Metal–Coenzyme Complexes

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Proton Shift upon One-Electron Reduction in Ruthenium(II)-Coordinated Pterins**

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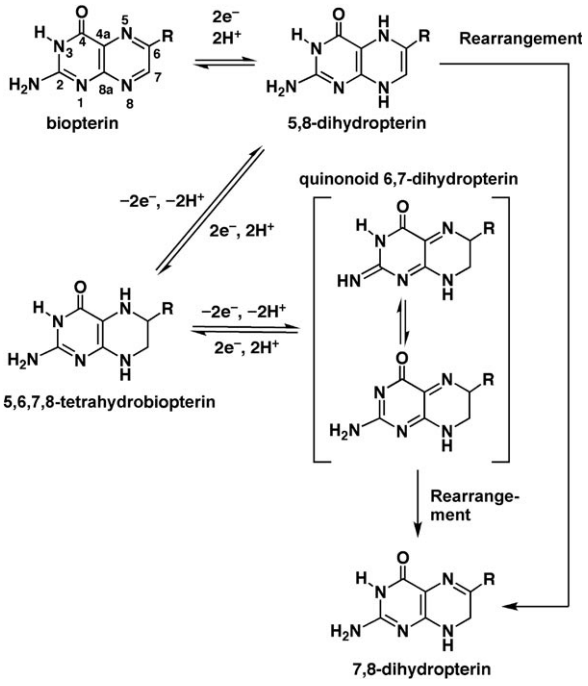
Pterins are ubiquitous heteroaromatic coenzymes that are involved in many biological redox reactions in the vicinity of various metal ions.[1–4] The redox processes of pterins proceed through proton-coupled electron transfer (PCET) involving the pyrazine moiety, in which up to four protons and electrons are manipulated in a concerted manner.[5] Such processes can convert fully oxidized biopterin into fully reduced 5,6,7,8-tetrahydrobiopterin.

In the course of the redox processes of pterins, as shown in Scheme 1, a 5,8-dihydropterin is formed as a two-electron-reduced species of the pterin.[6] In contrast, the two-electron oxidation of a 5,6,7,8-tetrahydrobiopterin gives a quinonoid 6,7-dihydropterin, which contains a C=N bond involving the carbon atom at the 2-position.[6,7] Both dihydropterins undergo thermal rearrangement to form a 7,8-dihydropterin as a thermodynamic sink.[7]



Scheme 1. PCET processes of pterins.

Fully oxidized pterins are known to release a proton from the nitrogen atom at the 3-position or the oxygen atom at the 4-position to coordinate to metal ions in a deprotonated imidate form (Scheme 2).[8] Subsequent protonation gives a neutral pterin ligand, which undergoes reduction. Recently, we reported the one-electron reduction of monoprotonated pterins coordinated to a ruthenium(II)–tris(2-pyridylmethyl)-amine (tpa) unit.[9] The reduction gives ruthenium-bound monohydropterin radicals in which an unpaired electron is delocalized over the PCET region of the pyrazine moiety. Herein, we report the unprecedented observation of a proton shift from the nitrogen atom at the 1-position of the pyrimidinone moiety to the nitrogen atom at the 8-position of the pyrazine moiety upon the one-electron reduction of novel monoprotonated pterins in ruthenium(II) complexes (Scheme 2).

$[Ru(Hdmp)(tpa)](ClO_4)_2$ (**1**; Hdmp = 6,7-dimethylpterin) and $[Ru(Hdmdmp)(tpa)](ClO_4)_2$ (**2**; Hdmdmp = *N*,*N*-dimethyl-6,7-dimethylpterin) were prepared through protonation of the corresponding precursor complexes [Ru(dmp)-(tpa)](ClO_4) (**3**)[9b] and [Ru(dmdmp)(tpa)](ClO_4) (**4**),[9,10] respectively, which have deprotonated, anionic pterin ligands, by adding 1 equivalent of $HClO_4$ in CH_3CN. Vapor diffusion of diethyl ether into the CH_3CN solutions of **1** and **2** gave single crystals suitable for X-ray crystallography.[11] ORTEP drawings of **1** and **2** are shown in Figure 1 (see also Figure S1 in the Supporting Information).

