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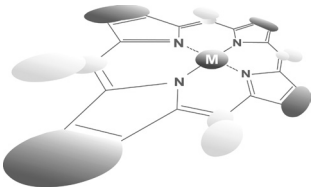

Porphyrin containing isoindoline nitroxides as potential fluorescence sensors of free radicals

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ABSTRACT: A series of new spin-labeled porphyrin containing isoindoline nitroxide moieties were synthesized and characterized as potential free radical fluorescence sensors. Fluorescence-suppression was observed in the free-base monoradical porphyrins, whilst the free-base biradical porphyrins exhibited highly suppressed fluorescence about three times greater than the monoradical porphyrins. The observed fluorescence-suppression was attributed to enhanced intersystem crossing resulting from electron-exchange between the doublet nitroxide and the excited porphyrin fluorophore. Notably, fluorescence-suppression was not as strong in the related metalated porphyrins, possibly due to insufficient spin coupling between the nitroxide and the porphyrin. Continuous wave EPR spectroscopy of the diradical porphyrins in fluid solution suggests that the nitroxyl-nitroxyl interspin distance is long enough and tumbling is fast enough not to detect dipolar coupling.

KEYWORDS: porphyrins, nitroxides, fluorescence, radicals, profluorescent probes, spin probes.

INTRODUCTION

Nitroxides are stable free-radical species which are currently utilized in a broad range of applications. As a result of their redox- and radical-trapping properties, nitroxides have been extensively used as electron paramagnetic resonance (EPR) spin labels for proteins, enzymes, nucleotides and other biological systems [1–4]. Their application as paramagnetic agents in the field of biology and medicine has recently allowed low frequency EPR imaging of large tissue samples or whole animals *in vivo* [5–8]. Although the piperidine- and pyrrolidine-based nitroxides are more widely used, some isoindoline nitroxides possess advantages over these commercially available species. The isoindoline nitroxides can exhibit enhanced bio-reductive stability [9], may possess increased thermal and chemical stability [10–12] and generate inherently narrower EPR line widths [13].

Nitroxides are commonly used as sensitive probes for monitoring processes involving free radicals. Profluorescent nitroxides [14], which possess a fluorophore tethered to a nitroxide moiety by a short covalent link, are quenchers of excited electronic states [15–23]. They exhibit low fluorescence due to electron exchange interactions between the excited molecule and the nitroxide radical (enhanced intersystem crossing from first excited singlet state to triplet state). Upon redox activity or radical trapping however, normal fluorophore emission is enabled and thus nitroxide-fluorophore adducts have been utilized as extremely sensitive probes for the detection of free-radical species. Most of the profluorescent nitroxides prepared to date contain linkages such as esters [15–17, 21, 24–28], amides [29–31] or sulfonamides [32–35] which are susceptible to hydrolysis. As scission of the nitroxide moiety from the fluorophore would restore fluorescence independently from any radical

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reactions of the nitroxide, our work has focused on the synthesis of nitroxide-fluorophore adducts *via* carbon-carbon bond formation [36–43]. Porphyrin fluorophores have attracted our interest due to their potential applications in biological systems, which arise because of the relatively long wavelength (~600–800 nm) at which porphyrins emit radiation *via* fluorescence. Biological systems typically exhibit background fluorescence at similar wavelengths to many common fluorophores, restricting the use of many profluorescent spin probes. As fluorescence by biological entities occurs at wavelengths below those of porphyrins, porphyrins make ideal fluorophore candidates for biological spin probes [44–46].

There are several reports describing the synthesis and properties of porphyrin-bound nitroxides. Spin-labeled porphyrins were first reported in the early 1970's when Asakura examined spin-labeled heme by EPR spectroscopy [47]. In subsequent years, Eaton and coworkers have extensively studied a large range of spin-labeled metalloporphyrins by EPR spectroscopy, with the principal focus on nitroxide-metalated exchange interactions [48–50]. More recently, Shultz has reported the synthesis of phenylnitroxide substituted zinc(II) porphyrins for the construction of coordination polymers with interesting magnetic properties [51]. The excited states of porphyrins, nitroxide-porphyrin hydrids and nitroxide-phthalocyanine systems have also been studied by time resolved EPR spectroscopy (TREPR) [52–54]. One aspect of spin-labeled porphyrins that has been largely overlooked is their suitability as fluorescence-suppressed spin probes for biological applications. Recently, Ishii and coworkers have reported phthalocyaninatosilicon covalently linked to TEMPO radicals as probes for detecting ascorbic acid in biological systems [55]. We have previously prepared porphyrin-nitroxide adducts linked through amide linkages [56]. Herein, we now wish to report the synthesis of a novel series of porphyrin-bound isoindoline nitroxides linked by carbon-carbon bonding at the *meso*-position of the porphyrin ring. Fluorescence spectroscopy and EPR experiments were used to evaluate the prepared compounds as potential fluorescence-suppressed spin probes.

