Hydrogen-Bond Promoted Intramolecular Electron Transfer to Photogenerated Ru(III): A Functional Mimic of Tyrosine$_Z$ and Histidine 190 in Photosystem II

Licheng Sun,*,† Mark Burkitt,‡ Markus Tamm,† Mary Katherine Raymond,§ Malin Abrahamsson,§ Denis LeGourriérec,§ Yves Frapart,‡ Ann Magnuson,‡ Ping Huang Kenéz,‡ Peter Brandt,† Anh Tran,† Leif Hammarström,*,§ Stenbjörn Styring,*,‡ and Björn Åkermark*,†,||

Contribution from the Department of Chemistry, Organic Chemistry, Royal Institute of Technology (KTH), S-100 44 Stockholm, Sweden, Department of Physical Chemistry, Uppsala University, P.O. Box 532, S-751 21 Uppsala, Sweden, and Department of Biochemistry, Center for Chemistry and Chemical Engineering, University of Lund, P.O. Box 124, S-221 00 Lund, Sweden

Received November 23, 1998

Abstract: As a model for redox components on the donor side of photosystem II (PS II) in green plants, a supramolecular complex **4** has been prepared. In this, a ruthenium(II) tris-bipyridyl complex which mimics the function of P_{680} in PS II, has been covalently linked to a tyrosine unit which bears two hydrogen-bonding substituents, dipicolylamine (dpa) ligands. Our aim is to mimic the interaction between tyrosine$_Z$ and a basic histidine residue, namely His190 in PSII, and also to use the dpa ligands for coordination of manganese. Two different routes for the synthesis of the compound **4** are presented. Its structure was fully characterized by ^{1}H NMR, COSY, NOESY, ^{13}C NMR, IR, and mass spectrometry. ^{1}H NMR and NOESY gave evidence for the existence of intramolecular hydrogen bonding in **4**. The interaction between the ruthenium and the substituted tyrosine unit was probed by steady-state and time-resolved emission measurements as well as by chemical oxidation. Flash photolysis and EPR measurements on **4** in the presence of an electron acceptor (methylviologen, MV^{2+}, or cobalt pentaminechloride, Co^{3+}) showed that an intermolecular electron transfer from the excited state of Ru(II) in **4** to the electron acceptor took place, forming Ru(III) and the methylviologen radical $MV^{+\bullet}$ or Co^{2+}. This was followed by intramolecular electron transfer from the substituted tyrosine moiety to the photogenerated Ru(III), regenerating Ru(II) and forming a tyrosyl radical. In water, the radical has a *g* value of 2.0044, indicative of a deprotonated tyrosyl radical. In acetonitrile, a radical with a *g* value of 2.0029 was formed, which can be assigned to the tyrosine radical cation. In both solvents the electron transfer is intramolecular with a rate constant $k_{ET} > 1 \times 10^7$ s^{-1}. This is 2 orders of magnitude greater than the one for a similar compound **3**, in which no dpa arm is attached to the tyrosine unit. Therefore the hydrogen bonding between the substituted tyrosine and the dpa arms in **4** is proposed to be responsible for the fast electron transfer. This interaction mimics the proposed His190 and tyrosine$_Z$ interaction in the donor side of PS II.

Introduction

Sustainable energy systems need to be developed in the future, and solar light is one very attractive energy source for such systems. Artificial photosynthesis has therefore attracted considerable attention in the past decades as a potential way of converting solar energy into fuel or electricity. In nature, solar light is absorbed by green plants and transformed into bioenergy by the reduction of carbon dioxide. Simultaneously, water is oxidized to molecular oxygen. Photosystem II (PSII) in green plants is the key enzyme in this complex process.[1] The conversion is initiated by the absorption of a photon by the light-harvesting system of PSII, leading to excitation of the central chlorophyll dimer P_{680}. An electron is then transferred from the excited-state P_{680}* to a series of electron acceptors: pheophytin, quinone$_A$, and quinone$_B$.[2] The transferred electron is later used to reduce carbon dioxide, while the chlorophyll unit P_{680} is regenerated from its photooxidized form P_{680}+ by electron transfer (ET) from a nearby manganese cluster.[3] This transfer is mediated by tyrosine$_Z$,[4] located 5−10 Å[4b,5] away from the manganese cluster and 10−15 Å from P_{680}.[6] After four

† Royal Institute of Technology.
‡ Lund University.
§ Uppsala University.
|| E-mail: bear@orgchem.kth.se.

(1) (a) Barber, J.; Andersson, B. *Nature* **1994**, *370*, 31−34. (b) Andersson, B.; Styring, S. In *Current Topics in Bioenergetics*; Lee, C. P., Ed.; Academic Press: San Diego, 1991; Vol. 16, 1−81.

(2) (a) Diner, B. A.; Babcock, G. T. In *Oxygenic Photosynthesis: The Light Reactions;* Ort, D. R., Yocum, C. F., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1996; pp 213−247, (b) Britt, R. D. In *Oxygenic Photosynthesis: The Light Reactions;* Ort, D. R., Yocum, C. F., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1996; pp 137−164.

(3) (a) Hoganson, C. W.; Babcock, G. T. *Science* **1997**, *277*, 1953−1957. (b) Tommos, C.; Babcock, G. T. *Acc. Chem. Res.* **1998**, *31*, 18−25.

(4) (a) Tommos, C.; Tang, X.-S.; Warncke, K.; Hoganson, C. W.; Styring, S.; McCracken, J.; Diner, B. A.; Babcock, G. T. *J. Am. Chem. Soc*. **1995**, *117*, 10325−10335. (b) Gilchrist, M. L.; Ball, J. A.; Randall, D. W.; Britt, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9545−9549.

(5) Svensson, B.; Vass, I.; Styring, S. *Z. Naturforsch*. **1991**, *46c*, 765−776.

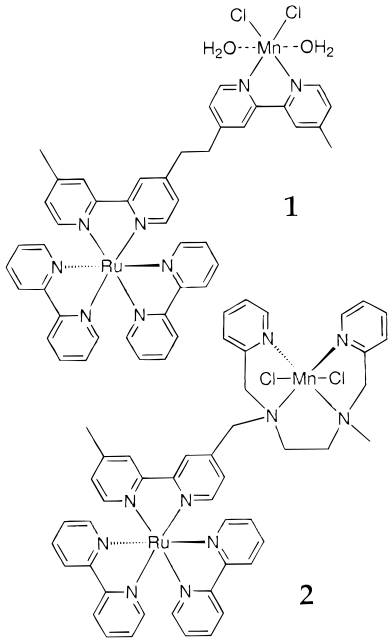
(6) (a) Svensson, B.; Etchebest, C.; Tuffery, P.; van Kan, P.; Smith, J.; Styring, S. *Biochemistry* **1996**, *35*, 14486−14502. (b) Kodera, Y.; Hara, H.; Astashkin, A. V.; Kawamori, A.; Ono, T. *Biochim. Biophys. Acta* **1995**, *1232*, 43−51.

10.1021/ja984048c CCC: $18.00 © 1999 American Chemical Society
Published on Web 07/14/1999

consecutive photoinduced ET events, the manganese cluster, which acts as a reservoir for oxidizing equivalents, recovers all four electrons by oxidizing two water molecules to molecular oxygen.[2,7] In recent years, model systems[8,9] for mimicking the manganese cluster have been studied extensively. Such systems have in general been mainly structural models, investigated by X-ray, EPR spectroscopy, and magnetic measurements. Fewer examples exist in which the OEC models have undergone electrochemical or light-induced processes.[10] However, to devise an artificial photochemical system that is capable of producing oxygen by oxidation of water,[11,12] electron transfer from a manganese complex to a photooxidized sensitizer must first be demonstrated.

