Metal Complexes as Photo- and Radiosensitizers

Hasrat Ali and Johan E. van Lier*

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

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Contents

Hasrat Ali was born in India and received his education at A.M.U. Aligarh where he obtained his Ph.D. in 1979 under the direction of Professor Shafiullah. After a short stay at Malti Chemical Industries in Nandesari, Baroda, India, he joined the MRC Group in the Radiation Sciences at the Medical Faculty of the Université de Sherbrooke, Québec, Canada, in 1982, and in 1992 was appointed Professeur Chargé D'enseignement. His research interests include the synthesis of photosensitizers. Presently he is also involved in the design of radiolabeled steroids for SPECT/PET imaging.

Johan E. van Lier was born in Amsterdam, The Netherlands, in 1942. He obtained his Ir. degree from the Technological University Delft in 1966 and his Ph.D. in 1969 under the direction of Professor Leland L. Smith from the Graduate School of Biomedical Sciences of the University of Texas at Galveston. In 1970 he joined the Medical Faculty of the Université de Sherbrooke, Québec, Canada, where he was appointed Full Professor in 1981. His main research interests include the development of second-generation photosensitizers for photodynamic therapy and radiopharmaceuticals for tumor imaging. He currently is the holder of the Jeanne and J.-Louis Lévesque Chair in Radiobiology, Head of the Department of Nuclear Medicine and Radiobiology, and Director of the new PET facility of the Sherbrooke University Hospital.

I. Metallophotosensitizers

A. Introduction

Light can be used for medical purposes by acting either directly on tissue, such as in the treatment of neonatal jaundice, or indirectly via the activation of a photosensitizer (light-absorbing molecule), as ex-

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ploited in photodynamic therapy (PDT). In this section, we review the recent developments in the chemistry of metallophotosensitizers (PS = photosensitizer) and some of their medical applications. Some metallophotosensitizers also have radiosensitizing properties and are discussed in this section only to avoid duplication of material. The nonmetalloPS are briefly discussed where necessary to establish relevance of the metalloanalogues to potential medicinal applications. Several indepth reviews on the current status of PDT have appeared,[1] covering drug development and formulation, light delivery and dosimetry, and action mechanism, and only a brief overview is provided below.

The use of light and dyes to treat medical conditions can be traced back to the ancient Egyptians who treated, over 4000 years ago, vitiligo with the combination of orally ingested plants and sunlight.[2] To date, we know that the success of the treatment resulted from a photodynamic reaction mediated by the psoralens present in the plants.[3] Furanocoumarins (psoralens) activated by UVA light (PUVA therapy) are now routinely used in modern medicine for the treatment of psoriasis, a common disease in humans.[4] The earliest systematic studies of photosensitized reactions are credited to Oscar Raab for his work on acridine and eosine dyes toward the end of the 19th century.[5] Raab discovered that these dyes had no effect on paramecia in the dark but rapidly killed the microorganisms upon exposure to light. Shortly thereafter, in 1903, Tappeiner reported the use of topically applied eosin and light as a treatment for skin cancer.[6] The photodynamic effect in humans, after systemic administration of a PS, was first observed in 1912 by Meyer-Betz, after injecting himself with 200 mg of hematoporphyrin (Hp). Subsequent exposure of small regions of skin on his arm to visible light resulted in severe sunburn reactions.[7] A few days after the experiment, Meyer-Betz went for a stroll in the rather dim winter sun resulting in extreme swelling and edema of his face. Ironically, this severe skin sensitivity is still a major side effect of patients treated with the only clinically approved photosensitizer, Photofrin. Since Photofrin consists of a mixture of dimeric and oligomeric Hp derivatives, which are readily formed during the purification of Hp, it is likely that the reaction endured by Meyer-Betz resulted from polymeric impurities in the Hp preparation rather than Hp itself. To date Photofrin is used for both palliative and curative treatment of various cancers in countries throughout the world.

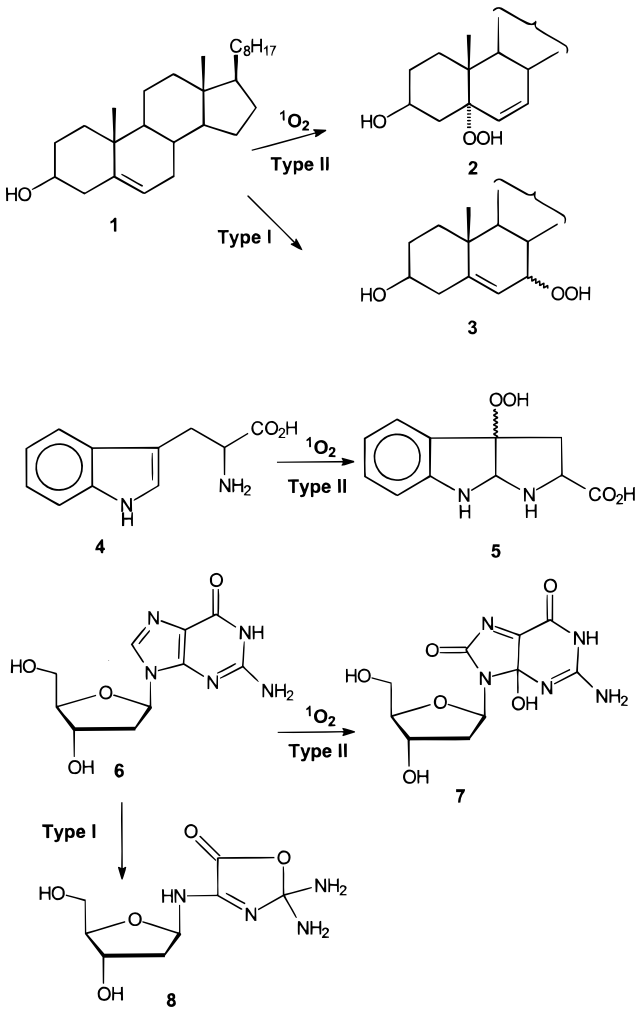
Clinical acceptance of this alternative cancer treatment protocol, combined with the potential use of PDT in many other medical indications, led to an extensive search for alternative PS with improved photophysical, chemical, and physiological properties. As a pharmaceutical, Photofrin has several shortcomings including chemical inhomogeneity, weak absorbance at the clinical excitation wavelength ($\epsilon = 10^3\ M^{-1}\ cm^{-1}$, at 640 nm) as well as prolonged skin retention. Sought-after features in second-generation PS include strong absorbance of red light (>650 nm), where tissue transparency is optimal, high quantum yields of triplet formation at sufficient high energy (>94 kJ mol^{-1}) to allow for singlet oxygen production, absence of dark toxicity, and favorable pharmacokinetics, i.e., selective retention in the selected target tissue combined with rapid clearance from the body. Evidently the dye should be chemically pure and large-scale synthesis from available starting materials, to yield a single isomeric product, should be facile. There are hundreds of naturally occurring and synthetic dyes that might fulfill such requirements,[8] but economics and proprietary constraints lead to a focused development of a few selected classes of PS.

Sensitizers currently under evaluation for PDT include several metallocomplexes. Most of them exhibit absorption maxima in the Q-band region above 600 nm, i.e., toward the red end of the visible spectrum, with molar extinction coefficients ranging from 10^3 to $10^5\ M^{-1}\ cm^{-1}$. Their photodynamic action is initiated by the absorption of a photon followed by many competing radiative and nonradiative reactions which ultimately result in the oxidation and degradation of vital biomolecules. Molecular oxygen plays a key role in propagating the initial molecular damage, resulting in vascular collapse, tissue destruction, and cell death.

The photophysical processes following photon excitation of the photosensitizer, leading to the formation of organic radicals and activated oxygen species, involves several stages. The first step, light absorption, results in the formation of an electronically excited PS species, usually in the singlet state ($S_0 + h\nu \rightarrow S_1$). Lifetimes of the S_1 state are in the nanosecond range, which is too short to allow for significant interaction with surrounding molecules. Accordingly, little photodynamic damage is to be expected from this initially formed short-lived excited species. The S_1 state stabilizes either via a radiative process, the singlet−singlet emission called fluorescence ($S_1 \rightarrow S_0 + h\nu$), or via nonradiative intersystem crossing (ISC) from the singlet to the triplet state ($S_1 \rightarrow T_1$ + heat). The energy of the T_1 species can be dissipated via a radiative triplet−singlet emission process, called phosphorescence ($T_1 \rightarrow S_0 + h\nu$). Lifetimes of the T_1 species are in the micro- to millisecond range, which is substantially longer than those of the S_1 species, and accordingly the T_1 excited sensitizer is more likely to participate in photodynamic reactions. Thus, the photodynamic effect mainly results from energy and/or electron transfer of the excited T_1 photosensitizer to an organic substrate or molecular oxygen.

Quenching mechanisms of the T_1 state of the sensitizer can be distinguished in Type I and Type II reactions.[9,10] The Type I reaction involves electron or hydrogen-atom transfer between the T_1 sensitizer and substrate molecules to yield radical ions and free radicals. The Type II reaction is defined as the interaction between the electronically excited triplet sensitizer and ground-state molecular oxygen ($^3O_2$), which also is a triplet.[11] The latter interaction usually involves energy transfer to yield singlet oxygen ($^1O_2$) since, at least under physiological conditions, electron transfer between the T_1 sensitizer and ground-state molecular oxygen to give the superoxide anion O_2^- is far less efficient.[12] Under in vitro conditions, in

Scheme 1



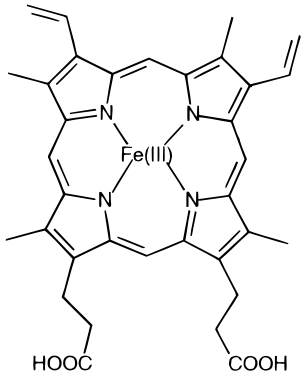
homogeneous oxygenated solutions, most photosensitizers currently under evaluation for PDT photooxidize substrate predominantly via a Type II pathway.[13−15] Accordingly, $^1O_2$ is considered the principal cytotoxic species during PDT even though direct evidence for its formation under in vivo conditions is not convincing.[16−18] In the absence of scavengers, the lifetime of $^1O_2$ in water is in the order of microseconds; however, its high reactivity combined with the abundant availability of unsaturated substrate molecules render its lifetime in the biological environment too short for detection.[19] Furthermore, binding of the sensitizer to cellular macromolecules could favor Type I hydrogen- or electron-transfer reactions which are not evident under in vitro conditions. For the overall photodynamic damage the initial reaction is of less importance since either Type I or Type II reactions can initiate similar radical chain reactions in the presence of oxygen. Both Type I and Type II pathways ultimately lead to the oxidative degradation of biomolecules with the nature and extent of the biological damage being determined by the localization of the sensitizer.

Singlet oxygen rapidly reacts with a variety of electron-rich substrates including cholesterol (**1**) and unsaturated fatty acids in lipid layers of membrane structures, amino acid residues such as cysteine, histidine, and tryptophan of protein structures, as well as nucleic acid bases of DNA, particularly guanine and thymine. Some typical $^1O_2$ (Type II) reaction products include the 5α-hydroperoxyl (**2**, major) and epimeric 6-hydroxyperoxyl (minor) derivatives from cholesterol,[20,21] a tricyclic hydroperoxyl derivative (**5**) from tryptophan[13] (**4**), and 4-hydroxy-8-oxo derivatives (**7**) from 2′-deoxyguanosine (**6**)[22] (Scheme 1). Cholesterol and 2′-deoxyguanosine give distinctly different products in Type I reactions including 7-hydroperoxyl derivatives (**3**) from cholesterol (**1**) and imidazole ring opening products (**8**) from 2′-deoxyguanosine (**6**) (Scheme 1).

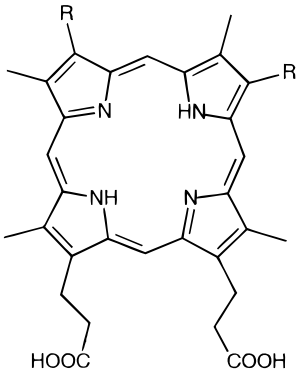
B. Porphyrins

1. Metalloporphyrins as Photosensitizers

The basic tetrapyrrole skeleton of porphyrin, found in many natural pigments, such as hemin (**9**), chlorophyll, and bacteriochlorophyll, is probably one of the oldest bioorganic structures known to man. On



9
Hemin



10: Hematoporphyrin (Hp) R = $CH(OH)CH_3$
11: Protoporphyrin IX (Pp IX) R = $CHCH_2$

the basis of their photophysical properties, both naturally occurring and synthetic tetrapyrrole derivatives have recently found specific biomedical applications, particularly in the field of PDT.[23] The electronic heart of the porphyrin structure is the inner 16-membered conjugated carbon ring with 18 π electrons that are responsible for the characteristic, porphyrin-type optical spectra. Generally, porphyrins show four absorption bands in the visible region and one very intense band, the Soret band, in the near-ultraviolet (∼400 nm). Formation of a metallopor-

phyrin complex results, in most cases, in the collapse of the four-banded spectrum to yield two absorption bands in the visible region, while the Soret band remains usually unaltered.[24] Among the naturally occurring porphyrins, hematoporphyrin (Hp) (**10**) and protoporphyrin IX (PpIX) (**11**) are readily available from mammalian blood.[25] An oligomeric mixture of Hp derivative (HpD), obtained during Hp−acetate hydrolysis, was first reported by Lipson et al. in 1961.[26] The material localized in tumor tissue, as evidenced from their fluorescence (610−630 nm). The active component was subsequently further purified,[27] revealing the presence of a complex mixture of Hp oligomers consisting of dimers[28] as well as 9-mer along with less oligomers.[29] Although ester and ether bond[30] linkages appear to prevail, side chains and methene (*meso*) position carbon−carbon linkages have also been proposed.[31] A standardized preparation is marketed as a lyophilized powder by QLT Phototherapeutics and Sanofi Pharmaceuticals under the commercial name Photofrin.[32] Currently, Photofrin is the only drug approved for clinical PDT as a potentially curative treatment of certain types of early-stage lung cancer and as a palliation of symptoms in patients suffering from esophageal or endobronchial nonsmall cell lung cancer.[23]

In aqueous solution, or in tumor tissue after injection, Hp and HpD form a new compound, with fluorescence emission at 570−590 nm.[33] Moan et al.[34] attributed this to the formation of Zn−porphyrins, possibly together with traces of Cu− and Co−porphyrins. In contrast to free porphyrins, Zn−porphyrin complexes are much less fluorescent and they are poor PS. The photosensitizing efficiency of ZnHp is about 50% lower than that of Hp, both with respect to formation of singlet oxygen and photoinactivation of cultured mammalian cells. On the other hand, the Cu^{2+}, Co^{2+}, and Fe^{2+} complexes lack photodynamic activity all together, reflecting their low quantum yields for singlet oxygen formation. Protoporphyrin IX is an efficient PS formed in most tissues as an intermediate in the biosynthesis of heme.[35] The first committed intermediate in the biochemical pathway leading to heme formation is 5-aminolaevulinic acid (ALA), which itself is not a PS.[35] Systemic administration of ALA results in PpIX accumulation in various diseased tissues. Dysplasia, neoplastic and nonmalignant inflammatory, and hyperproliferative lesions have been shown to accumulate efficacious photosensitizing concentration of PpIX following applications of exogenous ALA. Kennedy and Pottier[35] showed that topical applications of ALA in oil and water emulsions to lesions of superficial basal carcinoma resulted in selective PpIX accumulation within the lesions. Subsequent exposure to red light resulted in up to 90% complete clinical response. ALA-PDT (DUSA Pharmaceutical Inc., Toronto) is currently in evaluation as topical PDT agent for a variety of clinical indications.[36,37]

Palladium(II)−coproporphyrin I is a good PS for singlet oxygen generation.[38] The complex has been used as a photoactivable group in sequence-specific modifications of nucleic acids in oligonucleotides.[39] A 34-mer oligodeoxynucleotide was shown to be selectively modified at the G17 position upon photoirradiation in the presence of a complementary 17-mer oligonucleotide bearing Pd(II)−coproporphyrin I covalently linked to the 5′-end phosphate group. Pd(II)−coproporphyrin has also been used in phosphorescence-based immunoassays.[40] The PS has a molar extinction coefficient of 200 000 M^{-1} cm^{-1}, and fluorescence quantum yield of 0.17, resulting in a higher detection sensitivity than what can be achieved with the standard horseradish peroxidase method. Pt−*meso*-tetracarboxyphenylporphyrin and Pt−coproporphyrin were also investigated as labels for antibodies. The porphyrins were coupled to the protein by a 1,3-dicyclohexylcarbodiimide-mediated reaction in the presence of 1-hydroxybenzotriazole as a catalyst and activated by conversion to the *N*-hydroxysuccinimide ester.[41]

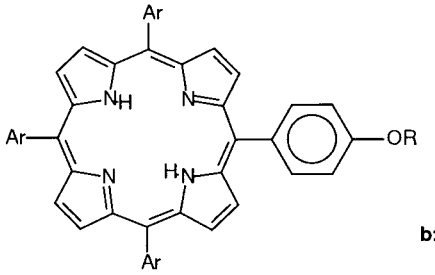
Tin−porphyrins are being considered as therapeutic agents for neonatal hyperbilirubinemia.[42] This condition is commonly treated by irradiation with broad-spectrum light containing wavelengths near 450 nm. Synthetic metalloporphyrins such as Sn−protoporphyrin (SnPP), Sn−*meso*-porphyrin (SnMP), and Sn−diiododeuterioporphyrin (SnI_2DP) are inhibitors of heme oxygenase, an activity which induces a decrease in the heme degradation to bilirubin. However, animal studies with SnPP and SnMP revealed that both dyes cause dermal erythema upon exposure of the skin of treated animals to UV irradiation.[44]

meso-Tetraphenylporphyrin (TPP) and *meso*-tetrapyridylporphyrin (TMPyP) featuring a wide variety of phenyl substituents are readily synthesized and metalated, and several derivatives have been studied as PS for PDT. *meso*-Tetra(4-sulfophenyl)porphyrin and lower sulfonated analogues are water soluble; however, substantial dark toxicity render the dyes unsuitable for medical applications.[45] TPP-substituted with hydroxyphenyl groups have also been prepared, and in particular, a chlorin analogue showed good photodynamic activity and is currently in clinical PDT trials.[46]

Water-soluble ruthenium porphyrins such as carbonyl(methanol)-*meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrinato−Ru(II) tetraacetate, carbonyl(ethanol)-*meso*-tetrakis(4-sulfonatophenyl)porphyrinato−Ru(II) tetrasodium salt, and carbonyl(methanol)-*meso*-tetrakis(4-carboxyphenyl)porphyrinato−Ru(II) were designed as DNA chain cleavers.[47] However, comparison in a mouse tumor model of their cytotoxic activity to that of *cis*-DPP did not show any significant prolongation of the survival time of the animals.

Platinum(II) complexes of TPP (**12**) or naturally occurring porphyrins (**13**−**15**) have also been reported, Charts 1 and 2.[48] Complexes with propionic acid substituents or 3-aminopropyl side chains at positions 6 and 7 of the porphyrin skeleton were prepared. The ligands were transformed into diaminedichloro−Pt(II) (**13**) and diaminedicarboxylato−Pt(II) (**14**, **15**) complexes, Chart 2. The water-insoluble dichloro−Pt(II) compound (**13e**) was converted into a water-soluble complex by replacing the chloride ligands for lactate anions (**13h**).

Chart 1



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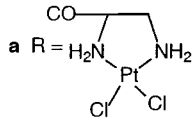
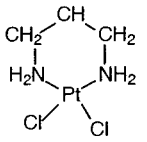
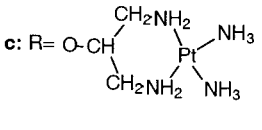
Ar= *p*-CH_3-C_6H_4



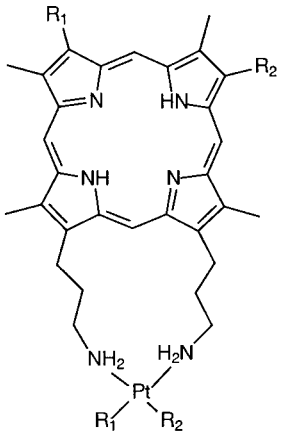
a R =



b: R=



c: R=



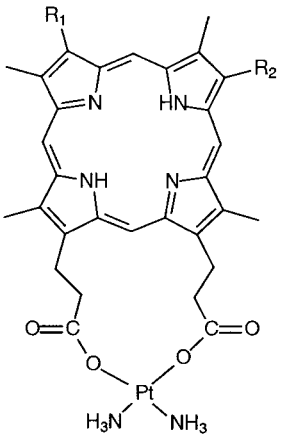
13

	R_1	R_2
a:	CH(OMe)Me	Cl,Cl
b:	CH(OCH_2Ph)Me	Cl,Cl
c:	CH[$(OCH_2CH_2)_2OMe$]Me	Cl,Cl
d:	$CH_2CH(OMe)_2$	Cl,Cl
e:	$(CH_2)_2OH$	Cl,Cl
f:	$(CH_2)_2O$glucosyl	Cl,Cl
g:	$(CH_2)O$galctosyl	Cl,Cl
h:	$(CH_2)_2OH$	Lactal

R_1=R_2

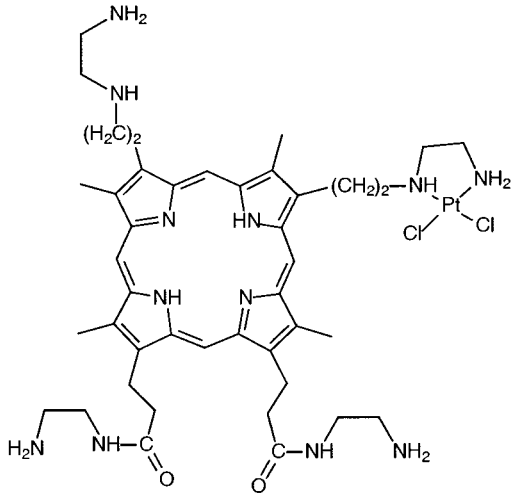
i: $CHMe(OCH_2)_7CH_3$
j: $CHMeOCH_2C(CH_3)_3$
k: $CHMeO(CH_2CH_2O)_{17}CH_3$
l: SO_3H
m: CH_2NMe_2
n: $CHCHCH_2NMe_2$
o: $CH_2NMe_3^+Cl^-$
p: $CHCHCH_2NMe_3^+Cl^-$

Chart 2



14

	R_1	R_2
a:	CH(OH)Me	CH(OH)Me
b:	CH(OH)Me	CH[$(OHp]_{2-5}$]Me
c:	CH(OH)Me	CH(OHp)Mel
d:	CH(OPh)Me	CH(OPh)Me
e:	CH_2(Ocyclohexyl)Me	CH_2(Ocyclohexyl)Me
f:	CH($OCH_2CH_2)_2$OMe}Me	CH($OCH_2CH_2)_2$OMe]Me
g:	$(CH_2)_2O$glucosyl	$(CH_2)_2O$glucosyl
h:	$(CH_2)_2O$galctosyl	$(CH_2)_2O$galctosyl

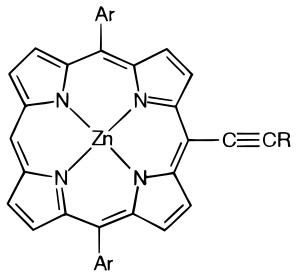


15

The photodynamic potential of the complexes was tested in vitro against a mammary tumor cell line and in vivo toward various transplantable murine carcinoma. The diamine dicarboxylato−Pt(II) complexes showed the highest antitumor activity, reflecting the combined effect of the cytotoxicity of the Pt complex and the photodynamic activity of the porphyrin moiety.

With the aim to develop steroid receptor-based PS, the synthesis of estrogen and progesterone derivatives conjugated to the Zn(II) complexes of 5,15-diphenylporphyrin[49] (**16**) and deuteroporphyrin IX dimethyl ester (**17**) have also been reported.[50]

The antiviral effects of Merocyanine 540 have been attributed to the presence of a barbituric acid group. Robinson and Morgan[51] synthesized a number of porphyrin and chlorin derivatives bearing thiobarbituric acid/barbituric acid groups on a porphyrin periphery. Compounds **18a** and **18d** were synthesized by the direct coupling of Ni *meso*-(2-formylvinyl)-octaethylporphyrin to 1,3-dibutyl-2-dibutyl-2-thiobarbituric acid or barbituric acid in the presence of



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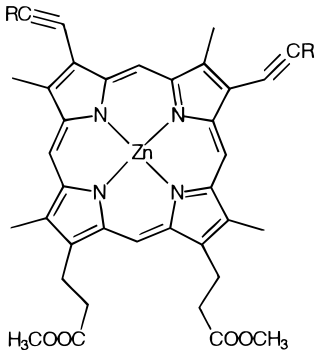
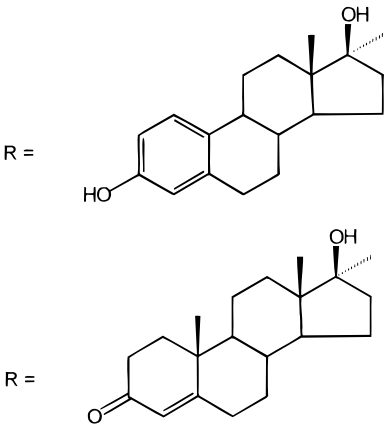


17



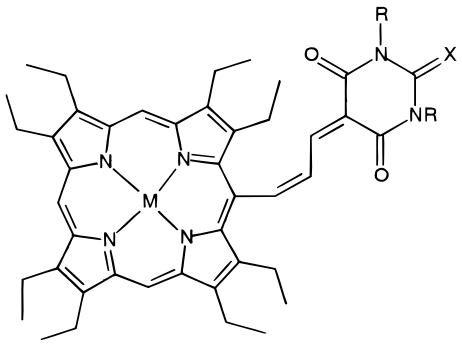
pyrrolidine, resulting in compounds with major absorption bands at 710, 505, and 404 nm, characteristic of the chlorin structure. Treatment of **18a** with concentrated H_2SO_4 gave the metal-free complex **18b**, which during silica gel purification underwent facile rearrangement to give the thiobarbituric acid chlorin (**19a**) (λ_{max} = 405, 660 nm), Chart 3. Insertion of zinc gave **18c**, which displays a chlorin-type electronic spectrum. Preliminary tumor response studies in RIF tumor-bearing mice suggested their potential use as PS for PDT, although more conclusive follow-up data are lacking.[51]

2. Boronated Metalloporphyrins

Boron neutron capture therapy (BNCT) is clinically evaluated for the treatment of cancer, particularly brain tumors.[52] Naturally occurring boron contains two stable isotopes ^{11}B (80% abundance) and ^{10}B (20% abundance). The ^{10}B isotope reacts with thermal neutrons (i.e., below 0.025 eV) to yield primary fission fragments (^4He, ^7Li) of relative low energy and high linear energy transfer (LET). The short travel range (ca. 10 μm) of the fragments combined with the local accumulation of B permits the destruction of selected tissue areas. Failure in early clinical trials of BNCT has been attributed to inadequate selectivity of ^{10}B for tumor tissues leading to excessive damage to normal tissues. Advances in the chemistry of stable cluster compounds provided new functional derivatives which can more selectively deliver boron to tumors. The apparent tumor affinity of porphyrins led to their evaluation as carriers for boron clusters. The synthesis of boronated derivatives of tetraphenylporphyrin and some naturally occurring porphyrins has been reported. They include coupling products between tetracarboranylporphyrins and four *closo*- or *nido*-carborane moieties attached directly via methylene or aromatic linkages to the porphyrin nucleus. The boron atoms are incorporated via an O-carborane cage, containing 10-B atoms per molecule. Most of the boron-containing porphyrins have photosensitizing properties, but their toxicity results from the overall structure rather than the porphyrin moiety alone.

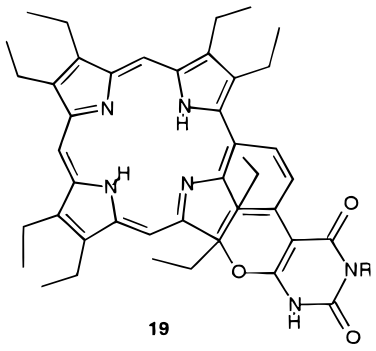
The first boronated tetraphenylporphyrin (BTPP) (**20**) developed for BNCT features four dicarbolide $[B_9C_2H_{11}]^-$ cages linked to the *o*-phenyl ring positions by anilide bonds.[53] Uptake studies in human glioma xenographs in nude mice demonstrated the feasibility

Chart 3



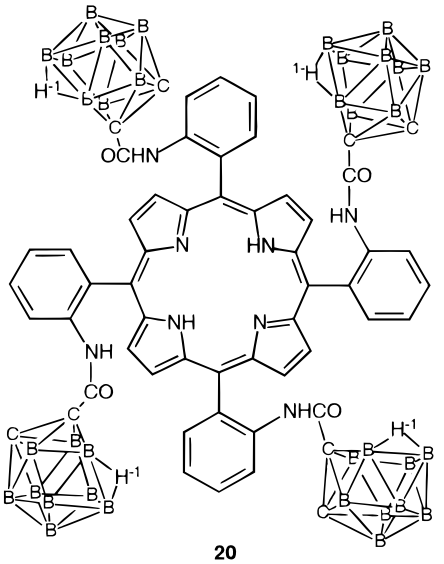
18

a: R = Butyl, M = Ni, X=S.
b: R = Butyl, M=2H, X=S.
c: R = Butyl, M=Zn, X=S.
d: R= H, M=Ni, X=O



19

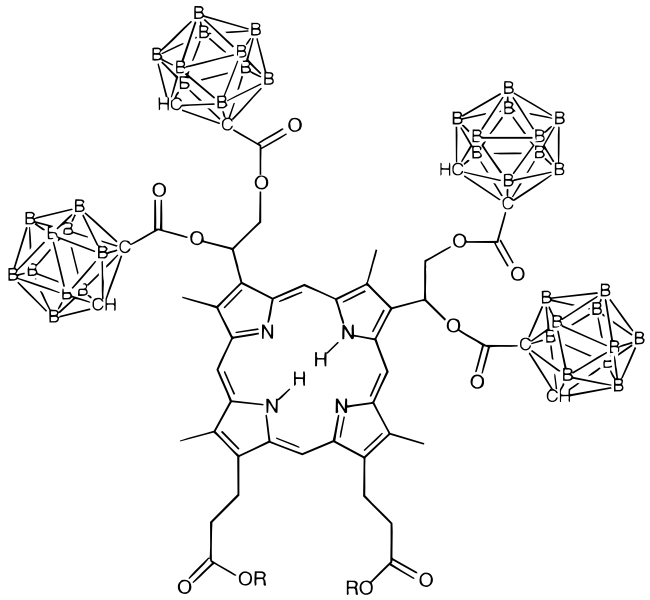
a: R = Butyl, X=S.
b: R= H, M=Ni, X=O



20

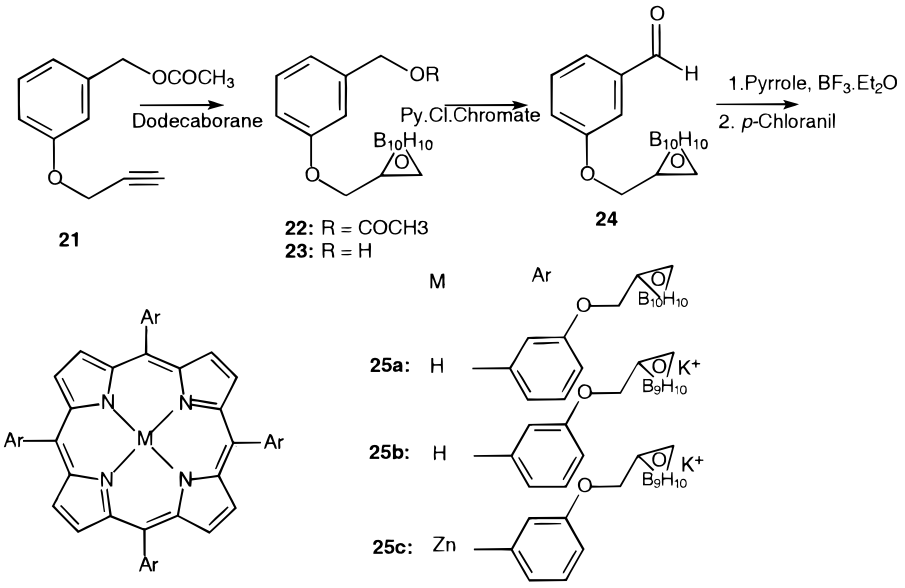
to achieve therapeutically useful boron concentrations (>20 μg/g of tissue). Other boronated TPPs were prepared by cyclization of a benzaldehyde precursor (**24**) containing a decarborane substituent (Scheme 2).[54] The latter was prepared by the treatment of **21** with decarborane to yield **22**, followed by hydrolysis of the acetyl group of **22** to give the free alcohol **23**, which was oxidized with pyridinium chlorochromate to give the aldehyde **24**. Cyclization of **24** with pyrrole in the presence of triethylorthoformate and $BF_3 \cdot Et_2O$, followed by treatment with *p*-chloranil, gave the boronated TPP **25a** in 42% yield. The carborane cage was degraded in KOH/MeOH/reflux/2 h to obtain the water-soluble anion **25b**, which upon insertion of Zn^{2+} gave **25c**. Although in vitro cell uptake experiments with **25b** were promising,[55] biodistribution studies in mice showed that both **25b** and **25c** are too toxic to allow for the administration of sufficient boron to tumor tissue.[56]

The compound that has been most widely studied for BNCT is the tetrakis-carborane carboxylate ester of deuteroporphyrin IX (BOPP) (**26a**).[57,58] The carborane ester **26a** was prepared in 85−90% yield, at room temperature, by reacting 4.5 equiv of the carborane carboxylic acid chloride with 2,4-bis(α,*β*-dihydroxyethyl)deuteroporphyrin IX dimethylester in dry CH_2Cl_2 in the presence of 4-(dimethylamino)-pyridine.[59] A trace amount of a bis-carboranyl compound was also formed. Overnight treatment with 25% aqueous HCl resulted in cleavage of the dimethyl esters to give the tetracarboranyl diacid **26b** in quantitative yield, without evidence of carboranyl cleavage. Filtration in tetrahydrofuran−H_2O (4:3) over ion-exchange resin in the K^+ form gave the highly water-soluble dipotassium salt **26c**. The visible spectrum of **26a** in CH_2Cl_2 consists of absorption maxima at 404 (Soret), 502, 536, 572, and 624 nm. The ability of BOPP to act both as a PS and a BNCT sensitizer was studied in vitro with a rat glioma cell line and in vivo with an intracerebral tumor model



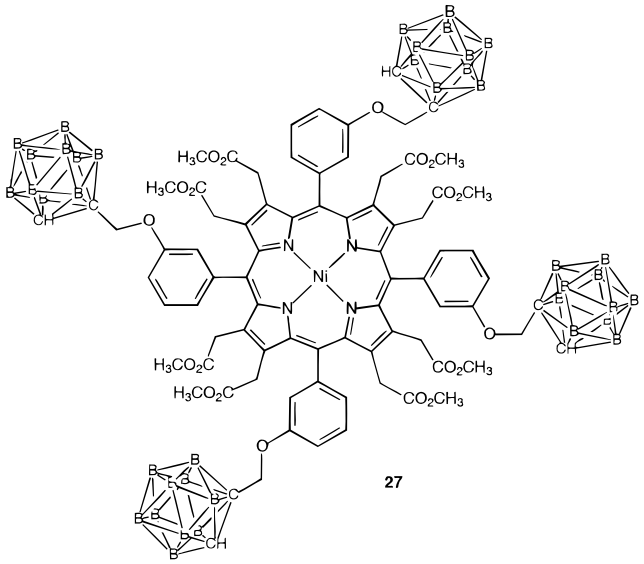
26a: R = CH_3
26b: R = H
26c: R= K

Scheme 2



in Wistar rats. The compound selectively localizes in the tumor to reach tumor-to-normal brain ratios as high as 400, suggesting the potential for selective tumor treatment while sparing normal brain tissue.[59] Confocal laser scanning microscopy of glioma cells showed that BOPP localizes in the mitochondria, which likely are the major cellular targets for the photodynamic action of the complex.[60] Studies in rats bearing intracerebral tumors showed, however, that BOPP is also too toxic at the levels required to obtain tumor concentrations useful for BNCT, making it unlikely that BOPP will enter clinical trials.[61] The analogous MnBOPP was advanced as a contrast agent for MRI of brain tumors.[62]

A more promising BNCT agent is the Ni−porphyrin complex Ni−TCP (**27**), featuring four carborane groups in a symmetric arrangement around the macrocycle.[63] This complex is not toxic at potential



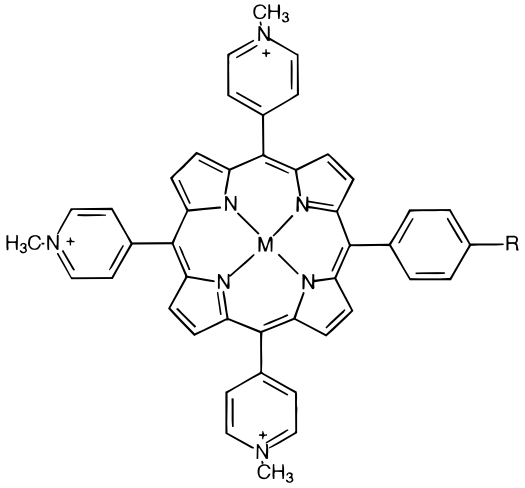
27

BNCT doses, with tumor-to-normal brain and tumor-to-blood boron differentials in mice reaching 10 and 250, respectively, at 4 days after administration. Boronated derivates of Hp that have been advanced include an amphiphilic compound prepared via substitution of the 3- and 8-hydroxyethyl groups featuring alkyl ethers of different chain lengths, with the carborane attached to the propionate side chains at the 13 and 17 positions of hematoporphyrin.[64] The boronated benzoporphyrin and purpurin-18-methyl ester have also been prepared, but their biological properties have not been detailed.[65] Finally, boronated porphyrins are also specific inhibitors of HIV protease.[66]

3. Cationic Metalloporphyrins

The design of specific DNA chain cleavers, as antiviral or antitumor agents, is a growing field of research.[67] Metalloporphyrins are versatile catalysts for oxidation reactions, capable of inducing the oxidative cleavage of DNA.[68] In particular, the cationic Mn−porphyrin complexes exert good affinity for nucleic acids and are capable of oxidizing the sugar C−H bonds of the desoxyribose units, resulting in efficient DNA cleavage.[69] Mechanistic studies indicate that Mn−oxo species likely are the reactive intermediates.[69,70]

Water-soluble, cationic metalloporphyrins bearing 4-nitrophenyl, 4-aminophenyl, 4-hydroxyphenyl, and/or pyridinium functions (**28**) on the *meso* position of the macrocycle exhibit varying cytotoxicity toward cancer cells in vitro, depending on the nature of the central atom (Mn, Fe, Zn, Ni) and on the number of pyridinium groups.[70] Derivatives based on the tris-



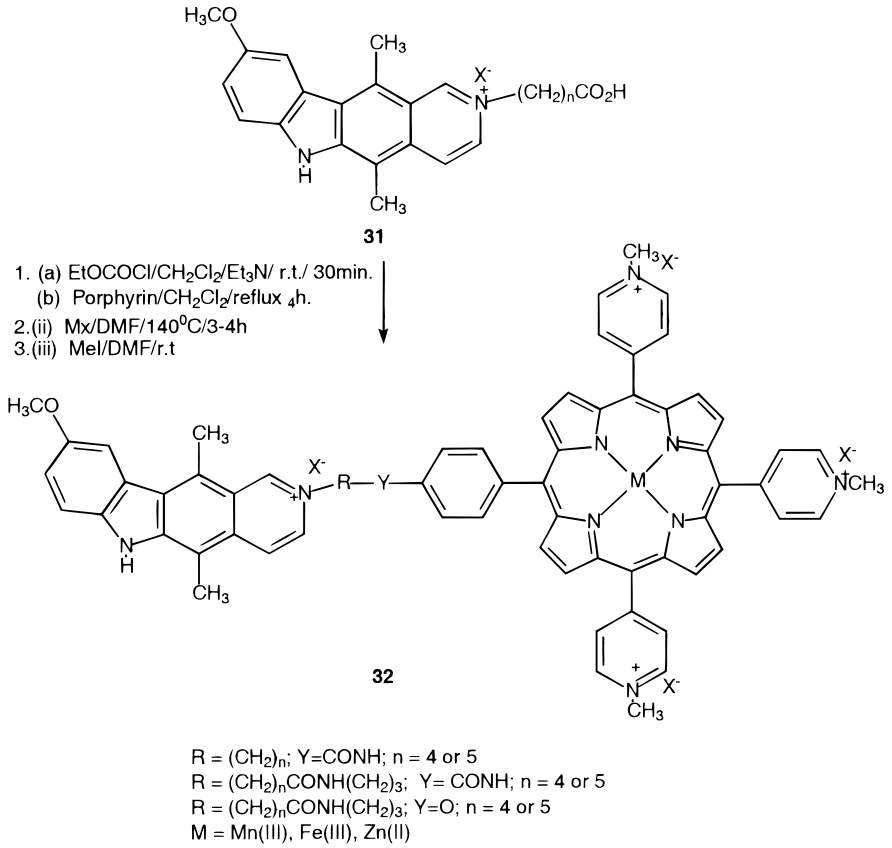
28: R= $(CH_3)_3$, NH_2, NO_2, OH
M= Mn(III), Fe(III), Zn(II), Ni(II),

29: R= CH_2NH_2

30: R= OX
X= H, COOEt, $(CH_2)_4COOEt$, $(CH_2)_4COO$ $(CH_2)_3OCPh_3$; $(CH_2)_3OH$; $(CH_2)_3NHCPh_3$; $(CH_2)_3NH_2$.
M= H_2, Ni(II), Mn(III)

(methylpyridinium)porphyrin motif were the most active, with the Mn complex exhibiting higher toxicity than the Fe analogue while the Zn and Ni complexes were inactive. Substituents on the fourth phenyl group also affect the activity with the 4-nitro analogue being the most cytotoxic, followed by the 4-amino and 4-hydroxy analogue. In the presence of high-intensity visible light and oxygen, such water-soluble porphyrins were found to induce single-strand scission in DNA.[71] Cu analogues of **29** were presented as the first example of DNA cleaving agents which utilize a porphyrin as a DNA recognizing element.[72] Recently a number of papers on structure−nuclease activity relationships of DNA cleavers, based on cationic metalloporphyrin−oligonucleotide conjugates, appeared.[73] Another series of water-soluble, cationic, unsymmetrical porphyrin ligands, **30**, containing *meso*-phenyl substituents terminating in an amine, alcohol, or acid function were prepared to allow linkage to various vectors (intercalators, peptides, or oligonucelotides).[74] Among such vectors, bleomycin has been regarded as a model compound for the development of DNA cleavers. Hybrid molecules consisting of both metalloporphyrin and ellipticine moieties were synthesized[75] in order to capitalize on the biological activities of both entities. Such complexes are prepared from 9-methoxyellipticine derivatives (**31**), which function as DNA intercala-

Scheme 3



R = $(CH_2)_n$; Y=CONH; n = 4 or 5
R = $(CH_2)_n CONH(CH_2)_3$; Y= CONH; n = 4 or 5
R = $(CH_2)_n CONH(CH_2)_3$; Y=O; n = 4 or 5
M = Mn(III), Fe(III), Zn(II)

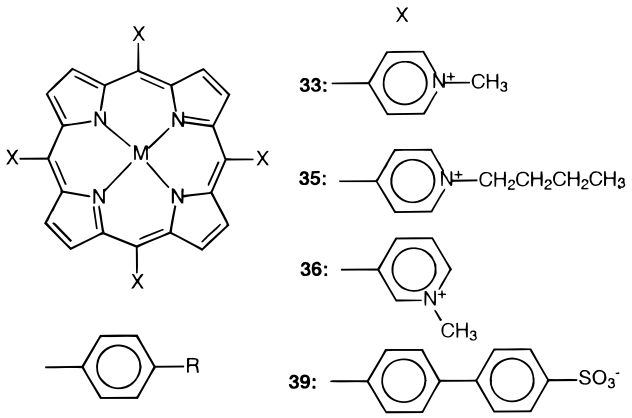
tors, and tris(4-*N*-methylpyridinium)metalloporphyrins (**30**) capable of catalyzing the oxidative cleavage of DNA. The latter feature a 4-aminophenyl or a 4-hydroxyphenyl group on the fourth *meso* position to allow for attachment of a spacer group. The effect of the length of the spacer (7−13 C atoms), its chemical nature (carboxamido or ether function), the position of the amino group between the two parts of the hybrid molecule, the number of intercalator moieties (ellipticinium) covalently attached to the metalloporphyrin, and the nature of the central metal atom (Mn, Fe, Zn) all affect the biological activity of these hybrid molecules (**32**), Scheme 3. These complexes have a particularly high affinity for double-stranded DNA resulting in cytotoxic effects, as demonstrated against murine leukemia cells in vitro. Iron complexes of **32** are more active than the analogous Mn complexes, while the corresponding Zn derivatives are almost inactive. These compounds were also found to inhibit the cytopathicity of human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus in MT-4 cells at concentrations ranging from 1.4 to 17 μg/mL. They also inhibit syncytium formation in persistent HIV-1-infected HUT-78 and uninfected Molt/cells, interfere with HIV-1 binding to cells, and suppress HIV-1-associated reverse transcript activity.[76]

4. Metalloporphyrins as Radiosensitizers

Several conflicting reports on the radiosensitizing activities of porphyrins have appeared, and a clear understanding of structural features that could explain such action does not exist.[77] Hematoporphyrin (Hp), which is the starting material for the clinically approved PDT preparation Photofrin, and a series of nonmetallic, water-soluble analogues were found to be inactive as a radiosensitizers by several researchers. In contrast, Zhao and co-workers[78] suggested that HPD increases the effect of ^{60}Co irradiation in transplanted rodent tumors by about 30%. In clinical experiments, hematoporphyrin was reported to improve the tumor response to ionizing radiation. Studies from Bellnier and Dougherty[79] and Winther et al.[80] indicated a simple additive effect while others suggested that the radiation and drug response may be synergistic. Clinical studies with substituted Hp in patients with melanoma and estrogenic sarcoma showed that a combination of radiation and phototherapy was more effective than PDT alone.[81−83]

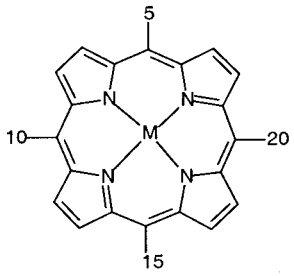
Both cationic and anionic metalloporphyrins do, however, exhibit radiosensitizing activity. A series of complexes featuring various metal ions, i.e., Co(III), Zn(II), Fe(III), Cu(II), Pd(II), Rh(III), Sn(IV), or Mn(III), were tested at 100 μM concentration against V79 cells.[83] This included the following cationic ligands, tetrakis(4-*N*-methylpyridyl)porphyrin [TMPyP] (**33**), tetrakis(4-*N*-trimethylaminophenyl)porphyrin [TMAP] (**34**), tetrakis(4-*N*-butylpyrdidyl)porphyrin [TBPyP] (**35**), and tetrakis(3-*N*-methylpyridyl)porphyrin [3-TMPyP] (**36**) and anionic ligands tetrakis-(4-sulfonatophenyl)porphyrin [TPPS] (**37**), tetrakis(4-carboxyphenyl)porphyrin [TCPP] (**38**), and tetrakis(bi-

phenyl)porphyrin sulfonate [TBPS] (**39**). The largest increase in radiation-induced cell kill was observed with **33** (M = Co) and **37** (M = Co) with sensitizer enhancement ratios (SER) of 2.4 and 2.3, respectively. The overall charge on the molecule did not correlate to the biological activities.



