Fan Liu, Yan-Chun Shen, Yao-Hua Ouyang, Guo-Ping Yan,* Si Chen, Hui Liu, Yan-Guang Wu, and Jiang-Yu Wu

School of Material Science and Engineering, Wuhan Institute of Technology, Wuhan 430074, China
*E-mail: guopyan2006@163.com
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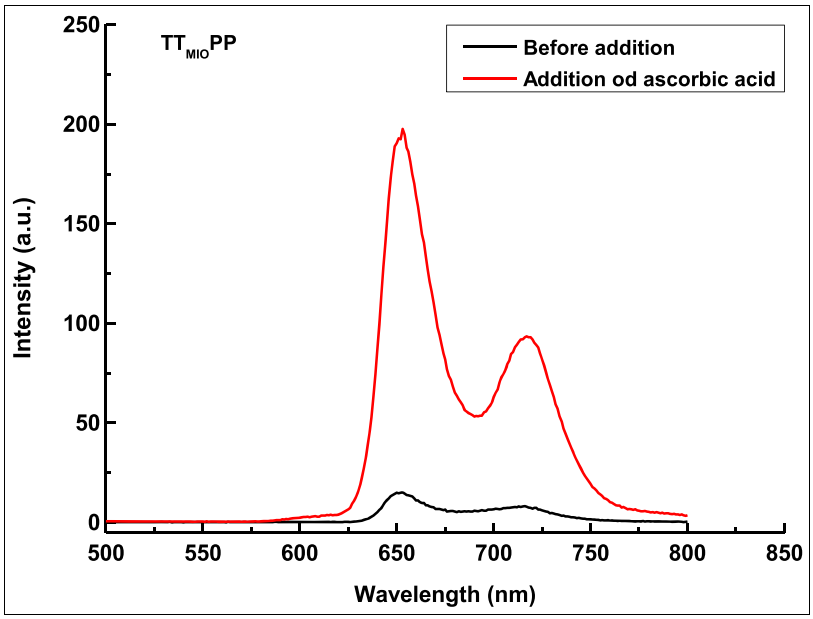

Isoindoline nitroxide-containing porphyrins were synthesized by the reaction of 5-phenyldipyrromethane and 5-(4′-nitrophenyl)-dipyrromethane with 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl using the Lindsey method. These spin-labeled porphyrins were further characterized by MS, UV, FTIR, ^{1}H-NMR, cyclic voltammetry, electron paramagnetic resonance (EPR), and fluorescence spectroscopy. The electrochemical assay demonstrated that these isoindoline nitroxides-containing porphyrins had similar electrochemical and redox properties as 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl. Electron paramagnetic resonance test exhibited these porphyrins possessed the hyperfine splittings and characteristic spectra of isoindoline nitroxides, with typical nitroxide g-values and nitrogen isotropic hyperfine coupling constants. Fluorescence spectroscopy revealed that these porphyrins indicated fluorescence suppression characteristic of nitroxide–fluorophore systems. Moreover, their reduced isoindoline nitroxide-containing porphyrins eliminated the fluorescence suppression and displayed strong fluorescence. Thus, these isoindoline nitroxide-containing porphyrins may be considered as the potential fluorescent and EPR probes.

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INTRODUCTION

Electron paramagnetic resonance (EPR) is the most effective method to detect exogenous paramagnetic species *in vivo*, such as nitroxides. Currently, the low frequency EPR imaging (EPRI) is receiving increased attention to be an important new clinical tool for non-invasive three-dimensional spatial mapping of tissue oxygenation and potentially multidimensional imaging of the spatial distribution of paramagnetic species in biological tissues [1–3]. Moreover, EPRI can be used to study on tumor hypoxia, tissue heterogenicity with respect to oxygen and redox status, and vascular deficiencies *in vivo*. Meanwhile, free radicals have played important roles as spin probes in EPRI with adequate steady-state concentration or biological half-life *in vivo* [4–6]

Most nitroxides have EPR lines that contain unresolved proton hyperfine interactions, typically from the alkyl environments surrounding the nitroxide moiety [7–9]. Tetramethyl isoindoline nitroxides can display superior EPR linewidths, compared to other classes of nitroxides [10–12]. 2,2,6,6-Tetramethylpiperidine-1-yloxyl (TEMPO) and 5-carboxy-1, 1, 3, 3-tetramethylisoindolin-2-yloxyl (CTMIO) are commonly used as the EPRI spin probes [13]. However, they are low molecular weight compounds and then have short retention time and nonspecific distribution in tumors. An ideal method to overcome these insufficiencies is to design and

