Synthesis and Properties of Novel Porphyrin Spin Probes Containing Isoindoline Nitroxides

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ABSTRACT:

We report the synthesis of novel spin-labelled porphyrins containing isoindoline nitroxides (TMIO-APTPP and TMIO-APTSPP) and their manganese complexes (Mn-TMIO-APTPP and Mn-TMIO-APTSPP). These compounds represent potential new tools for electron paramagnetic resonance (EPR) as well as novel spin probes. Both TMIO-APTPP and TMIO-APTSPP have characteristic UV absorption peaks of free base porphyrin, while the characteristic absorption peaks of their manganese complexes Mn-TMIO-APTPP and Mn-TMIO-APTSPP shifted to shorter wavelength. Electron Paramagnetic Resonance (EPR) spectroscopy indicated that these compounds all exhibit the hyperfine splittings characteristic of EPR spectra of tetramethylisoindoline nitroxides, the typical nitroxide g-values of approximately 2.006 and nitrogen isotropic hyperfine coupling constants (a_N values) of about 14G (293K). The observed linewidths (La) for TMIO-APTSPP (0.73G) and Mn-TMIO-APTSPP (0.65G) in distilled water are significantly narrower than for TMIO-APTPP (1.475G) and Mn-TMIO-APTPP (1.55G) in chloroform.

Key Words: Electron Paramagnetic Resonance (EPR); Porphyrin; Nitroxide; Isoindoline; Spin Probe

Abbreviations

EPR	electron Paramagnetic Resonance
TMIO	1,1,3,3-tetramethylisoindolin-2-yloxyl
CTMIO	5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl
$BF_3 \cdot O(Et)_2$	Boron trifluoride etherate
DDQ	2,3-dichloro-4,5-dicyanobenzoquinone
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
TEMPO	2,2,6,6-tetramethylpiperidine-1-yloxyl
UV-Vis	ultraviolet-visible
NMR	nuclear magnetic resonance
G	Gauss
MS	mass spectrometry
EI	electron impact
NPTPP	5-(4-nitrophenyl)-10,15,20-triphenylporphyrin
APTPP	5-(4-aminophenyl)-10,15,20-triphenylporphyrin
TMIO-APTPP	5-[4"-(1',1',3',3'-tetramethylisoindolin-2'-yloxyl-5'-amido)phenyl]-10,15, 20-trisphenylporphyrin
TMIO-APTSPP	5-[4"-(1',1',3',3'-tetramethylisoindolin-2'-yloxyl-5'-amido) phenyl]-10,15,20- tris(4"'-sulfonatophenyl)porphyrin, trisodium salt
Mn-TMIO-APTPP	5-[4"-(1',1',3',3'-tetramethylisoindolin-2'-yloxyl-5'-amido)phenyl]-10, 15,20- trisphenylporphyrin manganese (III)
Mn-TMIO-APTSPP	5-[4"-(1',1',3',3'-tetramethylisoindolin-2'-yloxyl-5'-amido)phenyl]-10,15, 20- tris(4"'-sulfonatophenyl)porphyrin manganese (III), trisodium salt

Introduction

Cancer is the genetic disease that arises from an accumulation of mutations that promote clonal cell selection with increasing aggressive behavior. Reactive oxygen species (ROS), such as oxygen- and organic-free radicals, as mediators can be involved in all three stages of carcinogenesis including the initiation, promotion and progression, directly causing damage to genomic DNA leading to mutation, activation of protooncogenes and inactivation of tumor suppressor genes [1,2]. On the other hand, hypoxia is an important physiological parameter in tumor growth and response to therapy, which results from an imbalance between oxygen delivery and oxygen consumption. Thus tumors have both a hypoxia microenvironment and a high-level redox status which differentiates them from normal tissues [3].

Electron Paramagnetic Resonance (EPR) spectroscopy is the most direct and powerful method for the detection and identification of free radicals and other species with unpaired electrons [4, 5]. Recently low frequency EPR instrumentation has allowed the use of large tissue samples or whole animals *in vivo* in the fields of biology and medicine since nitroxides free radicals have been used as the important biomedical spin probes in EPR technique in biomedical studies [6-8]. Moreover, Electron Paramagnetic Resonance (EPR) spectroscopy is a potential non-invasive modality potentially of use for the study of tumor hypoxia, tissue heterogenicity with respect to oxygen and redox status, and vascular deficiencies *in vivo* [4, 5].

