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Helix Induction by Dirhodium: Access to Biocompatible Metallopeptides with Defined Secondary Structure

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Abstract: The use of carboxylate side chains to induce peptide helicity upon binding to dirhodium centers is examined through experimental and computational approaches. Dirhodium binding efficiently stabilizes α helicity or induces α helicity in otherwise unstructured peptides for peptides that contain carboxylate side chains with *i*, *i*+4 spacing. Helix induction is furthermore possible for sequences with *i*, *i*+3 carboxylate spacing, though in this case the length of the side chains is crucial: ligating to longer glutamate side chains is strongly helix inducing, whereas ligating the shorter aspartate side chains destabilizes the helical structure. Further studies demonstrate that a dirhodium metallopeptide complex persists for hours in cellular media and exhibits low toxicity toward mammalian cells, enabling exploitation of these metallopeptides for biological applications.

Keywords: biocompatibility · rhodium · helical structures · metallopeptides · secondary structures

Introduction

Peptide secondary structure drives molecular functions such as binding to target proteins and aggregation into defined supramolecular materials. As such, there is a long-standing interest in chemistries that control secondary structure in a well-defined manner. The α-helix motif is particularly prevalent and well studied. Helix stabilization is an established method to improve or re-establish the binding affinity of peptides and hence, to improve the biological function.[1] To achieve structure stabilization, a variety of methods has been explored.[2–5] Non-natural amino acids have been explored in covalently-bridging distal amino acids to favor helix formation.[6] Covalent modifications by ring-closing metathesis[7–9] and 1,3-dipolar cycloaddition[10] have enabled the creation of helical structures in short peptide sequences. Wholly non-natural peptide mimics, such as β peptides and aryl-based oligomers, have also been shown to achieve improved helicity.[11–19] Although progress has been made in this area, the requirement for non-natural amino acids represents a considerable drawback.

We have begun a program to overcome the limitations associated with using non-natural amino acids by developing methods to directly influence the structure of canonical polypeptides. Control of peptide secondary structure with organic reagents through selective bioconjugation methodologies remains limited.[20–30] Alternatively, metal–polypeptide interactions are a long-standing area of study that enables the control of structure and function in metallopeptide complexes.[31–44]

We became interested in the use of dirhodium centers as ligands for carboxylate side chains to stabilize helical structure, as we believed this strategy could address a number of limitations of extant methods.[45,46] First, carboxylate side chains are largely unexplored as sites for selective reactivity or ligation.[45,47–52] Well-defined metal binding to natural polypeptides in water typically has been confined to histidine, cysteine, and methionine residues that contain "soft" ligands for monomeric metal centers (see Scheme 1).[43,44,53–59] Whereas selective binding of carboxylate residues in a fully deprotected peptide remains a challenge, dirhodium is well suited for carboxylate ligation, in part because carboxylates coordinate through a κ^2 orientation that bridges the Rh–Rh bond and engenders increased stability against ligand substitution. Nevertheless, we have demonstrated that dirhodium–peptide adducts can undergo controlled cleavage with appropriate external reagents.[60] Dirhodium complexes also catalyze bond-forming processes in aqueous solution,[61–63] and importantly, dirhodium metallopeptides retain catalytic activity towards diazo reagents.[64]

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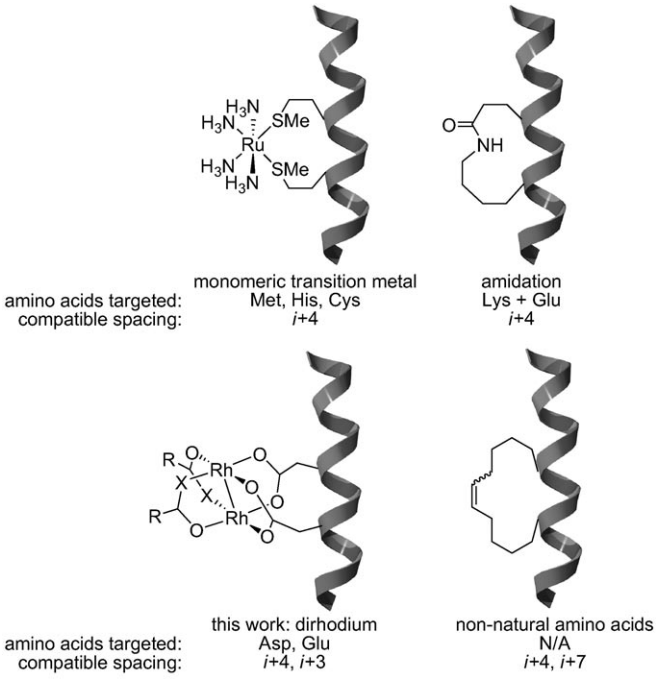


Scheme 1. Strategies to stabilize or induce α-helical secondary structure in peptides.