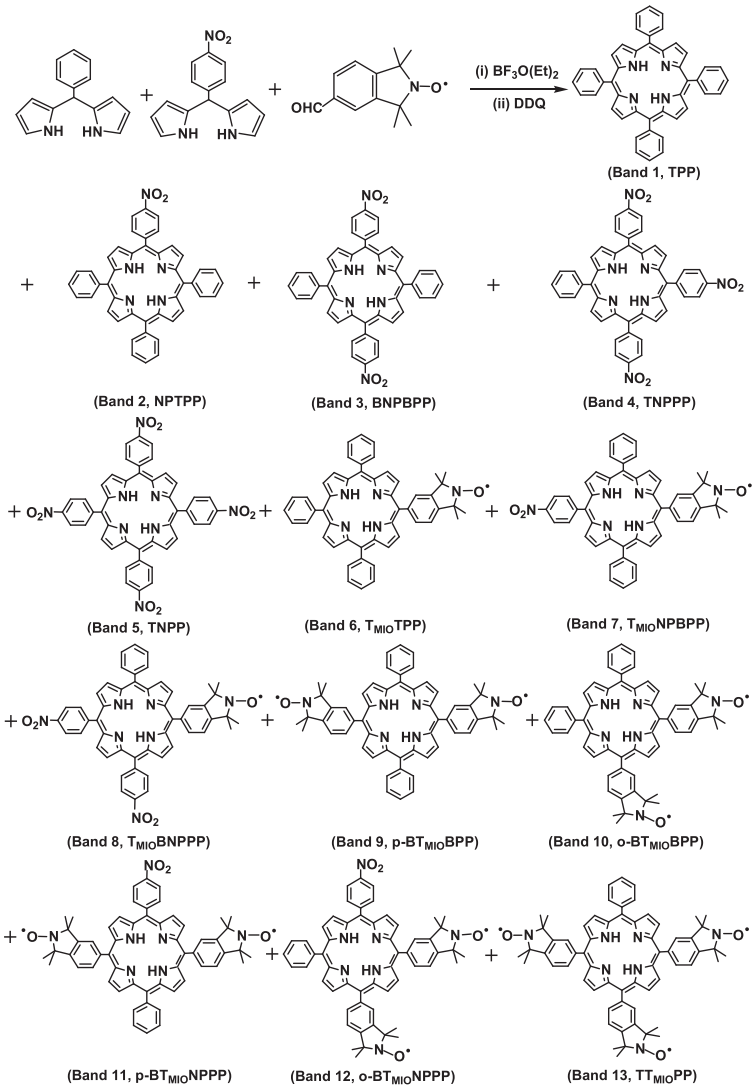
synthesize a nonionic, low-toxicity, and tumor-targeting spin probe [14–16]

Some porphyrins and their metal complexes play important roles in magnetic resonance imaging (MRI), photodynamic therapy (PDT), anticancer drug, and fluorescence imaging because of their preferential selective uptake and retention by tumor tissues [17–20] Meso-tetrakis[4-(carboxymethyleneoxy) phenyl] porphyrin (H_2T_4CPP) can accumulate in the Sarcoma 180 in mice and in mammary tumors of Sprague–Dawley rats [21]. Meso-tetrasulfonatophenyl porphyrin (TPPS) was found to be highly concentrated in Walker carcinosarcoma [22]. Several other groups have made efforts towards the synthesis of porphyrin-antitumor drug conjugates and studied on their tumor selectivity and antitumor activity [23]. The uptake mechanism of porphyrins is most likely that porphyrins are incorporated into the tumor cells via the receptor-mediated endocytosis of low-density lipoproteins (LDL) because the tumor cells express high levels of LDL receptors [21–24]

Spin-labeled porphyrins have attracted much interest since the early 1970s when Asakura et al. and Eaton et al. reported the EPR spectroscopies of spin-labeled heme and spin-labeled metalloporphyrins, respectively [25,26]. Recently, spin-labeled porphyrins have received more and more attention to the potential molecular magnetic materials and EPR probes in the study of porphyrin excited states [27–38]

In this work, a series of isoindoline nitroxide-containing porphyrins were synthesized by the reaction between 5-phenyldipyrromethane, 5-(4′-nitrophenyl)dipyrromethane and 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl (FTMIO) (Scheme 1). Subsequently, these porphyrins were characterized by MS, UV, ^{1}H-NMR, and FTIR.

Scheme 1. Synthetic route of isoindoline nitroxide-containing porphyrins.



Their properties including cyclic voltammetry, EPR, and fluorescence were also evaluated herein.

RESULTS AND DISCUSSION

Synthesis and characterization. Isoindoline nitroxide-containing porphyrins including $T_{MIO}TPP$, $T_{MIO}NPBPP$, $T_{MIO}BNPPP$, *p*-$BT_{MIO}BPP$, *o*-$BT_{MIO}BPP$, *p*-$BT_{MIO}NPPP$, *o*-$BT_{MIO}NPPP$, and $TT_{MIO}PP$ were synthesized by the reaction between 5-phenyldipyrromethane, 5-(4′-nitrophenyl)-dipyrromethane and FTMIO using the Lindsey method. The experimental data of FTIR, UV, MS, ^{1}H-NMR, cyclic voltammetry, EPR, and fluorescence provided the evidences for the formation of these isoindoline nitroxide-containing porphyrins. However, good quality ^{1}H-NMR spectra could not be measured due to the expected paramagnetic broadening that arises from the presence of the nitroxide radicals. (The experimental data were listed in the Supporting Information).

Evaluation of fluorescence suppression. Fluorescence spectra of isoindoline nitroxide-containing porphyrins and their diamagnetic hydroxylamine equivalents were carried out on a deoxygenated solution in methanol (10^{-6} mol/L). The optimum excitation wavelength was determined to be 414–426 nm. Addition of excess ascorbic acid to the solution of isoindoline nitroxide-containing porphyrins resulted in an increase in fluorescence intensity with a maximum intensity reached after 1 h. The further ascorbic acid was added; however, no further change was observed, indicating that all nitroxide groups (N—O·) had been reduced to hydroxylamine groups (N—OH).

The fluorescence suppression was observed at the fluorescence measurements between the nitroxides and their diamagnetic hydroxylamine equivalents. Spin-labeled porphyrins and their related hydroxylamines had two characteristic fluorescence emission peaks at 650–654 nm and 716–718 nm. In general, the intensities of the hydroxylamine of spin-labeled porphyrins were 2.34–13.13 times greater than that of the corresponding nitroxides (Fig. 1, Table 1). It appears that the typical pathways for fluorescence quenching in radical–fluorophore systems were effective in spin-labeled porphyrins.

