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4′-Aminomethyl-2,2′-bipyridyl-4-carboxylic Acid (Abc) and Related Derivatives: Novel Bipyridine Amino Acids for the Solid-Phase Incorporation of a Metal Coordination Site Within a Peptide Backbone

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This paper is dedicated to the memory of Professor Bruce W. Erickson (1942–1998)

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Abstract—The novel bipyridyl amino acid, 4′-aminomethyl-2,2′-bipyridyl-4-*c*arboxylic acid (**Abc**), and related Boc- and Fmoc-protected derivatives were synthesized to provide high-affinity bidentate metal-binding amino acid modules for the solid-phase peptide synthesis (SPPS) of metallopeptides. Since the bipyridyl group of Abc is inserted into the peptide mainchain and not in the sidechain, its presence in a peptide should impart distinct conformational constraints to the backbone geometry, influencing local secondary structure. To demonstrate its amenability for SPPS and its capacity for metal complexation, Abc was incorporated into the hexapeptide Ac-Ala-*Abc*-Ahx-Ahx-*Abc*-Gly-NH_2 (peptide **Aha**; where Ahx=aminohexanoic acid) and subsequently used as a tetradentate ligand to octahedrally coordinate and asymmetrically encapsulate a ruthenium(II) ion, creating a novel peptide-caged redox-active metal complex.

Introduction

In recent years, the rational and systematic investigation of biological electron and energy transfer has been greatly assisted by the use of de novo designed monomeric proline II,[1–4] β-sheet,[5] and α-helical peptide assemblies[6–9] or multimeric α-helical protein maquettes as models of redox-active proteins.[10–16] Common to these peptide assemblies is the pendant attachment of redox-active cofactors, electron donors/acceptors, or chromophores which serve to initiate or propagate electron/energy transfer events. A subset of these peptides employ ruthenium tris(bipyridyl) transition metal complexes ($Ru^{II}(bpy)_3$) as light-harvesting chromophores, which mimic primary excitation events in photosynthesis by generating metal-to-ligand charge transfer (MLCT) excited states upon irradiation with visible light.[17] To provide minimalist model systems to probe the effect of peptide sequence and outersphere structure on protein-bound metal complexes, we have developed a general strategy for the modular solid-phase incorporation of high affinity binding sites for ruthenium(II) or other metal ions into a designed peptide sequence, with metal complexation occurring in solution following cleavage of the peptide from the solid support.

Peptides containing a 2,2′-bipyridine (bpy or b) group can bind metal ions such as ruthenium(II) or osmium(II), creating redox-active octahedral metal complexes. This versatile bidentate ligand has been incorporated into peptides as a monoacid,[8,18] a diacid;[19] a diamine,[20] or an α-amino acid having the bpy group in the side chain.[21–23] This paper describes the synthesis of the novel amino acid 4′-aminomethyl-2,2′-bipyridine-4-carboxylic acid (**Abc**, **5**) and several of its derivatives. The attachment of the NH_2–, CH_2– and CO_2H groups in Abc require that a linear peptide containing an Abc residue will have the bipyridyl group not in a side chain but in the *main chain*. The presence of the bipyridyl amino acid in the peptide backbone should impart distinct conformational constraints within the context of a peptide, and should provide a potential ligand site for metals that undergo facile redox catalysis. The product of this strategy more closely resembles natural metallopeptides and is a step towards the generation of synthetically feasible systems for rationally probing the effect protein exostructure plays on metal cofactor reactivity, lability, and excited state properties.

Keywords: metallopeptide; metal binding site; ruthenium(II) tris(bipyridine); caged compounds; bipyridine.
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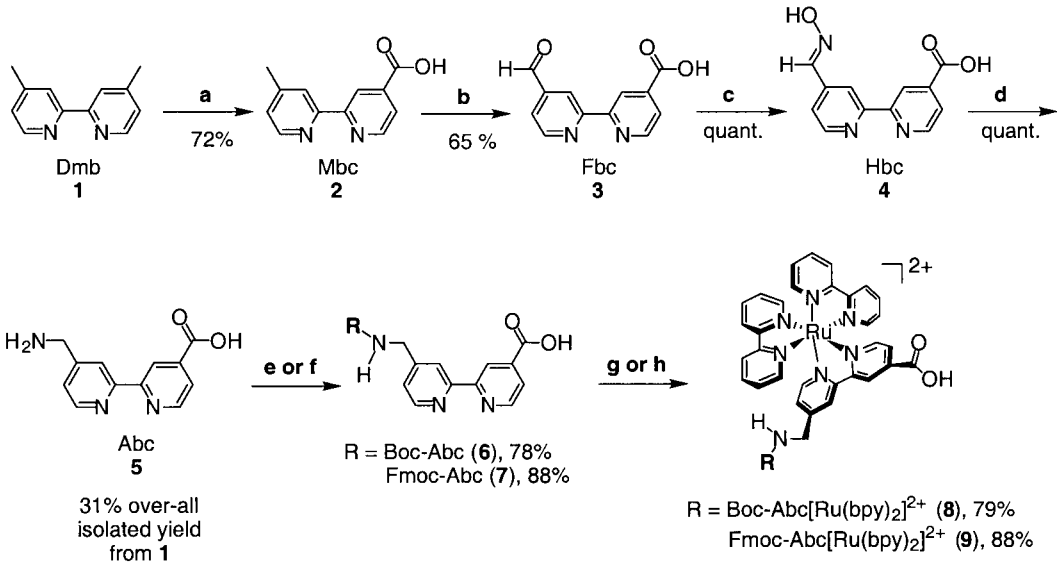


Figure 1. Synthesis and metal complexation of 4′-aminomethyl-2,2′-bipyridyl-4-carboxylic acid and Boc/Fmoc protected derivatives. Reagents and conditions: (a) SeO_2, Δ, dioxane, 16 h; then Ag_2O; (b) 5 equiv. SeO_2, dioxane, Δ, four days; (c) $NH_2OH{\cdot}HCl$, 1:1 pyridine/EtOH, Δ, 2 h; (d) H_2, 10% Pd/C EtOH:H_2O:HCl, 16 h; (e) Boc_2O, 1 N NaOH, dioxane; (f) Fmoc-OSu, 10% Na_2CO_3, dioxane, 0°C; (g) $Ru(bpy)_2Cl_2$, MeOH, Δ, 16 h; (h) $Ru(bpy)_2Cl_2$, EtOH/H_2O/dioxane, Δ, 16 h.

