Optimal photosensitizers for photodynamic therapy : the preparation and characterization of novel photosensitizers derived from mesoporphyrin

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Chapter 1

General Introduction

§ 1.1 Biological role of iron porphyrins

Iron and magnesium porphyrins play an essential role in living organisms e.g.: the colored oxygen carrying part of hemoglobin in blood is heme (Figure 1.1). Heme is the Fe^{2+} complex of protoporphyrin IX. Four hemes are bound to the imidazole nitrogen of each of a unique histidine residue in the four peptide chains (two α-chains and two β-chains) in hemoglobin.[1,2] Whereas free heme is immediately oxidized by air to the iron (III) form, it is completely stable when bound in the active sites of hemoglobin.

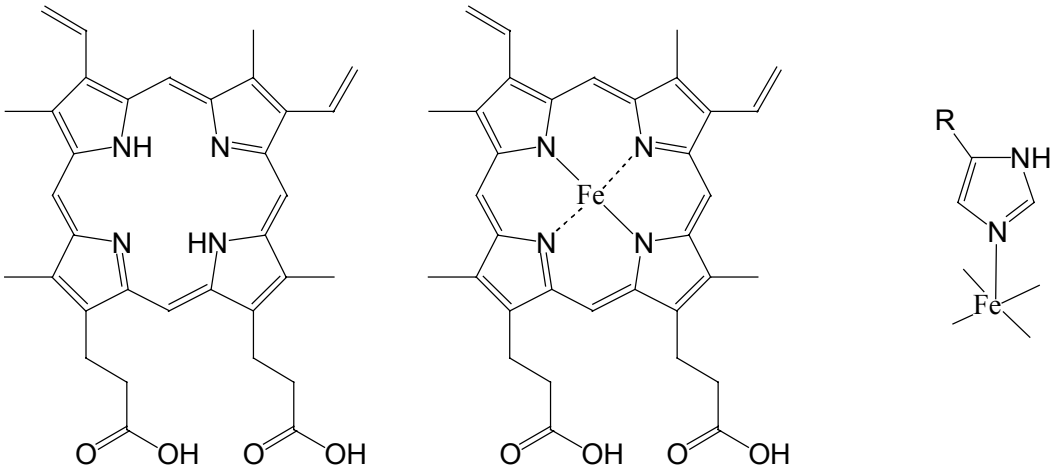


Figure 1.1 *Structure of protoporphyrin IX (left), heme (middle, Fe^{2+}-protoporphyrin IX) and coordination of histidine to the iron(II) in hemoglobin (right).*

The drastic difference in stability between the bound two-valent state and the instability of the free heme is nicely demonstrated by the denaturation of hemoglobin in blood with acetic acid. Due to denaturation the heme is set free and immediately oxidized by air to the Fe^{3+}-state. This form combines with the Cl^- ions in blood under formation of crystalline hemin where Cl^- forms the fifth ligand on Fe^{3+}, the so-called crystals of Teichmann (Figure 1.2). This process is carried out with cow and pig blood yearly on a multiton scale. This means that hemin is easily commercially available for a reasonable price as starting material for porphyrin research.

In our body hemoglobin is converted in our lungs into oxyhemoglobin, which has one of the oxygen atoms of O_2 as sixth ligand. This is transported to various organs, brain and muscles etc. where the free oxygen is released and is used as the oxidizer in the generation of the energy that powers our biological processes.

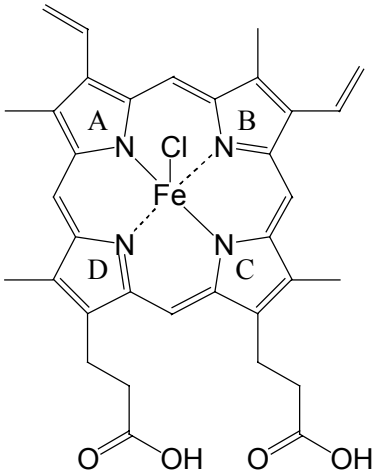


Figure 1.2 *Hemin is Fe^{3+}-protoporphyrin IX chloride*

Interestingly hemoglobin is paramagnetic (which means that it has unpaired electron spins), similarly oxygen is also paramagnetic. When these two paramagnetic species react they form the diamagnetic oxyhemoglobin.[1] It is interesting to realize that the oxygenation and the deoxygenation of hemoglobin are spin forbidden processes. In muscle cells the oxygen is bound in a similar way by the heme of myoglobin, which has only one peptide chain and one heme group. Myoglobin was the first protein of which the three-dimensional structure has been elucidated at atomic resolution with X-ray techniques.[3] The binding of the heme groups of myoglobin and hemoglobin with CO is much stronger than with O_2, even low concentrations of CO lead to irreversible binding and complete loss of oxygen carrying capacity resulting in death of the organism in question.