Electrochemical assay. The current–potential curves at a potential sweep rate of 0.1 V/s are given in Figure 2**.** It can be seen that two type reversible oxidation/reduction reactions appear at −1.5–1.6 V in the current–potential curve of CTMIO in the methanol solution. The reversible oxidation/reduction reactions include the change between nitroxide NO· and hydroxylamine N—OH and the conversion from nitroxide NO· to nitroso-onium ion. Figure 2 shows similar peaks for the oxidation and reduction reactions in $T_{MIO}TPP$, $T_{MIO}NPBPP$, $T_{MIO}BNPPP$, *p*-$BT_{MIO}BPP$, *o*-$BT_{MIO}BPP$, *p*-$BT_{MIO}NPPP$, *o*-$BT_{MIO}NPPP$, and $TT_{MIO}PP$ solutions as CTMIO, which demonstrated that isoindoline nitroxide-containing porphyrins possess similar electrochemical and redox properties as CTMIO.

Electron paramagnetic resonance spectroscopy. Samples were deoxygenated by bubbling argon through the solution prior to analysis. X-band EPR spectra were recorded at room temperature for $T_{MIO}TPP$, $T_{MIO}NPBPP$, $T_{MIO}BNPPP$, *p*-$BT_{MIO}BPP$, *o*-$BT_{MIO}BPP$, *p*-$BT_{MIO}NPPP$, *o*-$BT_{MIO}NPPP$, and $TT_{MIO}PP$ solutions (Fig. 3). These isoindoline nitroxide-containing porphyrins exhibited the characteristic EPR hyperfine splittings of tetramethyl isoindoline nitroxides, typical nitroxide *g*-value for a free electron of approximately 2.006 and nitrogen isotropic hyperfine coupling constants (a_N values, a_1 and a_2, 293 K) (Table 2), respectively. In contrast, CTMIO has the typical nitroxide *g*-value for a free electron of 2.00628 and a_N values (a_1 and a_2) of 14.57 and 14.48 G (293 K) under the same conditions.

SUMMARY

Isoindoline nitroxide-containing porphyrins were synthesized and evaluated as the potential fluorescence-suppressed spin probes. Compared with CTMIO, these isoindoline nitroxide-porphyrins possessed similar electrochemical and redox properties, and the characteristic EPR hyperfine splittings as well as superior EPR linewidth and resistance to reduction. Moreover, these porphyrins exhibited fluorescence suppression characteristic of nitroxide–fluorophore systems. Their reduced isoindoline nitroxide-porphyrins eliminated the fluorescence suppression and displayed the strong fluorescence properties. Thus, these isoindoline nitroxide-porphyrins may be considered as the potential fluorescence and EPR probes.

EXPERIMENTAL

Instrumentation and reagents. The compounds were characterized using a Voyager DE STR MALDI-TOF-MS Laser Time-of-Flight Mass Spectrometer (Applied Biosystems by Life Technologies Co., USA), a Varian Mercury-VX300 NMR spectrometer (Varian, Inc. Corporate, Palo Alto, CA), a UV–Vis spectrophotometer (UV-2800 series, Unico, Shanghai, China), a Nicolet IS10 Fourier transform-infrared (FT-IR) spectrophotometer (Thermo Fisher Scientific Inc., Madison, WI), and an

