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The synthesis and structural characterization of *N-para*-ferrocenyl benzoyl dipeptide esters: The X-ray crystal structure of *N*-{*para*-(ferrocenyl)benzoyl}-L-alanine-glycine ethyl ester

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Abstract

A series of*N-para*-ferrocenyl benzoyl dipeptide esters **2**–**5** were prepared by coupling *para*-ferrocenyl benzoic acid (**1**) to the dipeptide ethyl esters using the conventional 1,3-dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt) protocol. The dipeptides employed in the synthesis were Ala-Gly(OEt) (**2**), Ala-Ala(OEt) (**3**), Ala-Leu(OEt) (**4**) and Ala-Phe(OEt) (**5**). The compounds were fully characterized by a range of NMR spectroscopic techniques, electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (MS/MS). In addition the X-ray crystal structure of the L-alanine-glycine derivative **2** has been determined.

Keywords: Ferrocene; Bioorganometallic chemistry; Tandem mass spectrometry; Dipeptides; X-ray

1. Introduction

Bioorganometallic chemistry is a rapidly growing research area, connecting organometallic chemistry with the preparation and characterization of novel sensor compounds, peptide mimetic models and unnatural drugs [1–9]. 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. The ferrocenyl group can also be conjugated with biologically important compounds. The incorporation of a ferrocene group onto proteins has shown the mediation of electron transfer between electrodes and the protein redox site [3,4]. The synthesis and structural characterization of novel *N*-ferrocenoyl and *N*-ferrocenyl amino acid and peptide derivatives has been reported [10–28]. In addition ferrocene has been incorporated in drugs such as antibiotics, aspirin, anti-malarial drugs and anti-cancer drugs such as tamoxifen [29–32]. A review on the bioorganometallic chemistry of ferrocene has been published [33]. Ferrocenyl derivatives with low oxidation potentials are attracting increasing attention due to their ability to generate hydroxyl radicals by interaction with the ferricenium ion generated under physiological conditions that results in cytotoxic effects [34]. In addition, conjugating redox active organometallic compounds to biological assemblies has also allowed for the design of materials for biomolecular sensors and switches [35]. Three international conferences on bioorganometallic chemistry (ISBOMC), have been held in the past six years and have focused on structure, reactivity, and biological activity of ferrocene derivatives. Our main focus has been in the synthesis of ferrocene derivatives that have low oxidation potentials and can interact with biomolecules *via* secondary interactions. The compounds are composed of three key moieties: (i) an

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electroactive core; (ii) a conjugated linker that lowers the oxidation potential of the ferrocene moiety; and (iii) a dipeptide ester that can interact with other molecules *via* hydrogen bonding.

The synthesis and structural characterization of *N-para*, *N-meta* and *N-ortho*-ferrocenyl benzoyl amino acid ester derivatives has been reported [36–39]. We have also recently reported the synthesis and structural characterization of *N-meta*-ferrocenyl benzoyl dipeptide esters [40]. Here, we now report the synthesis and structural characterization of a series of novel *N-para*-ferrocenyl benzoyl dipeptide ester derivatives **2**–**5**. The ferrocene moiety is linked to the dipeptide esters through a *para*-benzoyl group. The dipeptides employed in the synthesis were Ala-Gly(OEt), Ala-Ala(OEt), Ala-Leu(OEt) and Ala-Phe(OEt). The compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, ESI mass spectrometry and tandem mass spectrometry. In addition, the X-ray crystal structure of *N*-{*para*-(ferrocenyl)benzoyl}-L-alanine-glycine ethyl ester **2** is reported.

