JOC*Article*

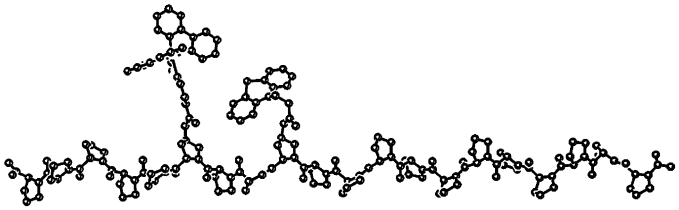
Synthesis and Characterization of Oligoproline-Based Molecular Assemblies for Light Harvesting

W. Steven Aldridge, III,[†] Brooks J. Hornstein,[‡] Scafford Serron,[†] Dana M. Dattelbaum,[‡] Jon R. Schoonover,[‡] and Thomas J. Meyer*,[†]

Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, and Los Alamos National Laboratory Los Alamos, New Mexico 87545

tjmeyer@unc.edu

Received February 24, 2006



Helical oligoproline arrays provide a structurally well-defined environment for building photochemical energy conversion assemblies. The use of solid-phase peptide synthesis (SPPS) to prepare four such arrays, consisting of 16, 17, 18, and 19 amino acid residues, is described here. Each array contains the chromophore $[Rub'_2m](PF_6)_2$ (b′ = 4,4′-diethylamidocarbonyl-2,2′-bipyridine; m = 4-methyl-2,2′-dipyridine-4′-carboxylic acid) and the electron transfer donor PTZ (phenothiazine). The arrays differ systematically in the distance between the redox-active metal complex and PTZ sites. They have been used in photophysical studies to provide insight into the distance dependence of electron transfer. (*J. Am. Chem. Soc*. **2004**, *126*, 14506−14514). This work describes the synthesis, purification, and characterization of the oligoproline arrays, including a general procedure for the synthesis of related arrays.

Introduction

Use of synthesis to control molecular structure is an essential element in the preparation of molecular assemblies for artificial photosynthesis. Controlled structures, such as those found in biological photosynthetic reaction centers, allow for the efficient separation of reduced and oxidized redox equivalents following visible excitation.[1−3] The synthesis of relevant models is important in the design of assemblies and in providing structures for probing fundamental mechanisms of electron and energy transfer.

Assemblies have been prepared based on porphyrins,[2] polymers,[3] dendrimers,[4] and peptides,[5] but the use of oligoprolines has special appeal due to the ease of synthesis based on sequential structures and the precise synthetic control provided

[†] University of North Carolina at Chapel Hill.

[‡] Los Alamos National Laboratory Los Alamos.

(1) (a) Alstrum-Acevedo, J. H.; Brennaman, K.; Meyer, T. J. *Inorg. Chem*. **2005**, *44*, 6802. (b) Minagawa, J.; Takahashi, Y. *Photosynthesis Res*. **2004**, *82*, 241−263. (c) Grotjohann, I.; Jolley, C.; Fromme, P. *Phys. Chem. Chem. Phys*. **2004**, *6*, 4743−4753. (d) Hang, M. H.; Dattelbaum, D. M.; Meyer, T. J. *Coord. Chem. Rev*. **2005**, *249*, 457−483.

(2) (a) Gust, D.; Moore, T. A. *Acc. Chem. Res*. **1993**, *26*, 198. (b) Li, F.; Yang, S. I.; Ciringh, Y.; Seth, J.; Martin, C. H. I.; Singh, D. L.; Kim, D.; Birge, R. R.; Bocian, J. D. F.; Holten, D.; Lindsey, J. S. *J. Am. Chem. Soc*. **1998**, *120*, 10001. (c) Collin, J.-P.; Harriman, A.; Heitz, V.; Odobel, J. F.; Sauvage, J.-P. *J. Am. Chem. Soc*. **1994**, *116*, 5679. (d) Hsiao, J.-S. Krueger, B. P.; Wagner, R. W.; Johnson, T. E.; Delaney, J. K.; Mauzerall, D. C.; Fleming, G. R.; Lindsey, J. S.; Ocean, D. F.; Donohoe, R. J. *J. Am. Chem. Soc*. **1996**, *118*, 11181. (e) Van Patten, P. G.; Shreve, A. P.; Lindsey, J. S.; Bonohoe, R. J. *J. Phys. Chem. B* **1998**, *102*, 4209.