As a common feature of **1** and **2**, one of the perchlorate anions forms a hydrogen bond with an NH group at the 1-position of the pterin ligand, as indicated by the close contact between one oxygen atom and N7 (Figure 1).[12] In the crystal of **1**, one of the perchlorate anions forms two hydrogen bonds to the neutral Hdmp ligand, one to the amino group at the 2-position (O···N 2.93(1)–3.046(9) Å) and one to the NH group at the 1-position (O···N 2.84(1)–2.88(1) Å).[13] This result clearly indicates that the proton is attached to the nitrogen atom at the 1-position in **1**. In the crystal of **2**, the NH group at the 1-position of the neutral Hdmdmp ligand forms a hydrogen bond with a perchlorate anion (O3···N7 2.94(1) Å) or a water molecule of crystallization (O···N 2.78(1) Å), confirming the protonation of the nitrogen atom at the 1-position in **2**.[13] These results indicate that the first protonation occurs at the nitrogen atom at the 1-position of the coordinated pterins, rather than the nitrogen atom at the 8-position. Prior to this work, it was thought that the first site of

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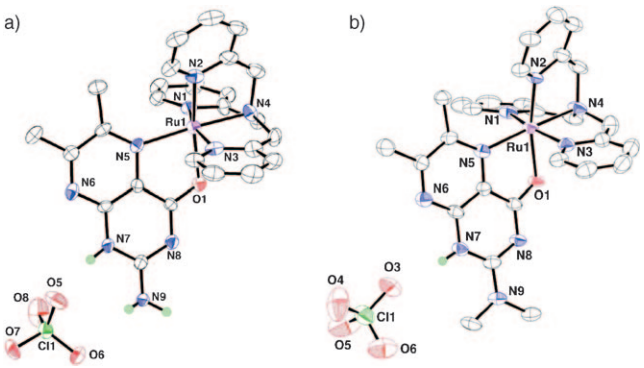


Figure 1. ORTEP drawings of a) **1** and b) **2** with thermal ellipsoids set at 50% probability. Only hydrogen atoms attached to nitrogen atoms are shown. Only the perchlorate anion hydrogen-bonded to the pterin ligand is shown.

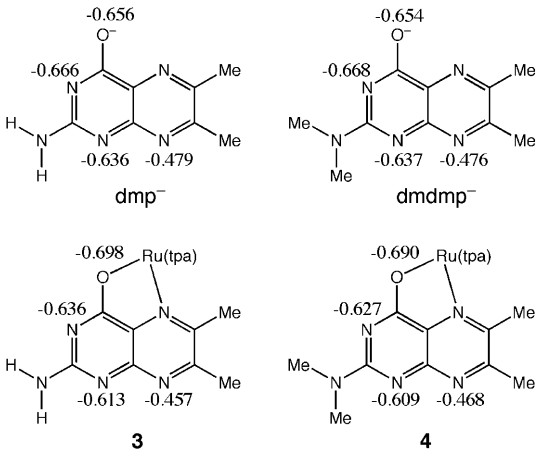


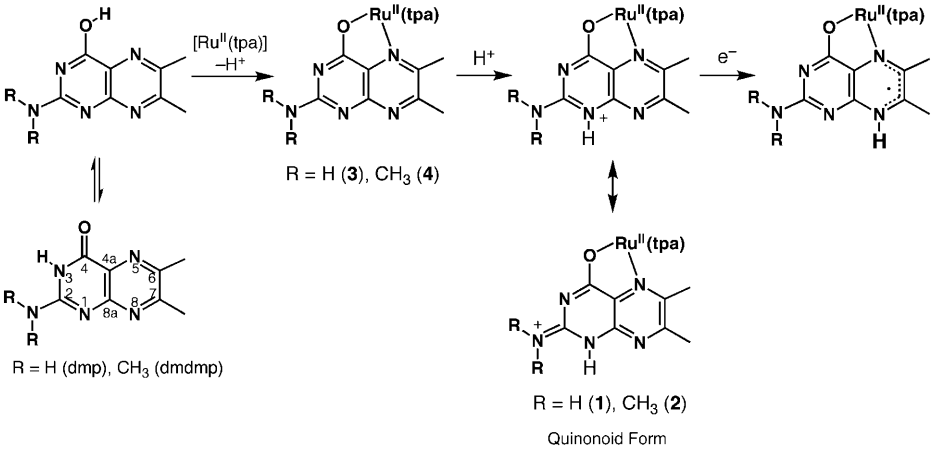
Figure 2. Atomic charges for dmp^-, $dmdmp^-$, **3**, and **4** obtained by DFT calculations with natural population analysis.