RESULTS AND DISCUSSION

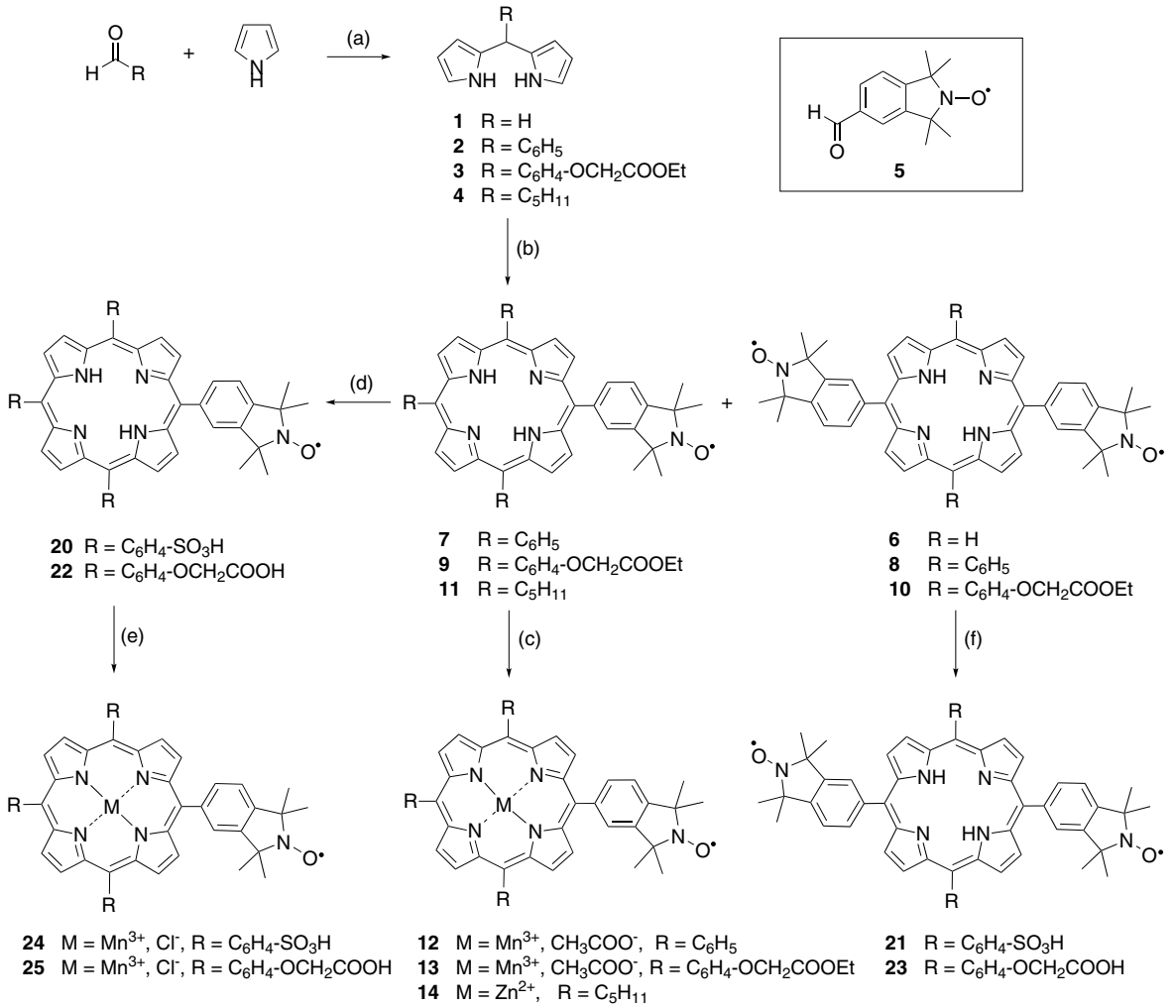
Synthesis

Spin-labeled porphyrins can be accessed by the acid catalyzed condensation of dipyrromethanes with nitroxide-containing aldehydes. Unsubstituted dipyrromethane **1** and 5-phenyl and 5-(4′-carboethoxymethyleneoxyphenyl) substituted derivatives **2** and **3** were prepared from pyrrole and the appropriate aldehyde using literature procedures [57–59]. 5-Pentyldipyrromethane (**4**) was synthesized using an adapted literature procedure (Scheme 1) [60]. The 5-phenyl and 5-(4′-carboethoxymethyleneoxyphenyl) substituted dipyrromethanes **2** and **3** were chosen because of their potential to provide water solubility either through sulfonation of the aromatic ring or ethyl ester hydrolysis to give the corresponding carboxylic acid. The *n*-pentyl substituted dipyrromethane **4** was selected for its high organic solubility.

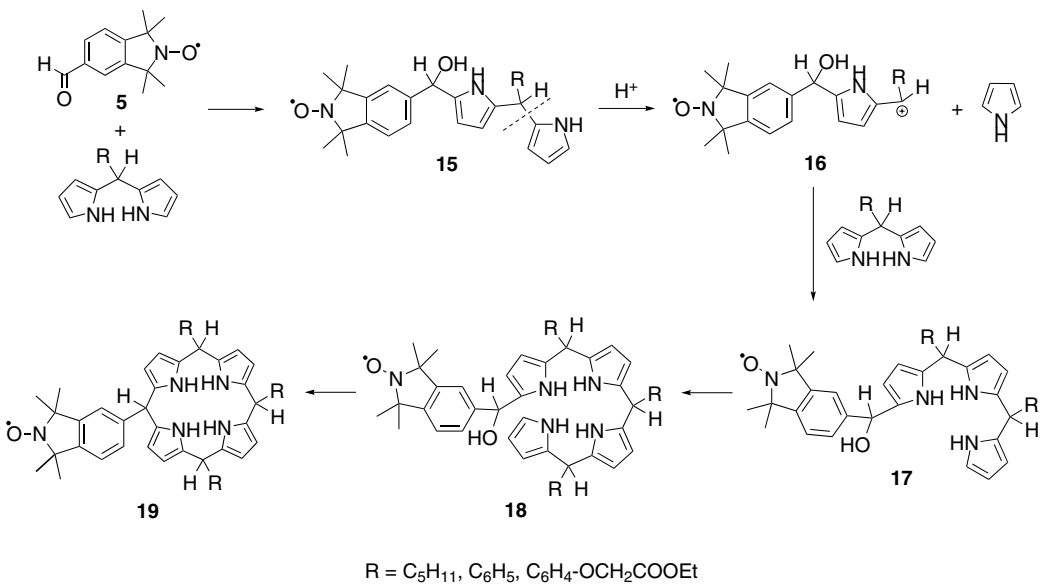
Reaction of dipyrromethane **1** (1.0 equiv.) with 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl (**5**) (1.0 equiv.) in the presence of trifluoroacetic acid (TFA), following by oxidation with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) gave exclusively the desired bis-nitroxide porphyrin **6** in low yield (9.6%) (Scheme 1). Interestingly, treatment of 5-phenyldipyrromethane (**2**) (1.0 equiv.) with aldehyde **5** (1.0 equiv.) in the presence of boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$) afforded more of the mono-nitroxide porphyrin **7** (8.3%) than the desired bis-nitroxide porphyrin **8** (1.5%) after oxidation with DDQ. Similarly, use of 5-(4′-carboethoxymethyleneoxyphenyl) dipyrromethane (**3**) under identical reaction conditions gave mono-nitroxide porphyrin **9** (8.7%) and bis-nitroxide porphyrin **10** (2%). The mono-porphyrin nitroxide **11** was also isolated as the sole reaction product (in 3.2% yield) from the acid-catalyzed condensation between 5-pentyldipyrromethane **4** and aldehyde **5**. The manganese(III) ion was inserted into mono-nitroxide porphyrins **7** and **9** using manganese(II) acetate (Mn^{2+} readily oxidizes to Mn^{3+} in the porphyrin) to give metalated porphyrins **12** and **13** in high yield (>90%). The use of zinc(II) acetate gave the mono-nitroxide porphyrin **14** in quantative yield (100%).

The observed preferential formation of mono-radical porphyrins **7** and **9** and exclusive formation of mono-radical porphyrin **11** can possibly be explained by acid catalyzed scrambling in the reactions between aldehyde **5** and the substituted dipyrromethanes **2**, **3** and **4**. Acid catalyzed scrambling has been reported to occur in the synthesis of porphyrins from unhindered 5-substituted dipyrromethanes and can result in products with unexpected substitution patterns, making the desired bis-substituted products difficult to obtain [57]. Acid catalyzed cleavage of the substituted dipyrromethane **15**, referred to as acidolysis, gives rise to a monopyrrolic carbocation **16** which can go on to react with another substituted dipyrromethane molecule. For reactions in which the bis-nitroxide products are not formed (or formed in very low yield), the resulting tripyrrolic species **17** can either undergo acidolysis followed by addition of another dipyrromethane molecule or react with a monopyrrolic acidolysis product to give **18**. The resulting tetrapyrrolic species **18** can then cyclize to form the porphyrinogen and subsequently be oxidized to the porphyrin **19** (Scheme 2).

