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Synthesis and Characterization of a Water-Soluble Metallopeptide That Resembles a Parallel $\beta$-Pleated Sheet

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The rational design of artificial proteins requires knowledge of the folding requirements for different structural motifs. Thus, the development of new model systems that mimic the properties of secondary protein structures has been the focus of much interest. However, with but few notable exceptions, the successful design of water soluble models for $\beta$-pleated sheets and $\beta$-turns has remained elusive due to their tendency to form insoluble aggregates in aqueous solution. Thus, the majority of these compounds are only amenable to study in organic solvents. Substitution-inert metal complexes have recently been used to help form such important protein structures as $\alpha$-helices, multihelical bundles, and proline helices in aqueous solution. Here, we explore the efficacy of applying this approach toward the design of water soluble metallo-peptide mimics.

We report the properties of a new metallopeptide that consists of two divalinyl peptides attached to the carboxylate groups of bipyridine, $L$. The tendency of such a small metallopeptide to suggest that the use of substitution-inert metal complexes has remained elusive due to their tendency to form $\beta$-sheetlike properties provides encouraging evidence for the rational design of artificial $\beta$-proteins.

Solution-phase methods were used to prepare the title compound. One equivalent of the ruthenium polypyridyl complex $[\text{Ru(bpy)}_2L]^2+$, bpy $= 2,2'$-bipyridine, $L = 3,5$-dicarboxy-2,2'$'$-bipyridine. This divalent ruthenium complex is indeed water soluble, and contains a parallel arrangement of interacting, extended peptide strands that is reminiscent of the parallel $\beta$-sheet structure. Circular dichroism results performed on a related compound support this assignment.

Table 1 summarizes the $^1$H NMR data acquired for $\Delta^l$III in aqueous solution. Variable-temperature $^1$D spectroscopy were used to probe the conformational effects caused by coupling two peptide chains to Ru(bpy)$_2$L. All of the amide (N-H) signals for the two singly-substituted compounds, I and II, show large temperature coefficients in $9:1$ H$_2$O:D$_2$O (d$\delta$/dT $\approx$ $-$9 ppb/K), indicating that an unaggregated and unordered conformation exists in the cases where only one peptide chain is coupled to the metal complex. In contrast, the amide signals for $\Delta^l$III show a more complicated behavior in which each amide proton experiences a different degree of solvent-shielding.

### Table 1. Chemical Shifts (278 K), $^3$J$_{\text{H},\text{C}}$ Coupling Constants, and Temperature Coefficients for the Amide Protons of $\Delta^l$III

<table>
<thead>
<tr>
<th>residue</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta_{310}$ (ppm)</td>
<td>9.870</td>
<td>9.086</td>
<td>8.957</td>
<td>8.702</td>
</tr>
<tr>
<td>$^3$J$_{\text{H},\text{C}}$ (Hz)</td>
<td>7.9</td>
<td>7.7</td>
<td>8.2</td>
<td>7.2</td>
</tr>
<tr>
<td>d$\delta$/dT (ppb/K)</td>
<td>$-$4.6</td>
<td>$-$7.5</td>
<td>$-$6.0</td>
<td>$-$9.1</td>
</tr>
</tbody>
</table>

were coupled using disopropylcarbodiimide and 1-hydroxynbenzotriazole in $1:1$ CH$_2$CN/DME. The reaction was monitored by reverse-phase HPLC using UV-vis diode array detection. Three product peaks were observed and separated by repetitive reverse-phase column chromatography using aqueous methanol eluents. The $^1$H NMR spectra show that these compounds correspond to each of the two mono-substituted metallopeptides, having only one divalinyl chain attached to L (I and II), and the desired bis(divalyl) complex (III). Semipreparative reverse-phase HPLC and circular dichroism (CD) spectroscopy were used to separate and identify the two diastereomeric forms of III. $^1$H NMR measurements (400 MHz and 500 MHz) were performed in $9:1$ H$_2$O:D$_2$O using presaturation solvent suppression. The 1-D spectrum of the $\Delta^l$-diastereomer consists of sharp, well-defined signals, indicating that the charged metallopeptide does not aggregate in aqueous solution. Individual residue assignments were made on the basis of 2-D TOCSY spectra which show four amide resonances appearing in the downfield region of the spectrum. NMR spectra could not be obtained for the uncomplexed ligand-peptide conjugate since it was found to be insoluble in aqueous solution at both neutral and acidic pH.

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(6) Nowick, J. S.; Powell, N.
(14) The two diastereomers were separated using a Whatman Magnum 9 semipreparative C$_8$ column ($9 	imes 50$ cm) using a binary elution gradient consisting of 10–90% methanol in 0.1% TFA.

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show the existence of extended peptide strands in this metallopeptide and support the assignment of the β-sheet structure. However, we caution that an unequivocal conformational assignment requires the observation of close inter-strand contacts from the NOESY data. The absence of such data in our spectra suggests that Δ-I III probably experiences some degree of conformational flexibility, as would be expected for such a small, acyclic polypeptide. It is of interest to note that no unambiguous assignment of such cross-chain, interresidue NOE’s has been reported for related β-sheet mimics. Nevertheless, analysis of the temperature coefficients, J N H-Cα, and NOESY data presented here demonstrate that Δ-I III contains a parallel arrangement of interacting, extended peptide strands reminiscent of the β-sheet structure.

Circular dichroism spectroscopy offers a convenient method to determine the conformational properties of polypeptides. Unfortunately, CD analysis could not be performed on Δ-I III as its spectrum is dominated by the chiral ruthenium center. Instead, measurements were performed on the related compound [RuIII(NH3)5L'(Val-Val–H)2]+ (IV), which contains peptide chains coupled to the carboxylate groups of L’ = 3,5-pyridinedicarboxylic acid. Thus, IV contains the essential stereo features of Δ-I III but has the advantage of possessing an achiral metal center. The CD spectrum of this compound consists of two strongly negative maxima at 225 nm (−22 120 mdeg cm2 dmol−1) and 197 nm (−18 850 mdeg cm2 dmol−1), which are consistent with the presence of the β-sheet and random coil conformations, respectively. The latter feature may be due to the presence of disordered endgroups. A zero crossing occurs at 188 nm and a positive band is seen at 267 nm (3430 mdeg cm2 dmol−1), which probably arises from the properties of the pyridine ligand.

In summary, the novel metallopeptide Δ-I III has been shown to display many characteristics of a β-sheet-like sheet including interacting peptide chains that display large vicinal coupling constants, short sequential NH-Cα distances, and solvent-shielded amide protons. This conclusion is supported by CD measurements performed on the achiral analog. The tendency of such small metallopeptides to display β-sheetlike properties provides encouraging evidence to suggest that the use of substitution-insert metal complexes may provide a new route toward the creation artificial β-proteins in aqueous solution.

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Figure 1. Schematic representation of Δ-I III showing the weak (---) and strong (--) NOE interactions observed for the peptide-based protons in 9:1 H2O/D2O (500 MHz, T = 278 K, τm = 500 ms, presaturation solvent suppression).