a deprotonated, fully oxidized pterin to be protonated was the nitrogen atom at the 8-position or the originally deprotonated nitrogen atom at the 3-position.[14]

The C–N bond lengths to the amino group at the 2-position in **1** (1.311(9)–1.32(1) Å) and **2** (1.326(8) and 1.34(1) Å) are shorter than that in **4** (1.309(9) Å).[15] The shorter bond lengths suggest that protonation of the nitrogen atom at the 1-position stabilizes the quinonoid forms of the coordinated pterins, with or without hydrogen bonding to the amino group at the 2-position (Scheme 2).[16]

Density functional theory (DFT) calculations were applied to clarify why protonation first occurs at the nitrogen atom at the 1-position. Atomic charges for optimized structures of dmp^- and $dmdmp^-$ (B3LYP/6-31G(d)), and of **3** and **4** (B3LYP/LANL2DZ) are shown in Figure 2. The results indicate that the nitrogen atom at the 1-position is intrinsically more negative than that at the 8-position. The optimized structures of **1** and **2**, which are protonated at the nitrogen atom at the 1-position (N1-H^+), have lower total energies than those of isomers of **1** and **2** that are protonated at the nitrogen atom at the 3-position (N3-H^+; +7.2 kcal mol^{-1} for **1**, +7.5 kcal mol^{-1} for **2**) or at the 8-position (N8-H^+; +6.9 kcal mol^{-1} for **1**, +7.5 kcal mol^{-1} for **2**), as shown in Figure 3. Thus, protonation occurs at the nitrogen atom at the 1-position prior to at the nitrogen atoms at the 3- or 8-positions.

Variable-temperature ^1H NMR spectroscopy measurements in CD_3CN were performed to shed light on the solution structures of the pterin ligands. In the case of **1**, a broad peak with an intensity corresponding to two protons was detected at $\delta = 6.39$ ppm, indicating free rotation of the amino group at the 2-position. Upon decreasing the temperature to 233 K, the peak split into two peaks, one of which broadened below 263 K (Figure S2 in the Supporting Information). This result is probably due to two-point hydrogen bonding of the amino group with a perchlorate anion, which makes one of the protons more acidic at lower temperatures. In sharp contrast, in the case of **3**, the singlet peak due to the amino group at the 2-position split into two peaks below 243 K, and its rotation barrier was estimated to be 12.9 kcal mol^{-1} at 263 K (Figure S3 in the Supporting Information). On the other hand, **2** exhibited one singlet at $\delta = 3.14$ ppm due to the two methyl groups of the *N*,*N*-dimethylamino group at the 2-position, which split into two peaks below 273 K (Figure S4 in the Supporting Information). The rotation barrier of the *N*,*N*-dimethylamino group at the 2-position of the Hdmdmp ligand in **2** was estimated to be 13.8 kcal mol^{-1} at 273 K.[17] The precursor **4** also exhibited similar peak splitting for the singlet ascribed to the methyl groups of the *N*,*N*-dimethylamino group below 263 K (Figure S5 in the Supporting Information), and the rotation barrier was estimated to be 13.0 kcal mol^{-1} at 263 K, which is slightly lower than that in **2**. This result is ascribed to the contribution of the quinonoid form of the Hdmdmp ligand, which is stabilized by protonation of the nitrogen atom at the 1-position.



Scheme 2. Formation and PCET processes of monoprotonated pterins coordinated to a ruthenium(II)–tpa unit.