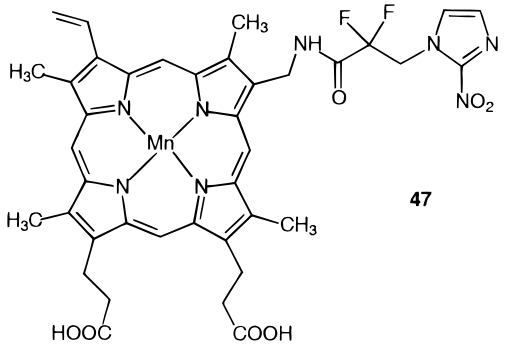
34: $(N^+CH_3)_2$
37: R= SO_3^-
38: CO_2^-

The introduction of a nitro and positively charged groups on the porphyrin moiety was expected to further increase the radiosensitization activities.[85] The Co(III) complex of **33**, **40**, and **41** and **37**, **44**, and **45**, the Cu(II) complex of **33**, **42**, and **45**, the Pt(II) complex of **33** and **41**, and the Ru(II)$(DMSO)_2$ complex were tested in vitro under hypoxic conditions and shown to have only marginal radiosensitizing activities.[86] The cationic Co(II) complexes showed the highest SER values although none of the complexes showed SER > 1.2. The Pt(II) and Ru(II) complexes of **37** were completely inactive as radiosensitizers. They do, however, bind to DNA (at *Bam*H1), as evidenced by their inhibition of DNA-damage repair, although they are far less effective than cisplatin.



40: 5,10,25-tris(4-MePy)-20-(4-Ph)porphyrin
41: 5,10,25-tris(4-MePy)-20-(4-NO_2Ph)porphyrin
42: 5,10,bis(4-MePy)-15,20-bis(4-NO_2Ph)porphyrin
43: 5,15,bis(4-MePy)-10,20-bis(4-NO_2Ph)porphyrin
44: 5(4-Py)-10,15,20-tris(4-SO_3Ph)porphyrin
45: 5(4-NH_2Ph)-10,15,20-tris(4-SO_3Ph)porphyrin
46: 5(4-NO_2Ph)-10-(4-Py)-,15,20-bis(4-SO_3Ph)porphyrin

The well-known radiosensitizing effect of nitroimidazoles prompted the evaluation of covalently linked nitroimidazole−metalloporphyrins such as **47**. At 1.5 mM concentration, this complex gave SER of 1.39 in cell culture, while in vivo assays in EMT-6 tumor-bearing Balb/c mice showed substantially improved tumor response to radiotherapy.[87]



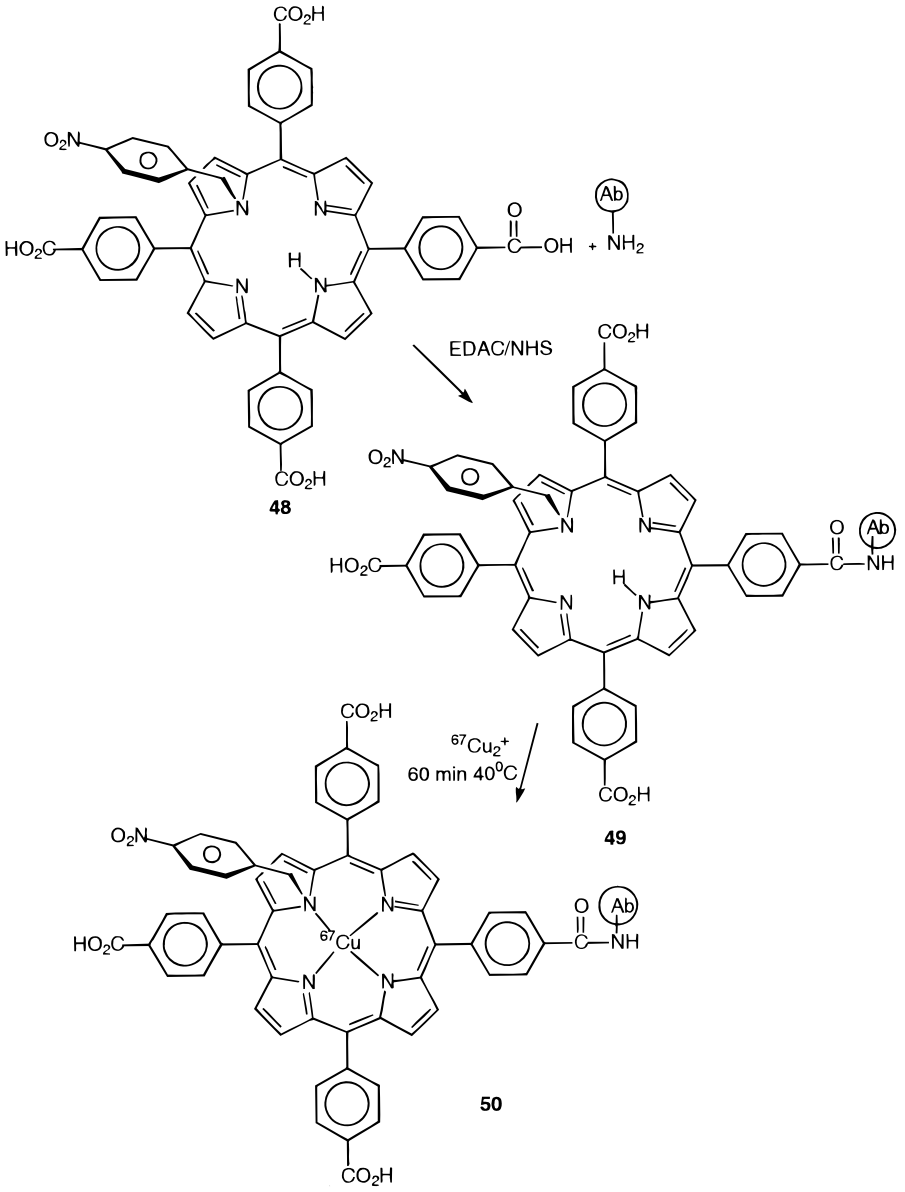
47

5. Radiolabeled Metalloporphyrins as Imaging Agents

The suggestion that porphyrins can be used for tumor detection dates back to 1924 when Policard reported their accumulation and fluorescence in tumor tissue.[88] The tumor-selectivity of porphyrin derivatives and their photodynamic action subsequently lead to their clinical use as sensitizers for PDT of various cancers. To evaluate the biodistribution pattern in order to optimize clinical treatment protocols, animal studies with ^{3}H- and ^{14}C-labeled analogues have been reported.[89] The main advantage of these labels is that the chemistry of the dyes is unaltered, although ^{3}H-labeled compounds may undergo exchange in vivo to give ambiguous results. However, these radionuclides are not suitable for scintigraphic in vivo imaging. With the development of nuclear medicine, both porphyrins and the structurally related Pc were studied as radionuclide carriers for tumor delineation. Wrenn et al.[90] developed a simple method to prepare ^{64}Cu tetrasulfophthalocyanine ($CuPcS_4$) and showed good selectivity for brain injury and experimental brain tumors in animals. Subsequently, a series of naturally occurring porphyrins labeled with ^{64}Cu ($t_{1/2}$ = 12.8 h; positron emitter) were administered to cancer patients in an attempt to visualize neoplasms. Compared to the promising results obtained with animals, localization in humans was found to be disappointing.[91] This remains, however, an active field of research, and more recently ^{64}Cu tetracarboxytetraphenylporphyrin was suggested as a potential radiopharmaceutical for inflamed lymph node detection.[92]

^{67}Cu−porphyrins (^{67}Cu: $t_{1/2}$ = 60 h; 185 keV) have also been evaluated as alternative bifunctional chelating agents for antibody labeling.[93] The commonly used bifunctional chelators diethylenetriaminepentaacetic acid (DTPA) and ethyldiaminotetraacetic acid (EDTA) form copper chelates that readily exchange with other metal ions attached to serum proteins. ^{67}Cu−porphyrins are nontoxic and exhibit good in vivo stability. They can readily be coupled to antibodies under mild condition via an amide linkage. To overcome the slow rate of metal complexation by porphyrins, an *N*-benzyl group has been added to the structure. Coupling of the *N*-benzylporphyrin **48** to an antibody (Ab) involves activation of the carboxylic group of the porphyrin with ethyldimethylaminopropylcarbodiimide (EDAC) and *N*-hydroxysuccimide (NHS) followed by a coupling reaction with the free amino groups of the antibody (Scheme 4).[94] The antibody−porphyrin conjugate **49** is then incubated

Scheme 4

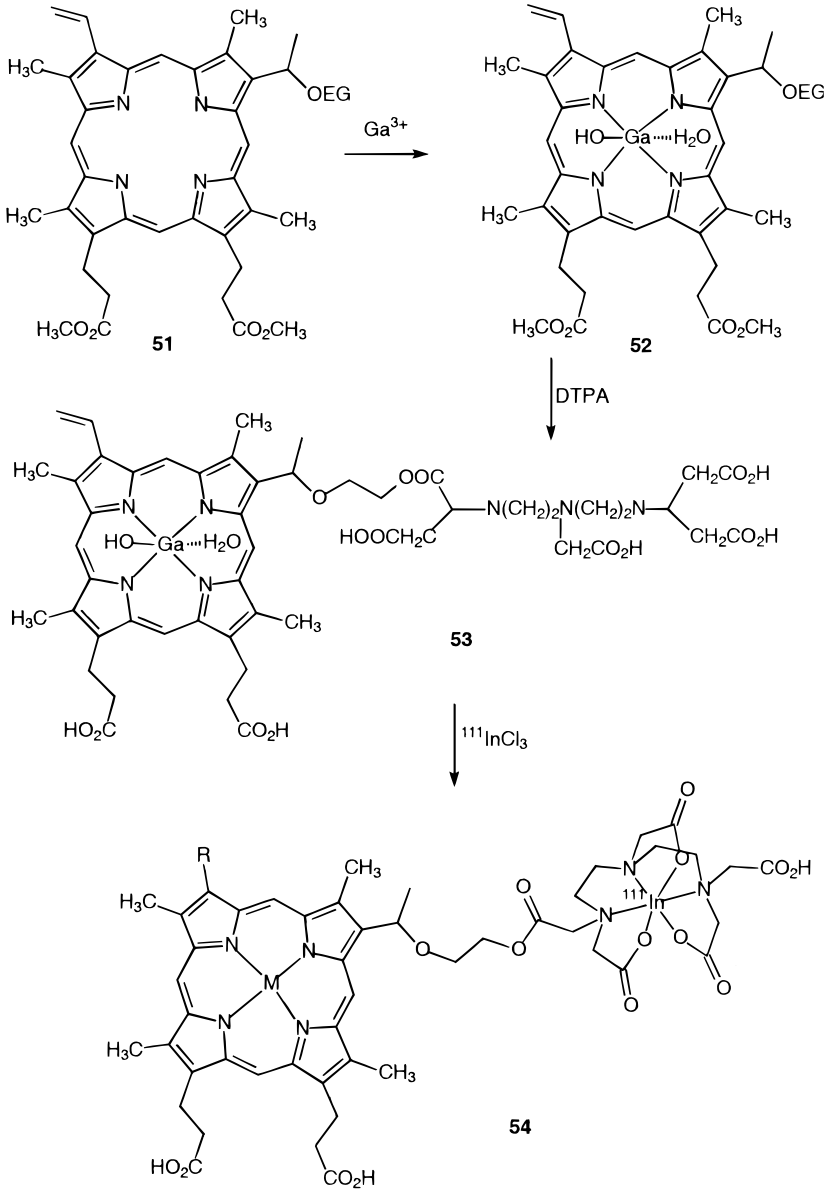


with ^{67}Cu−chloride at 40 °C for 1 h to give the radiolabeled complex **50** in 60% yield with a specific activity of 0.5 Ci/g and over 70% conservation of the immunoreactivity. The *N*-4-nitrobenzyl-5-(carboxyphenyl)-10,15,20-tris(4-sulfophenyl)porphyrin has also been used to radiolabel polyclonal antibodies and peptides.[93] This porphyrin has only one carboxy group available for coupling, thus avoiding possible cross-linking. An antirenal carcinoma antibody A6H was labeled in this manner with ^{67}Cu and its biodistribution studied in human RCC xenograft-bearing nude mice. At 45 h p.i., tumor-to-blood ratios reached 16, which is 4-fold higher than the ratio obtained with free ^{67}Cu−porphyrin.

In the case of tetraphenylporphyrins (TPP), direct chelation with ^{111}In ($t_{1/2}$ = 2.8 days; γ = 171 and 245 keV) is possible and several stable complexes were synthesized and evaluated for their biodistribution pattern.[95−97] Tetra(*N*,*N*,*N*-trimethylanilinium)porphyrin (TTAP), tetra(*N*-methyl-4-pyridyl)porphyrin (TMPyP), and tetra(4-sulfophenyl)porphyrin (T4SPP) were labeled with ^{111}In in 97% yield by heating at 120 °C with $^{111}InCl_3$ for 30 min. In rats, ^{111}In−TTAP revealed a maximum lymph node-to-muscle uptake ratio of 85:1 at 24−48 h p.i. Lymph nodes in rabbits were also clearly visualized by γ-scintigraphy at 48 h p.i. Similarly, ^{111}In−TMPyP clearly delineated malignant melanoma tumors in the hamster. The clinically approved photosensitizers Photofrin and DHE were also labeled with ^{111}In and evaluated for tumor uptake in animal models.[98]

The DTPA ester of the metal-free 4-[1-(2-hydroxyethyloxy)ethyl]-2-vinyldeuteroporphyrin-IX (ATN-0), the Ga(III) complex (ATN-2), and Mn(III) complex (ATN-10) were chelated with ^{111}In and evaluated for tumor scintigraphy.[99−102] The ATN-10 was included since the Mn(III) complex is not active as a photosensitizer and thus would avoid side effects such as skin photosensitivity. The synthetic steps involve hydrobromination of protoporphyrindimethylester to obtain a monobromoderivative, which upon treatment with ethylene glycol provides porphyrin **51** featuring an ether group at either the 7- or 12-position of the macrocycle. Metalation with $GaCl_3$

Scheme 5



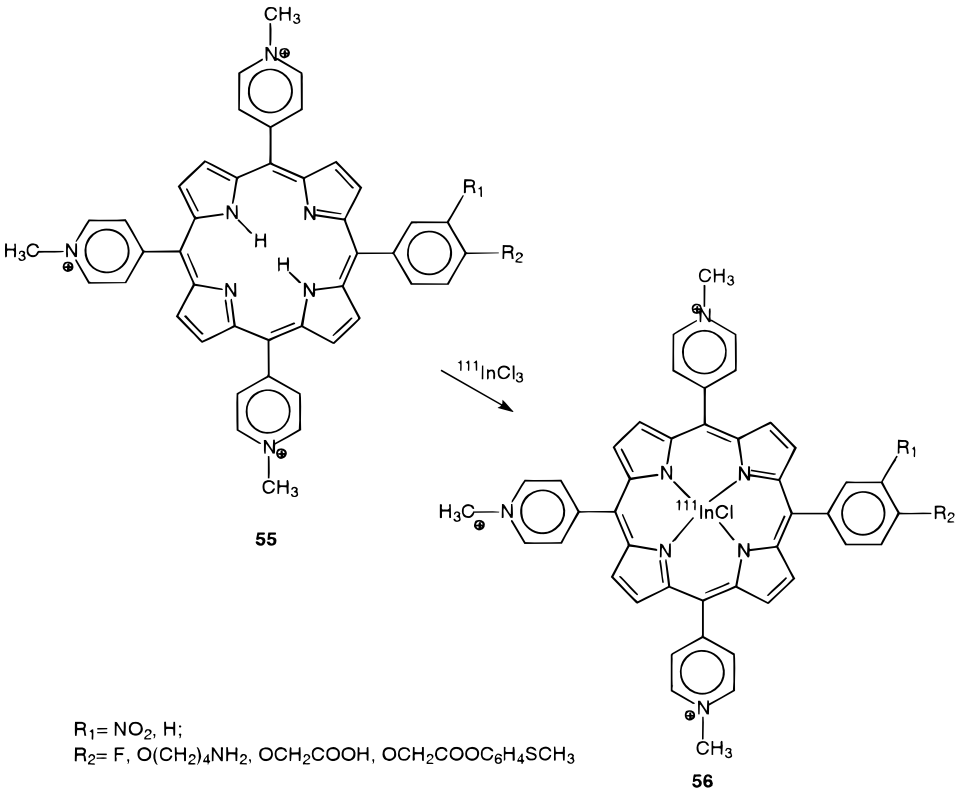
affords the Ga−porphyrin **52**, and final condensation with DTPA yields the coupled porphyrin ATN-2 (**53**), Scheme 5. Chelation of ATN-0, ATN-2, or ATN-10 with $^{111}InCl_3$ proceeds rapidly affording the radiolabeled porphyrins (**54**) in over 95% yield. Animal studies showed good tumor uptake of ^{111}In−ATN-2 and ^{111}In−ATN-10, whereas ^{111}In−ATN-0 is essentially void of tumor localizing properties. A correlation between the nature of various substituents R on the dicarboxyporphyrin complex **54** and tumor accumulation in an animal model revealed that among over 200 derivatives, ^{111}In−hematoporphyrin glutamic acid, ^{111}In−monoDTPA-EG-Ga-DP, and ^{111}In−bisDTPA-EG-Ga-DP showed good tumor accumulation.[99] Further modifications of the deuteroporphyrin IX side chains by replacing an ethylene glycol for a 2-vinyl group gave a derivative with optimal tumor selectivity.[100]

Water-soluble, monosubstituted positively charged arylporphyrins (**55**) have also been labeled with ^{111}In, Scheme 6. These derivatives (**56**) can be linked covalently to BSA and mAb. Labeling efficiency of anti-CEA mAb was >60% with good retention of immunoreactivity.[103]

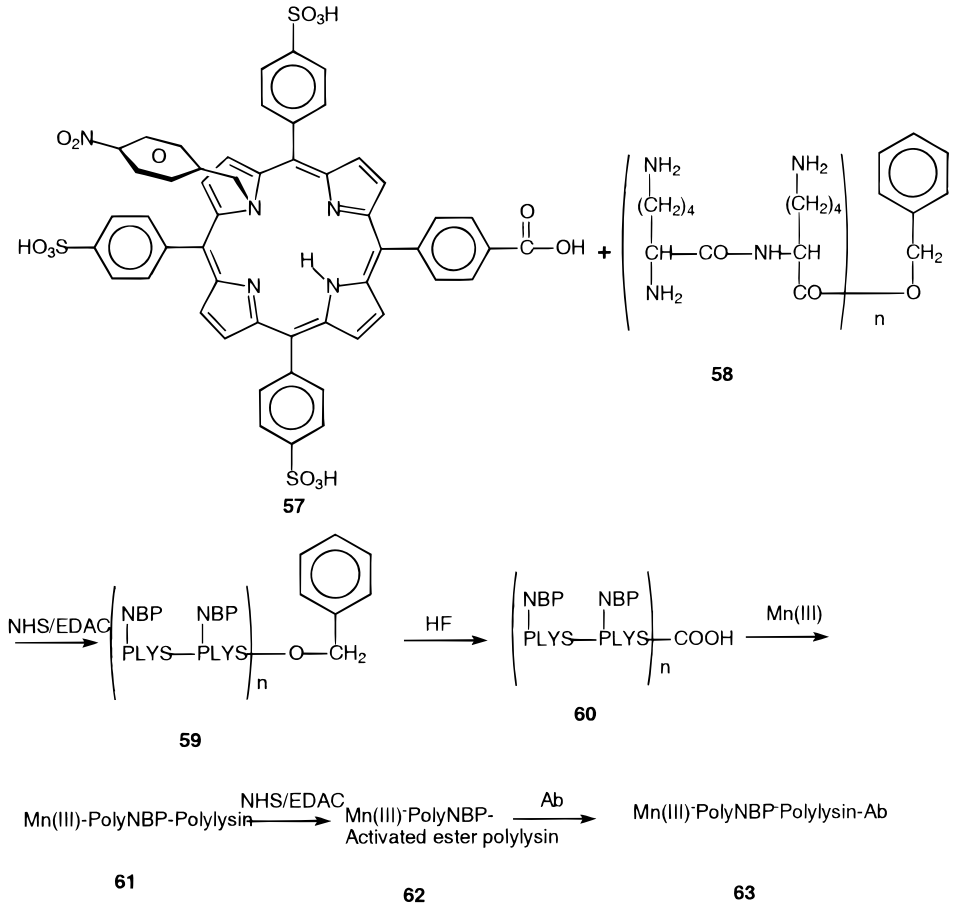
Several attempts have been made to label photosensitizers with ^{99m}Tc ($t_{1/2}$ = 6 h, γ = 140 keV). Hematoporphyrin derivatives (HPD) can be labeled with ^{99m}Tc in >90% yield by simply reducing TcO_4^- to TcO_2 with $SnCl_2$ followed by addition of an HPD solution (pH 7.4) at room temperature and 30 min incubation.[104] Tumors in different animal models were clearly visualized by scintigraphy using this preparation. Similar reaction conditions with bacteriochlorin-a, a potent long-wavelength photosensitizer, and ^{99m}Tc either as TcO_4^- or TcO_2 failed to yield labeled products, probably due to the constraint space of the macrocycle cavity.[105]

In addition to the above radionuclides, several other isotopes have also been used to prepare radioactive photosensitizers such as ^{57}Co−tetraphenylporphyrin (TPP),[106] ^{109}Pd−porphyrins and −HP,[107] ^{54}Mn−HPD.[108] Pheophorbide (Pheo), a porphyrin

Scheme 6

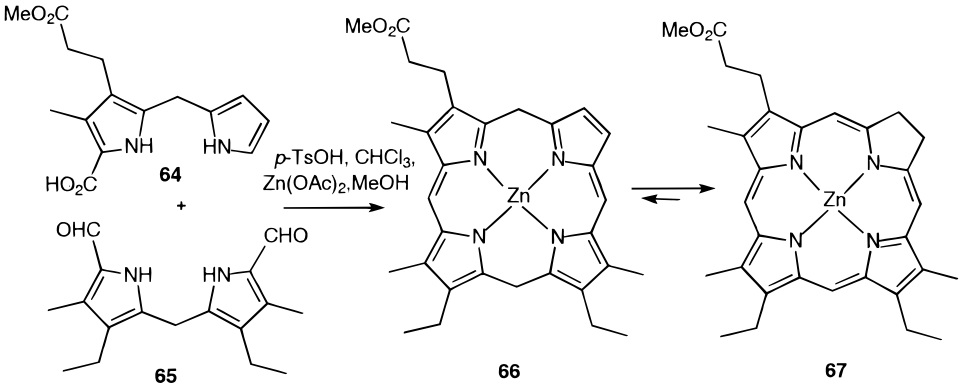


Scheme 7



produced from chlorophyll by elimination of the phytyl group and Mg, has a photodynamic action and affinity for malignant tumor tissues. ^{48}V-labeled Pheo, in which ^{48}V (a positron emitter, $t_{1/2}$ = 15.97 days) was inserted into the porphyrin ring, has been studied as a potential tumor PET scanning agent.[109]

Scheme 8



6. Metalloporphyrins as Contrast Agents

This section addresses the use of metallophotosensitizers as contrast agents for magnetic resonance imaging (MRI). Mn(III) is an interesting metal ion for medical application due to its good contrast-enhancing properties for MRI. High concentrations of manganese are, however, toxic. Mn(III) ions do form strong chelates with porphyrins to give stable complexes which are void of this unwanted activity. Synthetic tetraarylporphyrins incorporate the Mn ions tightly; they are not readily metabolized and therefore ideal for MRI. A visual contrast required at least 1 μg/g of manganese in selected tissues. To achieve this, the use of an oligopeptide carrier, such as polylysine, which is capable of chelating 100−200 metalloporphyrin molecules, has been suggested.[109] Attachment of the polylysine to the antibody involves a single lysine moiety, limiting possible damage to the antibody. The synthesis of a Mn−porphyrin−polylysine−antibody complex is depicted in Scheme 7. After protecting the carboxyl terminus of the polylysine (**58**), the material is reacted with the porphyrin (**57**) utilizing EDAC and NHS. The protecting group of the polylysine is removed by HF, the metal introduced, and the terminal carboxyl group reactivated with EDAC and NHS for conjugation to the antibody.[110]

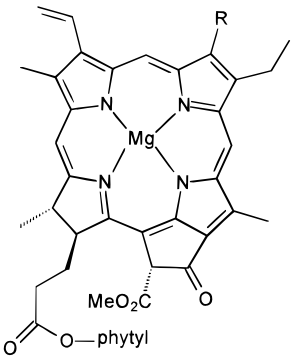
C. Chlorins and Bacteriochlorins

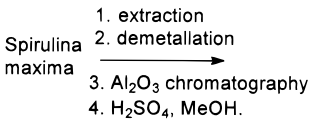
The level of saturation of the porphyrin macrocycle strongly affects the overall nature of the absorption spectrum. Chlorins (2,3-dihydroporphyrins) are distinguished from the parent porphyrins by the presence of one reduced peripheral double bond, and this change in symmetry leads to a strong absorption in the long-wavelength portion of the visible spectrum (λ_{max} = 650−680 nm). Much less intense bands appear in the central part of the visible spectrum. The tetrahydro system with two opposite reduced pyrrolic units is called a bacteriochlorin, while the regioisomer with an adjacent reduced pyrrolic unit is known as *iso*bacteriochlorin. Placing a metal in either porphyrin, chlorin, or bacteriochlorin results in a blue-shift of the Q-band.

All double bonds of the porphyrin macrocycle are potentially reducible; however, only a few of the porphyrin double bonds will react with a given reagent accordingly to their chemical stability. Among the various positions that can be reduced, the *meso*- and the β-carbons are the most reactive. Photochemical, electrochemical,[111] and other reduction methods have been used to convert porphyrins to chlorins, and although steric[112] as well as electronic factors[113] may affect selectivity, the reaction usually is nonregioselective and often reversible.

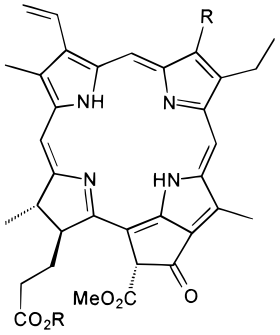
An example of a direct synthesis of a chlorin is the MacDonald 2 + 2 condensation of the dipyrromethane bisaldehyde **65** with dipyrromethane **64**, which under carefully controlled oxygen-free conditions gives porphodimethene. Upon metalation with Zn(II), the product **66** quantitatively tautomerizes over the course of several hours to metallochlorin **67**, and surprisingly only one of the two possible chlorin isomers is formed almost exclusively[114] (Scheme 8).

Chlorophyll *a* (**68**), *b* (**69**), and *c* are the most abundant among the naturally occurring tetrapyr-



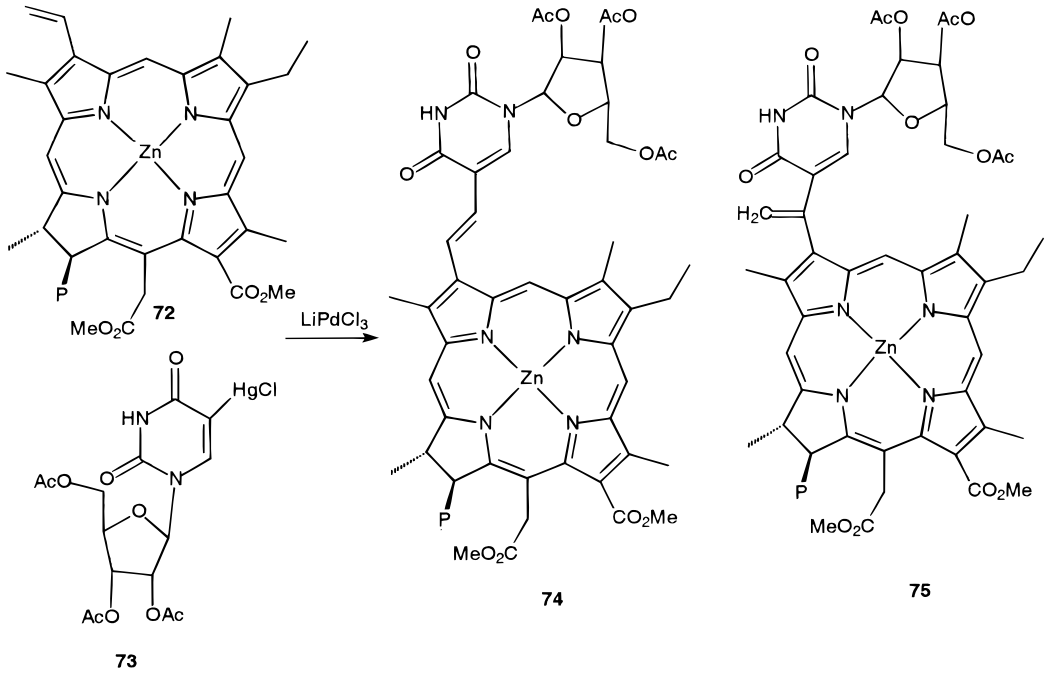


68: Chlorophyll a R= Me
69: Chlorophyll b R=CHO



70: R= Phytyl
71: R= Me

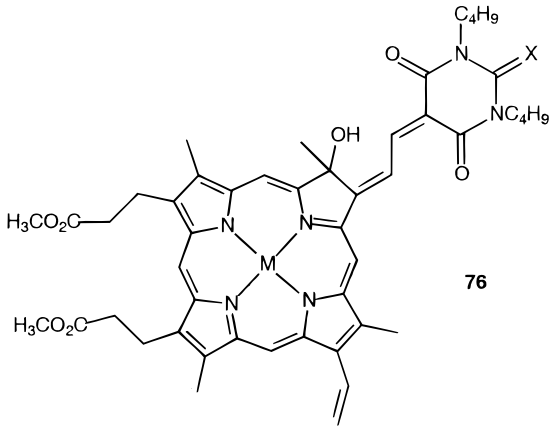
Scheme 9



rolic pigments, and they serve as starting materials for the preparation of different chlorins. Silkworm excreta, which contain relatively high concentrations of chlorophyll, have been used as folk medicine in the Far East for centuries. Recent work has shown that the acetone extract of silkworm has photodynamic properties and may contain porphyrin derivatives useful for PDT.[115] Extraction of the commercially available algae *Spirulina maxima* gives phenophytin (**70**) (demetalated chlorophyll *a*) as a single product in 0.3% yield.[116] Chlorophyll *a* and *b* bacteriochlorophyll (administered in Tween 80) sensitize the photodynamic destruction of SMT mammary tumors implanted in DBA/2 mice.[117] Bacteriochlorophyll is, however, a less efficient PS for PDT as compared to Photofrin II. A number of pheophorbide derivatives including some metal complexes have been screened for tumor retention in a golden hamster model.[118]

Several attempts have been made to modulate the biodistribution of chlorins through specific chemical modifications. A monovinylchlorin **72** has been coupled to the mercuric salt of uridine **73** by means of a Heck-type coupling reaction to yield porphyrin−nucleoside adducts **74** and **75**, Scheme 9.[119] The addition is not regiospecific and results in coupling with either carbon of the vinyl group, producing the various possible regioisomers. Reaction of the ring A and B isomers of photoprotoporphyrin with 1,3-dibutyl-2-thiobarbituric acid gives the corresponding thiobarbituric acid chlorin derivative **76** (M = 2H) and its positional isomer (structure is not shown), which upon metalation with Zn(II) gives **76** (M = Zn), a complex with a large red-shifted Q-band at 800 nm.[51]

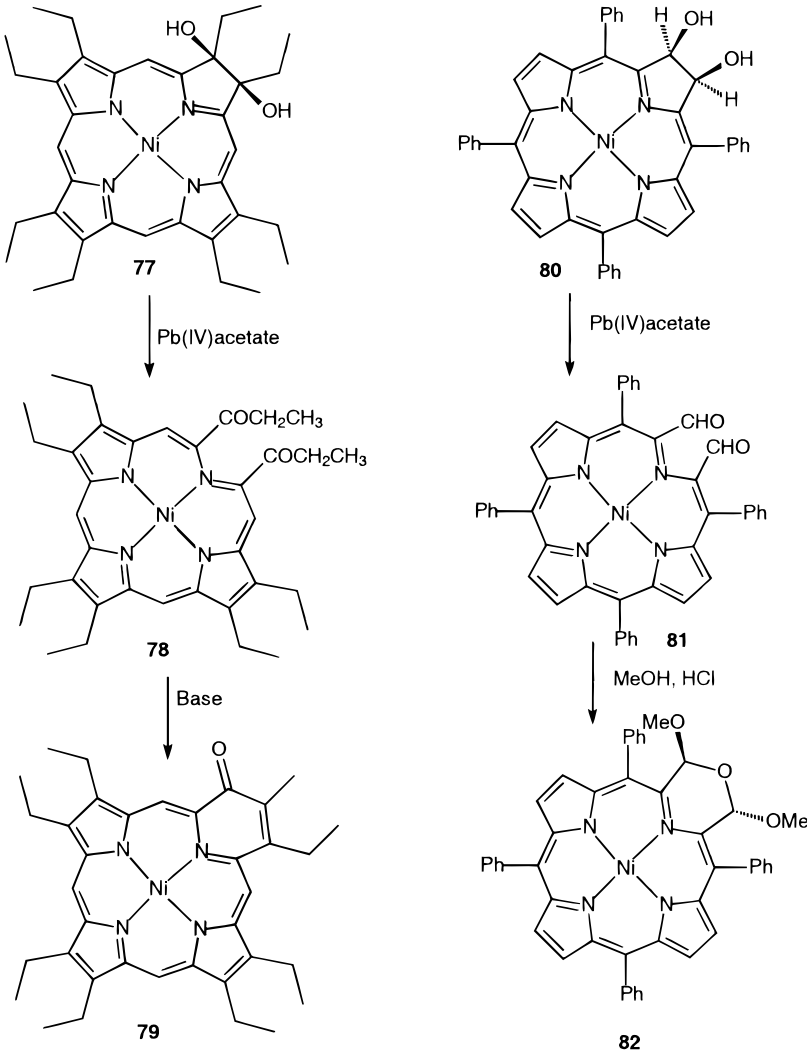
Secochlorins are chlorins in which the reduced peripheral double bond is cleaved. Two secochlorins



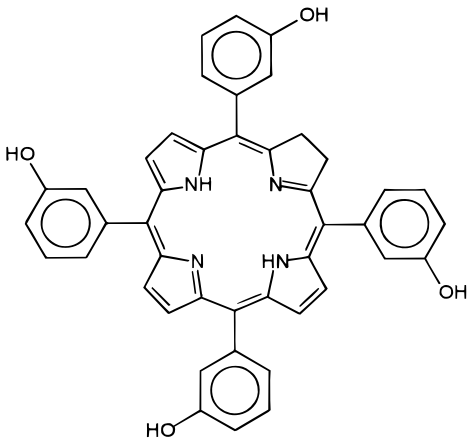
have recently been reported (Scheme 10).[120,121] Both pigments (**79**, **82**) are derived from the oxidative cleavage of the corresponding metallochlorin diol **77** or **80**. Treatment of the intermediate **78** with base induces an aldol condensation to give the novel homoporphyrinone system **79**. The intermediate pigment **81** undergoes an almost quantitative, intramolecular double-acetal formation upon treatment with methanol in the presence of acid to give a porphyrinoid **82**. The absorption spectrum of **82** resembles that of a metallochlorin with a considerable bathochromic shift of the Q-band.

Among the *meso*-tetraphenylporphyrins, the hydroxyphenyl analogues have been tested extensively as potential PS for PDT. Of the three possible isomers, the *ortho* isomer (a mixture of atropisomers) caused skin phototoxicity while the *meta* and *para* isomers showed increased capacity for tumor photonecrosis and more favorable organ selectivity as compared to HpD. To enhance red-light absorption, the corresponding chlorin and bacteriochlorin ana-

Scheme 10



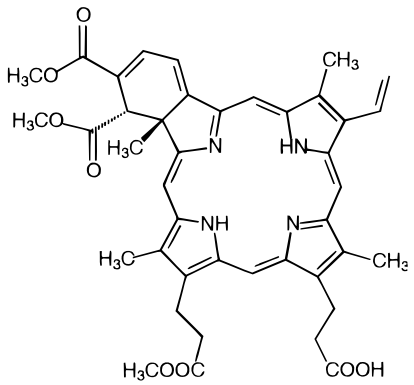
logues were prepared.[122] In vivo PDT activity increases in the following order, porphyrin < chlorin < bacteriochlorin. The *m*-tetrahydroxyphenyl chlorin (*m*-THPC, **83**) was selected for further studies in view



83 (*m*-THPC)

of its advantageous photophysical properties, stability, and lack of mutagenic activity.[123−126] *m*-THPC is undergoing clinical trials for head and neck cancer in Europe and the United States under the sponsorship of Scotia Pharmaceutical (U.K.).[127]

Benzoporphyrin derivatives, which are chlorins despite their name, are prepared by the Diels−Alder reaction of protoporphyrin dimethyl ester with dimethyl acetylenedicarboxylate.[128] Both isomeric products show a significant shift in the Q-band with high molar extinction coefficient (λ_{max} = 666 nm, log ϵ = 4.70). Base-catalyzed rearrangement of the 1:1 adduct (1,4-diene system) gave the conjugated analogue (1,3-diene system) with a methoxycarbonyl moiety arranged *cisoid* to the methyl group, while with stronger base the thermodynamically more stable *transoid* product (λ_{max} = 686 nm, log ϵ = 4.54) was formed.[129] Partial hydrolysis gave the monoacid which exhibits a far superior biological activity[130] as compared to the free diacid or diester. The monoacid **84** (BPD or Verteporfrin, QLT Phototherapeutics, Canada) in liposomal formulations is currently undergoing clinical evaluation for various PDT treatments including basal cell carcinoma, psoriasis, macular degeneration, and choroidal melanoma and skin cancer.[131,132]



84 (BPD, Verteporfin)

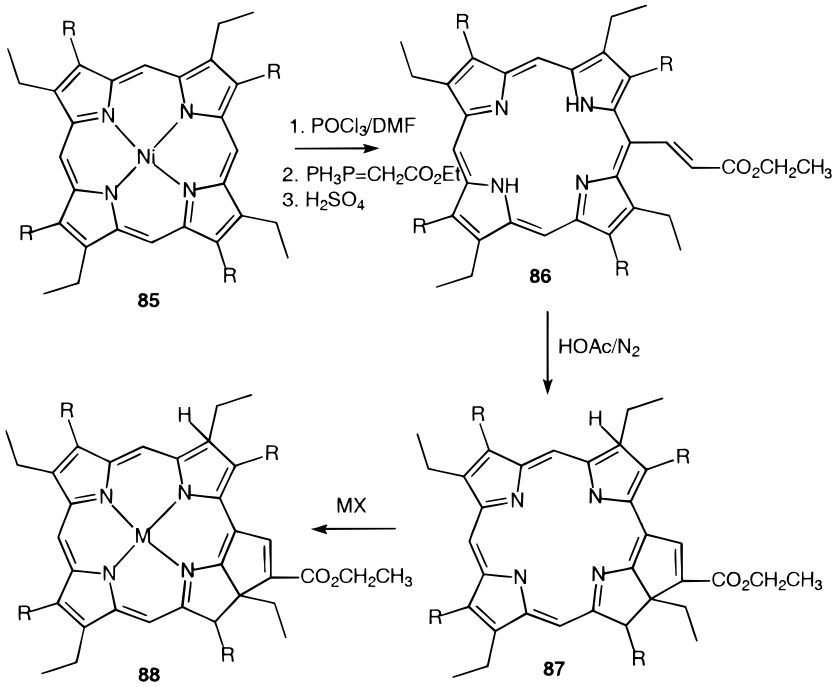
D. Purpurins, Benzochlorins, and Porphycenes

Purpurins have been known since the early 1940s as degradation products of chlorophyll, and their synthesis was first reported[133] in 1960. Recently, Morgan and Tertel[134] reported the synthesis of a number of new purpurin derivatives (**88**) and showed their potential as second-generation PS for PDT, Scheme 11. The metal-free derivatives exhibit a Q-band around 660 nm, while insertion of a Sn(IV) causes a red-shift of about 20−30 nm. Nickel *meso*-formyloctaethylporphyrin (**85**), on treatment with (carboethoxymethylene)triphenylphosphorane, gave Ni−*meso*-[β-(ethoxycarbonyl)vinyl]octaethylporphyrin which was demetalated to **86** using concentrated sulfuric acid. Upon reflux in glacial acetic acid under a nitrogen atmosphere, cyclization occurs, affording the corresponding (ethoxycarbonyl)octaethylpurpurin (**87a**) and -etiopurpurin (**88b**) in good yield. The Zn-(II) and Sn(IV) complexes **88** were prepared by reflux with zinc acetate or tin(II) chloride in CH_2Cl_2−MeOH. The ^{1}H NMR spectrum shows a one-proton singlet at δ 9.40 attributed to the olefinic proton of the cyclopentyl ring. One of the four-*meso* protons, as the proton adjacent to a reduced pyrrole ring, is shifted upfield to δ 8.71. The visible spectrum shows a characteristic Q-band at 695 nm. The structure was further supported by chemical transformations. Catalytic hydrogenation with 5% Pd/C results in reduction to the porphyrinogen **91**. Stirring a solution of **88** (M = 2H) in the presence of air and sunlight resulted in the cleavage of the isocyclic double bond to give the aldehyde **89**. Compounds **89** and **91** undergo facile metalation to yield **90** and **92**, Scheme 12. The spectra resemble those of chlorins (λ_{max} = 662 nm), but the ^{1}H NMR lacks the signal attributed to a cyclopentenyl proton. The less mobile fraction correspond to a purpurin, and a second compound with λ_{max} at 711 nm is indicative of a purpurin chromophore with conjugation at the periphery of the macrocycle.

Purpurins lack the symmetry found in most porphyrins. The stereoselectivity and specificity of purpurin formation from porphyrins usually results in the formation of several products. The preferential cyclization by acid proceeds exclusively at the carbon carrying the ethyl group toward the carbon carrying the methyl group, e.g., cyclization of *meso*-[β-ethoxycarbonyl)vinyl]coproporphyrin I tetramethyl ester (**93**) results in formation of purpurin **94**, Chart 4.[135] Such a selective rearrangement confirms the role of steric stress as the driving force of the reaction.