Results and Discussion

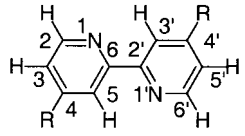
Bipyridyl amino acid synthesis

A dual oxidation strategy was employed to prepare the bipyridyl amino acid 4′-aminomethyl-2,2′-bipyridine-4-carboxylic acid (**5**, Abc, Fig. 1). First, 4,4′-dimethyl-2,2′-bipyridine (**1**) was selectively oxidized to the 4′-monocarboxylic acid derivative, 4′-methyl-2,2′-bipyridine-4-carboxylic acid (**2**) in 72% yield according to the method of McCafferty and coworkers.[8] Second, the 4′-methyl group of **2** was oxidized with excess selenium dioxide to the aldehyde acid 4′-formyl-2,2-bipyridine-4-carboxylic acid (**3**) in ~65% yield as assessed by ^1H NMR analysis of the crude product (Table 1). Oximation with hydroxylamine·HCl in ethanol/pyridine smoothly converted all **3** into 4′-((hydroxyimino)methyl)-2,2′-bipyridine-4-carboxylic acid (**4**). Fortuitously, excess hydroxylamine·HCl added to produce **4** reduced remaining unreacted selenium dioxide to metallic selenium, which precipitated and thus could be easily separated by filtration prior to extractive workup (dilute HCl, pH 3). Lastly, oxime acid **4** was transformed into the desired amino acid by catalytic hydrogenolysis followed by purification over octadecyl-silica solid-phase extraction media (Waters) to provide pure Abc (**5**) as the hydrochloride salt in 31% over-all isolated yield from **1**. Proton NMR chemical shifts for **3**, **4**, and **5** and related bipyridines are summarized in Table 1. Amino acid **5** was converted into both Boc and Fmoc-derivatives for use as modules for solid-phase peptide synthesis. Treatment of Abc·HCl salt with di-(*tert*-butyl)dicarbonate provided Boc-Abc-OH (**6**) in 78% yield. Similarly, reaction of Abc·HCl with Fmoc-succinimide furnished Fmoc-Abc-OH (**7**) in 88% yield.

Bipyridyl amino acid metal complexation

The metal complexation properties of bipyridyl SPPS

Table 1. Selected ^1H NMR chemical shifts for disubstituted derivatives of 2,2′-bipyridine



Entry	R-4	R-4′	Solvent	^1H Chemical Shift (δ, ppm)							
				H-3	H-5	H-6	H-3′	H-5′	H-6′	R-4′	R-4
Mbf	CH_3	CHO	$CDCl_3$	8.28	7.21	8.58	8.86	7.72	8.89	10.17	2.47
Mbh	CH_3	CHNOH	CD_3OD	8.18	7.35	8.51	8.44	7.63	8.65	8.18	2.49
Mba	CH_3	CH_2NH_2	$CDCl_3$	8.22	7.15	8.55	8.31	7.26	8.55	4.05	2.70
2	CO_2H	CH_3	$(CD_3)_2SO$	8.84	7.87	8.87	8.27	7.34	8.59	2.43	
3	CO_2H	CHO	$(CD_3)_2SO$	8.85	7.94	9.02	8.83	7.91	8.94	10.22	
4	CO_2H	CHNOH	$(CD_3)_2SO$	8.83	7.90	8.89	8.61	7.65	8.74	8.35	
5	CO_2H	CH_2NH_2	CF_3CO_2D/D_2O	8.90	8.33	9.08	8.81	8.16	9.02	4.68	
6	CO_2H	CH_2NHBoc	$(CD_3)_2SO$	8.82	7.84	8.79	8.32	7.31	8.63	4.24	
7	CO_2H	$CH_2NHFmoc$	$(CD_3)_2SO$	8.80	7.78	8.65	8.33	7.25	8.61	4.30	

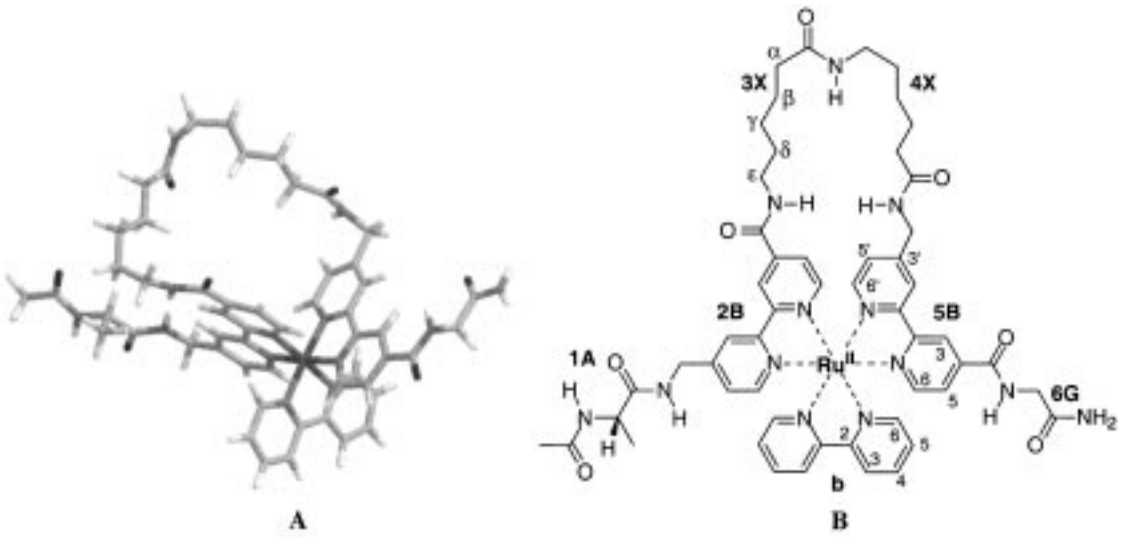


Figure 2. (A) An energy minimized model of the metallohexapeptide **11** generated using the CAChe molecular modeling program. The molecule of 2,2′-bipyridine (bpy) and both bpy moieties of the Abc residues of the apohexapeptide Ac-Ala-Abc-Ahx-Ahx-Abc-Gly-NH_2 (Aha, **10**) are octahedrally ligated to ruthenium(II). The N-terminal segment (Ac–Ala) is at the left, the bridging segment Ahx–Ahx) is at the top, the C-terminal segment (Gly–NH_2) is at the right, the bpy molecule is at the bottom, and both Abc residues and the octahedrally coordinated ruthenium(II) atom are above the bpy molecule. (B) Structural abbreviations for stereoisomers of the metallopeptide Ru^{II}(Aha)(bpy) (**11**). The six residues of Aha are numbered sequentially from the N terminus to the C terminus. For designating protons seen in ^{1}H NMR spectra, B=Abc and X=Ahx.

modules **6** and **7** were confirmed by synthesis of their respective ruthenium(II) octahedral mixed-ligand complexes. Reaction of **6** and **7** with dichlorobis(2,2′-bipyridine)ruthenium(II) (Rub_2Cl_2) afforded the bis-heteroleptic complexes **8** and **9**, respectively. The metal complexation reactions were performed in refluxing alcohol blends to minimize thermal decomposition of the carbamate protecting groups. The chloride salts of metal complexes **8** and **9** were preparatively purified by reversed-phase HPLC and subsequently converted to the organic-soluble PF_6^- salt by treatment of aqueous solutions of **8** and **9** with saturated aqueous NH_4PF_6. The chemical composition of **8** and **9** were verified by ^{1}H NMR and mass spectrometry. Furthermore, by UV/visible spectroscopy, Abc-metal complexes **8** and **9** exhibited metal-to-ligand charge transfer bands (MLCT) at λ=456 nm (ϵ=11,500 cm^{-1} M^{-1}) which are characteristic of heteroleptic tris(bipyridyl)ruthenium(II) octahedral coordination complexes.[24–26] The observation of an MLCT band nearly identical to that of $Ru^{II}(bpy)_3$ suggests that the presence of the aminomethyl and carboxylate groups do not adversely abrogate its potential as a phototrigger for electron transfer processes.