Because of the lack of sufficient amounts of naturally occurring paramagnetic

species and the short half-life of most free radicals, stable free radicals can be administered as imaging agents, and following their fate in the body can give useful insights into organ function and tissue status. Nitroxides provide a key modality for EPR studies in viable systems, as they are sensitive to other free radicals, to the redox state, and to oxygen levels. Moreover, nitroxides are remarkably stable free radicals that have been used as spin probes for many years, giving characteristic three line signals readily detected by EPR. In addition, nitroxides can be linked to carrier molecules to achieve tissue or organ selectivity [5, 9-11].

Although there are several classes of nitroxides known, such as imidazolines, pyrrolidines, piperidines and oxazolidines , we have focused on isoindoline-based nitroxides as these possess some advantages including narrower linewidth EPR performance, bio-reductive stability, and excellent thermal and chemical stability towards a wide variety of chemical environments, including basic and acidic solutions. The EPR advantages of the isoindoline system can be very clearly seen in the EPR spectra which contrast the spectra of the widely used 2,2,6,6-tetrmethyl-1-piperidinoxy (4-carboxy TEMPO), a commonly used commercially available nitroxide versus 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (5-carboxy-TMIO or CTMIO) partitioned in a mixed lipid/water matrix. In contrast to the 2,2,6,6-tetramethylpiperidine-1-yloxyl (TEMPO) system, the isoindoline nitroxide shows a well resolved third peak, allowing greatly increased accuracy in the quantification of the relative lipid/water levels via the standard computer simulation and deconvolution

techniques[12-16].

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. Although the exact mechanism of the uptake is still unknown, it is most likely that the porphyrins are incorporated into the tumor cell via receptor mediated endocytosis of low-density lipoproteins (LDL), since cancer cells express elevated levels of LDL receptors. The water-soluble meso-tetrasulfonatophenyl porphyrin (TPPS) was found to be highly concentrated in Walker carcinosarcoma [17, 18]. 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 [19]. Several other groups have made efforts towards the synthesis of porphyrin-antitumor drug conjugates and studied on their tumor selectivity and antitumor activity [18, 20].

Spin-labelled porphyrins have received much interest since the early 1970's when Asakura et al reported a study on the EPR spectroscopy of spin-labelled heme [21]. In subsequent years, Eaton et al have reported extensively on the EPR spectroscopy of a large range of spin-labelled metalloporphyrins with a principal focus on nitroxide-metal exchange interactions [22-25]. Recently, spin-labelled porphyrins have received attention as potential molecular magnetic materials and in the study of porphyrin excited states [26]. One aspect of spin-labelled porphyrins that has received less attention is their suitability as EPR probes.

In the work described here, a series of novel spin-labelled porphyrins based on isoindoline nitroxide was synthesized and characterized and their EPR characteristics determined.

Scheme 1

Experimental procedures

Materials

The compounds prepared were characterized using a Spectrum One infrared spectrophotometer, Lambda Bio40 UV/Vis spectrophotometer and a Bruker 400 NMR spectrometer. ESI mass spectrometry (ESI-MS) and High resolution EI+ mass spectra, were recorded by Dr Noel Davies and Marshall Hughes (Central Science Laboratory, University of Tasmania) on a Kratos Concept ISQ mass spectrometer utilizing a direct insertion probe and operating at 70 eV, 5.3 KV accelerating voltage. Silica gel (Chromatography neutral silica gel for column layer, 230-400mesh and 60A) was purchased from the QINGDAO HAIYANG CHEMICAL CO., LTD, China.

All chemicals and solvents were of analytical grade. Dry tetrahydrofuran was distilled from potassium/benzophenone and dry dichloromethane was distilled from calcium hydride, under argon, immediately prior to use. 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (5-carboxy-TMIO or CTMIO) [12] were prepared according to the literature.

