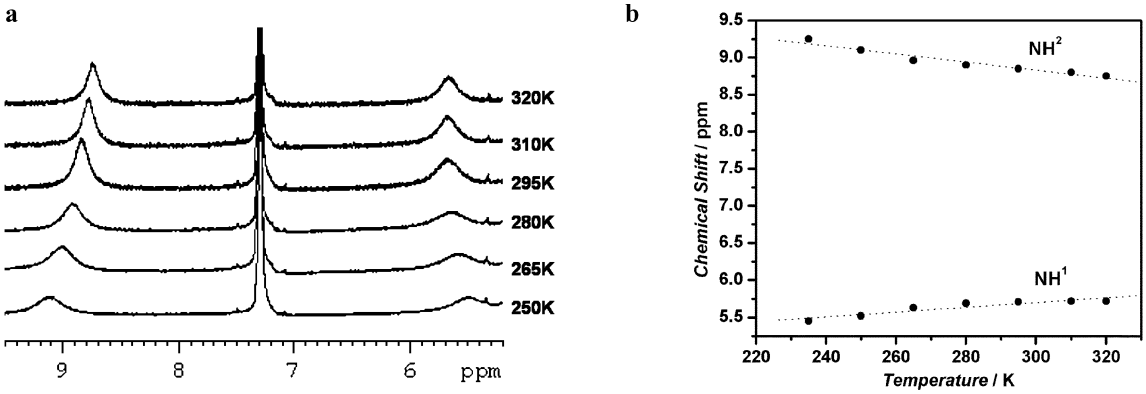


Fig. 4 (a) ^{1}H NMR spectra of compound **8** at different temperatures in 1 mM solution in $CDCl_3$. (b) Corresponding plot of chemical shift *vs.* temperature for compound **8**; **NH**^{1} (slope = +4.2 ppb K^{-1}) is the amide hydrogen attached to the Cp ring of ferrocene and **NH**^{2} (slope = −6.9 ppb K^{-1}) is the amide proton from alanine.

H-bonding is broken, the intramolecular H-bonding is strengthened. CD provides evidence that compounds **7** and **8** maintain their helicity in solution. The CD spectra for compounds **7** and **8** were measured in CH_2Cl_2 at a concentration of 1 mM and are shown in Fig. 5. As expected for the D and L forms, the two compounds give CD spectra that are the exact opposite of each other, indicating that a chiral rigid structure is maintained in solution. This necessitates the absence of free rotation about the Cp–Fe–Cp axis and the lack of free rotation of the podant ligand about the Cp–N bond. Thus, the presence of the intramolecular H-bonding between the amino acid substituents is crucial for maintaining the structure in solution and is seen as the major cause for chirality organization in these systems. Compound **7** is found to form a *P*-helical structure as indicated from the positive Cotton effect at the ferrocene function region (480 nm), as was observed by Metzler-Nolte for L-amino acid conjugates with 1,1′-ferrocenedicarboxylic acid. A negative Cotton effect of same intensity and in the same region was observed for **8**, which corresponds to the observation of Hirao's D-amino acid conjugates.[9]

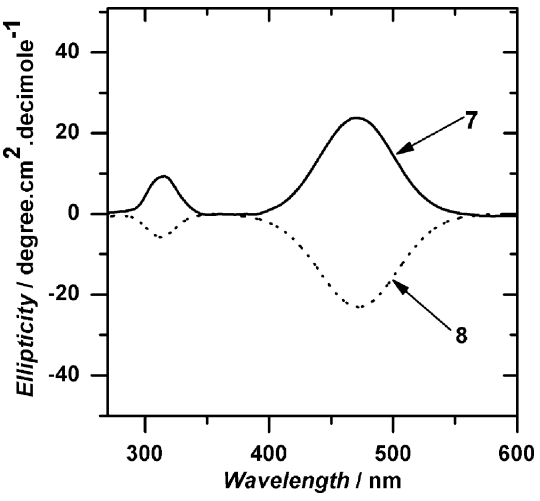


Fig. 5 CD spectra of 1,1′-bis(*tert*-butoxycarbonyl-L-alanine-amido)-ferrocene (**7**) and 1,1′-bis(*tert*-butoxycarbonyl-D-alanine-amido)-ferrocene (**8**) in 1 mM solution in CH_2Cl_2 showing opposite chirality with respect to ferrocene.