(3) (a) Watkins, D. M.; Fox, M. A. *J. Am. Chem. Soc*. **1996**, *118*, 4344. (b) Kaschak, D. M.; Len, T. J.; Waraksa, C. C.; Saupe, G. B.; Usami, H.; Mallouk, T. E. *J. Am. Chem. Soc*. **1999**, *121*, 3435. (c) Webber, S. E. *Chem. Rev*. **1990**, *90*, 1469. (d) Guillet, J. *Polymer Photophysics and Photochemistry*; Cambridge University Press: Cambridge, U.K., 1985. (e) Martin, T. J.; Webber, S. E. *Macromolecules* **1995**, *28*, 8845 (f) Hisada, K.; Ito, S.; Yamamoto, M. *Langmuir* **1996**, *12*, 3682. (g) Dupray, L. M.; Devenney, M.; Striplin, D. R.; Meyer, T. J. *J. Am. Chem. Soc*. **1997**, *119*, 10243. (h) Yatsue, T.; Miyashita, T. *J. Phys. Chem*. **1995**, *99*, 16047. (i) Jones, W. E.; Baxter, S. M.; Strouse, G. F.; Meyer, T. J. *J. Am. Chem. Soc*. **1993**, *115*, 7363. (j) Baxter, S. M. Jones, W. E. Danielson, E.; Worl, L. A.; Strouse, G. R.; Youhnathan, J.; Meyer, T. J. *Coord. Chem. Rev*. **1991**, *111*, 47. (k) Youhnathan, J. N.; Jones, W. E.; Meyer, T. J. *J. Phys. Chem*. **1991**, *95*, 488. (l) Strouse, G. F.; Worl, L. A.; Youhnathan, J. N.; Meyer, T. J. *J. Am. Chem. Soc*. **1989**, *111*, 1901. (m) Olmsted, J. I.; McClanahan, S. F.; Daneilson, E.; Youhnathan, J. N.; Meyer, T. J. *J. Am. Chem. Soc*. **1987**, *109*, 3297.

10.1021/jo0603936 CCC: $33.50
Published on Web 06/03/2006

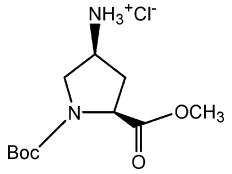


FIGURE 1. The modified proline monomer (Boc-Pra-OCH_3·HCl) that is coupled to $[Rub'_2m]^{2+}$ or PTZ and then incorporated into an oligoproline array.

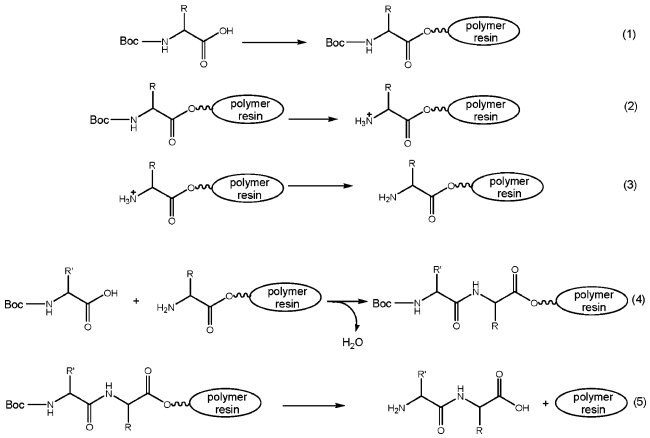
by solid-phase peptide synthesis (SPPS) techniques.[6] Attaching redox-active molecules to a modified amino acid monomer allows for the incorporation of the molecule into the oligomer backbone. This can have significant advantages over previous structural motifs in which the redox center is attached to the C- or N-terminus of the peptide.[7] Figure 1 shows the modified proline, Boc-Pra-OCH_3·HCl, used in this study. Once the redox-active molecules are coupled to the modified proline they are incorporated into the array with the use of SPPS techniques. This methodology results in a precisely controlled array that features a helical proline-II rod with the redox-active species extending from it.[8]

While the final product of this synthesis is similar (but not identical) to that of previous reports,[5a−e] this work provides details of the synthesis, purification, and characterization specifically for proline oligomers as well as the important precursors that are missing from the previous reports. In addition, this report brings together the synthetic techniques, with key references, for the construction of these and similar spatially oriented arrays built with the use of SPPS.

Results and Discussion

Synthesis of the oligoproline arrays was carried out in three phases: (1) synthesis of the modified proline monomer (Boc-Pra-OCH_3·HCl), (2) attachment of the redox-active modules [PTZ and $[Rub'_2m](PF_6)_2$] to the modified proline and, (3) building the array by using SPPS techniques. This strategy allows the precise control of the spatial relationship between the chromophore and the electron transfer donor, which is necessary for the systematic study of photoinduced electron transfer.[5a] Phases 1 and 2 of this synthetic strategy have been previously reported, but they are briefly mentioned here, with the appropriate references, for completeness. The remainder of this report will focus on the assembly and characterization of arrays **6**−**9**, for which there is little detailed information available.[5a]

