Available online at www.sciencedirect.com
ScienceDirect
Journal of Organometallic Chemistry 692 (2007) 1292–1299
Journal of Organometallic Chemistry
www.elsevier.com/locate/jorganchem

The synthesis and structural characterization of novel *N*-*meta*-ferrocenyl benzoyl dipeptide esters: The X-ray crystal structure and *in vitro* anti-cancer activity of *N*-{*meta*-ferrocenyl)benzoyl}-L-alanine-glycine ethyl ester

Alok Goel [a,b], David Savage [a], Steven R. Alley [a,b], Paula N. Kelly [a,b], Dermot O'Sullivan [b], Helge Mueller-Bunz [c], Peter T.M. Kenny [a,b,*]

[a] *School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland*
[b] *National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland*
[c] *Department of Chemistry, University College Dublin, Belfield, Dublin 4, Ireland*

Received 22 August 2006; received in revised form 25 September 2006; accepted 25 September 2006
Available online 6 October 2006

Abstract

A series of *N*-*meta*-ferrocenyl benzoyl dipeptide esters **2**–**5** have been prepared by coupling *meta*-ferrocenyl benzoic acid **1b** to the dipeptide ethyl esters using the conventional 1,3-dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt) protocol. The dipeptides employed in the synthesis were AlaGly(OEt) (**2**), AlaAla(OEt) (**3**), AlaLeu(OEt) (**4**) and AlaPhe(OEt) (**5**). The compounds were fully characterized by a range of NMR spectroscopic techniques, mass spectrometry (MALDI-MS, ESI-MS), and cyclic voltammetry (CV). In addition, the X-ray crystal structure and cytotoxicity of *N*-{*meta*-(ferrocenyl)-benzoyl}-L-alanine-glycine ethyl ester (**2**) towards lung cancer cells has been determined.

Keywords: Ferrocene; Bioorganometallic chemistry; ESI; CV; Dipeptides; X-ray; Cytotoxicity; Lung cancer

1. Introduction

Bioorganometallic chemistry has recently developed as a rapidly growing area, the main focus being the preparation of peptide mimetic models, macromolecular assemblies for molecular recognition, and anti-cancer drugs [1–13]. The conjugation of redox active organometallic compounds to biological assemblies has also allowed for the design of materials for biomolecular sensors and switches [14]. The organometallic compound ferrocene is a promising candidate for biological applications. Ferrocene can be conjugated with biologically important compounds due to its stability, electrochemical properties, and ease of use [1,2]. Ferrocenyl derivatives with low oxidation potentials are attracting increasing attention due to their ability to catalyze the production of reactive oxygenated species (ROS), under physiological conditions, that generates cytotoxic effects [2]. Some ferrocene derivatives have shown cytotoxicity against lung tumours [15].

In our laboratory, we have synthesized various ferrocene derivatives incorporating natural amino acids and dipeptides [16–22]. We have incorporated three key moieties in the synthesis of these unusual biological materials, namely: (i) an electroactive core, (ii) a conjugated linker that lowers the oxidation potential of the ferrocene moiety, and (iii) an amino acid or peptide derivative that can interact with other molecules via hydrogen bonding. The ferrocene

* Corresponding author. Address: School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland. Tel.: +353 1 7005689; fax: +353 1 7005503.
E-mail address: peter.kenny@dcu.ie (P.T.M. Kenny).

0022-328X/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jorganchem.2006.09.057

moiety is linked to the peptide derivative via a disubstituted benzoyl group. Herein, we report the synthesis and electrochemical properties of *N*-*meta*-ferrocenyl benzoyl dipeptide esters. In addition, we present a molecular and crystal structural study, and an anti-cancer activity study of *N*-{*meta*-(ferrocenyl)-benzoyl}-L-alanine-glycine ethyl ester (**2**).

