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Cyclic metallopeptides, cyclo[Gly-L-Cys(terpyPt^{II})]$_n$Cl$_n$†

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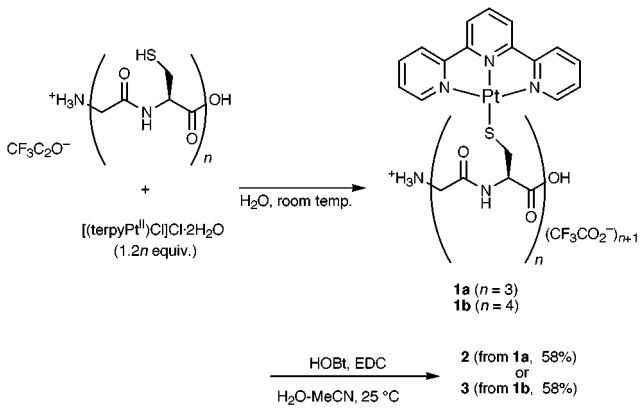
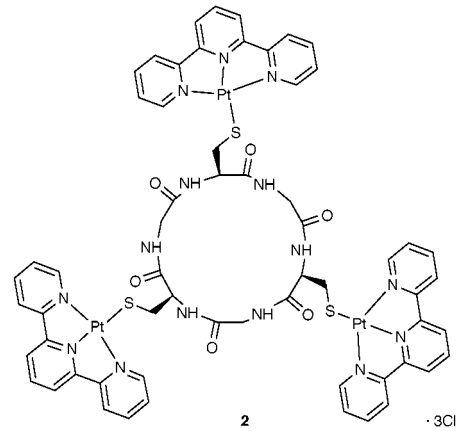
An efficient strategy for the liquid-phase synthesis of cyclic metallopeptides, cyclo[Gly-L-Cys(terpyPt^{II})]$_n$Cl$_n$ (n = 3, 4), has been developed, which could provide a powerful tool for arraying metal centers on cyclopeptide frameworks.

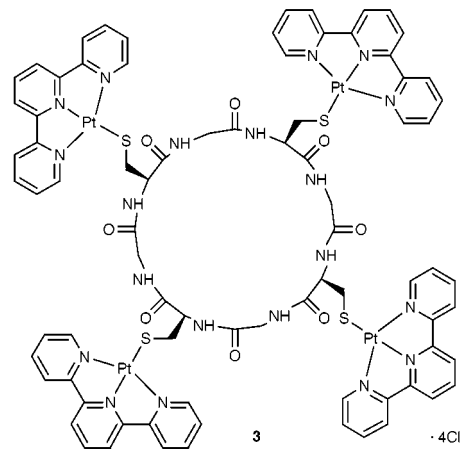
Cyclopeptides are a class of compounds with biological activity[1] and great potential as functional molecules.[2] The development of strategies for functionalizing cyclopeptides is a fundamental challenge in the emerging field of *de novo* protein design. One of the most exciting recent advances in this field is the development of a wide range of structural and functional capabilities for self-assembling nanotubes made from cyclic D,L-α-peptides and from cyclic β-peptides.[3] As another advantageous tool for the functionalization of peptides, metal binding sites have been engineered into peptides and proteins using the side chains of naturally occurring amino acids or unnatural metal binding sites incorporated at the residues, for model studies of protein folding and enzymes, biosensors and molecular architectures.[4] Our approach is based on the use of amino acids containing a metal coordinating site (*e.g.* the thiol group of Cys)§ as the components of cyclopeptides for constructing cyclopeptide–metal complex conjugates. Herein we describe an efficient strategy for the liquid-phase synthesis of cyclopeptides having a repeating Gly-L-Cys(terpyPt^{II}) sequence, cyclo[Gly-L-Cys(terpyPt^{II})]$_n$Cl$_n$, **2** (n = 3) and **3** (n = 4).§ Interest in the incorporation of a terpyPt^{II} complex onto L-Cys was initially aroused by its binding to DNA and antitumor properties.[5] In these peptides, positively charged Pt^{II} complexes are designed to align on the periphery of the macrocyclic peptide scaffold. We have found that these cyclic metallopeptides provide a novel structural motif in the receptor site for anionic guest molecules.