SCHEME 1



Synthesis of the protected proline follows established procedures that are provided in the Experimental section for the interested reader.[5e,g,9,10] Syntheses of the chromophore salt $[Rub'_2m](PF_6)_2$[11,12,13] and the electron donor $PTZ(CH_2)_2COOH$[14] were carried out as described previously. Well-established methods using N,N′-dicyclohexylcarbodiimide, *N*-methylmorpholine, and 4-(dimethylamino)pyridine were used to couple the redox active molecules to the modified amino acid monomers.[5b,e,10] Finally, saponification of methyl ester monomers was carried out to give the corresponding carboxylic acids Boc-Pra$[Rub'_2m]$-OH and Boc-Pra$[PTZ(CH_2)_2]$-OH.[5b,e10]

Once the precursors were assembled and characterized (Supporting Information), the residues were manually linked to Boc-Pro-OH or Boc-Pro-Pro-OH in a predetermined sequence. This is efficiently carried out with SPPS procedures, which are outlined in Scheme 1.[6] The N-protected amino acid is first attached to an insoluble polymeric resin, in this case, a methylbenzhydrylamine polymer, as a benzyl ester. Once attached, the amino acid is deprotected, neutralized, and then coupled to the C-terminus end of another amino acid (steps 2−4

(4) (a) Balzani, V.; Campagna, S.; Denti, G.; Juris, A.; Serroni, S.; Venturi, M. *Acc. Chem. Res*. **1998**, *31*, 26. (b) Jiang, D.-L.; Aida, T. *Nature* **1997**, *388*, 454. (c) Devadoss, C.; Bharathi, P.; Moore, J. S. *J. Am. Chem. Soc*. **1996**, *118*, 9635. (d) Stewart, G. M.; Fox, M. A. *J. Am. Chem. Soc*. **1996**, *118*, 4354.

(5) (a) Serron, S. A.; Aldridge, W. S., III; Fleming, C. M.; Danell, R. M.; Baik, M.-H.; Sykora, M.; Dattelbaum, D. M.; Meyer, T. J. *J. Am. Chem. Soc*. **2004**, *126*, 14506−14514. (b) Striplin, D. R.; McCafferty, D. G.; Wall, C. G.; Friesen, D. A.; Erickson, B. W.; Meyer, T. J. *J. Am. Chem. Soc*. **2004**, *126*, 5282−5291. (c) Slate, C. A.; Binstead, R.; Meyer, T. J.; Erickson, B. W. *Lett. Pept. Sci*. **1999**, *6*, 61−69. (d) Slate, C. A.; Striplin, D. R.; Moss, J. A.; Chen, P. Y.; Erickson, B. W.; Meyer, T. J. *J. Am. Chem. Soc*. **1998**, *120*, 4885−4886. (e) McCafferty, D. G. Friesen, D. A.; Danielson, E.; Wall, C. G.; Sanderholm, M. J.; Erickson, B. W.; Meyer, T. J. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8200−8204. (f) McCafferty, D. G.; Bishop, B. M.; Wall, C. G.; Hughes, S. G.; Mecklenberg, S. L.; Meyer, T. J.; Erickson, B. W. *Tetrahedron* **1995**, 51, 1093−1106. (g) McCafferty, D. G.; Friesen, D. A.; Slate, C. A.; Nakhle, B. M.; Graham. H. D.; Austell, T. L.; Vachet, R. W.; Mullis, B. H. Erickson, B. W. *Tetrahedron* **1995**, *51*, 9859−9872. (h) Isied, S. S.; Vassilian, A.; Magnuson, R. H.; Schwarz, H. A. *J. Am. Chem. Soc*. **1985**, *107*, 7432. (i) Isied, S. S.; Vassilian, A. *J. Am. Chem. Soc*. **1984**, *106*, 1726.

(6) (a) Merrifield, B. *Science* **1986**, *232*, 341. (b)Stewart, J. M.; Young, J. D. *Solid-Phase Peptide Synthesis*, 2nd ed.; Pierce Chemical Co.: Rockford, IL, 1984.

(7) (a) Mishra, A. K.; Chandrasekar, R.; Faraggi, M.; Klapper, M. H. *J. Am. Chem. Soc*. **1994**, *116*, 1414−1422. (b) Ogawa, M. Y.; Moreira, I.; Wishart, J. F.; Isied, S. S. *Chem. Phys*. **1993**, *176*, 589−600. (c) Ogawa, M. Y.; Wishart, J. F.; Young Z.; Miller J. R.; Isied, S. S. *J. Phys. Chem*. **1993**, *97*, 11456−11463.

(8) (a) Traub, W.; Shmueli, U. *Nature* **1963**, *198*, 1165−1166. (b) Cowan, P. M.; McGavin, S. *Nature* **1955**, *176*, 501−503.

(9) (a) Webb, T. R.; Eigenbrot, C. *J. Org. Chem*. **1991**, *56*, 3016−3020. (b) Abraham, D. J.; Molotoff, M.; Sheh, L.; Simmons, J. E. *J. Med. Chem*. **1983**, *26*, 549−554.