In our cells cytochromes transport electrons from electron-rich organic molecules to oxygen, which is reduced to H_2O.

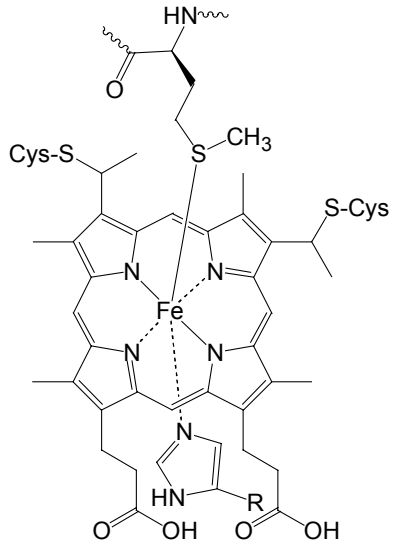


Figure 1.3 *Binding and coordination to heme in the reduced form of cytochrome c.*

Cytochromes contain heme groups that are coordinated to iron via the sulfur of a methionine residue and the nitrogen of a histidine residue. At the same time vinyl groups of the protoporphyrin groups have formed a covalent linkage with cystein residues of the protein (Figure 1.3).[4] The electron transport is vectorial: the Fe^{3+} of a cytochrome can take up an electron to form the Fe^{2+} complex, which transfers the electron to the Fe^{3+} of an other cytochrome while the Fe^{3+} of the former cytochrome is regenerated. In this way the electrons in our biological system are transported from electron-rich materials to the final oxidator, O_2. In the presence of traces of cyanide the Fe^{3+} form is irreversibly converted into Fe^{3+}-cyanide where the cyano group occupies the sixth ligand site. This form cannot take up an electron, which leads to a halt of the electron transport and the rapid death of the organism. The electron transport processes mediated by cytochromes take place in all living cells including those of plants, algae, cyanobacteria and photosynthetic bacteria.

§ 1.2 Biological role of magnesium porphyrin derivatives

In plants, algae and cyanobacteria magnesium containing porphyrins are present in the form of chlorophylls (Figure 1.4). They occur both in so-called antenna pigments and in the heart of photosystem I (PSI) and photosystem II (PSII). These membrane protein systems contain besides the chlorophyll system also carotenoids. Light is absorbed by the chlorophylls in the antenna pigments. The resulting electronic energy is transferred to photosystems I and II where the electronic energy is converted into redox equivalents, which serve the reduction of CO_2 in PSI and the oxidation of water in PSII. In this way oxygenic photosynthesis generates the electron rich organic molecules and the free oxygen, which serve both the material and energy needs of the organisms in biosphere. The products of the oxygenic photosynthesis either, contemporarily formed or as fossil materials (natural gas, oil and coal), are also the essential materials to allow the present worldwide economy to function. The processes involved in the use of electronic energy in the plant especially the photosynthetic reaction centers have to be very fine-tuned. If this tuning is not perfect a chlorophyll triplet state may be formed. This triplet species in the presence of air converts ground state triplet oxygen into singlet oxygen. Singlet oxygen is a very reactive oxygen species that destroys tissues. In order to prevent this as much as possible the photosynthetic reaction centers contain a carotenoid in their active site, which quenches the chlorophyll triplet state leading to both ground state

chlorophyll, ground state carotene and the excess energy by heat.[5,6] Similarly carotenoids can also quench singlet oxygen.

