Interaction of Ferrocenoyl-Dipeptides with 3-Aminopyrazole Derivatives: β-Sheet Models? A Synthetic, Spectroscopic, Structural, and Electrochemical Study

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The use of 3-aminopyrazole derivatives as β-sheet templates is investigated using a series of ferrocenoyl (Fc)-dipeptides (Fc-Gly_2-OEt, Fc-Ala_2-OBzl, Fc-Leu-Phe-OMe, Fc-Val-Phe-OMe, Fc-Phe_2-OMe, Fc-Leu_2-OMe, Fc-Val_2-OMe). The synthesis and full characterization are reported. The solid-state structures of Fc-Gly_2-OMe and Fc-Leu-Phe-OMe show extensive hydrogen bonding of the podand peptide substituents, resulting in the formation of supramolecular Fc-dipeptide assemblies. For Fc-Gly_2-OMe, this can be described as a parallel β-sheet, whereas intermolecular interactions in Fc-Leu-Phe-OMe result in the formation of supramolecular helical structures. The saturation titrations of Fc-dipeptides with 3-amino-5-methylpyrazole (3-AMP) and 3-trifluoroacetylamido-5-methylpyrazole (3-TFAc-AMP) show a 1:1 interaction of the Fc-peptide with the aminopyrazole derivatives. IR measurements in solution confirm binding to the top face of the Fc-dipeptide and the involvement of the Fc−C═O and the ester C═O groups in establishing H-bonding interactions with the 3-TFAc-AMP. However, binding constants in chloroform are low and range from 8 to 27 M^{-1}, which correspond to binding energies of 5−7 kJ mol^{-1}. In higher polarity solvents, such as acetonitrile or acetone, the binding constants are below 5 M^{-1}, emphasizing the limited utility of 3-AMP derivatives as β-sheet templates. Electrochemical measurements confirm the weak interactions between the various Fc-dipeptides and 3-TFAc-AMP. Typical shifts in the redox potential of the Fc moiety are in the range 0−20 mV. Attempts to modify 3-AMP at the 3-position by carbodiimide coupling with amino acid derivatives and, thus, enhance the binding to the Fc-peptides resulted in 2-amino acid substituted 3-AMP derivatives. Substitution at the 2-position blocks the binding site, and no interactions with Fc-dipeptides are observed.

Introduction

Controlling the architecture of peptide assemblies using specific interactions, such as hydrogen bonding or electrostatic interactions, is one of the most important innovations which may allow for the design of peptidic biomaterials having novel functions.[1] In particular, hydrogen bonding has been extremely useful in designing highly ordered peptidic systems, such as self-assembled peptide tubes,[2] and has been widely exploited in crystal engineering of nonbiological materials.[3]

Incorporating redox active organometallic moieties into a biological supramolecular assembly is particularly relevant, because it may allow for the design of novel biomaterials, which may act as biomolecular sensing and switching devices.[4] Modified ferrocenes and their analogues have long been exploited as redox probes and are able to respond to the structural changes that will take place upon substrate binding to an adjacent coordination site attached.[5] Recently, we reported a strategy that allows the incorporation of the ferrocene group into a peptidic framework under very mild conditions.[6] Subsequently, we showed that the redox potential of the ferrocenoyl group (Fc) attached to helical and nonhelical oligopeptides is influenced by the peptide's secondary structure.[7] As a logical next step, we wanted to investigate systems that have β-sheet-like structures and study the effect that this particular secondary structure exerts on the electrochemical properties of the ferrocene group. These studies are part of our larger efforts toward understanding electron transfer in structurally well-defined

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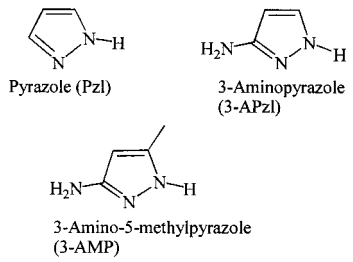
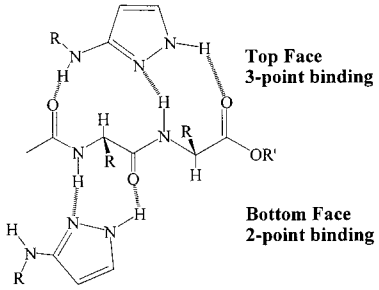
Chart 1



Scheme 1. Two- and Three-Point Binding of 3-Aminopyrazole (APzl) to an Acylated Dipeptide (According to Ref 11)



peptide assemblies. According to theoretical studies, the electron-transfer properties of peptides adopting a β-sheet structure are different from those of peptides having an α-helical structure.[8]

There are now several routes available allowing β-sheet formation even in small dipeptides.[9,10] We were intrigued by a novel strategy, recently reported by Schrader and Kirsten,[11] using 3-aminopyrazole (3-APzl) and 3-amino-5-methylpyrazole (3-AMP) as templates to induce β-sheet formation by forming hydrogen bonds with the peptide backbone (Chart 1).

It was shown that 3-APzl acts as a template by binding to various acylated dipeptides and forces them into the β-sheet conformation. It was reported that 2:1 complexes are formed by hydrogen bonding interactions between an aminopyrazole derivative and a series of Ac-dipeptides. Both binding sites were thought to be occupied (Scheme 1). Three-point binding to the top face of the peptide is preferred over two-point binding to the bottom face, resulting in the ability of the aminopyrazole derivative to distinguish between the two different faces of the acylated dipeptide. The bottom face only allows for a weak two-point binding, involving the acyl NH and the peptide C═O groups. Hence, the three-point site will be occupied preferentially over the bottom two-point binding site. Pyrazole (Pzl), a two-point binder, is unable to distinguish between the two faces of the molecule and will bind to both sites. This regioselective binding is crucial for proper peptide recognition.

We decided to make use of this strategy to investigate the interaction of APzl derivatives with Fc-peptides and to evaluate their ability to act as templates for stabilizing β-sheet structures. In a preliminary study,[6b] we reported the results of an investigation of the interaction of the parent 3-APzl substrate with three Fc-dipeptides, Fc-Gly_2-OEt (**7**), Fc-Ala_2-OBzl (**8**), and Fc-Leu-Phe-OMe (**9**). Surprisingly, we found that only the top-face binding site of the Fc-dipeptide interacted with the ligand. Binding constants in the range 9−21.5 M^{-1} were observed, indicating weak binding.[6b] To obtain stronger binding between the Fc-dipeptides and the 3-APzl derivatives, we decided to study Fc-dipeptides with high β-sheet propensity,[18] such as Fc-Val_2-OMe, and use aminopyrazole derivatives, such as 3-trifluoromethylacetyl-AMP, with a higher affinity for binding to the dipeptide backbone. The interaction between the ligand and the Fc-dipeptide can be quantified by ^{1}H NMR saturation titrations and IR spectroscopy, and the validity of the binding model can be assessed. Electrochemical studies of the Fc-dipeptides in the absence and presence of the β-sheet inducing ligands should provide information regarding the electronic effects of peptides in a β-sheet conformation on an attached redox probe.

The present paper gives a complete account of the preparation and characterization of several Fc-dipeptides, including the X-ray structures of Fc-Gly_2-OEt (**7**) and Fc-Leu-Phe-OMe (**9**). We report our findings on the interaction of Fc-dipeptides with various 3-APzl derivatives in solution (^{1}H NMR saturation titration experiments and IR spectroscopy). Our studies allow us to quantify the interaction and to assign the involvement of only one binding site at the Fc-dipeptide. Furthermore, we report results of our electrochemical studies investigating the electronic effects of the interaction between the Fc-dipeptides and the aminopyrazole ligands.

Experimental Section

General Procedure. All syntheses were carried out in air in CH_2Cl_2 unless otherwise indicated. CH_2Cl_2 and $CHCl_3$ (BDH, ACS grade) used for synthesis, FT-IR, and electrochemistry were dried (CaH_2) and distilled prior to use. Acetone, EtOAc, CH_3CN, MeOH, diethyl ether (BDH, ACS grade), hexanes (Fischer, HPLC grade), $CHCl_3$, and CH_2Cl_2 used for the purpose of purification were used as received. $CDCl_3$ and CD_3CN (Aldrich) were dried by and stored over molecular sieves (8−12 mesh, 4 Å effective pore size, Fisher) before use. Acetone-d_6 (MSD) was used as received. Pyrazole, APzl, AMP, DCC, HOBt, H-Gly-OEt·HCl, H-Phe-OMe·HCl, AcCl, TFAc-anhydride, TFA, H-Leu-OMe, H-Val-OMe (Aldrich), Boc-Gly-OH, H-Ala-OBzl·Tos, Boc-Ala-OH, Boc-Leu-OH·H_2O (Novabiochem), $MgSO_4$, $NaHCO_3$ (VWR), Boc-Val-OH (Advanced ChemTech), Boc-Phe-OH (AminoTech), and

