Hydroxyphthalocyanines as Potential Photodynamic Agents for Cancer Therapy

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A series of benzyl-substituted phthalonitriles, substituted at the 3-, 4-, and 4,5-positions, underwent varied condensations with phthalonitrile to give a series of protected (monohydroxy- and polyhydroxyphthalocyaninato)zinc(II) derivatives which were readily cleaved to give several hydroxyphthalocyanines (ZnPc) (phthalocyanine phenol analogues). Their efficacy as sensitizers for the photodynamic therapy (PDT) of cancer was evaluated on the EMT-6 mammary tumor cell line. In vitro, the 2-hydroxy ZnPc (**32**) was the most active, followed by the 2,3- and 2,9-dihydroxy ZnPc (**39** and **45**), with the 2,9,16-trihydroxy ZnPc (**33**) exhibiting the least activity. In vivo, the monohydroxy derivative **32** and the 2,3-dihydroxy derivative **39** were both efficient in inducing tumor necrosis at 1 μmol kg^{-1}, but complete tumor regression was poor, even at 2 μmol/kg. In contrast, the 2,9-dihydroxy isomer **45**, at 2 μmol kg^{-1}, induced tumor necrosis in all animals treated, with 75% complete regression. These results underline the importance of the position of the substituents on the Pc macrocycle to optimize tumor response and confirm the PDT potential of the unsymmetrical Pcs bearing functional groups on adjacent benzene rings.

Introduction

Photodynamic therapy (PDT) has been proposed as an alternative treatment modality to complement conventional protocols in the management of malignant tumors. Thousands of patients have been treated by PDT worldwide, and a large majority of them have favorably responded to treatment.[1] PDT using a mixture of hematoporphyrin derivatives (Photofrin) as the photosensitizer is in an advanced experimental phase in several centers, especially for the endoscopic irradiation of lung, bronchial, and esophageal tumors and for the photosterilization of the tumor bed after surgical resection of large neoplasm,[2] and is approved as a sensitizer for photodynamic treatment of esophageal and early lung cancer in the United States, France, The Netherlands, and Japan. It is well-known that Photofrin is far from being the ideal drug for PDT[3] mainly because of its complex, variable, and unstable composition,[4] its low extinction coefficient at 630 nm, and the onset of long-term toxic effects, since some components of Photofrin are retained in amounts as large as several micrograms per gram of tissue by liver and spleen.[4d] For these reasons it appears essential to develop new tumor photosensitizers in order to overcome the present limitations of Photofrin.

Many synthetic porphyrin derivatives have been tested for toxicity and phototoxicity in PDT studies, and polyhydroxyporphyrins, especially *meso*-tetra(hydroxyphenyl)porphyrin, *meso*-tetra(sodium sulfonated)porphyrins, and (3,4-dihydroxyphenyl)porphyrin, are among the most active compounds.[5] Phthalocyanine (Pc) derivatives, as porphyrin analogues, have attracted much interest because of their many advantages over Photofrin such as existing as single substances, having large extinction coefficients and absorption in the convenient 650−800-nm range, and having no dark toxicity. Pcs and naphthalocyanines (Ncs) form stable chelates with metal cations, and the photosensitizing properties of aluminum, gallium, zinc, and silicon Pcs[6] and Ncs[7] have been documented.

A very important property of a photosensitizing drug is its hydrophobic and hydrophilic properties. Both the porphyrin and Pc skeletons are essentially hydrophobic. Tumor localization can be improved by introducing polar substituents to confer amphiphilicity and improve selectivity. Sulfonic acid, carboxylic acid, phosphonic acid, hydroxyl, and quaternary ammonium salts are among some of the common functional groups which can be used for this purpose. The relationship between the chemical structure and the tumor-localizing activity of photosensitizers has been investigated in detail using cultured cells and different experimental tumors.[8] It appears that optimal cell uptake efficiency is imparted to the porphyrin-type macrocycles by the presence of two substituents in adjacent rings, yielding an amphiphilic molecule which retains a hydrophobic matrix.[9] It has been shown that water-soluble disulfonated Pcs, particularly the "adjacent" disulfonated Pc isomers,[9] can diffuse readily through a cellular membrane and demonstrated that such disulfonated Pcs are particularly efficient drugs for inducing direct cell kill during in vivo PDT.[10] Comparison of cellular uptake of disulfonated tetraphenylporphyrin with sulfonate groups on adjacent opposite phenyl rings also showed the former as being more efficient.[11] Some 2,9,16,23-tetrahydroxy Pcs[12] have shown good photodynamic potential under in vitro and in vivo assays. Unfortunately, both the sulfonated

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phthalocyanines (including sulfonated porphyrins) and tetrahydroxyphthalocyanines were only prepared as a mixture of different isomers. Furthermore, the mono- or the "adjacent" disulfonated Pcs were only available in small amounts by tedious separation by HPLC, and these Pcs still existed as a mixture of sodium 3- and 4-monosulfonated Pcs or a mixture of "adjacent" and "opposite" Pc isomers.[13] In this paper, we report new directed specific synthetic methods to prepare pure mono-, "adjacent" di-, tri-, and tetrahydroxy Pcs. Their photobiological potentials were evaluated on EMT-6 mammary tumor cells growing on monolayer or implanted intradermally in BALB/c mice.

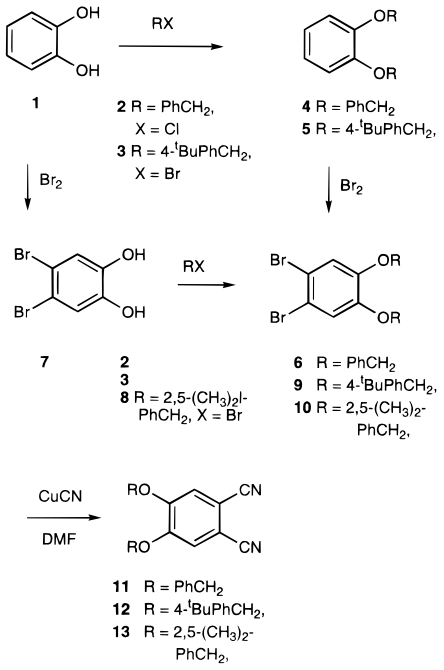
Results and Discussion

Chemistry. Because the self-condensation of hydroxyphthalonitrile cannot give the desired hydroxyphthalocyanines,[12a] the desired polyhydroxyphthalocyanines can only be obtained from the cleavage of the corresponding substituted phthalocyanines. Some published methods for the preparation of unsymmetrical Pcs include functionalization of preformed phthalocyanines and the mixed condensation of two different precursors.[14] Partial functional substitution of Pcs can be used to prepare unsymmetrical Pcs and to introduce hydrophilic groups. An example of this is the partial sulfonation of Pcs[13] which gives a very complex isomeric mixture. Time-consuming and tedious chromatographic separation procedures have to be used to isolate sulfonated Pcs from the complex mixture. Mixed condensation methods using two different precursors can also be used to prepare unsymmetrical Pcs and have similar disadvantages. These mixtures are very difficult to isolate by common chromatography due to the Pcs tendency toward aggregation.[15] There are some methods developed specifically for the preparation of unsymmetrical Pcs such as the solid-phase synthetic method[16,17] and the "sub-phthalocyanine" method,[18] but each needs several reaction steps and gives low yields. Thus, to prepare unsymmetrical Pcs a key aspect is the development of a suitable separation method to isolate these mixtures of Pcs.

Although flash chromatography does not efficiently separate these complex mixtures of Pcs, size exclusion chromatography is useful in the separation of these mixtures if there is a sufficient difference in size among these Pcs. The strategy of synthesis was to choose a suitable protecting group to prepare protected hydroxyphthalonitriles. The protecting group should be bulky enough to result in a large size difference among these substituted Pcs, so that it is possible to isolate these Pcs using gel permeation chromatography (size exclusive chromatography) (GPC). The protecting group should also be a good leaving group, because these unsymmetrical Pcs would be cleaved in the final step to give the designed polyhydroxyphthalocyanines.

Synthesis of Substituted Phthalonitriles. Since benzyl and substituted benzyl groups can be readily cleaved under a variety of conditions and are sufficiently bulky to provide one-isomer Pcs, separated by GPC, they were chosen to prepare the protected hydroxyphthalonitriles in this research. Thus, catechol (**1**) was treated in *N*,*N*-dimethylformamide (DMF) with benzyl chloride (**2**) and 4-*tert*-butylbenzyl bromide (**3**) to give

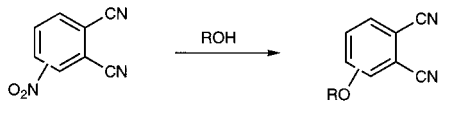
Scheme 1



1,2-bis(benzyloxy)benzene (**4**) and 1,2-bis(4-*tert*-butylbenzyloxy)benzene (**5**) in 92% and 90% yield, respectively (Scheme 1). Compound **4** could be easily brominated in dichloromethane to give 4,5-dibromo-1,2-bis(dibenzyloxy)benzene (**6**) in 83% yield, but unfortunately, when compound **5** reacted with bromine, the hydrogen bromide released from the bromination hydrolyzed the ether bond, which resulted in a complex mixture which included bromocatechol and 3,4,5-tribromo-1,2-bis(4-*tert*-butylbenzyloxy)benzene. An alternative route, including bromination followed by protection, was used to prepare substituted phthalonitriles. Treatment of catechol (**1**) with bromine in acetic acid gave 4,5-dibromocatechol (**7**)[19] in 85% yield. Mass spectroscopy of the crude products showed **7** as the main product but contaminated with 3,4,5- and 3,4,6-tribromocatechols which were very difficult to remove from the crude products. Flash column chromatography on silica gel, while very slowly eluting with benzene, yielded pure **7**. The reactions between **7** and benzyl chloride (**2**), **3**, and 2,5-dimethylbenzyl bromide (**8**) gave compound **6**, 4,5-dibromo-1,2-bis(4-*tert*-butylbenzyloxy)-benzene (**9**), and 4,5-dibromo-1,2-bis(2,5-dimethylbenzyloxy)benzene (**10**) in 90%, 85%, and 91% yield, respectively (Scheme 1). Treatment of **6**, **9**, or **10** with cuprous cyanide in refluxing DMF gave 4,5-bis(benzyloxy)phthalonitrile (**11**), 4,5-bis(4-*tert*-butylbenzyloxy)-phthalonitrile (**12**), or 4,5-bis(2,5-dimethylbenzyloxy)-phthalonitrile (**13**) in 60%, 45%, or 21% yield, respectively (Scheme 1).

Treatment of 4-nitrophthalonitrile (**14**) with diphenylmethanol (**15**) in DMSO gave 4-(diphenylmethoxy)-phthalonitrile (**16**) in 92% yield (Scheme 2).[20] The reactions between 3-nitrophthalonitrile (**17**), potassium carbonate, and *p*-*n*-butylbenzyl alcohol (**18**), benzyl alcohol (**19**), propargyl alcohol (**20**), or neopentyl alcohol (**21**) in DMSO gave 3-substituted phthalonitrile **22**, **23**, **24**, or **25** in 90%, 95%, 88%, or 90% yield, respectively (Scheme 2).[20] Treatment of **17** with sodium methoxide and methanol (**26**) in DMSO gave the sterically less

Scheme 2



14 4-NO_2 **15** R = $(Ph)_2CH$ **16** R = 4-$(Ph)_2CH$
17 3-NO_2 **18** R = *p*-*n*-BuPhCH$_2$ **22** R = 3-*p*-*n*-BuPhCH$_2$
19 R = $PhCH_2$ **23** R = 3-$PhCH_2$
20 R = $HCCCH_2$ **24** R = 3-$HCCCH_2$
21 R = $(CH_3)_3CCH_2$ **25** R = 3-$(CH_3)_3CCH_2$
26 R = CH_3 **27** R = 3-CH_3

demanding 3-methoxyphthalonitrile (**27**) in 90% yield (Scheme 2). These phthalonitriles were fully characterized by MS, IR, and different NMR techniques such as ^{1}H NMR, ^{13}C NMR, JMOD ^{13}C NMR, and proton−carbon correlation spectroscopy in attempts to assign different signals (Table 1).

Synthesis and Separation of Unsymmetrical Phthalocyanines. Mixed condensation methods were used to prepare unsymmetrical Pcs using two precursors. Since both phthalonitrile (**28**) and protected hydroxyphthalonitriles are hydrophobic, optimal reaction conditions are readily selected for these mixed condensations. Although a mixed condensation of two precursors in a 1:1 ratio gives a mixture of all Pc products, the use of one of the precursors in a large excess, such as a ratio of 10:1, promotes the possibility that one of these isomers becomes the main product. Thus, treatment of 4-(diphenylmethoxy)phthalonitrile (**16**) and phthalonitrile (**28**) in a ratio of 1:10 with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in 1-butanol at 100 °C for 1 h and followed by the addition of excess zinc acetate gave a mixture of [2-(diphenylmethoxy)-phthalocyaninato]zinc(II) (**29**) (50%) and zinc Pc with trace amounts of disubstituted Pcs (Scheme 3).

The same procedure was used to prepare [2,9,16-tris-(diphenylmethoxy)phthalocyaninato]zinc(II) (**30**) in 48% yield from **16** and **28** in a ratio of 10:1 (Scheme 3). Treatment of 3-(*p*-*n*-butylbenzyloxy)phthalonitrile (**22**) with **28** in a ratio of 1:10 using lithium in 1-octanol followed by addition of zinc acetate gave [1-(*p*-*n*-butylbenzyloxy)phthalocyaninato]zinc(II) compound (**31**) in 62% yield (Scheme 4). The cleavage of **29**−**31** by trifluoroacetic acid (TFA) and 1,2,4,5-tetramethylbenzene (TMB)[21] gave the corresponding (2-hydroxyphthalocyaninato)zinc(II) (**32**), (2,9,16-trihydroxyphthalocyaninato)zinc(II) (**33**), or (1-hydroxyphthalocyaninato)-zinc(II) (**34**) in 90%, 77%, or 85% yield, respectively (Schemes 3 and 4). The ^{1}H NMR spectrum of **32** showed a typical ABX splitting pattern with a doublet at 9.18 ppm (J = 8.0 Hz), a small doublet at 8.70 ppm (J = 1.6 Hz), and a doublet−doublet at 7.67 ppm (J = 8.0, 1.6 Hz) for the substituted benzene ring. There is a singlet at 10.70 ppm (OH), which disappeared after D_2O exchange.

4,5-Bis(benzyloxy)phthalonitrile (**11**) was first used to determine the possibility for this precursor to form Pcs. Treatment of **11**, DBU and zinc(II) in 1-butanol did give [2,3,9,10,16,17,23,24-octakis(benzyloxy)phthalocyaninato]zinc(II) (**35**) as a blue solid in 34% yield (Scheme 5). Similary, **11**−**13** with excess **28** gave 2,3-disubstituted Pcs **36**−**38** in 38%, 24%, and 18% yield, respectively (Scheme 5). The cleavage of **37**−**39** by TFA and TMB gave (2,3-dihydroxyphthalocyaninato)zinc(II) (**39**) in 58%, 84%, and 62% yield, respectively. Extensive flash chromatography followed by size exclusion chromatography[20] was used to purify all products (see Experimental Section).