(10) (a) Serron, S. A.; Aldridge, W. S., III; Danell, R. M.; Meyer, T. J. *Tetrahedron Lett*. **2000**, *41*, 4039−4042. (b) Hartshorn, C. M.; Maxwell, K. A.; White, P. S.; DeSimone, J. M.; Meyer, T. J. *Inorg. Chem*. **2001**, *40*, 601−606.

(11) (a) Omberg, K. M.; Smith, G. D.; Kavaliunas, D. A.; Chen, P.; Treadway, J. A.; Schoonover, J. R.; Palmer, R. A.; Meyer, T. J. *Inorg. Chem*. **1999**, *38*, 951. (b) Sasse, A. A. J. *Aust. J. Chem*. **1986**, *39*, 1053. (c) Sullivan, B. P.; Salmon, D. J.; Meyer, T. J. *Inorg. Chem*. **1978**, *17*, 3334.

(12) Smith, G. D.; Maxwell, K. A.; DeSimone, J. M.; Meyer, T. J.; Palmer, R. A. *Inorg. Chem*. **2000**, *39*, 893−898.

(13) Peek, B. M.; Ross, G. T.; Edwards, S. W.; Meyer, G. J.; Meyer, T. J.; Erickson, B. W. *Int. J. Pept. Protein Res*. **1991**, *38*, 114−123.

(14) (a) Hu, X.; Tierney, M. T.; Grinstaff, M. W. *Bioconjugate Chem*. **2002**, *13*, 83−89. (b) Tierney, M. T.; Grinstaff, M. W. 2000, 2, 3413−3416. (c) Godenfroi, E. K.; Wittle, E. L. *J. Org. Chem*. **1956**, *21*, 1163−1168. (d) Smith, N. L. *J. Org. Chem*. **1950**, *5*, 1125−1130.

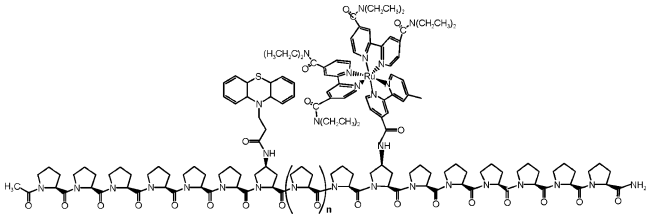


FIGURE 2. The linear structure of the assembled oligoproline arrays (for **6**, $n = 1$; for **7**, $n = 2$; for **8**, $n = 3$; and for **9**, $n = 4$).

in Scheme 1). The coupling reactions were carried out with 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-*N*-hydroxybenzotriazole (HOBt), dicyclohexylcarbodiimide (DCC), *N*,*N*-dimethylamonipyridine (DMAP), and *N*-methylmorpholine (NMM) as a base. Yields for the coupling reaction between unmodified prolines were between 97% and 99%, while those for the coupling reactions between proline and redox-active modified prolines were 20−30% for individual steps.

After the peptide is assembled, it is cleaved from the polymeric resin with a mixture of trifluoroacetic acid, thioanisole, 1,2-ethandiol, *m*-cresol, and trimethylsilyl bromide. In all cases, the nearly quantitative cleavage reaction was carried out at 0 °C for 1 h and the insoluble polymer resin was removed by filtration. The methods discussed above were used to synthesize arrays **6**−**9** which vary in the distance between the ruthenium(II) chromophore and the PTZ electron donor (Figure 2). The soluble peptide was then purified and characterized as described in the following section.

Alternatively, **7**−**9** can be synthesized from a common isolable intermediate, Boc-Pro_3-Pra(Rub'_2m)-Pro_6-NH-polymer resin. This intermediate was synthesized on a 0.3-mmol scale and was subsequently used in 0.1-mmol amounts for the synthesis of each of the larger peptides. Syntheses carried out with this intermediate resulted in products that were indistinguishable from those prepared without isolation of the common intermediate, as shown by HPLC and ESI-MS.

Each peptide was purified by size exclusion chromatography to remove any salts remaining from the cleavage procedure followed by semipreparative reversed-phase HPLC on an octadecylsilicate column. The effectiveness of this purification procedure is demonstrated in chromatograms of the crude and purified material (Figures S1−S4 of the Supporting Information). After the peptides were purified by semipreparative HPLC, they were characterized by electrospray ionization mass spectrometry (ESI-MS). In each case the expected molecular ion was observed. Mass spectra for **6**−**9** are shown in Figures S5−S8 of the Supporting Information.