The electrochemical behavior of compounds **6–8** was studied by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) in CH_2Cl_2. The electrochemical parameters for compounds **6–8** are summarized in Table 2. All three compounds exhibit only quasi-reversible behavior with a Faradic current ratio of close to unity by large peak separations. The halfwave potential, $E_{1/2}$, for the Boc-derivative is observed at 306 mV (*vs.* Ag/AgCl), while the redox potentials for compounds **7** and **8** are shifted to higher oxidation potential.

Table 2 Electrochemical data for compound **6–8**[a]

Compound	$E_{1/2}$ (mV)	ΔE_p (mV)	i_a/i_c
6	306 (±5)	361	1.06
7	366 (±5)	151	1.11
8	370 (±5)	154	1.10

[a] Measured using a GCE working electrode *vs.* Ag/AgCl, scan rate 0.1 V s^{-1}, compounds **6**, **7** and **8** at 0.1 M in CH_2Cl_2, 0.1 M TBAP. The $E_{1/2}$ of the Fc/Fc^+ couple under the experimental conditions is 448 (±5) mV (*vs.* Ag/AgCl).

Conclusion

In conclusion, we provide a new synthetic approach to bis-Boc protected 1,1′-diaminoferrocene, a very convenient derivative of unstable diaminoferrocene, and show that this novel ferrocene compound can be converted cleanly to amino acid conjugates. Thus resulting bioconjugates show a specific secondary structure through the involvement of strong intramolecular H-bonding in the solid state as well as in solution, forming a 14-membered ring as is seen in antiparallel β-sheet peptides. The chirality of the amino acid is found to change the chiral organization of the peptide strands with respect to ferrocene.

Experimental

Synthesis and characterization

All syntheses were carried out in air unless otherwise indicated. CH_2Cl_2 (BDH; ACS grade) used for synthesis was dried (CaH_2) and distilled prior to use. $CDCl_3$ (Aldrich) was dried (CaH_2) and stored over molecular sieves (8–12 mesh; 4 Å effective pore size; Fisher) before use. EDC·HCl, HOBt (Quantum), $MgSO_4$, and $NaHCO_3$ (VWR) were used as received. For column chromatography, a column with a width of 2.7 cm (ID) and a length of 45 cm was packed 18–22 cm high with 230–400 mesh silica gel (VWR). For TLC, aluminium plates coated with silica gel 60 F_{254} (EM Science) were used. NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer using a 5 mm broadband probe operating at 500.134 MHz (^{1}H) and 125.766 MHz ($^{13}C\{^1H\}$). Peak positions in both ^{1}H and ^{13}C spectra are reported in ppm relative to TMS. The ^{1}H NMR spectra are referenced to the residual $CHCl_3$ signal at δ 7.27. All $^{13}C\{^1H\}$ spectra are referenced to the $CDCl_3$ signal at δ 77.23.

Mass spectrometry was carried out on a VG Analytical 70/20 VSE instrument. Infrared spectra were obtained in KBr and recorded with a Perkin-Elmer model 1605 FT-IR (resolution: 4 cm^{-1}).