Synthesis of "Adjacently" Substituted Phthalocyanines. To prepare a desired polyhydroxy Pc with amphiphilic properties for PDT study, a pure "adjacently" substituted Pc has to be synthesized first. A mixed condensation of an unsubstituted phthalonitrile and a substituted phthalonitrile could only give a mixture of the "adjacent" and "opposite" substituted Pcs which could not be separated by gel permeation chromatography.

A phthalonitrile dimer intermediate ("half Pc") has been shown to be an intermediate in Pc formation.[22a] To prepare the "adjacently" substituted Pcs a "half-Pc" has to be prepared. The "half-Pc" can undergo further condensation with another phthalonitrile in a large excess to give the desired "adjacent" Pcs without the "oppositely" substituted Pc.[22b]

The first attempt to prepare the "adjacent" Pc was to treat 4,5-disubstituted phthalonitriles with lithium methoxide in methanol, and then commercially available phthalonitrile (**28**) was added in a large excess. Unfortunately, this route gave 2,3-disubstituted Pcs as the main product because the two benzyloxy groups inactivated both cyano groups so that monomer intermediates were the main product instead of the desired "half Pc", when a disubstituted phthalonitrile such as 4,5-bis(benzyloxy)phthalonitrile (**11**) was treated with lithium methoxide.

Thus, **28** was first treated with lithium methoxide in methanol for a few hours to give a mixture of intermediates with the desired unsubstituted "half-Pc" as the main product (Scheme 6). After evaporation of methanol under reduced pressure, the mixture was directly used to undergo a further mixed condensation with **11**, **16**, or 4,5-dimethoxyphthalonitrile (**40**) in a large excess in 1-octanol to give a mixture containing the "adjacently" substituted Pc but without the "oppositely" substituted Pc (Scheme 6). The isolation of this mixture could give a pure "adjacent" Pc. In this way the "adjacent" alkoxy-substituted Pcs **41**−**43** were isolated. Pcs **42** and **43** were cleaved with TFA/TMB as before to yield the 2,3,9,10-tetrahydroxyphthalocyanine (**44**) and 2,9-dihydroxyphthalocyanine (**45**) (see Experimental Section).

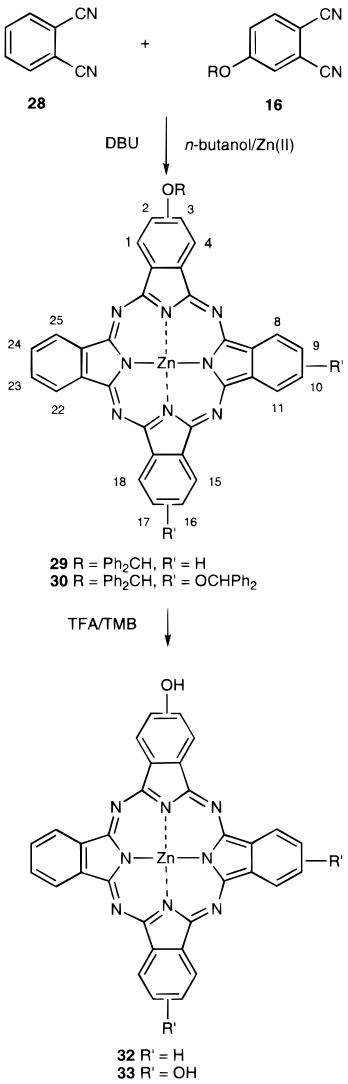
The ^{1}H NMR spectrum of a pure "adjacent" Pc, (2,3,9,10-tetramethoxyphthalocyaninato)zinc(II) (**41**), showed two doublets at 9.79 ppm (J = 7.4 Hz, 2H) and 9.71 ppm (J = 7.4 Hz, 2H) which were assigned to two hydrogens at the 15- and 25-positions and two hydrogens at the 18- and 22-positions of the Pc macrocycle, respectively. Two singlet signals at 9.24 and 9.19 ppm were assigned to the two hydrogens at the 1- and 11-positions and the 4- and 8-positions of the Pc molecule, respectively. Four hydrogens at the 15-, 16-, 22-, and 23-positions of the Pc gave multiplet signals. There were two singlet signals at 4.31 and 4.11 ppm which were assigned to two groups of hydrogens from the four methyl groups. ^{1}H NMR spectrum showed that the compound **41** is a pure adjacent Pc without contamination with the opposite Pc.

Synthesis of Single-Isomer 1,8,15,22-Tetrasubstituted Phthalocyanines. In one of our previous publications, an example in which 1,8,15,22-tetrakis(*p*-*n*-butylbenzyloxy)phthalocyanine was prepared from

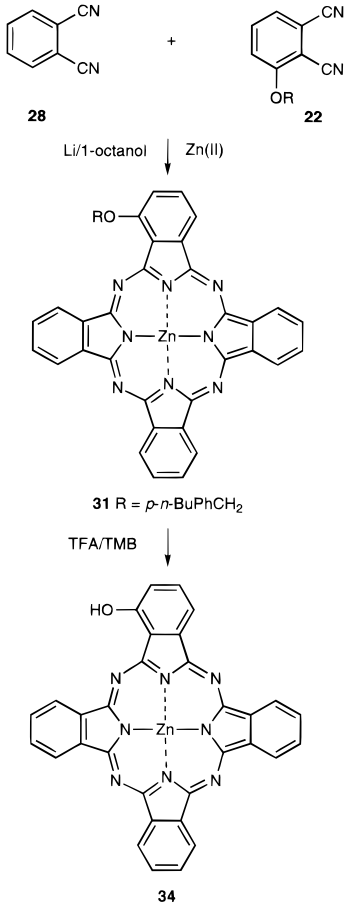
Table 1. ^{13}C NMR Chemical Shifts (ppm, δ) of Substituted Phthalonitriles

compd	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8
16	107.57	117.47	120.09	161.78	119.80	135.29	115.29	115.69
22	117.22	105.52	161.00	117.22	134.46	125.40	115.26	112.95
23	117.22	105.53	161.14	117.62	134.44	125.30	115.32	113.02
24	117.43	105.80	159.83	117.61	134.34	126.03	115.15	112.68
27	117.50	105.78	159.88	117.64	134.30	125.98	115.10	112.67

Scheme 3



Scheme 4

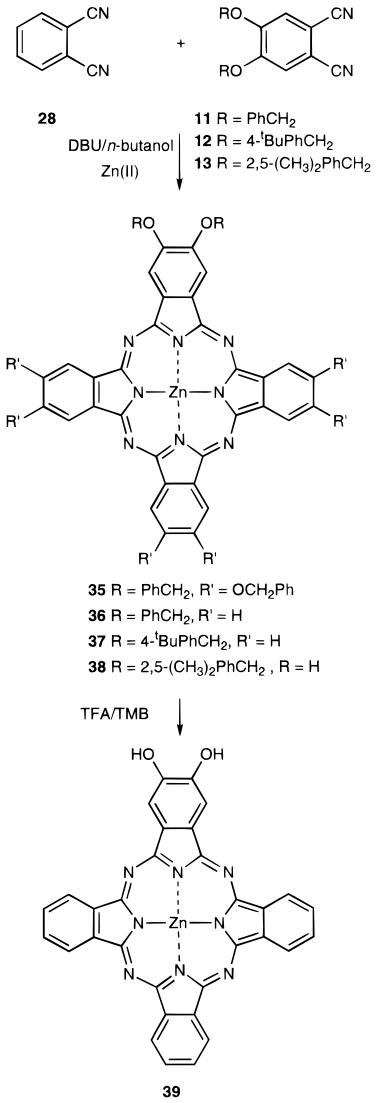


3-(*p*-*n*-butylbenzyloxy)phthalonitrile as a pure single isomer[20] was reported. In this paper more 1,8,15,22-tetrasubstituted Pcs were synthesized as pure single isomers. The effects of reaction temperature and size of substituents on the isomer distribution were also studied.

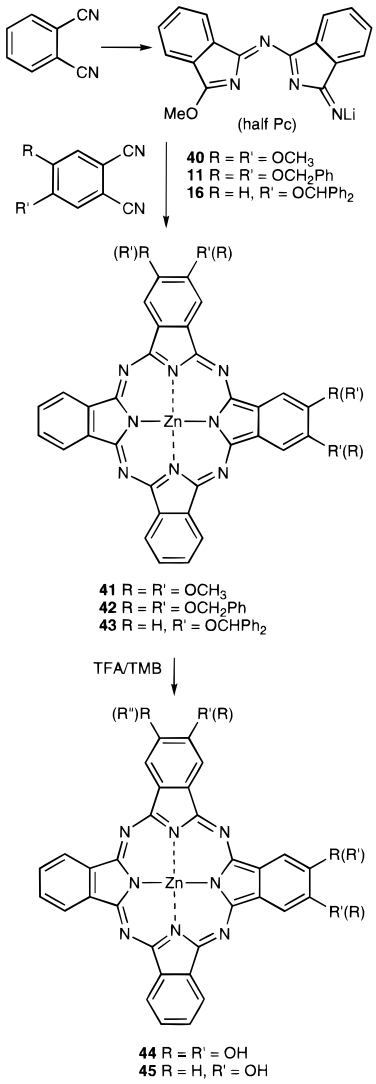
Treatment of phthalonitriles **22**−**25** and **27** with lithium in 1-octanol at different temperatures gave the corresponding 1,8,15,22-tetrakis(*p*-*n*-butylbenzyloxy) Pcs (**46**, **47**), 1,8,15,22-tetrakis(benzyloxy) Pcs (**48**, **49**, **50**), (tetrapropargyloxyphthalocyaninato)zinc(II) (**51**), 1,8,15,22-tetrakis(neopentyloxy) Pcs (**52**, **53**, **54**), and (1,8,15,22-tetramethoxyphthalocyaninato)zinc(II) (**55**) (Scheme 7). Cleavage of **47** in TFA/TMB as before gave 1,8,15,22-tetrahydroxyphthalocyanine (**56**) as a single isomer. The reaction temperature was believed to play an important role in the formation of single-isomer Pcs. Although a phthalonitrile intermediate with a bulky substituent can skew the reaction to give exclusively a Pc as a pure single isomer, it is believed that if the reaction temperature increases the intermediate will have enough energy to overcome steric interactions to form different isomers. To prove the supposition, the self-condensation of 3-(neopentyloxy)phthalonitrile was performed at different temperatures. As expected, when the reaction temperature is higher than 150 °C, Pc **54** was produced as a mixture of all possible isomers; when the reaction temperature was 60−80 °C, the Pc was produced as a pure single isomer (**53**); and when the reaction temperature was around 120 °C, a nonstatistical mixture of isomers was obtained. Self-condensations of other phthalonitriles gave similar results. ^1H NMR of compound **53** exhibited the desired doublet, triplet, and doublet generated by the hydrogens of the Pc macrocycle of the highly symmetrical molecule, and the ^{13}C NMR spectrum of **53** gave the 11 singlet signals which represent 11 groups of carbons in this symmetrical molecule. Compared to the spectra of **53**, the ^1H and ^{13}C NMR spectra of **54** gave multiplet signals.

The distribution of isomers depended on the bulky properties of substituents. Self-condensation of a 3-sub-

Scheme 5



Scheme 6

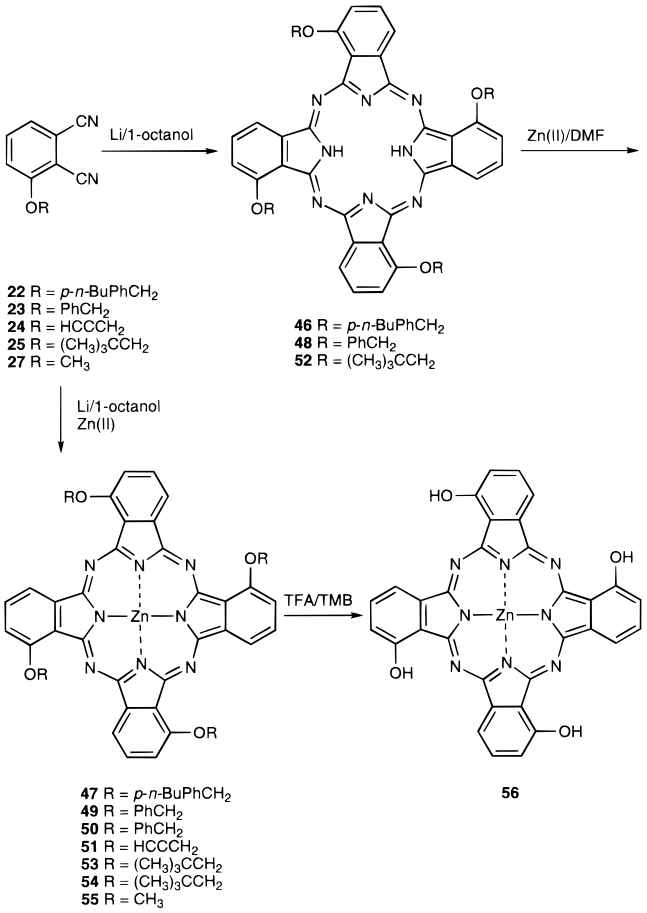


stituted phthalonitrile with a bulky substituent such as a *p*-*n*-butylbenzyloxy group gave the corresponding Pc as a pure single isomer even when the reaction was performed at 120−130 °C, but phthalonitriles with less bulky substituents such as neopentoxy or propargyloxy gave the Pc as a mixture of isomers at the same temperature. The results could be also used to explain why a high-temperature reaction gave a much lower yield. Instead of cyclizing to a Pc macrocycle, the strong steric interactions between two substituents may cause different intermediates to form linear polymers. FAB-MS spectra of the crude products show signals of linear polymers which possess higher molecular weights than the corresponding Pc.

Electronic effects are also considered to play a role in the reaction. It is believed that the electron-donating effects of the substituent could partially control the initial nuclear attack of alkoxy anion RO^- to only one of the two cyano groups to lead to only one intermediate which underwent further condensation to a Pc. 3-Methoxyphthalonitrile (**27**), the smallest hindered alkoxy-substituted phthalonitrile, was prepared for this study. It was believed that if the self-condensation of a 3-methoxyphthalonitrile could exclusively give one single isomer, it could be that the electronic effects played an important role in the condensation because there should exist enough space in a Pc macrocycle for two methoxy groups to exist in the "face-to-face" positions. Thus, to a solution of lithium in 1-octanol was added a solution of **27** in THF at 40−50 °C, and the solution was kept stirring overnight. Zinc acetate was added, and the reaction mixture was stirred for another 1−2 days. The reaction did give a blue zinc Pc in 50% yield. ^{1}H and ^{13}C NMR spectra of the Pc showed that the Pc, 1,8,15,-22-tetramethoxy zinc Pc (**55**), was a pure single isomer. Because the electron-donating effects of the methoxy group can affect ortho- and para-positions, it is obvious that the alkoxy anion RO^- favors attack at the cyano at the meta-position. The reactivities of the phthalonitrile will decrease as the reaction temperature decreases. When the temperature is low enough (40 °C) as used in this research, the cyano group at the 2-position will be totally inactive. Thus, at this temperature the alkoxy anion attacks only one of the two cyano groups and results in only one phthalonitrile monomer which undergoes further condensation to a Pc macrocycle.