The helical structure of the oligoprolines was investigated with far-UV circular dichroic spectroscopy. It is a useful tool for distinguishing a proline-I helix, which has *cis*-peptide bonds and is right-handed, from a proline-II helix, which has *trans*-peptide bonds and is left-handed.[8b,15a,16] The CD spectrum of each peptide (Supplementary Figure 2, published elsewhere)[5a] shows a strong negative band near 206 nm and a weak positive band near 226 nm, which is consistent with a proline-II helix.[5a,f,15] The spectrum for the shortest peptide (**6**) is slightly red-shifted compared to the larger oligoprolines, which is likely due to a steric interaction between the Ru chromophore and the PTZ electron transfer donor. Despite this subtle difference, all four spectra are nearly identical and provide strong evidence for a proline-II helix through the series, which is consistent with previous studies of oligoproline arrays.[5a,e]

Finally, UV−vis spectra were recorded in water (Figures S9−S12 of the Supporting Information). As expected, the visible spectra do not change throughout the series of oligoprolines. This result suggests that the distance between the chromophore and electron transfer donor does not have an effect on the local electronic structure of the chromophore, which makes these assemblies ideal for studying distance-dependent electron transfer.[5a]

This report provides details of the experimental procedures and characterization that were previously unavailable for four oligoproline arrays with varying distances between redox active centers. Although the procedures are specific to the four arrays, these methods, including solid-phase peptide synthesis techniques, provide a general approach to the synthesis of more complex helical oligoproline-based structures.

Experimental Section

Materials. β-(10-Phenothiazinyl)propionic acid (PTZ$(CH_2)_2$-COOH) was prepared according to literature methods,[14] and the modified amino acid Boc-Pra(PTZ)-OH was made by the methods of McCafferty et al.[5g] Bis(4,4′-diethylamidocarbonyl-2,2′-bipyridine)(4′-methyl-2,2′-bipyridine-4-carboxylic acid)ruthenium(II) ($[Rub'_2m](PF_6)_2$) was also synthesized according to literature procedures.[11−13] Details of these syntheses are provided in the Supporting Information.

Instrumentation. ^{1}H NMR (200 MHz) and UV−vis spectra were recorded on standard instruments. Hydrogenation was carried out at elevated pressure in a bomb. Thin layer chromatography (TLC) was conducted on silica gel 10 × 20 cm, 250 μm uniplates. All mass spectra were recorded on an ion trap mass spectrometer equipped with an electrospray ionization source.

Synthesis of *cis*-1-(1,1-Dimethylethoxycarbonyl)-4-amino-L-proline Methyl Ester, Boc-Pra-OCH$_3$·HCl.[5e,g,9,10] **A. *trans*-4-Hydroxy-L-proline Methyl Ester (1).** To a flame-dried 1 L round-bottom flask containing a suspension of *trans*-4-hydroxy-L-proline in methanol (50.0 g, 381 mmol, 131.13 g/mol) was added HCl gas by bubbling through a glass pipet from a lecture cylinder with vigorous stirring. HCl addition was continued for 5 min following dissolution of all solid. The glass pipet was removed and a Soxhlet extractor filled with 4 Å molecular sieves was attached. After refluxing for 2 h, the solvent was removed by rotary evaporation to yield a crystalline white product that was collected on a fritted glass funnel and washed with cold diethyl ether (3 × 25 mL). The solid was dried overnight in a vacuum desiccator (yield 55.3 g, 80%): ^{1}H NMR (200 MHz, D_2O) 4.7 (m, 2H), 3.84 (s, 3H), 3.53 (dd, 1H), 3.42 (m, 1H), 2.5 (m, 1H), and 2.3 ppm (m, 1H).

B. *trans*-1-(1,1-Dimethylethoxycarbonyl)-4-hydroxy-L-proline Methyl Ester (2). To a flame dried, three-necked round-bottom flask were added 100 mL of *N*,*N*-dimethylformamide, hydroxyproline methyl ester **1** (13.0 g, 0.071 mol, 181.6 g/mol), *N*-methylmorpholine (10.76 g, 0.107 mol, 101 g/mol), and 4-(dimethylamino)-pyridine (0.964 g, 7.9 mmol, 122 g/mol). A jacketed water condenser and an addition funnel were attached to the flask. After purging the system with argon for 15 min, the temperature of the solution was increased to 55 °C using a temperature controlled oil bath. Solid *tert*-butoxycarbonyl anhydride (17.2 g, 0.079 mol, 218.2 g/mol) was melted in a water bath, transferred to the addition funnel, and added dropwise to the solution under argon, over a period of 1 h. The reaction mixture was then heated at 55 °C for 12 h.

(15) (a) Woody, R. W.; *Adv. Biophys. Chem.* **1992**, *2*, 37. (b) Woody, R. W. *Peptides* **1985**, *7*, 15. (c) Okabayashi, H.; Isemura, T. Sakakibara, S. *Biopolymers* **1968**, *6*, 232.

(16) Hruby, V. J. *Conformation in Biology and Drug Design*; Academic Press: Orlando, FL, 1985; Vol 7.