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FcOH (Strem) were used as received. Et_3N (BDH, ACS grade) used in peptide and Fc-peptide couplings was used as received but was dried by molecular sieves when used in stoichiometric quantities. For column chromatography, a column with a width of 2.7 cm (i.d.) and a length of 45 cm was packed 18−22 cm high with 230−400 mesh silica gel (VWR). For TLC, aluminum plates coated with silica gel 60 F_{254} (EM Science) were used. NMR spectra were recorded either on a Bruker AMX-300 spectrometer operating at 300.135 MHz (^{1}H) and 75.478 MHz (^{13}C{^{1}H}), or on a Bruker AMX-500 spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C{^{1}H}). Peak positions in both ^{1}H and ^{13}C spectra are reported in ppm relative to TMS. ^{1}H NMR spectra of Fc-peptides are referenced to the CH_2Cl_2 resonance (δ 5.32 ppm) of an external standard ($CDCl_3/CH_2Cl_2$). ^{1}H spectra of all other compounds are referenced to the residual $CHCl_3$ signal. All ^{13}C{^{1}H} spectra are referenced to the $CDCl_3$ signal at δ 77.23 ppm. 2D-COSY and HMBC (heteronuclear multiple bond correlation) experiments were recorded to make proper spectral assignments of the ^{1}H and ^{13}C spectra. The dipeptides, Boc-Gly-Gly-OEt (**1**),[6b] Boc-Ala-Ala-OBzl (**2**),[6b] Boc-Leu-Phe-OMe (**3**),[6b,12c] Boc-Phe-Phe-OMe (**4**),[12d] Boc-Leu-Leu-OMe (**5**),[12e] and Boc-Val-Val-OMe (**6**),[12f] were prepared as described before. The ferrocenoyl-dipeptides Fc-Gly-Gly-OEt (**7**), Fc-Ala-Ala-OBzl (**8**), and Fc-Leu-Phe-OMe (**9**) were prepared as described before using EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) instead of DCC (1,3-dicyclohexylcarbodiimide) (vide infra).[6b] Their spectroscopic properties were identical to those described before.[6b]

General Procedure for the Preparation of Fc-Peptides (10−13). To a stirring mixture of FcOH (1 mmol) and HOBt (1.1 mmol) in CH_2Cl_2 (20 mL) at room temperature is added solid EDC (1.1 mmol), causing the orange slurry to slowly change into a clear solution. Boc-protected peptide (1.2 mmol) is treated with neat TFA (1 mL) for at least 30 min, and then the excess TFA is removed in vacuo. The remaining residue is dissolved in CH_2Cl_2 (5 mL), and Et_3N (1 mL) is added. The basic peptide solution is transferred to the reaction vessel containing the FcOH/EDC/HOBt mixture and is left stirring overnight. The reaction solution is washed consecutively with aqueous solutions of saturated $NaHCO_3$, 10% citric acid, saturated $NaHCO_3$, and finally distilled water. The organic phase is dried by anhydrous $MgSO_4$ and filtered and the solvent removed under reduced pressure, giving the crude orange product. Fc-Gly-Gly-OEt and Fc-Ala-Ala-OBzl were purified by dissolving the crude product in a small amount of CH_2Cl_2, filtering, and then crystallizing from CH_2Cl_2/hexane. Fc-Leu-Phe-OMe, Fc-Val-Phe-OMe, Fc-Phe-Phe-OMe, and Fc-Leu-Leu-OMe were purified by column chromatography in the solvent systems indicated below for TLC.

Characterization of Fc-Val-Phe-OMe (10). R_f = 0.6 (hexanes/acetone 2:1). Yield: 63% (0.147 g). Elemental Anal. Calcd for $C_{26}H_{30}FeN_2O_4$: C, 63.68; H, 6.15; N, 5.71. Found: C, 63.77; H, 6.33; N, 5.70. EI-MS (positive ion mode) accurate mass: M^+ calcd, 490.1555; found, 490.1554. FT-IR ($CHCl_3$): 1743 (C═O Phe, ester), 1676 (C═O Val), 1651 cm^{-1} (C═O Fc). ^{1}H NMR (δ, $CDCl_3$): 7.26 (3H, m, C*H*$_m$, C*H*$_p$ Phe), 7.12 (2H, d, J_{HH} = 6.2 Hz, C*H*$_o$ Phe), 6.23 (2H, d, J_{HH} = 7.8 Hz, N*H* Val, N*H* Phe), 4.90 (1H, dd, J_{HH} = 5.9, 7.8 Hz, C*H*$^\alpha$ Phe), 4.74 (1H, s, C*H*$_o$ Cp), 4.68 (1H, s, C*H*$_o$ Cp), 4.38 (3H, m, C*H*$_m$ Cp, C*H*$^\alpha$ Val), 4.21 (5H, s, *C*H unsubst Cp), 3.74 (3H, s, OC*H*$_3$), 3.14 (2H, m, C*H*$_2^\beta$ Phe), 2.18 (1H, m, C*H*$^\beta$ Val), 1.01 (3H, d, J_{HH} = 2.9 Hz, C*H*$_3^\gamma$ Val), 0.99 (3H, d, J_{HH} = 2.9 Hz, C*H*$_3^\gamma$ Val). ^{13}C{^{1}H} NMR (δ, $CDCl_3$): 171.8 (*C*═O), 171.3 (*C*═O), 170.6 (*C*═O), 135.7 (*C*$_i$ Phe), 129.4 (*C*H$_{Ar}$ Phe), 128.9 (*C*H$_{Ar}$ Phe), 127.5 (*C*H$_p$ Phe), 75.7 (*C*$_i$ Cp), 70.8 (*C*H$_m$ Cp), 70.0 (5C, *C*H unsubst Cp), 68.8 (*C*H$_o$ Cp), 68.2 (*C*H$_o$ Cp), 58.3 (OC*H*$_3$), 53.4 (*C*H$^\alpha$), 52.6 (*C*H$^\alpha$), 38.1 (*C*H$^\beta$), 31.4 (*C*H$^\beta$), 19.4 (*C*H$_3^\gamma$ Val), 18.4 (*C*H$_3^\gamma$ Val).

Characterization of Fc-Phe-Phe-OMe (11). R_f = 0.48 ($CHCl_3$/EtOAc/MeOH 20:4:1). Yield: 47% (0.213 g). Elemental Anal. Calcd for $C_{30}H_{30}FeN_2O_4$: C, 66.92; H, 5.62; N, 5.20. Found: C, 66.69; H, 5.61; N, 5.25. EI-MS (positive ion mode) accurate mass: M^+ calcd, 538.1555; found, 538.1561. FT-IR ($CHCl_3$): 1744 (C═O Phe$_2$, ester), 1677 (C═O Phe$_1$), 1648 cm^{-1} (C═O Fc). ^{1}H NMR (δ, $CDCl_3$): 7.31 (5H, m, C*H*$_{Ar}$ Phe$_1$), 7.17 (3H, m, C*H*$_m$, C*H*$_p$ Phe$_2$), 6.97 (2H, m, C*H*$_o$ Phe$_2$), 6.45 (1H, d, J_{HH} = 7.6 Hz, N*H* Phe$_2$), 6.05 (1H, d, J_{HH} = 7.4 Hz, N*H* Phe$_1$), 4.81 (2H, m, C*H*$^\alpha$ Phe$_1$, C*H*$^\alpha$ Phe$_2$), 4.66 (1H, s, C*H*$_o$ Cp), 4.51 (1H, s, C*H*$_o$ Cp), 4.36 (2H, br m, C*H*$_m$ Cp), 4.04 (5H, s, *C*H unsubst Cp), 3.72 (3H, s, OC*H*$_3$), 3.09 (4H, m, C*H*$_2^\beta$ Phe$_1$ & C*H*$_2^\beta$ Phe$_2$). ^{13}C{^{1}H} NMR (δ, $CDCl_3$): 171.5 (*C*═O), 170.9 (*C*═O), 170.8 (*C*═O), 137 (*C*$_i$ Phe), 135.5 (*C*$_i$ Phe), 129.6 (*C*H Phe), 129.4 (*C*H$_{Ar}$ Phe), 129.1 (*C*H$_{Ar}$ Phe), 128.8 (*C*H$_{Ar}$ Phe), 127.5 (*C*H$_p$ Phe), 127.3 (*C*H$_p$ Phe), 175.0 (*C*$_i$ Cp), 70.9 (*C*H$_m$ Cp), 70.0 (*C*H unsubst Cp), 69.1 (*C*H$_o$ Cp), 67.8 (*C*H$_o$ Cp), 54.1, 53.6, and 52.6 (OC*H*$_3$, *C*H$^\alpha$ Phe$_1$, *C*H$^\alpha$ Phe$_2$), 38.1 (*C*H$_2$ Phe), 37.9 (*C*H$_2$ Phe).

