Porphyrins incorporating heterocyclic *N*-oxides: (oxidopyridyl)porphyrins, porphyrin-*N*-oxides, and a tirapazamine–porphyrin conjugate

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Abstract: Porphyrins containing one to four 4-pyridyl groups as *meso*-substituents were synthesized via a mixed aldehyde condensation, and then "*N*-oxidized" with *m*-chloroperbenzoic acid to produce five novel (oxidopyridyl) porphyrins and seven porphyrin-*N*-oxides, which were characterized by analysis and spectroscopic methods, especially NMR; an X-ray crystal structure of 5-(1-oxido-4-pyridyl)-10,15,20-triphenylporphyrin was also obtained. Crystals of (oxidopyridyl)triphenylporphyrin are tetragonal, $a = b = 15.174(1)$, $c = 13.709(1)$ Å, $Z = 4$, space group *I*2*d*. The structure was solved by direct methods and refined by full-matrix least-squares procedures to $R = 0.031$ ($R_w = 0.026$) for 685 reflections with $I \geq 3\sigma(I)$. Sulfonation of two of the (oxidopyridyl)porphyrins was achieved readily with no loss of oxygen from the oxidopyridyl groups. Tirapazamine (3-amino-1,2,4-benzotriazine-1,4-di-*N*-oxide) was treated with triphosgene to yield the previously reported 2*H*-[1,2,4]oxadiazolo[3,2-*c*][1,2,4]benzotriazin-2-one-5-oxide (**1**); this reacts like an isocyanate and with 5-(4-aminophenyl)-10,15,20-triphenylporphyrin yields a tirapazamine-porphyrin conjugate (**2**).

Key words: porphyrin-*N*-oxides, (oxidopyridyl)porphyrins, tirapazamine.

Résumé : Faisant appel à une condensation mixte d'aldéhyde, on a synthétisé des porphyrines contenant d'un à quatre groupes 4-pyridyles comme substituants *méso* et on les a ensuite oxydées à l'aide d'acide *m*-chloroperbenzoïque pour produire cinq nouvelles (oxydopyridyl)porphyrines et sept *N*-oxydes de porphyrine que l'on a caractérisés par analyse et par spectroscopie, principalement la RMN; on a aussi déterminé la structure cristalline de la 5-(1-oxydo-4-pyridyl)-10,15,20-triphénylporphyrine par diffraction des rayons X. Les cristaux de la (oxydopyridyl)triphénylporphyrine sont tétragonaux, groupe d'espace *I*2*d*, avec $a = b = 15{,}174(1)$ et $c = 13{,}709(1)$ Å et $Z = 4$. On a résolu la structure par des méthodes directes et on l'a affinée par la méthode des moindres carrés (matrice entière) jusqu'à des valeurs de $R = 0{,}031$ et $R_w = 0{,}026$ pour 685 réflexions avec $I \geq 3\sigma(I)$. On a effectué la sulfonation de deux des oxydopyridylporphyrines facilement, sans perte d'oxygène à partir des groupes oxydopyridyles. On a traité la tirapazamine (le 1,4-dioxyde de la 3-amino-1,2,4-benzotriazine) avec du triphosgène pour obtenir la 5-oxyde de la 2*H*-[1,2,4]oxadiazolo[3,2-*c*][1,2,4]benzotriazine-2-one (**1**) rapportée antérieurement; celle-ci réagit comme un isocyanate et, avec la 5-(4-aminophényl)-10,15,20-triphénylporphyrine, elle fournit une tirapazamine–porphyrine conjuguée.

Mots clés : *N*-oxyde de porphyrine, (oxydopyridyl)porphyrine, tirapazamine.

[Traduit par la Rédaction]

Introduction

One advantage of porphyrin-based, cancer therapies is that neoplastic tissue has been reported to accumulate many porphyrins to a greater degree than many normal tissues; this phenomenon also occurs with other aromatic macrocycles (e.g., naphthalocyanines, phthalocyanines, and chlorins) (1, 2). The mechanisms of tumor localization of such aromatic macrocycles have been discussed and are complex (2–5).

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Heterocyclic *N*-oxides, most notably aromatic tirapazamine (6) and RB90740 (6, 7) have been investigated as radiosensitizers and hypoxia-selective agents. Thus, because of our ongoing interest in the development of porphyrin-based anticancer agents (8–10), we have pursued porphyrins incorporating heterocyclic *N*-oxides, as this might improve the delivery of heterocyclic *N*-oxides to neoplastic tissue. Herein, we describe the *N*-oxidation of several *meso*-pyridyl porphyrins to yield their corresponding (oxidopyridyl)porphyrins and their corresponding porphyrin-*N*-oxides, and the conjugation of tirapazamine to a porphyrin.

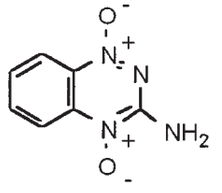
Experimental

All solvents were reagent grade or better. Unless otherwise indicated, all commercial chemicals were used as purchased from Aldrich Chemical Co., Strem, or Sigma Chemical Co. Pyrrole was dried over and distilled from CaH_2 or vacuum-transferred before use. Benzaldehyde, 4-pyridine-

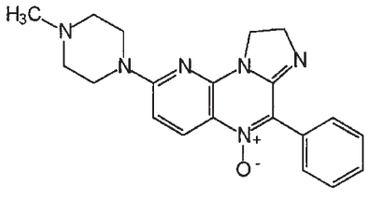
Table 1. Abbreviations used for the *meso*-pyridyl porphyrins, and corresponding (oxidopyridyl)porphyrins and porphyrin-*N*-oxides.

Porphryin precursor	Abbrev.	Oxidopyridyl abbrev.	Corresponding porphyrin-*N*-oxide abbrev.
5,10,15-Triphenyl-20-(4-pyridyl)porphyrin	TrPhPyP	OPyTrPhP	OPyTrPhP-21O, OPyTrPhP-23O
5,15-Diphenyl-10,20-bis(4-pyridyl)porphyrin	*t*-DPhBPyP[a]	*t*-BOPyDPhP	Not isolated
5,10-Diphenyl-15,20-bis(4-pyridyl)porphyrin	*c*-DPhBPyP	*c*-BOPyDPhP	*c*-BOPyDPhP-21O, *c*-BOPyDPhP-22O, *c*-BOPyDPhP-24O
5-Phenyl-10,15,20-tris(4-pyridyl)porphyrin	PhTrPyP	TrOPyPhP	TrOPyPhP-21O, TrOPyPhP-23O
5,10,15,20-Tetrakis(4-pyridyl)porphyrin	TPyP	TOPyP	Not isolated

[a]Present in small amounts as an impurity in TrPhPyP.



Tirapazamine



RB90740

carboxaldehyde, and trifluoroacetic acid (TFA) were freshly distilled before use. Tirapazamine (3-amino-1,2,4-benzotriazine-1,4,-di-*N*-oxide) was a gift from Dr. M. Tracy (SRI International, Menlo Park, Calif.). Reaction products were dried in vacuo at 78°C overnight, unless otherwise noted.

The alumina used for chromatography was Fisher, activity I, and was deactivated by adding water to give a standard range of activities (11). Unless otherwise noted, the silica gel used in the chromatographies was Merck Silica Gel 60, 230–400 mesh, whilst R_f values were measured on Merck silica TLC Al sheets (silica gel 60 F_{254}). Preparative TLCs of porphyrin free bases were performed with Merck silica gel 60, 0.5 mm plates (without indicator, to avoid metallation of the products). In general, the visualization of non-porphyrinic compounds was achieved with the aid of a UV lamp. Dialysis tubing (SpectraPor) used in the purification of the sulfonated porphyrins had a molecular weight cutoff of 1000.

^{1}H and ^{13}C NMR spectra were recorded on a BRUKER AC-200, a Varian XL-300, or a BRUKER WH-400 instrument, and UV-vis spectra on an HP8452A photo-diode array spectrophotometer (± 2 nm) with molar absorptivity (ϵ) values recorded for analytically pure porphyrins. IR spectra were recorded as KBr pellets or as a thin film on a KBr disk (from slow evaporation of a solution of the compound on the surface of a KBr disk) on an AIT Mattson Genesis Series FTIR instrument. Elemental analyses were performed by Mr. P. Borda of the Department of Chemistry (UBC). High- (HR-MS) and low-resolution (LR-MS) mass spectra were obtained on a KRATOS MS50 (EI), a KRATOS MS80 (CI), a KRATOS Concept IIHQ (LSIMS), or a BRUKER Biflex MALDI-TOF mass spectrometer. When analyzed with EI techniques, the mass spectra of free-base porphyrins often showed a peak at 61 *m*/*z* higher ($M^+ - 2H + Cu$) than the parent mass due to a gas phase metallation reaction with Cu from the ionization source.

Porphyrin precursors

The *meso*-pyridyl porphyrins were synthesized according to literature methods (8, 12); the abbreviations used for these porphyrins and the corresponding (oxidopyridyl)porphyrin and porphyrin-*N*-oxide derivatives are given in Table 1.

***N*-Oxidations**

Based on published procedures for the preparation of pyridine-*N*-oxide (13), excess *meta*-chloroperoxybenzoic acid (*m*-CPBA, 60–85%, the rest being benzoic acid and H_2O) was added to a stirred solution of the starting porphyrin in CH_2Cl_2 or CH_2Cl_2–MeOH at room temperature (r.t.). Addition of 1–2 equiv. aliquots of *m*-CPBA gave the (oxidopyridyl)porphyrins, while a single addition of several equivalents gave the corresponding porphyrin-*N*-oxides in low and variable yield. The reactions were monitored by TLC on silica (CH_2Cl_2:MeOH 100:1 to 10:1); Et_3N was added to the final reaction mixture, and the product was purified by chromatography after preadsorbing the product mixture on silica. With very polar eluent mixtures, metallation (with Zn from the fluorescent indicator in the silica) occurred on the TLC plate. In such cases, analytical TLC plates without indicator were used. Tables 2–6 summarize, respectively, ^{1}H NMR data for the (1-oxido-4-pyridyl)porphyrins, ^{1}H NMR data for some porphyrin-*N*-oxides, UV-vis data, elemental analyses, and mass spectrometry data; the ^{1}H NMR assignments are considered in Results and Discussion. IR data for the (oxidopyridyl)porphyrins and the two sulfonated derivatives (Table S1) and the porphyrin-*N*-oxides (Table S2) are available as supplementary material.[2]

If the porphyrin-*N*-oxides were not produced or their isolation was not pursued, the product was purified by first removing the solvent under reduced pressure, and the residue

[2]Copies of material on deposit may be purchased from: The Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, Canada, K1A 0S2. Tables of hydrogen atom coordinates have also also been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from: The Director, Cambridge Crystallographic Data Centre, University Chemical Laboratory, 12 Union Road, Cambridge, CB2 1EZ, U.K.