The solvent was removed under reduced pressure to yield a light brown solid that was dissolved in ethyl acetate; washed with H_2SO_4 (3 × 100 mL), aqueous saturated NaCl (2 × 100 mL), aqueous saturated $NaHCO_3$ (3 × 100 mL), and again with aqueous saturated NaCl (2 × 100 mL); and then dried over anhydrous Na_2SO_4. The ethyl acetate was removed in vacuo to afford the Boc-protected methyl ester **2** as a slightly off-white powder (yield 14.8 g, 85%). TLC indicated pure material [1:1 (v/v) hexane/ethyl acetate]: ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.45 (m, 2H), 3.75 (s, 3H), 3.55 (m, 2H), 2.55 (s, 1H), 2.3 (m, 1H), 2.07 (m, 1H), and 1.45 ppm (d, 9H).

C. *cis*-1-(1,1-Dimethylethoxycarbonyl)-4-(methylsulfonyl)-L-proline Methyl Ester (3). The Boc-protected methyl ester **2** (10 g, 40.8 mmol, 245.3 g/mol) was added to a 200 mL round-bottom flask charged with pyridine (45 mL). After stirring for 30 min in an ice bath, freshly distilled methanesulfonyl chloride (6.5 mL, 9.62 g, 84.0 mmol, 114.55 g/mol) was added dropwise over 20 min via an addition funnel. The ice bath was maintained for 3 h, after which the reaction was allowed to warm to room temperature and to continue for an additional 10 h. A brown solid was obtained from removal of the solvent under reduced pressure. The solid was dissolved in ethyl acetate (150 mL) and washed with 1 M $NaHCO_3$ (3 × 150 mL), aqueous saturated NaCl (3 × 100 mL), 1 M citric acid (3 × 150 mL), distilled water (2 × 200 mL), and again with aqueous saturated NaCl (2 × 100 mL). The ethyl acetate layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to yield the mesitylate **3** as a white solid (yield 9.5 g, 72%). TLC indicated pure material [19:1 (v/v) chloroform/methanol]: ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.2 (m, 1H), 4.35 (m, 1H), 3.7 (m, 5H), 3.0 (s, 3H), 2.55 (m, 1H), 2.2 (m, 1H), and 1.4 ppm (d, 9H).

D. *cis*-1-(1,1-Dimethylethoxycarbonyl)-4-azido-L-proline Methyl Ester (4). To a round-bottom flask containing 40 mL of *N*,*N*-dimethylformamide were added mesitylate **3** (10 g, 30.9 mmol, 323.4 g/mol) and sodium azide (10.1 g, 155 mmol, 65.0 g/mol). The solution was heated to 55 °C using a temperature-controlled oil bath and monitored by TLC [1:1 (v/v) ethyl acetate/hexanes]. If the reaction was incomplete after 12 h, the mixture was cooled to room temperature, an additional 5 g of sodium azide was added to the solution, and the temperature was returned to 55 °C for 8 h. The solvent was removed under reduced pressure to yield a viscous pale yellow oil that was dissolved in ethyl acetate (100 mL) and washed with distilled water (5 × 100 mL), 0.1 N HCl (1 × 100 mL), distilled water (1 × 100 mL), aqueous saturated NaCl (1 × 100 mL), 0.1 N HCl (1 × 100 mL), distilled water (1× 100 mL), and aqueous saturated NaCl (1 × 100 mL). The ethyl acetate layer was then dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to yield a light brown oil: ^{1}H NMR (200 MHz, D_2O) δ 4.3 (m, 1H), 4.07 (m, 1H), 3.67 (m, 4H), 3.4 (m, 1H), 2.4 (m, 1H), 2.1 (m, 1H), and 1.35 ppm (d, 9H).

E. *cis*-1-(1,1-Dimethylethoxycarbonyl)-4-amino-L-proline Methyl Ester, Boc-Pra-OCH$_3$·HCl. A solution of azide **4** (10 g, 37.0 mmol, 270.29 g/mol), 6 N HCl (2 mL), and ethanol (98 mL) was added to a Parr hydrogenation vessel containing 10% palladium on carbon (2 g) under a blanket of argon. The vessel was purged with 40 psi H_2 for 2 min followed by evacuation with an aspirator. This procedure was repeated three times before the reactor was sealed under 40 psi of H_2 and shaken in a Parr hydrogenator for 24 h. Following the hydrogenation, the palladium catalyst was removed by filtration through a pad of diatomacious earth. The solvent was removed under reduced pressure to yield a light brown oil. TLC indicated that the reaction incomplete [85:15 (v/v) chloroform/methanol]. The oil was dissolved in a small amount of ethanol and dripped into ice cold, vigorously swirling petroleum ether, causing selective precipitation of the amino proline as a white solid. The solid was collected on a fritted glass funnel and washed with cold diethyl ether (3 × 25 mL). The solvent was removed from the filtrate by rotary evaporation to yield a light-brown oil (yield 5.8 g, 56%). TLC of the oil showed both amino proline and unreacted azide starting material: ^{1}H NMR (200 MHz, D_2O) δ 4.45 (m, 1H), 4.0 (m, 2H), 2.8 (s, 3H), 3.58 (m, 1H), 2.8 (m, 1H), 2.15 (m, 1H), and 1.4 ppm (d, 9H).