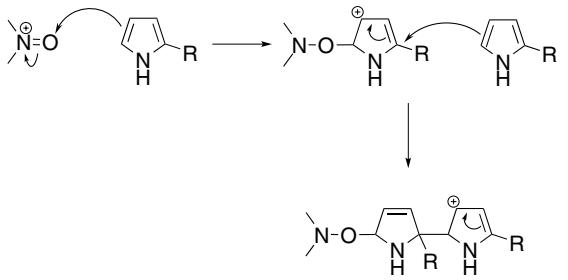
Despite the possible occurrence of acid catalyzed scrambling, both the mono-nitroxide and bis-nitroxide porphyrin products were only observed in low yield. Accelerated polymerization of dipyrromethane (Scheme 3) may be the cause, as the formation of an insoluble black



Scheme 1. (a) AcOH or TFA; (b) (i) 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl (**5**), DCM, (ii) $BF_3{\cdot}Et_2O$ or TFA, (iii) DDQ, **6** 9.6%, **7** 8.3%, **8** 1.5%, **9** 8.7%, **10** 2%, **11** 3.2%; (c) $Mn(OAc)_2$, MeOH or $Zn(OAc)_2$, $CHCl_3$, **12** 93%, **13** 90%, **14** 100%; (d) for **20** — (i) H_2SO_4, (ii) NaOH, 36%; for **22** — NaOH, 69%; (e) $MnCl_2{\cdot}4H_2O$, H_2O, **24** 60%, **25** 55%; (f) for **21** — (i) H_2SO_4, (ii) NaOH, 30%, for **23** — NaOH, 53%



Scheme 2. Proposed acid catalyzed scrambling of 5-substituted dipyrromethane during the attempted preparation of di-substituted porphyrins



Scheme 3. Proposed oxoammonium ion catalyzed polymerization of dipyrromethane

precipitate was observed over the course of the reaction. Light and oxygen are known to catalyze polymerization of pyrrole and pyrromethanes. Since light and oxygen were excluded from the reaction, it was speculated that this polymerization was catalyzed by the presence of the nitroxide moiety. Nitroxides are oxidized to oxoammonium ions in the presence of acids, such as TFA and $BF_3{\cdot}O(Et)_2$, which catalyze the polymerization of dipyrromethanes. In an attempt to avoid accelerated polymerization of dipyrromethane, the analogous reaction was performed using the secondary amine, 5-formyl-1,1,3,3-tetramethylisoindoline, in place of formyl-nitroxide **5**. Although accelerated polymerization of dipyrromethane was not observed, no porphyrin products were identified in the reaction mixture.

With the spin-labeled porphyrins in hand, attempts were made to improve their water solubility. Treatment of mono-nitroxide porphyrin **7** with sulfuric acid (H_2SO_4) gave the tri-sulfonated porphyrin **20** in moderate yield (36%) (Scheme 1). The di-sulfonated bis-nitroxide **21** could be obtained in a similar manner in 30% yield. Basic hydrolysis of the ester sidechains of porphyrins **9** and **10** afforded carboxylic acid-containing porphyrins **22** and **23** in reasonable yields (69% and 53% respectively). The manganese(III) ion was inserted into water-soluble mono-nitroxide porphyrins **20** and **22** using manganese(II) chloride (Mn^{2+} readily oxidizes to Mn^{3+} in the porphyrin) to give **24** and **25** in modest yields (36% and 69% respectively).

The formation of the porphyrin nitroxide targets was supported by experimental data, including ^{1}H NMR, IR, UV-vis, EPR, MS and fluorescence spectra. However, good quality ^{1}H NMR and ^{13}C NMR spectra could not be measured due to the expected paramagnetic broadening that arises from the presence of the nitroxide radical and various transition metal ions.

Evaluation of fluorescence suppression

Of primary interest for the fluorescence properties of the prepared nitroxide-porphyrin systems were the variations in fluorescence emissions between the nitroxides and their diamagnetic hydroxylamine equivalents. The ability of the nitroxide to quench the fluorescence of the porphyrin macrocycle can be used to assess the validity

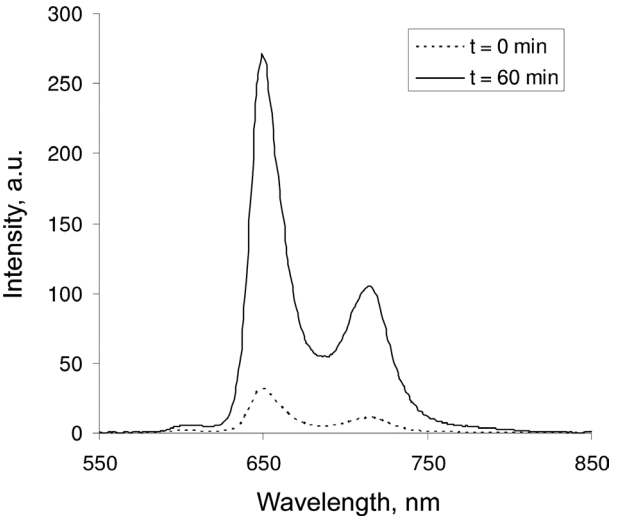
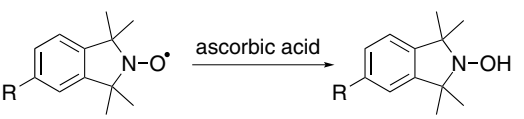


Fig. 1. Fluorescence spectra after addition of excess ascorbic acid to compound **7** in methanol

of these compounds as fluorescence-suppressed spin probes. The effect of the presence of either one or two nitroxide groups on the level of fluorescence suppression provides insight into the mechanism of fluorescence suppression and aids in the design of more sensitive spin probes.

Fluorescence measurements were carried out on deoxygenated solutions of the spin-labeled porphyrins in methanol (10^{-6} M). The optimum excitation wavelength was determined by UV-vis spectroscopy to be 414–426 nm. Addition of excess ascorbic acid to the solution of spin-labeled porphyrin resulted in an increase in fluorescence intensity with a maximum intensity reached after one hour (for example, see Fig. 1). At this point, treatment of the solution with additional ascorbic acid resulted in no further change, indicating that all nitroxide radicals had been reduced to the corresponding hydroxylamine groups (Scheme 4). The magnitudes of fluorescence suppression by the nitroxide for compounds **6–14** and **20–25** are listed in Table 1.