Synthesis of 5-(4-nitrophenyl)-10,15,20-triphenylporphyrin (1. NPTPP)

4-Nitrobenzaldhyde (0.59 g, 3.9mmol, 1 equiv), benzaldhyde (1.13 ml, 11.1 mmol, 3 equiv) and pyrrole (1.04 mL, 15 mmol, 4 equiv) were dissolved in freshly distilled dichloromethane (1000 mL) and the reaction vessel was purged with argon for 15 min. Boron trifluoride etherate ($BF_3 \cdot O(Et)_2$, 0.4 mL of 2.5 M stock solution in dichloromethane, 3.3 mM) was then added and the mixture was stirred for 1 h at room temperature under argon protected from light, whereupon 2,3-dichloro-4,5-dicyanobenzoquinone (DDQ, 2.6g, 15mmol) was added. The reaction was stirred for an additional 1 h and then the solvent was removed under reduced pressure. Column chromatography (Silica gel, dichloromethane/hexanes:1/1) gave, as the second band eluting from the column, the desired 5-(4-nitrophenyl)-10,15,20-triphenylporphyrin (0.52g, 20%). [25, 26] ^{1}H NMR ($CDCl_3$) δ: 8.86 (d, 2H, β-pyrrole), 8.85 (d, 4H, β-pyrrole), 8.69 (d, 2H, β-pyrrole), 8.54 (d, 2H, nitrophenyl), 8.31 (d, 2H, nitrophenyl), 8.19 (m, 6H, ortho phenyl), 7.71 (m, 9H, meta/para phenyls), -2.74 (s, 2H, pyrrole NH); EI MS found M^+ 659 for $C_{44}H_{29}N_5O_2$.

Synthesis of 5-(4-aminophenyl)-10,15,20-triphenylporphyrin (2. APTPP)

5-(4-Nitrophenyl)-10,15,20-triphenylporphyrin (0.26g, 0.39mmol) was dissolved in 10 mL of concentrated hydrochloric acid under argon. Tin (II) chloride dihydrate (0.28 g, 1.2 mmol) was added to the solution, and the reaction was heated to 65 ^{o}C for 4h. The porphyrin solution was cooled and added to 30 mL of cold water and was adjusted to pH 8 with concentrated ammonium hydroxide. The aqueous phase was extracted with

chloroform, which was dried over magnesium sulfate. The organic phase was concentrated under pressure and purified by silica column with dichloromethane as an eluent to give 5-(4-aminophenyl)-10,15,20-triphenylporphyrin (0.22 g, 89%). [26] ^{1}H NMR ($CDCl_3$) δ: 8.95 (d, 2H, β-pyrrole), 8.84 (d, 2H, β-pyrrole), 8.83 (s, 2H, β-pyrrole), 8.22 (m, 6H, ortho triphenyl), 8.00 (d, 2H, 4-aminophenyl), 7.76 (m, 9H, meta/para triphenyl), 7.06 (d, 2H, 4-aminophenyl), 4.02 (s, 2H, amino), -2.73 (s, 2H, pyrrole NH); Positive EI MS found M^+ 629 for $C_{44}H_{31}N_5$; ν_{max}(KBr)/cm^{-1} 3321 (NH), 2974 (aryl CH), 1618 and 1516 (aryl C-C); UV-Vis ($CHCl_3$) λ_{max} 420, 517, 554, 592, 649 nm.

Synthesis of 5-[4''-(1',1',3',3'-tetramethylisoindolin-2'-yloxyl-5'-amido)phenyl]- 10,15, 20-trisphenylporphyrin (3. TMIO-APTPP)

5-(4-Aminophenyl)-10,15,20-triphenylporphyrin (0.2g, 0.32mmol) and 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (0.15g, 0.64mmol, 2 equiv.) were dissolved in dry tetrahydrofuran (20mL). Dicyclohexylcarbodiimide (DCC, 0.13g, 0.64mmol, 2 equiv.) was added and the solution heated to reflux for 3 days. Dicyclohexylurea was removed by filtration and the product was concentrated under pressure and purified by silica column with dichloromethane as an eluent to give 5-[4''-(1',1',3',3'-tetramethylisoindolin-2'-yloxyl-5'-amido)phenyl]-10,15,20-trisphenylpor phyrin (TMIO-APTPP, 0.16g, 59%). ^{1}H NMR ($CDCl_3$) δ: 8.9 (br, 2H, β-pyrrole), 8.8 (br, 4H, β-pyrrole), 8.22 (m, 6H, ortho triphenyl), 8.1-7.9 (br, 4H, 4-aminophenyl and aryl