Irradiation of mixtures of octaethyl and etiopurpurins with light leads to oxidative cleavage of the

Scheme 11



a: R = CH_2CH_3
b: R = CH_3

Scheme 12

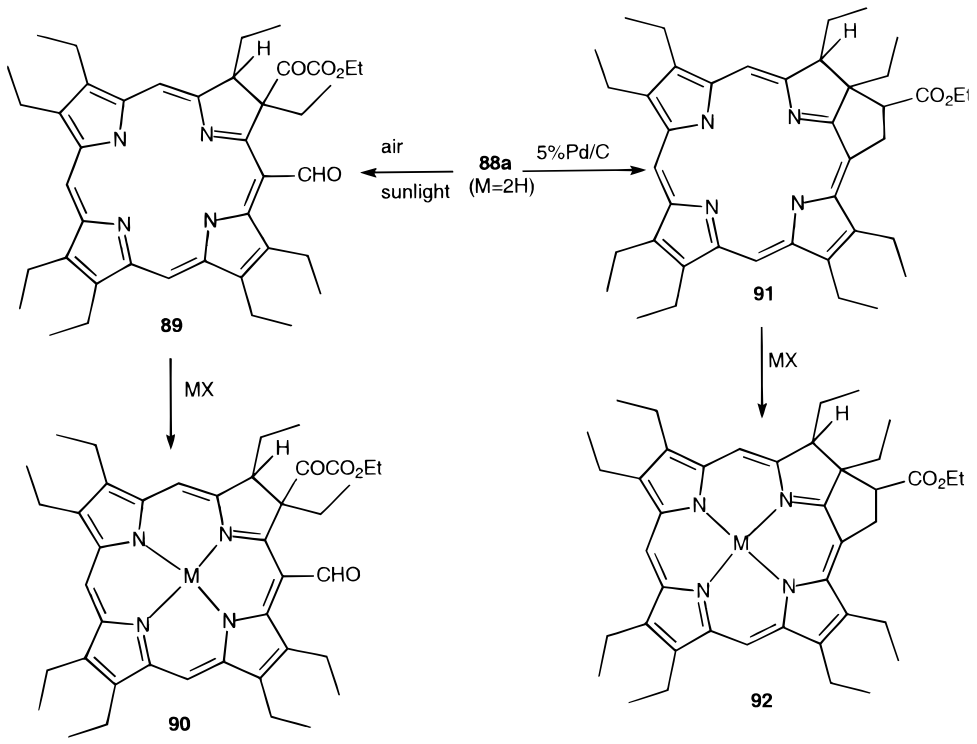
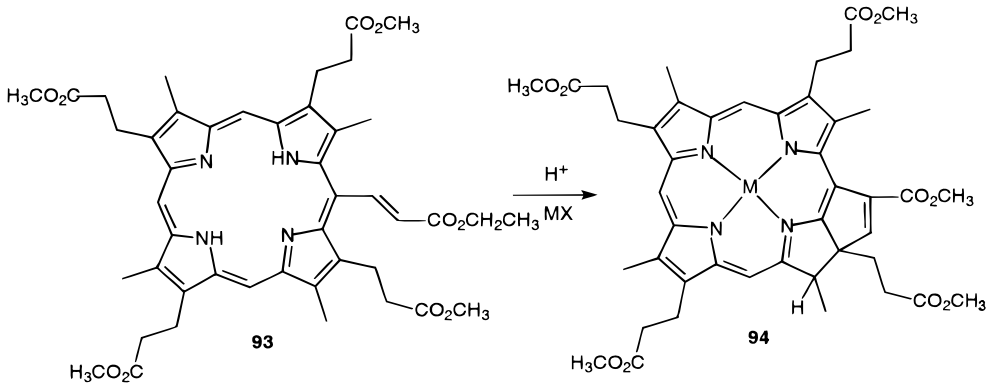


Chart 4

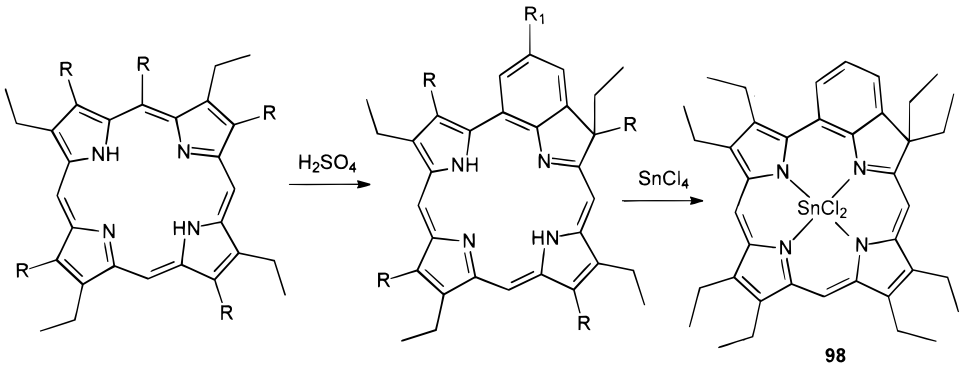


isocyclic ring to form the corresponding oxidized purpurin. The latter are still active generators of singlet oxygen.[136] The reduced purpurin is very stable and does not photobleach under steady-state irradiation. In general, purpurins have a high triplet quantum yield with long lifetime (~100 μs), and accordingly they are efficient generators of singlet oxygen.[136,137] Fluorescence lifetimes of purpurins are short (less than 1−2 ns) and their fluorescence yields are low despite the higher intensity of the Q-band absorption as compared to porphyrins.[136] Comparison of photodynamic properties on cell cultures[138] and animal tumor models,[139,140] using various formulations to facilitate drug delivery, showed that the metallopurpurins were more active than the metal-free analogues. The Sn(IV) complexes are more active than the Zn(II) purpurins, and in particular, the Sn(IV)−etiopurpurin analogue ($SnEt_2$) (**88b**) showed advantageous biological properties warranting its further development as a clinical PS.[138] $SnEt_2$ has been formulated in a proprietary emulsion for human use by Medvalent (California). This company currently supports a phase III clinical study to evaluate $SnEt_2$ for the treatment of some skin malignancies and AIDS related Kaposis' sarcoma.[142]

Benzochlorins feature a benzene ring fused to the tetrapyrrolic structure. They are synthesized from *meso*-substituted porphyrins, and a number of synthetic methods have been reported.[143] Benzochlorin derivatives of both octaethyl and etioporphyrins as well as the corresponding monosulfonated derivatives have been prepared. The metal-free acrylate **86** on reduction with diisobutylaluminumhydride yield the *meso*-3-propenylporphyrin derivatives **95**.

Treatment of **95** with concentrated sulfuric acid for 5 min at room temperature resulted in formation of the benzochlorin **96**, Scheme 13. Treatment of the free base with concentrated sulfuric acid gave the sulfonated derivative in high yield **97**. The metal-free **97** are easily metalated to **98**. The photodynamic

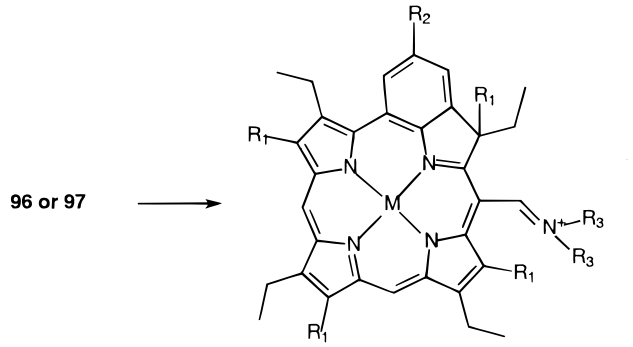
Scheme 13



properties of a series of benzochlorins were evaluated against murine leukemia L1210 cells in vitro and transplanted urothelial cell carcinoma in rats. Sulfonation reduced photodynamic response in the tumor model while insertion of Sn(IV), as in **98**, increased the effect.[145]

Benzochlorin derivatives featuring an iminium salt at the stable *meso* position exhibit a strong absorption band around 750 nm and are of particular interest for PDT.[146,147] Treatment of the Cu(II) octaethyl benzochlorin derivatives **96** or **97** with DMF/$POCl_3$ under typical Vilsmeier conditions gave the iminium salts **99** and **100**, respectively, Scheme 14.

Scheme 14

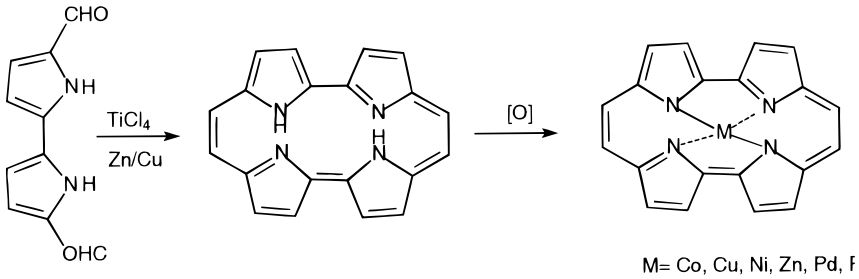


Due to their cationic nature, the absorption maxima shift to a lower wavelength. Using various *N*,*N*-dialkylformamides as part of the reagent allows for the preparation of iminium salts featuring other substituents (**99a**,**b** and **100a**,**b**). The Cu(II) iminium salt derivatives are unusual PS since their triplet state is not detectable. They are, however, capable of photoinactivating leukemia cells in vitro, and they also were shown to induce a complete PDT response in a rat bladder tumor model.[148−150] It has been speculated that the iminium group interacts with biomolecules, enhancing the triplet lifetime to allow electron-transfer reactions and the formation of radicals and radical ions.[151] It may also be possible that the redox properties of the Cu(II) promote transfer of a negative charge from the porphyrin to surrounding structures via the iminium group as part of the deactivation process. Removal of the coordinated Cu(II) drastically changed the pharmacokinetics of the Cu−benzoiminium derivative such that tumor response is only observed with short intervals between drug administration and PDT. In contrast, substituting the Cu(II) for a Zn(II) ion resulted in a marked increase in the in vivo photodynamic action of the dye.[151]

Boyle and Dolphin[152] synthesized 5,15-diphenyl-7-oxobenzochlorin in which ring closure collapse of the chiral center and extension of the conjugation results in the generation of a chiral center at the β-pyrrolic position. Treatment of metallo-5,15-diphenylporphyrin (Cu or Ni) with *N*,*N*-dimethylaminoacrolein−phosphorus−oxychloride gave 5,15-diphenyl-10-(2-formylvinyl)porphyrin (M = Cu, Ni), which using borontrifluoride−diethyl ether as catalyst was converted to two benzochlorin products. Demetalation gave a product with a broad absorption band at 746 nm.

Porphycenes are isomeric porphyrins which contain the same constituents as the parent [18]porphyrin-(1.1.1.1) but in different configurations. Synthesis of the first, well-characterized isomer, porphycene [18]-porphyrin-(2.0.20) (**103**), Scheme 15, via a reductive McMurray-type coupling of two 5,5′-diformylbipyrrole subunits (**101**), was reported in 1986 by Vogel et al.[153] The unsubstituted parent porphycene is a nonpolar compound with a porphyrin-like spectrum ($\lambda_{max} = 630$ nm, $\log \epsilon = 4.71$). Although the central cavity of porphycenes is smaller than that of porphyrins, they can form a stable, neutral complex with a wide range of monovalent, divalent, and higher-valent metal cations.[153,154] Porphycenes are poorly soluble in most organic solvents, which led to the synthesis of a number of β and *meso* substituted derivatives. Properties of the metal-coordinated porphycenes are similar to those of the unsubstituted analogues.[155] Octaethylporphycene is capable of photogenerating singlet oxygen, but its low solubility and short wavelength absorption maximum do not provide

Scheme 15



101 102 103

advantageous properties for PDT. Several nonmetalloporphycene analogues do show favorable photophysical properties and are being tested as PS for dermatological applications.[156]

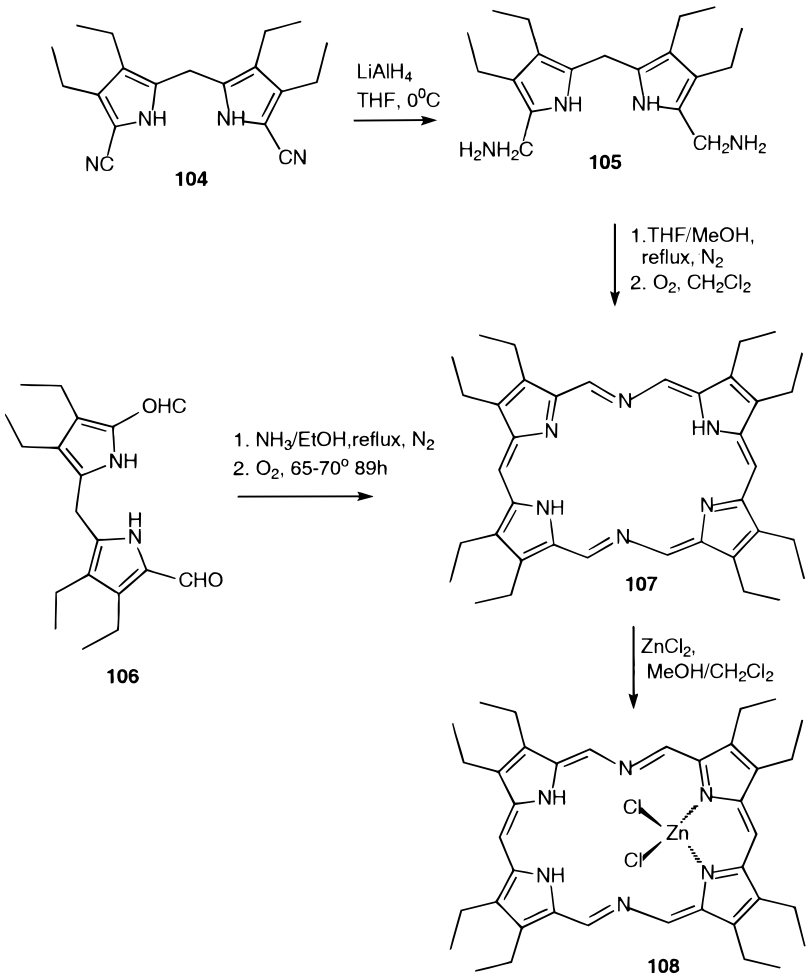
E. Expanded Porphyrins

1. Expanded Metalloporphyrins as Photosensitizers

Expanded porphyrins such as porphocyanines, hexaphyrins, and texaphyrins contain an increased number of bridging atoms separating the heterocyclic rings. They have similar physical properties as porphyrin congeners but contain an increased number of π-electrons, whereas the added atoms also provide for a larger central binding core.

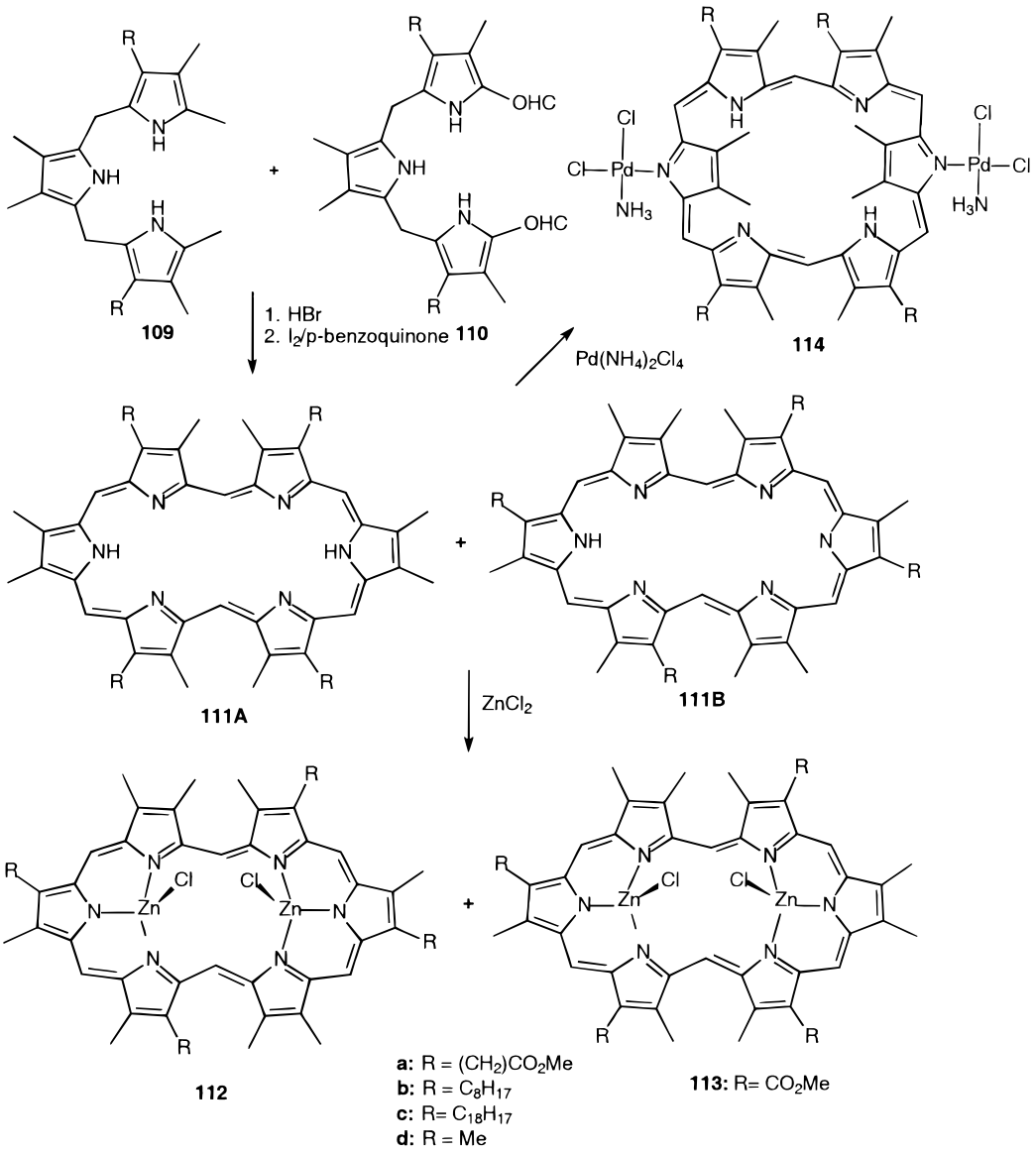
Porphocyanines are expanded porphyrins in which the central methine group of the expanded vinylogous bridge is replaced by a pair of imine linkages. This new class of aromatic tetrapyrroles incorporate some of the structural features of both porphyrins and phthalocyanines.[157,158] Octaethylporphocyanine **107** was obtained via a reaction sequence (Scheme 16) involving hydride reduction of the biscyanodipyrromethane **104** followed by self-condensation of the unstable intermediate bis(aminomethyl)dipyrromethane (**105**) in refluxing MeOH/THF and oxidation of the resulting macrocycle. The same product was obtained in higher yield (24%) by reacting bisformyldipyrromethane (**106**) in EtOH saturated with NH_3 in a sealed vessel, under rigorously anhydrous conditions. Similarly, 4,4′-bis[2(methoxycarbonyl)ethyl]-5,5′-diformylpyrromethane undergoes cyclocondensation.[158] Alternatively, a one-pot synthesis has also been reported in which the cyclo group is reduced by

Scheme 16



104 105 106 107 108

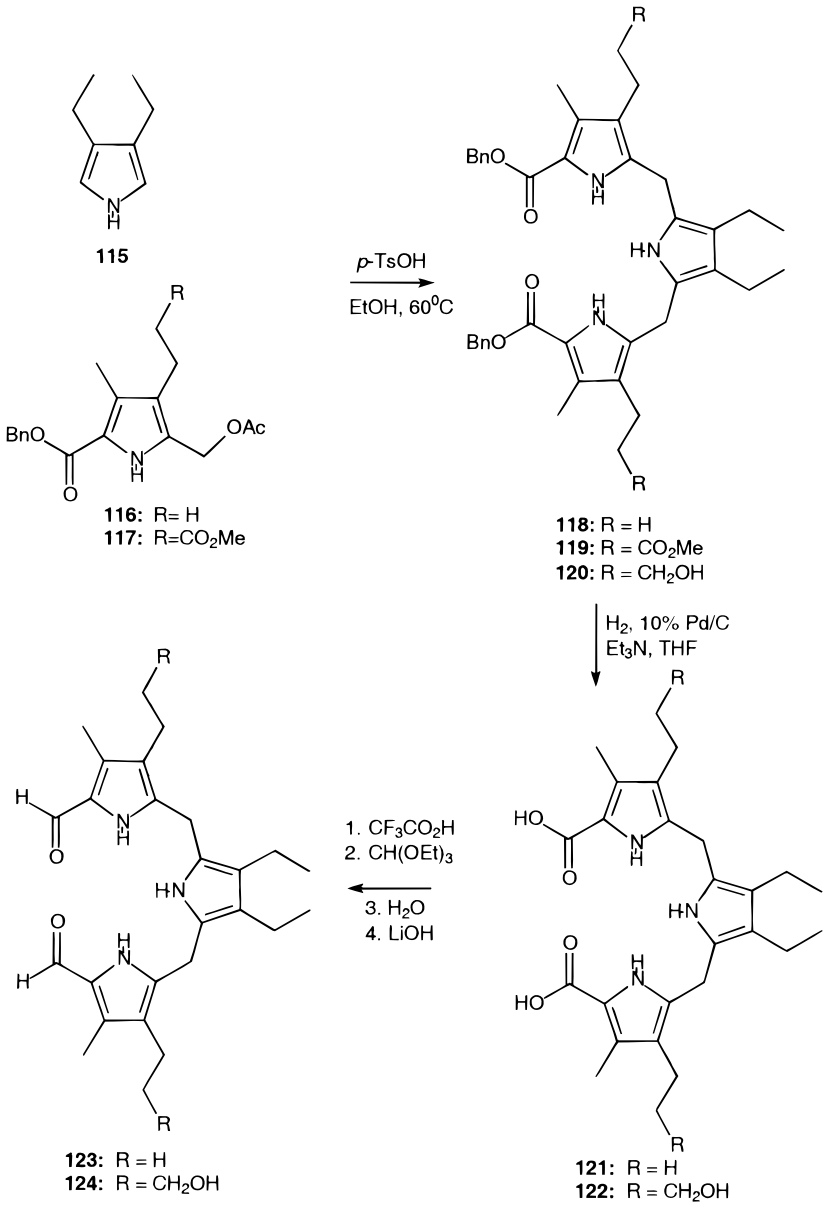
Scheme 17



$LiAlH_4$ to yield the aromatic porphocyanine.[159,160] In a similar manner, asymmetric macrocycles can be prepared via co-reduction of two appropriate substituted dipyrrolic subunits.[218] The optical spectrum of octaethyl porphocyanine **107** consists of a dominant Soret-like absorption peak at 457 nm ($\epsilon = 240\,000$) and Q-type absorption maxima at 529 ($\epsilon = 17\,000$), 633 ($\epsilon = 5800$), 728 ($\epsilon = 3200$), and 797 nm ($\epsilon = 27\,000$). This pattern is typical of the conjugated aromatic 22 π-electron system of the porphocyanine. Introduction of a phenyl substituent at a *meso*-position induces a bathochromic shift. ^{1}H NMR confirms the diamagnetic ring current. The single *meso*-bridge protons and the four other peripheral iminoprotons resonate as a singlet at $\delta = 11.95$ and 13.75 ppm, while the inner pyrrolic NH signals appear at $\delta = -5.75$ ppm.

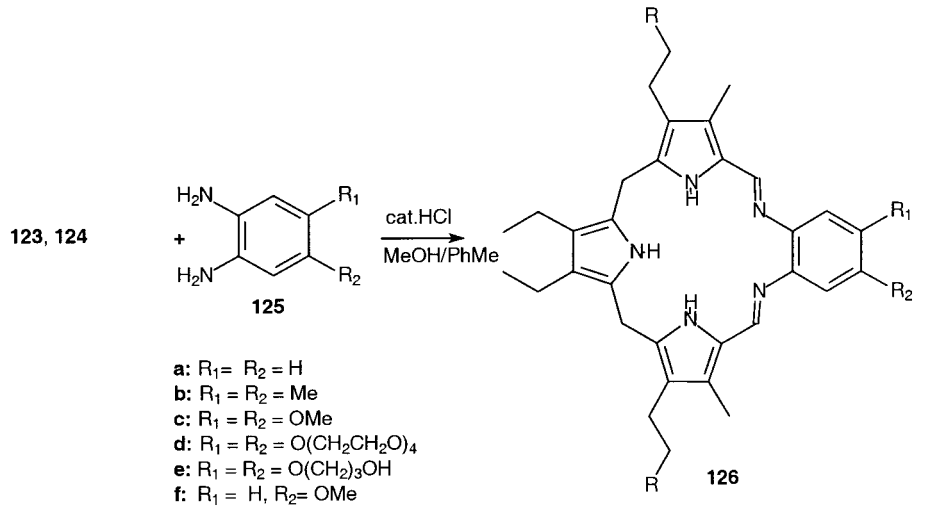
When methanolic solutions of the acetate salts of Cd^{2+}, Mn^{2+}, Co^{2+}, and Zn^{2+} were added to the porphocyanine (in CH_2Cl_2 or $CHCl_3$) at elevated temperature, spectral changes indicated that a metal complex **108** was formed. However, UO_2^+ cannot be accommodated by the porphocyanine cavity. The Zn(II) complex **108** was isolated as a crystalline solid in low yield. Zinc porphocyanine exhibited a markedly different electronic spectra as compared to the free porphocyanine with a split Soret band at 464 and 476 nm and a weaker Q-band at 736 nm. Addition of base induces a spectral change to a single Soret peak at 480 nm and a red-shifted Q-band at 762 nm. A single-crystal X-ray analysis showed the presence of one Zn-(II) ion in the macrocycle and confirmed the planarity of the molecule. The Zn(II) ion is completely encapsulated within the macrocyclic core, coordinated to two of the pyrrolic nitrogens in one-half of the macrocycle and to two chloride ions, in a tetrahedral fashion. All metal complexes induce the type II photooxidation of cholesterol to give the 5α-hydroperoxycholesterol, confirming their capacity to generate singlet oxygen. Reports on their photodynamic properties in preclinical studies are however limited. The larger core allows complexation with bulky metal oxides such as $^{99m}TcO_2$ to yield ^{99m}Tc-labeled porphocyanine. Radioimaging after administration of 20 mCi of the ^{99m}Tc complex to a 32-year old female patient showed a focus of abdominal accumulation of ^{99m}Tc.

Scheme 18



Hexaphyrin represents the first reported example of an expanded porphyrin.[162] Gossauer and co-workers[162,163] published a two-step process in which the di-α-free tripyrranes **109** and bisformyl **110** derivatives undergo an acid-catalyzed condensation. Oxidation with iodine/*p*-benzoquinone furnished the two isomeric expanded macrocycles **111A**,**B** in equal proportions, together with minor quantities of the corresponding pentaphyrins, Scheme 17. The isomeric macrocycles exhibit three distinct, low-field singlets assigned to the protons in the *Z*-configuration at the methine bridges, which integrate roughly to a 2:1:1 ratio. The UV−vis spectrum is characterized by three absorption bands at λ_{max} = 572 (ϵ = 76 000), 595 (ϵ = 47 000), and 789 (ϵ = 3981) nm. Acidification results in a spectral shift to yield one strong absorption band at 551 nm and a slightly red-shifted low-energy absorption at 798 nm. Treating the isomeric mixture (**111A**,**B**) with $ZnCl_2$ furnished the symmetrical bimetallic Zn(II) complexes **112** and **113** of C_{2v} point symmetry. Both complexes display strong absorption maxima in the UV−vis spectra at 574 nm (ϵ = 263 000) along with additional bands at 450, 601, and 810 nm. In acidic media, this "Soret-like" band is blue-shifted to 556 nm and doubled in intensity (ϵ = 410 000). The bispalladium complexes **114** were obtained via treatment of a mixture of hexaphyrins with ammonium tetrachloropalladate. Pd hexaphyrins have an unusual geometry in order to accommodate the square-planar geometry about the d^8 Pd centers. The two central pyrrolic rings coordinated to the metal atoms are rotated 180°, with concomitant *E*/*Z* isomerization of the two formal C=C bonds. Exchange of the labile ammonia ligands at the Pd centers with pyridine yields the corresponding bis(pyridyl)dipalladium complex. Interestingly, the electronic spectrum exhibits several intense absorptions peaks over a range from 276 to 840 nm, with two main peaks of equal intensities at 574 and 607 nm.

Scheme 19

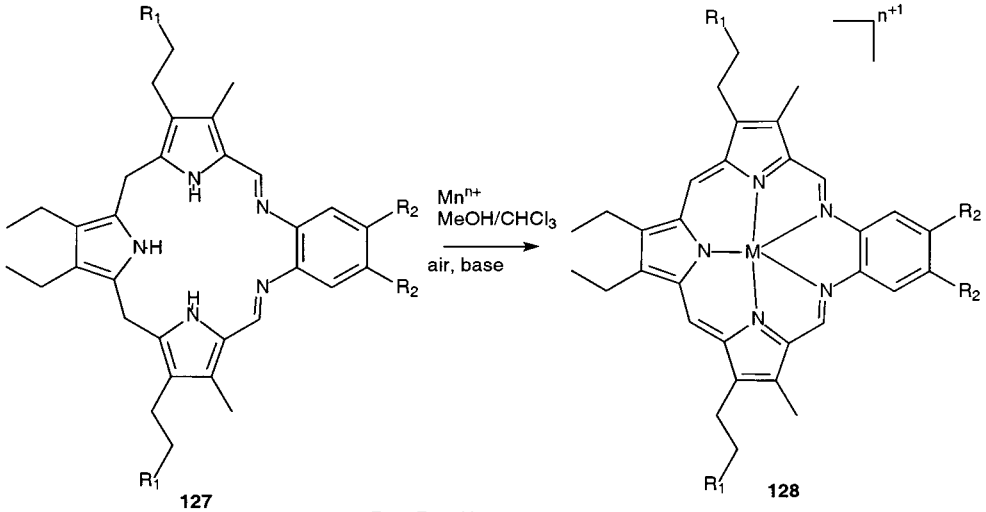


In 1987, Sessler et al. reported the synthesis of a new class of expanded porphyrins via a Shiff base condensation between a diformyltripyrrane and aromatic 1,2-diamine.[164] This new class of aromatic porphyrin-like macrocycles became known as the texaphyrins. They strongly absorb over the 730−770 nm range, i.e., where tissue transparency is optimal. The condensation of primary α,ω-diamines and heterocyclic dicarbonyl compounds is an established synthetic route for the preparation of multidentate Schiff base-type cyclic ligands. Earlier work on this condensation reaction deals with 2,6-dicarbonyl derivatives of pyridine as the heterocycle.[165,166] Mertes[167] reported in 1985 the structure of a tetrapyrrolic "accordion" macrocycle, which is considered the first account of a "truly" expanded porphyrin Schiff base complex. However, these complexes could not be converted to fully conjugated species due to the nature of the bridging tetraamino chains.[167]

The texaphyrin-type molecules reported by Sessler[164] possess a number of unique physical and chemical properties that make them of interest for a variety of biomedical applications, including as PS for PDT and contrast agents for MRI. Synthetic procedures include the acid-catalyzed condensation of 1 equiv of 3,4-diethylpyrrole[168] (**115**), and 2 equiv of the (acetoxymethyl)pyrrole[169] (**116**) produces tripyrrane (**118**). Debenzylation of **118** and Clezy formylation[170] provided the diacid tripyrrane **121**, which furnished the diformylpyrranes (**123**) in 68% yield. The latter are key intermediates for further synthesis of texaphyrins. Similarly, the (acetoxymethyl)pyrrole **117** with a (methoxycarbonyl)ethyl substituent at the pyrrole 3-position provided a tripyrrane[171] (**119**), which was converted to the bis(hydroxypropyl)-substituted diformyltripyrrane (**124**) in up to 80% yield (Scheme 18).

Acid-catalyzed condensation of diformyltripyrranes (**123**, **124**) and O-phenylenediamine derivatives (**125**) results in the formation of a tripyrrane macrocycle (**126**), containing a Schiff base structure, in 90% yield (Scheme 19). These compounds are nonaromatic, show absorbance only in the UV region of the electronic spectrum, and are essentially colorless. As such they behave more like "expanded porphyrinogens"[172] rather than true expanded porphyrins.[164] Although they are rather stable, oxidation of **127a** was effected by stirring the macrocycle in air-saturated chloroform−methanol containing a Brönsted base[173] to yield a solid, stable aromatic (green) product in 12% yield (**128a**) (Scheme 20). This product can be considered as an aromatic 22-π benzannulene containing both 18-π and 22-π electron delocalization pathways. A shift of the NH proton signal upfield by 10 ppm suggested that the strength of the diamagnetic ring current is similar to that observed in the porphyrins. The electronic spectrum showed a Soret-like band at 422 nm ($\epsilon = 60\,500$) and a Q-band at 752 nm ($\epsilon = 36\,400$). However, generalization of the above oxidative chemistry proved to be difficult. Furthermore, these aromatic texaphyrins showed no propensity to chelate metals. Treatment of **127a** with $ZnCl_2$ or $[Rh(CO_2)_2Cl]_2$ in benzene afforded a pink solid and a green microcrystalline solid.[162,174] ^{1}H NMR spectra of these complexes showed signals of internal pyrrole NH protons, suggesting

Scheme 20



a: $R_1 = R_2 = H$
b: $R_1 = R_2 = Me$
c: $R_1 = R_2 = OMe$
d: $R_1 = R_2 = O(CH_2CH_2O)_4$
e: $R_1 = R_2 = O(CH_2)_3OH$
f: $R_1 = H$, $R_2 = OMe$
h: $R_1 = H$, $R_2 = Cl$
i: $R_1 = H$, $R_2 = CO_2H$
j: $R_1 = H$, $R_2 = NO_2$

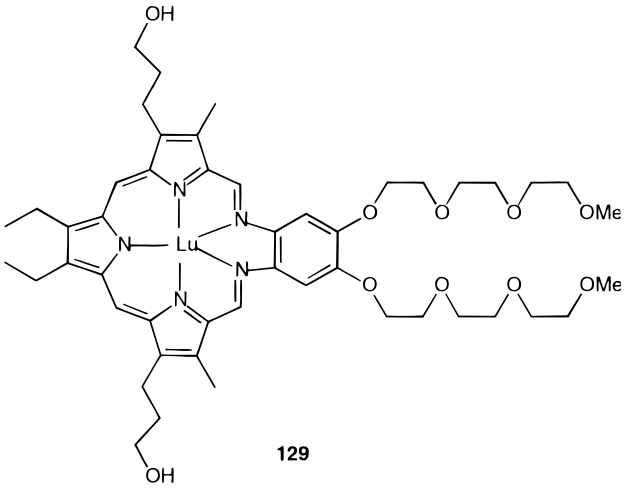
M^{2+} = Cd(II), Zn(II), Hg(II), Mn(II),etc.
M^{3+} = Gd(III), La(II), Lu(III), Ce(III), Ce(III),

that the metal was actually bound in a η^2 fashion. In marked contrast, when $CdCl_2$ was employed as the coordinating metal, under aerobic conditions, a strongly absorbing green material was obtained.

Another approach to form metal complexes with these reduced macrocycles is based on the oxidative insertion of a metal cation. During this process, the reduced ligand wraps around the metal ion, thus stabilizing the resulting metallotexaphyrin complex. This strategy is depicted in Scheme 20 using Cd(II) as the templating cation and air as the oxidant. The resulting CdCl−texaphyrin (**128a**, M = Cd) gives an optical spectrum, which resembles that of other aromatic pyrrole-containing macrocycles such as sapphyrins and pentaphyrins. The prominent Soret-like band at 427 nm (ϵ = 72 700) is substantially less intense than that of typical Cd−porphyrins (i.e., CdPy−octaethylporphyrin: λ_{max} = 421 nm; ϵ = 288 000). The ^{1}H NMR of **128a** exhibits the characteristic signals of aromatic systems with strong diamagnetic ring currents, i.e., H-signals shifted to lower field. The bridging methylene protons appear as a sharp singlet (δ = 11.3 ppm), typical for *meso*-protons. Oxidative metal insertion was shown to be useful with a number of different reduced texaphyrins precursors and with a wide range of other large cations, including those from the lanthanide series. The Ln(III) cation is coordinated to all five nitrogen atoms of the macrocycle and represents a true 1:1 adduct.

The photophysical properties of metallotexaphyrins (M = Zn, Cd, Mn, Sm, Eu) **128** parallel those of the corresponding metalloporphyrins. The diamagnetic texaphyrin Zn and Cd complexes **128a**,**i** show strong red-shifted optical absorptions in the 730−770 nm range, as well as high triplet quantum yield, and act as efficient PS for the production of singlet oxygen ($\phi > 0.6$). The fluorescence quantum yields for **128a**,**i** are only 0.1, while the quantum yields for triplet formation can approach unity. Several complexes containing paramagnetic metal ions, e.g., Mn (**128a**), Sm (**128b**), and Eu (**128b**), are nonluminescent and their triplet excited states could not be detected. Laser excitation of these compounds in aerated methanol gave no redox products (e.g., texaphyrin cation and superoxide anion), although the production of singlet oxygen ($\phi < 0.05$) could be detected. Thus, in particular, the diamagnetic texaphyrin complexes are highly efficient photosensitizers for singlet oxygen production of interest for PDT applications.

In vitro studies with the Cd−texaphyrin **128a** showed efficient photodynamic inactivation of human leukemia cells and both Gram-positive *Staphylococcus* and Gram-negative *Escherichia coli* bacteria.[175−178] However, the limited water-solubility and known Cd toxicity, limited in vivo follow-up studies with this material.[179] To overcome these concerns, texaphyrin-based PDT focused on the water-soluble Lu(III) derivative **129** (PCI-0123) (λ_{max} = 732 nm).[181,182] Compound **129** induced complete cures of implanted, fast-growing mouse mammary tumors (SMT-F) using a proper combination of drug dose, light fluence, and drug−light administration timing. The dye was also photoactive against other murine cancer models including the EMT6 mammary sarcoma and the B16



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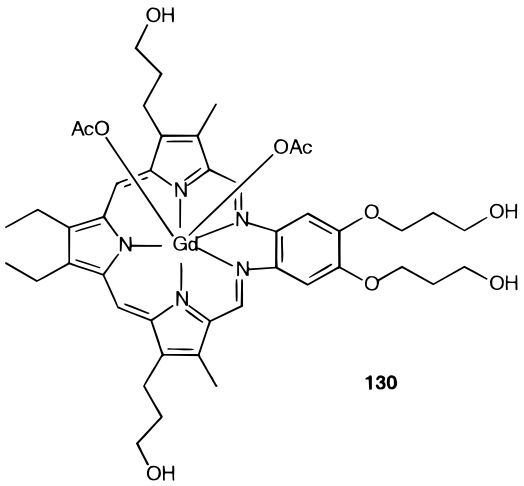

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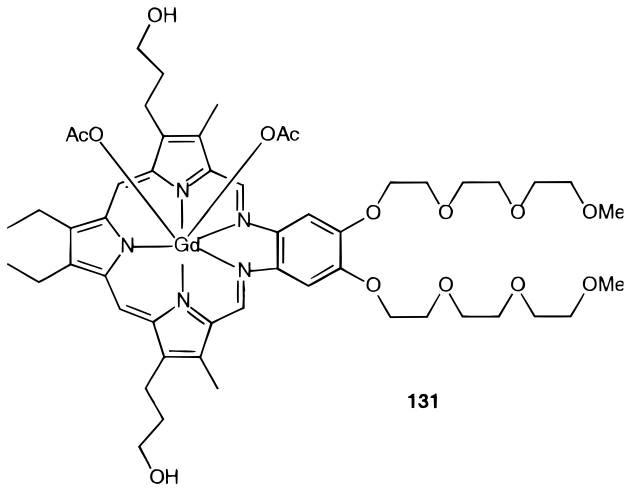


131

pigmented melanoma.[182,183] An additional application of texaphyrins has been proposed for site-selective hydrolysis and photolysis in antisense drug design. The water-soluble Eu−texaphyrin complex Eu(T_2B_2-Typh)(NO_3)$_2$ was shown to interact with RNA. The complex acts as a hydrolytic cleaving agent for uridyl uridine monophosphate (UpU) with a pseudo-first-order rate constant of 0.057 ± 2 h^{-1} at 37 °C and pH = 7.0 with 0.49 mM UpU and 0.3 mM Eu(T_2B_2 typh)-(NO_3)$_2$. Under identical conditions, a 0.26 mM solution of Eu(NO_3)$_2$ hydrolyzes UpU with a rate constant of 0.007 ± 3 h^{-1}. Attachment of a photoactivatable unit to a suitable oligodeoxynucleotide can generate a conjugate capable of photomodifying a complementary DNA target sequence. The advantage of texaphyrins over porphyrins for such an application are the same as that for PDT applications, i.e., excitation wavelength where tissues are most transparent and high $^1O_2$ quantum yields.[184] Functionalized Eu(III) and dysprosium(III) texaphyrins, conjugated to DNA, have also been shown capable of hydrolyzing complementary RNA oligomers in the absence of light, mimicking the catalytic action of a ribosome and supporting the potential use of such conjugates in antisense applications.[185] Compound **129** in its bisacetate are being evaluated[186] in a phase I clinical study (PCI-0123 or LuTex, Pharmacyclics, California) for PDT of pigmented melanoma, breast cancer, AIDS-related Kaposi's sarcoma, and basal cell carcinoma.[187]

2. Expanded Metalloporphyrins as Radiosensitizers

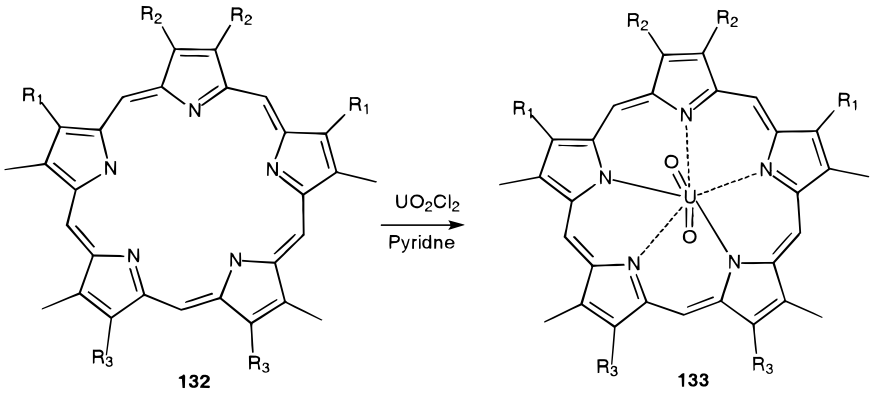
As discussed above, texaphyrins are large planar porphyrin-like macrocycles capable of coordinating a range of relatively large cations, including Gd(III) and other members of the trivalent lanthanide series (structures **130** and **131**). The 1:1 metal−ligand complexes are stable, easily reduced ($E_{1/2}$(red) $\approx$ 0.08 V vs normal hydrogen electrode), and form a long-lived π-radical cation upon exposure to hydrated electrons, reducing ketyl radicals, or superoxide ions. Their facile reduction, combined with good tumor uptake in animal models, led to the suggestion that these species could also function as radiosensitizers.[188] Complexes Gd−Tex^{2+} **130** and **131** were tested in vitro against L1210 and HT 29 cell line at 10^{-5}–10^{-3} M. Both preparations are effective with complex **131** featuring a SER = 1.92 against the HT 29 cell line. In vivo treatment at a dose of 40 μmol/kg (i.v.) prior to a single radiation dose of 30-Gy induced a significant growth delay of SMT-F tumors in DMA/2N mice.[189]

3. Radiolabeled Expanded Metalloporphyrins

Gossauer[190] demonstrated that pentaphyrin **132** formed a stable complex **133** with the uranyl (UO_2^{2+}) cation, Scheme 21. Furthermore, the uranyl center could be readily displaced upon treatment with acid without the destruction of the macrocycle. Later, Sessler and co-workers[191] prepared two similar uranyl complexes. A single-crystal diffraction study showed a saddle-shaped pentaporphyrin macrocyle with the uranyl cation centrally coordinated in a nearly pentagonal bipyramidal fashion. This complex is a unique example of a pyrrole-derived expanded porphyrin where well-defined actinide cation coordination is achieved.

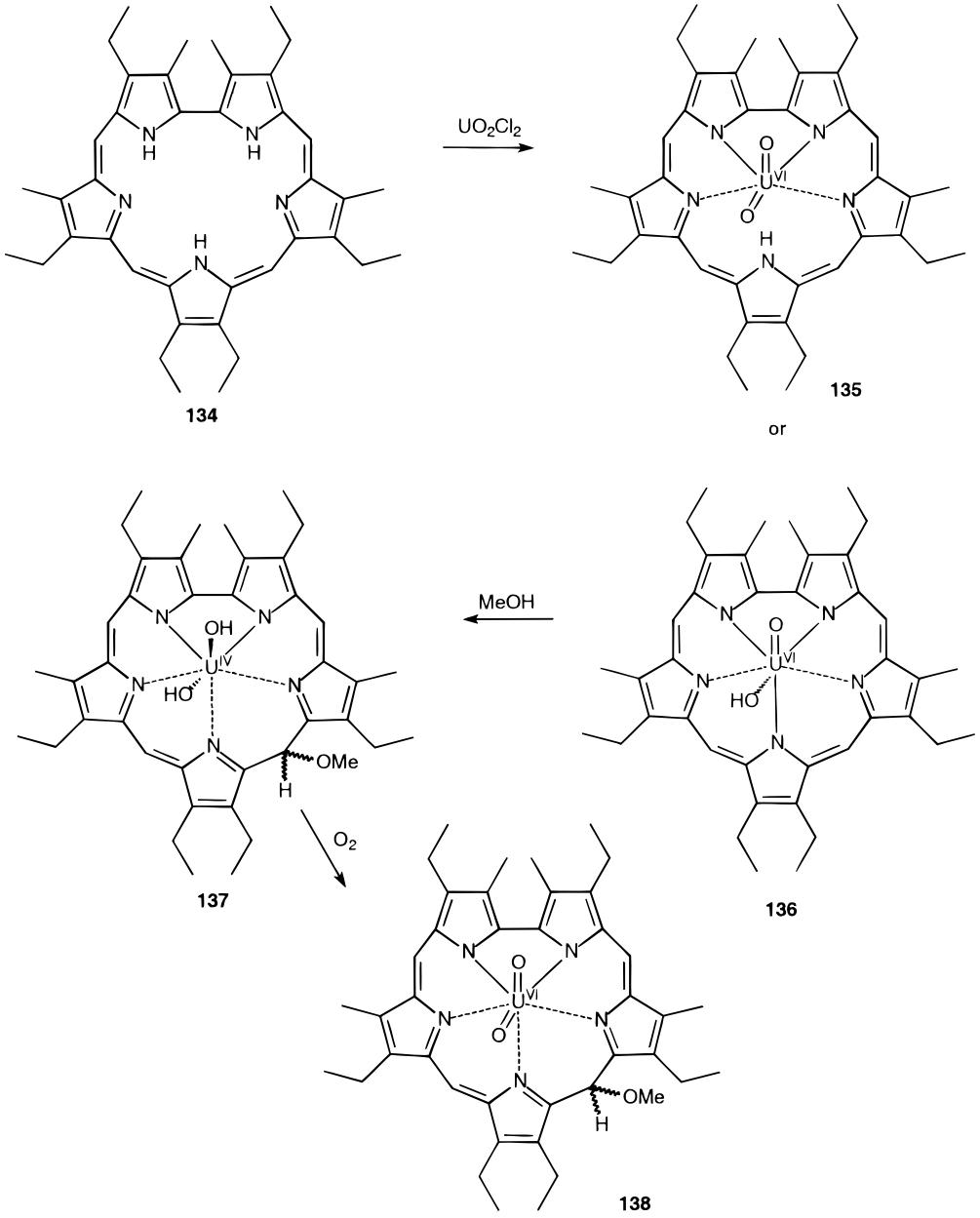
The related Sapphyrins, which contain a (1.1.1.1.0) arrangement of four carbon atoms (**134**), were first reported by Woodward et al.[192] Contrary to the earlier reports, Sessler et al.[193] showed that sapphyrin (**134**) can form a stable complex **138** with the uranyl cation, Scheme 22. The sapphyrin undergoes a rapid reaction with the uranyl cation in a mixture of methanol, pyridine, and triethylamine. The reaction likely

Scheme 21

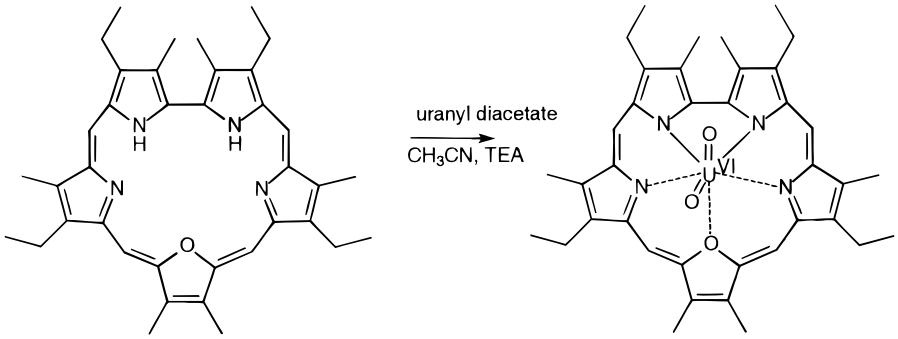


a: $R_1=CH_2CH_2CO_2Me$, $R_2=R_3=Me$
b: R=Me, $R_2=Et$, $R_3=n\text{-}Bu$
c: $R_1=R_3=CH_2CH_2CH_2OH$, $R_2=Et$

Scheme 22



Scheme 23



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proceeds via complexes such as **135** or **136** which react with MeOH at one of the *meso*-positions to give **137**, followed by air oxygen to yield the U(VI) complex **138**. The visible spectrum of the red complex exhibits two broad bands at 479 and 508 nm which are different from the usual Soret-like absorption of sapphyrin complexes. Mass spectrum, NMR, and X-ray analysis indicated the presence of a methanol group at the *meso* carbon of the final, oxidized complex **138**, explaining the disruption of the inherent 2-fold symmetry, the loss of overall aromaticity, and the absence of a Soret-like transition in the optical spectrum.

