3462 © 1990 The Chemical Society of Japan Bull. Chem. Soc. Jpn., 63, 3462—3466 (1990) [Vol. 63, No. 12

The Anion Radicals of Pheophytin *a* and Its Derivatives Studied by Means of CIDNP Technique

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(Received May 30, 1990)

We applied the Chemically Induced Dynamic Nuclear Polarization (CIDNP) technique to the photochemical reaction of pheophytin *a* (**P1**) and 4-methoxyphenol (**1**) in benzene-d_6. The observed CIDNP effects were explained in terms of reversible electron-transfer reactions from **1** to $^3(\mathbf{P1})^*$ and from $(\mathbf{P1})^{\cdot-}$ to $(\mathbf{1})^{\cdot+}$. The polarized signals of **P1** were induced from the in-cage recombination of the triplet radical ion pair.

The initial stage of photosynthesis in photosynthetic bacteria and green plants involves a highly efficient electron-transfer reaction, generating an anion-cation radical pair.[1,2] In the photosynthetic bacterial reaction centers, the cation radical of bacteriochlorophyll dimer and the anion radical of bacteriopheophytin are the initial components at the photoexcited charge separation stage. Recently evidence has also been accumulated to indicate the involvement of pheophytin *a* (**P1**) as the initial electron acceptor in the photosynthetic reaction center of green plants (PS II).[2] Thus the investigation of the electronic structure of the anion radical of **P1** will be of considerable importance because of its presumed unique role in the initial electron-transfer process.

ESR and ENDOR experiments have been performed for the characterization of $(\mathbf{P1})^{\cdot-}$.[3,4] However, owing to the broad ESR spectra, the limited resonances of the ENDOR experiments, and the complicated tetrapyrrole pigments, it is not necessarily easy to draw definite conclusions on the electronic structure of $(\mathbf{P1})^{\cdot-}$.

Chemically Induced Dynamic Nuclear Polarization (CIDNP) has been widely used to investigate a variety of radical reactions. We have applied previously the photo-CIDNP technique to study the photoinduced electron-transfer reactions of porphyrins. When phenol-linked porphyrin or tyrosine-linked pyropheophorbide *a* was irradiated in the presence of quinones, the corresponding quinone adducts were obtained in good yields. In these photoaddition reactions, the polarized signals due to both the quinone-linked porphyrin and the starting phenol-linked porphyrin were simultaneously observed.[5] Based on these results, the triplet radical pair consisting of phenoxyl radical and semiquinone radical was identified as the reaction intermediate. We have similarly investigated the reversible electron-transfer reaction between porphyrins and electron-rich phenols by the CIDNP technique, which was the first example of the successful detection of anion radicals of porphyrins under the steady state CIDNP conditions.[6] In this paper we applied the CIDNP technique to investigate photochemical behaviors of more complicated natu-