The synthetic route for the cyclopeptides is shown in Scheme 1. The linear peptides, H$_2$(Gly-L-Cys)$_n$OH·(CF$_3$CO$_2$) (n = 3 and 4), were prepared on a peptide synthesizer using standard Fmoc chemistry. Treatment of these peptides with 1.2n equiv. of [(terpyPt^{II})Cl]Cl·2H$_2$O in H$_2$O at room temperature afforded H$_2$[Gly-L-Cys(terpyPt^{II})]$_n$OH·(CF$_3$CO$_2$)$_{n+1}$ **1a** (n = 3) and **1b** (n = 4), in 88 and 97% yields, respectively, after purification by RP-HPLC [eluent: H$_2$O–MeCN (7:3) containing 0.1% TFA].§ The resulting linear peptides, **1a** and **1b**, were cyclized at a concentration of *ca.* 0.50 mM in H$_2$O–MeCN (7:3) at 25 °C for 48 and 72 h, respectively, in the presence of excess HOBt and EDC.§ Cyclo[Gly-L-Cys(terpyPt^{II})]$_n$Cl$_n$, **2** (n = 3) and **3** (n = 4) were obtained as red precipitates in 58% yield in both cases.[6,7] The products were highly pure even in the crude form. Whereas the ^{1}H NMR spectral patterns for the linear **1a** and **1b** were highly complicated, those for the cyclopeptides **2** and **3** were much more symmetrical and only one set of signals corresponding to a Gly-L-Cys(terpyPt^{II}) moiety was observed in each case. The electrostatic repulsion that would occur intramolecularly between the positively charged Pt^{II} complexes may complicate the solvent-dependent prefolding of the linear starting peptides, and at the same time may facilitate the intramolecular cyclization. Although the ESI MS data showed *m/z* 587 for both **2** and **3**, the rings of the cyclopeptides were clearly shown to be 18- and 24-membered, respectively, from the numbers of split lines observed in the high-resolution



Scheme 1





† Experimental and spectral data for **1a**,**b**, **2** and **3** are available from the RSC web site, see http://www.rsc.org/suppdata/cc/1999/2475/

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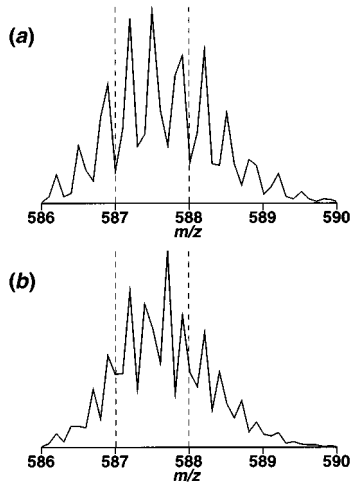
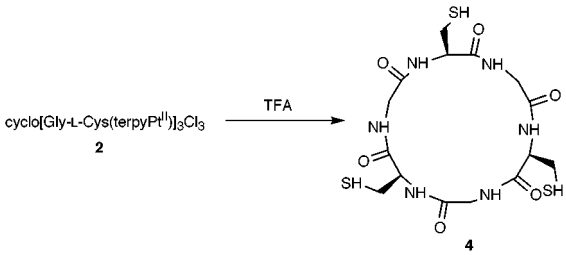


Fig. 1 High resolution ESI mass spectra for (*a*) **2** and (*b*) **3**.



Scheme 2

spectra (Fig. 1). To the best of our knowledge, this is the first example providing an efficient strategy for the liquid-phase synthesis of cyclic metallopeptides from linear peptides bearing functional metal complexes.

The terpyPt^{II} complex moieties of **2** were readily removed by treatment with TFA to afford the corresponding cyclopeptide, cyclo[Gly-L-Cys]$_3$ **4**, as indicated by its ESI MS data (*m/z* 479 [M − H$^+$]$^-$) (Scheme 2). Consequently, terpyrPt^{II} complexes can be regarded as both protecting and promoting groups for peptide cyclization.

These cyclic metallopeptides were found to act as positively charged anion receptors.[8] In these receptors, coulombic interactions were expected primarily to contribute to the attractive force for anion binding. We have examined the binding of cyclohexapeptide **2** to benzenetricarboxylate anions (1,2,3-, 1,2,4- and 1,3,5-tricarboxylates).[9] It was clearly demonstrated from its ^{1}H NMR studies that the cyclohexapeptide **2** selectively separated benzene-1,3,5-tricarboxylate from an equimolar mixture of the three tricarboxylates to form a 1 : 1 ternary complex in neutral water at room temperature.

We have demonstrated an efficient strategy for the liquid-phase synthesis of cyclopeptides containing a repeating Gly-L-Cys(terpyPt^{II}) sequence. These results raise the intriguing possibility that this strategy will provide a powerful tool for arraying metal centers on cyclopeptide frameworks, which could lead to structural as well as functional control of multinuclear metal complexes.

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Notes and references

§ Abbreviations used: Gly, glycine; Cys, cystein; Fmoc, fluoren-9-ylmethoxycarbonyl; RP-HPLC, reverse-phase high performance liquid chromatography; HOBt, 1-hydroxy-1*H*-benzotriazole monohydrate; EDC, *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide hydrochloride; ESI MS, electrospray ionization mass spectrometry.

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6 These metallopeptides were characterized by ^{1}H NMR, ESI MS and elemental analyses.
7 In the case with n = 2, the intermolecular coupling predominantly occurred to produce the linear octapeptide and then cyclooctapeptide **3** as the main product. The isolation of the desired cyclotetrapeptide as the minor product was not successful.
8 *Comprehensive Supramolecular Chemistry*, ed. J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vogtle, J.-M. Lehn and G. W. Gokel, Elsevier Science, Oxford, 1996, vol. 1.
9 The binding constants could not be determined accurately due to the low solubility of the complex with carboxylate anions in general solvents.

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