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Quo vadis porphyrin chemistry?

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Summary

This review summarizes recent developments in the area of porphyrin chemistry in the direction of biological applications. Novel synthetic methodologies are reviewed for porphyrin synthesis, porphyrin analog synthesis, stable porphyrinogens - calixpyrroles, expanded porphyrins. Unique biological properties of those compounds are desribed with focus on photodynamic therapy (PDT) and molecular recognition properties. Special attentions given to metalloporphyrins with potential to affect heme degradation and CO formation.

Key words

Porphyrins • Synthesis • Expanded porphyrins • Photosenzitizers • Molecular recognition • Metalloporphyrins

1. Porphyrin skelet in nature

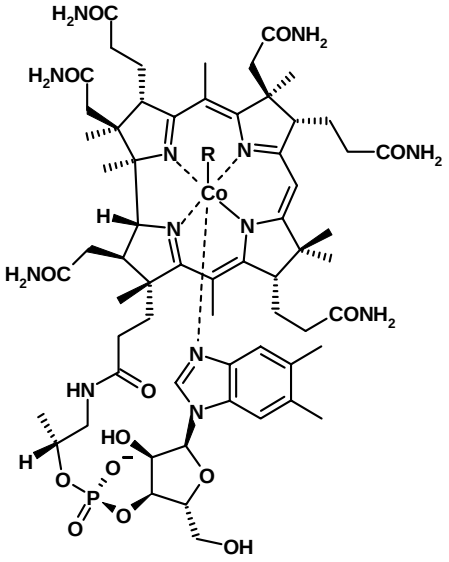
Naturally occuring porphyrins are synthesized by living matter. Among the best known natural structures utilizing porphyrin skelet are vitamin B_{12} (Fig. 1), chlorophyll (Fig. 3), uroporphyrins, coproporphyrins and heme (Fig. 2).

In the natural system, vitamin B_{12} is known to have a contracted porphyrin framework which is known as *corrin* (Battersby 1994).

Heme, iron-containing tetrapyrrole, is indispenseable for life. It is utilized by a whole host of proteins involved in numerous cellular processes such as oxygen transport (hemoglobin), respiration (cytochrome oxidase), vascular homeostasis (nitric oxide synthases), detoxification (cytochromes P450), and cell death (cytochrome c). Heme is produced in the mitochondrion by a complex cellular machinery comparising eight enzymes that are evolutionarily conserved from bacteria to humans.

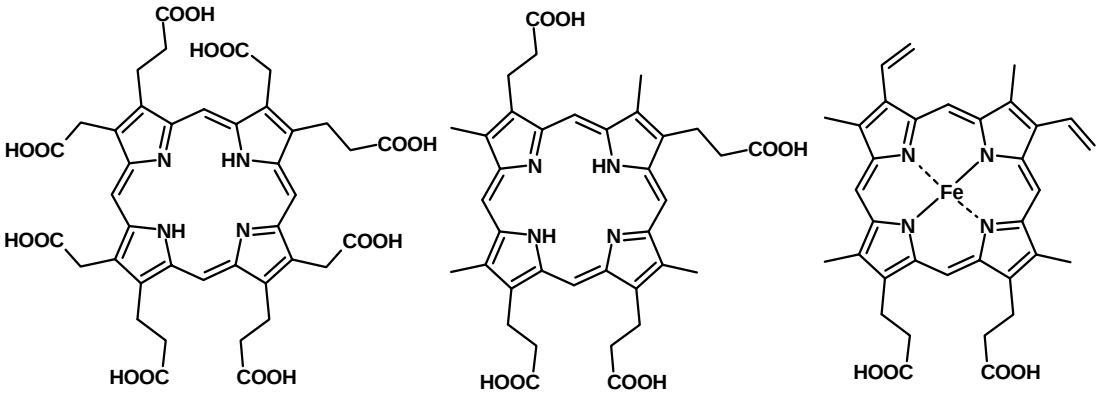
Hem is ferroprotoporphyrin complex. The basis of the structure is the porphyrin skelet, which is formed by four pyrroles linked with four methine bridges. The substituents, four methyls, two vinyls and two propionic side chains, in beta positions of pyrroles, can be arranged by fifteenth modality, but only one of these isomers, called Protoporphyrin IX, is present in living systems. The biological functions are ensured by its metallocomplex with iron.

Chlorophyll is one of the prevalent spread structures utilizing a porphyrin skelet. This structure is present in all green plants. In this structure, porphyrin form a complex with magnesium, and the magnesium complex is the key compound in photosynthesis. The main purpose of this magnesium complex is in absorption of irradiation. The absorption of the photons is attributed



Vitamin B_{12} (R = CN)
Coenzyme B_{12} (R = 5'-deoxyadenosyl)
Methylcobalamin (R = CH_3)

Fig. 1.



Uroporphyrin III | Coproporphyrin III | Heme (Protoporphyrin IX)

Fig. 2.

to π-electrons in conjugated double bonds of molecules of chlorophyll.

2. Synthetic porphyrins

These porphyrins form the second part of porphyrins, porphyrins which are not present in nature and human body. Therefore their synthesis in laboratory is the only way that they can be obtained. Nowadays, many porphyrins have been synthesized. These structures are derived from the simplest porphyrin called *porphyne* (Fig. 4).

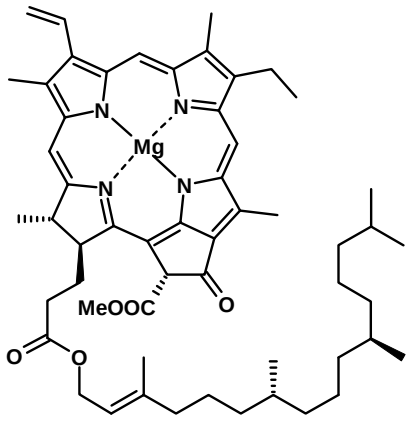


Fig. 3. Chlorophyll a

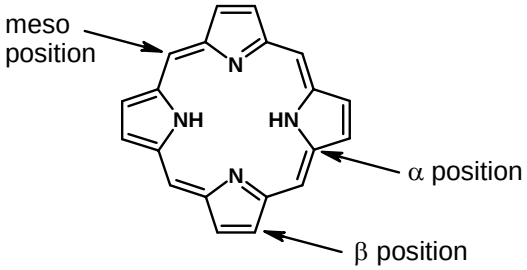


Fig. 4. Porphyne – the simplest porphyrin

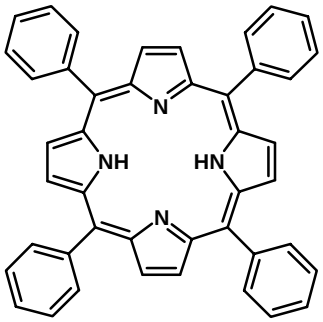


Fig. 5. *meso*-tetraphenylporphyrin

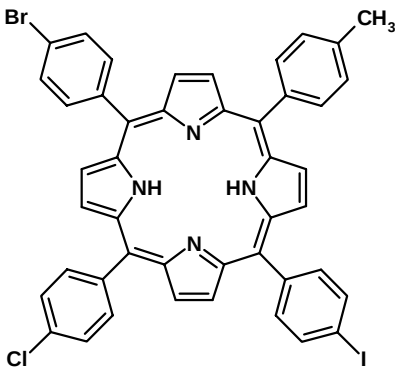


Fig. 6. Asymetric porphyrin

Porphyrins (which comes from the Greek πορφυροσ means "purple, scarlet") are based on 16-atom rings containing four nitrogen atoms. They are macrocycles that contain only sp^2-hybridized bridging meso carbon atoms within their framework. The structure is fully aromatic, contains 18 π-electrons. They are of perfect size to bind nearly all metal ions.

By substitution of hydrogens in the *meso*-position of some substitutents, the porphyrins are obtained. Depending on synthesis, the substituents in the *meso*-position can either be the same or different.

The basic porphyrin skelet can be synthesized by several routes based on condensation reactions between aldehydes, pyrroles, dipyrrylmethanes or similar precursors under acidic conditions and following oxidation. The first synthesis of porphyrin - tetraphenylporphyrin (TPP) (Fig. 5), was first accomplished using benzaldehyde and pyrrole in 1936 by Rothmund (Rothmund 1936). Since that time, a series of both symetrical and asymetrical porphyrins, has been prepared.

Symmetrical porphyrins are more easily synthesized than a asymetrical porphyrins. Their synthesis is based on condensation of pyrrol and aldehyde whereas various reaction conditions, for example *Adler-Longo conditions* (Adler *et al.* 1967, Dattagupta *et al.* 1981, Dattagupta *et al.* 1988, Kamogawa and Koga 1992, Tamiaki *et al.* 1993, Hombrecher and Ohm 1993, Matile *et al.* 1995, Neya and Funasaki 1997, Momenteau *et al.* 1983, Reddy and Chandrashekar 1992), or *Lindsey method* can be used (Lindsey *et al.* 1987, Lindsey and Wagner 1989, Deisenhofer *et al.* 1985, Barkigia *et al.* 1988, Groves and Nemo 1983, Bortolini and Meunier 1984, Bortolini *et al.* 1986, Medforth and Smith 1990, Wagner *et al.* 1991, Cornia *et al.* 1994, Anderson *et al.* 1998, Lindsey *et al.* 1994, Li *et al.* 1997, Onaka *et al.* 1993). As shown below, the first prepared symetrical porphyrin had four phenyl substituents in *meso*-positions (Rothmund 1936).

