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Antibacterial activities of ferrocenoyl- and cobaltocenium-peptide bioconjugates

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Abstract

The peptide and metallocene-peptide bioconjugates R-Arg-Arg-Phe-NH_2, R-Phe-Arg-Phe-NH_2 where R = H, Fe(Cp)(C_5H_4–CO), Co(Cp)(C_5H_4–CO)$^+$ and R′-Gly-Trp-Arg-Arg-Phe-NH_2, R′-Trp-Arg-Arg-Phe-NH_2, where R′ = n-C_5H_{11}CO, Fe(Cp)(C_5H_4–CO), Co(Cp)(C_5H_4–CO)$^+$, and Arg = L-arginine, Gly = L-glycine, Phe = L-phenylalanine, Trp = L-tryptophan were prepared by solid phase peptide synthesis (SPPS). The compounds were purified by RP-HPLC and characterized by ESI–MS and NMR spectroscopy. Antibacterial properties of the compounds were determined by minimum inhibitory concentration (MIC) tests against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Keywords: Antibacterial agents; Bioorganometallic chemistry; Cobaltocenium; Ferrocene; Peptides; Solid phase synthesis

1. Introduction

Bioorganometallic chemistry is a fast growing field and a number of novel organometallic compounds have been reported to exhibit important biological activities [1–3]. Ferrocene bioconjugates have the potential to be applied in electrochemical sensor devices due to the well-behaved redox chemistry of ferrocene. Medicinal applications of ferrocene is another area of active research. In the antimalarial compound ferroquine [4–8] and the antitumour agent ferrocifen [9–11], the introduction of the ferrocenyl group enhances the activity of the compounds and changes their pharmacodynamic profiles. Furthermore, the replacement of aromatic groups with a ferrocenyl group in penicillin and cephalosporine reportedly enhances the antibacterial potency of the antibiotics [12,13].

To tackle the serious problem of multiresistant bacteria, pharmaceutical companies have mainly focused on the modification of existing antibiotics, the major classes of which were introduced in the 1940–1960s. Almost four decades passed between the introduction of the fluoroquinolone class of antibiotics in 1962 and the next new structural class, the oxazolidinones in 2000 [14]. There is clearly a need for new types of antibiotics [15] and antimicrobial peptides (AMPs) form a class of prospective new antibacterial drugs. The diversity of AMPs, the relatively large size of certain types and their complicated conformational structures, for example the β-defensins, mean that AMPs are difficult to synthesize chemically [16]. Shorter and modified peptides with enhanced activities are sought after.

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Antibacterial peptides are usually cationic, having a net excess of positively charged amino acid residues. However, a significant proportion of hydrophobic residues are also present. This structural motif is linked to the mechanism of action, which is initiated by electrostatic binding to the negatively charged bacterial cell membrane. Studies of the antimicrobial activities of short, cationic peptides showed that the minimum structural requirement for antibacterial activity was the presence of two cationic charges and two units of bulk [17]. We have incorporated this motif in metallocene–peptide conjugates where the ferrocenyl moiety represents a unit of bulk and the cobaltocenium moiety a unit of bulk, as well as a unit of charge. The peptide sequences were varied from three to five amino acids in length and were made up of Arg (for its cationicity), Phe and Trp (for hydrophobic bulk). Gly was incorporated as a linker, as it does not contribute to charge or bulk. The metallocene moiety was attached at the N-terminus.

The use of metallocenes in SPPS has rarely been described before, the instability of ferrocenyl- and half-sandwich-peptides being problematic [18–22]. The attachment of ferrocene to DNA [23–27] and Mo carbonyls to [Leu^5]-enkephalin [28] have, however, been reported in the last couple of years. Very recently, the SPPS and cellular localization of a cobaltocenium-NLS peptide was reported by the Metzler-Nolte group [29]. We have developed a methodology that allows for metallocene peptide conjugates to be synthesized in relatively good yields and in high purities [30].

The antibacterial properties of the metallocene peptide bioconjugates were tested against the Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and the Gram positive *Staphylococcus aureus*.

2. Experimental

SPPS were performed manually at room temperature using an Fmoc-protection strategy on a Rink Amide resin (200 mg, 0.13 mmol). N-terminal Fmoc deprotection was achieved using 20% v/v piperidine in DMF over 10 min. The coupling steps made use of Fmoc-Aaa-OH or $C_5H_{11}CO_2H$ (4 equiv.)/TBTU (4 equiv.)/DIPEA (9 equiv.) in DMF and [Fe(Cp)(C_5H_4–COOH)] or [Co(Cp)(C_5H_4–COOH)]PF_6 (4 equiv.)/TBTU (4 equiv.)/HOBt · H_2O (4 equiv.)/DIPEA (9 equiv.) for 20–40 min. Arg and Trp side-chains were protected by Pbf and Boc, respectively. After the last coupling step or deprotection step the resin was washed with CH_2Cl_2 and dried. Peptide-ferrocenoyl amides were cleaved from the resin and the side-chains deprotected using a mixture of TFA, phenol and TIS (85:10:5). All other peptides were cleaved and deprotected with a TFA: H_2O:TIS (95:2.5:2.5) mixture over a period of 3 h. After filtration the peptides were precipitated by the addition of cold Et_2O, centrifuged, washed with cold Et_2O and air-dried. The peptides were then dissolved in H_2O/MeCN, filtered and lyophilized before being purified by reverse-phase HPLC (Varian C18 column, 60 Å/8 μm, 250 × 10.0 mm). The eluent consisted of MeCN/H_2O mixtures containing 0.1% TFA. The peptides were analyzed at 25 °C using an analytical Varian Microsorb C18 column (60 Å/8 μm, 250 × 4.60 mm) at 220 and 254 nm (Varian ProStar PDA detector). The solvent gradient was increased linearly from 5% MeCN/95% H_2O to 95% MeCN/5% H_2O over 30 min at a flow rate of 1 mL min^{-1} and decreased back to 5% MeOH at 35 min. Alternatively, a Merck Chromolith™ Performance 18e column (100 × 4.60 mm) column was used with a linear solvent gradient from 100% H_2O/0.1% TFA to 100% MeCN/0.1% TFA over 5 min at a flow rate of 4 mL min^{-1}. The purities of the HPLC traces were greater than 97%. Mass spectra were measured by positive ion electrospray mass spectrometry on a Finnigan TSQ 700 spectrometer. NMR spectra were recorded on a Bruker DRX-300 spectrometer, except for compounds **1** and **4** which were measured on a Bruker AM 360 spectrometer. The ^{1}H and ^{13}C chemical shifts were referenced to residual solvent signals. Two-dimensional H,H-COSY and H,C-HETCOR NMR experiments were conducted to aid assignments of signals.

2.1. Antibacterial activity

The antimicrobial activity of the peptides were determined against *E. coli* (ML-35), *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 25923) using the minimum inhibitory concentration (MIC) test. Serial 1:1 dilutions, from 100 to 0.098 μg mL^{-1}, of each peptide were made in 96-well microtiter plates, to a final volume of 100 μL of Tryptic soy broth (15% v/v in 10 mM sodium phosphate buffer, pH 7.4) with 2×10^5 colony forming units (CFU) mL^{-1} of the bacteria in the exponential phase. Plates were incubated at 37 °C overnight and the MIC was considered as the first well without visible growth. Each peptide was tested in parallel and in duplicate.

