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Superoxide Dismutase Mimetics: Synthesis and Structure–Activity Relationship Study of MnTBAP Analogues

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Abstract—Carboxylic ester and amide-substituted analogues of [5,10,15,20-tetrakis(4-carboxyphenyl)-porphyrinato]manganese(III) chloride (MnTBAP) were synthesized and assayed as potential superoxide dismutase (SOD) mimetics. The tetraester analogues **4a** and **4b** were found to have comparable SOD activity to the known SOD mimetic MnTBAP, while amides **4c**–**4e** exhibited reduced SOD activity. In the substituted methyl benzoate/acid and disubstituted porphyrin series, analogues **12c**, **12f**, and **12m** were found to have comparable to improved SOD activity relative to MnTBAP and analogues **12j**, **13a**, and **13d** exhibited improved activity in both the SOD and thiobarbituric acid reactive species (TBARS) assays relative to MnTBAP.

Introduction

Superoxide radicals ($O_2^{\bullet-}$) are produced as part of the normal metabolism of all aerobic cells. Superoxide is known to be important in the pathogenesis of many disease processes including lung, central nervous system and skeletal muscle diseases[1] and, by modulating the effects of nitric oxide, contributes to the pathogenesis of inflammatory[2] and vascular disorders[3] and the aging process.[4] A critical balance of enzymes defending against antioxidants is therefore required to maintain normal cell and organ function. Superoxide dismutases (SODs), a family of metalloenzymes, represent the first line of defense against the harmful effects of superoxide radicals by catalyzing the intra and extracellular conversion of $O_2^{\bullet-}$ into H_2O_2 plus O_2.

It has been recognized for years that simple metal chelates could react with superoxide and hydrogen peroxide.[5] These include the Mn-desferal[5a] and manganese complexes of lactate, citrate, or succinate.[5b] However, the rates of reaction of these chelates with superoxide were low and the complexes unstable. The search for more potent and stable metal chelates has resulted in the discovery of at least three classes of metal-containing SOD mimetics.[6] These include the salen,[6a] macrocyclic,[6b] and metalloporphyrins.[6c] The most stable of these complexes are the metalloporphyrins including MnTBAP. They have also been shown to be potent inhibitors of lipid peroxidation.[7] Lipid peroxidation is a natural occurring reaction with polyunsaturated fatty acids and reactive oxygen species. As the fatty acids degrade they accumulate as small aldehyde fragments that are associated with a number of disease processes and inflammation.[8] The stability of metalloporhyrins compared to other metal chelates made them attractive basis to develop a new series of Mn(III) porphyrins as SOD mimetics.

Although many newer SOD mimetic have greater in vitro activity than the MnTBAP (the prototype of this series), MnTBAP has been shown to possess very potent effects in vivo against oxidative stress.[9a] MnTBAP has an extensive and impressive amount of pharmacology. In cell cultures models, MnTBAP has been shown to be effective in micromolar levels against injury produced by paraquat, hydrogen peroxide, endotoxin, and the excitotoxic agents NMDA and kainic acid.[9a] In addition, MnTBAP is a potent inhibitory of apoptosis induced by staurosporine, ceramide, and growth factor

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withdrawal. MnTBAP also is very effective in animal models of injury such as paraquat-induced lung injury, carrageenan-induced lung injury, and bleomycin-induced lung injury. Recently, it was found to be more efficacious against acetaminophen toxicity than *N*-acetyl-L-cysteine.[9b] One of the most remarkable effects of MnTBAP was its ability to substitute for mitochondrial SOD in the mitochondrial knockout mouse and correct the dilated cardiomyopathy phenotype and extend its life span.[9c] MnTBAP has a 9.5-h plasma half-life in mice and was fairly non-toxic to mice with a reported LD_{50} of 100 mg/kg body weight.[9d] These features of MnTBAP were the incentive to develop a number of structurally related compounds reported in this paper.

Results and Discussion

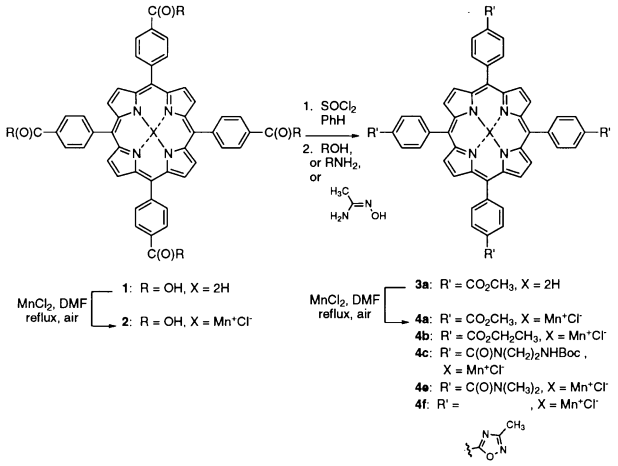
Chemistry

Utilizing MnTBAP (**2**) as our lead compound, we investigated the syntheses and biological evaluation of porphyrin analogues by substituting the carboxylic acid functionality in MnTBAP with carboxylic acid mimics (i.e., tetrazole, oxadiazole, etc.) and carboxylic acid derivatives (i.e., amides and esters). The esters **4a** and **4b**, amides **4c** and **4e**, and oxadiazole **4f** were prepared from the commercially available 5,10,15,20-tetrakis (4-carboxyphenyl)porphyrin (H_2TBAP) (Scheme 1). H_2TBAP (**1**) was metalated[10] by treatment with $MnCl_2$ in refluxing DMF to provide MnTBAP. Conversion of MnTBAP to the acid chloride followed by treatment of alcohol, amine, or amideoxime provided ester **4b**, amides **4c** and **4e**, and oxadiazole **4f**, respectively. Ester **4a** was prepared by converting H_2TBAP to the acid chloride first followed by quenching with anhydrous methanol and metalation.

