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Metal-directed assembly of cyclometallopeptides

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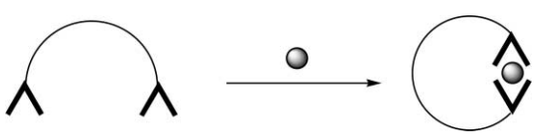
An undecapeptide predisposed to cyclisation as a result of the inclusion of a proline residue has been functionalised with tpy metal-binding domains at both the N and C termini, and shown to form a cyclometallopeptide upon reaction with iron(II) salts.

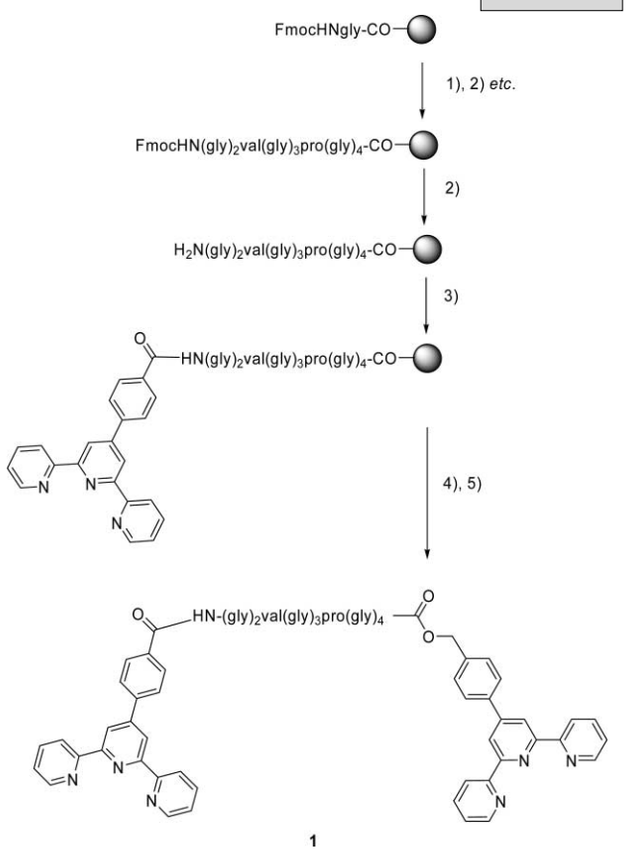
Metal-directed self-assembly is a powerful methodology in supramolecular chemistry, allowing the synthesis of a whole range of topologically and topographically novel species.[1–4] We are currently investigating the extension of the metal-directed self-assembly approach to the construction of unnatural bioaggregates.[5–7] Although metal-functionalised amino acids, peptides and proteins have long been studied, it is only relatively recently that *chemical* studies of *de novo* systems have become routinely viable. The modification of *de novo* peptide sequences with synthetic metal-binding domains offers exciting new possibilities in supramolecular design. Conventional modification of peptides and proteins involves covalent attachment of a metal-binding domain to an appropriate residue. We have become particularly interested in cyclic peptides, in which the conformational rigidity enhances specificity in molecular recognition processes, both as host and as guest species. This has lead us to design oligopeptides bearing metal-binding domains at both the N and the C termini. Although peptides functionalised at one terminus have been used for the assembly of protein bundles and other motifs,[8,9] there are very few reports of N,C-bis-functionalised systems.[10–12] In this Communication, we describe the synthesis of an N,C-bis-functionalised undecapeptide and its subsequent cyclisation upon treatment with iron(II).

We considered that a peptide functionalised with metal-binding domains at the N and C termini might be cyclised upon reaction with a metal ion commensurate with the donor set (Scheme 1). On the basis of modelling studies, we selected the undecapeptide $H_2N(Gly)_2Val(Gly)_3Pro(Gly)_4CO_2H$ as the spacer; the glycine (Gly) residues were chosen to provide maximum flexibility, the proline (Pro) to induce a β-turn and the valine (Val) to provide a convenient NMR spectroscopic spectator. For maximum complex stability with six-coordinate metal centres, we decided to attach terpyridine (tpy) metal-binding domains to the N and C termini and selected the molecule **1** as our primary target.



