Syntheses and Photodynamic Activities of Novel Trisulfonated Zinc Phthalocyanine Derivatives

Svetlana Kudrevich, Nicole Brasseur, Carole La Madeleine, Sandra Gilbert, and Johan E. van Lier*

MRC Group in the Radiation Sciences, Faculty of Medicine, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4

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The synthesis of water-soluble, unsymmetrical, trisulfonated zinc phthalocyanines ($ZnPcS_3$) as single products of the ring expansion of boron tri(4-sulfo)subphthalocyanine (SubPc) is reported. The novel, water-soluble trisulfo-SubPcB(OH) was prepared via hydrolysis of the tris(4-chlorosulfonyl)SubPcB(Br) which in turn was obtained from the condensation of 4-(chlorosulfonyl)phthalonitrile with BBr_3 in 1-chlorobenzene. A number of $ZnPcS_3$ analogues were prepared via the reaction of S_3SubPcB(OH) with different diiminoisoindoline derivatives of increasing hydrophobicity. The reaction proceeds at relative low temperature with acceptable yields. Metalation of free base Pc's with zinc acetate dihydrate afforded the corresponding zinc complexes. Photodynamic activities were measured against the EMT-6 mouse mammary tumor cell line and compared to those of the known $ZnPcS_3$ and $ZnPcS_4$. Added (*t*-Bu)benzo and (*t*-Bu)naphtho groups increased the in vitro cell photoinactivation efficacy of the $ZnPcS_3$, whereas addition of a fourth sulfobenzo or bulky diphenylpyrazino group decreased the activity of the parent molecule. The (*t*-Bu)naphthotrisulfobenzoporphyrazine induced the best in vivo photodynamic tumor control which, combined with its good solubility and broad absorption spectrum, renders this compound an interesting dye for photodynamic applications in medicine.

Introduction

Photodynamic therapy (PDT) of cancer involves the sensitization of neoplastic tissues to red light after systemic or topical administration of a photosensitizer preparation.[1] Preferential tumor retention of the photosensitizer combined with local illumination induces tumor regression without extensive damage to surrounding healthy tissues. Due to its mechanism of action, which may vary from direct tumor cell kill to vascular occlusion, PDT has potential for a number of other nononcologic indications involving cell hyperproliferation or neovascularization.[2]

A mixture of hematoporphyrin derivatives enriched in dimers and oligomers (Photofrin, QLT Photo-Therapeutics Inc.) has received approval for the PDT of selected cancers in many countries since 1993. Disadvantages linked to Photofrin, notably chemical heterogeneity, low red light absorption, and long-term cutaneous photosensitivity, invited an extensive search for new, chemically well-defined sensitizers with improved biological properties.[3] Among the second-generation photosensitizers advanced for PDT, the phthalocyanines (Pc) have received particular attention due to their high molar absorption coefficient ($\epsilon \approx 10^5$ M^{-1} cm^{-1}) in the red part of the spectrum (640−710 nm) allowing increased tissue penetration of the activating light. The biological potential of these photosensitizers has been well-documented.[4]

We have previously synthesized water-soluble zinc, gallium, and aluminum Pc with various degrees of sulfonation by either condensation or sulfonation methods[5] and shown that increasing the degree of sulfonation led to a decrease in photocytotoxicity under both in vitro and in vivo conditions and that this decrease was directly correlated to the hydrophilicity of the dye.[6] The amphiphilic disulfonated dye bearing two sulfonate groups on adjacent benzo rings exhibited the strongest in vitro photocytotoxicity, and this was related to greater membrane-penetrating properties.[7a−c] Furthermore, varying in vivo PDT protocols with these amphiphilic dyes (drug/light doses, timing of drug-illumination interval) permits modulation of tumor response via direct cellular effects or vascular stasis.[8] We have also demonstrated that the addition of tertiary butyl groups on sulfonated gallium Pc enhances the hydrophobic and amphiphilic properties of the drugs resulting in increased cell uptake and in vitro photocytotoxicity.[9] Meanwhile, the design of synthetic routes to water-soluble and amphiphilic Pc's carrying well-selected substituents remains a challenge. The limited availability of the latter type of compounds can be attributed to the difficulties encountered in isolating the desired products derived from the conventional synthetic approach, i.e., the condensation of two or more different phthalonitrile (diiminoisoindoline) derivatives. The complex statistically defined mixture obtained in this procedure requires time-consuming chromatographical separations, and the yields of desired products usually are very low.[5,10] Thus, efficient synthetic routes to each isomer are required. In this context three methods for the preparation of mono- and disubstituted lipophilic Pc's and their analogues are noteworthy:[9] condensation of an iminoisoindoline derivative with either a 1,3,3-trichloroisoindoline,[11] a sterically crowded diiminoisoindoline,[12] or a 1,3-bis[(3′-imino-1′-isoindolinydene)amino]-1,2,4-triazole (or its metal complex).[13] None of these methods has however been applied to the synthesis of Pc's featuring hydrophilic substituents, such as sulfo groups, on the benzo rings. Some amphiphilic Pc's, which are particularly sought as photosensitizers for medical applications, could not be obtained using the conventional condensation of two different precursors.

