Spectroscopic and Theoretical Study of Asymmetric 1,1′-Diaminoferrocene Conjugates of α-Amino Acids

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The synthesis and characterization of asymmetric 1,1′-diaminoferrocene conjugates of α-amino acids Boc-AA-NH-Fn-NH-Ac [AA = Gly (**10**), Ala (**11**), D-Ala (**12**), Val (**13**), Fn = 1,1′-ferrocenediyl] is reported. The conformational preferences of these organometallic peptidomimetics in solution are determined experimentally by circular dichroism spectroscopy, IR spectroscopy, and NMR spectroscopy and corroborated theoretically by DFT calculations.

Introduction

Understanding and control of secondary structures of proteins, peptides, and oligoamides is fundamentally important for designing functional peptidic materials,[1–4] i.e., in the field of molecular machines.[5] In these studies the use of molecular templates (scaffolds) is a widely exploited strategy.[6] These scaffolds nucleate or propagate a certain conformation from their ordered region through a substructure portion consisting of natural amino acids to form α-helices or *β*-sheet structures. In this context 1,1′-disubstituted ferrocene conjugates with amino acids or short peptides have been successfully used as redox-active scaffolds with an ideal distance between cyclopentadienyl rings (ca. 3.3 Å) to form turn elements on the basis of intramolecular hydrogen bonding interactions (IHBs).[6,7] Asymmetrically 1,1′-disubstituted and chiral ferrocene derivatives also play a major role in catalytic applications.[8–10] Symmetrically substituted ferrocene derivatives based on ferrocene-1,1′-dicarboxylic acid have been extensively investigated by Herrick, Hirao, Kraatz, and others (Scheme 1, type I)[11–23] as have conjugates of 1′-aminoferrocene-1-carboxylic acid (Scheme 1, type II).[24–34] Recently, the first symmetrical bioconjugates of 1,1′-diaminoferrocene have been reported by Kraatz et al. (Scheme 1, type III).[35] In symmetrical ferrocene bioconjugates two IHBs are simultaneously formed between the two amino acid substituents at the two cyclopentadienyl rings. The situation is more complicated in asymmetric conjugates with only one amino acid substituent attached to one cyclopentadienyl ring

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(1) Klok, H.-A. *Angew. Chem., Int. Ed.* **2002**, *41*, 1509–1513.
(2) Kanamori, D.; Okamura, T.; Yamamoto, H.; Ueyama, N. *Angew. Chem., Int. Ed.* **2005**, *44*, 969–972.
(3) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893–4012.
(4) Huc, I. *Eur. J. Org. Chem.* **2004**, 17–29.
(5) Kay, E. R.; Leigh, D. A.; Zerbetto, F. *Angew. Chem., Int. Ed.* **2007**, *46*, 72–191.
(6) Moriuchi, T.; Hirao, T. *Chem. Soc. Rev.* **2004**, *33*, 294–301.
(7) Van Staveren, D. R.; Metzler-Nolte, N. *Chem. Rev.* **2004**, *104*, 5931–5986.
(8) Arrayás, R. G.; Adrio, J.; Carretero, J. C. *Angew. Chem., Int. Ed.* **2006**, *45*, 7674–7715.
(9) Siemeling, U.; Auch, T.-C. *Chem. Soc. Rev.* **2005**, *34*, 584–594.
(10) Atkinson, R. C. J.; Gibson, V. C.; Long, N. J. *Chem. Soc. Rev.* **2004**, *33*, 313–328.
(11) Kuik, A.; Skoda-Földes, R.; Jánosi, L.; Kollár, L. *Synthesis* **2007**, 1456–1458.
(12) Chowdhury, S.; Sanders, D. A. R.; Schatte, G.; Kraatz, H.-B. *Angew. Chem., Int. Ed.* **2006**, *45*, 751–754.
(13) Chowdhury, S.; Schatte, G.; Kraatz, H.-B. *Eur. J. Inorg. Chem.* **2006**, 988–993.
(14) Moriuchi, T.; Nagai, T.; Hirao, T. *Org. Lett.* **2006**, *8*, 31–34.
(15) Orlowski, G. A.; Chowdhury, S.; Long, Y.-T.; Sutherland, T. C.; Kraatz, H.-B. *Chem. Commun.* **2005**, 1330–1332.
(16) Moriuchi, T.; Nagai, T.; Hirao, T. *Org. Lett.* **2005**, *7*, 5265–5268.
(17) Chowdhury, S.; Schatte, G.; Kraatz, H.-B. *Dalton Trans.* **2004**, 1726–1730.
(18) Van Staveren, D. R.; Weyhermüller, T.; Metzler-Nolte, N. *Dalton Trans.* **2003**, 210–220.
(19) Moriuchi, T.; Yoshida, K.; Hirao, T. *Org. Lett.* **2003**, *5*, 4285–4288.
(20) Moriuchi, T.; Nomoto, A.; Yoshida, K.; Hirao, T. *Organometallics* **2001**, *20*, 1008–1013.
(21) Moriuchi, T.; Nomoto, A.; Yoshida, K.; Ogawa, A.; Hirao, T. *J. Am. Chem. Soc.* **2001**, *123*, 68–75.
(22) (a) Georgopoulou, A. S.; Mongos, D. M. P.; White, A. J. P.; Williams, D. J.; Horrocks, B. R.; Houlton, A. *J. Chem. Soc., Dalton Trans.* **2000**, 2969–2974. (b) Nomoto, A.; Moriuchi, T.; Yamazaki, S.; Ogawa, A.; Hirao, T. *Chem. Commun.* **1998**, 1963–1964.
(23) Herrick, R. S.; Jarret, R. M.; Curran, T. P.; Dragoli, D. R.; Flaherty, M. B.; Lindyberg, S. E.; Slate, R. A.; Thornton, L. C. *Tetrahedron Lett.* **1996**, *37*, 5289–5292.
(24) Heinze, K.; Siebler, D. *Z. Anorg. Allg. Chem.* **2007**, *633*, 2223–2233.
(25) Mahmoud, K. A.; Kraatz, H.-B. *Chem.−Eur. J.* **2007**, *13*, 5885–5895.
(26) Heinze, K.; Wild, U.; Beckmann, M. *Eur. J. Inorg. Chem.* **2007**, 617–623.
(27) Barišić, L.; Rapić, V.; Metzler-Nolte, N. *Eur. J. Inorg. Chem.* **2006**, 4019–4021.
(28) Chowdhury, S.; Schatte, G.; Kraatz, H.-B. *Angew. Chem., Int. Ed.* **2006**, *45*, 6882–6884.
(29) Barišić, L.; Čakić, M.; Mahmoud, K. A.; Liu, Y.; Kraatz, H.-B.; Pritzkow, H.; Kirin, S. I.; Metzler-Nolte, N.; Rapić, V. *Chem.−Eur. J.* **2006**, *12*, 4965–4980.
(30) Heinze, K.; Beckmann, M. *Eur. J. Inorg. Chem.* **2005**, 3450–3457.
(31) Heinze, K.; Schlenker, M. *Eur. J. Inorg. Chem.* **2005**, 66–71.
(32) Barišić, L.; Dropučić, M.; Rapić, V.; Pritzkow, H.; Kirin, S. I.; Metzler-Nolte, N. *Chem. Commun.* **2004**, 2004–2005.
(33) Heinze, K.; Schlenker, M. *Eur. J. Inorg. Chem.* **2004**, 2974–2988.
(34) Okamura, T.; Sakauye, K.; Ueyama, N.; Nakamura, A. *Inorg. Chem.* **1998**, *37*, 6731–6736.
(35) Chowdhury, S.; Mahmoud, K. A.; Schatte, G.; Kraatz, H.-B. *Org. Biomol. Chem.* **2005**, *3*, 3018–3023.