C-H), 7.76 (m, 9H, meta/para triphenyl), 7.3-7.2 (br, 3H, 4-aminophenyl and aryl C-H), 2.2(s, 12H, CH_3), -2.73 (s, 2H, pyrrole NH); Positive EI MS found M^+ 845.36004 (-0.43 ppm from calc. for $C_{57}H_{45}N_6O_2$); ν_{max}(KBr)/cm^{-1} 2974 (aryl CH), 1677, 1595 (CONH), 1618 and 1516 (aryl C-C), 1340 and 1351 (NO), 1243 (C-O); UV-Vis ($CHCl_3$) λ_{max} 415, 515, 550, 590, 645 nm.

Synthesis of 5-[4''-(1',1',3',3'-tetramethylisoindolin-2'-yloxyl-5'-amido)phenyl]-10,15,20-tris(4'''-sulfonatophenyl)porphyrin, trisodium salt (4. TMIO-APTSPP)

5-(4-Aminophenyl)-10,15,20-triphenylporphyrin (0.2g, 0.24mmol) was dissolved in 50mL sulfuric acid (Baker 98% reagent grade) and heated with stirring to 70 ^oC for 4 days. The dark green solution was stirred under argon for 3 more days at room temperature and then poured into 50 mL of cold water with stirring. The dark green suspension was adjusted to pH 10 using 2M sodium hydroxide. The solvent was removed under pressure and the solid residue was extracted by methanol. The methanol solution was concentrated under pressure and purified by silica column chromatography using methanol/ethyl acetate (2:3) as eluent to give 5-[4''-(1',1',3',3'-tetramethylisoindolin-2'-yloxyl-5'-amido)phenyl]-10,15,20-tris(4'''-sulfonatophenyl)porphyrin, trisodium salt (0.14g, 68%). ^{1}HNMR (DMSO) δ: 8.9 (br, 2H, β-pyrrole), 8.8 (br, 4H, β-pyrrole), 8.2 (m, 6H, ortho triphenyl), 8.0 (m, 7H, meta triphenyl and aryl C-H), 7.9 (br, 3H, 4-aminophenyl and aryl C-H), 7.1 (br, 3H, 4-aminophenyl and aryl C-H), 2.0(s, 12H,

CH_3), -2.73 (s, 2H, pyrrole NH); Negative EI MS found $[M-Na]^-$ 1128.17735 (-8.53 ppm from calc. for $C_{57}H_{42}N_6O_{11}S_3\ Na_2$). Doubly charged m/z: 552.5 for $[M-2Na]^{2-}$; 541.5 for $[M-3Na+H]^{2-}$; triply charged m/z: 361 for $[M-3Na+H]^{2-}$; ν_{max}(KBr)/cm^{-1} 2972 (aryl CH), 1731 (SO_3), 1667, 1595 (CONH), 1556 and 1519 (aryl C-C), 1397 and 1367 (NO), 1243 (C-O), 1194 (C-S); UV-Vis (H_2O) λ_{max} 415, 513, 548, 590, 645 nm.

Synthesis of 5-[4''-(1',1',3',3'-tetramethylisoindolin-2'-yloxyl-5'-amido)phenyl]-10,15,20-trisphenylporphyrin manganese (III) (5. Mn-TMIO-APTPP)

To a refluxing solution of 5-[4''-(1',1',3',3'-tetramethylisoindolin-2'-yloxyl-5'-amido)phenyl]-10,15,20-trisphenylporphyrin (68 mg, 80 μmol) in methanol (5 mL) was added manganese acetate (94mg in 5mL of methanol, 4 mmol). The mixture was refluxed for 5 h and then allowed to cool. The solution was removed under reduced pressure. Column chromatography (Silica gel, methanol/ethyl acetate: 1/2) gave Mn-TMIO-APTPP (49.5 mg, 69%); Positive EI MS found M^+ 898 for $MnC_{57}H_{43}N_6O_2$; ν_{max}(KBr)/cm^{-1} 3423 (OH), 2924 (aryl CH), 1651, 1597 (CONH), 1537 and 1462 (aryl C-C), 1377 and 1342 (NO), 1235 (C-O); UV-Vis ($CHCl_3$) λ_{max} 385, 395, 419, 470, 565, 600 nm.