In summary, both electronic effects and steric interactions were considered to affect the isomer distribution. When the reaction temperature is low (40 °C) electronic effects control the initial nucleophilic attack of RO^- to

Scheme 7



one of two cyano groups and give only one intermediate which undergoes further condensation to a pure single-isomer Pc. This observation matches the experimental results that self-condensation of all 3-substituted phthalonitriles described at 40−60 °C or lower have given Pcs as single isomers. When the reaction temperature increases to 120−130 °C, the nucleophilic attack of RO^- can result in two intermediates. Now the steric interaction controls the isomer distribution, and only phthalonitriles with a very bulky substituent such as *p-n*-butylbenzyloxy can give the corresponding Pc as a pure isomer. Those phthalonitriles with less bulky substituents such as propargyloxy and neopentyloxy give Pcs as mixtures of isomers. When the reaction is performed at a very high temperature such as 170 °C, different intermediates can be obtained, and they can overcome the activation barrier of steric interactions to form different Pc isomers. Thus, all Pcs are obtained as mixtures of isomers, but only a much lower yield can be obtained because of the increase of byproducts such as linear polymers.

Photobiological Properties. 1. Cell Photoinactivation. Cell survival was estimated by means of the colorimetric MTT assay. The light dose (LD_{90}) at 1 μM dye, or drug concentration (LC_{90}) at a constant fluence of 2.4 J cm^{-2}, required to inactivate 90% of the cells is given in Table 2. No dark toxicity was observed with any of the dyes at the concentrations under study. The four compounds tested (**32, 33, 39, 45**) exhibited good photoactivities, especially after 24-h incubation with 1 μM drug, with LD_{90} between 0.15 and 0.48 J cm^{-2}.

Table 2. EMT-6 Cell Photoinactivation and Dye Mobility of Different ZnPcs

compound (substituents)	LD_{90}[a] (J cm^{-2})		LC_{90}[b] (μM)		
	1 h	24 h	1 h	24 h	R_f[e]
ZnPc (pyridinium salt)	0.14[c]	NA	NA	NA	0.0
32 (2-hydroxy)	0.40	0.15	0.25	0.12	0.62
39 (2,3-dihydroxy)	2.40	0.25	1.00	0.30	0.0[f]
45 (2,9-dihydroxy)	7.50	0.40	3.20	0.58	0.55
33 (2,9,16-trihydroxy)	19.00	0.48	4.50	0.80	0.47
$ZnPc(OH)_4$ (2,9,16,23-tetrahydroxy)	NA	NA	>100[d]	NA	NA

[a] LD_{90}, light dose (λ > 590 nm, fluence rate 10 mW cm^{-2}) required to kill 90% of cells after 1- or 24-h incubation with a fixed drug concentration of 1 μM (SEM < 20%). [b] LC_{90}, drug dose required to kill 90% of cells after 1- or 24-h incubation with a fixed fluence of 2.4 J cm^{-2} (SEM < 20%). [c] Datum taken from ref 23. [d] As determined by colony formation assay with Chinese hamster V-79 fibroblasts.[12b] [e] Dye mobilities relative to the solvent front (R_f) on silica gel TLC in ethyl acetate/methanol (50:50) and 3% triethylamine. [f] Compound **39** did not migrate, possibly due to air oxidation to the quinone.

After 1-h incubation with 0.1 μM drug, the monohydroxy derivative **32** gave a LD_{90} of 0.25 J cm^{-2} whereas **33, 39**, and **45** exhibited LD_{90} > 2.4 J cm^{-2}. Increasing the dye concentration to 1 μM augmented the differences in phototoxicities among the dyes with LD_{90} varying between 0.4 and 19 J cm^{-2}. The relative activities correlate to some extent with differences in hydrophobicities. The latter is known to affect the rate of cell uptake, explaining in part variations in phototoxicity, although differences in intracellular localization also constitute an important parameter.[9b] Relative mobilities (R_f) on silica gel TLC in ethyl acetate/methanol (50:50) and 3% triethylamine are included in Table 2 as a measure of relative hydrophobicity. The 2,3-dihydroxy ZnPc (**39**) did not migrate in our TLC system, possibly due to its facile conversion to a 2,3-quinoid species. Decreasing hydrophobicity of the unsubstituted parent ZnPc via the addition of hydroxyl groups onto the macrocycle, i.e., 2-hydroxy (**32**), and 2,9-dihydroxy (**45**), 2,9,16-trihydroxy (**33**), 2,9,16,23-tetrahydroxy ZnPc,[12b] parallels the observed pattern of decreasing phototoxicity, with LC_{90} (Table 2: 2.4 J cm^{-2} after 1 h) from 0.25, 3.2, 4.5, to >100 μM, respectively. The $ZnPc(OH)_4$ was completely void of cellular photoactivity, even after 1-h incubation at 150 μM followed by exposure to a 2.4 J cm^{-2} light dose.[12b] The highly insoluble unsubstituted ZnPc is active as a pyridinium salt solubilized in Cremophor,[23] exhibiting a photocytotoxicity comparable to that of the monohydroxy derivative **32**. Both isomeric dihydroxy ZnPc **39** and **45**, as well as **32**, appear to remain monomeric upon dilution in serum from either THF solutions or Cremophor emulsions, as judged by the absence of a blue shift of the Q-band. In fact, their absorption maxima in serum are slightly red-shifted (data not shown). Quantum yields of singlet oxygen of monomeric metallo phthalocyanines are around 0.5; however, the actual in vivo yields of this cytotoxic species may vary substantially due to differences between the various dyes in tendencies to aggregate in the aqueous environment.[6d]

2. Photodynamic Therapy. The EMT-6 tumor

Table 3. EMT-6 Tumor Response Induced in BALB/c Mice after ZnPc (650−700 nm)- and Photofrin (600−650 nm)-Mediated PDT (200 mW cm^{-2}, 400 J cm^{-2})

compound (substituents)	concn (μmol kg^{-1})	*n*	tumor response (%)		
			necrosis[a]	regression[b]	edema[c]
ZnPc[24] (pyridinium salt)	2	NA	NA	100	NA
32	1	8	100	12	++++
(2-hydroxy)	2	4	25	25	++++
39	1	8	62	25	+++
(2,3-dihydroxy)	2	4	25	0	+++
45	1	3	0	0	+
(2,9-dihydroxy)	2	8	100	75	++
Zn−Pc(OH)$_4$[12b] (2,9,16,23-tetrahydroxy)	10	9	NA	0	NA
Photofrin[5c]	5 (mg kg^{-1})	10	100	70	+++

[a] Necrosis, percent of animals showing flat and necrotic tumors within 2−3 days after PDT. [b] Regression, percent of animals showing complete tumor regression 3 weeks after PDT. [c] Edema is determined 24 h after PDT: light (+), medium (++), important (+++), extensive (++++).

response induced in mice after photosensitization with the monohydroxy (**32**), the two isomeric dihydroxy (**39**, **45**), and the trihydroxy (**33**) ZnPc are presented in Table 3. For comparison, earlier reported data for the unsubstituted ZnPc, the ZnPc(OH)$_4$, and Photofrin are also included. The monohydroxy derivative **32** and the 2,3-dihydroxy isomer **39** induce substantial tumor necrosis at 1 μmol kg^{-1}. However, this effect was accompanied by extensive edema and resulted in low tumor cure rates. Surprisingly, increasing the dye dose to 2 μmol kg^{-1} decreased the PDT efficiency, most likely as a result of the extensive inflammatory response which obscures tumor necrosis. In contrast, the 2,9-dihydroxy isomer **45**, while being totally inactive at 1 μmol kg^{-1}, induced tumor necrosis and cure in 100% and 75% of animals, respectively, at 2 μmol kg^{-1} (1.2 mg kg^{-1}). Furthermore, edema was less as observed with compounds **32** and **39** at 1 μmol kg^{-1}. To obtain a similar tumor response with Photofrin requires a 5 mg kg^{-1} drug dose.[5c] The highly insoluble, unsubstituted ZnPc shows good tumor response at 2 μM kg^{-1} if administered as a pyridinium salt formulated in a Cremophor emulsion.[24] For comparison, the ZnPc(OH)$_4$ failed to induce tumor cure under the same light conditions, even at 10 μmol kg^{-1}.[12b] This lack of activity of the tetrahydroxy Pc is in strong contrast with the high photodynamic activities of tetrahydroxyphenyl porphyrins and chlorins. These differences likely reflect the conformational flexibility of the latter molecules. Several reports[12b,5a] indicate that biologically active conformations of substituted porphyrins and phthalocyanines require a critical distance between oxygen atoms, a requirement which cannot be fulfilled with the rigidly locked hydroxyls of the ZnPc(OH)$_4$. In our hydroxy Pc series it thus appears that the presence of hydroxyl groups on two adjacent benzene rings, e.g., as in **45**, optimizes the in vivo photoactivity of the ZnPc molecule. Such structural arrangements augment amphiphilicity of the dye, a property which promotes uptake by tumor cells and intracellular localization at photosensitive sites.[9b,c]

Experimental Section

Chemistry. All organic solvents were dried by appropriate methods and distilled before use. All reagents were freshly distilled or were recrystallized and then dried under reduced pressure before use. Anhydrous potassium carbonate (K_2CO_3) and zinc acetate ($Zn(AcO)_2 \cdot 2H_2O$) were finely ground, dried at 110 °C under vacuum for 36 h, and then stored in sealed vials. Unless otherwise noted, Matheson high-purity argon was used to maintain inert atmosphere conditions, and magnetic stirring methods were utilized during distillation and reaction processes. Thin-layer chromatography (TLC) was performed using silica gel G as the adsorbent. Flash chromatography was performed using silica gel of particle size 20−45 μm. Gel permeation chromatography was performed using Bio-beads, SX-2, 200−400 mesh. Melting points (mp) were determined using a Kofler hot stage melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Pye Unicam SP3-200 or a Perkin-Elmer infrared spectrophotometer, and FT-IR was performed on a Unicam Mattson 3000 FT-IR spectrometer using KBr disks. The ultraviolet−visible spectra (UV−vis) were recorded on a Hewlett-Packard HP8451A diode array spectrophotometer. Mass spectra (MS) were performed by Dr. B. Khouw (York University, Toronto, Ontario, Canada) and recorded at 70 eV on a VG Micromass 16F or a Kratos MS-50 triple analyzer mass spectrometer in the EI mode. The FAB-MS spectra were obtained with a Kratos MS-50 triple analyzer mass spectrometer equipped with a FAB ion source of standard Kratos design and Ion Tech atom gun. Microanalyses were performed by Guelph Chemical Laboratories Ltd., Guelph, Ontario (only analyses above ±0.4% calculated values are given as Pcs are notoriously difficult to combust). Nuclear magnetic resonance (NMR) spectra for protons and carbons were recorded on either a Bruker AM300 NMR spectrometer or a Bruker ARX400 high-field Fourier transform instrument. The positions of signals are reported in δ units (parts per million relative to tetramethylsilane (TMS)). The splitting patterns of the signals of proton resonances are described as singlets (s), doublets (d), triplets (t), quartets (q), doublets of doublets (dd), multiplets (m), or broad signals (br). ^{13}C NMR resonances are reported as the proton-decoupled chemical shifts, and in most cases the JMOD or/and DEPT ^{13}C NMR technique was used to differentiate carbons.

1,2-Bis(benzyloxy)benzene (4). From a solution of 22 g (0.20 mol) of catechol (**1**) in 150 mL of DMF, dissolved oxygen was removed by repeated evacuation followed by the admission of argon. Benzyl chloride (**2**) (53.13 g, 0.42 mol) was added and the system again deoxygenized. Hydrated potassium hydroxide (84% KOH) (26.5 g, 0.4 mol) and 1 mL of Aliquate 336 (phase-transfer agent)[25] were then added, and the mixture was heated at 120 °C under argon for 4 h with vigorous stirring. Water (300 mL) and 200 mL of methylene chloride were added. The organic layer was separated, and the aqueous layer was extracted with two 50-mL portions of methylene chloride. The combined organic extracts were washed with 150 mL of water and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica gel, eluting with toluene. Evaporation of the solvent gave a colorless solid. Recrystallization from methylene chloride gave **4** as white crystals: yield 90%; mp 60−61 °C; IR (KBr, cm^{-1}) ν 3030, 2941, 1260 and 1020 (Ar−O−C),

770, 741; ^{1}H NMR ($CDCl_3$) δ 7.45 (d, J = 8 Hz, 4H), 7.39−7.30 (m, 6H), 6.97−6.86 (m, 4H), 5.16 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 149.10, 137.43, 128.47, 127.76, 127.32, 121.67, 115.34, 71.35; EI-MS *m*/*z* (rel intensity) 291 [$(M + 1)^+$, 42], 290 (M^+, 53), 199 [$(M - PhCH_2)^+$, 50], 91 ($PhCH_2^+$, 100). Anal. ($C_{20}H_{18}O_2$) C, H.

1,2-Bis(4-*tert*-butylbenzyloxy)benzene (5). A mixture of 5.5 g (0.05 mol) of **1**, 25 g (0.11 mol) of 4-*tert*-butylbenzyl bromide (**3**), 5.6 g of hydrated potassium hydroxide (84% KOH), and 1 mL of Aliquate 336 was stirred at 120 °C for 4 h under argon. To this mixture were added 50 mL of water and 50 mL of methylene chloride, and the water layer was extracted twice with 30 mL of methylene chloride. The organic layer was washed with 50 mL of water and dried over $MgSO_4$ overnight. Evaporation of the solvent gave a brown oil. The residue was purified by silica gel chromatography, eluting with ethyl acetate and hexane (3:97). Recrystallization of the crude product from acetone gave **5** as white crystalline needles: yield 88%; mp 65−66 °C; IR (KBr, cm $^{-1}$) ν 3080, 3040, 2970, 1260 and 1015 (Ar−O−C), 756, 700; ^{1}H NMR ($CDCl_3$) δ 7.46 (m, 8H), 6.98−6.94 (m, 2H), 6.91−6.87 (m, 2H), 5.14 (s, 4H), 1.33 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 150.75, 149.34, 134.51, 127.29, 125.42, 121.57, 115.51, 71.34, 34.61, 31.51; EI-MS *m*/*z* (rel intensity) 402 (M^+, 30), 255 {$[M - (CH_3)_3CPhCH_2)]^+$, 28}, 147 $[(CH_3)_3CPhCH_2)^+$, 100]. Anal. ($C_{28}H_{34}O_2$) C, H.