1,1′-Bis(*tert*-butoxycarbonylamino)ferrocene (6)

1,1′-Bis(azidocarbonyl)ferrocene (1.52 g, 10 mmol) was refluxed in *tert*-butanol at 80 °C for 8 h. After rotoevaporation of *tert*-butanol, the crude product was purified by flash column chromatography using hexane–ethyl acetate (3.5:1.0; $R_f = 0.35$), and dried under reduced pressure to give 1.70 g (72%) of compound **1**. HRMS (ES): calc. for $C_{20}H_{28}N_2O_4Fe$: 416.1449; found: 416.1410 $[M]^+$. FT-IR (KBr, cm^{-1}): 3316 (b, s, N–H), 1695 (s, C=O), 1542 (s, Amid I). UV-vis (CH_2Cl_2; λ in nm [ε in M^{-1} cm^{-1}]): 442 [154]. ^{1}H-NMR ($CDCl_3$, δ/ppm): 6.06 (br s, 2H, NH), 4.36 (s, 4H, *ortho*-H of Cp), 3.99 (s, 4H, *meta*-H of Cp), 1.49 (s, 18H, CH_3 of Boc). $^{13}C\{^1H\}$-NMR ($CDCl_3$, δ/ppm): 153.2 (C=O), 95.0 (*ipso*-C Fc), 79.3 (*ortho*-C of Fc), 68.3 (*ortho*-C of Fc), 64.4 (*meta*-C of Fc), 62.1 (*meta*-C of Fc), 62.0 (tertiary C of Boc), 27.1 (CH_3 of Boc).

1,1′-Bis(*tert*-butoxycarbonyl-L-alanine-amido)ferrocene (7)

1,1′-Bis(*tert*-butoxycarbonylamino)ferrocene (**6**) (0.42 g, 1 mmol) was dissolved in CH_2Cl_2 (2 ml) and cooled to 0 °C. TFA (2 mL) was added dropwise under a nitrogen atmosphere. Then the ice bath was removed and stirring was continued. After 30 min, CH_2Cl_2 (10 mL) was added and the solution was cooled before an excess Et_3N was added dropwise. To this, a solution of Boc-L-Ala-OH. (0.38 g, 2 mmol), HOBt (0.38 g, 2.5 mmol), and EDC·HCl (0.48 g, 2.5 mmol) in dry CH_2Cl_2) was added. The reaction mixture was stirred for 12 h at room temperature and then washed consecutively with saturated aqueous sodium bicarbonate, 10% aqueous citric acid, saturated sodium bicarbonate, and water. The organic phase was separated and dried over Na_2SO_4, filtered and then evaporated to dryness. After purification by flash column chromatography (hexane–ethyl acetate 3:1; R_f = 0.3) the product was obtained in 77% yield (0.43 g) as an orange crystalline solid. HRMS (ES): calc. for $C_{26}H_{38}N_4O_6Fe$: 558.2210; found: 558.2181 $[M]^+$. FT-IR (KBr, cm^{-1}): 3284 (b, s, N–H), 1671 (s, C=O), 1575 (s, C=O), 1549 (s, Amid II). UV-vis (CH_2Cl_2; λ in nm [ε in M^{-1} cm^{-1}]): 448 [250]. ^{1}H-NMR ($CDCl_3$, δ/ppm): 9.00 (1H, s, NH of Fc), 5.39 (1H, s, *ortho*-H of Fc), 5.11 (1H, d, $J = 7$ Hz, NH Ala), 4.36 (1H, m, α CH Ala), 4.11 (1H, s, *ortho*-H of Fc), 3.98 (1H, s, *meta*-H of Cp), 3.93 (1H, s, *meta*-H of Cp), 1.48 (9H, s, CH_3 of Boc), 1.35 (3H, d, $J = 7$ Hz, CH_3 of Ala). $^{13}C\{^1H\}$-NMR ($CDCl_3$, δ/ppm): 171.7 (C=O), 155.5 (C=O of Boc), 95.1 (*ipso*-C of Cp), 80.2 (tertiary C of Boc), 64.8 (*ortho*-C of Cp), 63.8 (*ortho*-C of Cp), 61.7 (*meta*-C of Cp), 60.0 (*meta*-C of Cp), 50.1(α-C of Ala), 27.6 (CH_3 of Boc), 17.6 (CH_3 of Ala).