Scheme 1 General scheme for the cyclisation of a peptide functionalised with metal-binding domains at both the N and C termini by coordination to a suitable metal centre.

The synthetic strategy was to prepare the peptide on resin, N-functionalise the resin-bound species with an appropriate tpy derivative and then cleave it from the resin and further functionalise the C terminus with a second tpy metal-binding domain. The use of solid-phase methods for the preparation of unnatural peptides containing bpy domains within the backbone has recently been reported.[13]



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Scheme 2 Synthesis of the N,C-bis-functionalised undecapeptide **1**. *Reagents and conditions*: (1) Fmoc amino acid, DMF, 1 equiv. TBTU, 1 equiv. Et^iPr_2N, 1 h, r.t.; (2) 20% piperidine in DMF, r.t., then next residue *via* step 1, 67% overall; (3) **2**, DMF, 1 equiv. TBTU, 1 equiv. Et^iPr_2N, 5 h, 91%; (4) TFA, CH_2Cl_2; (5) **4**, K_2CO_3, DMF, 40 °C.

Key synthetic details are presented in Scheme 2. The undecapeptide was prepared linearly using standard solid-phase methods† on Sasrin resin,[14] the C-terminus glycine was attached to the resin and, subsequently, the peptide synthesised by successive addition and deprotection of Fmoc-protected amino acids to give $H_2N(Gly)_2Val(Gly)_3Pro(Gly)_4CO$–Sasrin in 67% overall yield. In the final solid-phase reaction, 4′-(4-carboxyphenyl)-2,2′:6′,2″-terpyridine (**2**) was reacted with $H_2N(Gly)_2Val(Gly)_3Pro(Gly)_4CO$–Sasrin to give the N-terminus tpy-functionalised solid-phase species $(tpy)C_6H_4CONH(Gly)_2Val(Gly)_3Pro(Gly)_4CO$–Sasrin in 91% yield. Coupling of this acid to the resin-bound peptide proceeded smoothly under the standard conditions, but required a reaction time of 5 h at 40 °C for completion. Cleavage of the *N*-tpy functionalised undecapeptide from the resin with trifluoroacetic acid in CH_2Cl_2 yielded free peptide **3** in 16% overall yield from Fmoc-glycine.‡ Finally, the C-terminus tpy metal-binding domain was introduced as a benzyl ester by the reaction of **3** with 4′-(4-bromomethylphenyl)-2,2′:6′,2″-terpyridine (**4**) in basic conditions; the final N,C-bis-functionalised compound (**1**) was obtained as a poorly soluble foam (Scheme 2).§

We[15] and others[16,17] have previously shown that bis-tpy ligands with appropriate length spacers yield metallocycles

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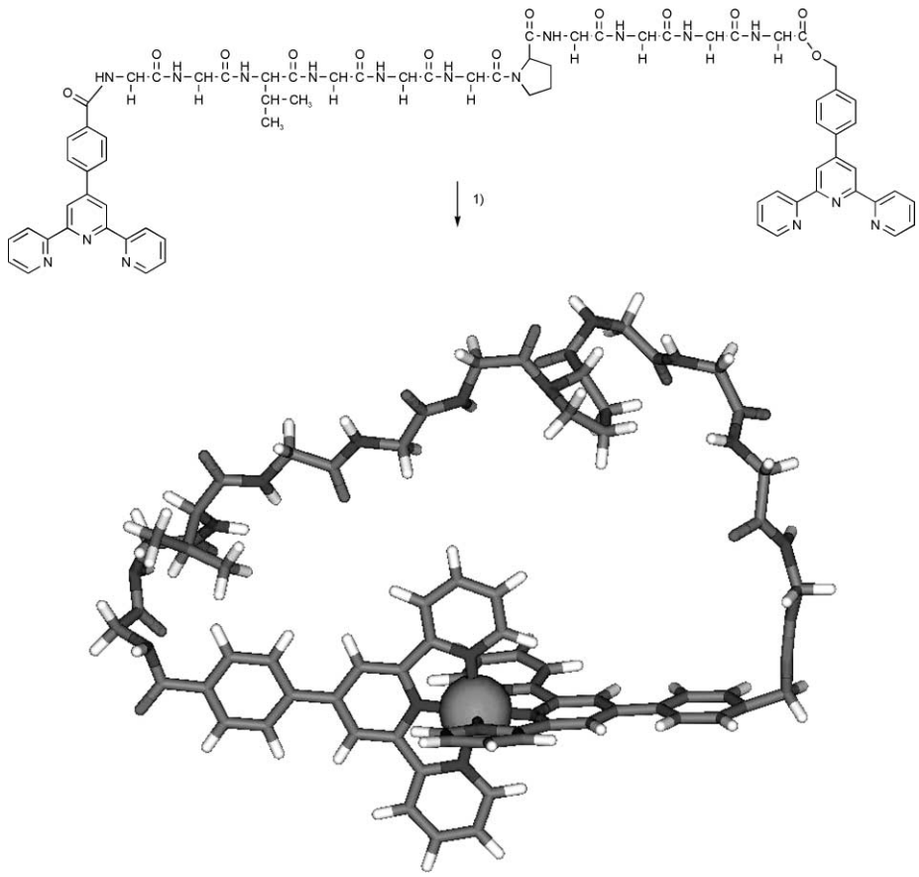