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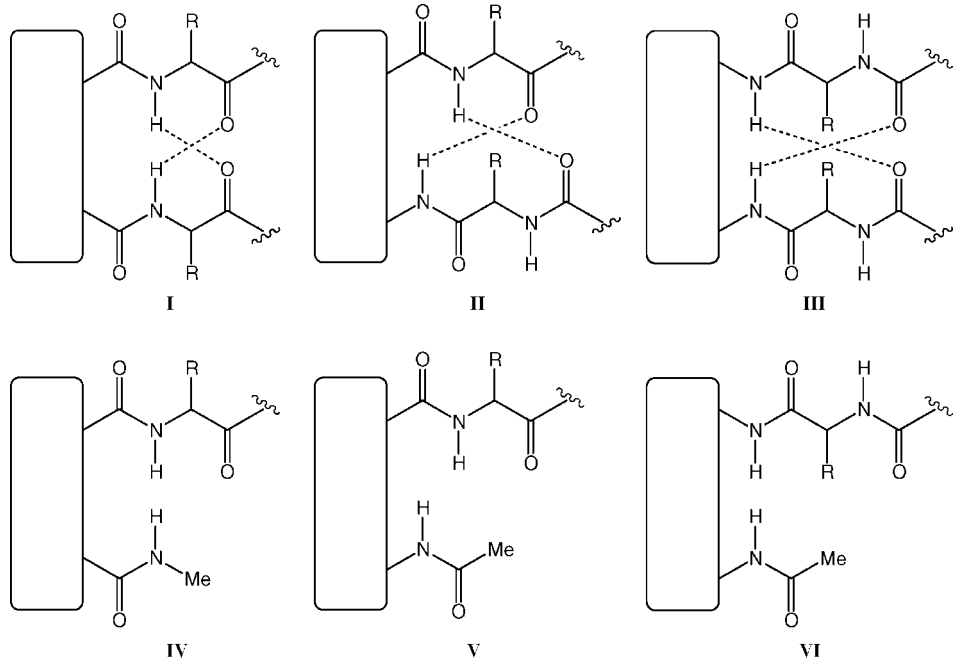
Scheme 1. Ferrocene-Derived Double-Strand Oligoamides (Types I, II, and III) and Single-Strand Oligoamides (Types IV, V, and VI)[a]



[a] The rectangle symbolizes a ferrocene-1,1′-diyl scaffold.

(Scheme 1, types IV, V, and VI). The asymmetric variants (types IV and V) have been recently investigated by us and are best described by an ensemble of conformers each having a single IHB in solution.[30,36] However, some conformations are significantly preferred over others in solution.[30,36]

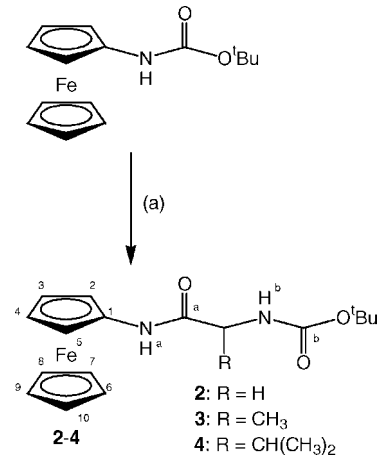
Here we describe our efforts to understand the folding and conformational preferences of asymmetric conjugates of type VI derived from 1,1′-diaminoferrocene and α-amino acids. To accomplish this task a combined experimental and theoretical investigation has been performed with CD, IR, and NMR spectroscopic techniques in combination with DFT calculations.

Results and Discussion

Synthesis of Reference Compounds 2, 3, and 4. For reference and comparison purposes the simple monosubstituted ferrocene derivatives **1**−**4** which are unable to engage in IHBs are very useful. *N*-Ferrocenylacetamide (Fc-NH-Ac, **1**) was prepared according to literature procedures.[33,37] The synthesis of amino acid conjugates of aminoferrocene **2**−**4** which serve as reference compounds for NMR analysis is depicted in Scheme 2. The Ala derivative **3** has been previously prepared.[29] Boc-protected aminoferrocene Fc-NH-Boc[29] is deprotected in situ with gaseous HCl and the resulting aminoferrocene[33] is subsequently coupled with Boc-protected amino acids Boc-Gly-OH, Boc-Ala-OH, and Boc-Val-OH, respectively, using standard EDC/HOBt coupling techniques [EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOBt = 1-hydroxybenzotriazole].[38] Relevant ^{1}H NMR spectroscopic data of **1**−**4** are summarized in Table 1.

Synthesis of Type VI Compounds 10, 11, 12, and 13. The synthesis of amino acid conjugates of 1,1′-diaminoferrocene is shown in Scheme 3. 1′-Acetamidoferrocene-1-carboxylic acid **5**[33,34] is transformed into azide **6** with use of sodium azide after activation with ethyl chloroformate and triethylamine in 56% isolated yield. *tert*-Butyl 1-(acetylamino)-1′-ferrocenylcarbamate **7** is obtained by Curtius rearrangement of azide **6** in *tert*-butyl alcohol at 70 °C. As a side-product the symmetrical urea

Scheme 2. Synthesis of Amino Acid Conjugates of Aminoferrocene 2–4[a]



[a] Reagents and conditions: (a) (1) HCl(g)/EtOAc; (2) EDC/HOBt, CH_2Cl_2; (3) Boc-AA-OH, CH_2Cl_2 (Boc-AA-OH = Boc-Gly-OH, Boc-Ala-OH, Boc-Val-OH).

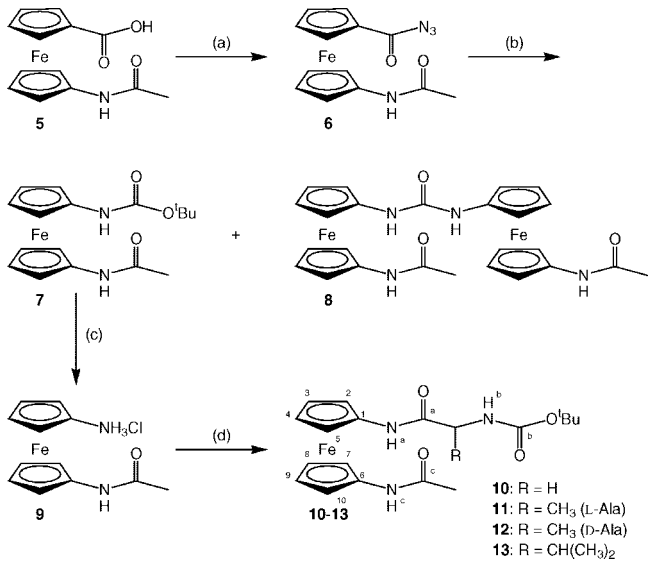
Table 1. Chemical Shift Data of Amide Protons of Reference Compounds 1–4 in $CDCl_3$[a]

	1	2	3	4
δ(NH, $CDCl_3$)/ppm	6.49	5.45; 6.68	5.55; 6.83	5.27; 7.14
δ(NH, [D_6]-DMSO)/ppm	9.28	7.00; 9.23	7.00; 9.28	6.78; 9.33
Δδ/ppm = δ(NH, [D_6]-DMSO) − δ(NH, $CDCl_3$)	2.79	1.55; 2.55	1.45; 2.45	1.51; 2.19

[a] In the sequence NH_b, NH_a. Atom numbering is shown in Scheme 2.