Synthesis of Chromophore-Substituted Proline [Boc-Pra(Rub′$_2$m)-OH](PF$_6$)$_2$. A. (2*S*,4*S*)-*N*$^\alpha$-(1,1-Dimethylethoxycarbonyl)-4-(bis(4,4′-diethylamidocarbonyl-2,2′-bipyridine)(4′-methyl-2,2′-bipyridine-4-carboxamide)ruthenium(II))-L-proline Methyl Ester Bishexafluorophosphate ([Boc-Pra(Rub′$_2$m)OCH$_3$](PF$_6$)$_2$) (5). This substituted amino acid was synthesized according to literature procedures.[5b,e,10] To a 200 mL round-bottom flask were added Boc-Pra-OCH$_3$·HCl (0.320 g, 1.14 mmol, 280 g/mol), [Rub′$_2$m](PF$_6$)$_2$[11,13] (1.50 g, 1.14 mmol, 1314.20 g/mol), *N*-methylmorpholine (NMM) (0.380 g, 3.76 mmol, 101 g/mol), and CH_2Cl_2 (70 mL). After the solids were dissolved, dicyclohexylcarbodiimide (DCC) (0.260 g, 1.26 mmol, 206.3 g/mol) and 4-(dimethylamino)pyridine (DMAP) (0.056 g, 4.5 mmol, 122 g/mol) were added. A septum was used to seal the flask and the reaction was stirred for approximately 20 h.

After 20 h, glacial acetic acid (4 drops) was added and the reaction was stirred for an additional 30 min. Precipitated solid dicyclohexyl urea was removed by filtration and the solvent was removed under reduced pressure to yield a red oil. The oil was dissolved in a minimum of 2:1 (v/v) acetonitrile/toluene and chromatographed on neutral alumina by elution with the same solvent mixture. Fractions containing the desired product were combined and again the solvent was removed under reduced pressure, leaving a red oil, which was dissolved in a minimum amount of acetonitrile and added dropwise to cold, vigorously stirring diethyl ether. This procedure resulted in the formation of an orange solid that was collected by vacuum filtration and washed with copious amounts of diethyl ether (yield 0.895 g, 51%): ^{1}H NMR (200 MHz, CD_3CN) δ 8.75 (s, 1H), 8.55 (s, 1H), 8.45 (s, 5H), 7.80 (m, 4H), 7.65 (m, 2H), 7.30 (m, 6H), 4.63 (m, 1H), 4.30 (m, 1H), 3.72 (s, 3H), 3.5 (m, 9H), 3.20 (q, 8H), 2.55 (m, 4H), 1.94 (m, 1H), 1.38 (d, 9H), 1.2 (t, 12H), and 1.05 ppm (t, 12H).

B. (2*S*,4*S*)-*N*$^\alpha$-(1,1-Dimethylethoxycarbonyl)-4-(bis(4,4′-diethylamidocarbonyl-2,2′-bipyridine)(4′-methyl-2,2′-bipyridine-4-carboxamide)ruthenium(II))-L-proline Bishexafluorophosphate ([Boc-Pra(Rub′$_2$m)-OH](PF$_6$)$_2$). In a 200-mL round-bottom flask, (Boc-Pra(Rub′$_2$m)OCH$_3$](PF$_6$)$_2$ (0.860 g, 0.559 mmol, 1539.7 g/mol) was dissolved in a 3:1 (v/v) methanol/water (60 mL) solution. After stirring in an ice bath for 20 min, lithium hydroxide monohydrate (0.070 g, 3.0 mmol, 23.9 g/mol) was added. The mixture was stirred in the ice bath for approximately 22 h, over which time the temperature increased to room temperature. The methanol was removed under reduced pressure and water (15 mL) was added. An orange solid was isolated by the addition of 1 M ammonium hexafluorophosphate. This material was purified by dissolution in methylene chloride (150 mL), drying over anhydrous sodium sulfate, reducing the volume to 3 mL under reduced pressure, and adding to cold diethyl ether. The resulting orange powder was collected on a medium porosity fritted glass funnel and stored in a vacuum desiccator (yield: 0.725 g, 85%): ^{1}H NMR (200 MHz, CD_3CN) δ 8.74 (s, 1H), 8.53 (s, 1H), 8.45 (s, 5H), 7.83 (m, 4H), 7.62 (m, 2H), 7.28 (m, 6H), 4.60 (m, 1H), 4.33 (m, 1H), 3.45 (m, 9H), 3.18 (q, 8H), 2.59 (m, 4H), 1.92 (m, 1H), 1.38 (d, 9H), 1.20 (t, 12H), and 1.05 ppm (t, 12H).