Scheme 3 Synthesis of the cyclometallaundecapeptide $[Fe(\mathbf{1})_2]^{2+}$. *Reagents and conditions*: (1) $[Fe(H_2O)_6][BF_4]_2$, EtOH–CH_2Cl_2, high dilution.

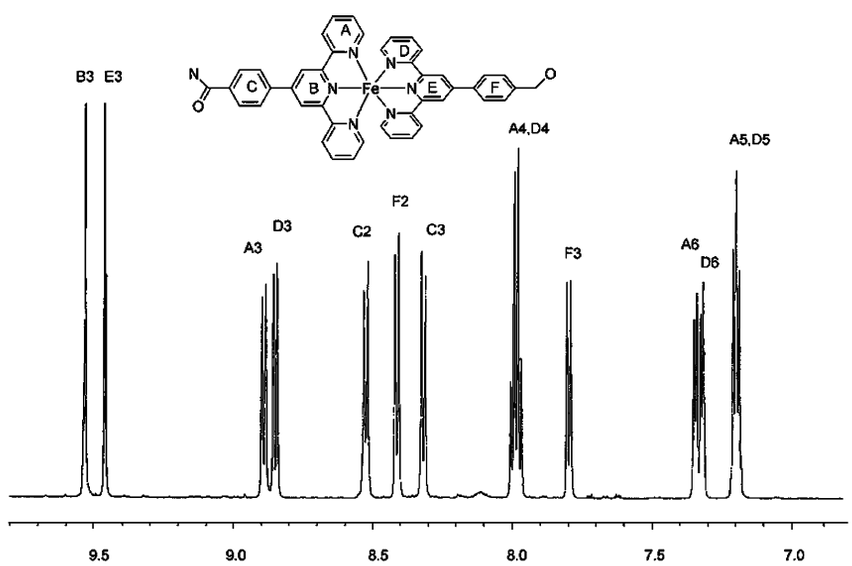

Fig. 1 The aromatic region of the 600 MHz ^{1}H NMR spectrum of a CD_3OD solution of $[Fe(\mathbf{1})_2]^{2+}$, showing the assignments of the two non-equivalent tpy domains obtained from COSY and TOCSY spectra.

upon treatment with labile first row transition metal ions, and modelling indicated that the spacer length in the undecapeptide would favour such a process. To further optimise the formation of the cyclic species, we performed the reactions at high dilution. Dropwise addition of **1** in EtOH–CH_2Cl_2 and $[Fe(H_2O)_6][BF_4]_2$ in EtOH to EtOH to give final concentrations of ~30 μM resulted in the formation of a violet solution, from which a purple solid was obtained upon evaporation to dryness. Purification was by HPLC, followed by silica chromatography, with a final HPLC purification step to give the analytically pure [1+1] complex $[Fe(\mathbf{1})](CF_3CO_2)_2$ as a violet solid (Scheme 3).¶ The complex was stable on the timescale of the purification and spectroscopic characterisation, but, on prolonged storage (~1 week) in solution, new species were observed by TLC analysis.