2.1.1. H-Arg-Arg-Phe-NH_2 (*1*)

White solid. ^{1}H NMR (360 MHz), [D_2O], 25 °C: $\delta = 8.23$ (d, $^3J = 8.4$ Hz, 1H; H^N), 7.36 (d, $^3J = 6.9$ Hz, 2H; $2 \times H^N$), 7.12–7.22 (m 5H; H^{δ}_{Phe}, H^{ε}_{Phe}, H^{ζ}_{Phe}), 4.43 (dd, $^3J = 6.0$ Hz, $^3J = 8.9$ Hz, 1H; H^{α}), 4.15 (t, $^3J = 7.0$ Hz, 1H; H^{α}), 3.89 (t, $^3J = 6.4$ Hz, 1H; H^{α}), 2.97–3.03 and 2.85–2.89 (m, 6H; H^{δ}_{Arg}, H^{β}_{Phe}), 1.3–1.8 (m, 8H; H^{β}_{Arg}, H^{γ}_{Phe}). ^{13}C NMR, [d_6]DMSO, 25 °C: $\delta =$ 173.5 (C═O), 171.6 (C═O), 157.2 (C^{ζ}_{Arg}), 138.1 (C^{γ}_{Phe}),

129.9 (C^{δ}_{Phe}), 128.2 (C^{ε}_{Phe}), 126.5 (C^{ζ}_{Phe}), 54.3 (C^{α}), 52.1 (C^{α}), 40.8 (C^{δ}_{Arg}), 37.9 (C^{β}_{Phe}), 28.7 and 28.1 (C^{β}_{Arg}), 24.5 and 23.6 (C^{γ}_{Arg}). ESI–MS (pos.) for $C_{21}H_{36}N_{10}O_3$ (476.3): *m/z*: 477.5 $[M + H]^+$, 239.5 $[M + 2H]^{2+}$.

2.1.2. [Fe(Cp)(η-C_5H_4-CO-Arg-Arg-Phe-NH_2)] (**2**)

Yellow solid. ^{1}H NMR (300 MHz), [d_6]DMSO, 25 °C: $\delta = 8.00$ (d, $^3J = 7.7$ Hz, 1H; H^N), 7.93 (d, $^3J = 7.9$ Hz, 1H; H^N), 7.81 (d, $^3J = 7.8$ Hz, 1H; H^N), 7.62 (m, 1H; H^{ε}_{Arg}), 7.50 (m, 2H; CO′NH_2), 7.12–7.24 (overlapping m; H^{δ}_{Phe}, H^{ε}_{Phe}, H^{ζ}_{Phe} and H^{η}_{Arg}), 4.90 (pseudo s, 1H; H_{Cp}), 4.87 (pseudo s, 1H; H_{Cp}), 4.42 (m, 2H; $2 \times H^{\alpha}$), 4.37 (pseudo s, 2H; H_{Cp}), 4.24 (m, 1H; H^{α}), 4.19 (s, 5H; H_{Cp}), 2.90–3.11 and 2.78–2.85 (m, 6H; H^{β}_{Phe}, H^{δ}_{Arg}), 1.4–1.6 (m, 8H; H^{β}_{Arg}, H^{γ}_{Arg}). ^{13}C NMR, [d_6]DMSO, 25 °C: $\delta = 172.7$ (C═O), 171.8 (C═O), 170.9 (C═O), 169.6 (C═O), 156.7 (C^{ζ}_{Arg}), 137.5 (C^{γ}_{Phe}), 129.1 (C^{δ}_{Phe}), 128.0 (C^{ε}_{Phe}), 126.2 (C^{ζ}_{Phe}), 75.7 ($C_{Cp\ ipso}$), 70.1 (C_{Cp}), 69.3 ($C_{Cp\ unsubstituted}$), 68.3 (C_{Cp}), 53.5 (C^{α}), 52.5 (C^{α}), 52.3 (C^{α}), 32.6, 29.1 and 28.4 (C^{β}_{Phe}, C^{δ}_{Arg}), 25.3 (C^{β}_{Arg}), 24.8 (C^{γ}_{Arg}). ESI–MS (pos.) for $C_{32}H_{44}FeN_{10}O_4$ (688.3): *m/z*: 689.5 $[M + H]^+$, 345.4 $[M + 2H]^{2+}$.

2.1.3. [Co(Cp)(η-C_5H_4-CO-Arg-Arg-Phe-NH_2)]CF_3CO_2 (**3**)

Yellow solid. ^{1}H NMR (300 MHz), [d_6]DMSO, 25 °C: $\delta = 8.81$ (d, $^3J = 7.4$ Hz, 1H; H^N_{Arg}), 8.21 (d, $^3J = 7.7$ Hz, 1H; H^N_{Arg}), 7.93 (d, $^3J = 8.0$ Hz, 1H; H^N_{Phe}), 7.78 (m, 1H; H^{ε}_{Arg}), 7.69 (m, 1H; H^{ε}_{Arg}), 7.50 and 7.12–7.26 (m, 16H; H^{δ}_{Phe}, H^{ε}_{Phe}, H^{ζ}_{Phe}, H^{η}_{Arg}, CO′NH_2), 6.44 (pseudo s, 1H; H_{Cp}), 6.29 (pseudo s, 1H; H_{Cp}), 5.94 (m, 2H; H_{Cp}), 5.85 (s, 5H; H_{Cp}), 4.41 (m, 2H; H^{α}_{Arg}, H^{α}_{Phe}), 4.26 (m, 1H; H^{α}_{Arg}), 2.96–3.13 and 2.77–2.85 (m, 6H; H^{β}_{Phe}, H^{δ}_{Arg}), 1.43–1.76 (m, 8H; H^{β}_{Arg}, H^{γ}_{Arg}). ^{13}C NMR, [d_6]DMSO, 25 °C: $\delta = 172.7$ (C═O), 171.1 (C═O), 170.9 (C═O), 161.8 (C═O), 156.8 (C^{ζ}_{Arg}), 137.6 (C^{γ}_{Phe}), 129.2 (C^{δ}_{Phe}), 128.0 (C^{ε}_{Phe}), 126.3 (C^{ζ}_{Phe}), 92.6 ($C_{Cp\ ipso}$), 86.1 (C_{Cp}), 86.0 ($C_{Cp\ unsubstituted}$), 84.5 (C_{Cp}), 83.8 (C_{Cp}), 53.6 (C^{α}), 53.4 (C^{α}), 52.5 (C^{α}), 40.4 (C^{δ}_{Arg},) 37.6 (C^{β}_{Phe}), 29.2 and 28.4 (C^{β}_{Arg}), 25.4 and 24.9 (C^{γ}_{Arg}). ESI–MS (pos.) for $C_{34}H_{44}CoF_3N_{10}O_6$ (804.3): *m/z*: 805.4 $[M + H]^+$, 691.5 $[M - TFA]^+$, 346.4 $[M - TFA + H]^{2+}$, 231.4 $[M - TFA + 2H]^{3+}$.