4,5-Dibromocatechol (7).[19] Column chromatography on silica gel was used to purify **7** by removing tribromocatechols: yield 85%; IR (KBr, cm^{-1}) ν 3600−3250 (OH); ^{1}H NMR (acetone-d_6) δ 8.60 (s, br, 2H), 7.14 (s, 2H); ^{13}C NMR (acetone-d_6) δ 146.94, 120.72, 113.58; EI-MS for $C_6H_4Br_2O_2$ *m*/*z* (rel intensity) 266, 268, 270 {(M^+, $(M + 2)^+$, $(M + 4)^+$, 25, 51, 26}.

1,2-Bis(benzyloxy)-4,5-dibromobenzene (6). Method I: A solution of 9.48 g (0.06 mol) of bromine in 15 mL of methylene chloride was slowly added to an ice-salt-cooled solution of 8.7 g (0.03 mol) of **4** in 15 mL of methylene chloride. The mixture was stirred for 2 h. The temperature was then raised to room temperature, and the mixture stirred for another 1 h. Excess bromine was neutralized with 10 mL of a 10% solution of sodium bisulfide. The solution was then washed with a 10% solution of sodium bicarbonate and water. The extract was dried overnight using $MgSO_4$. The residue was purified by silica gel chromatography, eluting with toluene and hexane (1:3). The crude product was then recrystallized from methylene chloride to give **6** as white crystals: yield 92%.

Method II: A mixture of 2.0 g (0.01 mol) of compound **7**, 4.0 g (0.032 mol) of benzyl chloride (**2**), 2.0 g of hydrated potassium hydroxide (84% KOH), and 0.5 mL of Aliquate 336 was heated to 120 °C for 3 h while stirring under argon. The mixture was cooled and then poured into 300 mL of ice−water. The mixture was extracted with three 100-mL portions of ether. The ether layer was dried with $MgSO_4$ overnight. The reaction residue was purified by column chromatography on silica gel, eluting with toluene and hexane (1:3). Recrystallization of the crude product from methylene chloride gave **6** as white crystals: yield 90%; mp 144.5−155.5 °C; IR (KBr, cm^{-1}) ν 3070, 3050, 2940, 2880, 1250 (Ar−O−C), 1200, 1050, 765, 710; ^{1}H NMR (acetone-d_6) δ 7.49 (d, J = 7 Hz, 4H), 7.41−7.32 (m, 8H), 5.20 (s, 4H); ^{1}H NMR ($CDCl_3$) δ 7.42−7.32 (m, 10H), 7.15 (s, 2H), 5.10 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 148.83, 136.23, 128.61, 128.14, 127.35, 119.41, 115.49, 71.59; EI-MS *m*/*z* (rel intensity) 446, 448, 450 {M^+, $(M + 2)^+$, $(M + 4)^+$, 38, 70, 35}, 357 {$(M - PhCH_2)^+$, 19}, 91 ($PhCH_2^+$, 100). Anal. ($C_{20}H_{16}Br_2O_2$) C, H.

4,5-Dibromo-1,2-bis(2,5-dimethylbenzyloxy)benzene (9). Method II via **7** was used to prepare 0.46 g of compound **9** from 0.2 g of **7** and 0.40 g of 2,5-dimethylbenzyl bromide (**8**): yield 91%; mp 114−115.5 °C; IR (KBr, cm^{-1}) ν 3020, 2920, 1375, 1250 and 1080 (Ar−O−C); ^{1}H NMR ($CDCl_3$) δ 7.21 (s, 2H), 7.20 (s, 2H), 7.09 (d, J = 8 Hz, 2H), 7.05 (d, J = 8 Hz, 2H), 5.03 (s, 4H), 2.33 (s, 6H), 2.31 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 151.01, 149.18, 136.01, 135.18, 130.28, 130.13, 129.22, 128.38, 119.21, 70.51, 20.96, 18.35; EI-MS *m*/*z* (rel intensity) 502, 504, 506 {(M^+, $(M + 2)^+$, $(M + 4)^+$, 22, 45, 21}, 383, 385, 387 {$[M - (CH_3)_2PhCH_2]^+$, $[M + 2 - (CH_3)_2PhCH_2]^+$, $[M + 4 - (CH_3)_2PhCH_2]^+$, 15, 30, 14}, 266, 268, 270 {$[M - 2(CH_3)_2PhCH_2]^+$, $[M + 2 - 2(CH_3)_2PhCH_2]^+$, $[M + 4 - 2(CH_3)_2PhCH_2]^+$, 18, 37, 17}, 119 {$(CH_3)_2PhCH_2^+$, 100}. Anal. Calcd ($C_{24}H_{24}Br_2O_2$): C, 57.14; H, 4.76. Found: C, 57.69; H, 4.32.

4,5-Dibromo-1,2-bis(*p*-*tert*-butylbenzyloxy)benzene (10). Method II via **7** was used to prepare 0.47 g of **10 (**from 0.20 g of **7** and 0.45 g of **3)** as white crystals in 85% yield: mp 83.5−84.5 °C; IR (KBr, cm $^{-1}$) ν 3018, 2920, 1376, 1250 and 1080 (Ar−O−C); ^{1}H NMR ($CDCl_3$) δ 7.41 (d, J = 8.2 Hz, 4H), 7.36 (d, J = 8.2 Hz, 2H), 7.19 (s, 2H), 5.09 (s, 4H), 1.30 (s, 18H); EI-MS *m*/*z* (rel intensity) 558, 560, 562 {M^+, $(M + 2)^+$, $(M + 4)^+$, 30, 59, 32}, 411, 413, 415 (9, 18, 8), 266, 268, 270 (13, 24, 12), 147 (100). Anal. ($C_{28}H_{32}Br_2O_2$) C, H.

4,5-Bis(benzyloxy)phthalonitrile (11). A mixture of 3.2 g (7.17 mmol) of compound **6** and 1.98 g (22.0 mmol) of CuCN in 60 mL of DMF was stirred at 150 °C for 8 h. The reaction mixture was cooled and then poured into 300 mL of concentrated ammonia solution. The mixture was stirred overnight and then filtered. The solid was washed with 10% aqueous ammonia and water and then extracted with 200 mL of acetone. The residue was then purified by column chromatography on silica gel, eluting with benzene. Recrystallization of the crude product from benzene gave white crystalline needles: yield 60%; mp 183.5−184.5 °C; IR (KBr, cm^{-1}) ν 3120, 3070, 2950, 2900, 2242 (CN), 1595, 1530, 1370, 1300, 1240 and 1095 (Ar−O−C), 898, 765, 745; ^{1}H NMR ($CDCl_3$) δ 7.45 (m, 10H), 7.17 (s, 2H), 5.24 (s, 4H); EI-MS *m*/*z* (rel intensity) 340 (M^+, 40), 249 (100), 158 (22), 91 (92). Anal. ($C_{22}H_{16}N_2O_2$) C, H, N.

4,5-Bis(4-*tert*-butylbenzyloxy)phthalonitrile (12). Similarly, **9** was used to prepare 0.20 g of compound **12** (from 0.56 g of **9)** as white crystals in 45% yield: mp 181−182 °C; IR (KBr, cm^{-1}) ν 3022, 2241 (CN), 1255 and 1094 (Ar−O−C), 770, 750; ^{1}H NMR ($CDCl_3$) δ 7.40 (d, J = 8.4 Hz, 4H), 7.30 (d, J = 8.4 Hz, 4H), 7.15 (s, 2H), 5.17 (s, 4H), 1.31 (s, 18H); EI-MS *m*/*z* (rel intensity) 453 [$(M + 1)^+$, 15], 305 [$(M - t\text{-}BuPhCH_2)^+$, 20], 147 ($t$-$BuPhCH_2^+$, 100). Anal. ($C_{30}H_{32}N_2O_2$) C, H, N.

4,5-Bis(2,5-dimethylbenzyloxy)phthalonitrile (13). Similarly, **10** was used to prepare 0.083 g of compound **13** (from 0.5 g of **10)** as white crystals: yield 21%; mp 188.5−190 °C; IR (KBr, cm^{-1}) ν 3020, 2240 (CN), 1260 and 1092 (Ar−O−C), 768, 748; ^{1}H NMR ($CDCl_3$) δ 7.23 (s, 2H), 7.15 (s, 2H), 7.10 (d, J = 7.0 Hz, 2H), 7.06 (d, J = 7.0 Hz, 2H), 5.09 (s, 4H), 2.28 (s, 6H), 2.27 (s, 6H); EI-MS *m*/*z* (rel intensity) 397 {$(M + 1)^+$, 18}, 277 {$(M - 2,5\text{-dimethylPhCH}_2)^+$, 18}, 119 (2,5-dimethylPhCH_2^+, 70). Anal. Calcd ($C_{26}H_{24}N_2O_2$): C, 78.70; H, 6.10; N, 7.06. Found: N, 6.42.

4-(Diphenylmethoxy)phthalonitrile (16). A previously described procedure[20] was used to prepare **16** (0.86 g) (from **14** (0.52 g) and diphenylmethanol (**15**) (0.55 g)) in 92% yield as white crystals: mp 156−157 °C; IR (KBr, cm^{-1}) ν 3080, 3020, 2880, 2230 (CN), 2220 (CN), 1575, 1468, 1295, 1245 and 1080 (Ar−O−C), 1040, 820, 790; ^{1}H NMR ($CDCl_3$) δ 7.67 (d, J = 9 Hz, 1H), 7.42−7.32 (m, 11H), 7.25 (dd, J = 9, 2.4 Hz, 1H), 6.28 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 161.09, 139.00, 135.15, 129.05, 128.64, 126.74, 121.14, 120.68, 117.44, 115.54, 115.17, 107.71, 83.16; EI-MS *m*/*z* (rel intensity) 310 (M^+, 6), 284 (48), 237 (32), 167 (Ph_2CH^+, 100), 152 (58), 115 (54), 89 (50), 82 (73), 63 (32), 51 (16). Anal. ($C_{21}H_{14}N_2O$) C, H, N.

3-(*p*-*n*-Butylbenzyloxy)phthalonitrile (22).[20] To a solution of 0.52 g of 3-nitrophthalonitrile (**17**) and 1.18 g of *p*-*n*-butylbenzyl alcohol (**18**) (7.2 mmol) in 10 mL of DMSO was added 0.44 g of potassium carbonate. The solution was stirred for 24 h at room temperature. TLC showed three spots (first, **18**; second, the product **22**; third, **17**). Potassium carbonate (0.44 g) and **17** (0.507 g) were added to this solution which was stirred for 48 h. Again, to the solution was added 0.3 g of potassium carbonate, and the solution was stirred for another 48 h. TLC did not show the presence of any remaining **17**. The reaction mixture was poured into 150 g of ice−water; the yellow precipitate was filtered, washed with cold water, and dried. The crude product was purified by flash column chromatography on silica gel, eluting with THF and hexane (8:92, v/v) to remove a small amount of **18** and then with THF

and hexane (25:75, v/v) to collect **22**. Evaporation of the solvent gave a white solid which recrystallized from benzene as white crystals: yield 95%; mp 70−71 °C; IR (KBr, cm^{-1}) ν 3080, 3020, 2950, 2920, 2850, 2250 (CN), 2220 (CN), 1575, 1470, 1295, 1280, 1250 (Ar−O−C), 1180, 1080, 1040, 840, 790; ^{1}H NMR ($CDCl_3$) δ 7.59 (t, J = 7.6 Hz, 1H), 7.54−7.16 (m, 6H), 5.24 (s, 2H), 2.63 (t, J = 7.7 Hz, 2H), 1.62−1.57 (m, 2H), 1.39−1.31 (m, 2H), 0.93 (t, J = 7.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 161.14, 143.68, 134.44, 131.75, 128.98, 127.34, 125.30, 117.62, 117.22, 115.32, 113.02, 105.53, 71.57, 35.38, 33.51, 22.34, 13.91; EI-MS m/z (rel intensity) 291 {$(M + 1)^+$, 18}, 247 (20), 147 ($C_4H_9PhCH_2^+$, 100), 127 (22), 116 (33), 104 (44), 91 (40), 78 (27), 64 (23), 51 (20). Anal. ($C_{19}H_{18}N_2O$) C, H, N.

3-(Benzyloxy)phthalonitrile (23). Similarly **19** was used to prepare **23** (0.63 g) in 90% yield (from 0.52 g of **17** and 0.32 g of benzyl alcohol (**19**)) as shining white crystals: mp 168−169 °C; IR (KBr, cm^{-1}) ν 3075, 3020, 2950, 2920, 2850, 2250 (CN), 2225 (CN), 1575, 1470, 1290, 1280, 1250 (Ar−O−C), 1185, 1080, 1040, 840, 790; ^{1}H NMR ($CDCl_3$) δ 7.62 (t, J = 7.9 Hz, 1H), 7.58−7.34 (m, 6H), 7.31 (d, J = 8.8 Hz, 1H), 5.23 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 161.00, 134.57, 134.46, 128.94, 128.69, 127.01, 125.40, 117.53, 117.22, 115.26, 112.95, 105.52, 71.48; EI-MS m/z (rel intensity) 235 {$(M + 1)^+$, 19}, 91 ($PhCH_2^+$, 100). Anal. ($C_{15}H_{10}N_2O$) C, H, N.

3-(Propargyloxy)phthalonitrile (24). Similarly, **20** was used to prepare 0.48 g of **24** from 0.52 g of **17** and 0.18 g of propargyl alcohol (**20**) as white crystals in 88% yield: mp 149−151 °C; IR (KBr, cm^{-1}) ν 3130, 3045, 2910, 2920, 2840, 2245 (CN), 2220 (CN), 1572, 1460, 1445, 1275, 1175, 1035, 1015; ^{1}H NMR ($CDCl_3$) δ 7.69 (t, J = 7.6 Hz, 1H), 7.45−7.41 (m, 2H), 4.91 (d, J = 2.1 Hz, 2H), 2.62 (t, J = 2.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 159.83, 134.34, 126.03, 117.61, 117.43, 115.15, 112.68, 105.80, 76.57, 75.94, 57.20; EI-MS m/z (rel intensity) 183 [$(M + 1)^+$, 25], 39 (100). Anal. ($C_{11}H_6N_2O$) C, H, N.

3-(Neopentyloxy)phthalonitrile (25).[26] Compound **25** was prepared by following the literature,[26] from the corresponding neopentyl alcohol and **17**.

3-Methoxyphthalonitrile (27).[27] To 1 mL of freshly distilled methanol (**26**) was added 0.02 g of sodium. To the solution was added 5 mL of DMSO and 0.8 g (5 mmol) of **17**, and the solution was stirred for 3 h. TLC analysis of the reaction mixture showed that some **17** remained; 1 mL of methanol and 0.4 g of potassium carbonate were added, and the solution was stirred until all of **17** reacted. The mixture was poured into 100 mL of ice−water to give a yellow precipitate, which was filtered, washed with cold water, and dried. After chromatography on silica gel, eluting with benzene, 0.80 g of **27** (90%) was obtained. Recrystallization of **27** from benzene gave shining crystals: mp 185.5−186.5 °C (lit.[27] mp 183−184 °C); IR (KBr, cm^{-1}) ν 3020, 2920, 2840, 2245 (CN), 2225 (CN), 1572, 1460, 1445, 1255 and 1075 (Ar−O−C), 1035, 1015; ^{1}H NMR ($CDCl_3$) δ 7.68 (t, J = 8.2 Hz, 1H), 7.38 (d, J = 8.1 Hz, 1H), 7.27 (d, J = 8.1 Hz, 1H), 4.03 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 159.88, 134.30, 125.98, 117.64, 117.50, 115.10, 112.67, 105.78, 56.30; EI-MS m/z (rel intensity) 158 (M^+, 100], 143 [$(M - CH_3)^+$, 25].