1,1′-Bis(*tert*-butoxycarbonyl-D-alanine-amido)ferrocene (8)

The synthetic procedure was identical to that reported for the synthesis of compound **7**. Column chromatography using hexane–ethyl acetate 3:1; R_f = 0.35. HRMS (ES): calc. for $C_{26}H_{38}N_4O_6Fe$: 558.2210; found: 558.2182 $[M]^+$. FT-IR (KBr, cm^{-1}): 3284 (b, s, N–H), 1671 (s, C=O), 1575 (s), 1548 (s, Amid II). UV-vis (CH_2Cl_2; λ in nm [ε in M^{-1} cm^{-1}]): 448 [240]. ^{1}H-NMR ($CDCl_3$, δ/ppm): 9.00 (1H, s, NH of Fc), 5.39 (1H, s, *ortho*-H of Cp), 5.11 (1H, d, $J = 7$ Hz, NH Ala), 4.36 (1H, m, α-CH of Ala), 4.11 (1H, s, *ortho*-H of Cp), 3.98 (1H, s, *meta*-H of Cp), 3.93 (1H, s, *meta*-H of Cp), 1.49 (9H, s, CH_3 of Boc), 1.35 (3H, d, $J = 7$ Hz, CH_3 Ala). $^{13}C\{^1H\}$-NMR ($CDCl_3$, δ/ppm): 171.7 (C=O), 155.6 (C=O of Boc), 95.2 (*ipso*-C of Fc), 80.7 (tertiary C of Boc), 65.6 (*ortho*-C of Cp), 64.5 (*ortho*-C of Cp), 62.5 (*meta*-C of Cp), 60.9 (*meta*-C of Cp), 50.8 (α-CH Ala), 28.4 (CH_3 of Boc), 17.5 (CH_3 of Ala).

CD measurements

CD spectra were recorded using a PiStar-180 spectropolarimeter (from Applied Biophysics) in the CH_2Cl_2 solution with the concentration (1.0×10^{-3} M) by using a quartz cell of path length 1 cm under an argon atmosphere at 25 °C.

X-Ray crystallography

Suitable crystals of compounds **6** (yellow; 0.14 × 0.11 × 0.09 mm), **7** (yellow; 0.25 × 0.20 × 0.15 mm) and **8** (reddish yellow; 0.25 × 0.20 × 0.13 mm) were obtained from hexane-layered solutions of the compounds in chloroform. All measurements were made on a Nonius KappaCCD 4-Circle Kappa FR540C diffractometer using monochromated Mo-K_α radiation ($\lambda = 0.71073$ Å) at −100 °C. An initial orientation matrix and cell was determined from 10 frames using ϕ scans. Data were measured using ϕ- and ω-scans and were processed using the standard Nonius software.[19–20] The structures were solved using direct methods (**6**: SHELXS-97[21]; **7,8**: SIR-97[22]) and refined by the full-matrix least-squares method on F^2 with SHELXL97-2.[23] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included at geometrically idealized positions (C–H bond distances 0.95/0.99 Å; N–H bond distances 0.88 Å) and were not refined. The isotropic thermal parameters of the hydrogen atoms were fixed at 1.2 times that of the preceding carbon or nitrogen atom.

Electrochemical measurements

The electrochemical experiments were carried out at room temperature using a CV-50W voltammetric analyzer. A gold electrode (diameter 50 μm) was used as the working electrode. 1 mM solutions of compounds **6–8** were prepared in 0.1 M tetrabutylammonium perchlorate (TBAP) in CH_2Cl_2. Cyclic voltammetry (CV) experiments were carried out at a scan rate of 100 mV s^{-1}. Differential pulse voltammetry (DPV) was carried out at 10 mV s^{-1}. A platinum wire (1 mm) was used as the counter electrode and a Ag/AgCl (BAS) was used as the reference electrode. The $E_{1/2}$ of the Fc/Fc+ couple under the experimental conditions is 448 (±5) mV (*vs*. Ag/AgCl).

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