2.1.4. H-Phe-Arg-Phe-NH_2 (**4**)

White solid. ^{1}H NMR (360 MHz), [d_4]Methanol, 25 °C: $\delta = 7.12$–7.28 (m, 10H; H^{δ}_{Phe}, H^{ε}_{Phe}, H^{ζ}_{Phe}), 4.60 (m, 1H; H^{α}), 4.33 (m, 2H; $2 \times H^{\alpha}$), 3.13 and 2.91 (m, 6H; H^{β}_{Phe}, H^{δ}_{Arg}), 1.70 (m, 2H; H^{β}_{Arg}), 1.54 (m, 2H; H^{γ}_{Arg}). ^{13}C NMR, [d_4]Methanol, 25 °C: $\delta = 173.2$ (C═O), 170.7 (C═O), 170.3 (C═O), 158.7 (C^{ζ}_{Arg}), 138.5 (C^{γ}_{Phe}), 135.8 (C^{γ}_{Phe}), 130.7, 130.5, 130.1, 129.5, 128.7 and 127.8 (C^{δ}_{Phe}, C^{ε}_{Phe}, C^{ζ}_{Phe}), 55.8 (C^{α}), 55.6 (C^{α}), 54.7 (C^{α}), 42.0 (C^{δ}_{Arg}), 39.0 and 38.5 (C^{β}_{Phe}), 30.3 (C^{β}_{Arg}), 25.7 (C^{γ}_{Arg}). ESI–MS (pos.) for $C_{24}H_{33}N_7O_3$ (467.3): *m/z*: 468.4 $[M + H]^+$, 235.0 $[M + 2H]^{2+}$.

2.1.5. [Fe(Cp)(η-C_5H_4-CO-Phe-Arg-Phe-NH_2)] (**5**)

Yellow solid. ^{1}H NMR (300 MHz), [d_6]DMSO, 25 °C: $\delta = 8.16$ (d, $^3J = 7.7$ Hz, 1H; H^N), 7.88 (d, $^3J = 8.1$ Hz, 1H; H^N), 7.81 (d, $^3J = 8.3$ Hz, 1H; H^N), 7.41 (m, 1H; H^{ε}_{Arg}), 7.38 (m, 2H; CO′NH_2), 7.06–7.28 (overlapping m; H^{δ}_{Phe}, H^{ε}_{Phe}, $H^{\zeta}_{Phe}$and H^{η}_{Arg}), 4.79 (pseudo s, 1H; H_{Cp}), 4.69 (pseudo s, 1H; H_{Cp}), 4.65 (m, 1H; H^{α}), 4.43 (m, 1H; H^{α}), 4.26 (pseudo s, 2H; H_{Cp}), 4.24 (m, 1H; H^{α}), 3.88 (s, 5H; H_{Cp}), 2.80 – 3.05 (overlapping m, 6H; H^{β}_{Phe}, H^{δ}_{Arg}), 1.38 – 1.65 (m, 4H; H^{β}_{Arg}, H^{γ}_{Arg}). ^{13}C NMR, [d_6]DMSO, 25 °C: $\delta = 172.5$ (C═O), 171.8 (C═O), 170.8 (C═O), 169.2 (C═O), 156.5 (C^{ζ}_{Arg}), 138.5 and 137.5 (C^{γ}_{Phe}), 129.0, 128.0, 127.9, 126.1 (C^{δ}_{Phe}, C^{ε}_{Phe}, C^{ζ}_{Phe}), 75.5 ($C_{Cp\ ipso}$), 69.9 (C_{Cp}), 69.2 ($C_{Cp\ unsubstituted}$), 68.5 (C_{Cp}), 67.7 (C_{Cp}), 54.2 (C^{α}), 53.4 (C^{α}), 52.3 (C^{α}), 37.5 and 36.7 (C^{β}_{Phe}), 29.1 (C^{β}_{Arg}), 24.8 (C^{γ}_{Arg}), C^{δ}_{Arg} obscured by solvent peak. ESI–MS (pos.) for $C_{35}H_{41}FeN_7O_4$ (679.3): *m/z*: 680.4 $[M + H]^+$.

2.1.6. [Co(Cp)(η-C_5H_4-CO-Phe-Arg-Phe-NH_2)]CF_3CO_2 (**6**)

Yellow solid. ^{1}H NMR (300 MHz), [d_6]DMSO, 25 °C: $\delta = 8.91$ (d, $^3J = 8.5$ Hz, 1H; H^N), 8.47 (d, $^3J = 7.6$ Hz, 1H; H^N), 7.93 (d, $^3J = 8.1$ Hz, 1H; H^N), 7.66 (m, 1H; H^{ε}_{Arg}), 7.11–7.46 (overlapping m; H^{δ}_{Phe}, H^{ε}_{Phe}, H^{ζ}_{Phe}, H^{η}_{Arg}, CO′NH_2), 6.29 (pseudo s, 1H; H_{Cp}), 6.25 (pseudo s, 1H; H_{Cp}), 5.88 (pseudo s, 2H; H_{Cp}), 5.52 (s, 5H; H_{Cp}), 4.85 (m, 1H; H^{α}), 4.46 (m, 1H; H^{α}), 4.28 (m, 1H; H^{α}), 2.79–3.17 (m, 6H; H^{β}_{Phe}, H^{δ}_{Arg}), 1.43–1.53 (m, 4H; H^{β}_{Arg}, H^{γ}_{Arg}). ^{13}C NMR, [d_6]DMSO, 25 °C: $\delta = 172.6$ (C═O), 170.9 (C═O), 170.8 (C═O), 161.4 (C═O), 156.8 (C^{ζ}_{Arg}), 138.2 and 137.6 (C^{γ}_{Phe}), 129.2 and 129.1 (C^{δ}_{Phe}), 128.3 and 128.0 (C^{ε}_{Phe}), 126.5 and 126.2 (C^{ζ}_{Phe}), 92.4 ($C_{Cp\ ipso}$), 86.0 (C_{Cp}), 85.8 ($C_{Cp\ unsubstituted}$), 84.2 (C_{Cp}), 83.6 (C_{Cp}), 54.3 (C^{α}), 53.5 (C^{α}), 52.6 (C^{α}), 37.6 and 37.0 (C^{β}_{Phe}), 29.1 (C^{β}_{Arg}), 25.0 (C^{γ}_{Arg}), C^{δ}_{Arg} obscured by solvent peak. ESI–MS (pos.) for $C_{37}H_{41}CoF_3N_7O_6$ (795.3): *m/z*: 796.4 $[M + H]^+$, 682.4 $[M - TFA]^+$, 341.9 $[M - TFA + H]^{2+}$.