To overcome this problem we reported a method for preparing monosulfonated Pc's and their derivatives via the Meerwein reaction, a procedure which gives selected

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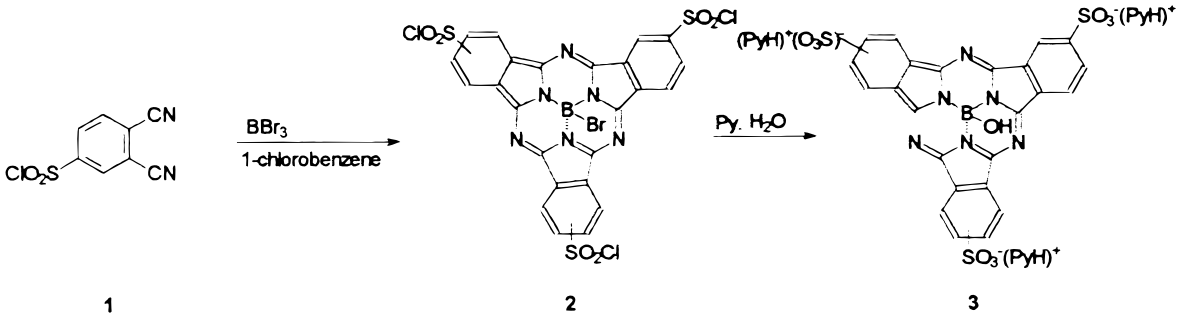
Scheme 1



1 2 3

positional isomers without chromatographical separations of polysulfonated mixtures.[14] However, di- and trisulfonated Pc's cannot be obtained via this route. We recently presented an alternative procedure for the preparation of unsymmetrical Pc's with sulfo substituents on three of the benzo rings and different lipophilic substituents on the forth ring.[15] We now report details of the syntheses of the zinc complexes of these dyes as well as their photodynamic properties against the experimental mouse mammary sarcoma EMT-6, both on cell monolayers and on tumors implanted intradermally in BALB/c mice.

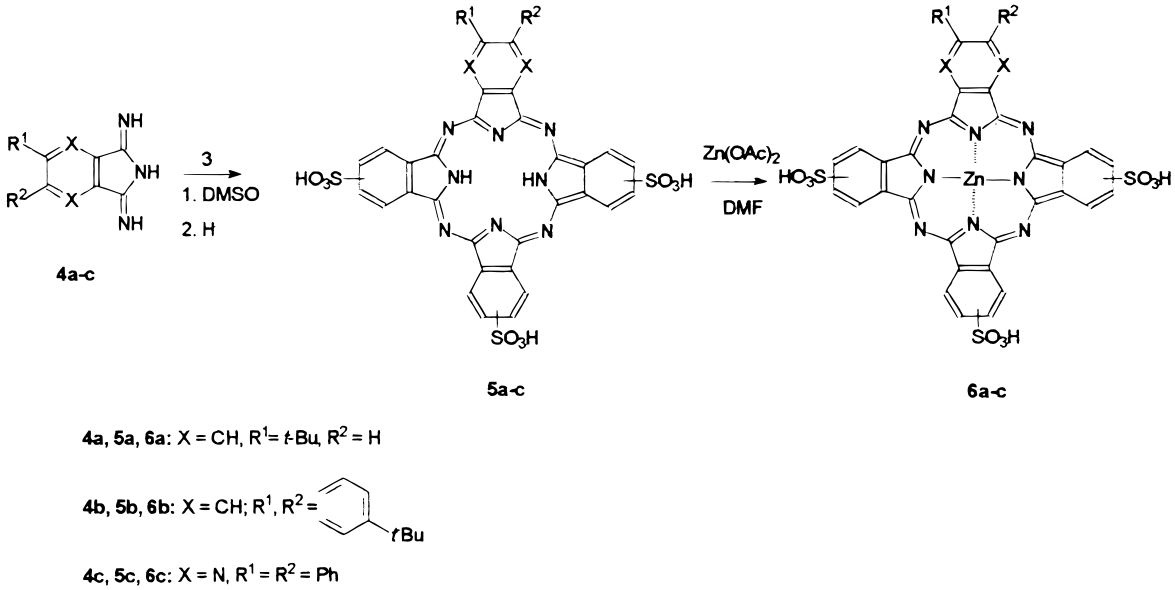
Results and Discussion

Synthesis. Preorganization of three phthalonitrile units as a subphthalocyanine (SubPc) of boron(III)[17] and subsequent conversion into a Pc macrocycle via reaction with various substituted diiminoisoindolines has proven to be an efficient procedure to obtain unsymmetrical Pc's featuring different lipophilic substituents, such as alkyl, alkoxy, alkylthio, amino, nitro, and crown ester groups.[18] However, this template reaction, employing SubPc's, has not previously been applied to the preparation of water-soluble Pc's and SubPc's substituted with hydrophilic moieties attached to the benzo rings.