Also pertinent to these studies, dirhodium complexes exhibit interesting functions in biological contexts.[65–75]

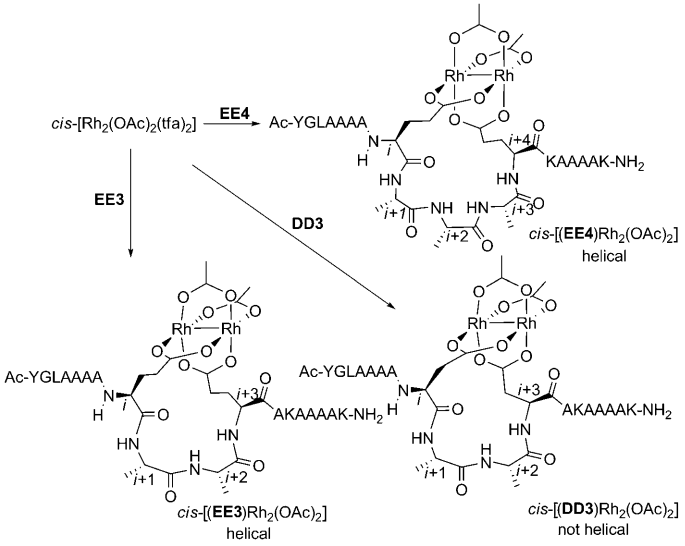
Second, previous studies of metal-mediated helix induction typically examine peptides under controlled conditions. Metallopeptides with non-biological metal centers ligated by natural amino acid side chains that are stable in the presence of diverse biomolecules are rare,[76,77] and the toxicity of metallopeptides is largely unexplored. Third, the extent of helical stabilization in metallopeptides is limited or difficult to predict, and the structural requirements for efficient helix induction are not fully understood. For example, simple hydrocarbon tethers have been successfully used to induce helicity,[9] but tethered peptides do not necessarily show significant increases in helicity—the impact of tether length, tether structure, and peptide sequence on the extent of increased helicity is not fully understood. Computational progress toward a predictive model for helical induction for hydrocarbon tethers has recently been reported.[78]

We report here metallopeptides that feature macrocyclic loops obtained by dirhodium chelation by natural carboxylate side chains. Depending on the identity and spacing of the carboxylate-containing residues within the polypeptide chain, helical secondary structure can be stabilized or induced in previously unstructured peptides. We present examples of helix induction upon binding carboxylate side chains with *i*, *i*+3 spacing—a previously unexplored structural class for helix induction. We further demonstrate the robust, biocompatible nature of a dirhodium metallopeptide by studies that were undertaken in living embryonic kidney cells (HEK-293). These studies highlight the broad potential of this ligation strategy for affecting biological structures and functions.

Results and Discussion

Sequence requirements for helix stabilization: To probe the impact of dirhodium binding on helical structure, we examined peptides, 18 amino acids in length, that have helical propensity due to an alanine-rich sequence (Table 1).[79] A complete turn of an α helix requires between three and four amino acids, so carboxylate side chains (Asp, Glu) were placed in *i*, *i*+3 or *i*, *i*+4 relationships. Bridging amino acids in an *i*, *i*+4 arrangement has been studied extensively for helix stabilization, but we are unaware of efforts at helix stabilization by means of an *i*, *i*+3 arrangement.[80] As expected, all free peptides exhibit some degree of helicity in water (Figure 1). Owing to the helix-disrupting properties of Asp residues, peptides containing this amino acid exhibit substantially less helicity than comparable Glu-containing sequences (see, **DD4**, **EE4**, Figure 1A).[81]

The peptides react with *cis*-$[Rh_2(OAc)_2(tfa)_2]$ (tfa = trifluoroacetate), to produce adducts with bridging dirhodium centers for both *i*, *i*+3 and *i*, *i*+4 carboxylate spacing



Scheme 2. Dihrodium metallopeptides with *i*, *i*+4 (**EE4**) and *i*, *i*+3 (**EE3** and **DD3**) residue spacing.

(Scheme 2). Circular dichroism (CD) spectroscopy was employed to assess the effect of dirhodium binding on helicity. In the *i*, *i*+4 series, both Asp (**DD4**) and Glu (**EE4**) peptides exhibit increased helicity on dirhodium binding (Figure 1A). The effect of binding is most pronounced in the Asp case; the helix-destabilizing influence of hydrogen bonding from the carboxylate side chain to the amide backbone is removed upon binding dirhodium.[81] Next, we considered peptides with *i*, *i*+3 carboxylate spacing. The free peptide **EE3** is helical in solution, and binding to a dirhodi-

Table 1. Helicity of free peptides and dirhodium–metallopeptide complexes.