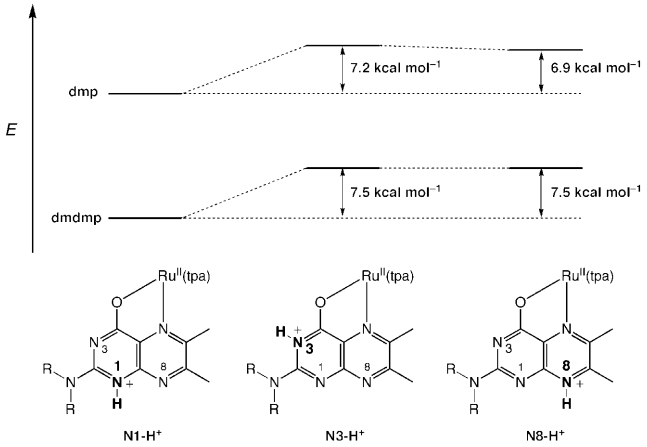


Figure 3. Relative energies of isomers of **1** and **2** that are protonated at the nitrogen atom at the 1-, 3-, or 8-position, obtained by DFT calculations.

Cyclic voltammograms (CV) of **1** and **2** in CH_3CN (0.1 M tetrabutylammonium (TBA) hexafluorophosphate as an electrolyte) showed reversible redox waves due to the ruthenium(II)/ruthenium(III) redox couple at $E_{1/2} = 0.55$ V ($\Delta E = 60$ mV) and $E_{1/2} = 0.52$ V ($\Delta E = 62$ mV) versus ferrocene/ferrocenium (Fc/Fc^+), respectively. In addition, quasi-reversible redox waves ($E_{pc} = -1.36$ V for **1**, $E_{pc} = -1.39$ V for **2**) due to reduction of the pterin ligands, which are protonated at the nitrogen atom at the 1-position, were observed (Figure S6 in the Supporting Information). In contrast, the CVs of **3** and **4** show reversible redox couples for the reduction of the dmp^- ($E_{1/2} = -2.04$ V) or $dmdmp^-$ ($E_{1/2} = -2.08$ V) ligand at 293 K, respectively (Table S3 in the Supporting Information).[9b]

In the course of the electrochemical reduction of **2** in CH_3CN (0.1 M $TBAPF_6$) at 253 K with a controlled potential of $E = -1.4$ V, we detected a well-resolved electron spin resonance (ESR) signal at $g = 2.0030$ with hyperfine splitting (Figure 4a). The spectrum (Figure 4b) simulated using the hyperfine coupling constants (hfcs) depicted in Figure 4c exhibits excellent agreement with the observed spectrum. DFT calculations at the UB3LYP/6-31G* level of theory made it possible to assign the hfcs of nitrogen and hydrogen nuclei (Figure 4d). The assignments indicate the existence of a proton at the nitrogen atom at the 8-position with an hfc of 8.30 G. Other hfcs are associated with the nitrogen atoms at the 5-position (8.50 G) and the 8-position (5.14 G), and the protons of the methyl groups at the 6-position (1.07 G) and the 7-position (5.61 G). In contrast, DFT calculations for possible isomers of **2** that are protonated at the nitrogen atoms at the 1- or 3-positions gave different sets of calculated hfcs: the hfcs of protons at the nitrogen atoms at the 1-position (0.12 G) or the 3-position (0.52 G) were too small to match the observed spectrum of **2** (Figure S7 in the Supporting Information). Thus, we concluded that the proton attached to the nitrogen atom at the 1-position of the pyrimidinone moiety in **2** shifted to the nitrogen atom at the 8-position of the pyrazine moiety upon one-electron reduction to form the pterin π-anion radical. This proton shift may induce the quasi-reversibility of the redox behavior observed

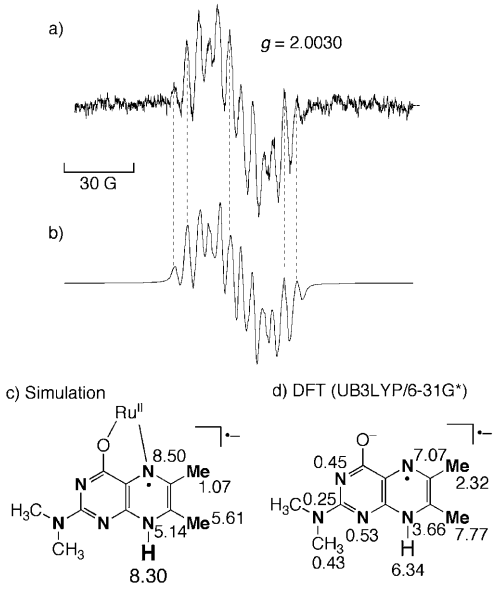


Figure 4. a) ESR spectrum of $[Ru(Hdmdmp^{-\bullet})(tpa)]^+$ generated in the course of electrochemical reduction at −1.4 V (versus Fc/Fc^+) in CH_3CN at 253 K. b) Simulation of the spectrum in (a) with a maximum-slope line width of 1.8 G. c) Hfcs [G] used for the simulated spectrum in (b). d) Hfcs [G] calculated for the DFT-optimized structure.