(36) Lapić, J.; Siebler, D.; Heinze, K.; Rapić, V. *Eur. J. Inorg. Chem.* **2007**, 2014–2024.
(37) Hall, D. W.; Richards, J. H. *J. Org. Chem.* **1963**, *28*, 1549–1554.
(38) *Fmoc Solid Phase Peptide Synthesis* Chan, W. C., White, P. D., Eds.; Oxford University Press: Oxford, UK, 2000.

Scheme 3. Synthesis of Amino Acid Conjugates of 1,1′-Diaminoferrocene 10–13[a]



[a] Reagents and conditions: (a) (1) $ClCOOEt/NEt_3$, acetone; (2) NaN_3, H_2O; (b) *tert*-BuOH, 70°C; (c) HCl(g)/EtOAc; (d) (1) EDC/HOBt, CH_2Cl_2; (2) Boc-AA-OH, CH_2Cl_2 (Boc-AA-OH = Boc-Gly-OH, Boc-Ala-OH, Boc-D-Ala-OH, Boc-Val-OH).

Table 2. UV/Vis and CD Spectroscopic Data of 11, 12, and 13 in CH_2Cl_2 and CH_2Cl_2/DMSO (20% v/v) (10^{-3} M)

	solvent	λ_{max}/nm (ϵ/$M^{-1}cm^{-1}$)	λ_{max}/nm (M_θ /deg $mM^{-1}cm^{-1}$)
11	CH_2Cl_2	447 (930)	469 (+30540)
	CH_2Cl_2/DMSO	447 (910)	469 (+28930)
12	CH_2Cl_2	448 (885)	469 (–30130)
	CH_2Cl_2/DMSO	448 (865)	469 (−28720)
13	CH_2Cl_2	448 (895)	469 (+34470)
	CH_2Cl_2/DMSO	448 (880)	469 (+23300)

derivative **8** is formed in ca. 10% yield.[39,40] Monitoring of this reaction by thin layer chromatography proved to be difficult because compounds **6** and **7** possess almost identical R_f values. Thus product mixtures are used in the following coupling step and the products of this reaction could be easily separated from **7** by TLC. To minimize formation of this undesired side-product reduction of reaction time and temperature proved to be beneficial. Boc-protected **7** is quantitatively deprotected by gaseous HCl in ethyl acetate and the resulting hydrochloride is treated with excess NEt_3 and coupled with Boc-protected natural amino acids Boc-Gly-OH, Boc-Ala-OH, Boc-D-Ala-OH, and Boc-Val-OH, respectively, using the EDC/HOBt protocol[38] to give Boc-Gly NH-Fn-NH-Ac (**10**, 50%), Boc-Ala-NH-Fn-NH-Ac (**11**, 57%), Boc-D-Ala-NH-Fn-NH-Ac (**12**, 54%), and Boc-Val-NH-Fn-NH-Ac (**13**, 58%). All amino acid conjugates were characterized by NMR, IR, UV/vis, and CD spectroscopy (if appropriate) as well as high-resolution mass spectrometry (Tables 2–4 and 6–8).

Conformational Analysis of 10, 11, 12, and 13. All amino acid conjugates **11**−**13** display the typical ferrocene absorption band around 448 nm in pure CH_2Cl_2 and CH_2Cl_2/DMSO mixtures (Table 2). CD spectroscopy provides evidence that chiral amino acid conjugates **11**−**13** possess helical ferrocene

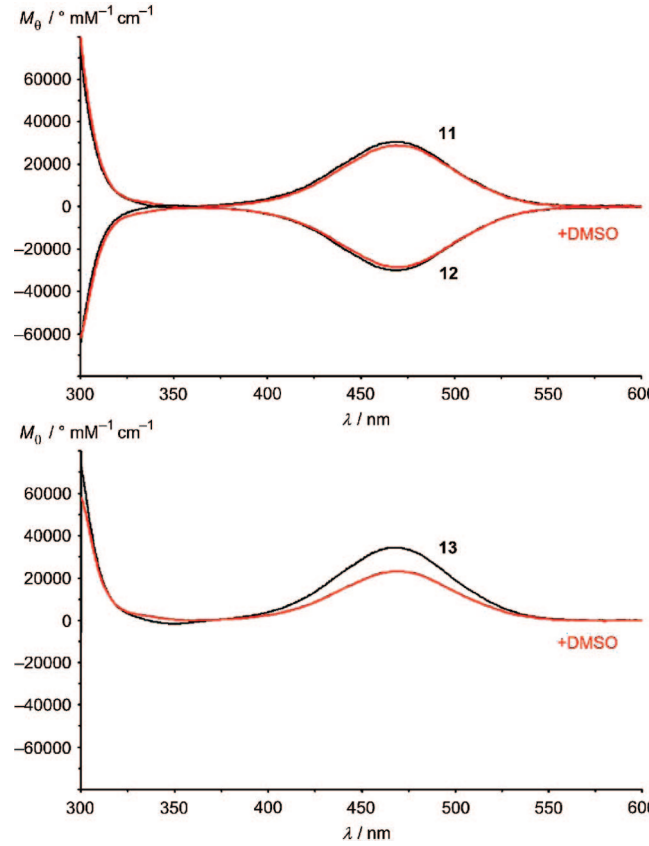


Figure 1. CD spectra of **11**, **12**, and **13** in CH_2Cl_2 (black) and CH_2Cl_2/DMSO (red) (20% v/v).