Synthesis of 5-[4''-(1',1',3',3'-tetramethylisoindolin-2'-yloxyl-5'-amido)phenyl]-10,15,20-tris(4'''-sulfonatophenyl)porphyrin manganese (III), trisodium salt

(6. Mn-TMIO-APTSPP)

To a refluxing solution of 5-(4-aminophenyl)-10,15,20-tris(4-sulfonatophenyl) porphyrin, trisodium salt (15 mg, 13 μmol) in distilled water (5 mL) was added manganese chloride (2.6mg in 5mL of distilled water, 13 mmol). The mixture was refluxed for 5 h and then allowed to cool. The solution was removed under reduced pressure. Column chromatography (Silica gel, methanol/ethyl acetate, 1:2) gave Mn-TMIO-APTSPP (8.6 mg, 55%); Negative EI MS found [M-Na+H] 1182.9 for $MnC_{57}H_{40}N_6O_{11}S_3Na_2$, $[M-3Na]^{3-}$ 1133.7 for $MnC_{57}H_{40}N_6O_{11}S_3$; ν_{max}(KBr)/cm^{-1} 3434 (OH), 2924 (aryl CH), 1727 (SO_3), 1631, 1580 (CONH), 1410 (aryl C-C), 1384 and 1340 (NO), 1222(C-O), 1204 (C-S); UV-Vis (H_2O) λ_{max} 380, 400, 415, 465, 565, 600 nm.

Electron paramagnetic resonance (EPR) 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. Electron paramagnetic resonance (EPR) studies of the nitroxides were conducted at the Centre for Magnetic Resonance (University of Queensland). X-band (9 GHz) EPR spectra were obtained on a Bruker Elexsys E500 multifrequency continuous wave EPR spectrometer fitted with an EIP 548B microwave frequency counter and a Bruker ER035M gaussmeter for microfrequency calibration. A

double Gunn diode X-band (9 GHz) microwave bridge and standard X-band rectangular TE102 microwave cavity were utilised 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.

Results and Discussion

Synthesis and characterization

Two novel water soluble spin-labelled porphyrins based on isoindoline nitroxide (TMIO-APTSPP and Mn-TMIO-APTSPP) and two novel hydrophobic spin-labelled porphyrins based on isoindoline nitroxide (TMIO-APTPP and Mn-TMIO-APTPP) were synthesized and studied in this work. As expected, the manganese porphyrin nitroxides complexes (Mn-TMIO-APTPP and Mn-TMIO-APTSPP) displayed different colors (dark green) to the free bass porphyrin nitroxides TMIO-APTPP and TMIO-APTSPP (purple). Experimental data including ^{1}H NMR, IR, UV, EPR and MS supported the formation of the porphyrin nitroxide targets and corresponding manganese complexes. However, the good quality ^{1}H NMR and ^{13}C NMR spectra cannot be measured due to paramagnetic broadening arising from the presence of the $R_2NO^{\cdot}$ group. The Mn-TMIO-APTPP and Mn-TMIO-APTSPP possess both free radical $R_2NO^{\cdot}$ and Mn^{3+} groups, further broadening the NMR spectra. Both TMIO-APTPP and TMIO-APTSPP have characteristic UV absorption peaks of free base porphyrin, while the characteristic

absorption peaks of their manganese complexes Mn-TMIO-APTPP and Mn-TMIO-APTSPP shifted to shorter wavelength.

Electron paramagnetic resonance (EPR) spectroscopy

Most nitroxides used as spin probes have EPR lines that contain unresolved proton hyperfine interactions, typically from the alkyl environments surrounding the nitroxide moiety. The signal-to-noise ratio decreases as unresolved hyperfine interactions broaden the nitroxide linewidth. Tetramethyl isoindoline nitroxides can display superior EPR linewidths, compared to other classes of nitroxides. 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 porphyrins containing isoindoline nitroxides solutions in chloroform or water (Figure 1, 2). The compounds all exhibit the typical nitroxide g-value for a free electron of approximately 2.006 and nitrogen isotropic hyperfine coupling constants (a_N values) of about 14G (293K).