A few years ago, we started to build such model systems based on tris-bipyridine ruthenium(II) as a substitute for chlorophyll. In our first studies it was shown that in a dinuclear Mn(II)−Ru(II) supramolecular model system **1**, an intramolecular ET from the coordinated manganese(II) monomer to a photogenerated ruthenium(III) was possible,[13,14] opening up the way to mimic the ET process in the donor side of PSII. We were also able to show that the distance between manganese and ruthenium is crucial. In **1** the intramolecular ET rate is fairly low, k_{ET} ca. 10^5 s^{-1}. However, when the distance was decreased as in **2**, the lifetime of the excited state was about 2 orders of magnitude shorter than that of **1** due to efficient quenching by the manganese (Chart 1). We were therefore unable to observe the initial ET from the excited Ru(II) of compound **2**. In PS II, the electron transfer is mediated by a phenolic unit, tyrosine$_Z$, perhaps in order to avoid quenching of the excited state of the chlorophyll unit by manganese, yet ensure rapid electron transfer to P_{680}+. The intermediate tyrosine$_Z$ may also be essential for

Chart 1



the oxidation of the manganese cluster in PS II by participating in hydrogen atom transfer from coordinated water.[3,4]

To build a supramolecular system based on similar principles, we have synthesized a compound **3**, in which L-tyrosine was covalently linked to a ruthenium tris-bipyridyl complex through an amide group.[14] We could show that a tyrosyl radical was generated by photoinduced ET from tyrosine to the photogenerated Ru(III). We could also show that this tyrosyl radical was capable of oxidizing a dinuclear manganese complex that has recently been prepared by Girerd and co-workers.[15,16]

Recently, it was found that tyrosine$_Z$ most probably is hydrogen-bonded to a histidine residue, His190 in the D1 polypeptide in PS II.[5,6a,17] It is also known that the EPR spectrum from the oxidized tyrosine represents the neutral, deprotonated radical. It has been proposed that the histidine mediates proton transfer from tyrosine$_Z$.[18,19] The exact mechanism for the electron transfer is not clear, but there are three reasonable alternatives. The first is that an electron is transferred from tyrosine$_Z$ to the photooxidized P_{680} to give a cation radical, which is subsequently deprotonated to the tyrosyl radical. The deprotonation should be very efficient because the radical cation is a strong acid. In the second alternative, tyrosine$_Z$ is first deprotonated to give a phenolate anion which should be a very efficient electron donor, giving the tyrosyl radical directly. Since histidine is a relatively weak base compared to phenolate, the relative concentration of phenolate should be low, however. The third alternative is that electron and proton transfers are coupled. This would be a very plausible mechanism if it is assumed that electron transfer takes place from a system in which strong

(7) (a) Yachandra, V. K.; Sauer, K.; Klein, M. P. *Chem. Rev.* **1996**, *96*, 2927−2950. (b) Dau, H.; Andrews, J. C.; Roelofs, T. A.; Latimer, M. J.; Liang, W.; Yachandra, V. K.; Sauer, K.; Klein, M. P. *Biochemistry* **1995**, *34*, 5274−5287. (c) Riggs-Gelasco, P. J.; Mei, R.; Ghanotakis, D. F.; Yocum, C. F.; Penner-Hahn, J. E. *J. Am. Chem. Soc.* **1996**, *118*, 2387−2399.

(8) (a) Pecoraro, V. L.; Baldwin, M. J.; Gelasco, A. *Chem. Rev.* **1994**, *94*, 807−826. (b) Caudle, M. T.; Riggs-Gelasco, P.; Gelasco, A. K.; Penner-Hahn, J. E.; Pecoraro, V. L. *Inorg. Chem.* **1996**, *35*, 3577−3584.

(9) (a) Frapart, Y.-M.; Boussac, A.; Albach, R.; Anxolabehere-Mallart, E.; Delroisse, M.; Verlhac, J.-B.; Blondin, G.; Girerd, J.-J.; Guilhem, J.; Cesario, M.; Rutherford, A. W.; Lexa, D. *J. Am. Chem. Soc.* **1996**, *118*, 2669−2678. (b) Berry, K. J.; Moubaraki, B.; Murray, K. S.; Nicols, P. J.; Schulz, L. D.; West, B. O. *Inorg. Chem.* **1995**, *34*, 4123−4133. (c) Kalsbeck, W. A.; Thorp, H. H. *J. Electroanal. Chem.* **1991**, *314*, 335−343. (d) Gamelin, D. R.; Kirk, M. L.; Stemmler, T. L.; Pal, S.; Armstrong, W. H.; Penner-Hahn, J. E.; Solomon, E. I. *J. Am. Chem. Soc.* **1994**, *116*, 2392−2399.

(10) Ruettinger, W.; Dismukes, G. C. *Chem. Rev.* **1997**, *97*, 1−24.

(11) (a) Watkinson, M.; Whiting, A.; McAuliffe, C. A. *J. Chem. Soc., Chem. Commun.* **1994**, 2141−2142. (b) Ashmawy, F. M.; McAuliffe, C. A.; Parish, R. V.; Tames, J. *J. Chem. Soc., Dalton Trans.* **1985**, 1391−1397. (c) Aurangzeb, N.; Hulme, C. E.; McAuliffe, C. A.; Pritchard, R. G.; Watkinson, M.; Bermejo, M.; Garcia-Deibe, A.; Rey, M.; Sanmartin, J.; Sousa, A. *J. Chem. Soc., Chem. Commun.* **1994**, 1153−1155.

(12) (a) Xu, X.; Shreder, K.; Iverson, B. L.; Bard, A. J. *J. Am. Chem. Soc.* **1996**, *118*, 3656−3660. (b) Kelly, L. A.; Rodgers, M. A. J. *J. Phys. Chem.* **1995**, *99*, 13132−13140.

(13) (a) Sun, L.; Hammarström, L.; Norrby, T.; Berglund, H.; Davidov, R.; Andersson, M.; Börje, A.; Korall, P.; Philouze, C.; Almgren, M.; Styring, S.; Åkermark, B. *Chem. Comm.* **1997**, 607−608. (b) Sun, L.; Berglund, H.; Davidov, R.; Norrby, T.; Hammarström, L.; Korall, P.; Börje, A.; Philouze, C.; Berg, K.; Tran, A.; Andersson, M.; Stenhagen, G.; Mårtensson, J.; Almgren, M.; Styring, S.; Åkermark, B. *J. Am. Chem. Soc.*, **1997**, *119*, 6996−7004. (c) Berglund-Baudin, H.; Sun, L.; Davidov, R.; Sundahl, M.; Styring, S.; Åkermark, B.; Almgren, M.; Hammarström, L. *J. Phys. Chem. A* **1998**, *102*, 2512−2518. (d) Hammarström, L.; Sun, L.; Åkermark, B.; Styring, S. *Biochim. Biophys. Acta* **1998**, *1365*, 193−199.

(14) Magnusson, A.; Berglund, H.; Korall, P.; Hammarström, L.; Åkermark, B.; Styring, S.; Sun, L. *J. Am. Chem. Soc.* **1997**, *119*, 10720−10724.

(15) Horner, O.; Anxolabéhére-Mallart, E.; Charlot, M.-F.; Tchertanov, L.; Guilhem, J.; Mattioli, T.; Boussac, A.; Girerd, J.-J. *Inorg. Chem.* **1999**, in press.

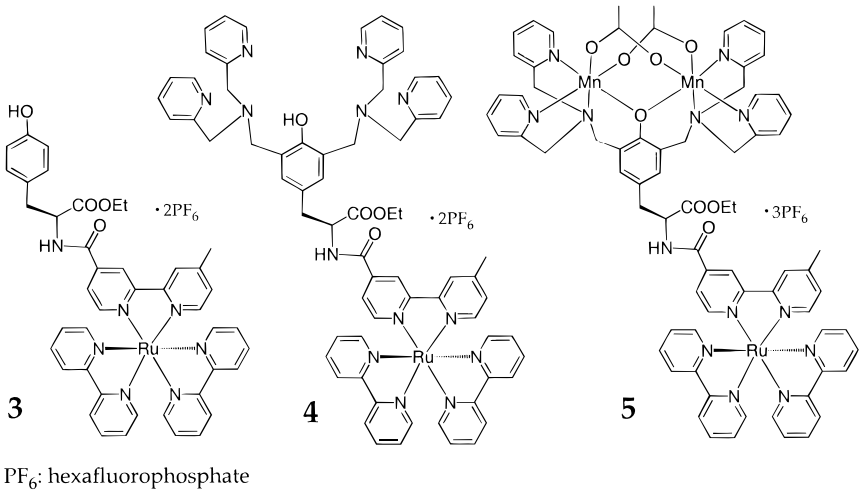
(16) Magnuson, A.; Frapart, Y.; Abrahamsson, M.; Horner, O.; Åkermark, B.; Sun, L.; Girerd, J.-J.; Hammarström, L.; Styring, S. *J. Am. Chem. Soc.* **1999**, *121*, 89−96.

(17) (a) Hays, A.-M. A.; Vassiliev, I. R.; Golbeck, J. H.; Debus, R. J. *Biochemistry* **1998**, *37*, 11352−11365. (b) Mamedov, F.; Sayre, R. T.; Styring, S. *Biochemistry* **1998**, *37*, 14245−14256.

(18) Berthomieu, C.; Hienerwadel, R.; Boussac, A.; Breton, J.; Diner, B. A. *Biochemistry* **1998**, *37*, 10547−10554.

(19) (a) Chu, H.-A.; Nguyen, A. P.; Debus, R. J. *Biochemistry* **1995**, *34*, 4, 5839- 5858 and references therein. (b) Stubbe, J.; van der Donk, W. A. *Chem. Rev.* **1998**, *98*, 705−762.