Table 2. ^{1}H NMR data for the (1-oxido-4-pyridyl)porphyrins.[a]

Porphyrin (MHz, solvent)	β-Pyrrole[b]	2,6-OPy 3,5-OPy[c]	2,6-Ph (or 2,6-SPh) 3,4,5-Ph (or 3,5-SPh)[d]	Pyrrole N-H
OPyTrPhP (200, $CDCl_3$)	8.92 d (2), 8.84 s (4), 8.82 d (2)	8.64 dd (2) 8.11 dd (2)	8.18 d (6) 7.76 m (9)	–2.82 s (~1.5)
t-BOPyDPhP (200, $CDCl_3$)	8.94 d (4), 8.83 d (4)	8.62 dd (4) 8.10 dd (4)	8.18 d (4) 7.80 m (6)	–2.87 s (~1.5)
c-BOPyDPhP (300, $CDCl_3$)	8.94 d (2), 8.93 s (2), 8.86 s (2), 8.83 d (2)	8.63 dd (4) 8.10 dd (4)	8.18 d (4) 7.78 m (6)	–2.85 s (~1.5)
TrOPyPhP (400, $CDCl_3$)	8.95 d (2), 8.94 s (4), 8.83 d (2)	8.63 dd (6) 8.10 dd (6)	8.18 d (2) 7.8 m (3)	–2.88 s (~1.5)
TOPyP (400, $CDCl_3$–CD_3OD)	8.95 s br (8)	8.67 dd (8) 8.19 dd (8)		None observed in $CDCl_3$–CD_3OD
OPyTrSPhP (200, DMSO-d_6)	9.05 d (2), 8.88 d (2), 8.85 s (4)	8.63 d (2) 8.17 d (2)	8.17 d (6) 8.05 d (6)	–2.96 s br (1.7)
c-BOPyBSPhP (200, DMSO-d_6)[e]	9.10 s (2), 9.05 d (2), 8.87 m (4)	8.64 d (4) 8.25 d (4)	8.18 d (4) 8.05 d (4)	–2.95 s br (1.6)

[a]Measured at r.t.; δ in ppm, signal pattern (number of protons). An H_2O signal was generally observed in the spectra.
[b]*J* values typically ~5 Hz (*meso*-phenyl derivatives) or ~6 Hz (*meso*-(4-sulfonatophenyl) derivatives).
[c]Typically, J_1 = 7 Hz, J_2 = 1 Hz.
[d]*J* values typically ~8 Hz.
[e]Impurity peaks appeared near δ ~7.8 and 8.4 (<0.5 H each by integration).

Table 3. ^{1}H NMR data for the porphyrin-*N*-oxides.[a]

Porphyrin	β-Pyrrole[b]	2,6-OPy 3,5-OPy[c]	2,6-Ph 3,4,5-Ph	β-Pyrrole-NO[d]	Pyrrole N-H
OPyTrPhP-23O	9.07 d (1), 9.00 d (1), 8.8 overlapping doublets (2), 8.75 d (1), 8.66 d (1)	8.63 d (2) 8.10 d (2)	8.5 m (6) 7.9 m (9)	7.5 s (2)	1.6 s br (>2)[e]
OPyTrPhP-21O	8.97 overlapping doublets (2), 8.91 d (1), 8.83 d (1), 8.65 m (2)	Under β-pyrrole signal at 8.65 Under 2,6-Ph signal at 8.17	8.23 d (2), 8.17 d (4) 7.8 m (9)	7.56 d (1), 7.50 d (1)	~1.7 s br (>2)[e]
TrOPyPhP-21O	9.06 overlapping doublets (2), 8.92 d (1), 8.85 d (1), 8.73 m (2)	8.64 m (6) 8.14 d (2), 8.07 d (4)	8.22 d (2) 7.83 m (3)	7.60 d (1), 7.54 d (1)	N-H peak not assigned because of large H_2O peak
TrOPyPhP-22O[f]	9.07 d, 9.00 d, 8.94 d, 8.92 d, 8.74 d, 8.63 d	8.6 m 8.14 m, 8.05 d	Under 8.14 m 7.8 m	7.61 s	N-H peak not assigned because of large H_2O peak

[a]Measured at r.t. in $CDCl_3$ (400 MHz); δ in ppm, signal pattern (number of protons). An H_2O signal was observed in all spectra.
[b]*J* values typically ~5 Hz.
[c]*J* values typically ~7 Hz.
[d]*J* values typically ~6 Hz; addition of D_2O changed these signals to broad multiplets.
[e]Integration includes H_2O peak; the peak disappeared when D_2O was added.
[f]Integrations did not match.

then being washed with acetone. The resulting solid contained mostly the (oxidopyridyl)porphyrin, while the filtrate contained the corresponding porphyrin-*N*-oxides and other reaction byproducts (e.g., large amounts of *m*-chlorobenzoic acid (*m*-CBA)). Excess *m*-CBA complicated the chromatography, and also could be removed by washing the reaction mixture with dilute aq. NaOH. In some cases, mixtures of porphyrins (e.g., *c*-DPhBPyP and PhTrPyP) were treated with *m*-CPBA to give mixtures of (oxidopyridyl)porphyrins and the corresponding porphyrin-*N*-oxides. The mixtures were separated by chromatography, but no yield was determined for the minor components, as the molar ratio of the compounds was not determined prior to the reaction.

Because of the polar solvent mixtures used in chromatography, the porphyrin samples were often contaminated with silica. To obtain analytically pure material, the porphyrin was allowed to crystallize slowly (days to weeks) from a CH_2Cl_2:MeOH (~10:1) solution in a vial placed in a chamber containing EtOH.

The (oxidopyridyl)porphyrins were deoxygenated under the conditions of EI or LSIMS mass spectrometry and had to be analyzed by MALDI-TOF mass spectrometry. The corresponding porphyrin-*N*-oxides were deoxygenated to a lesser degree, and in some cases the parent peaks were intense enough to be analyzed by HR-MS (EI).

5-(1-Oxido-4-pyridyl)-10,15,20-triphenylporphyrin (OPyTrPhP)

TrPhPyP (0.223 g, 0.36 mmol) was dissolved in CH_2Cl_2 (150 mL), and treated with excess *m*-CPBA (3 × 0.1 g aliquots)

Table 4. UV-vis data for the (1-oxido-4-pyridyl)porphyrins and porphyrin-*N*-oxides.

Porphyrin (solvent)	*N*-Oxide region (log ϵ)	Soret (log ϵ)	Q bands (log ϵ)
OPyTrPhP[a] (CH_2Cl_2:MeOH 20:1)	274 (4.32)	420 (5.60)	516 (4.35), 552 (3.95), 590 (3.80), 646 (3.67)
t-BOPyDPhP[b] (CH_2Cl_2:MeOH 10:1)	274, 310 (weak)	426	516, 556, 602, 648
c-BOPyDPhP[a] (CH_2Cl_2:MeOH 10:1)	272 (4.49)	422 (5.57)	518(4.30), 556 (4.00), 592 (3.85), 650 (3.59)
TrOPyPhP[a] (CH_2Cl_2:MeOH 10:1)	274 (4.61)	424 (5.65)	518 (4.33), 554 (4.07), 594 (3.87), 650 (3.65)
TOPyP[a] (CH_2Cl_2:MeOH 10:1)	274 (4.66)	424 (5.63)	518 (4.3), 554 (4.03), 594 (3.85), 650 (3.56)
OPyTrPhP-23O[c] (CH_2Cl_2)	282 (4.30)	420 (5.14)	518 (3.77), 544 (3.76), 594 (3.85), 684 (3.41)
OPyTrPhP-21O (CH_2Cl_2)	280	420	516, 548, 596, 684
OPyTrPhPZn-23O[d,e] (CH_2Cl_2:MeOH 5:1)	272 (4.35), 322 (4.26)	436 (5.27)	574 (3.89), 622 (3.91)
OPyTrPhPZn-21O (CH_2Cl_2:MeOH 5:1)	276, 330	438	574, 622
c-BOPyDPhP-O mixture (CH_2Cl_2)	280, 342 (weak)	422	514, 550, 594, 688
TrOPyPhP-21O (CH_2Cl_2)	283, 334 (weak)	424	518, 548, 600, 685
TrOPyPhP-22O (CH_2Cl_2)	280, 348 (weak)	424	518, 552, 598, 685
OPyTrSPhP[f] (H_2O)	258 (4.32), 306 (4.27)	414 (5.59)	516 (4.14), 554 (3.84), 580 (3.81), 636 (3.57)
c-BOPyBSPhP (H_2O)	258, 306	418	520, 560, 586, 646

[a]Soret ϵ determined at 2×10^{-6} M; ϵ of other bands determined at 2×10^{-5} M.
[b]The C, H, and N elemental analyses were ~0.5% too low (see Table 5); ϵ values were not determined.
[c]The C and N elemental analyses were ~0.5 and 1.26% too low, respectively (see Table 5).
[d]Soret ϵ determined at 5×10^{-6} M; ϵ of other bands determined at 5×10^{-5} M.
[e]Prepared from a known concentration of OPyTrPhP-23O and excess $Zn(OAc)_2$.
[f]ϵ determined at 1.0×10^{-6} M.

Table 5. Elemental analyses for the (1-oxido-4-pyridyl)porphyrins and porphyrin-*N*-oxides.[a]

Porphyrin (formula)	C (%)	H (%)	N (%)
OpyTrPhP ($C_{43}H_{29}N_5O$)	81.53 (81.75)	4.63 (4.88)	11.09 (11.10)
OpyTrPhP-23O ($C_{43}H_{29}N_5O_2 \cdot 1H_2O$)	77.58 (77.12)	4.69 (4.65)	10.52 (9.26)
t-BOPyDPhP ($C_{42}H_{28}N_6O_2 \cdot 2H_2O$)	73.67 (73.12)	4.71 (4.24)	12.27 (11.86)
c-BOPyDPhP ($C_{42}H_{28}N_6O_2 \cdot H_2O$)	75.66 (75.83)	4.54 (4.41)	12.60 (12.73)
c-BOPyDPhP-O mixture ($C_{42}H_{28}N_6O_3 \cdot 1.5H_2O$)	72.93 (72.71)	4.52 (4.46)	12.60 (12.73)
TrOPyPhP ($C_{41}H_{27}N_7O_3 \cdot 1.5H_2O$)	71.09 (71.04)	4.37 (4.31)	14.15 (13.93)
TOPyP ($C_{40}H_{26}N_8O_4 \cdot 2H_2O$)	68.56 (68.55)	4.03 (3.98)	15.99 (15.75)
OpyTrSPhP ($C_{43}H_{26}N_5Na_3O_{10}S_3 \cdot 11H_2O$)	45.46 (45.72)	4.26 (4.11)	6.16 (6.12)
c-BOPyBSPhP ($C_{42}H_{26}N_6Na_2O_8S_2 \cdot 4.5H_2O$)	54.02 (54.28)	3.78 (3.66)	9.00 (8.80)

[a]Calculated (found); not obtained for OPyTrPhP-23O, TrOPyPhP-21O, and TrOPyPhP-22O.

over 1–2 h while the solution was stirred. Et_3N (20 mL) was then added, and the product was preadsorbed on silica and chromatographed on silica ($CHCl_3$: pyridine 10:1). The solvent was removed from the main product band to yield 0.128 g (56% yield) of OPyTrPhP. The reaction was scaled up to ~0.5 g of TrPhPyP with similar results. Slow solvent evaporation from a CH_2Cl_2–MeOH solution of the porphyrin yielded analytically pure crystals which were studied by X-ray crystallography.

5-(1-Oxido-4-pyridyl)-10,15,20-triphenylporphyrin-21-oxide (OPyTrPhP-21O), and -23-oxide (OPyTrPhP-23O)

From reactions in which several equivalents of *m*-CPBA were added all at once to a solution of TrPhPyP in CH_2Cl_2:MeOH, two additional products were isolated (~5% yield each) after OPyTrPhP was first isolated chromatographically. The two bands following that of OPyTrPhP were collected; the solvent was removed, and a second chromatography column silica (CH_2Cl_2:THF:MeOH 100:5:2.5) was used to separate two isomers. The less polar band was OPyTrPhP-23O, and the more polar band was OPyTrPhP-21O.