2. Results and discussion

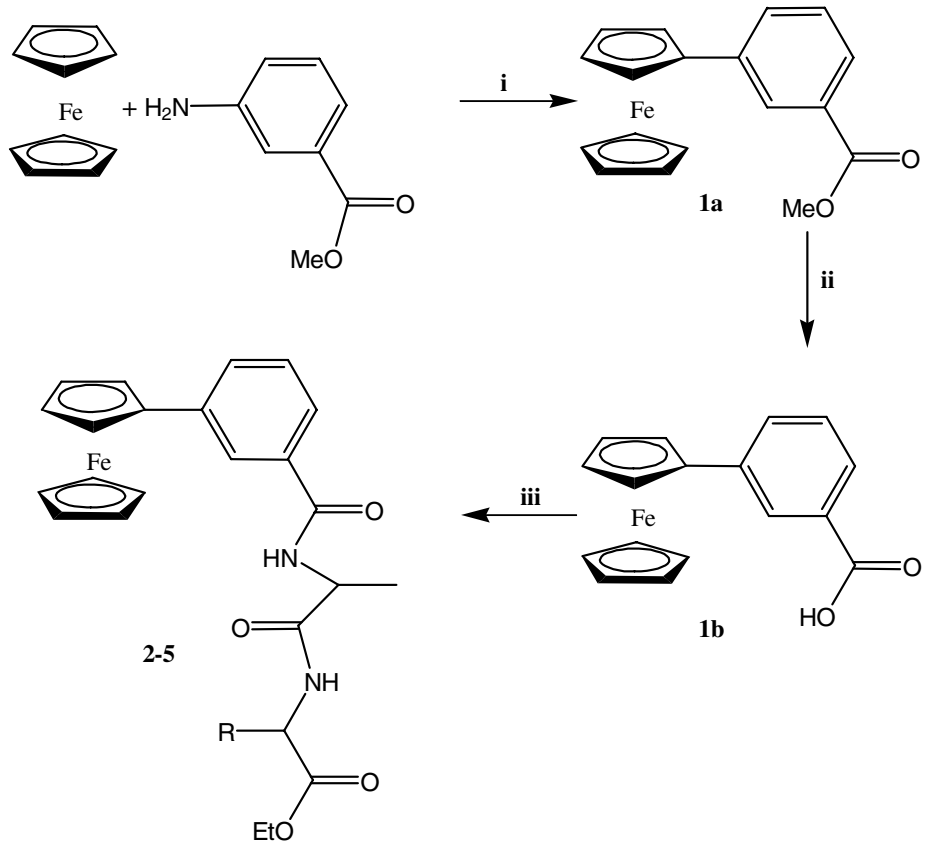
2.1. Synthesis

The conventional 1,3-dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt) protocol was employed in the preparation of the dipeptide ethyl esters and to also couple *meta*-ferrocenyl benzoic acid (**1b**) to the dipeptides AlaGly(OEt) (**2**), AlaAla(OEt) (**3**), AlaLeu(OEt) (**4**) and AlaPhe(OEt) (**5**) as previously reported [18,20] and is outlined in Scheme 1. The compounds **2**–**5** were purified by column chromatography, using 2:3 petroleum ether (40–60 °C): ethyl acetate as the eluant, to obtain yellow/orange colored crystals. The yields obtained were in the 51–56% range, and all compounds gave analytical and spectroscopic data in accordance to the proposed structures. Compounds **2**–**4** were reasonably stable, however compound **5** underwent slow decomposition over a period of time. It is envisaged that the phenyl group of the dipeptide chain on the *meta* position causes steric hindrance to the unsubstituted cyclopentadienyl ring and makes this compound slightly unstable.

Compounds **2**–**5** were fully characterized by a range of NMR spectroscopic techniques, cyclic voltammetry, and mass spectrometry. Crystals of sufficient quality for X-ray diffraction studies were also obtained for compound **2**. However, it is important to note that the crystals decomposed if completely dried. Hence, crystals were kept in the mother liquor until ready for crystallographic analysis. Thus, it is not surprising that compound **2** co-crystallizes with a molecule of ethyl acetate (*vide infra*).

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 mono substituted ferrocene benzoyl moiety [16–22]. The protons in the *ortho* position of the (η^5-C_5H_4) ring appear in the region δ 4.62 to δ 4.67 whereas the protons in the *meta* position occur in the range δ 4.17 to δ 4.31. The (η^5-C_5H_5) ring appears in the region δ 3.8 to δ 4.0. The protons of the *meta*-disub-



Scheme 1. Synthesis of *N*-*meta*-ferrocenyl benzoyl dipeptide esters **2**–**5**. (i) $NaNO_2$, HCl, 5 °C; (ii) NaOH/MeOH; (iii) DCC, HOBt, triethylamine, Ala-dipeptide ethyl esters.

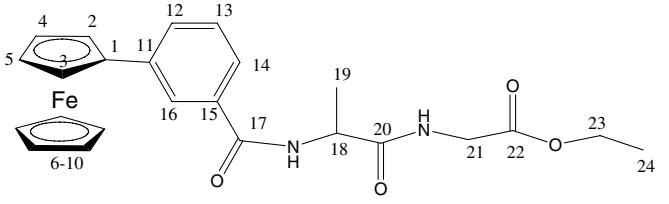
stituted benzoyl group appear as a triplet, doublet, doublet and singlet in the region δ 7.18 to δ 7.8. For example, in the case of *N*-{*meta*-(ferrocenyl)-benzoyl}-L-alanine-glycine ethyl ester **2**, the aromatic protons are present as a triplet, two doublets and a singlet at δ 7.27, δ 7.48, δ 7.54, and δ 7.8, respectively. The *meta* and *ortho* protons on the (η^5-C_5H_4) ring are present at δ 4.31 and δ 4.67. The L-alanine methyl group is present as a doublet at δ 1.48 and the ethyl ester methyl group appears as a triplet at δ 1.14.

The ^{13}C NMR spectra of compounds **2**–**5** show signals in the region δ 66.8 to δ 84.5 indicative of a monosubstituted ferrocene benzoyl subunit. The *ipso* carbon of the (η^5-C_5H_4) ring appears in a very narrow range of δ 84.4 to δ 84.5. This signal is absent in the DEPT 135 spectra. The carbon atoms of the aromatic ring are non-equivalent and therefore six signals are visible in the region δ 124.5–140.8. The quaternary carbons of the aromatic ring and the methylene carbon atoms of derivatives **2**–**5** were identified by DEPT-135. A complete assignment of the ^{1}H and ^{13}C NMR spectra of *N*-{*meta*-(ferrocenyl)-benzoyl}-L-alanine-glycine ethyl ester (**2**) is presented in Table 1.

2.3. Mass spectrometry

Since the introduction of soft ionization techniques such as matrix assisted laser desorption ionization (MADLI) and electrospray ionization mass spectrometry (ESI-MS), a wide range of thermolabile and non-volatile compounds can be subjected to mass spectrometric analysis [23,24].

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



Site	^{1}H NMR	^{13}C NMR	HMQC
1		84.5	
2,3	4.67		67
4,5	4.31		69.8
6–10	3.8–4.0		70.1
11		140.8	
12	7.48		124.5
13	7.27		129.0
14	7.54		129.8
15		134.2	
16	7.8		125.1
17		167.8	
18	4.74		49.5
19	1.48		18.8
20		170.0	
21	3.8–4.0		41.8
22		173.0	
23	4.14		62
24	1.14		14.5