Figure 1

Figure 2

Figure 1 and 2 show the four spin-labeled porphyrins possess the characteristic EPR hyperfine splittings of tetramethyl isoindoline nitroxides and there are clearly significant differences in linewidth. The observed linewidths (La) for TMIO-APTSPP (0.73G) and Mn-TMIO-APTSPP (0.65G) in distilled water are significantly narrower than for

TMIO-APTPP (1.475G) and Mn-TMIO-APTPP (1.55G) in chloroform (Table 1). Moreover, the manganese complex Mn-TMIO-APTSPP displays narrower linewidths and sharper lines than TMIO-APTSPP. A small reduction in linewidth can result in a significant increase in line-height, with a corresponding increase in the signal-to-noise ratio. Therefore Mn-TMIO-APTSPP is expected to have somewhat lower detection limits than the corresponding TMIO-APTSPP. This may be of relevance in biological applications, resulting in lower effective doses of the nitroxide for imaging purposes or physico-chemical measurements (e.g. oximetry).

Table 1

In comparison to 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl (0.21G), EPR lines of TMIO-APTSPP and Mn-TMIO-APTSPP are wider and shorter due to greater anisotropy arising from slower molecular motions in these large molecules. Moreover, TMIO-APTSPP and Mn-TMIO-APTSPP possess water solubility and, they therefore may have applications as biological probes. However, for these simple experimental conditions, the EPR signal of manganese does not appear as expected in X-band (9 GHz) EPR spectra, which maybe result from weak interaction by manganese. Low temperature EPR may be used to more fully monitor this metal/nitroxide coupling.

Conclusion

Two novel water soluble spin-labelled porphyrins based on isoindoline nitroxide (TMIO-APTSPP and Mn-TMIO-APTSPP) and two novel hydrophobic prophyrins

nitroxides (TMIO-APTPP and Mn-TMIO-APTPP) were synthesized and characterized. Both TMIO-APTPP and TMIO-APTSPP have characteristic UV absorption peaks of free base porphyrin, while the characteristic absorption peaks of their manganese complexes Mn-TMIO-APTPP and Mn-TMIO-APTSPP shifted to shorter wavelength. The compounds all exhibit the hyperfine splittings characteristic EPR spectra of tetramethyl isoindoline nitroxides, the typical nitroxide g-values of approximately 2.006 and nitrogen isotropic hyperfine coupling constants (a_N values) of about 14G (293K). The observed linewidths (La) for TMIO-APTSPP (0.73G) and Mn-TMIO-APTSPP (0.65G) in distilled water are significantly narrower than for TMIO-APTPP (1.475G) and Mn-TMIO-APTPP (1.55G) in chloroform. Moreover the manganese complex Mn-TMIO-APTSPP displays the narrower linewidths and sharper lines than TMIO-APTSPP. Thus the water soluble TMIO-APTSPP and Mn-TMIO-APTSPP are well suitable as candidates for biological spin probes using EPR spectroscopy.

Acknowledgments

We thank QUT's Postdoctoral Research Fellowship Scheme and the Australian Research Council for their financial support.