Replacing one of the pyrrole units of sapphyrin by a furan gives the monooxasapphyrin **139**, Scheme 23. Treatment with uranyl diacetate in the presence of triethylamine leads to the formation of a stable, in-plane aromatic uranyl complex **140**.[194] The UV−vis spectrum is typical of the sapphyrin structure with one strong absorption maximum at 483 nm and three weaker Q-type bands at 624, 647, and 708 nm. The significant bathochromatic shift (29 nm) of the Soret band, together with the Q-type band shifts, suggests that the monooxasapphyrin skeleton remained intact upon metal insertion.

Finally, some evidence exists that a large, nonaromatic, decappyrolic macrocycle containing 40 π electrons can form a coordination complex with the uranyl cation $(UO_2)^{2+}$ to yield a bisuranyl compound with a twisted figure-eight conformation.[195]

4. Expanded Metalloporphyrins as Contrast Agents

Since gadolinium(III) is used as a contrast-enhancing metal ion for MRI, it was anticipated that Gd(III) texaphyrins also could function as MRI contrast agents.[196] Administration of the Gd−Tex^{2+} complex **130** (40 μmol/kg) to SMT-F tumor-bearing Balb/c mice indeed revealed contrast enhancement of the tumor (94% at 10 min to 7% at 24 h p.i).

The solubility of the Gd−texaphrin complex was enhanced via the coupling of two Gd complexes with a spacer chain consisting of gluconolated 1,4,8,11-tetraazacyclotetradecane (**141**), Chart 5.[197] In addition to providing two Gd ions per molecule, the space also provides additional binding sites for the chelated metal ion. The system was found to have a longitudinal (T_1) relaxation of ca. 40 mM^{-1} s^{-1} at room temperature, neutral pH, and 500 MHz of applied field. Under the same conditions, the monomer **130** displayed a relaxation rate of 8 mM^{-1} s^{-1}. The augmented relaxivity renders the system of interest as an improved texaphyrin-type MRI contrast-enhancing agent. This also suggested that increasing the molecular size influences the per-Gd(III)-based relaxivities of the Gd(III)−texaphyrin. To test the hypothesis, two water-soluble polymeric systems were prepared, i.e., **142** and **143**, both featuring poly-L-lysine backbones, with **143** functionalized with *S*-gluconolactone to increase water solubility, Chart 6. Both complexes displayed per-metal-center relax-

Chart 5

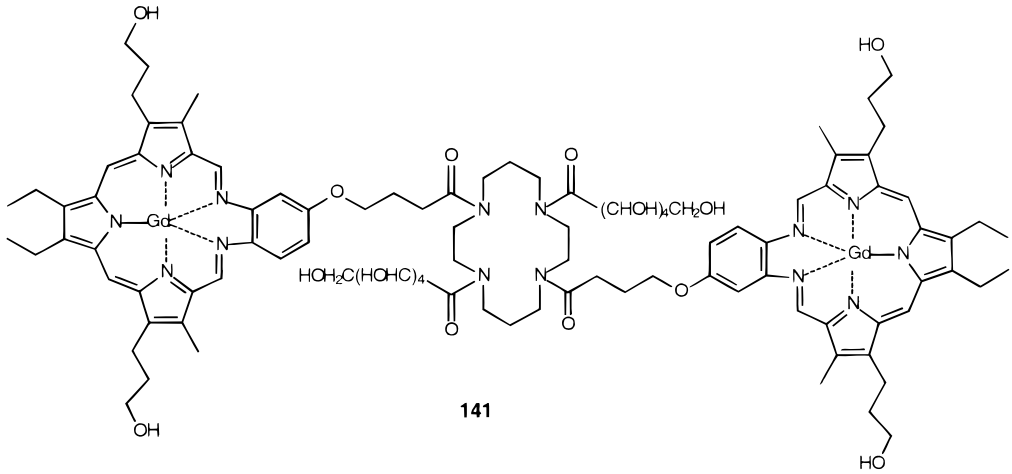

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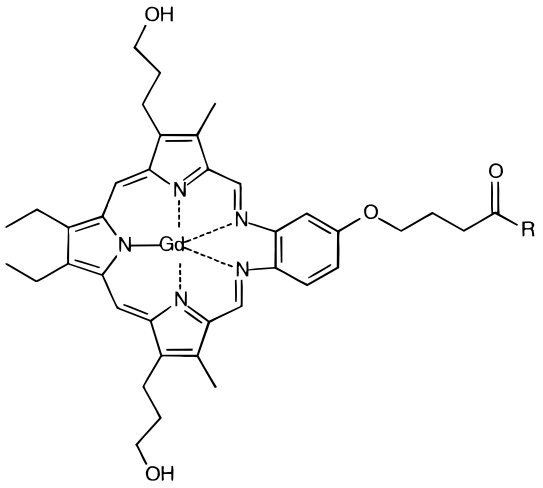
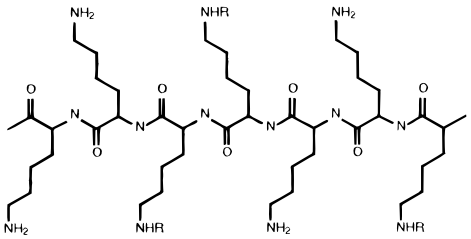
Chart 6





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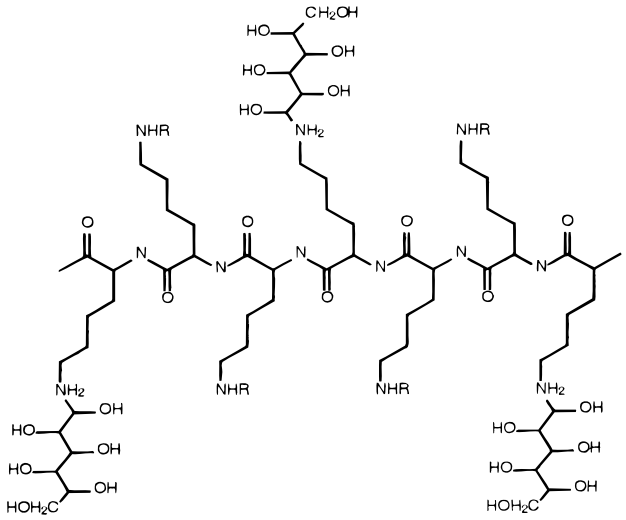


143

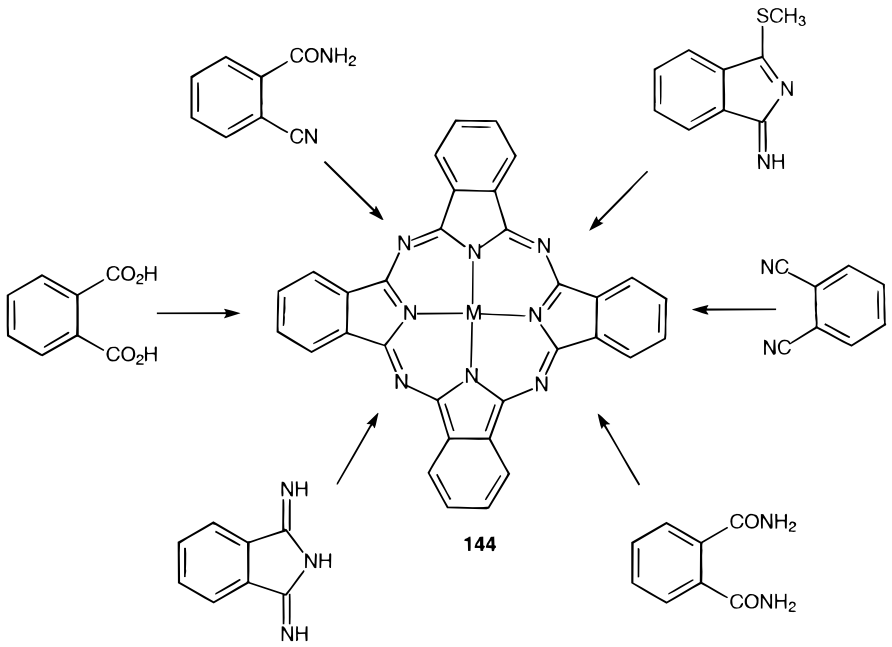
ivities of ca. 90 and 315 mM^{-1} s^{-1}, respectively, that are substantially enhanced relative to those obtained for monomeric Gd(III) adducts. Thus, these new systems have a high potential to find applications as improved MRI contrast agents.

F. Phthalocyanines

1. Phthalocyanines as Photosensitizers

The word phthalocyanine (Pc) is derived from the Greek terms for naphtha (rock oil) and cyanine (dark blue). This blue pigment was first observed in 1907 by Braun and Tcherniac as a byproduct of the preparation of *o*-cyanobenzamide from phthalamide and acetic anhydride.[198] The next reported preparation of Pc was by de Diesbach and von der Weid in 1927 from the reaction of *o*-dibromobenzene and copper cyanide in pyridine.[199] They also observed the exceptional stability of the products to alkaline, sulfuric acid, and heat. Linstead et al.[200] were the first to determine the structure of Pc in 1933. They cornered the name "phthalocyanine" and reported the formation of several metallo-Pc (MPc) complexes. The numerous commercial uses of Pc as colorants for paint, inks, textiles, as well as catalysts of redox and photographic reactions, semiconductors, and photoelectric cells, lead to an extensive patent literature on the preparation of derivatives soluble both in organic solvent or in water.[201] Monomeric MPc have characteristic absorption spectra:[202] a Soret band at approximately 350 nm, a small band around 600 nm and a narrow, very strong absorption peak (Q-band) around 670 nm, with a molar extinction coefficient in the range of 10^5 M^{-1} cm^{-1}. Absorption maxima of water-soluble MPc may vary slightly with pH. Such variations are attributed to changes in the axial ligand coordinated to the metal center rather than protonation of macrocycle sites.[203] In addition to being red-shifted, Pc light absorption is almost 2 orders of magnitude stronger than the highest Q-band absorption of Hp. The photophysical properties of the Pc dyes are strongly influenced by the presence and nature of the central metal ion. Complexation of Pc with transition metals gives dyes with short triplet lifetimes (in the ns range) resulting in variations in yield (ϕ_t) and lifetime (τ_t) of the photoexcited triplet state of MPc. Closed shell, diamagnetic ions, such as Zn^{2+}, Al^{3+}, and Ga^{3+}, give Pc complexes with both high triplet yields ($\phi_t > 0.4$) and long lifetimes (i.e., tetrasulfonated $ZnPcS_4$, $\tau_t = 490$ Fs; $AlPcS_4$, $\tau_t = 400$ Fs).[204] Thus, the latter complexes are expected to exhibit strong photochemical and photodynamic activities. The quantum yields of singlet oxygen generation by $ZnPcS_4$ is 0.70, and most other MPcS (*S* = sulfonate) give yields of at least 0.4. MPcS aggregate in aqueous buffer at pH 7.4, resulting in loss of photochemical inactivity; however, in the presence of detergents, they are monomeric and photoactive.[205]

Scheme 24



144

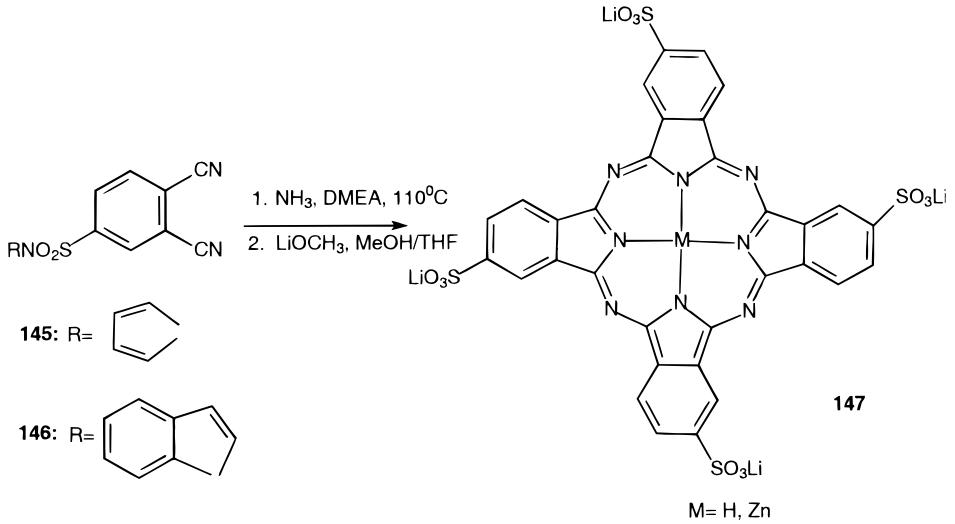
The first biomedical application of Pc relates to the observation that uranium and copper complexes of PcS_4 were retained in experimental murine brain tumors.[206] With the recent development of clinical PDT, Pc were advanced as second-generation PS for PDT and an extensive literature on their biological properties as photodynamic drugs evolved.[204]

The preparation of unsubstituted MPc involves a straightforward, one-step condensation reaction.[201] Scheme 24 represents a general method for the synthesis of the Pc macrocycle (**144**) from phthalic acid and its various derivatives. The symmetrical, nonsubstituted MPc are highly insoluble in most organic solvents or aqueous media and need to be formulated as emulsions or liposomes for biological applications. A unilamellar liposomal ZnPc preparation has been studied extensively for PDT in Balb/c mice bearing the MS-2 fibrosarcoma.[207] The dye is transported by lipoproteins and cleared from the serum via the bile−gut pathway. ZnPc is eliminated rapidly from most tissues, whereas the tumor reaches a maximum concentration only after 1−2 days, with tumor-to-muscle ratios of about 7.[207] Subsequently, the same authors investigated the possibility of manipulating ZnPc biodistribution by its complexation with human low-density lipoprotein (LDL) and formulation in DPPC liposomes.[207] Association of the dye with LDL increases maximum tumor concentrations and results in high tumor/muscle ratios. Isle et al.[209] developed a liposomal ZnPc formulation consisting of a mixture of 1-palmitoyl-2-oleoylphosphatidylchlorine (POPC) and 1,2-dioleoylphosphatidylserine (OOPS) (9:1). The advantageous biodistribution of this preparation in animal models[210] resulted in subsequent proposals for its clinical use.[211] A simple, alternative approach to formulate ZnPc through noncovalent binding to bovine serum albumin (BSA) has also been reported.[212] Analysis of serum fractions from treated animals showed that ZnPc readily redistributed over the high-density lipoprotein fraction (HDL) after intravenous administration. At a ZnPc dose equivalent to 0.5 mol kg^{-1} followed 24 h later by PDT, tumor control was demonstrated in two different models, the EMT-6 tumor in Balb/c mice and the human colon T380 carcinoma in nude mice.[212]

To overcome the solubility problems of ZnPc while retaining its symmetry and single-isomeric nature, the fluorinated analogue was evaluated for PDT.[213] Hexadecafluorinated zinc phthalocyanine ($ZnPcF_{16}$) can be conveniently synthesized, in one step, from commercially available tetrafluorophthalonitrile by condensation with $Zn(OAc)_2$ at 160 °C for 3 h.[214] The fluorine atoms on $ZnPcF_{16}$ impact sufficient solubility on the molecule to allow for the preparation of stable, aqueous emulsions and offer the potential for F-MR imaging or spectroscopy. Comparison of biodistribution pattern in EMT-6 tumor-bearing mice of ZnPc and $ZnPcF_{16}$ formulated in Cremophor revealed that the $ZnPcF_{16}$ reached higher tumor concentrations than the ZnPc, particularly 48 h postinjection. This was reflected in the tumor response, which remained extensive even if light was applied as much later as 72 h postinjection.[213]

AlClPc, which features an axially coordinated Cl ion, is somewhat more soluble in organic solvent than ZnPc. An ethanolic solution of AlClPc was shown to be phototoxic toward V-79 cells.[215−217] A 10% Cremophor EL emulsion in PBS was used to formulate AlClPc for in vivo PDT of murine EMT-6 tumors.[218] Complete tumor control was possible at a low dose of 0.25 μmol kg^{-1}, indicating that AlClPc is more efficient as a PS for PDT than its sulfonated analogues. Like Photofrin and $AlPcS_4$, the nonsubstituted AlClPc also induced tumor destruction via an indirect mechanism involving initial damage of the tumor vasculature.[218] Water-soluble conjugates of AlClPc were prepared by axial coordination of poly(ethylene glycol) (PEG) or poly(vinyl alcohol) (PVA) to the central Al(III) ion. Both dye preparations induced complete EMT-6 tumor regression at a similar drug (0.25 μmol kg^{-1}) and light dose as that

Scheme 25

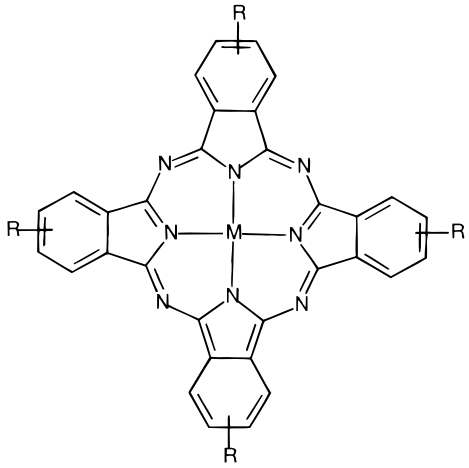


previously observed with the AlClPc−Cremophor emulsion.[219]

The water-soluble, sulfonated Zn−, Al−, and Ga−PcS_n ($n = 1-4$) have received particular attention as PDT agents since they do not require an additional vehicle for in vivo administration.[204] Sulfonated MPcS can simply be prepared by treatment of the unsubstituted MPc with oleum.[220] A complex mixture of many isomeric, differently sulfonated products is obtained, although the reaction can be controlled to some extent by varying the temperature and reaction time.[220] The central metal ion plays an important role in the product distribution. With ZnPc as a substrate, good yields of a relatively clean mixture of mono- and disulfonated ZnPcS are obtained after a 30 min treatment at 50 °C, whereas similar yields of mono- and disulfonated products with the GaCl or AlClPc requires substantially longer reaction times and higher temperatures. The complexity of the reaction mixtures renders the procedure impracticable for the preparation of single-isomeric products. It is, however, possible, using reverse-phase HPLC with mixtures of phosphate-buffered water and methanol, to separate the isomeric products in sufficient amounts to allow for biological screening. The degree of sulfonation of the various fractions can be determined by an oxidative degradation procedure with nitric acid or ceric ammonium nitrate.[220] The resulting mixture of phthalimide and sulfophthalimide fragments are quantified by HPLC to yield the average number of sulfonato groups per Pc. Direct sulfonation results in substitution at both 3- and 4-positions of the phthalic subunit, contributing to the high complexity of the reaction mixture. The HPLC degradation assay of MPcS prepared in this manner revealed the presence of equal amounts of 3- and 4-sulfophthalimide. In contrast, MPcS obtained by the condensation method allows for the use of phthalic subunits which are only substituted at the 4-position. A more hydrophobic AlPcS, i.e., the phthalimidomethyl AlPcS, was obtained by introducing phthalimide and *para*-formaldehyde to the reaction mixture during the sulfonation step.[221,222]

The conventional condensation route to prepare MPcS involves the tetramerization reaction of the monosodium salt of 4-sulfophthalic acid and heating at >200 °C in the presence of metal salt, urea, and catalyst.[223] Recently, Leznoff et al.[224] reported an improvement on the method through the use of indole and pyrrole moieties which have sulfonamides as blocking groups. Thus, 1-(3,4-dicyanophenylsulfonyl)-pyrrole (**145**) or 1-(3,4-dicyanophenylsulfonyl)indole (**146**) upon treatment with ammonia in 2-*N*,*N*-dimethylaminoethanol (DMAE) gave the protected phthalocyanine-2,9,16,23-tetrasulfonamide in over 40% yield, Scheme 25. In the presence of $Zn(OAc)_2$, the analogous ZnPc was obtained in up to 68% yield. Base cleavage of the sulfonamide groups using lithium methoxide in THF/MeOH gave the free H_2PcS_4 and Zn analogue (**147**) (M = 2H or Zn) in quantitative yields.

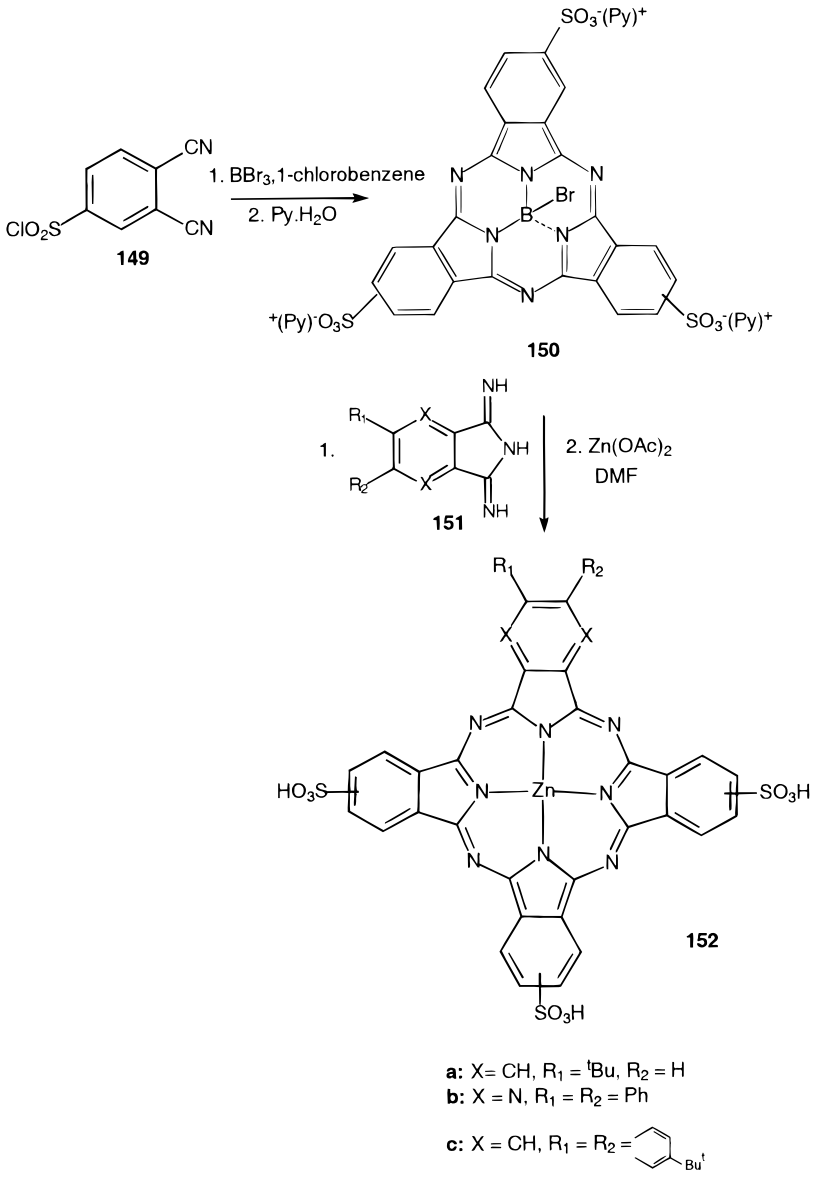
Differently sulfonated products can be obtained using mixtures of 4-sulfophthalic acid and substituted phthalonitrile.[220] Varying the substrate ratio in the reaction mixture allows the preparation of $MPcS_n$ ($n = 1-4$) with selected degree of sulfonation. Also, phthalic acid can be replaced with a number of other reactants such phthalic anhydride, phthalamide, or phthalonitrile. Similarly, 3- and 4-R-



148

M= Zn, ClGa, ClAl
R = $(SO_3Na)_n$ (H, ^{t}Bu, Cl, I, $NO_2)_{4-n}$

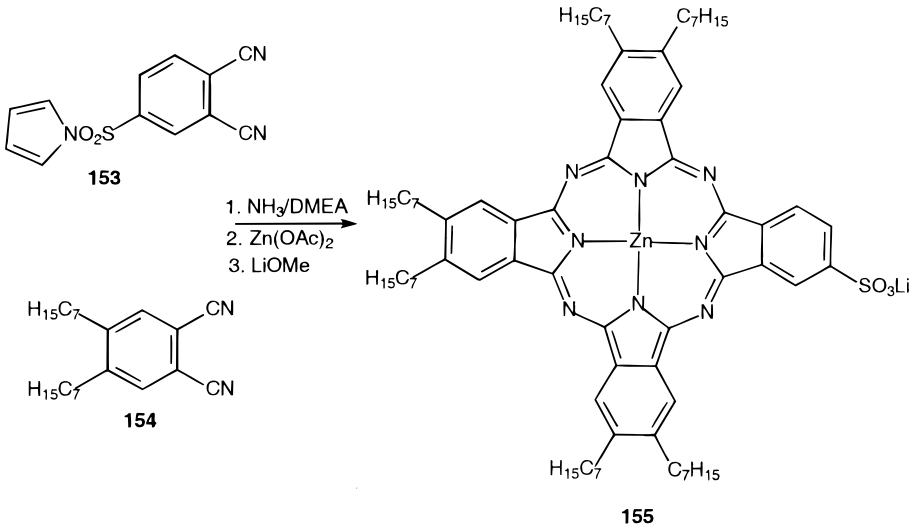
Scheme 26



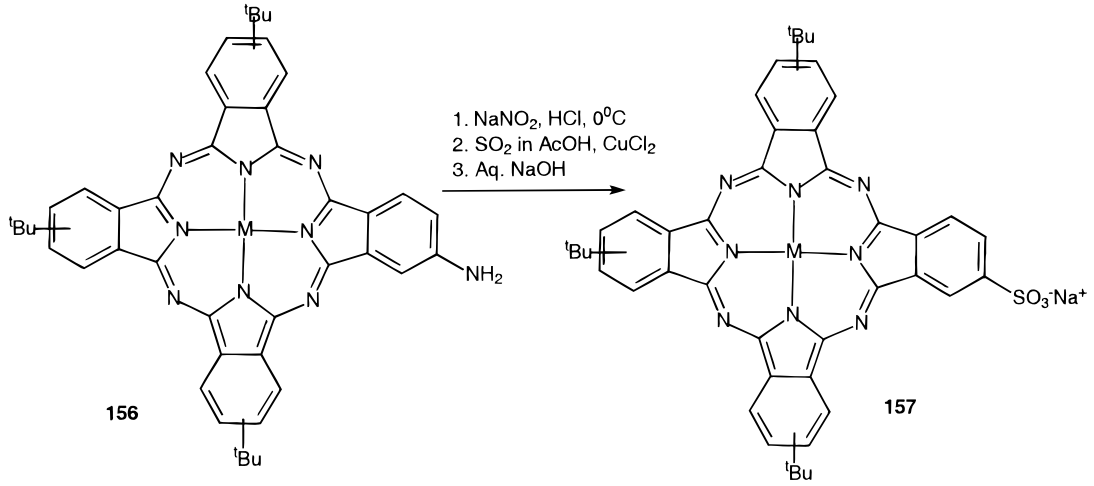
substituted phthalic acid derivatives (R = 4-*tert*-butyl, 4-chloro, 4-nitro, 4-iodo) have been used in the mixed condensation reaction with 4-sulfophthalic acid. Condensation of these compounds in the presence of sulfophthalic acid always gives complex mixtures of PcSR (**148**) with different S/R ratios.

The potential of sulfonated Pc for clinical PDT prompted a search for alternate synthetic approaches to yield well-characterized compounds as single-isomeric products. The synthetic route via the ring expansion of a boron SubPc is particularly attractive in this regard, since the number of possible isomers in the final products is low. Meller and Ossko in 1972 attempted to prepare boron-containing Pc via the reaction of phthalonitrile with haloboranes.[225] Surprisingly, the reaction did not furnish the desired tetrameric Pc derivative but rather a "contracted" trimeric compound, referred to as a sub-phthalocyanine (SubPc). Since the initial discovery of SubPc, several examples of such complexes have been reported including SubPc featuring *tert*-butyl substituents, halogen (F, Cl, Br, I), and hexakis(alkylthio) as well as complexes with different axial ligands (e.g., F, Cl, Br, Ph) at the boron center.[226] The first application of SubPc, as intermediates for the synthesis of Pc, was reported by Kobayashi et al.[227] This group showed that treatment of the tri-*tert*-butyl BSubPc with succinimide or diiminoisoindoline gave the analogous, unsymmetrically substituted Pc in 13% and 8−20% yield, respectively. Most MPc prepared in this manner are, however, not water soluble. A similar procedure to obtain a trisulfonated SubPc was recently reported.[228] The reaction involves treatment of 4-(chlorosulfonyl)phthalonitrile (**149**) with a commercially available solution of BBr_3 in chloromethane to give the pyridinium salt of tris(4-chlorosulfonyl)SubPcBBr (**150**) as golden purple crystals (from water/pyridine) in 60% yield, Scheme 26. The ring expansion with diiminoisoindoline derivatives (**151**) can be achieved at low temperature in DMSO (50 °C) to yield the corresponding trisulfonated H_2PcS_3 in over 30% yield. The latter are

Scheme 27



Scheme 28



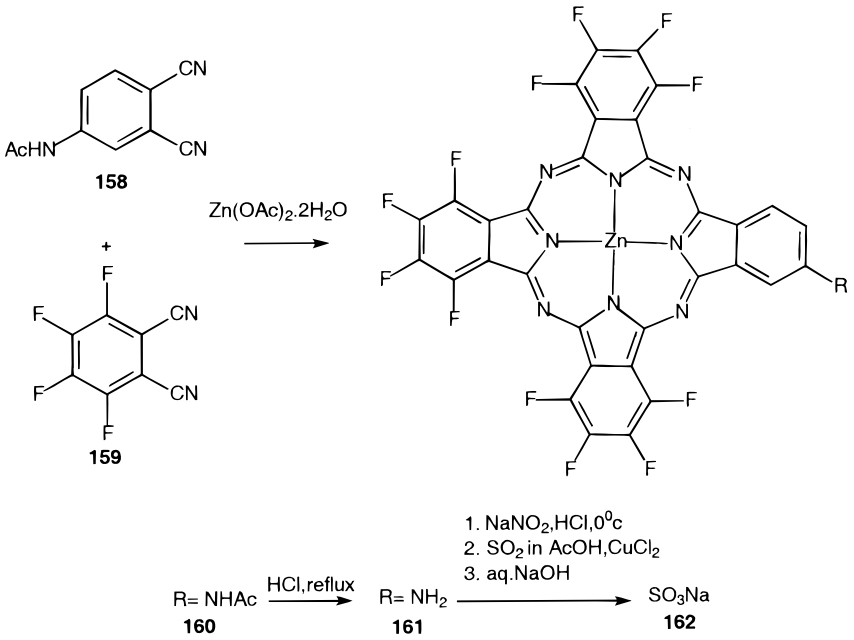
readily metalated with $Zn(OAc)_2$ in dry DMF (**152**). Photodynamic activities of a series of substituted $ZnPcS_3$ prepared in this manner were measured against the EMT-6 mouse mammary tumor cell line.[229] Adding a (*t*-Bu)benzo (**152a**) or a (*t*-Bu)-naphtho (**152c**) group to $ZnPcS_3$ increased the in vitro cell photoinactivation efficacy, whereas addition of a fourth sulfobenzo ($ZnPcS_4$) or bulky diphenylpyrazino group decreased the activity of the parent ($ZnPcS_3$) molecule. In vivo, PDT with the (*t*-Bu)naphtho derivatives, i.e., the Zn (*t*-Bu) naphthalo(tris)sulfobenzo)porphyrazine (Zn−NSBP) (**152c**), induced the best tumor control, which combined with its good solubility and broad absorption spectrum (λ_{max} 706 and 678 nm) renders this compound an interesting PS for medical applications.

A method for the synthesis of monosulfonated MPcS via a mixed condensation of 1-(3,4-dicyanophenylsulfonyl)pyrrole (**153**) and phthalonitrile has also been reported.[224] The products are, however, highly insoluble and difficult to separate by chromatography. To increase the solubility, **153** was condensed with 4,5-diheptylphthalonitrile (**154**) to give 9,10,-16,17,23,24-hexakis-(1-heptyl)phthalocyanine-2-*N*-pyrrolyl)sulfonamide in 14% yield. Treatment with $Zn(OAc)_2$ in DMF gave 9,10,16,17,23,24-hexakis(1-heptyl)phthalocyanine-2-*N*-(*N*-pyrrolyl)sulfonamide Zn(II) in 86% yield. Hydrolysis with lithium methoxide in methanol afforded lithium 9,10,16,17,23,24-hexakis(1-heptyl)phthalocyanine-2-sulfonate Zn(II) (**155**) in 85% yield, Scheme 27.

An alternative route for the synthesis of monosulfonated Pc via substitution of a monoamino Pc, using the Meerwein procedure, has also been reported.[230] The monodiazonium salt of 11,18,25-tri(*tert*-butyl)-4-diazaPc (obtained from monoamine **156** and sodium nitrile in HCl) on treatment with SO_2 in AcOH in the presence of $CuCl_2$ as catalyst gave 11,18,25-tri-(*tert*-butyl)-4-(chlorosulfonyl)Pc (55% yield) along with a chlorinated derivative (25% yield). The chlorosulfonyl complex was hydrolyzed in aqueous NaOH to yield the monosulfonated MPc (**157**) (Scheme 28).

An attempt to prepare the 4-aminododecafluoro ZnPc in a similar manner failed, since sodium sulfide induced a nucleophilic substitution of the aromatic F atoms. To overcome the problem, the 4-amino group was protected as an acetamido group (**158**), reacted in a mixed condensation with tetrafluorophthaloni-

Scheme 29

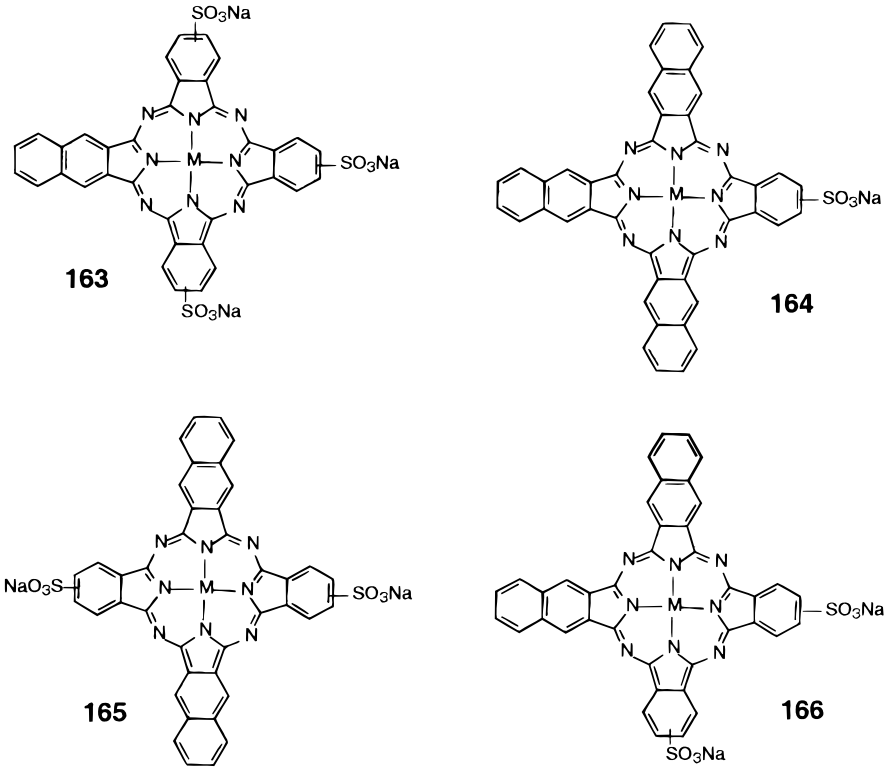


trile (**159**), cleaved in acidic conditions to give the 3-amino ZnPc (**161**), and subsequently the pure 3-monosulfo ZnPc (**162**) in good yield, Scheme 29. Compared to the hexadecafluorinated $ZnPcF_{16}$, the monosulfonated analogue $ZnPcF_{12}S_1$ (**162**) showed improved pharmacokinetics in tumor-bearing mice including lower liver and spleen retention and higher tumor-to-nontarget ratios. However, at 1 μmol/kg, **162** induced toxic shock after light treatment, likely resulting from extensive cellular effects.[231] Other analogues of $ZnPcF_{16}$ that have been advanced include the water-soluble fluorinated ZnPc bearing different numbers of additional sulfophenyl substituents. In vitro, these derivatives are more photoactive than $ZnPcS_4$; however, no in vivo activities are reported.[232]

Varying the degree of sulfonation of substituted MPc significantly affects their in vitro and in vivo phototoxicity[233] as well as their photochemical properties.[234] However, as long as the molecules are in their monomeric form, the quantum yield for singlet oxygen is not affected. Thus, the observed differences in the capacity to generate singlet oxygen between differently sulfonated $GaPcS_n$ correlates well with the different tendencies of the dyes to dimerize or aggregate. In contrast, at the cellular level, the mono- and disulfonated GaPcS, which exhibit the highest tendencies to aggregate, are the most active in terms of photoinactivation of V-79 cells, whereas the $GaPcS_4$ was found to be totally inactive.[235] Obviously the increased photoactivity of the lower sulfonate derivatives does not result from different photochemical properties but rather reflects their better cell-penetrating properties. Similar pattern were observed with the analogous $ZnPcS_n$.[236] Complex isomeric mixtures of Pc appear to be less aggregated than pure, single-isomeric Pc, resulting in striking differences in their biological behavior. For example, an isomeric mixture of $ZnPcS_4$ obtained via direct sulfonation of ZnPc was found to be 10-fold more active than the homogeneous $ZnPcS_4$ prepared via the condensation of 4-sulfophthalic acid. In vitro, structure−photodynamic activity relationships of a series of 4-substituted ZnPc showed that partition coefficients correlated with dye uptake by the cells but not necessarily with their photodynamic activities.[237] A parabolic relationship was observed for the latter, with the adjacently, disulfonated ZnPc showing the highest phototoxicity. Under in vivo conditions, similar relationships are observed, although differences between the various sulfonated Pc are less pronounced. Overall, $MPcS_2$, bearing sulfonates on adjacent benzo rings, showed the best tumoricidal activity.

Various studies have demonstrated the photodynamic efficiency of AlPcS, both in vitro and in vivo.[238] As with Ga and Zn analogues, the disulfonated AlPcS is the most active among the sulfonated derivatives.[239] After in vitro incubation with V-79 cells, fluorescence microscopy revealed that the dye was uniformly distributed in the cytoplasm but absent in the nucleus. Tumor necrosis induced in animal tumor models, following PDT with a mixture of differently sulfonated AlPcS, resulted from initial disruption of the vasculature rather than a direct effect against tumor cells, similar to the action of Photofrin. However, using $AlPcS_2$ bearing sulfonates on adjacent rings, in morphological studies Peng et al.[240] showed that tumor necrosis early after PDT induced minimum impairment of the tumor vasculature. They concluded that tumor response with $AlPcS_2$ involved direct tumor cell cytotoxicity, and this was subsequently confirmed by pathological[241] and ex vivo tumor cell survival studies.[242] Introduction of an additional substituent *t*-Bu on the nonsulfonated benzene ring of $GaPcS_2$ further enhances the photodynamic efficacy of the dye.[243] Cellular targets of MPc photosensitization vary depending on the nature and solubility of the Pc, and both membrane and subcellular damage has been demonstrated.[244] In addition

Chart 7



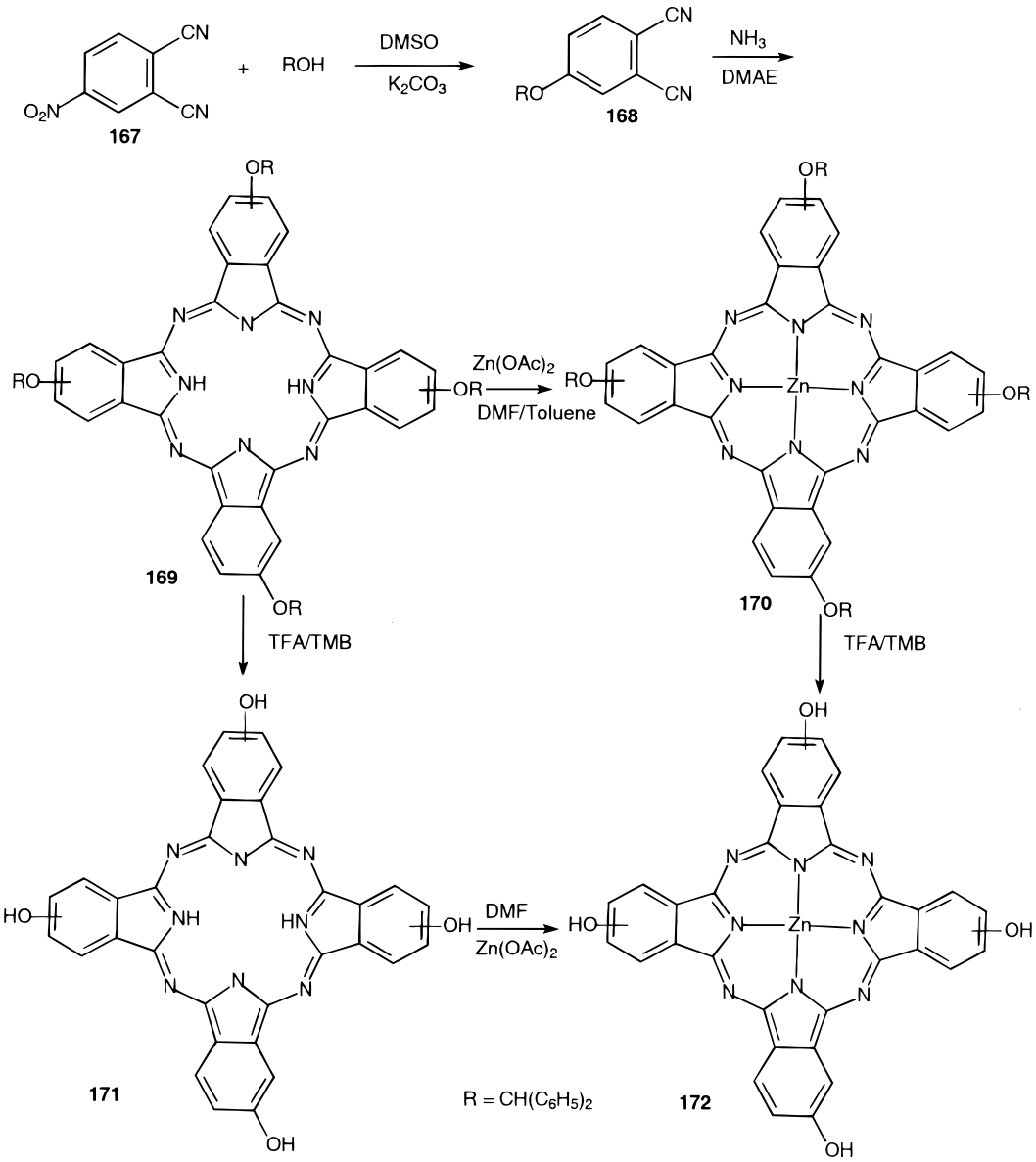
to their potential use for tumor-PDT, sulfonated MPc have been evaluated as PS for the photodynamic inactivation of viruses and the sterilization of blood products.[245,246]

Binding interactions between human serum albumin and differently sulfonated $AlPcS_n$ were studied using optical and ESR spectroscopy.[247] Such studies showed that slelected $AlPcS_n$ components may be involved in different intreactions with serum albumin (affinities and number of bound molecules) and, hence, result in altered transport, tissue release/accumulation, and body retention. It also has been demonstrated that cell uptake of the monomeric (photoactive) $AlPcS_n$ is optimal for preparations consisting of various regioisomers or of several differently sulfonated components.[248] Such data suggest that mixed isomeric preparations may be more efficient as PDT agents than isomerically pure compounds.