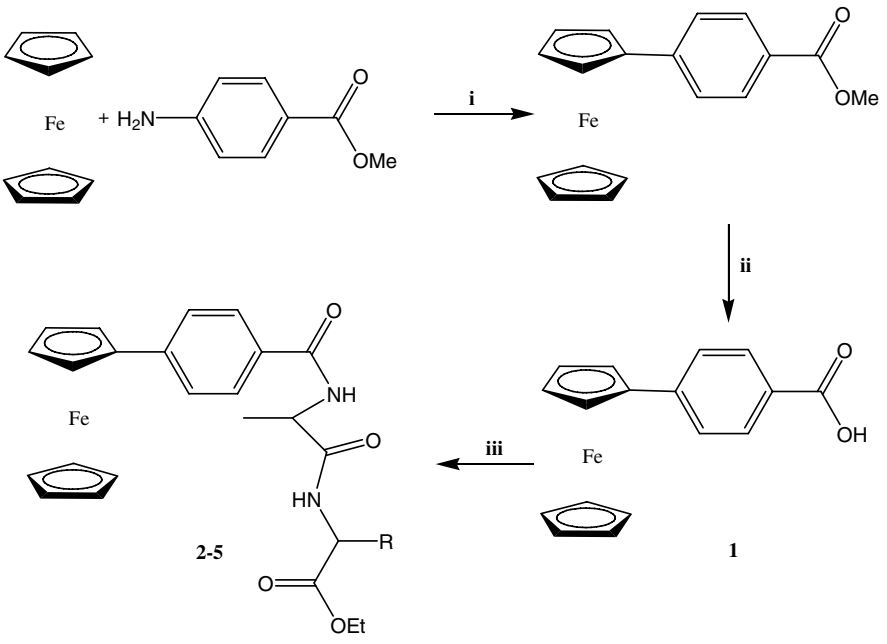
2. Results and discussion

2.1. Synthesis

para-Ferrocenyl benzoic acid **1** was prepared as previously reported [36]. Conventional peptide chemistry was employed in the preparation of the dipeptide ethyl esters. Equimolar quantities of the *N-Boc* protected alanine 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 *para*-ferrocenyl benzoic acid using equimolar amounts of DCC and catalytic amounts of HOBt (Scheme 1). Purification by silica gel column chromatography furnished the yellow/orange coloured products in yields of 59–65% and all gave analytical and spectroscopic data in accordance with the proposed structures. Correct micro-analysis data (to within 0.5% error for carbon) could not be obtained for compound **2**. It was later discovered (*vide infra*) by X-ray analysis that the compound co-crystallizes with ethyl acetate and water. The *N-para*-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. In addition, electrospray ionization mass spectrometry (ESI) in conjunction with tandem mass spectrometry (MS/MS) was employed in the analysis. Crystals of sufficient quality for X-ray diffraction studies were obtained for *N*-{*para*-(ferrocenyl)benzoyl}-L-alanine-glycine ethyl ester **2**.

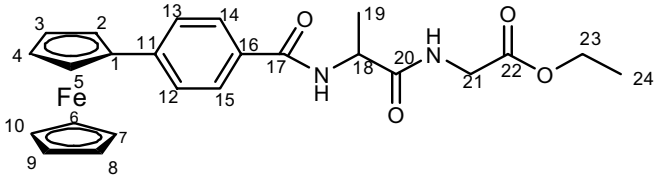
2.2. ^{1}H and ^{13}C NMR spectroscopic analysis

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 monosubstituted ferrocenyl benzoyl moiety [36–40]. The (η^5-C_5H_5) ring appears in the region δ 3.83–3.96. The protons in the *ortho* position of the (η^5-C_5H_4) ring appear in the region δ 4.63–4.74, whereas the protons in the *meta* position occur in the



Scheme 1. Synthesis of*N-para*-ferrocenyl benzoyl dipeptide esters **2**–**5**; AlaGly(OEt) (**2**), AlaAla(OEt) (**3**), AlaLeu(OEt) (**4**), AlaPhe(OEt) (**5**). (i) $NaNO_2$, HCl, 5 °C, (ii) NaOH/MeOH, H_2O, (iii) DCC, HOBt, Et_3N, dipeptide ethyl ester.

Table 1
^{1}H and ^{13}C spectroscopic data for **2**



Site	^{1}H NMR	^{13}C NMR	HMQC
1		85.4	
2,5	4.63		67.2
3,4	4.32		70.1
6–10	3.96		70.2
11		144.4	
12,13	7.44		126.2
14,15	7.66		127.6
16		131.0	
17		167.5	
18	4.72		49.5
19	1.47		18.7
20		170.0	
21	3.99		41.8
22		173.0	
23	4.15		62.0
24	1.21		14.5

range δ 4.24–4.32. The protons of the *para*-disubstituted benzoyl group appear as two doublets in the region δ 7.43–7.67. For example in the case of *N*-{*para*-(ferrocenyl)-benzoyl}-L-alanine-glycine ethyl ester derivative **2**, the aromatic protons are present as two doublets at δ 7.44 and δ 7.66. The unsubstituted C_5H_5 ring appears as a singlet in the ^{1}H NMR spectrum at δ 3.96 whereas the *ortho* and *meta* protons on the substituted Cp ring are present at δ 4.63 and δ 4.32, respectively. The N*H* protons are present in the range δ 6.71–6.8 and a doublet at δ 3.99 ($J = 5.2$ Hz) corresponds to the glycine methylene protons. The doublet at δ 1.47 ($J = 7.2$ Hz) is due to the methyl group of the alanine residue.

The ^{13}C NMR spectra of compounds **2–5** show signals in the region δ 66.9–85.4 indicative of a monosubstituted ferrocenyl benzoyl subunit. The *ipso* carbon of the (η^5-C_5H_4) ring appears in a very narrow range of δ 83.5–85.4. This signal is absent in the DEPT 135 spectra as are the two quaternary carbon atoms of the benzoyl group. The carbon atoms of the aromatic ring are visible in the region δ 125.6–144.4. The methylene carbon atoms of the derivatives were identified by DEPT-135. A complete assignment of the ^{1}H and ^{13}C NMR spectra of compound **2** is presented in Table 1.

