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The synthesis and structural characterization of novel *N-meta*-ferrocenyl benzoyl dipeptide esters

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Abstract

A series of novel *N-meta*-ferrocenyl benzoyl dipeptide esters (**2**–**5**) have been prepared by coupling *meta*-ferrocenyl benzoic acid (**1**) to the dipeptide ethyl esters using the conventional 1,3-dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt) protocol. The dipeptides employed were GlyGly(OEt) (**2**), GlyAla(OEt) (**3**), GlyLeu(OEt) (**4**) and GlyPhe(OEt) (**5**). The compounds were fully characterized by a range of NMR spectroscopic techniques and by mass spectrometry (MALDI-MS, ESI-MS).

Keywords: Ferrocene; Bioorganometallic chemistry; Dipeptides; MALDI

Research in the area of bioorganometallic chemistry has developed as a rapidly growing area, connecting organometallic chemistry with the preparation of novel sensor compounds, peptide mimetic models and unnatural drugs [1–7]. The stability of the ferrocenyl group and its derivatives, in addition to its spectroscopic, electrochemical properties and ease of use make it suitable for biological applications and conjugation with biologically important compounds. The synthesis and structural characterization of novel *N*-ferrocenoyl and *N*-ferrocenyl amino acid and peptide derivatives has been reported [8–21]. In addition ferrocene has been incorporated in drugs such as antibiotics, aspirin, anti-malarial drugs and anti-cancer drugs such as tamoxifen [22–25]. A review on the bioorganometallic chemistry of ferrocene has recently been published [26].

In this communication, we report the synthesis and structural characterization of a series of novel *N-meta*-ferrocenyl dipeptide ethyl ester derivatives (**2**–**5**). The ferrocenyl moiety is linked to the dipeptide esters via a *meta*-benzoyl group. We recently, reported the synthesis and structural characterization of a series of *N-para*- and *N-meta*-ferrocenyl benzoyl amino acid ester derivatives [27–29]. The *N-meta*-ferrocenyl dipeptide ethyl ester derivatives are composed of three key moieties: (i) an electroactive core, (ii) a conjugated linker that can act as a chromophore and (iii) a dipeptide ethyl ester derivative that can interact with other molecules via hydrogen bonding.

meta-Ferrocenyl benzoic acid (**1**) was prepared as previously reported [29]. Conventional peptide chemistry was employed in the preparation of the dipeptide ethyl esters. Equimolar quantities of the *N-Boc* protected glycine was reacted with the amino acid ethyl ester hydrochloride salts of glycine, L-alanine, L-leucine and L-phenylalanine under basic conditions in the presence of dicyclohexylcarbodiimide (DCC), catalytic amounts of 1-hydroxybenzotriazole (HOBt) in dichloromethane (DCM) at 0 °C yielding the protected dipeptide ethyl esters. Deprotection of the amino terminal was achieved using TFA. The deprotected dipeptide ethyl esters were then coupled to *meta*-ferrocenyl benzoic acid using equimolar amounts of DCC and catalytic

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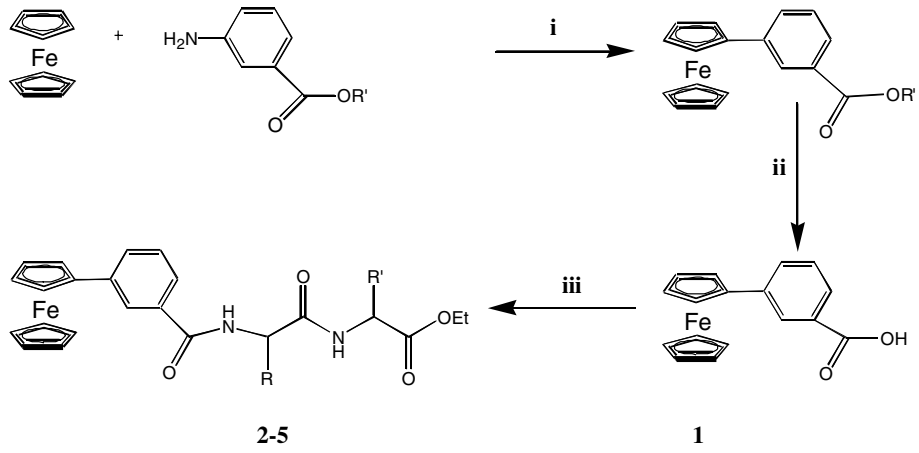


Scheme 1. Synthesis of the *N*-ferrocenyl benzoyl dipeptide esters (**2**–**5**): GlyGly(OEt) (**2**), GlyAla(OEt) (**3**), GlyLeu(OEt) (**4**) and GlyPhe(OEt) (**5**). (i) $NaNO_2$, HCl, 5 °C, (ii) NaOH/MeOH, H_2O, (iii) DCC, HOBt, Et_3N, dipeptide ethyl ester.