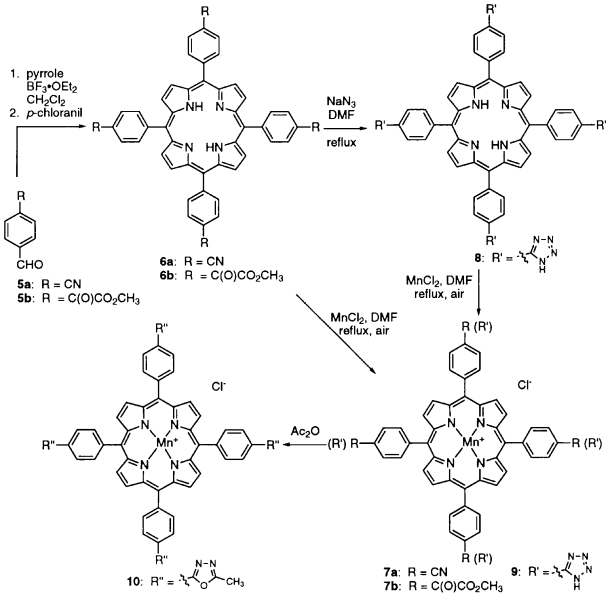
The preparation of tetrazole[11] porphyrin **9** and ketoester porphyrin **7b** began with the construction of the porphyrin core **6a** and **6b**, from aldehydes **5a** and **5b**, respectively, according to a general procedure reported by Lindsey et al.[12] (Scheme 2). Thus, condensation of aldehyde **5a** or **5b** with pyrrole, catalyzed by $BF_3{\cdot}OEt_2$, followed by oxidation with *p*-chloranil provided cyanophenyl porphyrin **6a** and ketoester porphyrin **6b**, respectively. Porphyrins **6a** and **6b** were then metalated to provide porphyrins **7a** and **7b**, respectively. Further functionalization of cyanophenyl **6a** by treatment with NaN_3 provided tetrazole **8** that was metalated to provide porphyrin **9**. The Mn-tetrazole porphyrin **9** was transformed to oxadiazole porphyrin **10** by treatment with acetic anhydride.[11] The physical properties of porphyrin derivatives **4**, **7**, **9**, and **10** are shown in Table 1.

Porphyrin analogues bearing salicylic acid groups at the *meso* positions and tetraphenylporphyrin analogues with electron donating or electron donating groups in the pendant aromatic rings were prepared and evaluated next **11**–**13** (Fig. 1). Analogues **11a**, **11b**, **12m**, **12n**, **12o**, and **12p** were prepared from commercially available aldehydes by methods analogous to those shown in Scheme 2. However, the requisite aldehydes needed for the preparation of porphyrin analogues **12a**–**j** and **13a**–**b** were not commercially available and were prepared from the corresponding toluic acids as shown in Scheme 3. Thus, esterification of the acid **14** to ester **15**, bisbromination of **15**, followed by hydrolysis provided the corresponding aldehyde **16**.

Once the aldehydes **16** were on hand, their condensation with pyrrole provided the corresponding porphyrins (Scheme 4, Table 2). Metalation of the porphyrins was accomplished according to the procedure reported by Longo et al.[10] Under these conditions, by products resulting from the partial hydrolysis of the ester group(s), **11a**, **12c**, **12d**, **12k**, and **13b**, were formed. These by-products were subsequently isolated and their biological activity was investigated. Porphyrin **12g** was also isolated as a byproduct during metalation of tetramethyl 4′,4″,4‴,4⁗-tetrafluoro-3′,3″,3‴,3⁗-(21*H*,23*H*-porphine-5,10,15,20-tetrayl)tetrabenzoate.



Scheme 1.



Scheme 2.

Table 1. Physical properties of porphyrins **4**, **7**, **9**, and **10**

Compd	R	Mp (°C)	λ_{max} (nm)	$\varepsilon^a \times 10^5$ ($cm^{-1}M^{-1}$)	FABMS (m/z)
MnTBAP	CO_2H	>300	468.0	0.88	843
4a	CO_2CH_3	>330	466.0	1.1	899
4b	$CO_2CH_2CH_3$	—	466.0	1.5	955
4c	$CONH(CH_2)_2NHBoc$	>375	466.5	—	–
4d	$CONH(CH_2)_2NH_2{\cdot}HCl$	—	466.5	0.50	1012
4e	$CON(CH_3)_2$	—	466.5	0.56	952
4f	3-Methyl-1,2,4-oxadiazol-5-yl	—	466.5	1.1	995
7a	CN	>310	464.5	1.4	767
7b	$COCO_2CH_3$	>300	466.5	1.1	1012
9	Tetrazol-5-yl	>320	467.5	0.42	939
10	2-Methyl-1,3,4-oxadiazol-5-yl	>300	466.5	1.6	995

[a]Extinction coefficient of porphyrins dissolved in ethanol unless otherwise noted.

Biology

The porphyrin analogues were screened using assays for SOD and peroxidase activity. Superoxide dismutase activity was measured by cytochrome c reductase as previously described in the literature.[13] Briefly, buffer consisted of 50 mM potassium phosphate containing 0.1 mM EDTA at pH 7.8. Cytochrome c was added to give a final concentration of 10 μM of the oxidized form. Xanthine was added to give a final concentration of 50 μM. Xanthine oxidase (2 nM) was added to start the formation of superoxide. Rates of reduction of cytochrome c were followed at 550 nm spectrophotometrically for 1 min. The amount of metalloporphyrin that inhibited the rate of cytochrome reduction by one-half was 1 SOD unit of activity.

The ability of mimetics to inhibit lipid peroxidation (TBARS, IC_{50} μM) was assessed as previously described.[7] Iron and ascorbate were used to initiate lipid peroxidation in tissue homogenates and the formation of thiobarbituric acid reactive species (TBARS) was measured.[14a,b]

The aerobic growth of *Escherichia coli* strain JI 132, which lacks superoxide dismutase activity, was monitored as described by Faulkner et al.[15] Briefly, *E. coli* were cultured in minimal medium contained 0.2% glucose, and 0.5 mM each of leucine, threonine, proline, arginine, and histidine, plus M9 salts in tap water, pH adjusted to 7.0. The compounds were assessed for efficacy (X+) or lack of efficacy (X−) by their ability to preserve aerobic growth in the SOD null *E. coli* (JI132) as assessed by turbidity measurement at 700 nm.

Our biological results for the monosubstituted phenyl derivatives (carboxylic acid mimics and carboxylic acid derivatives) are summarized in Table 3. The ester analogues **4a** and **4b** exhibited SOD activities that were comparable to MnTBAP while the amides generally showed reduced SOD activities relative to MnTBAP. The amide analogues **4d** and **4e** showed improved inhibition in the TBARS assay relative to MnTBAP or the ester analogues. Of the three heterocyclic analogues prepared, oxadiazole **10** exhibited comparable SOD activity and better inhibition in the TBARS assay relative to MnTBAP. The tetrazole derivative **9** was found to be highly toxic to cultured cells (results not shown here).