On the other hand, the **asymetrical porphyrins** are much less synthetically accesible. Ther preparation is based on various approaches as a *Adler-Longo conditions, Lindsey method, 2+2 Porphyrin synthesis* (Sessler and Johnson 1987, Hombrecher *et al.* 1992, Chambron *et al.* 1995, Wang and Bruce 1996, Wilson and Anderson 1996, Proess *et al.* 1992, Ravikanth *et al.* 1998, Abdalmuhdi and Chang 1985, Lecas *et al.* 1984, Lecasnawrocka *et al.* 1984, Maruyama *et al.* 1988, Osuka *et al.* 1989, Sessler and Capuano 1990, Sessler *et al.* 1993, Benson *et al.* 1990, Hombrecher and Horter 1991, Chang and Abdalmuhdi 1983, Sessler *et al.* 1986, Sessler and Piering 1987, Sessler *et al.* 1990, Pandey *et al.* 1992), *3+1 Porphyrin synthesis* (Boudif and Momenteau 1994, Boudif and Momenteau 1996, Chandrasekar and Lash 1996, Lin and Lash 1995, Sessler *et al.* 1996, Lash *et al.* 1998, Hayes and Lash 1998, Berlin *et al.* 1996, Lash and Chaney 1996) or *synthesis of porphyrins from linear tetrapyrrols* (Clezy and Van 1984, Wijesekera and Dolphin 1990, Hin *et al.* 1990, Pandey *et al.* 1992, Lash and Roper 1994, Lin *et al.* 1997, Mansuy 1992).

An example of an asymetric porphyrin prepared by Lindsey (Lee *et al.* 1995). containing four different *meso*-substituents is shown below (Fig. 6).

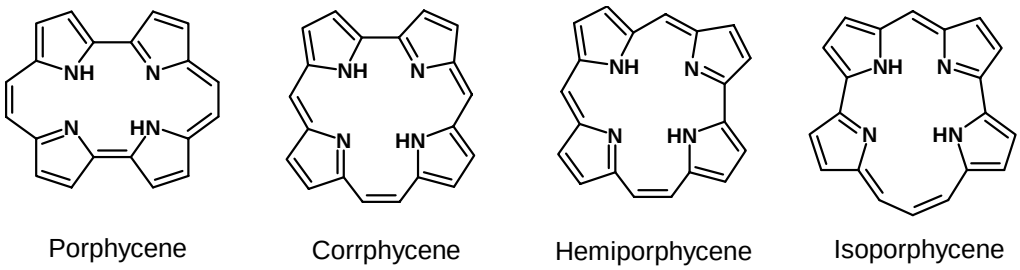


Fig. 7. Porphyrin analogues

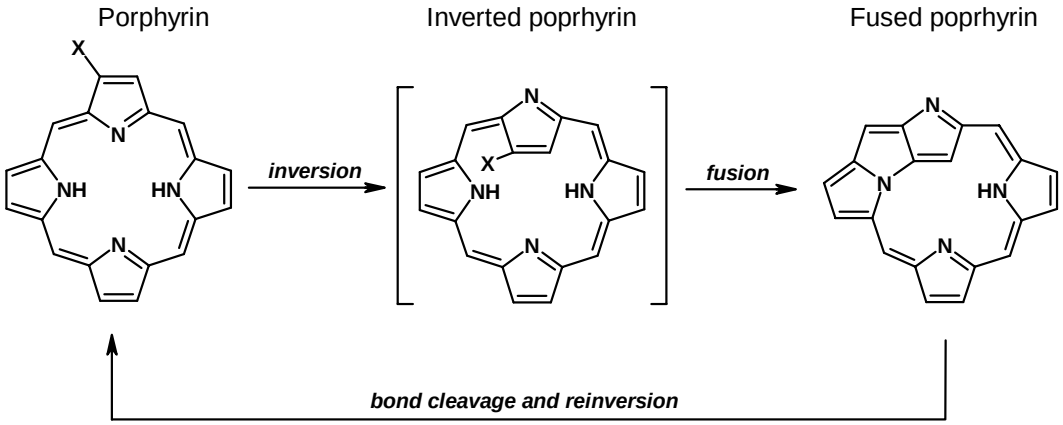


Fig. 8. Inverted and fused porphyrins

3. Analogues of porphyrin

From a single porphyrin, several isomers which can be derived differ by the position of the methine link between pyrrole rings. The study of artificial porphyrin analogs started in 1960s. The first isomer of this type, *porphycene*, ([18]porphyrin-(2.0.2.0) which differs in the pyrrole linking carbon chain ([18]porphyrin-(1.1.1.1), was synthesized by Vogel *et al.* in 1986 (Vogel *et al.* 1986, Gosmann and Franck 1986)

Since then, the other configurational isomers containing the same $C_{20}H_{14}N_4$ composition, such as *corrphycene* ([18]porphyrin-(2.1.0.1) (Sessler *et al.* 1994, Aukauloo and Guilard 1994), *hemiporphycene* ([18]porphyrin-(2.1.1.0) (Callot *et al.* 1995), *isoporphycene* ([18]porphyrin-(3.0.1.0) (Vogel 1996) (Fig. 7) and so on, have been reported.

4. Inverted, confused and fused porphyrins

First, the terms *confusion*, *inversion*, and *fusion* must be defined. In the normal porphyrin framework, α and α‘ linkage is ordinary. *Confusion* is defined as a linkage at the α and β (β‘) positions of pyrroles or other hetero-pentacycles. *Inversion* means that the pyrrole or other pentacycle rings are turning round and *inverted* is a state of pyrrole NH pointing outward. *Fusion* is used for the formation of a tripentacyclic ring by connection of a pyrrole ring to a neighbouring *inverted* pyrrole with its nitrogen (Fig. 8).

N-confused porphyrin (NCP) is a porphyrin isomer that is different largely from the parent porphyrin, particularly in the physical, chemical, structural, and coordination properties. Introduction of the *confused* pyrrole into the *normal* and *expanded* porphyrins leads to generation of the *confused* porphyrinoids, which have rich structural diversity.

The first NCP was synthesized through the Rothemund type reaction, namely, the acid-catalyzed condensation of pyrrole and benzaldehyde, with concurrent formation of *normal* porphyrin. In 1994, Latos-Grazyński *et al.* and another working groups independently isolated a completely different isomer of [18]-(1.1.1.1) type (Latos-Grazyński 1999, Sessler 1994, Geier *et al.* 1999, Furuta *et al.* 1994, Chmielewski *et al.*1994) (Fig. 9).