Table 3. IR Spectroscopic Data in CH_2Cl_2 (ca. 10^{-3} M) and HR-EI Mass Spectrometric Data of 10, 11, and 13

	10	**11**	**13**
ν_{NH}(free)/cm^{-1}	3428 (m)	3426 (m)	3426 (m)
ν_{NH}(assoc)/cm^{-1}	3328 (m)	3333 (m)	3308 (m)
ν(amide I)/cm^{-1}	1710 (sh), 1679 (vs)	1710 (sh), 1679 (vs)	1710 (sh), 1679 (vs)
ν(amide II)/cm^{-1}	1534 (s), 1505 (sh)	1535 (s), 1499 (m)	1563 (s), 1532 (m), 1500 (sh)
molecular formula	$C_{19}H_{25}N_3O_4Fe$	$C_{20}H_{27}N_3O_4Fe$	$C_{22}H_{31}N_3O_4Fe$
m/z (obs)	415.1194	429.1331	457.1662
m/z (calcd)	415.1218	429.1351	457.1664

moieties in CH_2Cl_2 solution as Cotton effects are observed at the ferrocene absorption band at 469 nm (Table 2). Generally, the CD maxima are found at lower energy than the UV/vis maxima which has been ascribed to the electronic splitting of the ferrocene absorption band.[41] As expected for enantiomers the CD spectra of L-Ala and D-Ala derivatives **11** and **12** are mirror images of each other (Figure 1, top). Positive Cotton effects have been associated with *P*-helical ferrocene units while negative Cotton effects are considered to be indicative of *M*-helical ferrocene chromophores.[6,42] As can be seen from Figure 1, L-amino acids induce *P*-helicity of the ferrocene as has also been shown by Kraatz for symmetrical 1,1′-disubstituted ferrocene derivatives Fn(-NH-Ala-Boc)$_2$ of L-Ala and D-Ala.[35] This is also consistent with findings for type I, type II, type IV, and type V conjugates. The helicity is induced by intramolecular hydrogen bonds (IHBs) between the two substituents at the

(39) Mahmoud, K.; Long, Y.-T.; Schatte, G.; Kraatz, H.-B. *J. Organomet. Chem.* **2004**, *689*, 2250–2255.
(40) Lapić, J.; Pavlović, G.; Siebler, D.; Heinze, K.; Rapić, V. *Organometallics* **2008**, *27*, 726–735.
(41) Falk, H.; Krasa, C.; Schlögl, K. *Monatsh. Chem.* **1969**, *100*, 1552–1563.
(42) Kirin, S. I.; Kraatz, H.-B.; Metzer-Nolte, N. *Chem. Soc. Rev.* **2006**, *35*, 348–354.

Table 4. Solvent and Concentration Dependent Chemical Shift Data of Amide Protons of 10, 11, and 13[a]

	10	11	13
δ(NH, $CDCl_3$)/ppm	5.52 (t); 7.88 (s); 8.14 (s)	5.33 (d); 8.08 (s); 8.19 (s)	5.25 (d); 7.98 (s); 7.98 (s)
δ(NH, [D_6]-DMSO)/ppm	6.98 (t); 9.08 (s); 9.16 (s)	6.99 (d); 9.07 (s); 9.22 (s)	6.75 (d); 9.09 (s); 9.27 (s)
$\Delta\delta$/ppm = δ(NH, [D_6]-DMSO) − δ(NH, $CDCl_3$)	1.46; 1.20; 1.02	1.66; 0.99; 1.03	1.50; 1.11; 1.29
v.r. (reference compound)	0.94 (**2**); 0.47 (**2**), 0.37 (**1**)	1.14 (**3**); 0.40 (**3**); 0.37 (**1**)	0.99 (**4**); 0.51 (**4**); 0.46 (**1**)
δ_0(NH, $CDCl_3$)/ppm[b]	5.23; 7.26; 7.56	5.03; 7.53; 7.59	5.06; 7.40; 7.45

[a] In the sequence NH_b, NH_a, NH_c. Atom numbering is shown in Scheme 3. [b] Measured in the concentration range 1–70 mM and extrapolated to infinite dilution by linear regression.

Table 5. DFT Calculated Relative Energies and (N)H···O Distances of Hydrogen Bonds of Conformers of 11

		intrachain hydrogen bond			interchain hydrogen bond		
	E_{rel}/kJ mol^{-1}	NH	CO	OH/Å	NH	CO	OH/Å
A−M	16.7				c	b	1.86
A−P	2.3				c	b	1.87
B−M	9.4	a	b	1.84	c	a	1.92
B−P	0.0	a	b	1.95	c	a	1.92
C−M	2.0				a	c	1.90
C−P	4.0				a	c	1.86
D−M	6.9				b	c	1.98
D−P	19.5				b	c	1.94
open1	22.6	a	b	1.86			
open2	12.7	a	b	1.97			
open3	20.8						
open4	9.4	a	b	1.96			

Table 6. ^{1}H NMR Data of 10, 11, and 13 in $CDCl_3$

	10	11	13
NH_a	8.14 (s, 1H)	8.19 (s, 1H)	7.98 (s, 1H)
NH_b	5.52 (t, 1H, ^3J = 5.65 Hz)	5.33 (br d, 1H)	5.25 (br d, 1H)
NH_c	7.88 (s, 1H)	8.08 (s, 1H)	7.98 (s, 1H)
Cp-H	4.48 (s, 2H)	4.56 (s, 2H)	4.55 (s, 2H)
	4.45 (s, 2H)	4.37 (s, 1H)	4.35 (s, 1H)
	4.02 (s, 4H)	4.29 (s, 1H)	4.29 (s, 1H)
		3.98 (s, 4H)	3.99 (s, 4H)
CH_α	3.83 (d, 2H, ^3J = 5.65 Hz)	4.09 (m, 1H)	3.91 (pt, 1H)
$COCH_3$	2.06 (s, 3H)	2.07 (s, 3H)	2.08 (s, 3H)
CCH_3	1.45 (s, 9H)	1.44 (s, 9H)	1.44 (s, 9H)
R		1.39 (d, 3H, ^3J = 7.15 Hz)	2.14 (br m, 1H)
			1.00 (d, 3H, ^3J = 6.06 Hz)
			0.97 (d, 3H, ^3J = 6.56 Hz)

ferrocene. Addition of the coordinating solvent DMSO (20% v/v), which might compete for hydrogen bonds, leaves the Cotton effects almost unchanged indicating that the average helicity of the ferrocene remains constant even in the presence of the hydrogen bond disrupting solvent DMSO.

To elucidate preferred folded conformations with IHBs or association phenomena detailed IR and NMR spectroscopic analyses were performed.[43,44] In CH_2Cl_2 solution ($c = 10^{-3}$ M) signals for NH stretching vibrations are observed around 3427 and 3320 cm^{-1}, both with medium intensity indicative of free and hydrogen-bonded NH groups, respectively (Table 3). However, intra- and intermolecular hydrogen bonds are indistinguishable by IR spectroscopy.

In the ^{1}H NMR spectra of **10**−**13** NH_b amide protons are easily assigned on the basis of their distinct coupling patterns to the H_α protons of the amino acid substituent, i.e., a triplet is observed for the glycine derivative **10** and doublets are observed for alanine and valine derivatives **11**−**13**. For **10** and **11** the chemical shifts of NH_a and NH_c are sufficiently different and NOE cross peaks between N<u>H</u>$_c$/COC<u>H</u>$_3$ and N<u>H</u>$_a$/C<u>H</u>$_\alpha$ allowed the assignment of the signals to individual NH_a and NH_c protons. The signals for NH_a and NH_c are observed at lower field while the resonance of NH_b is found below δ 7 ppm, suggesting participation of NH_a and NH_c in hydrogen bonding and a practically nonhydrogen-bonded NH_b group (Table 4).