Design and synthesis of the linear apohexapeptide *Aha*

In natural proteins, multiple sidechain residues complex metal ion cofactors for optimal tailoring of coordination geometry and reactivity. Similarly, multiple Abc ligands appropriately spaced within the context of a single polypeptide might be expected to serve as a multidentate ligand for encapsulation of a single metal ion. To demonstrate the utility of Abc (**5**) in solid-phase peptide synthesis, a heptapeptide containing two Abc residues was designed and synthesized to serve as a tetradentate caging peptide ligand for a ruthenium(II) ion. This peptide was carefully designed to form a tight chelating environment around ruthenium(II) ion, in effect asymmetrically encapsulating the metal in a rigid peptide cage. Encapsulation of ruthenium complexes with caging ligands or incorporation of the complex into rigid matrices such as plastics, zeolites or silica gel has been shown to stabilize metal-to-ligand (MLCT) excited states relative to ligand field states.[27,28] This SPPS modular strategy represents an approach for controlling excited state properties based on controlled structural variations in an encapsulating multidentate ligand.

(N terminus)-**Abc**-(bridge)-**Abc**-(C terminus)

↓

(Ac-Ala)-**Abc**-(Ahx-Ahx)-**Abc**-(Gly-NH_2)

Peptide **Aha** (**10**)

Peptide Aha (**10**) is a minimalist tetradentate model peptide capable of intramolecular occupation of four of the six coordination sites of an octahedral metal–ion complex (Fig. 2). Thus Aha can serve as a tetradentate ligand of the bridged bis(bpy) type. The arrangement of amino acids in Aha comprises five segments: N-terminus, bpy ligand, bridge, bpy ligand, and C-terminus. Aha contains two bpy-containing Abc residues flanking the Ahx–Ahx bridging tether composed of two C-6 aminohexanoic acid residues (Ahx). The design intent was to minimize the possible number of geometric conformational isomers which would be expected to form upon octahedral metal coordination, thus the Ahx–Ahx bridge was designed to be just long enough to form *cis*-bridged meridonal metal complexes but too short to form *trans*-bridged facial metal complexes. Fig. 2 illustrates an energy-minimized molecular model of the metallopeptide Ru^{II}(Aha)(bpy) which illustrates that the two seven atom Ahx spacers of Aha are conformationally flexible enough for the two peptide-based bipyridines to bind Ru(II) along two meridonal planes.

As simple surrogates for longer peptide segments, each

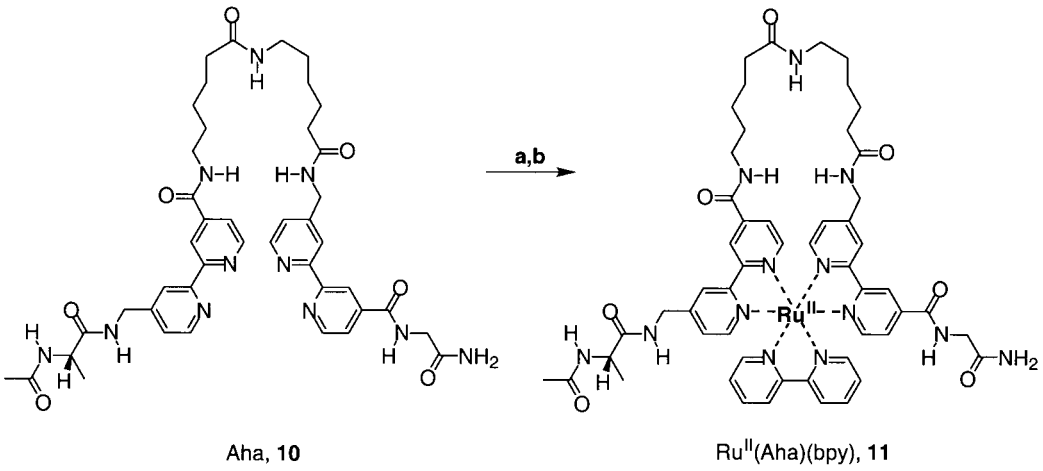


Figure 3. Reagents and conditions: (a) $RuCl_2(DMSO)_4$, EtOH/*i*PrOH, Δ; (b) 2,2′-bipyridine, EtOH/*i*PrOH/H_2O, Δ.

terminal segment was designed to contain a single amino-acid residue. In order to minimize their chemical reactivity, the amino group of the N-terminal L-alanine residue was blocked as the acetamide (Ac–Ala) and the carboxylic acid group of the C-terminal glycine residue was blocked as the carboxamide (Gly–NH_2). The C-terminal Gly residue was included to facilitate attachment of the bipyridine **8** or **9** to the sterically hindered methylbenzhydrylamine (MBHA) resin since direct coupling of Abc-OH to MBHA resin proved sluggish (data not shown). Lastly, the N-terminal Ala residue was included to distinguish it from the C-terminal Gly residue by NMR. The chiral α-carbon atom of the L-alanine residue provides a second chiral site in addition to the Δ or Λ chirality of the octahedrally ligated metal complex. Therefore it was predicted that the (Δ,Λ) and (Λ,L) diastereomers of the geometric isomers of metallopeptide Ru^{II}(Aha)(bpy) might be separable chromatographically.

The acetylated hexapeptide amide Aha (**10**) was prepared by the Boc/TFA strategy from Boc-Abc-OH and other Boc-amino acids using conventional reagents and procedures for manual solid-phase peptide synthesis. Coupling times and yields of **8** to the Gly-MBHA resin were markedly improved (from 43% over 16 h to 87% over 4 h) by addition of stoichiometric amounts of the acylation catalyst 4-(dimethylamino)pyridine (DMAP).[8] Each coupling step was monitored for completeness by quantitative ninhydrin analysis.[29] Following assembly, apopeptide **10** was cleaved from the resin with anhydrous HF and purified to homogeneity by solid-phase extraction.

Synthesis of the caged metallohexapeptide Ru^{II}(Aha)(bpy)

Ruthenium(II) was selected as the most suitable metal ion to form a complex with apopeptide **10** due to its inertness to ligand exchange and its potential use as a redox-active chromophore for the study of excited-state electron and energy transfer processes in peptides (Fig. 3).[1,2,8,30,31] Initial attempts to prepare the bis-bipyridyl complex Ru^{II}(Aha)Cl_2 via reductive complexation of $Ru^{III}Cl_3$ under standard conditions ($Ru^{III}Cl_3$, DMF, TEA, LiCl, reflux) resulted in significant thermal decomposition of **10**. However, this was avoided by performing the complexation reaction in lower boiling alcohol blends using the labile complex $Ru^{II}Cl_2(DMSO)_4$ as the ruthenium(II) source.[32] Subsequent conversion of Ru^{II}(Aha)Cl_2 to the heteroleptic tris(bipyridyl) complex Ru^{II}(Aha)(bpy) (**11**) was performed in the same reaction vessel by subsequent addition of 2,2′-bipyridine. The onset of an MLCT absorbance band at ~465 nm for **11** served as a convenient spectroscopic probe to monitor the reaction progress.

Bridging two of the bipyridine (bpy) groups in a $Ru^{II}(bpy)_3$ complex by a tether of restricted length was expected to preclude the formation of *trans*-substituted facial complexes and encourage the formation of *cis*-bridged meridonal geometric isomers due to the restricted orientation of the bridged ligands. Fig. 4 shows three possible *cis*-geometric isomers of (Δ,L)-Ru^{II}(Aha)(bpy) and one impossible *trans*-isomer. The four pyridine rings of Aha are numbered sequentially from the N-terminus to the C-terminus. Isomers A(=A′), B(=B′), and C(=C′) each have a *cis* bridge between pyridine rings 2 and 3. The Ahx–Ahx bridge in Aha is 14 atoms long, which is sufficiently long to form the *cis* bridges of isomers A, B, and C but too short to form the *trans* bridge in isomer D(=D′). Thus isomers A, B, and C are geometrically possible but D is not. Each of these three (Δ,L) isomers will be accompanied by the corresponding (Λ,L) isomer, which differs only in the chiral twist of the bpy ligands around the ruthenium atom. So six isomers of Ru^{II}(Aha)(bpy) are predicted, namely (Δ,L)-A, (Λ,L)-A, (Δ,L)-B, (Λ,L)-B, (Δ,L)-C, and (Λ,L)-C. Because the L-alanine residue of Aha is chiral, the diastereomeric pair (Δ,L)-A and (Λ,L)-A are not mirror images.