Characterization of Fc-Leu-Leu-OMe (12). R_f = 0.36 (hexanes/EtOAc 1:1). Yield: 58% (0.096 g). Elemental Anal. Calcd for $C_{24}H_{34}FeN_2O_4$: C, 61.28; H, 7.29; N, 6.00. Found: C, 61.46; H, 7.35; N, 5.84. EI-MS (positive ion mode) accurate mass: M^+ calcd, 470.1868; found, 470.1864. ^{1}H NMR (δ, $CDCl_3$): 6.43 (1H, d, J_{HH} = 7.4 Hz, N*H* Leu$_2$), 6.08 (1H, d, J_{HH} = 7.7 Hz, N*H* Leu$_1$), 4.70 (2H, d, J_{HH} = 2.0 Hz, C*H*$_o$ Cp), 4.63 (2H, m, C*H*$^\alpha$ Leu$_1$ & C*H*$^\alpha$ Leu$_2$), 4.38 (2H, d, J_{HH} = 1.9 Hz, C*H*$_m$ Cp), 4.22 (5H, s, *C*H unsubst Cp), 3.76 (3H, s, OC*H*$_3$), 1.67 (6H, m, C*H*$_2^\beta$C*H*$^\gamma$ Leu$_1$ & C*H*$_2^\beta$C*H*$^\gamma$ Leu$_2$), 1.01 (6H, d, J_{HH} = 6.0 Hz, (C*H*$_3$)$_2$ Leu), 0.93 (6H, d, J_{HH} = 5.6 Hz, (C*H*$_3$)$_2$ Leu). ^{13}C{^{1}H} NMR (δ, $CDCl_3$): 173.3 (*C*═O, Leu$_2$), 172.4 (*C*═O, Fc), 170.8 (*C*═O, Leu$_1$), 75.4 (*C*$_i$ Cp), 70.9 (2C, *C*H$_m$ Cp), 70.0 (5C, *C*H unsubst Cp), 68.5 (*C*H$_o$ Cp), 68.4 (*C*H$_o$ Cp), 52.5 (OCH$_3$), 51.6 (*C*H$^\alpha$ Leu), 51.0 (*C*H$^\alpha$ Leu), 41.6 (*C*H$^\gamma$ Leu$_1$ & *C*H$^\gamma$ Leu$_2$), 25.0 (*C*H$_2^\beta$ Leu$_1$ & *C*H$_2^\beta$ Leu$_2$), 23.2 (*C*H$_3^\gamma$ Leu), 23.0 (*C*H$_3^\gamma$ Leu), 22.4 (*C*H$_3^\gamma$ Leu), 22.1 (*C*H$_3^\gamma$ Leu).

Characterization of Fc-Val-Val-OMe (13). R_f = 0.41 (hexanes/EtOAc 1:2). Yield: 57% (0.251 g). Elemental Anal. Calcd for $C_{22}H_{30}FeN_2O_4$: C, 59.74; H, 6.84; N, 6.33. Found: C, 59.91; H, 6.95; N, 6.29. EI-MS (positive ion mode) accurate mass: M^+ calcd, 442.1555; found, 442.1549. FT-IR ($CHCl_3$): 1739 (C═O Val$_2$, ester), 1678 (C═O Val$_1$), 1648 cm^{-1} (C═O Fc). ^{1}H NMR (δ, $CDCl_3$): 6.38 (2H, br d, N*H* Val$_1$, N*H* Val$_2$), 4.73 (2H, t, J_{HH} = 1.9 Hz, C*H*$_0$ Cp), 4.59 (1H, dd, J_{HH} = 4.9, 8.7 Hz, C*H*$^\alpha$ Val$_2$), 4.44 (1H, dd, J_{HH} = 7.0, 8.6 Hz, C*H*$^\alpha$ Val$_1$), 4.38 (2H, t, J_{HH} = 1.9 Hz, C*H*$_m$ Cp), 4.23 (5H, s, *C*H unsubst Cp), 3.78 (3H, s, OC*H*$_3$), 2.21 (2H, septet, J_{HH} = 6.8 Hz, C*H*$^\beta$ Val$_1$, C*H*$^\beta$ Val$_2$), 1.05 (6H, dd, J_{HH} = 2.2, 6.8 Hz, (C*H*$_3$)$_2$, Val), 0.96 (6H, t, J_{HH} = 6.9 Hz, (C*H*$_3$)$_2$ Val). ^{13}C{^{1}H} NMR (δ, $CDCl_3$): 172.4 (*C*═O), 171.9 (*C*═O), 170.7 (*C*═O), 75.8 (*C*$_i$ Cp), 70.8 (2C, *C*H$_m$ Cp), 69.9 (5C, *C*H unsubst Cp), 68.7 (*C*H$_o$ Cp), 68.3 (*C*H$_o$ Cp), 58.5 (*C*H$^\alpha$ Val), 57.5 (*C*H$^\alpha$ Val), 52.3 (OCH$_3$), 31.5 (*C*H$^\beta$ Val), 31.2 (*C*H$^\beta$ Val), 19.5 (*C*H$_3^\gamma$ Val), 19.2 (*C*H$_3^\gamma$ Val), 18.7 (*C*H$_3^\gamma$ Val), 18.1 (*C*H$_3^\gamma$ Val).

Preparation and Characterization of 3-Trifluoroacetylamido-5-methylpyrazole (TFAc-AMP; 14). The literature procedure[11b] was modified. To a cooled (0 °C) solution of 3-AMP (359 mg, 3.7 mmol) in CH_2Cl_2 (18 mL) was added Et_3N (800 μL, 5.74 mmol), followed by the dropwise addition of trifluoroacetic anhydride (620 μL, 4.4 mmol). After it was stirred overnight, the reaction mixture was filtered through a cotton pad, and the solvent was removed in vacuo. The oily residue was purified by column chromatography (EtOAc; R_f = 0.35). **14** crystallizes from EtOAc by slow evaporation to give colorless X-ray quality crystals. Yield: 0.220 g, 31%. Elemental Anal. Calcd for $C_6H_6N_3OF_3$: C, 37.32; H, 3.13; N, 21.76. Found: C, 37.44; H, 3.06; N, 21.52. EI-MS accurate mass for $C_6H_6N_3OF_3$: M^+ calcd, 193.0463; found, 193.0456. ^{1}H NMR (δ, CD_3CN): 10.60 (1H, br s, N*H*), 10.14 (1H, br s, N*H*), 6.40 (1H, s, C*H* AMP), 2.27 (3H, s, C*H*$_3$ AMP). ^{13}C-{^{1}H} NMR (CD_3CN): 155.3 (1C, q, J_{CF} = 37.8 Hz, CF_3CO), 146.2 (1C, s, *C*3 AMP), 141.6 (1C, s, *C*5 AMP), 117.0 (1C, q, J_{CF} = 287.0 Hz, *C*F$_3$), 97.5 (1C, s, *C*H AMP), 11.1 (1C, s, *C*H$_3$).

^{1}H NMR Titrations of Fc-Dipeptides with 3-Amino-5-methylpyrazole Derivatives. For the NMR titration experiments, all ^{1}H NMR spectra were referenced to the CH_2Cl_2 signal (δ 5.32) of an external standard ($CH_2Cl_2/CDCl_3$) placed in the NMR tube before each titration. All spectra were carried out at 296 ± 1 K. A 30° pulse with a sweep width of 8.632 ppm (O1 = 4865.34 Hz) was used. 128 scans with a delay of 0.1 s and 32K data points per scan were collected for each spectrum. NMR samples of Fc-peptide in $CDCl_3$ in the concentration range 5.7−6.5 mM were prepared by microliter addition of stock Fc-peptide solution to an NMR tube containing neat $CDCl_3$. An initial spectrum of the free Fc-peptide was collected, and then aliquots of ligand solution were added from a stock solution via microsyringe (10 and 50 μL, Hamilton; 500 μL, SGE). When small aliquots of ligand solution were added, the NMR tube was capped, shaken vigorously, and then injected into the spectrometer. After the magnetic field was

reshimmed, a spectrum was collected. When a large volume of ligand solution was added, or when the volume in the NMR tube became too large to mix by shaking, the NMR tube was capped, inverted several times, and then injected into the spectrometer. After the magnetic field was reshimmed, the spectrum was collected, and if the amide protons did not resolve into doublets (triplets for **1**), the sample was allowed to come to equilibrium before the next spectrum was collected. The amide proton of the terminal residue of Fc-peptide was monitored as the mole ratio (m.r.) of ligand to Fc-peptide increased, and the chemically induced shift (CIS) values were plotted versus m.r. Dilutions of the solutions were taken into account. The association constant K_A for the interaction and the chemical shift δ of the fully complexed amide proton were determined by nonlinear regression.[6b] Each titration experiment was repeated at least three times under identical conditions.

Variable Temperature ^{1}H NMR Experiments. NMR samples of Fc-peptide or Fc-peptide with APzl derivative (1:1 mixture) in $CDCl_3$ are prepared from stock solutions. The amide proton resonances of the Fc-peptide are monitored as the temperature of the solution is raised and lowered. δ values are referenced to the CH_2Cl_2 resonance at δ 5.32 of an external standard (CH_2Cl_2/$CDCl_3$) placed in the NMR tube at the start of the experiment. Differences in chemical shifts ($\Delta\delta$) are plotted versus the temperature, and the slope of the line indicates the temperature dependence of each amide proton.

FT-IR Experiments. All ligand and Fc-peptide sample solutions are prepared by transferring stock solutions via microsyringe to a vial containing dry $CHCl_3$. The total volume of the solution is in the range 1−2 mL, and the concentration of solutions is 6.0 mM, unless otherwise specified. All Fc-peptide/ligand samples are prepared by adding solid ligand to a vial, adding $CHCl_3$, and then transferring an equimolar quantity of stock Fc-peptide solution to the vial and thoroughly mixing. The total volume of the solution is in the range 5−6 mL, and the concentration of Fc-peptide and ligand in the 1:1 mixture is between 5.0 and 5.5 mM. After a background spectrum of the empty liquid cell (KBr) and a solvent spectrum are collected, the sample solution is injected into the cell and a spectrum is collected. The cell is thoroughly rinsed with $CHCl_3$, the Fc-peptide/ligand solution is injected, and a spectrum of the 1:1 solution is collected. The solvent spectrum is subtracted from the initial spectrum of the 1:1 solution, followed by a baseline correction. In the case of Fc-peptide/3-TFAc-AMP mixtures, Fourier self-deconvolution (FSD) is also applied.