2.3. Mass spectrometry

Soft ionization techniques such as electrospray ionization (ESI) mass spectrometry permit the analysis of thermolabile and non-volatile analytes [41]. Electrospray ionization (ESI) in conjunction with tandem mass spectrometry (MS/MS) was employed in the analysis of compounds **2–5** and

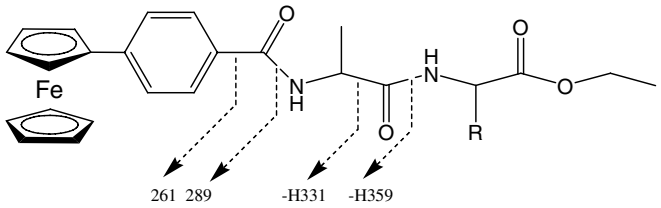


Fig. 1. Product ions observed in the MS/MS spectra of compounds **2–5**.

confirmed the correct relative molecular mass for all the compounds. Examination of the mass spectra revealed the presence of both radical-cations as well as $[M+H]^+$ species. Intense adducts due to sodium and potassium were also present 22 kDa and 38 kDa higher than the protonated molecular ion species. Similar observations were made in the analysis of the ferrocenyl benzoyl amino acid ester derivatives [36–39]. Sequence specific fragment ions were not observed or were of low intensity in the mass spectra and therefore tandem mass spectrometry was employed to confirm the integrity of the structures. Sequence specific ions were observed in all the MS/MS spectra for compounds **2–5** confirming that the alanine residue was linked to the benzoyl spacer group. Important product ions were present at *m/z* 261, *m/z* 289, *m/z* 331 and *m/z* 359 (Fig. 1). The ions at *m/z* 261 and *m/z* 289 were previously observed in the FAB, MALDI and ESI mass spectra of the ferrocenyl benzoyl amino acid ester derivatives and are due to the ferrocenylphenyl and ferrocenylbenzoyl subunits, respectively [36–39]. However, the expected a_1 and b_1 product ions at *m/z* 332 and *m/z* 360 were not observed, instead $a_1 - 1$ and $b_1 - 1$ product ions were observed at *m/z* 331 and *m/z* 359, respectively. Accurate mass measurement of the MS/MS spectrum for compound **3** gave an elemental composition of $C_{19}H_{17}N_1O_1Fe_1$ and a molecular mass of *m/z* 331.0659 for the ion at nominal mass *m/z* 331. This corresponds to a ppm error of 0.1. Obviously a hydrogen atom has also been lost during the fragmentation process. This is unusual as these a_1 and b_1 fragment ions are usually produced without loss of a hydrogen atom [42]. The MS/MS spectrum of *N*-{*para*-(ferrocenyl)benzoyl}-L-alanine-alanine ethyl ester **3** is presented in Fig. 2.

2.4. X-ray crystallographic studies of dipeptide derivative 2

There are numerous crystal structures of ferrocene amino acid/peptide conjugates reported in the Cambridge Structural Database (CSD), however structures incorporating a ferrocene benzoyl moiety are relatively scarce. We have previously reported single crystal X-ray structures of a number of ferrocene benzoyl amino acid derivatives [36,37,39]. Herein, we report a single crystal structure of the ferrocene benzoyl dipeptide **2**. An ORTEP plot for the asymmetric unit is shown in Fig. 3 with selected structural parameters, such as torsion angles, hydrogen bonds and short contacts are listed in Tables 2–4 and the crystallographic details given in the footnote.