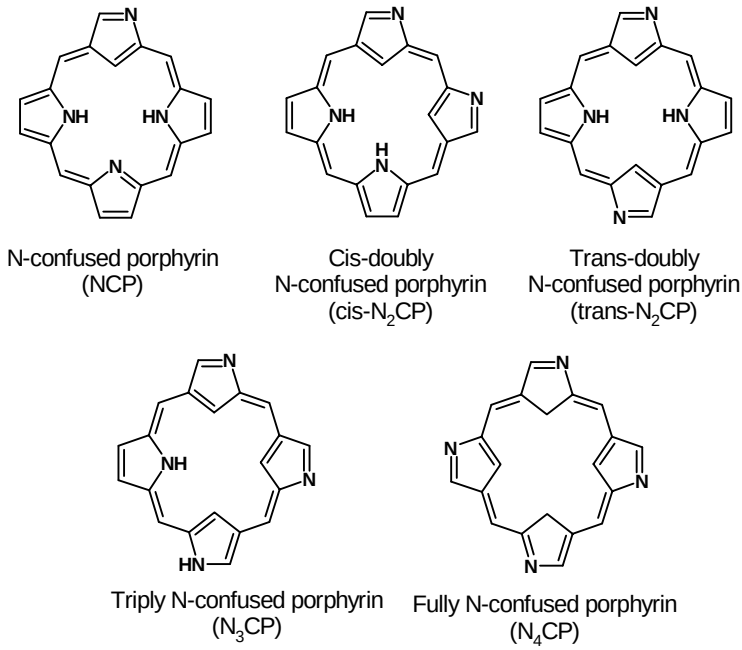


Fig. 9. Confused porphyrins

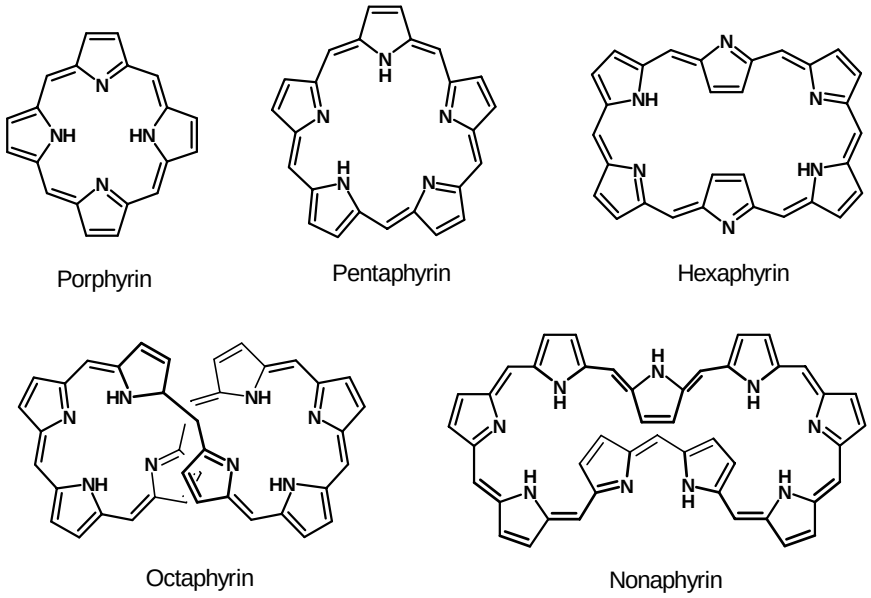


Fig. 10. Expanded porphyrins

5. Contracted and expanded porphyrins

On the other hand, increasing attention has been paid to a class of porphyrin analogs with different core sizes, namely, *expanded* (Fig. 10) and *contracted* (Fig. 11) porphyrins. The higher homolog with all methine-bridges, *pentaphyrin*, was reported by Gossauer in 1983 and shown to sustain a 22 π-aromatic periphery (Gossauer 1983).

In 1964, Johnson *et al.* synthesized the first contracted porphyrin with an 18 π-electron system, *corrole*, wherein one of the *meso*-carbons was missing in the skeleton, by the cyclization of a tetrapyrrolic precursor (Johnson and Kay 1964). Efficient one-pot syntheses of *meso*-substituted corroles were reported recently (Gross *et al.* 1999, Gryko and Jadach 2001). In 1966, Woodward reported the first example of an expanded porphyrin with a 22 π-electron system,

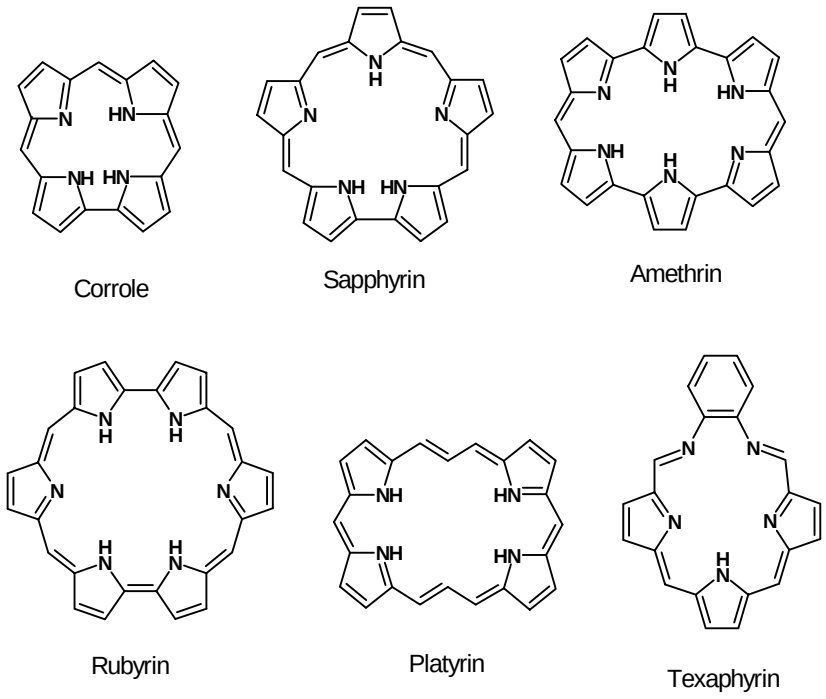


Fig. 11. Contracted porphyrins

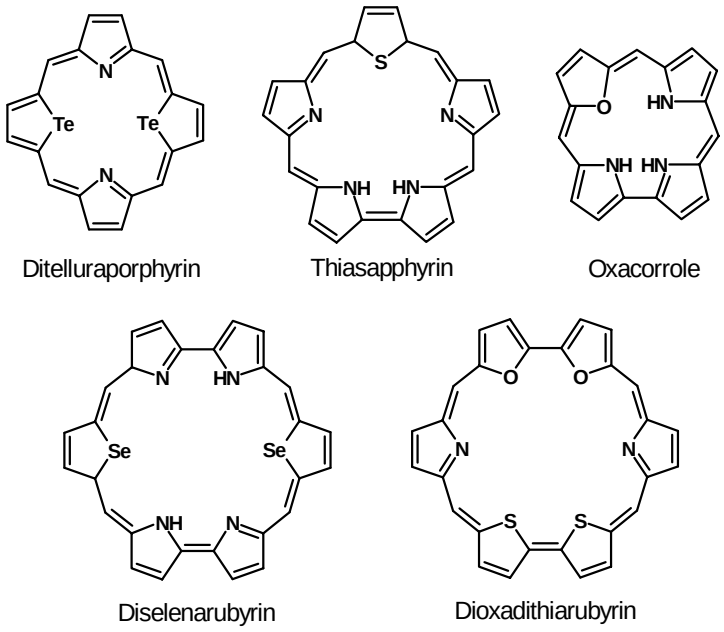


Fig. 12. Porphyrins with heteroatoms

sapphyrin, which contained five pyrrole rings and four *meso*-carbons (Woodward 1966).

6. Porphyrins with heteroatoms

Porphyrin analogs containing heteroatoms such as O, S, Se and Te have also been synthesized (Fig. 12, 13) by the groups of Lee and Latos-Grażyński (Heo *et al.* 1996, Heo and Lee 1996, Lee and Kim 1997, Lee *et al.* 1999, Yoon and Lee 2000, Sprutta and Latos-Grażyński 1999, Pacholska *et al.* 2000, Sprutta and Latos-Grażyński 2001, Pushpan *et al.* 2001).

Furthermore, Lash and co-workers reported syntheses of a series of CNNN- and CNCN-core

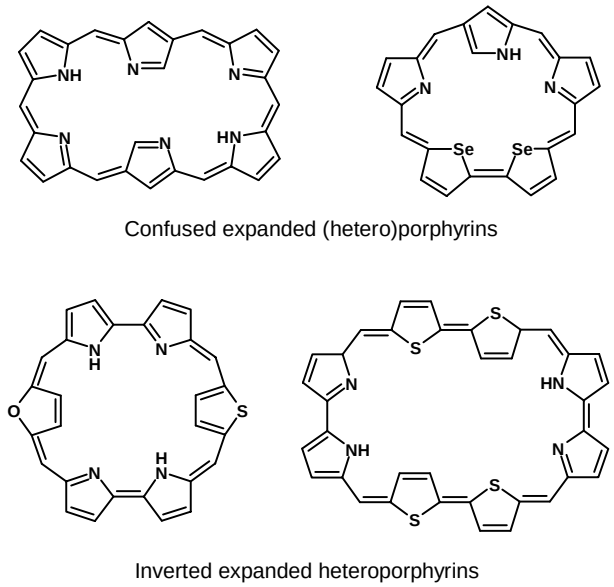


Fig. 13. Confused and expanded porphyrins with heteroatoms

porphyrins including 'true' carbaporphyrins, which contain a cyclopentadienyl unit in the macrocycle (Fig. 14) (Lash and Hayes 1997, Hayes *et al.* 1998, Lash *et al.* 1999).

7. Calixpyrrols

Calix[n]pyrroles are porphyrin analogs that contain pyrroles bridged exclusively by sp^3 *meso* carbon centers. In contrast to porphyrins they are not planar and display remarkable anion-binding properties (Sessler *et al.* 2001). The most simple calixpyrrol – porphyrinogen (Fig. 15) can be seen as a reduced form of porphyne.

Examples of anion binding (Gale *et al.*1996) of chlorine and fluorine are shown below (Fig. 16). Picture **a** shows X-ray structure binding of chlorine and picture **b** – shows X-ray structure binding of fluorine anion.