The fluorescence observed for the hydroxylamines of biradical porphyrins **6**, **8**, **10**, **21** and **23** was 16–28 times greater than that of the corresponding nitroxides. Interestingly, the hydroxylamines of monoradical porphyrins **7**, **9**, **11**, **20** and **22** displayed only 5–9 times more fluorescence than their corresponding nitroxides. Thus, the presence of two nitroxides covalently linked to the porphyrin fluorophore increased the level of the fluorescence suppression. The analogous fluorescence experiments were attempted on the spin-labeled metalated porphyrins **12–14**, **24** and **25**. The addition of ascorbic acid had no significant effect on the fluorescence emission of compounds **12–14** which



Scheme 4. Reduction of nitroxide with ascorbic acid

Table 1. Fluorescence emission wavelengths and suppression ratios for compounds **6–14** and **20–25**

Compound	Fluorescence λ_{em}, nm	Fluorescence suppression ratio[a]
Biradical porphyrin		
6	649, 715	25
8	651, 715	28
10	654, 715	20
21	649, 714	26
23	653, 719	16
Monoradical porphyrin		
7	649, 715	9
9	654, 715	7
11	649, 715	8
20	650, 718	5
22	656, 717	5
Monoradical metalloporphyrin		
12	653, 715	1
13	654, 715	1
14	648, 715	1
24	656, 715	3
25	659, 715	3

[a] Fluorescence suppression ratio = hydroxylamine fluorescence intensity at λ_{em}/nitroxide fluorescence intensity at λ_{em}.

was attributed to the very low solubility of these compounds in aqueous solution. A 3-fold increase in fluorescence emission was, however, observed for metalated porphyrins **24** and **25** upon the addition of ascorbic acid. It therefore appears that the typical pathways for fluorescence quenching in radical-fluorophore systems are less effective in spin-labeled metalated porphyrins. If the interaction between the excited porphyrin fluorophore and the nitroxide radical was very small, then the level of fluorescence-suppression would also be very small. From preliminary TREPR measurements on spin-labeled metalated porphyrins, performed by Ishii, it appears that this is the most likely explanation for the observed fluorescence behavior [61].

EPR investigations

Most nitroxides used as spin probes produce EPR spectra consisting of three resonances which arise from ^{14}N hyperfine coupling. ^{1}H hyperfine couplings arising from the alkyl environments surrounding the nitroxide radical are usually unresolved. The signal-to-noise ratio decreases as unresolved hyperfine interactions increase the linewidth of the ^{14}N hyperfine resonances. Tetramethyl isoindoline nitroxides can give narrower EPR linewidths, compared to other classes of nitroxides [13]. Samples for EPR studies were prepared at concentrations of ≤2 mM to eliminate intermolecular effects. X-band

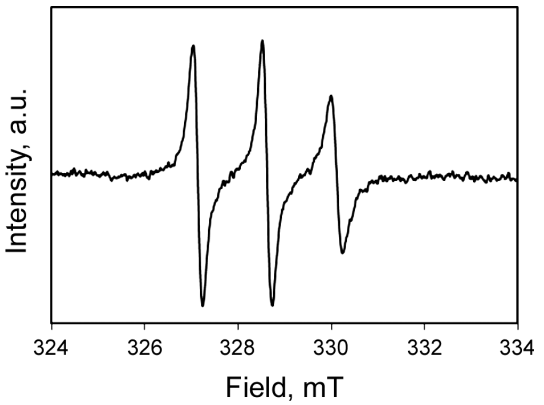


Fig. 2. X-band EPR spectrum biradical porphyrin **21** (ν = 9.255 GHz) in methanol at 298 K

EPR spectra were recorded at room temperature in solutions of chloroform, toluene, methanol or water.

The spin-labeled porphyrins **6**, **11**, **14** and **21** possess the characteristic EPR hyperfine splittings of tetramethyl isoindoline nitroxides. Notably, the biradical porphyrins **6** and **21** showed the characteristic EPR spectra of tetramethyl isoindoline nitroxides (three lines) and not the same EPR characteristics (five lines arising from weak antiferromagnetic exchange coupling) displayed by more proximate isoindoline biradicals, such as bis(1,1,3,3-tetramethylisoindolin-2-yloxyl-5-yl)methane and 1,1-bis-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)ethane [62]. Apparently, the free rotation of the nitroxide prevents the overlap of the π orbitals of the porphyrin ring and the nitroxide moieties, thus eliminating the antiferromagnetic exchange coupling. The inter-spin distance is long enough, and the tumbling fast enough, that dipolar splitting is not observed in the fluid solution spectra. The parameters for the doubly-spin labeled porphyrin **21** were similar to values for 1,1,3,3-tetramethylisoindolin-2-yloxyl (TMIO) reported in the literature (Fig. 2). The aN/gβ of 1.5 mT in methanol is slightly larger than the literature value for TMIO in toluene, which is 1.41 mT [63]. The increased value of aN/gβ in polar solvents is typical of nitroxide radicals. The g value for the spin-labeled porphyrin **21** in methanol (2.0055) is within experimental uncertainty of the literature value for TMIO in toluene (g = 2.00497). Some solvent dependence of the g value may also be present.

EXPERIMENTAL

General

^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer and referenced to the relevant solvent peak. Low and high resolution mass spectra were recorded at the Australian National University (ANU) using either a Micromass autospec double focusing magnetic sector mass spectrometer (EI+ spectra) or a Bruker Apex 3

fourier transform ion cyclotron resonance mass spectrometer with a 4.7 T magnet (ESI+ spectra). Formulations were calculated in the elemental analysis programs of Mass Lynx 4.0 or Micromass Opus 3.6. Fourier transform infrared (FTIR) spectra were recorded on a Nicolet 870 Nexus Fourier Transform Infrared Spectrometer equipped with a DTGS TEC detector and an ATR objective. Melting points were measured on a GallenKamp Variable Temperature Apparatus by the capillary method and are uncorrected. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer. UV-vis spectroscopy was performed with a Varian Cary 50 spectrophotometer. X-band continuous wave (CW) EPR spectra were obtained using a Bruker Biospin Elexsys E500 EPR spectrometer fitted with a super high Q cavity. The magnetic field and microwave frequency were calibrated with a Bruker ER036M Teslameter and an EIP 548A frequency counter, respectively. The EPR spectrum for compound **21** was obtained using a Varian E9 spectrometer using air saturated methanol.

Synthesis

Dichloromethane (DCM) was freshly distilled from calcium hydride. Dipyrrolmethane (**1**) [57], 5-phenyldipyrromethane(**2**)[58],5-(4′-carboethoxymethyleneoxyphenyl) dipyrromethane (**3**) [59] and 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl (**5**) [64] were synthesized according to literature procedures.

5-pentyldipyrromethane (4). A solution of hexanal (1.00 mL, 10.0 mmol) and pyrrole (28.0 mL, 400 mmol) was deoxygenated with argon for 10 min. Trifluoroacetic acid (TFA, 80.0 µL, 1.00 mmol) was added and the solution was stirred for 15 min. The solution was then diluted with DCM (300 mL), washed with aqueous sodium hydroxide (0.1 M, 200 mL) and water (200 mL), dried (Na_2SO_4) and filtered. The DCM was removed under reduced pressure and excess pyrrole was removed by vacuum distillation. Chromatography of the resulting light brown oil (SiO_2; 20:80:1 *n*-hexane/ethyl acetate/TEA) gave compound **4** as a yellowish oil (1.10 g, 5.08 mmol, 55%). The spectroscopic data obtained for **4** was in agreement with that previously reported [60].