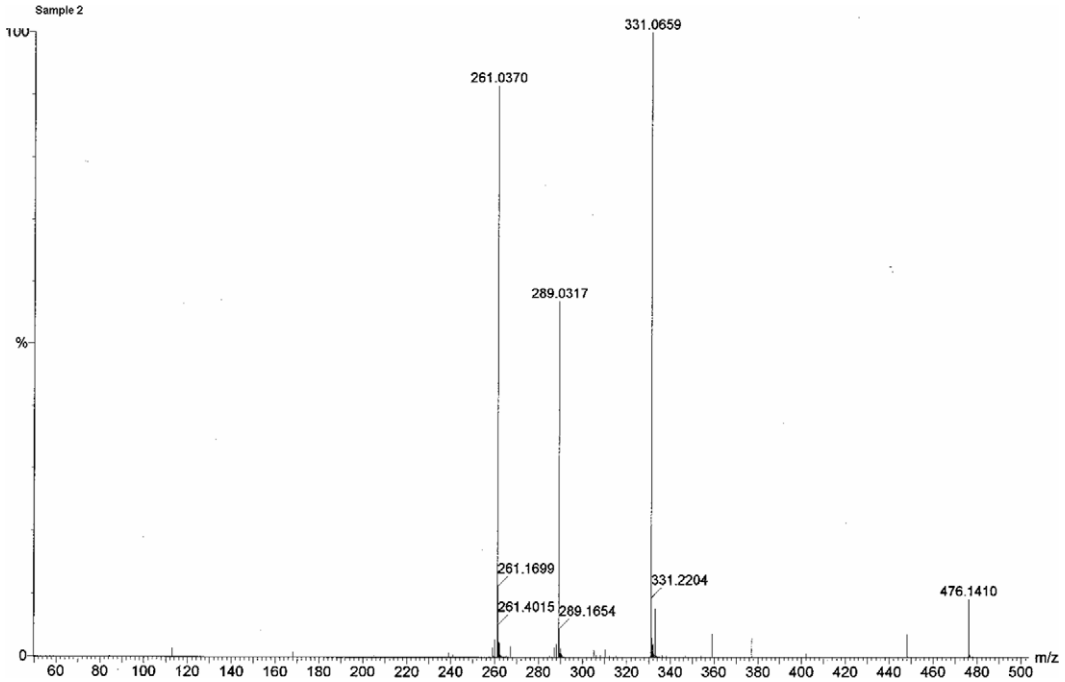


Fig. 2. MS/MS spectrum of*N*-{*para*-(ferrocenyl)benzoyl}-L-alanine-alanine ethyl ester **3**.

Table 2
Torsion angles (°)

	A	B	C	D
O4–C27–C68–C30			16.6(14)	
O8–C26–C36–C59		6.5(12)		
O12–C29–C38–C88	15.5(12)			
O13–C2–C14–C62				13.9(12)
C22–C13–C58–C80		3.2(15)		
C49–C25–C41–C56			19.8(15)	
C50–C19–C45–C71				5.6(14)
C79–C53–C78–C46	7.5(16)			

Table 3
Hydrogen bonds

D–H...A	H...A (Å)	DHA (°)
N1–H1A...O7	2.10	142.2
N2–H2A...O8	1.99	167.9
N3–H3A...O4	2.32	162.1
N4–H4A...O4	2.14	131.2
N5–H5A...O11	2.01	143.4
N6–H6A...O12	2.08	166.2
N7–H7B...O14	1.97	163.2
N8–H8A...O13	1.99	167.1

Table 4
Short contacts

Contact (molecules)	Length (Å)	Length-VdW (Å)
O12...H40A (A...B)	2.28	−0.44
O8...H7A (B...C)	2.40	−0.32
O3...H70B (C...B)	2.44	−0.28
O4...N4 (C...B)	2.80	−0.27
H22A...C47 (A...B)	2.65	−0.24
O7...N1 (B...C)	2.85	−0.22
O8...N2 (B...C)	2.86	−0.21
O2...O100	2.83	−0.21
O6...H5AC	2.52	−0.21

2.4.1. Molecular and crystal structure study of **2**

The dipeptide derivative **2** crystallizes in the tetragonal space group $P4_3$. The unit cell comprises four crystallographically independent molecules of *N*-{*para*-(ferrocenyl)benzoyl}-L-alanine-glycine ethyl ester (A, B, C and D), one molecule of ethyl acetate and one molecule of water (this explains the incorrect microanalysis results obtained using the chemical formula $C_{24}H_{26}N_2O_4Fe$ without including ethyl acetate and water molecules; correct results are obtained using the chemical formula $C_{25}H_{28}N_2O_{4.75}Fe$). The water molecule has a short contact O100...O2 of 2.83 Å with the ester group of molecule C and ethyl acetate has a short contact O6–H5AC of 2.52 Å with the ester group of molecule B. Molecules B are packed along the crystallographic *a*-axis and the molecules C are packed along the *b*-axis. Whereas, both molecules D and A are packed along the *c*-axis in a head to tail arrangement. Molecules B and C are linked by two hydrogen bonds C=O4 (C)... H4A–N4 (B) (O...H 2.14 Å, O...H–N 131.20°) and C=O4 (D)...H3A–N3 (B) (O...H 2.319 Å, O...H–N 162.10°) and a short contact O3–H70B, 2.44 Å between an amide group of C and a glycyl hydrogen of B, and molecules A and B are linked *via* a short contact H22A–C47, 2.65 Å between a hydrogen of the cyclopentadiene ring (B) and the methyl carbon of the alanine subunit (A). Molecules D and A form four intermolecular amide...amide hydrogen bonds with four other molecules of the same