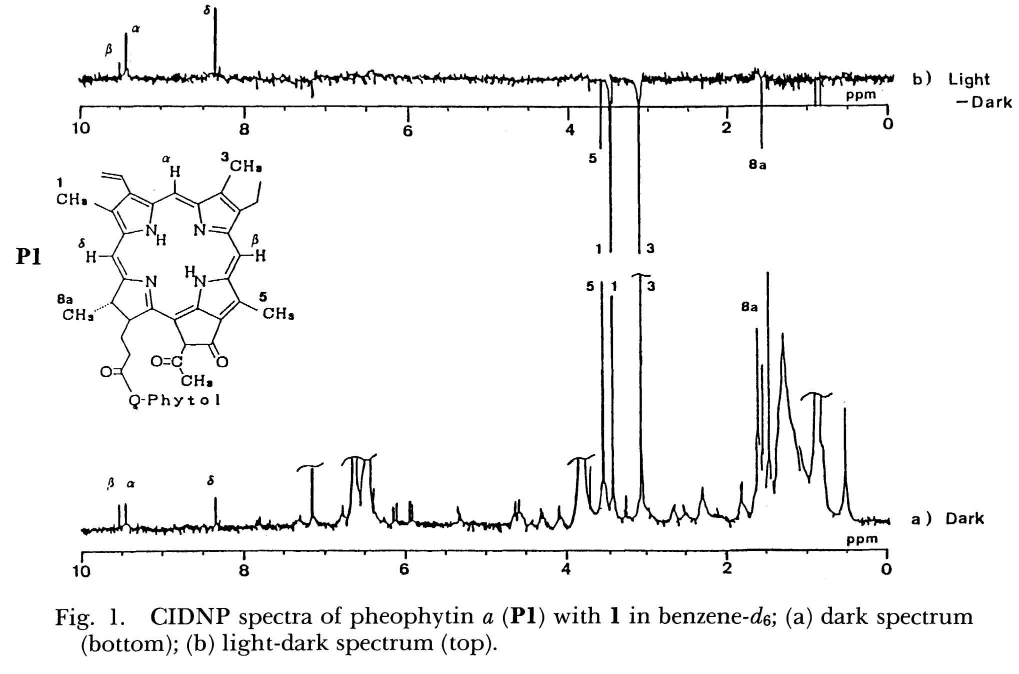

Fig. 1. CIDNP spectra of pheophytin *a* (**P1**) with **1** in benzene-d_6; (a) dark spectrum (bottom); (b) light-dark spectrum (top).

rally occurring photosynthetic pigments such as pheophytin *a* (**P1**) and its derivatives.

Results and Discussion

In Fig. 1, the CIDNP spectra observed in a benzene-d_6 solution of **P1** (1 mM) and 4-methoxyphenol **1** (10 mM) are given, (a; bottom, dark spectrum) and (b; top, light-dark spectrum). Enhanced absorption signals were observed for the three meso-protons (α, β, and δ-H) and emission signals for the peripheral methyl protons (1-. 3-, 4b-, 5-, and 8a-positions). At the low concentrations of **1** (<3 mM), these CIDNP signals were not clearly detected. In solvents such as methanol-d_4 and acetonitrile-d_3, the weak polarizations due to **1** were observed.

According to the Kaptein's rule,[7] the polarized direction of the CIDNP effects is determined by several parameters, including the spin multiplicity of the precursor (μ), the magnetic properties (electron g-factor differences (Δg) and hyperfine coupling constants (a_i)) of the radical intermediates, and the type of the reaction (ε) by which the polarized products are formed.

Judging from the Kaptein's rule,[7] the observed CIDNP effects were reasonably explained by assuming the formation of the triplet radical ion pair: ${}^3\overline{(\mathbf{P1})^{\cdot-}, (\mathbf{1})^{\cdot+}}$ (g values are 2.0030 (±0.0001) for $(\mathbf{P1})^{\cdot-}$,[3] and 2.0035 for $(\mathbf{1})^{\cdot+}$;[8] thus $\Delta g<0$ for **P1**). Electron transfer from **1** to ${}^3(\mathbf{P1})^*$ ($\mu>0$), in-cage electron return ($\varepsilon>0$) in the triplet radical ion pair, and $a_i<0$ for the meso proton of **P1**, give rise to net effect: $\Gamma_{ne}=\Delta g\cdot a_i\cdot\mu\cdot\varepsilon=-\cdot-\cdot+\cdot+>0$; the enhanced absorption is expected. On the other hand for the peripheral methyl proton, $a_i>0$, and thus net effect: $\Gamma_{ne}=\Delta g\cdot a_i\cdot\mu\cdot\varepsilon=-\cdot+\cdot+\cdot+<0$; the enhanced emission is expected.

$$\mathbf{P} \xrightarrow{h\nu} {}^1(\mathbf{P})^* \xrightarrow{\text{isc}} {}^3(\mathbf{P})^* \quad (1)$$

$${}^3(\mathbf{P})^*+\mathbf{1} \longrightarrow {}^3\overline{(\mathbf{P})^{\cdot-}, (\mathbf{1})^{\cdot+}} \quad (2)$$

$${}^3\overline{(\mathbf{P})^{\cdot-}, (\mathbf{1})^{\cdot+}} \overset{\text{isc}}{\rightleftarrows} {}^1\overline{(\mathbf{P})^{\cdot-}, (\mathbf{1})^{\cdot+}} \quad (3)$$

$${}^1\overline{(\mathbf{P})^{\cdot-}, (\mathbf{1})^{\cdot+}} \longrightarrow \mathbf{P}^{\dagger}+\mathbf{1} \quad (4)$$

$${}^3\overline{(\mathbf{P})^{\cdot-}, (\mathbf{1})^{\cdot+}} \longrightarrow (\mathbf{P})^{\cdot-}+(\mathbf{1})^{\cdot+} \quad (5)$$

Scheme 1.

The signal * denotes an excited state; the signal † represents nuclear polarization.