With the aim to develop Pc with a multiple choice of red-shifted excitation wavelengths, analogues consisting both of phthalo and naphthalo moieties, were prepared.[249] These metallonaphthalosulfobenzoporphyrazines (M-NSBP) can be perceived as hybrid molecules of $MPcS_4$ and naphthalocyanines. They feature spectral characteristic of both parent molecules resulting in composite spectra that vary in shift according to the number of naphthalene or sulfobenzene units (i.e., compounds **163**−**166**), Chart 7. The monosodium salt of 4-sulfophthalic acid, naphthalene-2,3-dicarboxylic acid (equimolar ratio), together with $Zn(OAc)_2$, $AlCl_3$, or $GaCl_3$ in the presence of urea, ammonium molybdate, and ammonium chloride were heated at 270−280 °C for 20 min either alone or in sulfolane. M-NSBP were purified on a C-18 reverse-phase column, whereby their characteristic spectral fingerprints were used to facilitate their detection.[250] The presence of both sulfobenzo as well as naphtho groups on the same macrocycle enhances the amphiphilicity, a property which has been shown to improve cell penetration and photodynamic potency. PDT with the amphiphilic disulfonated AlOH−NSBP **166** of EMT-6 tumor-bearing Balb/c mice induced a significant tumor response, while the monosulfonated derivative **164** was much less active.[251]

Substituting the MPc macrocycle with hydroxyl groups increases the solubility and structure−activity relationships between the site of substitution and photodyanamic activity have been observed.[252] The synthesis of a series of ZnPc substituted with hydroxyl groups fused directly to the Pc skeleton and via aliphatic spacer chains permitted this study. The parent tetrahydroxy $ZnPc(OH)_4$ was prepared via the self-condensation of dicyano precursor **168** in dimethylaminoethanol (DMAE), which gave the 2,9,16,-23-tetrasubstituted Pc (**169**) as a mixture of isomers in 25% yield, Scheme 30.[253] Refluxing the metal-free Pc **169** with $Zn(OAc)_2$ in DMF and toluene gave 2,9,-16,23-tetra(diphenylmethoxy) Zn(II)Pc (**170**) in 90% yield. Alternatively, **170** can be prepared in high yield in one step directly from **168** using 1,8-diazobicyclo-[5.4.0]undec-7-ene (DBU) as a base and $Zn(OAc)_2$ at 100 °C. Cleavage of the protected, metal-free **169** or the ZnPc **170** under reflux in TFA in the presence of polyalkylbenzene gave the 2,9,16,23-$H_2Pc(OH)_4$ (**171**) or -Zn(II)$Pc(OH)_4$ (**172**) in 84% yield. The metal-free $H_2Pc(OH)_4$ (**171**) can also be metalated by treatment with the metal salt to yield **172**. The $Pc(OH)_4$ are readily purified by gel-permeation chromatography.

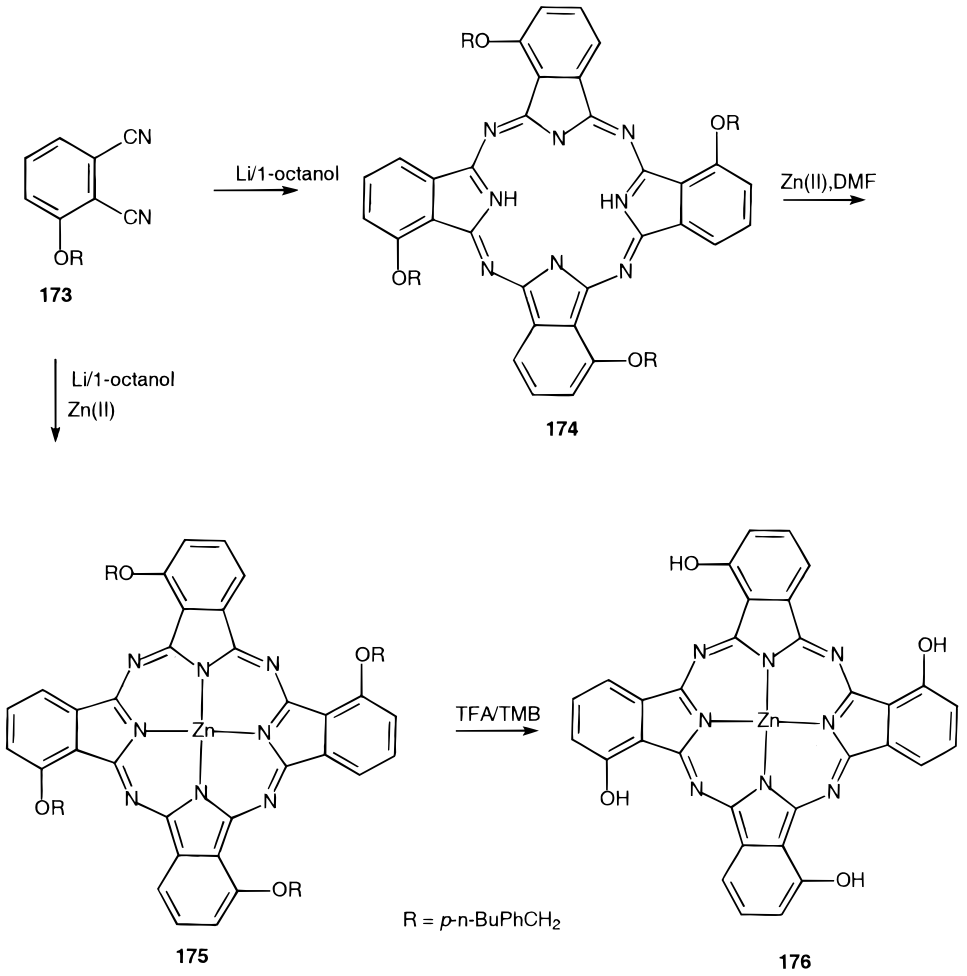
Scheme 30



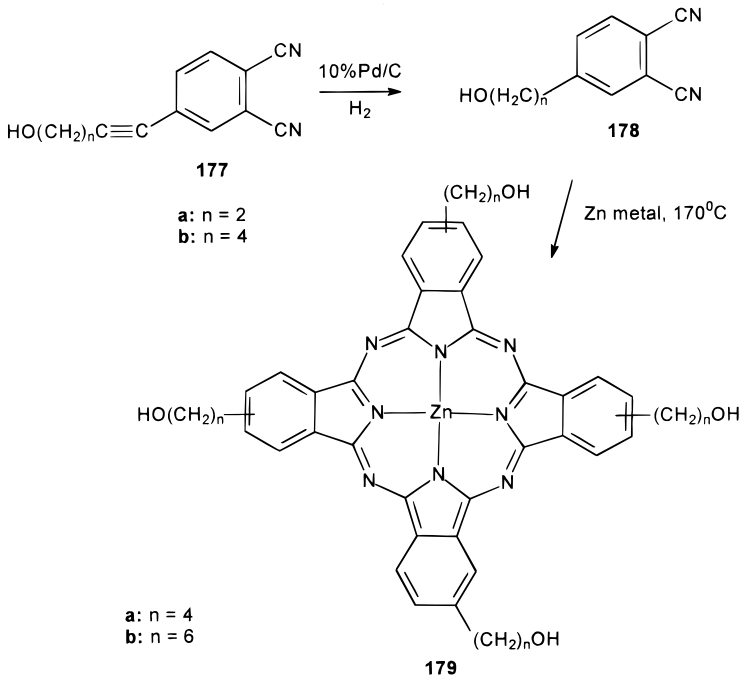
Both electronic effects and steric interactions affect the isomer distribution during Pc formation. Synthesis of a single isomer of 1,8,15,22-tetrahydroxy Pc has been achieved[254] by treatment of **173** with lithium in 1-octanol to yield Pc **174**, which upon treatment with $Zn(OAc)_2$ gave the ZnPc **175**, Scheme 31. Cleavage with TFA and 1,2,4,5-tetramethylbenzene (TMB) gave a single isomer of $ZnPc(OH)_4$ (**176**). Increasing the reaction temperature augmented the complexity of the mixture. The increased energy apparently permits the intermediate to overcome steric interactions and to change between different conformations. Similarly, self-condensation of **173** at 150 °C gave a mixture of all possible isomers; however, at 60−80 °C, only one Pc was produced as a single isomer, while at 120 °C a nonstatistical mixture was obtained. ^{13}C NMR of **176** gave singlet signals of all different carbon atoms, while the ^1H NMR exhibited the desired doublet, triplet, and doublet−doublet of hydrogens of the symmetrical Pc macrocycle. The distribution of isomers in the reaction mixture depends on the VDW volume of the 3-substituent of the phthalonitrile. At the same temperature, a bulky 3-substituent gave a single isomer whereas less bulky groups, such as a neopentoxy or propargyloxy group, gave mixtures of Pc isomers. Electronic effects also play a role in the reaction. Thus, addition of a solution of Li in 1-octanol to 3-methoxyphthalonitrile in THF at 40−50 °C, stirred overnight, followed by the addition of $Zn(OAc)_2$ gave a pure, single-isomeric product in 50% yield.

To study the effect of distance and freedom of movement of the four hydroxyl groups on photodynamic activities, $ZnPc(OH)_4$ with the OH groups attached via aliphatic chains of three and six carbon atoms were prepared.[252] Palladium-catalyzed alkynation of 4-iodophthalonitrile with propyn-1-ol and hex-5-yn-1-ol products **177a** and **177b** were obtained. Catalytic hydrogenation of 10% palladium on charcoal gave 4-(propylhydroxy)phthalonitrile (**178a**) and

Scheme 31

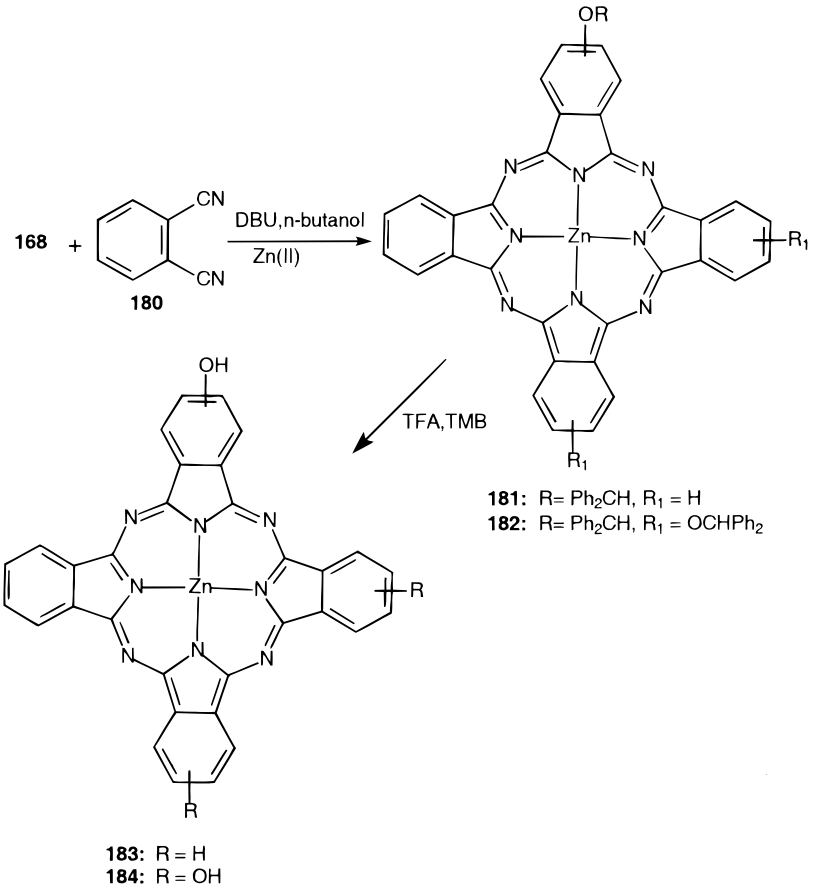


Scheme 32



4-(hexylhydroxy)phthalonitrile (**178b**), both of which condensed with Zn metal at 170 °C to give ZnPc-$(ProH)_4$ (**179a**) and ZnPc$(HexOH)_4$ (**179b**), respectively, Scheme 32. The tetrahydroxy ZnPc derivatives

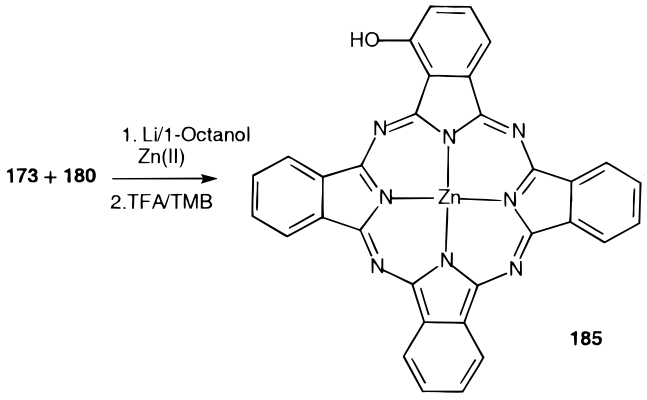
Scheme 33



were tested for their photodynamic potency in vitro against V-79 cells and in vivo on EMT-6 tumor-bearing Balb/c mice. The tetraalkylhydroxy ZnPc are effective PS in vivo with the tetrapropylhydroxy compound exhibiting about twice the activity of the tetrahexylhydroxy analogue. In vitro, these differences were accentuated by an order of magnitude while the tetrahydroxy compound lacking spacer chains was inactive in both systems.[252]

Mixed condensation reactions[254] with two different precursors have been used to prepare unsymmetrical Pc using two different precursors. Treatment of 4-(diphenylmethoxy)phthalonitrile (**168**) and phthalonitrile (**180**) in a ratio of 1:1 gives a mixture of all possible Pc products. However, in a ratio of 1:10 with DBU in 1-butanol at 100 °C for 1 h, followed by the addition of excess $Zn(OAc)_2$, the reaction gave 2-(diphenylmethoxy)Zn(II)(Pc) (**181**) as a major product (50%) together with ZnPc and trace amounts of the disubstituted ZnPc (Scheme 33). The same procedure was used to prepare the 2,9,16-tris(diphenylmethoxy)Zn-(II)Pc (**182**) in 48% yield, using **168** and **180** in a ratio of 10:1. Cleavage of protected **181** and **182** with TFA/TMB gave the corresponding Zn(II)Pc(2-OH) and 2,9,-16-Zn(II)Pc$(OH)_3$. Treatment of 3-(*p*-*n*-butylbenzyloxy)-phthalonitrile (**173**) and phthalonitrile **180** in a ratio of 1:10 in a similar manner followed by deprotection of the hydroxy group gave Zn(II)Pc(1-OH) (**185**) in 62% yield, Scheme 34. The ^{1}H NMR spectrum of **185** showed a typical ABX splitting pattern with a doublet at 9.18 ppm, a small doublet at δ 8.70 ppm, and a doublet−doublet at δ 7.67 ppm representing aromatic protons from the substituted benzene ring.

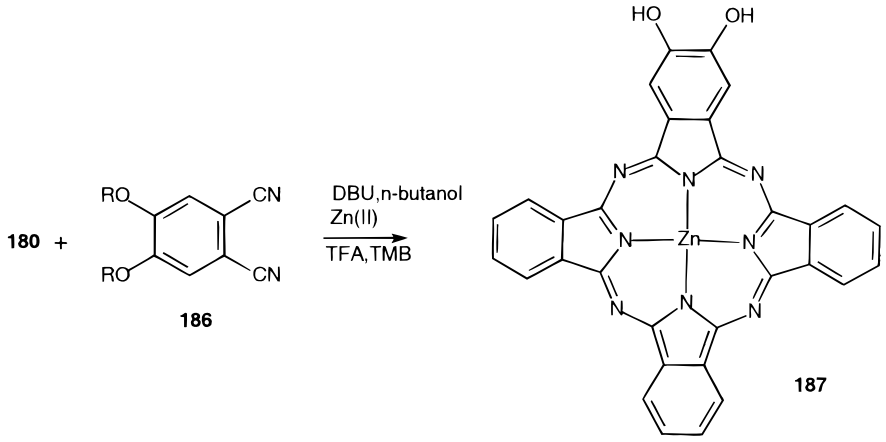
Scheme 34



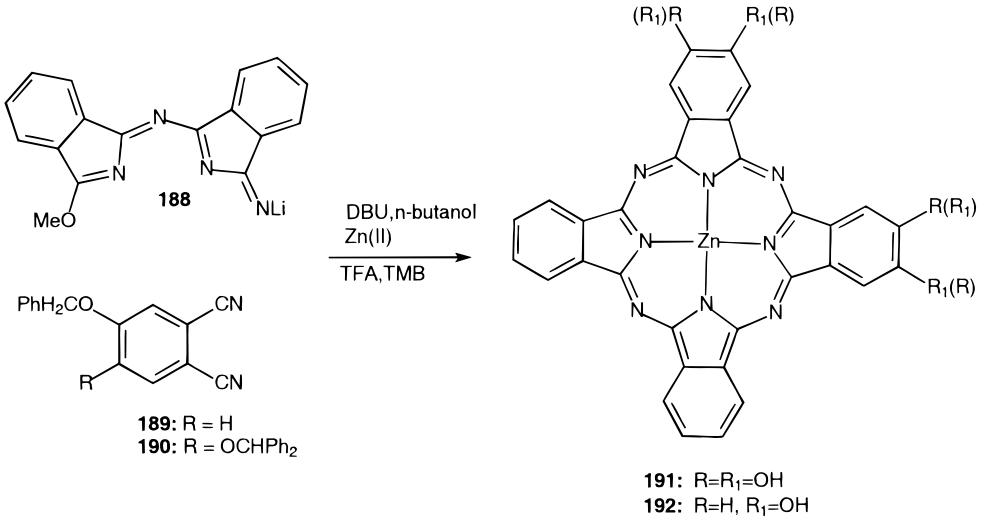
The mixed condensation of **180** with **186** gave a protected 2,3-disubstituted Pc, which following metallation, cleavage by TFA/TMB and chromatography gave Zn(II)Pc$(2,3\text{-}OH)_2$ (**187**) in >50% yield, Scheme 35.

Mixed condensation of unsubstituted and substituted phthalonitrile give "adjacent" and "opposite" substituted Pc which are difficult to separate by chromatography. The Pc cyclization reaction has been shown to proceed via a phthalonitrile dimer intermediate (half-Pc). If the latter is in a large excess, the half-Pc undergoes further condensation with the

Scheme 35



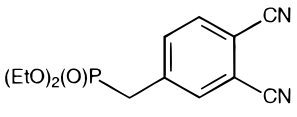
Scheme 36



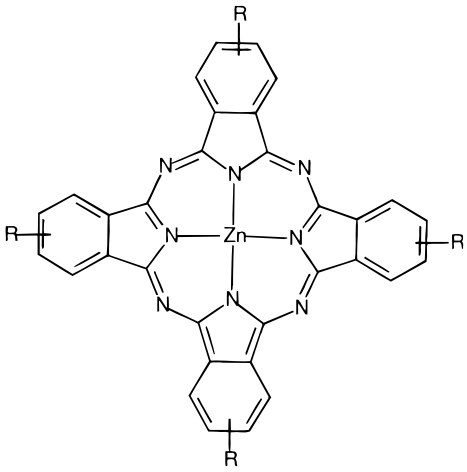
phthalonitrile monomer to give the desired adjacent Pc as a single product. The reaction of phthalonitrile (**180**) with lithium methoxide in methanol gave the half-Pc **188** as the main product.[254] The mixture was subjected to further mixed condensation with a large excess of monomer **189** or **190** in 1-octanol to give the adjacent substituted Pc as the only product, which upon cleavage of the ether linkages with TFA/TMB, gave 2,3,9,10-Pc(OH)$_4$ (**191**) and 2,9-ZnPc(OH)$_2$ (**192**), Scheme 36. The activity of the hydroxy ZnPc as PS was evaluated on the EMT-6 tumor cell line and compared to the unsubstituted ZnPc. In vitro, ZnPc(2-OH) (**183**) was the most active followed by 2,3- and 2,9-ZnPc(OH)$_2$ (**187**, **192**) with 2,9,16-ZnPc(OH)$_3$ (**184**) exhibiting the least activity. In vivo, both the monohydroxy and the 2,3-hydroxy derivative induced a poor PDT response at 2 μmol kg^{-1}, while the 2,9-dihydroxy isomer induced 75% tumor regression under the same conditions. The ZnPc(OH)$_4$, even at 10 μmol kg^{-1}, failed to induce a tumor response.[254]

Several water-soluble ZnPc substituted with phosphate groups were also reported as posible PDT agents. Synthesis of ZnPc substituted with phosphate groups directly on the macrocyle involves condensation of diethyl 3,4-dicyanobenzylphosphonate, obtained by alkylic bromination of 3,4-dicyanotoluene with NBS/benzoylperoxide in CCl_4. Treatment with triethyl phosphite in refluxing acetonitrile gave **193** in 42% yield via the Arbuzov rearrangement.[256] Condensation of **193** Zn(OAc)$_2$ gave the ZnPc, which was hydrolyzed to tetra(1-phosphoethoxymethyl)ZnPc tetrasodium salt (**194**) in aqueous NaOH.[255] The symmetric tetraphosphonato ZnPc (**195**) was formed by direct condensation of 4-diethoxyphosphinyl phthalonitrile with Zn(OAc)$_2$ at 170 °C for 3 h, followed by hydrolysis in 6 N HCl to yield the water-soluble **195**.[256]

The synthesis of a phosphate derivative with butyl spacer chains involves treatment of 4-iodo-1,2-dicyanobenzene with but-3-yn-1-ol and palladium catalyst under Heck conditions to yield 4-(1-hydroxybut-3-ynyl)-1,2-dicyanobenzene, which was catalytically hydrogenated to give 4-(1-hydroxybutyl)-1,2-dicyanobenzene in 92% yield. The latter compound was reacted with diethyl chlorophosphate in pyridine for 16 h to give diethyl 4-(3,4-dicyanophenyl)butyl phosphate in 65% yield. Heating with Zn(OAc)$_2$ at 130 °C for 3 h gave a blue/green solution, which was treated with aqueous NaOH, neutralized with dil. HCl, and purified by reverse-phase HPLC to yield tetra[1-(*O*-



193



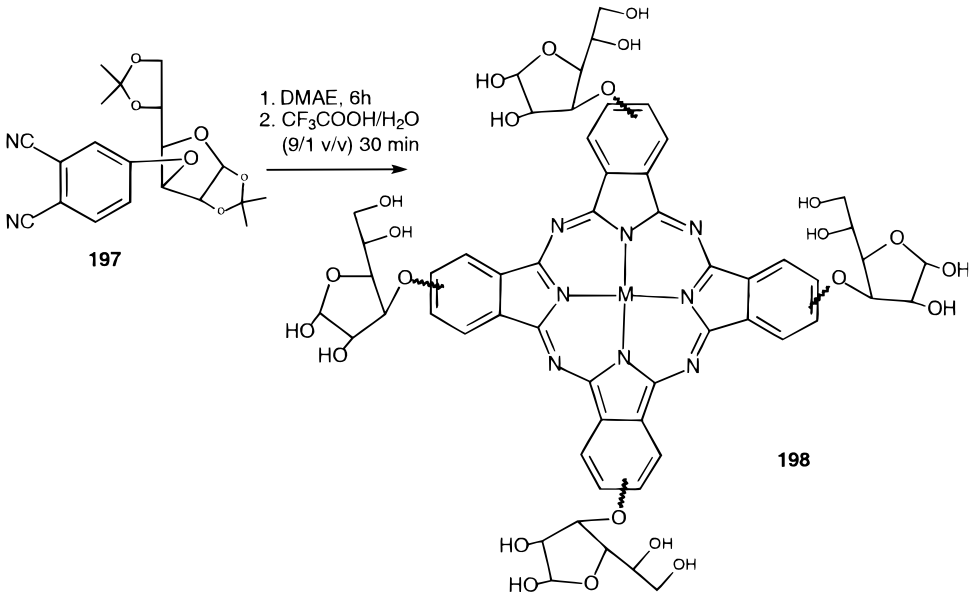
194: R= PO_3H_2
195: R= $(CH_2)P(O)(OEt)(ONa)$
196: R= $(CH_2)_4OP(O)(OEt)(ONa)$

ethylphosphatobutyl)]tetrasodium ZnPc salt (**196**) as a major product, along with products resulting from hydrolysis of the butoxy−phosphorus bonds (one−three *O*-ethylphosphato groups).[257] The photodynamic activity of **196** against the EMT-6 tumor in Balb/c mice is similar to that observed with the tetrapropylhydroxy ZnPc (**179a**), but the phosphato analogue **196** has the advantage of being very water soluble, facilitating its formulation.[255]

Another class of water-soluble Pc, substituted with biocompatible groups, is the glycosylated MPc.[258] Condensation of 4-nitrophthalonitrile and 1,2:5,6-diisopropylidene-D-glucofuranose in the presence of $Zn(OAc)_2$ gave the protected glycosylated ZnPc in 61% yield. Treatment with trifluoroacetic acid/water (9:1) gave the unprotected ZnPc **198**, Scheme 37, which in DMSO exhibits a spectrum typical of monomeric MPc with maximum absorption bands at 354, 618, and 685 nm. Apparently the attached nonionizable units provide steric hindrance which prevent the aggregation of the Pc and increases their solubility in water.

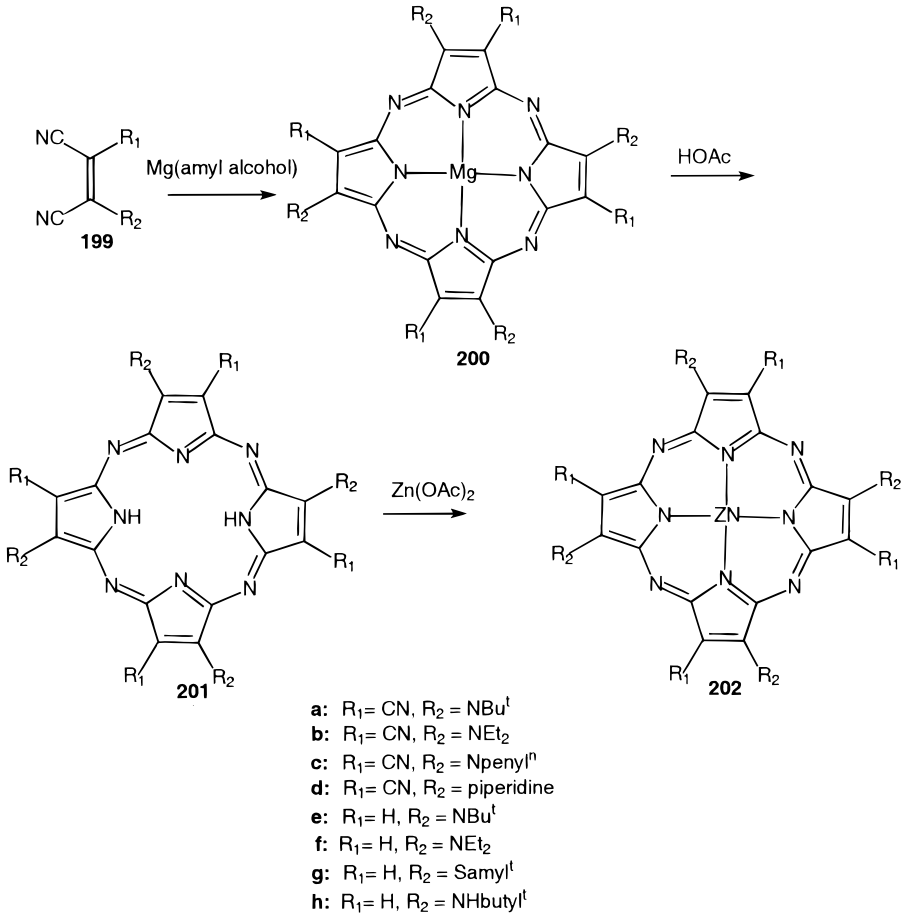
The first synthesis of a tetraazaporphyrin was reported in 1952.[259] The free dye has absorption properties similar to those of porphyrin (λ_{max} = 620 nm). Attachment of electron-donating substituents results in a red-shift, whereas metalation results in a blue-shift (λ_{max} = 591 nm). The red-shifted analogues are particularly attractive PS for PDT. A series of alkylated Zn tetraazaporphyrins **202** were synthesized[260] according to the method of Kopranenkov[261] by the treatment of dicyano **199** with magnesium pentyloxide in pentyl alcohol, Scheme 38. Subsequent treatment of the Mg complex **200** with glacial acetic acid gave the metal-free compound **201**, which was converted to the corresponding zinc complexes upon treatment with $Zn(OAc)_2$ in DMF. The presence of an electron-withdrawing cyano group among the substituents (i.e., **202a**−**h**) has a marked influence on the visible absorption spectrum. Thus, compound **202b** has a λ_{max} at 750 nm while its corresponding analogue lacking a CN group shows a peak at 705 nm. Similarly, **202d** absorbs at 764 nm and the corresponding CN-free analogue at 705 nm. The shift is more pronounced in the case of an amino substituent, i.e., **202f** has a λ_{max} at 710 nm compared to the *tert*-amylthio analogue **202g** at 649 nm.[262] Evaluation of the series **202a**−**h** for their ability to photoinactivate V-79 Chinese hamster fibroblasts in vitro showed that the *N*,*N*-diethylamino **202f** has the highest PDT potential.[260,262] Coordination of Zn^{2+} in the center cavity results in little change in the biological activity, but attachment of both long aliphatic chains or cyclic amines and cyano groups in the periphery of the porphyrazines causes a loss in activity. Also, the removal of the cyano moiety has a

Scheme 37

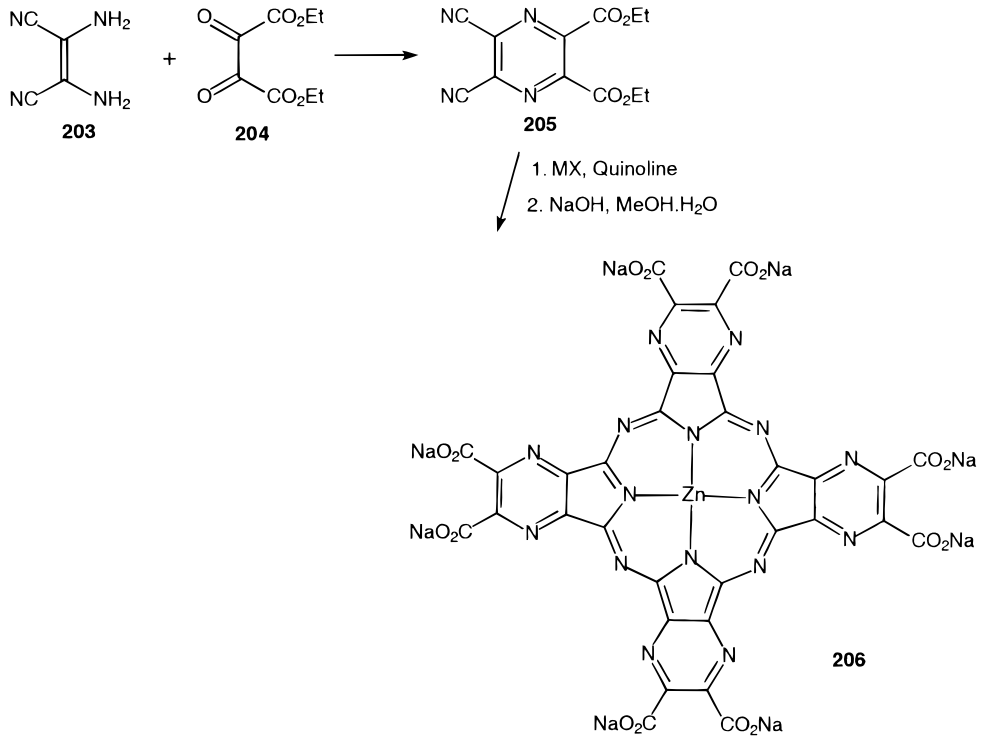


197 198

Scheme 38



Scheme 39



negative effect on the photodynamic efficacy of the compounds. It has been postulated that in the absence of the cyano group, the ability of the porphyrin to quench singlet oxygen is highly increased.

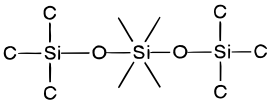
AzaPcs, which feature additional N atoms in the macrocycles have also been synthesized as potential PDT agents. The starting material 2,3-dicyano-5,6-diethoxycarbonylpyrazine (**205**) was synthesized by

the condensation of diaminomaleodinitrile (**203**) with diethyl dioxysuccinate (**204**) in the presence of acetic acid, Scheme 39. The condensation of *o*-dinitrile (**205**) with $Zn(OAc)_2$ afforded tetra-2,3-(5,6-diethoxycarbonylpyrazino)porphyrazine Zn(II), which upon mild hydrolysis yields the water-soluble octacarboxy Zn complex **206**.[263]

The use of group IV metalloPc allows facile structural modifications via the attachment of axially ligated substituents. Such derivatives are single-isomeric products of varying solubility with reduced tendencies to aggregate. The synthesis of the key intermediates, i.e., the dichloro SiPc ($PcSiCl_2$) via the reaction of *O*-phthalonitrile with either silicone tetrachloride or hexachlorodisiloxane in quinoline, was first reported in 1965.[264] Subsequently, a more convenient reaction was proposed using *O*-cyanobenzamide and 1,3-diiminoisoindoline as precursors.[265] Treatment of $PcSiCl_2$ with equal volumes of pyridine and concentrated ammonia or sulfuric acid gave $PcSi(OH)_2$. Thermal dehydration of the latter gave a polymer, $HO(PcSiO)_xH$.[266] The reaction, i.e., $xPc(SiOH)_2 \rightarrow HO(PcSiO)_xH + (x-1)H_2O$, entails the cleavage of Si−O bonds in the presence of Si−N bonds. $PcSi(OH)_2$ condenses with itself and with PcAlOH, $(C_6H_5)_3SiOH$, C_6H_5OH, and $C_6H_5CH_2OH$:

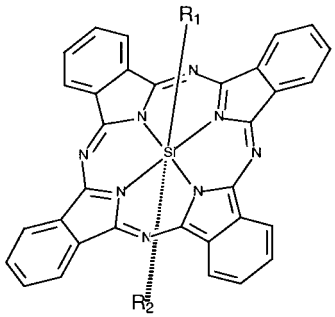
$$PcSi(OH)_2 + 2HOR \rightarrow PcSi(OR)_2 + 2H_2O$$

The success of the reaction depends on the acidity of the HOR reactant, SiOR bond strength, the solubility of $PcSi(OH)_2$ in organic solvents, and the reaction temperature.[267] The bulk of the siloxy group in the siloxysilicon compounds and the nature of the Pc ring substituents suggest a *trans*-octahedral arrangement. Thus, the backbone of $Pc[OSi(C_6H_5)_3]_2$ may be assumed to be of the type:



This structure is unusual in that it contains both tetracoordinate and hexacoordinate silicon in the same unit. The Si−N bonds in the SiPc show a marked chemical resistance. For example, the Si−N bonds in $PcSi(OH)_2$ are stable in concentrated H_2SO_4, refluxing alcohol, or aqueous ammonia and pyridine mixtures. The four N-atoms of the Si−N bonds are positioned symmetrically around the Si atom within the ring structure, and demetalation requires the rupture of all four bonds. Introduction of an axial trialkylsiloxy group on the central Si atom augments the solubility of the complex. A variety of SiPc containing two or more axial groups have been synthesized by cyclization of a ring precursor around the silicon followed by ligand exchange. The last step involves heating $SiPc(OH)_2$ with a chlorosilane derivative in the presence of a base to yield the axially substituted SiPc in high yield as deep blue compounds.

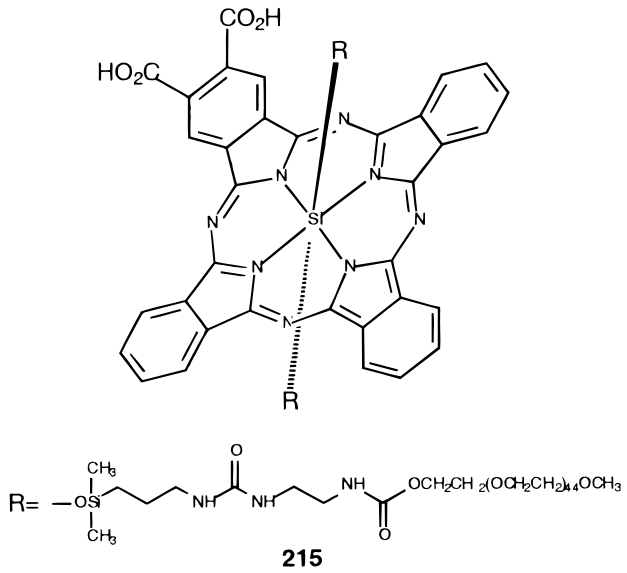
Kenney et al. prepared several SiPc (**207**−**214**) with one or two amino-bearing ligands.[268] The $HOSiPcOSiR_3$ containing a single siloxy ligand is prepared by reacting $CH_3SiPcOH$ with the corresponding CH_3OSiR_3 to yield the intermediate $CH_3SiPcOSiR_3$. The latter is photolyzed in triethylamine saturated with H_2O and toluene to yield the analogous $HOSiPcOSiR_3$. The $SiPc(OSiR_3)_2$ containing two axial siloxy ligands can be prepared from $SiPc(OH)_2$ by direct reaction with the selected siloxy compound.



207: $R_1 = OSi(CH3)2(CH_2)_3N(CH_3)_2$, $R_2 = CH_3$
208: $R_1 = OSi(CH_3)_2(CH_2)_3N(CH_3)_2$, $R_2 = OH$
209: $R_1 = OSi(CH_3)_2(CH_2)_3N(CH_3)_2{+}I^-$, $R_2 = OH$
210: $R_1 = R_2 = OSi(CH_3)_2CH_2)_3N(CH_3)_2{+}I^-$
211: $R_1 = OSi(CH_3)_2(CH_2)_3N(C_2H_5)(CH_2)_2N(CH_3)_2$, $R_2 = OH$
212: $R_1 = R_2\ OSi(CH_3)_2(CH_2)_3N(CH_3)_2$
213: $R_1 = R_2\ OSi(CH_3)_2(CH_2)_3N(C_2H_5)(CH_2)_2N(CH_3)_2$
214: $R_1 = R_2\ OSi(CH_3)_2C(CH_3)_2CH(CH_3)_2$

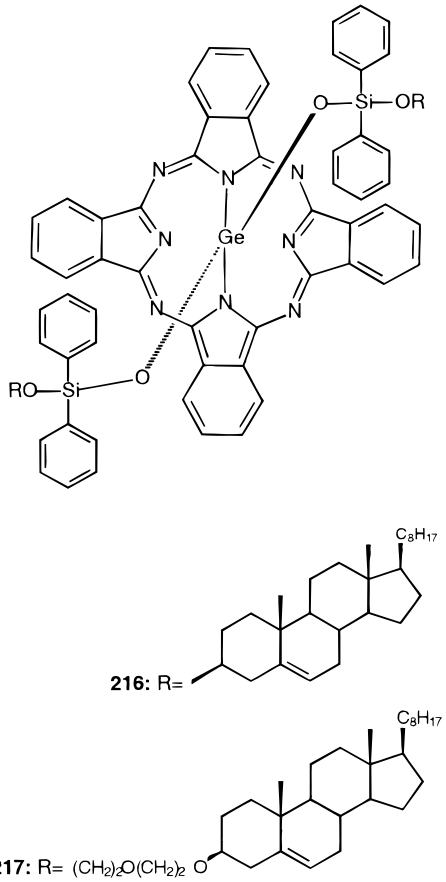
In air-saturated solution, the triplet lifetimes of axially substituted SiPc are in the range of 100−200 μs. All triplet states are quenched in the presence of oxygen and efficiencies to generate singlet oxygen vary from $\phi = 0.2$ to 0.5. They show good photodynamic activities against V79 Chinese hamster cells, erythrocyte ghosts, and liver microsomes in vitro and different mouse tumors in vivo.[269] The monosubstituted $HOSiPcOSi(CH_3)_2(CH_2)_3N(CH_3)_2$ (**208**, Pc-4) showed particularly promising activities and was retained for further evaluation. An in vitro structure active study compared Pc-4 (**208**) with three other SiPc having one or two aminosiloxy axial ligands.[270] The presence of a small axial ligand such as OH was found to be nonessential for efficient photosensitization. Second, the presence of two aminosiloxy ligands was found to provide at least as good photosensitization as one such ligand. In vivo studies on the RIF-1 tumor in mice indicate that both the mono- and diamine-ligated SiPc are at least as effective as Photofrin in PDT protocols.[271] Pc-4 (**208**) was also shown effective in killing HIV and other enveloped viruses as well as blood borne parasites, rendering this a candidate drug for the photosterilization of red blood cells and platelet concentrates.[272] Two types of SiPc, featuring axial cholesterol moieties either directly linked to the central silicon ion or via SiO-*o*-bridges, have also been reported.[273]

La Jolla Blue (**215**) is a commercially available SiPc dye that contains free carboxyl groups which allow linkage via a NHS ester to biomolecules.[274] The dye has absorption and fluorescence emission peaks in the near-infrared (680 and 705 nm, respectively) and does not aggregate in solution. The compound lacks nonspecific binding affinity for serum components, which is a desirable feature for diagnostic applications such as a probe for the labeling and detection of DNA.



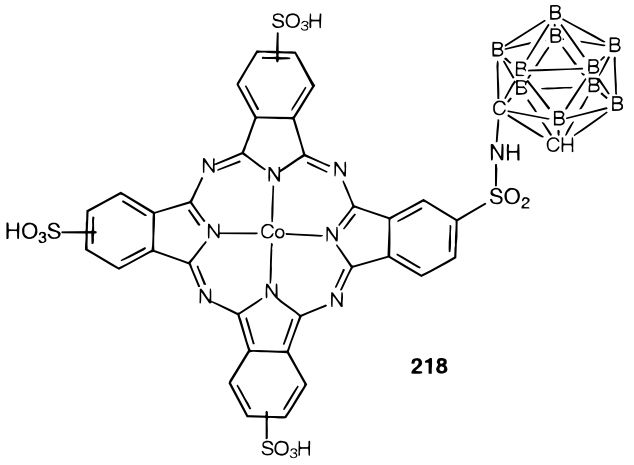
215

Several axially substituted GePc have also been prepared as possible photosensitizer for PDT.[275] Axial substituents includes cholesterol derivatives, which were selected to promote interaction with membrane structures. The dicholesteryl-substituted GePc (**216**) was prepared from $GePc(OH)_2$ which in turn was obtained from the dichloride derivative by hydrolysis in basic conditions using diphenylsilanediol as a spacer. Alternative spacers groups were used to



enhance the overall amphiphilicity of the complex (**217**). These complexes show a good quantum yield for singlet oxygen (ϕ 0.5) in organic solvent but require liposomal formulation for PDT applications.

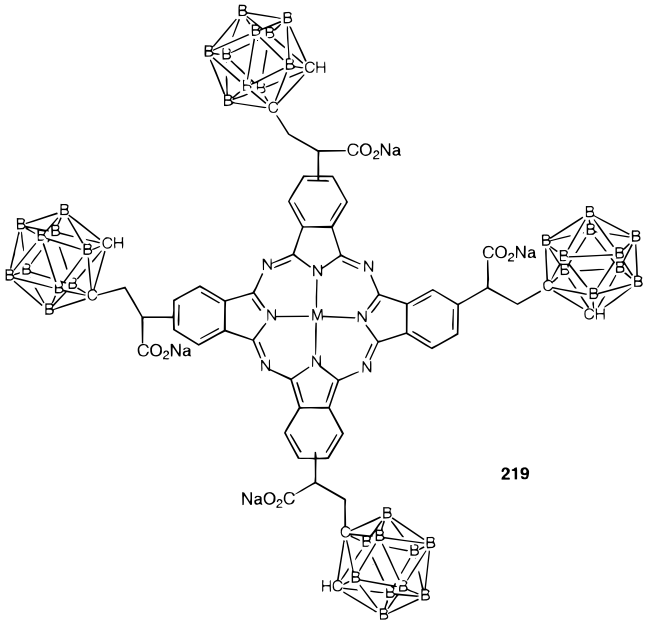
The potential tumor-localization properties of MPc provoked the synthesis of boronated Pc for BNCT. The first water-soluble mono-boronated-Pc complex **218** was synthesized by Soloway et al.[276] starting from the tetrasulfonated CoPc. Kahl and Li[277] sub-



218

sequently prepared a MPc with four *closo*-carborane cages covalently bound to the periphery of the Pc macrocycle.