Purification and characterization of four isomers of the metallohexapeptide Ru^{II}(Aha)(bpy)

Analysis of the metal complexation reaction mixture by reversed-phase HPLC and mass spectrometry (MS) showed that six closely eluting isomers of Ru^{II}(Aha)(bpy) formed, in accordance with prediction. They were named **11a–f** to denote their increasing elution order on analytical reversed-phase HPLC. By FAB-MS, each of these isomers

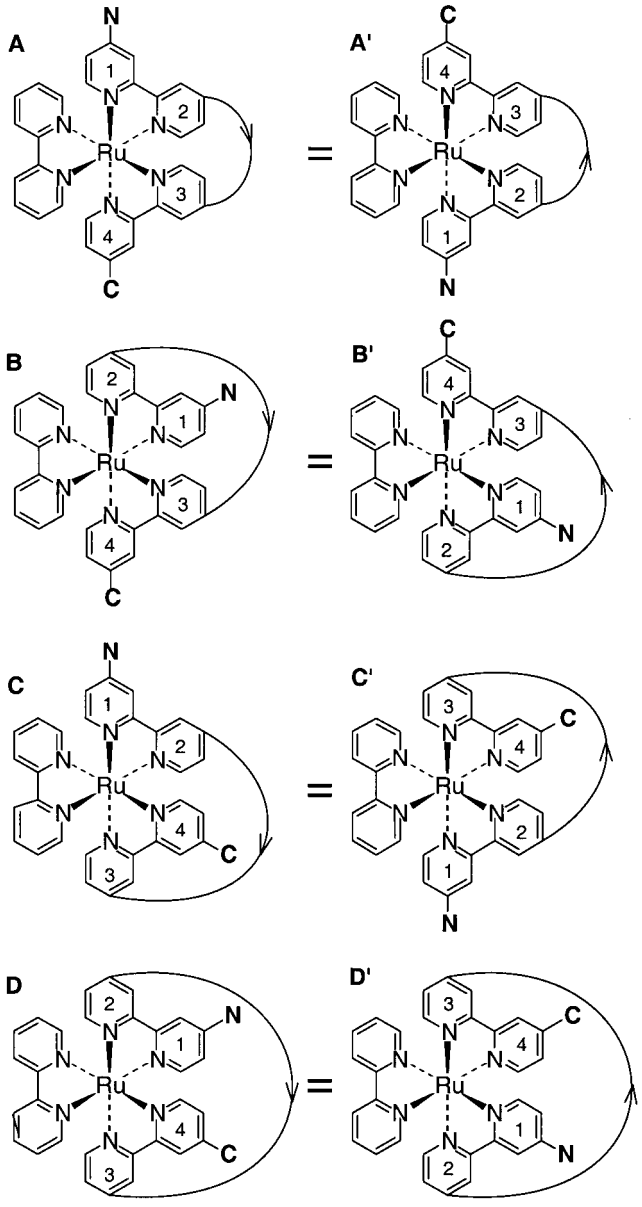


Figure 4. Geometric isomers of (Δ,L)-Ru^{II}(Aha)(bpy). The four pyridine rings of Aha are numbered from N to C. By rotation around a horizontal symmetry axis, isomer A=A′, B=B′, and C=C′. The Ahx–Ahx bridge in Aha is long enough to form the *cis* bridges of isomers A, B, and C but too short to form the *trans* bridge of isomer D=D′.

gave the expected mass for the desired metallopeptide Ru^{II}(Aha)(bpy). In addition, a trace amount of $Ru^{II}(bpy)_3$ was observed. Of the six isomers of Ru^{II}(Aha)(bpy) detected, four (**11a–d**) comprised ~95% of the total material, and only minor amounts (<5%) of two additional isomers (**11e–f**) were detected. The product distribution for isomers **11a–d** was 2:3:1:1, respectively. Preparatively, isomers **11a–b** eluted together, as did isomers **11c–d**. However, **11a**,**b**,**c** and **d** were separately isolable following repeated preparative reversed-phase HPLC. Of the six possible *cis*-bridged diastereomeric isomers predicted, only four *cis*-meridonal isomers of Ru^{II}(Aha)(bpy) formed in substantial quantities. Conformational constraints imposed by the restricted bridging sequence of Aha coupled with intramolecular templating encountered by the second coordinating bipyridine following initial metal capture is the likely origin of this isomer selectivity. Similar examples of chiral biasing of octahedral metal complex assembly have recently been observed in systems where bipyridine ligands have been covalently attached to conformationally constrained non-peptide template molecules.[33–36] A more rigid peptide scaffold or one that incorporates three bipyridines for tripodal hexadentate coordination might be expected to impart even more dramatic geometric isomer biasing during metal coordination.

The UV/visible absorption spectra for each of the four Ru^{II}(Aha)bpy isomers **11a–d** in water were nearly identical and typical of heteroleptic tri(bipyridyl)ruthenium(II) complexes.[37,38] The visible and near-UV regions of the spectra were dominated by MLCT bands arising from $d\pi \rightarrow \pi^*$ transitions to the lowest lying π^* acceptor levels localized on one of the three bipyridyl ligands. Two of the three ligands contain C- and N-terminal amide groups

Table 2. Proton assignments from the ^{1}H NMR spectra (500 MHz, D_2O) for the early-eluting stereoisomers **11a** and **11b** of the metallopeptide Ru^{II}(Aha)(bpy)$(Tfa)_2$ (Abbreviations: Aha=CH_3CO-Ala-Abc-Ahx-Ahx-Abc-Gly-NH_2; bpy=b; A=Ala; B=Abc; X=Ahx; G=Gly; Tfa=CF_3CO_2. For multiplicity, b=broad; d=doublet; m=multiplet; p=pentet; q=quartet; s=singlet; t=triplet. For the numbering of the residues and the numbering or lettering of their atoms, see Fig. 2)