To address the question which NH group preferentially participates in hydrogen bonds, NH chemical shifts were also measured in different solvents ["variation ratio" (v.r.) method].[29] For interpretation of observed δ_{NH} values it has proven useful to compare them to those of reference compounds **1**−**4**. The standard compounds possess NH groups in a comparable chemical environment but without any intramolecular hydrogen bond (non-hydrogen-bonded reference state) in non-coordinating solvents (e.g., in $CDCl_3$). The fully hydrogen bonded reference state is simulated by measuring the ^{1}H NMR spectrum in the strongly coordinating solvent [D_6]-DMSO (Tables 1 and 4).[44] Chemical shift variation from [D_6]-DMSO to $CDCl_3$ ($\Delta\delta_{NH}$) provides a measure of the extent to which an amide proton participates in an intramolecular hydrogen bond. If the shift variation of a particular NH proton is distinctly smaller than that of the hydrogen bond free reference, the NH proton is considered to be intramolecularly hydrogen bonded in $CDCl_3$ solution. The variation ratio v.r. = $\Delta\delta_{NH}$(sample)/$\Delta\delta_{NH}$(reference) describes the involvement of the considered proton in an intramolecular hydrogen bond. Small v.r. values indicate strong hydrogen bonds while larger v.r. values suggest weak hydrogen bonds. V.r. values significantly smaller than one are observed for NH_a and NH_c indicating strong hydrogen bonds while NH_b has a v.r. value around one ruling out a strong involvement of this particular proton in hydrogen bonds (Table 4).

Intermolecular hydrogen bonding of NH_a and NH_c can be safely excluded on the basis of concentration-dependent ^{1}H NMR spectra in the concentration range 1–70 mM (Figure 2). All NH chemical shifts are only weakly dependent on the concentration (Figure 2). Extrapolation of the chemical shift values to infinite dilution gives $\delta_0(NH_a) > 7$ and $\delta_0(NH_c) > 7$

(43) Vass, E.; Holósi, M.; Besson, F.; Buchet, R. *Chem. Rev.* **2003**, *103*, 1917–1954.
(44) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512–523.
(45) Heinze, K.; Beckmann, M. *J. Organomet. Chem.* **2006**, *691*, 5576–5584.
(46) Lin, L.; Berces, A.; Kraatz, H.-B. *J. Organomet. Chem.* **1998**, *556*, 11–20.
(47) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, G. E.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M. ; Toyota, K.; Fukuda, R.; Hasegawa, J. ; Ishida, M.; Nakajima, T. ; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E. ; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C. ; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K. ; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M. ; Gill, P. M. W.; Johnson, B. ; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, Revision B.03; Gaussian, Inc.: Pittsburgh, PA, 2003.

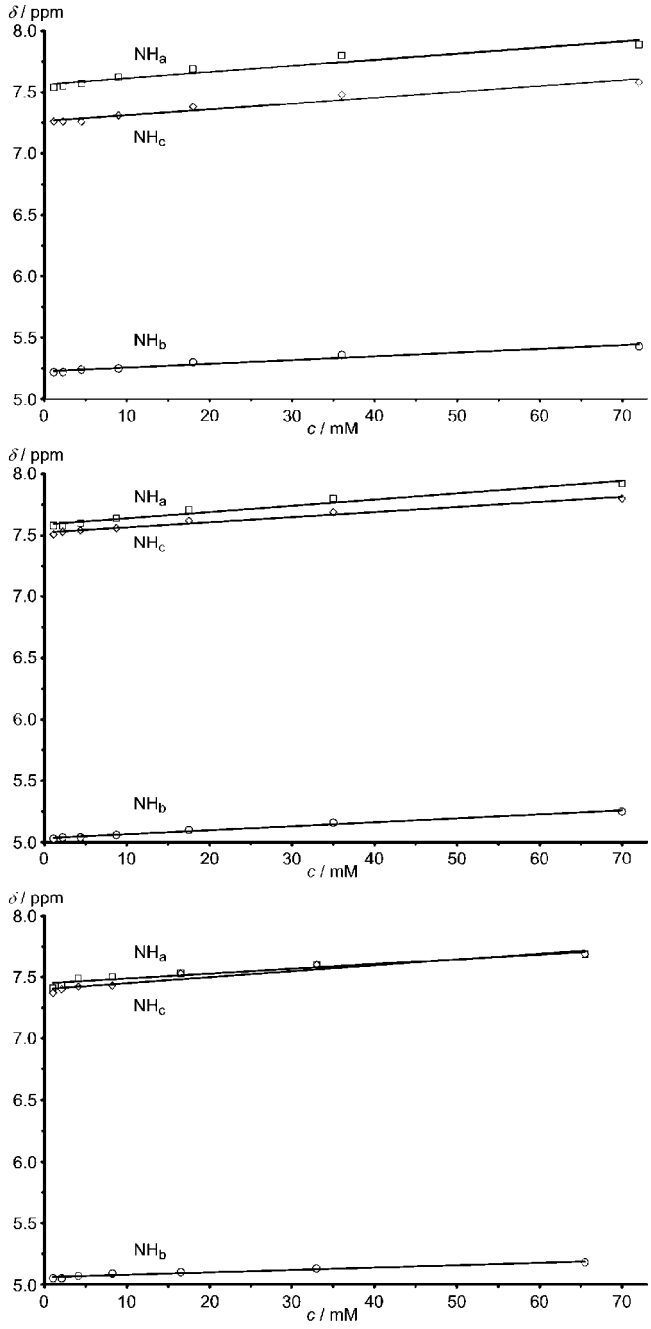


Figure 2. Concentration-dependent ^{1}H NMR chemical shifts of amide protons of **10**, **11**, and **13** in $CDCl_3$ (the solid lines correspond to linear regression fits).

confirming the intramolecular nature of the hydrogen bonds involving NH_a and NH_c.

The CD data suggested that the IHBs which render the ferrocene chromophores helical are still intact in the presence of DMSO. The proton chemical shifts of the cyclopentadienyl rings of **11** and **13** in [D_6]-DMSO confirm this interpretation as the protons H^2/H^5 as well as the protons H^7/H^{10} are diastereotopic (Table 7). This is only possible if the chirality information of the amino acid substituent is transferred to the other cyclopentadienyl ring via IHBs. This interpretation is also consistent with the analogous stereodiscrimination of the cyclopendienyl carbon nuclei in chiral conjugates **11** and **13** (Table 8). A fully comparable situation has been observed for ferrocene amino acid derivatives of type V.[30]

In summary, the combined experimental data suggest IHBs involving NH_a and NH_c resulting in *P*-helical ferrocene units

Table 7. ^{1}H NMR Data of 10, 11, and 13 in [D_6]-DMSO

	10	**11**	**13**
NH_a	9.16 (s, 1H)	9.22 (s, 1H)	9.27 (s, 1H)
NH_b	6.98 (t, 1H, ^3J = 5.79 Hz)	6.99 (d, 1H, ^3J = 6.94 Hz)	6.75 (d, 1H, ^3J = 8.79 Hz)
NH_c	9.08 (s, 1H)	9.07 (s, 1H)	9.09 (s, 1H)
Cp−H	4.51 (s, 2H)	4.53 (s, 1H)	4.55 (s, 1H)
	4.47 (s, 2H)	4.51 (s, 1H)	4.52 (s, 1H)
	3.87 (s, 4H)	4.49 (s, 1H)	4.48 (s, 1H)
		4.40 (s, 1H)	4.42 (s, 1H)
		3.86 (s, 2H)	3.86 (s, 2H)
		3.85 (s, 2H)	3.85 (s, 2H)
CH_α	3.57 (d, 2H, ^3J = 5.79 Hz)	3.94 (m, 1H)	3.74 (pt, 1H)
$COCH_3$	1.89 (s, 3H)	1.89 (s, 3H)	1.89 (s, 3H)
CCH_3	1.39 (s, 9H)	1.38 (s, 9H)	1.38 (s, 9H)
R		1.20 (d, 3H, ^3J = 7.15 Hz)	1.95 (br m, 1H)
			0.87 (d, 6H, ^3J = 6.73 Hz)

for L-amino acid substiuents while NH_b is practically devoid of hydrogen bonds. However, these findings are hard to explain by a single preferred conformation in solution. To address this question theoretical modeling studies were performed for the alanine derivative **11**.