Chart 2



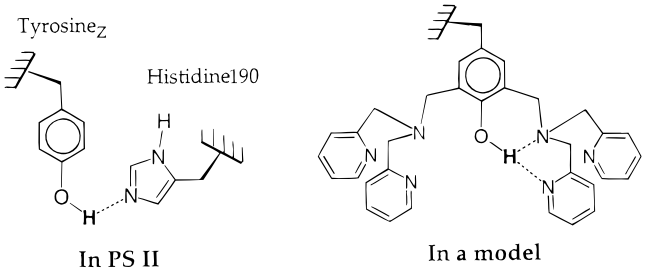


Figure 1. Schematic presentation of the hydrogen bonding around redox active tyrosine in photosystem II and in a model system **4**.

hydrogen bonding between the phenol and the histidine aids the electron transfer by increasing electron density on the tyrosine. The hydrogen bonding should also promote transfer of the proton because it should very readily move along the hydrogen bond from a position close to the oxygen to a position close to the histidine nitrogen.

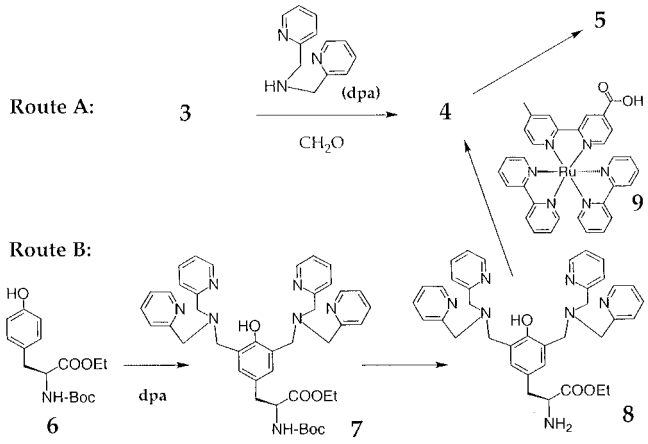
In an attempt to study these questions we have now synthesized complex **4**, in which two dpa arms are connected to the *o*-positions of the phenol group of L-tyrosine. The dpa arms form a strong hydrogen bond to the phenol hydroxy group and also act as an internal base. Of course, they also form a site for coordinating manganese ions to the complex,[20] giving **5**.

In this paper we present the synthesis and characterization of complex **4** (Chart 2). We have been able to demonstrate intramolecular electron transfer from the tyrosine analogue to the photogenerated Ru(III). We have compared the rate of electron transfer with that of the unsubstituted tyrosine analogue in **3**, which lacks the hydrogen-bonding substituents, and find it to be much faster in **4**. We have also been able to show that the photogenerated tyrosyl residue in **4** is a radical cation in acetonitrile, a moderately polar, aprotic solvent. In contrast, a neutral tyrosyl radical was observed in aqueous solution, closely mimicking the photoevents at the donor side of PS II (Figure 1).

Results and Discussion

Synthesis. The compound **4** was prepared by two different routes, shown in Scheme 1. In route A, compound **3**, which has been prepared earlier,[14] was subjected to a Mannich reaction with dipicolylamine(dpa) (see Scheme 1, route A), to give the substituted tyrosine−ruthenium compound **4**, which was subsequently purified by column chromatography. The progress of this reaction could easily be followed by ^{1}H NMR. The two aromatic doublet peaks of the tyrosine moiety in the starting material **3** merged into a singlet at 7 ppm, an area where no other protons gave a signal in the reaction mixture. The purification of the crude **4** by column chromatography proved to be difficult due to the necessary excess of dpa as starting material. The problem was overcome by purification of the hydrochloric salt of **4** on Al_2O_3 using a hydrochloric acid in methanol as eluent. The product was then obtained by neutralizing the product containing fractions with a dilute aqueous solution of sodium bicarbonate. Compound **4** was characterized by ^{1}H NMR, COSY, NOESY, ^{13}C NMR, IR, and mass spectrometry. The ^{1}H NMR shows the phenolic proton at 11 ppm, indicating a strong hydrogen bond to the dpa arms. This observation is supported by the NOESY spectrum[21] in DMSO which shows an interaction between the phenolic proton, the benzylic CH_2 groups, and two of the pyridine protons from the dpa arms. Also the absorption of the phenolic OH in the IR at 3405 cm^{-1} supports the existence of a hydrogen bond. Electrospray ionization mass spectrometry (ESI-MS) spectrum of **4** from acetonitrile gave a mono charged peak at *m*/*z* 1386 [M −

Scheme 1. Two Different Routes to Synthesize Complex **4** and Its Manganese Complex **5**



(20) (a) Pessiki, P. S.; Khangulov, S. V.; Ho, D. M.; Dismukes, G. C. *J. Am. Chem. Soc.* **1994**, *116*, 891. (b) Khangulov, S. V.; Pessiki, P. J.; Barynin, V. V.; Ash, D. E.; Dismukes, G. C. *Biochemistry* **1995**, *34*, 2015. (c) Sun, L.; Raymond, M. K.; Magnuson, A.; LeGourriérec, D.; Tamm, M.; Abrahamsson, M.; Kenéz, P. H.; Mårtensson, J.; Stenhagen, G.; Hammarström, L.; Styring, S.; Åkermark, B. *EUCHEM Conference, Artificial Photosynthesis*, May 16−20, 1998, Sigtuna, Sweden.

(21) See Supporting Information.

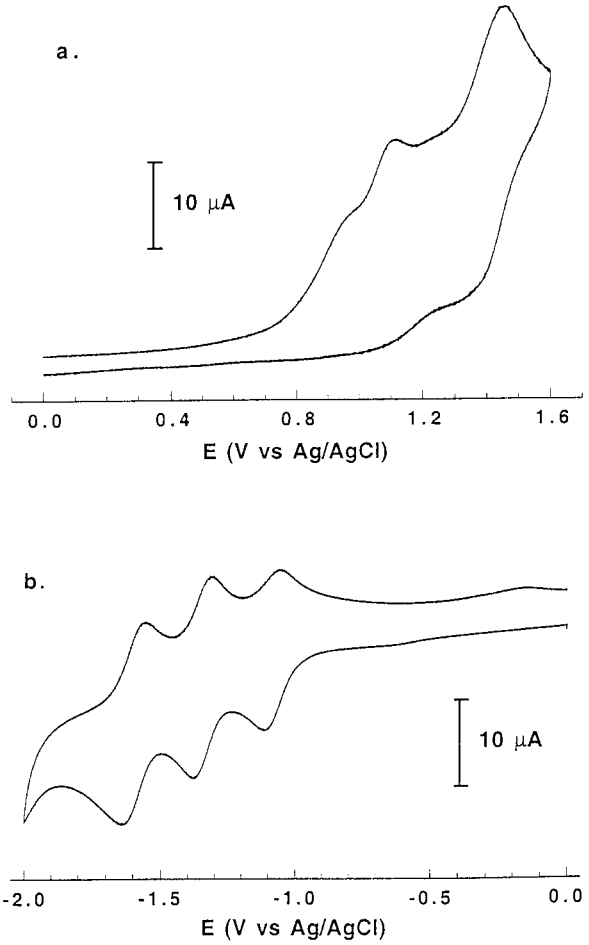


Figure 2. Cyclic voltammetry of 1.12 mM **4** in 0.1 M $TBABF_4/CH_3CN$ at a glassy carbon working electrode (∅ 3 mm): (a) scan from 0.0 to +1.6 V; (b) scan from 0.0 to −2.0 V. A Ag/AgCl electrode (saturated with LiCl in CH_3CN) is used as reference electrode. Scan rate 100 mV/s.

$PF_6^-]^+$ and doubly charged peaks at *m*/*z* 693 [M − PF_6^- + $H^+]^{2+}$, and *m*/*z* 621 [M − $2PF_6^-]^{2+}$. Fast atom bombardment mass spectrometry (FAB-MS) using 1-thioglycerol as matrix, showed the mono charged peaks at *m*/*z* 1532 [M + $H^+]^+$ and 1386 [M − $PF_6^-]^+$. These results clearly prove the assigned structure.

An alternative approach to the synthesis of compound **4** is described in Scheme 1, route B. In this route, the L-tyrosine ester **6** was subjected to the Mannich reaction with dpa to give compound **7**. Deprotection of the amino group gave the compound **8** which was then linked to the ruthenium tris-bipyridyl carboxylic acid **9** [22] to give **4**. Also on this pathway the complex turned out to be difficult to purify, because the main side product, the starting material **8**, had physical properties similar to those of the product **4**. It was found that the addition of small amounts of acetic acid to the eluent during column chromatography made the product **4** eluable while **8** remained on the column. Unfortunately, the yield of this amide coupling is still unsatisfactory. Attempts to perform the coupling by other established methods, for example, via an active ester, gave even lower yield. Nevertheless, both routes A and B led to the same compound **4**.