Both HPLC and ^{1}H NMR spectroscopic analyses of freshly prepared solutions of the violet product indicate the presence of a single solution species. The ^{1}H NMR spectrum has been fully assigned by COSY and TOCSY methods, and a number of pertinent features are observed. Signals for two non-equivalent tpy systems are observed (Fig. 1) and the chemical shifts are typical for those in $[Fe(tpy)_2]^{2+}$ complexes, indicating that both the N and C termini are coordinated. The assignment of B3 and E3 follows from ROESY contacts to A3 and D3,

respectively. Similarly, C2 and F2 are assigned by ROESY contacts to B3 and E3, respectively. The α-CH of the proline gives rise to two signals in a 9 : 1 ratio, corresponding to *trans* and *cis* conformations. This is the ratio that is commonly found in linear peptides, indicating that there are no excessive additional strains within the complex. ROESY experiments indicate a series of through-space interactions of glycine α-CH_2 and valine methyl groups with the phenylene protons of the N-terminus ligand and the protons in the 5 and 6 positions on the terminal tpy rings. All of these data support the existence of a single solution species in which both tpy domains are coordinated to iron. The overwhelming evidence is for a [1+1] complex, as two isomers (head-to-head and head-to-tail) are expected to be formed for a [2+2] stoichiometry.

We had difficulties in obtaining ES-MS data for these complexes, but, eventually, collected satisfactory MALDI-TOF and FAB mass spectrometric data. As mentioned above, the complex was purified by multiple HPLC steps and isolated as a trifluoroacetate salt. The FAB mass spectrum shows characteristic and intense peaks assigned to [Fe(**1**) + K + OH + CF_3CO_2]$^+$, [Fe(**1**) + Na + OH + CF_3CO_2]$^+$ and [Fe(**1**) + OH]$^+$, with the latter being the base peak in the spectrum.

In conclusion, we have demonstrated that bis-functionalised peptides bearing metal-binding domains at each end may be prepared and that treatment with an appropriate metal ion results in the formation of a cyclometallopeptide. We are currently extending these studies to the preparation of cyclometallopeptides containing the octeatride sequence.

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Notes and references

† Reactions were performed manually. Fmoc (9-fluorenylmethoxycarbonyl) amino acid (0.25–2.25 mmol) in DMF (1–3 cm^3) was treated with 1 equivalent each of 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and *N*-ethyl-*N*,*N*-diisopropylamine, and the solution added to the resin. It was allowed to react for 1 h, after which time, the resin was washed with DMF and isopropanol. Completion of each coupling step was monitored by addition of 2,4,6-trinitrobenzenesulfonic acid and *N*-ethyl-*N*,*N*-diisopropylamine in DMF, followed by examination of the resin under a microscope. Free amino groups react and give a red colouration; after coupling, the resin has to be completely colourless before carrying the synthesis through to the next step. If any free amino groups were detected, the coupling step was repeated. The new Fmoc-protected N-terminal residue was deprotected with 20% piperidine in DMF, followed by washing with DMF, isopropanol, DMF and *tert*-butyl methyl ether. Yields were calculated from the weight of the dried resin.

‡ **3**: MS (MALDI-ToF): *m*/*z* 1063 (**3**), 1089 (**3** + Na), 1121 (**3** + Fe); ^{1}H NMR (250 MHz, CD_3OD): δ 8.88–8.79 (m, 6H, $H^{T3',T3,T6}$), 8.37 (t, 2H, H^{T4}), 8.01 (m, 4H, $H^{phenylene}$), 7.81 (t, 2H, H^{T5}), 4.30–3.40 (m, 22H, α-CH, δ-CH_2^{Pro}), 2.03–1.95 (m, 5H, β- and γ-CH_2^{Pro}, β-CH^{Val}), 0.97 (m, 6H, CH^{Val}).