DFT Modeling of 11. By using DFT calculations (B3LYP, LanL2DZ[24,26,30,31,33,36,40,45,46]) several local minima could be located on the energy landscape of **11**. Conformations of type A, B, C, and D possess interchain hydrogen bonds while intrachain hydrogen bonds $NH_a\cdots CO_b$ (γ-turns[43]) were observed in conformations of type B, open1, open2, and open4 (Figure 3, Table 5). γ-Turns have also been observed in amino acid conjugates of ferrocene-1,1′-dicarboxylic acid.[14] Type A conformation represents "one half" of the experimentally observed conformation of Kraatz's symmetrical 1,1′-disubstituted ferrocene derivative Fn(-NH-Ala-Boc)$_2$.[35] In conformations with energies below 6 kJ mol^{-1} (A-P, B-P, C-P, and C-M) NH_c and NH_a act as hydrogen atom donors while NH_b is scarcely involved in hydrogen bonding (conformations D-P and D-M only). These data explain the experimental findings that both NH_a and NH_c are strongly hydrogen bonded while NH_b is not (Tables 4 and 5). These two IHBs are achieved through an ensemble of conformations (A-P, B-P, C-P, and C-M) and not by a single conformer with two IHBs being present simultaneously. Three different conformers have also been observed in the solid state of symmetrical Fn(-NHBoc)$_2$.[35] One of these conformers of Fn(-NHBoc)$_2$ exhibits an IHB similar to that of conformers B and C of **11**. In addition most of the low-energy conformations of **11** possess a *P*-helical ferrocene moiety that nicely fits to the observed positive Cotton effect at 469 nm in the CD spectra (Figure 1, Table 2).

Experimental Section

General. The syntheses were carried out under argon. CH_2Cl_2 used for synthesis and FT-IR spectroscopy was dried (P_2O_5), distilled over CaH_2, and stored over molecular sieves (4 Å). EDC and HOBt (Aldrich) and amino acids (Merck) were used as received. Products were purified by preparative thin layer chromatography on silica gel (Merck, Kieselgel 60 HF_{254}), using the mixtures CH_2Cl_2/EtOAc or CH_2Cl_2/MeOH. Melting points were determined with a Buechi apparatus. IR spectra were recorded as CH_2Cl_2 solutions with a Bomem MB 100 mid FT-IR spectrophotometer. ^{1}H and ^{13}C{^{1}H} NMR spectra were recorded on a Varian EM 360 or Varian Gemini 300 spectrometer in CD_2Cl_2, $CDCl_3$, and [D_6]-DMSO solutions with Me_4Si as internal standard or on a Varian Unity Plus 400 spectrometer. FAB and HR-FAB mass spectra were

Table 8. ^{13}C NMR Data of 10, 11, and 13 in $CDCl_3$ (assignments are based on HSQC and HMBC spectra)

	10	11	13
CO_a	168.4 (s)	171.8 (s)	170.8 (s)
CO_b	155.9 (s)	155.8 (s)	156.2 (s)
CO_c	169.6 (s)	169.6 (s)	169.6 (s)
Cp−C	C_{ipso} n.o. 65.5 (2 s), 63.8 (s), 63.5 (s)	94.7 (s), 94.4 (s), 65.5 (s), 65.4 (s), 65.3 (s), 65.2 (s), 64.4 (s), 63.8 (s), 63.4 (s), 63.2 (s)	C_{ipso} n.o. 65.6 (2 s), 65.3 (s), 65.2 (s), 64.5 (s), 64.0 (s), 63.6 (s), 63.4 (s)
C_α	44.8 (s)	50.9 (s)	60.9 (s)
CH_3(Boc)	28.3 (s)	28.3 (s)	28.4 (s)
C_q(Boc)	80.5 (s)	80.4 (s)	80.2 (s)
CH_3(Ac)	23.9 (s)	23.9 (s)	23.9 (s)
R		18.3 (s, CH_3)	30.7 (s, C_α) 19.4 (s, CH_3) 18.0 (s, CH_3)

recorded on a JEOL JMS-700. CD spectra were recorded with CD spectrophotometer Jasco-810. *N*-Ferrocenylacetamide (**1**) was prepared according to the literature.[33,37] NMR data for Fc-NH-Ala-Boc (**3**) were taken from ref 29.

Computational Method. Density functional calculations were carried out with the Gaussian03/DFT[47] series of programs. The B3LYP formulation of density functional theory was used employing the Lanl2DZ basis set.[47] All points were characterized as minima ($N_{imag} = 0$) by frequency analysis.