5,10-bis(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrin (6). A solution of 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl (**5**) (145 mg, 664 µmol) and dipyrromethane (**1**) (97 mg, 664 µmol) in freshly distilled DCM (66 mL) was purged with argon for 15 min. TFA (3.50 µL) was then added and the mixture was stirred under argon and protected from light. After 20 h, 2,3-dichloro-5, 6-dicyano-*p*-benzoquinone (DDQ) (50.0 mg, 220 µmol) was added and the mixture was stirred for a further 30 min then concentrated to 10 mL under reduced pressure. Column chromatography (SiO_2; DCM) gave **6** (32.0 mg, 46.6 µmol, 9.6%). UV-vis ($CHCl_3$): λ_{max}, nm 409, 505, 539, 575, 636. MS (EIMS): *m/z* 686.33606 $[M]^+$, required for $C_{44}H_{42}N_6O_2$ 686.33692. $g = 2.00590$, $a_N/g\beta = 1.41$ mT (toluene, 293 K).

5,10,15-trisphenyl-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrin (7) and 5,15-bisphenyl-10,20-bis(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrin (8). A solution of 5-formyl-1,1, 3,3-tetramethylisoindolin-2-yloxyl (**5**) (218 mg, 1.00 mmol) and 5-phenyldipyrromethane (**2**) (222 mg, 1.00 mmol) in freshly distilled DCM (100 mL) was purged with argon for 15 min. Boron trifluoride diethyl etherate (2.5 M solution in DCM, 133 µL, 0.33 mmol) was then added and the mixture was stirred under argon at room temperature and protected from light. After 1 h, DDQ (174 mg, 75.0 mmol) was added and the mixture was stirred for an additional 1 h. The solvent was then removed under reduced pressure. Purification by column chromatography (SiO_2, DCM) afforded **7** (20.0 mg, 27.5 µmol, 8.3%) and **8** (6.20 mg, 7.20 µmol, 1.5%). **Compound 7.** IR (KBr): ν, cm^{-1} 2923, 2853, 1597, 1467, 1370, 1350, 1281, 1120. UV-vis ($CHCl_3$): λ_{max}, nm 420, 520 (ε 6663, 2% $CHCl_3$/MeOH), 545, 600, 650. ^1H NMR (400 MHz, $CDCl_3$): δ_H, ppm 9.0-8.8 (8H, bs, β-pyrrole), 8.2 (8H, m, *ortho* phenyl), 8.0 (1H, bs, *meta* phenyl), 7.8-7.7 (9H, bm, *meta*/*para* triphenyl), 2.2 (12H, s, CH_3), -2.75 (2H, s, pyrrole NH). MS (LSIMS): *m/z* 727.33132 $[M + H]^+$, required for $C_{50}H_{41}N_5O$ 727.33111. **Compound 8.** IR (KBr): ν, cm^{-1} 2923, 2853, 1597, 1467, 1370, 1350, 1281, 1120. UV-vis ($CHCl_3$): λ_{max}, nm 420, 520, 545, 600, 650. ^1H NMR (400 MHz, $CDCl_3$): δ_H, ppm 8.9-8.8 (8H, bs, β-pyrrole), 8.2 (8H, m, *ortho* phenyl), 8.0 (2H, bs, *meta* phenyl), 7.8-7.6 (6H, bm, *meta*/*para* bisphenyl), 2.2 (24H, s, CH_3), -2.75 (2H, s, pyrrole NH). MS (LSIMS): *m/z* 839.40928 $[M + H]^+$, required for $C_{56}H_{50}N_6O_2$ 838.39952. A molar extinction coefficient for tetraphenylporphyrin of 18,900 M^{-1}. cm^{-1} at 515 nm has previously been reported [65].

5,10,15-tris(4″-carboethoxymethyleneoxyphenyl-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrin (9) and 5,15-bis(4″-carboethoxymethylene-oxyphenyl)-10,20-bis(1′,1′,3′,3′-tetramethyl-isoindolin-2′-yloxyl-5′-yl)porphyrin (10). 5-(4′-carboethoxymethyleneoxyphenyl)dipyrromethane (**3**) (324 mg, 1.00 mmol) was treated with 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl (**5**) (218 mg, 1.00 mmol) as described above. Purification by column chromatography (SiO_2, DCM) afforded **9** (30.0 mg, 29.0 µmol, 8.7%) and **10** (10.4 mg, 10.0 µmol, 2%) as purple solids. **Compound 9.** IR (KBr): ν, cm^{-1} 2925, 2856, 1756, 1734, 1604, 1505, 1464, 1377, 1259, 1080. UV-vis ($CHCl_3$): λ_{max}, nm 415, 515, 550, 590, 645. ^1H NMR (400 MHz, $CDCl_3$): δ_H, ppm 9.0-8.8 (8H, bs, β-pyrrole), 8.15 (6H, d, *ortho* phenyl), 8.0 (1H, bs, *meta* phenyl), 7.7, 7.5 (2H, bs, *ortho* phenyl), 7.3 (6H, d, *meta* phenyl), 4.9 (6H, s, OCH_2CO), 4.4 (6H, q, OCH_2), 2.2 (12H, s, CH_3), 1.5 (9H, s, CH_3), -2.75 (2H, s, pyrrole NH). MS (LSIMS): *m/z* 1033.42571 $[M + H]^+$, required for $C_{62}H_{59}N_5O_{10}$ 1033.42619. **Compound 10.** IR (KBr): ν, cm^{-1} 2974, 2927, 2855, 1759, 1738, 1605, 1505, 1471, 1376, 1358, 1281, 1248, 1198, 1176. UV-vis ($CHCl_3$): λ_{max}, nm 420, 520, 550, 600, 650. ^1H NMR (400 MHz, $CDCl_3$): δ_H, ppm 9.0-8.8 (8H, bs, β-pyrrole), 8.2 (4H,

bs, *ortho* phenyl), 8.0 (2H, bs, *meta* phenyl), 7.35 (4H, d, *ortho* phenyl), 7.25 (4H, d, *meta* triphenyl), 4.9 (4H, s, OCH_2CO), 4.4 (4H, q, OCH_2), 2.2 (24H, s, CH_3), 1.5 (6H, s, CH_3), -2.75 (2H, s, pyrrole NH). MS (LSIMS): *m/z* found 1043.47060 $[M + H]^+$, required for $C_{64}H_{63}N_6O_8$ 1043.47074.