Electrochemical Studies. All electrochemical experiments were carried out using a CV-50W voltammetric analyzer (BAS) at room temperature (20 ± 2 °C). No special precautions were taken to exclude oxygen. Chloroform was dried over CaH_2 and distilled under nitrogen prior to use. Tetrabutylammonium perchlorate (TBAP) was used as the supporting electrolyte (0.1 M). For the cyclic voltammetry studies, a glassy carbon working electrode (BAS, diameter 2 mm) and a platinum wire counter electrode were used. The glassy carbon working electrode was polished with 3 μm, followed by 1 μm, and then finished with 0.5 μm alumina prior to use to remove any surface contaminants. The reference electrode was a Ag/AgCl electrode (BAS). *iR* compensation was applied. Backgrounds of the solvent containing 0.1 M TBAP were collected before each set of experiments and then subtracted from the spectra. A scan was taken before and after the addition of a 10-fold excess of aminopyrazole derivative. The experiment was repeated at least 10 times to get reliable values for $E_{1/2}$.

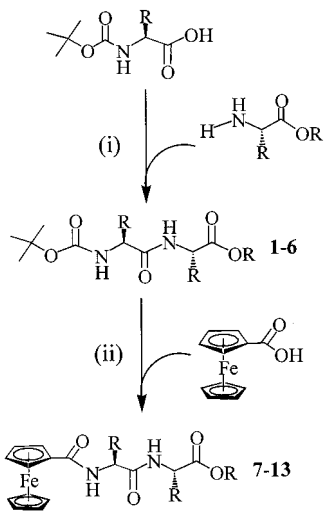
X-ray Crystallography. Suitable crystals of **7** were obtained by layering a CH_2Cl_2 solution of **7** with hexanes. Yellow needles suitable for X-ray crystallography were deposited after 2 days. Crystals of **9** were obtained from CH_2Cl_2. NMR measurements on these crystals confirmed the presence of CH_2Cl_2 in the crystals. However, once mounted, these crystals rapidly lost crystallinity even at −100 °C. We therefore decided to grow **9** from a solution of 1,2-dichloroethane, which was layered with hexanes. This gave suitable orange crystals of **9**. Both compounds were mounted onto glass fibers. Data for **7** and **9** were measured using a Siemens Smart CCD (for **9**: Bruker P4 SMART equipped with a rotating anode and a 1000 CCD) diffractometer using Mo Kα radiation (graphite monochromated) with ω scans. Both structures were solved using direct methods.[13a] For **7** and **9**, all non-hydrogen atoms were refined anisotropically using full-matrix least-squares on F^2.[13b] The final R value for **7** was $R = 0.0324$ for 6157 reflections with $I > 2\sigma(I)$ (9027 total reflections). For **9**, the final R value was 0.0749 for 11 725 reflections with $I > 2\sigma(I)$ (22 997 total reflections). Crystallographic details have been summarized in Table 1.

Table 1. Crystallographic Data for Fc-Gly$_2$-OEt (**7**) and Fc-Leu-Phe-OEt (**9**)

	7	**9**
chemical formula	$C_{35}H_{42}Fe_2N_4O_{8.5}$	$C_{29}H_{36}Cl_2FeN_2O_4$
fw	766.43	603.35
space group	$C2/c$	$P2_1$
a, Å	47.192(3)	17.5020(15)
b, Å	8.5076(5)	16.4060(17)
c, Å	17.3694(10)	21.789(2)
β, deg	91.7180(10)	102.398(2)
V, Å^3	6970.5(7)	6110.5(10)
Z	8	8
T, K	293(2)	193(2)
λ, Å	0.71073	0.71073
ρ_{calc}, g cm^{-1}	1.461	1.312
μ (Mo Kα), cm^{-1}	0.891	0.703
R^a	0.0324	0.0749
R_w	0.0681	0.1724

[a] $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $R_w = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^4)]^{1/2}$. $w = [\sigma^2(F_o^2) + (0.0879P)^2 + 0.0229P]^{-1}$ where $P = [\max(F_o^2,0) + 2F_c^2]/3$.

Scheme 2. Synthesis of Ferrocenoyl Dipeptides **7**−**13**[a]



[a] (i) (a) EDC (or DCC), HOBt, CH_2Cl_2; (b) Et_3N and Amino Acid Ester, CH_2Cl_2; (ii) (a) TFA, 30 min; (b) Et_3N, CH_2Cl_2; (c) EDC, HOBt, CH_2Cl_2, 30 min.

Results and Discussion

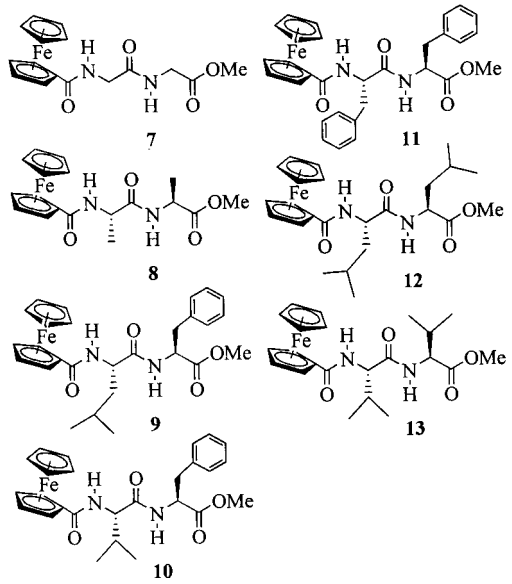
Synthesis and Characterization of Fc-Dipeptides. Boc-dipeptide esters **1**−**6** were produced in moderate to excellent yield from Boc-amino acids and amino acid esters using either DCC or EDC (see the Experimental Section) in the presence of HOBt in basic CH_2Cl_2, using the carbodiimide protocol.[12] After the Boc group was removed by reaction with neat TFA, the dipeptides were coupled to FcOH using the EDC/HOBt procedure (Scheme 2), resulting in the formation of the desired Fc-dipeptides (**7**−**13**) (see Chart 2). The crude products were purified by column chromatography, producing yellow-orange crystalline solids in moderate yield.

Compounds **7**−**13** were characterized by ^{1}H and ^{13}C{^{1}H} NMR spectroscopy, mass spectrometry (MS), and elemental analysis. ^{1}H NMR assignments were made on the basis of chemical shift, relative integration, signal multiplicity, comparison with similar compounds, and 2D-NMR experiments such

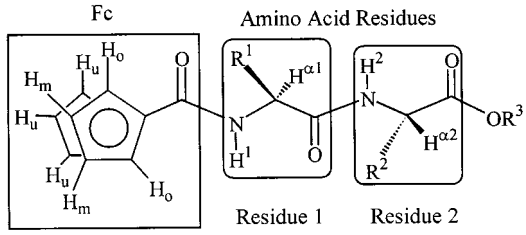
Table 2. Selected Chemical Shift Values (δ, ppm, Relative to TMS) for Fc-Dipeptides **7**−**13** (See Chart 2)

compound	NH^1	NH^2	$H^{\alpha 1}$	$H^{\alpha 2}$	$H^{\beta 1}$	$H^{\beta 2}$	$^{Cp}H_o$	$^{Cp}H_m$	$^{Cp}H_u$
Fc-G_2-OEt (**7**)	6.39	6.64	4.08	4.11			4.72	4.38	4.24
Fc-A_2-OBzl (**8**)	6.24	6.66	4.66	4.66	1.46	1.46	4.66	4.37	4.21
Fc-LF-OMe (**9**)	6.00	6.57	4.61	4.85	1.68	3.12	4.73, 4.64	4.39	4.20
Fc-VF-OMe (**10**)	6.23	6.23	4.38	4.90	2.18	3.14	4.74, 4.68	4.38	4.21
Fc-FF-OMe (**11**)	6. 05	6.45	4.81	4.81	3.09	3.09	4.66, 4.51	4.36	4.04
Fc-LL-OMe (**12**)	6.08	6.43	4.63	4.63	1.67	1.67	4.70	4.38	4.22
Fc-VV-OMe (**13**)	6.38	6.38	4.44	4.59	2.21	2.21	4.73	4.38	4.23

Chart 2



Scheme 3. Numbering Scheme for Fc-Dipeptides



as ^{1}H-^{1}H COSY. Scheme 3 illustrates the naming/numbering used in the discussion and the Experimental Section for all Fc-dipeptides prepared in this work.

The ^{1}H NMR properties of **7**−**13** are summarized in Table 2. The ^{1}H and ^{13}C{^{1}H} signals produced by the Fc moiety follow the typical signal pattern of a monosubstituted Fc group with the unsubstituted Cp ring producing a singlet near 4.2 ppm, with the meta proton signals further downfield and the ortho protons furthest downfield. The Cp meta protons are relatively far from the stereocenter of the first amino acid residue, and so these diastereotopic protons appear together as a broadened singlet like in all Fc-peptides synthesized. The Cp ortho protons, however, are close enough to the chiral center that it is possible to distinguish these protons spectroscopically. For **10** and **11**, the signals are separated, while in the case of **12** and **13** both protons appear coincidentally as one signal. It was important to unequivocally assign the NH proton resonances for all Fc-peptides, because the interpretation of NMR titration data depends on these assignments. Hence, we carried out HMBC experiments for **7**−**13**. For all systems, the proton signal of amide NH^1 exhibits long-range coupling to the Cp carbon atoms and the carbon atom of the peptide carbonyl, whereas the proton signal of the amide NH^2 shows coupling with the carbon of the ester carbonyl but not with the Cp carbons. Likewise, NH^1 does not show any long-range coupling with the ester C═O.