The porphyrin-*N*-oxides metallated rapidly when mixed with $Zn(OAc)_2$ in CH_2Cl_2–MeOH to give OPyTrPhPZn-21O and OPyTrPhPZn-23O (see Table 4 for UV-vis data). Attempts to grow crystals of the free-base porphyrin-*N*-oxides or Zn-porphyrin-*N*-oxides were unsuccessful.

5,15-Bis(1-oxido-4-pyridyl)-10,20-diphenylporphyrin (*t*-BOPyDPhP)

t-BOPyDPhP was prepared in the same manner as OPyTrPhP. The *m*-CPBA was added in 1–2 equiv. aliquots to a solution of an impure sample of TrPhPyP containing a small amount of *t*-DPhBPyP. The products were purified by column chromatography on silica; OPyTrPhP was eluted from the column with $CHCl_3$:pyridine (10:1), and *t*-BOPyDPhP was eluted with $CHCl_3$:pyridine (10:3). The yield was not determined. Because only small amounts of *t*-DPhBPyP were obtained from the mixed-aldehyde synthesis (8, 12), the synthesis of the corresponding porphyrin-*N*-oxide was not pursued.

5,10-Bis(1-oxido-4-pyridyl)-15,20-diphenylporphyrin (*c*-BOPyDPhP)

c-BOPyDPhP was synthesized in the same manner as OPyTrPhP. To a solution of *c*-DPhBPyP (0.5 g, 0.8 mmol,

Table 6. Mass spectrometry data for (1-oxido-4-pyridyl)porphyrins and porphyrin-*N*-oxides.

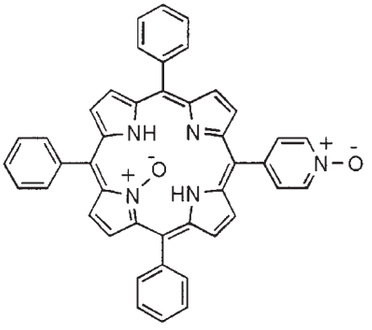
Porphyrin	LR-MS, m/z[a] (intensity %, assignment)	Formula HR-MS, calcd. (found)
OPyTrPhP[b]	632 (88, M^{+1}), 616 (36, $M^{+1} - O$)	Not obtained
OPyTrPhP-21O[c]	692 (2, $M^+ - O - 2H + Cu$), 676 (5, $M^+ - 2O - 2H + Cu$), 647 (4, M^+), 631 (10, $M^+ - O$), 615 (100, $M^+ - 2O$)	$C_{43}H_{29}N_5O_2$ 647.232 12 (647.232 07)
OPyTrPhP-23O[c]	692 (1, $M^+ - O - 2H + Cu$), 677 (2, $M^+ -2O - 2H + Cu$), 647 (1, M^+), 631 (20, $M^+ - O$), 615 (100, $M^+ - 2O$)	$C_{43}H_{29}N_5O_2$ 647.232 12 (647.229 65) $C_{43}H_{29}N_5O$ 631.237 18 (631.237 01)
t-BOPyDPhP[b,d]	650 (100, M^{+1}), 634 (62, $M^{+1} - O$), 614 (19, $M^{+1} - 2O$)	Not obtained
c-BOPyDPhP[b,d]	649 (100, M^{+1}), 633 (86, $M^{+1} - O$), 617 (27, $M^{+1} - 2O$)	Not obtained
c-BOPyDPhP-NO mixture[c]	677 (5, $M^+ - 2O - 2H + Cu$), 693 (2, $M^+ - O -2H + Cu$), 664 (1, M^+), 648 (5, $M^+ - O$), 632 (29, $M^+ - 2O$), 616 (100, $M^+ - 3O$)	$C_{42}H_{28}O_3N_6$ 664.222 29 (664.219 42) $C_{42}H_{28}O_2N_6$ 648.227 36 (648.225 16)
TrOPyPhP[b,d]	666 (87, M^{+1}), 650 (100, $M^{+1} - O$), 634 (66, $M^{+1} - 2O$), 618 (33, $M^{+1} - 3O$)	Not obtained
TrOPyPhP-21O[c]	710 (1, $M^+ - 2O - 2H + Cu$), 694 (7, $M^+ - 3O - 2H + Cu$), 678 (8, $M^+ - 4O - 2H + Cu$), 665 (1, $M^+ - O$), 649 (11, $M^+ - 2O$), 633 (80, $M^+ - 3O$), 617 (100, $M^+ - 4O$)	$C_{41}H_{27}O_3N_7$ 665.217 53 (665.217 19) $C_{41}H_{27}O_3N_7$ 649.222 60 (649.222 10)
TrOPyPhP-22-NO[c]	678 (20, $M^+ - 4O -2H + Cu$), 665 (2, $M^+ - O$), 649 (9, $M^+ - 2O$), 633 (10, $M^+ - 3O$), 617 (100, $M^+ - 4O$)	$C_{41}H_{27}O_3N_7$ 665.217 53 (665.215 11) $C_{41}H_{27}O_3N_7$ 649.222 60 (649.221 75)
TOPyP[b]	683 (51, M^{+1}), 666 (84, $M^{+1} - O$), 650 (99, $M^{+1} - 2O$), 635 (100, $M^{+1} - 3O$), 619 (79, $M^{+1} - 4O$)	Not obtained
c-BOPyBSPhP[f]	875 (0.1, $M^{+1} + Na$), 859 (0.1, $M^{+1} + Na - O$), 853 (0.1, M^{+1}), 837 (0.1, $M^{+1} - O$)	Not obtained

[a] M^{+1} implies $(M + H)^+$.
[b] MALDI-TOF mass spectroscopy.
[c] EI mass spectroscopy.
[d] Dimer peaks (5–10% intensity, due to combinations of the species listed in the LR-MS column) are observed.
[e] (+)CI mass spectroscopy.
[f] (+)LSIMS mass spectroscopy.

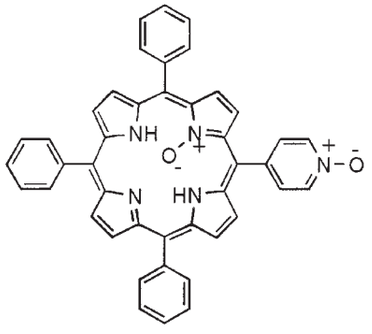
but containing a small percentage of PhTrPyP) in CH_2Cl_2:MeOH (125:25 mL) was added excess *m*-CPBA (4 × ~1.25 g aliquots), and the reaction monitored by TLC. Et_3N (~10 mL) was added; the product was preadsorbed on silica and intially chromatographed on silica (CH_2Cl_2:pyridine:MeOH 20:1:2 to 20:1:3). The product bands were poorly resolved, possibly because of large amounts of residual *m*-CPBA and *m*-CBA; the porphyrin-containing fractions were washed with dilute aq. NaOH. The product mixture was rechromatographed on silica (CH_2Cl_2:pyridine:MeOH 10:1:2.5); *c*-BOPyDPhP eluted first (~0.27 g, ~50% yield), followed by a mixture of porphyrin-*N*-oxides, and then a small amount (~20 mg) of TrOPyPhP. A *c*-BOPyDPhP sample of analytical purity was obtained by slow crystallization from a solution of the compound in CH_2Cl_2:MeOH.

5,10-Bis(1-oxido-4-pyridyl)-15,20-diphenylporphyrin-21-oxide (*c*-BOPyDPhP-21O), -22-oxide (*c*-BOPyDPhP-22O), and -24-oxide (*c*-BOPyDPhP-24O)

A mixture of porphyrin-*N*-oxides was isolated (~30 mg, ~5%) from the reaction of *c*-DPhBPyP with a large excess of *m*-CPBA (see above). Attempts to separate the mixture on



OPyTrPhP-23O



OPyTrPhP-21O

silica (CH_2Cl_2:pyridine:MeOH 20:1:2.5) were unsuccessful. There are three possible porphyrin-*N*-oxides isomers: *c*-BOPyDPhP-21O, *c*-BOPyDPhP-22O, and *c*-BOPyDPhP-24O.

The ^{1}H NMR data given below support the presence of at least two of these. The two doublets for the β-pyrrole-NO protons (δ 7.57 and 7.51) indicate the presence of *c*-BOPyDPhP-21O, while the corresponding singlet at δ 7.60 indicates the presence of either *c*-BOPyDPhP-22O or *c*-BOPyDPhP-24O, or both. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05 (d, J = 5 Hz), 8.99 (overlapping ds, J = 5 Hz), 8.93 (d, J = 5 Hz), 8.91 (d, J = 5 Hz), 8.85 (d, J = 5 Hz), 8.74 (d, J = 5 Hz), 8.65 (m), 8.22 (dd, J_1 = 6 Hz, J_2 = 1.5 Hz), 8.15 (m), 8.08 (d), 7.8 (m), 7.60 (s), 7.57 (d, J = 6 Hz), 7.51 (d, J = 6 Hz). Because the ratio of products is unknown, the ^{1}H NMR signals are not assigned; general δ_H values for the (oxidopyridyl)porphyrins and porphyrin-*N*-oxides can be found in Tables 2 and 3.

5,10,15-Tris(1-oxido-4-pyridyl)-20-phenylporphyrin (TrOPyPhP)

TrOPyPhP was produced in the same manner as OPyTrPhP. PhTrPyP was present in small amounts in the sample of *c*-DPhBPyP used above, and TrOPyPhP was isolated from the reaction mixture. The yield was not determined. A sample of analytical purity was obtained by slow crystallization from CH_2Cl_2–MeOH.

5,10,15-Tris(1-oxido-4-pyridyl)-20-phenylporphyrin-21-oxide (TrOPyPhP-21O), and -22-oxide (TrOPyPhP-22O)

TrOPyPhP-21O and TrOPyPhP-22O were prepared in the same manner as the porphyrin-*N*-oxides of OPyTrPhP. The product was purified by preparative TLC on silica (CH_2Cl_2:MeOH 10:1 to 5:1). Only ~1 mg of each porphyrin-*N*-oxide was obtained (<5% yield).

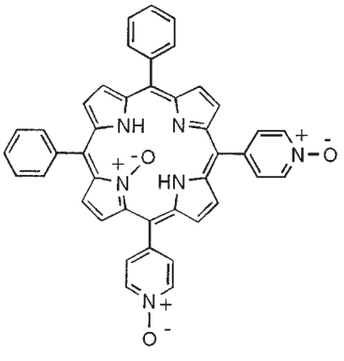
5,10,15,20-Tetrakis(1-oxido-4-pyridyl)porphyrin (TOPyP)

Oxidation of TPyP

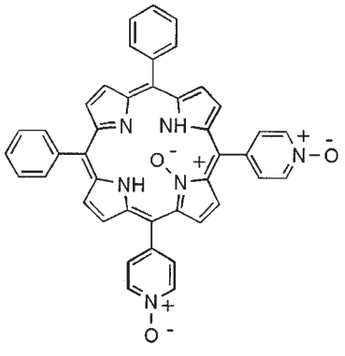
TOPyP was prepared in the same manner as OPyTrPhP. TPyP (0.35 g, 0.57 mmol) was dissolved in CH_2Cl_2:MeOH (150:25 mL) and excess *m*-CPBA (6 × 0.5 g) was added in aliquots over 3 h to the stirred mixture. TLC showed at least four intermediates as the reaction progressed. Et_3N (~20 mL) was added to the reaction mixture and the solvent was removed. The residual solid was rinsed with MeOH, acetone, and H_2O, and air-dried. Further purification was achieved by dissolving the product in H_2O:TFA (20:1), neutralizing the solution (NaOH), and subsequently filtering off the crude TOPyP precipitate; total yield 0.275 g (0.40 mmol, ~70%). Analytically pure TOPyP was obtained by slow crystallization from CH_2Cl_2:MeOH. Alternatively, small amounts of the product could be chromatographed on Al_2O_3(III) (CH_2Cl_2:MeOH 20:1). TOPyP was insoluble in $CDCl_3$, and thus its ^{1}H NMR spectrum was measured in $CDCl_3$–CD_3OD.