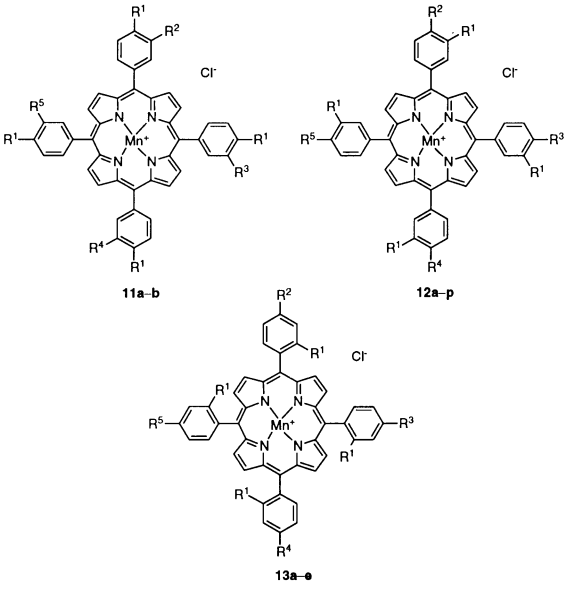
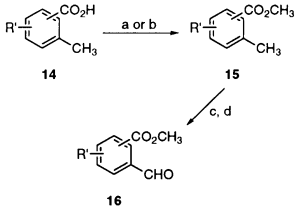
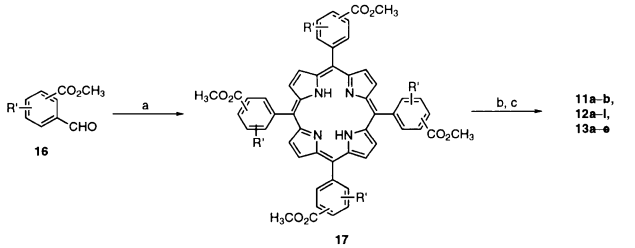


Figure 1.



Scheme 3. (a) $(COCl)_2$, cat. DMF, CH_2Cl_2; CH_3OH, Et_3N; (b) $(CH_3O)_2SO_2$, K_2CO_3, acetone; (c) NBS, CCl_4, initiator; (d) $AgNO_3$, H_2O, acetone.



Scheme 4. (a) Pyrrole, $BF_3{\cdot}OEt_2$, CH_2Cl_2; *p*-chloranil; (b) $MnCl_2$, DMF, reflux; (c) Claisen's base, MeOH.

The biological data for the substituted MnTBAP analogues are also shown in Table 3. Several novel porphyrin derivatives that were synthesized showed enhanced SOD activity relative to MnTBAP (vide infra). Relative to MnTBAP, porphyrin analogues **12** and **13a** exhibited enhanced SOD activity while porphyrin analogues **12a**, **12b**, **12f**, **12g**, **12j**, **12o**, **13a**, **13b**, and **13d** exhibited enhanced acitivity in the TBARS assay. The activity of analogues **12c**, **12f**, **12n**, and **13d** in the SOD assay was comparable to that of MnTBAP. Within the same series of compounds, the tetraester porphyrin analogues **12f**, **12j**, **13a**, and **13d** showed enhanced activity in the SOD and TBARS assays relative to their corresponding acid analogues. In the *E. coli* assay, the tetraacids **12e**, **12l**, and **13c** showed activity while the tetraester analogues **12b**, **12j**, **13a**, and **13d**, respectively, did not.

Conclusion

Several novel porphyrin derivatives synthesized showed enhanced activities in the SOD and/or TBARS assays relative to MnTBAP. Derivatives prepared in the first series, carboxylic acid mimics and derivatives, have shown comparable to reduced SOD activity but enhanced activity in the TBARS assay relative to the lead compound MnTBAP. In the second series of analogues prepared (substituted MnTBAP analogues), compounds **12j** and **13a** exhibited enhanced activity in both the SOD and TBARS assays. Analogues **12a**, **12b**, **12f**, **12j**, **12o**, **13b**, and **13d** exhibited enhanced activity in the TBARS assay relative to MnTBAP. SOD activity was not predictive of lipid peroxidation or rescue of null SOD *E. coli* (i.e., analogues **12c**, **12e**, and **12j**). Further in vitro and in vivo biological evaluations of these compounds continue in an attempt to elucidate the reasons for these observations. We are also exploring additional porphyrin derivatives of differing substitution in order to further improve these biological findings. This work will be reported in due course.

Experimental

Proton NMR spectra were obtained on a Bruker AC 300 spectrometer at 300 MHz and were referenced to tetramethylsilane as an internal standard. IR spectra were prepared in a pressed disc of KBr or as a film on NaCl plates and acquired with a total of four accumulations at a resolution of 4.00 cm^{-1} on a single beam Perkin–Elmer Spectrum 1000 FT-IR. Melting points were obtained on a Mel-Temp II apparatus and are uncorrected. FAB mass spectra were performed by M-Scan. Elemental analyses were performed by Quantitative Technologies, Inc. of Whitehouse, NJ, USA. HLPC analyses were obtained using a Microsorb C18 Column with UV detection at 230 nm (unless otherwise specified). HPLC grade methylene chloride and anhydrous DMF were used.

5,10,15,20-Tetrakis(methyl 4-benzoate)porphyrin (3a)

A solution of TBAP (559 mg, 1.32 mmol), thionyl chloride (5 mL), and a drop of pyridine was heated at reflux for 4 h. The excess thionyl chloride was evaporated off then the residue was dissolved in anhydrous MeOH. The solution was cooled to 0 °C then Et_3N (0.4 mL) was added dropwise to provide, after purification by flash chromatography, porphyrin **3a** as a purple solid (388 mg, 61%): ^1H NMR ($CDCl_3$) δ 8.83 (s, 8H), 8.45 (d, 8H), 8.30 (d, 8H), 4.15 (s, 12H), −2.80 (br s, 2H).

Table 2. Physical properties of porphyrins **11**–**13**

Compd	R^1	R^2	R^3	R^4	R^5	Mp (°C)	λ_{max} (nm)	$\varepsilon^a \times 10^5$ ($cm^{-1}M^{-1}$)	FABMS (*m*/*z*)
11a	OH	CO_2CH_3	CO_2H	CO_2H	CO_2H	>300	472.0	0.46	921
11b	OH	CO_2H	CO_2H	CO_2H	CO_2H	>300	472.0	—	907
12a	OCH_3	CO_2CH_3	CO_2CH_3	CO_2CH_3	CO_2CH_3	>300	467.0	1.3	—
12b	OH	CO_2CH_3	CO_2CH_3	CO_2CH_3	CO_2CH_3	>300	467.5	1.1	963
12c	OH	CO_2H	CO_2CH_3	CO_2CH_3	CO_2CH_3	>300	467.5	1.3	949
12d	OH	CO_2H	CO_2H	CO_2CH_3	CO_2CH_3	>300	468.5	1.0[b]	935
12e	OH	CO_2H	CO_2H	CO_2H	CO_2H	>320	467.5	0.66	944
12f	CO_2CH_3	F	F	F	F	>300	465.0	1.3	971
12g	CO_2CH_3	$N(CH_3)_2$	F	F	F	>300	468.0	1.3	996
12h	CO_2H	F	F	F	F	>300	467.5	1.0	915
12i	CO_2H	$N(CH_3)_2$	F	F	F	>300	467.0	0.94	940
12j	NO_2	CO_2CH_3	CO_2CH_3	CO_2CH_3	CO_2CH_3	>300	464.0	1.4[c]	1079
12k	NO_2	CO_2H	CO_2CH_3	CO_2CH_3	CO_2CH_3	>300	464.0	1.2[c]	1065
12l	NO_2	CO_2H	CO_2H	CO_2H	CO_2H	>300	465.0	1.3[c]	1023
12m	NO_2	CH_3	CH_3	CH_3	CH_3	—	465.0	0.77	—
12n	NO_2	OH	OH	OH	OH	—	472.5	1.8	911
12o	F	OCH_3	OCH_3	OCH_3	OCH_3	>300	468.0	0.90	859
12p	F	OH	OH	OH	OH	>300	469.5	0.52	803
13a	F	CO_2CH_3	CO_2CH_3	CO_2CH_3	CO_2CH_3	>300	462.5	1.7	971
13b	F	CO_2H	CO_2CH_3	CO_2CH_3	CO_2CH_3	>300	463.0	0.39	957
13c	F	CO_2H	CO_2H	CO_2H	CO_2H	>300	463.0	1.5	915
13d	OCH_3	CO_2CH_3	CO_2CH_3	CO_2CH_3	CO_2CH_3	>300	467.0	1.5	1019
13e	OCH_3	CO_2H	CO_2H	CO_2H	CO_2H	>300	467.0	1.5	963