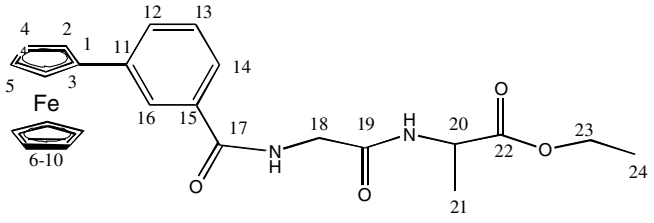
amounts of HOBt (Scheme 1). Purification by silica gel column chromatography furnished the pure products in yields of 50–57% and all gave analytical and spectroscopic data in accordance with the proposed structures [30]. The *N*-*meta*-ferrocenyl benzoyl dipeptide ethyl esters (**2**–**5**) were characterized by a combination of ^{1}H NMR, ^{13}C NMR, DEPT-135 and ^{1}H–^{13}C COSY (HMQC) spectroscopy, matrix assisted laser desorption ionization (MALDI) **2, 4, 5** and electrospray ionization mass spectrometry **3** (ESI).

All the proton and carbon chemical shifts for compounds **2**–**5** were unambiguously assigned by a combination of DEPT-135 and ^{1}H–^{13}C COSY (HMQC). The ^{1}H and ^{13}C NMR spectra for compounds **2**–**5** showed peaks in the ferrocene region characteristic of a mono substituted ferrocene benzoyl moiety [27–29]. The protons in the *ortho* position of the (η^5-C_5H_4) ring appear in the region δ 4.62–4.71, whereas the protons in the *meta* position occur in the range δ 4.27–4.35. The (η^5-C_5H_5) ring appears in the region δ 3.96–4.05. The protons of the *meta*-disubstituted benzoyl group appear in the region δ 7.2–7.96. For example, in the case of the L-alanine derivative **3,** the aromatic protons are present as a triplet, two doublets and a singlet at δ 7.36, δ 7.64, and δ 7.96, respectively. The (η^5-C_5H_5) ring appears as a singlet in the ^{1}H NMR spectrum at δ 4.05, whereas the *meta* and *ortho* protons on the (η^5-C_5H_4) ring are present at δ 4.35 and δ 4.71, respectively. The glycine N*H* proton appears as a triplet at δ 7.31, whereas the L-alanine N*H* appears as a doublet at δ 7.09. The L-alanine methyl group is present as a doublet at δ 1.47 and the ethyl ester methyl group appears as a triplet at δ 1.3.

The ^{13}C NMR spectra of compounds **2**–**5** show signals in the region δ 67.02–84.7 indicative of a monosubstituted ferrocene benzoyl subunit [27–29]. The *ipso* carbon of the (η^5-C_5H_4) ring appears in a very narrow range of δ 84.4–84.7. This signal is absent in the DEPT 135 spectra. The carbon atoms in the *ortho* position of the (η^5-C_5H_4) ring are present at δ 67.02–67.14, whereas the *meta* carbon atoms appear in the range δ 69.71–69.87. The carbon atoms of the (η^5-C_5H_5) ring appear in the range δ 70.08–70.26. The carbon atoms of the aromatic ring are non-equivalent and therefore six signals are visible in the region δ 124.7–140.7. The methylene carbon atoms of the derivatives **2**–**5** were identified by DEPT-135. A complete assignment of the ^{1}H and ^{13}C NMR spectra of compound **3** is presented in Table 1.

Since the introduction of soft ionization techniques such as fast-atom bombardment mass spectrometry (FABMS), matrix assisted laser desorption ionization (MADLI) and electrospray ionization mass spectrometry (ESI-MS), a wide range of thermolabile and non-volatile compounds

Table 1
^{1}H and ^{13}C spectroscopic data for **3**



Site	^{1}H NMR	^{13}C NMR	HMQC
1		84.4	
2,3	4.71		67
4,5	4.35		69.7
6–10	4.05		70.1
11		134.1	
12	7.64		129.8
13	7.36		124.7
14	7.64		129
15		140.7	
16	7.96		125.1
17		168.3	
18	4.19–27		44
19		169.1	
20	4.62		48.8
21	1.47		18.6
22		173.1	
23	4.19–27		62
24	1.3		14.5