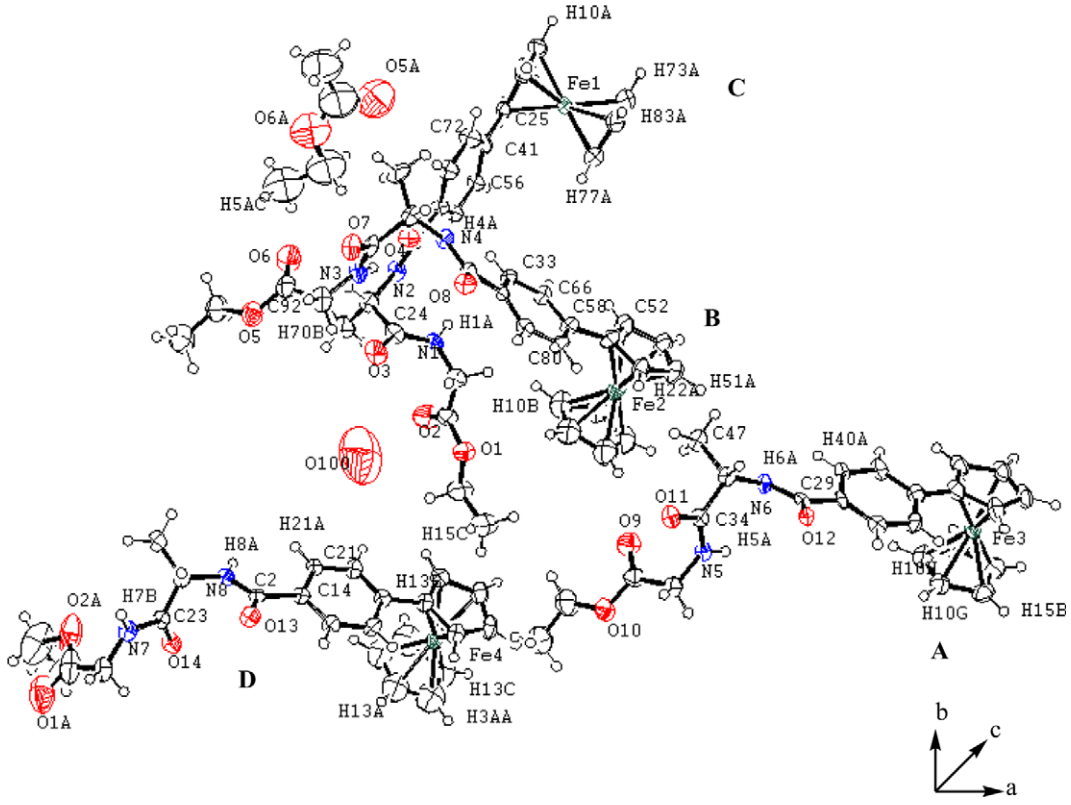


Fig. 3. Molecular drawing using ORTEP: displacement ellipsoids are drawn at the 50% probability level.

crystallographic type and generate independent double helices along the *c*-axis. Molecules B and C also form the intermolecular amide...amide hydrogen bonds with the other molecules but two with the same crystallographic type and two with each other (*vide supra*). Hydrogen bonding interactions are summarized in Tables 3 and 4 shows a list of select intermolecular contacts that stabilize the structure.

The cyclopentadienyl (Cp) rings of molecules A, B and D are staggered, and the extent varies significantly with C1*n*...Cg...Cg′...C2*n* torsion angles ($n = 1$–5) ranging from 25.45° to 44.97°. The Cp ring of the molecule C is almost eclipsed with C1*n*...Cg...Cg′...C2*n* in the range 7.75–9.89°. Centroids of the Cp rings are equidistant from the iron core in all four molecules, Fe...Cg/Cg′ distances are in the range of 1.628(10)–1.660(10) Å with 174.20–178.24° Cg...Fe...Cg′ angle. All four molecules in the unit cell have the peptide chains directed towards their unsubstituted Cp rings. It is interesting to note that the ethyl ester groups in molecules A, B and C are pointing away from the unsubstituted ring (*transoid*) similar to the L-alanine derivative [36], while in molecule D the ethyl ester group is oriented towards the unsubstituted Cp ring (*cisoid*). The phenyl groups are coplanar with the substituted Cp rings except in molecule C where the phenyl group is significantly twisted and has a torsion angle C49–C25–C41–C56 of 19.8 (15)°. Perhaps, the rigid arrangement created by the two hydrogen bonds between B and C (*vide supra*) forces the phenyl group slightly out of plane. The amide C=O bond length in D and A are in the range of 1.243(10)–1.256(10) Å as expected, however B and C have a wider range of 1.200(10)–1.290(10) Å. The amide groups are also twisted in all molecules with respect to the phenyl groups, the torsion angles range from 6.5(12)–16.60(14)°. Twisting of the amide groups is likely due to the rigid framework of strong intermolecular hydrogen bonds present in the structure. The twisting of the amide groups is not uncommon and has been observed previously in semi-rigid systems [23]. The amide CO–NH bond lengths are similar in all the cases and are in the range 1.306(11)–1.359(11) Å, as expected.