Synthesis of the Oligoproline Arrays 6−9. A. CH$_3$CO-Pro$_6$-Pra(PTZ)-Pro$_2$-Pra(Ru^{II}b′$_2$m)$^{2+}$-Pro$_6$-NH$_2$ (6). Assembly of each array was carried out under low ambient light conditions. The absence of light is required to avoid decomposition of PTZ$^+$ formed by photochemical excitation and electron transfer on the array.[6a] Manual solid-phase peptide synthesis was employed to assemble the 16-residue peptide (**6**) using Boc-Pro-OH, Boc-Pro-Pro-OH, Boc-Pra(Rub′$_2$m)-OH, Boc-Pra(PTZ)-OH and methylbenzhydrylamine (MBHA) resin. The reagents used in each of the coupling reactions were HBTU (4.0 equiv), NMM (4.0 equiv), HOBT (4.0 equiv), and DMAP (0.4 equiv). The coupling reactions used to incorporate Boc-Pro-OH and Boc-Pro-Pro-OH took place over 1 h

with 4.0 equiv of the amino acid residue. Only 2 equiv of the ruthenium and phenothiazine redox-active amino acids were incorporated in coupling steps that lasted for at least 24 h. Following the coupling of these redox-active amino acids, any unreacted amino groups were capped during a 35-min capping reaction using a 1:1 (v/v) mixture of acetic anhydride/methylene chloride. After the addition of the phenothiazine residue, all Boc-Pro-OH and Boc-Pro-Pro-OH couplings were allowed a 2 h reaction time. Prior to cleavage, the resin-bound peptide was *N*-terminally acetylated with a mixture of 50% (v/v) acetic anhydride/methylene chloride and stored in a vacuum desiccator. Cleavage from the resin was carried out with a mixture of trifluoroacetic acid (TFA, 7.48 mL), thioanisole (1.2 mL), 1,2-ethanedithiol (0.6 mL), *m*-cresol (0.2 mL), and trimethylsilyl bromide (TMSBr, 1.35 mL). This solution was cooled in an ice bath for 5 min before its addition to a 20-mL scintillation vial containing the dry, bound peptide. After stirring for 2 h and slowly warming to room temperature, the resin was filtered in vacuo on a medium porosity fritted glass funnel and washed with water (5 × 20 mL) and TFA (5 × 10 mL). Water (250 mL) was added to the filtrate and the solution was washed with diethyl ether (5 × 200 mL) to remove the organic cleavage reagents. TFA was removed by rotary evaporation and the water was removed by lyophilization. Extraneous salts from the cleavage procedure were removed by size exclusion chromatography using Sephadex G10 and elution with water. The desalted peptide was purified by semipreparative reversed-phase HPLC on an octadecyl-silica column (C18) eluted over 60 min with a linear gradient from 14% to 42% acetonitrile in 0.06% TFA/water. Fractions containing the desired peptide were freed of solvent by evaporation under reduced pressure and lyophilization to yield the final product as a flocculent orange powder (yield: 0.065 g): ESI-MS calcd for $[C_{149}H_{190}N_{30}O_{23}SRu]^{2+}$ 2902.47 Da, found 2904 Da.

B. CH_3CO-Pro_6-Pra(PTZ)-Pro_3-Pra($Ru^{II}b'_2m)^{2+}$-Pro_6-NH_2 (7), CH_3CO-Pro_6-Pra(PTZ)-Pro_4-Pra($Ru^{II}b'_2m)^{2+}$-Pro_6-NH_2 (8), and CH_3CO-Pro_6-Pra(PTZ)-Pro_5-Pra($Ru^{II}b'_2)^{2+}$-Pro_6-NH_2 (9). These arrays were synthesized in a manner similar to that of **6**. Analytical data for each is provided in the Supporting Information.

Acknowledgment. This work was supported by the Department of Energy research grant DE-FGO2-96ER 14607 (to T.J.M.), the National Science Foundation grant CHE-9705724 and Los Alamos National Laboratory (Laboratory Directed Research and Development Program project number 20020222).

Supporting Information Available: Synthesis of PTZ-substituted proline Boc-Pra(PTZ)-OH; synthesis of the ruthenium chromophore bis(4,4′-diethylamidocarbonyl-2,2′-bipyridine)(4′-methyl-2,2′-bipyridine-4-carboxylic acid)ruthenium(II) ($[Rub'_2m](PF_6)_2$); Figures S1−S4, analytical HPLC chromatograms of **6**−**9** before (A) and after (B) semipreparative HPLC purification; Figures S5−S8, ESI mass spectrum of **6**−**9** measured after purification; Figures S9−S12, UV−vis absorbance spectra of **6**−**9** in water at 298 K. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0603936