The *N*-*meta*-ferrocenyl benzoyl dipeptide esters were not amenable to electron ionization (EI) or chemical ionization (CI) studies, therefore ESI was employed in the analysis of compounds **2** and **4** whereas compound **3** and **5** were analyzed by MALDI. Both ionization techniques confirmed the correct relative molecular mass for the compounds and examination of the mass spectra revealed the presence of intense radical-cation species. The formation of the radical-cation molecular ion species was further confirmed by the detection of the sodium adducts $[M+23]^+$ and potassium adducts $[M+39]^+$ for each of the compounds analyzed. Similar observations were made in the analysis of related ferrocenyl benzoyl amino acid and peptide derivatives. Fragment ions were not observed in the MALDI spectra of compounds **3** and **5**. Sequence specific fragment ions were also not observed or were of low intensity in the ESI mass spectra of compounds **2** and **4** and therefore tandem mass spectrometry was employed to confirm the integrity of the structures. Sequence specific ions were observed in the MS/MS spectrum for compound **4** 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. The ions at *m*/*z* 261 and *m*/*z* 289 were previously observed in the FAB, MALDI and ESI mass spectra of related ferrocenyl benzoyl derivatives and are due to the ferrocenylphenyl and ferrocenylbenzoyl subunits, respectively [16–19]. 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 **4** gave an elemental composition of $C_{19}H_{17}N_1O_1Fe_1$ and a molecular mass of *m*/*z* 331.0662 for the ion at nominal mass *m*/*z* 331. This corresponds to a ppm error of 0.8. This indicates that a hydrogen atom has been lost during the fragmentation process. A similar observation was made in the analysis of the related *para* derivatives [21,22].

2.4. Electrochemistry

Compounds **2**–**5** exhibited quasi-reversible cyclic voltammograms, similar to ferrocene under the same conditions, and $E^{0\prime}$ values were in 46–59 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^+) [25]; Fc-Ala-Ph-OMe, $E^{0\prime} = 190$ mV (vs Fc/Fc^+) [26] are explicable in terms of substituent effects. The benzoyl moiety offers extended conjugation to the pi electrons of the Cp rings and makes these derivatives easier to oxidize, thus making them suitable as anti-cancer agents (vide infra).

2.5. X-ray crystallographic studies of 2

A number of crystal structures of ferrocene peptide conjugates have been reported in the Cambridge Structural Database (CSD); however, structures incorporating a fer-

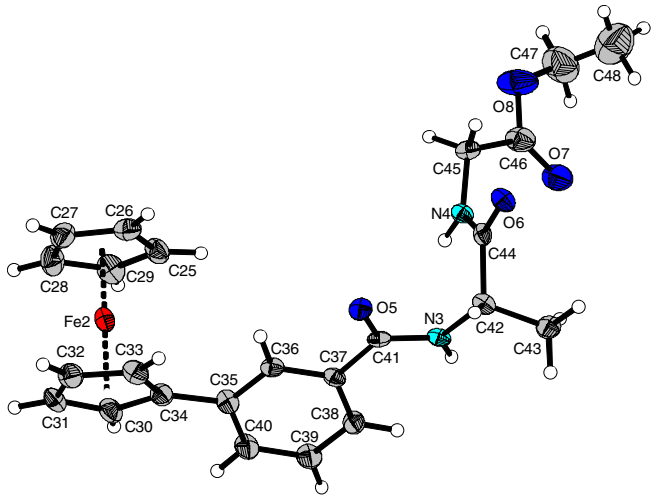


Fig. 1. Molecular drawing of B using ORTEP: displacement ellipsoids are drawn at the 50% probability level.

rocene benzoyl moiety and peptides are relatively rare. We have previously reported single crystal X-ray structures of a number of ferrocene benzoyl amino acid derivatives [16,17,19] and a ferrocene benzoyl dipeptide [22]. Herein, we report a single crystal structure of another ferrocene benzoyl dipeptide derivative **2**. A molecular drawing of molecule B using ORTEP is shown in Fig. 1 with selected structural parameters such as torsion angles, hydrogen bonds and short contacts listed in Tables 2–4 and the crystallographic details given in the footnote.

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

Compound **2** crystallizes in the orthorhombic space group $P2_12_12_1$ (no. 19). The unit cell has two crystallographically independent molecules of *N*-{*meta*-(ferrocenyl)benzoyl}-L-alanine-glycine ethyl ester (A and B) and two crystallographically independent molecules of ethyl acetate (C and D). Molecules A are packed along the crystallographic *c*-axis and molecules B are packed along the *a*-axis; ethyl acetate molecules C and D are packed along the *c* and *a*-axis, respectively. Each A molecule forms four intermolecular amide···amide hydrogen bonds with two B molecules (and vice versa) and that generates a helical structure along the *b*-axis with alternating B and A molecules packed in a head to tail arrangement along their own crystallographic axis (Fig. 2). The helix has a pitch of 16.7111(14) Å, which is equivalent to the length of the crystallographic *b*-axis.

Table 2
Torsion angles (°)

	A	B
C6–C10–C11–C16	20.7(6)	
C30–C34–C35–C40		25.9(7)
C20–N2–C21–C22	65.0(5)	
C44–N4–C45–C46		102.1(4)

Table 3
Hydrogen bonds

D–H...A	H...A (Å)	DHA (°)
N1–H1N1...O5	1.96	164.3
N2–H1N2...O6	1.99	151.1
N3–H1N3...O1	1.97	164.1
N4–H1N4...O2	2.03	145.3

Table 4
Short contacts

Contact	(Molecules)	Length (Å)	Length-VdW (Å)
O1...H38	(A...B)	2.38	−0.34
O3...H53A	(A...C)	2.51	−0.21
C35...H49B	(B...D)	2.70	−0.20
O6...H55A	(B...C)	2.51	−0.21
O9...H56C	(D...C)	2.44	−0.28