Electrochemistry Studies. The electrochemical behavior of **4** (route B) was studied by cyclic voltammetry in both acetonitrile and water. In acetonitrile, see Figure 2a and 2b, there is an oxidation with peak potential 1.32 V vs SCE and three reversible reduction peaks at negative potentials, the first one with peak potential −1.22 V vs SCE. These are typical for oxidation of the metal and the reduction of the three ligands in $Ru(bpy)_3^{2+}$ complexes. In Figure 2a the reduction peak of Ru(III) looks small compared to the oxidation peak, but scanning only over the potential range around the Ru peak gave equal peak hights. The two irreversible oxidation peaks at 0.84 and 1.01 V vs SCE were assigned to the oxidation of the tyrosine and the dpa arm, respectively. This conclusion is based on the shape and peak potential obtained in acetonitrile for the free dpa arm (1.02 V vs SCE) and complex **3** (0.85−0.95 V vs SCE). These data imply that the tyrosine is easier to oxidize than the dpa arm and that both of them can be oxidized by the Ru(III) complex.

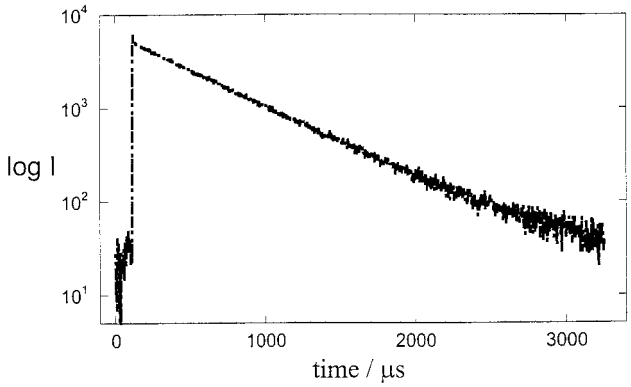


Figure 3. Time-resolved emission decay profile of compound **4** in N_2-purged acetonitrile at room temperature, $\lambda_{ex} = 327$ nm, $\lambda_{em} = 610$ nm.

In water solution at pH 7 (not shown) only one irreversible oxidation peak with a peak potential 0.79 V (0.74 V for **3**) vs SCE was obtained (the redox processes of the $Ru(bpy)_3^{2+}$ moiety are outside the potential window in water). This was assigned to the oxidation of tyrosine due to similarities in the shape and peak potential of earlier published results for free tyrosine and **3**.[16,23] For the free dpa arm in water, we obtained an irreversible oxidation with the peak potential 0.89 V vs SCE, i.e., a much lower potential than in acetonitrile. Because oxidized amines undergo fast secondary reactions, the observed irreversible peak potentials may lead to poor estimates of the true oxidation potentials.[24,25] The oxidation potential for the dpa arm in **4** may therefore be even lower than 0.89 V, making it difficult to decide whether the dpa or the tyrosine unit is the most readily oxidized.

Emission Studies and Flash Photolysis. In acetonitrile, complex **4** displayed a MLCT absorption band at 456 nm that is typical of $Ru(bpy)_3^{2+}$ type compounds.[26] The emission maximum (λ_{EM}) of **4** in water is 675 nm, and in acetonitrile 656 nm. An emission decay curve for **4** was obtained by single-photon counting (Figure 3). Complex **4** displayed a single-exponential decay in acetonitrile and in water with lifetimes $\tau = 1350$ ns and 470 ns, respectively. In both solvents, **4** had a similar excited-state lifetime as the compound **3**, in which the

(22) 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.

(23) Harriman, A. *J. Phys. Chem.* **1987**, *91*, 6102.

(24) Clark, C. D.; Hoffman, M. Z. *J. Phys. Chem.* **1996**, *100*, 14688.

(25) Chow, Y. L.; Danen, W. C.; Nelsen, S. F.; Rosenblett, D. H. *Chem. Rev.* **1978**, *78*, 243.

(26) For properties of Ru(II)−polypyridyls, especially concerning photoinduced electron transfer, see: (a) Juris, A.; Balzani, V.; Barigelletti, F.; Campagna, S.; Belser, P.; von Zelewsky, A. *Coord. Chem. Rev.* **1988**, *84*, 85. (b) Meyer, T. J. *Acc. Chem. Res.* **1989**, *22*, 163. (c) Kalyanasundaram, K. *Photochemistry of Polypyridine and Porphyrin Complexes*; Academic Press: London, 1992, Chapter 6.

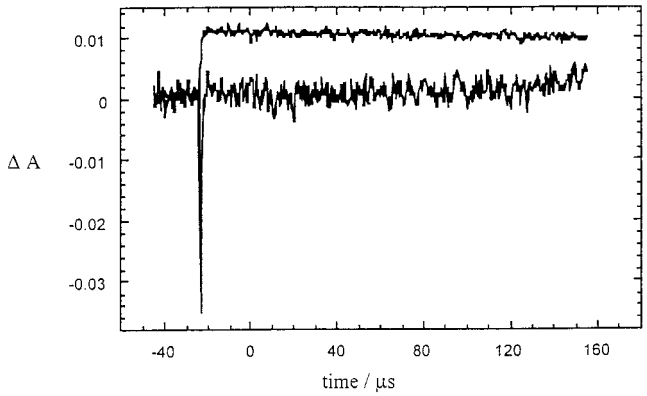


Figure 4. Transient absorption curves at 600 nm (top) and 450 nm (bottom) of **4** (4×10^{-5} M) and methylviologen (MV·2Cl, 1.0×10^{-2} M) in water at room temperature. The Ru(III) to Ru(II) recovery is on the same time scale as the Ru(II)* excited-state decay (the spike in the 450 nm one).

ligand contains an unsubstituted tyrosine unit ($\tau = 1150$ ns in acetonitrile and 370 ns in water). Neither of the tyrosine moieties quenched the Ru-excited state, as shown with a reference compound with alanine instead of tyrosine.[14] The shorter excited-state lifetime in water is a solvent effect on the Ru−chromophore itself, typical for these Ru-complexes.[26]

To measure the photoinduced intramolecular ET rate from the tyrosine moiety to the photogenerated ruthenium(III), flash photolysis measurements were made. Application of a 460 nm laser pulse (<30 ns duration) to complex **4** in water in the presence of the electron acceptor methylviologen (MV^{2+}) resulted in a rapid loss in absorption at 450 nm and an increase at 600 nm (Figure 4). The bleaching at 450 nm reflects the loss of Ru(II), due to excitation and the oxidative quenching of the excited state, Ru(II)* (reaction 1). The absorption observed at 600 nm is caused by the methylviologen radical cation, $MV^{+\bullet}$, formed upon reduction by Ru(II)*.

$$\text{Ru(II)*} + MV^{2+} \rightarrow \text{Ru(III)} + MV^{+\bullet} \qquad (1)$$

The decay of the $MV^{+\bullet}$ absorption occurred much more slowly than the recovery of the Ru(II) signal, leading to the significant conclusion that Ru(II) recovery does not simply occur via the reverse of reaction 1. Recovery of Ru(II) must, therefore, involve electron transfer from the tyrosine phenolate moiety. Flash photolysis experiments in acetonitrile gave a similar result (see Figure 5). The rate of Ru(II) recovery was in fact limited by the quenching reaction 1, that had a pseudo-first-order rate constant of 1×10^7 s^{-1} at the highest concentration of MV^{2+} used. This gives that the ET reaction that regenerates Ru(II) has a rate constant $k_{ET} > 1 \times 10^7$ s^{-1} both in acetonitrile and in water, which means that the electron transfer must be intramolecular.

Thermodynamically, both the dpa arm and the tyrosine unit can work as electron donors. To exclude the possibility of the dpa arm as a primary electron donor, a control experiment was performed. Instead of **4**, a compound in which the ruthenium(II) tris-bipyridyl complex was directly linked to a dpa ligand via a single carbon bond[27] was used in the flash photolysis. The result showed that the ET rate constant from the dpa ligand to the photogenerated Ru(III) was 3 orders of magnitude lower, ca. 10^4 s^{-1}, despite the short Ru−dpa distance. Therefore, the primary electron donor in **4** is not likely to be the dpa arm but

(27) Berg, K. E.; Tran, A.; Raymond, M. K.; Abrahamsson, M.; Wolny, J.; Redon, S.; Andersson, M.; Sun, L.; Styring, S.; Hammarström, L.; Toftlund, H.; Åkermark, B., manuscript in preparation.