*Acid-catalyzed condensation of pyrrole and 4-pyridinecarboxaldehyde-*N*-oxide*

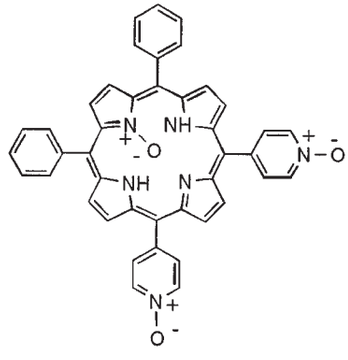
Attempts were also made to prepare TOPyP via acid-catalyzed condensation of pyrrole and 4-pyridinecarboxaldehyde-*N*-oxide. Pyrrole (0.3 mL, 0.4 mmol) and 4-pyridinecarboxal-



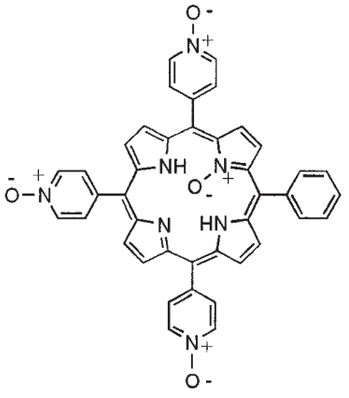
c-BOPyDPhP-21O



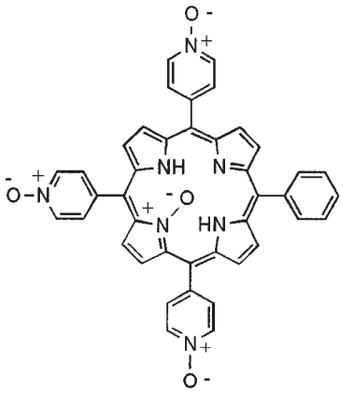
c-BOPyDPhP-22O



c-BOPyDPhP-24O



TrOPyPhP-21O



TrOPyPhP-22O

dehyde-*N*-oxide (0.05 g, 0.4 mmol) were dissolved in propionic acid (2 mL), and the solution was refluxed for ~50 min. UV-vis spectroscopy indicated that a porphyrin had formed (Soret 418 nm), but TLC analysis of the mixture on silica (CH_2Cl_2–MeOH) indicated numerous side products and so further isolation was not attempted. Attempts to synthesize TOPyP with Lindsey-type conditions in CH_2Cl_2 with pyrrole and 4-pyridinecarboxaldehyde (4 or 8 mM), using TFA or BF_3·MeOH and subsequent oxidation with 2,3,5,6-tetrachloro-1,4-benzoquinone (TCQ), yielded only traces of porphyrin by UV-vis spectroscopy.

TPyP synthesis via Lindsey conditions

When Lindsey conditions (14) were applied to the synthesis of TPyP using trichloroacetic acid, TFA, or BF_3·MeOH catalysis, TPyP was produced when aliquots of the reaction mixture were treated with TCQ, which showed that the precursor porphyrinogen was forming. However, the porphyrinogen was eventually consumed by a side reaction that gave a very polar, unidentified, blue compound. The rate of the side reaction increased with increasing acid strength and concentration.

Sulfonations

Sodium 5-(1-oxido-4-pyridyl)-10,15,20-tris(4-sulfonatophenyl)porphyrin (OPyTrSPhP)

Based on the literature procedure for the sulfonation of 5,10,15,20-tetraphenylporphyrin (TPhP) (8), OPyTrPhP (0.535 g, 0.85 mmol) was dissolved in conc. H_2SO_4 (10 mL), and the solution was heated at 110°C for 4 h. The reaction was monitored by first neutralizing an aliquot of the mixture (using aq. Na_2CO_3), then analyzing by TLC on silica (CH_2Cl_2:MeOH 100:1 to 5:1). The reaction mixture was cooled with an ice-bath, and cold, distilled H_2O (50 mL) was added, with the pH of the solution being adjusted to 7–8 with aq. NaOH and aq. Na_2CO_3. The solvent was removed and the porphyrin washed from the residue with MeOH. Then the MeOH was removed and the porphyrin subsequently dialyzed in dialysis tubing in a beaker of distilled H_2O to yield 0.51 g (~53% yield) of OPyTrSPhP·$11H_2O$. The ^{1}H NMR signals were assigned with the help of a ^{1}H-2D-COSY spectrum.

*Sodium 5,10-bis(1-oxido-4-pyridyl)-15,20-bis(4-sulfonatophenyl)porphyrin (*c*-BOPyBSPhP)*

c-BOPyDPhP (0.10 g, 0.15 mmol) was sulfonated in the same manner used for OPyTrSPhP. The reaction was monitored by TLC; an intermediate band appeared between that of the starting material and the baseline, and heating was continued ~0.5 h after the intermediate had disappeared (6 h total). After dialysis of the worked up product, its ^{1}H NMR spectrum revealed the presence of an impurity. Redissolving the product in conc. H_2SO_4 and heating at 100°C for another 2 h with the same work-up procedure did not remove the impurity (i.e., it is not a monosulfonatophenyl product). Attempts to purify by chromatography on silica MeOH or by recrystallization from MeOH–acetone were unsuccessful. The results of the elemental analysis (Table 5) suggest that the composition of the impurity does not differ significantly from that of *c*-BOPyBSPhP. Careful TLC analysis of the sample of *c*-BOPyDPhP used here showed the presence of another compound with an R_f nearly identical to that of *c*-BOPyDPhP; the presence of the impurity was not evident in the ^{1}H NMR spectrum ($CDCl_3$).

2*H*-[1,2,4]Oxadiazolo[3,2-*c*][1,2,4]benzotriazin-2-one-5-oxide (1)

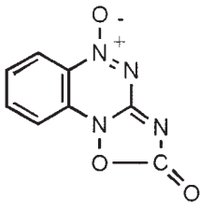
Based on a literature reaction between phosgene and tirapazamine (15), a suspension of tirapazamine (0.3 g, 1.7 mmol) in dry toluene (5 mL) was heated to 90°C, and triphosgene (0.173 g, 0.58 mmol) was added. Within 5 min, the suspension turned from orange to yellow and TLC indicated near quantitative conversion to **1** (R_f = 0.75 silica (CH_2Cl_2:MeOH ~50:1)). (When the solution was heated for ~2 h, TLC indicated that some **1** had been reconverted to tirapazamine (R_f = 0.18).) The reaction mixture was cooled, and the precipitate was filtered off and washed with hexanes to yield 0.324 g (1.59 mmol, 94% yield) of **1** as a yellow–brown solid, which contained a small amount of tirapazamine (by TLC). The product was used without further purification in subsequent reactions. Compound **1** was reconverted to tirapazamine when exposed to H_2O vapor or when co-spotted with H_2O on a TLC plate, and tirapazamine was isolated in variable amounts from subsequent reactions involving **1**. IR (KBr pellet): 3460 w br, 3170 w, 3067 w, 1814 vs, 1598 s, 1541 vs, 1437 vw, 1386 s,1344 s, 1226 m,

Table 7. ^{1}H NMR data for the benzotriazine-dioxide compounds.[a]

Compound (solvent)	-CON*H*	Benzotriazine ring *H*[b]	Other substituent
Tirapazamine (acetone-d_6)[c]		8.28 t of d (2), 7.98 t of d (2), 7.64 t of d (2)	7.38 s br (2, -NH_2)
1 (acetone-d_6)		8.47 d (1), 8.27 t of d (1), 7.97 d of d (1), 7.80 t of d (1)	
2 ($CDCl_3$)	9.71 s (1), 8.81 s (1)	7.27 t(1), 7.04 m (2), 6.63 t (1)	8.91 s br (4), 8.84 s (4), β-pyrrole *H*; 8.35 d (2, *J* 8, 2,6-RNHPh-*H*); 8.25 m (6, 2,6-Ph-*H*); 8.00 d (2, *J* 8, 3,5-RNHPh-*H*); 7.78 m (9, 3,4,5-Ph-*H*); –3.19 s br (2, pyrrole N-*H*)

[a]Measured at r.t. at 400 MHz; δ in ppm, signal pattern (number of protons).
[b]Typical *J* values for doublets or triplets 8–9 Hz; for a doublet or triplet of doublets $J_1 \sim 9$, $J_2 \sim 1$ Hz.
[c]The ^{1}H NMR data are similar to those reported in the literature (recorded in TFA), ref. 15*b*.

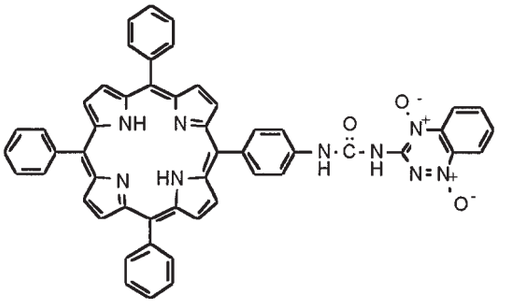
1196 m, 1149 m, 1110 m, 772 m, 734 m; IR of tirapazamine (thin film on a KBr disk): 3411 w, 3274 w br, 3098 w, 1594 vs, 1408 vs, 1362 vs, 1105 vs, 1025 w, 828 w, 723 w. For **1**, LR-MS (+CI): 205 (30, M^{+1}), 163 (100, M^{+1} – NCO), 147 (75, M^{+1} – NCO – O). HR-MS (+CI) calcd. for $C_8H_5N_4O_3$: 205.03616; found 205.03622. The ^{1}H NMR data for **1** (not reported previously) and for tirapazamine appear in Table 7.