Compared to Pc's, SubPc's—i.e., the lower homologues of Pc composed of three diiminoisoindoline units—have been much less studied. This is mainly due to the relative instability of these compounds and the ensuring difficulties often encountered with their purification.[18a,b,d,h] Meller and Ossko reported the synthesis of boron(III) SubPc's with an axial halogen, SubPcB(X), where X = F, Cl.[17a] In their experiments, SubPc's were formed in the reaction of BF_3, BCl_3, or $PhBCl_2$ with phthalonitrile in boiling 1-chloronaphthalene. Subsequently it was shown that in this reaction formation of periferally halogenated byproducts is inevitable, since an electrophilic substitution reaction of free halogen, generated from BX_3 (X = F, Cl) or $PhBCl_2$ with the macrocycle, is catalyzed by unreacted BX_3. Separation of resulting mixture is difficult.[17b] Synthesis of unsubstituted and tri-*tert*-butyl-substituted SubPc's with an axial bromine, i.e., SubPcB(Br), was also described.[18b,19] These compounds were obtained by reacting bromophenylboranes (Ph_2BBr or $PhBBr_2$) with an appropriate phthalonitrile in 1-chloronaphthalene. However, attempts to repeat the synthesis of *t*-Bu_3SubPcB(Br) yielded instead *t*-Bu_3SubPcB(Ph) containing an axial phenyl group.[17b,20] For the synthesis of tris(4-chlorosulfonyl)SubPcB(Br) (**2**) from 4-(chlorosulfonyl)phthalonitrile (**1**),[21] we used a commercially available 1 M solution of BBr_3 in dichloromethane (Scheme 1). Formation of halogenated byproducts is unlikely with these reactants. The reaction was conducted in 1-chlorobenzene, which has a lower boiling point as compared to 1-chloronaphthalene, i.e., the solvent previously used for such cyclotrimerization. In contrast to all described analogues, which were synthesized at much higher temperatures (e.g., at reflux temperature of 1-chloronaphthalene),[17a] the reaction leading to **2** started readily at room temperature and was completed in 1 h at 40 °C, to yield the SubPc in high purity and over 60% yield. The assigned structure was confirmed by FAB-MS, UV−vis, NMR, and IR spectroscopic data. No peaks corresponding to tris(chlorosulfonyl)SubPcBBr derivatives brominated on the benzo rings could be detected in the mass spectra of **2**. Compound **2** is soluble in most organic solvents (i.e., chloroform, toluene, methanol). The chlorosulfonyl groups of **2** are susceptible to rapid hydrolysis accompanied by destruction of the macrocycle, and the compound should be protected from atmospheric air. If stored in a hermetically sealed container and shielded from light, the sulfonyl chloride **2** remains unchanged for months. TLC of **2** on silica gel or alumina plates using chloroform as eluant revealed two spots corresponding to the C_1 and C_3 type isomers.[20] Unfortunately, we also detected rapid destruction of the compound on the adsorbent. Attempted preparative chromatography on a silica gel column using chloroform as eluant provided only about 10% of recovered material. However, it was possible to use unpurified **2** directly in the next step of the reaction sequence. Since the chlorosulfonyl moieties of **2** may react with the imino groups of diiminoisoindolines, **2** was first converted to the sulfo acid derivative. Attempts to hydrolyze **2** in concentrated or diluted HCl, diluted aqueous solution of NaOH, or ammonia led to destruction of the boron SubPc macrocycle. The SubPc revealed to be stable in a mixture of water/pyridine (2:1) in which it was dissolved and hydrolyzed to afford the pyridinium salt **3** as golden-purple crystals in 60% yield. The material was characterized by combustion analysis and spectroscopic data. Compound **3** is soluble in water, DMF, DMSO, and methanol. The UV−vis spectrum of **3** in water features a sharp absorption maximum at 569 nm, which is characteristic for SubPc's. HPLC analysis of an aqueous solution of the salt **3** revealed a single peak with t_R 12 min, which is similar to the t_R of tetrasulfonated metallo Pc in this system.[5]

The stepwise reaction of SubPc ring enlargement is highly dependent on the reactants (SubPc and diiminoisoindoline) and often leads to a low yield of the desired trisubstituted Pc's due to the formation of cleavage products from the SubPc followed by statistical

Scheme 2



condensation of fragments.[18f,g] The polarity of the solvent and the temperature are important factors for the ring-expansion reaction of SubPc. To date, almost all such syntheses were conducted in a mixture of chloronaphthalene and DMSO at 70−90 °C. Because of the limited solubility of salt **3**, we tried different polar solvents. The use of DMF and alcohols, including *N*,*N*-dimethylaminoethanol, at temperatures from 0 to 90 °C, led to decomposition of starting material without formation of Pc products. Pure, dry DMSO appeared to be a suitable solvent, which allowed us to study the effect of variables, such as the nature of precursors and the reaction temperature, on the rate of the ring expansion of **3**. The choice of the substituents on the forth ring of the Pc was dictated by the desired solubility and spectral properties of the final products. Substituted 1,3-diiminoisoindolines **4a**−**c** were obtained by treatment of the corresponding dinitriles with sodium methylate and ammonia in methanol: **4a** from 4-*tert*-butylphthalonitrile, **4b** from 6-*tert*-butyl-2,3-dicyanonaphthalene, and **4c** from 5,6-diphenyl-2,3-dicyanopyrazine.[22] Attempts to perform this reaction with precursors other than 1,3-diiminoisoindolines, including *o*-dinitriles and 1-imino-3-thioisoindoline,[23] failed. The reactions of **3** with diiminoisoindoline derivatives **4a**−**c** were all accomplished in dry DMSO, at relatively low temperatures. Thus, compound **3** reacts with **4a** at room temperature and with **4c** at 50 °C to yield trisulfo-Pc in up to 31% yield. Most earlier reported ring-expansion reactions of lipophilic SubPc's with diiminoisoindolines require high temperatures. This may result in decomposition of the SubPc to yield complex mixtures containing, besides PcS_3, the other statistical distribution Pc's in variable ratios.[18f,g] Also, the presence of excess diiminoisoindoline in the reaction mixture (>70 °C) may result in self-condensation to give the corresponding tetrasubstituted metal-free Pc derivatives. By combining a SubPc with electron-withdrawing sulfo substituents (**3**), an electron-donating type diiminoisoindoline (**4a**), and extremely polar solvent, we achieved the ring expansion at room temperature thereby excluding most of the above side reactions. During the preparation of **5a,c** no tetrasulfo-Pc was detected. The relatively slow reaction of **3** with the less polar, annelated diiminoisoindoline derivative **4b** only proceeded at 70 °C to yield the trisulfo-Pc analogue **5b** as the main product together with a small amount of tetrasulfo-Pc (about 10% of Pc mixture) (Scheme 2). Compound **3**, dissolved in DMSO, slowly degrades at room temperature in the dark. In a few days **3** disappears completely without any trace of Pc products, whereas heating of this solution to 70 °C leads to the formation of tetrasulfo-Pc in about 5% yield. These results are in accord with the SubPc expansion mechanism suggested recently by Sastre et al.[18h] In our case, it is obvious that cleavage of the SubPc is not thermal and that, along with the solvent, diiminoisoindoline participates in the initial macrocycle rupture. The reactivity of diiminoisoindoline appears to be the dominant parameter guiding the cyclization step. Completion of the ring-expansion reaction was detected spectrophotometrically: the intensity of the absorption bands, characteristic for the metal-free Pc **5a**, benzonaphthoporphyrazine **5b**, and azaphthalocyanine **5c** (around 650−700 nm), gradually increased during the course of the reaction, whereas the Q-band of the boron SubPc (around 570 nm) gradually disappears. The trisulfo-Pc pyridinium salts **5a**−**c** were precipitated from DMSO upon the addition of methanol and/or chloroform and isolated by filtration to yield almost pure products. Multiple redissolvation of the pyridinium salts in water, followed by reprecipitation with HCl, gave analytical samples of the corresponding sulfo acids. Compound **5b** required additional chromatographical purification to remove the tetrasulfo-Pc byproduct. HPLC analysis[5] of **5a**−**c** showed the presence of a single fraction ($t_R \sim 20$ min), in each case consisting of three poorly resolved peaks of the type isomers, corresponding to the trisulfonated Pc's. Free base compounds **5a**−**c** were metalated with zinc acetate dihydrate in dry DMF affording the zinc complexes **6a**−**c**, which are soluble in water as well as in methanol and DMF.