Table 3. Amide Absorptions (υ, cm^{-1}) for Fc-Dipeptides **7**−**13** in $CHCl_3$ Solution in the Absence and Presence of Equimolar Amounts of TFAc-AMP (**14**)[a]

compound	$C{=}O_{ester}$	$C{=}O_{amide}$	$C{=}O_{Fc-C=O}$
Fc-G_2-OEt (**7**)	1744	1681	1648
7 + TFAc-AMP (**14**)	1741	1681	1646
Fc-A_2-OBzl (**8**)	1740	1681	1646
8 + 3-AMP	1738	1680	1644
8 + TFAc-AMP (**14**)	1738	1678	1644
Fc-LF-OMe (**9**)	1743	1676	1651
9 + TFAc-AMP (**14**)	1724	1675	1641
Fc-VF-OMe (**10**)	1743	1676	1651
10 + TFAc-AMP (**14**)	1726	1675	1644
Fc-FF-OMe (**11**)	1744	1677	1647
11 + TFAc-AMP (**14**)	1725	1676	1645
Fc-LL-OMe (**12**)	1742	1678	1647
12 + TFAc-AMP (**14**)	1726	1676	1641
Fc-VV-OMe (**13**)	1739	1678	1647
13 + TFAc-AMP (**14**)	1724	1677	1642
13 + 2-(Boc-Aib)-AMP (**15**)	1740	1677	1647

[a] For **8** and **13**, the interaction with equimolar amounts of 3-AMP and 2-(Boc-Aib)-AMP is reported. The concentration for all systems is in the range 5.0−5.5 mM.

The IR spectra of all Fc-peptides display three characteristic absorptions in the C═O near 1740, 1680, and 1650 cm^{-1} (see Table 3). The band assignments are made by comparison with IR spectra of Boc-dipeptides and Fc-amino acids. Fc-amino acid esters, such as Fc-Gly-OEt, show two carbonyl bands at 1739 and 1655 cm^{-1}. Boc-Gly_2-OEt exhibits three bands at 1741, 1713, and 1686 cm^{-1}. With this information, we were able to assign the carbonyl bands in **7**−**13**. The signal near 1740 cm^{-1} is assigned to the ester group. The signals at 1680 and 1650 cm^{-1} are assigned to the peptide amide and the Fc-amide, respectively. Importantly, the carbonyl bands are sensitive to the presence of H-bonding and will be used to qualitatively evaluate H-bonding to 3-APzl derivatives (vide infra).

For our studies, it was of paramount importance to ensure that the Fc-dipeptides are in fact able to undergo H-bonding interactions with small molecules. To evaluate the steric effects of the Fc-moiety, which may reduce the ability of the acceptor and donor groups (Fc-CO or NH^1) adjacent to the Fc-group to engage in H-bonding because of the possibility of steric congestion, we decided to carry out detailed crystallographic studies of compounds **7** and **9**. However, on the basis of our work with Fc-amino acids, we did not expect a significant hindrance in the H-bonding ability.

We were fortunate to be able to obtain single crystals of suitable quality of compounds **7** and **9** for X-ray crystallography.

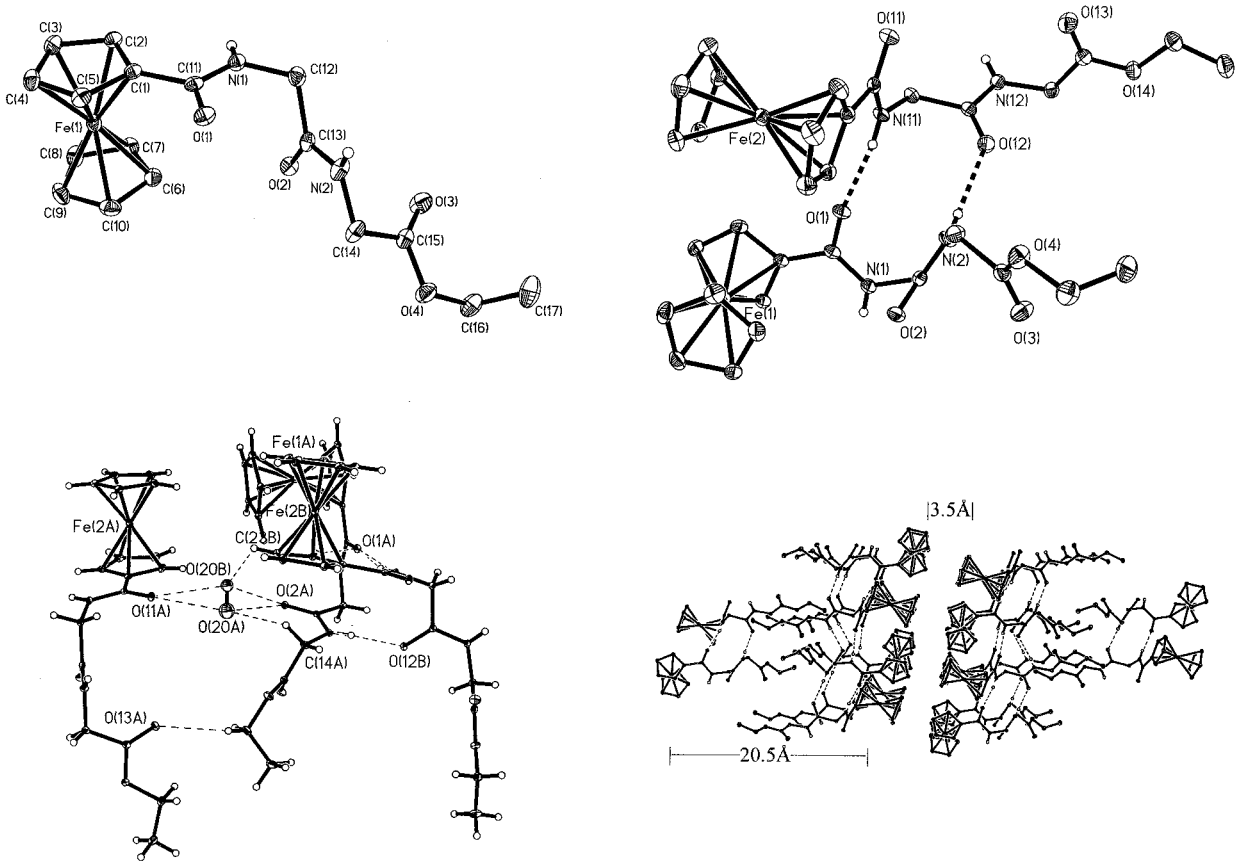


Figure 1. (a) Molecular structure of Fc-Gly_2-OEt (**7**). The ellipsoids are shown at the 30% probability level. Only one of the two molecules in the asymmetric unit is shown. The hydrogen atoms are omitted for clarity. (b) Two adjacent molecules interacting in a head-to-head fashion engaging in H-bonding, resembling the interaction found in parallel β-sheets. (c) Parallel β-sheet layers held together by H-bonding to an interstitial water molecule (disordered over two positions). Bond lengths include d(O(11A)—O(20A)) = 2.765 Å and d(O(2A)—O(20B)) = 2.848 Å. In addition, there is a close C═O···H—C contact of 2.566 Å between the carbonyl oxygen O(13A) and the H atom on C(16A) and a close H_2O···H—C contact of 2.247 Å between O(20A) and the H atom on C(14A). (d) Projection looking at the β-sheet. The water molecules holding together the β-sheets have been omitted for clarity. Two β-sheets form a tail-to-tail bilayer with a thickness of 20.5 Å, with the podand peptide tails on the inside and the Fc head groups on the outside. The bilayers themselves form an extended layered structure with an interlayer distance of 3.5 Å.

Table 4. Selected Bond Lengths (Å) and Angles (deg) for **7**[a]

Bond Lengths, Å	
av Fe(1)—C_{Cp}	2.048(2)
av Fe(1)—C_{CpR}	2.048(2)
O(1)—C(11)	1.240(2)
N(1)—C(11)	1.331(2)
C(1)—C(11)	1.477(2)
Bond Angles, deg	
O(1)—C(11)—N(1)	120.96(15)
O(1)—C(11)—C(1)	119.81(15)
N(1)—C(11)—C(1)	119.23(15)
Torsion Angles, deg	
Φ	ψ
−120	+116 (N-term)
−125	+97 (C-term)

[a] Bond angles and lengths are given only for one molecule.