2.5. Electrochemistry

Compounds **2**–**5** exhibited quasi-reversible cyclic voltammograms, similar to ferrocene under the same conditions, and $E^{0\prime}$ values were in 137–140 mV (vs Fc/Fc^{+}) range. Lower oxidation potential values as compared to the corresponding dipeptide derivatives lacking a benzoyl moiety, Fc-Ala-Ala-OMe, $E^{0\prime} = 230$ mV (vs Fc/Fc^{+}) [43]; Fc-Ala-Ph-OMe, $E^{0\prime} = 190$ mV (vs Fc/Fc^{+}) [23] are explicable in terms of substituent effects. The benzoyl moiety affords extended conjugation and makes these derivatives easier to oxidize.

3. Conclusions

The novel *N-para*-ferrocenyl benzoyl dipeptide esters **2**–**5** were prepared in good yields and fully characterized by a range of spectroscopic techniques. These compounds display some unusual characteristics both in the mass spectra and crystal structure studies. The mass spectra of all compounds showed intense peaks due to radical-cations and the MS/MS spectra of the radical-cation species showed unusual fragmentation patterns. The product ions corresponding to the a_1 and b_1 ions occur with loss of a hydrogen atom and appear one Dalton less than expected. The X-ray crystal structure shows that compound **2** co-crystallizes with ethyl acetate and water, displaying the ability of this derivative to trap small molecules and its potential as a receptor in sensor applications. The potential applications of these derivatives are in the anti-cancer and the biosensor area.

4. Experimental

4.1. General procedures

All chemicals were purchased from Sigma/Aldrich and used as received. Commercial grade reagents were used without further purification, however, solvents were purified prior to use. Melting points were determined using a Griffin melting point apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet 405 FT-IR spectrometer and UV–Vis spectra on a Hewlett–Packard 8452A diode array UV–Vis spectrophotometer. NMR spectra were obtained on a Bruker AC 400 NMR spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. The ^{1}H and ^{13}C NMR chemical shifts (ppm) are relative to TMS and all coupling constants (J) are in Hertz. Electrospray ionization mass spectra and tandem mass spectra were obtained on either an Applied Biosystems QSTAR or a Micromass Q-ToF Ultima quadrupole time of flight mass spectrometer.

Single-crystal analysis was made with a Bruker SMART APEX CCD area detector using graphite monochromatised Mo Kα radiation ($\lambda = 0.71073$ Å) at 153(2) °C. Data-reduction was performed using SAINT [44]. Intensities were corrected for Lorentz and polarization effects and for absorption using SADABS [45]. The space group was determined from systematic absences and checked for higher symmetry. A full sphere of data was obtained using the ω-scan method. The structure was solved by direct methods using SHELXS [46] and refined on F^2 using all data by full-matrix least-squares procedures with SHELXL-97 [47]. All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were included in calculated positions with isotropic displacement parameters 1.2 times the isotropic equivalent of their carrier atoms. The correct absolute structure was determined using the Flack x parameter [48]. The functions minimized were $\sum w(F_o^2 - F_c^2)$, with $w = [\sigma_2(F_o^2) + (aP)^2 + bP]^{-1}$, where $P = [\max(F_o)^2 + 2F_c^2]/3$.

4.2. General procedure for the synthesis of N-{para-(ferrocenyl)benzoyl} dipeptide esters 2–5

4.2.1. N-{para-(ferrocenyl)-benzoyl}-L-alanine-glycine ethyl ester (2)

L-Alanine-glycine ethyl ester hydrochloride (0.2 g, 0.95 mmol) was added to a solution of *para*-(ferrocenyl) 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 an orange solid. (0.27 g, 59%). The crystals were of sufficient quality for an X-ray diffraction study.

m.p. 75–77 °C, $E^{0\prime} = 137$ mV (vs Fc/Fc$^+$), $[\alpha]_D^{20} = +5°$ (c 2 EtOH).

Analysis:	found:	C, 61.63; H, 5.68; N, 5.9%.
$C_{24}H_{26}N_2O_4Fe$	requires:	C, 62.35; H, 5.67; N, 6.06.
$C_{25}H_{28}N_2O_{4.75}Fe$	requires:	C, 61.49; H, 5.78; N, 5.74.

Mass spectrum: found: $[M]^{+\cdot}$ 462.1201, $C_{24}H_{26}N_2O_4Fe$ requires: 462.1242.

IR ν_{max}(KBr): 3326, 1748, 1627, 1609, 1560, 1512, 1195 cm^{-1}.

UV–Vis λ_{max} MeCN; 352 (ε 2400), 450 (ε 720) nm.

^{1}H NMR (400 MHz) δ ($CDCl_3$): 7.66 (2H, d, $J = 8$ Hz, ArH), 7.44 (2H, d, $J = 8$ Hz, ArH), 6.71–6.80 (2H, m, –CON*H*–, –CON*H*–), 4.72 {1H, quint, –C*H*(CH_3)}, 4.63 {2H, t, $J = 1.6$ Hz, *ortho* on (η^5-C_5H_4)}, 4.32 {2H, t, $J = 1.6$ Hz, *meta* on (η^5-C_5H_4)}, 4.15 (2H, q, $J = 7.2$ Hz, –O*C*$H_2$$CH_3$), 3.99 (2H, d, $J = 5.2$ Hz, –NHCH_2CO–), 3.96 {5H, s, (η^5-C_5H_5)}, 1.47 {3H, d, $J = 7.2$ Hz, –CH(CH_3)}, 1.21 (3H, t, $J = 7.2$ Hz, OCH_2CH_3).