2.1.7. C_5H_{11}-C(O)-Gly-Trp-Arg-Arg-Phe-NH_2 (**7**)

White solid. ^{1}H NMR (300 MHz), [d_6]DMSO, 25 °C: $\delta = 10.83$ (s, 1H; $H^{\varepsilon 1}_{Trp}$), 8.17 (m, 1H; H^N_{Gly}), 8.07 (pseudo d, $^3J = 7.5$ Hz, 1H; H^N), 7.76 (m, 2H; $2 \times H^N$), 7.57 (d, $^3J = 7.8$ Hz, 1H; H^{δ}_{Trp}), 6.95–7.33 (m; H^{δ}_{Phe}, H^{ε}_{Phe}, H^{ζ}_{Phe}, $H^{\varepsilon 3}_{Trp}$, H^{ζ}_{Trp}, H^{η}_{Trp}, H^{ε}_{Arg}, CO′NH_2), 4.55 (m, 1H; $H^{\alpha}_{Phe/Trp}$), 4.42 (m, 1H; $H^{\alpha}_{Phe/Trp}$), 4.15 (m, 2H; $2 \times H^{\alpha}_{Arg}$), 3.78 (dd, $^3J = 5.9$ Hz, $^2J = 16.4$ Hz, 1H; H^{α}_{Gly}), 3.54 (dd, $^3J = 5.1$ Hz, $^2J = 16.4$ Hz, 1H; H^{α}_{Gly}), 2.98–3.17 and 2.77–2.85 (overlapping m, 8H; H^{β}_{Phe}, H^{β}_{Trp}, H^{δ}_{Arg}), 2.07 (t, $^3J = 7.4$ Hz, 2H; H^{α}_{Hex}), 1.2–1.67 (m, 14H; H^{β}_{Arg}, H^{γ}_{Arg}, H^{β}_{Hex}, H^{γ}_{Hex}, H^{δ}_{Hex}), 0.84 (t, $^3J = 6.8$ Hz, 3H; H^{ε}_{Hex}). ^{13}C NMR, [d_6]DMSO, 25 °C: $\delta = 172.7$ (C═O), 172.8 (C═O), 172.0 (C═O), 170.8 (C═O), 169.4 (C═O), 156.8 (C^{ζ}_{Arg}), 156.7 (C^{ζ}_{Arg}), 137.8 and 136.1 (C^{γ}_{Phe}, $C^{\varepsilon 2}_{Trp}$), 129.2, 128.0, 127.3, 126.2,

123.7, 120.8, 118.3, 118.2 (C^{δ}_{Phe}, C^{ε}_{Phe}, C^{ζ}_{Phe}, C^{δ}_{Trp}, $C^{\varepsilon 3}_{Trp}$, C^{ζ}_{Trp}, C^{η}_{Trp}), 109.7 (C^{γ}_{Trp}), 53.8 (C^{α}), 37.5 ($C^{\beta}_{Phe/Trp}$), 35.1(C^{α}_{Hex}), 30.9 ($C^{\gamma}_{Hex/Arg}$), 24.8 ($C^{\beta}_{Arg/Hex}$/C^{δ}_{Hex}), 21.9 ($C^{\gamma}_{Hex/Arg}$), 13.8 (C^{ε}_{Hex}), C^{δ}_{Arg} obscured by solvent peak, $1\times C{=}O_{amide}$, $4\times C^{\alpha}$ and $C^{\beta}_{Arg/Hex}$/C^{δ}_{Hex} unresolved. ESI–MS (pos.) for $C_{40}H_{59}N_{13}O_6$ (817.5): *m/z*: 818.6 $[M+H]^+$, 410.0 $[M+2H]^{2+}$.

2.1.8. [Fe(Cp)(η-C_5H_4-CO-Gly-Trp-Arg-Arg-Phe-NH_2)] (**8**)

Yellow solid. ^{1}H NMR (300 MHz), [d_6]DMSO, 25 °C: $\delta = 10.85$ (s, 1H; $H^{\varepsilon 1}_{Trp}$), 8.29 (d, $^3J = 7.6$ Hz, 1H; H^{N}), 8.16 (m, 2H; $2\times H^{N}$), 7.95 (d, $^3J = 7.4$ Hz, 1H; H^{N}), 7.89 (d, $^3J = 8.0$ Hz, 1H; H^{N}), 7.61 (d, $^3J = 7.8$ Hz, 1H; H^{δ}_{Trp}), 7.54 (m, 2H; CO′NH_2), 7.49 (br s, 1H; H^{ε}_{Arg}) and 6.93–7.34 (overlapping m; H^{δ}_{Phe}, H^{ε}_{Phe}, H^{ζ}_{Phe}, $H^{\varepsilon 3}_{Trp}$, H^{ζ}_{Trp}, H^{η}_{Trp} and H^{η}_{Arg}), 4.76 (m, 2H; H_{Cp}), 4.62 (m, 1H; H^{α}), 4.44 (m, 1H; H^{α}), 4.35 (pseudo s, 2H; H_{Cp}), 4.28 (m, 2H; $2\times H^{\alpha}$), 4.20 (s, 5H; H_{Cp}), 3.92 (dd, $^3J = 6.0$ Hz, $^2J = 16.3$ Hz, 1H; H^{α}_{Gly}), 3.62 partly obscured by H_2O peak (m, 1H; H^{α}_{Gly}), 2.90–3.18 and 2.78–2.86 (overlapping m, 8H; H^{β}_{Phe}, H^{β}_{Trp}, H^{δ}_{Arg}), 1.38–1.72 (m, 8H; H^{β}_{Arg}, H^{γ}_{Arg}). ^{13}C NMR, [d_6]DMSO, 25 °C: $\delta = 172.7$ (C═O), 171.8 (C═O), 171.2 (C═O), 170.8 (C═O), 170.1 (C═O), 169.5 (C═O), 156.7 (C^{ζ}_{Arg}), 137.6 and 136.0 (C^{γ}_{Phe}, $C^{\varepsilon 2}_{Trp}$), 129.1, 128.0, 127.2, 126.2, 123.7, 120.8, 118.4, 118.2, 111.3, 109.8 (C^{δ}_{Phe}, C^{ε}_{Phe}, C^{ζ}_{Phe}, C^{γ}_{Trp}, C^{δ}_{Trp}, $C^{\varepsilon 3}_{Trp}$, C^{ζ}_{Trp}, C^{η}_{Trp}), 75.6 ($C_{Cp\,ipso}$), 70.0 (C_{Cp}), 69.4 ($C_{Cp\,unsubstituted}$), 68.2 (C_{Cp}), 68.1 (C_{Cp}), 53.6 (C^{α}), 53.5 (C^{α}), 52.4 (C^{α}), 52.3 (C^{α}), 42.2 (C^{δ}_{Arg}), 37.6 (C^{β}_{Phe}), 29.0, 28.8 and 27.6 (C^{β}_{Arg}, C^{β}_{Trp}), 24.9 and 24.7 (C^{γ}_{Arg}). ESI–MS (pos.) for $C_{45}H_{57}FeN_{13}O_6$ (931.4): *m/z*: 466.9 $[M+2H]^{2+}$.

2.1.9. [Co(Cp)(η-C_5H_4-CO-Gly-Trp-Arg-Arg-Phe-NH_2)]CF_3CO_2 (**9**)