The crystal structure of **7** is shown in Figure 1. Selected bond distances and angles are given in Table 4. The asymmetric unit contains two independent molecules of **7**. In addition, the asymmetric unit contains a water molecule, disordered over two positions. Compound **7** exhibits features common to many monosubstituted ferrocene amides,[14] including the coplanarity of the two Cp rings with small Cp—Fe—Cp bent angles (2.1° and 1.9° for the two molecules). The Cp and amide planes are virtually coplanar with small torsion angles allowing the interaction between the π-systems of the Cp ring and the amide group. The Cp—C(O) distances of 1.477(2) and 1.484(2) Å for the two molecules within the asymmetric unit are within the range of those for other simple Fc-amides. Similarly, the amide C—O and C—N bond distances are normal, compared to those of other Fc-amides. The two independent molecules of **7** are held together by H-bonding involving the Cp-amide carbonyl and NH groups (d(O(1)—N(11)) = 3.073 Å and d(N(2)—O(12)) = 2.992 Å) (Figure 1b). Compound **7** forms a layered structure with H-bonding between adjacent units and layers through a disordered water molecule (Figure 1c). The Fc-diglycine molecules are organized in such a way that their arrangement can be described as a parallel β-sheet (Figure 1d). In fact, the torsion angles Φ and ψ in **7** are $\Phi = -120°$ and $\psi = +116°$, which are close to the literature values of $\Phi = -119°$ and $\psi = +113°$ for parallel β-sheets.[27] Two Fc-dipeptide β-sheets form a bilayer with the peptide substituents pointing toward each

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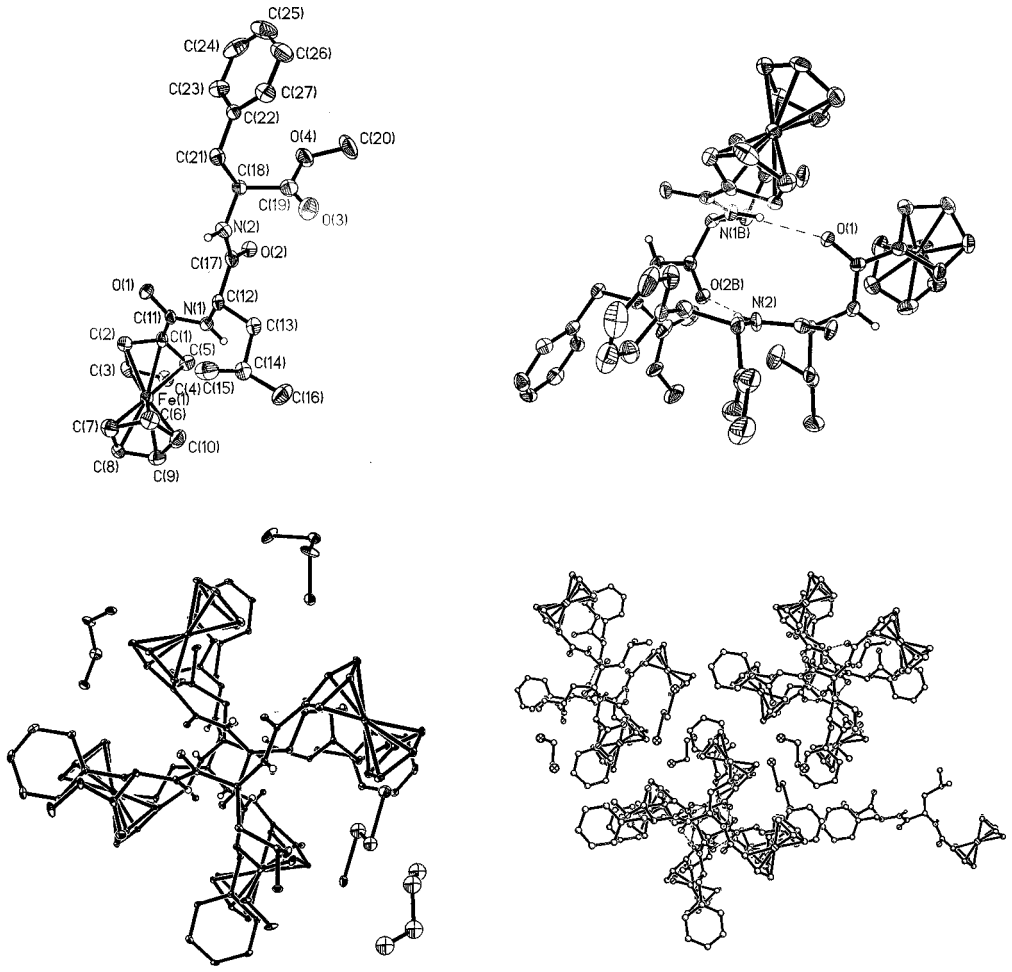

Figure 2. (a) Molecular structure of Fc-Leu-Phe-OMe (**9**). The ellipsoids are shown at the 30% probability level. The hydrogen atoms are omitted for clarity. (b) Two molecules of **9** H-bonding in a head-to-head fashion. For clarity, the solvent molecule ($C_2H_4Cl_2$) and its interaction with **9** is not shown here. (c) View down the helical axis of the supramolecular structure formed by four molecules of **9**. The width of the helix (Fe−Fe distance) is 12.7 Å, and its height is 14.6 Å. (d) Packing of the helicates of **9**. View down the helical axis.

other. The bilayer thickness is 20.5 Å, and its separation from the adjacent bilayer is 3.5 Å. Studies of diglycine show that it crystallizes in a layered structure with strong N−H···O═C H-bonds linking molecules within a layer, giving it a parallel β-sheet-like structure.[15] A second group of H-bonds joins the layers. Essentially, the H-bonding ability of diglycine is not significantly perturbed by the presence of the Fc group. In fact, the Gly_2 substituent controls the arrangement of the molecules in the solid state, resulting in a strongly H-bonded structure similar to that found in diglycine. Strongly H-bonded networks leading to an antiparallel assembly of the Fc-dipeptide were recently reported for Fc-Ala-Pro-py.[16] Extensive H-bonding involving the amide N−H of **7** is present in solution. In a saturated solution of **7** (3 M) in $CDCl_3$, the two amide signals of a saturated solution of **7** exhibit a modest temperature dependence (G1, −8.5 ppb/K; G2, −8.2 ppb/K). Higher temperatures will decrease the ability for H-bonding interactions, thus resulting in a shift closer to the "unperturbed" non-H-bonded signal.

The molecular structure of Fc-Leu-Phe-OMe (**9**) is shown in Figure 2. Selected bond distances and angles are given in Table 5. The two Cp rings in **9** are coplanar with a Cp−Fe−Cp bent angle of 4.5°. The torsion angle between the Cp and amide planes is 18.9°. This larger torsion angle can be explained by considering the larger steric requirements of the isobutyl side chain of Leu. Similar torsion angles have been observed in other Fc-amides having bulky side chains. The Cp−C(O), amide C−O, and C−N distances are all within the range of those for related structures (d(C1−C11) = 1.485(10) Å; d(O1−C11) = 1.215(8) Å; d(N1−C11) = 1.362(10) Å). The H-bonding in **9** has similarities to that of **7** in that it involves the interaction of two Fc-amides (Fc−C═O···H−N−C(O)−Fc; d(O(1)−N(1B)) = 2.977 Å) and two peptide amides ($C{=}O_{Phe}{\cdots}H{-}N_{Phe}$; d(O(2B)−N(2)) = 2.983 Å) of two adjacent molecules (Figure

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(25) The ferrocene/ferrocenium couple had a peak separation of 85 mV under these experimental conditions and a ratio of peak currents of 0.95.
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Table 5. Selected Bond Lengths (Å) and Angles (deg) for **9**[a]

Bond Lengths, Å	
av Fe(1)−C_{Cp}	2.040(9)
av Fe(1)−C_{CpR}	2.043(8)
O(1)−C(11)	1.215(8)
N(1)−C(11)	1.362(10)
C(1)−C(11)	1.485(10)
Bond Angles, deg	
O(1)−C(11)−N(1)	121.2(7)
O(1)−C(11)−C(1)	122.3(7)
N(1)−C(11)−C(1)	116.5(6)
Torsion Angles, deg	
Φ	ψ
−119	+109 (N-term)
−122	+111 (C-term)

[a] Bond angles and lengths are given only for one molecule.

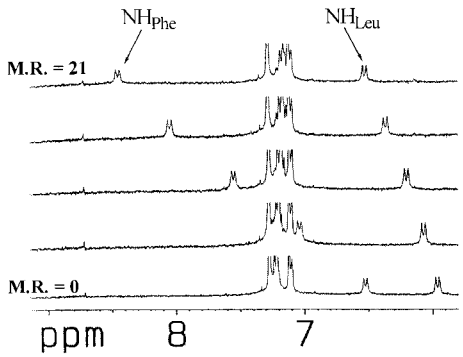


Figure 3. ^{1}H NMR study of the interaction of Fc-Leu-Phe-OMe (**9**) with 3-AMP in $CDCl_3$ at various mole ratios of 3-AMP to **9**. Please note the strong downfield shift of the Phe NH resonance as the concentration of 3-AMP is increased.

2b). In the interaction, the directionality of the interaction is such that it can be described as a parallel sheetlike structure. In addition, the ester carbonyl oxygen is involved in H-bonding to a molecule of solvent, which has been omitted from Figure 2b. Interestingly, the intermolecular H-bonding interactions enable the formation of supramolecular helicates, with the Fc groups on the outside of the center of the helix (Figure 2c) and the isobutyl group on the inside of the helix. Presumably, this supramolecular structure is adopted because of the steric requirements of the Leu and Phe groups. The solvent molecules are occupying positions between individual helicate stacks. The packing of the helices is shown in Figure 2d.

In summary, our X-ray crystallographic studies and those carried out by others[6,16] clearly show that extensive H-bonding is present in **7** and **9**. It can be concluded that the Fc group does not interfere with the peptides' ability to engage in intermolecular hydrogen bonding. The Fc−C═O, the peptide amide N−H, and the ester C═O group are availabile for binding to substrates, such as 3-AMP derivatives.