General Preparation and Separation of Unsymmetrical Substituted Pcs. A mixture of 0.5 g (3.9 mmol) of **28** and 1.21 g (3.9 mmol) of **16** in 15 mL of *n*-butanol was heated to 100 °C and stirred until the phthalonitriles dissolved. To the solution was added 1.5 mL of DBU, and the solution was stirred for 1 h. Zinc acetate (0.1 g) was added, and the solution was stirred at 100−110 °C overnight. The reaction mixture was cooled to 60 °C, and 100 mL of methanol and water (1:1) was added to give a blue precipitate. The precipitate was collected by centrifugation, washed with methanol and hexane, and dried. TLC analysis of the crude products showed several colored spots. Half of the crude products were dissolved in 10 mL of THF, preabsorbed on 10 g of flash silica gel, and put onto a flash silica gel column. Elution with THF and hexane (from 3:97 to 20:80, v/v) gave different fractions which were separately purified by another flash column chromatography on silica gel to give [2-(diphenylmethoxy)phthalocyaninato]-zinc(II) (**29**), [2,9- and 2,16-bis(diphenylmethoxy)phthalocyaninato]zinc(II), [2,9,16-tris(diphenylmethoxy)phthalocyaninato]-zinc(II) (**30**), or 2,9,16,23-tetrakis(diphenylmethoxy) zinc Pc.

An alternative isolation was performed only by size exclusion chromatography columns on Bio-beads gel (SX-2). The crude product (20 mg) was put onto a column and eluted with THF. A few different blue-colored bands could be seen on the column. These bands were collected and purified by another size exclusion column chromatography, respectively. In general, three or four columns were needed to obtain a pure Pc.

The ideal isolation method was achieved by a combination of flash chromatography and gel permeation chromatography. The crude product was put onto a silica gel column and first eluted with benzene to collect a mixture of tetrasubstituted zinc Pc and **30** (with small amounts of disubstituted zinc Pcs). Further elution with benzene and ethyl acetate (1:1) gave a mixture enriched in disubstituted zinc Pcs together with small amounts of **29** and zinc Pc. The last eluted with ethyl acetate gave **29** with small amounts of zinc Pc and disubstituted zinc Pcs. The three fractions were purified separately by size exclusion column chromatography on Bio-beads gel (SX-2) and eluted with THF to give **29** (6%), disubstituted zinc Pcs (13%), **30** (12%), and zinc Pc (17%) in a total yield of 48% (calculation based on **16**). Because a fast silica gel column could give several fractions and each of them enriched in one of the products, and another gel permeation column could give an almost pure Pc, the method is convenient, time-saving, and used to separate other mixtures of Pcs. FAB-MS: for tetrakis-(diphenylmethoxy) zinc Pc, $C_{84}H_{56}N_8O_4Zn$ m/z (rel intensity) 1304, 1305 {M^+, $(M + 1)^+$, 88, 95}, 1137 {$(M - Ph_2CH)^+$, 60}, 970 {$(M - 2Ph_2CH)^+$, 40}, 803 {$(M - 3Ph_2CH)^+$, 32}, 636 {$(M - 4Ph_2CH)^+$, 20}; for **30**, $C_{71}H_{46}N_8O_3Zn$ m/z (rel intensity) 1122 (M^+, 100), 955 {$(M - Ph_2CH)^+$, 65}, 788 {$(M - 2Ph_2CH)^+$, 38}, 621 {$(M - 3Ph_2CH)^+$, 32}; for bis(diphenylmethoxy) zinc Pcs, $C_{58}H_{36}N_8O_2Zn$ m/z (rel intensity) 940 (M^+, 85), 773 {$(M - Ph_2CH)^+$, 80}, 606 {$(M - 2Ph_2CH)^+$, 100}; for **29**, $C_{45}H_{26}N_8$-OZn m/z (rel intensity) 758 (M^+, 78), 591 {$(M - Ph_2CH)^+$, 60}.

[2-(Diphenylmethoxy)phthalocyaninato]zinc(II) (29). A mixture of 0.24 g (0.78 mmol) of **16**, 1.00 g (7.8 mmol) of **28**, and 1 mL of DBU in 20 mL of 1-butanol was heated to 100 °C for 1 h. To the solution was added zinc acetate, and the solution was stirred for 20 h. The mixture was cooled to room temperature, and 50 mL of methanol and water (1:1) was added. The precipitate was collected by centrifugation and purified to give 0.25 g of **29** as a blue solid in 42% yield: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 672 (5.20), 640 (4.38), 606 (4.45), 344 (4.78); IR (KBr, cm^{-1}) ν 3010, 1250 (Ar−O−C), 1120, 1085 (Ar−O−C); ^{1}H NMR (pyridine-d_5) δ 9.70−9.68 (m, 6H), 9.55 (d, J = 8.2 Hz, 1H), 9.52 (s, 1H), 8.21−8.16 (m, 6H), 8.11 (dd, J = 8.2, 2.0 Hz, 1H), 7.98 (d, J = 8 Hz, 4H), 7.48 (t, J = 7.8 Hz, 4H), 7.32 (t, J = 6.3 Hz, 2H), 6.98 (s, 1H); FAB-MS m/z (rel intensity) 758 (M^+, 78), 591 {$(M - Ph_2CH)^+$, 60}. Anal. Calcd ($C_{45}H_{26}N_8OZn$): C, 71.10; H, 3.45; N, 14.75. Found: N, 15.30.

[2,9,16-Tris(diphenylmethoxy)phthalocyaninato]zinc-(II) (30). The procedure described for the preparation of **29** was repeated with a different ratio to prepare 0.12 g of **30** as a blue solid (from 0.03 g of **28** and 0.24 g of **16** in 1:10 ratio) in 40% yield: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 676 (5.12), 608 (4.22), 348 (4.70); IR (KBr, cm^{-1}) ν 3030, 2862, 1250 and 1085 (Ar−O−C); ^{1}H NMR (pyridine-d_5) δ 9.74−9.71 (m, 2H), 9.57−9.51 (m, 6H), 8.20−8.17 (m, 2H), 8.13−8.06 (m, 3H), 7.96 (d, J = 8 Hz, 12H), 7.50−7.20 (m, 21H). Anal. ($C_{71}H_{46}N_8O_3Zn$) C, H, N.

[1-(*p*-*n*-Butylbenzyloxy)phthalocyaninato]zinc(II) (31). Lithium (0.2 g) was dissolved in 20 mL of 1-octanol. To the solution was added a solution of 0.5 g (3.9 mmol) of **28** and 0.11 g (0.39 mmol) of **22** in 4 mL of THF. The solution was stirred at 100 °C for 5 h. To the solution was added zinc acetate (0.15 g), and the solution was stirred overnight. The mixture was cooled to room temperature and quenched with methanol and water. The crude product was purified by gel permeation chromatography to give 0.18 g of **31** as a blue solid in 62% yield: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 676 (5.18), 646 (4.24), 610 (4.45), 342 (4.73); IR (KBr, cm^{-1}) ν 3010,

2920, 2862, 1255 and 1085 (Ar−O−C); ^{1}H NMR (pyridine-d_5) δ 9.73−9.69 (m, 6H), 9.46 (d, J = 7.8 Hz, 1H), 8.32 (d, J = 8.0 Hz, 2H), 8.25−8.18 (m, 7H), 7.86 (d, J = 8.0 Hz, 1H), 7.63 (d, J = 8.0 Hz, 2H), 5.92 (s, 2H), 2.83 (t, J = 7.5 Hz, 2H), 1.77−1.73 (m, 2H), 1.45−1.41 (m, 2H), 0.96 (t, J = 6.9 Hz, 3H); FAB-MS *m/z* (rel intensity) 738, 739 {M^+, $(M + 1)^+$, 91, 100}, 591 {$(M - R)^+$, 60}. Anal. ($C_{43}H_{30}N_8OZn$) C, H, N.

(2-Hydroxyphthalocyaninato)zinc(II) (32). A previously described procedure[20] was used to prepare **32** by the cleavage of compound **29** (76 mg, 0.1 mmol) with trifluoroacetic acid (TFA) and 1,2,4,5-tetramethylbenzene (TMB). This reaction gave **32** (53 mg, 0.09 mmol) as a blue solid in 90% yield: UV−vis (THF) λ_{max} (log ϵ) 672 (5.05), 604 (4.42), 346 (4.72); IR (KBr, cm^{-1}) ν 3650−3300 (OH); ^{1}H NMR (DMSO-d_6) δ 10.70 (s, 1H), 9.36−9.35 (m, 6H), 9.18 (d, J = 8 Hz, 1H), 8.70 (d, J = 1.6 Hz, 1H), 8.24−8.21 (m, 6H), 7.67 (dd, J = 9.5, 1.6 Hz, 1H); FAB-MS *m/z* (rel intensity) 592 (M^+, 100), 593 (94), 594 (90), 595 (88); HRMS (FAB) *m/z* calcd for $C_{32}H_{16}N_8OZn$ 592.0738, found 592.0710.

(2,9,16-Trihydroxyphthalocyaninato)zinc(II) (33). Similarily, the cleavage of **30** (112 mg, 0.1 mmol) gave **33** (48 mg, 0.077 mmol) in 77% yield: UV−vis (THF) λ_{max} (log ϵ) 678 (4.95), 610 (4.32), 348 (4.69); IR (KBr, cm^{-1}) ν 3650−3300 (OH); ^{1}H NMR (DMSO-d_6) δ 10.69 (s, br, 3H), 9.39−9.37 (m, 2H), 9.21−9.16 (m, 3H), 8.72−8.69 (m, 3H), 8.23−8.21 (m, 2H), 7.66−7.64 (m, 3H); FAB-MS *m/z* (rel intensity) 624, 625, 626 {M^+, $(M + 1)^+$, $(M + 2)^+$, 88, 93, 100}. Anal. ($C_{32}H_{16}N_8O_3Zn$) C, H, N.

(1-Hydroxyphthalocyaninato)zinc(II) (34). Cleavage of **31** (74 mg, 0.1 mmol) with TFA as above gave **34** (50 mg, 0.085 mmol) as a blue solid in 85% yield: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 686 (5.11), 622 (4.48), 338 (4.75); IR (KBr, cm^{-1}) ν 3650−3300 (OH); ^{1}H NMR (DMSO-d_6) δ 10.70 (s, br, 1H), 9.53 (br, 1H), 9.38−9.27 (m, 6H), 8.95 (br, 1H), 8.25−8.21 (m, 6H), 8.09 (br, 1H); FAB-MS *m/z* (rel intensity) 592 (M^+, 100), 593 (90), 594 (94), 595 (87). Anal. ($C_{32}H_{16}N_8OZn$) C, H, N.

[2,3,9,10,16,17,23,24-Octakis(benzyloxy)phthalocyaninato]zinc(II) (35). A mixture of 100 mg of compound **11** and 0.5 mL of DBU in 10 mL of 1-butanol was heated to 100 °C under argon for 1 h. To the mixture was added zinc acetate (0.1 g), and the solution was stirred overnight at 120 °C under argon. The mixture was cooled to room temperature, and 20 mL of methanol and water (1:1) was added. The blue precipitate was collected by centrifugation and was washed with methanol and then hexane. The blue solid was purified by flash column chromatography on silica gel, eluting with benzene and THF (3:1) to give pure 0.035 g of **35** in 34% yield: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 672 (5.10), 608 (4.35), 356 (4.78); IR (KBr, cm^{-1}) ν 3020, 1250 and 1082 (Ar−O−C); ^{1}H NMR (pyridine-d_5) δ 9.34 (s, 8H), 7.86 (d, J = 8 Hz, 16H), 7.47 (t, J = 7.5 Hz, 16H), 7.39 (t, J = 7.5 Hz, 8H), 5.58 (s, 16H); FAB-MS *m/z* (rel intensity) 1424 (M^+, 38), 1333, 1242, 1151, 1060 {$(M - 4PhCH_2)^+$, 100}, 969, 878. Anal. ($C_{88}H_{64}N_8O_8Zn$) C, H, N.

[2,3-Bis(benzyloxy)phthalocyaninato]zinc(II) (36). A mixture of 60 mg (0.18 mmol) of compound **11**, 276 mg (2.16 mmol) of **28**, and 1.0 mL of DBU in 50 mL of 1-butanol was heated to 100 °C for 1 h under argon. To the mixture was added zinc acetate (0.15 g), and the solution was stirred at 100−110 °C overnight. The mixture was then cooled to room temperature, and 100 mL of methanol and water (1:1) was added to this mixture to give a blue precipitate. The precipitate was collected by centrifugation, washed with methanol and hexane, and extracted with ethyl acetate for 24 h in a Soxhlet extractor. The reaction residue was purified by size exclusion chromatography (SX-2), eluting with THF. Further purification was performed on a short flash silica gel column, eluting with hexane and THF (3:1): yield 38%; mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 668 (5.20), 638 (4.44), 604 (4.49), 348 (4.75); IR (KBr, cm^{-1}) ν 3020, 2840, 1080 (Ar−O−C); ^{1}H NMR (pyridine-d_5) δ 9.73−9.70 (m, 4H), 9.62 (d, J = 7.2 Hz, 2H), 9.36 (s, 2H), 8.23−8.16 (m, 6H), 7.92 (d, J = 6.5 Hz, 4H), 7.50 (t, J = 7.4 Hz, 4H), 7.39 (t, J = 7.2 Hz, 2H), 5.77 (s, 4H); FAB-MS *m/z* (rel intensity) 788 (M^+, 100), 697 ($M - PhCH_2^+$, 35), 606 ($M - 2PhCH_2^+$, 28). Anal. ($C_{46}H_{28}N_8O_2Zn$) C, H, N.

[2,3-Bis(*p-tert*-butylbenzyloxy)phthalocyaninato]zinc(II) (37). Similarily, **12** gave **37** (21 mg) (from 45 mg of **12** and 128 mg of **7**) in 24% yield: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 666 (5.05), 638 (4.38), 604 (4.40), 342 (4.65); IR (KBr, cm^{-1}) 3020, 1080 (Ar−O−C); ^{1}H NMR (pyridine-d_5) δ 9.79−9.76 (m, 4H), 9.64 (d, J = 7.8 Hz, 2H), 9.50 (s, 2H), 8.26−8.16 (m, 6H), 7.89 (d, J = 8 Hz, 4H), 7.66 (d, J = 8 Hz, 4H), 5.73 (s, 4H), 1.34 (s, 18H); FAB-MS *m/z* (rel intensity) 900 (M^+, 100), 753 (55), 606 (32). Anal. ($C_{54}H_{44}N_8O_2Zn$) C, H, N.