The maxima in the electronic spectra of the naphthobenzoporphyrazine **6b** and Pc aza analogue **6c** in methanol (DMF) are indicative of the asymmetrical character of these macrocycles (Figure 1). Although only one major absorption maximum is observed for **6c** around 680 nm, this band is broad relative to that of ZnPc (e.g., **6a**), with resolved satellite. Two well-

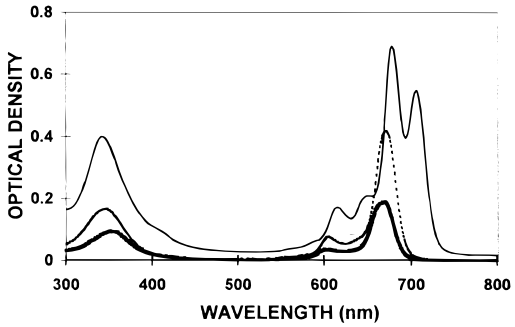


Figure 1. UV−vis electronic spectra of **6a** (dotted trace), **6b** (regular trace), and **6c** (bold trace) at 5 μM in MeOH.

Table 1. Photoinactivation of EMT-6 Cells and Chromatographic Mobilities of Differently Substituted ZnPc

compd	LD_{90}[a] (J cm^{-2}) 1 h	LD_{90}[a] (J cm^{-2}) 24 h	HPLC t_R[b] (min)
6a	>30	2.5	27
6b	21	2.5	29
6c	>30	19.5	26
$ZnPcS_3$	29	5	21
$ZnPcS_4$	>30	14	18

[a] Light dose to inactivate 90% of cells incubated with 1 μM dye for a period of 1 or 24 h. [b] Weighted average retention time of regional isomers on a reversed-phase C-18 HPLC column.[27]

resolved bands, red-shifted comparatively to the Q-band of ZnPc, were detected in the UV−vis spectrum of **6b**. These observations are in agreement with the 2-fold orbital degeneracy of the excited electronic state of the metallo Pc's when the group of symmetry of the macrocycle varies from D_{4h} to C_{2v}.[10,14] The electronic spectra of complexes **6a**−**c** in water are similar: the broad bands around 630 nm are indicative of complete dimerization of Pc's in this solvent.

Biological Properties. The efficiency of the differently substituted sulfo-Pc's to photoinactivate cells in vitro is summarized in Table 1. Survival of control cells exposed to red light only or incubated with drug without further exposure to light was 100%. When cells were incubated with 1 μM dye for 1 h, followed by light exposure, phototoxicity was low, with only the *tert*-butylnaphthotrisulfo derivative **6b** and the $ZnPcS_3$ scoring LD_{90} < 30 J cm^{-2}. After longer exposure to the dyes (24 h) cells exhibited marked photosensitivity, with the *tert*-butylbenzo (**6a**) and the *tert*-butylnaphtho (**6b**) derivatives showing the highest phototoxicities (LD_{90} = 2.5 J cm^{-2}), followed by the $ZnPcS_3$ (LD_{90} = 5 J cm^{-2}), with the $ZnPcS_4$ and diphenylbenzo derivative **6c** exhibiting the lowest activities (LD_{90} = 14 and 19.5 J cm^{-2}, respectively). The slightly higher hydrophobicity of the unsymmetrically substituted Zn phthalocyanines **6a,b**, as compared to the $ZnPcS_3$ and $ZnPcS_4$ (Table 1, t_R values) correlates with increased photoinactivating efficacy of the former dyes. We previously showed that the increased amphiphilicity of adjacently disulfonated metallo PcS_{2a} as compared to the tri- and tetrasulfonated analogues correlated well with increased cell uptake and augmented phototoxicity.[7] In the case of compound **6c**, steric hindrance due to the presence of the bulky diphenylpyrazino substituent may account for reduced cellular uptake and lower cytotoxicity.