The use of nonpolar solvent such as benzene-d_6 was quite essential for observing these CIDNP effects, since the key step may be the two competitive processes; the coupling dependent in-cage electron return (Eqs. 3 and 4 in Scheme 1) and the diffusion from the triplet radical ion pair (Eq. 5 in Scheme 1). Probably, in methanol-d_4 or acetonitrile-d_3 a large part of the triplet radical ion pair tends to diffuse apart.

The free energy ($-\Delta G^\circ$) for the formation of the solvent separated ion pair (${}^3\overline{(\mathbf{P1})^{\cdot-}, (\mathbf{1})^{\cdot+}}$) was estimated to be 0.3—−0.1 eV.[9] In accord with the electron transfer mechanism, the CIDNP effects due to **P1** were detected only with the use of **1** as electron donor but not observed with the less electron-donating phenols, such as phenol (**2**) or 4-methylphenol (**3**).[10]

For methyl pyropheophorbide a (**P2**) and methyl 9-hydroxydeoxopyropheophorbide a (**P3**), similar CIDNP effects were observed (Figs. 2 and 3). The emission signal of the methylene protons at the 10-

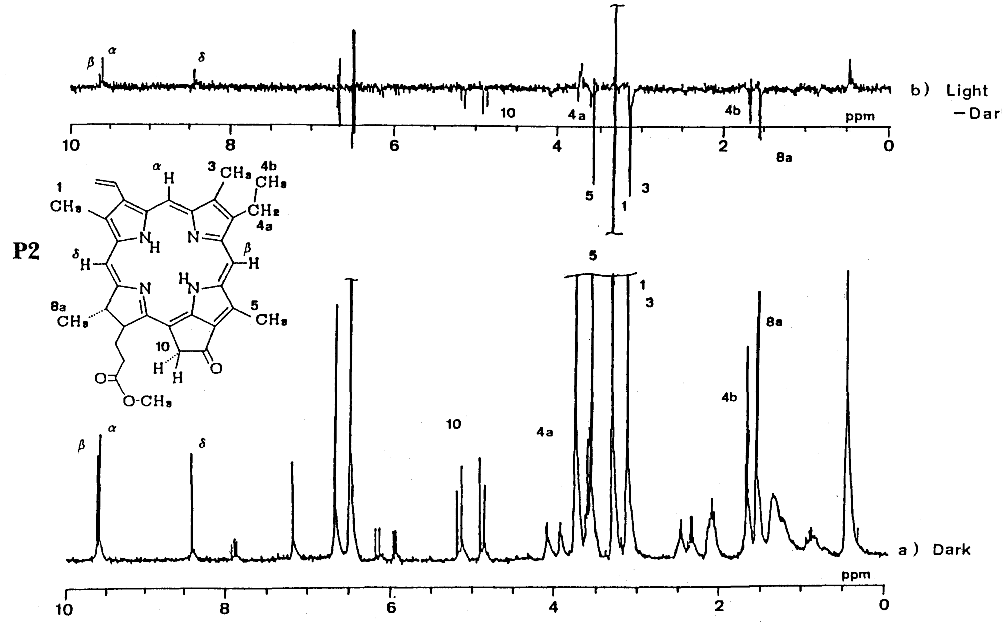

Fig. 2. CIDNP spectra of methyl pyropheophorbide *a* (**P2**) with **1** in benzene-d_6; (a) dark spectrum (bottom); (b) light-dark spectrum (top).