can be subjected to mass spectrometric analysis [31–34]. The *N-meta*-ferrocenyl benzoyl dipeptide esters were not amenable to electron ionization (EI) or chemical ionization (CI) studies, therefore MADLI was employed in the analysis of compounds **2, 4** and **5,** whereas compound **3** was analyzed by ESI-MS. MALDI confirmed the correct relative molecular mass for the compounds and examination of the mass spectra revealed the presence of intense radical-cations. The formation of the radical-cation molecular ion species was further confirmed by the detection of the sodium adduct $[M + 23]^+$ and potassium adduct $[M + 39]^+$ for each of the compounds analyzed. This is not common for the analysis of peptides and proteins by MADLI-MS, as the formation of $[M + H]^+$ is favored. The vast majority of analytes subjected to analysis by soft ionization techniques such as FAB and MALDI furnish protonated molecular ion species as a result of proton transfer reactions between the analyte and matrix, and/or cation adduction. It has been reported that the molecular radical cation of ferrocene and not the protonated molecular ion is generated during MALDI analysis [35]. Fragment ions were not observed or were of very low intensity in the MALDI spectra. This is in contrast to the analysis of the related *para*-ferrocenyl benzoyl amino acid derivatives by FABMS, where important fragment ions were observed [27,28]. Compound **3** was analyzed by electrospray ionization mass spectrometry (ESI-MS). The ESI mass spectrum displayed an intense $[M + Na]^+$ species at *m/z* 485 and a potassium adduct was also present at *m/z* 501. Fragment ions were not observed or were of very low intensity.

In conclusion, the novel *N-meta*-ferrocenyl benzoyl dipeptide ethyl esters **2–5** were prepared in good yields using standard organic peptide synthetic protocols. The compounds were characterized by a range of NMR spectroscopic techniques and by MALDI and ESI mass spectrometry.

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[30] *Synthesis of compound* **3**. Glycine-L-alanine ethyl ester hydrochloride (0.2 g, 1.0 mmol) was added to a solution of *meta*-ferrocenoyl benzoic acid (0.3 g, 1.0 mmol), 1-hydroxybenzotriazole (0.2 g, 1.5 mmol), triethylamine (0.5 ml), and dicyclohexylcarbodiimide (0.45 g, 2.1 mmol) in 50 ml of dichloromethane at 0 °C. After 30 min, the temperature was raised to room temperature and the reaction was allowed to proceed for 48 h. The precipitated *N*,*N*′-dicyclohexylurea was removed by filtration and the filtrate was washed with water, 10% potassium hydrogen carbonate, 5% citric acid, dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by column chromatography {eluant 2:3 petroleum ether (40–60 °C): ethyl acetate}. Recrystallization from petroleum ether (40–60 °C): ethyl acetate furnished the title compound as orange needles. (0.229 g, 50%). M.p. 87–89 °C, $E'^0 = 131$ mV, $[\alpha]_D^{20} = -18°$ (c 2, EtOH). Mass spectrum: found: $[M + Na]^+$ 485.3, $C_{24}H_{26}N_2O_4FeNa$ requires: 485.12. IR ν_{max}(KBr): 3358, 3257, 3095, 2932, 1735, 1687, 1639, 1561, 1528, 1509 cm^{-1}. UV–Vis λ_{max} EtOH; 325 (ε 1290), 442 (ε 370) nm. ^1H NMR (400 MHz) δ ($CDCl_3$): 7.96 (1 H, s, ArH), 7.64 (2H, d, $J = 8.4$ Hz, ArH), 7.36 (1H, t, $J = 8.4$ Hz, ArH), 7.31 (1H, t, $J = 7.2$ Hz, –CON*H*–), 7.09 (1H, d, $J = 7.2$ Hz, –CON*H*–), 4.71 {2H, t, $J = 2$ Hz, *ortho* on (η^5-C_5H_4)}, 4.62 {1H, quint, $J = 7.6$ Hz, –C*H*(CH3)}, 4.35 {2H, t, $J = 2$ Hz, *meta* on (η^5-C_5H_4)}, 4.19–4.27 (4H, m, –NHCH_2CO–, –OCH_2CH$_3$), 4.05 {5H, s,(η^5-C_5H_5)}, 1.47{3H, d, $J = 7.6$ Hz, –CH(CH_3)}, 1.30 (3H, t, $J = 7.6$ Hz, –

OCH_2CH_3) ^{13}C NMR (100 MHz) δ ($CDCl_3$): 173.1, 169.1, 168.3, 140.7, 134.1, 129.8, 129.0, 125.1, 124.7, 84.4, 70.1, 69.7, 67.0, 62.0 (−ve DEPT), 48.8, 44.0 (−ve DEPT), 18.6, 14.5. (Elemental analysis: found, C, 62.06; H, 5.62; N, 5.93, $C_{24}H_{26}N_2O_4Fe$ requires, C, 62.35; H, 5.67; N, 6.06).

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