Yellow solid. ^{1}H NMR (300 MHz), [d_6]DMSO, 25 °C: $\delta = 10.85$ (s, 1H; $H^{\varepsilon 1}_{Trp}$), 9.00 (m, 1H; H^{ε}_{Arg}), 8.30 (m, 2H; $2\times H^{N}$), 8.04 (d, $^3J = 8.0$ Hz, 1H; H^{N}), 7.93 (d, $^3J = 8.1$ Hz, 1H; H^{N}), 7.52–7.64 (m, 4H; H^{N}, H^{ε}_{Arg}, CO′NH_2), 6.94–7.34 (overlapping m; H^{δ}_{Phe}, H^{ε}_{Phe}, H^{ζ}_{Phe}, H^{δ}_{Trp}, $H^{\varepsilon 3}_{Trp}$, H^{ζ}_{Trp}, H^{η}_{Trp} and H^{η}_{Arg}), 6.25 (m, 2H; H_{Cp}), 5.92 (pseudo s, 2H; H_{Cp}), 5.85 (s, 5H; H_{Cp}), 4.65 (m, 1H; H^{α}), 4.47 (m, 1H; H^{α}), 4.25 (m, 2H; $2\times H^{\alpha}$), 3.96 (dd, $^3J = 6.2$ Hz, $^2J = 16.8$ Hz, 1H; H^{α}_{Gly}), 3.77 (dd, $^3J = 5.43$ Hz, $^2J = 16.5$ Hz, 1H; H^{α}_{Gly}), 2.8–3.2 (overlapping m, 8H; H^{β}_{Phe}, H^{β}_{Trp}, H^{δ}_{Arg}), 1.35–1.75 (m, 8H; H^{β}_{Arg}, H^{γ}_{Arg}). ^{13}C NMR, [d_6]DMSO, 25 °C: $\delta = 172.7$ (C═O), 171.5 (C═O), 171.1 (C═O), 170.8 (C═O), 168.2 (C═O), 161.9 (C═O), 156.7 (C^{ζ}_{Arg}), 137.5 and 136.0 (C^{γ}_{Phe}, $C^{\varepsilon 2}_{Trp}$), 129.1, 128.0, 127.3, 126.2, 123.8, 120.8, 118.4, 118.2, 111.3, 109.8 (C^{δ}_{Phe}, C^{ε}_{Phe}, C^{ζ}_{Phe}, C^{γ}_{Trp}, C^{δ}_{Trp}, $C^{\varepsilon 3}_{Trp}$, C^{ζ}_{Trp}, C^{η}_{Trp}), 92.5 (C_{Cp} ispo), 86.0 (C_{Cp}), 84.0 ($C_{Cp\,unsubstituted}$), 83.7 (C_{Cp}), 53.5 (C^{α}), 53.4 (C^{α}), 52.4 ($2\times$ C^{α}), 52.3 (C^{α}), 37.7 (C^{β}_{Phe}), 29.2, 29.0 and 27.8 (C^{β}_{Arg}, C^{β}_{Trp}), 25.0 and 24.7 (C^{γ}_{Arg}), C^{δ}_{Arg} obscured by solvent peak, $1\times C{=}O_{amide}$ unresolved. ESI–MS (pos.) for $C_{47}H_{57}CoF_3N_{13}O_8$ (1047.4): *m/z*: 1048.5 $[M+H]^+$, 934.7 $[M-TFA]^+$, 467.9 $[M-TFA+H]^{2+}$, 312.4 $[M-TFA+2H]^{3+}$.

2.1.10. C_5H_{11}-C(O)-Trp-Arg-Arg-Phe-NH_2 (**10**)

White solid. ^{1}H NMR (300 MHz), [d_6]DMSO, 25 °C: $\delta = 10.79$ (s, 1H; $H^{\varepsilon 1}_{Trp}$), 8.10 (d, $^3J = 7.7$ Hz, 1H; H^{N}_{Arg}), 7.99 (m, 2H; H^{N}_{Arg}, H^{N}_{Trp}), 7.90 (d, $^3J = 7.8$ Hz, 1H; H^{N}_{Phe}), 7.57 (m, 2H; CO′NH_2), 7.46 (s, 1H; H^{ε}_{Arg}), 6.9–7.3 3 m (H^{δ}_{Phe}, H^{ε}_{Phe}, H^{ζ}_{Phe}, H^{δ}_{Trp}, $H^{\varepsilon 3}_{Trp}$, H^{ζ}_{Trp}, H^{η}_{Trp} and H^{η}_{Arg}), 4.40–4.55 (m, 2H; H^{α}_{Trp}, H^{α}_{Phe}), 4.2–4.3 (m, 2H; $2\times H^{\alpha}_{Arg}$), 2.8–3.1 (overlapping m, 8H; H^{β}_{Phe}, H^{β}_{Trp}, H^{δ}_{Arg}), 2.02 (t, $^3J = 7.5$ Hz, 2H; H^{α}_{Hex}), 1.3–1.7 (m, 10H; H^{β}_{Arg}, H^{γ}_{Arg}, H^{β}_{Hex}), 1.16 (m, 2H; H^{γ}_{Hex}), 1.06 (m, 2H; H^{δ}_{Hex}), 0.77 (t, $^3J = 6.9$ Hz, 3H; H^{ε}_{Hex}). ^{13}C NMR, [d_6]DMSO, 25 °C: $\delta = 172.7$ (C═O), 172.6 (C═O), 172.1 (C═O), 171.2 (C═O), 170.9 (C═O), 156.7 (C^{ζ}_{Arg}), 137.6 and 136.1 (C^{γ}_{Phe}, $C^{\varepsilon 2}_{Trp}$), 129.2, 128.0, 127.2, 126.3, 123.5, 120.8, 118.4, 118.1 and 111.3 (C^{δ}_{Phe}, C^{ε}_{Phe}, C^{ζ}_{Phe}, C^{δ}_{Trp}, $C^{\varepsilon 3}_{Trp}$, C^{ζ}_{Trp}, C^{η}_{Trp}), 110.2 (C^{γ}_{Trp}), 53.6 (C^{α}), 53.5 (C^{α}), 37.6 (C^{β}_{Phe}), 35.2 (C^{α}_{Hex}), 30.7 (C^{δ}_{Hex}), 29.1 and 29.0 (C^{β}_{Arg}), 28.0 (C^{β}_{Trp}), 24.9 and 24.8 (C^{γ}_{Arg}, C^{β}_{Hex},), 21.8 (C^{γ}_{Hex}), 13.8 (C^{ε}_{Hex}), C^{δ}_{Arg} obscured by solvent peak. ESI–MS (pos.) for $C_{38}H_{56}N_{12}O_5$ (760.5): *m/z*: 761.5 $[M+H]^+$, 381.4 $[M+2H]^{2+}$.

2.1.11. [Fe(Cp)(η-C_5H_4-CO-Trp-Arg-Arg-Phe-NH_2)] (**11**)

Yellow solid. ^{1}H NMR (300 MHz), [d_6]DMSO, 25 °C: $\delta = 10.86$ (s, 1H; $H^{\varepsilon 1}_{Trp}$), 8.19 (d, $^3J = 7.2$ Hz, 1H; H^{N}), 8.06 (d, $^3J = 7.6$ Hz, 1H; H^{N}), 7.92 (d, $^3J = 8.0$ Hz, 1H; H^{N}), 7.73 and 7.57 (m, 2H; CO′NH_2, H^{N}, H^{ε}_{Arg}), 7.49 (br s, 1H; H^{ε}_{Arg}) and 7.00–7.31 (overlapping m; H^{δ}_{Phe}, H^{ε}_{Phe}, H^{ζ}_{Phe}, H^{δ}_{Trp}, $H^{\varepsilon 3}_{Trp}$, H^{ζ}_{Trp}, H^{η}_{Trp} and H^{η}_{Arg}), 4.77 (pseudo s, 1H; H_{Cp}), 4.71 (m, 1H; H^{α}), 4.68 (pseudo s, 1H; H_{Cp}), 4.44 (m, 1H; H^{α}), 4.35 (m, 1H; H^{α}), 4.29 (pseudo s, 2H; H_{Cp}), 4.24 (m, 1H; H^{α}), 3.82 (s, 5H; Cp), 3.0–3.2 and 2.8–2.9 (overlapping m, 8H; H^{β}_{Phe}, H^{β}_{Trp}, H^{δ}_{Arg}), 1.4–1.7 (m, 8H; H^{β}_{Arg}, H^{γ}_{Arg}). ^{13}C NMR, [d_6]DMSO, 25 °C: $\delta = 172.7$ (C═O), 172.3 (C═O), 171.2 (C═O), 170.8 (C═O), 169.4 (C═O), 156.7 (C^{ζ}_{Arg}), 137.5 and 136.2 (C^{γ}_{Phe}, $C^{\varepsilon 2}_{Trp}$), 129.1, 127.9, 127.1, 126.2, 123.8, 120.9, 118.4, 118.2, 111.4, 110.4 (C^{δ}_{Phe}, C^{ε}_{Phe}, C^{ζ}_{Phe}, C^{γ}_{Trp}, C^{δ}_{Trp}, $C^{\varepsilon 3}_{Trp}$, C^{ζ}_{Trp}, C^{η}_{Trp}), 75.6 ($C_{Cp\,ipso}$), 69.9 (C_{Cp}), 69.1 ($C_{Cp\,unsubstituted}$), 68.4 (C_{Cp}), 67.9 (C_{Cp}), 53.9 (C^{α}), 53.6 (C^{α}), 52.3 (C^{α}), 52.1 (C^{α}), 37.6 (C^{β}_{Phe}), 29.1 and 27.2 (C^{β}_{Arg}, C^{β}_{Trp}), 25.0 and 24.7 (C^{γ}_{Arg}). C^{δ}_{Arg} obscured by solvent peak. ESI–MS (pos.) for $C_{43}H_{54}FeN_{12}O_5$ (874.4): *m/z*: 875.6 $[M+H]^+$, 438.5 $[M+2H]^{2+}$.