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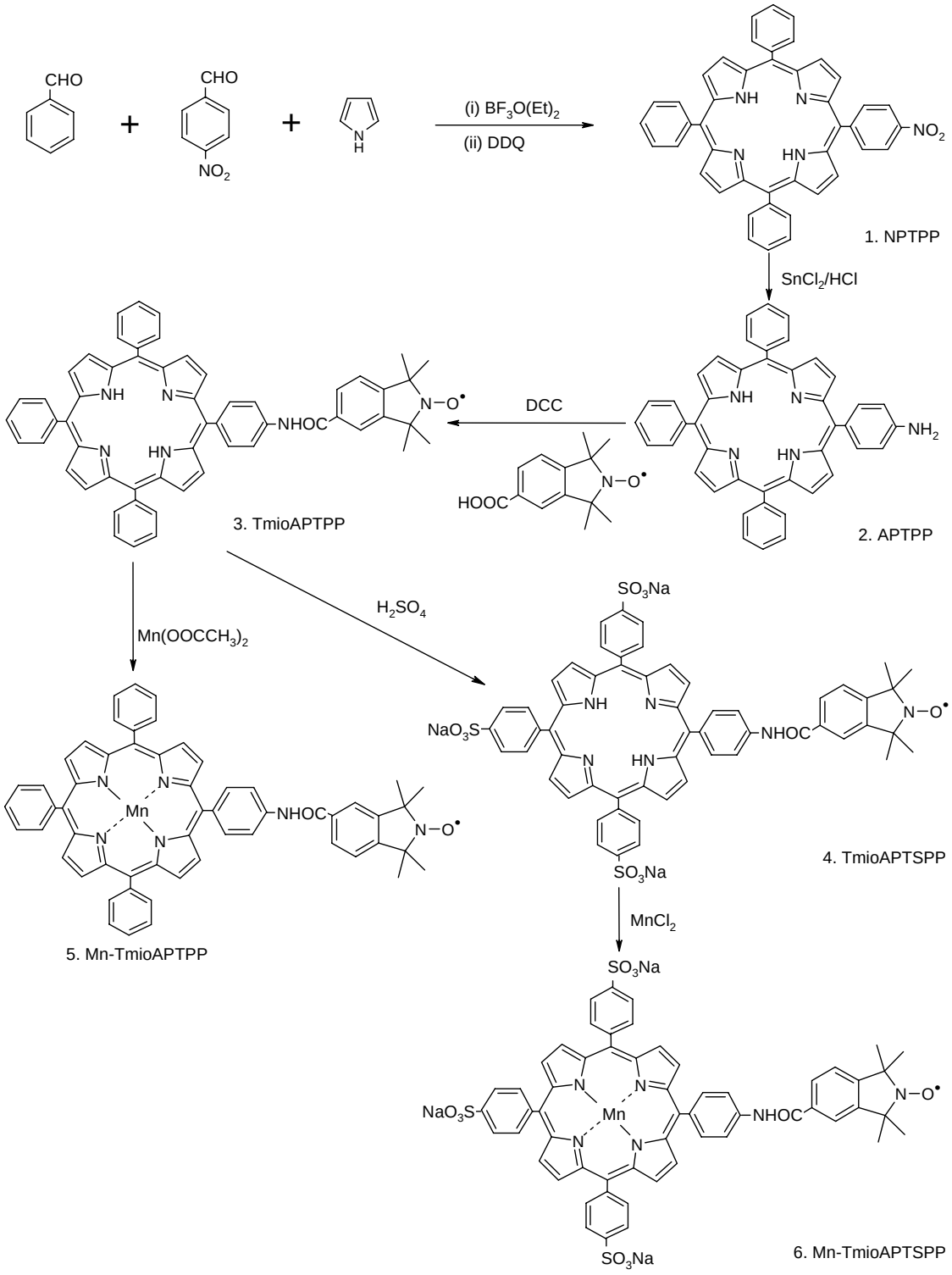