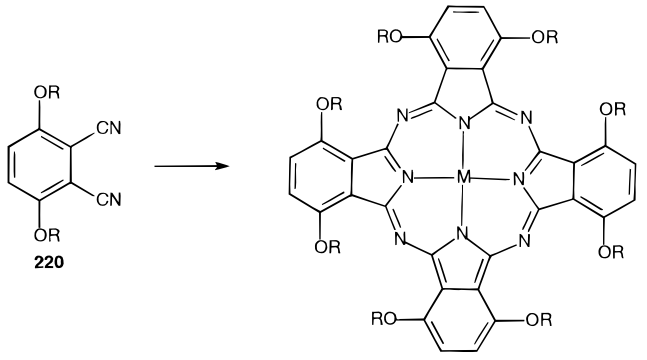
The starting material, dimethyl malonate, which is readily obtained from 4-nitrophthalonitrile, reacts with propargyl bromide to furnish propargyl phthalonitrile derivative in 83% yield. Coupling with borane was achieved by condensation of dodecarborane ($B_{10}H_4$) with the ethynyl group to give the carborane phthalonitrile derivative. Solid-state condensation of the latter with $Co(II)Cl_2$ was achieved by heating at 200 °C for 2 h to give the carborane CoPc (**219**) in 27% yield. Attempts to hydrolyze the



219

ester under mild or extreme acidic conditions failed; however, basic conditions ($NaOCH_3$ in absolute ethanol) were successful and gave the tetra-acid upon acidification. The highly water-soluble sodium salt was obtained by cation exchange. No degradation of the carborane cage to the *nido* open

Scheme 40



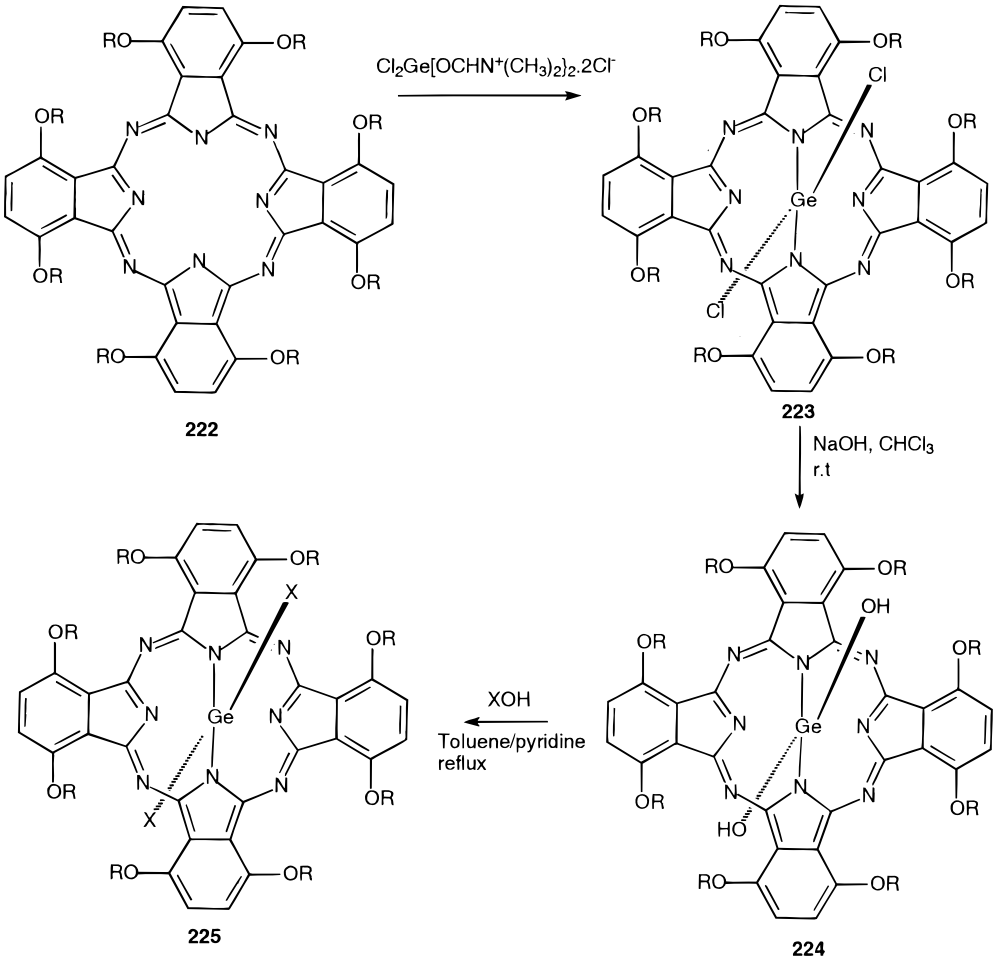
cage occurred under these conditions. The synthesis of other boronated Pc, whereby the boron cage is added to the preformed, derivatized Pc, has also been explored.

The MPc Q-band can be further red-shifted upon substitution of the benzene rings, particularly at the 3- and 6-positions. The lithium alkoxide-catalyzed cyclic tetramerization of 3,6-dialkoxy-4,5-dichlorophthalonitriles, 1,4-dialkoxyphthalonitriles (**220**), gave the corresponding metal-free octaalkoxy Pc derivatives which upon treatment with various metal ions yield the 3,6-substituted MPc (**221**), Scheme 40. Dissolved in toluene, these compounds show Q-band absorption maxima in the 710−770 nm range of the visible spectrum.[278]

A series of octasubstituted GePc featuring both axial and peripheral substituents have been reported.[279] The metal-free octaalkoxy Pc was metalated using a $GeCl_4$−DMF complex. Hydrolysis of the Cl atoms to give hydroxyl groups occurs in 1 N NaOH at room temperature. Further substitution of the hydroxyl groups by various substituted alcohols gave a series of octaalkoxy GePc (**225**), Scheme 41.

A series of isomerically pure 1,4,11,15,18,22,15-octaalkyl ZnPc **227** was obtained by condensation of the appropriate phthalonitrile with zinc acetate in the presence of DBU, Scheme 42.[279] All derivatives show absorption maxima around 703 nm and the fluorescence emission spectrum peak in cyclohexane

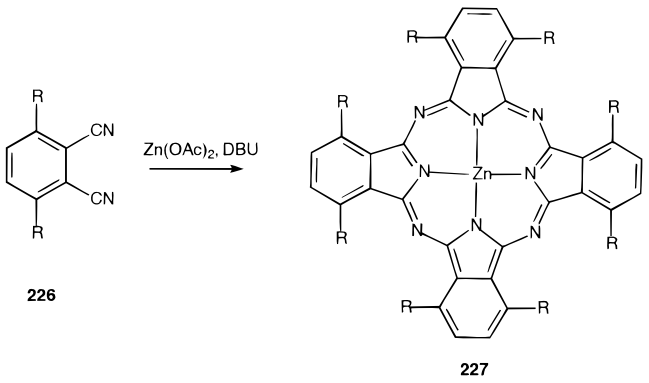
Scheme 41



R= $CH_2CH_2OCH_3$.

X= OCH_3, $OC_{16}H_{13}$, $SC_{12}H_{25}$, $OSiPh_3$, $OSiMe_2Tex$, $OC_2H_4OC_2H_4OBu$, $OC_2H_4OC_2H_4OAc$, 2-ONaphthyl, 2-OCH_2Naphthyl, $OSiPh_2O$-cholesterol

Scheme 42



a: R = methyl, **b:** R = ethyl, **c:** R = butyl, **d:** R= pentyl, **e:** R = hexyl, **f:** R = heptyl, **g:** R = octyl, **h:** R = nonyl, **i:** R = decyl

around 710 nm. These derivatives are sensitive to photooxidation leading to the formation of 3,6-bis-decylphthalimide, a process which is inhibited by singlet-oxygen quenchers. One of the analogues, **227i** formulated in cremophor, induced a photodyanamic response in tumor-bearing Balb/c mice involving both random cell death and apoptosis.

2. Cationic Phthalocyanines as Photosensitizers

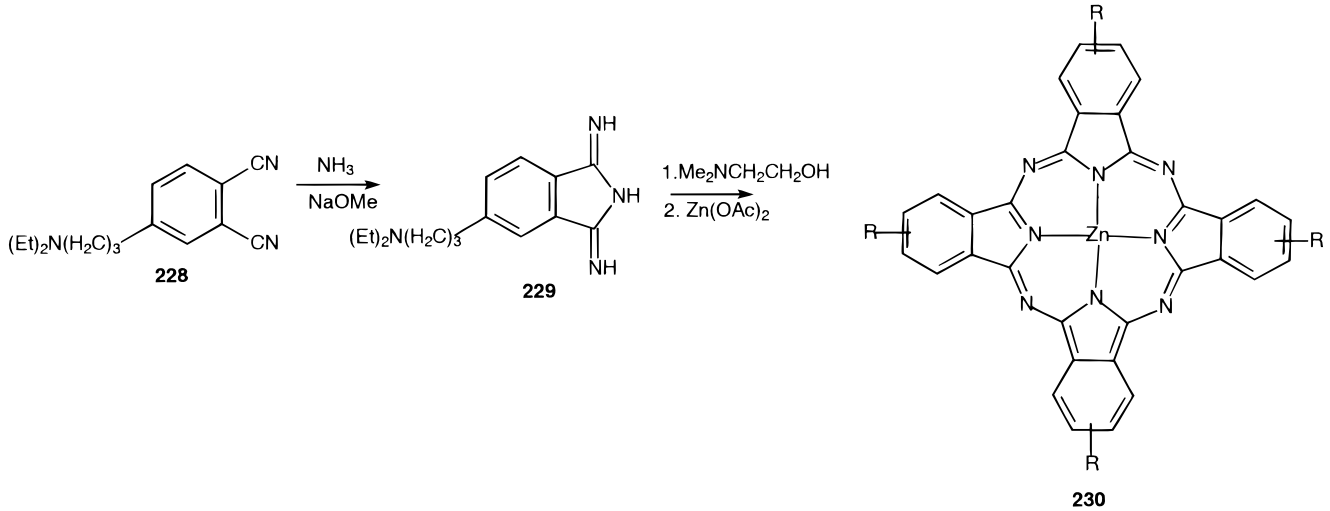
Positive charged dyes are believed to localize in mitichondria, and several positively charged MPc were prepared as potential photodynamic agents. Synthesis of Pc **230**, featuring four tertiary amine substituents, involves the following steps.[281] The 4-iodophthalonitrile is reacted with 3-*N*,*N*-diethyl-aminoprop-1-yne in the presence of bis(triphenylphos-phine)Pd(II)Cl_2 and Cu(I)iodo followed by catalytic hydrogenation to yield 4-(3-*N*,*N*-diethylaminopropyl)-phthalonitrile (**228**). Treatment of the later with NH_3 and $NaOCH_3$ gave 5-(3-*N*,*N*-diethylaminopropyl)-1,3-diiminoisoindoline (**229**), which upon heating under-went self-condensation to give the dark blue 2,9,16,-23-tetra(3-*N*,*N*-diethylaminopropyl)Pc in 28% yield. Heating of the metal-free Pc with anhydrous Zn-$(OAc)_2$ in toluene/2-methoxyethanol (1:1) gave 2,9,-16,23-tetra(3-*N*,*N*-diethylaminopropyl)ZnPc (**230a**), Scheme 43. Quaternization of the four amino groups occurred on treatment with excess iodomethane in toluene at room temperature to give 2,9,16,23-tetra-(3-*N*,*N*-diethylaminopropyl)ZnPc tetraiodide **230b** in 97%. The photodynamic activity against V-79 cells in vitro showed that cationic ZnPc **230b** was less photoactive than its neutral counterpart **230a**.

In another approach to obtain a positive-charge Pc, 4-NO_2-phthalonitrile was reacted with 2-dimethyl-aminoethanol to yield 4-(2-dimethylaminoethoxy)-phthalonitrile (**231**), which after cyclotetramerization with or without $Zn(OAc)_2$ gave tetrasubstituted Pc **232** in quantitative yield, Scheme 44.[282]

Quaternized pyridinoporphyrazines (**233**) carry their positive charges on the macrocycle. Their synthesis involves the reaction of 2,3-dicyanopyridine in *N*,*N*-diaminoethanol in the absence or presence of a metal salt (M = Cd, Zn, Cu) and ammonia. Preparation of the Mg, Al(OH), and Ga(OH) metal complexes requires high temperatures and was con-ducted in sealed ampules. Quaternization of this compound to yield tetra-2,3-pyridinoporphyrazines **233** can be achieved by alkylation with dimethyl sulfate in DMF.[283]

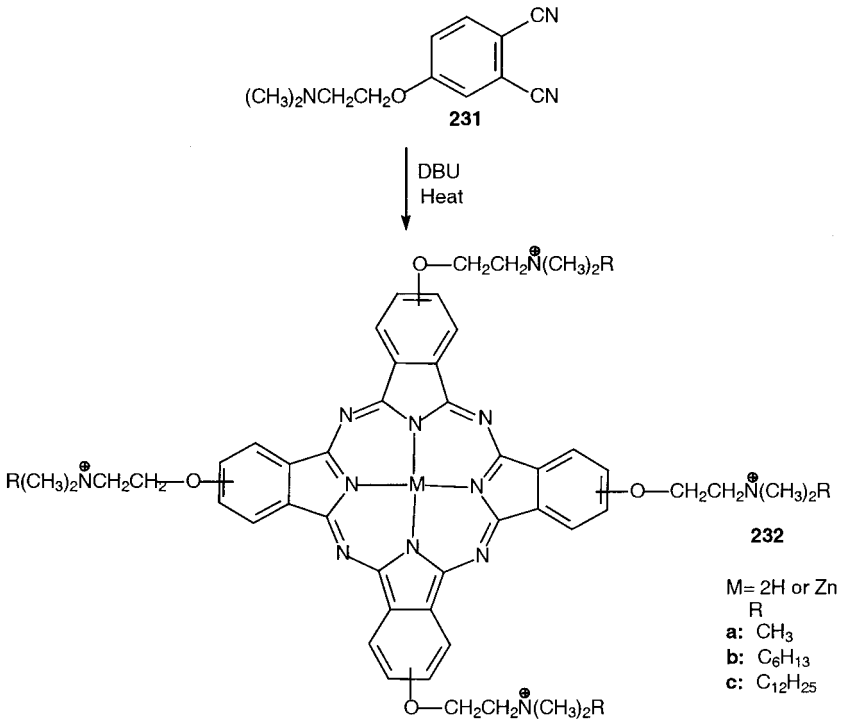
A similar reaction of 3,4-dicyanopyridine in *N*,*N*-diaminoethanol with metal salt and ammonia gave tetra-3,4-pyridineporphyrazines, which likewise were quaternized by refluxing in dimethyl sulfate. In vitro testing on V-79 cells showed that the quaternized metallopyridinoporphyrazines induce substantial dark toxicity and that light exposure gave little addition effect. The interaction of the AlCl complex of **233** with DNA was investigated by absorption/emission and circular-dichroic spectroscopy. The data suggest that the porphyrazine molecule binds to the outside of the

Scheme 43

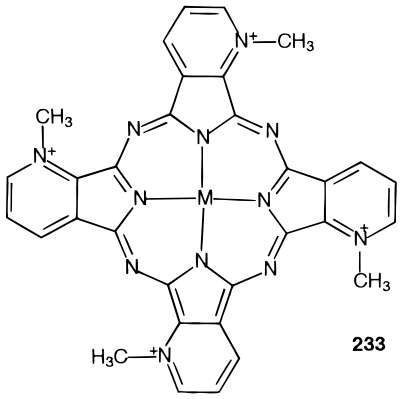


R
a: $(ET)_2N(CH_2)_3$,
b: $Me(Et)_2N^+(CH_3CH_3I^-$,
c: $Me(Et)_2N^+(CH_2)_3Cl^-$

Scheme 44



DNA helix, involving interactions which are not limited to electrostatic forces only.[284]



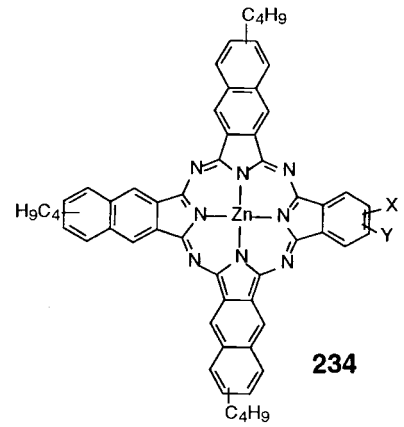
A series of positively charged Zn(II) naphthobenzoporphyrazines (Zn−NBP), bearing between one and eight positively charged methylpyridinium substituents, were also prepared.[285] These unsymmetrical complexes were synthesized by statistical tetramerization of two differently substituted dicarbodinitriles, i.e., of 6-(1,1-dimethylethyl)-2,3-naphthalenedicarbonitrile, with 4-(3-pyridyloxy)- or 4,5-bis(3-pyridyloxy)-1,2-benzenedicarbonitrile in *n*-pentanol under reflux in the presence of lithium pentalonate. After purifying the metal-free compounds, **234**−**238** were converted to the Zn(II) complex with $Zn(OAc)_2$ in DMF, Chart 8. With the exception of the adjacent and opposite derivatives, all five possible derivatives are easily separated using flash chromatography on silica gel. Varying the molar ratios of the reactants permitted modulation of the relative yield of the products. N-Alkylation with an excess of methyl iodide gave the positively charged porphyrazines. The alkylated products are soluble in organic solvents such as DMF and DMSO. Water solubility increases with the number of alkylated pyridyloxybenzo units. All complexes exhibit characteristic absorptions in the Soret- and Q-band region. A bathochromic shift in the Q-band from 680 to 760 nm is observed in polar organic solvents with increasing numbers of linear annelated naphthalene rings. The trinaphthobenzo compound **234** and the naphthotribenzo **238** compounds show a split in the Q-band due to the lower symmetry of the aromatic macrocycle. The dinaphthodibenzo compound exhibit three absorption maxima in the Q-band region. Their capacity to produce singlet oxygen is similar to that of ZnPc, but their photostability decreases with the number of annelated naphthalene rings.

3. Radiolabeled Phthalocyanines as Imaging Agents

Like porphyrins, selected Pc derivatives exhibit tumor retention in animal models, which led to their evaluation as carriers of clinically accepted radioisotopes such and ^{99m}Tc. Direct labeling of sulfonated Pc with ^{111}In did not give stable complexes. Therefore, a series of mono-, di-, and tetra-DTPA-substituted ZnPc derivatives (**239**) were prepared and complexed with ^{111}In. These negatively charged complexes (**240**) revealed significant liver and spleen retention in an animal model, but tumor uptake was marginal, Chart 9.[286]

Direct labeling of Pc derivatives with various oxidized ^{99m}Tc and ^{99}Tc likewise was not successful. Instead, these complexes can be prepared in situ via the condensation at 200 °C of 4-sulfophthalic acid in the presence of pertechnetate (TcO_4^-), urea, catalyst, and reducing agent. The reduced technetium, likely TcO_2, forms a 1:1 complex with the three imidazole nitrogens of the PcS_4 macrocycle to yield an out-of-plane Tc complex. A second radioactive Pc complex formed during the condensation likely represents a sandwich-type $^{99}Tc(PcS_4)_2$ dimer. Comparative scin-

Chart 8



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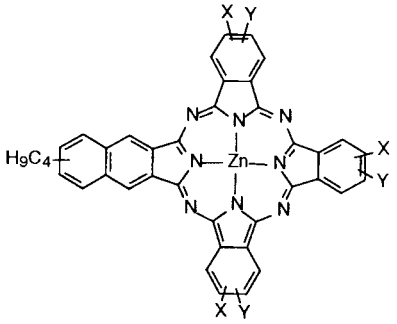

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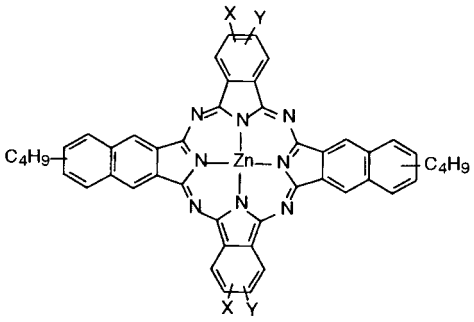

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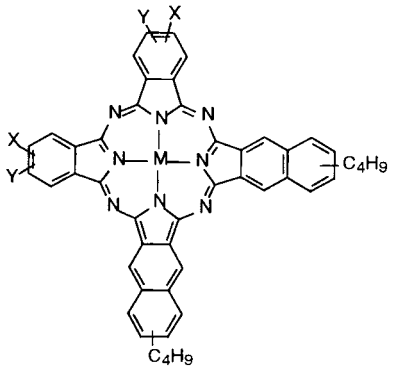

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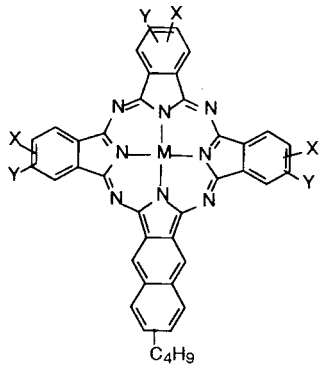
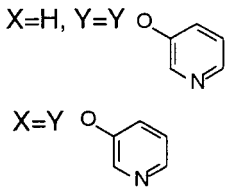
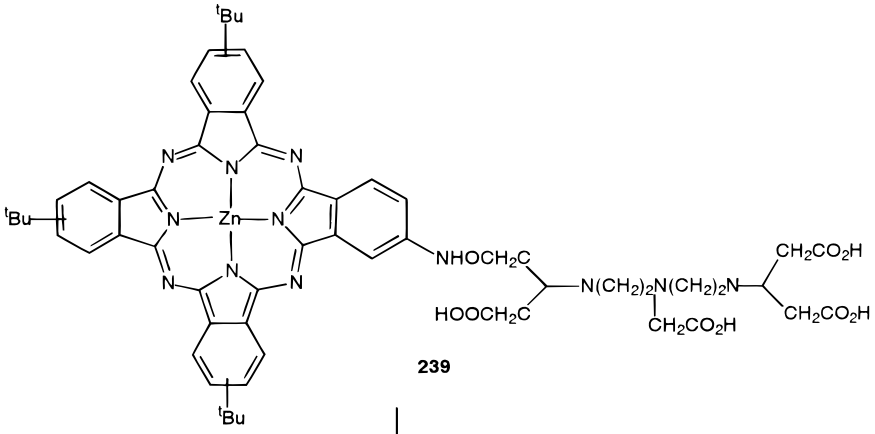




238

Chart 9

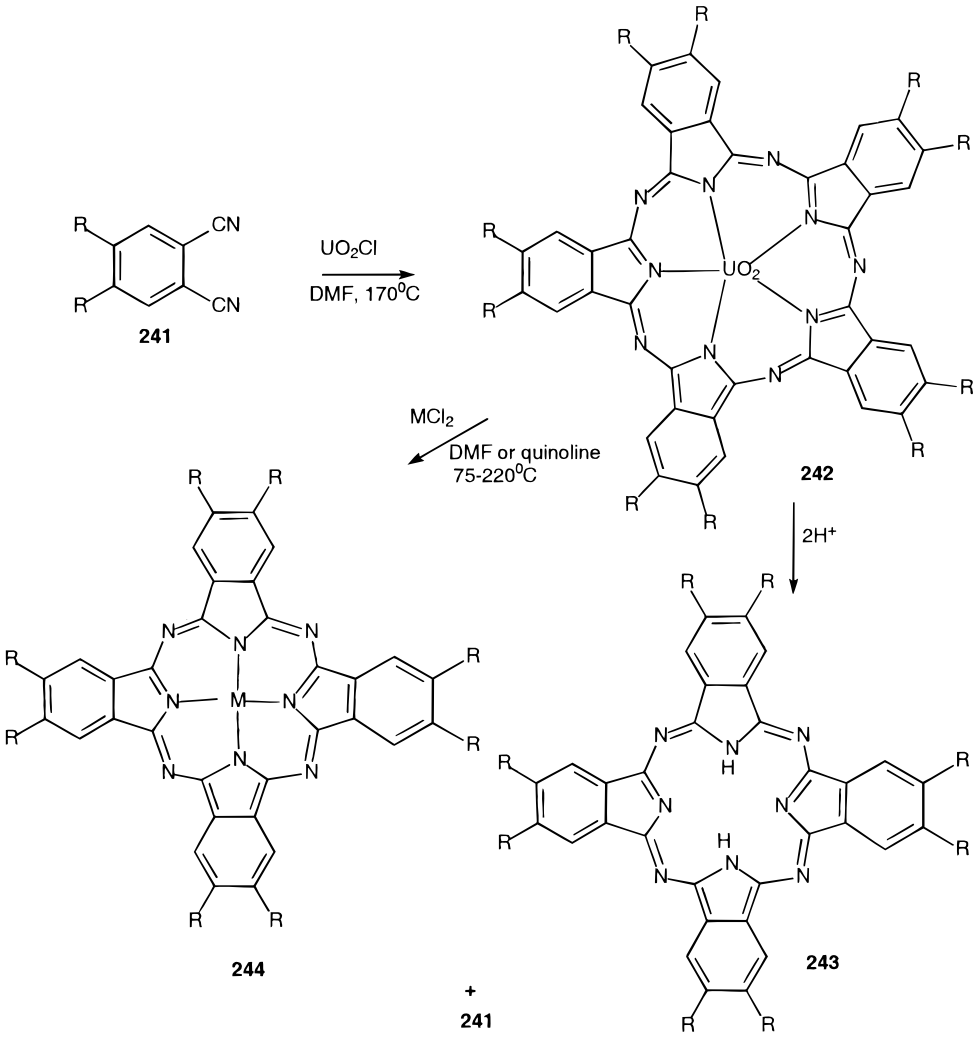


239

$ZnPcR_3$-DTPA-^{111}In

240

Scheme 45



tigraphic studies of the $^{99m}Tc-PcS_4$ complexes and free $^{99m}TcO_4^-$ provided for a rapid evaluation of the in vivo stability of the complex.[287]

The metal-free, water-soluble H_2PcS can be labeled directly with $^{67}Ga^{3+}$ ($t_{1/2}$ = 3.2 days; γ = 93 keV) in low yield (10−13%). Higher yields can be obtained by the in situ synthesis of PcS via the condensation of 4-sulfophthalic acid with $^{67}GaCl_3$.[288] To study the effect of lipophilic substituents on the in vivo behavior, ^{67}GaPcRS derivatives featuring both long aliphatic chains (R) and sulfonate groups (S) were prepared via the condensation of 4-octadecyloxyphthalonitrile with $^{67}GaCl_3$. Subsequent treatment of the ^{67}Ga−4-tetraoctadecyloxy−Pc with oleum yielded a mixture of sulfonated analogues, which gave good ^{67}Ga tumor uptake in an animal model.[289]

Several ZnPc derivatives were radiolabeled in order to establish structure−activity relationship in regard to cell uptake and phototoxicity. The highly lipophilic [^{125}I]ZnPcI$_4$ was prepared from 4-aminophthalonitrile, which was converted to [^{125}I]iodophthalonitrile by the diazotization method and subsequently condensed in the presence of zinc acetate. Water-soluble $ZnPcS_{1-4}$ were labeled with ^{65}Zn ($t_{1/2}$ = 34.9 weeks; γ = 112 MeV) via the direct insertion of ^{65}Zn in the corresponding metal-free H_2PcS.[290]

The water-insoluble uranyl Pc was prepared from an anhydrous uranyl complex salt. These salts are made by reacting an inorganic uranyl salt with a Lewis base-type nitrogen- or sulfur-containing organic compound, e.g., DMF or DMSO.[291]

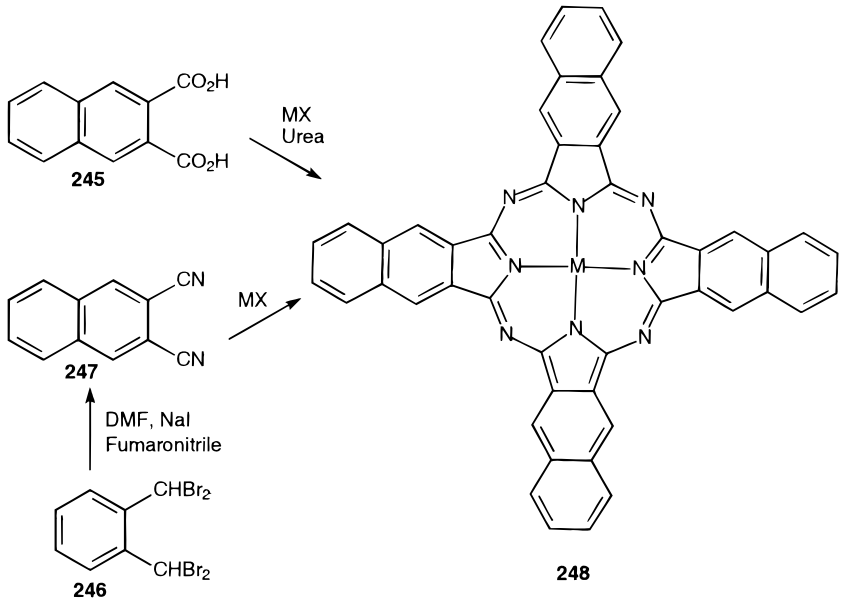
$$UO_2(NO_3)_2 \cdot 6H_2O + 2DMF \rightarrow UO_2(NO_3)_2 \cdot 2DMF + 6H_2O$$

The uranyl complex is mixed with a stoichiometric quantity of Li_2Pc at room temperature, under anhydrous conditions, resulting in substitution of the 2Li by UO_2.

$$Li_2Pc + UO_2(NO_3) \cdot 2DMF \rightarrow UO_2Pc + 2LiNO_3 + 2DMF$$

The analogous water-soluble Pc was prepared by heating a mixture of an inorganic uranyl salt and a sulfonated LiPc in water. The monouranyl Pc or its sulfonated derivatives containing fissionable uranium isotopes are chemically nontoxic since the uranyl ion is strongly bonded in the Pc cavity and does not dissociate from the complex under in vivo conditions. The sulfonated $^{235}UO_2PcS$ was retained in mouse brain tumors at 50:1 ratios, as compared

Scheme 46



to surrounding healthy brain tissue. Activation of the ^{235}U by a beam of slow neutrons provides the potential of local radiation therapy resulting from ^{253}U fission products.

The reaction of *O*-dicyanobenzene (**241**) with anhydrous uranyl chloride does not yield a cyclic, four-subunit Pc complex. Rather, it yields an expanded, cyclic five-subunit pentakis(2-iminoisoindoline) complex—a "superphthalocyanine" (SPc) (**242**), Scheme 45.[291] Such conjugated nitrogenous macrocycles with 22 π-electron ($4n + 2$ aromatic) inner rings are otherwise not readily accessible. Their formation is, however, evident in view of the general tendency of the uranyl ion to form a pentagonal bipyramidal or hexagonal bipyramidal coordination geometry with a long U−N bond length (2.5−2.6 Å vs 1.85−2.05 Å in Pc). X-ray diffraction studies confirm a pentagonal bipyramidal coordination geometry about the uranium atom with only 0.20 Å average deviation for each of the five coordinated N atoms from their average bond length. The macrocycle is severely and irregularly buckled, which is thought to arise from steric strain inherent to the inner ring of 20 atoms coordinated around the uranyl atom. The electronic spectra of the SPc generally consist of an intense red-shifted band at 914 nm (compared to ZnPc at 680 nm) with a shoulder at 810 nm and a second intense band at 420 nm, which are analogous to the Q- and Soret-bands observed in the electronic spectra of metalloporphyrins and Pc. The use of substituted phthalonitrile in the uranyl-centered condensation reaction was also investigated as a means to prepare the more soluble pentamethyl, decamethyl, and deca-*n*-butyl $SPcUO_2$.[292] However, substitution of 1,2-dicyanobenzene with electron-withdrawing groups (3,4,5,6-tetrachloro or 3,4,5,6-tetrafluoro) or steric bulk (3,6-dimethoxy) prohibit formation of the SPc macrocycle.

The severe strain within the SPc macrocycle is also evident from other chemical and physical properties. The reaction of $SPcUO_2$ with acids, using conditions which readily demetalate MPc and porphyrin coordination complexes, leads to an unusual contraction reaction. Attempts to replace the uranyl ion in $SPcUO_2$ with Cu^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}, or Fe^{3+} results in the formation of the corresponding four-subunit metal complex (**244**).[293] The larger Sn^{2+} and Pb^{2+} ions also induce ring contraction. This tendency of the SPc ligand to undergo contraction to the four-subunit Pc suggests that the uranyl ion plays a significant role in stabilizing the expanded macrocycle. ^1H NMR data indicate that in solution, as in the solid state, the SPc macrocycle is highly distorted from planarity. This results in a decreased shielding of the benzo protons in the SPc, as compared to the Pc, and the apparent impairment of the π-electron system.

To study the biodistribution and tumor uptake of PcS_n as function of the degree of sulfonation, ^{14}C-labeled $GaPcS_2$ and S_3 were prepared.[294] Synthesis of $[^{14}C]GaPcS_n$ via condensation of $[^{14}C]$phthalic acid and 4-sulfophthalic acid in the presence of $GaCl_3$ followed by chromatography gave a sufficient amount of the di- and trisulfo derivatives to conduct biodistribution studies in tumor bearing mice. The highest tumor uptake and tumor-to-blood ratios were observed with the trisulfonated $[^{14}C]GaPcS_3$.

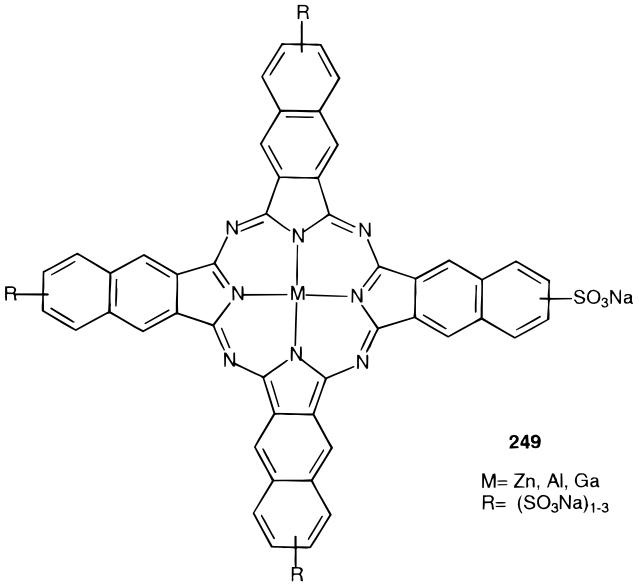
G. Naphthalocyanines

Further conjugation of the Pc macrocycle with a benzo ring on each of the isoindole subunits gives the naphthalocyanine macrocycle (NPc, **248**) with strong red-shifted Q-band absorption maxima around 740−780 nm, Scheme 46. NPc are dark green crystalline compounds with a blue to purple luster. They do not easily sublimate and are usually purified by recrystallization from high-boiling solvents. Two major isomeric classes can be identified, 1,2-NPc and 2,3-NPc.[295] 1,2-NPc consist of several isomers, which are difficult to separate and are formed in varying proportions during their preparation, depending on the orientation of the naphthalene. Most reported work, however, deals with 2,3-NPc, which exists only in one possible configuration.[296] 2,3-NPc are less stable than Pc; they readily decompose in the pres-

ence of light and oxygen. NPc are of considerable interest due to the extension of the π-electron system, which as compared to Pc results in a 80−100 nm red-shift of the Q-band and affects electronic properties such as redox potential, electrical conductivity, photoconductivity, and catalytic activity. NPc have been studied for applications in electrochemistry, Q-switch dyes in lasers, optical recording media, and more recently as PS for PDT. Several derivatives featuring different central metal ions and macrocycle substituents were developed to modulate absorbance properties or to increase their solubility in aqueous or organic solvents. A typical MNPc (**248**) synthesis involves heating naphthalene-2,3-dicarboxylic acid (**245**) with urea and metal salt.[296] Alternatively, **248** can be prepared from 2,3-dicyanonaphthalene and a metal salt.[297] The dicyano **247** can be obtained from the reaction of α,α,α′,α′-tetrabromoxylene with fumaronitrile in DMF-containing NaI. Sulfonation by treatment of the MNPc with oleum proceeds at a lower temperature than that required for the corresponding MPc.

MNPc, which lack axial ligands, tend to form H-aggregates in solution with concurrent loss of photodynamic properties.[297] The synthesis of several octaalkoxy and octaalkyl NPc substituted symmetrically about the macrocycle have been reported.[299−301] Substitution closest to the point of fusion to the porphyrazine ring causes the largest bathochromic shifts (λ_{max} 810−870 nm), and furthermore, alkoxy groups were shown to bring about a larger spectral shift than alkyl groups.[301] SiNPc featuring two axial substituents together with periphery ring substituents display a characteristic J-type molecular arrangement in solid films and typical monomeric spectra in solution.[302]

Differently sulfonated Zn− and Al−NPc (**249**), prepared by treatment of MNPc in oleum, were studied for their photodynamic properties.[205,303,304]

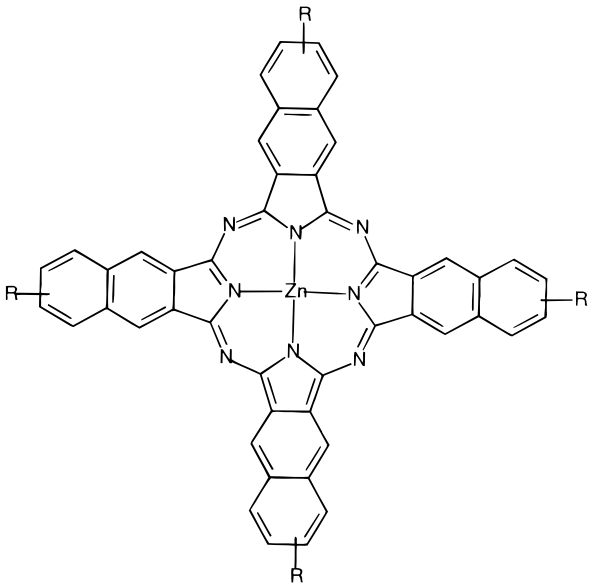


249

M= Zn, Al, Ga
R= $(SO_3Na)_{1-3}$

Their capacity to generate a singlet is 1.6−3 times less as that reported for the analogous sulfonated MPc. The phototoxicity and cell uptake was studied in vitro with V-79 and NHIK 3025 cells. Whereas all underlying parameters leading to photosensitized cell killing were found to be similar for both sulfonated AlPc and AlNPc (i.e., cell uptake and capacity to generate $^1O_2$), the AlNPc was found to be photoinactive.[303] Among the ZnNPc only the least sulfonated derivative produced some phototoxic effects whereas the lack of activity of the higher sulfonated derivatives was attributed to intracellular aggregation of the dye.[304]

To examine the effect of side-chain substituents on the PDT efficacy of the parent ZnNPc, a series of different amido substituents ZnNPc bearing was prepared.[305−307] The 6-amino-2,3-dicyanonaphthalene was acetylated with various acid chlorides to yield a series of eight different amido derivatives in 50−60% yield. Heating with $Zn(OAc)_2$ in sealed glass tubes gave the corresponding 2,11,20,29-tetraamido-substituted Zn−NPc (**250**) in 20−25% yield. All aryl- or alkyl-substituted ZnNPc are sensitive to photooxidation; they are soluble in various organic solvents and exhibit a Q-band transition in DMF at 770 nm. The quantum yield for $^1O_2$ varies from 0.3 to 0.36 and is about 1.4 times lower than that observed for the corresponding ZnPc. The ZnNPc were incorporated in DPPC liposomes and administered to mice bearing the Lewis lung carcinoma. The best tumor response following light treatment was observed with the tetrabenzamido derivative (**250d**) followed by the unsubstituted ZnNPc and the tetraacetylamido derivative (**250a**). Action mechanisms appeared to



250

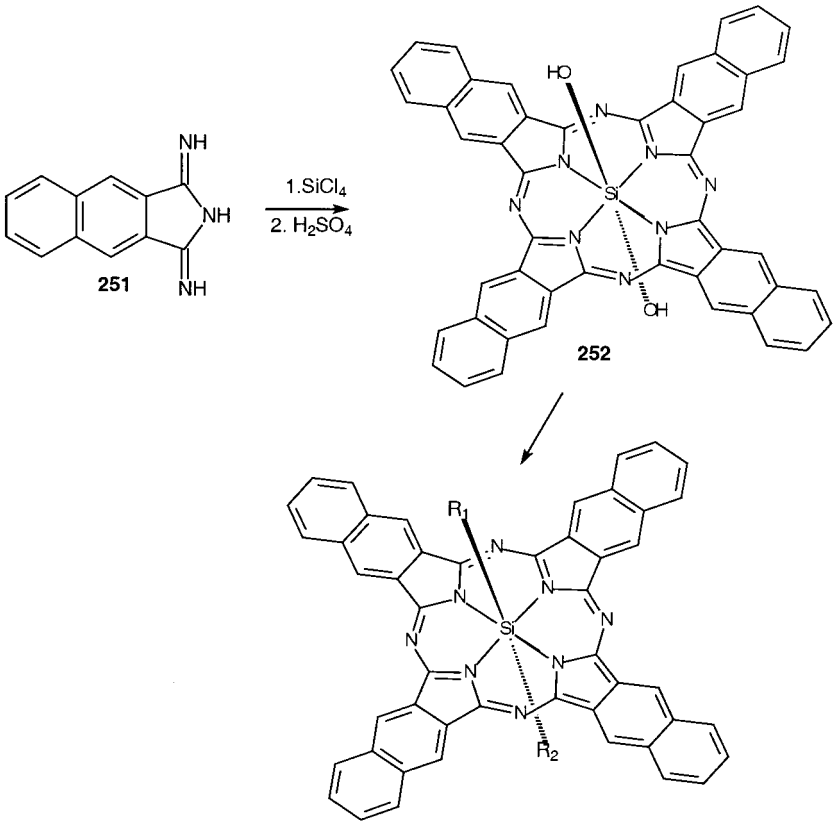
R

a: $NHCOCH_3$
b: NH_2
c: OCH_3
d: $NHCOC_6H_5$
e: $NHCOC_6H_4OCH_3$
f: $NHCOC_5H_{11}$
g: $NHCOC_{11}H_{23}$

involve direct effects on the neoplastic cells rather than vascular damage.[307]

For biological application, axial substitution of the central metal ion is particularly interesting since this

Scheme 47



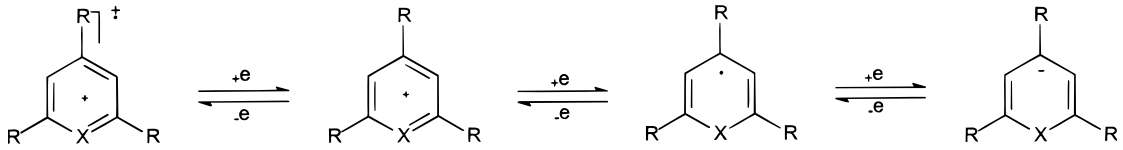
253: R_1= R_2 $OSi(CH_3)_2C(CH_3)_3$
254: R_1= R_2 $OSi(CH_3)_2C(CH_3)_2CH(CH_3)_2$
255: R_1 = R_2 $OSi(C_6H_{13})_3$
256: R_1= R_2 $OSi(CH_3)_2C_{18}H_{37}$
257: R_1 = R_2 $OSi(i\text{-}C_4H_9)_2n\text{-}C_{18}H_{37}$
258: R_1= $OSi(CH_2)_2(CH_2)_3N(CH_3)_2$ R_2 = $OSi(n\text{-}C_{10}H_{22})_3$
259: R_1= R_2 $OCH_2CH_2OCH_3$

provides complexes that remain monomeric in solution.[308] In particular, Si(IV) complexes of NPc have been studied in detail as PS for biomedical applications.[309] Similarly, NPc complexes with Ge(IV) as the central ion also show strong, monomeric absorption peaks in the near-infrared (779 nm).[311] SiNPc with only one substituent tend to form aggregates in solution, whereas SiNPc with two axial ligands bound to the central metal ion show monomeric properties. The photoinduced interactions of a series of siloxanes $(R_3SiO)_2$SiNPc with various quenchers in dichloromethane[310] showed that the dye can act both as electron-donating and -accepting sensitizers, depending on the electron affinity of the quencher. In addition to their diminished tendencies to aggregate, axially substituted SiNPc also exhibit greater stability toward photooxidation as compared to Al− and Zn−NPc. Combined, these features likely contribute to their good photodynamic properties.

A key intermediate in the synthesis of substituted SiNPc is the dichloro derivative $SiNPcCl_2$, prepared from the reaction of $SiCl_4$ and 1,3-diiminobenz[*f*]-isoindole.[308,309] Further ligand exchange of $SiNPcCl_2$ with NaO-*n*-C_8H_{17} yields SiNPc(O-*n*-$C_8H_{17})_2$. SiNPc-(O-*n*-$C_8H_{17})_2$ is an attractive intermediate for further axial substitution due to its high solubility in organic solvents, permitting ligand exchange in a homogeneous solution under mild conditions.

Among the disubstituted SiNPc, SiNPc[OSi(i-Bu)$_2$-*n*-$C_{18}H_{37}]_2$ or *iso*BOSiNc (**257**) has been extensively studied as a PS for PDT.[312,313] The compound can be prepared by ligand exchange of the HOSi(i-Bu)$_2$-*n*-$C_{18}H_{37}$ with the axial ligands of the intermediate SiNPc(O-*n*-$C_8H_{17})_2$.[309] Administered (*i.v.*) as a DPPC liposomal preparation to Balb/c mice bearing the MS-2 fibrosarcoma, the dye redistributes over the serum lipoprotein fraction and reaches optimal tumor concentrations 24 h postinjection. Tumor necrosis following PDT is extensive and linearly depending on the fluence rate.[314] *iso*BOSiNc (**257**) was also shown to be effective against the B16 pigmented melanoma transplanted in mice, although no selectivity in tumor vs skin uptake was observed.[315] The use of such far-red-absorbing PS is particularly promising for PDT of highly pigmented tumors since absorption of excitation light is not limited by melanin absorption. Recently, the photodynamic activity of *iso*BOSiNc (**257**) was compared to that of an unsymmetric analogue, SiNPc[OSi(*n*-$C_{10}H_{21})_3$][OSi-$(CH_3)_2N(CH_3)_2$], or DAP−SiNc (**258**) in two cell variants of B_{16} melanoma.[316] Upon excitation with 776 nm diode laser light, DAP−SiNc (**258**) appeared to

Scheme 48



260: X= O, S, Se, Te

be a markedly more efficient photosensitizer than *iso*BOSiNc, likely reflecting higher cell uptake and less intracellular aggregation. Highly pigmented melatonic B16F1 cells were, however, less sensitive to the photoinactivating action of the dye as compared to the amelatonic B78H1 clone, probably due to the protective action of melanin through optical filtering as well as the shorter triplet lifetime of the dye in the B16F1 cellular environment. SiNPc substituted with two methoxyethyleneglycol axial ligands (**259**) induced only a minor PDT response in B16 pigmented melanoma in mice reflecting poor localization of the dye in the tumor.[317] Uptake of **259** by Lewis lung carcinoma in mice was almost 5-fold higher and resulted in efficient tumor response to PDT.