5,10,15-tri-*n*-pentyl-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrin (11). A solution of 5-formyl-1,1,3,3-tetramethylisoindolin-2-yloxyl (**5**) (353 mg, 1.62 mmol) and 5-pentyldipyrromethane (**4**) (350 mg, 1.62 mmol) in freshly distilled DCM (300 mL) was purged with argon for 15 min. TFA (53.0 µL) was then added and the mixture was stirred under argon protected from light. After 20 h, DDQ (523 mg, 2.30 µmol) was added and the mixture was stirred for a further 30 min then evaporated to dryness. Column chromatography (SiO_2; $CHCl_3$) gave **11** (37.0 mg, 52 µmol, 3.2%). UV-vis ($CHCl_3$): λ_{max}, nm (log ε) 420 (5.60), 488 (3.61), 520 (4.23), 556 (4.07), 598 (3.74), 656 (3.89). MS (LSIMS): *m/z* 709.47149 $[M + H]^+$, required for $C_{47}H_{59}N_5O$ 709.47196. g = 2.00589, $a_N/g\beta$ = 1.41 mT (toluene, 293 K).

5,10,15-trisphenyl-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrinato manganese(III) acetate (12). To a refluxing solution of 5,10,15-trisphenyl-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrin (**7**) (10.0 mg, 14.0 µmol) in methanol (5 mL) was added a solution of manganese(II) acetate (17.0 mg, 50.0 mmol) in methanol (5 mL). The mixture was refluxed for 5 h, allowed to cool and then concentrated *in vacuo*. Column chromatography (SiO_2; methanol/ethyl acetate 1:2) gave **12** (10.0 mg, 13.0 µmol, 93%). IR (KBr): ν, cm^{-1} 2957, 2925, 2853, 1731, 1599, 1558, 1488, 1375, 1342, 1233, 1072. UV-vis ($CHCl_3$): λ_{max}, nm 380, 400, 425, 470, 565, 600. MS (ESMS): *m/z* 779.2452, $[M + H]^+$ required for $C_{50}H_{37}MnN_5O$ 779.2451.

5,10,15-tris(4″-carboethoxymethyleneoxyphenyl)-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)-porphyrinato manganese(III) acetate (13). To a refluxing solution of 5,10,15-tris(4″-carboethoxymethyleneoxyphenyl-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrin (**9**) (10.0 mg, 9.70 µmol) in methanol (5 mL) was added a solution of manganese(II) acetate (17.0 mg, 50.0 mmol) in methanol (5 mL). The mixture was refluxed for 5 h, allowed to cool and the solvent removed under reduced pressure. Column chromatography (SiO_2; methanol/ethyl acetate 1:2) gave **13** (9.50 mg, 8.70 µmol, 90%). IR (KBr): ν_{max}, cm^{-1} 2927, 2857, 1759, 1605, 1501, 1443, 1380, 1357, 1207, 1179. UV-vis ($CHCl_3$): λ_{max}, nm 385, 405, 420, 470, 570, 605. MS (EIMS): *m/z* 1086 $[M]^+$.

5,10,15-tri-*n*-pentyl-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrinato zinc(II) (14). To a refluxing solution of 5,10,15-tri-*n*-pentyl-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrin (**11**) (20.0 mg, 28.0 µmol) in $CHCl_3$ (3 mL) was added zinc(II) acetate (0.50 mL of a saturated solution in methanol). The mixture was stirred for 5 min then allowed to cool. The solution was diluted with $CHCl_3$ (5 mL), washed with sat. $NaHCO_3$ (5 mL) and then H_2O (5 mL), dried (Na_2SO_4), filtered, and evaporated to dryness to yield **14** (22.0 mg, 28.0 µmol, 100%). UV-vis ($CHCl_3$): λ_{max}, nm (log ε) 424 (5.54), 556 (4.12), 600 (3.82). MS (LSIMS): *m/z* $[M]^+$ 770.37921, required for $C_{47}H_{56}N_5OZn$ 770.37763. g = 2.00590, $a_N/g\beta$ = 1.41 mT (toluene, 293 K).

5,10,15-tris[(4‴-sulfuric acid)phenyl]-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrin (20). 5,10,15-trisphenyl-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrin (**7**) (20.0 mg, 27.5 µmol) was dissolved in 5 mL of sulfuric acid (98% reagent grade) and heated at 70 °C with stirring for 4 days. The dark green solution was stirred under argon for a further 3 days at room temperature and then poured into 10 mL of ice with stirring. The dark green solution was adjusted to pH 10 with 2 M sodium hydroxide solutions and the solvent was removed. The resultant solid was abstracted with methanol and then concentrated *in vacuo*. Purification by column chromatography (SiO_2; methanol/ethyl acetate 1:2) gave **20** (10.0 mg, 10.0 µmol, 36%). IR (KBr): ν, cm^{-1} 3435, 2961, 2927, 2858, 1648, 1561, 1412, 1384, 1346, 1223, 1195, 1126, 1039. UV-vis ($CHCl_3$): λ_{max}, nm 415, 515, 550, 600, 650. MS (EIMS): *m/z* 967 $[M + H]^+$.

5,15-bis[(4‴-sulfuric acid)phenyl]-10,20-bis(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrin (21). A sample of 5,15-bisphenyl-10,20-bis(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrin (**8**) (10.0 mg, 24.0 µmol) was treated with sulfuric acid as described for compound **20** to give **21** as a purple solid (3.60 mg, 3.60 µmol, 30%). IR (KBr): ν, cm^{-1} 3436, 2956, 2925, 2854, 1636, 1561, 1460, 1412, 1384, 1354, 1220, 1124, 1041. UV-vis ($CHCl_3$): λ_{max}, nm 420, 515, 550, 595, 650. MS (EIMS): *m/z* 999 $[M+H]^+$. g = 2.0055, $a_N/g\beta$ = 1.50 mT (methanol, 298 K).

5,10,15-tris(4″-carboxymethyleneoxyphenyl)-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)-porphyrin (22). 5,10,15-tris(4″-carboethoxymethyleneoxyphenyl-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrin (**9**) (30.0 mg, 29.0 µmol) was dissolved in a mixture of aqueous sodium hydroxide (3 M, 3 mL) and methanol (3 mL). The mixture was heated at 50 °C with stirring for 2 h. The solution was stirred under argon for a further 5 h at room temperature and then adjusted to pH 10 with 2 M aqueous sulfuric acid. The solvent was removed and the resulting solid was extracted with methanol and then concentrated *in vacuo*. Purification by column chromatography (SiO_2; ethanol) gave **22** (20.0 mg, 20.0 µmol, 69%). IR (KBr): ν, cm^{-1} 3433, 2956, 2925, 2854, 1605, 1508, 1472, 1382, 1351, 1285, 1234, 1178, 1119, 1054. UV-vis ($CHCl_3$): λ_{max}, nm 420, 515, 550, 600, 650. MS (EIMS): *m/z* 949 $[M + H]^+$.