[a]Extinction coefficient of porphyrins dissolved in ethanol unless otherwise noted.
[b]Compound dissolved in ethanol with 50 μL 0.1 N NaOH (100 mL total volume).
[c]Compound dissolved in methanol.

[5,10,15,20-Tetrakis(methyl 4-benzoate)porphyrinato]manganese (III) chloride (4a). A solution of porphyrin **3a** (288 mg, 0.32 mmol), $MnCl_2$ (202 mg, 1.61 mmol) in DMF (30 mL) was heated at reflux. The extent of reaction was monitored by UV–Vis spectroscopy; the disappearance of the free porphyrin Soret band (typically around 417 nm) and the emergence of the metalated porphyrin Soret band (typically around 466 nm) would indicate that metalation was complete. Once metalation was complete, the reaction mixture was evaporated off, adsorbed onto silica gel, and purified by column chromatography to provide **4a** as a green solid: mp $>330\,^{\circ}C$; FABMS m/z 899 $[C_{52}H_{36}MnN_4O_8]^+$. UV (EtOH) λ_{max}, nm (ε) 466.0 (1.1×10^5). HPLC 94%. Anal. calcd for $C_{48}H_{27}ClMnN_4O_8\cdot H_2O$: C, 64.30; H, 4.15; N, 5.77. Found: C, 64.41; H, 3.73; N, 5.74.

[5,10,15,20-Tetrakis(ethyl 4-benzoate)porphyrinato]manganese(III) chrloride (4b). Following the procedure for the preparation of porphyrin **3a**, porphyrin **4b** was prepared by converting MnTBAP (**2**) to the acid chloride and quenching the acid chloride with anhydrous ethanol: FABMS m/z 955 $[C_{56}H_{44}MnN_4O_8]^+$; HPLC 93%. UV (EtOH) λ_{max}, nm (ε) 46.0 (1.5×10^5).

5,10,15,20-Tetrakis(*N*,*N*-dimethyl-4-benzamide)porphyrinato]manganese(III) chrloride (4e). Following the procedure for the preparation of porphyrin **3a**, porphyrin **4e** was prepared by converting MnTBAP (**2**) to the acid chloride and quenching the acid chloride with dimethylamine: FABMS m/z 952 $[C_{56}H_{48}MnN_8O_4]^+$. HPLC $>99\%$. UV (EtOH) λ_{max}, nm (ε) 466.5 (0.56×10^5).

{5,10,15,20-Tetrakis[4-(4-methyl-2,3,5-oxadiazolyl)phenyl]porphyrinato}manganese(III) chloride (4f). Porphyrin **4f** was prepared in a similar manner as porphyrin **3a** by converting MnTBAP (**2**) to the acid chloride and quenching the acid chloride with *N*-hydroxy-acetamidine. FABMS m/z 995 $[C_{56}H_{36}MnN_{12}O_4]^+$. HPLC 93%. UV (EtOH) λ_{max}, nm (ε) 466.5 (1.1×10^5).

5,10,15,20-Tetrakis(4-cyanophenyl)porphyrin (6a). In a foil-covered 2-L 3-neck flask equipped with a reflux condenser, magnetic stirrer, and a N_2 inlet was added 4-formyl benzonitrile (1.13 g, 98%, 8.46 mmol), pyrrole (0.6 mL, 98%, 8.46 mmol), and CH_2Cl_2 (850 mL). The reaction mixture was stirred for 15 min at room temperature then $BF_3\cdot OEt_2$ (105 μL, 0.85 nmol) was added and the extent of reaction was followed by UV–Vis spectrometry. After 2 h, *p*-chloranil (1.56 g, 6.37 mmol) was added and the reaction mixture was heated at reflux for 2 h. The reaction mixture was evaporated to a volume of 50–100 mL, then adsorbed onto silica gel (2.8 g). Purification by column chromatography provided **6a** (700 mg) in 46% yield as a violet solid. ^1H NMR ($CDCl_3$) δ 8.83 (s, 8H), 8.35 (d, 8H), 8.10 (d, 8H), −2.85 (br s, 2H).

{5,10,15,20 - Tetrakis[4 - cyanophenyl]porphyrinato}manganese (III) chloride (7a). A solution **6a** (0.19 g, 0.26 mmol) and $MnCl_2$ (0.18 g, 1.4 mmol) in DMF (20 mL) was heated at reflux for 4–5 h. The reaction mixture was allowed to cool to room temperature then DMF was evaporated under reduced pressure. The crude product was adsorbed onto silica gel (1.5 g) then was purified by column chromatography to provide of **7a** (0.20 g, 95%) as a black solid: mp $>310\,^{\circ}C$. FABMS m/z 767 $[C_{48}H_{24}MnN_8]^+$. HPLC 99%. UV (EtOH) λ_{max}, nm (ε) 464.5 (1.4×10^5). Anal. calcd for $C_{48}H_{24}ClMnN_8\cdot CH_3OH$: C, 70.47; H, 3.38; N, 13.42. Found: C, 70.46; H, 3.85; N, 13.11.