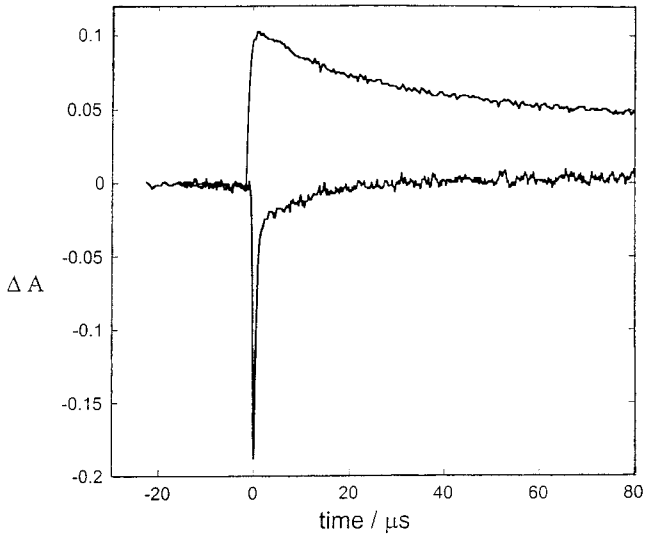


Figure 5. Transient absorption curves at 600 nm (top) and 450 nm (bottom) of deoxygenated acetonitrile solution of **4** (4×10^{-5} M) in the presence of $MV{\cdot}2PF_6$ (1.0×10^{-2} M) at room temperature. The intensity of the slow component of the Ru(II) recovery at 450 nm increases as the sample is subjected to more light.

the tyrosine unit which is hydrogen-bonded to it. This conclusion was supported by direct observation of the photogenerated phenoxyl radicals in the EPR experiments (see below).

In the flash photolysis experiments, the $MV^{+\bullet}$ signal at 600 nm decayed following second-order kinetics with a rate constant of ca. 5×10^9 M^{-1} s^{-1}. The decay was attributed to the recombination between $MV^{+\bullet}$ and the tyrosine radical.[14] A buildup of the methylviologen radical was observed after repeated flashes to a solution of **4** in acetonitrile (but not in water). This is probably due to the partial photodecomposition of the electron donor which would otherwise convert $MV^{+\bullet}$ to MV^{2+}. Another control experiment, using $Ru(bpy)_3^{2+}$ and bis-(picolyl)ethylamine instead of **4**, also gave a buildup of methylviologen radical, suggesting that the dpa ligand can serve as an irreversible electron donor in acetonitrile.

Phenoxyl Radicals. To study the light-induced formation of tyrosyl radicals from **4**, a series of EPR experiments were conducted. Continuous, in situ laser flashing of an aqueous solution of **4** in the presence of the sacrificial electron acceptor $[Co(NH_3)_5Cl]^{2+}$ resulted in the generation of a neutral phenoxyl radical, as evidenced by a characteristic EPR signal (Figure 6), having an isotropic *g* value of 2.0044 and a peak-to-trough line width of approximately 8.7 G. This photochemical reaction proceeds in several steps. First, the Ru(II) moiety transfers an electron from its MLCT state to the sacrificial electron acceptor, $[Co(NH_3)_5Cl]^{2+}$, resulting in the formation of $[Co(H_2O)_6]^{2+}$ and Ru(III). The Ru(III) moiety retrieves an electron from the dpa-substituted tyrosine moiety in **4**, and a phenoxyl radical is formed after loss of one proton. The high *g* value (*g* ca. 2.0044) is strong evidence that the observed spectrum represents a neutral (i.e., deprotonated) phenoxyl radical (see below).

In a similar experiment with **4** in pure acetonitrile, using MV^{2+} as an external electron acceptor instead of $[Co(NH_3)_5Cl]^{2+}$, a protonated phenoxyl radical ($PhOH^{+\bullet}$) was detected. Also here, Ru(III) was formed in the first step together with a methylviologen radical ($MV^{+\bullet}$) after an intermolecular ET. The $MV^{+\bullet}$ radical has a large, wide EPR spectrum with many hyperfine lines. To observe radical species formed from **4** after a laser pulse, we conducted kinetic EPR experiments which permit their detection even in the presence of the strong $MV^{+\bullet}$ signals. To accomplish this, time-mode EPR measurements at

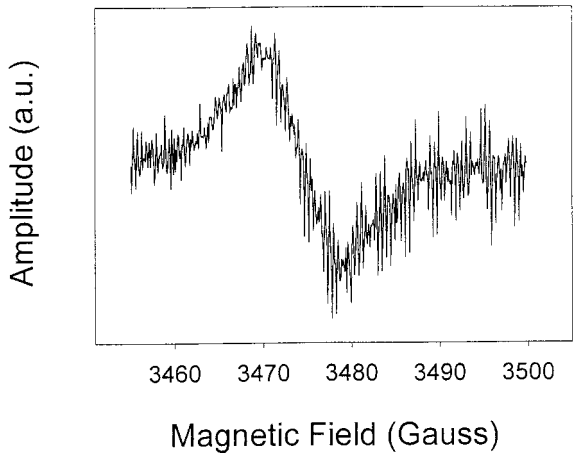


Figure 6. EPR spectrum recorded during the continuous laser-flashing of an aqueous solution (10% DMSO) of **4** in the presence of $[Co(NH_3)_5Cl]^{2+}$. The radical EPR signal has a *g* value of 2.0044 indicative of the formation of a neutral phenoxyl radical. The laser flashes were delivered with 5 Hz flash frequency and the "spikes" in the spectrum are due to the transient decay of a fraction of the formed radical between the flashes. For EPR settings see the Experimental Section.

field positions at which the methylviologen radical was determined to give zero signal intensity were used to construct a "kinetic" spectrum (see Figure 7A). This revealed the presence of an underlying signal from a short-lived ($t_{1/2} \approx 40$ ms) species (see Figure 7B). The signal had a peak-to-trough width of approximately 16 G and an isotropic *g* value of 2.0029. This *g* value is characteristic of a protonated phenoxyl radical ($PhOH^{+\bullet}$),[28] which was generated by intramolecular ET from dpa-substituted tyrosine to the Ru(III). It has been reported that upon increasing the acidity to below pH 0, phenoxyl radicals are converted to phenol radical cations. Phenol radical cations have also been observed in nonaqueous solvents. The phenol radical cation has a pK_a of -2.0 in water and should be rapidly deprotonated in aqueous solution, as observed. Phenoxyl radicals are reported to have *g* values of 2.0044 ± 0.0004, and those for phenol radical cations have values of 2.0032 ± 0.0004, strongly supporting our conclusion that the observed radical with *g* value of 2.0029 is a protonated phenoxyl radical.[28]

In summary, these results establish that the tyrosine moiety is the intramolecular donor to the photogenerated Ru(III), as observed also for the complex **3**, where the tyrosine is unsubstituted. The electron transfer from the ligand to the photogenerated Ru(III) in **4** is more than 2 orders of magnitude faster than in **3**.[29] We propose that the reason for this is strong intramolecular hydrogen bonding between the phenolic hydroxyl group and the dpa arms in **4**. Because of the hydrogen bond, the electron density on the tyrosine is increased, facilitating electron transfer. Since the electron-transfer rate in the parent complex **3** goes up as the pH increases,[30] partial deprotonation of the phenol in **4** is also possible.

These results thus show for the first time that a photoinduced electron-transfer process from a tyrosine unit to a photooxidized sensitizer, here Ru(III), is coupled with a proton-transfer process, generating a tyrosine centered radical (see Scheme 2). In water solution, the electron transfer is very fast and the result is a deprotonated radical. Thus the phenolic proton has been removed from **4** (Scheme 2, left pathway). In acetonitrile, the electron transfer is again very fast, but the proton stays in the close vicinity of the phenolic oxygen in **4** after its oxidation. It is probable that this is due to very strong hydrogen bonding in a network such as that indicated in Scheme 2 (pathway on the right) which retains the phenolic proton despite the very acidic radical formed. Together, these results suggest that the fast electron transfer to the photooxidized Ru-center from the phenolic moiety in **4** is aided by a hydrogen bond network, involving the nitrogens in the dpa arms, which immediately accept the proton when the electron leaves the phenolic center.