Entry	Peptide[a]	Sequence/yield of metallopeptide [%]	$[\Theta]_{220}$[b]	f_H[c,d]	f_H(TFE)[d,e]	Rel. helicity [%][e]
bis-carboxylate peptides as ligands for dirhodium diacetate metal centers						
1	**EE3**	Ac-YGKAAAAEAAEAKAAAAK-NH_2	−11.5	39	44	70
2	[(**EE3**)$Rh_2(OAc)_2$]	70	−13.8	45	51	82
3	**DD3**	Ac-YGKAAAADAADAKAAAAK-NH_2	−5.3	21	24	39
4	[(**DD3**)$Rh_2(OAc)_2$]	80	−1.5	10	12	19
5	**DE3**	Ac-YGKAAAADAAEAKAAAAK-NH_2	−8.0	29	32	52
6	[(**DE3**)$Rh_2(OAc)_2$]	67	−0.9	11	10	16
7	**ED3**	Ac-YGKAAAAEAADAKAAAAK-NH_2	−11.4	38	43	70
8	[(**ED3**)$Rh_2(OAc)_2$]	59	−1.7	11	12	20
9	**EE3G**	Ac-YGKAAAAEAGEAKAAAAK-NH_2	−2.5	13	15	24
10	[(**EE3G**)$Rh_2(OAc)_2$]	80	−9.6	33	38	61
11	**EE4**	Ac-YGKAAAAEAAAEKAAAAK-NH_2	−12.3	41	46	75
12	[(**EE4**)$Rh_2(OAc)_2$]	65	−17.3	55	62	100
13	**DD4**	Ac-YGKAAAADAAADKAAAAK-NH_2	−5.7	22	25	41
14	[(**DD4**)$Rh_2(OAc)_2$]	73	−16.0	51	58	94
15	**EE4G**	Ac-YGKAAAAEAGAEKAAAAK-NH_2	−1.9	12	13	21
16	[(**EE4G**)$Rh_2(OAc)_2$]	73	−8.3	30	34	54
17	**ED4G**	Ac-YGKAAAAEAGADKAAAAK-NH_2	−1.1	9	10	17
18	[(**ED4G**)$Rh_2(OAc)_2$]	57	−12.1	40	46	74
19	**DD4G**	Ac-YGKAAAADAGADKAAAAK-NH_2	−1.1	9	11	17
20	[(**DD4G**)$Rh_2(OAc)_2$]	70	−10.8	37	41	67
21	**sEE4**	Ac-KAEAAAEAK-NH_2	−0.4	9	8	13
22	[(**sEE4**)$Rh_2(OAc)_2$]	58	−11.6	48	44	71
metallopeptides with other coordination spheres						
23	[(**EE3**)$Rh_2(pyrr)_2$]-*iso* A	50	−12.6	42	47	76
24	[(**EE3**)$Rh_2(pyrr)_2$]-*iso* B	29	−10.4	36	40	65
25	[(($\mathbf{EE3}$)$_2$)Rh_2]-*iso* A	25	−7.7	26	31	51
26	[(($\mathbf{EE3}$)$_2$)Rh_2]-*iso* B	25	−7.6	25	31	50
27	[(($\mathbf{EE4}$)$_2$)Rh_2]-*iso* A	30	−9.8	31	38	62
28	[(($\mathbf{EE4}$)$_2$)Rh_2]-*iso* B	24	−11.0	34	42	68
29	*trans*-[(**EE4**)$Rh_2(OAc)_2$]	19	0.3	5	6	10
30	*trans*-[(**EE4G**)$Rh_2(OAc)_2$]	65	0.5	5	6	9

[a] All metallopeptides have *cis* geometry at the dirhodium center unless stated otherwise. [b] Residual molar ellipticity in $[\mathrm{deg\,cm^2\,dmol\text{-}res^{-1}} \times 10^3]$. [c] Helicity content calcd as $f_H = ([\Theta]_{220} - [\Theta]_c)/([\Theta]_{inf} - [\Theta]_c)$, where $[\Theta]_c = 2220 - 53T$, $[\Theta]_{inf} = (-44000 + 250T)(1 - k/N_p)$, T is the temperature in degrees Celsius, $k = 3.0$ and N_p is the number of peptide units. Because each peptide has an unstructured YG tag at the N terminus, the calculated helicity values are likely to underestimate the helicity of the rest of the peptide. [d] Relative helicity calculated relative to [(**EE4**)$Rh_2(OAc)_2$]. [e] TFE = trifluoroethanol. Helicity content calculated as a fraction of $[\Theta]_{inf} - [\Theta]_{220}$ for **EE4** in 100% trifluoroethanol.

um center results in increased helical content, in line with *i, i*+4 examples discussed above (Figure 1D). However, the structural requirements for helix stabilization in *i, i*+3 peptides are clearly stricter than those for the *i, i*+4 case: all sequences with at least one Asp residue binding to dirhodium (peptides **DD3**, **ED3**, and **DE3**) display complete disruption of helicity upon binding to dirhodium (Figure 1B). The data suggest that the extra methylene unit in the Glu side chain is necessary to allow proper positioning at a dirhodium center. Although residues at *i, i*+3 spacing are proximal in space in a helical structure, the side chains project in different directions and we are not aware of any previous examples of helix induction or stabilization through metal binding in an *i, i*+3 fashion.

Helix induction: Creating secondary structure upon metal binding in otherwise unstructured peptides potentially allows metal-based switching of molecular function. The polyalanine sequences discussed above contain at least some helical bias in the unbound state, and upon dirhodium binding appear more strongly helical. Inducing helicity in unstructured peptides is a more stringent test, and to probe helix induction in otherwise random-coil peptides, we introduced glycine—a powerful helix disrupting residue—into our peptide sequences at the *i*+2 position.[82] All of the glycine-containing peptides exhibit a random-coil structure in aqueous solution. CD analysis of the metallopeptide adducts, however, indicates a helical structure (Figure 1C). For peptides with *i, i*+4 spacing, as before, good helix induction was observed regardless of whether the binding residues are Asp or Glu. A glycine-containing peptide with *i, i*+3 carboxylate spacing (**EE3G**) was also prepared. The peptide **EE3G** is a random coil in the unbound state but becomes helical following ligation to dirhodium, as evidenced by the appearance of a negative feature at $\lambda = 220$ nm (Figure 1D). This result extends the possibilities for metal-induced helicity beyond the *i, i*+4 systems reported with other metals to Glu-Xaa-Xaa-Glu sequences. The assignment of helical structure to the adduct [(**EE3G**)$Rh_2(OAc)_2$] is further supported by NMR spectroscopy experiments. Due to the repetitive nature of our sequences, unambiguous assignment of peaks was not possible at the periphery of the **EE3G** sequence (Y1–A5 and K13–K18). Nonetheless, peaks in the key dirhodium-binding region were readily identified