[2,3-Bis(2,5-dimethylbenzyloxy)phthalocyaninato]zinc(II) (38). Similarily, **13** gave **38** (30 mg) (from 79 mg of **13** and 256 mg of **7**) in 18% yield: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 664 (5.00), 638 (4.39), 602 (4.38), 338 (4.74); IR (KBr, cm^{-1}) ν 3022, 1080 (Ar−O−C); ^{1}H NMR (pyridine-d_5) δ 9.77−9.74 (m, 4H), 9.67 (d, J = 7.3 Hz, 2H), 9.56 (s, 2H), 8.48−8.14 (m, 6H), 7.67 (s, 2H), 7.16 (d, J = 7.5 Hz, 2H), 7.11 (d, J = 7.5 Hz, 2H), 5.75 (s, 4H), 2.56 (s, 6H), 2.30 (s, 6H); FAB-MS *m/z* (rel intensity) 844 (M^+, 100), 725 (42), 606 (36). Anal. ($C_{50}H_{36}N_8O_2Zn$) Calcd: C, 70.97; H, 4.23; N, 13.24. Found: N, 13.88.

(2,3-Dihydroxyphthalocyaninato)zinc(II) (39). A mixture of 40 mg of compound **36** and 200 mg of TMB in 10 mL of TFA was refluxed for 24 h under argon. The solvent was then evaporated under vacuum, and the residue was washed with hexane. The blue solid was dissolved in 4 mL of THF, and the solution was applied to a gel permeation column (SX-2), eluting with THF to give 14 mg of **39** as a blue solid in 58% yield. Cleavage of **37** and **38** gave **39** in 84% and 62% yield, respectively: UV−vis (THF) λ_{max} (log ϵ) 672 (5.06), 608 (4.30), 346 (4.67); IR (KBr, cm^{-1}) ν 3650−3300 (OH); ^{1}H NMR (DMSO-d_6) δ 10.34 (s, br, 2H), 9.47−9.37 (m, 6H), 8.76 (s, 2H), 8.30−8.20 (m, 6H); FAB-MS *m/z* (rel intensity) 608 (M^+, 100), 609 (92), 610 (90), 611 (78), 612 (76), 613 (53). Anal. ($C_{32}H_{16}N_8O_2Zn$) Calcd: C, 63.01; H, 2.64; N, 18.38. Found: N, 17.90.

(2,3,9,10-Tetramethoxyphthalocyaninato)zinc(II), an "Adjacent" Pc (41). To 20 mL of freshly distilled methanol were added 0.07 g of lithium (10 mmol) and 2.56 g (20 mmol) of **7**. The solution was refluxed for 2 h under argon. To 2 mL of the dark-green solution was added 1.84 g (10 mmol) of 4,5-dimethoxyphthalonitrile (**40**)[28] in 10 mL of 1-octanol. The mixture was heated to 100 °C and stirred overnight. Zinc acetate was added to the mixture, which was stirred for another 8 h. The mixture was poured into 50 mL of methanol and water (1:1) to give a blue precipitate which was collected by centrifugation. The residue was passed through a silica gel column to remove some impurities by eluting with THF and hexane (1:1). Further purification was performed by size exclusion column chromatography on Bio-beads gel (SX-2), and two main fractions, **41** and (2,3-dimethoxyphthalocyaninato)zinc(II), were collected in 28% and 22% yield, respectively. Compound **41** is a blue solid: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 668 (5.11), 640 (4.32), 604 (4.34), 354 (4.68); IR (KBr, cm^{-1}) ν 3010, 2960, 2862, 1375, 1085 (Ar−C−O); ^{1}H NMR (pyridine-d_5) δ 9.79 (d, J = 7.4 Hz, 2H), 9.71 (d, J = 7.4 Hz, 2H), 9.24 (s, 2H), 9.19 (s, 2H), 8.25−8.18 (m, 4H), 4.31 (s, 6H), 4.11 (s, 6H); FAB-MS *m/z* (rel intensity) 696 (M^+, 95), 697 (75), 698 (84), 699 (100), 700 (65). Anal. ($C_{36}H_{24}N_8O_4Zn$) C, H, N. (2,3-Dimethoxyphthalocyaninato)zinc(II) is also a blue solid: mp >320 °C; ^{1}H NMR (pyridine-d_5) δ 9.78−9.73 (m, 8H), 8.24−8.22 (m, 6H), 4.31 (s, 6H); FAB-MS for $C_{34}H_{20}N_8O_2Zn$ *m/z* (rel intensity) 636 (M^+, 100), 637 (90), 638 (96).

[2,3,9,10-Tetrakis(benzyloxy)phthalocyaninato]zinc(II), an "Adjacent" Pc (42). To 30 mL of freshly distilled methanol were added 0.07 g of lithium (10 mmol) and 2.56 g (20 mmol) of **7**. The solution was refluxed for 2 h under argon. To 1 mL of the solution was added 1.13 g (3.33 mmol) of **11** in 10 mL of 1-octanol. The mixture was heated to 80 °C and stirred overnight. Zinc acetate (0.35 g) was added to the mixture, which was stirred for another 8 h. The mixture was poured into 50 mL of methanol and water (1:1) to give a blue precipitate which was collected by centrifugation. Further

purification was performed by size exclusion column chromatography on Bio-beads gel (SX-2), and two main fractions, **42** and **36**, were collected in 18% and 12% yield, respectively. Compound **42** is a blue solid: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 672 (5.03), 638 (4.20), 608 (4.26), 354 (4.67); IR (KBr, cm^{-1}) ν 3020, 2862, 1850 (Ar−C−O); ^{1}H NMR (pyridine-d_5) δ 9.83−9.72 (m, 4H), 9.52 (s, 2H), 9.50 (s, 2H), 8.25−8.15 (m, 6H), 7.92−7.88 (m, 8H), 7.35−7.20 (m, 12H), 5.93 (s, 4H), 5.88 (s, 4H); FAB-MS *m/z* (rel intensity) 1000 (M^+, 90), 909 {(M − $PhCH_2)^+$, 80}, 818 {(M − $2PhCH_2)^+$, 61}, 727 {(M − $3PhCH_2)^+$, 50}, 636 {(M − $4PhCH_2)^+$, 32}. Anal. ($C_{60}H_{40}N_8O_4Zn$) C, H, N.

[2,9-Bis(diphenylmethoxy)phthalocyaninato)zinc(II) (43). The procedure previously described for the preparation of **41** was used to prepare **43** from **16** and **28**: yield 30%; mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 676 (5.20), 606 (4.40), 346 (4.79); IR (KBr, cm^{-1}) ν 3010, 1250, 1120, 1085 (Ar−O−C); ^{1}H NMR (pyridine-d_5) δ 9.74−9.70 (m, 4H), 9.62−9.59 (m, 4H), 8.23−8.20 (m, 4H), 8.16−8.8.13 (m, 2H), 7.97−7.95 (m, 8H), 7.53−7.30 (m, 12H), 7.05−6.98 (m, 2H); FAB-MS *m/z* (rel intensity) 940 (M^+, 100), 773 {(M − $Ph_2CH)^+$, 66}, 606 {(M − $2Ph_2CH)^+$, 52}. Anal. ($C_{58}H_{36}N_8O_2Zn$) Calcd: C, 73.93; H, 3.85; N, 11.89. Found: C, 72.95.

(2,3,9,10-Tetrahydroxyphthalocyaninato)zinc(II) (44). Cleavage of **42** (100 mg, 0.1 mmol), as for **32**, gave **44** (27 mg, 0.042 mmol) as a blue solid in 42% yield: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 676 (5.06), 610 (4.32), 344 (4.67); IR (KBr, cm^{-1}) ν 3650−3300 (OH); ^{1}H NMR (DMSO-d_6) δ 10.64 (s, br, 2H), 10.57 (s, br, 2H), 9.57−9.40 (m, 4H), 8.68 (s, 1H), 8.58 (s, 1H), 8.30−8.20 (m, 4H); FAB-MS *m/z* (rel intensity) 640 (M^+, 100). Anal. ($C_{32}H_{16}N_8O_4Zn$) C, H, N.

(2,9-Dihydroxyphthalocyaninato)zinc(II) (45). Cleavage of **43** (94 mg, 0.1 mmol) by TFA gave **45** (45 mg, 0.088 mmol) as a blue solid in 88% yield: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 676 (4.99), 610 (4.25), 344 (4.60); IR (KBr, cm^{-1}) ν 3650 (br, OH); ^{1}H NMR (DMSO-d_6) δ 10.74 (s, br, 2H), 9.40−9.35 (m, 2H), 9.20−9.17 (m, 2H), 8.76−9.71 (m, 2H), 8.25−8.08 (m, 6H), 7.80−7.75 (m, 2H); FAB-MS *m/z* (rel intensity) 608 (M^+, 100). Anal. ($C_{32}H_{16}N_8O_2Zn$) Calcd: C, 63.01; H, 2.64; N, 18.38. Found: C, 63.60.

1,8,15,22-Tetrakis(*p*-*n*-butylbenzyloxy)phthalocyanine (46). Lithium (0.30 g) was suspended in 30 mL of 1-octanol. The suspension was heated to 170 °C and stirred for 4 h. To the homogeneous solution, cooled to 40 °C, was added 0.35 g (1.2 mmol) of **22** in 4 mL of dried THF. The temperature was raised to 60 °C, and the solution was stirred for 2 h. Then, the temperature was raised to 100 °C, and the mixture stirred for 12 h. The temperature was further raised to 120 °C, and the solution was stirred for another 2 h. The mixture was cooled to room temperature, and the reaction was quenched with methanol and water (1:1) to form a blue precipitate. The precipitate was collected by centrifugation, washed successively with water, methanol, and hexane, and dried to give 126.7 mg (40%) of a dark-green solid ,**46**: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 724 (4.70), 690 (4.71), 658 (4.02), 627 (2.95), 395 (3.46), 351 (3.96); IR (KBr, cm^{-1}) ν 3310 (N−H), 3010, 2960, 2920, 2860, 1665, 1530, 1440, 1385, 1240 (Ar−O−C), 1175, 1145, 1110, 1010, 925, 850; ^{1}H NMR (pyridine-d_5) δ 9.47 (d, J = 7.5 Hz, 4H), 8.33 (d, J = 7.8 Hz, 8H), 8.25 (t, J = 7.5 Hz, 4H), 7.90 (d, J = 8.0 Hz, 4H), 7.65 (d, J = 7.7 Hz, 8H), 5.93 (s, 8H), 2.83 (t, J = 7.5 Hz, 8H), 1.83−1.73 (m, 8H), 1.51−1.42 (m, 8H), 0.99 (t, J = 7.5 Hz, 12H), −4.2 (s, br, 2H); FAB-MS *m/z* (rel intensity) 1164 {(M + 2)$^+$, 68}, 1017 {[(M + 2) − R]$^+$, 56}, 870 {[(M + 2) − 2R]$^+$, 23}, 723 {[(M + 2) − 3R]$^+$, 100}, 578 {[(M + 2) −4R]$^+$, 91}. Anal. ($C_{76}H_{74}N_8O_4$) C, H, N.

[1,8,15,22-Tetrakis(*p*-*n*-butylbenzyloxy)phthalocyaninato]zinc(II) (47). Lithium (0.60 g) was suspended in 60 mL of 1-octanol. The mixture was heated to 170 °C and stirred for 4 h. To the homogeneous solution, cooled to 40 °C, was added 0.52 g (1.79 mmol) of **22** in 5 mL of THF. The solution was slowly heated to 100 °C and underwent a color change from colorless to green. To the green solution was added 326 mg (1.79 mmol) of zinc acetate, and the solution was stirred for 12 h at 100 °C. The temperature was raised to 120 °C, and the solution was stirred for another 2 h. The mixture was cooled to room temperature and quenched with methanol and water (1:1) to form a shining blue precipitate. The precipitate was collected by centrifugation, washed with water, methanol and hexane, successively, and dried. The blue solid was examined under a microscope and exhibited crystalline needles. Further recrystallization from THF gave 0.44 g (80%) of shining, blue, square crystals, **47**.

Compound **47** can also be prepared from **46**: A mixture of 80 mg (0.069 mmol) of **46** and 50 mg of zinc acetate in 5 mL of DMF and toluene (1:1) was refluxed for 24 h. To the mixture was added 10 mL of water, and the solution was centrifuged to separate a shining, blue solid. The solid was washed with water, methanol, and hexane and dried to give 76 mg (90%) of shining, blue **47** as crystalline needles or square crystals: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 696 (5.34), 665 (4.37), 626 (4.46), 369 (4.45); IR (KBr, cm^{-1}) ν 3010, 2940, 2920, 2840, 1580, 1485, 1335, 1260, 1235, 1130, 1090, 1060, 1040, 1020, 800, 760, 735; ^{1}H NMR (pyridine-d_5) δ 9.43 (d, J = 7.5 Hz, 4H), 8.33 (d, J = 6.6 Hz, 8H), 8.22 (t, J = 7.6 Hz, 4H), 7.87 (d, J = 8.0 Hz, 4H), 7.65 (d, J = 7.6 Hz, 8H), 5.91 (s, 8H), 2.83 (t, J = 7.6 Hz, 8H), 1.83−1.73 (m, 8H), 1.51−1.39 (m, 8H), 0.98 (t, J = 7.3 Hz, 12H); JMOD ^{13}C NMR (pyridine-d_5) δ 156.85, 154.18, 154.07, 143.01, 142.25, 135.20, 130.91, 129.20, 128.62, 126.41, 116.79, 113.68, 71.40, 35.84, 34.20, 22.72, 14.25; FAB-MS *m/z* (rel intensity) 1226 {(M + 2)$^+$, 100}, 1079 {[(M + 2) − R]$^+$, 88}, 932 {[(M + 2) − 2R]$^+$, 96}, 785 {[(M + 2) − 3R]$^+$, 84}, 638 {[(M + 2) − 4R]$^+$, 68}. Anal. ($C_{76}H_{72}N_8O_4Zn$) C, H, N.

1,8,15,22-Tetrakis(benzyloxy)phthalocyanine (48). As for **46**, compound **23** (0.94 g) was used to prepare 0.39 g of **48** at 100 °C as a blue solid in 42% yield: UV−vis (THF) λ_{max} (log ϵ) 726 (5.01), 692 (5.05), 662 (4.12), 632 (3.18), 352 (2.75), 318 (2.99); IR (KBr, cm^{-1}) ν 3310 (N−H), 3010, 2960, 2920, 2860, 1665, 1530, 1440, 1385, 1240, 1175, 1145, 1110, 1010, 925, 850; ^{1}H NMR (pyridine-d_5) δ 9.41 (d, J = 7.5 Hz, 4H), 8.43 (d, J = 8 Hz, 8H), 8.18 (t, J = 7.5 Hz, 4H), 7.99−7.90 (m, 12H), 7.55 (t, J = 7.5 Hz, 4H), 5.73 (s, 8H), −2.2 (br, 2H); FAB-MS *m/z* (rel intensity) 940 {(M + 2)$^+$, 92}, 849 {[(M + 2) − R]$^+$, 70}, 758 {[(M + 2) − 2R]$^+$, 65}, 667 {[(M + 2) − 3R]$^+$, 80}. Anal. ($C_{60}H_{42}N_8O_4$) C, H, N.