5,10,15,20-Tetrakis[4-(2,3,4,5-tetrazolyl)phenyl]porphyrin (8). A solution of tetrabenzonitrile porphine **6a** (0.30 g, 0.42 mmol), NaN_3 (0.24 g, 3.67 mmol), NH_4Cl (0.18 g, 3.37 mmol), and DMF (25 mL) was heated at 120 °C for 3 days. Additional NaN_3 (0.17 g, 2.59 mmol) and NH_4Cl (0.11 g, 2.05 mmol) were added to push the reaction to completion. The DMF was evaporated under reduced pressure then cold H_2O (10 mL) was added. The resulting solution was acidified with 6 N HCl then CH_2Cl_2 (10 mL) was added. The resulting precipitate was collected and dried under vacuum to provide **8** (0.37 g) in 99% yield as a green solid, which

Table 3. In vitro biological activity data for porphyrins **4**–**13**

Compd	SOD[a] (units/mg)	TBARS[b] (IC_{50} μM)	*E. coli*[c]
MnTBAP	41 ($n=3$)[d]	45	X+
4a	36 ($n=2$)	56 ($n=5$)	X−, ppt[e]
4b	23	59	X−
4c	8	NA[f]	X−, ppt
4d	6.2 ($n=3$)	4.0 ($n=3$)	X−
4e	2	4.9	X−
4f	2	20	X+
7a	6	0.28	X−
7b	NA	7.2	X−
9	Xo Inh[g]	NA	X−
10	36	1.6	X−, ppt
11a	2.9	NA	X+
11b	7	NA	X±
12a	27	0.85 ($n=2$)	X−, ppt
12b	ppt	18	X−, ppt
12c	40 ($n=3$)	208 ($n=3$)	X+
12d	5	54	X+
12e	3	47 ($n=2$)	X+
12f	38	12	
12g	31 ($n=2$)	29 ($n=4$)	
12h	NA	NA	X±
12i	NA	NA	
12j	58	0.6	X±
12k	NA	8.4	X+
12l	7.6 ($n=2$)	NA	X+, X±
12m	56	NA	X−
12n	35 ($n=2$)	NA	X+
12o	21	6.4	X−
12p	7	NA	X−
13a	56	6.9	X−
13b	31	15	X−
13c	2.5 ($n=2$)	69 ($n=2$)	X+ ($n=2$)
13d	42 ($n=2$)	12	X±
13e	NA	NA	X±

[a]Units of superoxide dismutase (SOD) activity defined as the amount of compound that inhibits 50% the reduction of cytochrome c.
[b]The concentration of compound that inhibits iron/ascorbate-mediated brain homogenate lipid peroxidation by 50%.
[c]Positive (X+) or negative (X−) *E. coli* aerobic growth.
[d]*n*, number of repetitions.
[e]ppt, compound precipitated out of the assay medium.
[f]NA, not active.
[g]The compound interferes with the assay by inhibiting xanthine oxidase which is utilized in the assay.

was used in the metalation step without further purification. ^{1}H NMR (DMSO-d_6) δ 7.97 (s, 8H), 7.49 (m, 16H).

{5,10,15,20-Tetrakis[4-(2,3,4,5-tetrazolyl)phenyl]porphyrinato}manganese(III) chloride (9). A solution of **8** (0.35 g, 0.4 mmol) and $MnCl_2$ (0.25 g, 2 mmol) in DMF (20 mL) was heated at reflux for 4–5 h. The reaction mixture was allowed to cool to room temperature then DMF was evaporated under reduced pressure. The crude product was adsorbed onto silica gel (1.5 g) then was purified by column chromatography to provide of **9** as a dark green solid; mp $>320\,^{\circ}$C. FABMS m/z 939 $[C_{48}H_{28}MnN_{20}]^+$. HPLC 84%. UV (EtOH) λ_{max}, nm (ε) 467.5 (0.42×10^5).

Methyl 2-methoxy-3-methylbenzoate (15a). To a magnetically stirred solution of 2-hydroxy-4-methyl benzoic acid (25.5 g, 168 mmoL), finely ground anhydrous K_2CO_3 (60 g, 434 mmol) and acetone (300 mL) was added $(MeO)_2SO_2$ (47.6 mL, 503 mmol). The solution was stirred at room temperature for 18 h then heated at reflux until analysis by TLC indicated the reaction was complete (1–2 h). The reaction mixture was cooled to room temperature, filtered, and the excess K_2CO_3 cake was thoroughly washed with acetone. The filtrate was evaporated and the residue was redissolved in EtOAc (300 mL) then Et_3N (70 mL) was added. The reaction mixture was stirred at room temperature for 30 min, transferred to a separatory funnel and washed successively with H_2O (200 mL), 2 N HCl (until slightly acidic), H_2O (200 mL), and brine (200 mL) then dried (Na_2SO_4), filtered, and evaporated under reduced pressure. Chromatography of the residue on silica provided **15a** (30 g) in 95% yield. ^{1}H NMR ($CDCl_3$) δ 7.73 (d, 1H), 6.79 (overlapping d and s, 2H), 3.91 (s, 3H), 3.86 (s, 3H), 2.39 (s, 3H).

Methyl 2-fluoro-5-methylbenzoate (15b). Following the procedure for the preparation of **15a** and using 2-fluoro-5-methyl benzoic acid (5.03 g, 32.6 mmol), finely ground anhydrous K_2CO_3 (14.2 g, 102.7 mmol), acetone (150 mL), and $(MeO)_2SO_2$ (3.80 mL, 40.1 mmol), ester **15b** was isolated in 94% yield (5.41 g). ^{1}H NMR ($CDCl_3$) δ 7.73 (dd, 1H, $J=2.31$, 6.87 Hz), 7.30 (m, 1H), 7.01 (dd, 1H, $J=8.55$, 10.65 Hz), 3.93 (s, 3H), 2.35 s).

Methyl 3-fluoro-4-methylbenzoate (15c). Following the procedure for the preparation of **15a** and using 3-fluoro-4-methyl benzoic acid (9.5 g, 61.6 mmoL), finely ground anhydrous K_2CO_3 (25.8 g, 186.7 mmol), acetone (150 mL), and $(MeO)_2SO_2$ (7.0 mL, 74.0 mmol), ester **15c** was isolated in 72% yield (7.42 g). ^{1}H NMR ($CDCl_3$) δ 7.72 (dd, 1H, $J=1.83$, 7.83 Hz), 7.65 (dd, 1H, $J=1.32$, 10.11 Hz), 7.26 (dd, 1H, $J=5.82$, 7.62 Hz), 3.91 (s, 3H), 2.33 (d, 3H, $J=1.89$ Hz).