Binding Studies. (a) ^{1}H NMR Spectroscopy. We have studied the interaction between Fc-peptides **7**−**13** and 3-AMP derivatives by ^{1}H NMR and FT-IR spectroscopies for the purpose of evaluating the utility of 3-AMP derivatives for inducing a β-sheet-like arrangement. ^{1}H NMR resonances of protons involved in H-bonding experience downfield shifts as H-bonding strength increases. Thus, the extent to which an H-bond donor is H-bonded can be monitored by ^{1}H NMR spectroscopy. In peptide systems, the extent of intermolecular H-bonding depends on the concentration of the peptide in solution, the solvent, and the presence of substrates able to H-bond to the peptide, such as 3-APzl derivatives. Hence, ^{1}H

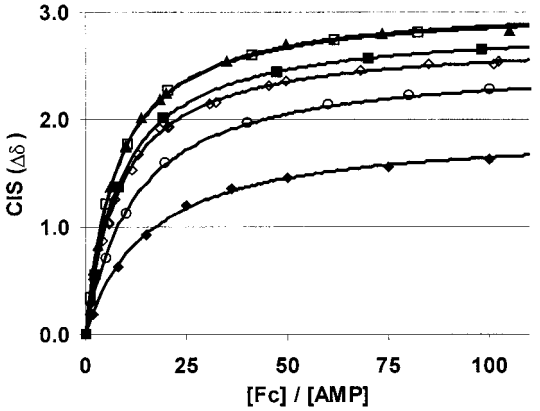


Figure 4. Interaction of Fc-dipeptides **7**−**13** with 3-AMP. Shown are the changes in δ (in ppm) for the C-terminal NH upon addition of 3-AMP to a $CDCl_3$ solution of the Fc-dipeptide. Experimental values (**7** (◆), **9** (◇), **10** (■), **11** (□), **12** (▲), **13** (○)) and the fits (solid lines) are shown. Please note that the curves for the interaction of **11** (□) and **12** (▲) overlap.

NMR experiments, in which the ratio of substrate to peptide is increased (saturation titrations), can provide information about the degree of H-bonding and the stoichiometry of the interaction and can be used to calculate association constants (K_A).[6b]

Figure 3 shows a stack plot of an NMR saturation titration experiment of **9** with 3-AMP. Upon addition of increasing amounts of 3-AMP, the NH^2 amide proton exhibits a strong downfield shift. The chemical shift of NH^1 is less strongly influenced. From this, a plot of the chemically induced shift (CIS) in ppm versus the ratio of ligand to Fc-dipeptide is constructed (Figure 4).

This behavior fits the two-state binding model (1:1) developed before for the interaction of **7**−**9** with 3-APzl (eqs 1 and 2).

$$\mathrm{Fc_{free}} + \text{3-AMP} \rightleftharpoons \mathrm{Fc_{bound}} \tag{1}$$

$$K_A = \frac{[\mathrm{Fc_{bound}}]}{[\text{3-APzl}][\mathrm{Fc_{free}}]} \tag{2}$$

$$\delta_{obs} = \alpha_{bound} d_{bound} + \alpha_{free}\delta_{free} \tag{3}$$

The observed chemical shift for the amide protons represents the time-averaged signal for the Fc-dipeptide in the complexed (δ_{bound}) and uncomplexed (δ_{free}) states, according to eq 3 (α_{bound} = fraction bound; α_{free} = fraction free). From this, we obtained K_A for the interaction by nonlinear regression,[17] as described before. Table 6 summarizes the K_A's for the interactions of **7**−**13** with 3-APzl, 3-AMP, 3-TFAc-AMP (**14**), and 2-Boc-Aib-AMP (**15**).

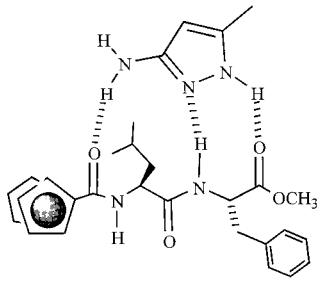
For all systems investigated, the ^{1}H NMR titration data fit the two-state (1:1) binding model in which the ligand binds only to the top face of the Fc-dipeptide (see Scheme 4). Our studies do not support binding of an additional ligand molecule to the bottom face of the Fc-dipeptide, as originally proposed by Schrader and Kirsten.[11] Instead, Fc-dipeptides exclusively bind 3-aminopyrazole derivatives on the top-face binding site. The IR spectroscopic study of the interaction provides additional support for this binding model (vide infra). This three-point binding site involves the Fc−C═O oxygen, the peptide N−H, and the ester carbonyl oxygen. Importantly, this group of atoms is also involved in H-bonding in the crystal structures of **7** and **9**. The crystal structure of **14** also shows the suitability of the complementary N−H/N/NH_2 set (vide supra). It is expected that the amide, which is part of the top face, will undergo the largest

Table 6. Association Constants (K_A) for the Interaction of Fc-Peptides **7**−**13** with 3-APzl, 3-AMP, 3-TFAc-AMP (**14**), and 2-Boc-Aib-AMP (**15**) in $CDCl_3$ Solution Measured at 23 ± 1 °C (Unless Otherwise Noted)[a]

	K_A (M^{-1})			
Fc-peptide	3-APzl	3-AMP	3-TFAc-AMP (**14**)	2-Boc-Aib-AMP (**15**)
Fc-Gly_2-OEt (**7**)	9[b]	8		
Fc-Ala_2-OBzl (**8**)	13			
Fc-Leu-Phe-OMe (**9**)	21.5[b]	17.5		0
Fc-Val-Phe-OMe (**10**)		17.5		
Fc-Phe_2-OMe (**11**)		20		
Fc-Leu_2-OMe (**12**)		19		
Fc-Val_2-OMe (**13**)		20	27[c]	0
Fc-Val_2-OMe (**13**)			4[d]	
Fc-Val_2-OMe (**13**)			2[e]	

[a] K_A values are calculated by nonlinear regression of the titration data using SigmaPlot.[17] (Error ≤10%). [b] See ref 6b. [c] Saturation titration in a mixture of $CD_3CN/CDCl_3$ (3:8). [d] Saturation titration in CD_3CN. [e] Saturation titration in acetone-d_6.

Scheme 4. Ligand Binding to the Top Face of the Fc-Dipeptide Involving the Fc—C═O, the Amide N—H, and the Ester C═O



chemically induced shift (CIS) if it engages in interactions with the 3-APzl derivatives. At this point, it is important to draw attention to the differences in the peptide side chains. The amino acids Gly and Ala have small side chains. The resulting dipeptides Gly_2 and Ala_2 are flexible and do not show an inherent preference for being part of a β-sheet conformation. Leu, Phe, and Val, having sterically more demanding side chains, show a high preference for being part of a β-sheet conformation.[18] Thus, we would expect lower binding constants for **7** and **8**. Comparing the interactions of 3-APzl and 3-AMP, our results show that the interactions of 3-AMP with the Fc-dipeptides **7**−**9** are slightly weaker than those for 3-APzl. The binding constants of 3-APzl and 3-AMP with **7** are the lowest of all Fc-dipeptides investigated (K_A = 9 and 8 M^{-1} for 3-APzl and 3-AMP, respectively). A reasonable explanation for this is the flexibility of the peptide backbone, which, in solution, may be in a conformation not suitable for binding and may have to undergo conformational changes, resulting in a lower net energy gain for the interaction. The presence of Leu, Phe, and Val in the Fc-dipeptides enables a stronger interaction, as seen in the larger K_A's for these complexes, ranging between 17.5 M^{-1} for the interaction of **10** with 3-AMP and 20 M^{-1} for the interaction of **13** with 3-AMP. These residues have a high preference for the β-sheet structure, which has the peptide in an extended conformation. This conformation is expected to be ideal for the interaction between the Fc-dipeptide and the 3-APzl derivatives. The K_A's, and thus the binding energies, are in a narrow region, suggesting that, as long as the peptide is in an extended conformation, the specific residues do not influence the binding to a large extent. As expected, for ligands such as 2-Boc-Aib-AMP (**15**), which has its binding site blocked, no interactions were observed.

We were particularly interested in evaluating the binding of 3-TFAc-AMP (**14**), which had been reported to be a good β-sheet inducer.[11] The substitution of the trifluoroacetyl group at the 3-amino position increases the H-bonding ability of the exocylic NH group,[19] and thus, stronger interactions and therefore larger binding constants are expected. Unfortunately, **14** has a very low solubility in $CHCl_3$, but it is very soluble in MeCN and acetone. Thus, we were unable to carry out titrations in $CHCl_3$ and had to resort to a mixture of $CDCl_3$ and CD_3CN (8:3) in order to obtain satisfactory solubility of the ligand. We decided to study the interaction of Fc-Val_2-OMe (**13**) with **14**, because this dipeptide has the highest propensity to form a β-sheet and should exhibit the strongest interaction with **14**. In addition, it would allow us a direct comparison with Schrader's results obtained for Ac-Val_2-OMe. However, in this solvent mixture, we obtained a K_A of only 27 M^{-1}. This binding constant is surprisingly low, given the reported high binding constant for the interaction of **14** with Ac-Val_2-OMe of 890 M^{-1}. Saturation titrations of **13** with **14** in neat acetone-d_6 and CD_3CN resulted also in small CIS values. This weak interaction between Fc-dipeptide and ligand may be due to competition for the H-bonding sites between the solvent and the ligand, resulting in low K_A's (<5 M^{-1}). Thus, we conclude that whereas 3-AMP derivatives can be useful as β-sheet templates in chlorinated solvent, they will not be useful in higher polarity solvents. Peptide solvation is of great importance and will influence the secondary structure that a peptide adopts in solution.[20] In particular, solvent molecules will influence the strength of hydrogen bonding between individual peptide strands and may in fact act as competitors,[21] inhibiting binding of the 3-AMP derivatives. In summary, only weak binding was observed with binding constants that were significantly below expectation.