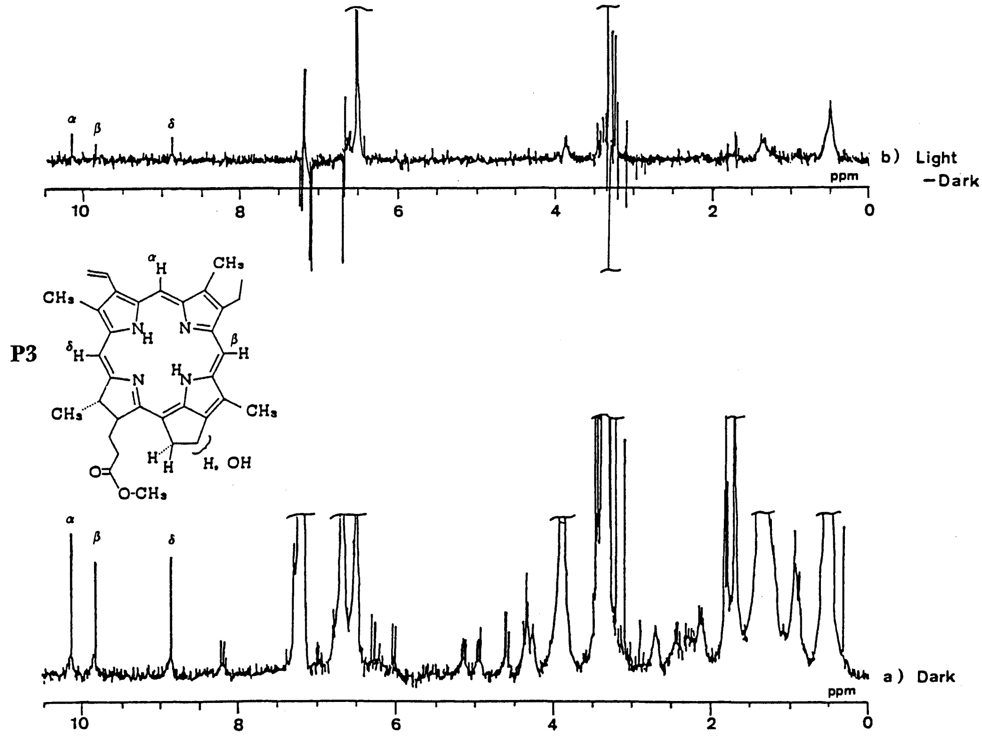

Fig. 3. CIDNP spectra of methyl 9-hydroxydeoxopyropheophorbide *a* (**P3**) with **1** in benzene-d_6; (a) dark spectrum (bottom); (b) light-dark spectrum (top).

position of **P2** is clearly observed as a doublet of doublet at 4.90 and 5.15 ppm. The CIDNP effects observed for **P3** were rather week, compared with **P1** and **P2**. Since the reduction potential of **P3** is lower than that of **P1** and **P2**, the electron transfer from **1** to ^{3}(**P3**)* will be less favorable.[11]

An apparent advantage of this CIDNP technique is its high resolution due to 400 MHz ^{1}H NMR, which makes the assignment of the polarized signals straightforward. Of particular interest is the comparison of the relative intensities of the polarized signals. For example, the relative intensities of the polarization of the three meso protons in **P1** are in a ratio of 1.9 : 1 : 1.4 for the α-, β-, and δ- protons. This ratio which was determined by the relative heights of the polarized signals normalized to the dark signals, was not affected by changing the light-pulse time or the total repetition time. Since three (Δg, μ, and ε) of the four parameters are always the same, the magnitudes of the polarization may reflect the hyperfine coupling constant (a_i) of the radical intermediate.[6] The spin-lattice relaxation time, T_1, of the meso protons under the same conditions without light was measured to be 1.2, 1.4, and 1.2 s for the α-, β-, and δ-protons, respectively. Accordingly, the different degree of polarization at the α-, β-, and δ-positions in **P1** may reflect the different spin densities at the meso positions in (**P1**)$^{\cdot-}$.[12] Similar polarization ratio (2.2 : 1 : 2.8 for the α-, β-, and δ-protons) was observed for **P2**. In contrast, the relative intensities of the α-, β-, and δ- protons in **P3** were in a ratio of 1.4 : 1 : 1.0, presumably suggesting roughly equal spin densities at the three meso positions. These results indicate that the presence or the absence of the carbonyl group at C-9 position has a small but decisive influence on the spin distributions in the one-electron reduced pheophytin.

In 1982, the hyperfine coupling constants of electrochemically generated (**P1**)$^{\cdot-}$ were determined by ENDOR and TRIPLE resonance technique.[4] The reported ratio of the hyperfine coupling constants for the three meso protons is 1 : 1.16 : 1.79, which seems different from the present results. There may be several reasons for this discrepancy. The present CIDNP experiment was carried out in nonpolar benzene-d_6 solution, while the ENDOR experiments were done in polar dimethoxyethane solution in the presence of tetra-*n*-butylammonium perchlorate (5X10^{-3} M) as supporting electrolyte. Our results rest on the relative heights of the polarized signals which are apparently dependent upon a variety of parameters including solvent, temperature, and relaxation process. Finally, it may be pointed out that in the ENDOR and TRIPLE resonance experiments, the resonance assignments to the respective protons of complicated tetrapyrrole pigments is not necessarily definitive.