2.1.12. [Co(Cp)(η-C_5H_4-CO-Trp-Arg-Arg-Phe-NH_2)]CF_3CO_2 (**12**)

Yellow solid. ^{1}H NMR (300 MHz), [d_6]DMSO, 25 °C: $\delta = 10.92$ (s, 1H; $H^{\varepsilon 1}_{Trp}$), 8.88 (d, $^3J = 8.4$ Hz, 1H; H^{N}_{Phe}),

8.49 (d, $^3J = 7.6$ Hz, 1H; H^N_{Arg}), 8.06 (d, $^3J = 7.6$ Hz, 1H; H^N_{Arg}), 7.92 (d, $^3J = 8.1$ Hz, 1H; H^N_{Phe}), 7.80 (d, $^3J = 7.5$ Hz, 1H; $H^{\delta 1}_{Trp}$), 7.67 (m, 2H; $CO'NH_2$), 7.50 (br s, 1H; H^{ε}_{Arg}), 6.99–7.31 (overlapping m, H^{δ}_{Phe}, H^{ε}_{Phe}, H^{ζ}_{Phe}, H^{ε}_{Trp}, H^{ζ}_{Trp}, H^{η}_{Trp}, H^{ε}_{Arg} and H^{η}_{Arg}), 6.25 (pseudo s, 1H; H_{Cp}), 6.20 (pseudo s, 1H; H_{Cp}), 5.85 (pseudo s, 2H; H_{Cp}), 5.35 (s, 5H; H_{Cp}), 4.92 (m, 1H; H^{α}_{Trp}), 4.44 (m, 1H; H^{α}_{Phe}), 4.35 (m, 1H; H^{α}_{Arg}), 4.23 (m, 1H; H^{α}_{Arg}), 3.0–3.1 and 2.81–2.85 (m, 8H; H^{β}_{Phe}, H^{β}_{Trp}, H^{δ}_{Arg}), 1.4–1.6 (m, 8H; H^{β}_{Arg}, H^{γ}_{Arg}). ^{13}C NMR, [d_6]DMSO, 25 °C: $\delta = 172.8$ (C=O), 171.4 (C=O), 171.2 (C=O), 170.9 (C=O), 161.3 (C=O), 156.8 (C^{ζ}_{Arg}), 137.6 and 136.2 (C^{γ}_{Phe}, $C^{\varepsilon 2}_{Trp}$), 129.2, 128.0, 126.3, 124.0, 121.1, 119.0, 118.4, 111.5 (C^{δ}_{Phe}, C^{ε}_{Phe}, C^{ζ}_{Phe}, $C^{\delta 1}_{Trp}$, $C^{\varepsilon 3}_{Trp}$, C^{ζ}_{Trp}, C^{η}_{Trp}), 127.1 ($C^{\delta 2}_{Trp}$), 110.4 (C^{γ}_{Trp}), 92.6 ($C_{Cp\ ipso}$), 86.0 (C_{Cp}), 85.6 ($C_{Cp\ unsubstituted}$), 84.3 (C_{Cp}), 83.6 (C_{Cp}), 53.9 (C^{α}), 53.6 (C^{α}), 52.4 (C^{α}), 52.4 (C^{α}), 37.7 (C^{β}_{Phe}), 29.2 and 29.1 (C^{β}_{Arg}), 27.7 C^{β}_{Trp}), 25.1 and 24.8 (C^{γ}_{Arg}), C^{δ}_{Arg} obscured by solvent peak. ESI–MS (pos.) for $C_{45}H_{54}CoF_3N_{12}O_7$ (990.4): *m/z*: 877.5 $[M - TFA]^+$, 439.4 $[M - TFA + H]^{2+}$, 293.3 $[M - TFA + 2H]^{3+}$.

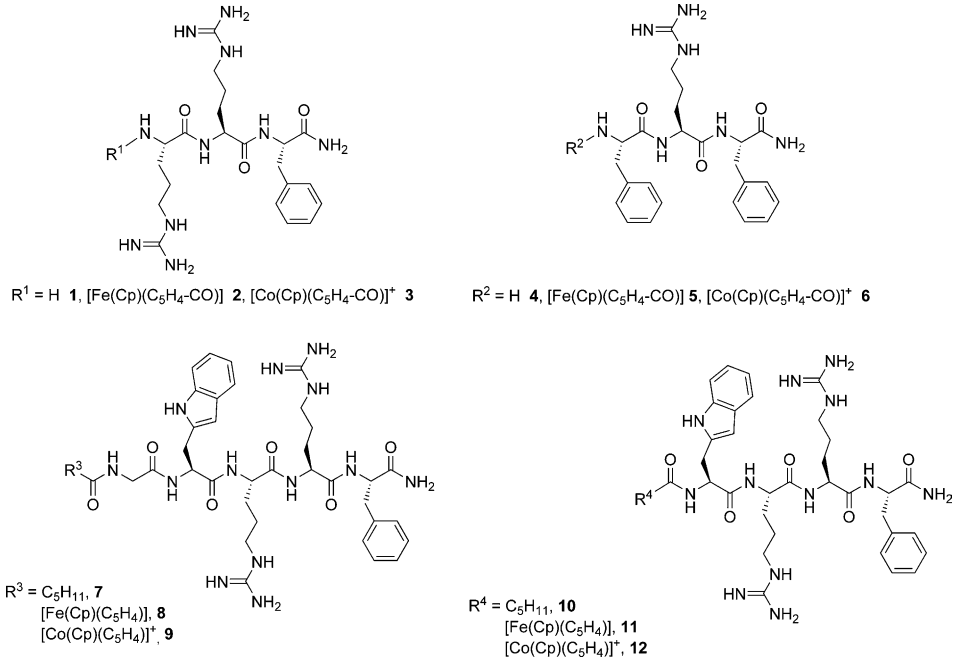
3. Results and discussion

The structures of the compounds prepared are shown in Scheme 1. The cobaltocenium bioconjugates were isolated as trifluoroacetate salts.