Tumor response induced by the differently substituted ZnPc under various conditions of drug and light doses is presented in Table 2. The time intervals between dye administration and irradiation, i.e., 24 and 48 h, were selected based on the earlier reported uptake maxima of sulfonated Pc in an animal tumor model.[24] Compounds **6a**,**b** were the most photoactive with efficiency comparable to that of the $ZnPcS_4$, whereas compound **6c** and the $ZnPcS_3$ were almost inactive. PDT performed at 400 J cm^{-2} with compounds **6a**,**b** and $ZnPcS_4$ injected at 5 μmol kg^{-1} 24 h before PDT induced tumor regression in about 90% of mice. However, for compound **6a** this effect was accompanied by severe damage to the muscle surrounding the tumor, resulting in 10% mortality. At a dose of 20 μmol kg^{-1}, **6a**,**b** and $ZnPcS_4$ induced mortality after PDT (400 J cm^{-2}, 24 h pi). The best tumor response was observed with **6b** when PDT was performed 2 days pi. Muscle damage was avoided with this protocol, while tumor regression occurred in about 90% of animals. Compounds **6a** and $ZnPcS_4$ were much less efficient under the same conditions. Lowering the drug dose of compound **6b** to 2 μmol kg^{-1} or the light dose to 300 J cm^{-2} reduced the cure rate to 60% and 43%, respectively. Overall, compound **6b** appeared the most effective in the series. For comparison, Photofrin, the clinically used photosensitizer, gave a comparable tumor control rate (70%) at 5 mg kg^{-1} (400 J cm^{-2}, 24 h pi).[25] The relative efficiency of the unsymmetrically substituted ZnPc dyes to photoinactivate cells in vitro or to induce tumor regression in mice after PDT follows a similar pattern: **6b** ≥ **6a** > **6c**. This was not the case for the $ZnPcS_3$ and $ZnPcS_4$, the former being more photoactive in vitro but less efficient in vivo than the latter. These results illustrate the inadequacy of in vitro studies to predict the PDT efficacy of new drugs to exert in vivo tumor control. Previous reports on the PDT efficacy of $ZnPcS_4$ vary substantially, and the extent of isomers in the preparation and the wavelength of the therapeutic light appear to be critical.[26] Replacing one of the four sulfo groups of $ZnPcS_4$ by a tertiary butyl group (**6a**) increases the amphiphilic nature of the dye which coincides with its increased cellular photoefficiency but which does not affect significantly in vivo photoactivity. However, when the sulfobenzo group is replaced by a *tert*-butylnaphtho group (**6b**), both the in vitro and in vivo photoactivities are improved. Moreover, this structural change results in an higher degree of conjugation with the appearance of an additional absorption band at 706 nm (Figure 1), as compared to the characteristic peak of the sulfonated ZnPc's around 680 nm.[10] This extended absorption spectrum provides flexibility in terms of choice of the therapeutic wavelengths and the use of convenient solid-state diode lasers, rendering this compound a versatile candidate drug for PDT.

Conclusions

Novel amphiphilic asymmetrical trisulfo-substituted phthalocyanines, i.e., (4-*tert*-butyl)tri(4-sulfo)Pc (**6a**), (6-*tert*-butyl)naphthotris(4-sulfobenzo)porphyrazine (**6b**), and (5,6-diphenyl-2,3-pyrazino)tris(4-sulfobenzo)porphyrazine (**6c**), were synthesized via the ring-expansion reaction of hydroxyboron tri(4-sulfo)SubPc with the corresponding diiminoisoindoline derivatives. The SubPc precursor is a water-soluble compound, unique for this class of compounds, which was prepared by hydrolysis of the tris(4-chlorosulfonyl)SubPcB(Br). Both acidic and basic solutions destroy the latter compound; instead a mild procedure of hydrolysis in water−pyridine mixture was developed. Formation of trichloro-

Table 2. EMT-6 Tumor Response in BALB/c Mice Treated with Differently Substituted ZnPc and Light

compd	drug dose (μmol kg^{-1})	light dose[a] (J cm^{-2})	time pi (h)	n	tumor response[b] (%) necrosis	tumor response[b] (%) regression	edema[d]
6a	5	400	24	10	100	90[c]	++++
	5	300	24	8	12	0	++
	5	400	48	8	37	37	+++
	2	400	24	6	50	33	++
6b	5	400	24	7	100	86	++++
	5	300	24	7	71	43	+++
	5	400	48	8	87	87	++
	2	400	24	5	60	60	++
6c	5	400	24	4	25	0	+
$ZnPcS_3$	5	400	24	8	12	0	++
$ZnPcS_4$	5	400	24	11	91	91	+++
	2	400	24	8	12	12	++
	5	400	48	8	50	38	++
PII[e]	5 mg kg^{-1}	400	24	10	70	70	+++

[a] Fluence rate, 200 mW cm^{-2}. [b] Tumor response was identified as necrosis when the tissue appeared as flat and necrotic, macroscopically observed within 2−3 days after PDT, or as regression, i.e., absence of a palpable tumor 3 weeks after PDT. [c] Excluding 10% mortality. [d] Determined 24 h post-PDT: (+) light, (++) medium, (+++) extensive, (++++) extensive edema and damage to the muscle surrounding the tumor. [e] Reference 25.

sulfonyl-substituted SubPc from 4-(chlorosulfonyl)-phthalonitrile in chlorobenzene occurs readily at room temperature, which is unprecedented for all reported cyclotrimerization reactions of this kind, whereas the use of low-boiling chlorobenzene, instead of previously employed 1-chloronaphthalene, facilitates the isolation of the product. The ring-expansion reactions of trisulfo-SubPcB(OH) were conducted in DMSO. The reaction rate and the composition of products largely depend on the nature of the diiminoisoindoline precursor and the reaction temperature. For instance, electron-donating 4-*tert*-butyldiiminoisoindoline reacts with trisulfo-SubPc at room temperature. The electron-withdrawing sulfo substituents on the SubPc macroring and the relative low reaction temperatures favor a clean reaction, and all trisulfo-substituted Pc's were obtained as major products which were readily isolated from the reaction mixture. Conventional metalation of free base Pc's with zinc acetate dihydrate afforded the corresponding zinc complexes which are soluble in water as well as in polar organic solvents.