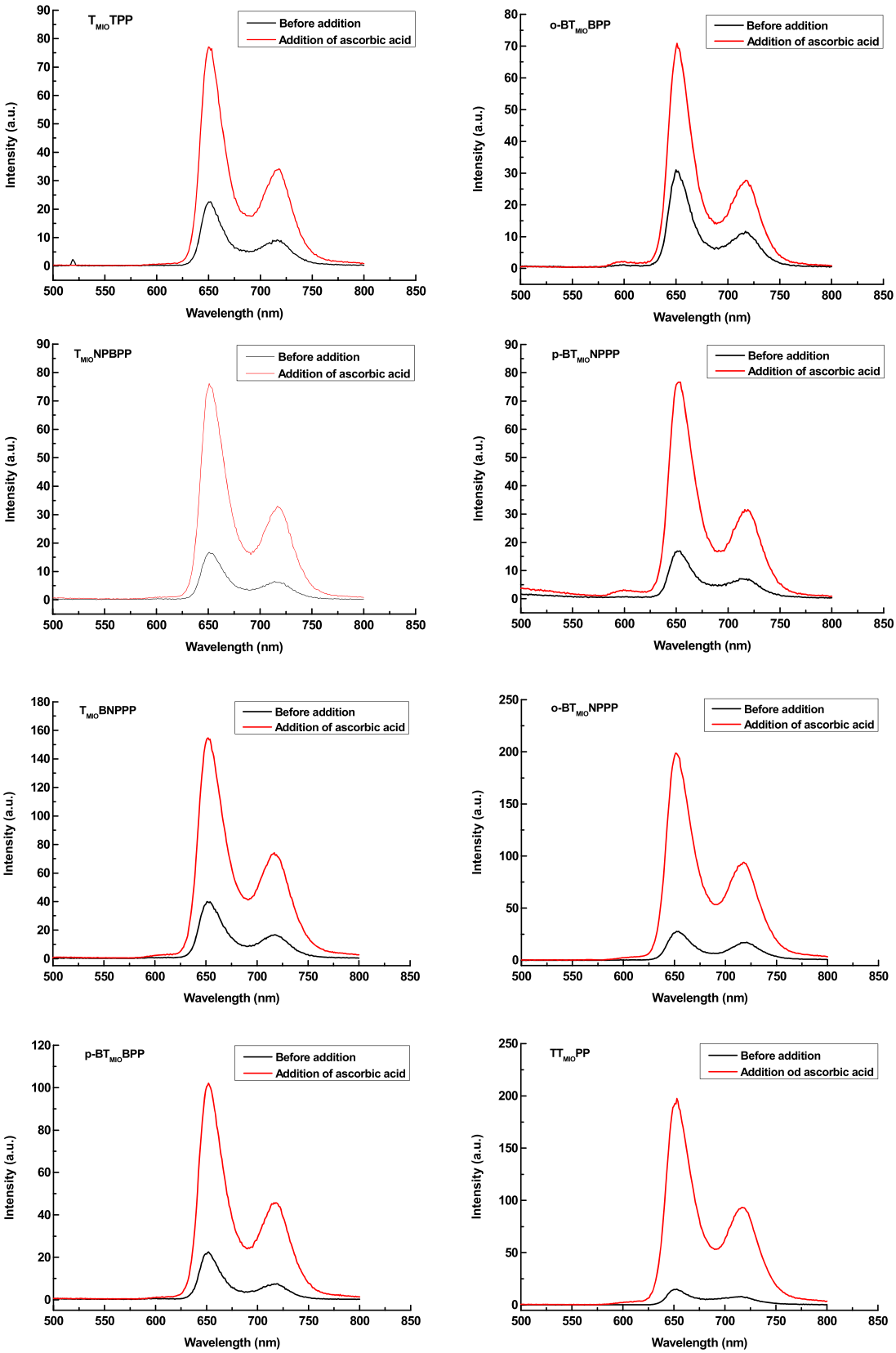


Figure 1. Fluorescence spectra obtained before and after addition of excess ascorbic acid to $T_{MIO}TPP$, $T_{MIO}NPBPP$, $T_{MIO}BNPPP$, *p*-$BT_{MIO}BPP$, *o*-$BT_{MIO}BPP$, *p*-$BT_{MIO}NPPP$, *o*-$BT_{MIO}NPPP$, and $TT_{MIO}PP$, respectively. [Color figure can be viewed at wileyonlinelibrary.com]

Table 1

Fluorescence measurement between isoindoline nitroxide-labeled porphyrins and their related hydroxylamines.

Sample solution	Fluorescence emission peak λem (nm)	Fluorescence suppression ratio
$T_{MIO}TPP$	650, 718	3.42, 3.84
$T_{MIO}NPBPP$	651, 717	4.54, 5.37
$T_{MIO}BNPPP$	652, 717	3.90, 4.46
p-$BT_{MIO}BPP$	652, 718	4.54, 6.25
o-$BT_{MIO}BPP$	651, 718	2.34, 2.47
p-$BT_{MIO}NPPP$	654, 716	4.56, 4.57
o-$BT_{MIO}NPPP$	651, 718	7.42, 5.57
$TT_{MIO}PP$	653, 717	13.13, 11.73

XT4-100× microscopic melting point apparatus (Beijing Electrooptics Science Factory, Beijing, China).

All chemicals and solvents were of analytical grade. 5-Phenyldipyrromethane [28], 5-(4′-nitrophenyl) dipyrromethane [28], 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl (FTMIO) [17], and 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (CTMIO) [17] were synthesized by the methods cited in the literatures.

Synthesis of isoindoline nitroxide-containing porphyrins. A solution of 5-phenyldipyrromethane (111.1 mg, 0.5 mmol, 1 equiv.), 5-(4′-nitrophenyl) dipyrromethane (133.1 mg, 0.5 mmol, 1 equiv.), and 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl (FTMIO, 220 mg, 1.01 mmol, 2 equiv.) in freshly distilled dichloromethane (DCM, 100 mL) was purged with argon for 15 min. Boron trifluoride etherate ($BF_3{\cdot}O(Et)_2$, 0.133 mL of 2.5 M stock solution in DCM, 0.0825 mmol/L) was then added, and the mixture was stirred at room temperature under argon shielded from light. The mixture was stirred for 1 h, and then 2,3-dichloro-4,5-dicyanobenzoquinone (DDQ, 0.175 g, 0.075 mmol) was added. The mixture was stirred for an additional 1 h, and subsequently the solvent was removed under reduced pressure. Column chromatography (length: 60 cm, external diameter: 2.55 cm outer diameter: 2.7 cm; chromatography silica gel SiO_2: 230–400 mesh (60A), The first eluent: DCM/n-hexane, v/v: 3/1; the second eluent: ethyl acetate/DCM/n-hexane, v/v: 1/3/4) gave a series of porphyrins as follows: 5,10,15,20-tetraphenylporphyrin (**Band 1**, TPP, 4.2 mg, 1.37%), 5-(4′-nitrophenyl)-10,15,20-trisphenylporphyrin (**Band 2,** NPTPP, 3.1 mg, 0.94%), 5,15-bis(4′-nitrophenyl)-10,20-bisphenylporphyrin (**Band 3,** BNPBPP, 2.5 mg, 0.71%), 5,10,15-tris(4′-nitrophenyl)-20-phenylporphyrin (**Band 4,** TNPPP, 1.1 mg, 0.29%), 5,10,15,20-tetra(4′-nitrophenyl)porphyrin (**Band 5,** TNPP, 4.6 mg, 1.2%), 5-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)-10,15,20-trisphenylporphyrin (**Band 6**, $T_{MIO}TPP$, 9.2 mg, 2.53%), 5-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)-15-(4′-nitrophenyl)-10,20-bisphenylporphyrin (**Band 7**, $T_{MIO}NPBPP$, 2.4 mg, 0.62%), 5-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)-10,15-bis (4′-nitrophenyl)-20-phenylporphyrin (**Band 8**, $T_{MIO}BNPPP$, 2.7 mg, 0.66%), 5,15-bis (1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)-10,20-bisphenylporphyrin (**Band 9**, p-$BT_{MIO}BPP$, 1.0 mg, 0.23%), 5,10-bis (1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)-15,20-bisphenylporphyrin (**Band 10**, o-$BT_{MIO}BPP$, 2.3 mg, 0.54%), 5,15-bis (1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)-10-(4′-nitrophenyl)-20-phenyl-porphyrin (**Band 11**, p-$BT_{MIO}NPPP$, 1.8 mg, 0.41%), 5,10-bis (1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)-15-(4′-nitrophenyl)-20-phenyl-porphyrin (**Band 12**, o-$BT_{MIO}NPPP$, 0.8 mg, 0.18%), 5,10,15-tris (1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)-20-phenylporphyrin (**Band 13**, $TT_{MIO}PP$, 2.5 mg, 0.52%).