This mimics the situation in PS II where the phenolic proton in $tyrosine_Z$ most likely is hydrogen-bonded to Histidine 190 (Figure 1)[17−19] which also aids in fast electron transfer from

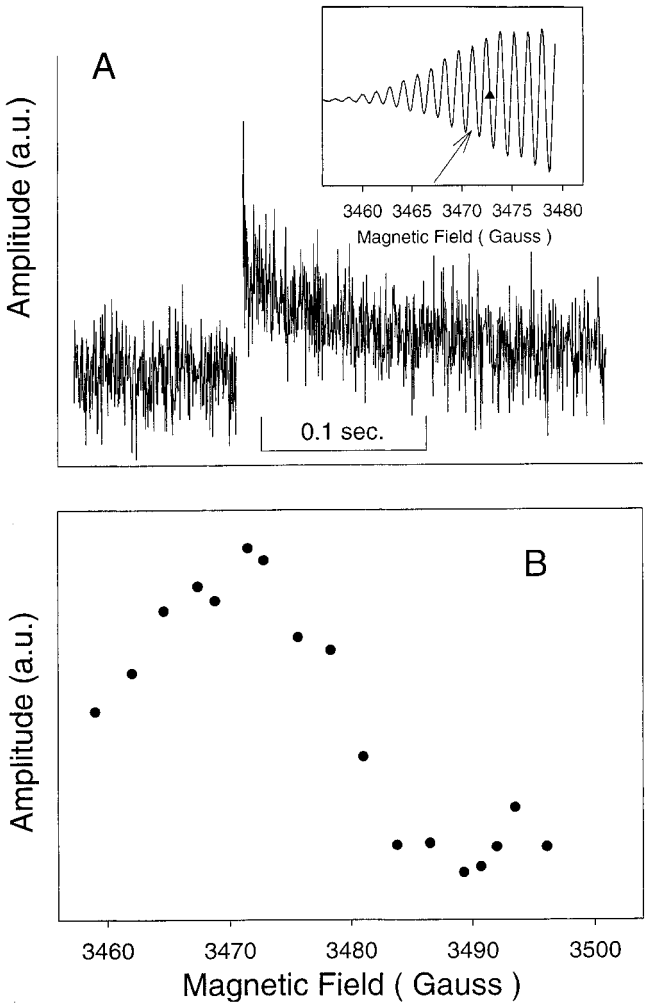


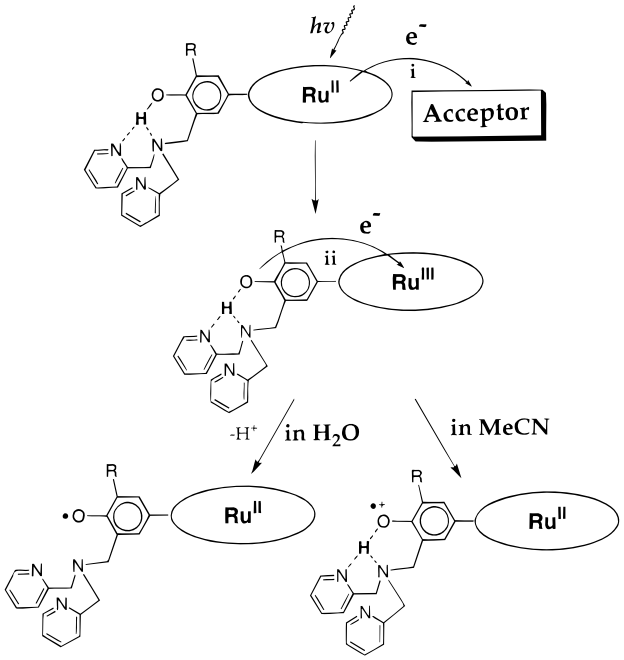
Figure 7. (A) Flash-induced induction and decay of the radical species in **4** after a single flash to a mixture of **4** in the presence of methylviologen ($MV^{\bullet}2PF_6$) in acetonitrile. The inset shows the spectrum of the methylviologen radical and the arrowmarked dot indicates the field position for the transient spectrum shown. The transient signal represents the average of 250 flash-induced events. (B) Kinetic spectrum of the radical species formed in A. The spectrum is constructed from the amplitude of the transient signals (Figure 7A) measured at different field positions chosen where the amplitude of the methylviologen radical signal was minimal or zero (A, inset). The approximate *g* value for the radical species is 2.0029, indicative of the formation of a protonated phenoxyl radical. See Experimental Section for details.

(28) (a) Dixon, W. T.; Murphy, D. *J. Chem. Soc., Faraday Trans. 2* **1976**, *72*, 1221−1230. (b) Barry, B. A.; Babcock, G. T. *Chem. Scr.* **1988**, *28A*, 117−122.

(29) Molecular mechanics calculations (see Supporting Information) predict different conformations of **4** in water and in acetonitrile, which may result in different ET rates. However, that cannot be proven because of the rate limitation imposed by the diffusion-controlled intermolecular electron transfer from excited Ru(II) to methylviologen, faster rates than ca. 1×10^7 s^{-1} for the intramolecular electron transfer from tyrosine to photogenerated Ru(III) cannot be measured.

(30) The electron-transfer rate increases from $k = 5 \times 10^4$ s^{-1} at pH 7 to $k = 7.6 \times 10^4$ s^{-1} at pH 8.2 and $k = 1.3 \times 10^5$ s^{-1} at pH 9.3.

Scheme 2. Proposed Photoinduced Electron Transfer Pathways of Model Compound **4** in the Presence of an Electron Acceptor $[Co(NH_3)_5Cl]^{2+}$ in Water or Methylviologen ($MV^{\cdot}2PF_6$) in Acetonitrile Solution



the tyrosine$_Z$. In this case the result is the neutral radical as judged from the spectral properties of tyrosine$_Z^{ox}$. However, the phenolic proton still resides in the vicinity and is spectroscopically observed to participate in a disordered or bifurcated hydrogen bond.[31] It seems clear that the hydrogen bond is weaker around tyrosine$_Z$ than in **4** since the radical which is formed in PS II is neutral and not cationic.

Experimental Section

General Methods. ^{1}H NMR and ^{13}C NMR spectra were measured using a Bruker-400 MHz spectrometer for 1D or a Bruker-500 MHz spectrometer for 2D. The electrospray ionization mass spectrometry (ESI-MS) experiments were performed on a ZacSpec mass spectrometer (VG Analytical, Fisons Instrument). Electrospray conditions were needle potential, 3kV; acceleration voltage, 4kV; bath and nebulizing gas; nitrogen. The liquid flow was 75 μL per min using a syringe pump (Phoenix 20, Carlo Erba, Fisons instrument). The solvent was pure acetonitrile. Precise mass measurements were obtained by the use of poly(ethylene glycol) (PEG) as an internal standard. The fast atom bombardment mass spectrometry (FAB-MS) experiments were run in a matrix of 1-thioglycerol. Absorption and steady-state emission spectra were recorded with Varian Cary 5E UV−vis−NIR and SPEX Fluorolog II Systems spectrometers. X-Band EPR spectra were recorded on a Bruker ESP380 spectrometer equipped with an Oxford Instruments temperature controller. Time-resolved emission measurements were conducted using single-photon counting equipment, employing a mode-locked Nd:YAG laser to pump a DCM dye laser. The output from the dye laser was frequency doubled to 327 nm and used to excite the samples, and the instrumental response function (fwhm) was 200 ps. The emission was observed around 610 nm by means of an interference filter with a 10-nm bandwidth. All solutions used for photophysical measurements were deoxygenated by purging with nitrogen for fifteen minutes before measurements and then by being kept under an atmosphere of nitrogen. Acetonitrile was of spectroscopic grade, and water was Millipore M-Q quality. In the flash photolysis experiments, an excimer laser pumped a dye laser to produce excitation pulses of 30-ns width at 460 nm. Transient absorption measurements were made at 450 and 600 nm. All solutions were bubbled with nitrogen.

Computational Methods. Molecular mechanics modeling was carried out using MacroModel version 6.0 with the MM3* force field. The force field was extended to include new parameters for the Ru−polypyridine moiety.[32] A conformational search was carried out using a Monte Carlo method[33] together with a truncated Newton−Raphson minimization algorithm. The number of Monte Carlo steps taken was 10 000 and a derivative convergent criteria of 0.05 kJÅ^{-1} mol^{-1} was used. Solvation (water and chloroform) was included in the minimizations using the GB/SA solvation model[34] implemented in MacroModel.

Electrochemistry Study. Electrochemical experiments were done in water and in acetonitrile. The experiments in acetonitrile were performed in an argon-filled glovebox. The electrolytes used were 0.2 M KCl in water and 0.1 M tetrabutylammonium tetrafluoroborate (Aldrich, $TBABF_4$) in acetonitrile. The $TBABF_4$ was vacuum-dried at 140 °C for 48 h, and acetonitrile (Aldrich, 99.8%) was used as received. The glassware was oven-dried. The electrochemical measurements were carried out by using a three-electrode system connected to an Eco Chemie model Autolab/GPES electrochemical interface. The working electrode was a freshly polished glassy carbon disk (diameter 3 mm), and the counter electrode consisted of a platinum wire. The reference electrode was commercial Ag/AgCl in 3 M KCl (0.207 V vs NHE) in water and Ag/AgCl in saturated LiCl (Merck) in acetonitrile. Both the reference and counter electrodes were separated from the solution by a salt bridge in contact with the working electrode. A ferrocenium/ferrocene couple was used as an internal reference in nonaqueous media. All data obtained in acetonitrile are reported vs SCE. The half potential of the reversible Ru(III/II) peak for $Ru(bpy)_3^{2+}$ was assigned to be 1.3235 V vs SCE according to the value reported by Bard et al.[35] By using the Ru(III/II) value obtained for $Ru(bpy)_3^{2+}$ and the half potential for the internal reference the data could be reported vs SCE.