8. Calixphyrins

Calixphyrins are a class of hybrid molecules that lie at the structural crossroads between porphyrins and calixpyrroles. Calixphyrins encompass all porphyrin analogs that contain a mixture of sp^2- and sp^3-hybridized bridging *meso* carbon centers. In the case of hybrid systems containing four pyrroles, calix[4]phyrins, this definition encompasses systems with one, two, and three sp^2-hybridized bridging *meso* carbons. This leads to partial interruptions in the conjugation pathway of the molecule, introduces novel structural features, and leads to interesting anion and cation recognition properties (Sessler *et al.* 2001). There are known *porphomethenes* (one sp^2-hybridized *meso* carbon atom), *porphodimethenes* (two sp^2-hybridized *meso* carbon atoms, arranged in either a "cis-" or "trans-like" (i.e., 5,10 or 5,15) fashion across the macrocycle), *isoporphyrins* (three sp^2-hybridized *meso* carbon atoms, one NH hydrogen atom), and *phlorins* (three sp^2-hybridized *meso* carbon atoms, three NH hydrogen atoms) (Fig. 17).

In addition to porphyrins, the calixphyrins can also form expanded species (Fig. 18).

9. Applications

Photodynamic theraphy (PDT)

History of PDT

While the term PDT is relatively new, this binary modality of treating diseases can be traced far back in history. The ancient Egyptians used the combination of orally ingested plants (containing light-activated psoralens) and sunlight to successfully treat vitilago over 4000 years ago (Edelson 1988). The use of ultraviolet light and psoralens for the treatment of psoriasis (PUVA) has been accepted throughout the world (Baden 1984). Contemporary PDT began when Raab described, in 1900, the action of acridine dyes and light on *Paramecia*, where he showed that these unicellular organisms could be effectively killed with this combination (Raab 1900). Trappeiner treated, in 1903, a skin cancer with topically applied eosin and light (Tappeiner 1903). In 1913 Meyer-Betz injected himself with 200 milligrams of hematoporphyrin (1) and registered no ill effects until he exposed himself to sunlight, whereupon he suffered extreme swelling, this photosensitivity remained for several months (Laurens 1933, Meyer-Betz 1913). In 1925 Policard examined the ability of porphyrins to produce a phototoxic effect (Policard 1925) and is indeed, the most recent photoactive based drug therapies utilize porphyrin-based chromophores in combination with visible light. Phototherapy was dormant for several decades, although the idea that light could be a therapeutic modality was well explored. For instance, a book published in 1933 lists over a thousand papers exploring UV light for the treatment of a wide variety of ailments, which included arthritis, colitis, lupus, and mental diseases (Gauvain 1933). The usefulness of high dose light might, at first

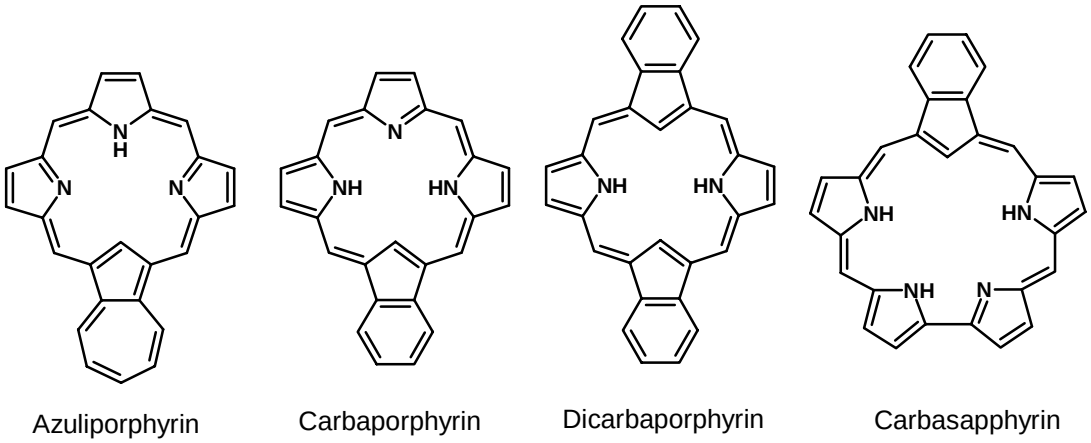


Fig. 14. Carbaporphyrins

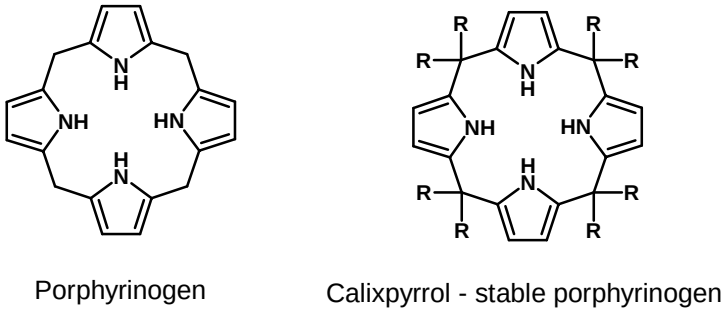


Fig. 15.

sight, not seem rational for the treatment of such diseases but in the case of auto-immune disorders, the immuno-suppressing nature of UV light is now well established (Luger and Schwartz 1995).

Photodynamic therapy (PDT), a new treatment modality, involves administration of a tumor-localizing photosensitizing agent (PS) followed by activation of the agent by light of a specific wavelength resulting in a sequence of photochemical and photobiological processes that cause irreversible photodamage of tumor tissues. *The hallmark of PDT is intracellular oxidative stress mediated by reactive oxygen species* (Fig. 19).

In order to achieve the most efficient photosensitizing effect on tumor cells, the sensitizer must enter the cell and become closely associated with the subcellular structure(s). Photosensitizers may enter cells either directly through the plasma membrane or by endocytosis. Uptake over the plasma membrane may occur by simple or facilitated diffusion or by an active transport mechanism. The incubation parameters and mode of delivery as well as the chemical nature of the photosensitizer (molecular size, charge, water-lipid partition coefficient, concentration), the type and physiological state of the cell, the environmental conditions and the nature of the carrier can all influence subcellular localization, creating a number of potential targets for photodamage (Gomer 1991, Henderson and Dougherty 1992).

Mechanism of the tumor localising effect in PDT

(i) Cancer cells, in common with other rapidly proliferating cells, may have an increased requirement for cholesterol for membrane biosynthesis. They may therefore upregulate the expression of the low-density lipoprotein (LDL) receptor (which recognises the apoB/E lipoprotein) (Maziere *et al.* 1991). It is known that lipoproteins are major carriers of lipophilic porphyrins in the bloodstream (Jori *et al.* 1984) and may therefore be a means of entry of these compounds into cells.

(ii) A decreased intratumoral pH may affect the ionization of porphyrin species with weakly acidic pK values, thus retaining them within tumours (Pottier and Kennedy 1990).

(iii) Tumours often contain increased numbers of lipid bodies and particularly neutral lipid droplets, in addition their cell membranes may be more hydrophobic than