TPP (Band 1) [21,31]: MS found $M + H^+$ m/z 615 (calc. 614 for $C_{44}H_{30}N_4$). ^1H NMR ($CDCl_3$) δ: 8.88 (d, 2H, β-pyrrole), 8.225 (d, 4H, β-pyrrole), 8.208 (d, 2H, β-pyrrole), 7.76 (m, 8H, ortho-phenyl), 7.742, 7.208 (m, 12H, meta/para phenyls), −2.79 (s, 2H, pyrrole NH); ν_{max} (KBr)/cm^{-1} 2975, 2928 (C—H), 1597, 1557, and 1472 (C—C), 1374 and 1351 (N—O); UV–Vis [chloroform ($CHCl_3$)] λ_{max} 417, 514, 549, 590, 645 nm.

NPTPP (Band 2) [22]: MS found M^+ m/z 659.7 (calc. 659 for $C_{44}H_{29}N_5O_2$); ^1H NMR ($CDCl_3$) δ: 8.86 (d, 2H, β-pyrrole), 8.74 (d, 4H, β-pyrrole), 8.64 (d, 2H, β-pyrrole), 8.41 (d, 2H, nitrophenyl), 8.22 (d, 2H, nitrophenyl), 7.77 (m, 6H, ortho-phenyl), 7.25 (m, 9H, meta/para phenyls), −2.79 (s, 2H, pyrrole NH); UV–Vis [chloroform ($CHCl_3$)] λ_{max} 418, 516, 550, 591, 645 nm.

BNPBPP (Band 3) [22]: MS found $M + H^+$ m/z 705.9 (calc. 704 for $C_{44}H_{28}N_6O_4$); ^1H NMR ($CDCl_3$) δ: 8.86 (d, 2H, β-pyrrole), 8.74 (d, 4H, β-pyrrole), 8.64 (d, 2H, β-pyrrole), 8.41 (d, 4H, nitrophenyl), 8.22 (d, 4H, nitrophenyl), 7.77 (m, 4H, ortho-phenyl), 7.25 (m, 6H, meta/para phenyls), −2.79 (s, 2H, pyrrole NH); UV–Vis [chloroform ($CHCl_3$)] λ_{max} 420, 516, 552, 593, 654 nm.

TNPPP (Band 4) [22]: MS found $M + H^+$ m/z 750.4 (calc. 749 for $C_{44}H_{27}N_7O_6$); ^1H NMR ($CDCl_3$) δ: 8.86 (d, 2H, β-pyrrole), 8.74 (d, 4H, β-pyrrole), 8.64 (d, 2H, β-pyrrole), 8.41 (d, 6H, nitrophenyl), 8.22 (d, 6H, nitrophenyl), 7.77 (m, 2H, ortho-phenyl), 7.25 (m, 3H, meta/para phenyls), −2.79 (s, 2H, pyrrole NH); UV–Vis [chloroform ($CHCl_3$)] λ_{max} 422, 514, 552, 590, 645 nm.

TNPP (Band 5) [22]: MS found $M + H^+$ m/z 795.5 (calc. 794 for $C_{44}H_{26}N_8O_8$); ^1H NMR ($CDCl_3$) δ: 8.86 (d, 2H, β-pyrrole), 8.74 (d, 4H, β-pyrrole), 8.64 (d, 2H, β-pyrrole), 8.41 (d, 8H, nitrophenyl), 8.22 (d, 8H, nitrophenyl), −2.79 (s, 2H, pyrrole NH); UV–Vis [chloroform ($CHCl_3$)] λ_{max} 418, 515, 554, 593, 644 nm.

$T_{MIO}TPP$ (Band 6): MS found $M + H^+$ 727.44 (calc. 726 for $C_{50}H_{40}N_5O$); ^1H NMR ($CDCl_3$, δ, ppm): 8.9–8.8 (br, 8H, β-pyrrole), 8.2 (m, 8H, ortho phenyl), 8.0 (br, 1H, meta phenyl), 7.8–7.7 (br, m, 9H, meta/para triphenyl), 1.4 (s, 12H, CH_3), −2.75 (s, 2H, pyrrole NH); ν_{max}