1

5-(4-*N*-(*N*′-(1,4-dioxido-1,2,4-benzotriazin-3-yl)aminocarbonyl)aminophenyl)-10,15,20-triphenylporphyrin (2)

To a solution of 5-(4-aminophenyl)-10,15,20-triphenylporphyrin (APhTrPhP) (8) (31 mg, 0.05 mmol) in dry DMF (3 mL) was added **1** (11 mg, 0.055 mmol), and the mixture was stirred at r.t. in the dark for 26 h. TLC (silica (CH_2Cl_2:MeOH 100:2–3)) showed a new product (R_f = 0.4), some APhTrPhP (R_f = 0.65), but little **1**; so another 1.0 equiv. of **1** was added, and the mixture was stirred another 24 h. The solvent was removed, and the desired product was isolated from several minor products by preparative TLC on silica (CH_2Cl_2:MeOH 100:2–3). The isolated product was washed with hexanes, dissolved in CH_2Cl_2:MeOH (20:1), and the solution was washed with H_2O. The solvent was removed to yield 20 mg of a purple powder (~50%). IR (KBr pellet, cm^{-1}): 3312 w, 1709 m, 1597 m, 1526 vs, 1405 m, 1332 m, 1311 m, 1225 m, 1181 w, 964 m, 799 m, 727 m, 701 m; UV-vis (2.4×10^{-6} M, CH_2Cl_2:MeOH (100:1)) corresponding to the tirapazamine moiety: 280 (log ε: 4.65), 478 (4.26) nm, corresponding to the porphyrin core: 418 (5.81), 514 (4.54), 550 (4.28), 586 (4.16), 646 (4.03). LR-MS (+LSIMS): 834 (0.25, M^{+1}), 818 (1, M^{+1} – O), 802 (0.25 M^{+1} – 2O), 656 (12, M^{+1} – tirapazamine). HR-MS (+LSIMS) calcd. for $C_{52}H_{36}N_9O_3$: 834.29411, found: 834.29456; calcd. for $C_{52}H_{36}N_9O_2$: 818.29919, found 818.29953. Anal. calcd. for $C_{52}H_{35}N_9O_3 \cdot 0.5H_2O$: C 74.10, H 4.30, N 14.96; found: C 73.96, H 4.18, N 14.41%. The ^{1}H NMR data for **2** appear in Table 7.



2

X-ray crystallographic analysis of OPyTrPhP

Crystallographic data appear in Table 8. The final unit-cell parameters were obtained by least squares on the setting angles for 25 reflections with $2\theta = 45.4 – 80.4°$. The intensities of three standard reflections, measured every 200 reflections throughout the data collection, showed only small random fluctuations. The data were processed[3] corrected for Lorentz and polarization effects and absorption (based on azimuthal scans).

The space group ambiguity (space groups $I4_1md$ and $I2d$ are possible) was resolved by trial and error. The structure was solved by direct methods, the coordinates of the non-hydrogen atoms being determined from *E*-maps or from subsequent difference Fourier syntheses. The molecule is centred at a point of crystallographic S_4 symmetry, thus the pyridine-*N*-oxide ligand and the N-H protons are statistically disordered. Position C(9)/N(2) was refined as 0.75C and 0.25N; the N and C atoms are too close to resolve. The oxygen position is 1/4 occupied and the N-H proton position is half occupied. All non-hydrogen atoms were refined with anisotropic thermal parameters. The full-occupancy hydrogen atoms were refined isotropically and the N-H and C(9)-H hydrogen atoms were fixed in calculated positions with C—H = 0.98 Å, N—H = 0.91 Å and $B_H = 1.2B_{\text{bonded atom}}$. A correction for secondary extinction was applied (Zachariasen type, isotropic, Gaussian), the final value of the extinction

[3]*teXsan*: Crystal structure analysis package. Unix version 1.7. Molecular Corporation. The Woodlands, Tex., U.S.A. 1995.

Table 8. Crystallographic data for OpyTrPhP.[a]

Compound	OPyTrPhP
Formula	$C_{43}H_{29}N_5O$
fw	631.73
Crystal system	Tetragonal
Space group	*I*2*d* (no. 122)
a, Å	15.174(1)
c, Å	13.709(3)
V, Å^3	3156.3(9)
Z	4
ρ_{calc}, g/cm^3	1.329
F(000)	1320
μ(Cu K_α), cm^{-1}	6.41
Crystal size, mm	0.20 × 0.35 × 0.45
Transmission factors	0.87–1.00
Scan type	ω-2θ
Scan range, deg in ω	1.00 + 0.20 tan θ
Scan speed, deg/min	32 (up to 9 scans)
Data collected	
$2\theta_{max}$, deg	155
Crystal decay, %	Negligible
Total reflections	998
Total unique reflections	998
Reflections with $I \geq 3\sigma(I)$	685
No. of variables	143
R	0.031
R_w	0.026
gof	2.2
Max Δ/σ (final cycle)	0.0008
Residual density, e/Å^3	–0.07 to 0.09

[a]Temperature 294 K, Rigaku AFC6S diffractometer, Cu K_α radiation (λ = 1.54178 Å), graphite monochromator, takeoff angle 6.0°, aperture 6.0 × 6.0 mm at a distance of 285 mm from the crystal, stationary background counts at each end of the scan (scan/background time ratio 2:1), $\sigma^2(F^2) = [S^2(C + 4B)]/Lp^2$ (S = scan rate, C = scan count, B = normalized background count), function minimized $\Sigma w(|F_o| - |F_c|)^2$ where $w = 4F_o^2/\sigma^2(F_o^2)$, $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $R_w = (\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2)^{1/2}$, and gof = $[\Sigma w(|F_o| - |F_c|)^2/(m - n)]^{1/2}$. Values given for R, R_w, and gof are based on those reflections with $I \geq 3\sigma(I)$.

coefficient being 1.54(5) × 10^{-6}. A parallel refinement of the mirror image gave marginally larger residuals (by a factor of 1.001). Neutral atom scattering factors for all atoms and anomalous dispersion corrections for the non-hydrogen atoms were taken from ref. 16. Final atomic coordinates and equivalent isotropic thermal parameters, bond lengths and bond angles appear in Tables 9–11, respectively. Infrared spectroscopy data, hydrogen atom parameters, anisotropic thermal parameters, torsion angles, intermolecular contacts, and least squares planes are included as supplementary material.[2]

Results and discussion

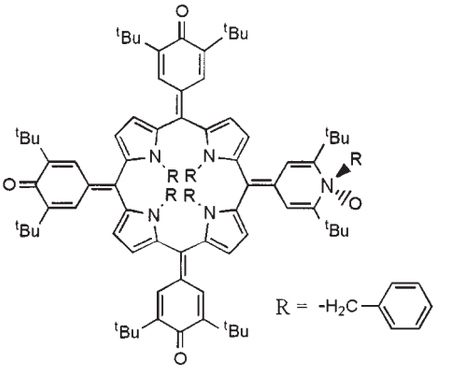
Porphyrin-*N*-oxides and (1-oxido-4-pyridyl)porphyrins

Porphyrin-*N*-oxides have been reported in the literature. Octaethylporphyrin-*N*-oxide (OEP-O) was first prepared by Bonnet et al. (17, 18) by treating OEP with hypofluorous acid (in 64% yield) or a peroxyacid (26% using *m*-CPBA, to 68% using permaleic acid). In studies relating to the active site of cytochrome P-450 enzymes, metal complexes of OEP-O (Ni, Mn, Co, Cu) (19–22), an Fe tetramesitylporphyrin-*N*-oxide complex Fe(TMP-O) (23–26), and a Ti tetraphenylporphyrin-*N*-oxide complex Ti(TPhP-O) (27) have been reported. With OEP-O, the metal was introduced after *N*-oxidation, while Fe(TMP-O) was produced by treating Fe(TMP) with *m*-CPBA, and Ti(TPhP-O) was prepared by photolysis of Ti(TPhP)(O_2). TMP-O was produced by oxidation of TMP, or demetallation of Fe(TMP-O) with HCl–HOAc (23), and Ti(TPhP-O) spontaneously demetallated in ethanol (27). In the metal complexes, the oxygen remains on one face of the porphyrin but, in the free-base porphyrin-*N*-oxides, the oxygen rapidly moves through the center of the porphyrin in a fluxional inversion process (19).

In contrast, (oxidopyridyl)porphyrins have not been reported, although a related compound (**3**) was obtained by Milgrom et al. (28) from the aerial oxidation of a (1-benzyl-4-pyridyl)porphyrin, this procedure leading also to further benzylation of the macrocyclic nitrogens.

Tetraarylporphyrin syntheses

The precursor *meso*-phenyl/pyridyl porphyrins and (aminophenyl)triphenylporphyrin were synthesized according to published procedures (8). The synthesis of (oxidopyridyl)porphyrins via acid-catalyzed condensation of pyrrole, benzaldehyde and 4-pyridinecarboxaldehyde-*N*-oxide was also attempted, but a significant percentage of the oxidopyridyl groups was deoxygenated in refluxing propionic acid. However, acid-catalyzed condensation of pyrrole and 4-pyridinecarboxaldehyde-*N*-oxide did produce at least one porphyrin, probably TOPyP, but this compound was much more conveniently prepared by *N*-oxidation of TPyP.



3

The synthesis of TPyP, via Lindsey conditions was hampered by a side reaction that produced an unidentified, blue compound. It has been noted that under Lindsey conditions, numerous heterocyclic aldehydes fail to give porphyrins but no explanation was suggested (14). *N*-Substitution on the pyridine nitrogen (as in *N*-oxides, and alkyl or acyl pyridinium salts) greatly activates the pyridine ring to nucleophilic addition at the α and γ positions (29). Thus, in situ formation of a pyridinium salt via protonation of (or BF_3 coordination to) the pyridine lone pair under high [H^+] and subsequent reaction (at the pyridine ring and aldehyde substituent) with pyrrole could explain the results obtained here. The side re-

Table 9. Final atomic coordinates (fractional) and B_{eq} (10^3 Å^2).[a]

Atom	*x*	*y*	*z*	B_{eq}	Occupancy
O(1)	0.5590(4)	0.3378(6)	0.1417(9)	8.0(3)	1/4
N(1)	0.0680(1)	0.3847(1)	0.2468(2)	4.68(5)	
N(2)	0.4789	0.3672	0.1491	6.61	1/4
C(1)	0.0344(2)	0.3027(2)	0.2670(2)	4.78(6)	
C(2)	0.1059(2)	0.2406(2)	0.2692(2)	5.60(8)	
C(3)	0.1804(2)	0.2851(2)	0.2478(3)	5.65(7)	
C(4)	0.1574(2)	0.3759(2)	0.2349(2)	4.71(7)	
C(5)	0.2170(2)	0.4450(2)	0.2217(2)	4.74(7)	
C(6)	0.3096(2)	0.4202(2)	0.1961(2)	4.95(7)	
C(7)	0.3305(2)	0.3948(2)	0.1022(3)	6.10(9)	
C(8)	0.4147(2)	0.3695(3)	0.0789(3)	7.4(1)	
C(9)	0.4789(2)	0.3672(2)	0.1491(3)	6.61(9)	3/4
C(10)	0.4600(2)	0.3928(3)	0.2407(3)	7.2(1)	
C(11)	0.3762(2)	0.4195(3)	0.2645(3)	6.90(9)	

[a] $B_{eq} = (8/3)\pi^2\Sigma\Sigma(U_{ij}a_ia_j(\mathbf{a}_i\mathbf{a}_j))$.

Table 10. Bond lengths (Å) with estimated standard deviations in parentheses.[a]

Bond	Length	Bond	Length
O(1)—N(2)	1.299(7)	N(1)—C(1)	1.372(3)
N(1)—C(4)	1.374(3)	N(2)—C(8)	1.369(4)
N(2)—C(10)	1.346(4)	C(1)—C(2)	1.438(4)
C(1)—C(5)′	1.398(4)	C(2)—C(3)	1.349(4)
C(3)—C(4)	1.433(4)	C(4)—C(5)	1.396(3)
C(5)—C(6)	1.496(4)	C(6)—C(7)	1.381(4)
C(6)—C(11)	1.378(4)	C(7)—C(8)	1.372(4)
C(9)—C(10)	1.346(5)	C(10)—C(11)	1.375(4)

[a] Symmetry operation: (′) $-1/2 + y$, $1/2 - x$, $1/2 - z$.