In another study a series of SiNPc substituted with different axial ligands, including bis(*tert*-butyldimethylsiloxy) (**253**), bis(dimethylthexylsiloxy) (**254**), bis(tri-*n*-hexylsiloxy) (**255**), and bis(dimethyloctadecylsiloxy) (**256**), prepared via substitution of the $SiNPc(OH)_2$ precursor with the corresponding chlorosilane ligands, were compared for their photodynamic properties against the EMT-6 tumor in Balb/c mice.[318] All four dyes showed limited phototoxicity in vitro, combined with substantial dark toxicity. Surprisingly, in vivo all four dyes showed excellent photodynamic activity, particularly the bis(dimethylthexylsiloxy) derivatives (**254**).[319]

The photophysical properties of bis(tri-*n*-hexylsiloxy)SiNPc (**259**) revealed that energy levels of the SiNPc T_1 state and $^1O_2$ are sufficiently close to permit reversible energy transfer between the excited- and ground-state species. However, the long natural lifetime of the T_1 state (331 μs, in benzene) ensures effective singlet oxygen population in aerated systems.[319]

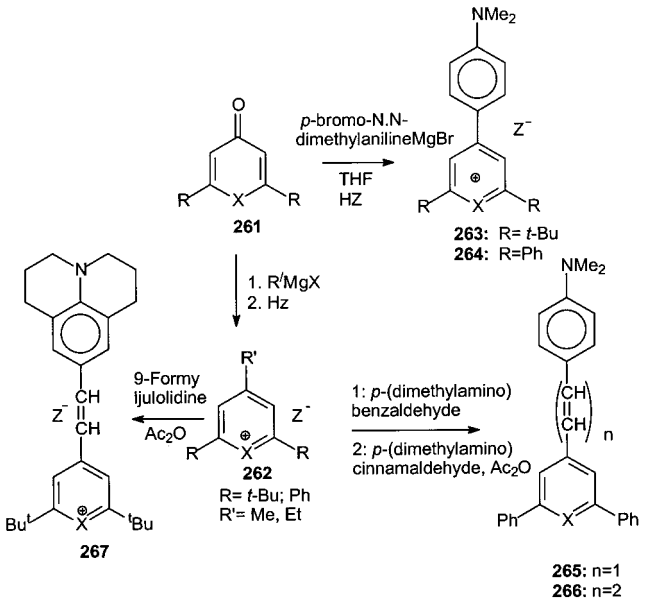
H. Miscellaneous Photosensitizers

1. Chalcogenapyrylium Dyes

Chalcogenapyrylium (CP) dyes are structurally related to rhodamine-123 (Rh-123). It has been noted for a long time that the positively charged rhodamine dyes are selectively taken up by tumor cells; however, their application for PDT is limited due to their weak absorption above 600 nm and poor quantum yield for singlet oxygen formation.[321] In contrast, the λ_{max} of water-soluble CP dyes can be modulated over 200 nm by varying the chalcogen atom to well above 800 nm. The parent chalcogen structure (**260**) features either an oxygen, sulfur, selenium, or tellurium atom. The chalcogen atom X is particularly important for the reactivity of the complex since it can stabilize an adjacent carbon ion or carbon radical center. The latter also affect the electronic spectrum and redox potential of the heterocyclic nucleus. The molecular structure of **260** allows for various resonance structures including cation/dication, cation/radical, and radical/anion[322] (Scheme 48).

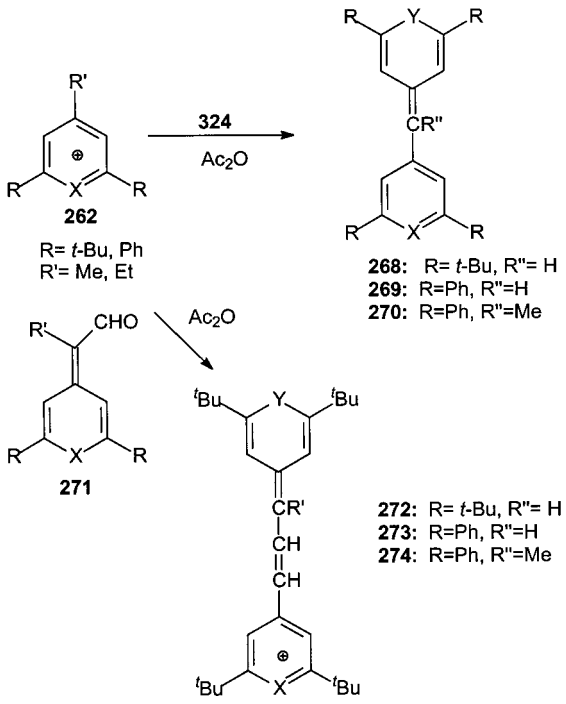
Incorporating various heteroatoms in the ring structure of methine and polymethine dyes, i.e., pyrylium, thiapyrylium, and selenopyrylium nuclei, induces a large bathochromic shift. Synthesis of methine dyes involves the following reaction sequence. Telluropyrone **261** (R = Ph) is converted to the pyrylium salt **262** (R = Ph) in four steps. Condensation of **262** with telluropyrone **260** and aldehyde in acetic anhydride gives mono- and trimethine dyes.[322,323] Additional derivates **263**, **264** have been prepared by reacting **261** with the Grignard reagent of *p*-bromo-*N*,*N*-dimethylaniline, followed by dehydration of the alcohol. The styryl dyes **265** and **266** were prepared by the condensation in Ac_2O of **262** with *p*-(dimethylamino)benzaldehyde or *p*-(dimethylamino)cinnamaldehyde, respectively. Similarly, **262a** reacts with 9-formylijulolidine in Ac_2O and compound **267** was obtained (Scheme 49). Con-

Scheme 49

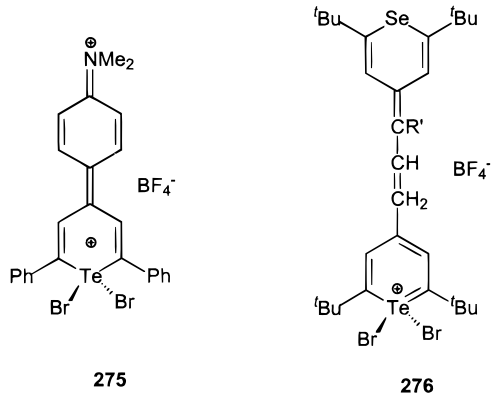


straining the alkyl groups of the *N*,*N*-dialkylanilino substituent shifts the absorption maximum to longer wavelengths. This spectral shift is attributed to the reinforced conjugation and steric shielding of the lone pair of electrons of the nitrogen atom with the aromatic ring. Heating of **262** with an appropriate chalcogenapyrone or aldehyde gave bischalgogen derivatives such as **268**−**274**, Scheme 50.

Scheme 50



Substitution of the O atom in CP dyes for heavier chalcogens induces sequential bathochromic shifts of the absorption maxima in the following order O < S < Se < Te.[327] This shift empirically correlates with an increase in the electropositive character of the heteroatom. The nature of the counterion does not affect the absorption spectra of the dyes. Phenyl vs *t*-Bu substituents at the 2- and 6-positions show the effect of conjugative interaction with the chalcogenapyrylium ring, whereas methyl substituents in the methine backbone, i.e., **276** vs unsubstituted analogues of **275**, reveal steric and electronic effects on the ring structure. Organotellurium compounds fea-

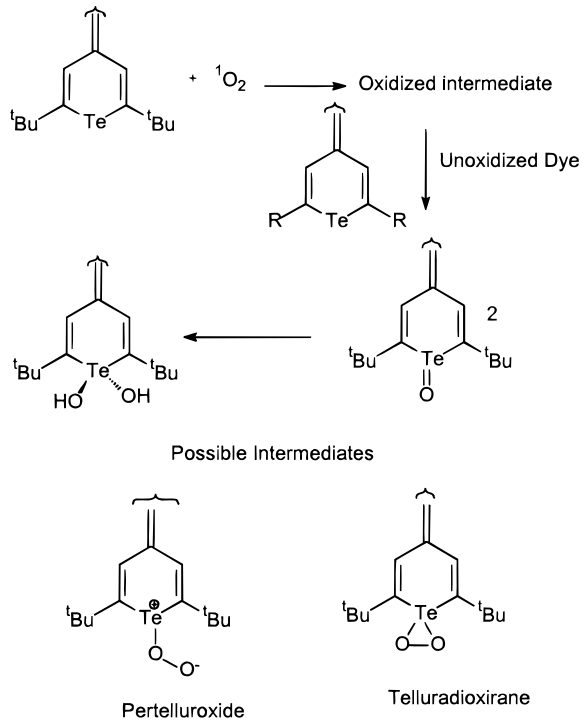


turing the Te ion in a lower oxidation state easily exchange bound halogen atoms of other halogens. The oxidative addition of halogens to tellurapyrylium dyes gives 10-Te-4 telluranes with hypsochromic-shifted absorption maxima. For example, bromination via the treatment of tellurapyrylium (**264**, X = Te) with Br_2 in CH_2Cl_2 gave tellurapyrylium dibromide **275** featuring a shift in the absorption maximum from 496 to 611 nm.

Oxidative bromination of **272** (Y = Se, X = Te) also resulted in the addition of two halogen atoms to the Te center to yield **276** (R′ = H); limiting the reagent to 2 mol equiv of Br_2 resulted in the addition of only one Br atom. Absorption spectra of all dihalide dyes exhibit hypsochromic shifts relative to the tellurapyrylium dyes, reflecting loss of the Te $5p_z$ orbital. The difference in absorption maxima between dichloride and dibromide analogues is 28 nm, suggesting that the Te−Cl bond is stronger than the Te−Br bond. The Te−I bonds is weak, and the absorption spectra of the diiodotellurapyrylium dyes are similar to those of the parent molecules.[324]

Irradiation of an air-saturated aqueous solution of CP dyes results in rapid disappearance of the chromophore and formation of a new product. In the absence of molecular oxygen, the photochemical reaction does not occur, whereas treatment with hydrogen peroxide gives similar products to those arising from the photochemical reactions. In air-saturated methanol, these dyes produce singlet oxygen with a quantum yield of $\phi = 0.13$. The tellurapyrylium dyes react 20−80 times faster with oxidants as compared to the lighter chalcogen analogues. The mechanism of their photooxidation is comparable to the oxidation of sulfides to sulfoxide by singlet oxygen. The final photoproducts are hydrated forms of telluroxides, resulting from rearrangement of an initially formed pertelluroxide or telluradioxirane intermediate (Scheme 51).[325,326] These oxidized de-

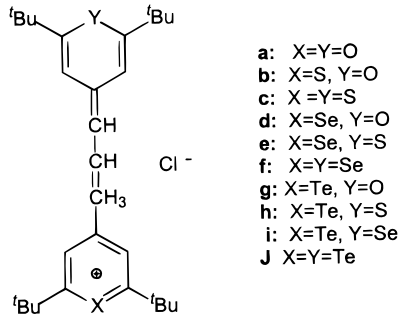
Scheme 51



rivatives have also been detected in vitro in cell cultures treated with tellurapyrylium dyes and light.[327]

Tellurapyrylium dyes strongly absorb in the near-infrared region of the visible spectrum with molar extinction coefficients above $10^5\ M^{-1}\ cm^{-1}$. A number of cationic selena and tellurapyrylium dyes were tested for their photodynamic properties. Treatment of isolated mitochondria from rat mammary adenocarcinomas with several CP dyes (**277**) inhibited the

cytochrome *c* oxidase activity.[327] Addition of various scavengers, including catalase for hydrogen peroxide, superoxide dismutase (SOD) for the superoxide anion, and mannitol for the hydroxy radical, did not protect the cytochrome *c* oxidase activity, suggesting that singlet oxygen is the active cytotoxic species. In particular, the Se and Te dyes are effective in producing singlet oxygen, a capacity that coincides with their efficacy to inhibit cytochrome *c* oxidase. Among a series of CP dyes, the Te/Se derivative **277i** showed the best selective phototoxicity against glioma tumor vs normal cells in culture.[328−331] The authors specu-

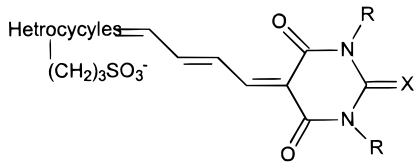


277

late that the high tissue penetration of near-infrared (800 nm) excitation light renders these dyes good candidate drugs for the PDT of brain tumors.

2. Merocyanine Dyes

Merocyanine 540 (MC 540) (**278**: X = Se; R = C_4H_9; heterocycle = Benzoxazole) has been used as a PS for the extracorporal photoinactivation of leukemia cells and enveloped viruses.[332] The biocidal activity



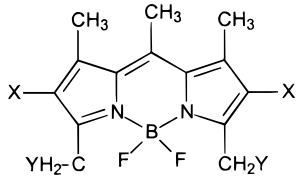
278

of photoexcited MC 540 has been attribute to singlet oxygen, even though the quantum yield for singlet oxygen is low ($\phi = 0.007$).[333] Replacement of the O atom in the oxazol ring for a heavier atom facilitates intersystem crossing and improves the singlet-oxygen yield. Substitution of Se for oxygen to give the selone dyes results in doubling of the biocidal activity.[334] The Se is conjugated with the chromophore and causes a substantial bathochromic shift in the absorption spectrum. Other structural modifications, which have been explored to improve the biological efficacy of merocyanine dyes, include addition of lipophilic substituents on the electron-deficient barbiturate moiety (i.e., substitute R = C_2-C_{10} chains) and modifications of the electron-donor heterocyclic moiety.[334] Such substitutions increase the overall polarity, which reduces the phototoxicity toward mammalian cells and viruses. Changing the substituents of the heterocyclic nitrogen from sulfopropyl to sulfobutyl provided no benefits. Replacement of O atom by S or Se, i.e., benzoxazole, benzothioazole, and benzoslenazole, did not enhance antiviral activity but yielded a 25-fold gain in the capacity to kill leukemia cells. Placing a single methoxy substituent on the back ring also improved the antileukemia action and provided a modest gain in the antiviral effect.

A dramatic enhancement in photodynamic activity was obtained by expanding the aromatic back ring from benzene to naphthalene.[334] Both the naphth-[2,3-*d*]- and naphth[2,1-*d*]-heteroazole showed a multilog increase in their capacity to inactivate tumor cells in vitro. A strong isomer effect was also observed. In sharp contrast to the high activities of the naphth[1,2-*d*] and [2,1-*d*] series, dyes derived from naphth[1,2-*d*]oxazole and naphth[1,2-*d*]thiazole were much less active. An attempt to further enhance the activity by expanding the R substituent did not improve antiviral or cellular phototoxicity. Replacing the S atom by an O atom at the 2-position of the barbiturate moiety enhances the overall lipophilicity of the dye and reduces the biological activity.[335] This is in line with the well-known difference in pharmacological properties between barbiturate and thiobarbiturate drugs.

3. Quasiaromatic Heterocycles

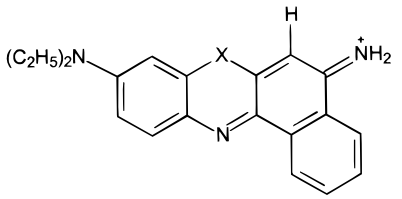
Quasiaromatic heterocycles (QAH) such as 1,3,5,7,8-pentamethylpyrromethene boron difluoride complexes (PMP−BF_2) (**279**) are interesting laser dyes, which compared to the aromatic analogues show a large bathochromic shift of their absorption maxima.



They exhibit high extinction coefficients (**281**: $\epsilon_{377} = 10^5$), large fluorescent quantum yields ($Q_f = 0.7-1.0$), and good photochemical stability. They are presumably planar since they show aromatic protons among the ^{1}H NMR signals. The water-soluble 2,6-disodium disulfonate salt, PMPDS−BF_2 (**279**), was tested as a possible PS for PDT of ovarian cancer. Human ovarian cells incubated at a dye concentration of 0.004 μg/mL showed a good response when exposed to light, but no further reports detailing biological activities of this class of dyes has appeared.[336]

4. Nile Blue Dyes

The benzophenoxazines, including several Nile blue analogues (**282**−**284**), are a unique group of dyes that localize selectively in animal tumors. Uptake and



retention of these dyes by cells in culture revealed that the lysosomes are the main site of localization and that an ion-trapping process is probably respon-

sible for the high accumulation of these dyes in tumor cells.[337] Benzophenoxazine dyes featuring the heavier chalcogen atoms S and Se have also been reported.[338] Changing the O atom in **282** for a S (**283**) or a Se atom (**284**) results in more lipophilic, red-absorbing chromophores and an increase in the capacity to generate singlet oxygen, i.e., $\phi(^1O_2)$ 0.005, 0.025, and 0.65, respectively. Under conditions whereby the Se complex **284** photoinactivates 97% of EMT-6 cells in culture, the S-analogue **283** only kills 5% of the cells whereas the O-analogue **284** is inactive. Adding halogens to the 6-position further increased the $^1O_2$ yield and enhanced the in vitro photodynamic potential of the Nile Blue analogues.

II. Metalloradiosensitizers

A. Introduction

Radiotherapy, either alone or in combination with chemotherapy, is an important treatment modality in cancer management. Although direct interaction between radiation and biomolecules may occur, the principal initial reaction is the radiolysis of the abundant water molecules present in the tissues. The primary radiolysis products of oxygenated aqueous solutions can be summarized as follows, with their yields (*G*-values) for γ-rays in parentheses.[339]

$$H_2O \rightarrow \underset{(2.7)}{\bar{e}_{aq}} + \underset{(0.55)}{H^\bullet} + \underset{(0.45)}{H_2} + \underset{(2.7)}{HO^\bullet} + \underset{(2.7)}{H_3O^+} + \underset{(0.7)}{H_2O_2}$$

Both hydrated electrons and hydrogen atoms/radicals react at a diffusion-controlled rate with molecular oxygen to yield the more stable superoxide ion. Among the different radical, ionized, and excited species, the $^\bullet$OH radical is by far the most reactive. Reactions between biomolecules and $^\bullet$OH proceed either by hydrogen abstraction or $^\bullet$OH addition. They represent a major pathway of indirect damage to cellular constituents. Direct interaction of γ-rays and biomolecules induces electron migration and chemical rearrangement reactions. The relative contributions between indirect and direct damage to biomolecular targets in oxygenated solutions have been estimated at 60% and 40%, respectively.[340] Oxygen plays a key role in propagating the initial damage and may, therefore, be considered the classical radiation dose-modifying agent or sensitizer. Its presence during radiation treatment is essential to exert an optimal physiological response. Oxygen molecules react with intermediate organic radicals in a nonreversible manner to yield organic (hydro)peroxides, resulting in "fixed" biological damage. In the absence of oxygen, more of the short-lived organic radicals revert to the original stable molecules and the radiation damage remains reversible and can be repaired.

Among the cellular components, DNA has been identified as the primary target in radiation-related cell impairment.[341−343] The indirect radiation damage involves the formation of DNA-centered radicals, generated by the reaction of $^\bullet$OH radicals derived from hydrolysis of water. Fixation of this damage occurs when the DNA radical reacts with molecular oxygen. In the second pathway, DNA is ionized directly, interaction with O_2 results in electron abstraction to give a radical species, which may induce a strand break or lead to further oxidative damage. In either case, the damage is only persistent in the presence of oxygen and when the subsequent chemical modifications are not repaired.

The effectiveness of radiation to induce cell kill is measured by cell survival curves, i.e., the percentage of living cells as a function of the radiation dose.[344] Cells exposed to ionizing radiation under N_2 or O_2 give different survival curves such that the dose required to kill 90% of the cells (LD_{90}) is approximately 3 times greater in deoxygenated (hypoxic) medium as compared to normal aerobic conditions. This ratio, which varies with the type of radiation but which is generally independent of the survival level, is called the oxygen enhancement ratio (OER). Likewise, the effect of radiosensitizers (RS) on cell survival in the absence of oxygen is referred to as the sensitizer enhancement ratio (SER).

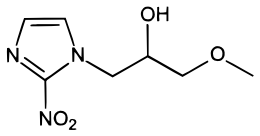
Many tumors contain hypoxic and necrotic areas, usually separated by some distance (150−200 μm) from the vascular system.[345,346] The degree of hypoxia within tumors varies according to the proximity of blood vessels.[347−349] In a rapidly growing tumor, vascularization does not keep up with cell proliferation. As the distance between the capillaries increases, the $[O_2]$ decreases due to O_2 consumption by the cells and limitations in O_2 diffusion, thus yielding hypoxic conditions and eventually necrotic regions. Hypoxia can also result from the intermittent interruption of blood flow because of irregularities and occlusions in the tumor vasculature.[347,349]

The clinical relevance of hypoxia in radio- and photochemotherapy is that the lack of oxygen will render hypoxic cells, which may represent up to 30% of the tumor mass, radioresistant. Approaches to chemically modify cell sensitivity toward radiation include the use of electron-affinic sensitizers, which act as oxygen mimics, and thiol-binding agents which increase the effective concentration of oxygen through competition with the oxygen fixation process. Thus, the basic requirements for an efficient radiosensitizer are the ability to undergo a one-electron reduction, preferential uptake by hypoxic tissues, and lack of toxicity toward aerobic cells.

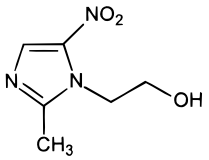
Many nitro-heterocycles and aromatic substrates used as antibacterial, anticancer, and chemotherapeutic agents can also function as RS. Their action mechanism often involves the reduction of a nitro group, presumably by metallonitro-reductases, via single or multiple reduction steps of 1, 2, 4, or 6 electrons.[350] In the case of nitroimidazoles (NO_2Im), three different types of reduction products are produced including the radical anion from the one-electron transfer, azonitroso derivatives from a two-electron reduction, and hydroxylamine derivatives from a four-electron reduction step.[351] These metabolites are considerably more cytotoxic, suggesting a potential application of the parent compounds as chemotherapeutic agents for the treatment of hypoxic tumors.

B. Radiosensitizers

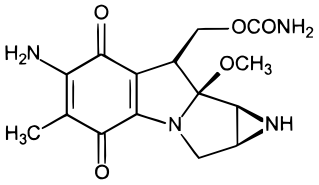
The principal classes of RS, which show enhanced radiotoxicity toward hypoxic cells as compared to aerobic cells, include (i) nitroimidazoles[352] (NO_2Im) such as misonidazole (**285**) and metronidazole (**286**), (ii) quinones[353] such as mitomycin C (**287**), the prototype of many bioreductive alkylating agents[354,355] and benzotriazine di-*N*-oxides[356] such as tirapazamine (SR 4233) (**288**), (iii) 5-nitrothiophene derivatives,[357] and (iv) 5-nitrofurane derivatives.[358]



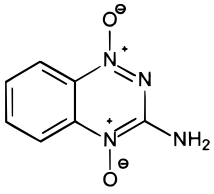
Misonidazole (**285**)



Metronidazole (**286**)

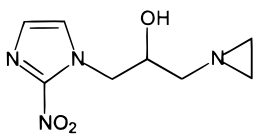


Mitomycin C (**287**)

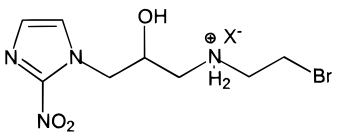


Tirapazamine (**288**)

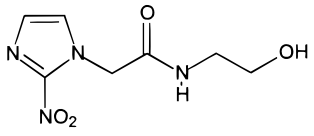
Mitomycin C is one of the most active drugs currently available for the treatment of nonsmall cell lung carcinoma.[359,360] The NO_2Im derivative misonidazole (**285**) has undergone the most extensive clinical evaluation as RS. Unfortunately, as was the case with many of the subsequently developed RS drugs, neurotoxicity prohibited its use at the required therapeutic dosage.[361] In a search for compounds with greater sensitizing potency and less side effects, a series of 2-NO_2Im derivatives containing an aziridine[362] or haloethylamino group[363] were developed. This yielded the powerful, dual-functional, cytotoxic agent α-[(1-aziridinyl)methyl]-2-nitro-1*H*-imidazole-1-ethanol (**289**, RSU 1069), which functions both as a hypoxic cell radiosensitizer and alkylating agent.[363] However, in early phase I trials, gastric toxicity (emesis) was observed and clinical evaluation was not further pursued.[364] The haloethylamino NO_2Im derivatives act as prodrugs of the corresponding aziridinyl agent. Thus, α-([(2-bromoethyl)amino]methyl)-2-nitro-1*H*-imidazole-1-ethanol[365] (**290**, RB 6145), i.e., the prodrug of RB 1069 (**289**), acts as a RS or as a bioreductively activated cytotoxin.[363] The equally active but less toxic analogue etanidazole (**291**) is in phase III trials.[366]



RSU 1069 (**289**)

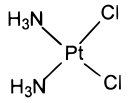


RB 6145 (**290**)

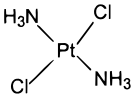


etanidazole (**291**)

Although most anticancer drugs are organic in nature, several metallocompounds have also found important medical application. Inorganic ions can participate in biological processes in different ways; they can (i) trigger a control mechanism, (ii) control the conformation of biological macromolecules and participate in template reaction, (iii) act as Lewis acids and change the proton concentration, and of particular importance for radiosensitizing activity (iv) they can function as redox catalyst. *cis*-Diaminedichloroplatinum(II) (*cis*-DDP or cisplatin) (**292**) and various analogues are used extensively in cancer therapy, either alone or in combination with other drugs or ionizing radiation.[367] The first report on the synergism between *cis*-DDP and radiation concerned lethality in mice exposed to X-rays.[368] This interaction received a great deal of attention due to the potential clinical impact. Only the *cis*-isomer of DDP is active as a chemotherapeutic agent, although both the *cis*- and *trans*-isomer are capable of radiosensitizing mammalian cells toward ionizing radiation. *cis*-DDP is believed to induce cytotoxicity via both intra- as well as interstrand cross-linking in DNA. The inactive *trans*-DDP (**293**) can only inflict the latter type of damage. A second generation of Pt complexes, e.g., carboplatin and iproplatin, appear to act both as chemotherapeutic and radiosensitizing agents.[369] In general, however, the persistence of severe side effects remains a major obstacle to the more widespread use of cisplatin agents.



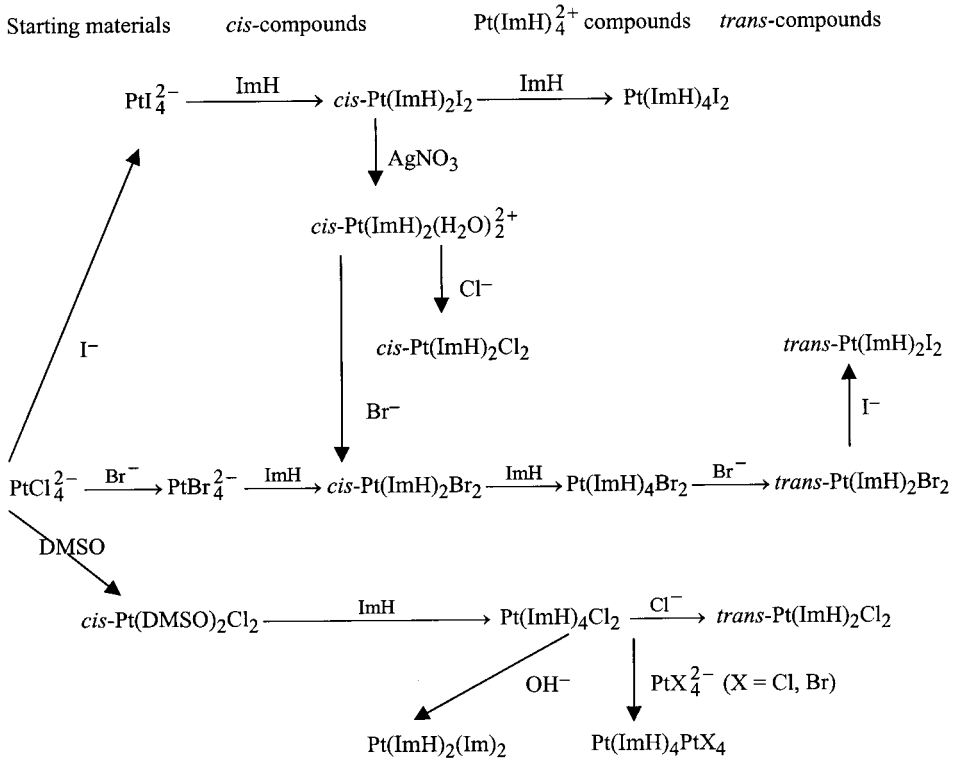
cis-DPP(**292**)



trans-DPP(**293**)

The pronounced anticancer properties of Pt complexes prompted a search for other metal complexes with biological activities. In particular, transition metals (e.g., Fe, Co, Ni, Cu) were thought to offer advantages over Pt because of their different (i) redox properties, (ii) reactivity toward thiols, and (iii) DNA-binding affinities. Transition-metal ions, with their labile d-electron system, can participate in biological redox processes for a number of reasons: (i) a range of accessible oxidation states enables the metal ion to transfer electrons, (ii) the redox potential can be varied by alterating the ligand or the geometry of the complex, (iii) metal-mediated atom transfer may occur, (iv) stable paramagnetic states are common, facilitating the reaction with radical substrates, (v) the degree of paramagnetism (spin state) can be varied, and (vi) metal ions can accommodate both neutral and anionic ligands. Many factors can influence the redox potential of metal complexes and subsequently their biological reactivity. Thus, knowledge of the redox potentials of metal ions is essential to understand their reactivity, either alone as organic complexes or as metalloenzymes. Incorporating a

Scheme 52. Synthetic Procedures for Several Pt Compounds (modified from ref 371)

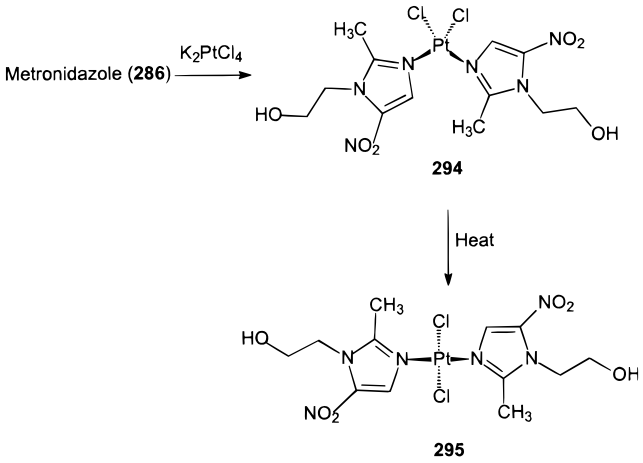


transition metal into the design of radiosensitizers can create an electron-affinic compound, while the fixed geometry of the ligands, imposed by the d orbitals of the metal ion, can be used to optimize specific interactions.[370]

The synthesis of a number of coordination compounds of Pt(II) and Pd(II) with imidazole (ImH) has been reported,[371] including the following Pt complexes *cis*- and *trans*-$Pt(ImH)_2X_2$, $Pt(ImH)_4X_2$, $Pt(ImH)_2(Im)_2$, and $Pt(ImH)_4PtX_4$, where X = Cl, Br, or I, and the following Pd complexes *trans*-$Pd(ImH)_2X_2$, $Pd(ImH)_4X_2$, and $Pd(ImH)_4PdX_4$, where X = Cl or Br, and *cis*-$Pd(ImH)_2X_2$, where X = Cl, Br or $X_2 = C_2O_4$, as well as $Pd(Im)_2$. In all cases, the metal ions are coordinated in a square-planar geometry. The polymeric $Pd(Im)_2$ structure exists as a bidentate compound, bridging two imidazolato anions. None of these Pt and Pd complexes show significant cytotoxicity. The various synthetic pathways to prepare the above Pt complexes are summarized in Scheme 52.[371]

The addition of the NO_2 group lowers the Im basicity, e.g., the pK_a of metronidazole (**286**) is 7.0 vs 2.4 for Im. Metronidazole (metro) (**286**), marketed as Flagyl, is an analogue of 5-NO_2Im with important therapeutic applications. Pt complexes of metro (**286**) include *cis*-$Pt(metro)_2Cl_2$ (**294**), which was obtained by addition of K_2PtCl_4 to a suspension of **286** in H_2O at 50 °C (90% yield). Heating of **294** at its melting point (180 °C) or in ethanolic suspension resulted in transformation to *trans*-$Pt(metro)_2Cl_2$ (**295**) in quantitative yield, a rare example in Pt chemistry[372] (Scheme 53). The molecular structures of **294** and **295** were established by X-ray crystallography, revealing that only the thermodynamically favored *trans*-isomer was produced. The geometry about the Pt is closely square planar, with bond angles in the range of 88.9−90.9°. Similarly, the reaction of K_2PtCl_4 with misonidazole (**285**) to yield the *trans*-$Pd(II)(miso)Cl_2$ has also been reported.[373] Pt(II) complexes, featuring two sensitizer ligands in either the *cis*- or *trans*-configuration, do not show improved radiosensitizing activity over the free ligands. The reaction of K_2PtCl_4 with unsubstituted 2- and 4-NO_2Im proceeds more slowly and requires a high temperature (95 °C).

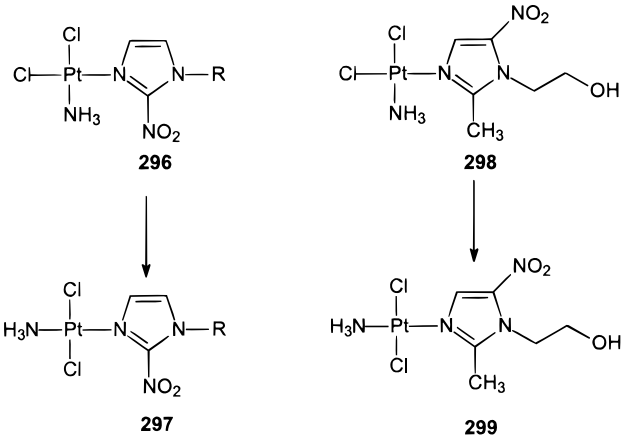
Scheme 53



The synthesis of a range of PtL_2X_2-related complexes, where L is a substituted 5-NO_2Im and X_2 is a dihalide, were prepared and characterized by ^{195}Pt NMR spectroscopy.[374,375] The complexes show an unusual loss of planarity between the nitro group and the imidazole ring, with a dihedral angle of 45.6°. Coordination of the NO_2Im ligand to Pt(II) lowers the wavelength of the $\pi-\pi^*$ electronic absorption band

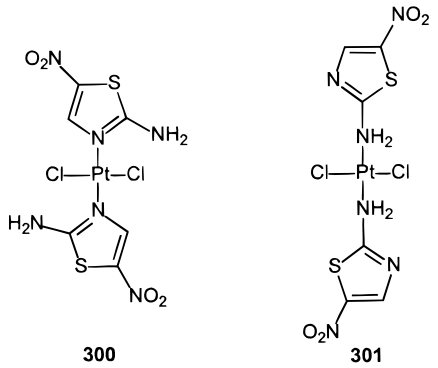
and reduces the polarographic reduction potential by ca. 0.15−0.2 V. The differences in antitumor activities between the *cis*- and *trans*-isomers of the Pt complexes are less pronounced as compared to those observed for the parent Pt−NH_3 isomers.

The monocomplexes of *cis*-$PtCl_2(NH_3)$(miso) (**296a**), *cis*-$PtCl_2(NH_3)$(etanidazole) (**296b**), and $PtCl_2(NH_3)$-(metro**)** (**298**) were obtained by reacting equivalent amounts of the corresponding NO_2Im and K[$PtCl_3$-(NH_3)].[376,377] Conversion to the *trans*-$PtCl_2(NH_3)$-

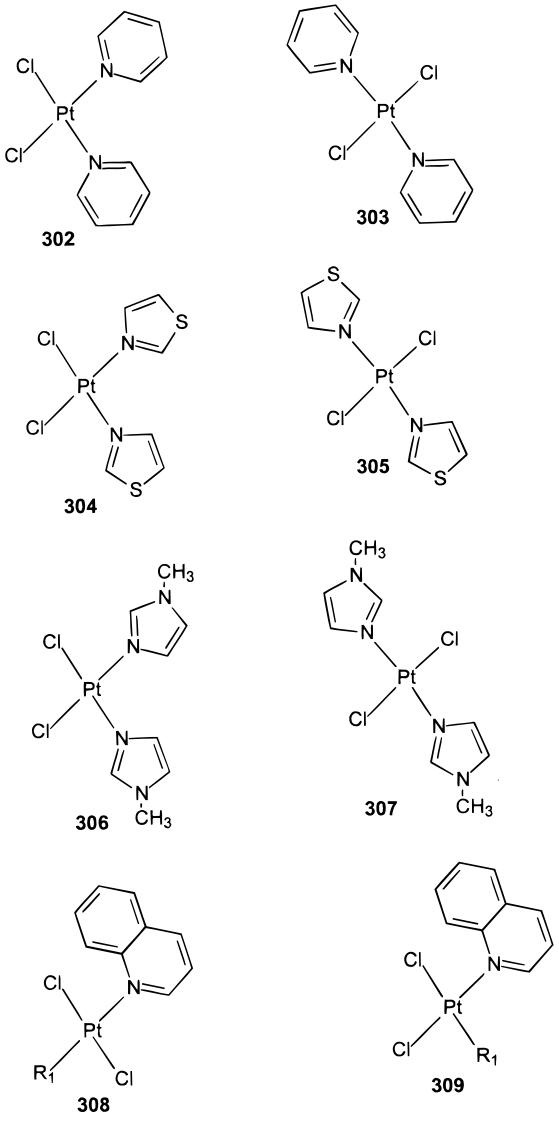


(miso) (**297a**) and *trans*-$PtCl_2(NH_3)$(metro) (**299**) from the *cis*-analogue **296a** and **298**, using X-ray diffraction to confirm the stereochemistry, has also been reported.[39] The monocomplexes are more effective RS than the analogous bis-complexes. They inhibit endonuclease activity, whereas both the *cis*- and *trans*-bis-complexes, at equivalent concentrations, lack this activity. This may relate to an increase in the electrochemical reduction potential of the nitro groups of the latter. Also, the presence of an amine in **296b** and **298** may enhance the DNA-binding affinity. Both **296b** and **297** inhibit *Bam*HI and *Eco*RI restriction enzyme activity on isolated plasmid DNA.[378] Among four closely related Pt complexes of 4(5)NO_2Im, i.e., $PtCl_2$(5-NO_2Im)$_2$, $PtCl_2$(4-NO_2Im)$_2$, $PtCl_2(NH_3)$(5-NO_2-Im), and $PtCl_2(NH_3)$(4-NO_2Im), the complexes containing 5-NO_2Im exhibited the highest radiosensitizing activity, but only the $Pt(Cl_2)(NH_3)$(4-NO_2Im) was found to bind to DNA.[379]

Under in vitro conditions, the 2-amino-5-nitrothiazole (ANT) is less toxic than misonidazole (**285**) and only slightly less active as a radiosensitizer.[380] The reaction of ANT with K[$PtCl_2(NH_3)$] gave a single complex with one radiosensitizer ligand, the *cis*-$PtCl_2$-(NH_3)ANT. The reaction with K_2PtCl_4 gave a number of products including two *trans*-di-ANT complexes **300** and **301**, in which the Pt is coordinated to different atoms of the ANT moiety.[381] Bonding either involves the ANT-amine group or the thiazole ring nitrogen. Complex **300** is a better radiosensitizer, with OER values in CHO cells of 1.6 vs 1.15 for **301**. The relative order of inhibition of the restriction enzyme activity on plasmid DNA[380] of a series of Pt−NO_2Im complexes showed that the monocomplexes are weaker inhibitors as compared to the very active *cis*-DPP while the bis-complexes are inactive.[382]



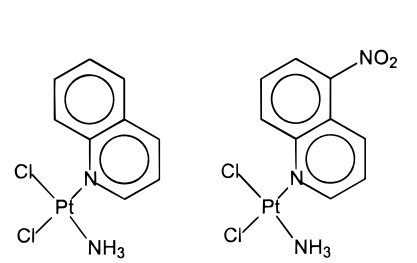
Changing the amine in *trans*-DDP (**293**) for planar pyridine (Py) ligands, as in *trans*-$PtCl_2(Py)_2$ (**302**), enhances the cytotoxicity by an order of magnitute.[383] A series of *trans* complexes featuring planar ligands of formula *trans*-$PtCl_2$(L)(L′), where (i) L = L′ = pyridine (**302**, **303**), thiazole (**304**, **305**), *N*-methyl Im (**306**, **307**), and (ii) L = quinoline (**308**, **309**) and L′



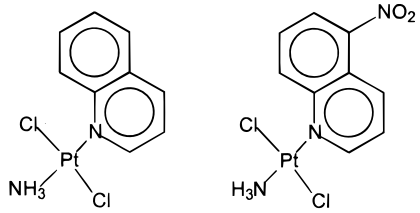
= substituted sulfoxide R′R″SO, where R′ = Me and R″ = Me, CH_2Ph, and Ph, and (iii) L = quinoline and

L′ = NH_3, were prepared[384] and compared for their in vitro cytotoxicity.[385] This study confirmed that the *trans*-geometry with planar ligands is the preferred conformation for biological activity. In the case of *cis*/*trans*-$PtCl_2(NH_3)$(quinoline), both isomers show the same cytotoxicity as *cis*-DDP, which is 10-fold less active than the *trans*-DDP isomer. The *trans*-$PtCl_2(Py)_2$ inhibits DNA synthesis, and accordingly DNA binding is likely implicated in the action mechanism. However, in vivo studies, using both murine L12110 and P388 leukemia cell lines, showed that *trans*-$PtCl_2(Py)_2$ did not inhibit tumor growth.

Another class of nitroaromatic Pt complexes, which were designed to improve sensitizing or cytotoxic properties of NO_2Im derivatives, are nitroquinoline (NQ) complexes featuring an NO_2 group at either the 5- or 6-position of quinoline.[386] Their larger planar surfaces as compared to NO_2Im enhances DNA binding while their redox potentials remain in the appropriate range to function as a radiosensitizers. *cis*-Pt−5-NQ (**311**) and *cis*-Pt−6-NQ (**312**) exhibit similar sensitizing activities against Chinese hamster ovary cells (V79) in vitro (SER of 1.7, at 15 and 40 μM, respectively). The free NQ ligands showed little



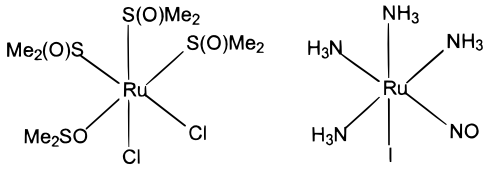
310 **311** **312**



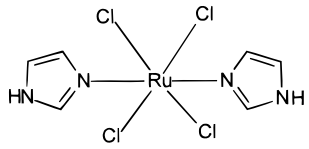
313 **314**

activity. The toxicity of *cis*-Pt−5-NQ (**311**) is greater than that of *cis*-Pt−6-NQ (**312**) with LD_{90} of 5 and 35 μM, respectively, while their redox potentials are similar (−260 and −280 mV). Both complexes show greater radiosensitizing ability in hypoxic than oxic conditions while the *trans*-Pt−5-NQ (**314**) is a more efficient RS under hypoxic conditions than the *cis*-isomer. The *trans*-isomer of the (unsubstituted) 5-NQ **313** is considerably more toxic than the *cis*-isomer **310**. These Pt complexes bind to DNA as evidenced by the inhibition of *Bam*HI and *Eco*RI restriction enzyme activity of plasmid DNA. Both Pt−NQ complexes inhibit *Bam*HI to a greater extent than the corresponding NO_2Im complexes. For the complex $PtCl_2NH_3L$, activities decrease in the following order: L = 5-NQ > 6-NQ > misonidazole > metronidazole > etanidazole > 5-NO_2Im > 4-NO_2Im.

The potential medicinal use of Ru complexes has been extensively explored via their interactions with molecules of biological interest. Ruthenium-based RS complexes exhibit strong DNA-binding properties.[387] Among a series of $Ru(NH_3)_xA_{6-x}$ complexes, where A is an anionic ligand, $Ru(NH_3)Cl_3$ showed the best binding affinity. Structurally unique complexes **315**−**317** also exhibit RS activity.