^{13}C NMR (100 MHz) δ ($CDCl_3$): 173.0, 170.0, 167.5, 144.4, 131.0, 127.6, 126.2, 85.4, 70.2, 70.1, 67.2, 62.0 (−ve DEPT), 49.5, 41.8 (−ve DEPT), 18.7, 14.5.

4.2.2. N-{para-(ferrocenyl)-benzoyl}-L-alanine-L-alanine ethyl ester 3

For compound **3**, L-alanine-L-alanine ethyl ester hydrochloride (0.2 g, 0.89 mmol) was used as a starting material. 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. (0.26 g, 61%).

m.p. 69–71 °C, $E^{0\prime} = 140$ mV (vs Fc/Fc$^+$), $[\alpha]_D^{20} = -1°$ (c 2, EtOH).

Analysis: found: C, 62.93; H, 6.18; N, 6.04%.
$C_{25}H_{28}N_2O_4Fe$ requires: C, 63.04; H, 5.92; N, 5.88.

Mass spectrum: found: $[M]^{+\cdot}$ 476.1398, $C_{25}H_{28}N_2O_4Fe$ requires: 476.1398.

IR ν_{max} (KBr): 3326, 2936, 1741, 1629, 1609, 1543, 1507 cm^{-1}.

UV–Vis λ_{max} EtOH; 353 (ε 2640), 449 (ε 840) nm.

^1H NMR (400 MHz) δ ($CDCl_3$): 7.67 (2H, d, $J = 8.4$ Hz, ArH), 7.43 (2H, d, $J = 8.4$ Hz, –ArH), 6.97 (2H, t, $J = 8$ Hz, –CON*H*–), 4.74 {1H, quint, $J = 7.2$ Hz, –C*H* (CH_3)}, 4.62 {2H, t, $J = 1.6$ Hz, *ortho* on (η^5-C_5H_4)}, 4.5 (1H, quint, $J = 7.2$ Hz, –C*H*(CH_3)}), 4.31 {2H, t, $J = 1.6$ Hz, *meta* on (η^5-C_5H_4)}, 4.15 (2H, q, $J = 7.2$ Hz, –OC*H*$_2$$CH_3$), 3.96 {5H, s, ($\eta^5$-$C_5H_5$)}, 1.47 {3 H, d, $J = 7.2$ Hz, –CH(C*H*$_3$)}, 1.36 {3H, d, $J = 7.2$ Hz, –CH(C*H*$_3$)}, 1.24 (3H, t, $J = 7.2$ Hz, –OCH$_2$C*H*$_3$).

^{13}C NMR (100 MHz) δ ($CDCl_3$): 173.1, 172.5, 167.3, 144.3, 131.2, 127.7, 126.2, 83.8, 70.2, 70.1, 67.2, 61.9 (−ve DEPT), 49.5, 48.7, 19.3, 18.5, 14.6.

4.2.3. N-{*para-(ferrocenyl)-benzoyl*}-L-alanine-L-leucine ethyl ester **4**

L-Alanine-L-leucine ethyl ester hydrochloride (0.2 g, 0.75 mmol) was used as a starting material. 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 an orange solid (0.234 g, 64%).

m.p. 62–64 °C, $E^{0\prime} = 138$ mV (vs Fc/Fc^+), $[\alpha]_D^{20} = +4°$ (c 1.8, EtOH).

Analysis: found: C, 64.97; H, 6.87; N, 5.7%.
$C_{28}H_{34}N_2O_4Fe$ requires: C, 64.87; H, 6.61; N, 5.4.

Mass spectrum: found: $[M]^{+\cdot}$ 518.1873, $C_{28}H_{34}N_2O_4Fe$ requires: 518.1866.

IR ν_{max}(KBr): 3336, 2958, 1737, 1655, 1629, 1562, 1510 cm^1.

UV–Vis λ_{max} EtOH; 354 (ε 2350), 450 (ε 740) nm.