Detection of the Neutral Phenoxyl Radical. To a saturated aqueous solution of $[Co(NH_3)_5Cl]^{2+}$ was added a solution of DMSO and water (10:90, v/v) and **4** (2 mM). The combined solution was transferred by aspiration to a quartz EPR flat-cell, positioned, and pretuned on a 10% aqueous solution of DMSO in the EPR cavity. A spectrum was recorded immediately upon the commencement of continuous laser-flashing (532 nm, 5 Hz, 1.5 W) into the spectrometer cavity from a GCR-200 series Nd:Yag laser (Spectra-Physics, CA). The following EPR spectrometer settings were used: modulation amplitude, 5 G; time constant, 20 ms; sweep time, 84 s; receiver gain, 1×10^5; microwave power, 18.3 mW; microwave frequency, 9.771 GHz. A solution of diphenylpicrylhydrazyl (in 10% aqueous solution of DMSO), transferred to the flat-cell without retuning, was used as a g marker ($g = 2.0036$).

Detection of the Protonated Phenoxyl Radical. The reaction mixture, containing **4** (2 mM) and methylviologen ($MV^{\cdot}2PF_6$) in acetonitrile, was transferred by using aspiration to an aqueous flat-cell positioned in the EPR cavity to obtain the spectrum of the methylviologen radical. The sample was subjected to continuous laser-flashing at 5 Hz (as above) for 1 min, after which an EPR spectrum of the resulting methylviologen radical cation was recorded by using the following instrument settings: modulation amplitude, 0.71 G; time constant, 20 ms; sweep time, 84 s; receiver gain, 2×10^4 microwave power, 17 mW; microwave frequency, 9.770 GHz. The magnetic field positions at which the multilined spectrum of the methylviologen radical crosses the baseline (i.e., has no microwave absorption) were noted. These field positions were used as static field settings in the construction of a "kinetic EPR" spectrum in the search for underlying, transient species (i.e., species other than the methylviologen radical). Without

(31) (a) Tommos, C.; McCracken, J.; Styring, S.; Babcock, G. T. *J. Am. Chem. Soc.* **1998**, *120*, 10441−10452. (b) Dorlet, P.; DiValentin, M.; Babcock, G. T.; McCracken, J. L. *J. Phys. Chem. B* **1998**, 102, 8239−8247. (c) Force, D. A.; Randall, D. W. and Britt, R. D. *Biochemistry* **1997**, *36*, 12062−12070.

(32) Brandt, P.; Norrby, T.; Åkermark, B.; Norrby, P.-O. *Inorg. Chem.* **1998**, *37*, 4120−4127.

(33) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379. Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1990**, *112*, 1419.

(34) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127.

(35) Tokel-Takvoryan, N. E.; Hemmingway, R. E.; Bard, A. J.; *J. Am. Chem. Soc.* **1973**, *95*, 6582.

retuning the instrument, the reaction mixture was replaced with fresh solution for each magnetic field position employed. Time-mode EPR spectra were obtained at each field position following the application of a single laser flash using the following instrument settings: modulation amplitude, 0.71 G; time constant, 0.16 ms; sweep time, 0.328 s; receiver gain, 1.25×10^5; microwave power, 17 mW; microwave frequency, 9.7692 GHz. For each static field position used, traces from 250 sequential laser flashes (523 nm, 5 Hz, 1.5 W) were accumulated. The EPR recording was triggered to commence 500 μs after each flash by means of a personal computer. A solution of diphenylpicrylhydrazyl in acetonitrile was used as a *g* marker (as above). An EPR "spectrum" was then constructed by plotting the initial signal amplitude observed at each static field position.

Materials. L-Tyrosine ethyl ester hydrochloride, 4,4′-dimethyl-2,2′-bipyridine, 2,2′-bipyridine (bpy), 4,4′-bipyridine, rutheniumtrichloride, silvertriflate, triethylamine, ammonium hexafluorophosphate, *p*-cresol, and paraformaldehyde were purchased from Aldrich and used as received. Silica gel 60 (230−400 mesh, Merck, Darmstadt, Germany) and aluminum oxide (Aldrich) were used for column chromatography. *cis*-Dichloro-bis(bpy)ruthenium (*cis*-$Ru(bpy)_2Cl_2 \cdot 2H_2O$) was prepared by the method reported by Meyer et al.[36] All solvents were dried by standard methods. Methylviologen ($MV \cdot 2PF_6$) was made by changing the counterions from methylviologen ($MV \cdot 2Cl$, Aldrich) followed by two times recrystallization (2×) from ethanol/water (90/10). *N*,*N*-Di-(2-picolyl)amine (dpa) was made by reaction of picolyl amine and picolyl aldehyde followed by reduction with sodium borohydride.[37]

2,6-Bis[[*N*,*N*-di(2-pyridylmethyl)amino]methyl]-4-methylphenol (Hbpmp). This compound was made by following a modification of the literature method.[37] To *N*,*N*-di(2-picolyl)amine (2.50 g, 12.5 mmol) in EtOH/H_2O (15 mL/45 mL) was added *p*-cresol (0.54 g, 5.0 mmol) and paraformaldehyde (0.75 g, 25.0 mmol). To the mixture was added 0.3 mL of 2 N HCl, and the two-phase reaction mixture was heated under reflux for 20 h and then allowed to cool to room temperature. CH_2Cl_2 (100 mL) and H_2O (100 mL) were added to the reaction mixture, and the organic phase was separated after washing with another 100 mL of H_2O and dried over anhydrous sodium sulfate. A yellow oil was obtained after evaporation of solvent. Medium-pressure column chromatography on silica gel with gradient eluents CH_2Cl_2 and CH_2Cl_2/MeOH (94/6) afforded a pale yellow oil (2.25 g, 85% yield) which became a solid upon standing, mp 112 °C (uncorrected). ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm 2.23 (s, 3H, -CH_3), 3.77 (s, 4H, ph-CH_2-N), 3.86 (s, 8H, N-CH_2-py), 6.98 (s, 2H, ph-H), 7.11 (m, 4H, py-H), 7.48 (d, J = 7.8 Hz, 4H, py-H), 7.59 (m, 4H, py-H), 8.50 (m, 4H, py-H). ^{13}C NMR (100 MHz, $CDCl_3$), δ in ppm 20.6, 54.8, 59.8, 121.9, 122.9, 123.8, 127.3, 129.7, 136.5, 148.9, 153.6, 159.4.

NH_2-CH(COOEt)-Hbpmp (8). To a suspension of dpa (500 mg, 2.51 mmol) and paraformaldehyde (100 mg, 3.33 mmol) in 2 mL of ethanol and 6 mL of water were added HCl (0.5 m, 0.5 mL) and the tyrosine derivative **6** (300 mg, 0.970 mmol).[38] The mixture was heated under reflux for 4 days, cooled to room temperature and all volatile fractions were evaporated in vacuo. The remaining highly viscous oil was dissolved in 5 mL CH_2Cl_2 at 0 °C, 5 mL of trifluoroacetic acid were added and the mixture was stirred for additional 12 h while it warmed to room temperature. The solvents were evaporated in vacuo and 100 mL of aqueous ammonia, saturated with NaCl, were added. The mixture was extracted with 5 × 50 mL CH_2Cl_2, the combined organic phases were dried over Na_2SO_4 and again the solvent was subsequent evaporated in vacuo. Column chromatography was performed on silica gel (30 g) with the following eluents: CH_3CN/CH_3COOH = 20:1, 100 mL; $CH_3CN/H_2O/CH_3COOH$ = 100:10:1, 200 mL; CH_3CN/NH_3(aqueous) = 10:1, 200 mL; eluating the desired compound as the last fraction. This fraction was concentrated in vacuo to 30 mL and extracted with 3 × 50 mL CH_2Cl_2. The combined extracts were dried over Na_2SO_4, and after evaporation of the solvent 510 mg of (83%) **8** was isolated as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm 1.12 (t, J = 7.0 Hz, 3H, $CO_2CH_2CH_3$), 1.70 (br s, 2H, NH_2), 2.72 (dd, J = 7.9 and 13.4 Hz, 1H, CHCH_2Tyr), 2.93 (dd, J_1 = 4.9 and 13.4 Hz, 1H, CHCH_2Tyr), 3.59 (m, 1H, CHCH$_2$Tyr), 3.74 (s, 4 H, TyrCH_2N), 3.81 (s, 8H, PyrCH_2N), 4.04 (q, J = 7.0 Hz, 2H, CO_2CH_2-CH_3), 6.99 (s, 2H, arom. Tyr-CH), 7.06 (m, 4H, Pyr-CH), 7.43 (m, 4H, Pyr-CH), 7.54 (m, 4H, Pyr-CH), 8.46 (m, 4H, Pyr-CH), 10.95 (s, 1H, TyrOH). ^{13}C NMR (100.6 MHz, $CDCl_3$, DEPT) δ in ppm 14.03 (+), 40.28 (−), 54.55 (−), 55.29 (+), 59.61 (−), 60.54 (−), 121.78 (+), 122.75 (+), 123.97 (C_{quart}), 126.58 (C_{quart}), 129.87 (+), 136.33 (+), 148.70 (+), 154.68 (C_{quart}), 159.05 (C_{quart}), 174.96 (C_{quart}). ESI-MS found: m/z 654 (**8** plus Na^+ requires 654).