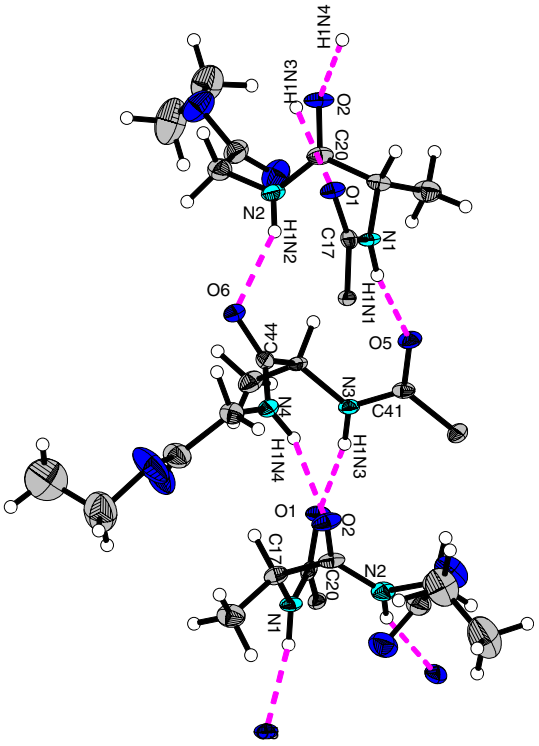


Fig. 2. Hydrogen bonding interactions between molecules B and A; [*meta*-ferrocenyl] phenyl groups are represented by their *ipso*-carbons only.

It is interesting to note that the corresponding *para* derivative [22] and the ferrocene dipeptide conjugates lacking the benzoyl moiety (Fc-Ala-Phe-OMe and Fc-Leu-Phe-OMe) also form helical structures [25,26]; however, Fc-Gly-Gly-OMe forms parallel β-sheet-like structures [27]. It is envisaged that the ferrocenyl benzoyl groups have a minimal effect on the molecular assembly of these conjugates, and even a small group like the methyl group on the peptide chain has a significant effect on the molecular assembly in the solid state. The helical assembly in the Ala-Gly(OEt) **2** derivative may have applications in recognition of biomolecular species by electrochemical methods, as the

pendant ferrocenyl benzoyl group is redox active and can provide an electrical signal.

In addition to hydrogen bonding interactions, molecules B and A also have a short contact O1…H38, 2.38 Å; also ethyl acetate molecules make short contacts with molecules B and A and with each other. Hydrogen bonding interactions are summarized in Tables 3 and 4 shows a list of selected intermolecular contacts that stabilize the structure.

The cyclopentadienyl (Cp) rings in both molecules B and A are nearly eclipsed and the centroids of the Cp rings are equidistant from the iron core; the peptide chains are directed towards their unsubstituted Cp rings and the ester groups are pointing away from the unsubstituted rings (*transoid*), similar to the L-alanine derivative [16]. The key difference between molecules B and A is in the degree of rotation around the bond between the methylene carbon of the glycine subunit and the amide nitrogen of the alanine subunit. The dihedral angle C20–N2–C21–C22 between the substituents in molecule B is 65.0(5)° as compared to the corresponding dihedral angle C44–N4–C45–C46 of 102.1(4)° in molecule A. The phenyl rings in both molecules B and A are twisted out of the plane of the Cp rings, with torsion angles C6–C10–C11–C16 and C30–C34–C35–C40 of 20.7(6)° and 25.9(7)°, respectively. It is likely that the rigid arrangement created by the hydrogen bonds between B and A (vide supra) forces the phenyl group out of the Cp ring's plane.

The amide C═O bond lengths in B and A are in the range of 1.243(4)–1.251(4) Å as expected, whereas all ester C═O bonds are of same length, 1.199(5) Å. The amide CO–NH bond lengths are similar in all the cases and are in the 1.319(5)–1.346(5) Å range. The amide groups are twisted in all molecules with respect to the phenyl groups, the torsion angles are in the 13.7(5)–14.9(6)° range. Twisting of the amide groups is likely due to the rigid framework of strong intermolecular hydrogen bonds present in the structure. Twisting of the amide groups is not uncommon and has been observed previously in rigid systems [26].

2.6. In vitro anti-cancer activity of 2

We had previously tested an *ortho*-ferrocenylbenzoyl amino acid derivative of glycine for its *in vitro* anti-cancer activity towards lung cancer cells (H1299, highly invasive/superinvasive; H1299 carboplatin resistant variant). This compound was found to be cytotoxic and had IC_{50} value of 48 μM. Therefore other derivatives, including compound **2** that represents the Ala-dipeptide series, were evaluated for their anti-cancer activity against lung cancer cell lines. Preliminary results show that the cytotoxicity of compound **2** is ca. 2 times higher than the *ortho*-glycine derivative, the IC_{50} value being 26 μM (RSD 20%); the corresponding *ortho* analog of **2** has an IC_{50} value of 21 μM (RSD 20%). The results are depicted in Fig. 3.

The possible mechanism for their anti-cancer activity originates from the low redox potential of these derivatives

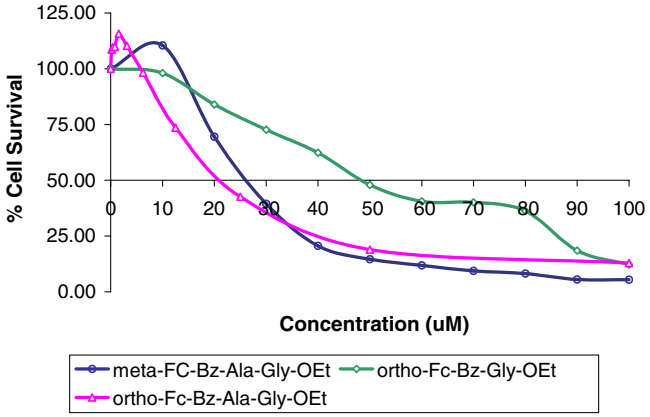


Fig. 3. Cytotoxicity of compound **2**, the *ortho* analog of **2**, and the corresponding *ortho*-glycine derivative.