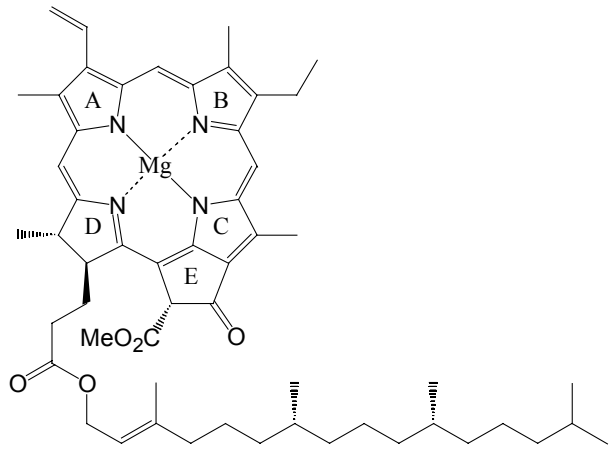


Figure 1.4 *Structure of chlorophyll a, compared with the structure of heme, an additional five-membered ring E is introduced. The D-ring has an sp^3-sp^3 single bond and the vinyl group on ring B is reduced into an ethyl group.*

The essential role of carotenoids in photosynthesis is dramatically demonstrated by the use of herbicides that either destroy the existing carotenoid or prevent *de novo* biosynthesis of carotenoids. Application of these herbicides on plants that are exposed to daylight leads within one day to the death of the plants involved[7]. This triplet formation occurs with metal free porphyrins and with the magnesium containing chlorophylls. Fortunately most heavy metal ions like iron II and iron III also shorten the life-time of the triplet state porphyrins to such a degree that no singlet oxygen formation takes place. In the case of Zn^{2+}-porphyrins the lifetime of the triplet state is shortened but not as drastic as in the heavier metal ions. In this way light absorbed by hemoglobin or other heme pigments does not result in this lethal action of light on living cells. However persons or animals with genetic defects that lead to the formation of free porphyrins in their bodies are highly light-sensitive[8].

§ 1.3 Photodynamic therapy

The lifetime of singlet oxygen in water is very short (3.1 μs).[9] This means that the damage of tissues occurs near to the place where the singlet oxygen is formed. If the porphyrin can be localized in tumors and locally irradiated this should result in efficient cancer therapy. Already mentioned earlier is the fact that hemin is commercially available for reasonable

prices at ton scale. Hemin is the right source to produce systems that may act as sensitizers in photodynamic therapy. First the iron has to be removed, which can be done by reducing the Fe^{3+} form with iron powder in the presence of concentrated sulfuric acid. In this highly acidic solution Fe^{2+} is removed from the porphyrin core and free protoporphyrin IX is formed (Figure 1.5).

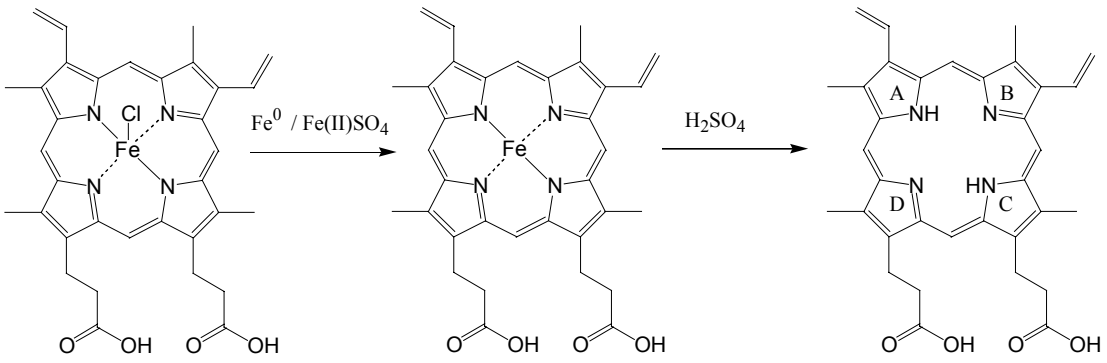


Figure 1.5 *Preparation of protoporphyrin IX from hemin*

Protoporphyrin IX does not fulfill the requirements for an efficient photosensitizer to generate singlet oxygen because singlet oxygen reacts with the vinyl groups on its A- and B-rings; leading to destruction of protoporphyrin IX itself. The solution is to treat protoporphyrin with H_2O under acid catalysis. The vinyl groups are electron rich and react with protons to form a carbenium ion (see Figure 1.6).