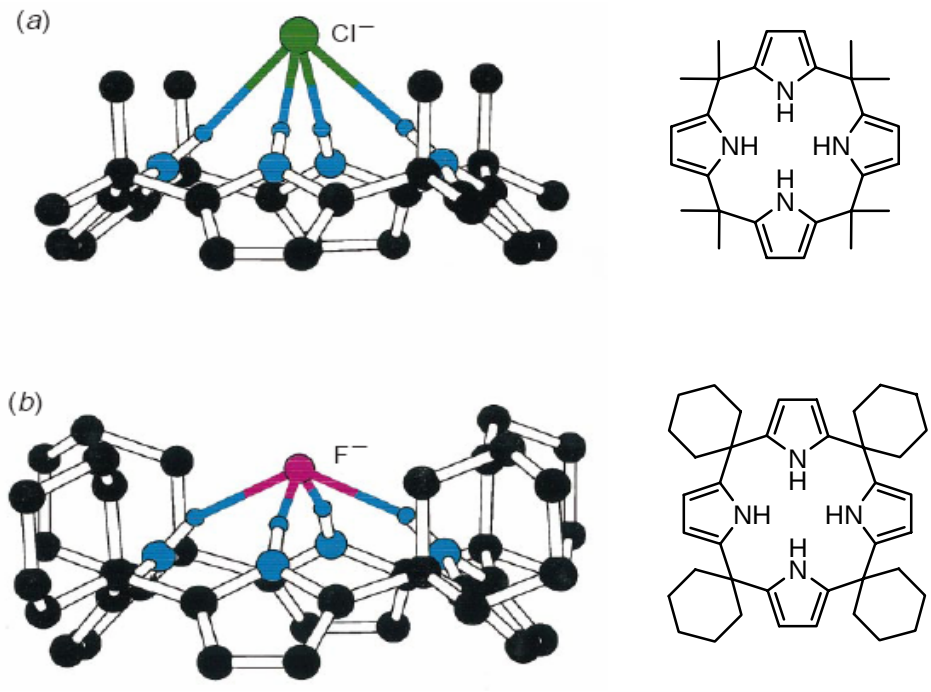


Fig. 16. Calixpyrrols aniont binding

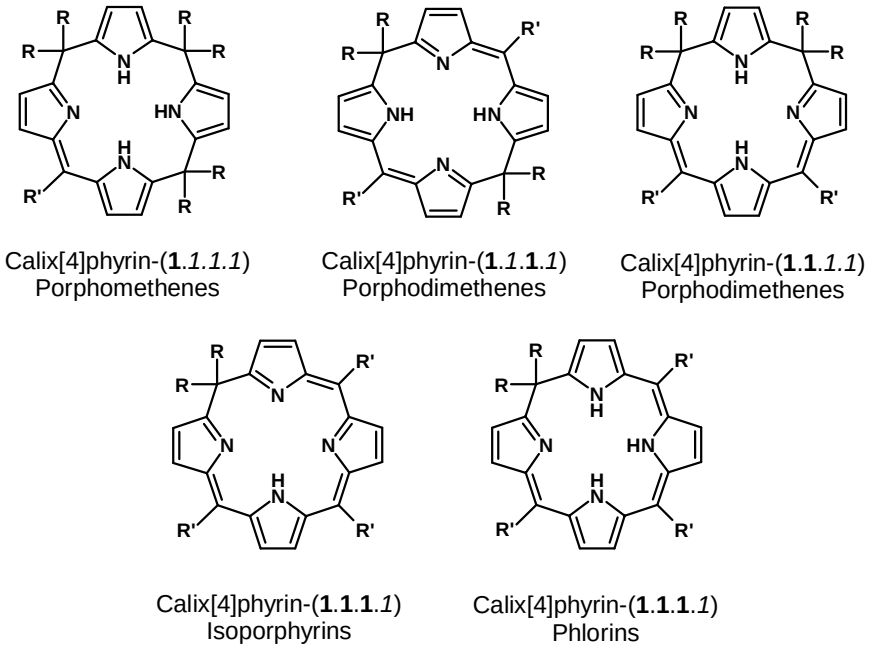


Fig. 17. Calix[4]phyrins

those of normal cells. Both phenomena might explain the accumulation of hydrophobic photosensitisers (Freitas 1990).

(iv) A combination of "leaky" tumour vasculature and reduced lymphatic drainage might encourage the build-up of porphyrins (whether as aggregates or protein complexes) in the interstitial space (Bugelski *et al.* 1981).

(v) Tumour cells may have increased capabilities for phagocytosis or pinocytosis of porphyrin aggregates (Jori 1989).

(vi) Tumour-associated macrophages (TAM) may be largely responsible for the concentration in tumours (Korbelik 1992), Korbelik *et al.* have found that TAM may contain up to nine times the porphyrin levels present in tumour cells (Korbelik *et al.* 1991). Many experimental tumours can comprise up to 80 % TAM (Milas *et al.* 1987).

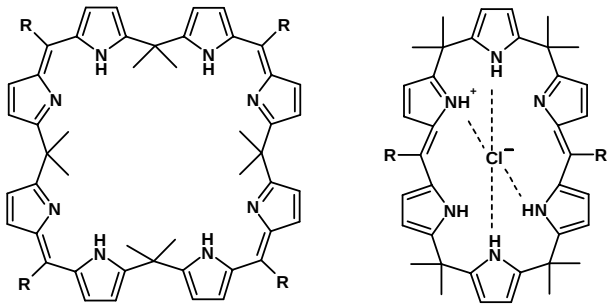


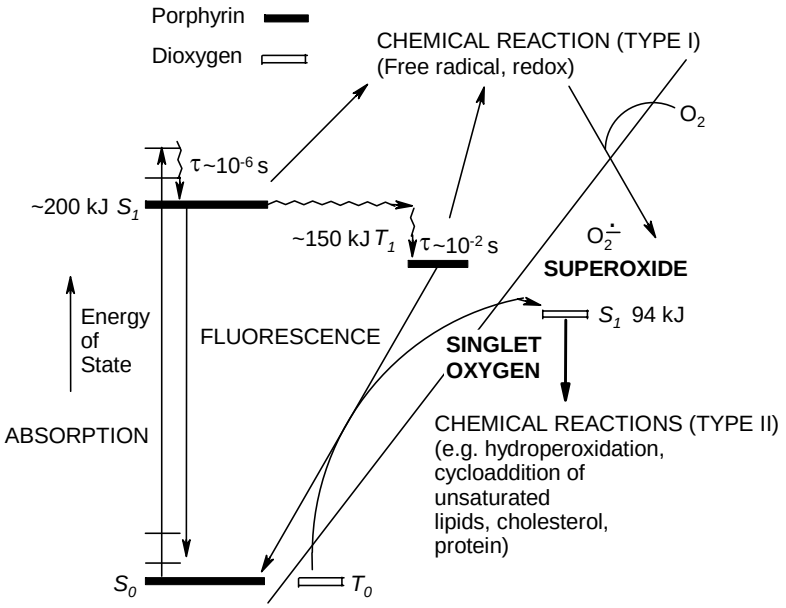
Fig. 18. Expanded calixphyrins

And even in human cancers TAM can make up 20-50 % of the cellular content. A high macrophage content is also a common factor with all the other sites of photosensitiser accumulation listed above.

Photodynamic therapy induces a highly complex series of changes in cells. The sequence of events in PDT are shown in following figure, fromwhich it can be seen that complete establishment of the protocol requires wider study of biochemical and photochemical phenomena (Fig. 20).

It is likely to affect multiple cell targets, of which cell membranes and mitochondria are of particular importance (Kessel and Luo 1999). But which may also include lysosomes, endoplasmic reticulum, DNA and microtubules (Henderson and Dougherty 1992, Morgan and Oseroff 2001, Berg and Moan 1997). Following exposure, cells experience a rapid increase in calcium concentration accompanied or followed by other electrolyte changes as membrane damage progresses. Sublethal damage may, via various signal transduction pathways, result in apoptosis characterized by a drop in mitochondrial potential, concurrent with a drop in ATP level and a decrease in cell respiration, translocation of phosphatidylserine of the plasma membrane, DNA fragmentation, appearance of apoptotic bodies and eventually loss of plasma membrane integrity (Carre *et al.* 1999). The signaling cascades involved in this process are under investigation. The involvement of components of signalling network such as cell surface death receptor Fas (Ahmad *et al.* 2000). tumor necrosis factor (TNF) and TNF-related apoptosis-inducing ligand TRAIL (Granville *et al.* 2001) as well as downstream molecules such as caspases (Granville *et al.* 1997) and Bcl-2 family members (Srivastava *et al.* 2001) have been demonstrated in various PDT-induced models of cell death. Recently, protein phosphorylation as an important regulator of the apoptotic process has been highlighted (Anderson 1997). Apoptotic signalling cascade in photosensitized human epidermal carcinoma cells was mediated by two-stage activation of the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) (Chan *et al.* 2000).

Very essential for in vivo efficacy of PDT is the selective retention of PS in neoplastic tissues. It is determined, among other factors, by the hydrophobicity and aggregated state of the PS, decreased pH in tumors, tumor neovascular effects, poorly developed tumor lymphatics, differences in the stromal cells and heterogeneity of the cells within the tumor (Hasan and Parrish 1996). The asymmetry of charge distribution has also been suggested as an explanation for the higher uptake of PS (Kessel *et al.* 1987). The underlying mechanism reveals a complex interaction of direct and indirect antitumor effects triggered by PDT, which may act to mediate tumor destruction. A direct tumor cell killing results from lethal events initiated by reactive oxygen species. Indirect PDT effects represent necrosis resulting from damage of tumor-associated vasculature with subsequent infarctive death of the tumor cells and initiating a post-treatment immune response directed against tumor cells (Henderson and Dougherty 1992, Dougherty *et al.* 1998). The effects of PDT were found to be modulated by dose, or dose rate changes, conjugations of photosensitizers to lipoproteins or liposomes, or by the addition of chemotherapeutic agents. The response of different tumors to PDT is highly variable, ranging from high sensitivity to extreme resistance. Factors such as photosensitiser localization properties at different levels (tumor tissue, cellular and intracellular distribution) and tumor oxygenation/vascularity have been identified as the parameters determining tumor sensitivity to PDT. However, a number of other physiological properties characterizing individual tumors may exert a marked influence on the therapeutic outcome. One such property appears to be tumor immunogenicity, since immune reaction induced by PDT against treated tumors can substantially contribute to the cure. Local level of nitric oxide (NO), which directly influences multiple events participating in the antitumor effect of PTD, is another



State energies are represented by thick lines: ▬ porphyrin senzitizer, ▭ dioxygen.
Reactive dioxygen intermediates are in bold type.

Fig. 19. Generation of excited porphyrin states and reactive dioxygene species

important, but less recognized parameter (Ali and Olivo 2003). The relevance of this radical, whose production varies considerably in different cancers, to the process of PDT mediated tumor destruction, has been the subject of recent studies.

Many reports in the current literature are confusing, and often apparently contradictory. There is clearly scope for much greater understanding and future studies should more systematically address phenomena in a range of cell types, photosensitizers, and treatment conditions.