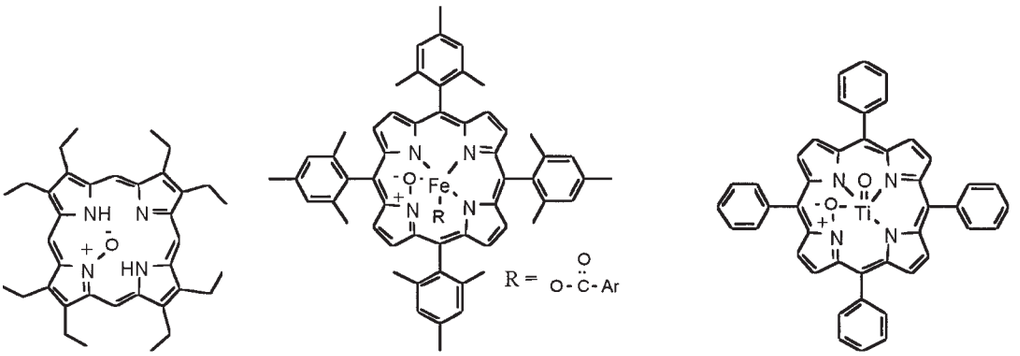
action did not occur using weaker acids (e.g., dichloroacetic acid), but these were not able to catalyze the formation of a porphyrinogen, and thus no TPyP was observed when TCQ was added.

N-Oxidations

N-Oxidation of the pyridyl substitutents was accomplished using *m*-CPBA; the oxidations were sluggish at 0°C, but proceeded readily at r.t. When the peroxyacid was added in small aliquots, only the (oxidopyridyl)porphyrins were obtained, while the single addition of *m*-CPBA generated the corresponding porphyrin-*N*-oxides in low but variable yield, as pyrrole nitrogens were also oxidized. Use of the same conditions with TPhP gave no significant amount of TPhP-oxide, and continued addition of *m*-CPBA only led to the degradation of the porphyrin. As an oxidopyridyl group is more electron-withdrawing than a phenyl group, the (oxidopyridyl)porphyrins are more resistant to oxidative degradation, and thus the (oxidopyridyl)porphyrin-*N*-oxides could be isolated.

Thermal deoxygenation is commonly observed with heterocyclic *N*-oxides (13), but no deoxygenation was observed (by ^{1}H NMR studies) when a solid sample of OPyTrPhP was heated at 100°C overnight in vacuo. Porphyrin-*N*-oxides deoxygenate at milder conditions (18), and were thus kept below 30°C. Deoxygenation was observed in the mass spectra of the (oxidopyridyl)porphyrins and their *N*-oxides. The elemental analyses of these compounds, and the presence of H_2O peaks in their ^{1}H NMR spectra were consistent with water being present; of note, pyridine-*N*-oxide itself is hygroscopic and freely soluble in water (13).

The porphyrin-*N*-oxides are much more soluble in weakly polar solvents (CH_2Cl_2, $CHCl_3$) than their parent oxidopyridyl compounds, and are soluble in MeOH. OPyTrPhP has good solubility in CH_2Cl_2 or $CHCl_3$, but with increasing numbers of oxidopyridyl groups, increasing amounts of MeOH are required as a cosolvent to ensure dissolution. For



OEP-O Fe(TMP-O) Ti(TPhP-O)

Fig. 1. ORTEP view of OPyTrPhP; 33% probability thermal ellipsoids are shown for the non-hydrogen atoms. The disorder is not shown for the sake of clarity.

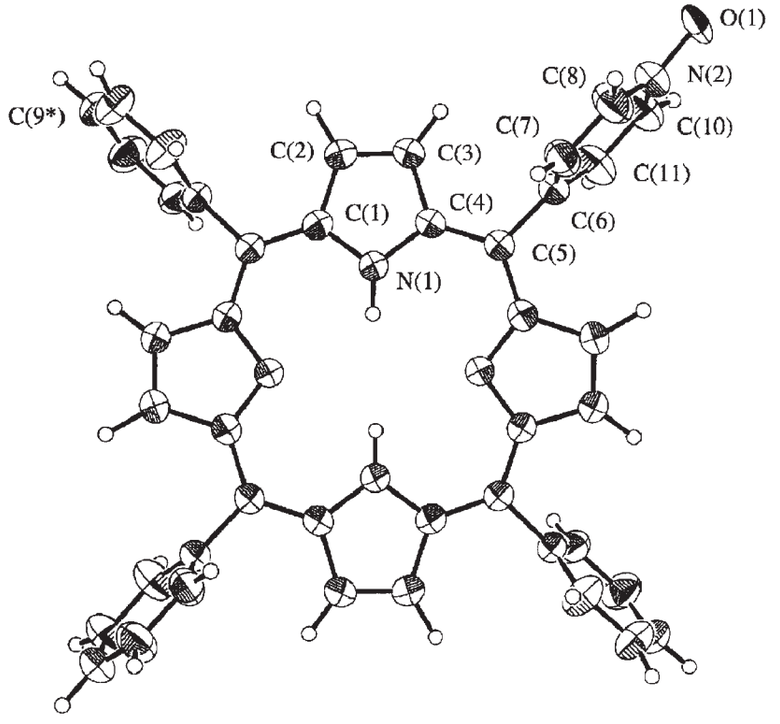


Table 11. Bond angles (deg) with estimated standard deviations in parentheses.[a]

Bonds	Angle	Bonds	Angle
C(1)-N(1)-C(4)	107.7(2)	O(1)-N(2)-C(8)	128.3(6)
O(1)-N(2)-C(10)	111.8(5)	C(8)-N(2)-C(10)	119.8(2)
N(1)-C(1)-C(2)	108.6(2)	N(1)-C(1)-C(5)′	125.2(2)
C(2)-C(1)-C(5)′	126.1(2)	C(1)-C(2)-C(3)	107.4(3)
C(2)-C(3)-C(4)	107.7(2)	N(1)-C(4)-C(3)	108.6(2)
N(1)-C(4)-C(5)	125.7(2)	C(3)-C(4)-C(5)	125.5(2)
C(1)″-C(5)-C(4)	125.1(2)	C(1)″-C(5)-C(6)	118.1(2)
C(4)-C(5)-C(6)	116.8(2)	C(5)-C(6)-C(7)	120.3(3)
C(5)-C(6)-C(11)	122.1(3)	C(7)-C(6)-C(11)	117.7(3)
C(6)-C(7)-C(8)	120.6(3)	C(9)-C(8)-C(7)	120.4(3)
C(8)-C(9)-C(10)	119.8(3)	C(9)-C(10)-C(11)	120.2(3)
C(6)-C(11)-C(10)	121.3(4)		

[a]Symmetry operations: (′) –1/2 + *y*, 1/2 – *x*, 1/2 – *z*; (″) 1/2 – *y*, 1/2 + *x*, 1/2 – *z*.

example, TOPyP is insoluble in $CDCl_3$ but dissolution (required for ^{1}H NMR measurement) occurs readily on addition of a few drops of CD_3OD. The difference in solubility may be related to less aggregation in the porphyrin-*N*-oxides because of steric effects of the macrocyclic oxygens; also, these oxygens may well be involved in hydrogen-bonding.

X-ray crystallography

The X-ray structure of OPyTrPhP is shown in Fig. 1. The morphology of the porphyrin core and the orientation of the aryl groups do not differ significantly from those of TPhP (30). The porphyrin ring is nearly planar, and the dihedral angle of the oxidopyridyl group and the porphyrin plane is 78°. The N(2)—O(1) bond length (1.299(7) Å) is significantly shorter than that in pyridine-*N*-oxide (1.34 Å), but lies within the range of N—O distances in a variety of substituted pyridine-*N*-oxides (13, 19).

^{1}H NMR spectra

^{1}H NMR spectroscopy (Tables 2, 3) was very useful for characterization of the (oxidopyridyl)porphyrins and enabled the porphyrin-*N*-oxide isomers to be identified.

Oxidopyridyl groups

Compared to the δ_H values for the unoxidized pyridyl group, the 2-*H* and 6-*H* protons of the oxidopyridyl groups were shifted upfield by ~0.4 ppm, while the 3-*H*- and 5-*H*-oxidopyridyl protons were shifted <0.1 ppm (see Fig. 2). Similar shift changes, observed for pyridine-*N*-oxide versus pyridine (13), were rationalized in terms of additional electron density at the 2, 4, and 6 positions resulting from mesomeric structures (Fig. 3). The splitting patterns and δ values for the phenyl and sulfonatophenyl signals of the (oxidopyridyl)porphyrins correspond well to those of the phenyl-(pyridyl)- and (pyridyl)(sulfonatophenyl)porphyrins, respectively (8).

Pyrrole-*N*-oxides

The β-pyrrole protons of the pyrrole-*N*-oxide of the porphyrin-*N*-oxides are shifted upfield to δ ~7.5 compared to those of the nonoxidized pyrroles (Fig. 4, Δ ~ 1.4 ppm). Similar δ values were noted for the β-pyrrole-oxide positions in TMP-O (δ 7.49) (23) and Ti(TPhP-O) (δ 7.26) (27). Based

Fig. 2. The aromatic region of the ^{1}H NMR spectrum TrPhPyP and OPyTrPhP in $CDCl_3$.

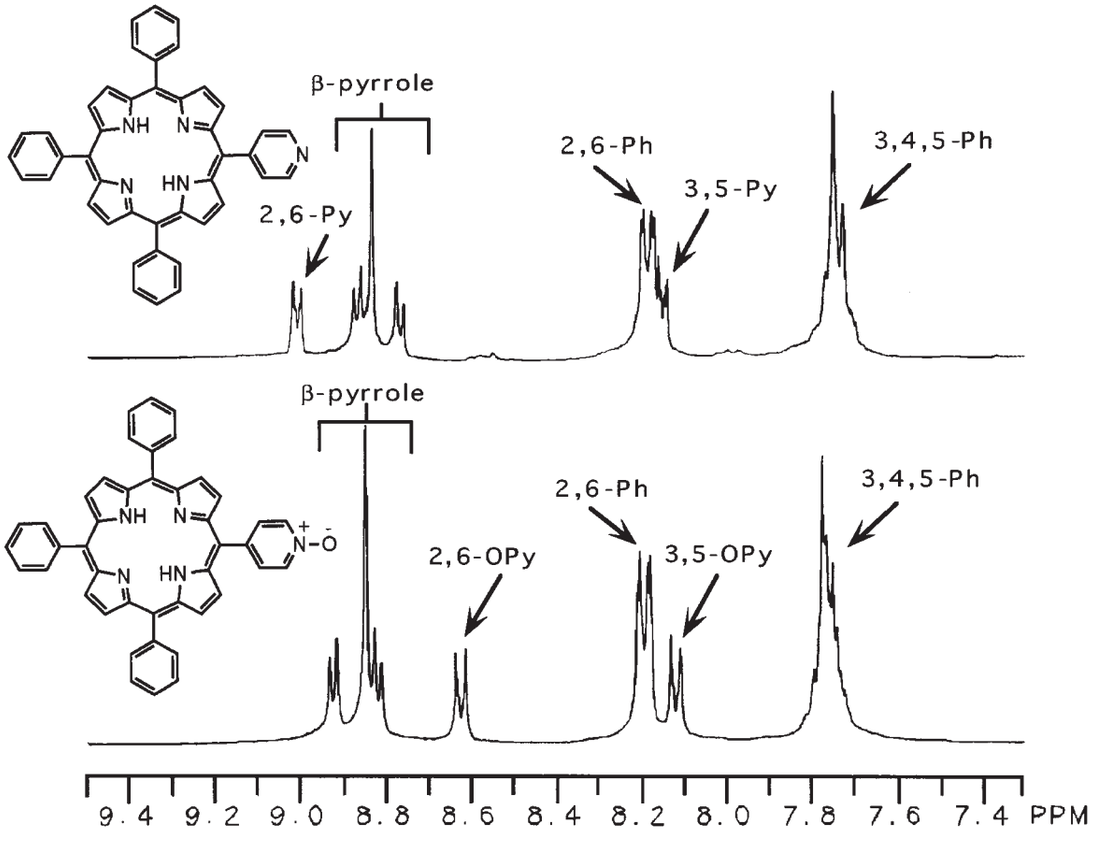
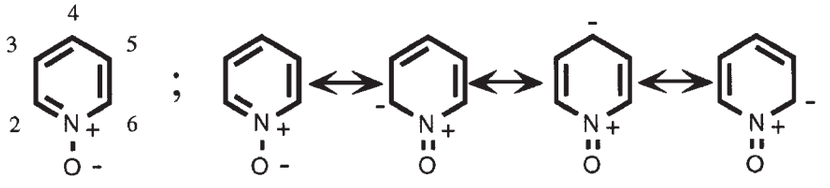