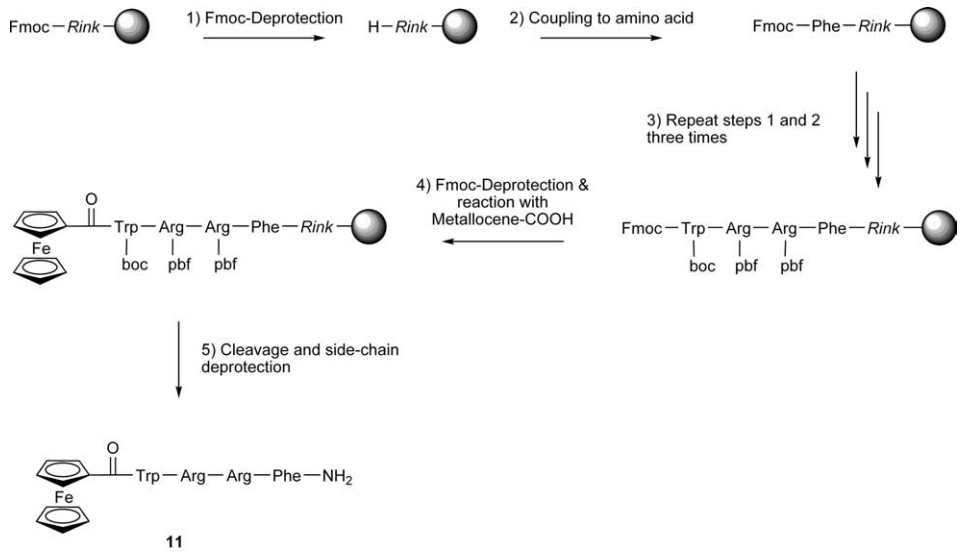
The ferrocene and the cobaltocenium groups were introduced at the N-terminus by reacting ferrocene carboxylic acid (commercially available) and cobaltocenium carboxylic acid hexafluorophosphate (prepared by the literature method [31]), respectively, with the free amino group of the peptide, while the peptide was attached to the solid support [30]. The SPPS of **11** is outlined in Scheme 2, and exemplifies the general procedure used for the preparation of the compounds described. After cleavage from the Rink amide resin the C-terminus was amidated, thus removing the carboxylate negative charge, increasing the overall cationicity of the peptide conjugate. The side-chain protecting groups were also removed during the cleavage step. In the case of compounds **7** and **10**, the N-terminus was capped with a hexanoate group by using *n*-hexanoic acid in the last coupling step, the lipophilicity of the hexanoate chain being similar to ferrocene.

The amino acid sequence ranged from three to five residues long. The side-chains of Phe and Trp and the ferrocenyl and cobaltocenium moieties were considered to be units of bulk, while the free N-terminal amino group, the guanidinium side-chain of Arg, and the cobaltocenium cation were counted as units of charge. Arg rather than Lys was included in the peptide sequences selected as it has been shown to confer better antibacterial activity [32]. The compounds were purified by RP-HPLC and characterized by ESI–MS (positive ion detection) and NMR.

Peptides **1**–**3** differ from the corresponding peptides **4**–**6** by the N-terminal amino acid, which is a polar Arg in the former set and a hydrophobic Phe in the latter set. Not surprisingly, peptides **1**–**3** have shorter retention times than the corresponding peptides **4**–**6** (Table 1). The retention times of the hexanoate-capped peptides (**7** and **10**) are very similar to that of the



Scheme 1.



Scheme 2. SPPS of **11**.

Table 1
Summary of HPLC retention times, R_t, and ESI–MS data for **1**–**12**

	R_t[a] (min)	Exact $mass_{calculated}$	$[M + H]^+$	$[M + 2H]^{2+}$
1	9.306 (1.661[b])	476.3	477.5	239.5
2	14.577 (2.556[b])	688.3	689.5	345.4
3	10.361	805.4	346.4[c]	231.4[d]
4	12.733	467.3	468.4	235.0
5	19.197 (3.305[b])	679.3	680.4	
6	14.039	795.3	341.9[c]	
7	16.143	817.5	818.6	410.0
8	16.388	831.4	–	466.9
9	12.638	1047.4	467.9[c]	312.4[d]
10	17.021	760.5	761.5	381.4
11	17.571	874.4	875.6	438.5
12	12.949	990.4	439.4[c]	293.3[d]

[a] Varian Microsorb C18 column (60 Å/8 μm, 250 × 4.60 mm), linear solvent gradient from 5% MeCN/95% H_2O/0.1% TFA to 95% MeCN/5% H_2O/0.1% TFA over 30 min, 1 mL min^{-1} flow rate.
[b] Merck Chromolith™ Performance 18e column (100 × 4.60 mm), linear solvent gradient from 100% H_2O/0.1% TFA to 100% MeCN/0.1% TFA over 5 min, 4 mL min^{-1} flow rate.
[c] $[M - CF_3COO + H]^{2+}$.
[d] $[M - CF_3COO + 2H]^{3+}$.

ferrocenoyl derivatives (**8** and **11**, respectively), indicating that the polarity of the compounds are similar. The cobaltocenium analogues are, as expected from the charged group, more polar and thus have lower retention times. Compounds **7**–**9** differ from their respective counterparts **10**–**12** by the presence of one Gly residue and the pentamers are only slightly more polar than their respective tetramer compounds. Overall, the retention times increase in the order: free N-terminal amino peptide < cobaltocenium derivative < hexanoate-capped peptide ≈ ferrocenoyl derivative.

The peptides could readily be identified by their $[M + H]^+$ and/or $[M + 2H]^{2+}$ m/z peaks in the ESI mass spectra (Table 1). In addition, the ESI mass spectra of ferrocenoyl-peptides usually displayed a peak at 213 m/z. This can be ascribed to the Fe(Cp)(C_5H_4–CO) moiety which fragments from the parent peptide bioconjugate [30,33]. This fragment is, however, not observed in the ESI mass spectum of ferrocene carboxylic acid, nor can the analogous Cc(Cp)(C_5H_4–CO) fragment be detected. The lower stability of the ferrocenoyl-peptides with respect to the cobaltocenium-analogues is also manifested in the synthetic conditions used when the bioconjugates are cleaved from the resin. Decomposition, that is loss of a ferrocenoyl moiety, occurs when a TFA/H_2O/TIS cleavage mixture is used. However, this problem can be circumvented by the use of phenol rather than water. The ferrocenoyl-peptides also undergo decomposition upon standing in [d_6]DMSO for prolonged periods.

The ^1H NMR resonances of the metallocene groups gave the typical pattern of intensity of 1:1:2:5 (Fig. 1).