(b) Infrared Spectroscopy. The amide group of peptides possesses several IR active modes that are sensitive to the presence of H-bonding. The two amide bands most commonly used to investigate the H-bonding in peptides and proteins are the amide I and amide A bands.[22] The amide I band is a strong, sharp absorption usually found near 1650 cm^{-1}, which is mostly a C═O stretching vibration with minor contributions from the C−N stretching vibration and a CCON deformation mode. The amide A band, usually found near 3300 cm^{-1}, arises solely from the NH stretching vibration and is very sensitive to H-bonding, often shifting up to several hundred wavenumbers upon interaction with good H-bond acceptors. Unfortunately, because it is a weak absorption and is significantly broadened, its utility as a technique for low concentration studies aimed at obtaining fine structural data is limited. Although it is much less sensitive to H-bonding interactions, shifting only 5−25 cm^{-1} upon interaction with donor groups, monitoring the amide I band is very useful for our purposes and has found wide applications for assessing secondary structural motifs in peptide and proteins caused by the presence of H-bonding.[23] Weakening of the C═O bond strength because of H-bonding will result in a corresponding change in amide I band position to lower wavenumbers.[24] Our Fc-dipeptides **7**−**13** possess three distinct carbonyl groups (ester, peptide-amide, Fc-amide) and produce three well separated IR bands at about 1740 (ester), 1680 (peptide-amide), and 1650 (Fc-amide) cm^{-1} (see Table 3), which all were assigned by comparison with Boc-dipeptides and Fc-amino acid esters.

Compound **14** is expected to show the strongest binding to the Fc-dipeptides. However, it presents experimental difficulties in that it has very low solubility in $CHCl_3$. It was noted,

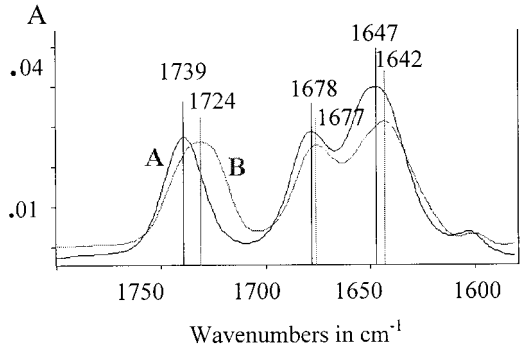


Figure 5. IR spectrum of Fc-Val_2-OMe (**13**) in $CHCl_3$ (5 mM) in the absence (A) and presence (B) of 3-TFAc-AMP (**14**).

however, that it can be solubilized in the presence of Fc-dipeptide. Thus, we carried out FT-IR experiments by monitoring the amide I and ester C═O bands of **7**−**13** in $CHCl_3$ and then adding 1 equiv of solid **14**.

Figure 5 shows the result of this addition experiment. Before the addition of a ligand, compound **13** exhibits three bands at 1739, 1678, and 1647 cm^{-1} in the amide I region. When 1 equiv of **14** is added to this solution, the signal assigned to the ester carbonyl shifts to 1724 cm^{-1}, and the Fc-carbonyl band shifts to 1642 cm^{-1}, indicating the involvement of these two carbonyl groups in H-bonding to **14**. These two groups are located on the same "side" of the molecule and are part of the three-point binding site made up of the Fc—CO, the ester, and the peptide NH group. Thus, our IR measurements provide important structural information about the nature of the interaction between the Fc-dipeptides and 3-APzl derivatives, which previously was inferred only from ^{1}H NMR measurements. The other Fc-dipeptides **9**−**12** also undergo shifts of the ester and Fc-carbonyl bands upon addition of **14**, whereas the band due to the peptide carbonyl remains virtually unchanged (Table 3). The magnitude of these shifts is comparable for **9**−**12**. The shifts experienced by **7** and **8** are small, indicating weak binding. This is in line with our NMR results on the interaction with the related 3-AMP. This ligand is able to interact only weakly with the flexible compounds **7** and **8**, resulting in low K_A's. Interactions with the Fc-dipeptides **9**−**13**, which prefer an extended conformation and are prone to β-sheet formation, are much stronger. All of our attempts in observing the interaction of 3-AMP with the Fc-dipeptides by IR failed, and no shifts in the amide I band were observed for any of the peptides. Importantly, we are unable to observe an interaction between the Fc-dipeptides **13** and **14** in the acetonitrile/chloroform solvent mixture. Our NMR titrations have shown that the interaction of **13** with **14** is of the same order of magnitude as the interaction with 3-AMP, suggesting that IR is not sufficiently sensitive to detect the resulting small changes in the position of the amide I band of the adduct.

(c) Electrochemical Studies. All Fc-dipeptides exhibit a fully reversible one-electron oxidation wave in chloroform solution in the potential range +129 to +159 (± 3) mV versus the ferrocene/ferrocenium couple (see Table 7). The separation between the oxidation and reduction peaks is between 70 and 90 mV for all systems investigated.[25] The ratio of oxidation and reduction peak currents is close to 1. It is immediately obvious that there are variations of the redox potential of the Fc moiety with the peptide substituent. It appears that the amino acid residue directly bound to the Fc group is largely reponsible for the observed redox potential. For example, the redox potential of Fc-V_2-OMe (**10**) is 159 mV, and that of Fc-VF-OMe (**13**) is 153 mV. All other Fc-dipeptides (**7**−**9**, **11**, and **12**) having non-V amino acids attached to the Fc moiety exhibit lower redox potentials in a range, thus making it easier to oxidize the Fc moiety.

Table 7. Cyclic Voltammetric Studies of the Interaction of Fc-Peptides with APzl and TFA-AMP in Chloroform[a]

	$E_{1/2}$,[b] mV		
compound	no ligand[c]	3-APzl	3-TFA-AMP
Fc-G_2-OEt (**7**)	129 (70)		131 (81)
Fc-A_2-OBzl (**8**)	133 (86)		130 (83)
Fc-LF-OMe (**9**)	132 (70)		146 (91)
Fc-VF-OMe (**10**)	153 (78)	158(121)	159 (88)
Fc-F_2-OMe (**11**)	143 (89)	154(157)	155 (91)
Fc-V_2-OMe (**13**)	159 (85)	173(117)	165 (94)

[a] 0.1 M TBAP in $CHCl_3$ at 20 ± 2 °C; glassy carbon working electrode, Pt counter electrode, Ag/AgCl references. [b] Versus ferrocene/ferrocenium; peak separation values follow in parentheses.

The addition of a large excess of 3-APzl to solutions of **10**, **11**, and **13** resulted in a cathodic shift of the redox potential by up to 14 (± 3) mV for **13**. Upon addition of 3-Apzl to solutions of **7** and **8**, no changes of the redox potential were observed. This is in line with the weakness of the interaction between the 3-APzl and these two flexible Fc-dipeptides **7** and **8** ($<5\ M^{-1}$). Addition of 3-TFA-AMP (**14**) to solutions of **10**, **11**, and **13** resulted in cathodic shifts of the redox potentials of up to 14 (± 3) mV. Again, the interaction with **7** and **8** did not result in any changes. Thus, ligation causes an increase in the electron density on the ferrocene group, causing a cathodic shift in the redox potential. Our observations compare favorably with those made by Beer and co-workers using neutral ferrocenoyl amides as anion selective receptors. When the interactions between the ferrocenoyl receptor and the anion were weak ($0-10\ M^{-1}$), addition of the anion did not influence the oxidation potential of the Fc group. Only for stronger interactions between the Fc receptor and the anion ($>10\ M^{-1}$) were changes observed in the oxidation of the Fc group.[5e,26] Shifts of 20 mV have been observed on complexation through H-bonding of carboxylic acids to the pyridine moiety of a ferrocenoyl amide. For the related bis-amido pyridine complex, a cathodic shift of up to 85 mV was observed.[5f]

Summary and Conclusions

In this paper, we give a full account of our work on the synthesis of ferrocenoyl dipeptides, including their full characterization. For the purpose of studying the electronic properties of peptides in a β-sheet conformation, we studied the interaction of Fc-dipeptides with aminopyrazole derivatives, a recently proposed β-sheet template. The ability of Fc-dipeptides to engage in H-bonding interactions with small molecules has been established in crystallographic studies of two representative members of a series of Fc-dipeptides. In both systems, the ferrocenoyl C═O group and the peptide amide N—H are both involved in H-bonding with neighboring molecules of Fc-dipeptide. A particularly interesting system is Fc-Gly_2-OEt (**7**). The podand diglycine substituent establishes a H-bonding pattern not unlike that in free diglycine, crystallizing as parallel β-sheets. The Fc group does not disrupt the ability of the diglycine chains to establish their native H-bonding interactions. In saturation titration studies, it is shown that the 3-AMP derivatives interact with the top face of the Fc-dipeptides by H-bonding. 3-AMP derivatives, which possess an additional substituent in the 2-position blocking the binding site, do not interact. However, the ability of 3-APzl derivatives to induce β-sheet conformation in peptides appears very limited. In fact, only weak interactions

are present in chloroform, ranging from 8 to 27 M^{-1}. In stronger donor solvents, such as acetonitrile or acetone, no evidence for the interaction between the Fc-dipeptides and ligands via H-bonding is found.

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Supporting Information Available: CIF files for compounds **7** (11 pages) and **9** (41 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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