^1H NMR (400 MHz) δ ($CDCl_3$): 7.66 (2H, d, $J = 8$ Hz, ArH), 7.43 (2H, d, $J = 8$ Hz, ArH), 6.93 (1H, d, $J = 7.2$ Hz, –CON*H*–), 6.84 (1H, d, $J = 7.2$ Hz, –CON*H*–), 4.77 {1H, quint, $J = 4.8$ Hz, –C*H*(CH_3)}, 4.62 {2H, t, $J = 2$ Hz, *ortho* on (η^5-C_5H_4)}, 4.50–4.52 [1H, m, –C*H*{CH_2CH-$(CH_3)_2$}], 4.31 {2H, t, $J = 2$ Hz, *meta* on (η^5-C_5H_4)}, 4.16 (2H, q, $J = 7.2$ Hz, –OC*H*$_2$$CH_3$), 3.96 {5H, s, ($\eta^5$-$C_5H_5$)}, 1.52–1.62 [3H, m, –CH{C*H*$_2$C*H*$(CH_3)_2$}], 1.47 {3H, d, $J = 6.8$ Hz, –CH(C*H*$_3$)}, 1.22 (3H, t, $J = 7.2$ Hz, –OCH$_2$C*H*$_3$), 0.83 [6H, t, $J = 4.4$ Hz, -CH-{$CH_2CH(CH_3)_2$}].

^{13}C NMR (100 MHz) δ ($CDCl_3$): 173.1, 172.8, 167.4, 144.3, 131.2, 127.6, 126.2, 83.8, 70.2, 70.1, 67.2, 61.8 (−ve DEPT), 51.4, 49.4, 41.6 (−ve DEPT), 25.2, 23.2, 22.2, 19.2, 14.6.

4.2.4. N-{*para-(ferrocenyl)-benzoyl*}-L-alanine-L-phenylalanine ethyl ester **5**

L-Alanine-L-phenylalanine ethyl ester hydrochloride (0.2 g 0.67 mmol) was used as a starting material. 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. (0.25 g, 65%).

m.p. 169–171 °C, $E^{0\prime}$ 139 mV (vs Fc/Fc^+), $[\alpha]_D^{20} = +9°$ (c 1.9, EtOH).

Analysis: found: C, 67.23; H, 6.10; N, 5.30%.
$C_{31}H_{32}N_2O_4Fe$ requires: C, 67.40; H, 5.84; N, 5.07.

Mass spectrum: found: $[M]^{+\cdot}$ 552.1708, $C_{31}H_{32}N_2O_4Fe$ requires: 552.1711.

IR ν_{max} (KBr): 3316, 3268, 1753, 1655, 1624, 1546, 1516 cm^{-1}.

UV–Vis λ_{max} EtOH; 352 (ε 2730), 449 (ε 890) nm.

^1H NMR (400 MHz) δ (DMSO): 8.25 (1H, d, $J = 7.6$ Hz, –CON*H*–), 8.14 (1H, d, $J = 7.6$ Hz, –CON*H*–), 7.64 (2H, d, $J = 8.4$ Hz, ArH), 7.46 (2H, d, $J = 8.4$ Hz, ArH), 7.04–7.07 (5H, m, ArH), 4.74 {2H, t, $J = 1.6$ Hz, *ortho* on (η^5-C_5H_4)}, 4.36 {1H, quint, $J = 7.2$ Hz, C*H*(CH_3)}, 4.24–4.28 {3H, m, –C*H*(CH_2Ph), *meta* on (η^5-C_5H_4)}, 3.83–3.88 {7H, m, –OC*H*$_2$$CH_3$, ($\eta^5$-$C_5H_5$)}, 2.81–2.85 {2H, m, –CH(C*H*$_2$-Ph)}, 1.14 {3H, d, $J = 7.6$ Hz, –CH(C*H*$_3$)}, 0.92 (3H, t, $J = 7.2$ Hz, –OCH$_2$C*H*$_3$).

^{13}C NMR (100 MHz) δ (DMSO): 173.0, 171.7, 166.2, 143.1, 137.4, 129.5, 128.6, 128.0, 126.9, 125.6, 83.5, 69.9, 66.9, 60.8 (−ve DEPT), 54.1, 48.8, 41.0 (−ve DEPT), 18.1, 14.3.

4.3. Crystallographic footnotes for **2**

Crystallographic data: chemical formula $C_{25}H_{28}N_2O_{4.75}Fe$, formula weight 488.34 g mol^{-1}, orange needle, crystal size = $0.51 \times 0.05 \times 0.04$ mm^3, tetragonal, space group $P4_3$, unit cell dimensions $a = 24.3576(8)$ Å ($\alpha = 90°$), $b = 24.3576(8)$ Å ($\beta = 90°$), $c = 16.5836(11)$ Å ($\gamma = 90°$), $V = 9838.9(8)$ $Å^3$, $Z = 16$, density = 1.319 g cm^{-3}, $\mu = 0.649$ mm^{-1}, 56 709 reflections collected in the range 1.49–26.00°, 19 282 independent reflections, 1180 parameters, R factor = 0.1064, $wR_2 = 0.2165$.

5. Supplementary material

Crystallographic data for the structural analysis have been deposited as CIF file, with the Cambridge Crystallographic Data Centre, as supplementary publication number 295390 for **2**. Copies of these data can be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CBZ 1EZ, UK (fax: +44 1223 336 033; email: deposit@ccdc.cam.ac.uk).

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