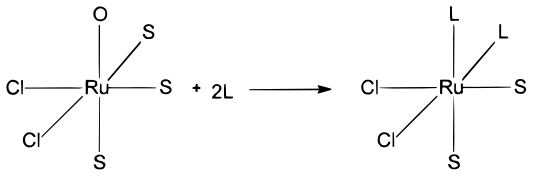


315 **316**

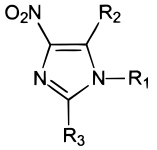


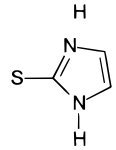
317

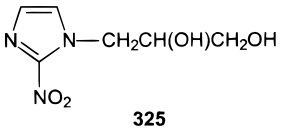
Introduction of an imidazole group to yield a *trans*-$[RuCl_4(Im)_2]^{2-}$ complex (**317**) increases the electron affinity of the ligand resulting in stronger radiosensitizing properties.[388] A series of Ru(II) complexes of formula $RuCl_2(DMSO)_2L_n$, where DMSO = S-bonded DMSO and L = a 4-NO_2Im derivative such as 4-NO_2-Im (**318**), RSU-1170 (**323**), RSU-3083 (**320**), and RSU-3100 (**321**), NMe-4-NO_2−Im (**319**), and 1-Me-5-(2′-thioimidazolyl)-4-NO_2Im (**324**, RSU-3159), or a 2-NO_2Im derivative of misonidazole (**285**) and desmethylmisonidazole (**325**), were obtained from the precursor complex *cis*-$RuCl_2(DMSO)_4$ by substitution of two sulfoxide ligands.[389] The spectral data (XPS, ^{1}H NMR or IR) showed that the diamagnetic, hexacoordinated complexes have a cis structure (i.e., cis, cis, cis), with both DMSO ligands being S-bonded. The ligand is attached via an Im nitrogen or the oxygen of the NO_2 group. $RuCl_2(DMSO)_2$ (RSU-3159) features S-bonded DMSO ligands and a coordinated thioether chelated via the nitrogen of the NMe group.[388] To study the effect on the sensitizing potentials of halide and sulfoxide ligands in Ru(II) complexes, the following series was prepared:[389] $RuCl_2(TMSO)_mL_n$, where TMSO = tetramethylene-sulfoxide and L = 2- or 4-NO_2Im (m = 1−3 and n = 1 or 2), and $RuBr_2(DMSO)_2L_n$, where L = 4-NO_2Im (n = 1 or 2). $RuCl_2(TMSO)_4$ contains no oxygen-bonded sulfoxide and most likely features *trans* geometry. The *trans*-$RuBr_2(DMSO)_4$ complex contains only one S-bonded sulfoxide and serves as an intermediate for the preparation of $RuBr_2(DMSO)_2$(4-NO_2Im)$_2$ and $RuBr_2(DMSO)_2$(NMe-4-NO_2Im). The latter contain S-bonded sulfoxide only and the products are pure *trans*-structures, containing a chelated Im (via the Im-nitrogen and oxygen of the NO_2 group), *cis*-sulfoxide, and *trans*-bromide ligands. Substitution of the NO_2Im complex for etanidazole (SR-2508) (**291**) gives a five-coordinate, trigonal bipyramidal structure.



(O or S implies Oxygen- or sulfur-bonded DMSO)
L=NO_2Im



	R_1	R_2	R_3
318	H	H	H
319	CH_3	H	H
320	CH_3	$NHCH_2CH_2OH$	CH_3
321	CH_3	OPh	H
322	CH_3	Cl	H
323	$CH_2CH(OH)CH_2N$ 	H	CH_3
324	CH_3		H



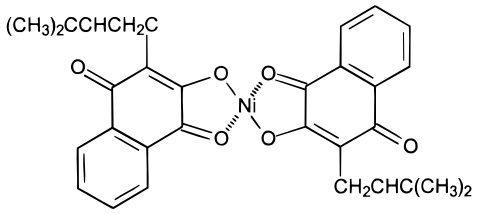
325

Among a series of Ru(II)−NO_2Im complexes, $RuCl_2(DMSO)_2(4\text{-}NO_2Im)_2$ is the most effective RS, exhibiting a higher activity than the free NO_2Im for CHO cell inactivation in vitro. At 200 μM, the complex induced a SER of 1.6 under hypoxic conditions, compared to 1.4 for 2-NO_2Im and 1.2−1.4 for most other complexes.[390] The complex also showed lower toxicity than the free NO_2Im at similar concentrations. The enhanced radiosensitizing effect was attributed to the metal's ability to target the sensitizer to DNA and/or to change the reduction potential (from ~685 mV for the free ligand to ~355 mV for the complexes). $RuCl_2(TMSO)_2$ (SR-2508), which contains only a single NO_2Im ligand (i.e., SR-2508), has a high SER value of 1.5. Substituting TMSO for DMSO increases both the lipophilicity and the reduction potential (by 10−15 mV) of the complex while the SER value remains the same. Substitution of Cl^- by Br^- decreases the reduction potential by 30 mV and reduces the radiosensitizing ability accordingly. Preliminary experiments with $RuCl_2(TMSO)_2(4\text{-}NO_2\text{-}Im)_2$ and $RuCl_2(TMSO)_2$(etanidazole)$_2$ in a mouse tumor model showed that these complexes also exhibit in vivo radiosensitizing properties.

The Rh(II)carboxylates constitute another interesting series of complexes with antitumor activity.[391] They feature a dimeric structure with bridging carboxylates. Upon incubation with both aerobic and hypoxic V79 cells, Rh(II)carboxylates deplete the thiol pool and increase the radiation sensitivity.[392] The hypoxic sensitization (SER = 1.9−2.1) was moderately higher than the oxic response (SER = 1.4−1.8). The Rh(II)metronidazole complex $Rh_2(OOCH_3)_4(metro)_2$[393] and a $Rh_2(OOCH_3)_4(2\text{-}NO_2Im)$ derivative (RSU-1130) were found to be better RS for hypoxic V70 cells as compared to free metronidazole or the corresponding Pt complexes.[375]

Copper−imidazole complexes have good radiosensitizing properties with toxicity ranging between that of the Cu(II) ion and the free ligand.[393] A 1:1 complex of [1-(2′-hydroxyethyl-2-methyl)-5-NO_2Im] with Cu(II) (or NiII) was prepared by mixing equimolar amounts of metronidazole and Ni(II)sulfate or Cu(II)acetate in aqueous medium at −5 °C.[394,395] Cobalt-60 irradiation of thymidine in the presence of these complexes led to a significantly higher level of thymidine glycol formation as compared to irradiation in the presence of free metronidazole. The enhanced radiosensitization is probably due to the higher reduction potential of the metal complex resulting in abundant formation •OH radicals. The latter also react with the nitro group of the metal complex, resulting in the destruction of the NO_2Im moiety.[396]

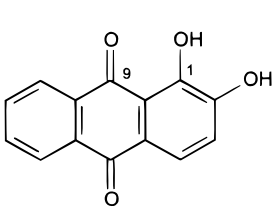
Lapachol is a naphthoquinone with antimalarial activity[397] that was first isolated from tropical hard wood in 1857. After Rao et al.[398] reported antitumor activity, several groups embarked on a search for Lapachol analogues with enhanced biological activity. Complexation of Lapachol or related 2-hydroxy-1,4-naphthoquinones with metal ions increases the radiosensitizing potential of the ligand.[399] Coordination of a metal ion with 2-hydroxy-1,4-naphthoquinones (L) occurs through the deprotonated hydroxyl group and the adjacent quinone oxygen (**326**). Chelates with



326

several metals (ML_n, n = 2 or 3) were tested for radiosensitizing potencies, which were found to decrease in the following order: M = Ni > Cu > Co > Zn. The Ni complex (**326**) has a redox potential of −370 mV and is the most active complex (SER = 3). Contrary to misonidazole, the toxicity of (**326**) is only slightly greater under hypoxic than aerobic conditions and thiol depletion does not occur. The observation that **326** caused 5-fold higher single-strand breaks under hypoxic conditions than in air was unexpected.[399]

The stable complexes of Cu(II) and Ni(II) with 1,2-dihydroxy-9,10-anthraquinone (DHA) (**327**), of the type $M(DHA)_3$ (**328**), catalyze the transfer of an electron from NADH to O_2 via NADH dehydrogenase. Owing to their high redox potential, the Cu(II) complexes are more effective in reducing molecular oxygen to the superoxide anion as compared to the corresponding Ni(II) complexes.[400]



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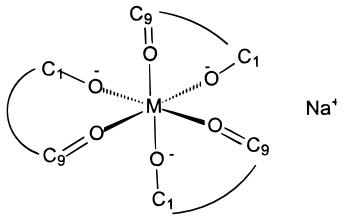


328

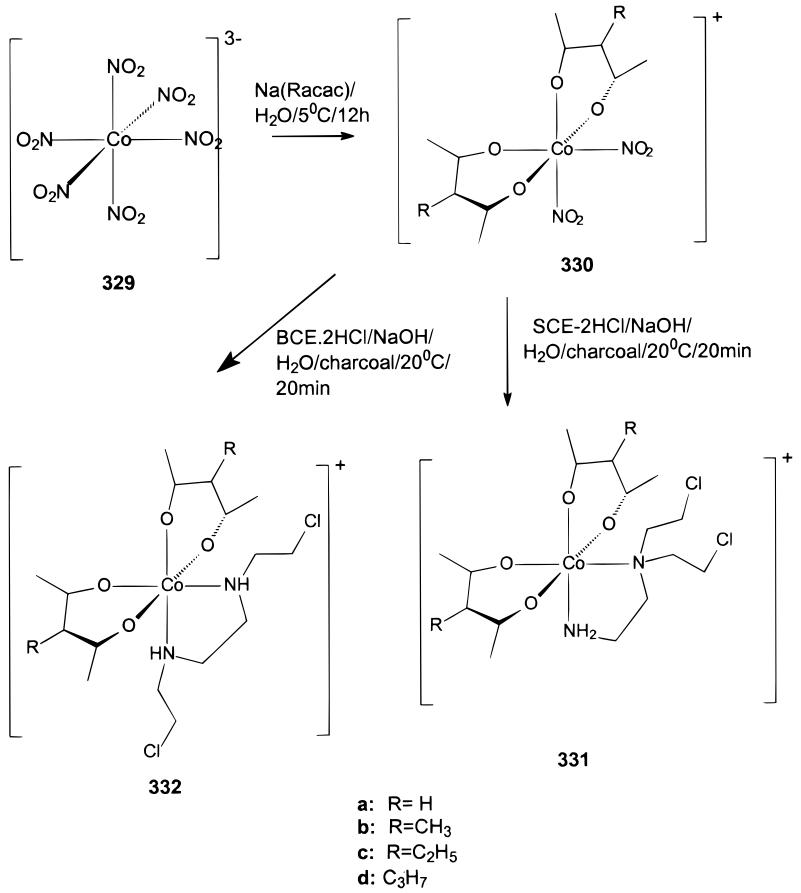
The reduction of Co(III) to Co(II) in Co chelates results in enhanced sensitivity to radiation as compared to complexes with stable metal ions. A series of complexes featuring a Co−amine system showed good radiosensitizing activity against hypoxic EMT6 cells. Several Co(III) and Fe(III) complexes were evaluated[401] including $Na_3[Co(NO_2)_6]$, *mer*-$Co(NH_3)_3(NO_2)_3$, *cis*-$[Co(NH_3)_4(NO_2)_2]NO_3$, $[Co(NH_3)_6]Cl_3$, *cis*- and *trans*-$[Co(NH_3)_4(NO_2)_2]CH_3CO_2$, *trans*-$[Co(NH_3)Cl_2]HSO_4$, *mer*-$Co(NH_3)_3(CN)_3$, Co(diethylenetriamine)$(NO_2)_3$, Na[Co(acetylacetonate)$_2(NO_2)$], *mer*-$Co(NH_2CH_3)_3(NO_2)_3$, *cis*-[Co(1,10-phenanthroline)$_2(NO_2)_2NO_3$, Fe(cyclopentadienide)$_2CCl_3CO_2\cdot 2CCl_3CO_2H$, Fe(methylcyclopentadienide)$_2CCl_3CO_2\cdot CCl_3CO_2H$, and Co(cyclopentadienide)$_2CCl_3CO_2\cdot 2CCl_3CO_2H$. The most active complexes contain a coordinated nitro ligand, and *trans*-$Co(NH_3)_4(NO_2)_2$ complexes were found to be more effective RS than the corresponding *cis* complexes.

Molecules containing the $RN(CH_2CH_2Cl)_2$ (nitrogen mustards) motive are well-known DNA cross-linking agents with potent cytotoxic activities. Their action relates to the electron density on the mustard nitrogen, which controls their alkylating reactivity.[402] Coordination of the nitrogen lone electron pair to Co(III) suppresses its toxicity since the electrons are no longer available to act as a nucleophile. Such Co(III) complexes are kinetically inert, and the nitrogen mustard ligands are displaced only very slowly, unless the Co(III) is reduced to the much more labile Co(II) state. Since the Co(III)−Co(II) reduction potential falls within the range of cellular reductants, i.e., −0.20 to −0.04 V, net chemical or metabolic one-electron reduction of the Co(III) complexes is to be expected under physiological conditions. The resulting Co(II) species undergo facile ligand substitution by water molecules, releasing free nitrogen mustard.

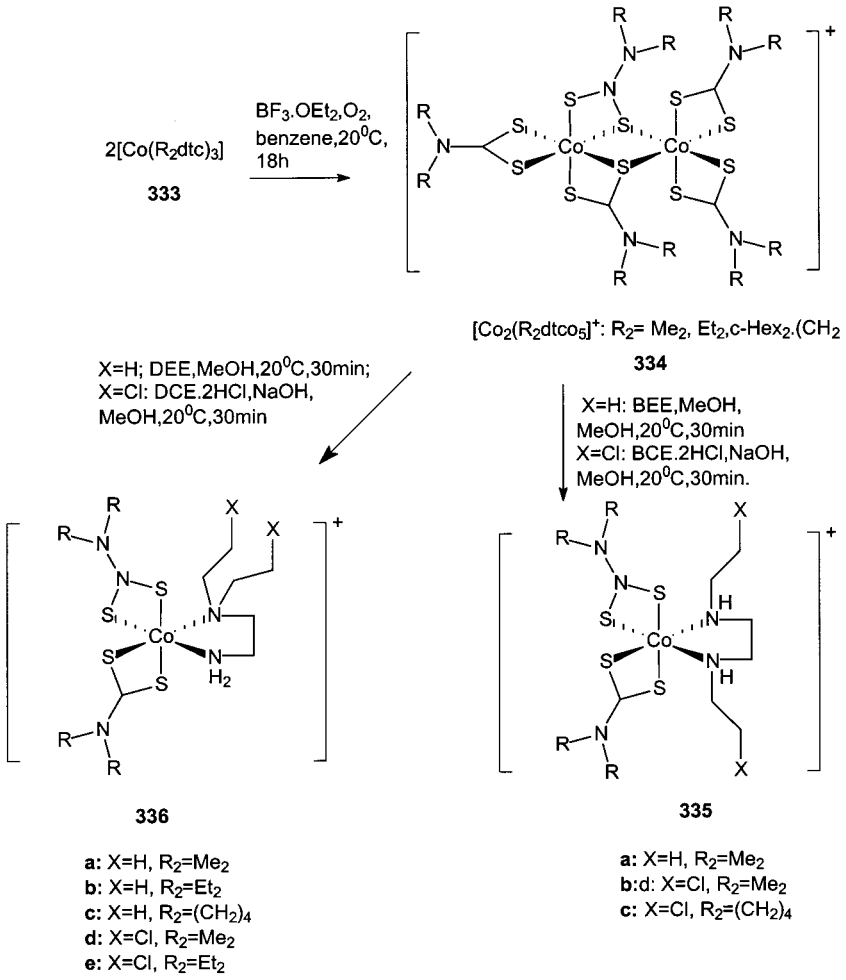
$$[Co(III)L_6]^{3+} \overset{O_2/O_2^-}{\rightleftharpoons} [Co(II)L_6]^{2+} \xrightarrow{H_2O} [Co(II)(H_2O)_6]^{2+} + 6L$$

Ware et al.[403] developed Co(III) complexes containing coordinated nitrogen mustards and demonstrated that they act as hypoxia-selective cytotoxins. Two series of Co(III) complexes of the bidentate bisalkylating ligands *N*,*N*′-bis(2-chloroethyl)ethylenediamine (BCE) and *N*,*N*-bis(2-chloroethyl)ethylenediamine (DCE) have been reported.[404] The preparation of these complexes requires a cobalt ligand system which undergoes relatively rapid substitution at the Co(III) center. Nitrogen mustards are unstable in the active free-base form, and Co(III) complexes can be prepared by treatment of $Na_3[Co(NO_2)_6]$ (**329**) with

Scheme 54



Scheme 55



Na(acac) (acac = pentane-2,4-dionato anion) or Na(Meacac) (Meacac = 3-methyl-2,4-dionato anion) to yield *trans*-Na[Co(acac)$_2$(NO$_2$)$_2$] (**330a**) and *trans*-Na[Co(Meacac)$_2$(NO$_2$)$_2$] (**330b**), respectively. Reaction of free-base BCE or DCE with **330** produces the desired Co(III) nitrogen mustard complexes **331** and **332**, respectively (Scheme 54). The complexes were isolated as ClO_4^- salts and characterized by IR and ^{1}H and ^{13}C NMR spectroscopy, revealing the two characteristic chiral nitrogen atoms. Rapid precipitation of BCE products from solution, followed by ^{1}H and ^{13}C NMR analysis, reveals three diastereoisomers, arising from the stereochemistry at the nitrogen-donor atoms, which become chiral upon coordination to cobalt. However, slow crystallization produces a single isomer, in high yield, as a stereochemically pure product. The X-ray crystal structure of [Co(Clacac)$_2$ (BCE)]ClO_4 confirmed the *cis*-geometry and *R*/*S* stereochemistry.[403a]

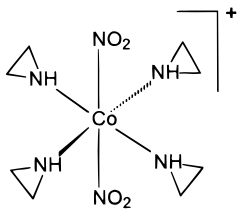
Cyclic voltammetry showed that varying the alkyl group in the auxiliary ligands alters the reduction potentials of the complexes (within the same series) over a range of about 150 mV. The complexes exhibited hypoxia-selective cytotoxicity[404] against CHO fibroblasts and UV4 cells in vitro. In both BCE and DCE series, the pattern of cytotoxicity of the Co complexes paralleled that of the free ligands, suggesting that the cytotoxicity of the complexes is due to release of the free ligands. The unsymmetrical DCE−Co complexes were an order of magnitude more cytotoxic than the corresponding free BCE compounds. Although the unsubstituted acac/DCE complex lacked hypoxia-selectivity against DNA repair-deficient UV4 cells, the methyl and ethyl analogues showed substantial selectivity. The results indicate a narrow range of biologically active reduction potentials with an optimum value close to that for the methyl analogue ($E_{1/2}$ = −305 mV). The methyl analogue also showed hypoxia-selectivity against repair-proficient cell lines (e.g., AA8 and EMT6). Furthermore, high activity against EMT6 cells in intact spheroids suggests that the released DCE is capable of back-diffusion from the hypoxic core of the spheroid. This work showed that metal complexes of nitrogen mustards have significant selectivity toward hypoxic mammalian cells and that they represent a promising new class of hypoxia-selective cytotoxins.

The kinetic basis for the in vitro sensitizing activity of the Co(III)−nitrogen mustard complex SN 24771 (NSC 675352) (**331b**) has been investigated by pulse radiolysis.[405] The rate constants for the one-electron reduction of **331b** by model reductants exhibited a marked dependency on the reduction potential of the reductant, with values up to several orders of magnitude slower than those reported for misonidazole. Following one-electron reduction of the Co(III) to the Co(II) complex (species I), consecutive conversion to

further transient species (species II and III) occurs with first-order rate constants of 120 ± 10 s^{-1} and 10 ± 2 s^{-1}, resulting in the release of the ligands. Neither of these subsequent processes are inhibited by the addition of O_2 up to a concentration of 0.5 mmol L^{-1}, suggesting that the sensitizing action of **331b** most likely arises from a mechanism other than simple redox cycling between Co(III) and Co(II) by molecular oxygen. If the measured low rate constants of one-electron reduction of **331b** by standard reductants (as compared to the reduction of nitroaromatics) is mirrored by biological reductants, then it is likely that hypoxic sensitization may occur through competition between **331b** and O_2 for these reductants.

Compound **331b** shows a 5−30-fold selectivity for hypoxic cells in culture, depending on the cell line and experimental conditions. Cytotoxicity of **331b** in cell suspensions was inhibited by very low oxygen concentrations. In this respect, **331b** resembles organic bioreductive drugs such as quinones and nitroaromatic compounds. The C_{50} value (O_2 required for 50% inhibition of one log cell kill) was ca. 0.02% O_2 at 1 h. However, intact spheroids were much more sensitive to **331b** than could be accounted for by the survival curve for single-cell suspensions. The extensive killing observed in multicellular spheroids is consistent with the release of a diffusible nitrogen mustard liberated upon reduction of the complex.

The synthesis of an alternative series of potentially radiosensitizing Co(III) complexes, derived from the lead compound **331b** by replacing the $Racac^-$ ligand with monoanionic, bidentate dithiocarbamato R_2dtc ligands, were also reported[406] (Scheme 55). Ligands were coordinated through two sulfur atoms to yield a complex of the formula $[Co(R_2DTC)_2(DCE)]^+$, which retained an overall cationic charge. The R substituents were varied to tailor properties such as redox potential and lipophilicity. Co(III)dithiocarbamate (DTC) complexes that have been reported include a complex with three R_2DTC ligands as well as the mustard ligands DCE and BCE and the corresponding nonalkylating analogues of DEE and BEE (*N*,*N*′- and *N*,*N*-diethylethylenediamine). The precursors, i.e., the binuclear Co(III) complexes $[Co_2(R_2DTC)_5]^+$ (**334**), were obtained from the reaction of $Co(R_2dtc)$ (**333**) with $BF_3{\cdot}OEt_2$. Treatment of the binuclear precursors with diamine mustard-donor ligands gave the final products **335** and **336**. Cyclic voltammetry



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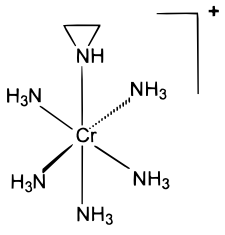


338

of the complexes in acetonitrile revealed quasi-reversible behavior for the Co(III)/Co(II) couple, with $E_{1/2}$ increasing in the order DCE > DEE > BCE > BEE. Radiosensitized cell killing was not appreciably enhanced under hypoxic conditions for any of the dithiocarbamato complexes. Combined with the instability of the parent complex, this implies that these compounds are not suitable as bioreductive anticancer drugs.

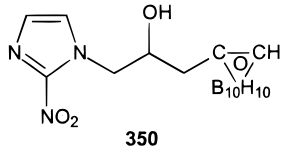
Aziridine is a potent alkylating agent that binds to DNA and as such possesses the basic properties for a potential role in cancer chemotherapy. A number of organic molecules containing the aziridine moiety are indeed used as anticancer agents.[407] The relatively stable aziridine group is activated by hydrolysis, which releases the potent alkylating aziridinium ion. The reactivity can be masked by complexation with metal ions, such as Co(III) or Cr(III), resulting in formation of a rather inert complex. One-electron reduction to the Co(II) or Cr(II) complex enhances instability, resulting in release of the cytotoxic ligand.[408] The synthesis of such complexes involves a direct substitution of an aziridine (Az) ligand for a halide ligand via the reaction of *cis*-$[Co(en)_2Cl_2]Cl$ (en = ethylenediamine), *cis*-$[Co(NH_3)(en)_2Br]Br_2$, or *cis*-$[Co(trien)Cl_2]Cl$ (trien = triethylenetetramine) in neat aziridine, producing *cis*-$[Co(en)_2(Az)Cl]Cl_2$ (**339**), *cis*-$[Co(NH_3)(en)_2(Az)]Br_3$ (**340**), or *cis*-$[Co(trien)(Az)Cl]Cl_2$ (**341**), respectively. *trans*-$[Co(Az)_4(NO_2)_2]Br{\cdot}2H_2O{\cdot}LiBr$ (**342**) was prepared similarly from $Na_3[Co(NO_2)_6]$. Triflato(trifluoromethanesulfonato) complexes *cis*-$[Co(NH_3)_4(OSO_2CF_3)_2](OSO_2CF_3)$ (**343**), *mer*-$[Co(NH_3)_3(OSO_2CF_3)_3]$ (**344**), and *cis*-$[Co(NH_3)(en)_2(OSO_2CF_3)](OSO_2CF_3)_2$ (**345**) were prepared by treatment of the corresponding halide complexes with neat triflic acid. The triflato ligands in **343** and **345** were substituted by aziridine to give *cis*-$[Co(NH_3)_4(Az)_2]Cl_3$ (**346**) and **340**, respectively. $[Co(NH_3)_5(Az)]Cl_3$ (**347**), *cis*-$[Co(en)_2(Az)_2]Br_3$ (**348**), and $[Cr(NH_3)_5(Az)](OSO_2CF_3)_3$ (**349**) were produced in a similar manner from known triflato precursor complexes. The stereochemistry of the aziridine complexes was assigned from ^1H and ^{13}C NMR spectroscopy and single-crystal X-ray analysis. *trans*-tetrakis$[(Az)NO_2)]_2$−Co(III) bromide−LiBr dihydrate was shown to crystallize in the orthorhombic space group *Ccca* with cell constants $a = 9.121(2)$ Å, $b = 15.836(2)$ Å, $c = 12.764(1)$ Å, $V = 1836.7(5)$ Å^3, and $Z = 4$. Cyclic voltammetry of **339**, **340**, **342**, **346**, and **347** showed that the Co(III) complexes were reduced to Co(II) species in an irreversible process, even at fast scan rates. Testing of the complexes for cytotoxic activities in hypoxic cell cultures showed activity, suggesting facile reduction of the Co(III) ion and release of aziridine under physiological conditions.

A complex of Co(III) coordinated with a nitro group and a bis(2-chloroethyl)amine, i.e., nitro-bis(2,4-pentanedionato)[bis(2-chloroethyl)amine]Co(III) [Co(BCA)], was also prepared[409] and the activity compared to that of nitro-bis(2,4-pentanedionato)(pyridine)Co(III) [Co(Py)]. Co(BCA) was significantly more toxic toward EMT6 cells than Co(Py). Under hypoxic conditions, Co(BCA) is an efficient RS (SER of 2.4), capable of increasing the life span of tumor-bearing mice (L1210 leukemia) at a drug dose 20-fold less than the lethally toxic dose.

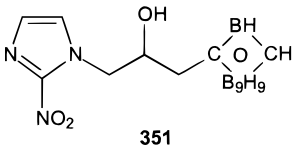
C. Boronated Radiosensitizers

The selective retention of substituted 2-nitroimidazoles in poorly vascularized hypoxic tumor tissue

was used to improve boron delivery for neutron activation therapy. ^{10}B atoms, as carborane groups, have been linked to 2-NO_2Im to yield derivatives **350** and **351**.[410] The synthesis involves the reaction of

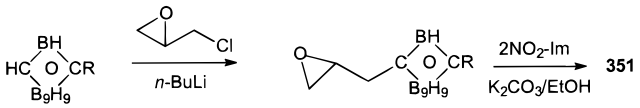


350



351

m-carborane **352** with *n*-BuLi and epichlorohydrin to yield **354a**, which without purification was treated with 2-NO_2Im. Although the desired product **351** was formed, the yield was low and purification difficult. An alternative reaction sequence commences with the thermal rearrangement of *o*-carborane to yield the trimethylsilyl-*m*-carborane **353**, which upon monoalkylation with *n*-BuLi and treatment with epichlorohydrin in THF gave **354b**. Facile removal of the trimethylsilyl protecting group and coupling with 2-NO_2Im gave **351** in 75% yield. Water solubility of



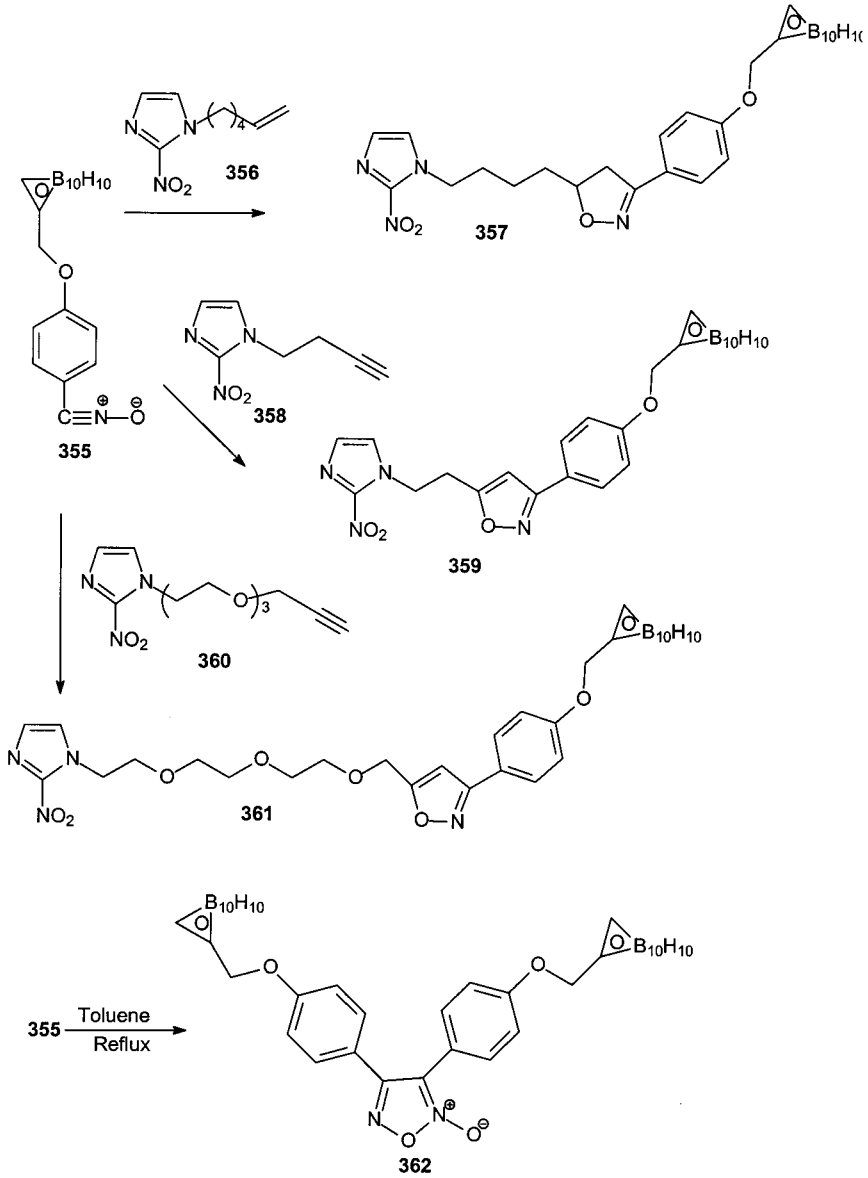
352: R=H
353: R=$SiMe_3$

354
354a: R=H
354b: R=$SiMe_3$

the carboranyl-2-NO_2Im **351** is, however, very low. The *o*-carboranyl derivative readily gives the anionic *nido*-derivative at room temperature in pyrrolidine, i.e., the water-soluble 1-(3′-*o*-[*nido*]carboranyl-2′-hydroxy)propyl-2-NO_2Im) with an octanol/water partition coefficient of 0.05. The latter compound exhibits hypoxia-selective toxicity in vitro and in a rodent tumor model at a dose level similar to that of misonidazole. Attempts to convert the *m*-carboranyl derivative to the anionic *nido*-carborane derivative in pyrrolidine failed, even at elevated temperature and longer reaction time.

In most cases, carboranes are readily formed by the prolonged reaction of alkynes and decaborane ($B_{10}H_{14}$) in the presence of boiling Lewis bases. However,

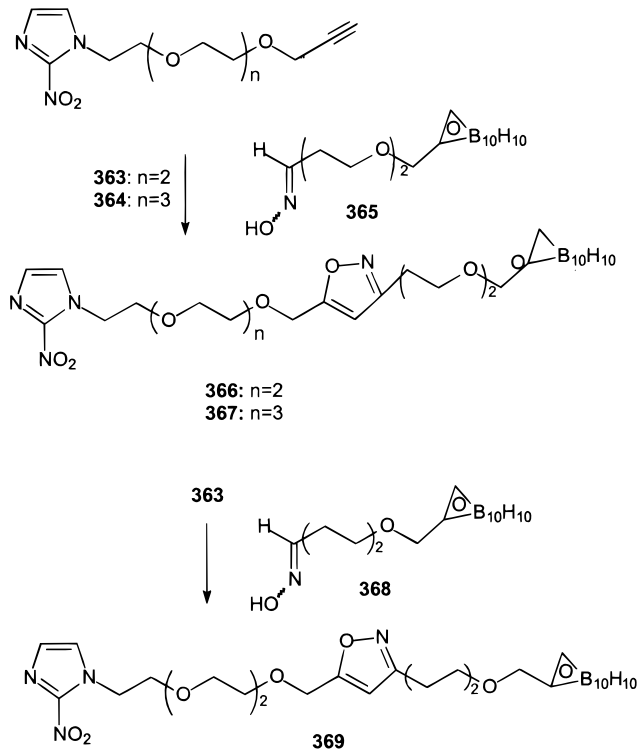
Scheme 56



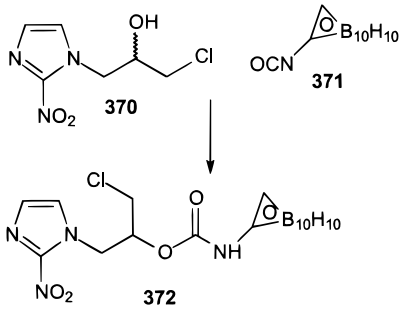
direct treatment of 1-but-3-ynyl-2-NO_2Im with $B_{10}H_{14}$ gave polar degradation products. Instead a mild procedure was developed for the 1,3-dipolar cycloaddition of the 4-(carboranylmethoxy)benzonitrile *N*-oxide (**355**) and nitroimidazolylalkenes and nitroimidazolylalkynes (Scheme 56).[411,412] The potassium salt of 2-NO_2Im is reacted with 6-bromohex-1-ene and but-3-ynyl tosylate in hot DMF to give the alkene and alkyne derivatives. Oxidative elimination of the carboranyl oxime in aqueous sodium hypochlorite gives an intermediate nitrile oxide **355**, which is reacted with nitroimidazolylalkene **356** and nitroimidazolylalkynes **358** and **360** to yield the dihydroisoxazole- and isoxazole-linked compounds **357**, **359**, and **361** (Scheme 56). The formation of the intermediate is rapid and products are stable. When compound **355** was heated in boiling toluene, a 1,2,5-oxadiazole-2-oxide **362** was formed. These complexes were not further pursued for biological applications due to their low water solubility.

To increase their solubility, multiple oxyethylene units (OCH_2CH_2O) have been used as spacer chains between the carborane and nitrile moieties. A polyether spacer chain was introduced via the reaction of 3-{2-[2-(2-chloroethoxy)ethoxy]ethoxy}prop-2-yne with 2-NO_2Im. The oxidation by sodium hypochlorite of oxime **368** (equimolar mixture of geometrical isomers) gave the corresponding nitrile oxide that reacted in situ in a two-phase aqueous dichloromethane system with the nitroimidazolealkyne **363** to give the nitroimidazole−isoxazole−carborane **369** in good yield. In a similar manner, the bis-ether-linked carborane−oxime **365** and the dipolar cycloaddition product of nitroimidazolealkynes **363**, **364** containing three and four ether groups were synthesized. Treatment of the mixture of stereoisomers **365** with sodium hypochlorite gave the corresponding nitrile oxide, which was coupled in situ with the alkynes **363** and **364**, to give **366** (13%) and the long-chain analogue **367** (36%) (Scheme 57).

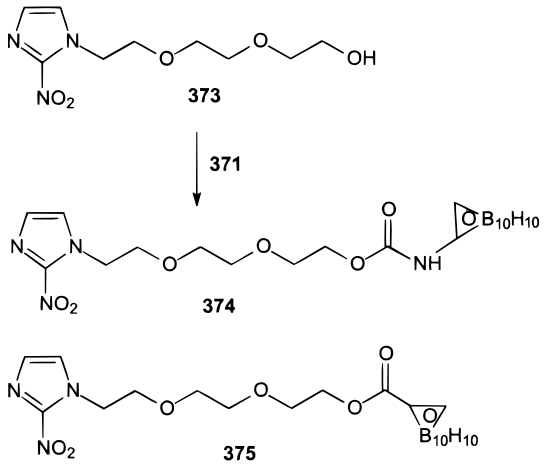
Scheme 57



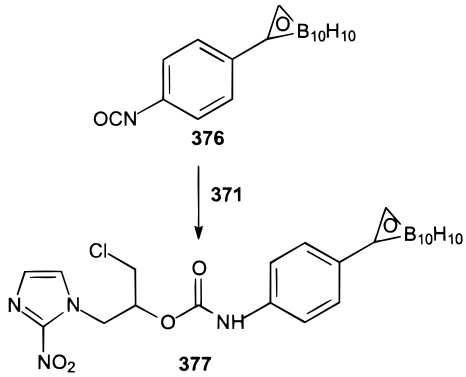
Carbamates, which are generally stable under physiological conditions, have also been used for the coupling of carborane and NO_2Im. Treatment of the isocyanate **371** with 1-(chloromethyl)-2-(2-NO_2Im-1-yl)ethanol (**370**) gave the carbamate-linked nitroimidazolylcarborane **372** in moderate yield. Better yields



were obtained when a primary alcohol was used.[413] Thus, the treatment of **371** with **373** gave the NO_2-Im−borane complex **374**. The corresponding ester-linked product **375** was isolated in low yield.



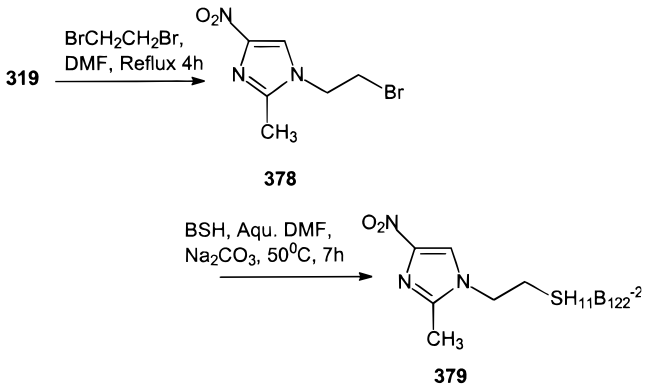
Coupling was also accomplished via a carboranylphenyl*iso*cyanate intermediate **376**. Treatment of 4-(1,2-dicarba-*closo*-dodecaboran(12)-1-yl)aniline with phosgene under basic conditions afforded the phenylisocyanate **376**, which reacted smoothly with the secondary nitroimidazole alcohol **370** to furnish the carbamate-linked nitroimidazolylcarborane **377**.[414]



The biodistribution of the nitroimidazole−carboranes **367** and **374** was examined in a murine tumor model using ^{11}B NMR. At 0.8 mmol/kg (i.p.) injected dose, tumor and brain ^{11}B uptake was detectable, although higher ^{11}B levels were reached in the liver.[415] Early tumor/brain activity ratios reached 2−3 with maximum liver/tumor ratios of 5−6. To study the biodistribution in more detail, the isoxazole-linked nitroimidazole−carborane was also labeled with tritium.[416]

An ester-linked boronated acylmetronidazole was also reported. The initial experiment included coupling of **286** with bromoacetyl bromide followed by coupling of bromoacetyl with $Cs-10B_{12}H_{11}SH$ (BSH, borocaptate). The resulting boranated acylmetronidazole was, however, unstable at pH 6.0. A more stable thioether linkage was prepared (Scheme 58)

Scheme 58

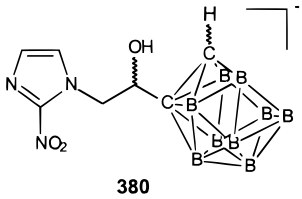


via the reaction of 1,2-dibromoethane with 2-methyl-5-NO_2Im (**319**) to yield 1-(2-bromoehtyl)-2-methyl-5-NO_2Im (**378**), which upon Cs salt coupling with BSH gave the desired 1-[2[(undecahydro-*closo*-dodecaborato)thio]ethyl)-2-methyl-5-NO_2Im (**379**).[417]

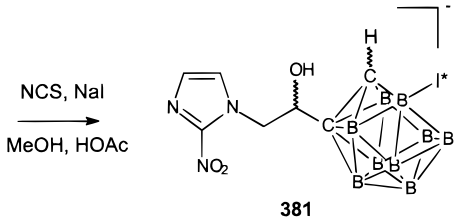
D. Radiolabeled Metalloradiosensitizers as Imaging Agents

Knowledge of the existence and extent of hypoxic regions of tumors in cancer patients is important information for the selection of an optimal treatment protocol. As discussed earlier, nitroimidazole (NO_2-Im) derivatives are electron-affinic molecules which are retained in oxygen-poor tissues through participation in anaerobic, metabolic pathways. Accordingly, there has been considerable interest in the use of radiolabeled NO_2Im derivatives for noninvasive imaging of hypoxia. Most NO_2Im derivatives are lipophilic, enabling them to diffuse readily across cell membranes. The potential to visualize hypoxic tissues has been successfully demonstrated with several radiohalogenated derivatives.

Apart from nuclear imaging of medical conditions, radiolabeled sensitizers have been used to study the pharmacokinetics of the parent drugs. Thus, boronated−NO_2Im complexes, developed for neutron capture therapy, have been labeled with γ-emitting radionuclides such as radioiodine ^{131}I or ^{123}I.[418] Iodination of the diastereoisomeric *nido*-carboranyl derivative **380** with 1.5 equiv of *n*-chlorosuccinimide (NCS) and 1.1 equiv of NaI in MeOH contain 10% HOAc to yield **381** was accomplished in 95% yield.



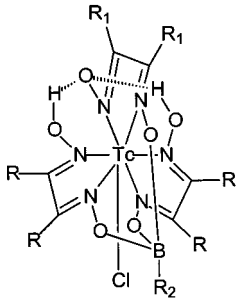
380



381

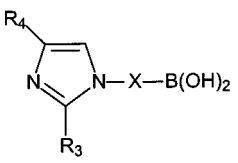
Radioiodination with NCA ^{131}I, using similar conditions, resulted in formation of the diasteroisomeric isomers in 93% yield.

A boronic acid adduct of technetium dioxime ($^{99m}Tc-BATO$) was developed as an organ perfusion radiopharmaceutical.[419] Coupling of $^{99m}Tc-BATO$ with NO_2Im derivates gave the first examples of ^{99m}-Tc complexes which could undergo enzymatic reduction. A number of NO_2Im substituted with alkyl and aryl boronic acids were synthesized,[420] chelated with ^{99m}Tc, and tested for their capacity to localize in hypoxic tissues.[421] The preparation of the corresponding complexes with the long-lived ^{99}Tc isotope permitted chemical characterization. Several $^{99}Tc-BATO$ compounds of the general formula $TcCl(dioxime)_3BR$ (**382**), containing a boron cap R, which incorporates a 2- or 4-NO_2Im, were prepared using $TcCl(dioxime)_3$ or $Tc(dioxime)_3(\mu\text{-}OH)SnCl_3$ [dioxime = dimethyl glyoxime (DMG) or cyclohexanedione dioxime (CDO)] as starting material. The nitroreductase enzyme,



382

R = CH_3 or $(CH_2)_3$
R_1 = Subsstiteunts containing the NO_2Im

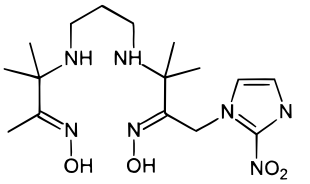


	R_3	R_4	X
a:	NO_2	H	CH_2-*p*-C_6H_4
b:	NO_2	H	CH_2CH_2-p-C_6H_4
c:	H	NO_2	CH_2-p-C_6H_4
d:	CH_3	NO_2	CH_2-p-C_6H_4
e:	NO_2	H	$CH_2CH_2CH_2$
f:	NO_2	H	$CH_2CH(OH)CH_2OCH_2$-p-C_6H_4
g:	NO_2	H	$CH_2CONHCH_2$-p-C_6H_4

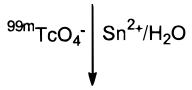
xanthine oxidase, in the presence of hypoxanthine, was shown to reduce the NO_2Im group of $^{99}TcOH(DMG)_3BBNO_2$ and $^{99}TcOH(DMG)_3BprenNO_2$ under anaerobic conditions. However, compared to misonidazole, the rate of reduction (both electrochemically and enzymatically) is slow, limiting the suitability of these compounds for imaging of hypoxic regions in vivo.

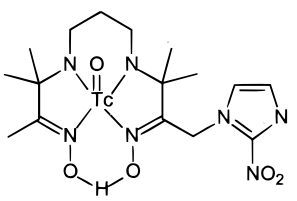
A 2-NO_2Im derivative of the well-known Tc(V)−oxopropyleneamine oxime (PnAO) complex BMS-181321, i.e., ^{99m}TcO(PnA-1-(2-nitroimidazole) (**384**), was prepared from 3,3,9,9-tetramethyl-1-(2-nitro-1*H*-imidazol-1-yl)-4,8-diazaundecane-2,10-dione dioxime (**383**).[421] The analogous long-lived ^{99}Tc complex was synthesized by the stannous tartrate reduction of TcO_4^- or by ligand exchange from $[TcO(ethylene\ glycol)_2]^-$. The identity of the compound was established using chromatographic analysis, spectroscopy, and X-ray crystallography. The ligand **383** is chiral and forms two Tc complexes as enantiomeric pairs, which were separated by HPLC, Scheme 59. Upon coordination to the Tc complex, the nitro redox potential of the NO_2Im moiety is slightly more positive. The rate of reduction of the Tc−NO_2Im−PnAO complex BMS-181321 is faster than that of the Tc−NO_2Im−BATO complexes, suggesting that the former has potential applications as a hypoxia imaging agent in cardiac and neurological conditions. BMS-181321 was preferentially retained in ischemic, but viable canine myocardium and its uptake was shown to be inversely related to the regional myocardial blood flow. Furthermore, the trapping of this ^{99m}Tc−nitroimidazole complex in myocardium was shown to be inversely related to the level of available oxygen.[422] Although enhanced retention of BMS-181321 was detectable by ex vivo SPECT imaging, an unfavorable heart-to-liver ratio was observed with in vivo planar imaging in a canine model, which may limit its use in clinical myocardial imaging.[423] The chemical modification of BMS-181321 (**383**) to ^{99m}Tc−5-oxaamineoxime nitroimidazole (BMS-194796) was recently shown to improve ischemic myocardia target-to-background ratios.[424]

Scheme 59



383





384

A recent finding with ^{99m}Tc complexes lacking the NO_2IM moiety suggests the latter may not be required for hypoxia selectivity (EORTC/NCI Conference, Amsterdam 1998).

In conclusion, this review reflects on the important role that metals can exert on the biological activity of both photo- and radiosensitizers.

III. Acknowledgments

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