	Isomer **11a**			Isomer **11b**		
Proton	Chemical shift (δ, ppm)	Number of protons	Multiplicity (Hz)	Chemical shift (δ, ppm)	Number of protons	Multiplicity (Hz)
2B3	8.75	1	s	8.84	1	s
5B3	8.84	1	s	8.84	1	s
2B3′	8.53	1	s	8.53	1	s
B3	8.50	2	d 7.7	8.50	2	d 7.7
5B3′	8.43	1	s	8.43	1	s
b4	7.99–8.06	2	m	7.99–8.06	2	m
5B6	7.95	1	d 6.0	7.95	1	d 6.0
2B6	7.88	1	d 6.0	7.89	1	d 5.8
(2, 5)B6′	7.74–7.79	2	m	7.74–7.79	2	m
b6	7.67–7.71	2	m	7.67–7.71	2	m
5B5	7.64	1	d 6.0	7.64	1	d 6.0
2B5	7.53	1	d 5.9	7.53	1	d 5.9
(2, 5)B5′, b5	7.24–7.37	4	m	7.24–7.37	4	m
5Bα	4.55	2	s	4.56	2	s
2Bαb	4.48	1	d 15.7	4.49	1	d 16.8
2Bαa	4.42	1	d 15.7	4.42	1	d 16.8
1Aα	4.22	1	q 7.3	4.26	1	q 7.0
6Gα	4.12	2	s	4.12	2	s
3Xϵb	3.40–3.43	2	m	3.48	1	d 12, t 6
3Xϵa	–	–	–	3.63	1	d 13, t 6
4Xϵb	2.80	1	d 14.0, t 7.0	2.84	1	d 13.4, t 6.7
4Xϵa	2.75	1	d 13.5, t 6.7	2.68	1	d 13.8, t 7.0
4Xαb	2.19	1	d 13, t 7	2.19	1	d 13, t 7
4Xαa	2.17	1	d 13, t 7	2.17	1	d 13, t 7
3Xα	2.16	2	t 6.2	2.16	2	t 6.2
Ac	1.96	3	s	2.00	3	s
(3, 4)Xβ, 4Xδ	1.51–1.61	6	m	1.51–1.61	6	m
3Xδ	1.36–1.50	2	m	1.36–1.50	2	m
1Aβ	1.36	3	d 7.3	1.38	3	d 7.3
4Xγ	1.26	2	p 7.4	1.26	2	p 7.4
3Xγ	1.12–1.23	2	m	1.12–1.23	2	m

emanating from the 4 and 4′ position, the remaining bpy ligand contains no substituents at these positions. Since the bipyridyl units surrounding the metal center are of two general types the energy differences between the lowest lying π^* acceptor levels on the different bipyridyl ligands translate into overlapping MLCT bands which were broadly centered around 456 nm.

The proton resonances seen in the 500 MHz ^{1}H NMR spectra of **11a–d** are consistent with each of these four compounds being a different isomer of the metallopeptide Ru^{II}(Aha)(bpy). Using the structural abbreviations given in Fig. 2, the NMR assignments of these four isomers are shown in Table 2 (**11a**+**11b**) and 3 (**11c**+**11d**). These proton assignments were aided by reference to the ^{1}H NMR spectrum of the apopeptide **10**, which only exhibited geminal coupling for the benzylic protons of Abc2 and for the ϵ-methylene protons of Ahx3. Each of the four isomers also exhibited geminal coupling for the α-methylene protons of Ahx4. In addition, the **11a** and **11b** isomers showed additional geminal coupling for the ϵ-methylene protons of Ahx4. These results suggest that the central tetrapeptide (Abc-Ahx-Ahx-Abc) of the Aha ligand is relatively rigid and that its main-chain geometry is restricted by coordination to the ruthenium atom. In general, these ^{1}H NMR spectra show that **11a** and **11b** are nearly identical, as are **11c** and **11d**, but **11a/b** exhibit distinctly different resonances than do **11c/d** and thus appear to be each one of the (Δ,L)/(Λ,L) diastereomers (Fig. 4). X-Ray crystallographic studies are in progress to determine the absolute configuration of these metallopeptide complexes. Results from the electrochemical and photophysical characterization of complexes **11a–d** will be published elsewhere.

Conclusions

The novel bipyridyl amino acid, 4′-aminomethyl-2,2′-bipyridyl-4-*c*arboxylic acid (**Abc**), and related Boc- and Fmoc-protected derivatives were synthesized to provide high-affinity bidentate metal-binding amino acid modules for the solid-phase peptide synthesis (SPPS) of metallopeptides. To demonstrate its utility in SPPS, two Abc residues were incorporated into the hexapeptide Aha (**10**). Although the Abc sidechain pyridyl nitrogens were left unprotected during SPPS, no side reactions were observed during peptide coupling, deprotection, or cleavage stages. This peptide was subsequently used as a tetradentate ligand to octahedrally coordinate and asymmetrically encapsulate a ruthenium(II) ion, creating a novel peptide-caged metal complex Ru^{II}(Aha)(bpy) (**11**). Though in solution the bridging sequences between ligating Abc residues in Aha are conformationally flexible, Aha induced asymmetric

octahedral ruthenium(II) complexation, creating diastereoisomers of **11** which could be easily resolved by standard HPLC methods. Thus in one reaction a family of isomeric ruthenium(II)-metallopeptides can be easily generated and separated for comparative photophysical analyses. Metallopeptides prepared by SPPS with Abc (**5**) have real potential as initiators of protein folding, electron/energy transfer probes, inducers of asymmetric catalysis, or site-specific DNA intercalation agents.

Experimental

Materials and methods

4′-Methyl-2,2′-bipyridine-4-carboxylic acid (Mbc) was prepared from 4,4′-dimethyl-2,2′-bipyridine (GFS Chemicals) according to literature procedures.[8] Methylbenzydrylamine-*copoly*-(styrene-1% divinylbenzene) (MBHA resin), Boc-Ahx-OH, Boc-Ala-OH, and Boc-Gly-OH, and the peptide coupling reagents (1-benzotriazoleoxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and (1-benzotriazoleoxy)tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) were purchased from Advanced Chemtech. All other chemicals and solvents were purchased reagent-grade from Aldrich and used without further purification. Melting points were determined using a Thomas–Hoover apparatus and are uncorrected. Analytical and preparative HPLC were performed with a Rainin Dynamax chromatograph monitored at 230 nm. Octadecyl-silica (Vydac C18) was used both analytically (4.6 mm×250 mm column eluted at 1.0 mL/min) and preparatively (12.5 mm×250 mm column eluted at 5.0 mL/min). ^{1}H NMR spectra were recorded with a Bruker AC200, WM250, or Bruker AMX500 spectrometer; ^{13}C NMR were recorded on a Varian XL400 spectrometer. Molecular modeling experiments were performed using Cache v3.0 modeling software on a Macintosh PowerPC. Prior to energy minimization, the six bonds between ligating nitrogen atoms and the ruthenium(II) atom were set at 2.055 Å and each amide bond was initially set in the *trans* conformation. High-resolution mass spectra were obtained by fast-atom bombardment with a JEOL HX110HF mass spectrometer by the Mass Spectrometry Laboratory for Biotechnology at North Carolina State University. Other mass spectra were recorded with a Finnigan MAT double-focusing FAB mass spectrometer by the laboratory of Professor Gary Glish, University of North Carolina at Chapel Hill. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

4′-Formyl-2,2′-bipyridine-4-carboxylic Acid (3). A 1 L three-neck round bottom flask equipped with a mechanical stirrer, argon inlet, and reflux condenser was charged with 4′-methyl-2,2′-bipyridine-4-carboxylic acid[8,18] (**1**, 4.00 g, 18.7 mmol) and dioxane (500 mL). Sand (15 g) followed by selenium dioxide (10.37 g, 93.5 mmol, 5.0 equiv.) were added and the mixture heated at a reflux under an argon atmosphere for four days. The mixture was filtered hot though a pad of diatomaceous earth (Celite), washed with dioxane (200 mL), and the solvent was removed by rotary evaporation. The resulting residue was dried overnight under vacuum to afford crude **3** as a solid (3.86 g, 65% yield by NMR) which was used for the following reaction without further purification: ^{1}H NMR (200 MHz, $(CD_3)_2SO$) δ 7.91 (dd, 1H, $J_{5',6'}$=4.8 Hz, $J_{3',5'}$=1 Hz, H-5′), 7.94 (dd, 1H, $J_{5,6}$=4.8 Hz, $J_{3,5}$=1 Hz, H-5), 8.83 (bs, 1H, H-3′), 8.85 (bd, 1H, $J_{3,5}$=1 Hz, H-3), 8.94 (dd, 1H, $J_{5',6'}$=4.8 Hz, $J_{3',6'}$=1 Hz, H-6′), 9.02 (d, 1H, $J_{5,6}$=4.9 Hz, H-6), and 10.22 ppm (s, 1H, CH═O).