[1,8,15,22-Tetrakis(benzyloxy)phthalocyaninato]zinc-(II) (as a single isomer) (49). Using the procedure previously described for the preparation of **47** (method I), **23** (0.13 g) was used to prepare **49** at 100 °C as cubic crystals in 68% yield: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 696 (5.24), 664 (4.30), 624 (4.26), 368 (4.12); IR (KBr, cm^{-1}) ν 3010, 2920, 2840, 2820, 1575, 1480, 1335, 1262, 1230, 1130, 1090, 1060, 1040, 1020, 800, 760, 735; ^{1}H NMR (pyridine-d_5) δ 9.35 (d, J = 7.4 Hz, 4H), 8.40 (d, J = 7.2 Hz, 8H), 8.15 (t, J = 7.6 Hz, 4H), 7.85−7.78 (m, 12H), 7.67 (t, J = 7.4 Hz, 4H), 5.90 (s, 8H); JMOD ^{13}C NMR (pyridine-d_5) δ 156.05, 154.08, 154.00, 142.15, 138.51, 135.02, 130.61, 129.10, 128.40, 126.21, 116.49, 113.47, 71.27; FAB-MS *m/z* (rel intensity) 1002 {(M + 2)$^+$, 100}, 911 {[(M + 2) −R]$^+$, 65}, 820 {[(M + 2) − 2R]$^+$, 64}, 729 {[(M + 2) − 3R]$^+$, 45}, 638 {[(M + 2) − 4R]$^+$, 40}. Anal. ($C_{60}H_{40}N_8O_4$-Zn) C, H, N.

[1,8,15,22-Tetrakis(benzyloxy)phthalocyaninato)zinc-(II) (as a mixture of isomers) (50). The above-described procedure for the preparation of **49** was repeated at 160 °C, and compound **50** was obtained in 8% yield: ^{1}H NMR (pyridine-d_5) δ 9.48−9.28 (m, 4H), 8.48−8.36 (m, 8H), 8.22−8.08 (m, 4H), 7.89−7.62 (m, 16H), 5.88 (s, br, 8H). Compound **50** exhibited the same FAB-MS spectrum as **49**.

[1,8,15,22-Tetrakis(propargyloxy)phthalocyaninato]-zinc(II) (a mixture of isomers) (51). As for **47** (method I), **24** (0.15 g) was used to prepare **51** at 120 °C as a blue solid in 26% yield: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 702 (5.14), 666 (4.20), 620 (4.06), 360 (4.12); IR (KBr, cm^{-1}) ν 3010, 2920, 2840, 2820, 1575, 1480, 1335, 1262, 1230, 1130, 1090, 1060, 1040, 1020, 800, 760, 735; ^{1}H NMR (pyridine-d_5) δ 9.70−9.41 (m, 4H), 8.34−8.11 (m, 4H), 7.91−7.73 (m, 4H), 5.75−5.58 (m, 8H), 2.80−2.73 (m, 4H); FAB-MS *m/z* (rel intensity) 792 (M^+,

30). Anal. ($C_{44}H_{24}N_8O_4Zn$) Calcd: C, 66.61; H, 3.05; N, 14.12. Found: N, 15.00.

1,8,15,22-Tetrakis(neopentyloxy)phthalocyanine (as a single isomer) (52). Using the procedure previously described for the preparation for **47**, the self-condensation of 0.88 g of 3-neopentoxyphthalonitrile (**25**)[26] gave 0.26 g of **52** at 60−70 °C as a blue solid in 30% yield: mp >320 C; UV−vis (THF) λ_{max} (log ϵ) 726, 698, 664, 628, 354, 320; IR (KBr, cm^{-1}) ν 3285 (N−H), 2945, 2840, 1575, 1480, 1335, 1255 (Ar−O−C), 1230, 1130, 1090, 1060, 1020, 735; ^{1}H NMR (pyridine-d_5) δ 9.74 (d, J = 7.8 Hz, 4H), 8.41 (t, J = 7.7 Hz, 4H), 7.66 (d, J = 7.8 Hz, 4H), 4.57 (s, 8H), 1.70 (s, 36H), −2.80 (s, br, 2H); FAB-MS m/z (rel intensity) 858 (M^+, 100). Anal. ($C_{52}H_{58}N_8O_4$) C, H, N.

[1,8,15,22-Tetrakis(neopentyloxy)phthalocyaninato]-zinc(II) (as a single isomer) (53). As for **52** (method I), **25** was used to prepare **53** at 60−70 °C in 65% yield as crystalline needles: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 698 (5.24), 662 (4.30), 622 (4.26), 365 (4.12); IR (KBr, cm^{-1}) ν 3010, 2920, 2840, 2820, 1575, 1480, 1335, 1255 (Ar−O−C), 1230, 1130, 1090, 1060, 1040, 1020, 800, 760, 735; ^{1}H NMR (pyridine-d_5) δ 9.71 (d, J = 7.4 Hz, 4H), 8.33 (t, J = 7.7 Hz, 4H), 7.76 (d, J = 7.4 Hz, 4H), 4.37 (s, 8H), 1.72 (s, 36H); JMOD ^{13}C NMR (pyridine-d_5) δ 158.10, 154.97, 154.73, 142.85, 132.00, 126.47, 116.90, 113.25, 79.70, 33.32, 27.35; FAB-MS m/z (rel intensity) 920 (M^+, 100). Anal. ($C_{52}H_{56}N_8O_4Zn$) Calcd: C, 67.71; H, 6.12; N, 12.14. Found: N, 11.47.

[1,8,15,22-Tetrakis(neopentyloxy)phthalocyaninato)-zinc(II) (mixture of isomers) (54). As for **47** (method I), **27** was repeated at >130 °C, and **54** was obtained as a blue solid in 24% yield. Compound **54** exhibited the same FAB-MS as **53** but gave multiple absorption peaks in its ^{1}H NMR spectrum: ^{1}H NMR (pyridine-d_5) δ 9.74−9.51 (m, 4H), 8.41−8.21 (m, 4H), 8.00−7.75 (m, 4H), 4.40−4.32 (m, 8H), 1.70−1.35 (m, 36H).

(1,8,15,22-Tetramethoxyphthalocyaninato)zinc(II) (55). As for **47** (method I), **27** was used to prepare 0.35 g of **55** (from 0.63 g of **27**) at 50 °C in 50% yield as blue crystals: mp >320 °C; UV−vis (THF) λ_{max} (log ϵ) 694 (5.24), 662 (4.37), 624 (4.43), 368 (4.37); IR (KBr, cm^{-1}) ν 3010, 2920, 2840, 2820, 1575, 1480, 1335, 1262, 1230, 1130, 1090, 1060, 1040, 1020, 800, 760, 735; ^{1}H NMR (pyridine-d_5) δ 9.55 (d, J = 8.0 Hz, 4H), 8.18 (d, J = 8.0 Hz, 4H), 7.69 (t, J = 8.0 Hz, 4H), 4.58 (s, 12H); JMOD ^{13}C NMR (pyridine-d_5) δ 156.85, 155.07, 154.89, 142.93, 132.03, 127.04, 117.04, 113.81, 56.88; FAB-MS m/z (rel intensity) 696 (M^+, 65), 697 {$(M + 1)^+$, 100}, 698 {$(M + 2)^+$, 90}, 699 {$(M + 3)^+$, 80}. Anal. ($C_{36}H_{24}N_8O_4Zn$) C, H, N.

(1,8,15,22-Tetrahydroxyphthalocyaninato)zinc(II) (56). To 109 mg (0.086 mmol) of **55** in 5 mL of TFA was added 46 mg (0.35 mmol) of TMB. The mixture was refluxed for 15 h. The TFA was evaporated, and the residue was washed with hexane a few times. The blue solid was dissolved in THF, and the solution was put onto a gel permeation column, eluting with THF. Evaporation of the solvent gave 46.8 mg (85%) of **56** as a blue solid: UV−vis (THF) λ_{max} (log ϵ) 719 (4.85), 686 (4.12), 646 (4.13), 344 (4.06); IR (KBr, cm^{-1}) ν 3500−3100 (br signal, phenolic group), 1650, 1520, 1430, 1360, 1275, 1215, 1060, 925, 840; ^{1}H NMR (DMSO-d_6) δ 10.56 (s, 4H), 8.57 (d, J = 7.5 Hz, 4H), 8.25 (t, J = 7.5 Hz, 4H), 7.82 (d, J = 7.6 Hz, 4H); FAB-MS m/z (rel intensity) 640 (M^+, 100). Anal. ($C_{32}H_{16}N_8O_4Zn$) Calcd: C, 59.88; H, 2.51; N, 17.46. Found: N, 16.89.

Biological Studies. 1. Dye Formulation. ZnPcs were first diluted in a minimal volume of THF; Cremophor EL (polyethoxylated castor oil; BASF, Toronto, Canada; 10% final) and 1,2-propanediol (3% final) were added under sonication, whereafter THF was evaporated under vacuum. The solution was then diluted with phosphate-buffered saline (PBS; pH 7.4) and filtered (0.2 μm). Pc concentration of stock solutions was determined spectroscopically upon dilution in THF by measuring the absorbance at the λ_{max} around 670−680 nm.

2. Cell Photoinactivation. EMT-6 mouse mammary tumor cells were maintained in Waymouth's medium supplemented with 15% fetal bovine serum and 1% L-glutamine (Gibco, Canada), according to an established protocol.[29] The photocytotoxicity test was conducted by means of the colorimetric MTT assay.[30] Briefly, 15×10^3 EMT-6 cells/well were inoculated in 100 μL of Waymouth's growth medium in 96-multiwell plates and incubated overnight at 37 °C in an atmosphere containing 5% CO_2. The cells were rinsed twice with PBS and incubated for 1 or 24 h at 37 °C with 100 μL of a 1 μM dye solution prepared by diluting the Cremophor emulsion in Waymouth 1% FBS. After incubation, the cells were rinsed twice with PBS, refed with 100 μL of Waymouth 15% FBS, and exposed to red light. The light source consisted of two 500-W tungsten/halogen lamps (GTE Sylvania, Canada) fitted with a circulating, refrigerated, aqueous rhodamine filter. The fluence rate calculated over the absorbance peaks of the dyes (660−700 nm) was 10 mW cm^{-2} for a total fluence of 0.1−20 J cm^{-2}. The cells were incubated at 37 °C overnight before assessing cell viability. Fifty microliters of a 5-fold diluted MTT stock solution (5 mg mL^{-1} PBS) in Waymouth 15% FBS was added to each well. After 3 h, 100 μL of sodium dodecyl sulfate (SDS; 10% in 0.01 N HCl) was added in the wells. Plates were incubated overnight at 37 °C, whereafter the absorbance was read at 595 nm by means of a microplate reader (BioRad, Mississauga, Ontario, Canada). The average absorbance of the control wells without cells was subtracted. The average absorbance of the control cells which were incubated with dye-free Waymouth 1% FBS was taken as 100% cell survival. The light doses (LD_{90}) required to inactivate 90% of the cells incubated with 1 μM dye were extrapolated from the survival curves. Eightfold replicates were run per light dose, and the experiment was repeated at least three times.

3. Photodynamic Therapy. All experiments were performed on male BALB/c mice (18−22 g) (Charles River Breeding Laboratories, Montreal, Canada) following a protocol approved by the Canadian Council on Animal Care and an in-house ethics committee. The animals were allowed free access to water and food throughout the course of the experiments. Before tumor implantation, hair on the hind legs and back of the mice was removed by shaving and chemical depilating (Nair, Whitehall, Mississauga, Canada). A tumor was implanted on each hind thigh by intradermal injection of 2×10^5 EMT-6 cells suspended in 0.05 mL of Waymouth growth medium; 6−8 days after tumor cell inoculation (tumor size: 3−5-mm diameter, 2−3-mm thickness), animals were given an intravenous injection of 1 or 2 μmol kg^{-1} dye prepared as a Cremophor emulsion (0.2 mL/20 g) and treated with red light 24 h later. One tumor served as a control, while the other was illuminated with 650−700-nm light generated by a 1000-W xenon lamp, equipped with a 10-cm circulating water filter to eliminate possible hyperthermic effects and two glass filters (Corion LL-650 and LS-700). The fluence rate over a 8-mm diameter beam was 200 mW cm^{-2} for a total fluence of 400 J cm^{-2}. Under these conditions an increase of tumor temperature of approximately 2 °C was recorded, as monitored by means of a microthermocouple inserted at the base of the tumor. A positive tumor response was assigned to tumors which appeared macroscopically as flat and necrotic tissues within a few days after PDT. A complete tumor regression is defined as the absence of a palpable tumor at 3 weeks after PDT.

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References

(1) Dougherty, T. J. Photodynamic Therapy. Yearly review. *Photochem. Photobiol.* **1993**, *58*, 895−900.
(2) Fisher, A. M.; Murphree, A. L.; Gomer, C. J. Clinical and Preclinical Photodynamic Therapy. *Laser Surg. Med.* **1995**, *17*, 2−31 (review).

(3) (a) Byrne, C. J.; Marshallsay, L. V.; Sek, S. Y.; Ward, A. P. In *Photodynamic Therapy of Neoplastic Disease;* Kessel, D., Ed.; CRC Press: Boca Raton, FL, 1990; Vol. 2, pp 133−144. (b) Zhou, C. New Trends in Photobiology, Mechanism of Tumor Necrosis Induced by Photodynamic Therapy. *J. Photochem. Photobiol., B: Biol.* **1989**, *3*, 299−318 (review).

(4) (a) Berenbaum, M. C.; Bonnett, R.; Scourides, P. A. In vivo Biological Activity of the Components of Haematoporphyrin Derivative. *Br. J. Cancer* **1982**, *45*, 571−581. (b) Bonnett, R.; Berenbaum, M. C. HPD − a Study of its Components and their Properties. *Adv. Exp. Med. Biol.* **1983**, *160*, 241−250. (c) Mironov, A. F.; Nizhnik, A. N.; Nockel, A. Y. Hematoporphyrin Derivative: An Oligomeric Composition Study. *J. Photochem. Photobiol., B: Biol.* **1990**, *4*, 297−306. (d) Byne, C. J.; Marshallsay, L. V.; Ward, A. D. The Composition of Photofrin II. *J. Photochem. Photobiol., B: Biol.* **1990**, *6*, 13−27. (e) Kessel, D. Sites of Photosensitizition by Derivatives of Hematoporphyrin. *Photochem. Photobiol.* **1986**, *44*, 489−493. (f) Pandey, R. K.; Siegel, M. M.; Tsao, R.; McReynolds, J. K.; Dougherty, T. J. Fast atom bombardment mass spectral analyses of Photofrin II and its synthetic analogues. *Biomed. Environ. Mass Spectrom.* **1990**, *19*, 405−414. (g) Pandey, R. K. L.; Shiau, F.-Y.; Dougherty, T. J.; Smith, K. M. Regioselective synthesis of ether linked porphyrin dimers and trimers related to Photofrin II. *Tetrahedron* **1991**, *47*, 9571−9584.