Scheme 1. Synthetic route to porphyrins containing isoindoline nitroxides

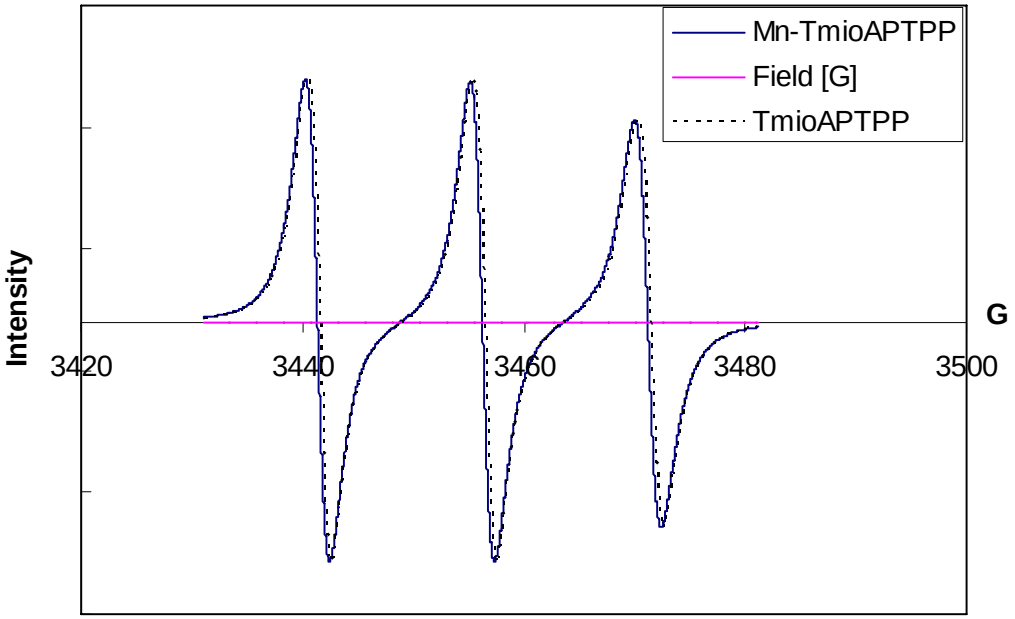

Figure 1. Comparison of X-band EPR spectra of nitroxides TMIO-APTPP (dashed line) and Mn-TMIO-APTPP (solid line) after at 293 K

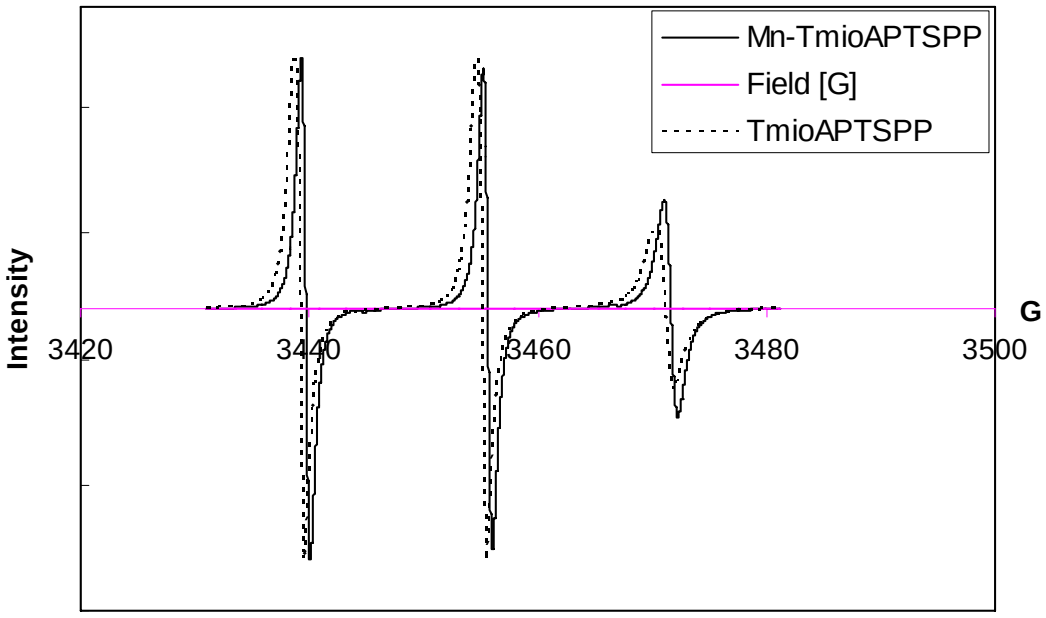

Figure 2. Comparison of X-band EPR spectra of nitroxides TMIO-APTSPP (dashed line) and Mn-TMIO-APTSPP (solid line) in water at 293 K

Table 1. EPR data of spin-labelled porphyrins

Spin-labelled porphyrin	Microwave frequency	g-value for a free electron	nitrogen isotropic hyperfine coupling constant a_N (G, 293 K)	Linewidth parameters La
CTMIO [12]		2.00585	14.445±0.289 ($CHCl_3$)	0.21±0.004
TMIO-APTPP	9.705991e+09	2.00625	13.710±0.274 ($CHCl_3$)	1.475±0.03
Mn-TMIO-APTPP	9.705663e+09	2.00653	13.725±0.274 ($CHCl_3$)	1.55±0.03
TMIO-APTSPP	9.701823e+09	2.00623	14.960±0.299 (water)	0.73±0.01
Mn-TMIO-APTSPP	9.70275e+09	2.00610	14.960±0.299 (water)	0.65±0.01