Differences in in vitro phototoxicity correlated well with changes in hydrophobicity resulting from the various substituents added to the parent $ZnPcS_3$, with the exception of the bulky diphenylpyrazino substituent which likely interferes with cell uptake. In vivo PDT tumor response with the differently substituted $ZnPcS_3$ did not follow the same structure−activity pattern as observed in the in vitro assay. However, the highest activity in both in vitro and in vivo assays was observed with the same analogue, i.e., the (*t*-Bu)naphtho compound **6b**. Solubility and advantageous spectral properties further render this compound an interesting candidate drug for the PDT of tumors.

Experimental Section

Materials. General. FAB-MS were obtained on an LG Autospec Q mass spectrometer from the Faculty of Medicine, Laval University (Québec). Electrospray MS, MALDI, and SIMS were obtained from the Department of Chemistry, University of British Columbia (Vancouver). ^{1}H and ^{13}C NMR spectra were taken on a Brucker AC-300 (300 MHz) spectrometer using DMSO-d_6 as a solvent. UV−vis spectra were recorded with a Hitachi U-2000 spectrophotometer. IR spectra were obtained on a Perkin-Elmer 1600 spectrometer.

TLC was performed on 0.25-mm thick POLYGRAM SIL G/UV-254 plates (Macherey-Nagel, Germany). Seppak cartridges (CSC, Montreal), packed with silica gel or C-18 particles, were used for purification of the analytical samples. Analytical HPLC was conducted on a 0.94- × 25-cm column (CSC, Montreal) packed with ODS-2, C-18 reversed-phase particles and operated with a linear gradient from 100% aqueous sodium phosphate buffer (pH 7) to 100% methanol over a period of 25 min followed by isocratic elution with 100% methanol for 10 min, at 1.5 mL min^{-1}. Eluted Pc's were detected by their absorbance at 670−700 nm, SubPc's at 566 nm. The 1 M solution of boron(III) tribromide in dichloromethane was purchased from Aldrich. All solvents were HPLC grade and were used without further purification unless otherwise stated. 4-*tert*-Butylphthalonitrile was purchased from TCI America, 6-*tert*-butyl-2,3-dicyanonaphthalene from Aldrich. $ZnPcS_{2a}$, $ZnPcS_3$, and $ZnPcS_4$ were obtained by the condensation of 4-sulfophthalic acid and phthalic acid in the presence of zinc acetate dihydrate.[5]

For compounds **5a**−**c**, original nomenclature for the phthalonitrile and 2,3-dicyanonaphthalene was retained.

Bromo{2,9,16(17)-tris(chlorosulfonyl)-7,12:14,19-diimino-21,5-dinitrilo-5*H*-tribenzo[*c*,*h*,*m*][1,6,11]triazacyclopentadecinato(2−)-*N*^{22},*N*^{23},*N*^{24}}boron (Bromoboron 2,9,16(17)-tris(chlorosulfonyl)subphthalocyanine) (2) (Scheme 1). 4-(Chlorosulfonyl)phthalonitrile (**1**) (2 g, 8.83 mmol) was dissolved in dry, freshly distilled 1-chlorobenzene (5-mL). A 5 mL solution of 1 M boron(III) tribromide in dichloromethane (1.26 g, 5 mmol of BBr_3) was added dropwise. The mixture immediately turned dark purple and was stirred for 15 min at room temperature, heated to 40 °C, and maintained at this temperature for 2 h. After cooling to room temperature, the solvent was evaporated under reduced pressure. The crude compound **2** (dark purple powder, free of starting material, soluble in $CHCl_3$, toluene, and CCl_4, gives magenta-colored solutions) was used in the next step without further purification. The samples used for spectroscopic characterization were rapidly purified in the dark on a normal-phase Seppak, conditioned with $CHCl_3$ by loading the concentrated solution of **2** in $CHCl_3$ and subsequent elution with the same solvent followed by evaporation of the solvent without heating. The purity of the sample was verified by silica gel TLC in $CHCl_3$, developed in the dark, revealing two close spots at R_f 0.62 and 0.54. MS (FAB): *m*/*e* 678 (5, M^+ − BBr), 452 (10, M^+ − BBr − (chlorosulfonyl)phthalonitrile), 226 (100). ^{1}H NMR (DMSO-d_6): δ 7.31 (m, 3H), 7.76 (m, 6H). ^{13}C NMR (DMSO-d_6): δ 119.99, 123.42, 123.67, 127.36, 128.72, 130.66, 131.74, 132.07, 154.09. UV−vis λ_{max} ($CHCl_3$): 569, 226 nm. IR (KBr): 1475−1350 s, 1220−1175 cm^{-1} s (S═O, sulfonyl chloride).