Desirable Properties For PDT Drug

The drug (photosensitizer) is the essential part in PDT. An ideal drug should have the following properties:
(i) Proper absorption wavelenagth: Due to light absorption by endogenous chromophores, mainly hemoglobin and light scattering, the effective light penetration through tissue is very poor in the low wavelength region of the visible spectrum (Wilson 1989). As the wavelength increases, the effective light penetration increases as well. Experiments indicate the light penetrates effectively through tissue in the red to the near infrared region (≥650 nm) (Wainwright 1996, Lown 1997). As a result, the ideal drug is one that exhibits a strong absorption in such a region (≥650 nm).

(ii) High preference for accumulation in the tumor: The drug must have a selectivity for enrichment in the tumorous tissue vs the normal tissue. Since singlet oxygen is also detrimental to the healthy tissues, a differentiation of drug concentration between biological compartments must be achieved before the irradiation. This ensures that the efficient destruction of the diseased tissue takes place while the healthy tissue remains intact or experiences less ill effect.
(iii) Low dark toxicity and quick metabolization: The PDT drug itself should be non-toxic in the absence of light. The drug should be excreted or metabolized quickly in a way that does not generate toxic metabolites of any kind after the treatment is complete.
(iv) From the standpoint of chemical synthesis, the drug should be made from readily available materials and the protocol of synthesis should be simple and able to be scaled up to an industrial scale. It should contain groups, such as phenyl group which allows easy derivatization or variation in order to optimize various properties of the drug.
(v) It should exhibit some preferred physical or photophysical properties for drug administration, such as good solubility in water and in the body's tissue fluid, easy formulation (Woodburn *et al.* 1994), high quantum yield of triple formulation, with a triplet energy greater than 94 kJ/mol, and high singlet oxygen quantum yield.

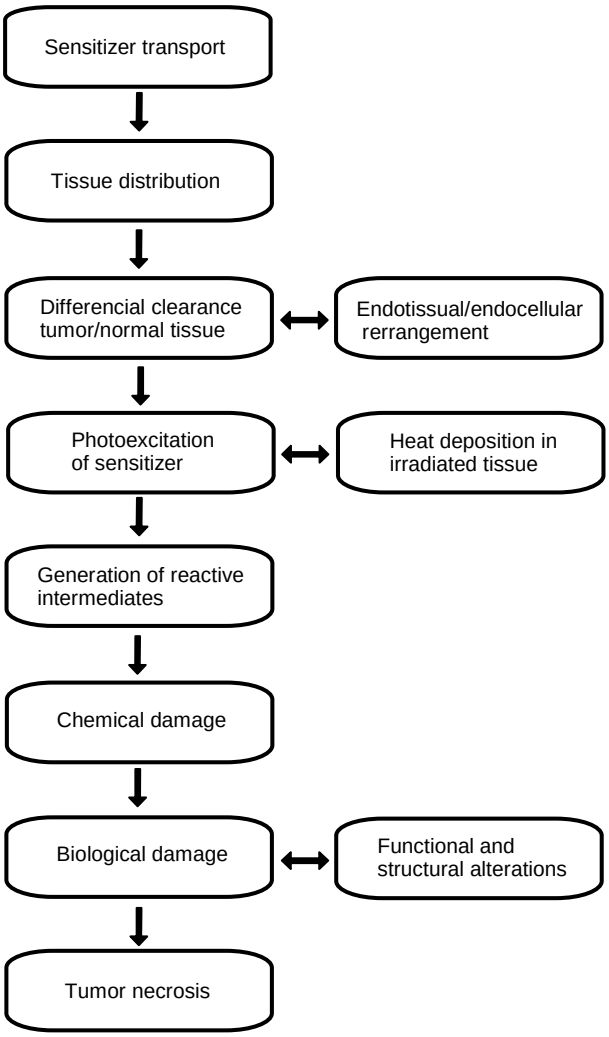


Fig. 20. Sequence of events in PDT

Structure of photosenzitizers

All sensitisers to date are based upon porphyrin-like molecules e.g. porphyrins, chlorins, bacteriocholins and pthalocyanines. The in-vivo photodynamic properties of tetrapyrrolic pigments such as porphyrins has been known since the early 1900's when Meyer-Betz self-administered haematoporphyrin (Hp) to determine its biological effect. To date the water soluble haematoporphyrin derivative (HpD) I, and its purified form commercialised under the trade name Photofrin II, have been used extensively in clinically treating a variety of malignancies. In particular, Photofrin II and complex mixture of porphyrins originated by chemical modification of haematoporphyrin (Hp) (Byrne *et al.* 1990) has been recently approved for the PDT of specific tumors at a clinical level in several countries (McDonald and Dougherty 2001). At present, a few thousand patients have been treated by PDT with Photofrin worldwide with objectively positive results (Schmidt-Erfurth *et al.* 1997, Stewaert *et al.* 1998). HpD is formed by the treatment of haematoporphyrin with a mixture of acetic and sulphuric acids to give a complex mixture of dimers and oligomers. The active component of HpD is believed to be either the dihaematoporphyrin ether II or di-haematoporhyrin ester (DHE). Clinical trials using HpD have proven PDT to be an effective cancer therapy and has shown considerable success in many human tumors. Further various expanded porphyrins have been synthesized and investigated for medical applications such as photodynamic therapy (PDT) (Bonnett 1995). On the following picture some photosenzitizers are shown (Fig. 21).

Saccharide recognition

Porphyrins represent an important class of naturally occurring compounds with unique optical properties. Porphyrins exhibit characteristic sharp and intense absorption maxima in the visible region of spectra (Soret band) and also in fluorescence, both of these properties are very advantageous for analytical applications. The introduction of suitable *meso*-substituent the planar porphyrin core allows to obtain three dimensional cage, cavity and cleft structures, which are effective for substrate entrapping. Taking into account all these factors porphyrin can be considered as perspective sensing molecule for recognition of bioanalyts. Water-soluble porphyrins have been recently extensively studied, mainly due to their possible madico-biological applications. The use of porphyrins and their derivatives (Fig. 22) for molecular recognition of saccharides is a very promising approach in such intriguing problem as molecular recognition of saccharides and modern bioanalytical chemistry (Lu 2006, Dukh *et al.* 2003, Rusin *et al.* 2001, Rusin *et al.* 2002, Murakami *et al.* 1994, Král *et al.* 2000, James *et al.* 1996).

Other applications

We have recently demonstrated (Králová *et al.* 2003) application of designed positively charged porphyrins for antisense and antigen application in terms of facilitated oligonucleotide transport. Leading structures are summarized below (Fig. 23).

Metalloporphyrins in connection with poly(ethylene glycol) (PEG) units have been used as