and their ability to catalyze the generation of reactive oxygenated species (ROS), under physiological conditions, that can oxidatively modify cellular components (e.g., DNA), disturb the redox balance in the cell, and/or interfere with the redox-related cellular signalling pathways. Several mechanisms may be involved in the production of ROS, including a Fenton-type reaction that generates hydroxyl radicals from the superoxide dismutation product, hydrogen peroxide [28]:

$$\mathrm{Fc(II)-Bz-AG-OEt} + \mathrm{H_2O_2} \rightarrow \mathrm{Fc^{+}(III)-Bz-AG-OEt} + {}^{\cdot}\mathrm{OH} + \mathrm{OH^{-}}.$$

Since, preliminary results imply some structure activity relationship, it is envisaged that the peptide chain of these derivatives may have a secondary mode of action. The actual mechanism is not clear, however it is plausible that the lipophilic ferrocenyl benzoyl moiety anchors the cell membrane and the peptide chain blocks the openings of the channels in the cell membrane, leading to cell death [29]; the extent of blocking may depend on the chain length or steric factors, or both. These preliminary results are encouraging and further studies are in progress to elucidate the mechanism of action and for the selection of a series that has maximum cytotoxicity against lung cancer cells.

3. Conclusion

A series of novel *N*-*meta*-ferrocenyl benzoyl dipeptide esters **2**–**5** were synthesized and fully characterized. The potential applications of these derivatives are in the anti-cancer and the biosensor area. Preliminary *in vitro* bioassay results show that these derivatives are cytotoxic and their cytotoxicity is higher than the corresponding amino acid derivatives. The helical molecular assembly of **2** coupled with its low oxidation potential may be useful in sensing biomolecular species and may have applications as materials for biomolecular sensor devices.

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 8452 A 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. Matrix assisted laser desorption ionization (MALDI) mass spectra were obtained on a Bruker Ultraflex TOF/TOF mass spectrometer employing a nitrogen laser at 337 nm. Electrospray ionization mass spectra were performed on either a Bruker Esquire ion trap mass spectrometer or a Micromass Q-ToF Ultima quadrupole time of flight mass spectrometer.

Cyclic voltammograms were recorded in acetonitrile (Sigma-Aldrich), with 0.1 M tetraethylammonium perchlorate (TEAP) as a supporting electrolyte, using a CH Instruments electrochemical analyzer (Pico-Amp Booster and Faraday Cage). The experiments were carried out at room temperature. A three-electrode cell consisting of a glassy carbon working-electrode, a platinum wire counter-electrode and an $Ag|Ag^+$ reference electrode was used. The glassy carbon electrode was polished with 0.3 μm alumina followed by 0.05 μm alumina, between each experiment to remove any surface contaminants. Sample solutions (1 mM) containing tetraethylammonium perchlorate (0.1 mol, TEAP) were prepared in acetonitrile. Typically, the sample solution (5 mL) was pipetted into an electrochemical cell, the electrodes were inserted into the receptor solution and the cyclic voltammograms was recorded by scanning voltage in a predefined range (e.g., 0.0–0.5 V) at a scan rate of 100 mV s^{-1}. A CV scan of a sample of ferrocene (1 mM) with tetraethylammonium perchlorate (0.1 mol) in acetonitrile was also recorded before each experiment to obtain $E^{0\prime}$ (Fc/Fc^+), and the $E^{0\prime}$ values obtained for the test samples were referenced to the Fc/Fc^+ couple.

Crystal data were collected at 100 (2) K using a Brüker SMART APEX CCD area detector diffractometer. A full sphere of the reciprocal space was scanned by ϕ–ω scans. Pseudo-empirical absorption correction based on redundant reflections was performed by the program SADABS [30]. The structures were solved by direct methods using SHELXS-97 [31] and refined by full-matrix least-squares on F^2 for all data using SHELXL-97 [32]. All hydrogen atoms were located in the difference Fourier map and allowed to refine freely with isotropic temperature factors. Anisotropic temperature factors were used for all non-hydrogen atoms.

4.2. General procedure for the synthesis of the starting materials

4.2.1. meta-Ferrocenyl ethyl benzoate (*1a*)

Concentrated hydrochloric acid (6 ml) was added with intermittent cooling to a solution of ethyl-3-aminobenzoate (3 g, 18.2 mmol) in 15 ml of water. A solution of sodium nitrite (1.4 g, 20.3 mmol) in 15 ml of water was then added slowly to this mixture with stirring, keeping the temperature below 5 °C furnishing a pale yellow solution. The resulting diazo salt was added to a solution of ferrocene (3.8 g, 20.4 mmol) in diethyl ether (90 ml) and allowed to react for 12 h. The reaction mixture was then washed with water, the ether layer was dried over $MgSO_4$, and the solvent was removed in vacuo to yield the crude product. The crude product was purified using column chromatography {eluant, 2:3 petroleum ether (40–60 °C): diethyl ether} to obtain an orange solid **1a**, m.p. 74–76 °C; I.R. ν_{max} (KBr) 1719 cm^{-1}; UV–Vis λ_{max} (MeCN): 354 (ε 1289), 453 (ε 240) nm. ^{1}H NMR (400 MHz) δ DMSO-d_6): 8.03 (1H, s, ArH), 7.84 (1H, d, $J = 8$ Hz, ArH), 7.79 (1H, d, $J = 8$ Hz, ArH), 7.46 (1H, t, $J = 8$ Hz, ArH), 4.84 {2H, s, *ortho* on (η^5-C_5H_4)}, 4.40 {2H, s, *meta* on (η^5-C_5H_4)}, 4.34 (2H, q, $J = 7.2$ Hz, –OCH_2CH$_3$), 4.03 {5H, s, (η^5-C_5H_5)}, 1.34 (3H, t, $J = 7.2$ Hz, –OCH$_2$CH_3). ^{13}C NMR (100 MHz) δ DMSO-d_6): 165.8, 138.8, 131.4, 129.5, 129.2, 127.2, 125.8, 83.1, 68.6, 68.2, 67.0, 65.5 (−ve DEPT), 13.3.