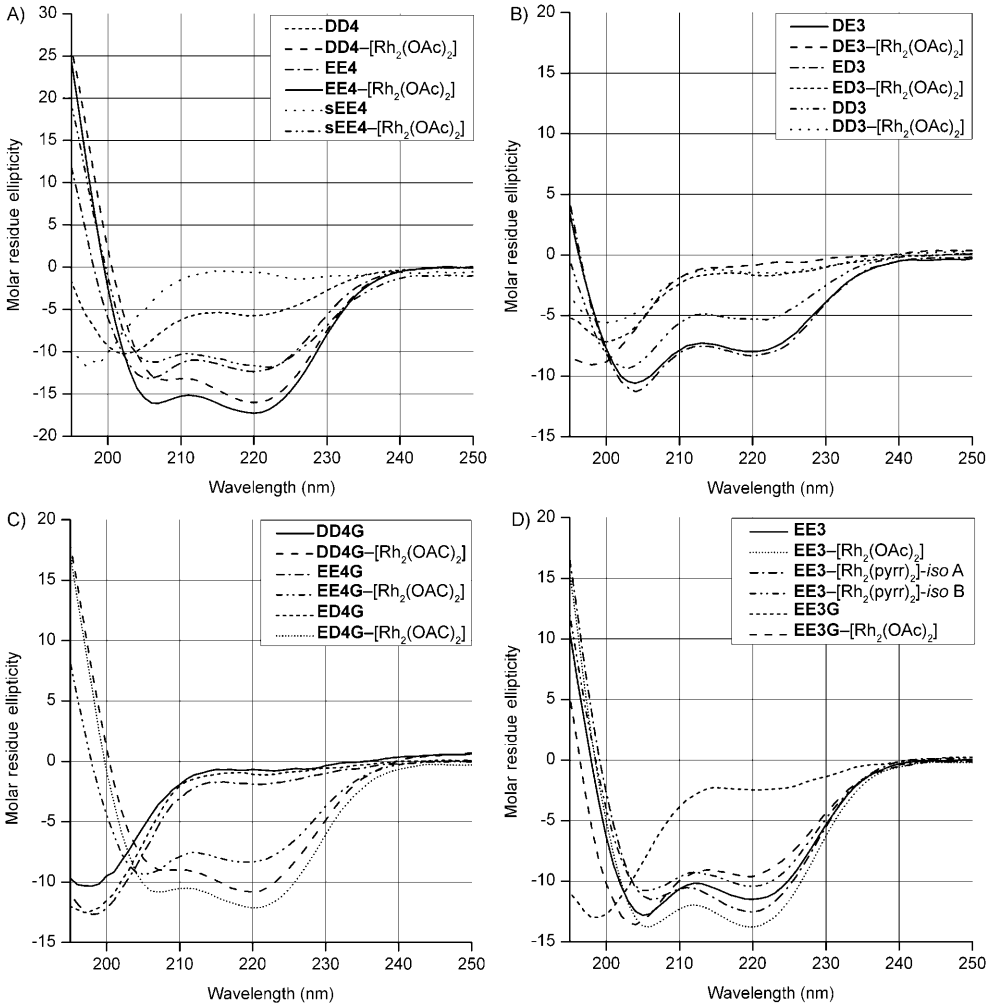


Figure 1. Circular dichroism spectra demonstrating the effect of dirhodium binding on the secondary structure of bis-carboxylate peptides. For sequences, see Table 1.

through COSY experiments. Within the dirhodium-binding region (A6–A12), the $^3J_{HN-H\alpha}$ coupling constants are all less than 6 Hz, consistent with a helical structure. In addition, unambiguous long-range αN *i, i*+3 NOEs were observed, together with short-range *i, i*+1 interactions (αN and NN) to residues in proximity to dirhodium-bound glutamates (Figure 2).[83]

The ability of dirhodium complexes to induce peptide helicity has proven to be quite general. For example, a significantly shorter peptide, KAEAAAEAK (**sEE4**), which is too short to exist with defined secondary structure in the free state, adopts a helical structure upon dirhodium binding (see Table 1, entry 21 and 22 and Figure 1A). In addition, variation of the dirhodium-reagent structure is tolerated both in the synthesis and in the secondary structure of metallopeptides. For example, the bis-amidate complex *cis*-[Rh_2-$(tfa)_2(pyrr)_2$] (pyrr = pyrrolidonate) reacts cleanly with the peptide **EE3** to afford the bis-carboxylate, bis-amidate product as two stereoisomers, which are separable by preparative HPLC (Scheme 3). Both isomers display helicity similar to the corresponding bis-acetate complex (Figure 1D).