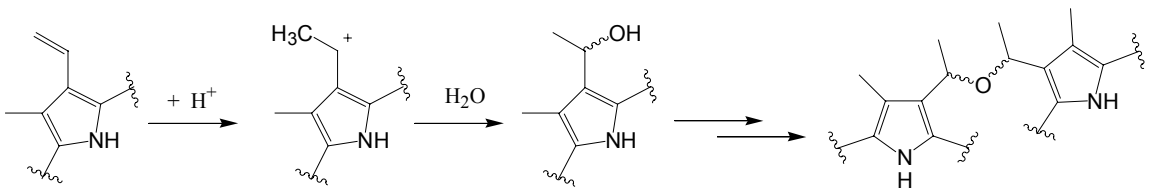


Figure 1.6 *Reactions of the vinyl groups of protoporphyrin under acidic circumstances leading to hematoporphyrin and photofrin©.*

H_2O can add to this carbenium ion to form a secondary alcohol. When both vinyl groups of the protoporphyrin are converted in this way hematoporphyrin is obtained. Further reaction will lead to an ether bond that links two porphyrins together. Also the carbenium function can attack a vinyl group linking porphyrins together via carbon-carbon bonds. Commercially Photofrin© is used as the basis of the present photodynamic therapy. It is a complex mixture that contains three or four porphyrin nuclei linked together. It should be realized that when only two porphyrins are linked together already 10 different dimers are involved.

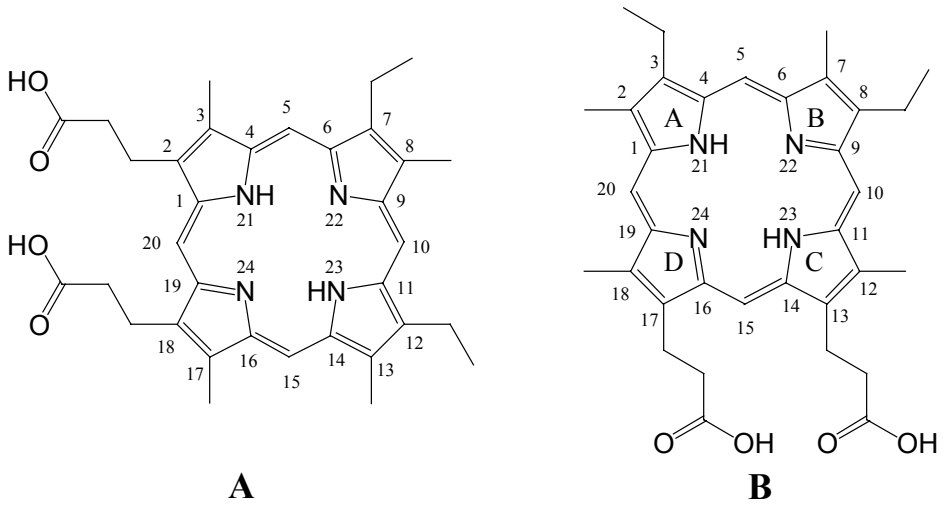
Photofrin© is used clinically as the basis for photodynamic therapy. However it has many drawbacks. First the system does not discriminate very well between tumors and healthy tissue. It is also present in the skin such that the patient remains very sensitive towards light during a long time. Secondly it is not a single pure compound and it is also acid sensitive. Acid induces the reversible dehydration of hydroxyethyl groups leading to the formation of vinyl functions. Thirdly, the light absorption of Photofrin© is far from optimal: it has an absorption maximum at 630 nm in the red part of the visible spectrum, while the penetration of light into tissue becomes appreciable at wavelengths longer than 650 nm (in the far red) with a maximum around 800 nm (near IR).[10,11]

The aim of this thesis is to develop a photosensitizer that is:

A) Non-toxic in the dark,
B) Stable both in the dark and in the light and especially towards the singlet oxygen that is generated by irradiating the system.
C) It should be rapidly cleared out of the body. In this way the patient will not be light sensitive after the photodynamic light treatment.
D) The required systems should have the easily available protoporphyrin IX as starting material for its synthesis.
E) It should preferably have an absorption maximum at wavelengths longer than 650 nm.