5,15-bis(4″-carboxymethyleneoxyphenyl)-10,20-bis(1′,1′,3′,3′-tetramethyl-isoindolin-2′-yloxyl-5′-yl)-porphyrin (23). A sample of 5,15-bis(4″-carboethoxymethylene-oxyphenyl)-10,20-bis(1′,1′,3′,3′-tetramethyl-isoindolin-2′-yloxyl-5′-yl)porphyrin (**10**) (20.0 mg,

19.0 μmol) was treated with sodium hydroxide as described for compound **22** to afford the **23** as a purple solid (10.3 mg, 10.0 μmol, 53%). IR (KBr): ν, cm^{-1} 3435, 2956, 2925, 2853, 1721, 1638, 1568, 1413, 1384, 1354, 1243, 1123, 1081. UV-vis ($CHCl_3$): λ_{max}, nm 420, 515, 550, 595, 650. MS (EIMS): *m/z* 985 $[M - H]^+$.

5,10,15-tris[(4‴-sulfuric acid)phenyl-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)-]porphyrinato, trisodium salt, manganese(III) chloride (24). To a refluxing solution of 5,10,15-tris[(4‴-sulfuric acid)phenyl]-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrin (**20**) (10.0 mg, 10.4 μmol) in distilled water (5 mL) was added a solution of manganese(II) chloride tetrahydrate (2.1 mg, 10.4 μmol) in water (5 mL). The mixture was refluxed for 4 h, allowed to cool and the solvent removed under reduced pressure. Purification by column chromatography (SiO_2; methanol/ethyl acetate 1:1) gave **24** (6.70 mg, 6.20 μmol, 60%). IR (KBr): ν, cm^{-1} 3436, 2972, 2926, 2858, 1656, 1640, 1463, 1439, 1408, 1384, 1341, 1220, 1194, 1128, 1084, 1040. UV-vis ($CHCl_3$): λ_{max}, nm 380, 400, 425, 465, 565, 600. MS (EIMS): *m/z* 1085 $[M]^+$.

5,10,15-tris(4″-carboxymethyleneoxyphenyl)-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)porphyrinato, manganese(III) chloride (25). To a refluxing solution of 5,10,15-tris(4″-carboxymethyleneoxyphenyl)-20-(1′,1′,3′,3′-tetramethylisoindolin-2′-yloxyl-5′-yl)-porphyrin (**22**) (10.0 mg, 10.5 μmol) in distilled water (5 mL) was added a solution of manganese(II) chloride tetrahydrate (2.10 mg, 10.5 μmol) in water (5 mL). The mixture was refluxed for 4 h, allowed to cool and the solvent removed under reduced pressure. Purification by column chromatography (SiO_2; methanol/ethyl acetate 1:1) gave **25** (5.80 mg, 5.80 μmol, 55%). IR (KBr): ν, cm^{-1} 3428, 2956, 2925, 2853, 1721, 1636, 1660, 1547, 1462, 1384, 1157, 1119, 1052. UV-vis (H_2O): λ_{max}, nm 380, 405, 425, 470, 600.

Fluorescence spectroscopy

All solutions for fluorescence spectroscopy were prepared in HPLC grade methanol and deoxygenated with argon prior to use. Spin-labeled porphyrins had to be first dissolved in a minimum volume of $CHCl_3$ or distilled water 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 two minutes over a one-hour period.

CONCLUSION

New porphyrin containing isoindoline nitroxides have been synthesized as potential fluorescence-suppressed spin probes. Fluorescence spectroscopy revealed that the free-base monoradical porphyrins prepared exhibited fluorescence suppression characteristic of nitroxide-fluorophore systems, while free-base biradical porphyrins exhibited highly suppressed fluorescence about three times as great as monoradical porphyrins. However, the related zinc or manganese porphyrins did not exhibit the expected fluorescence suppression. The absence of fluorescence-suppression is an indication of weak nitroxide-porphyrin interaction and possibly occurs due to insufficient spin coupling between the nitroxide and the porphyrin. EPR spectroscopy of the diradical porphyrins in solution suggests that the nitroxyl-nitroxyl interspin distance is long enough and tumbling is fast enough not to detect dipolar coupling.

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REFERENCES

1. Smirnov AI. In *Nitroxides: Applications in Chemistry, Biomedicine, and Materials Science,* Wiley-VCH: Weinham, 2008; pp 121–159.
2. Hyodo F, Soule BP, Matsumoto K, Matusmoto S, Cook JA, Hyodo E, Sowers AL, Krishna MC and Mitchell JB. *J. Pharm. Pharmacol.* 2008; **60**: 1049–1060.
3. Knight JA. *Free Radicals, Antioxidants, Aging and Disease*, AACC Press: USA, 1999.
4. Yan G-P, Peng L, Jian S-Q, Li L and Bottle SE. *Chin. Sci. Bull.* 2008; **53**: 3777–3789.
5. Gallez B and Swartz HM. *NMR Biomed.* 2004; **17**: 223–225.
6. Jackson SK, Madhani M, Thomas M, Timmins GS and James PE. *Toxic. Lett*. 2001; **120**: 253–257.
7. Swartz HM, Khan N, Buckey J, Comi R, Gould L, Grinberg O, Hartford A, Hopf H, Hou H, Hug E, Iwasaki A, Lesniewski P, Salikhov I and Walczak T. *NMR Biomed.* 2004; **17**: 335–351.
8. Swartz HM and Berliner LJ. In *In Vivo EPR (ESR): Theory and Application*, Berliner LJ. (Ed.) Kluwer Academic: New York, 2003; p 18.