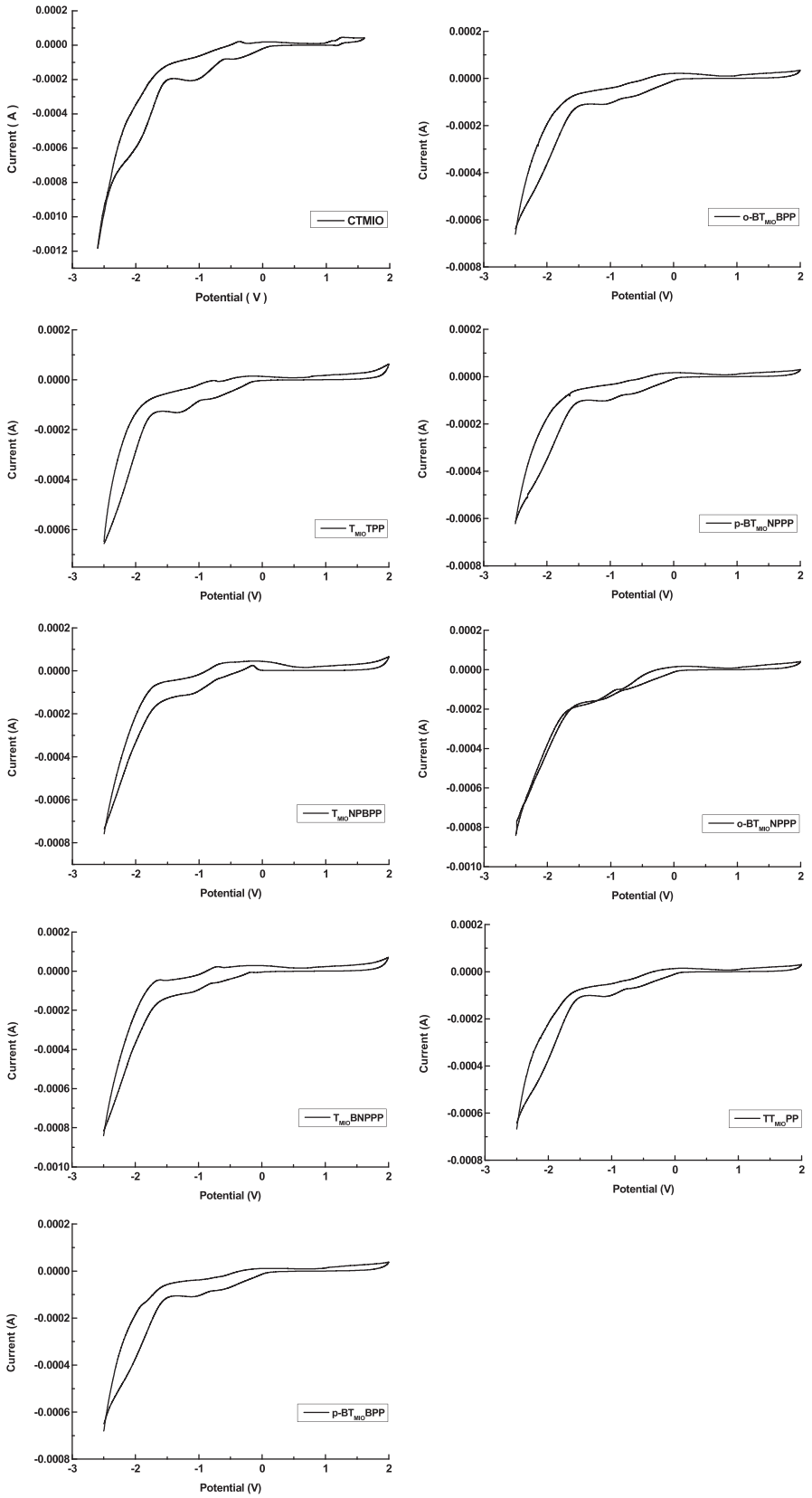


Figure 2. Current–potential curves of the solutions of $T_{MIO}TPP$, $T_{MIO}NPBPP$, $T_{MIO}BNPPP$, *p*-$BT_{MIO}BPP$, *o*-$BT_{MIO}BPP$, *p*-$BT_{MIO}NPPP$, *o*-$BT_{MIO}NPPP$, and $TT_{MIO}PP$, respectively.