4.2.2. meta-Ferrocenyl benzoic acid (*1b*)

Sodium hydroxide (0.3 g, 7.5 mmol) was added to *meta*-ferrocenyl ethyl benzoate (2.4 g, 7.2 mmol) in a 1:1 mixture of water/methanol and was refluxed for 3 h. Concentrated HCl was added until pH 2 was reached. The solution was allowed to cool and filtered to obtain an orange solid **1b**, m.p. 159–161°C; I.R. ν_{max}(KBr): 3450, 1688 cm^{-1}; UV–Vis λ_{max} (CH_2Cl_2): 350 (ε 720), 436 (ε 290) nm; ^{1}H NMR (400 MHz) δ DMSO-d_6): 13.2 (1H, s, –COO*H*), 8.03 (1H, s, ArH), 7.80(1H, d, $J = 8$ Hz, ArH), 7.77 (1H, d, $J = 8$ Hz, ArH), 7.43 (1H, t, $J = 8$ Hz, ArH), 4.84 {2H, s, *ortho* on (η^5-C_5H_4)}, 4.39 {2H, t, *meta* on (η^5-C_5H_4)}, 4.03 {5H, s, (η^5-C_5H_5)}; ^{13}C NMR (100 MHz) δ DMSO-d_6): 167.8, 140.0, 131.2, 130.7, 129.1, 127.1, 126.5, 84.0, 69.8, 69.6, 68.1, 66.8.

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

4.3.1. N-{meta-(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 *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 a yellow solid (0.236 g, 51%). The crystals were of sufficient quality for an X-ray diffraction study and had m.p. 151–153 °C; $E^{0\prime} = 50$ mV (vs Fc/Fc^+); $[\alpha]_D^{20} = -18^\circ$ (*c*2, EtOH); UV–Vis λ_{max} MeCN; 234 (ε 580), 445 (ε 100) nm; I.R. ν_{max}(KBr): 3300, 2937, 1751, 1657, 1632, 1580, 1543, 1199 cm^{-1}; ^1H NMR (400 MHz) δ ($CDCl_3$): 7.80 (1H, s, ArH), 7.54 (1H, d, $J = 8$ Hz, ArH), 7.48 (1H, d, $J = 8$ Hz, ArH), 7.27 (1H, t, $J = 8$ Hz, ArH), 6.83–6.87 (2H, m, –CON*H*–), 4.74 {1H, quint, $J = 7.2$ Hz, –C*H*(CH_3)}, 4.67 {2H, s, *ortho* on (η^5-C_5H_4)}, 4.31 {2H, s, *meta* on (η^5-C_5H_4)}, 4.14 (2H, q, $J = 7.2$ Hz, -OC$H_2$$CH_3$), 3.80–4.00 {7H, m, ($\eta^5$-$C_5H_5$), –NHC$H_2$CO–}, 1.48 {3H, d, $J = 7.2$ Hz, –CH(CH_3)}, 1.14 (3H, t, $J = 7.2$ Hz, –OCH$_2$CH_3); ^{13}C NMR (100 MHz) δ ($CDCl_3$): 173.0, 170.0, 167.8, 140.8, 134.2, 129.8, 129.0, 125.1, 124.5, 84.5, 70.1, 69.8, 67.1, 67.0, 62.0 (−ve DEPT), 49.5, 41.8 (−ve DEPT), 18.8, 14.5.

Analysis: found: C, 62.25; H, 5.85; N, 6.36.

$C_{24}H_{26}N_2O_4Fe$ requires: C, 62.35; H, 5.67; N, 6.06.

Mass spectrum: found: $[M+Na]^+$ 485.20.

$C_{24}H_{26}N_2O_4FeNa$ requires: 485.12.

4.3.2. *N*-{*meta*-(*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 yellow needles. (0.224 g, 53%), m.p. 58–60 °C; $E^{0\prime} = 50$ mV (vs Fc/Fc^+); $[\alpha]_D^{20} = -37^\circ$ (*c*1.9, EtOH); UV–Vis λ_{max} EtOH; 325 (ε 1540), 443 (ε 430) nm; IR ν_{max}(KBr):3276, 2986, 2939, 1739, 1637, 1584, 1543, 1400, 1128 cm^{-1}; ^1H NMR (400 MHz) δ ($CDCl_3$): 7.83 (1H, s, ArH), 7.50–7.56 (2H, m, ArH), 7.26 (1H, t, $J = 8$ Hz, ArH), 7.06 (2H, t, $J = 7.6$ Hz, –CON*H*–), 4.78 {1H, quint, $J = 7.2$ Hz, –C*H*(CH_3)}, 4.62 {2H, t, $J = 1.2$ Hz, *ortho* on (η^5-C_5H_4)}, 4.50 [1H, quint, $J = 7.2$ Hz, –C*H*(CH_3)], 4.26 {2H, t, $J = 1.6$ Hz, *meta* on (η^5-C_5H_4)}, 4.14 (2H, q, $J = 7.2$ Hz, –OC$H_2$$CH_3$), 3.96 {5H, s, ($\eta^5$-$C_5H_5$)}, 1.49 {3H, d, $J = 6.8$ Hz, –CH(CH_3)}, 1.37 {3H, d, $J = 6.8$ Hz, –CH(CH_3)}, 1.22 (3H, t, $J = 7.2$ Hz, –OCH$_2$CH_3); ^{13}C NMR (100 MHz) δ (DMSO-d_6): 173.1, 172.5, 167.6, 140.7, 134.3, 129.8, 128.9, 125.1, 124.6, 84.4, 70.1, 69.7, 67.1, 67.0, 62.0 (−ve DEPT), 49.6, 48.7, 19.4, 18.5, 14.5.

Analysis: found: C, 62.96; H, 6.13; N, 5.62.