Fig. 3. Mesomeric forms of pyridine-*N*-oxide.



solely on inductive effects, such shifts are opposite to those expected by incorporation of an O-atom, and are unlikely due to partial negative charges for mesomeric forms (cf. Fig. 3), as these would tend to be delocalized over the whole macrocycle. An out-of-plane tilt of the pyrrole-*N*-oxide could explain the upfield shift, as the β-pyrrole protons would then experience less deshielding from the induced ring current. However, in the solid state, the pyrrole-*N*-oxide ring in OEP-O is tipped out of the porphyrin plane only 6.1° (20), which is similar to that of 6.6° found in two of the pyrrole rings of TPhP (30), and such a small tilt in solution is unlikely to explain the large $\Delta\delta$. In the dynamic porphyrin inversion process, in which the oxygen atom moves through the center of the porphyrin (19), the pyrrole groups may be tilted far enough for such a loss of deshielding, and in support of this a similar loss of deshielding is noted for the methylene groups in the Ni(II) complex of OEP-O, where a large pyrrole-*N*-oxide tilt (38.3°) is observed and inversion does not occur (19, 20). A large tilt of the pyrrole-*N*-oxide unit is also expected in Ti(TPhP-O) where a $\Delta\delta$ of ~2 is observed (27).

As in the corresponding pyridyl(phenyl)- and (oxidopyridyl)(phenyl)porphyrin series, the β-pyrrole protons show a singlet when situated between two idendical *meso*-substituents, and an AB spectrum when between two different *meso*-subsituents (8). The rest of the β-pyrrole region is complex because of the inequivalence introduced by the proximity of the oxidopyrrole group and different *meso*-subsitutents.

The inner N-H protons of the porphyrin-*N*-oxides appear as broad singlets at δ ~1.6, which disappear in the presence of D_2O. Compared to the (oxidopyridyl)porphyrins (where δ_{NH} is ~–2.85), the induced ring current is presumably slightly disrupted because of the out-of-plane pyrrole-*N*-oxide ring. The inner N-H signals of OEP-O and TMP-O appear at δ 0.8 (19) and δ 1.75 (23), respectively.

In the ^{1}H NMR spectrum of TOPyP in $CDCl_3$–CD_3OD, the β-pyrrole protons appear as a broad singlet and no N-H

Fig. 4. The aromatic region of the ^{1}H NMR spectrum of OPyTrPhP-23O and -21O.

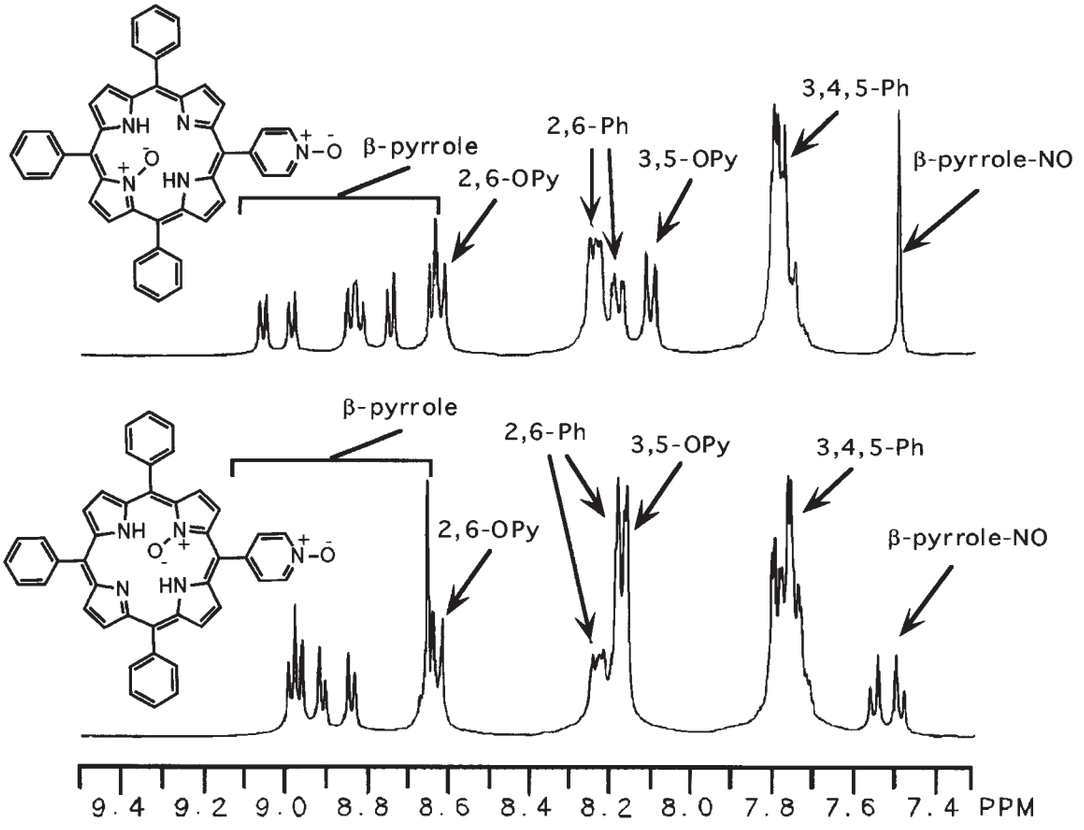
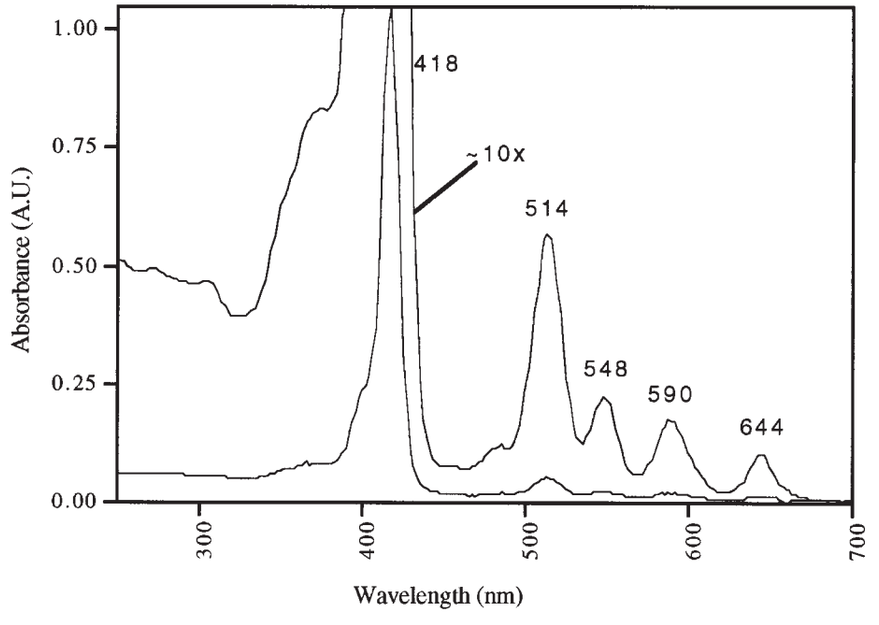


Fig. 5. UV-vis spectrum of TPhPyP (2.3×10^{-6} M) in CH_2Cl_2.



signals are seen. Similar data were obtained when D_2O or CD_3OD was added to the $CDCl_3$ solutions of TrPhPyP, OPyTrPhP, *t*-DPBPyP, *t*-BOPyDPhP, *c*-DPhBPyP, or *c*-BOPyDPhP, and reflect H/D exchange with the N-H protons. The β-pyrrole protons are weakly coupled to the N-H protons but rapid tautomerism prevents any observable

Fig. 6. UV-vis spectrum of OPyTrPhP (2.6×10^{-6} M) in CH_2Cl_2.

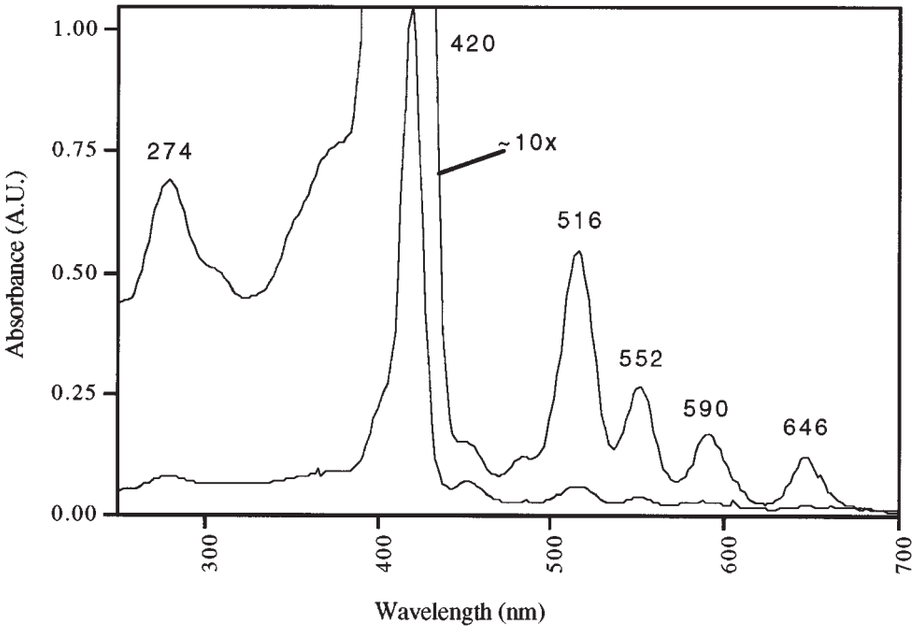
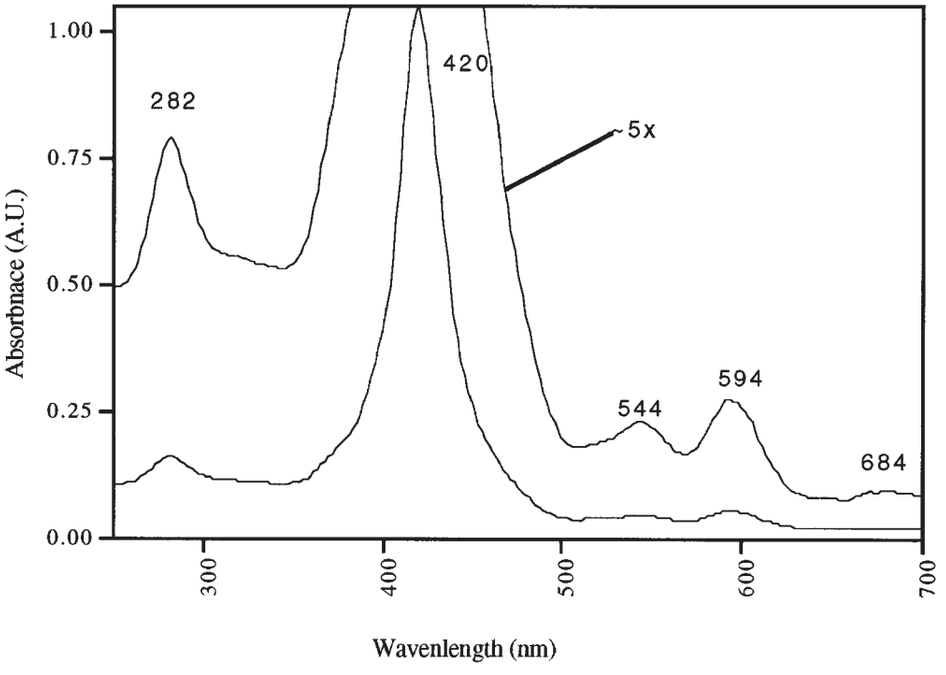


Fig. 7. UV-vis spectrum of OPyTrPhP-23O (~7.6×10^{-6} M) in CH_2Cl_2. (At ~2.8×10^{-5} M, an additional, small peak is observed at 518 nm.)