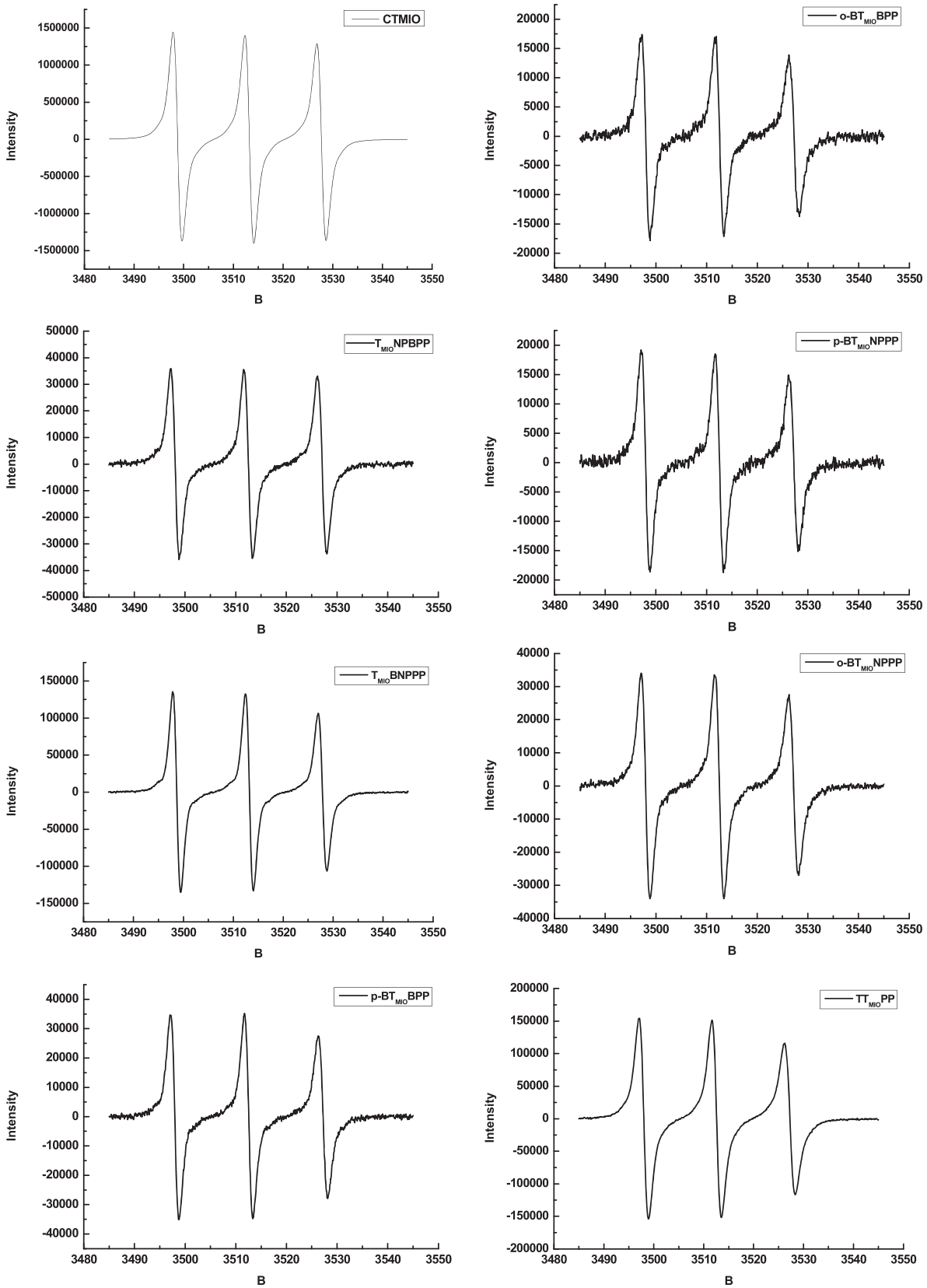


Figure 3. EPR spectra of $T_{MIO}TPP$, $T_{MIO}NPBPP$, $T_{MIO}BNPPP$, *p*-$BT_{MIO}BPP$, *o*-$BT_{MIO}BPP$, *p*-$BT_{MIO}NPPP$, *o*-$BT_{MIO}NPPP$, and $TT_{MIO}PP$ in DCM at room temperature.

Table 2

Characteristic EPR data of isoindoline nitroxide-containing porphyrins.

Sample solution	*g*-value	a_N values (G)	
		a_1	a_2
CTMIO	2.00628	14.57	14.48
T_{MIO}TPP	2.00618	14.43	14.44
T_{MIO}NPBPP	2.00653	14.37	14.57
T_{MIO}BNPPP	2.00645	14.57	14.67
p-BT_{MIO}BPP	2.00646	14.57	14.77
o-BT_{MIO}BPP	2.00650	14.67	14.57
p-BT_{MIO}NPPP	2.00650	14.57	14.67
o-BT_{MIO}NPPP	2.00648	14.57	14.77
TT_{MIO}PP	2.00645	14.67	14.57

(KBr)/cm^{-1} 2963, 2923 (C—H), 1631 and 1400 (C—C), 1384, 1260, and 1091 (N—O), 1020 (C—N); UV–Vis [chloroform ($CHCl_3$)] λ_{max} 421, 514, 551, 590, 647 nm.

T_{MIO}NPBPP (Band 7): MS found M + H^+ m/z 772.85 (calc. 771 for $C_{50}H_{39}N_6O_3$); ^{1}H NMR ($CDCl_3$, δ, ppm): 9.04–8.8 (br, 8H, β-pyrrole), 8.37 (m, 8H, ortho phenyl), 8.18 (d, 2H, nitrophenyl), 7.93 (br, 1H, meta phenyl), 7.36 (m, 6H, meta/para phenyls), 1.4 (s, 12H, CH_3), −2.75 (s, 2H, pyrrole NH); ν_{max} (KBr)/cm^{-1} 2963, 2923, 2852 (C—H), 1646 and 1401 (C—C), 1384, 1261, and 1094 (N—O), 1020 (C—N); UV–Vis [chloroform ($CHCl_3$)] λ_{max} 418, 516, 552, 591, 646 nm.

T_{MIO}BNPPP (Band 8): MS found M + H^+ m/z 817.47 (calc. 816 for $C_{50}H_{38}N_7O_5$); ^{1}H NMR ($CDCl_3$, δ, ppm): 8.6 (br, 8H, β-pyrrole), 7.66 (m, 8H, ortho phenyl), 7.39 (d, 4H, nitrophenyl), 7.38 (br, 1H, meta phenyl), 7.3 (m, 3H, meta/para phenyls), 1.4 (s, 12H, CH_3), −2.75 (s, 2H, pyrrole NH); ν_{max} (KBr)/cm^{-1} 2963, 2853 (C—H), 1645, 1609, and 1412 (C—C), 1384, 1261, and 1026 (N—O), 1020 (C—N); UV–Vis [chloroform ($CHCl_3$)] λ_{max} 421, 514, 549, 589, 641 nm.

p-BT_{MIO}BPP (Band 9): MS found M + H^+ 840.01 (calc. 838 for $C_{56}H_{50}N_6O_2$); ^{1}H NMR ($CDCl_3$, δ, ppm): 8.94 (br, 8H, β-pyrrole), 8.63 (m, 8H, ortho phenyl), 8.26 (br, 2H, meta phenyl), 7.80 (m, 6H, meta/para phenyls), 1.3 (s, 24H, CH_3), −2.75 (s, 2H, pyrrole NH); ν_{max}(KBr)/cm^{-1} 2962, 2923, 2851 (C—H), 1661, 1646, 1399 (C—C), 1384, 1261, and 1095 (N—O), 1021 (C—N); UV–Vis ($CHCl_3$) λ_{max} 418, 515, 550, 591, 647 nm.