$C_{25}H_{28}N_2O_4Fe$ requires: C, 63.04; H, 5.92; N, 5.88.

Mass spectrum: found: $[M]^+$ 476.14.

$C_{25}H_{28}N_2O_4Fe$ requires: 476.14.

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

For the compound **4** L-alanine-L-leucine ethyl ester hydrochloride (0.2 g, 0.75 mmol) was used as 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 (0.203 g, 56%), m.p. 110–112 °C; $E^{0\prime} = 46$ mV (vs Fc/Fc^+); $[\alpha]_D^{20} = -25^\circ$ (*c*1.8, EtOH); UV–Vis λ_{max} EtOH; 323 (ε1430), 442 (ε 410) nm; I.R. ν_{max}(KBr): 3286, 2963, 1750, 1637, 1537, 1450, 1180 cm^{-1}; ^1H NMR (400 MHz) δ DMSO-d_6): 8.32 (1H, d, $J = 7.6$ Hz, –CON*H*–), 8.08 (1H, d, $J = 7.6$ Hz, –CON*H*–), 7.77 (1H, s, ArH), 7.50 (2H, t, $J = 8$ Hz, ArH), 7.18 (1H, t, $J = 8$ Hz, ArH), 4.65 {2H, t, $J = 2$ Hz, *ortho* on (η^5-C_5H_4)}, 4.34 {1H, quint, $J = 6.4$ Hz, –C*H*(CH_3)}, 4.17 {2H, t, $J = 1.6$ Hz, *meta* on (η^5-C_5H_4)}, 3.97–4.05 [1H, m, C*H*{$CH_2CH(CH_3)_2$}], 3.83–3.87 (2H, m, –OC$H_2$$CH_3$), 3.81 {5H, s, ($\eta^5$-$C_5H_5$)}, 1.24–1.52 [3H, m, CH{CH_2C*H*(CH_3)$_2$}], 1.15 {3H, d, $J = 7.2$ Hz, CH(CH_3)}, 0.95 (3H, t, $J = 7.2$ Hz, –OCH$_2$CH_3), 0.69 [3H, d, $J = 6.4$ Hz, CH{CH_2C*H*(CH_3)$_2$}], 0.64 [3H, d, $J = 6.4$ Hz, CH{CH_2C*H*(CH_3)$_2$}]; ^{13}C NMR (100 MHz) δ (DMSO-d_6): 173.1, 172.8, 166.3, 139.5, 134.5, 129.1, 128.6, 125.5, 124.7, 84.4, 69.8, 69.4, 66.8, 60.7 (−ve DEPT), 50.8, 48.9, 40.0 (−ve DEPT), 24.6, 23.1, 21.8, 18.2, 14.4.

Analysis: found: C, 64.68; H, 6.67; N, 5.46.

$C_{28}H_{34}N_2O_4Fe$ requires: C, 64.87; H, 6.61; N, 5.40.

Mass spectrum: found: $[M]^+$ 518.1860.

$C_{28}H_{34}N_2O_4Fe$ requires: 518.1868.

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

For compound **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 an orange solid (0.203 g, 52%), m.p. 61–63 °C; $E^{0\prime} = 59$ mV (vs Fc/Fc^+); $[\alpha]_D^{20} = -19^\circ$ (*c*1.9, EtOH); UV–Vis λ_{max} (EtOH); 327 (ε 1440), 442 (ε 390) nm; I.R. ν_{max}(KBr): 3325, 2930, 1739, 1637, 1579, 1532, 1499, 1208 cm^{-1}; ^1H NMR (400 MHz) δ ($CDCl_3$): 7.82 (1H, s, ArH), 7.58 (1H, d, $J = 8$ Hz, ArH), 7.46 (1H, d, $J = 8$ Hz, ArH), 7.29 (1H, t, $J = 8$ Hz, ArH), 7.10–7.12 (3H, m, ArH), 7.02–7.04 (2H, m, ArH), 6.67 (1H, d, $J = 7.2$ Hz, –CON*H*–), 6.50 (1H, d, $J = 7.2$ Hz, –CON*H*–), 4.78–4.80 [1H, m, –NHC*H*(CH_3)], 4.63– 4.66 {3H, m, *ortho* on (η^5-C_5H_4), –NHC*H*(CH_2Ph)}, 4.29 {2H, s, *meta* on (η^5-C_5H_4)}, 4.14 (2H, q, $J = 7.2$ Hz, –OC$H_2$$CH_3$), 3.98 {5H, s, ($\eta^5$-$C_5H_5$)}, 3.08 {1H, dd, $J_{ax} = 2.0$ Hz, $J_{ab} = 5.6$ Hz, CH(CH_2Ph)}, 3.03 {1H, dd, $J_{max} = 2.0$ Hz, $J_{ab} = 5.6$ Hz, CH(CH_2Ph)}1.62 {3H, d, $J = 6.4$ Hz, –CH(CH_3)}, 1.21 (3H, t, $J = 7.2$ Hz, –OCH$_2$CH_3); ^{13}C NMR (100 MHz) δ ($CDCl_3$): 172.2, 171.6, 167.5, 140.8, 136.1, 134.2, 129.8,

129.7, 129.0, 127.5, 125.1, 124.5, 84.5, 70.1, 69.8, 67.1, 67.0, 62.1 (−ve DEPT), 53.8, 49.6, 38.3 (−ve DEPT), 18.8, 14.5.

Analysis: found: C, 65.96; H, 5.85; N, 5.14.

$C_{31}H_{32}N_2O_4Fe$ requires: C, 67.40; H, 5.84; N, 5.07.

Mass spectrum: found: $[M]^+$ 552.169.

$C_{31}H_{32}N_2O_4Fe$ requires: 552.171.