In summary, the laser-excitation of **P1**, **P2**, and **P3** in the presence of 4-methoxyphenol **1** in benzene-d_6 showed the CIDNP effects of these naturally occurring pigments. The effects are reasonably interpreted by taking into consideration of the reversible electron transfer from **1** to $^3(\mathbf{P})^*$ and from $(\mathbf{P})^{\bar{\cdot}}$ to $(\mathbf{1})^{\dagger}$.

Experimental

The ^{1}H NMR spectra were recorded on 400 MHz JEOL JNM GX-400 instruments. Cyclic voltammetry was performed with a PAR model 174. Redox potentials (V vs. Ag/AgClO$_4$) were measured on Pt electrode in 1 mM solution of DMF (**P1**, **P2**, or **P3**) or acetonitrile (phenols) containing 0.1 M tetraethylammonium perchlorate at room temperature.

CIDNP Experiments. The laser-induced photo-CIDNP experiments were done by using a JEOL GX-400 NMR Spectrometer and G40H5PI Probe (a specially modified NMR probe, having a quartz rod which served as a light guide to the sample). The laser beam (a NEC Argon Laser GLC-3300, 4W) was directed via prisms at the edge of the rod with a computer-controlled mechanical shutter which allowed the synchronization of light and radio frequency (RF) pulses (Fig. 4). The presaturation pulse technique and subtraction method were used to obtain clear CIDNP signals.[13] The presaturation pulse was done by the broadband RF irradiation to saturate a complete spectrum and the pseudo-random pulse which was only the repetition of six pulses with arbitrary pulse width and interval (Fig. 5). The following pulse sequence was used: presaturation random pulse (0.1 s)-light pulse (usually 0.3 s)-observation pulse (flip angle, $\theta=30\,^\circ$). The total repetition time for the whole cycle was 15 s, which was so long that no serious sample heating occurred due to light absorption. The number of accumulations was 200. FID signals with and without light were collected separately and a difference spectrum was taken by subtracting dark signals from light signals to complete the cancellation of unpolarized signals. For the CIDNP experiments, a benzene-d_6 solution of pigment (**P1**, **P2**, or **P3**) (1 mM) and 4-methoxyphenol (**1**) (10 mM) was deoxygenated in the NMR sample tube by bubbling argon gas (99.99%) into the solution for 3 min immediately before irradiation.

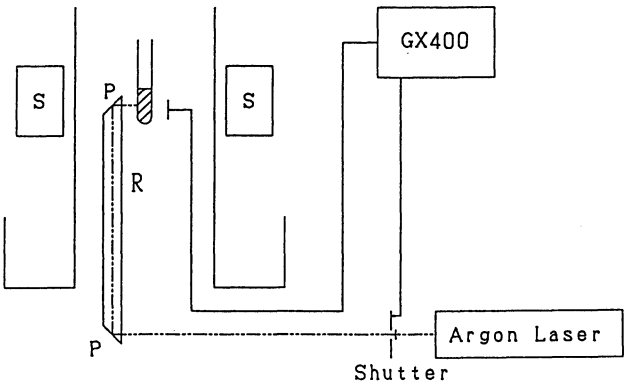

Fig. 4. Experimental set-up for the laser photo-CIDNP experiment at the JEOL GX400 NMR spectrometer. S; superconducting solenoid. P; prism. R; quartz rod.

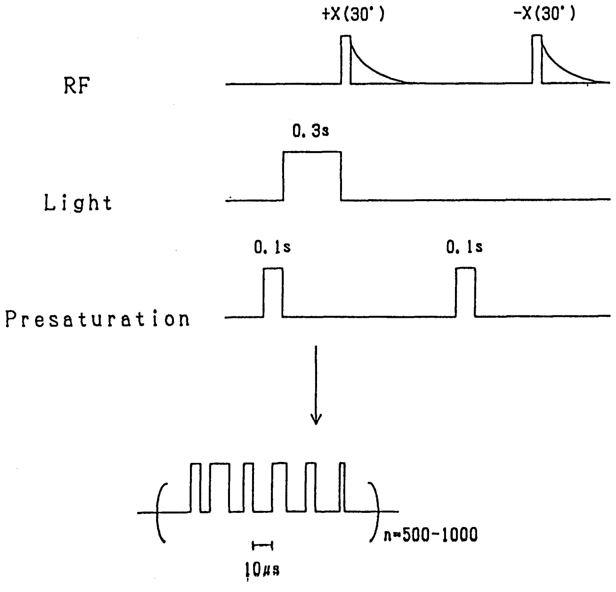

Fig. 5. Pulse sequence for the CIDNP measurement.