Methyl 4-formyl-2-methoxybenzoate (16a). A magnetically stirred solution of **15a** (24.43 g, 135.6 mmol), NBS (54.41 g, 305.7 mmol), and CCl_4 (1 L) was exposed to a 100-watt lamp for 6 h. Analysis by TLC indicated that mono-, di- and tribrominated benzoates were formed and the dibromide was the major product. The reaction mixture was quenched with H_2O (200 mL), washed with saturated $Na_2S_2O_3$ (500 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was dissolved in acetone/H_2O (200 mL, 83:17) then $AgNO_3$ (47.25 g, 275.2 mmol) was added. The flask was covered with foil to avoid decomposition of the $AgNO_3$. The reaction mixture was stirred at room temperature for 2–3 h then the AgBr salts were filtered off through a pad of Celite. The filtrate was diluted with EtOAc (400 mL), transferred to a separatory funnel, then washed successively with saturated $NaHCO_3$ (300 mL), H_2O (300 mL), and brine (300 mL). The organic extract was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. Chromatography of the residue on silica gel provided aldehyde **16a** (11.92 g) in 42% yield. ^{1}H NMR ($CDCl_3$) δ 10.05 (s, 1H), 7.9 (d, 1H, H-4), 7.5 (overlapping s and d, 2H), 3.99 (s, 3H), 3.92 (s, 3H).

Methyl 2-fluoro-5-formylbenzoate (16b). A magnetically stirred solution of **15b** (4.70 g, 27.9 mmol), NBS (10.5 g, 58.9 mmol), AIBN (226 mg, 1.4 mmol), and CCl_4 (350 mL) was Heated at reflux for 20 h. Analysis by TLC indicated that mono-, di- and tribrominated benzoates were formed and the dibromide was the major product. The reaction mixture was evaporated and the residue was redissolved in EtOAc (250 mL), washed successively with H_2O (150 mL), saturated $Na_2S_2O_3$ (150 mL), H_2O (150 mL), and brine (150 mL). The organic extract was then dried (Na_2SO_4), filtered and evaporated. The residue was dissolved in acetone/H_2O (200 mL, 83:17) then $AgNO_3$ (12.2 g, 71.8 mmol) was added. The flask was covered with foil to avoid decomposition of the $AgNO_3$. The reaction mixture was stirred at room temperature overnight then the AgBr salts were filtered off from the solution. The filtrate was diluted with EtOAc (400 mL), transferred to a separatory funnel then, washed successively with saturated $NaHCO_3$ (200 mL), H_2O (200 mL), and brine (200 mL). The organic extract was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. Chromatography of the residue on silica gel provided aldehyde **16b** (2.89 g) in 57% yield. ^{1}H NMR ($CDCl_3$) δ 10.01 (s, 1H), 8.49 (dd, 1H, $J=6.9$, 2.16 Hz), 8.09 (ddd, 1H, $J=8.5$, 4.5, 2.16 Hz), 7.31 (dd, 1H, $J=8.5$, 9.96 Hz), 3.98 (s. 3H).

Methyl 3-fluoro-4-formylbenzoate (16c). Following the procedure for the preparation of **16b** and using **15c** (2.11 g, 12.5 mmol), NBS (6.31 g, 35.4 mmol), AIBN (118 mg, 0.70 mmol), and CCl_4 (150 mL). The resulting crude dibromide was treated with $AgNO_3$ (6.15 g, 36.2 mmol) in acetone/H_2O (100 mL, 83:17). After purification by column chromatography, compound **16c** was obtained in 85% yield (1.94 g). ^{1}H NMR ($CDCl_3$) δ 10.42 (s, 1H), 7.93 (m, 2H), 7.84 (d, 1H, $J=11.3$ Hz), 3.97 (s, 3H).

5,10,15,20-Tetrakis(4-carboxymethyl-3-methoxyphenyl)-porphyrin (17a). Following the procedure for the preparation of **6a** and using aldehyde **16a** (1 g, 5.1 mmol), porhyrin **17a** (102 mg) was isolated in 8% yield as a violet solid. ^{1}H NMR ($CDCl_3$) δ 8.90 (s, 8H), 8.19 (d, 4H), 7.88 (d, 4H), 7.86 (s, 4H), 4.11 (s, 12H), 4.00 (s, 12H), −2.83 (br s, 2H).

5,10,15,20-Tetrakis(3-carboxymethyl-4-fluorophenyl)porphyrin (17b). Following the procedure for the preparation of **6a** and using aldehyde **16b** (1.16 g, 6.4 mmol),

porhyrin **17b** (580 mg) was isolated in 40% yield as a violet solid. ^{1}H NMR ($CDCl_3$) δ 8.83 (s, 8H), 8.78 (d, 4H, J = 6.78 Hz), 8.34 (m, 4H), 7.57 (dd, 4H, J = 8.6, 10.1 Hz), 4.01 (s, 12H), −2.86 (s, 2H).

5,10,15,20-Tetrakis(4-carboxymethyl-2-fluorophenyl)porphyrin (17c). Following the procedure for the preparation of **6a** and using aldehyde **16c** (1.16 g, 10.0 mmol), porhyrin **17c** (228 mg) was isolated in 10% yield as a violet solid. ^{1}H NMR ($CDCl_3$) δ 8.83 (s, 4H), 8.25 (m, 8H), 4.15 (s, 12H), −1.25 (br s, 2H).

5,10,15,20-Tetrakis(3-carboxymethyl-4-hydroxyphenyl)-porphyrin (17d). Following the procedure for the preparation of **6a** and using methyl 3-formyl-6-hydroxy benzoate (1.65 g, 9.1 mmol), porphyrin **17d** (300 mg) was isolated in 14% yield as a violet solid. ^{1}H NMR ($CDCl_3$) δ 11.16 (4H), 8.87 (s, 8H), 8.67 (s, 4H), 8.30 (d, 4H), 7.39 (d, 4H), 3.96 (2, 12H).

5,10,15,20-Tetrakis(4-carboxymethyl-3-hydroxyphenyl)-porphyrin (17e). Following the procedure for the preparation of **6a** and using methyl 4-formyl-2-hydroxy benzoate (1.01 g, 10.0 mmol), porphyrin **17e** (554 mg) was isolated in 42% yield as a violet solid. ^{1}H NMR ($CDCl_3$) δ 11.13 (4H), 8.90 (s, 8H), 8.20 (d, 4H), 7.89 (s, 4H), 7.73 (d, 4H), 4.15 (s, 12H), −2.85 (br s, 2H).

5,10,15,20-Tetrakis(4-carboxymethyl-3-nitrophenyl)porphyrin (17f). Following the procedure for the preparation of **6a** and using methyl 4-formyl-2-nitro benzoate (1.05 g, 5.0 mmol), porphyrin **17f** (678 mg) was isolated in 56% yield after repeated purification by column chromatography as a violet solid. ^{1}H NMR ($CDCl_3$) δ 8.86 (s, 8H), 8.75 (s, 4H), 8.54 (d, 4H), 8.21 (d, 4H), 4.14 (s, 12H), −2.89 (br s, 2H).