4′-((Hydroxyimino)methyl)-2,2′-bipyridine-4-carboxylic acid (4). Solid hydroxylamine hydrochloride (3.86 g, 55.5 mmol) was added to a solution of crude **3** (3.86 g, 16.9 mmol) in ethanol (30 mL) and pyridine (30 mL). The solution was stirred at a reflux for 2 h under an argon atmosphere, then filtered hot through a Celite pad. The pad was washed with hot pyridine followed by ethanol, with the organics combined and the solvent was removed by rotary evaporation. The remaining solid was vacuum dried overnight, suspended in dilute aqueous TFA (pH 3.8, 35 mL) with sonication, filtered again, washed with aqueous HCl (pH 3), and dried under vacuum overnight to afford **4** as a solid (3.12 g) which was used for the subsequent reaction without further purification: ^{1}H NMR (200 MHz, $(CD_3)_2SO$) δ 7.65 (d, 1H, $J_{5',6'}$=5.1 Hz, H-5′), 7.90 (d, 1H, $J_{5,6}$= 4.9 Hz, H-5), 8.61 (s, 1H, H-3′), 8.35 (s, 1H, CH═N), 8.74 (d, 1H, $J_{5',6'}$=5.0 Hz, H-6′), 8.83 (s, 1H, H-3), and 8.89 ppm (d, 1H, $J_{5,6}$=5.1 Hz, H-6).

4′-Aminomethyl-2,2′-bipyridine-4-carboxylic acid hydrochloride (Abc·HCl, 5). Under an argon atmosphere, a 250-mL Parr hydrogenation vessel containing 10% Pd-on-carbon (1.04 g) was charged with a solution of **4** (3.12 g, 12.83 mmol) in ethanol (80 mL). Water (80 mL) and 12 N HCl (3.6 mL) were added and the mixture was hydrogenated at 40 psi for 16 h. After filtration through Celite, propylene oxide (4 mL) was added and the solution was heated at a reflux under Ar for 1 h. The solvent was reduced to half volume by rotary evaporation, then lyophilized. The remaining solid was dissolved in water (50 mL), titrated to pH 3 with 1.2 N HCl, and purified by solid-phase extraction using Waters Sep-Pack octadecyl-silica media (20 g) prewashed with acetonitrile and aqueous HCl (pH 3). After sample application, the desired compound was eluted with aqueous HCl (pH 3) over five 50-mL portions. Lyophilization afforded pure Abc·HCl (**5**) as a light pink solid (2.14 g, 8.00 mmol): mp 200–203°C (dec); ^{1}H NMR (200 MHz, 2:1 (v/v) D_2O/CF_3CO_2D) δ 4.68(s, 2H, CH_2), 8.16 (d, 1H, $J_{5',6'}$=5 Hz, H-5′), 8.33 (d, 1H, $J_{5,6}$=5 Hz, H-5), 8.81 (s, 1H, H-3′), 8.90 (s, 1H, H-3), 9.02 (d, 1H, $J_{5',6'}$=6 Hz, H-6′), and 9.08 ppm (d, 1H, $J_{5,6}$=5 Hz, H-6); ^{13}C NMR (400 MHz, D_2O) δ 41.7, 121.4, 121.6, 124.5, 124.7, 145.2, 146.8, 148.0, 149.0, 151.7, 152.0, and 170.0 ppm; MS (calcd for $C_{12}H_{11}N_3O_2$: 229.0851 Da) 229.0849 Da; anal. (calcd for $C_{12}H_{12}ClN_3O_2$: C 54.25, H 4.55, N 15.81) C 53.90, H 4.78, N 15.75.

4′-(1,1-Dimethylethoxycarbonylaminomethyl)-2,2′-bipyridine-4-carboxylic acid (6). Solid Abc·HCl (0.500 g, 1.89 mmol) suspended in 1.0 N NaOH (12 mL) and dioxane (2 mL) was cooled to 0°C in an ice bath. A solution of di(*tert*-butyl)dicarbonate (1.231 g, 5.64 mmol) in dioxane (10 mL) was added in one portion, the ice bath was removed, and the mixture was stirred under argon for 16 h. Dioxane was removed by rotary evaporation and the

aqueous solution was extracted with EtOAc (2×5 mL). The EtOAc washes were combined and extracted with 1.0 N NaOH (2×5 mL). The aqueous washes were combined, acidified to pH 4 with 1.2 N HCl, and extracted with EtOAc (5×30 mL). The organics were dried over anhydrous Na_2SO_4 and solvent was removed by rotary evaporation. The remaining residue was suspended in isopropanol (6 mL), filtered, and the desired product was precipitated from solution by the subsequent addition of hexanes (60 mL). The precipitate was filtered and vacuum dried to provide pure Boc-Abc (**6**, 0.482 g, 1.47 mmol, 78% yield): mp 180°C (dec); ^{1}H NMR (200 MHz, $(CD_3)_2SO$) δ 1.40 (s, 9H, $(CH_3)_3C$), 4.24 (d, 1H, J_{CH2-NH}=6.7 Hz, CH_2), 7.31 (dd, 1H, $J_{5',6'}$=5.0 Hz, $J_{3',5'}$=1.2 Hz, H-5′), 7.61 (t, 1H, J_{CH2-NH}=6.4 Hz, N-H), 7.84 (dd, 1H, $J_{5,6}$=5.2 Hz, $J_{3,5}$=1.6 Hz, H-5), 8.32 (bs, 1H, H-3′), 8.63 (d, 1H, $J_{5',6'}$=5.0 Hz, H-6′), 8.79 (d, 1H, $J_{5,6}$=5.4 Hz, H-6), and 8.82 ppm (bs, 1H, H-3); ^{13}C NMR (400 MHz, $(CD_3)_2SO$) δ 28.2, 42.8, 78.2, 118.6, 119.9, 122.5, 123.3, 143.5, 149.3, 149.7, 150.0, 155.0, 155.8, 166.5, and 166.9 ppm; MS (calcd for $C_{17}H_{19}N_3O_4$: 329.1375 Da) 329.1390 Da; anal. (calcd for $C_{17}H_{19}N_3O_4$: C 62.00, H5.81, N12,76) C 61.89, H 5.84, N 12.71.