9. Marx L, Chiarelli R, Guiberteau T and Rassat A. *J. Chem. Soc., Perkin Trans. 1* 2008; **8**: 1181–1182.
10. Moad G, Rizzardo E and Solomon DH. *Macromolecules* 1982; **15**: 909–914.
11. Griffiths G, Rizzardo and Solomon DH. *Tetrahedron Lett.* 1982; **23**: 1309–1312.
12. Gillies DG, Sutcliffe LH and Wu X. *J. Chem. Soc., Faraday Trans.* 1994; **90**: 2345–2349.
13. Shen J, Bottle SE, Khan N, Grinberg O, Reid D, Micallef A and Swartz H. *Appl. Magn. Reson*. 2002; **22**: 357–368.
14. Blinco JP, Fairfull-Smith KE, Morrow BJ and Bottle SE. *Aust. J. Chem.* 2011; **64**: 373–389.
15. Blough NV and Simpson DJ. *J. Am. Chem. Soc.* 1988; **110**: 1915–1917.
16. Green SA, Simpson DJ, Zhou G, Ho PS and Blough NV. *J. Am. Chem. Soc.* 1990; **112**: 7337–7346.
17. Gerlock JL, Zacmanidis PJ, Bauer DR, Simpson DJ, Blough NV and Salmeen IT. *Free Rad. Res. Comm.* 1990; **10**: 119–121.
18. Kieber DJ and Blough NV. *Anal. Chem.* 1990; **62**: 2275–2283.
19. Kieber DJ and Blough NV. *Free Rad. Res. Comm.* 1990; **10**: 109–117.
20. Herbelin SE and Blough NV. *J. Phys. Chem. B* 1998; **102**: 8170–8176.
21. Coenjarts C, Garcia O, Llauger L, Palfreyman J, Vinette AL and Scaiano JC. *J. Am. Chem. Soc.* 2003; **125**: 620–621.
22. Ivan MG and Scaiano JC. *Photochem. Photobiol.* 2003; **78**: 416–419.
23. Aspee A, Garcia O, Maretti L, Sastre R and Scaiano JC. *Macromolecules* 2003; **36**: 3550–3556.
24. Yang X-F and Guo X-Q. *Analyst* 2001; **126**: 1800–1804.
25. Yang X-F and Guo X-Q. *Anal. Chim. Acta* 2001; **434**: 169–177.
26. Moad G, Shipp DA, Smith TA and Solomons DH. *J. Phys. Chem. A* 1999; **103**: 6580–6586.
27. Ballasteros OG, Maretti L, Sastre R and Scaiano JC. *Macromolecules* 2001; **34**: 6184–6187.
28. Dang Y-M and Guo X-Q. *Appl. Spectrosc.* 2006; **60**: 203–207.
29. Toniolo C, Crisma M and Formaggio F. *Biopolymers* 1998; **47**: 153–158.
30. Pispisa B, Palleschi A, Stella L, Venanzi M and Toniolo C. *J. Phys. Chem. B* 1998; **102**: 7890–7898.
31. Pispisa B, Mazzuca C, Palleschi A, Stella L, Venanzi M, Wakselman M, Mazaleyrat J-P, Rainaldi M, Formaggio F and Toniolo C. *Chem.--Eur. J.* 2003; **9**: 4084–4093.
32. Hideg E, Kalai T, Hideg K and Vass I. *Biochemistry* 1998; **37**: 11405–11411.
33. Kalai T, Hideg E, Vass I and Hideg K. *Free Radical Biol. Med.* 1998; **24**: 649–652.
34. Lozinsky E, Martin VV, Berezina TA, Shames AI, Weis AL and Likhtenshtein GI. *Biochem. Biophys. Methods* 1999; **38**: 29–42.
35. Bilski P, Hideg K, Kalai T, Bilska MA and Chignell CF. *Free Radical Biol. Med*. 2003; **34**: 489–495.
36. Micallef AS, Blinco JP, George GA, Reid DA, Rizzardo E, Thang SH and Bottle SE. *Polym. Degrad. Stab.* 2005; **89**: 427–435.
37. Keddie DJ, Johnson TE, Arnold DP and Bottle SE. *Org. Biomol. Chem*. 2005; **3**: 2593–2598.
38. Blinco JP, McMurtrie JC and Bottle SE. *Eur. J. Org. Chem.* 2007; **48**: 4638–4641.
39. Blinco JP, George GA and Bottle SE. *Polym. Prepr.* 2007; **48**: 629–630.
40. Micallef A, Bottle S, Blinco J and George G. In *ACS Symposium Series, Polymer Durability and Radiation Effects*, Vol. 978, Celina MC and Assink RA. (Eds.) American Chemical Society: Portland, 2008; pp 59–69.
41. Fairfull-Smith KE, Blinco JP, Keddie DJ, George GA and Bottle SE. *Macromolecules* 2008; **41**: 1577–1580.
42. Fairfull-Smith KE and Bottle SE. *Eur. J. Org. Chem.* 2008; **32**: 5391–5400.
43. Blinco JP, Keddie DJ, Wade T, Barker PJ, George GA and Bottle SE. *Polym. Degrad. Stab.* 2008; **93**: 1613–1618.
44. Ishii K and Kobayashi N. *Coord. Chem. Rev.* 2000; **198**: 231–250.
45. Saiful IS, Fujisawa J, Kobayashi N, Ohba Y and Yamaguchi S. *Bull. Chem. Soc. Jpn.* 1999; **72**: 661–667.
46. Ishii K, Fujisawa J, Ohba Y and Yamauchi S. *J. Am. Chem. Soc.* 1996; **118**: 13079–13080.
47. Asakura T, Leigh Jr JS, Drott HR and Yonetani TB. *Proc. Natl. Acad. Sci. USA* 1971; **68**: 861–865.
48. Braden GA, Trevor KT, Neri JM, Greenslade DJ and Eaton GR. *J. Am. Chem. Soc.* 1977; **99**: 4854–4855.
49. Rakowsky MH, More KM, Kulikov AV, Eaton GR and Eaton SS. *J. Am. Chem. Soc.* 1995; **117**: 2049–2057.
50. Rakowsky MH, Zecevic A, Eaton GR and Eaton SS. *J. Magn. Reson*. 1998; **131**: 97–110.
51. Shultz DA, Gwaltney KP and Lee H. *J. Org. Chem.* 1998; **63**: 769–774.
52. Fujisawa J, Ohba Y and Yamauchi S. *J. Phys. Chem. A* 1997; **101**: 434–439.
53. Fujisawa J, Ishii K, Ohba Y, Yamauchi YS, Fuhs M and Möbius K. *J. Phys. Chem. A* 1997; **101**: 5869–5876.
54. Ishii K, Fujisawa J, Adachi A, Yamaguchi S and Kobayashi N. *J. Am. Chem. Soc.* 1998; **120**: 3152–3158.
55. Ishii K, Kubo K, Sakurada T, Komori K and Sakai Y. *Chem. Commun.* 2011; doi: 10.1039/c1cc10817d.

56. Yan G-P, Bischa D and Bottle SE. *Free Rad. Biol. Med.* 2007; **43**: 111–116.
57. Wang QM and Bruce DW. *Synlett.* 1995; 1267–1268.
58. Laha JK, Dhanalekshmi S, Taniguchi M, Ambroise A and Lindsey JS. *Org. Proc. Res. Dev.* 2003; **7**: 799–812.
59. Mareček V, Jänchenová H, Stibor I and Budka J. *J. Electroanal. Chem.* 2005; **575**: 293–299.
60. Rucareanu S, Mongin O, Schuwey A, Hoylet N and Gossauer A. *J. Org. Chem.* 2001; **66**: 4973–4988.
61. Ishii K, Bottle SE, Shimizu S, Smith CD and Kobayashi N. *Chem. Phys. Lett.* 2003; **370**: 94–98.
62. Smith CD, Bott RC, Bottle SE, Micallef AS and Smith G. *J. Chem. Soc., Perkin Trans. 2* 2002; **3**: 533–537.
63. Bolton R, Gillies DG, Sutcliffe LH and Wu X. *J. Chem. Soc., Perkin Trans. 2* 1993; **11**: 2049–2052.
64. Bottle SE, Gillies DG, Hughes DL, Micallef AS, Smirnov AI and Sutcliffe LH. *J. Chem. Soc., Perkin Trans. 2* 2000; **7**: 1285–1291.
65. Barnett GH, Hudson MF and Smith KM. *J. Chem. Soc. Perkin Trans. I* 1975; 1401–1403.