4.3.5. Crystallographic footnotes for ***2***

Crystallographic data: chemical formula $C_{28}H_{34}N_2O_6Fe$, formula weight 550.42 g mol^{-1}, orange needle, crystal size = $0.60 \times 0.20 \times 0.10$ mm^3, tetragonal, space group $P4_3$, unit cell dimensions $a = 16.0466(14)$ Å ($\alpha = 90°$), $b = 16.7111(14)$ Å ($\beta = 90°$), $c = 20.3902(17)$ Å ($\gamma = 90°$), $V = 5467.8(8)$ Å^3, $Z = 8$, density = 1.337 g cm^{-3}, $\mu = 0.595$ mm^{-1}, 36 839 reflections collected in the range 1.58–26.00°, 10 588 independent reflections, 675 parameters, R factor = 0.0604, $wR_2 = 0.1471$, Flack parameter = 0.031(17).

5. Supplementary material

CCDC 616784 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

Acknowledgements

D.S. thanks the Irish American Partnership and Dublin City University for the funding of a studentship award 1999–2002. This research was partly supported by the National Institute for Cellular Biotechnology under the Programme for Research in Third Level Institutions (PRTLI, round 3, 2001–2006).

References

[1] R.H. Fish, G. Jaouen, Organometallics 22 (2003) 2166.
[2] E.W. Neuse, J. Inorg. Organomet. Poly. Mat. 15 (1) (2005) 3.
[3] V. Degani, A. Heller, J. Am. Chem. Soc. 110 (1988) 2615.
[4] M. Kira, T. Matsubara, H. Shinohara, M. Sisido, Chem. Lett. (1997) 89.
[5] H. Fink, N.J. Long, A.J. Martin, G. Opromolla, A.J.P. White, D.J. Williams, P. Zanello, Organometallics 16 (1997) 2646.
[6] H. Plenio, C. Aberle, Organometallics 16 (1997) 5950.
[7] A. Nomoto, T. Moriuchi, S. Yamazaki, A. Ogawa, T. Hirao, J. Chem. Soc. Chem. Commun. (1998) 1963.
[8] T. Itoh, S. Shirakami, N. Ishida, Y. Yamashita, T. Yoshida, H.-S. Kim, Y. Wataya, Bioorg. Med. Chem. Lett. 10 (2000) 1657.
[9] T. Moriuchi, A. Nomoto, K. Yoshida, T. Hirao, Organometallics 20 (2001) 1008.
[10] S. Chowdhury, D.A.R. Sanders, G. Schatte, H.B. Kraatz, Angew. Chem., Int. Ed. 45 (5) (2006) 751.
[11] S.K. Dey, H.B. Kraatz, Bioconj. Chem. 17 (1) (2006) 84.
[12] S.I. Kirin, U. Schatzschneider, X.D. Hatten, T. Weyhermueller, N. Metzler-Nolte, J. Organomet. Chem. 691 (16) (2006) 3451.
[13] S.I. Kirin, H.B. Kraatz, N. Metzler-Nolte, Chem. Soc. Rev. 35 (4) (2006) 348.
[14] E.C. Constable, Angew. Chem., Int. Ed. 30 (4) (1991) 407.
[15] V.N. Babin, P.M. Raevskii, K.G. Shchitkov, L.V. Snegur, S. Yu. Neerasov, Mendeleev Chem. 39 (1995) 17.
[16] D. Savage, J.F. Gallagher, Y. Ida, P.T.M. Kenny, Inorg. Chem. Commun. 5 (2002) 1034.
[17] D. Savage, G. Malone, J.F. Gallagher, Y. Ida, P.T.M. Kenny, J. Organomet. Chem. 690 (2005) 383.
[18] D. Savage, N. Neary, G. Malone, S.R. Alley, J.F. Gallagher, P.T.M. Kenny, Inorg. Chem. Commun. 8 (2005) 429.
[19] D. Savage, G. Malone, S.R. Alley, J.F. Gallagher, A. Goel, P.N. Kelly, H. Mueller-Bunz, P.T.M. Kenny, J. Organomet. Chem. 691 (2006) 463.
[20] D. Savage, S.R. Alley, J.F. Gallagher, A. Goel, P.N. Kelly, P.T.M. Kenny, Inorg. Chem. Commun. 9 (2006) 152.
[21] D. Savage, S.R. Alley, A. Goel, T. Hogan, Y. Ida, P.N. Kelly, L. Lehmann, P.T.M. Kenny, Inorg. Chem. Commun. 9 (2006) 1267.
[22] A. Goel, D. Savage, S.R. Alley, T. Hogan, P.N. Kelly, S. Draper, C.M. Fitchett, P.T.M. Kenny, J. Organomet. Chem. 691 (2006) 4686.
[23] K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida, Rapid Commun. Mass Spectrom. 2 (1988) 151.
[24] J.B. Fenn, J. Am. Soc. Mass Spectrom. 4 (1993) 524.
[25] W. Bauer, K. Polborn, W. Beck, J. Organomet. Chem. 579 (1999) 269.
[26] D.R. van Staveren, T. Weyhermuller, N. Metzler-Nolte, Dalton Trans. (2003) 210.
[27] P. Saweczko, G.D. Enright, H.B. Kraatz, Inorg. Chem. 40 (2001) 4409.
[28] R. Huang, A. Wallquist, D.G. Covell, Biochem. Pharmacol. 69 (2005) 1009.
[29] Z. Zhong, M.D. Wheeler, X. Li, M. Froh, P. Schemmer, M. Yin, H. Bunzendaul, B. Bradford, J.J. Lemasters, Curr. Opin. Clin. Nutr. Metabol. Care 6 (2) (2003) 229.
[30] G.M. Sheldrick, SADABS, Brüker AXS Inc., Madison, WI, 2000.
[31] G.M. Sheldrick, SHELXS-97, University of Göttingen, 1997.
[32] G.M. Sheldrick, SHELXL-97-2, University of Göttingen, 1997.