5,10,15,20-Tetrakis(4-fluoro-3-methoxyphenyl)porphyrin (17i). Following the procedure for the preparation of **6a** and using 3-flouro-4-methoxybenzaldehyde (1.50 g, 9.6 mmol), porphyrin **17i** (735 mg) was isolated in 38% yield after purification by column chromatography as a violet solid. ^{1}H NMR ($CDCl_3$) δ 8.87 (s, 8H), 7.96 (dd, 4H, J = 1.4, 11.7 Hz), 7.87 (d, 4H, J = 8.3 Hz), 7.29 (dd, 4H, J = 8.4, 8.6 Hz), 4.12 (s, 12H), −1.25 (br s, 2H).

5,10,15,20-Tetrakis(4-carboxymethyl-2-methoxyphenyl)-porphyrin (17k). Following the procedure for the preparation of **6a** and using methyl 4-formyl-3-methoxy benzoate (1.23 g, 6.3 mmol), porphyrin **17k** (333 mg) was isolated in 22% yield after purification by column chromatography as a violet solid. ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 8H), 8.10 (m, 12H), 4.12 (s, 12H), 3.62–3.61 (four singlets corresponding to rotamers, 12H), −2.65 (br s, 2H). FABMS m/z 967 $[C_{56}H_{46}N_4O_{12}+H]^+$.

[5-(3-Carboxymethyl-4-hydroxyphenyl)-10,15,20-tris(3-carboxy-4-hydroxyphenyl)porphyrinato]-manganese(III) chloride (11a) and [5,10,15,20-tetrakis(3-carboxy-4-hydroxyphenyl)porphyrinato]-manganese(III) chloride (11b). Following the procedure for the preparation of **9** and using porphyrin **17d** (100 mg, 0.103 mmol), porphyrins **11a** (33 mg) and **11b** (121 mg) were isolated after purification by column chromatography as green solids. For porphyrin **11a**: mp > 300 °C; IR (KBr) 3134, 1674, 1624, 1484, 1384, 1290, 1212, 1089, 804 cm^{-1}. FABMS m/z 921 $[C_{49}H_{30}MnN_4O_{12}]^+$. HPLC 99%. UV (EtOH) λ_{max}, nm (ε) 472.0 (0.46×10^5). For porphyrin **11b**: mp > 300 °C; IR (KBr) 3422, 3159, 1625, 1558, 1483, 1429, 1342, 1208, 805 cm^{-1}. FABMS m/z 907 $[C_{48}H_{28}MnN_4O_{12}]^+$. HPLC 98%. UV (EtOH) λ_{max}, nm (ε) 472.0.

[5,10,15,20-Tetrakis(2-carboxymethyl-3-methoxyphenyl)-porphyrinato]manganese(III) chloride (12a). Following the procedure for the preparation of **9** and using porphyrin **17a** (100 mg, 0.103 mmol), porphyrin **12a** (59 mg) was isolated in 54% yield after purification by column chromatography a green solid: mp > 325 °C. HPLC > 99%. UV (EtOH) λ_{max}, nm (ε) 467.5 (1.3×10^5). Anal. calcd for $C_{56}H_{44}ClMnN_4O_{12}\cdot H_2O$: C, 62.66; H, 4.32; N, 5.22. Found: C, 62.21; H, 4.69; N, 5.07.

[5,10,15,20-Tetrakis(4-carboxymethyl-3-hydroxyphenyl)-porphyrinato]manganese(III) chloride (12b), [5-(4-carboxy-3-hydroxyphenyl)-10,15,20-tris(4-carboxymethyl-3-hydroxyphenyl)porphyrinato]-manganese(III) chloride (12c) and [5,10-bis(4-carboxy-3-hydroxyphenyl)-15,20-bis(4-carboxymethyl-3-hydroxyphenyl)porphyrinato]manganese(III) chloride (12d). Following the procedure for the preparation of **9** and using porphyrin **17e**, porphyrins **12b**, **12d**, and **12d** were isolated after purification by column chromatography as green solids. For porphyrin **12b**: mp > 300 °C. FABMS m/z 963 $[C_{52}H_{36}MnN_4O_{12}]^+$. HPLC 88%. UV (EtOH) λ_{max}, nm (ε) 467.5 (1.1×10^5). For porphyrin **12c**: mp > 300 °C. FABMS m/z 949 $[C_{51}H_{34}MnN_4O_{12}]^+$. HPLC 98%. UV (EtOH) λ_{max}, nm (ε) 467.5 (1.3×10^5). For porphyrin **12d**: mp > 300 °C. IR (KBr) 3423, 1677, 1618, 1439, 1341, 1290, 1202, 1094, 948 cm^{-1}. FABMS m/z 935 $[C_{50}H_{32}MnN_4O_{12}]^+$. HPLC 98%. UV (EtOH) λ_{max}, nm (ε) 468.5 (1.0×10^5). Anal. calcd for $C_{50}H_{32}ClMnN_4O_{12}\cdot NH_4OH$: C, 59.68; H, 3.71; N, 6.96. Found: C, 59.83; H, 3.80; N, 6.82.

[5,10,15,20-Tetrakis(4-carboxy-3-hydroxyphenyl)porphyrinato]manganese(III) chloride (12e). Saponification of a mixture of porphyrins **12b**–**12d** provided porphyrin **12e** after purification by column chromatography as a green solid: mp > 320 °C. FABMS m/z 944 $[C_{48}H_{28}ClMnN_4O_{12}+H]^+$. UV (EtOH) λ_{max}, nm (ε) 467.5 (0.66×10^5).

[5,10,15,20-Tetrakis(3-carboxymethyl-4-fluorophenyl)-porphyrinato]manganese(III) chloride (12f) and [5-(4-dimethylaminophenyl)-10,15,20-tris(4-carboxymethyl-3-methoxyphenyl) porphyrinato]-manganese(III) chloride (12g). Following the procedure for the preparation of **9** and using porphyrin **17b** (470 mg, 0.51 mmol), porphyrins **12f** (149 mg, 29%) and **12g** were isolated after purification by column chromatography as green solids. Porphyrin **12g** was a by product resulting from the nucleophilic substitution the 4-fluoro position in **12f**: For porphyrin **12f**: mp > 300 °C. FABMS m/z 971 $[C_{52}H_{32}F_4MnN_4O_8]^+$. HPLC 97%; UV (EtOH) λ_{max}, nm (ε) 467.0 (1.3×10^5). For porphyrin **12g**: mp > 300 °C. FABMS m/z 996 $[C_{54}H_{38}F_3MnN_5O_8]^+$. HPLC 96%. UV (EtOH) λ_{max}, nm (ε) 468.0 (1.3×10^5).