Materials. Pheophytin *a* (**P1**) was obtained by removing Mg^{2+} from chlorophyll *a*, which was extracted from spinach and purified according to the reported methods.[14] Methyl pyropheophorbide *a* (**P2**) was obtained from **P1** by the standard method.[14] Methyl 9-hydroxydeoxopyropheophorbide *a* (**P3**) was obtained as a mixture of isomers at C-10 (cis : trans=3 : 2) by the reduction of **P2** with sodium borohydride in dioxane at 98% yield.[15] **P3**: ^{1}H NMR (CDCl$_3$) δ=9.87 (1H, s, α), 9.63 (1H, s, β), 8.90 (1H, s, δ), 8.25 (1H, dd, $J_{X,A}$=17.97Hz, $J_{X,B}$=11.23Hz, 2X), 6.52 (1H, d, $J_{9,10a}$=6.35Hz, cis-9), 6.50 (d, 1H, $J_{9,10b}$=6.35Hz, trans-9), 6.36 (1H, dd, $J_{A,B}$=1.47Hz, $J_{A,X}$=17.97Hz, 2A), 6.18 (1H, dd, $J_{B,A}$=1.47Hz, $J_{B,X}$=11.23Hz, 2B), 5.40 (1H, dd, $J_{10b,9}$=6.35Hz, $J_{10b,10a}$=16.11Hz, trans-10b), 5.30 (1H, dd, $J_{10a,9}$=6.35Hz, $J_{10a,10b}$=16.11Hz, cis-10a), 4.73 (1H, d, $J_{10b,10a}$=16.11Hz, cis-10b), 4.69 (1H, m, 8), 4.60 (1H, d, $J_{10a,10b}$=16.11Hz, trans-10a), 4.47 (1H, m, 7), 3.87 (2H, q, J=7.81Hz, 4a), 3.56 (3H, s, Me), 3.56 (3H, s, 5-Me), 3.41 (3H, s, 1-He), 3.41 (3H, s, 3-Me), 2.78 (1H, m, 7′), 2.60 (1H, m, 7″), 2.42 (1H, m, 7′), 2.24 (1H, m, 7″), 1.86 (3H, d, J=7.33Hz, trans-8Me), 1.86 (3H, d, J=7.84Hz, cis-8Me), 1.77 (3H, J=7.32Hz, 4b), −1.4 (1H, br, NH), −3.22 (1H, br, NH).

We are grateful to Dr. S. Okazaki for his permission to use the PAR model 174.

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9) The overall free energy ($-\Delta G^\circ$) is calculated by Eq. 6;

$$-\Delta G^\circ = -E_{1/2}{}^{\mathrm{ox}}(\mathbf{1}/(\mathbf{1})^{\dot{+}}) + E_{1/2}{}^{\mathrm{red}}(\mathbf{P1}/(\mathbf{P1})^{\dot{-}}) + E_{\mathrm{T}} + 14.38/\varepsilon R;^{16)} \qquad (6)$$

where $E_{1/2}{}^{\mathrm{ox}}$ is the oxidation potential of **1**, $E_{1/2}{}^{\mathrm{red}}$ is the reduction potential of **P1**, E_{T} is the triplet energy of **P1** (1.34 eV),[17] ε is the dielectronic constant of benzene (2.27),[18] and R is the center-to-center distance of the geminate radical ion pair, which is usually considered in a range of 6—10 Å.[19] Since we could not measure accurate redox potentials of $(\mathbf{P1})^{\dot{-}}$ and $(\mathbf{1})^{\dot{+}}$ in benzene, the $-\Delta G^\circ$ value was estimated on the basis of the oxidation peak potential of **1** (0.67 V vs. ferrocene/ferrocenium) in acetonitrile and the reduction potential of **P1** (−1.37 V vs. ferrocene/ferrocenium) in DMF.

10) The oxidation peak potentials of **2** and **3** were 1.27 and 1.07 V (vs. ferrocene/ferrocenium) in acetonitrile, respectively.

11) The reduction potentials of **P2** and **P3** were −1.46 and −1.74 V (vs. ferrocene/ferrocenium) in DMF, respectively.

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