§ **1** (46.2 mg, 23.6 μmol) in EtOH (125 cm^3) and CH_2Cl_2 (125 cm^3), and $[Fe(H_2O)_6][BF_4]_2$ (11.9 mg, 35.3 μmol) in EtOH (250 cm^3) were simultaneously added dropwise to EtOH (750 cm^3) over 10 h at room temperature. The solution turned violet. After stirring for another 11 h at room temperature, the solvent was removed *in vacuo*. The crude product was purified by HPLC chromatography (silica, MeCN–saturated aqueous KNO_3–H_2O 14 : 2 : 1, then 14 : 2 : 7) to give a clean (by TLC) product that was further purified by HPLC to give [Fe(**1**)]$(CF_3CO_2)_2$ as a violet solid (3.7 mg, 2.22 μmol, 9.4% for the last 2 steps and purification). MS (FAB): *m*/*z* 1609 ([Fe(**1**) + OH + CF_3COO + K]$^+$), 1592 ([Fe(**1**) + OH + CF_3COO + Na]$^+$), 1457 ([Fe(**1**) + OH]$^+$), 1117. ^{1}H NMR (600 MHz, CD_3OD): δ 9.53 (2H, s, H^{B3}), 9.45 (2H, s, H^{E3}), 8.89 (d, 2H, J = 8.0 Hz, H^{A3}), 8.85 (d, 2H, J = 7.9 Hz, H^{D3}), 8.52 (d, 2H, J = 8.4 Hz, H^{C2}), 8.41 (d, 2H, J = 8.2 Hz, H^{F2}), 8.31 (d, 2H, J = 8.3 Hz, H^{C3}), 7.98 (m, 4H, J = 5.9 Hz, $H^{A4,D4}$), 7.79 (d, 2H, J = 8.3 Hz, H^{F3}), 7.32 (m, 4H, $H^{A6,D6}$), 7.19 (t, 4H, J = 6.5 Hz, $H^{A5,D5}$), 4.55 [m, 0.1H, α-$CH^{Pro(cis)}$], 4.34 [dd, 0.9H, J = 8.3, 5.7 Hz, α-$CH^{Pro(trans)}$], 4.24–4.15 (m, 3H, α-$CH^{Val,Gly}$), 4.10–3.84 (m, 16H, α-CH_2^{Gly}), 3.68–3.60 (m, 2H, δ-CH_2^{Pro}), 2.23 (m, 2H, β-$CH^{Pro,Val}$), 2.09 (m, 1H, γ-CH^{Pro}), 1.95 (m, 2H, β- and γ-CH_2^{Pro}), 1.04 (d, 3H, J = 6.8 Hz, CH_3^{Val}), 1.02 (d, 3H, J = 6.8 Hz, CH_3^{Val}). A ROESY experiment showed the following through-space signals: δ 4.20 (α-$CH^{Val\ or\ Gly}$) to 8.31 (H^{C3}); δ 3.90 (α-CH_2^{Gly}) to 8.52 (H^{C2}), 8.31(H^{C3}), 7.32 (very weak, $H^{A6,D6}$) and 7.19 (very weak, $H^{A5,D5}$); δ 1.03 (CH_3^{Val}) to 8.31 (weak, H^{C3}).

¶ A mixture of **4** (11.6 mg, 28.8 μmol), **3** (40.4 mg, 28.8 μmol) and K_2CO_3 (15.9 mg, 115 μmol) in DMF (10 cm^3) was stirred at 90 °C for 3.5 h, after which time, the solvent was removed *in vacuo*. The residue was suspended in a mixture of MeOH (25 cm^3) and CH_2Cl_2 (25 cm^3) and filtered. Acetic acid (0.013 cm^3) was added and the solvent then removed *in vacuo* to give **1** as a white foam, which was too insoluble for further HPLC purification (56.4 mg). ^{1}H NMR (300 MHz, CD_3OD): 8.63 (m, 12H, $H^{T3',T3,T6}$), 8.10–7.85 (m, 12H, $H^{T4,T5,phenylene}$), 7.43 (t, 2H, H^{T5}), 5.38 (s, 2H, H^{benzyl}), 4.10–3.50 (m, 22H, α-CH, δ-CH_2^{Pro}), 2.20–1.85 (m, 5H, β- and γ-CH_2^{Pro}, β-CH^{Val}), 0.97 (m, 6H, CH_3^{Val}).

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