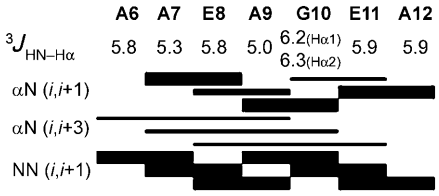
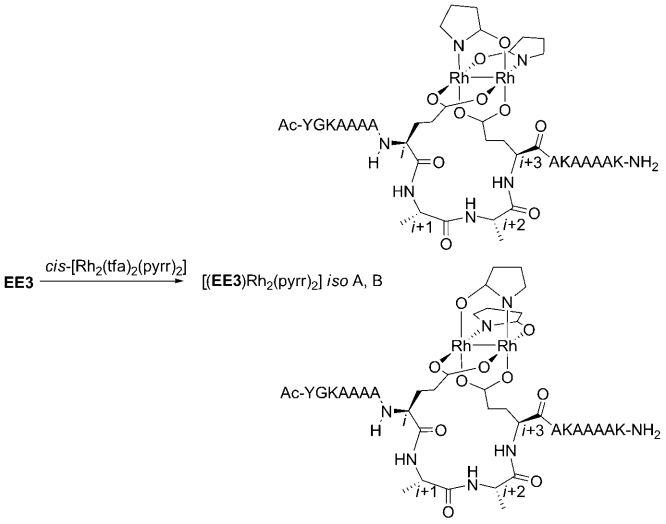


Figure 2. $^3J_{HN-HA}$ coupling constants and NOE connectivity for the metal-binding region of the [(**EE3G**)$Rh_2(OAc)_2$] complex. The NOE intensity is represented by the thickness of the bars.

Bis-peptide complexes with dirhodium: In addition to 1:1 dirhodium/helix adducts, we have been able to produce adducts with 1:2 dirhodium/peptide stoichiometry by employing the reagent [$Rh_2(tfa)_4$].[84] Treatment of peptide **EE4** with [$Rh_2(tfa)_4$] results in the formation of a [(**EE4**)$_2Rh_2$] adduct. The CD spectrum indicates helical structure for the bis-peptide adduct as well as, identifies the coordination geometry to be *cis* (Figure 3). The observation of *cis* binding is opposite to what might be predicted on the basis of the



Scheme 3. Synthesis of a peptide–dirhodium complex with amidate ligands. The identity of the product stereoisomers has not been assigned.

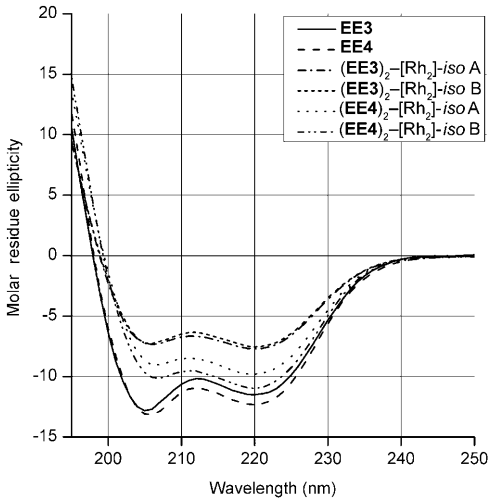
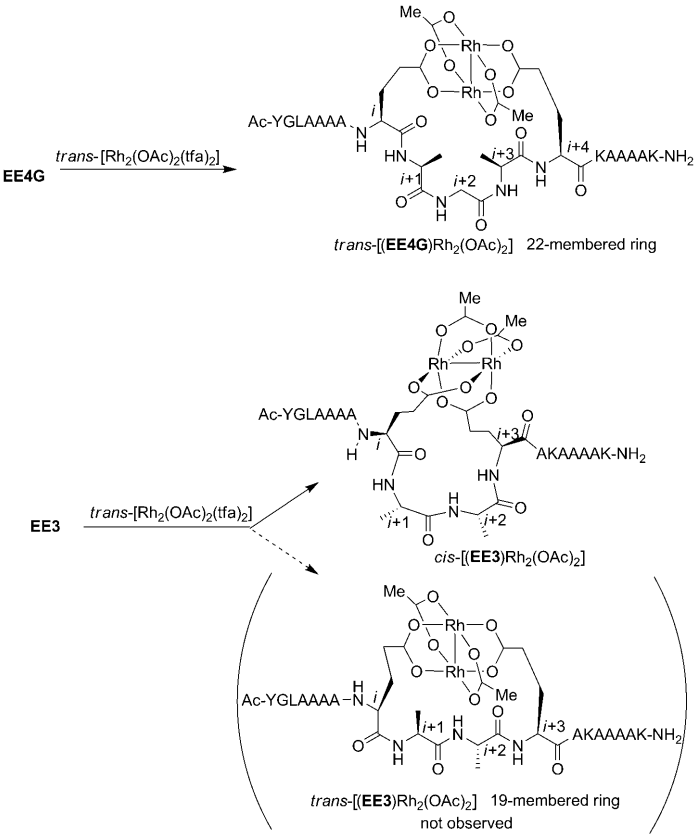


Figure 3. Circular dichroism spectra of bis-peptide–dirhodium complexes.

trans ligand effect. Dirhodium complexes of the type $[Rh_2(RCO_2)(tfa)_3]$ are known to react in ligand-exchange processes to give the *trans*-bis-tfa product.[85] In the case at hand, ligand loss from a monodentate (peptide)–$Rh_2(tfa)_3$ structure should form a *trans* product after intramolecular ligation to afford the chelate structure. Potential explanations for this phenomenon include fast intermolecular ligations relative to intramolecular *trans*-chelate formation, *cis–trans* isomerization of an intermediate *trans* complex, associative displacement of a *cis*-trifluoracetate ligand by the second chelating-carboxylate ligand, and trifluoroacetate displacement due to a competing *cis*-ligand effect by a dissociative interchange (I_d) mechanism.[86] Further mechanistic investigations will be necessary to identify the origin of this result.