in the CV of **2**. In addition, the ESR spectroscopy analysis indicates that the unpaired electron is delocalized over the PCET region (5-, 6-, 7-, and 8-positions) of the pyrazine moiety, as observed for $[Ru(H_2dmp^{\bullet})(tpa)](ClO_4)$ and $[Ru(H_2dmdmp^{\bullet})(tpa)](ClO_4)$, which contain one-electron-reduced species of doubly protonated pterin ligands.[9] The increase of the charge and spin density in the pyrazine ring of the pterin ligand upon reduction is consistent with that observed upon reduction of free pterins.[18] Thus, the ruthenium-coordinated pterin retains the fundamental characteristics of free pterins, even though the g values of the ruthenium-bound pterin radicals are shifted owing to spin–orbit coupling.[19]

DFT calculations at the UB3LYP/LANL2DZ level of theory on the one-electron-reduced forms of **1** and **2** were performed to verify the proton shift discussed above. The relative energies of optimized structures of the one-electron reduced species $[Ru(Hdmp^{\bullet-})(tpa)]^+$ and $[Ru(Hdmdmp^{\bullet-})(tpa)]^+$ are summarized in Figure 5. In each case, the forms protonated at the nitrogen atom at the 8-position (N8-H$^{\bullet}$) had the lowest energy, compared to the forms protonated at the nitrogen atom at the 1-position (N1-H$^{\bullet}$; +6.4 kcal mol^{-1} for **1**, +5.7 kcal mol^{-1} for **2**) or the 3-position (N3-H$^{\bullet}$; +13.9 kcal mol^{-1} for **1**, +13.2 kcal mol^{-1} for **2**). These results lend credence to the proton shift upon one-electron reduction of **1** and **2**.

To close, we have demonstrated that the ruthenium(II)-coordinated pterins in **1** and **2** are protonated at the nitrogen atom at the 1-position. This protonation, which is assisted by hydrogen-bond formation, stabilizes the quinonoid forms of the ligands in crystals and solutions of **1** and **2**. The one-electron reduction of the coordinated pterins induces a unique proton shift from the nitrogen atom at the 1-position

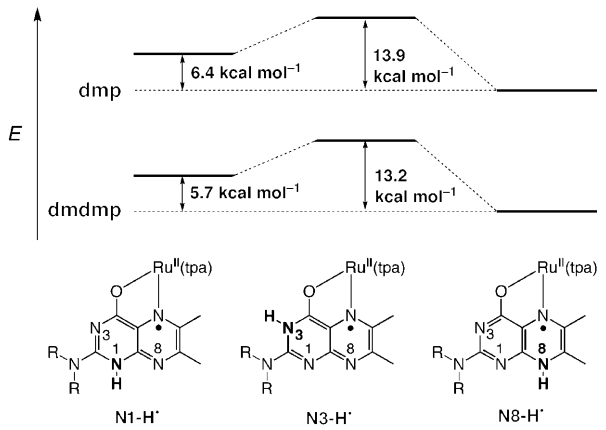


Figure 5. Relative energies of π-anion radical isomers of **1** and **2** that are protonated at the nitrogen atom at the 1-, 3-, or 8-position, obtained by DFT calculations.

to that at the 8-position to give the corresponding π-anion radicals. This transformation is a novel peri (1,8) rearrangement in a heteroaromatic system in the course of a redox reaction. These findings shed new light on the PCET processes of pterins, with respect to how the pterins accept protons and electrons.