o-BT_{MIO}BPP (Band 10): MS found M + H^+ 840.16 (calc. 838 for $C_{56}H_{50}N_6O_2$); ^{1}H NMR ($CDCl_3$, δ, ppm): 8.91 (br, 8H, β-pyrrole), 8.63 (m, 8H, ortho phenyl), 8.2–8.0 (br, 2H, meta phenyl), 7.8 (m, 6H, meta/para phenyls), 1.8–0.9 (s, 24H, CH_3), −2.75 (s, 2H, pyrrole NH); ν_{max}(KBr)/cm^{-1} 2923, 2853 (C—H), 1631, 1400 (C—C), 1384, 1261, and 1094 (N—O), 1021 (C—N); UV–Vis ($CHCl_3$) λ_{max} 418, 515, 550, 592, 647 nm.

p-BT_{MIO}NPPP (Band 11): MS found M + H^+ 884.95 (calc. 883 for $C_{56}H_{49}N_7O_4$); ^{1}H NMR ($CDCl_3$, δ, ppm): 8.31 (br, 8H, β-pyrrole), 8.0 (m, 8H, ortho phenyl), 7.2 (d, 2H, nitrophenyl), 6.8 (br, 2H, meta phenyl), 6.3 (m, 3H, meta/para phenyls), 1.6–0.8 (s, 24H, CH_3), −2.75 (s, 2H, pyrrole NH); ν_{max}(KBr)/cm^{-1} 2962, 2921, 2850 (C—H), 1630, 1401 (C—C), 1384, 1261, and 1094 (N—O), 1020 (C—N); UV–Vis [chloroform ($CHCl_3$)] λ_{max} 420, 515, 550, 590, 646 nm.

o-BT_{MIO}NPPP (Band 12): MS found M + H^+ 884.79 (calc. 883 for $C_{56}H_{49}N_7O_4$); ^{1}H NMR ($CDCl_3$, δ, ppm): 8.8 (br, 8H, β-pyrrole), 8.28 (m, 8H, ortho phenyl), 8.0 (d, 2H, nitrophenyl), 7.7 (br, 2H, meta phenyl), 7.5 (m, 3H, meta/para phenyls), 1.6–0.8 (s, 24H, CH_3), −2.75 (s, 2H, pyrrole NH); ν_{max}(KBr)/cm^{-1} 2962, 2923 (C—H), 1634, 1399 (C—C), 1384, 1261, and 1091 (N—O), 1021 (C—N);UV–Vis [chloroform ($CHCl_3$)] λ_{max} 420, 516, 551, 591, 647 nm.

TT_{MIO}PP (Band 13): MS found M + H^+ 951.93 (calc. 950 for $C_{62}H_{60}N_7O_3$); ^{1}H NMR ($CDCl_3$, δ, ppm): 8.6 (br, 8H, β-pyrrole), 8.3 (m, 8H, ortho phenyl), 7.8 (br, 3H, meta phenyl), 7.3 (m, 3H, meta/para phenyls), 1.6–0.8 (s, 36H, CH_3), −2.75 (s, 2H, pyrrole NH); ν_{max}(KBr)/cm^{-1} 2963, 2924, 2852 (C—H), 1630, 1401 (C—C), 1384, 1261, and 1094 (N—O), 1021 (C—N); UV–Vis [chloroform ($CHCl_3$)] λ_{max} 419, 515, 549, 590, 645 nm.

Fluorescence spectroscopy. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer (Varian, Inc. Corporate, Palo Alto, CA, United States of America). All solutions (10^{-6} mol/L) for fluorescence spectroscopy were prepared in HPLC grade methanol and deoxygenated with argon prior to use. Isoindoline nitroxide-containing porphyrins had to be first dissolved in a minimum volume of $CHCl_3$ and then diluted with methanol to the desired concentration. A deoxygenated and saturated solution of ascorbic acid in methanol was prepared for experiments. Fluorescence suppression experiments were performed by adding and mixing excess ascorbic acid (~50 μL of the saturated solution) to the deoxygenated nitroxide sample at t = 0. Spectra were recorded automatically every 2 min over a 1-h period.

Electrochemical experiments. The electrochemical examinations were performed in a one-compartment, three electrode cell with the use of a CHI660C potentiostat (Shanghai Chenghua, Shanghai, China) under the control of a computer at room temperature. Electrochemical behaviors were investigated with a platinum disc electrode (1.96×10^{-3} cm^2) that was polished and cleaned before each experiment. An Ag/AgCl (0.1 mol/L KCl) electrode was used as the reference electrode, and a platinum wire was used as the counter electrode. The solution in acetonitrile (1 mmol/L) was de-gassed by the bubbling of dry argon before each electrochemical experiment, and a slight argon overpressure was maintained during the experiment. The platinum disc was dried and then put into a sample solution

of acetonitrile with 0.1 mol/L TBAF to obtain the cyclic voltammograms with a potential sweep rate of 0.1 V/s at room temperature.

Electron paramagnetic resonance spectroscopy. Samples for EPR studies were prepared at concentrations of ≤0.5 mM to eliminate intermolecular effects. Samples were deoxygenated by bubbling argon or nitrogen through the solution prior to analysis. X-band EPR spectra were recorded at room temperature for isoindoline nitroxide-containing porphyrin solutions in dichloromethane on a Brucker EPR A300. A double Gunn diode X-band (9 GHz) microwave bridge and standard X-band rectangular TE102 microwave cavity were utilized for all spectra. Nitrogen isotropic hyperfine coupling constants (a_N value) and g value for a free electron are quoted within the spectroscopic description given with each nitroxide synthesis.

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