***trans*-chelating dirhodium metallopeptides**: Because the dirhodium bis-acetate complexes described here can exist in *cis* or *trans* isomeric forms, we were interested in the effects of coordination stereochemistry on helix induction. Models indicate that only *cis* coordination should be compatible with a helical structure, and this is confirmed by experiment. Treatment of peptide **EE4G** with the isomeric *trans*-$[Rh_2(OAc)_2(tfa)_2]$ complex[85] results in the formation of the bridged dirhodium complex, *trans*-[(**EE4G**)$Rh_2(OAc)_2$] (Scheme 4). The structure of this complex can be inferred



Scheme 4. Synthesis of metallopeptides from dirhodium precursors that have *trans* geometry.

from mass spectrometry and the fact that *trans*-[(**EE4G**)$Rh_2(OAc)_2$] exhibits HPLC run times and a CD spectrum different from that of the *cis* isomer (Figure 4). Consistent with expectations from modeling, the CD spectrum of *trans*-[(**EE4G**)$Rh_2(OAc)_2$] exhibits none of the features above $\lambda = 200$ nm that would imply helical structure (Figure 4). The *trans*-dirhodium linkage appears to destroy any helical propensity, despite the significant helical bias of the free peptide.

Dimetal complexes with *trans*-oriented bridging carboxylates, such as *trans*-[(**EE4G**)$Rh_2(OAc)_2$], are quite rare. A single previous example employed a calix[4]arene to template the formation of a *trans* linkage.[87] In the case at hand, the *i, i*+4 linkage may be close to the minimum spacing nec-

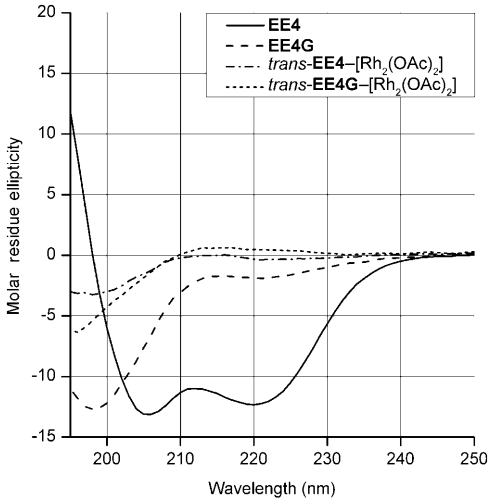


Figure 4. Circular dichroism spectra of *trans*-dirhodium–peptide complexes.

essary to span the dirhodium center with *trans* ligation. The *trans*-linked structure is a 22-membered ring. Treating the similar peptide **EE3** with *trans*-$[Rh_2(OAc)_2(tfa)_2]$ produced none of the expected *trans*-$[(\mathbf{EE3})Rh_2(OAc)_2]$, but afforded *cis*-$[(\mathbf{EE3})Rh_2(OAc)_2]$ instead. The shorter 19-membered ring present in dirhodium–**EE3** adducts is apparently too short for facile formation of a *trans* complex, and a *trans*-to-*cis* isomerization occurs during the ligation process. Even slight changes in peptide structure affect the efficacy of the formation of the *trans* adduct. The peptide **EE4** has a single amino acid substitution (G9A), and treatment of this peptide with *trans*-$[Rh_2(OAc)_2(tfa)_2]$ results in the formation of the *trans* adduct in low yield (19%) along with significant amounts of the *cis* complex.

Computational analysis of dirhodium metallopeptide helicity: To better understand the influence of side-chain length and carboxylate spacing on helix induction and disruption, we performed a molecular-mechanics computational study of dirhodium-bound helices. Models of idealized, alanine-rich helices were ligated to a constrained $[Rh_2(OAc)_2]$ fragment,[88] and the conformation space was explored with a Monte-Carlo algorithm native to Spartan'04.[89] After quantitatively aligning the identified low-energy structures to an idealized polyalanine helix, (Figure 5) it was found that the minimized structures of *i*, *i*+4 metallopeptides (**EE4** and **DD4**) overlay closely to the idealized polyalanine helix. Minimized structures of the *i*, *i*+3 complexes (**EE3** and **ED3**) align quite well with the idealized α helix, which provides further support for the assignment of α-helical secondary structure for *cis*-$[(\mathbf{EE3G})Rh_2(OAc)_2]$ and speaks against a 3_{10} helical assignment. In contrast, the minimized structures of *i*, *i*+3 complexes with **DD3** and **DE3** peptides align poorly with the idealized helix.