§ 1.4 Nomenclature of porphyrins[12]

Simple porphyrins of biological origin have trivial names such as protoporphyrin IX; its structure is depicted in figure 1.5. The systematic IUPAC nomenclature of protoporphyrin IX is 7,12-divinyl-3,8,13,17-tetramethylporphyrin-2,18-dipropionic acid (see IUPAC rule TP-1.7).[12] Most of the novel porphyrins described in this thesis are derived from mesoporphyrin IX (or mesoporphyrin). In figure 1.7 the structure of mesoporphyrin is depicted. Its systematic IUPAC name is 7,12-diethyl-3,8,13,17-tetramethylporphyrin-2,18-dipropionic acid. The systematic IUPAC numbering is given in figure 1.7A. However also a semi-systematic numbering exists. This is displayed in figure 1.7B. For the new mesoporphyrin derivatives the semi-systematic nomenclature based on mesoporphyrin is used. In chapter 4 the numbering of the various porphyrin derivatives is based on the semi-systematic IUPAC numbers with an extension that allows a simple assignment of the various ^{1}H NMR resonances.



A **B**

Figure 1.7 *Structure and numbering of mesoporphyrin:*
A) *according to the systematic IUPAC nomenclature*
B) *according to the semi-systematic IUPAC nomenclature*

§ 1.5 Outline of the thesis

In **Chapter 2** is discussed how nickel (II) mesoporphyrin which is easily accessible via well known reactions starting from protoporphyrin IX can be converted into mesoporphyrins with acrylonitrile groups on a mesoposition. Via acid induced reaction these systems could be converted into novel quino[4,4a,5,6-*efg*] annulated 8-deethyl-7-demethylmesoporphyrin. These unique systems show outstanding properties as sensitizers for photodynamic therapy in the far-red region of the visible spectrum.

In **Chapter 3** Ni(II) mesoporphyrin with a meso acrylonitrile side-chain was treated with the Vilsmeier reagent derived from methylphenylformamide. This process leads to an other completely novel 2'-cyano-8'-formyl-N'-methyl tetrahydroacrido-[4,5,5a,6-*bcd*] annulated 2,3,-dihydromesoporphyrin. Once more these systems which are completely different from those described in chapter two show outstanding properties to serve as basis for photodynamic therapy in the far-red region of the visible spectrum. Similar treatment with the Vilsmeier reagent derived from dimethylformamide was also investigated and it was found that the two Vilsmeier reagents differ in their initial attack on the appending acrylonitrile function. Whereas the Vilsmeier reagent derived from the aromatic formamide has its initial attack on carbon two of the acrylonitrile group; the initial attack of the Vilsmeier reagent is on the nitrile function. The latter reaction leads to simple conversion into similar systems as

described in chapter two. In this way the number of possible quino-annulated porphyrin derivatives is greatly expanded.

In **Chapter 4** very interesting ^{1}H-NMR-properties of protoporphyrin, mesoporphyrin and deuteroporphyrin are described. As mentioned before mesoporphyrin is obtained by hydrogenating the vinyl groups of protoporphyrin into ethyl groups. In deuteroporphyrin the vinyl groups on rings A and B of protoporphyrin have been completely removed.

The study of these molecules as potential systems for photodynamic therapy was started before the systems described in chapter 2 and 3 were discovered. In order to increase the solubility in water of this system first the amidation of the propionic functions on ring C and D with the amino group of protected glycine derivatives were attempted. After the failure of the direct introduction of the glycine group an indirect method was tried in which the reaction of the propionic acid chlorides were treated with 2,2-dialkoxy ethyl amine, which after deprotection and oxidation of the free aldehyde group should lead to the required glycine derivatives. However the ^{1}H-NMR-spectra to characterize the first bis-2,2-diethoxyethylamide derivatives showed such interesting properties that we studied a whole series of similar proto- and mesoporphyrin derivatives, all of them in the free base, and the Zn^{2+} coordinated form. The results of this study are presented and discussed in Chapter four.

In **Chapter 5** a general discussion about the properties of the systems described in chapters 2 and 3 is given. A future outlook for the possibilities of the use of the above-described systems in clinical photodynamic cancer treatment is given.

§ 1.6 References

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