(5) (a) Berenbaum, M. C.; Akande, S. L.; Bonnett, R.; Kaur, H.; Ioannou, S.; White, R. D.; Winfield, U. J. meso-Tetra(hydroxyphenyl)porphyrins, a New Class of Potent Tumour Photosensitizer with Favourable Selectivity. *Br. J. Cancer* **1986**, *54*, 717−725. (b) James, D. A.; Arnold, D. P.; Parsons, P. G. Potency and Selective Toxicity of Tetra(hydroxyphenyl)- and Tetrakis(dihydrophenyl)porphyrins in Human Melamoma Cells, with and without Exposure to Red Light. *Photochem. Photobiol.* **1994**, *59*, 441−447. (c) Brasseur, N.; Lewis, K.; Rousseau, J.; van Lier, J. E. Measurement of Tumor Vascular Damage in Mice with ^{99m}Tc-MIBI Following Photodynamic Therapy. *Photochem. Photobiol.* **1996**, *64*, 702−706.

(6) (a) Spikes, J. D. Phthalocyanines as Photosensitizers in Biological System and for the Photodynamic Therapy of Tumors. *Photochem. Photobiol.* **1986**, *43*, 691−699. (b) Ben-Hur, E. In *From Photophysics to Photobiology*; Favre, A., Tyrrell, R., Cadet, J., Eds.; Elsevier: New York, 1987; p 407. (c) van Lier, J. E. In *Light in Biology and Medicine*; Douglas, R. H., Moan, J., Dall'Acqua, F., Eds.; Plenum Press: New York, 1988; Vol. 1, p 133. (d) van Lier, J. E. Phthalocyanines as Sensitizers for PDT of Cancer. In *Photodynamic Therapy of Neoplastic Disease*; Kessel, D., Ed.; CRC Press: Boca Raton, FL, 1990; pp 279−290. (e) Chan, W. S.; Marshall, J. F.; Svensen, R.; Bedwell, J.; Hart, I. R. Effect of Sulfonation on the Cell and Tissue Distribution of the Photosensitizer Aluminum Pc. *Cancer Res.* **1990**, *50*, 4533−4538. (f) Boyle, R. W.; Paquette, B.; van Lier, J. E. Biological Activities of Phthalocyanines XIX. Effcct of Hydrophobic Phthalimidoethyl Groups on the In Vivo Phototoxicity and Mechanism of Photodynamic Action of Sulphonated Aluminum Phthalocyanines. *Br. J. Cancer* **1992**, *65*, 813−817. (g) Barr, H.; Tralau, C. J.; Boulos, P. B.; McRobert, A. J.; Lewis, M. R.; Phillips, D.; Bown, S. G. Selective Necrosis in Dimethylhydrazine-Induced Rat Colon Tumors Using Phthalocyanine Photodynamic Therapy. *Gastroenterology* **1990**, *98*, 1532−1537.

(7) (a) Brasseur, N.; Nguyen, T. L.; Langlois, R.; Ouellet, R.; Marengo, S.; Houde, D.; van Lier, J. E. Synthesis and Photodynamic Activities of Silicon 2,3-Naphthalocyanine Derivatives. *J. Med. Chem.* **1994**, *37*, 415−420. (b) Brasseur, N.; Ouellet, R.; Lewis, K.; Potter, W. R.; van Lier, J. E. Photodynamic Activities and Skin Photosensitivity of the *bis*(Dimethylthexylsiloxy) Silicon 2,3-Naphthalocyanine in Mice. *Photochem. Photobiol.* **1995**, *62*, 1058−1065. (c) Cuomo, V.; Jori, G.; Rihter, B.; Kenney, M. E.; Rodgers, M. A. Liposome-Delivered Si (IV) − Naphthalocyanine as a Photodynamic Sensitizer for Experimental Tumors: Pharmacokinetic and Phototherapeutic Studies. *Br. J. Cancer* **1990**, *62*, 966−970. (d) Henderson, B. W.; Mayhew, E. Experience with the Liposomal Delivery of the Photosensitizer *iso* BoSiNc. *Photodyn. Ther.: Mechanisms II* **1990**, *1203*, 12135. (e) Margaron, P.; Tempête, C.; Dendane, Y. M.; Gaspard, S.; Giannotti, C.; Werner, G.-H. Activité Photosensibilisatrice in vitro de Naphthalocyanines Métallées Hydrosolubles. *Crit. Rev. Acad. Sci. Paris* **1989**, *309* (II), 1159−1164. (f) Paquette, B.; Ali, H.; Langlois, R.; van Lier, J. E. Biological Activities of Phthalocyanines-XI. Phototoxicity of Sulfonated Aluminum Naphthalocyanines Towards V-79 Chinese Hamster Cells. *Photochem. Photobiol.* **1990**, *55*, 313−317. (g) Yates, N. C.; Moan, J.; Western, A. Water-Soluble Metal Naphthalocyanines-Near-IR Photosensitizer I: Cellular Uptake, Toxicity and Photosensitizing Properties in NHIK 3025 Human Cancer Cells. *J. Photochem. Photobiol., B: Biol.* **1990**, *4*, 379−390.

(8) Boyle, R. W.; Dolphin, D. Structure and Biodistribution Relationships of Photodynamic Sensitizers. *Photochem. Photobiol.* **1996**, *64*, 469−485 (review).

(9) (a) Brasseur, N.; Ali, H.; Langlois, R.; van Lier, J. E. Biological activities of phthalocyanines −VII. Photoinactivation of V-79 chinese hamster cells by selectively sulfonated gallium phthalocyanines. *Photochem. Photobiol.* **1987**, *46*, 739−744. (b) Paquette, B.; Ali, H.; Langlois, R.; van Lier, J. E. Biological activities of phthalocyanines −VIII. Cellular distribution in V-79 chinese hamster cells and phototoxicity of selectively sulfonated aluminum phthalocyanines. *Photochem. Photobiol.* **1988**, *47*, 215−220. (c) Margaron, P.; Grégoire, M.-J.; Ščasnár, V.; van Lier, J. E. Structure−photodynamic activity relationships of a series of 4-substituted zinc phthalocyanines. *Photochem. Photobiol.* **1996**, *63*, 217−223.

(10) (a) Margaron, P.; Madarnas, P.; Ouellet, R.; van Lier, J. E. Biological Activities of Phthalocyanines. XVII Histopathologic Evidence for Different Mechanisms of EMT-6 Tumor Necrosis Induced by Photodynamic Therapy with Disulfonated Aluminum Phthalocyanine or Photofrin. *Anticancer Res.* **1996**, *16*, 613−620. (b) Chan, W.-S.; Brasseur, N.; La Madeleine, C.; van Lier, J. E. Evidence for different mechanisms of EMT-6 Tumor Necrosis by Photodynamic Therapy with Disulfonated Aluminum Phthalocyanine or Photofrin: Tumor Cell Survival and Blood Flow. *Anticancer Res.* **1996**, *16*, 1887−1892.

(11) (a) Boekelheide, K.; Eveleth, J.; Tatum, A. H.; Winkelman, J. W. Microtubule assembly inhibition by porphyrins and related compounds. *Photochem. Photobiol.* **1987**, *46*, 657. (b) Kessel, D.; Thompson, P.; Saatio, K.; Nautwi, K. D. Tumor localization and photosensitization by sulfonated derivatives of tetraphenylporphine. *Photochem. Photobiol.* **1987**, *45*, 787.

(12) (a) Rosenthal, I.; Ben-Hur, E.; Greenberg, S.; Conception-Lam, A.; Drew, D. M.; Leznoff, C. C. The Effect of Substituents on Phthalocyanine Photocytotoxicity. *Photochem. Photobiol.* **1987**, *46*, 959−963. (b) Boyle, R. W.; Leznoff, C. C.; van Lier, J. E. Biological Activities of Pcs-XVI: Tetrahydroxy- and Tetraalkyl Hydroxy Zinc Pcs. Effect of Alkylchain Length on In Vitro and In Vivo Photodynamic Activities. *Br. J. Cancer* **1993**, *67*, 1177−1181.

(13) Ali, H.; Langlois, R.; Wagner, J. R.; Brasseur, N.; Paquette, B.; van Lier, J. E. Biological Activities of Phthalocyanines-X. Synthesis and Analyses of Sulfonated Phthalocyanines. *Photochem. Photobiol.* **1988**, *47*, 713−717.

(14) (a) Margaron, P.; Langlois, R.; van Lier, J. E.; Gaspard, S. Photodynamic Properties of Naphthasulfonenzoporphyrazines, Novel Asymmetric, Amphiphilic Phthalocyanine Derivatives. *J. Photochem. Photobiol., B: Biol.* **1992**, *14*, 187−199. (b) Kliesch, H.; Weitemeyer, A.; Müller, S.; Wöller, D. Synthesis of Pcs with One Sulfonic Acid, Carboxylic Acid, or Amine Group. *Liebigs Ann.* **1995**, 1269−1273. (c) Gaspard, S.; Margaron, P.; Tempête, C.; Tran-Thi, T. H. Mixed Acenannellated Metallotetraazaporphins: A New Class of Amphiphlic Photosensitizers for the Photodynamic Therapy of Cancer. *J. Photochem. Photobiol., B: Biol.* **1990**, *4*, 419−423.

(15) Nevin, W. A.; Liu, W.; Greenberg, S.; Hempstead, M. R.; Marcuccio, S. M.; Melnik, M.; Leznoff, C. C.; Lever, A. B. P. Synthesis, Aggregation, Electrocatalytic Activity, and Redox Properties of a Tetranuclear Cobalt Pc. *Inorg. Chem.* **1987**, *26*, 891−899.

(16) (a) Leznoff, C. C.; Hall, T. W. The Synthesis of a Soluble, Unsymmetrical Pc on A Polymer Support. *Tetrahedron Lett.* **1982**, 3023−3026. (b) Hall, T. W.; Greenberg, S.; McArthur, C. R.; Khouw, B.; Leznoff, C. C. The Solid-Phase Synthesis of Unsymmetrical Pcs. *Nouv. J. Chim.* **1982**, *6*, 653−658.

(17) Wöhrle, D.; Krawczyk, G. Polymeric Bound Porphyrins and their Precursors-2. Solid-Phase Synthesis of a Monosubstituted Phthalocyanine. *Polym. Bull., Berlin* **1986**, *15*, 193−200.

(18) (a) Kobayashi, N.; Kondo, R.; Nakjima, S. I.; Osa, T. New Route to Unsymmetrical Pc Analogues by the Use of Structurally Distorted SubPcs. *J. Am. Chem. Soc.* **1990**, *112*, 9640−9641. (b) Kobayashi, N. A Rigid, Laterally Bridged Binuclear SubPc: the First Dimer of Aromatic Macrocyclic Complexes Containing Boron. *J. Chem. Soc., Chem. Commun.* **1991**, 1203−1205. (c) Kietaibl, H. Die Kristall-Und Molekülstruktur Eines Nevartigen Phthalocyaninähnlichen Borkomplexes. *Monatsh. Chem.* **1974**, *105*, 405. (d) Kasuga, K.; Idehara, T.; Handa, M. Preparation of Unsymmetrical Pc by Means of A Ring Expansion of SubPc. *Inorg. Chim. Acta* **1992**, *196*, 127−128. (e) Kudrevich, S. V.; Gilbert, S.; van Lier, J. E. Syntheses of Trisulfonated Phthalocyanines and Their Derivatives Using Boron(III) Subphthalocyanines as Intermediates. *J. Org. Chem.* **1996**, *61*, 5706−5707.

(19) Kohn, M. Bromination of Catechol. *J. Am. Chem. Soc.* **1951**, *73*, 480.

(20) Leznoff, C. C.; Hu, M.; McArthur, C. R.; Qin, Y.; van Lier, J. E. Synthesis of 1,8,15,22- and 2,9,16,23-Tetrahydroxyphthalocyanines. *Can. J. Chem.* **1994**, *72*, 1990−1998.

(21) (a) Hanessian, S.; Liak, T. J.; Vanasse, B. Facile Cleavage of Benzyl Ethers by Catalytic Transfer Hydrogenation. *Synthesis* **1981**, 396−397. (b) Yoshino, H.; Tsuchiya, Y.; Saito, I.; Tsujii, M. *Chem. Pharm. Bull.* **1987**, *35*, 3438.

(22) (a) Oliver, S. W.; Smith, T. D. Oligomeric Cyclization of Dinitriles in the Synthesis of Pcs and Related Compounds: the Role of the Alkoxide Anion. *J. Chem. Soc., Perkin Trans. II* **1987**, 1579−1582. (b) Nolan, K. J. M.; Hu, M.; Leznoff, C. C. "Adjacent" Substituted Phthalocyanines. *Synlett* **1997**, 593−594.
(23) Allémann, E.; Brasseur, N.; Kudrevich, S. V.; La Madeleine, C.; van Lier, J. E. Photodynamic Activities and Biodistribution of Fluorinated Zinc Phthalocyanine Derivatives in the Murine EMT-6 Tumour Model. *Int. J. Cancer* **1997**, *72*, 289−294.
(24) Boyle, R. W.; Rousseau, J.; Kudrevich, S. V.; Obochi, M. O. K.; van Lier, J. E. Hexadecafluorinated Zinc Phthalocyanine: Photodynamic Properties against EMT-6 Tumour in Mice and Pharmacokinetics Using ^{65}Zn as a Radiotracer. *Br. J. Cancer* **1996**, *73*, 49−53.
(25) Ohta, K.; Jacquemin, L.; Sirlin, C.; Bosio, L.; Simon, J. Influence of the Nature of the Side Chains on the Mesomorphic Properties of Octasubstituted Phthalocyanine Derivates. Annelides XXIX. *New J. Chem.* **1988**, *12*, 751−754.
(26) Leznoff, C. C.; Drew, D. M. The Use of Bisphthalonitriles in the Synthesis of Side-Strapped 1,11,15,25-Tetrasubstituted Phthalocyanines. *Can. J. Chem.* **1996**, *74*, 307−318.
(27) Derkacheva, V. M.; Iodko, S. S.; Kaliya, O. L.; Luk'yanets, E. A. Influence of Substituents on the Basicity of Copper Phthalocyanines. *Zh. Obshch. Khim.* **1981**, *51*, 2319−2324.
(28) Metz, J.; Schneider, O.; Hanack, M. Synthesis and Properties of Substituted (Phthalocyaninato)iron and -cobalt Compounds and their Pyridine Adducts. *Inorg. Chem.* **1984**, *23*, 1065−1071.
(29) Rockwell, S. C.; Kallman, R. F.; Fajardo, L. F. Characteristics of a Serially Transplanted Mouse Mammary Tumour and its Tissue-Culture-Adapted Derivative. *J. Natl. Cancer Inst.* **1972**, *49*, 735−749.
(30) Tada, H. R.; Shibo, O.; Kuroshima, K.; Koyama, M.; Tsukamoto, K. An Improved Colorimetric Assay for Interleukin 2. *J. Immunol. Methods* **1986**, *93*, 157−165.

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