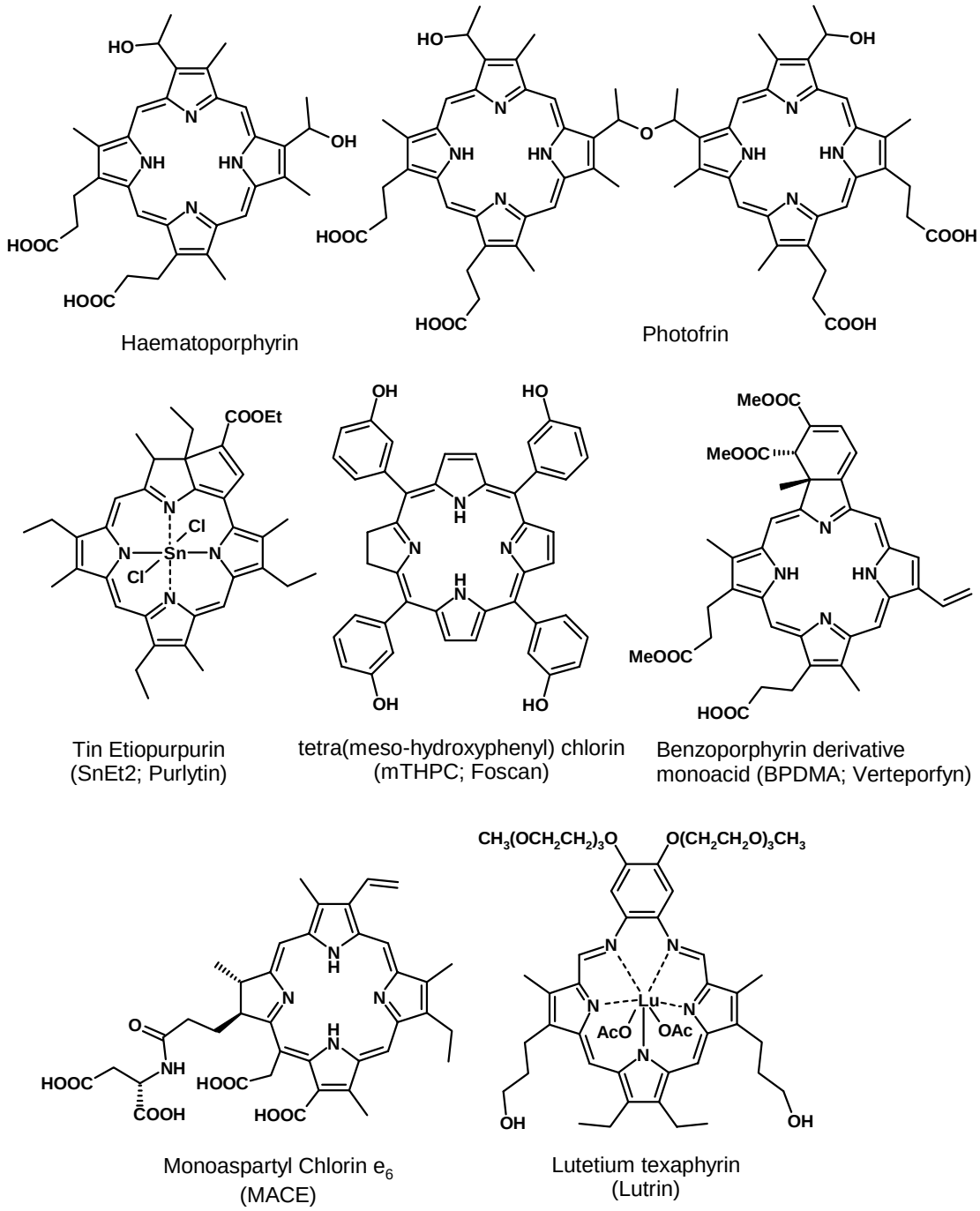


Fig. 21. Photosensitizers approved for use in PDT

oxygen carriers (Tsuchida *et al.* 2006). This system is based on (PEG) conjugated recombinant human serum albumin (HSA) incorporating the synthetic iron-porphyrin (FeP) [PEGylated albumin-heme, PEG(HSA–FeP)] and is a unique albumin-based oxygen carrier as a red blood cell (RBC) substitute.

One rational approach to designing tumor-targeting platinum(II) complexes (Sohn *et al.* 2003) is to introduce a suitable carrier ligand which tends to accumulate in the tumor tissue. Some porphyrins are known to selectively accumulate in the tumor tissue. The tumor-targeting properties of porphyrins are known to be dependent on their hydrophobicity and hydrophilicity balance. In general, the insolubility of most porphyrin derivatives in aqueous solution causes serious problems in biological applications, but some amphiphilic porphyrins are known to selectively accumulate in tumor tissues. A systematic variation of the amphiphilic properties requires a regiochemical arrangement of hydrophobic and hydrophilic substituents in the structure.

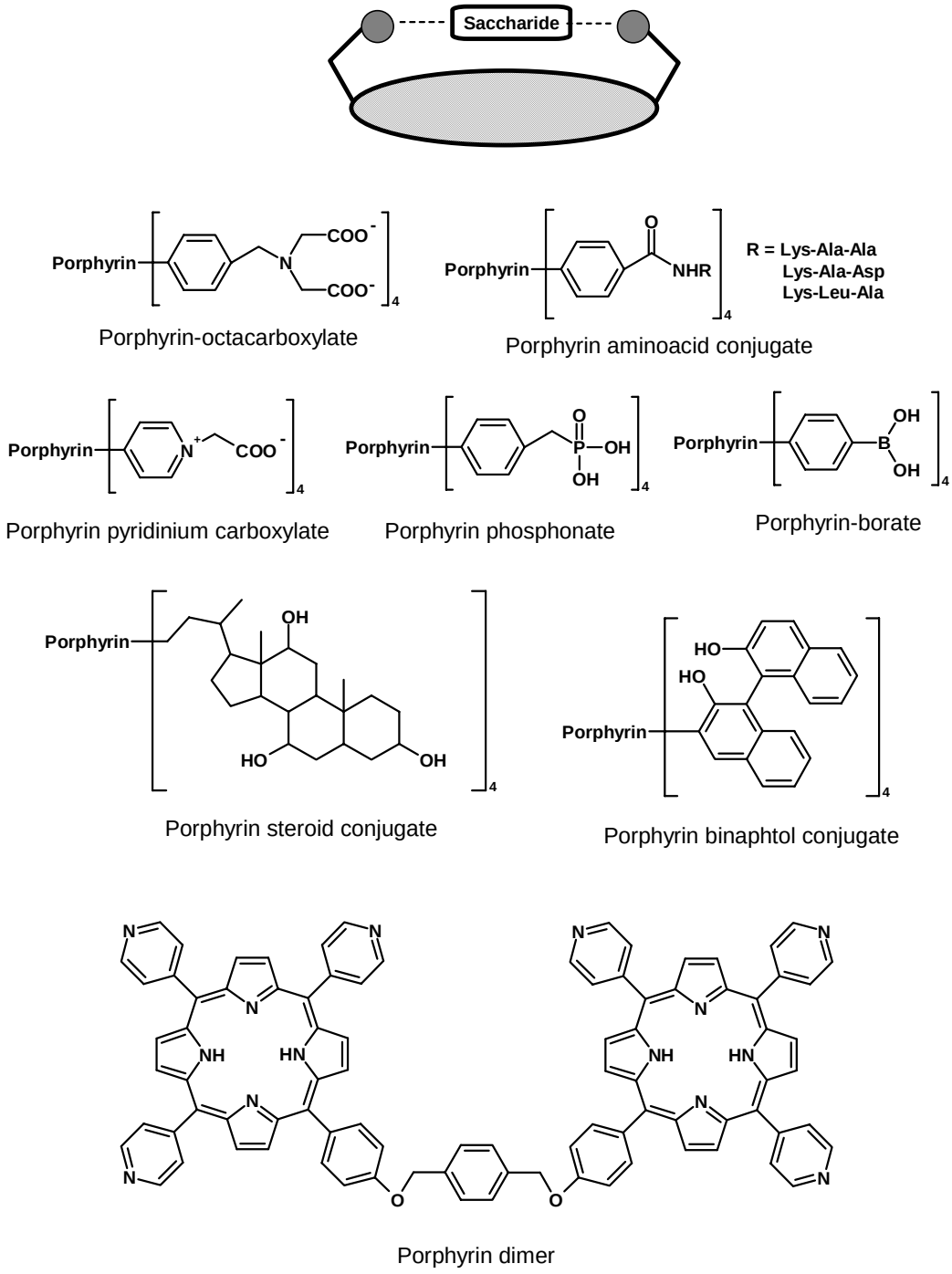


Fig. 22. Synthetic receptors for saccharides

A new series of platinum (II) complexes (Sohn *et al.* 2003) of pegylated hematoporphyrin derivatives with controlled hydrophobic/hydrophilic balance was synthesized by introducing different kinds of poly(ethylene glycol) and amine ligands to the porphyrin ring (Fig. 24).

The antitumor activity of the porphyrin–platinum(II) conjugates was assayed *in vitro* and *in vivo* against the leukemia L1210 cell line and various human tumor cell lines. The present complexes exhibited high antitumor activity and improved water solubility as well as considerable lipophilicity.

Porphyrin-peptide conjugates bearing a nuclear localizing sequence SV40 or a fusogenic peptide (HIV-1Tat 40-60 or octa-arginine) linked by low molecular weight poly(ethylene glycol) have been prepared (Vicente *et al.* 2006) and utilized in *in vitro* studies using human HEp2 cells. The porphyrins were designed to