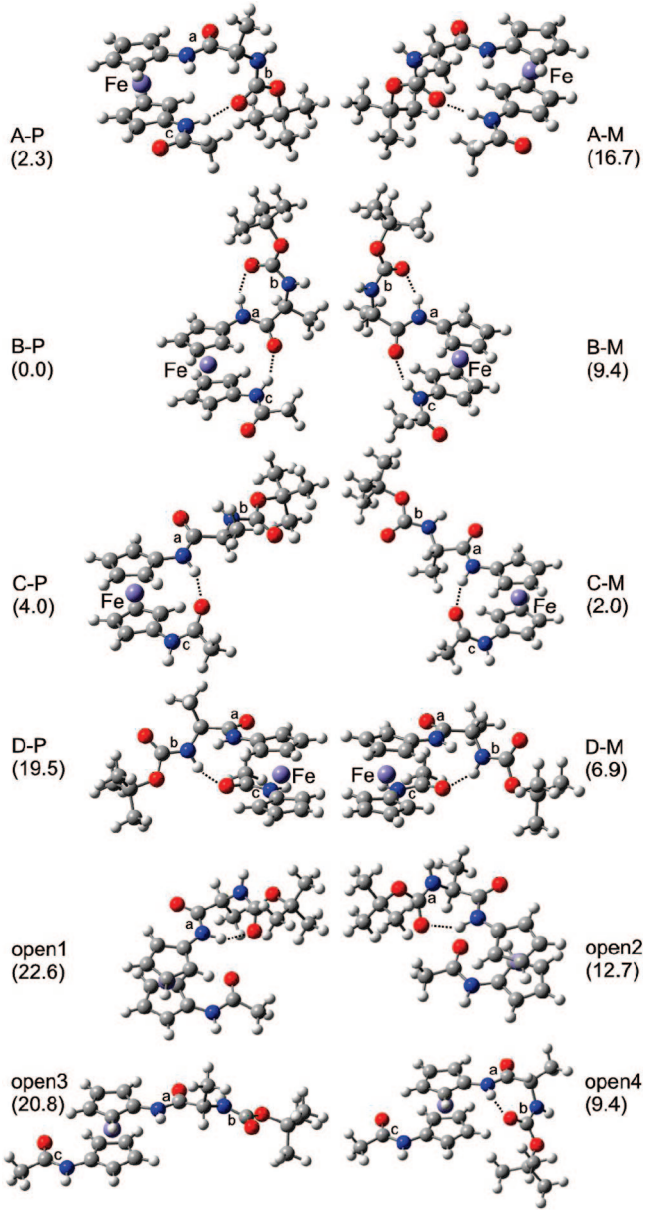


Figure 3. DFT-optimized geometries of **11** along with their relative energies (in kJ mol^{-1}) in parentheses.

Synthesis of Fc-NH-AA-Boc (2: AA = Gly; 4: AA = L-Val). A suspension of Fc-NH-Boc (45 mg, 0.157 mmol) in ethyl acetate (2 mL) was cooled to 0 °C and treated with gaseous HCl for 1 h. After being stirred at room temperature for 4 h the mixture was evaporated in vacuo to dryness to give yellow ferrocenylammonium chloride (34 mg, 91%). The hydrochloride was treated with NEt_3 in CH_2Cl_2 (pH ∼ 8) and coupled with Boc-AA-OH (0.314 mmol) by using the standard EDC/HOBt method. The reaction mixture was stirred for 1 h at room temperature, then washed three times with a saturated aqueous solution of $NaHCO_3$, 10% aqueous solution of citric acid, and H_2O. After drying over Na_2SO_4 and evaporating the solvent in vacuo the crude products were TLC-purified with CH_2Cl_2/CH_3OH (10:0.5) as eluent. **Fc-NH-Gly-Boc (2):** yellow crystals (31 mg, 58%). Mp 79–81 °C. HR-MS (EI): *m*/*z* 358.1007 (calcd for $C_{17}H_{22}N_2O_3Fe$ 358.0980). IR (CH_2Cl_2): ν 3421 (m, NH_{free}), 3322 (vw, NH_{assoc}), 1695 (s, C═O, COO^{t}Bu, amide I), 1538 (s, amide II) cm^{-1}. ^{1}H NMR ([D_6]-DMSO): δ 9.23 (s, 1H, NH^a), 7.00 (t, 1H, $^3J_{HH}$ = 5.64 Hz, NH^b), 4.57 (s, 2H, $H^{2,5}$), 4.09 (s, 5H, H^{6-10}), 3.93 (s, 2H, $H^{3,4}$), 3.56 (d, 2H, $^3J_{HH}$ = 6.06 Hz, H_α), 1.39 (s, 9H, $C(CH_3)_3$) ppm. ^{1}H NMR ($CDCl_3$): δ 6.68 (s, 1H, NH^a), 5.45 (br s, 1H, NH^b), 4.97 (s, 2H, $H^{2,5}$), 4.69 (s, 5H, H^{6-10}), 4.18 (s, 2H, $H^{3,4}$), 3.63 (br s, 2H, H_α), 1.59 (m, 9H, $C(CH_3)_3$) ppm. ^{13}C NMR ($CDCl_3$): δ 167.7 (s, CO^a), 156.5 (s, CO^b), 94.9 (s, C^1), 80.6 (s, $C(CH_3)_3$), 69.6 (s, C^{6-10}), 64.9 (s, $C^{3,4}$), 61.6 (s, $C^{2,5}$), 45.3 (s, C_α), 28.3 (s, $C(CH_3)_3$) ppm. **Fc-NH-Val-Boc (4):** yellow crystals (39 mg, 61%). Mp 191–192 °C. Anal. Calcd for $C_{20}H_{28}N_2O_3Fe$: C, 60.01; H, 7.05; N, 7.00. Found: C, 59.71; H, 7.14; N, 6.92. HR-MS (EI): *m*/*z* 400.1441 (calcd for $C_{20}H_{28}N_2O_3Fe$ 400.1449). IR (CH_2Cl_2): ν 3424 (m, NH_{free}), 3309 (vw, NH_{assoc}), 1690 (s, C═O, COO^{t}Bu, amide I), 1535 (s, amide II) cm^{-1}. ^{1}H NMR ([D_6]-DMSO): δ 9.33 (s, 1H, NH^a), 6.77 (d, 1H, $^3J_{HH}$ = 8.58 Hz, NH^b), 4.60 (s, 2H, $H^{2,5}$), 4.09 (s, 5H, H^{6-10}), 3.93 (s, 2H, $H^{3,4}$), 3.71 (pt, 1H, H_α), 1.91 (m, 1H, H_α), 1.38 (s, 9H, $C(CH_3)_3$), 0.88 (d, 3H, $^3J_{HH}$ = 6.6 Hz, $CH_{3,Val}$), 0.86 (d, 3H, $^3J_{HH}$ = 6.54 Hz, $CH_{3,Val}$) ppm. ^{1}H NMR ($CDCl_3$): δ 7.13 (s, 1H, NH^a), 5.27 (br s, 1H, NH^b), 4.96 (s, 2H, $H^{2,5}$), 4.32 (s, 5H, H^{6-10}), 3.63 (br s, 3H, H_α + $H^{3,4}$), 2.08 (m, 1H, H_α), 1.43 (s, 9H, $C(CH_3)_3$), 0.94 (d, 3H, $^3J_{HH}$ = 6.96 Hz, $CH_{3,Val}$), 0.92 (d, 3H, $^3J_{HH}$ = 6.99 Hz, $CH_{3,Val}$) ppm. ^{13}C NMR ($CDCl_3$): δ 170.1 (s, CO^a), 156.2 (s, CO^b), 94.8 (s, C^1), 80.1 (s, $C(CH_3)_3$), 69.3 (s, C^{6-10}), 64.7 (s, $C^{3,4}$), 61.5 (s, $C^{2,5}$), 61.2 (s, CH_α), 30.4 (s, C_α), 28.3 (s, $C(CH_3)_3$), 19.4 (s, $CH(CH_3)_2$), 18.2 (s, $CH(CH_3)_2$) ppm.