$[Ru(bpy)_2[4\text{-Me-}4'\text{-CONH-CH(COOEt)(Hbpmp)}]](PF_6)_2$ (4). Route A. To compound **3**[14] (0.177 g, 0.15 mmol) in EtOH/H_2O (1.2 mL/1.5 mL) *N*,*N*-di(2-picolyl)amine (0.150 g, 0.75 mmol) and paraformaldehyde (0.023 g, 0.75 mmol) were added, followed by addition of 0.05 mL 2 N HCl, and the two-phase reaction mixture was heated under reflux for 20 h under argon. After the solution was cooled to room temperature, a solution of NH_4PF_6 (2 g) in 20 mL of H_2O and 20 mL of CH_2Cl_2 was added to the reaction mixture, and the organic phase was isolated. To this organic solution, which contained the product **4** and the excess dpa, a 20 mL solution of 0.2 N HCl and NH_4PF_6 (1 g) were added. The organic phase was washed with a solution of 30 mL of H_2O and Na_2CO_3 (3 g) and then with 30 mL of water. The organic phase was finally separated and dried over anhydrous sodium sulfate. After evaporation of the solvent, a red solid was obtained. Further purification was conducted by three successive flash column chromatographies on Al_2O_3 with eluents of MeOH/2M HCl (95:5, v/v). The desired fractions were combined and concentrated to about 10 mL and then neutralized by adding an aqueous solution of Na_2CO_3. Extraction with CH_2Cl_2, drying over Na_2SO_4, and evaporation of the solvent afforded **4** (0.149 g, 65% yield) as a red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ in ppm 1.01 (t, J = 7.0 Hz, 3H, $CO_2CH_2CH_3$), 2.51 (s, 3H, Me), 3.01−3.12 (m, 2H, CHCH_2Tyr), 3.55−3.72 (m, 12H, CH_2N), 3.99 (q, J = 7.0 Hz, 2H, $CO_2CH_2CH_3$), 4.62−4.74 (m, 1H, CHCH$_2$Tyr), 7.09 (s, 2H, Tyr), 7.19 (dd, J = 4.8 and 7.0 Hz, 4H, Pyr), 7.39 (d, J = 7.4 Hz, 4H, Pyr), 7.43−7.57 (m, 4H, Bpy), 7.63−7.72 (m, 8H, Bpy, Pyr), 7.72−7.77 (m, 1H, Bpy), 7.80−7.85 (m, 1H, Bpy), 8.09−8.19 (m, 5H, Bpy), 8.39 (dd, J = 4.8 and 5.4 Hz, 4H, Pyr), 8.69 (d, J = 19.8 Hz, 1H, Bpy), 8.77−8.86 (m, 5H, Bpy), 9.05 (d, J = 19.8 Hz, 1H, Bpy), 9.45 (br s, 1H, NH), 10.95 (s, 1H, TyrOH). ^{13}C NMR (100.6 MHz, DMSO-d_6, DEPT) δ in ppm 13.77 (+), 20.61 (+), 35.77 (−), 53.78 (−), 54.84 (+), 58.63 (−), 60.56 (−), 121.55 (+), 122.05 (+, Pyr), 122.49 (+, Pyr), 123.48 (C_{quart}), 124.34 (+), 125.39 (+), 126.13 (C_{quart}), 127.80 (+), 128.85 (+), 129.82 (+), 136.51 (+, Pyr), 137.89 (+), 140.80 (C_{quart}), 148.53 (+, Pyr), 149.79 (C_{quart}), 149.84 (C_{quart}), 150.28 (+), 150.97 (+), 151.82 (+), 154.14 (C_{quart}), 155.48 (C_{quart}), 156.27 (C_{quart}), 156.31 (C_{quart}), 156.36 (C_{quart}), 156.40 (C_{quart}), 156.45 (C_{quart}), 157.31 (C_{quart}), 157.34 (C_{quart}), 158.50 (C_{quart}, Pyr), 162.91 (C_{quart}), 162.97 (C_{quart}), 171.02 (C_{quart}). IR (cm^{-1}) 1669 (ν(C═O)), 1732 (ν(C═O)), 3405 (ν(OH)). Electrospray ionization mass spectrometry (ESI-MS) spectrum of **4** from acetonitrile gave a mono charged peak at m/z 1386 (calcd for $[M - PF_6^-]^+$, 1386) and doubly charged peaks at m/z 693 (calcd for $[M - PF_6^- + H^+]^{2+}$, 693) and m/z 621 (calcd for $[M - 2PF_6^-]^{2+}$, 621). Fast atom bombardment mass spectrometry (FAB-MS) using 1-thioglycerol as matrix, showed the mono charged peaks at m/z 1532 (calcd for $[M + H^+]^+$, 1532) and 1386 (calcd for $[M - PF_6^-]^+$, 1386).

Route B. To Ru-complex−acid **9** (300 mg, 0.33 mmol) was added freshly distilled $SOCl_2$ (5 mL). The resulting red solution was stirred for 2 h under reflux and then cooled to room temperature. The $SOCl_2$ was evaporated in vacuo, and CH_3CN (10 mL) was added followed by **8** (210 mg, 0.33 mmol) dissolved in CH_3CN (5 mL). After 30 min, K_2CO_3 (800 mg, 5.79 mmol) was added, and the resulting suspension was stirred for 24 h at room temperature. The mixture was then diluted with water (100 mL) and extracted with CH_2Cl_2 (3 × 80 mL). The combined organic phases were concentrated by evaporation to 10 mL and subsequently chromatographed on 30 g silica gel (eluent: $CH_3CN/H_2O/KNO_{3(aq)}/CH_3COOH$ = 100:10:5:2). To the obtained shiny red

(36) Sullivan, B. P.; Salmon, D. J.; Meyer, T. J. *Inorg. Chem.* **1978**, *17*, 3334.

(37) Lubben, M.; and Feringa, B. L. *J. Org. Chem.* **1994**, *59*, 2227−2233.

(38) Houlihan, F.; Bouchard, J.; Frechet, J. M.; Willson, C. G. *Can. J. Chem.* **1985**, *63*, 153−162.

(39) Due to the low solubility of the complex **4** in water, it has not been possible to perform NOESY studies to verify this result.

fraction, was added aqueous K_2CO_3 (20 mL), followed by evaporation of the highly volatile parts. After dissolving of NH_4PF_6 (2.00 g, 12.27 mmol), the mixture was extracted with CH_2Cl_2 (6 × 50 mL). Evaporation of the organic solvents and washing of the obtained red solid with ethyl acetate (50 mL) and hexane (50 mL) gave **4** (266 mg, 53%). The NMR spectra turned out to be identical with the ones from the other pathway.

Acknowledgment. This work was financially supported by grants from the Knut and Alice Wallenberg Foundation, the Swedish Natural Science Research Council, the Swedish Research Council for Engineering Sciences, the European TMR Program (TMR Network CT96-0031), and the Nordic Energy Research Program. We thank Drs. Gunnar Stenhagen and Jerker Mårtensson for performing the ESI-MS and FAB-MS measurement, Dr. Anna Börje for help with the 2D NMR, Mr. Jiutian Zheng for synthesis of dpa and Hbpmp, and Drs. Pierre Mialane and Heimo Schmitt for help with the IR.

Supporting Information Available: A conformational search of **4** in water and in acetonitrile by molecular mechanics calculation and a NOESY spectrum of **4** in DMSO-d_6 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA984048C