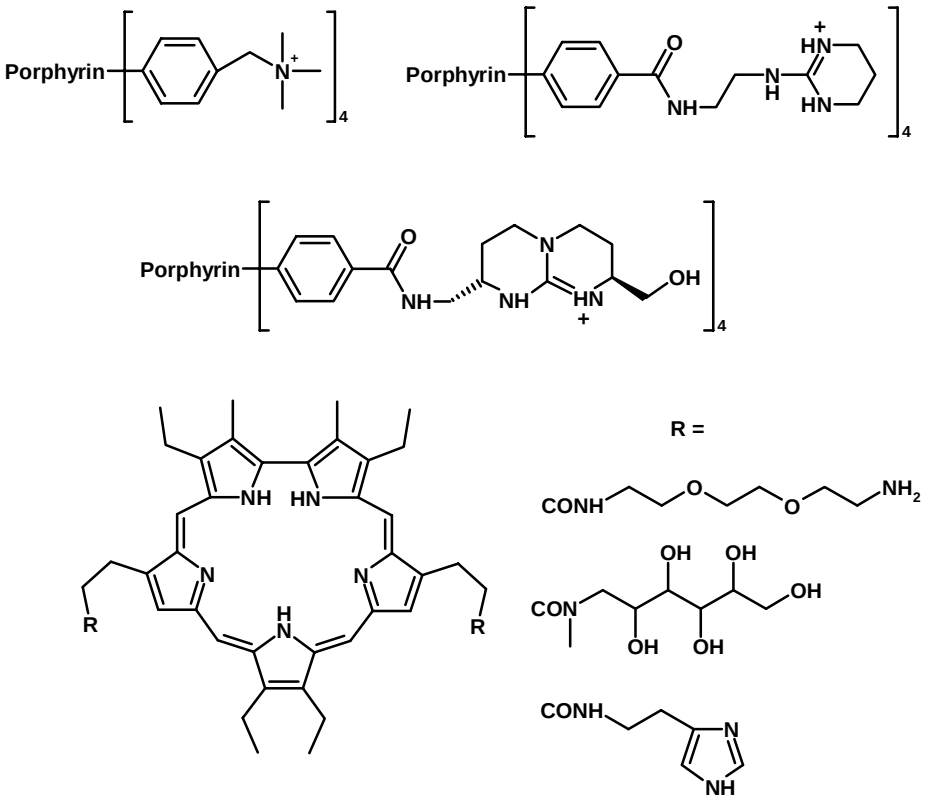


Fig. 23. Oligonucleotide transport agents

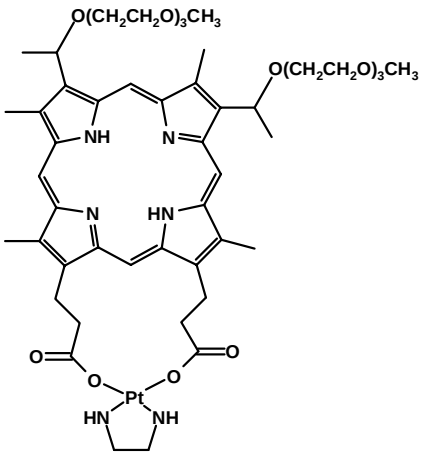


Fig. 24. Platinum (II) complex of pegylated hematoporphyrin

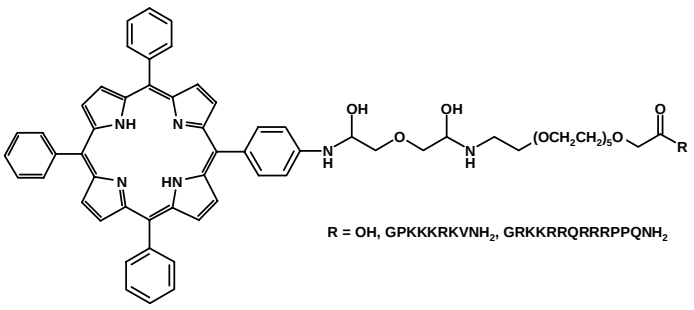


Fig. 25. Porphyrin-peptide conjugate bearing a nuclear localizing sequence SV40 or a fusogenic peptide

contain a peptide sequence (NLS or fusogenic peptide) linked by a low molecular weight PEG in order to minimize intramolecular interactions between the porphyrin and the peptide moieties and to enhance their water solubility (Fig. 25).

Previous studies have shown that PEG-drug conjugates display enhanced water solubility, serum life, and tumor accumulation. The studies show that the cellular uptake of the conjugates depends significantly on the nature and sequence of amino acids in the peptide and on the nature of the substituents on the porphyrin macrocycle. The fusogenic peptide sequences HIV-1Tat 40-60 and octa-arginine were the most effective in delivering the conjugates to the cells.

The new tri(ethyleneglycol)-derivatized Mn(III) porphyrins were synthesized (Dewhirst *et al.* 2006) with

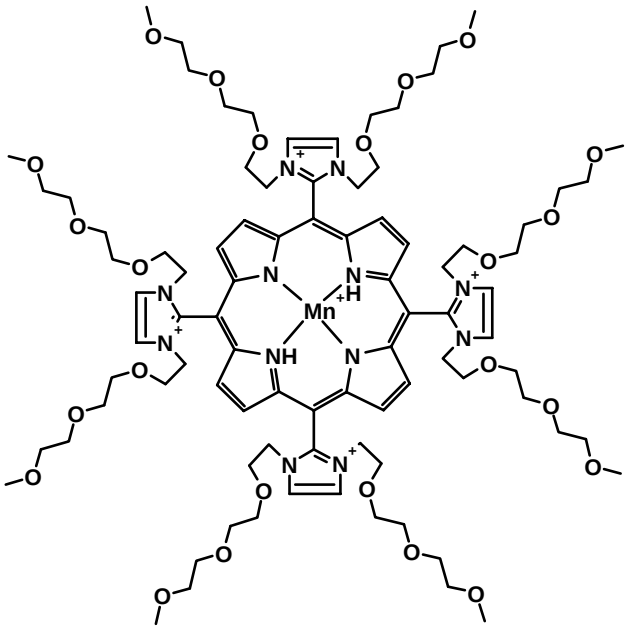


Fig. 26. Tri(ethyleneglycol)-derivatized Mn(III) porphyrin

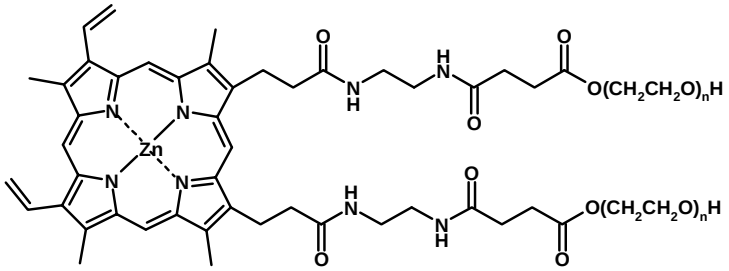


Fig. 27. Pegylated zinc protoporphyrin (PEG-ZnPP)

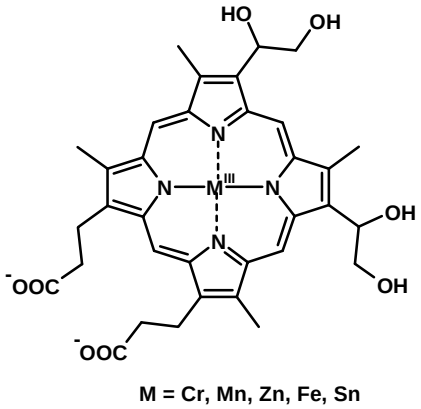


Fig. 28. Metallodeuteroporphyrin IX-2,4-bisethylene glycol

the aim of increasing their bioavailability, and blood-circulating half-life (Fig. 26).

Substitution with 1-(2-(2-(2-methoxy-ethoxy)ethoxy)ethyls in ortho positions of meso pyridyl and imidazolyl substituents significantly increased blood-circulating half-life and decreased unfavorable interactions with biological molecules. The presence of oxygen atoms in substituents on pyridyls and imidazolyls eliminated their surfactant-like properties. Consequently, they were not toxic in a simple model of oxidative stress,

SODdeficient E. coli. They possess the highest ability to disproportionate $O_2^{\bullet-}$ among *meso*-substituted porphyrins.

Selective delivery of 10B to tumours is one of the major remaining problems in boron neutron capture therapy (BNCT) of cancer. Because the porphyrins are selectively accumulated in tumours, they were used in connection with carboran units. The solubility was ensured with PEG substitution. Thus two series of carborane-carrying porphyrins (Threadgill *et al.* 2003) were constructed, with additional functionality for attachment of uncharged potentially water solubilising polyethers. *Meso*-substituted porphyrins carrying carboranes and oligo(ethylene glycol) units have been used for potential applications in boron neutron capture therapy.

Zinc protoporphyrin (ZnPP) was conjugated with poly(ethylene glycol) (PEG) with a molecular weight of 5000 kDa, to make ZnPP, a water-soluble compound (PEG-ZnPP), and to improve its tumor-targeting efficiency (Maeda *et al.* 2002), (Maeda *et al.* 2003), (Maeda *et al.* 2004) (Fig. 27).

The divalent zinc cation was chelated into the protoporphyrin ring to obtain PEG-ZnPP. PEG-ZnPP became highly water-soluble, and formed multimolecular associations with molecules larger than 70 kDa in aqueous media. PEG-ZnPP inhibited splenic microsomal HO activity *in vitro* in a competitive manner in the presence of hemin, with an apparent inhibitory constant of 0.12 μM. Most important, PEG-ZnPP injected intravenously significantly suppressed intratumor HO activity in a murine solid tumor model, which suggests that tumor-targeted inhibition of HO is possible with the use of PEG-ZnPP.

A number of metallo-deuteroporphyrins have been synthesized and tested for their ability to modulate HO (Maines 2005). For example, zinc deuteroporphyrin IX 2,4-bis glycol (Fig. 28) dramatically inhibits heme oxygenase activity. This structure which was prepared and tested in 1988 (Martásek *et al.* 1988) showed the highest inhibition of HO from prepared metallocomplexes. The zinc metallocomplex has been intensively explored in the field of HO (Atzori *et al.* 2004).

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The financial support from the Ministry of Education of the Czech Republic (grants no. MSM 0021620806 and 1M683785002), the Grant Agency of the Czech Republic (grants No. GA301/04/1315, GA303/05/0335) is gratefully acknowledged.

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