Upon closer inspection, a qualitative correlation exists between the experimental relative helicity (Table 1) and the computed atomic deviation from an idealized helix (Figure 5). The trend is consistent with general polymer theory which states that, for entropic reasons, helical propensity increases with ring size.[90–92] To better localize the origin of the differences in the metal-binding region, we examined dihedral angles and hydrogen-bonding distances (see Table S-2 in the Supporting Information). For all adducts that appear helical by CD analysis, the structural deviations over the central nine amino acid region are minimal (i.e., hydrogen-bond distance and dihedral angle deviations $\leq$ 0.1 Å and $\leq$ 3.2°, respectively). In contrast, models for adducts of **DD3** and **DE3** afford low-energy structures with hydrogen-bonding distances and Φ/Ψ angles with much

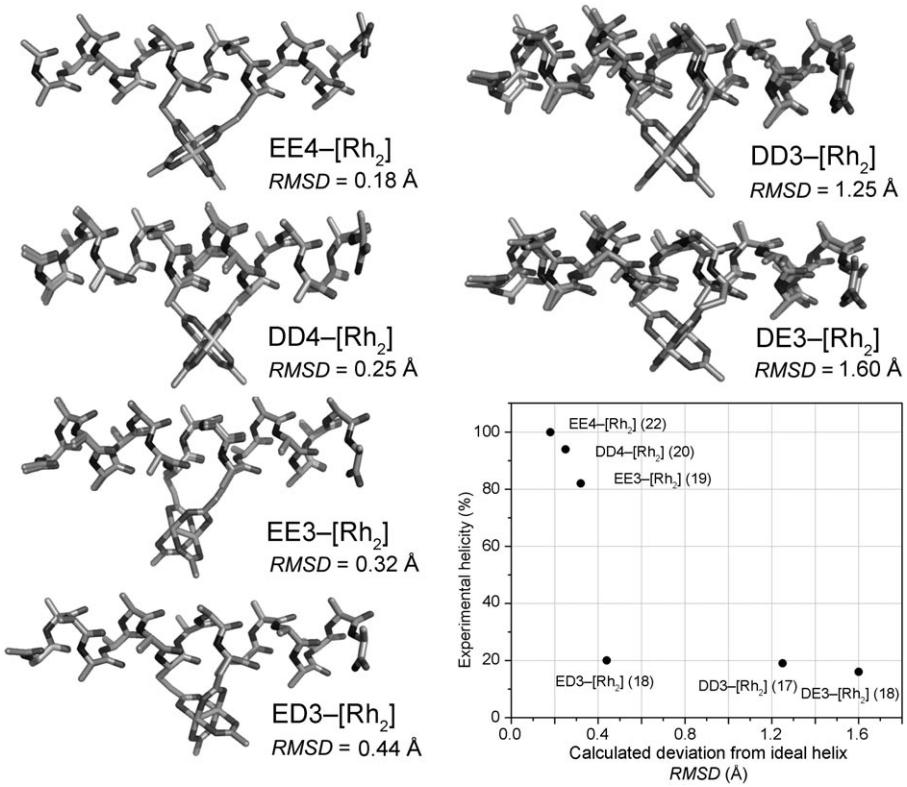


Figure 5. Superimpositions of the low-energy conformations of peptide–$Rh_2(OAc)_2$ complexes and an idealized polyalanine helix as identified by Monte Carlo simulations, and qualitative correlation between experimentally determined relative helicity (Table 1) and root-mean-squared deviation (RMSD, [Å]) obtained from the alignment analysis. Numbers in parentheses indicate the ring size of the peptide–rhodium complex.

larger *RMSD* (0.6–0.7 Å and 4.8–9.3°, respectively). The **ED3**–Rh_2 complex represents an intermediate example for which our computational analysis suggests relatively small deviation from an idealized helix whereas CD analysis indicates a random coil. Nevertheless, the alignment *RMSD* (0.44 Å) for this complex is larger than that of any of the complexes that appear helical by experiment. The experimental helicity is correlated both with ring size and with calculated deviation from an idealized helix. The relationship to the ring size is non-linear, and the structures with a ring size less than 19 are all strongly non-helical.

Metallopeptides in a biological environment: Stabilization of peptide secondary structure, and of α helicity in particular, is an important tool to probe, understand, and control biological interactions. Indeed, it has been demonstrated that stabilized helices result in improved binding affinity for protein targets, an approach that is actively pursued for the treatment of human disease.[93] Metallopeptides are an alternative approach to bioactive polypeptides with predictable and robust secondary structure. Whereas current methods for helix stabilization require non-natural amino acid backbones or side chains, dirhodium reagents can be designed to bind directly to natural side chains. The advantages of natural peptide substrates include facile structure optimization through biosynthesis-based library generation without unnatural amino acids.[94,95] Unfortunately, transition metals are often toxic to cells and undergo facile ligand exchange in biologically relevant environments. To our knowledge, no reported metallopeptide structures have demonstrated stability or low toxicity in cellular media.

In undertaking a study of biological stability, we were aware that biological pH is near the upper end of the stability range for dirhodium–carboxylate linkages. We have observed significant decomposition of the metallopeptides at or above pH 8, primarily though hydrolysis of the dirhodium–carboxylate linkage to release free peptide.[60] To simultaneously examine toxicity and stability, we chose a representative helical metallopeptide, [(**EE4G**)$Rh_2(OAc)_2$], and dosed living human embryonic kidney stem cells (HEK-293) in 10% fetal bovine serum and Dulbecco's modified eagle medium (DMEM) buffer. The cells were incubated at 37°C and aliquots were taken over a period of 24 h for analysis of metallopeptide concentration. The concentration of metallopeptide is plotted in Figure 6A. We observe an initial drop in peptide concentration, likely attributable to non-specific adhesion. After the initial drop, little decomposition was observed over the full 24 h of the experiment. In addition to HPLC analysis, ESI/MS analysis of the aliquot taken after 24 h confirms the presence of the starting dirhodium complex (Figure S-2 in the Supporting Information).