Experimental Section

1: Et_2O vapor was deposited at room temperature onto a solution containing **3** and $HClO_4$ (60%, 1 equiv) in CH_3CN to give single crystals of **1**. UV/Vis (CH_3CN): $\lambda_{max} = 311$, 344, 390, 437, 498 nm. ^{1}H NMR (CD_3CN): $\delta = 2.83$ (s, 3H; 7-CH_3), 3.26 (s, 3H; 6-CH_3), 4.60 (s, 2H; CH_2 (ax)), 5.04, and 5.23 (ABq, $J_{AB} = 16$ Hz, 4H; CH_2 (eq)), 6.38 (br s, 2H; NH_2), 7.01 (t, 6 Hz, 1H; py-*H*5 (ax)), 7.14 (t, 7 Hz, 2H; py-*H*5 (eq)), 7.21 (d, 7 Hz, 1H; py-*H*3 (ax)), 7.38 (d, 8 Hz, 2H; py-*H*3 (eq)), 7.50 (t, 8 Hz, 1H; py-*H*4 (ax)), 7.71 (td, $J = 8$ and 2 Hz, 2H; py-*H*4 (eq)), 8.11 (d, $J = 6$ Hz, 2H; py-*H*6 (eq)), 8.86 ppm (d, $J = 5$ Hz, 1H; py-*H*6 (ax)).

2: The same method was employed as described above, using **4** instead of **3**. UV/Vis (CH_3CN): $\lambda_{max} = 358$, 440, 493 nm. ^{1}H NMR (CD_3CN): $\delta = 2.83$ (s, 3H; 7-CH_3), 3.13 (s, 6H; N(CH_3)$_2$), 3.25 (s, 3H; 6-CH_3), 4.59 (s, 2H; CH_2 (ax)), 5.04 and 5.21 (ABq, $J_{AB} = 16$ Hz, 4H; CH_2 (eq)), 7.01 (td, $J = 7$ and 2 Hz, 1H; py-*H*5 (ax)), 7.14 (t, 6 Hz, $J = 2$ *H*; py-H5 (eq)), 7.20 (d, $J = 7$ Hz, 1H; py-*H*3 (ax)), 7.38 (d, $J = 8$ Hz, 2H; py-*H*3 (eq)), 7.49 (td, $J = 8$ and 1 Hz; py-*H*4 (ax)), 7.70 (td, $J = 8$ and 2 Hz, 2H; py-*H*4 (eq)), 8.11 (dd, $J = 5$ and 2 Hz, 2H; py-*H*6 (eq)), 8.85 ppm (d, $J = 5$ Hz, 1H; py-*H*6 (ax)).

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[10] Perchlorate salts of metal complexes with organic ligands are potentially explosive. They should be handled with great care in small quantities.

[11] Crystal data for **1**: $C_{28}H_{26.75}Cl_2N_{9.75}O_{9.13}Ru$, $M_r = 817.89$, triclinic, $P\bar{1}$ (No. 2), $a = 12.403(6)$, $b = 18.005(9)$, $c = 32.08(2)$ Å, $\alpha = 92.655(8)$, $\beta = 96.468(9)$, $\gamma = 102.141(6)°$, $V = 6040(6)$ Å^3, $T = -150$ °C, $Z = 8$, $R(R_w) = 0.098(0.2745)$ ($I > 2\sigma(I)$), GOF = 1.15. Crystal data for **2**: $C_{29.5}H_{31}Cl_2N_{9.75}O_{9.5}Ru$, $M_r = 846.11$, monoclinic, $P2_1/n$ (No. 14), $a = 23.534(7)$, $b = 10.550(3)$, $c = 30.064(9)$ Å, $\beta = 106.351(4)°$, $V = 7163(4)$ Å^3, $T = -150$ °C, $Z = 8$, $R(R_w) = 0.105(0.282)$ ($I > 2\sigma(I)$), GOF = 1.16. CCDC-686790 (**1**) and -686791 (**2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[12] There is no interaction between the other perchlorate anion and the pterin ligand.

[13] Single crystals of **1** and **2** contain four and two crystallographically independent molecules, respectively. In the case of **2**, one independent molecule forms a hydrogen bond with a perchlorate anion, and the other independent molecule forms a hydrogen bond with a water molecule of crystallization. See Figure S1 in the Supporting Information.

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[19] Atomic charges for the one-electron-reduced forms of **3** and **4** obtained by DFT calculations were not consistent with the ESR spectroscopy results. The DFT calculations suggest that the nitrogen atom at the 8-position is still less negative than that at the 1-position in the one-electron-reduced species, with or without the ruthenium(II)–tpa unit. See Figure S8 in the Supporting Information.