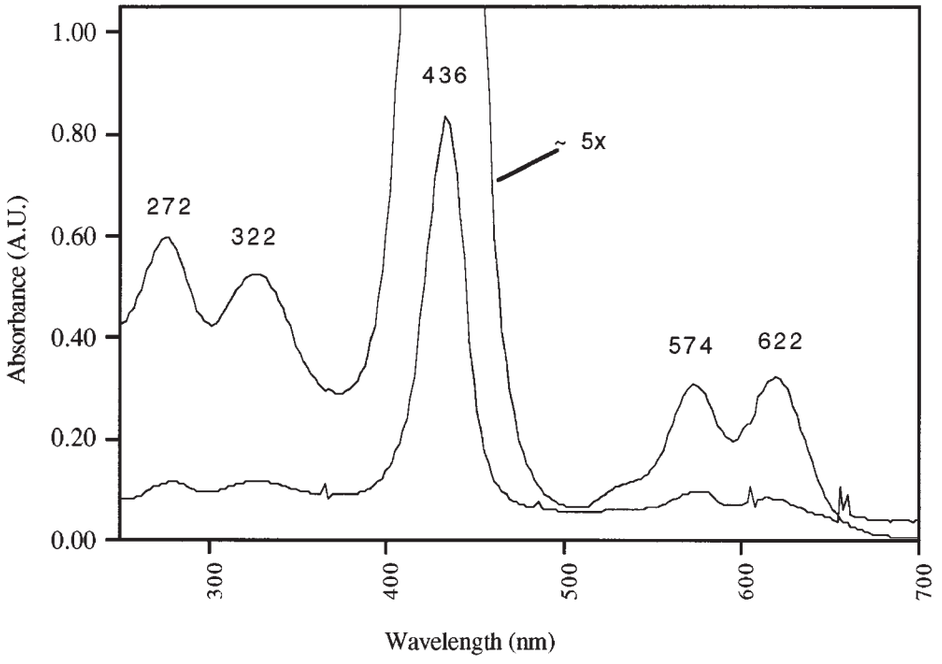


splitting at room temperature; with N-D, the tautomerism is slower (k_H/k_D ~ 12.1 at 35°C) (31), and coupling to the D-atoms broadens the β-pyrrole signals.

UV-vis spectroscopy

Pyridine-*N*-oxide displays an absorbance maximum (log ϵ > 4) in the 255–285 nm region and a weaker (log ϵ ~ 2) absorbance maximum near 350 nm; in polar solvents, the longer wavelength band is blue-shifted and can be hidden by the shorter wavelength absorption maximum (13). The shorter wavelength maximum appears at ~275 nm for the (oxidopyridyl)porphyrins and the corresponding porphyrin-*N*-oxides (see Table 4); and the corresponding longer wavelength band is sometimes observed in the ~310–350 nm

Fig. 8. UV-vis spectrum of OPyTrPhPZn-23O (~1.0×10^{-5} M) in CH_2Cl_2.



region, although this is usually masked by the "background" absorbance from the porphyrin moiety (see Figs. 5 and 6). With the exception of the UV region, the spectra of the (oxidopyridyl)porphyrins are similar to those of free-base tetraarylporphyrins.

The presence of the Soret bands for the porphyrin-*N*-oxides suggests that the conjugation of the macrocycle is uninterrupted (18); however, the Soret bands are broadened, and the Q bands are not as intense as those in the parent (oxidopyridyl)porphyrins (Fig. 7). The Soret and Q-bands in the spectra of OPyTrPhPZn-21O and -23O are similarly broad and diminished in intensity, but the peaks at 320–330 nm are more prominent (see Fig. 8). There is no evidence for a band assignable to any electronic transition of the pyrrole-*N*-oxide group.

Infrared spectroscopy

The *N*-oxide moiety exhibits an intense, characteristic band in the 1200–1350 cm^{-1} region attributed to ν(N-O) (13), and the IR spectra of the (oxidopyridyl)porphyrins show an intense band in the 1221–1261 cm^{-1} region due to the pyridyl-*N*-oxide stretch; for the sulfonated derivatives, the values are about 20 cm^{-1} lower than for the corresponding nonsulfonated porphyrins (see Table S1)[2]. Porphyrin- *N*-oxide stretches for OEP-O (18) and TMP-O (23) appear at 1265 and 1273 cm^{-1}, respectively. Intense bands for the porphyrin-*N*-oxides synthesized here are in the range 1238–1261 cm^{-1}, and these likely result from overlap of the porphyrin- and pyridine-*N*-oxide stretches. The other spectral bands common to the porphyrin ring structure can be assigned according to the literature (32).

Mass spectrometry

The oxidopyridyl groups were deoxygenated using EI and LSIMS techniques and no moleular ion peak was seen, but this could be observed using MALDI-TOF mass spectrometry (using a dihydroxybenzoic acid matrix) (Table 6). Some of the parent peaks for the porphyrin-*N*-oxides were observed under EI ionization conditions (see Fig. 9), and HR-MS analyses were obtained for these species (Table 6).

Sulfonation reactions

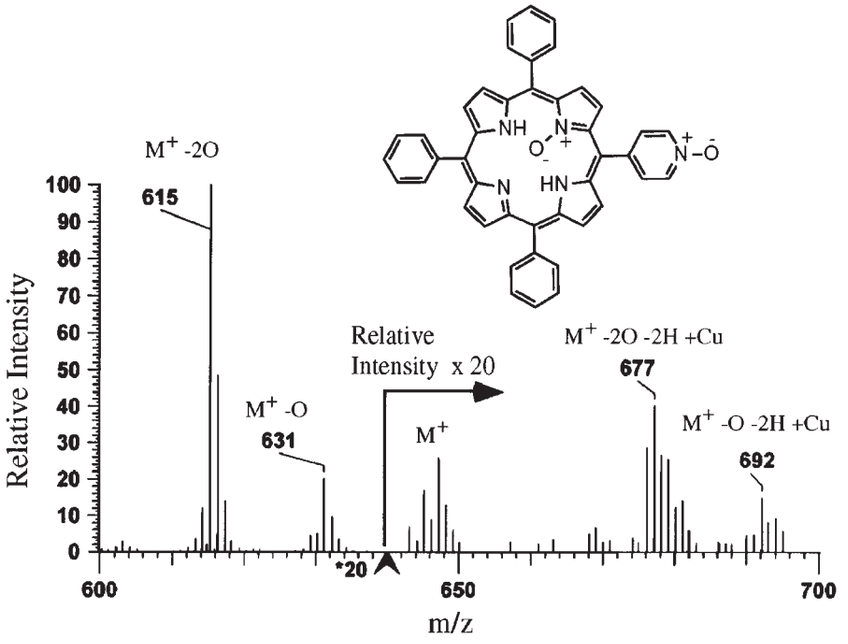
Sulfonation of the phenyl groups of OPyTrPhP and *c*-BOPyDPhP was achieved readily in hot, conc. H_2SO_4. Like the pyridyl subsituent, the oxidopyridyl group was not sulfonated under these conditions, and the *N*-oxide was retained. The pyridine nitrogen (or oxygen in the oxidopyridyl group) is certain to be protonated under the sulfonation conditions, thus making the pyridyl group unreactive towards electrophilic addition.

The sulfonated porphyrins, when isolated by solvent removal from an aqueous solution, retained several mole equivalents of water as judged by elemental analysis. This behavior for sulfonated porphyrins has been noted previously by our group (9).

Tirapazamine chemistry

Patents and publications on the biological activity of tirapazamine have appeared (33–35), and the desire to incorporate tirapazamine into a porphyrin was noted in the introduction. The use of triphosgene, a crystalline compound (36), in place of the highly toxic, gaseous phosgene, for reaction with tirapazamine, provided an effective, rapid route to **1**, although the product contained a small amount of tirapazamine. Compound **1** had previously been made in a 4 h reaction using phosgene, and its structure proposed (15) on IR data: ν(C=O) 1814 cm^{-1}, ν(C=N) 1541 cm^{-1}, and the absence of ν(NCO) bands at 2273–2000 cm^{-1} (37). Reaction of an amine with phosgene typically generates an isocyanate, and despite its cyclic structure, **1** does react like an

Fig. 9. Low-resolution mass spectrum (EI) of OpyTrPhP-21O.



isocyanate and produce ureas and carbamates when mixed with amines and alcohols, respectively, undergoing a ring-opening process (15, 33). Also typical of an isocyanate, **1** reacts with water (even on a TLC plate) to regenerate the amine tirapazamine.

The porphyrin bearing an amino group, APhTrPhP, was mixed with **1** to yield **2** in ~50% isolated yield. An IR peak seen at 1225 cm^{-1} due to ν(NO) was not observed in the aminophenyl porphyrin (38). Peaks attributed to tirapazamine appeared in the UV-vis spectrum, but these were in a region of relatively high background absorbance from the porphyrin core.

Conclusions

Porphyrins containing one to four pyridyl groups were synthesized and *N*-oxidized with *m*-CPBA to produce five novel (oxidopyridyl)porphyrins and seven porphyrin-*N*-oxides (including three from a *c*-BOPyBPhP-O mixture). Only three free-base porphyrin-*N*-oxides have been reported previously (OEP-O, TMP-O, and TPhP-O). The (oxidopyridyl)porphyrins and porphyrin-*N*-oxides were well characterized and an X-ray crystal structure of OPyTrPhP was obtained. Sulfonation of OPyTrPhP and *c*-BOPyDPhP was achieved readily under standard conditions with no loss of oxygen from the oxidopyridyl groups.

The product **1** from tirapazamine and triphosgene reacts like an isocyanate, reacting with porphyrins bearing amines and alcohols; the best results were obtained using APhTrPhP to produce the novel porphyrin conjugate **2**. The electrochemical and in vitro evaluation of these porphyrins incorporating *N*-oxide functionalities (39) will be published elsewhere.

Acknowledgments

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