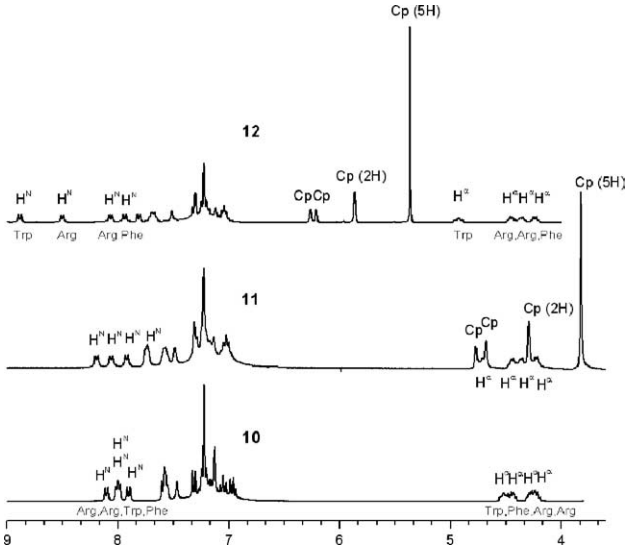


Fig. 1. ^{1}H NMR of **10**–**12** (H^{N}, H^{α} and Cp region).

For cobaltocenium, these resonances appeared in a region (5.3–6.5 ppm) clear of other resonances, while the ferrocene cyclopentadienyl chemical shifts (3.9–4.9 ppm) overlap that of the amino acid alpha protons. Signals due to the alpha protons were typically found in the region of 4.2–4.9 ppm, except for Gly ($\delta \approx 3.8$ and 3.6 ppm). Two-dimensional H,H-COSY experiments elucidated the coupling of the α protons to the amide protons (δ 7.1–9.0 ppm) and to the side-chain protons. The amide protons were generally resolved as doublets with $^{3}J \approx 8$ Hz. The side-chain NH-indolyl proton of Trp was detected downfield at 10.4 ppm. All other signals for the amino acid side-chains were readily assigned from two-dimensional spectra or by comparison to the literature values.

The ^{13}C NMR spectra displayed the carbonyl carbon resonances in the region of 169–174 ppm, but values as low as 161 ppm were found for the N-terminal carbonyl group of the cobaltocenium peptides (**3**, **6**, **9** and **12**). Ferrocenoyl Cp resonances were very similar across the series: δ_{ave} 75.6 (Cp_{ipso}), 70.0, 69.3 ($Cp_{unsubstituted}$), 68.4 and 68.0 ppm. Cobaltocenium bioconjugates gave analogous patterns of Cp resonances, but these were shifted downfield: δ_{ave} 92.5 (Cp_{ipso}), 86.0, 85.4 ($Cp_{unsubstituted}$), 84.2 and 83.7 ppm. Alpha carbon resonances were detected at 52–56 ppm.

The peptides and peptide bioconjugates were on the whole not very active against the bacteria tested (Table 2). Efficient antibacterial compounds have MIC values in the range of 0.1–10 $\mu g\,mL^{-1}$ [16]. The best results reported here have MICs of 50 $\mu g\,mL^{-1}$ (**10** and **11**); all other values were equal to or greater than 100 $\mu g\,mL^{-1}$. The R-Arg-Arg-Phe-NH_2 compounds (**1**–**3**) and the R-Phe-Arg-Phe-NH_2 derivatives (**5**–**6**) do not show any

Table 2
MIC results

Compound[a,b,c]		M_w	Units of charge	Units of bulk	MIC (μmol L^{-1})		
					E. coli	*P. aeruginosa*	*S. aureus*
1	H-RRF-NH_2	476.58	3	1	>210	>210	>210
2	FcC(O)-RRF-NH_2	688.60	2	2	>145	>145	>145
3	Cc^{+}C(O)-RRF-NH_2	804.71	3	2	>124	>124	>124
4	H-FRF-NH_2	467.56	2	2	>214	>214	>214
5	FcC(O)-FRF-NH_2	679.59	1	3	>147	>147	>147
6	Cc^{+}C(O)-FRF-NH_2	795.70	2	3	>126	>126	>126
7	*n*-C_5H_{11}C(O)-GWRRF-NH_2	817.98	2	2	>122	122	122
8	FcC(O)-GWRRF-NH_2	931.86	2	3	107	107	107
9	Cc^{+}C(O)-GWRRF-NH_2	1047.97	3	3	>95	>95	>95
10	*n*-C_5H_{11}C(O)-WRRF-NH_2	760.93	2	2	131	66	131
11	FcC(O)-WRRF-NH_2	874.81	2	3	57	114	57
12	Cc^{+}C(O)-WRRF-NH_2	990.92	3	3	>101	>101	>101

[a] Fc = Fe(Cp)(C_5H_4–).
[b] Cc^{+} = Co(Cp)(C_5H_4–)$^{+}$, counterion is ($CF_3CO_2^-$).
[c] F, phenylalanine, G, glycine, R, arginine, W, tryptophan.

antibacterial activity at the maximal concentration tested. These two groups differ by having a Phe (bulk unit) interchanged with an Arg (charge unit). The extra bulk conferred by the metallocene groups, as well as the charge provided by the cobaltocenium seems to improve the antibacterial properities with respect to the unmetallated peptides. This trend is observed for the R′-Gly-Trp-Arg-Arg-Phe-NH_2 (**7**–**9**) sequences in the order of $R' = n\text{-}C_5H_{11}CO$, $Fe(Cp)(C_5H_4\text{-}CO)$, $Co(Cp)(C_5H_4\text{-}CO)^+$, and overall the longer sequences (**7**–**12**) were somewhat better than the shorter peptides (**1**–**6**). Introduction of the bulky, hydrophobic Trp residue in compounds **7** to **12** results in an overall improvement in activity. With the exception of two MIC results, the effect of the additional Gly residue in **7**–**9** as opposed to **10**–**12** does not produce a significant change in antibacterial activity between the two sets. On the whole, the compounds are completely unselective for the three bacterium species under the test conditions, except for **10** which gave the best MIC against *P. aeruginosa*. In contrast, the ferrocenoyl analogue, **11**, is better for *E. coli* and *S. aureus* than for *P. aeruginosa*.

The MIC results reported here cannot readily be correlated to the number of bulk and charge units present in the peptide or peptide bioconjugate.

4. Conclusion

We have described the successful synthesis of novel metallocene-peptide bioconjugates by SPPS. Although the overall activity of most of the compounds, against selected Gram positive and Gram negative bacteria, is not impressive, their antibacterial potency is certainly enhanced by the presence of the metallocene group. By modification of the primary amino acid sequence it should be possible to further improve the antibacterial activity. Work along these lines is in progress in our laboratories.

5. Abbreviations

Peptides are consistently written from N- to C-terminus in standard peptide nomenclature using standard three-letter codes for amino acids: Aaa, L-amino acid, ATCC, American Type Culture Collection, AMP, antimicrobial peptides, Boc, *tert*-butoxycarbonyl, DIPEA, *N*,*N*-diisopropylethylamine, Fmoc, fluorenylmethoxycarbonyl, Hex, $C^{\varepsilon}H_3\text{-}C^{\delta}H_2\text{-}C^{\gamma}H_2\text{-}C^{\beta}H_2\text{-}C^{\alpha}H_2\text{–}C(O)\text{–}$, HOBt, *N*-hydroxybenzotriazole, NLS, nuclear localization signal (H-Pro-Lys-Lys-Lys-Arg-Lys-Val-OH), Pbf, 2,2,4,6,7-pentamethyldihydrobenzofurane-5-sulfonyl, TBTU, 2-(1H-benzotriazol-1-yl)1,1,3,3-tetramethyl uronium, TFA, trifluoroacetic acid, TIS, triisopropylsilane.

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Appendix A. Supplementary data

(1) HPLC traces at 254 nm (2) ESI mass spectra and (3) ^1H NMR spectra of compounds **1**–**12**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2005.07.007.

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