[5,10,15,20-Tetrakis(3-carboxyl-4-fluorophenyl)porphyrinato]manganese(III) chloride (12h). To a solution of porphyrin **12g** (130 mg, 0.13 mmol) in MeOH (10 mL) was added Claisen's base (0.25 mL, excess) and the solution was heated at reflux for 3 h. The solution was then cooled to room temperature then acidified with 6 N HCl. The reaction mixture was then evaporated off and the residue was adsorbed onto silica gel. Purification by column chromatography provided tetraacid porphyrin **12h** (80 mg) in 65% yield as a green solid: mp $>300\,^{\circ}C$. FABMS m/z 915 $[C_{48}H_{24}F_4MnN_4O_8]^+$. HPLC 96%. UV (EtOH) λ_{max}, nm (ε) 4637.5 (1.0×10^5).

[5-(4-Dimethylaminophenyl)-10,15,20-tris(4-carboxy-3-methoxyphenyl)porphyrinato]manganese(III) chloride (12i). Saponification of **12g** provided **12i** as a green solid: mp $>300\,^{\circ}C$. FABMS m/z 940 $[C_{50}H_{30}F_3MnN_5O_8]^+$. HPLC 91%. UV (EtOH) λ_{max}, nm (ε) 467.0 (0.94×10^5).

[5,10,15,20-Tetrakis(4-carboxymethyl-3-nitrophenyl)porphyrinato]manganese(III) chloride (12j) and [5-(4-carboxy-3-nitropheyl)-10,15,20-tris(4-carboxymethyl-3-nitrophenyl)porphyrinato]manganese(III) chloride (12k). Following the procedure for the preparation of **9** and using porphyrin **17f** (280 mg, 0.27 mmol), porphyrins **12j** (210 mg, 69%) and **12k** (34 mg, 11%) were isolated after purification by column chromatography as green solids. For porphyrin **12j**: mp $>300\,^{\circ}C$. IR (KBr) 3438, 2962, 1736, 1540, 1437, 1353, 1297, 1131, 1071, 824 cm^{-1}. FABMS m/z 1079 $[C_{52}H_{32}MnN_8O_{16}]^+$. HPLC >99%. UV (MeOH) λ_{max}, nm (ε) 464.0 (1.4×10^5). For porphyrin **12k**: mp $>300\,^{\circ}C$. IR (KBr) 3422, 2923, 1736, 1612, 1540, 1384, 1297, 1131, 1072, 802 cm^{-1}. FABMS m/z 10710659 $[C_{51}H_{30}MnN_8O_{16}]^+$. HPLC 96%. UV (MeOH) λ_{max}, nm (ε) 464.0 (1.2×10^5).

[5,10,15,20-Tetrakis(4-carboxy-3-nitrophenyl)porphyrinato]manganese(III) chloride (12l). Saponification of porphyrin **12j** provided porphyrin **12l** as a green solid: mp $>300\,^{\circ}C$. IR (KBr) 3424, 1719, 1612, 1535, 1353, 1072, 1013, 802 cm^{-1}. FABMS m/z 1023 $[C_{48}H_{24}MnN_8O_{16}]^+$. HPLC 98%. UV (MeOH) λ_{max}, nm (ε) 465.0 (1.3×10^5).

[5,10,15,20-Tetrakis(4-carboxymethyl-3-nitrophenyl)porphyrinato]manganese(III) chloride (12o). Following the procedure for the preparation of **9** and using porphyrin **17i** (192 mg, 0.24 mmol), porphyrin **12o** (150 mg) in 71% yield after purification by column chromatography as a green solid: mp $>300\,^{\circ}C$. IR (KBr) 3449, 2932, 1578, 1517, 1304, 1273, 1132, 1016, 943, 804 cm^{-1}. FABMS m/z 859 $[C_{48}H_{32}F_4MnN_4O_4]^+$. HPLC 94%. UV (MeOH) λ_{max}, nm (ε) 468.0 (0.9×10^5). Anal. calcd for $C_{48}H_{32}ClF_4MnN_4O_4{\cdot}CH_3OH$: C, 63.47; H, 3.91; N, 6.04. Found: C, 63.70; H, 3.99; N, 5.89.

[5,10,15,20-Tetrakis(4-carboxymethyl-2-fluorophenyl)-porphyrinato]manganese(III) chloride (13a) and 5-(4-carboxy-2-fluorophenyl)-10,15,20-tris(4-carboxymethyl-2-fluorophenyl) porphyrinato]-manganese(III) chloride (13b). Following the procedure for the preparation of **9** and using porphyrin **17c** (228 mg, 0.25 mmol), porphyrins **13a** (124 mg, 47%) and **13b** (50 m, 20%) were isolated after purification by column chromatography as green solids. Porphyrin **13b** was a by-product, resulting from the mono-hydrolysis of the ester functionality. For porphyrin **13a**: mp $>300\,^{\circ}C$. IR (KBr) 3436, 2953, 1728, 1437, 1414, 1297, 1221, 1092, 1010, 763 cm^{-1}. FABMS m/z 971 $[C_{52}H_{32}F_4MnN_4O_8]^+$. HPLC 96%. UV (EtOH) λ_{max}, nm (ε) 462.5 (1.7×10^5). For porphyrin **13b**: mp $>300^{\circ}$. IR (KBr) 3417. 2950, 1728, 1384, 1298, 1222, 1011, 765 cm^{-1}. FABMS m/z 957 $[C_{51}H_{30}F_4MnN_4O_8]^+$. HPLC 94%. UV (EtOH) λ_{max}, nm (ε) 463.0 (0.39×10^5).

5,10,15,20-tetra(4-carboxy-2-fluorophenyl) porphyrinato]manganese(III) chloride (13c). Saponification of **13a** provided **13c** as a green solid: mp $>300\,^{\circ}C$. FABMS m/z 915 $[C_{48}H_{24}F_4MnN_4O_8]^+$. HPLC 94%. UV (EtOH) λ_{max}, nm (ε) 463.0 (1.5×10^5).

5,10,15,20-Tetrakis(4-carboxymethyl-2-methoxyphenyl)-porphyrinato]manganese(III) chloride (13d). Following the procedure for the preparation of **9** and using porphyrin **17k** (321 mg, 0.33 mmol), porphyrin **13d** (294 mg) was obtained in 47% yield after purification by column chromatography as a green solid: mp $>300\,^{\circ}C$. FABMS m/z 1019 $[C_{56}H_{44}MnN_4O_{12}]^+$. HPLC 92%. UV (EtOH) λ_{max}, nm (ε) 467.0 (1.5×10^5).

5,10,15,20-Tetrakis(4-carboxy-2-methoxyphenyl)porphyrinato]manganese(III) chloride (13e). Saponification of **13d** provided **13e** as a green solid: mp $>300\,^{\circ}C$. FABMS m/z 963 $[C_{52}H_{36}MnN_4O_{12}]^+$. HPLC 96%. UV (EtOH) λ_{max}, nm (ε) 467.0 (1.5×10^5).

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