1′-Acetamidoferrocene-1-carboxazide (6). 1′-Acetamido-1-ferrocenecarboxylic acid (**5**, 1.5 g, 0.005 mol) was suspended in water (0.99 mL) and sufficient acetone was added to dissolve it. After cooling to 0 °C, NEt_3 (0.84 mL) in acetone (10.8 mL) was added. While maintaining the temperature at 0 °C, a solution of ethyl chloroformate (0.63 mL) in the same solvent (2.8 mL) was added and the mixture was stirred for 30 min at 0 °C. Thereafter, a solution of sodium azide (517.3 mg, 7.96 mmol) in water (1.8 mL) was added. The mixture was stirred for 90 min at 0 °C, poured

into ice–water, and extracted with dichloromethane. The extracts were washed with a 5% aqueous solution of $NaHCO_3$ and a saturated aqueous solution of NaCl, dried over Na_2SO_4, and evaporated in vacuo at room temperature to dryness to give red crystals (880 mg, 56%). Mp 123–125 °C. HR-MS (EI): *m/z* 312.0319 (calcd for $C_{13}H_{12}N_4O_2Fe$ 312.0309). IR (CH_2Cl_2): ν 3431 (m, NH_{free}), 3335 (w, NH_{assoc}), 2135 (s, N_3), 1711 (s, C═O, $COCH_3$), 1685 (s, C═O, CON_3), 1530 (s, amide II) cm^{-1}. ^1H NMR ($CDCl_3$): δ 6.86 (s, 1H, NH), 4.80 (pt, 2H, $H^{7,10}$), 4.67 (pt, 2H, $H^{2,5}$), 4.50 (pt, 2H, $H^{8,9}$), 4.02 (pt, 2H, $H^{3,4}$), 2.09 (s, 3H, CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ 176.4 (s, *C*ON_3), 168.5 (s, *C*OCH_3), 96.2 (s, C^6), 73.7 (s, $C^{8,9}$), 73.3 (s, C^1), 71.4 (s, $C^{7,10}$), 66.5 (s, $C^{3,4}$), 63.0 (s, $C^{2,5}$), 23.9 (s, CH_3) ppm.

***tert*-Butyl 1′-Acetamidoferrocenylcarbamate (7).** A solution of 1′-acetamidoferrocene-1-carboxazide (**6**, 500 mg, 1.6 mmol) in ^{t}BuOH (25 mL) was heated at 70 °C for 3 h. The reaction mixture was evaporated to dryness and purified by preparative thin layer chromatography, using dichlormethane/ethyl acetate (10:1) as eluent, to give **7** as the major product and the urea derivative **8** as the side product. **7:** yellow crystals (510 mg, 89%). Mp 64–67 °C. HR-MS (EI): *m/z* 358.0977 (calcd for $C_{17}H_{22}N_2O_3Fe$ 358.0980). IR (CH_2Cl_2): ν 3431 (m, NH_{free}), 3328 (w, NH_{assoc}), 1711 (s, C═O, COO′Bu), 1678 (s, C═O, $COCH_3$, amide I), 1531 (s, amide II) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.41 (s, 1H, NH^a), 5.80 (s, 1H, NH^c), 4.52 (m, 4H, $H^{2,5,7,10}$), 4.16 (m, 4H, $H^{3,4,8,9}$), 2.06 (s, 3H, $COCH_3$), 1.48 (s, 9H, $C(CH_3)_3$) ppm. ^{13}C NMR ($CDCl_3$): δ 168.9 (s, CO^c), 153.7 (s, CO^a), 97.4 (s, C^1), 93.6 (s, C^6), 80.4 (s, *C*$(CH_3)_3$), 67.9, 66.4, 65.1, 61.6 (s, $C^{2,5}$, $C^{3,4}$, $C^{7,10}$, $C^{8,9}$), 28.4 (s, C(*C*$H_3)_3$), 23.8 (s, CO*C*H_3) ppm. **8:** yellow crystals (57 mg, 10%). Mp 144–146 °C. IR (CH_2Cl_2): ν 3422 (m, NH_{free}), 3313 (w, NH_{assoc}), 1677 (s, C═O, $COCH_3$, amide I), 1536 (s, amide II) cm^{-1}. ^1H NMR ([D_6]-DMSO): δ 9.13, 7.52 (s, 1H, NH^a, NH^c), 4.39 (s, 4H, $H^{2,5}$, $H^{7,10}$), 3.89 (s, 4H, $H^{3,4}$, $H^{8,9}$), 1.89 (s, 3H, CH_3) ppm. ^{13}C NMR ([D_6]-DMSO): δ 168.3 (s, CO^c), 153.8 (s, CO^a), 97.9, 96.1 (s, C^1, C^6), 64.8, 62.1 (s, $C^{2,5}$, $C^{3,4}$, $C^{8,9}$, $C^{7,10}$), 23.9 (s,CH_3) ppm.

General Synthesis of Boc-AA-NH-Fn-NH-Ac (10–13). A suspension of **7** (58.2 mg, 0.162 mmol) in ethyl acetate (3 mL) was cooled to 0 °C and treated with gaseous HCl for 1 h. Thereafter, the mixture was evaporated in vacuo to dryness to give yellow-orange solid 1′-acetamidoferrocenylammonium chloride **9**. The hydrochloride **9** was treated with NEt_3 in CH_2Cl_2 (pH∼8) and coupled with Boc-AA-OH (0.324 mmol) by using the standard EDC/HOBt method. After being stirred for 1 h at room temperature, the mixture was subjected to standard aqueous workup, followed by TLC purification (CH_2Cl_2/CH_3OH, 10:0.5). **Boc-Gly-NH-Fn-NH-Ac (10):** yellow crystalline material after standing in the refrigerator (33.4 mg, 50%). Mp 171 °C. Anal. Calcd for $C_{19}H_{25}N_3O_4Fe$: C, 54.95; H, 6.07; N, 10.12. Found: C, 55.05; H, 6.19; N, 10.04. **Boc-L-Ala-NH-Fn-NH-Ac (11):** yellow crystals (56.6 mg, 57%). Mp 83–85 °C. Anal. Calcd for $C_{20}H_{27}N_3O_4Fe \cdot 0.5H_2O$: C, 54.81; H, 6.44; N, 9.59. Found: C, 54.83; H, 6.19; N, 9.42. **Boc-D-Ala-NH-Fn-NH-Ac (12):** yellow crystals (50 mg, 54%). Analytical data (NMR, IR) are identical with those of **11**. **Boc-L-Val-NH-Fn-NH-Ac (13):** yellow crystals (173.8 mg, 58%). Mp 80–82 °C. Anal. Calcd for $C_{22}H_{31}N_3O_4Fe$: C, 57.78; H, 6.83; N, 9.19. Found: C, 57.37; H, 6.97; N, 8.88.

Conclusion

In summary it has been shown that asymmetric 1,1′-diaminoferrocene conjugates of α-amino acids (Scheme 1, type VI) form an ensemble of conformations in weakly coordinating solvents as well as in DMSO solution with intramolecular hydrogen bonds (IHBs) involving the NH groups closest to the ferrocene unit. The majority of the low-energy conformations possess a *P*-helical ferrocene chromophore for L-amino acid substituents so that chirality organization is already achieved by attaching one amino acid substituent at the 1,1′-diaminoferrocene central unit. The average strength of IHBs within the subset of compounds investigated in this report (**10**, **11**, and **13**) as estimated by v.r. values is almost independent of the amino acid employed, i.e., the steric demand of the amino acid side chain R.

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Supporting Information Available: Gaussian fits of CD spectra of **11**, **12**, and **13** and the DFT calculated Cartesian coordinates of all conformers of **11**. This material is available free of charge via the Internet at http://www.pubs.acs.org.

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