Bis(2,2′-bipyridine)(4′-(1,1-dimethylethoxycarbonylaminomethyl)-2,2′-bipyridine-4-carboxylic acid)ruthenium(II) bis(hexafluorophosphate) (8). Solid Boc-Abc (100 mg, 0.29 mmol) and *cis*-dichlorobis(2,2′-bipyridine)-ruthenium(II) (126 mg, 0.24 mmol) were suspended in methanol (15 mL). The dark-purple reaction mixture was heated at a reflux under argon overnight with stirring. The resulting dark-orange solution was cooled to room temperature and solvent was removed by rotary evaporation. The resulting solid was dissolved in water (15 mL) and filtered. The filtrate was cooled in an ice bath and acidified to ~pH 3 with 1% aqueous HPF_6. Saturated aqueous NH_4PF_6 (~0.5 mL) was added and the orange precipitate which formed was collected on a medium porosity glass fritted funnel, washed with purified water (three 1 mL portions), and dried overnight in a vacuum desiccator to yield pure Boc-Abc$(Rub_2)\cdot(PF_6)_2$ (**8**, 198 mg, 0.19 mmol, 79% yield) as a bright orange solid: ^{1}H NMR (250 MHz, $(CD_3)_2SO$) δ 1.39 (br s, 9H, $(CH_3)_3C$), 4.387 (brs, 2H, Abc-CH_2), 7.360 (d, 1H, J=5.5 Hz, Abc-5′), 7.42–7.57 (m, 4H, b-5), 7.595 (t, 1H, J=5.8 Hz, N–H), 7.704 (brd, 4H, J=5.7 Hz, b-6), 7.777 (d, 1H, J=5.1 Hz, Abc-6′), 7.846 (d, 1H, J=5.9 Hz, Abc-5), 7.913 (d, 1H, J=5.9 Hz, Abc-6), 8.174 (brt, 4H, J=7.6 Hz, b-4), 8.836 (d, 4H, J=8.0 Hz, b-3), 8.916 (s, 1H, Abc-3′), and 9.020 ppm (s, 1H, Abc-3); anal. (calcd for $C_{37}H_{35}N_7O_4F_{12}P_2Ru$: C 43.03, H3.42, N9.53) C 43.13, H 3.46, N 9.50.

4′-(9-Fluorenylmethoxycarbonylaminomethyl)-2,2′-bipyridine-carboxylic acid (7). A solution of Abc·HCl (0.375 g, 1.42 mmol) in 10% Na_2CO_3 (5 mL) and dioxane (1 mL) was cooled to 0°C with stirring. A solution of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (0.884 g, 2.62 mmol) in dioxane (2 mL) was added in one portion, the ice bath was removed, and the mixture stirred under argon for 2 h. Dioxane was removed by rotary evaporation and the residue was acidified to pH 4 with 1.2 N HCl. The precipitate which formed was collected by filtration, washed with water, and dried under vacuum overnight to afford solid Fmoc-Abc-OH (**7**) and the related salt Fmoc-Abc-ONa in a 4:3 molar ratio (0.596 g, 1.25 mmol, 88% yield): mp 171°C (dec); ^{1}H NMR (250 MHz, $(CD_3)_2SO$) δ 4.25 (t, 1H, J_{CH-CH2}=7.3 Hz, Fmoc H-9), 4.31 (d, 2H, J_{CH2-NH}=5.8 Hz, Abc CH_2), 4.37 (d, 2H, J_{CH-CH2}=7.6 Hz, Fmoc-CH_2), 7.25 (d, 1H, $J_{5',6'}$~4.5 Hz, H-5′), 7.30 (t, 2H, J=7.6 Hz), 7.40 (t, 2H, J=7.3 Hz), 7.71 (d, 2H, J=7.6 Hz), 7.78 (d, 1H, $J_{6,5}$=5.1 Hz, H-5), 7.88 (d, 2H, J=7.7 Hz), 8.10 (t, 1H, J_{CH_2}–NH=6.2 Hz, N-H), 8.33 (s, 1H, H-3′), 8.61 (d, 1H, $J_{5',6'}$=4.8 Hz, H-6′), 8.65 (d, 1H, $J_{5,6}$=4.8 Hz, H-6), and 8.80 ppm (s, 1H, H-3); ^{13}C NMR (400 MHz, $(CD_3)_2SO$) δ 40.2, 43.1, 46.8, 65.6, 118.6, 120.2, 120.3, 122.3, 123.6, 125.2, 127.1, 127.7, 140.8, 143.9, 146.8, 149.2, 149.9, 155.4, 155.6, 156.5, 167.7, and 176.9 ppm; negative-ion MS (calcd for $C_{27}H_{20}N_3O_4$ (Fmoc-Abc-O$^-$anion): 450.1454 Da) 450.1456 Da; anal. (calcd for $(Fmoc\text{-}Abc\text{-}OH)_4\cdot(Fmoc\text{-}Abc\text{-}ONa)_3\cdot(H_2O)_9$: C 63.78, H 4.91, N 8.26, Na 3.39) C 63.79, H4.73, N 8.33, Na 3.52.

Bis(2,2′-bipyridine)(4′-(9-fluorenylmethoxycarbonylaminomethyl)-aminomethyl-2,2′-bipyridine-4-carboxylic acid) ruthenium(II) bis(hexafluorophosphate) (9). Solid Fmoc-Abc (100 mg, 0.21 mmol) was dissolved in dioxane (2 mL) and diluted with 70% ethanol/water (10 mL). Solid *cis*-dichlorobis(2,2′-bipyridine)ruthenium(II) (91 mg, 0.18 mmol) was added and the dark purple reaction mixture was heated at a reflux for 16 h under an argon atmosphere. The resulting red–orange solution was cooled to temperature, and the ethanol and dioxane were removed by rotary evaporation. Water (10 mL) was added and the solution was filtered through a medium porosity glass fritted funnel. The filtrate was cooled in an ice bath, acidified to ~pH 3 with aqueous 1% HPF_6 and treated with saturated aqueous NH_4PF_6 (~0.5 mL). The orange solid which precipitated was collected on a medium frit funnel, washed with water (2×1 mL), and vacuum dried to afford Fmoc-Abc$(Rub_2)\cdot(PF_6)_2$ (**9**, 186 mg, 0.16 mmol, 85% yield): ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 4.215 (t, 1H, J=6.1 Hz, Fmoc-CH), 4.38–4.44 (m, 4H, Abc-CH_2 and Fmoc-CH_2), 7.16–7.29 (m, 3H), 7.340 (brq, 2H, J=7.6 Hz), 7.474 (t, 1H, J=6.6 Hz), 7.49–7.58 (m, 2H), 7.782 (d, 1H, J=5.9 Hz, Abc-6′), 7.847 (d, 1H, J=5.8 Hz, Abc-5), 7.862 (d, 2H, J=6.8 Hz), 7.900 (d, 1H, J=5.7 Hz, Abc-6), 7.986 (t, 1H, J=5.9 Hz, N–H), 8.12–8.22 (m, 4H, b-4), 8.82–8.88 (m, 4H, b-3), 8.953 (s, 1H, Abc-3′), and 9.023 ppm (s, 1H, Abc-3); MS ((M+2) calcd for $C_{47}H_{37}N_7O_4Ru$: 433 Da) 433 Da; anal. (calcd for $C_{47}H_{37}N_7O_4$ $F_{12}P_2Ru$: C 48.87, H 3.23, N 8.52) C 48.55, H 3.48, N 8.22.