In conjunction with the metallopeptide stability study, we examined the viability of HEK-293 cells in the presence of metallopeptide. Cell viability and population size assays by using a hemocytometer and trypan blue stain were performed over a period of 24 h (Figure 6A). The metallopeptide-treated cells continue to reproduce over the first 2 h and exhibit a minimal increase in the number of dead cells. After 6 h, cell population remained at 220 K and a small increase in dead cells (12%) was observed. Over the remaining time, the treated cells recovered to levels comparable to the control despite the continued presence of metallopeptide (≈10 μM) in the media. Cell viability data was further confirmed by brightfield and fluorescence microscopy (Figure 6B–D and Figure S-3 in the Supporting Information). With the caveat that the issue of cell permeability is not directly addressed here, these studies do provide a foundation

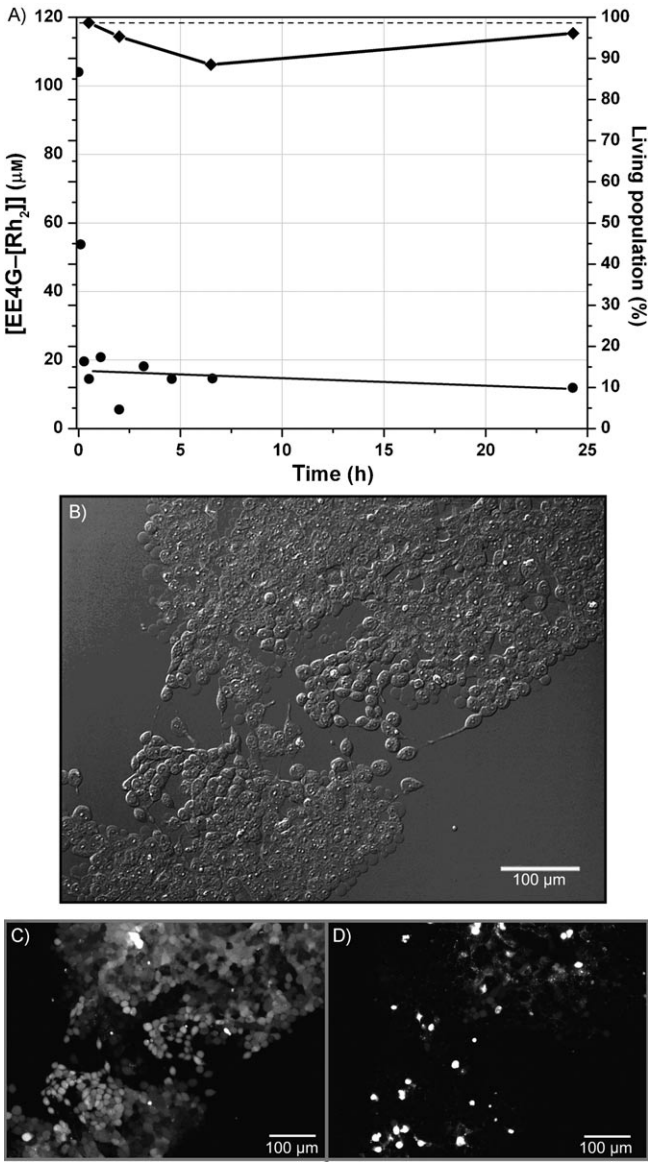


Figure 6. Biocompatibility analysis of [(**EE4G**)$Rh_2(OAc)_2$] (Table 1, entry 18) (100 μM) incubated with HEK-293 cells. A) [(**EE4G**)$Rh_2(OAc)_2$] stability time course (left axis, ●) and cell viability (right axis, ◆) of control (dashed line) and metallopeptide-treated (solid line) cells. Representative brightfield (B) and fluorescence microscopy images (C, D) at 20× magnification of cells treated with [(**EE4G**)$Rh_2(OAc)_2$] after 28 h. Live/dead viability assay for mammals (Invitrogen) was used to visualize living (C) and dead (D) cells. A two-color combined image is available in the Supporting Information (Figure S3-B).

for applications of dirhodium metallopeptides in a biological environment.

Conclusion

This work demonstrates that dirhodium–carboxylate ligation can have powerful helix-inducing properties. Helix induction upon dirhodium binding is extremely general for bis-carboxylates with *i, i*+4 spacing, and this work also expands the scope of known metal-binding topologies to include helix induction in peptides with *i, i*+3 spacing. Salient features of the work include the generality and functional-group compatibility of the synthetic method and the serum stability and low toxicity of the metallopeptides to living cells. In addition, we have shown that carboxylate ligation is compatible with the presence of commonly employed metal-binding side chains (Met and His).[60] These attributes combine to make dirhodium ligation an attractive platform for examining the alteration of peptide function through control of secondary structure. The tolerance of our method for other metal-binding side chains is particularly significant as it may make it possible to switch the structure of a peptide among multiple states through the use of complementary metal-ligation strategies.

The helical structure created upon binding peptides to dirhodium defines a strategy to create a well-defined ligand environment around a metal center. Experimental and computational evidence suggest that peptide helicity is correlated with chelate ring size. At the same time, the synthesis of metallopeptides with dirhodium precursors that bear different ancillary (non-peptide) ligands, such as pyrrolidonate, affords an opportunity to tune the electronics of the dirhodium center. Taken together, these capabilities enable a modular approach to affect the function of a dirhodium center by altering the electronic and steric properties of the ligand sphere.

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