Hydroxy{2,9,16(17)-trisulfo-7,12:14,19-diimino-21,5-dinitrilo-5*H*-tribenzo[*c*,*h*,*m*,][1,6,11]-triazacyclopentadecinato(2−)-*N*^{22},*N*^{23},*N*^{24}}boron, Tripyridinium Salt (Hydroxyboron(III) 2,9,16(17)-trisulfosubphthalocyanine, tripyridinium salt) (3) (Scheme 1). Crude compound **2** (2.2

g) was dissolved in a mixture of 30 mL of pyridine and 60 mL of water and stirred for 12 h in the dark, at room temperature. The solvent was evaporated under reduced pressure, and the residue (dark purple solid) was redissolved in a minimal amount of water (about 10 mL) to yield a purple solution which was filtered (no residue) and poured into 300 mL of cold acetone. The resulting dark purple slurry was centrifuged, and the slightly pink supernatant was discarded. The pellet was washed with acetone and dried in vacuo (1 mmHg, 30−40 °C) for 6 h to yield **3** (1.75 g, 60% yield based on **3** pentahydrate, according to combustion analysis data, and starting compound **1**) as golden-purple crystals. Compound **3** is soluble in water, DMSO, and methanol (magenta-colored, light-sensitive solutions). Anal. ($C_{39}H_{38}N_9BO_{15}S_3$) (pentahydrate) N, S; C: calcd, 47.81; found, 47.30. H: calcd, 3.91; found, 3.47. SIMS: *m/e* 625 (4, $M^+ - BOH$), 207 (100). UV−vis λ_{max} (H_2O) (log ϵ): 569 (4.85), 515 (sh), 226 nm (4.4). IR: 1135−1187 s, 1036−1029 s, 676−644 cm^{-1} s (S=O). HPLC: single peak, t_R 12 min.

(4-*tert*-Butyl)tri(4-sulfo)phthalocyanine (5a) (Scheme 2). A solution of **3** (0.2 g, 0.2 mmol) in 10 mL of anhydrous DMSO was added to a solution of the diiminoisoindoline derivative **4a** (0.4 g, 2 mmol) in 10 mL of anhydrous DMSO. The mixture was shielded from light, kept at room temperature for 1 h, diluted with 75 mL of methanol, and stirred at room temperature for another 2 h. The blue phthalocyanine precipitate was filtered, repeatedly washed with methanol, and dried. The blue solid was redissolved in a minimal amount of water and reprecipitated upon addition of concentrated HCl. The precipitate was filtered, washed with diluted HCl and acetone, and dried in vacuo (1 mmHg, 100 °C) for 6 h to yield 52 mg (32%) of **5a**. An analytical sample was purified on a C-18 Seppak conditioned with 5 mM phosphate buffer (pH 5), washed with the same buffer, and phthalocyanine was eluted with methanol. Anal. ($C_{36}H_{26}N_8S_3O_9$) C, H, S; N: calcd, 13.82; found, 13.03. MS (electrospray): 811 (M^+). UV−vis λ_{max} (methanol), (log ϵ): 690 (5.5), 656 (5.48), 633 (4.8), 340 nm (4.95). HPLC (detector 656 nm): three close peaks, t_R 21−24 min.

6-*tert*-Butylnaphthotris(4-sulfobenzo)porphyrazine (5b) (Scheme 2). A solution of **3** (0.2 g, 0.2 mmol) and **4b** (0.5 g, 2 mmol) in 20 mL of anhydrous DMSO was kept in the dark at 50 °C for 48 h. The reaction was followed spectrophotometrically, and upon completion a small amount of chloroform was added. The resulting dark green mixture was centrifuged, and the waxy precipitate was redissolved in methanol, reprecipitated with a few drops of chloroform, and centrifuged. This procedure was repeated until the UV−vis spectrum of the compound was constant. The sulfo acid **5b** (21 mg, 12%) was obtained from the pyridinium salt upon dissolution in water and precipitation with concentrated HCl. This material, analyzed by HPLC, contained about 10% tetrasulfo-Pc (single peak, t_R 10 min). This impurity was eliminated as follows. A short silica gel column was purged with a mixture of ethanol, methanol, and concentrated aqueous ammonia (8:3:2). The Pc mixture, dissolved in a minimal amount of aqueous methanol, was loaded on a silica gel column, and pure **5b** was eluted using the same alcohol−ammonia mixture, whereas tetrasulfo-Pc remained on the column. The solvent was evaporated; the residue was redissolved in a minimal amount of water and salted out with brine. Anal. ($C_{40}H_{25}N_8S_3O_9 \cdot Na_3$) (sodium salt) S; C: calcd, 55.81; found, 55.12. H: calcd, 3.47; found, 2.98. N: calcd, 11.02; found, 10.51. MS (electrospray): 859 ($M^+ - 1$). UV−vis λ_{max} (methanol) (log ϵ): 728 (4.57), 692 (5.05), 656 (5.01), 625 sh, 610 sh, 340 nm (4.64). HPLC (detector 656 nm): three close peaks, t_R 22−24 min.

5,6-Diphenyl-2,3-pyrazinotris(4-sulfobenzo)porphyrazine (5c) (Scheme 2). **5c** was synthesized as described for **5b**, using **3** (0.2 g, 0.2 mmol) and **4c** (0.564 g, 2 mmol) as starting materials. The reaction was conducted at 70 °C and took 36 h to complete, to yield **5c** (55 mg, 31%). The phthalocyanine precipitate was filtered and washed with acetone. Further purification of this compound was performed as described for **5a**. Anal. ($C_{42}H_{26}N_{10}S_3O_{10}$) (monohydrate) C, H, N. Anal. ($C_{42}H_{35}N_{10}S_3O_{16} \cdot Na_3$) (sodium salt heptahydrate) C, N; H: calcd, 3.20; found, 4.18. S: calcd, 8.74; found, 8.23. MS (electrospray): 908 (M^+). UV−vis λ_{max} (methanol) (log ϵ): 677 (5.15), 659 (5.11), 605 (4.78), 382 nm (4.51). HPLC (detector 677 nm): three close peaks, t_R 20−24 min.