Ac-Ala-Abc-Ahx-Ahx-Abc-Gly-NH_2 (Aha, 10). This acetylated hexapeptide amide was assembled on MBHA resin (0.62 mmol/g, 0.5 mmol) using manual solid-phase methods. Boc-Gly-OH, Boc-Ahx-OH, and Boc-Ala-OH (each 2 mmol, 4 equiv.) were coupled for 1 h using the coupling agent BOP (4.4 equiv.), *N*-methylmorpholine (NMM, 8.8 equiv.), and 1-hydroxybenzotriazole (HOBt, 4.4 equiv.). Boc-Abc-OH (1.5 equiv.) was coupled for 15 h by using the acylation catalyst DMAP (1.5 equiv.), the coupling agent PyBOP (2.0 equiv.), NMM (3.2 equiv.), and HOBt (2.0 equiv.). Coupling efficiency was monitored by ninhydrin analysis.[29] The Ala-Abc-Ahx-Ahx-Abc-Gly-NH-resin was acetylated on resin with Ac_2O/NMM/CH_2Cl_2 (4:1:4, v/v) then cleaved from the support with anhydrous HF (10 mL, 1 h, 4°C) in the absence of

Table 3. Proton assignments from the ^{1}H NMR spectra (500 MHz, D_2O) for the late-eluting stereoisomers **11c** and **11d** of the metallopeptide Ru^{II}(Aha)(bpy)$(Tfa)_2$ (Abbreviations: Aha=CH_3CO-Ala-Abc-Ahx-Ahx-Abc-Gly-NH_2; bpy=b; A=Ala; B=Abc; X=Ahx; G=Gly; Tfa=CF_3CO_2. For multiplicity, b=broad; d=doublet; m=multiplet; p=pentet; q=quartet; s=singlet; t=triplet. For the numbering of the residues and the numbering or lettering of their atoms, see Fig. 2)

	Isomer **11a**			Isomer **11b**		
Proton	Chemical shift (δ, ppm)	Number of protons	Multiplicity (Hz)	Chemical shift (δ, ppm)	Number of protons	Multiplicity (Hz)
2B3	8.80	1	s	8.77	1	s
5B3	8.79	1	s	8.79	1	s
2B3′	8.49	1	s	8.49	1	s
5B3′	8.40	1	s	8.40	1	s
b3	8.52	2	d 8.4	8.52	2	d 8.4
b4	8.03	2	bt 8.1	8.03	2	bt 8.1
2B6	7.94	1	d 4.7	7.93	1	d 4.6
5B6	7.81	1	d 5.6	7.81	1	d 5.6
2B6′	7.78–7.80	1	bm	7.78	1	bm
b6, 5B6′	7.69–7.73	3	bm	7.69–7.73	3	bm
2B5	7.50	1	d 4.4	7.49	1	d 4.5
5B5	7.66	1	d 5.9	7.66	1	d 5.9
2B5′	7.37	1	d 6.0	7.37	1	d 6.0
5B5′	7.27	1	d 5.6	7.26	1	d 5.6
b5	7.34	2	d 7.2, d 6.0	7.34	2	d 7.2, d 6.0
5Bα	4.54	2	bs	4.54	2	bs
2Bαb	4.44	1	d 16.1	4.44	1	d 16.1
2Bαa	4.41	1	d 16.1	4.41	1	d 16.1
1Aα	4.22	1	q 7.0	4.20	1	q 7.0
6Gα	4.09	2	s	4.09	2	s
3Xϵ	3.34–3.50	2	m	3.34–3.50	2	m
4Xϵ	2.64–2.74	2	m	2.46–2.56	2	m
4Xαb	2.38	1	d 14.2, t 7.1	2.38	1	d 14.2, t 7.1
4Xαa	2.30	1	d 14.2, t 7.1	2.30	1	d 14.2, t 7.1
3Xα	2.03–2.15	2	m	2.03–2.15	2	m
Ac	1.98	3	s	1.96	3	s
(3, 4)Xβ, (3, 4)Xδ	1.40–1.65	8	m	1.40–1.65	8	m
1Aβ	1.36	3	d 7.3	1.36	3	d 7.2
4Xγ	1.08–1.20	2	m	1.08–1.20	2	m
3Xγ	1.20–1.28	2	m	1.20–1.28	2	m

scavengers. Cold diethyl ether was added (30 mL) and the precipitated peptide/resin mixture was transferred to a fritted glass filter, washed with cold diethyl ether (5× 30 mL), then extracted into 10% acetic acid (4×30 mL) and water (4×30 mL). The combined aqueous extracts were lyophilized to afford pure Aha (**10**, 120 mg, 0.14 mmol) as determined by analytical HPLC and quantitative amino-acid analysis (97%): ^{1}H NMR (500 MHz, 4:1 (v/v) D_2O/CF_3CO_2D) δ 1.65 (p, J=7.5 Hz, 2H, γ-Ahx), 1.71 (p, J=7.1 Hz, 2H, γ-Ahx), 1.79 (d, J=7.1 Hz, 3H, β-Ala), 1.86 (p, J=7.0 Hz, 2H, δ-Ahx), 1.91–2.02 (m, 6H, δ-Ahx and β-Ahx), 2.38 (s, 3H, Ac), 2.71 (t, J=6.3 Hz, 2H, α-Ahx), 3.56 (t, J=6.9 Hz, 2H, ϵ-Ahx4), 3.72 (dt, J=13.4 Hz, J=7.0 Hz, 1H, ϵ-Ahx3), 3.78 (dt, J=13.4 Hz, J=7.0 Hz, 1H, ϵ-Ahx3), 4.50 (s, 2H, α-Gly), 4.70 (q, J=7.1 Hz, 1H, α-Ala), 5.06 (s, 2H, α-Abc5), 5.06 (d, J=18.0 Hz, 1H, α-Abc2), 5.12 (d, J=18.0 Hz, 1H, α-Abc2), 8.27 (d, J=6.1 Hz, 1H, 5-Abc), 8.28 (d, J=6.1 Hz, 1H, 5-Abc), 8.29 (d, J=5.1 Hz, 1H,5′-Abc), 8.35 (d, J=5.1 Hz, 1H,5′-Abc), 8.81 (s, 1H,3′-Abc), 8.85 (s, 1H,3′-Abc), 8.94 (s, 1H, 3-Abc), 9.01 (s, 1H, 3-Abc), 9.11 (d, J=6.1 Hz, 1H, 6-Abc), 9.12 (d, J=6.1 Hz, 1H, 6-Abc), 9.25 (d, J=5.1 Hz, 1H,6′-Abc), and 9.29 ppm (d, J=5.1 Hz, 1H, 6′-Abc); High-resolution FAB-MS (calcd for $C_{43}H_{53}N_{11}O_7Na$ (M−H+Na): 858.403 Da) 858.397 Da.

(Ac-Ala-Abc-Ahx-Ahx-Abc-Gly-NH_2)(2,2′-bipyridine)-ruthenium(II) bis(trifluoroacetate) (Ru^{II}(Aha)(bpy)-$(Tfa)_2$, 11). A solution of $RuCl_2((CH_3)_2SO)_4$[32] (12.7 mg, 0.026 mmol) in chloroform (0.78 mL) was added dropwise to a suspension of Aha (20 mg, 0.024 mmol) in 3:7 (v/v) isopropanol/ethanol (300 mL). The mixture was heated at a reflux under argon for four days, after which water (10 mL) and 2,2′-bipyridine (24.6 mg, 0.16 mmol) were added, and the mixture was heated at a reflux an additional 16 h under argon. Solvents were removed by rotary evaporation and the resulting red solid was purified by preparative reversed-phase HPLC with linear gradients of acetonitrile/water 0.1% TFA, yielding four major isomers of **11** (**11a**–**d**) and two minor isomers (**11e**–**f**, see text) each with FAB-MS (calcd for $C_{53}H_{61}N_{13}O_7Ru$ (M^+) 1093.4 Da) 1093.7 Da. The ^{1}H NMR data for compounds **11a**,**b** and **11c**,**d** in D_2O are reported in Tables 2 and 3, respectively.

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