Zinc 4-*tert*-Butyltri(4-sulfo)phthalocyanine (6a) (Scheme 2). Compound **5a** (50 mg, 0.06 mmol) and zinc acetate dihydrate (13 mg, 0.6 mmol) were suspended in 10 mL of dry DMF. The mixture was stirred at 60 °C for 1 h. After the resulting solution was cooled to room temperature, the solvent was evaporated under reduced pressure; the residue was dissolved in a minimal amount of water and precipitated upon addition of concentrated HCl. The precipitate was isolated, washed with ethanol, and dried; then the procedure was repeated to yield 43 mg (82%) of **6a**. Additional purification of the analytical sample was performed using reversed-phase preparative chromatography; the phthalocyanine was eluted with water−methanol. Anal. ($C_{36}H_{24}N_8S_3O_9Zn$) S. Anal. ($C_{36}H_{21}N_8S_3O_9Na_3Zn$) (sodium salt) C, H, N. Anal. ($C_{36}H_{27}N_8S_3O_{12}Na_3Zn$) (sodium salt trihydrate) C, H, S; N: calcd, 11.27; found, 10.51. MS (electrospray): 874.5 (M^+). UV−vis λ_{max} (methanol) (log ϵ): 672 (4.9), 606 (4.19), 346 nm (4.57) (Figure 1). HPLC (detector 672 nm): three close peaks, weighted t_R 27 min.

Zinc 6-*tert*-Butylnaphthotris(4-sulfobenzo)porphyrazine (6b) and Zinc 5,6-Diphenyl-2,3-pyrazinotris(4-sulfobenzo)porphyrazine (6c) (Scheme 2). **6b,c** were synthesized as described for **6a**, using 10-fold excess of zinc acetate dihydrate. Since the solubility of these compounds in DMF is much higher as compared to **6a**, metalation (monitored by UV−vis spectroscopy) was completed within 10 min. The resulting solution was taken to dryness, the residue was redissolved in water, precipitated upon addition of concentrated HCl, washed with 1 N HCl (compounds **6b**,**c** are both soluble in methanol), and dried over potassium carbonate. Further purification was conducted as described for **6a**.

6b: yield 89%. Anal. ($C_{40}H_{26}N_8S_3O_9Zn$) S. Anal. ($C_{40}H_{29}N_8S_3O_{13}Na_3Zn$) (sodium salt tetrahydrate) C, H, N. MS (electrospray): 925 (M^+). UV−vis λ_{max} (methanol) (log ϵ): 706 (5.05), 678 (5.15), 650 (4.59), 615 (4.5), 342 nm (4.87) (Figure 1). HPLC (detector 678 nm): three close peaks, weighted t_R 29 min.

6c: yield 93%. Anal. ($C_{42}H_{22}N_{10}S_3O_9Zn$) S. Anal. ($C_{42}H_{19}N_{10}S_3O_9Na_3Zn$) (sodium salt) N; C: calcd, 48.59; found, 47.60. H: calcd, 1.84; found, 1.34. MS (MALDI): 973 ($M^+ + 1$). MS (electrospray): 972 (M^+). UV−vis λ_{max} (DMF) (log ϵ): 680 (4.6), 608 (3.9), 360 nm (4.32); λ_{max} (water) 670 (sh), 630 (4.11), 340 nm (4.25). HPLC (detector 680 nm): three close peaks, weighted t_R 26 min.

Dye Formulation. The concentration of stock solutions prepared in PBS (pH 7.4) was determined spectroscopically after dilution in MeOH (**6a**−**c**) or in DMF ($ZnPcS_3$, $ZnPcS_4$).

Cell Photoinactivation (Table 1). EMT-6 mouse mammary tumor cells were maintained in Waymouth's medium supplemented with 15% fetal bovine serum (FBS) and 1% L-glutamine (Gibco, Canada), according to an established protocol.[16] The photocytotoxicity test was conducted by means of the colorimetric MTT assay,[7c] which measures mitochondrial dehydrogenase activity in viable cells. Comparison of this method with a clonogenicity assay, using different PDT protocols, showed that both methods are in close agreement.[6b,7d] 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 and 5% CO_2. The cells were rinsed twice with PBS and incubated for 1 or 24 h at 37 °C with 100 μL of the drug prepared at 1 μM in Waymouth's 1% FBS. After incubation, the cells were rinsed twice with PBS, refed with 100 μL of Waymouth's 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 1.5−30 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's 15% FBS was added to each well. After 3 h, 100 μL of sodium dodecyl sulfate (10% SDS 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 blank wells in which cells were omitted was subtracted from the reading of each well. The average absorbance of the control cells, which were incubated with dye-free Waymouth 1% FBS, was taken as 100% cell survival. The light dose required to inactivate 90% of the cells (LD_{90}) incubated with 1 μM dye was extrapolated from the survival curves; 8-fold replicates were run per light dose, and the experiment was repeated at least three times.

Animal Experiments. General. All experiments were performed on male BALB/c mice (18−22 g) (Charles River Breeding Laboratories, Montreal, Canada). These experiments were conducted 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 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's growth medium.

Photodynamic Therapy (Table 2). Mice were used 6−8 days after tumor cell inoculation (tumor size: 3−5-mm diameter, 2−3-mm thickness). Animals were given an intravenous injection of 2−20 μmol kg^{-1} dye prepared in PBS (0.2 mL/20 g) and treated with red light 24 or 48 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 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 300−400 J cm^{-2}.

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